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EDITORIAL

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The Role of Hyaluronic Acid Injection for the Treatment of Tendinopathy

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SUMMARY
Hyaluronic acid has carved out an essential, though sometimes discussed, role in the treatment of joint degenerative pathology. Recent studies, first in vitro, then preclinical, have paved the way for use in tendon pathology. Clinical experience to date has shown extremely encouraging results in different tendinopathy frameworks such as tenosynovitis, insertional tendinopathies and tendon mid-portion.

KEY WORDS
Hyaluronic acid; injection therapy; tendinopathy; tendon pathology; tenocytes; therapeutic option.

INTRODUCTION

Hyaluronic acid (HA) plays a key role in the joints, where it maintains the functional and metabolic interaction between synovial membrane, synovial fluid, cartilage and, indirectly, subchondral bone.
In the past, this molecule was extracted from rooster combs after grinding and chemical treatment, while today is mainly produced through bacterial fermentation processes (1, 2).
Most of the physiological effects of exogenous HA and its viscosity depend primarily on the molecular weight of the molecule, consequently influencing its possible applications (3, 4).
Intra-articular hyaluronic acid (HA) injections are widely used in the conservative treatment of osteoarthritis and could delay surgical treatment (5, 6).
The efficacy is related to possible disease-modifying effects, secondary to modulation of inflammatory response and to the direct and indirect effect on synovial tissue, bone and cartilage (7-16).
Recently, the interest about the applications of HA in tendon pathologies is increasing. This could be in part related to the emerging preclinical evidences on the interaction with tenocytes and tendon behaviour and in part to the increased use of ultrasound (US). Indeed, US allows better accuracy, helping to achieve better outcomes also for less approachable locations. Our US-guided technique permitted a real-time visualization of the needle during the procedure, ensuring the correct distribution of the product, with low-risk of failure to inject in the desired area.
In this brief editorial, we present, according to ethical standard of the journal (17), the state-of-art of the growing body of evidences in the field and the possible implications in clinical practice.

IN VITRO AND PRE-CLINICAL STUDIES

In recent years, several Authors have investigated the effects of HA on tendon cells relating to biomechanics, cell regeneration and proliferation.
Multiple findings support the role of HA in gliding resistance of tendon sheets decrease (18-21). The antiadhesive effect seems dose-dependent (20, 22).
Similarly, HA may determine increase tenocyte proliferation and vitality in a dose-dependent manner not related to molecular weight (23).
Anti-inflammatory properties of HA and its therapeutic biomolecular targets were also investigated.
Nakamura *et al.* compared the effects of corticosteroids and HA in human tendon fibroblasts from rotator cuff tears after surgical lesion. The Authors found that, both HA and corticosteroids induce anti-inflammatory and anti-adhesive effects on tendon and synovial fibroblast, whether cortico-

steroid cause biomechanical weakening of the torn rotator cuff tendons, causing apoptosis of the tendon fibroblasts at the ruptured sites (24).
Po-Ting Wu *et al.* showed that high molecular weight HA significantly downregulated the mRna and protein expression of MMP-1 and 3 in a dose-dependent manner, two major endopeptidases implicated in pain generation and tendinopathy cleaving ECM proteins and collagen (25).
Osti *et al.* tested different HA formulations, observing that HA enhanced viability, proliferation and expression of collagen type I in tendon derived cells from H_2O_2-induced oxidative stress, decreasing cytotoxicity, reducing Nrf2 expression and enhancing catalase recovery (26).
Several trials focused on the ability of HA to decrease adhesion formation in different animal models (27-29).
In a model of Achilles tendon rupture, healing time in the HA group was shorter probably due to the early termination of the inflammatory phase (30). Furthermore, the repetitive administration of sodium hyaluronate during the Achilles-tendon healing process could regulate angiogenesis increasing VEGF and type 4 collagen expression (31).
Repeated peri-patellar injections of HA in detrained patellar tendon may limit detrained-associated damage in tenocytes and maintain tenocyte anabolic activity during detraining (32). In a second study, Frizziero *et al.* showed that repeated peri-patellar injections of HA may maintain e structural and functional properties of patellar tendon and enthesis in detrained rats (33).

CLINICAL STUDIES

Rotator cuff tendinopathy

Meloni *et al.* found that ultrasound-guided HA injections in supraspinatus tendinosis may determine improvement in symptoms and disability until 9 months of follow-up compared to placebo (34).
Similarly, a 5-week HA injection protocol showed efficacy compared to placebo in rotator cuff partial tears in a placebo-controlled trial (35, 36).
Merolla *et al.* compared ultrasound-guided subacromial injections of HA and physiotherapy, founding that both treatments determine pain relief and clinical scores amelioration in the short term, while only HA group maintained a significant improvement at 12 weeks of follow-up (37).
Özgen observed that HA injection and physical therapy present similar effects in short and long term for supraspinatus tendinopathy (38). Flores *et al.* found that the combination of HA with an exercise protocol is superior to exercise only, leading to an earlier return to pre-injury activity and the need of less rehabilitation sessions (39).
Frizziero *et al.* found that both HA and low-energy ESWT are effective in improving joint function and reducing pain in patients with non-calcific rotator cuff tendinopathy until 3 months of follow-up, with no clinically significant difference (40).

Plantar fasciopathy

Two recent studies evaluated the effects of HA on plantar fascia pathology. Kumai *et al.* found that 5 ha injections determine symptoms relief with a dose-dependent improvement (41).
Raeissadat *et al.* compared ultrasound-guided Injection of high molecular weight HA versus corticosteroid observing that both corticosteroid and HA were effective in improving pain and function and decreasing plantar fascia thickness. However, corticosteroids seem to have a faster trend in the short term with no significant difference 24 weeks after the treatment between the groups (42).

Epicondylitis

In the study of Petrella *et al.* patients that received HA injections had significantly greater improvement in VAS pain at rest and after grip testing than control placebo group, that persisted to 1 year follow up (43).
Khan *et al.* observed that a single injection is effective in management of moderate pain (VAS score < 7), but not severe lateral epicondylitis (44).
Tosun *et al.* compared the effects of a combined HA- chondroitin sulphate injection versus a corticosteroid injection founding that both treatments were effective in reducing pain and improving function in short-term while HA was superior in long term follow-up (45).

Achilles tendinopathy

Lynen *et al.* compared safety and efficacy of 2 HA peritendinous injections respect to ESWT in mid-portion Achilles tendinopathy. HA injections showed greater outcome in short- and long-term with higher pain relief and function improvement until 6 months of follow-up (46).
Similarly, Fogli *et al.* and Frizziero *et al.* found that three US-guided HA injections induce prompt improvement in pain (NRS), symptoms and function (VISA-A, VISA-P and EQ-5D-5L) and US parameters (47, 48).
Good results were also reported in Ayyaswamy *et al.* with a single peritendinous injection of HA for non-insertional Achilles tendinopathy (49).

Patellar tendinopathy

Kumai found that a single HA was effective and safe in patellar entesopathy a week after treatment (50).
In accordance to Achilles tendinopathy, Fogli *et al.* and Frizziero *et al*. found that three US-guided HA injections induce prompt improvement in pain (NRS), symptoms and function (VISA-A, VISA-P and EQ-5D-5L) and US parameters (47, 48).
Kaux *et al*. compared platelet-rich plasma injections and hyaluronic acid injections under US guidance, evidencing that even both treatments could ameliorate symptoms, PRP group had significant improvement in quadriceps strength while HA seemed to have a prompt effect in pain-relief (51).

Tenosynovitis

Callegari *et al*. examined the efficacy and safety of ultrasound-guided HA and corticosteroid injection and compared with open surgery for the treatment of trigger fingers. Injection therapy was associated with a shorter recovery time, with a consequent reduced absence from sports and work activities and fewer complications (52). In accordance, other Authors found that HA achieved similar effect as steroid injection in trigger finger with a long-lasting functional improvement (MHQ scores continued to increase in the HA group at 3 months follow-up) without adverse events, until 6 months of follow-up (53, 54).
Orlandi *et al*. compared the 6 months outcome of three different ultrasound guided percutaneous injection treatment for de Quervain's disease (steroid alone, steroid with saline, steroid with HA). At 6-month follow-up, patients treated with steroids and HA injections had significantly better VAS score, quick DASH score and retinaculum thickness compared to other groups (55).

PERSPECTIVES

Therapies for tendinopathies keep changing as research in this field progresses. To date, different injective substances have been investigated: platelet-rich plasma, high volume image-guided injections, hyaluronic acid, and prolotherapy, as a suitable option beside the commonly used eccentric loading rehabilitation regimen (56).
Preclinical and clinical findings appear to be promising for hyaluronic acid, especially for Achilles and patellar tendon pathology.
Considering that pre-clinical findings suggest dose-dependent effect and most of clinical studies used repeated injections protocol, it seems that more than 1 injection should be considered in clinical practice to maximize the efficacy.
Actually, no clear indication about the correct molecular weight could be provided. In *in vitro* studies all the molecules were effective, while no comparison in clinical trials has never be performed to our knowledge. However, the rationale for HA in tendon is the possible effect on tendon cells, collagen structure and resistance gliding and we speculate that low to medium molecular weight may be prefer, considering that no "viscosupplementation" effect is necessary.
Further studies with large cohorts of patients for adequately long follow-up periods are needed to reinforce the present positive clinical results.

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ORIGINAL ARTICLE

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Randomized Trial of Hymovis® *versus* Synvisc® on Matrix Metalloproteinases in Knee Osteoarthritis

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SUMMARY

Background. This study aims to evaluate the effects of HYADD 4-G, a not chemically cross-linked HA (Hyaluronic Acid) derivative, and hylan G-F 20, a cross-linked HA product, on synovial matrix metalloproteinases and cytokines in patients suffering from knee osteoarthritis.

Methods. 31 patients were randomized to receive HYADD 4-G or hylan G-F 20. Synovial fluid was collected before and one week after the first injection. Activity of MMP-3, MMP-2, MMP-13, IL-6 and other cytokines were measured. Changes in synovial fluid neutrophils and lymphocytes were analyzed. The VAS, the WOMAC questionnaire, and a Physician Global Assessment (PhGA VAS) were recorded.

Results. A trend towards a greater decrease in MMP-3 activity was observed in the Hymovis® group. Active MMP-2 and MMP-13 decreased in both groups. IL-6 levels also decreased significantly in both groups. Median change in neutrophils from baseline was significantly different between the treatment groups. No differences between the treatment groups were observed for the WOMAC, VAS and PhGA VAS scores. No serious adverse events were reported.

Conclusions. These findings demonstrate that intra-articular HA injections in patients with knee osteoarthritis tend to protect cartilage structural integrity reducing the activity of key proteolytic enzymes implicated in cartilage degradation as well as inflammatory mediators.

KEY WORDS

Hyaluronic acid; knee; metalloproteinases; osteoarthritis; sodium hyaluronate; cytokines.

INTRODUCTION

Osteoarthritis (OA) is the most common degenerative musculoskeletal disease (1), with an estimated prevalence ranging from 5% for hip OA, 33% for hand OA and up to 50% for knee OA in subjects over 65 years of age (2). Joint tissue integrity can be lost on a traumatic basis or, more frequently, because of chronic inflammation mediated by soluble factors including cytokines, chemokines and degrading enzymes (3). Among the latter, tissue remodeling matrix metalloproteases (MMPs) are key players in OA pathogenesis. They include collagenases (MMP-1, 8, -13), gelatinases A and B (MMP-2 and -9 respectively) and the stromelysins (MMP-3, -10, -11). When activated, MMPs recognize specific sequences in extracellular matrix proteins and mediate their cleavage (4, 5). Collagenases cleave the collagen triple helix, which can be then further degraded by the gelatinases. Stromelysins have broad specificity against non-collagen matrix components such as fibronectin, elastin, laminin, and aggrecan.

All are greatly over-expressed in OA joints. The imbalance between them and their endogenous inhibitors (α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs)), are among the major causes of the degradation of type II collagen in OA (6).

MMP-3 activity is linked specifically to proteoglycan loss. In addition, MMP-3 contributes to the activation of proM-

MP-13, producing an amplification effect. Moreover, MMP-3 expression in synovial tissue of OA patients was positively correlated with the severity of OA, highlighting its contribution in OA pathogenesis (7). OA is also characterized by synovial inflammation (8). Many inflammatory mediators, including IL-1α, TNF-β, and IL-6, are involved in the development and maintenance of OA by inducing the expression of genes encoding for inflammatory proteins and cartilage-degrading enzymes, such as MMPs.

Common OA symptoms include pain and/or stiffness, which in later stages of the disease can be present also at rest. Symptomatic treatment primarily consists of non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular injections of corticosteroids. However, the short-term effect along with the associated side-effects of these treatments, in particular in an aged population, has generated interest in alternative treatments, such as viscosupplementation through intra-articular hyaluronic acid (HA) injections to restore the viscoelastic properties of synovial fluid (SF) (9-11).

Previous *in vitro* studies have shown that HA exerts an inhibitory effect on several MMPs (12, 13) and HA treatment has been recently shown to reduce MMP-2 levels in SF of patients affected by knee OA (14).

However, the effects of HA preparations may differ depending on method of production, treatment schedule, molecular weight, half-life within the joint, rheological properties and pharmacodynamics (15).

Hymovis®, a CE-marked and FDA-approved viscosupplementation preparation for intra-articular injection in OA patients is a gel made of linear HA partially chemically modified with an alkyl chain. The main component of Hymovis® is the partial hexadecylamide of HA, named HYADD4p5 (16).

The polymer structure of this derivative suggests that the alkyl side chain could selectively insert into the hydrophobic pocket of the catalytic site of the MMP and that the HA carboxyl group could act as a zinc-coordinating moiety in the MMP catalytic domain. *In vitro* studies confirmed that Hymovis® exerts an inhibitory effect on several active MMPs (17) and a strong inhibition of MMP activity and expression (*i.e.* MMP-8 and MMP-13) by human OA chondrocytes and synovial fibroblasts has been described (11). These effects have not been yet confirmed in human studies. The aim of the present open-label pilot study was to evaluate the effect of a single intra-articular injection of Hymovis® in comparison with Synvisc®, a gel mixture of cross-linked HA and high molecular weight HA, on local generation of cartilage-degrading MMPs in the synovial fluid of patients with knee OA through the measurement of concentrations of specific metalloproteinases (MMP-2, MMP-3, and MMP-13).

METHODS

Study population

The study was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice, UNI EN ISO 14155:2011 and was approved by the Ethical Committee of the authors' affiliated institution (Comitato Etico delle Aziende Sanitarie della Regione Umbria, CEAS Umbria obtained on 21st of May 2014). Patients were recruited at a single center and gave their written informed consent.

Inclusion criteria were: age > 50 years, diagnosis of idiopathic knee OA according to the American College of Rheumatology diagnostic criteria (18) at least 1 year before inclusion, presence of osteophytes confirmed by radiography, morning stiffness of ≤ 30 minutes duration or crepitus on motion, Kellgren-Lawrence stage II-IV confirmed by an X-ray performed within 6 months prior to inclusion, presence of knee effusion confirmed by ultrasonography (US) at the time of enrollment, absence of intraarticular corticosteroids for at least 3 months and/or HA for at least 6 months prior to enrolment.

Exclusion criteria were: inflammatory rheumatic disorders, secondary OA, contraindications to intra-articular injections, therapy with systemic NSAIDs or corticosteroid in the week prior first treatment injection, cognitive impairment, hemoglobin levels < 12 g/dL in males and < 11 g/dL in females in the 3 months prior to enrolment, oral anticoagulant therapy, pregnancy, infectious diseases. Rescue therapy was allowed according to the physician's opinion but not within 24 hours prior to any visit.

The sample size for this study was estimated as a minimum of 26 patients to be enrolled, based on the MMP-2 activity levels reported in a previous publication (17), considering a 90% power, a significance level of 0.05, and a 30% drop-out rate.

The study meets the ethical standards of the journal. In particular, all experimental procedures were carried out according to the journal guidelines (19).

Study design

Primary endpoint

The primary endpoint of the study was the effect of a single administration of Hymovis® on the activity of metalloproteinase MMP-3 in the SF of the knee compared to Synvisc®.

Secondary endpoints

Secondary endpoints being measured to determine the effect of one intra-articular injection of Hymovis® were the levels of expression and activity of the metalloproteinase

MMP-3, MMP-2 and MMP-13, as well as cellular count and the expression of pro-inflammatory cytokines in the SF.
To determine the effect of Hymovis® treatment, endpoints being measured were the reduction of pain perceived by the patient, the joint mobility and patient's physical functioning, and treatment safety.

Treatment

Eligible patients were randomized to receive open-label treatment with either Hymovis® (Fidia Farmaceutici SpA, Abano Terme, Italy) or Synvisc® (Sanofi, Bridgewater, NJ, USA). Patients randomized to the Hymovis® treatment arm received two intra-articular (i.a.) injections administered one week apart (days 0 and 7) while patients randomized to the Synvisc® treatment arm received three intra-articular injections administered one week apart (days 0, 7 and 14) (**figure 1**).

Ultrasonography-guided intra-articular injection was performed according to standard techniques. Prior to injection at visit 1 (baseline, day 0) and at visit 2 (day 7) a small sample of SF (3-5 ml) was collected.

Laboratory assessment

SF was collected in sterile Vacutainer tubes containing heparin for MMPs measurement or 0.18% K3EDTA for cell count.
To assess MMP levels, samples were pre-treated using hyaluronidase 250 U/mL at 37 °C for 10 min (5) to reduce sample viscosity and improve homogeneity, centrifuged, and the supernatant was used for MMP testing. If necessary, samples were diluted to fit the standard curve. Three replicates were analyzed for each time point. Active MMP-3 levels were measured using a Sensolyte® 520 fluorimetric kit (Anaspec, Fremont, California, USA; cat. AS-71152)

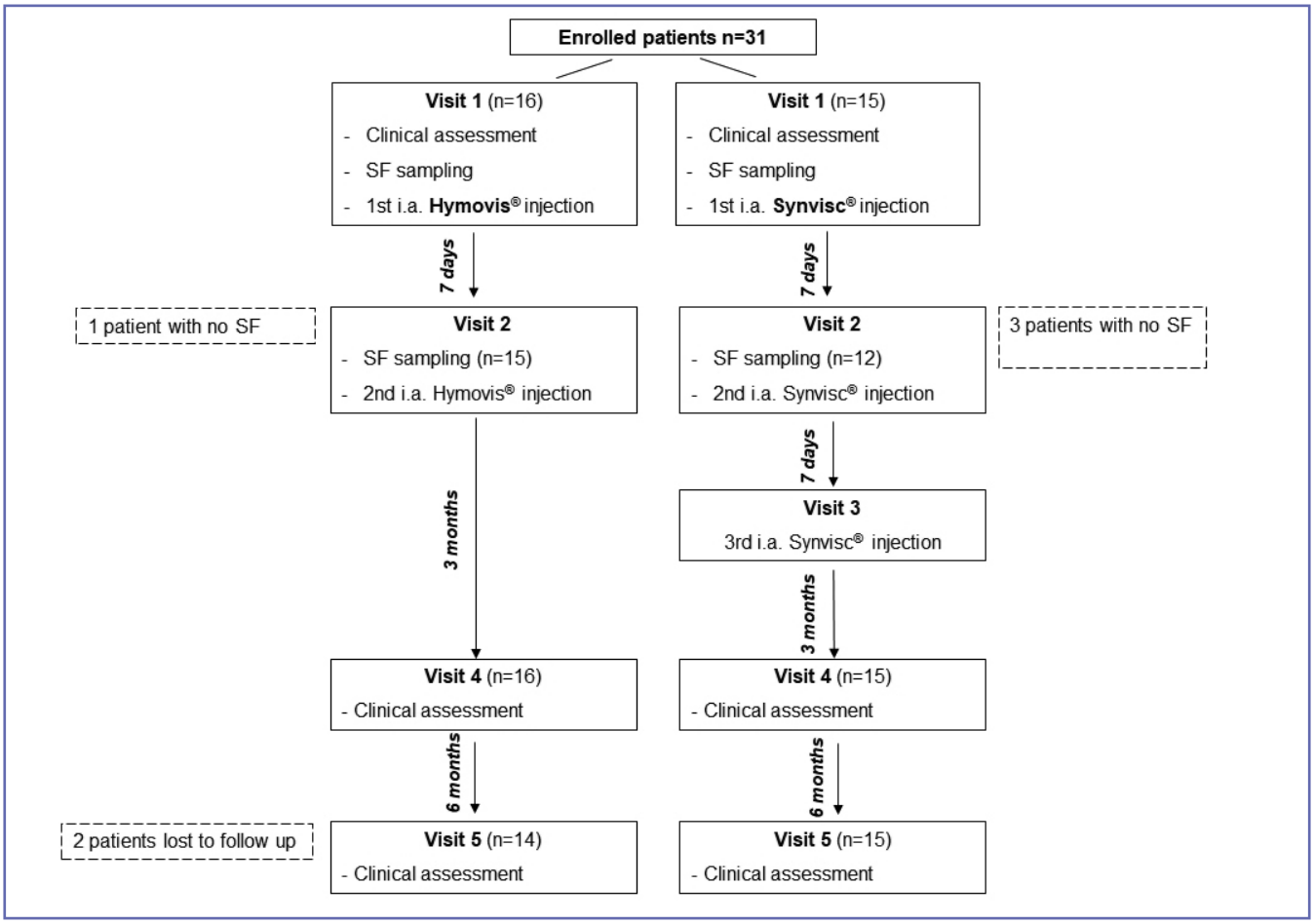

Figure 1. Flow chart of the study. Sixteen patients were treated with Hymovis® and 15 with Synvisc®. Reassessment followed after 1 week. Two patients in the Hymovis® group were lost to follow up after the 3-month follow-up visit.

that uses a 5-FAM (fluorophore) and QXL®520 (quencher) labelled fluorescence resonance energy transfer (FRET) peptide substrate for measurement of enzyme activity, showing high specificity for MMP-3. Fluorescence recovery upon cleavage of the FRET peptide by MMP-3 was monitored at excitation/emission = 490 nm/520 nm. Active MMP-3 was quantified against a standard curve built using human MMP-3 enzyme, pre-activated with 4-aminophenylmercuric acetate (APMA).

Total MMP-3 levels were assessed using a Sensolyte® MMP-3 ELISA kit (Anaspec, cat. AS-72103) and quantified against a standard curve built using human MMP-3.

The active and total MMP-2 and MMP-13 were measured using the Sensolyte® Plus 520 MMP-2 fluorimetric kit (Anaspec, cat. AS-72224) and Sensolyte® Plus 520 MMP-13 fluorimetric kit (Anaspec, cat. AS-72019), respectively. Both kits (ELISA assay), use a specific monoclonal anti-human MMP antibody to pull down MMP-2 or MMP-13, respectively, from the mixture. MMP activity is then quantified by a 5-FAM/QXLTM 520 FRET peptide. Total MMP-2 and MMP-13 in synovial fluid was quantified against a standard curve built using human MMP-2 or MMP-13 respectively, pre-activated using APMA. A validation of the recovery of MMP-13 activity, total MMP-13 level, and active and total MMP-2 activity was performed after hyaluronidase treatment, because a specific validation of this kit in synovial fluid has not been reported.

Cytokine levels (IL-1β, IL-6, IL-8, TNFα and RANTES) were measured by flow cytometry using a FlowCytomix kit (Bender MedSystems,Vienna, Austria), according to the manufacturer's instructions.

For cell count, after hyaluronidase incubation (250 U/ml at 37 °C for 10 min) (20), 10 µL SF was added to 190 µL of Stromatol® solution (Mascia Brunelli Srl, Milan, Italy) and total cell number was counted by optical microscopy using a Bürker chamber (Neuroprobe Inc, Gaithersburg, Maryland, USA) with a 40x objective. Differential count was determined in smears of cytocentrifuged, hyaluronidase-treated SF after May-Grünwald staining. SF samples were also analyzed by flow cytometry (FC 500, Beckman Coulter, Miami, Florida, USA) for platelet and platelet-leukocyte aggregates counts.

Clinical assessment

At baseline (visit 1, day 0), visit 2 (day 7), visit 4 (month 3) and visit 5 (month 6) patients self-reported knee pain on a 100 mm visual analogue scale (VAS) with 0 mm indicating no pain and 100 mm unbearable pain and answered the 24-item Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire. This consists of three subscales: pain (five questions), stiffness (two questions), and physical function (17 questions). The WOMAC 3.1 Index measures the patient's response to each of the 24 questions on a 5-point Likert scale with higher scores indicating greater symptom severity (0=none to 4=extreme). The WOMAC normalized subscale and total scores were then calculated. Finally, the physician global assessment (PhGA) of how the treated knee affected the patient's status at the above time points was also assessed on a 0-100 mm VAS scale where 0 indicated "not at all" and 100 indicated 'extremely'.

Statistical methods

The analysis included data from all patients who received at least one injection of the study products and for whom a sample of synovial fluid at visit 1 (day 0) and visit 2 (day 7) was collected.

Descriptive statistics for changes and percent changes from baseline were used to compare treatment groups and pre and post treatment values. Non-parametric tests were used to make comparisons between treatment groups (the Mann-Whitney U test) and to compare values between baseline and after treatment within treatment group (the Wilcoxon signed rank test). Comparison against baseline at all time points was adjusted as per the Bonferroni method to 0.05/2=0.025. To minimize the high variability of the percent changes from baseline of active MMP-3, the Chauvenet's criterion was adopted. A Mann-Whitney U test was performed to compare treatment groups without detected outliers.

VAS, PhGA and WOMAC scores were analysed using the same non-parametric tests mentioned above. Data are expressed as mean ± standard error of the mean (SEM). A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Thirty-one patients (10 males, 21 females, age 72.6 ± 1.8 years, range 53-88) were recruited (**table I**). Of these, 16 were allocated to receive Hymovis® and 15 Synvisc®. Two patients in the Hymovis® group were lost to follow up after the 3-month follow-up visit. In addition, synovial fluid assessment (specifically for active MMP-3 levels) was not performed in 4 patients at visit 2 (1 Hymovis® and 3 Synvisc®) due to lack of a sufficient amount of US detectable SF. Therefore, 15 patients were included in the Hymovis® group and 12 patients in the Synvisc® group for the assessment of laboratory data.

Mean duration of OA was 5.7 ± 0.9 years. At baseline, 74% of patients had Kellgren-Lawrence grade 3, 16% grade 2

Table I. Baseline characteristics of patients included in the study.

	Hymovis® (n=16)	Synvisc® (n=15)	All patients (n=31)
Mean age (years)	73.9 ± 2.7	71.2 ± 2.4	72.6 ± 1.8
Sex:			
Male	7 (43.7%)	3 (20.0%)	10 (32.3%)
Female	9 (56.3%)	12 (80%)	21 (67.7%)
Mean body weight (kg)	67.0 ± 2.7	67.5 ± 2.5	67.3 ± 1.8
Mean BMI (kg/m^2)	24.9 ± 0.9	24.7 ± 0.6	24.8 ± 0.5
Mean duration of OA (years)	6.2 ± 1.5	5.2 ± 1.1	5.7 ± 0.9
Kellgren-Lawrence:			
Grade 2	3 (18.8%)	2 (13.3%)	5 (16.1%)
Grade 3	11 (68.8%)	12 (80.0%)	23 (74.2%)
Grade 4	2 (12.4%)	1 (6.7%)	3 (9.7%)
Mean interval between treatments 1 and 2 (days)	8.1	7.0	7.6

OA osteoarthritis; BMI body mass index. Data are expressed as mean ± SEM.

and 10% grade 4. 97% had crepitus on motion, 74% had clinically evident swelling and 68% morning stiffness.

During the study, 5 patients received prohibited medications, 2 in the Hymovis® group (steroid injection and NSAIDs) and 3 in the Synvisc® group (2 patients NSAIDs and one patient oxycodone). In all cases, medications were administered after receiving the last study injection.

Synovial fluid biomarkers

Platelet count and platelet-leukocyte aggregates, as well as total cell count, did not change significantly after treatment with Hymovis®. At microscopic analysis, a significant decrease in percentage of neutrophils (20.9 ± 2.2% *vs* 14.6 ± 2.9%, p=0.046) was observed following Hymovis® treatment while not in the Synvisc® group. The median change from baseline was significantly different between the treatment groups (Hymovis® -7.0% *vs* Synvisc® -3.0%, p=0.024).

Table II shows changes in total and active MMP-2, MMP-3 and MMP-13 levels for both treatment groups. No significant difference was observed for median active MMP-3 levels: percent change from baseline was -5.3% (range: -61.7% to +100.9%) for Hymovis® and +8.8 (range: -48.1% to +82.9%) for Synvisc®. A trend towards a greater decrease in MMP-3 activity was observed in the Hymovis® group, as active MMP-3 decreased in 9/15 and increased in 6/15 Hymovis® patients, compared with a decrease in 4/12 and increase in 8/12 Synvisc® patients. Reductions in active MMP-2 and active MMP-13 were observed in both groups. Overall there were no significative differences in total MMP-2, MMP-3 or MMP-13 between the treatment groups or within each treatment group.

Active MMP-2 levels tended to decrease in both treatment groups (**table II**), while active MMP-3 level decreased, although not significantly, in Hymovis® group and not in Synvisc® group. This trend was also confirmed when analyzing the percent variation against baseline adopting the Chauvenet's criterion, which detected one patient in the Hymovis® group as an outlier. After excluding this patient, a significant difference between treatment groups was evidenced (p=0.042) in favor of Hymovis® where a median decrease in activity of -10.6% was observed.

Moreover, the active MMP-3/pro-MMP-3 and the active MMP-2/pro-MMP-2 ratio, an index of MMP activity, tended to decrease (p=0.09 and p=0.14, respectively) after Hymovis® treatment (**figure 2**) while it did not change in the Synvisc® group (p=0.24 and p=0.62, respectively).

For MMP-13, no difference in active form and in active MMP-13/pro-MMP-13 ratio was observed in the two treatment groups (**table II**).

A significant decrease in IL-6 levels was observed at visit 2 compared to baseline in both groups, without difference between the two treatments (Hymovis® group: 3866 ± 1162 *vs* 1321 ± 387 pg/mL; Synvisc® group: 3908 ± 1045 *vs* 1849 ± 332 pg/mL). IL-6 levels decreased from baseline in 10/15 Hymovis® patients, and in 10/12 Synvisc® patients. None of the other inflammatory cytokines measured, IL-1β, TNF-α, IL-8 and RANTES, showed difference between treatment groups or when comparing post-treatment values to baseline within each treatment group.

Table II. Matrix Metalloproteinase (MMP) levels (ng/ml) at baseline and 7 days after treatment with Hymovis® or Synvisc®.

	Hymovis® (n=15)			Synvisc® (n=12)		
	baseline	Visit 2	p value (change within each group)	baseline	Visit 2	p value (change within each group)
MMP-2						
Active MMP-2	13.5 ± 1.2	10.0 ± 1.5	*0.084*	13.7 ± 1.7	9.4 ± 2.5	*0.26*
Pro MMP-2	636.5 ± 82	700 ± 107	*0.63*	553 ± 60	585 ± 73.5	*0.63*
Active/pro	0.025 ± 0.003	0.018 ± 0.003	*0.14*	0.016 ± 0.03	0.007 ± 0.037	*0.30*
MMP-3						
Active MMP-3	105.1 ± 8.2	84.9 ± 7.8	*0.25*	86.3 ± 7.1	91.7 ± 6.7	*0.42*
Pro MMP-3	1429 ± 332	1621 ± 303	*0.49*	1589 ± 521	1371 ± 425	*0.33*
Active/pro	0.13 ± 0.026	0.07 ± 0.01	*0.09*	0.09 ± 0.01	0.11 ± 0.01	*0.24*
MMP-13						
Active MMP-13	21.7 ± 2.3	20.9 ± 1.6	*0.97*	26.4 ± 2.8	22.3 ± 1.6	*0.23*
Pro MMP-13	50.9 ± 4.7	60.1 ± 6.1	*0.072*	47.4 ± 4.4	47.9 ± 4.7	*0.93*
Active/pro	0.47 ± 0.06	0.41 ± 0.06	*0.30*	0.59 ± 0.07	0.52 ± 0.06	*0.51*

Data are expressed as mean ± SEM.

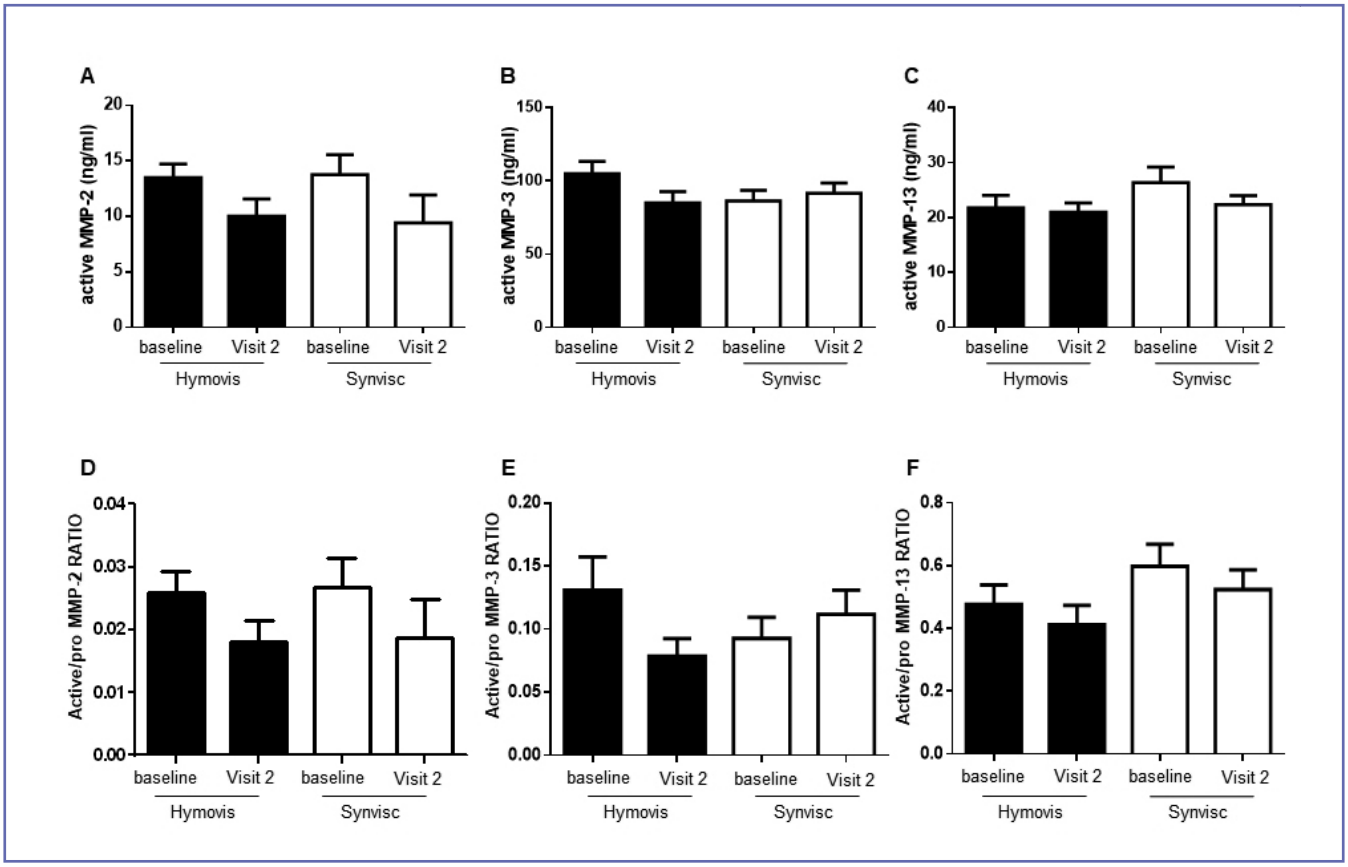

Figure 2. Levels of metalloproteinases after Hymovis® and Synvisc® injection compared to baseline. Active MMP-2 (A), MMP-3 (B), MMP-13 (C) levels and their ratio *versus* proenzyme form (D, E, F). Data are reported as means ± SEM.

Clinical assessment

No differences between the treatment groups or after treatment in the treatment arms were observed for the WOMAC scores.

The percentage of Hymovis® treated patients with increased or decreased VAS perceived pain values at 3 and 6 months as well that concerning PhGA are shown in **figure 3.**

No serious adverse events, adverse device effects or device deficiencies were reported. There were 4 adverse events, 3 patients reported increased knee pain which was considered possibly related to administration (1 on Hymovis®, 2 on Synvisc®) and one patient in the Hymovis® group reported flu symptoms which were not considered to be related to treatment.

DISCUSSION

Viscosupplementation with HA-based formulations was shown to have variable degrees of efficacy in the therapy of knee OA (21-24). HA suppressed interleukin IL-1β-induced MMP activity in OA synovial tissue explants *in vitro* (25) and decreased the levels of MMP-2 in synovial fluid of OA patients (20). This finding suggested that viscosupplementation with HA could provide clinical benefit by blocking MMP activity in the OA joint. Hymovis®, an intra-articular formulation of HA, is a valid therapeutic option in the treatment of osteoarthritis based on its viscosupplementation properties, displaying a beneficial effect for up to 12 months (26). The current study aimed to investigate the effects of a single intra-articular injection of Hymo-

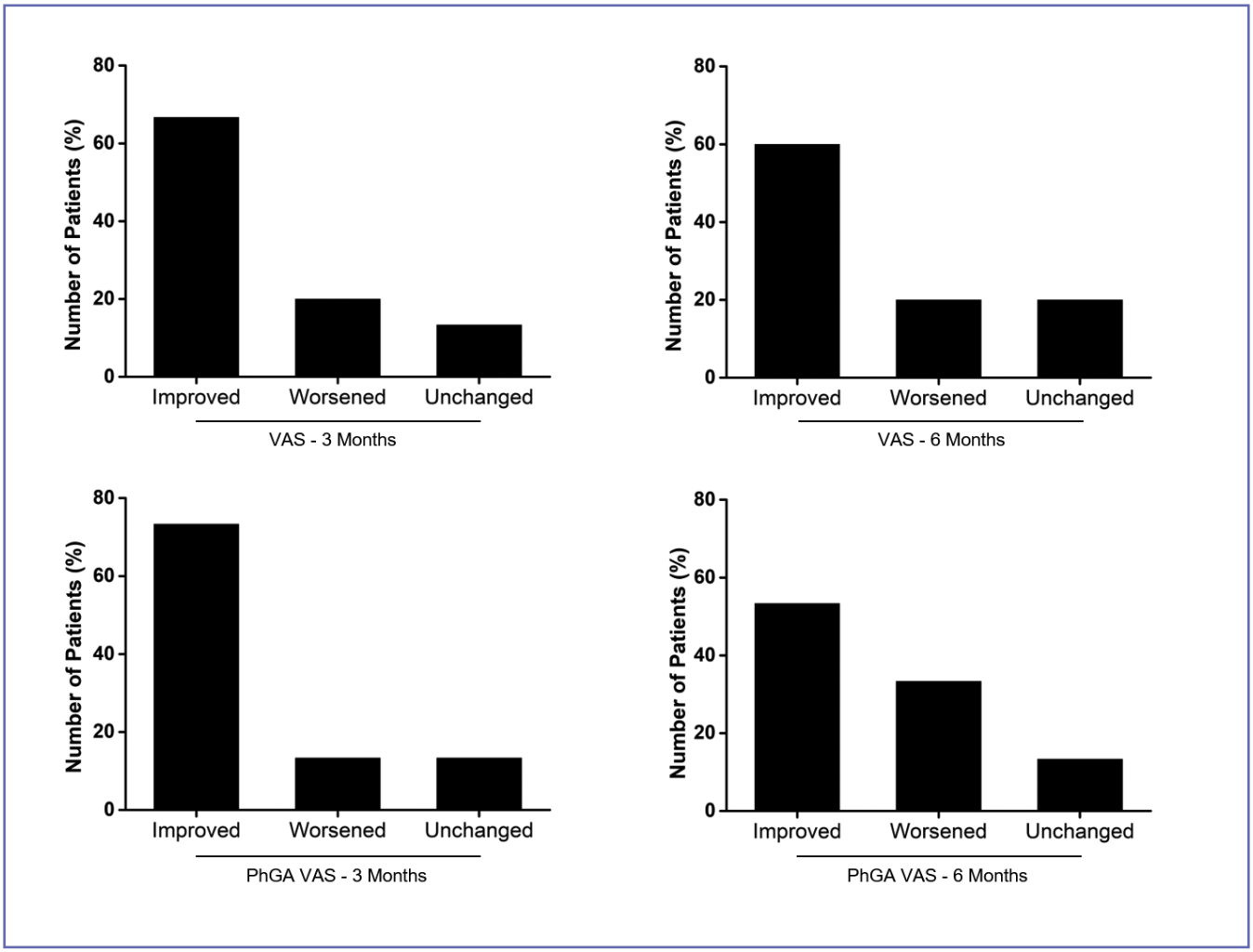

Figure 3. Number of patients (%) considered improved, worsened or unchanged in visual analogue scale (VAS) and physician global assessment (PhGA) at 3 and 6 months from baseline. VAS scores were compared using Wilcoxon pair data test.

vis® on the expression and activity of metalloproteinases in the synovial fluid of patients with OA of the knee and demonstrated that an i.a. injection of Hymovis® may have both symptom and chondroprotective effects in OA by decreasing MMPs. 60% of Hymovis® treated patients, in fact, had a decrease in active MMP-3 levels one week after the first intra-articular injection. Although not statistically significant, this trend was confirmed when excluding an outlier patient in the Hymovis® group using Chauvenet's method. Hymovis® treated patients also showed a reduction in active MMP-2 and active MMP-13 levels (67% and 53% of patients respectively), as well as a reduction of the active MMP-2/pro-MMP-2 ratio and the active MMP-3/pro-MMP-3 ratio indices of MMP activity. The dynamic balance of active-pro MMPs is the crucial factor in the regulation of MMPs' enzymatic activity, as the role of MMPs in pathophysiologic conditions does not depend on the absolute concentration of MMPs, but also on the active/proMMPs ratio.

These results are consistent with a previous *in vitro* investigation that demonstrated that HYADD-4G, a lead compound in a class of alkyl derivatives of HA, showed the highest inhibition potency towards all human MMPs and especially against MMP-13 and MMP-8 (17).

The significant reduction in neutrophils and IL-6 levels observed in the Hymovis® group may indicate a reduction in the inflammatory state in the synovial fluid of these patients.

Following treatment, there were no significant differences in OA related symptoms between the treatment groups. No serious adverse events, adverse device effects or device deficiencies were reported.

Our findings suggest that viscosupplementation using Hymovis® reduces pain in patients with knee osteoarthritis and protects structural integrity of cartilage by decreasing MMPs. A first limitation of the present study consists in the relatively small number of individuals being studied. Yet, our findings are consistent with previous reports, adding strength to our conclusions. Secondarily, the study was open-label, and this could have introduced a bias. The mechanism as well as the potential therapeutic value of HA suppression of MMPs and the chondroprotective effects of viscosupplementation remain to be elucidated by further preclinical and clinical data.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests. NG is an employee of Fidia Farmaceutici SpA. All the authors state, however, that Fidia Farmaceutici SpA did not participate in the decision to submit this manuscript for publication.

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ORIGINAL ARTICLE

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Muscle Injuries: 2020 Update of the I.S.Mu.L.T. Classification

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LEVEL OF EVIDENCE: 5

SUMMARY
It is becoming increasingly important to have a common terminology, as international and shared as possible, beginning with the classification of injuries. This can then aid in correct diagnosis, a focus prognosis, and appropriate therapy. The most adopted classification system within practice must thus be both accurate and complete but also clinically useful for those less familiar in injury management.
Muscle injuries are divided into two main categories according to the mechanism of onset: direct muscle injuries caused by the impact of a blunt body against the muscular belly and in indirect muscle injuries, caused by a phenomenon of overstretching or eccentric contraction causing internal disruption on the muscle-tendon unit.
For the clinician who faces these pathologies, it is essential to utilize a classification system which considers both the clinical findings and information from diagnostic investigations, such as ultrasound and MRI. The multi-modal approach to muscle injury assessment (clinical, ultrasound and possibly resonance imaging) seems essential for a correct clinical framework and the assessment of the severity of injury, to subsequently determine the most appropriate therapeutic pathway. In recent years, numerous classifications have been published, to attempt to arrive at a standardized common language, but in most cases it has failed to make all the professional figures involved in the dialogue on rehabilitation. For an appropriate classification system, it is important to adopt correct terminology and avoid terms such as 'elongation' or 'strain'.
The objective of this paper is not to deeply address the diagnostic and therapeutic approach of muscle injuries, but to renew the standard scientific language related to muscle injuries among the various professional figures involved in rehabilitation starting from the already shared I.S.Mu.L.T. 2014 muscle injuries classification (1).

KEY WORDS
Muscle injury; classification; sport injury; muscle trauma; prognosis.

INTRODUCTION

Muscle injuries are common, particularly during activities that involve high speed running, throwing and jumping (1, 2). Despite a wide variability between the different sports, the literature data report for them an overall amount of 10-55% of all acute injuries in athletes (3). These injuries frequently involve the lower limbs, particularly the hamstrings, quadriceps (rectus femoris) and gastrocnemius (medial head) muscle groups (4).

Recent scientific literature have demonstrated that the risk of a muscle injury is the result of complex interactions between different factors (5). Risk factors can be divided into intrinsic (related to athlete's characteristics) and extrinsic ones (related to the external environment) and they can be distinguished as modifiable and unmodifiable factors. The aim of injury prevention programs is to reduce or eliminate modifiable risk factors and control or over-compensate for non-modifiable risk factors (6). Age is an important predisposing factor for muscle injuries as a consequence of age related changes within the muscle, such as the re-arrangement of motor units and the denervation processes (7). A positive anamnesis for previous muscle injuries is related to a higher risk for other muscle lesions, both in the same muscular group and in other districts, because of the development of fibrotic tissues and/or acquired biomechanical alterations (8). Reduced muscle flexibility, joint range of motion and muscle strength are considered as modifiable risk factors, although the evidence is equivocal (9). Also, psycho-social factors (*e.g.* stressful events) appear to play a significant role for injuries (10). Higher levels of competition, lower recovery time between matches, higher intensity and overall volume of training time are associated with increased risk for muscle lesions (11). Match play carriers a much higher risk for muscle injury than training, for both adult professional and young players (12).

The diagnosis of a muscle injury is usually based on anamnestic data and clinical examination, supported by diagnostic imaging (13). The anamnesis focuses on the type of pain perceived, its localization and onset, the traumatic mechanism and the timing of the match or training. Furthermore, clinicians should investigate about history of previous muscle injuries of the same muscle group or adjacent joint muscles. Clinical examination is based on inspection, palpation, assessment of the range of motion, stretching and functional tests (14).

Diagnostic imaging is very important, not only for the early diagnosis and classification of the injury, but also to check the healing status, any complications during the treatment process, and to support prediction of return to play (RTP) times (15-16). Magnetic resonance imaging (MRI) is indicated when clinical tests and ultrasound information conflict (especially in professional athletes or in the case of muscles difficult to study in ultrasound such as psoas, obturators), as well as in the case of suspected subtotal or complete muscle injury, tendon involvement or bone-tendon unit avulsions (17).

Within the literature, there are a few classification systems which have been scientifically validated based on research demonstrating an association between the muscle injury grade classification and RTP times (18, 19). In order to promote an international terminology which could classify muscle injuries and aid in correct injury diagnosis, prognosis and rehabilitation treatment, the Italian Society of Muscles, Ligaments and Tendons (I.S.Mu.L.T.) proposed a new classification of muscle injuries in 2014 (1). According to the mechanism of injury, muscle injuries are distinguished in to two distinct categories: direct and indirect injuries. A direct injury is defined as involving an external force applied to the muscle and the injury therefore depends on the impact intensity, the state of contraction of the muscle, the traumatic moment and the injured muscle. For indirect injuries, typical of non-contact sports, the main cause of injury is due to internal eccentric muscular force generation, with the main injury site being the muscle tendon junction (MTJ) (20). The structural damage to the muscle fibres may be caused by a singular contraction or by the cumulative effect of multiple contractions (21).

A correct therapeutic approach is crucial to avoid complications and to prevent re-injury. Early complications include compartment syndrome, phlebitis and deep vein thrombosis. Late complications include fibrosis, intermuscular effusion, intramuscular cyst, calcification, intramuscular ossification and muscle hernia (22).

Most muscle injuries are managed conservatively with excellent results, with surgery advocated only for much larger tears. Conservative approaches include many different treatment modalities including POLICE (Protection, Optimal Loading, Ice, Compression, Elevation) protocols, stretching, functional rehabilitation and physical therapies, although there is no consensus on gold-standard management (23, 24). A pharmacological and nutritional approach is fundamental to control pain and to promote optimal healing by supporting and optimizing rehabilitation treatments and physiotherapy (25, 26). Contrary to the evidence (27), injection treatments for muscle injuries (*e.g.* platelet-rich plasma) have recently become popular, particularly in professional athletes, with the view to accelerating RTP times. The main indications for surgery are complete lesions and subtotal lesions associated with persistent pain and loss of strength after conservative management (28).

Whichever therapeutic approach is utilised, there should be a strong focus on incorporating re-injury prevention practices, considering all injury risk factors and their interactions,

through the use of a holistic approach to reduce the risk of re-injury. This should be for both the injured muscle, as well for reducing the risk of other musculoskeletal injuries (29). Prevention programs should be based on multimodal exercise protocols including eccentric training, high speed running, core stability, endurance, flexibility, proprioception, agility and coordination (30, 31). Moreover, a specific warm-up before a training session or match can significantly reduce the risk of injury (32). The study was conducted according to the journal's standards (33).

Rationale for an update of the I.S.MU.L.T. classification of muscle injuries

The I.S.Mu.L.T. classification and guidelines for muscle injuries have provided the scientific world very useful indications and suggestions on epidemiology, diagnosis, classification and management of muscle injuries (1). However, recent reviews of the literature has highlighted the need for an update of this classification system, with a particular focus on three key area.

Firstly, there is a need to focus more precisely on the injury location, distinguishing between proximal, middle and distal sites of the muscle. Indeed, research has highlighted that muscle injuries which occur in the proximal area of the hamstring or rectus femoris muscles are associated poorer prognosis, whilst injury of the triceps surae muscle to the distal regions is associated with longer recovery time (34).

Secondly, it is important to distinguish between the damage tissue type. In particular, ultrasound and MRI can aid in distinguishing between myofascial, muscle fibers, MTJ, and tendon involvement (35).

Thirdly, recent research reports that distinguishing between primary injury and a re-injury is crucial for prognosis and recovery time, and can therefore influence the therapeutic approach (36, 37). Muscle re-injury rates are between 12-43% across different sports. The recovery and RTP times after a re-injury are typically longer than for a primary injury (38). Non-modifiable risk factors for muscle re-injury are age (especially for triceps surae and hamstring) and number of previous injuries of the same or other muscles. Modifiable risk factors include muscle strength deficits, reduced flexibility and muscle fatigue (39). Such modifiable risk factors are directly related to the pathological modifications that usually occurs after an injury, such as stiffness, muscle atrophy, scar tissue formation, biomechanical alterations and neuromuscular control deficits (40). They can also be due to an inadequate rehabilitation management, characterized by an overly aggressive rehabilitation process, incomplete rehabilitation, an underestimation of the previous injury, an overly long immobilization process or an incorrect treatment approach (41).

Updated classification

Classification

As we know, muscle injuries are divided in two main categories according to injury mechanism: direct and indirect injuries.

Direct injuries

Direct muscle injuries are divided into: lacerations and contusion. Lacerations are caused by blunt objects that cause cuts, whilst contusions are caused by direct impact not causing cuts.

Contusions are classified as minor, moderate and severe depending on identified functional deficits assessed by the capacity to engage the muscle and execute its specific action. It is important to re-assess the athlete 24 hours after the injury, as the blunt trauma can be initially overestimated due to the algic component, which limits early movement (1).

Indirect injuries

Indirect muscle injuries occur without any contact with other players or objects outside the field of play. The athlete essentially injures them self and these types of injury can be divided in two categories:

- *non-structural injuries:* no anatomic damage or tear, divided into 4 sub-categories;
- *structural injuries:* characterized by anatomical damage and classified into 3 sub-categories (13).

Non-structural muscle injuries

Non-structural muscle injuries are the most common injury type, but the diagnosis and treatment can be insidious. In football, they constitute 70% of all muscle injuries and although they do not involve structural muscle damage, they are still responsible for more than 50% of muscle injury burden from sports. If neglected they can lead to subsequent structural injuries.

Four non-structural muscle injury subcategories

- Sub-group 1A is fatigue induced muscle disorder, typical of repetitive movements, inconstant playing surface or by excessive workload volumes.
- Subgroup 1B is caused by exercise induced muscle damage resulting in delayed onset of muscle soreness (DOMS).
- Subgroup 2A is caused by spinal problems, often difficult to diagnose, such as minor intervertebral defects, which irritate the corresponding spinal nerve causing altered muscle tone of the respective muscle. In these cases, resolution of the muscle injury requires treating the spinal problem.

- Subgroup 2B arises from an imbalance in neuromuscular control especially altered reciprocal muscle inhibition. Sensory information from the muscle is carried by ascending pathways to the brain. The afferent signals enter the spinal cord with the alpha motoneurons of the associated muscle, but also give branches capable of stimulating the interneurons in the spinal cord that act in an inhibitory way on the alpha motoneurons of the antagonist muscles. Thereby simultaneous inhibition of the alpha motor neurons to antagonistic muscles (reciprocal inhibition) occurs to support muscle contraction of agonistic muscle. A dysfunction of these neuromuscular control mechanisms can lead to significant impairment of normal muscle tone and can result in neuromuscular disorders, when the inhibition of antagonistic muscles is impaired (*e.g.* decreased) and the agonist must over-contract to compensate (13).

Structural muscles injuries

Structural muscle injuries are divided into 3 sub-categories accordingly the severity of the anatomical damage:

- 3A. Partial minor tear – a tear of the primary muscle fascicles inside a secondary muscle (1).
- 3B. Partial moderate tear – a tear of at least one secondary muscle fascicle with 50% structural damage of the investigated muscle cross sectional area.
- 4. Sub-total tear or complete tear - a tear of at least 50% structural damage of the investigated muscle cross sectional area. Otherwise complete tear of whole muscle or MTJ.

In addition, considering the I.S.Mu.L.T. classification of 2014, it seems necessary to consider the location of the muscle injury according to its site: proximal, middle, distal.

The classification of muscle structural injuries including the injured location along the muscle**:**
Proximal site (P); Middle site (M); Distal site (D).
Ultrasound and particularly MRI scans can help to identify involvement of different damaged tissues:

- MF: Myofascial (muscle fibers and fascia);
- MT: Muscle fibers or MTJ;
- T: Tendon.

Myofascial injuries have a more benign prognosis than MTJ and tendon injuries. This for several reasons including i) from the biomechanical point of view, myofascial injuries undergo less traction and stress than the MTJ and tendons and ii) anatomically, the fascia is richly vascularized and therefore has the possibility of *"restitutio ad integrum"* (restoration to original condition) or better, after a muscle injury.

Anatomically, the MTJ is the muscle portion in which the tendon fibers intertwine until it merges with the muscle fibers. It is the *"locus minor resistentiae"* (location of least resistance) following specific and eccentric sports movements, which subject the muscle to biomechanical stress. As such, the MTJ is a frequent site of muscle injuries. Physiologically, this is in part due to a histological feature of the myotendinous tissue, which is rich in fast type II fibers, which utilise predominantly anaerobic metabolism. This is important for injury diagnosis and relevant for prognosis, as injuries at the MTJ have longer recovery times than injuries within the muscle belly. Furthermore, injuries with tendon involvement typically have the worst prognosis and may require surgical repair (19). Therefore, it is useful to classify the site of injury, as it is an important prognostic factor.
As described previously, muscle injuries which occur in the proximal area of hamstrings muscle or rectus femoris muscle have longer prognosis compared with muscles injuries at the middle of the muscle, despite similar structural damage. Furthermore, the triceps surae muscles has longer prognosis if the tear is located at the distal level.
A further consideration for these developed guidelines, was the patient's anamnestic history regarding previous injuries. It is important to understand if the injury is a primary injury or first, second or third re-injury (36).
A further innovation of the guidelines is the consideration of injury occurrences:

- Primary injury (R0);
- First re-injury (R1);
- Second re-injury (R2);
- Third re-injury (R3).

We believe recording of the injury as either primary or as a re-injury and the number of re-injuries is essential, as muscle re-injuries are associated with worse prognosis and possibly emphasis a previously failed functional recovery process.
A muscle re-injury is another new injury from non-contact mechanisms in the same location of the previous injury or in the same muscle, occurring within 2 months after RTP (37). RTP times for muscle re-injuries are typically longer than for primary injuries, Recent epidemiological studies on elite football players report muscle re-injury rates of 13–16% for structural muscle injuries and 12% for non-structural injuries. Muscle re-injury rates vary between 12-43% across different sports, including soccer, Australian football, athletics and rugby and result in delayed RTP times. Again, risk factors for muscle injuries can be categorised into modifiable and non-modifiable factors (38).
A few authors reported that muscle re-injuries occur due to multiple co-existing injury risk factors. Deficits in muscle flexibility are considered to associated with muscle re-in-

Table I. New classification system based on the extent of the tear, tear location, tissue involvement and injury number.

Tear extent	Tear location	Tissue involved	Re-injury
3A: Partial minor tear	P: Proximal	MF: Myofascial	R0: Primary injury
3B: Partial moderate tear	M: Middle	MT: Muscle fibers	R1: First re-injury
4: Subtotal or complete tear	D: Distal	and myotendon	R2: Second re-injury
		T: tendon	R3: Third re-injury

juries, but not primary muscle injury. Eccentric hamstring strength deficits are associated with higher risk of muscle re-injury. According to the literature, hamstring muscle re-injuries are commonly grade 3A and 4, thought due to miss-diagnosis and failure to identify the severity of the initial injury. Medial gastrocnemius, hamstring and rectus femoris muscles are most susceptible to re-injury (39). The symptoms and clinical signs of the re-injury are similar to the primary injury, so the same considerations of imaging and conservative treatment indications are needed. Muscle re-injury practices should consider the risk factors which may have predisposed the athlete to the initial muscle injury, correct diagnosis of the injury, reduced immobilization times, correct exercises selection, a full and complete functional recovery process as well as management of the athlete after RTP (40). Rehabilitation and/or re-injury prevention programs should include eccentric strengthening at longer muscle lengths, as evidence suggests a protective effect for re-injury upon RTP. Half of muscle re-injured occur during the first month after RTP, thought due to incomplete functional recovery (41).

This new proposed classification considers the magnitude of the tear, localisation of the tear, involved tissue and numbers of re-injury can be seen in **table I**.

Clinical examples using this update classification system:

- *long head biceps femoris* **3B, P, T, R2**: second re-injury with partial moderate tear at proximal site with tendon tissue involvement;
- *adductor longus* **4, P, MT, R0**: primary injury with subtotal or complete tear of the proximal myotendinous junction;
- *recuts femoris* **3A, M, MF, R1**: first re-injury with partial minor tear at middle site with myofascial involvement.

CONCLUSIONS

To conclude, we believe the proposed classification system is complete, considering the anamnestic, clinical and localization aspects of muscle injuries, which should support clinicians in determining muscle injury prognosis. Such an integrated classification can support the scientific community to have consistent methods of reporting muscle injuries. This could aid in the development of more targeted and evidence-based treatments to support athletes to RTP early and safely, as well as minimizing the risk for muscle re-injury.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Adding Injectable Chitosan Poloxamer to Platelet Rich Plasma (PRP) Has no Beneficial Effect in Knee OA. A prospective Experimental Study in Guinea Pigs

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SUMMARY

Background. Short term symptomatic relief with single dose PRP in early knee OA has been established. We studied the effect of Chitosan as an additive to PRP, in an attempt to improve and prolong the effects.

Methods. 42 Dunkin Hartley guinea pigs were divided into 4 groups of 10 each; 2 animals were used for preparation of allogeneic PRP. Disease control group (DC) had 10 animals where no intervention was done in either knee. Groups G1, G2 and G3 (10 animals each) were given single intra-articular injections of PRP, Chitosan gel alone and Chitosan + PRP in one knee respectively. Isotonic saline injection was given in the contralateral knees in all 3 groups to act as control. Five animals from each group (subgroup DC.3, G1.3, G2.3 and G3.3) were euthanized at three months and the remaining five (subgroup DC.6, G1.6, G2.6 and G3.6) at six months post intervention. Upon euthanasia, knee joint synovial fluid was taken for cartilage oligomeric protein (COMP) estimation by ELISA, and histologic assessment of articular cartilage and synovium was done using Mankin score.

Results. The mean synovitis scores was significantly lower in both the PRP and Chitosan PRP group compared to disease control and Chitosan gel alone groups at 3 months ($p < 0.05$) signifying effectiveness of PRP for synovitis; at 6 months there was no difference between the 3 intervention groups and disease control group, implying that the effects of PRP wear off. There was no significant difference between Chitosan PRP and PRP groups at 3 or 6 months in terms of mean total articular cartilage score and COMP levels. The above findings imply that Chitosan addition did not improve or prolong the effects of PRP.

Conclusions. Addition of chitosan poloxamer gel did not offer any additional advantage in prolonging efficacy of PRP in a Guinea pig Knee OA model. Nevertheless, the efficacy of single dose PRP, with or without Chitosan, has been again demonstrated in the short term, due to decreased synovitis scores.

KEY WORDS

Chitosan; poloxamer gel; platelet rich plasma; guinea pigs; animal study; COMP.

INTRODUCTION

In the 21st century, Platelet Rich Plasma (PRP) has emerged as a promising treatment option for early osteoarthritis of knee. The positive effects of PRP have been noted by multiple authors (1, 2, 3); however, the downside is that the effect tends to deteriorate after periods of time varying from 6 to 12 months (3).

PRP has been combined with many different biomaterials like gelatin hydrogels, Chitosan, PLGA (poly lactic-co-glycolic acid) mesh and β-tricalcium phosphate scaffolds for prolonged and sustained release of growth factors, with varying degrees of success. The combination of PRP with these different biomaterials has been demonstrated to be significantly better than PRP alone in various *in vitro* and in vivo studies for various applications like wound healing, healing of bone defects and for osteoarthritis knee (4-9).

Chitosan has been shown to improve some platelet properties. It increases platelet aggregation and adhesion and increases the release of growth factors (10, 11). Chitosan scaffolds activated with PRP are safe and nontoxic (12) and have displayed controlled and sustained release of growth factors (12, 13). Many in vivo studies have also demonstrated the improved efficacy of Chitosan and PRP combination for wound healing and healing of bone defects (14, 15). Chitosan and PRP combination scaffolds have been successfully used in focal cartilage defect models in rabbit where better quality of cartilage tissue with PRP+ Chitosan implants has been noted compared to controls (16, 17). Dwivedi *et al.* (17) had used injectable Chitosan PRP which solidifies in the defect to behave as a scaffold. Injectable Chitosan gels have been used in healing of abdominal defects and in preventing peritoneal adhesions (18, 19). In the present study we explored the potential of injectable Chitosan and injectable Chitosan + PRP combination for use in Osteoarthritis model. Considering the positive effects in cartilage healing demonstrated by Chitosan + PRP scaffolds in focal defects, we hypothesized that this combination may enhance and prolong the effect of PRP in cartilage healing in osteoarthritis, and prospectively designed a study in a Guinea Pig model of OA. A comparative experimental study was done to determine whether Chitosan poloxamer + PRP is better than PRP alone for osteoarthritis in guinea pig knee, based on histopathological analysis of synovium and cartilage.

MATERIAL AND METHODS

Selection of animals for study and grouping

Forty-two male Dunkin-Hartley guinea pigs, 5 months of age and weighing approximately 600 to 800 grams were chosen for the study after institutional animal ethics committee clearance (IAEC number 547). The Dunkin Hartley guinea pigs represent a naturally occurring model of osteoarthritis, where the progression of osteoarthritis is affected by increasing age and weight gain (20). 2 animals were used for preparation of allogeneic PRP (Donor Group). The remaining 40 animals constituted the experimental group. The study animals were divided into 4 groups randomly as described and shown in **figure 1**. Each knee of the 40 animals was considered a separate entity and therefore a total of 80 knees were available for the study. Therapeutic intervention was done on one knee (randomly selected) and the contra-lateral knee was taken as control for each animal in study all groups.

Group DC: Disease control group – This group had 10 animals, which were further divided into subgroups of 5 animals each, which were sacrificed at 3 and 6 months (DC.3 and DC.6) respectively. Both knees received no intervention, giving a total of 20 knees.

Group G1: PRP Injection group – This group contained 10 animals. These were further divided into subgroups containing 5 animals each, which were sacrificed at 3 and 6 months (G1.3 and G1.6) respectively. Single PRP injection was given in the intervention knee and same amount of saline was injected in the control knee.

Group G2: Chitosan Injection group – This group contained 10 animals. These were further sub divided into groups containing 5 animals each, which were sacrificed at 3 and 6 months (G2.3 and G2.6) respectively. Single injection of Chitosan gel was given in the intervention knee and same amount of saline was injected in the control knee.

Group G3: Chitosan-PRP combination injection group – This group contained 10 animals. These were further sub divided into groups containing 5 animals each which were sacrificed at 3 and 6 months (G3.3 and G3.6) respectively. A single injection of Chitosan poloxamer PRP gel was given in the intervention knee and same amount of saline was injected in the control knee.

The animals were housed according to subgroups in groups of 5 each in open rectangular metal floor pen of 45 x 90 cm size. The bedding was made with straw with free access to food and water and maintained on a 12 hr light/12 hr dark cycle (8, 20). All standard ethical guidelines as stated by this journal were followed (21).

The mean synovitis scores, the mean total articular scores and mean COMP concentrations of control arm of various groups (DC, G1, G2 and G3) at 3 months and 6 months were calculated (**table I**). The control arms were comparable to each other (p value > 0.05 for all comparisons) at 3 months and 6 months (**table II**), implying that Osteoarthritis progression in all groups was similar and comparable.

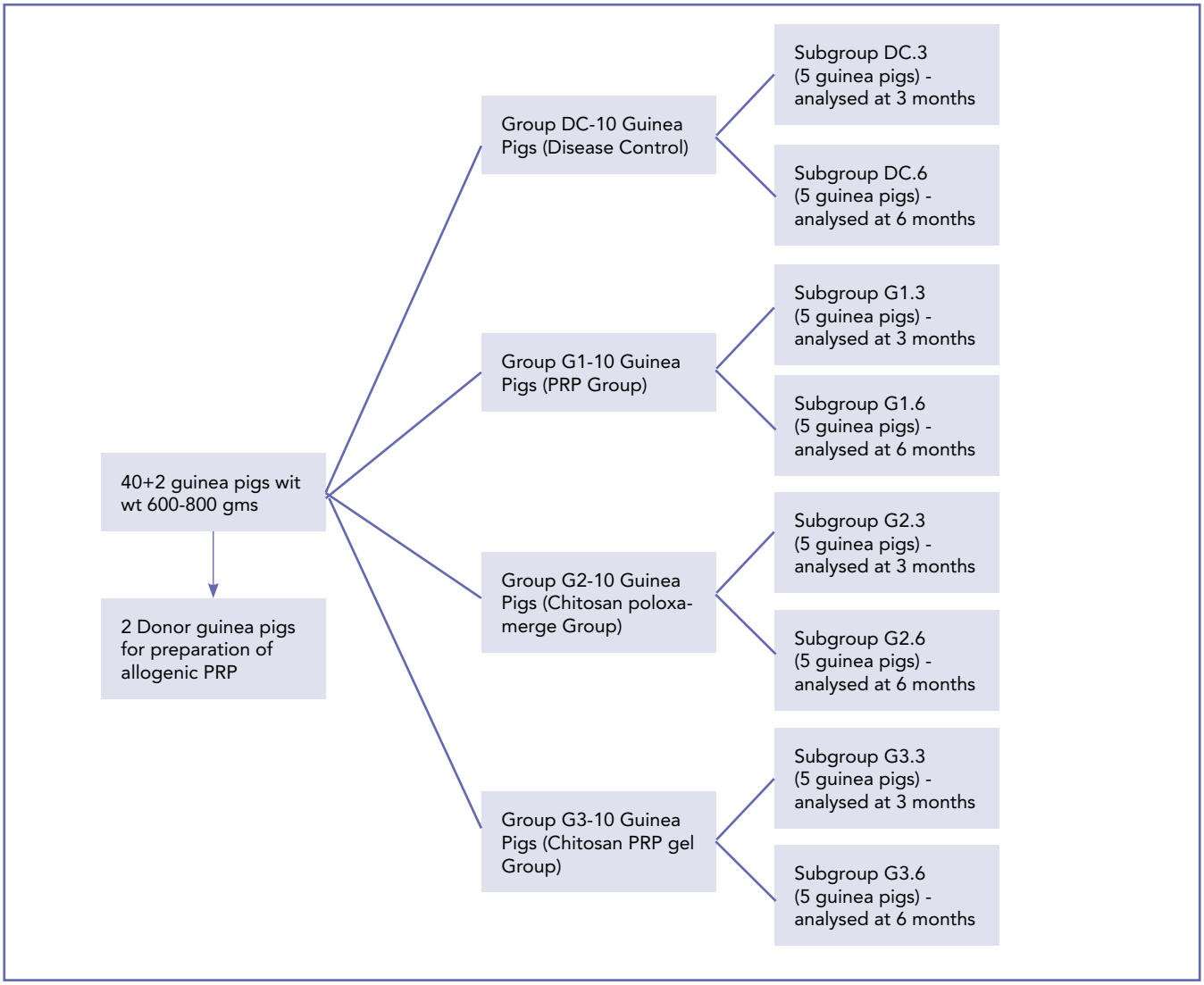

Figure 1. Pictorial representation of the distribution of animal among various groups. Group DC-Disease control group, Group 1 – PRP group, Group 2 – Chitosan only group, Group 3 – Chitosan PRP group.

Table I. Mean Synovitis score, Mean Total Articular scores and mean COMP scores of CONTROL Arm of all 8 subgroups.

Sl No	Group	Synovitis score (Mean ± Std deviation)	Total Articular Score (Mean ± Std deviation)	Mean synovial COMP Levels
1	DC.3	3.40 ± .894	8.60 ± 3.209	7990.00 ± 2403.258
2	G1.3	3.00 ± 2.828	8.80 ± 1.924	6818.00 ± 2258.942
3	G2.3	3.80 ± 1.924	9.00 ± 2.646	6898.60 ± 568.507
4	G3.3	3.20 ± 1.304	9.40 ± 2.881	5676.60 ± 1117.429
5	DC.6	6.20 ± 1.643	12.60 ± 1.140	10084.60 ± 1365.321
6	G1.6	5.60 ± 1.817	13.00 ± 3.000	14976.00 ± 1585.257
7	G2.6	5.80 ± 1.643	11.20 ± 1.643	12622.20 ± 1356.354
8	G3.6	6.40 ± 1.342	11.80 ± 1.483	14557.20 ± 2294.318

Table II. Table showing p values for comparison among CONTROL arm of various groups for mean synovial score, mean articular score and mean synovial COMP levels.

Subgroup 1 for comparison	Subgroup 2 for comparison	P value for comparison of mean synovial scores	P value for comparison of mean articular cartilage scores	P value for mean synovial COMP levels
DC.3	G1.3	1.000	1.000	.997
DC.3	G2.3	1.000	1.000	.998
DC.3	G3.3	1.000	.997	.943
G1.3	G2.3	.989	1.000	1.000
G1.3	G3.3	1.000	.999	.998
G2.3	G3.3	.997	1.000	.997
DC.6	G1.6	.997	1.000	.802
DC.6	G2.6	1.000	.954	.991
DC.6	G3.6	1.000	.996	.904
G1.6	G2.6	1.000	.878	.983
G1.6	G3.6	.987	.976	1.000
G2.6	G3.6	.997	.999	.998

Preparation of platelet rich plasma (PRP)

We used allogeneic blood obtained from donor animals via cardiac puncture for preparation of PRP. Double spin technique was used for preparation of PRP, as it has been shown to yield higher concentration of platelets compared to single spin technique (22). Blood was collected in vials containing acid citrate dextrose (ACD) containing 0.48% w/v citric acid, 1.32% w/v sodium citrate and 1.47% dextrose. 1 ml of this anticoagulant was added to each 10 ml vial. Drawn blood was analyzed for platelet count; then it was subjected to double centrifugation. The centrifugation protocol used was 20 minutes of soft spin at 800g which separated the blood into a red cell layer and a buffy coat. The buffy coat was pipetted out and subjected to hard spin for 15 minutes at 2200 g. This yielded platelet poor plasma overlying platelet pellet at the bottom of the centrifugation tube. Two-third of the platelet poor plasma was removed, and the rest was evenly dissolved with the platelet pellet to produce a homogenous solution. While preparing PRP for Chitosan PRP group, PRP was concentrated doubly (see reason below) obtained by removing five-sixth of the platelet poor plasma and dissolving the platelet pallet with the rest of plasma (20, 23). 15 ml whole blood produced approximately 3 ml of allogenic PRP. The obtained PRP was subjected to platelet count for quality control, done manually using a Nuebaeur chamber. The normal guinea pig platelet count reference range is 3.4^{-10} x 10^5/mm (24); the PRP prepared had platelet counts at least 2 to 3 times the baseline (25).

The allogeneic PRP thus produced was injected into the joint immediately after preparation.

Activation of PRP

The PRP was activated by adding $CaCl_2$ prior to injection; 1 part 0.025 M $CaCl_2$ was added to 4 parts of allogenic PRP. Fresh $CaCl_2$ solution was prepared for each injection by dissolving 27.78 mg of anhydrous $CaCl_2$ in 100 ml distilled water. This solution was mixed in the ratio 1:4 with PRP injection in group 1. No activation of PRP was done for Group 3 (Chitosan PRP group) as Chitosan has been shown to be an activator of PRP (10).

Preparation of Injectable Chitosan poloxamer gel

The Chitosan solution (1.33% w/w) was prepared by mixing Chitosan (Sigma Aldrich™ medium molecular weight) in acetic acid solution (0.25% w/v) with continuous stirring until complete dissolution. The required amount of poloxamer 188 (Sigma Aldrich™) was added to Chitosan solution to obtain a 26.6% (w/v) solution and the partially dissolved solution was kept in the refrigerator (at around 4 °C) until the entire polymer completely dissolved (approximately 24 h). The Chitosan poloxamer solution so obtained was sterilized by autoclaving at 121 °C (250 F) for 20 minutes at 15 psi (26). The ratio of Chitosan and poloxamer in the final preparation were 1% w/w and 20% w/v respectively. The rheological properties and gelling of Chitosan poloxamer solution before and after sterilization were checked. The force shear graphs obtained before sterilization and after sterilization were linear in nature and represented Newtonian type of fluid. There was no change in the rheological properties of the preparation after sterilization. The transition temperature of solution to gel was 30 to 32 °C.

Preparation of Injectable PRP-Chitosan poloxamer gel combination

The guinea pig knee is small and accommodates around 100 microliters of fluid. As we had to combine PRP with Chitosan solution, the volume of both injected would have resulted in lesser volume/dose of PRP. In order to counter this and ensure adequate Platelet numbers in the PRP, we used a double concentrate PRP for group G3. The initial steps of PRP preparation were same which included double spin technique. Double concentrate PRP was then obtained by removing five-sixth of the platelet poor plasma and dissolving the platelet pellet in the rest of plasma. The Chitosan PRP group had half the volume of PRP in contrast to PRP alone group. The sterilized Chitosan solution was added to the double concentrate PRP solution under sterile conditions in the ratio 3:1 (Chitosan: PRP). To counter the dilution we used a double concentrate PRP in the chitosan PRP group and did not use an activator in the chitosan PRP group as chitosan is itself an activator of PRP. The preparation was kept at room temperature before injecting.

Joint injections

PRP and other preparations were instilled in the knee joint chosen for therapy through the inferior patellar tendon with a 26-gauge needle and syringe (100 microliters); the same amount of normal saline was injected in the other knee of the animal (23, 27). During the procedure the guinea pigs were anesthetized with a mixture of Xylazine (5 mg/kg) + ketamine (50 mg/kg) + acepromazine (1 mg/kg) given at a dose of 0.1 ml/kg i. m injection and monitored. The procedure was conducted using sterile conditions. After the procedure the animals were monitored until they recovered from the anesthesia.

Collection of synovial fluid

We collected synovial fluid samples from each knee to perform Cartilage Oligomeric Protein (COMP) (a marker of cartilage degradation) level measurements. The synovial fluid samples were collected by the technique described previously after euthanasia of animals (23, 27). Through the inferior patellar tendon 200 microlitres isotonic saline was injected intra articularily. The limb was subjected to 10 cycles of flexion and extension of knee joint to evenly distribute the injected fluid. With the same approach and a 26 gauge needle the knee was aspirated.100 micro liters of sample was collected from each knee and the collected fluid was then subjected to centrifugation at 2000 rpm for 10 minutes to remove cell debris and the supernatant was collected and stored at -20 °C for analysis. The samples were collected at the time of sacrificing the animal, before the joints were processed for histopathology.

Analysis of synovial fluid for COMP levels

The collected synovial fluid aliquots from each knee were thawed and subjected to quantitative COMP levels analysis by Enzyme Linked Immunosorbent Assay (ELISA) by a blinded observer. The ELISA kits (MyBiosource Elisa kits) use double sandwich ELISA technique and the kit had a sensitivity of 0.05 ng/ml. The kit was stored at -20 °C until usage. The samples were analyzed following the steps as given in the user manual provided by the manufacturer.

Collection and analysis of synovial tissue and joint cartilage

Under xylazine and ketamine anesthesia, euthanasia was performed on the animal by intra peritoneal injection of pentobarbital (100 mg/kg).The animal was observed for the loss of respiratory drive and loss of vital parameters before starting the tissue harvest (23, 28). The harvested tissue was then fixed in 10% formaldehyde and subjected to histological analysis.

The harvested tissues were fixed in 10% formalin and both knee joints for each animal were assessed. The samples were processed using saturated solution of EDTA in 0.1 M phospahate buffer. A pH of approximately 7.6-7.8 was maintained. The solution was replaced once every week until calcium precipitated was no longer detected. This took approximately 8-12 weeks. Further, paraffin wax blocks were made (2, 29). For articular cartilage staining toluidine blue stain was used and scoring was done using semi quantitative Mankin score which has been validated by the Osteoarthritis Research Society International (OARSI) histopathology initiative in guinea pigs (29). The score has 5 components which included articular cartilage score (0-8), proteoglycan content (0-6), cellularity (0-3), tidemark (0-1) and osteophytes (0-3) with a maximum score of 21 and a minimum score of 0 (29). Synovial tissue was stained with heamatoxylin and eosin and was analyzed according to a semi-quantitative score given by Pelletier *et al.* and validated and recommended by the OARSI histopathology initiative (29).The scoring has 3 components which include synovial hyperplasia (0-2), villous hyperplasia (0-3) and degree of cellular infiltration (0 or 5). The minimum score was 0 and maximum was 10 (29). An independent blinded observer did all scorings.

STATISTICS

The collected data was subjected to statistical analysis using SPSS v20 software. The descriptive statistics for all 8 subgroups (*e.g.* Mean, standard deviation *etc.*) were calculated for weight, platelet counts in whole blood and PRP, synovitis scores, articular cartilage scores and COMP levels.

The data was confirmed to be normally distributed. Weight gain, total synovial scores, total articular scores and COMP levels were compared among various groups using one way analysis of variance and post hoc Tukey HSD tests. A p value of < 0.05 was considered significant.

RESULTS

The mean initial weights at the start of experiment in all subgroups, the mean weight at sacrifice at 3 months and 6 months, and the mean weight gain of animal subgroups sacrificed at 3 months and 6 months were all comparable (**figure 2**), with no significant difference among the groups. The p values for these comparisons are listed in **table III**. These animals were thus comparable in terms of osteoarthritis (29).

The baseline platelet counts in source blood used for the PRP injection of group G1 and group G3 were 585,000/µl and 640,000/µl. The platelet counts in PRP injection prepared for group G1 was 1,875,000/ µl and PRP injection prepared for group G3 was 4,362,500/µl. As the PRP used for injection in group G3 was mixed with Chitosan poloxamer preparation in the ratio 1:3, the platelet concentration in final preparation was 1090625/µl. The final PRP concentration in group G1 after activation with $CaCl^2$ was 1,500,000/µl.

The mean synovitis scores for intervention arm of all groups are represented in **figure 3**. At 3 months, the scores for both

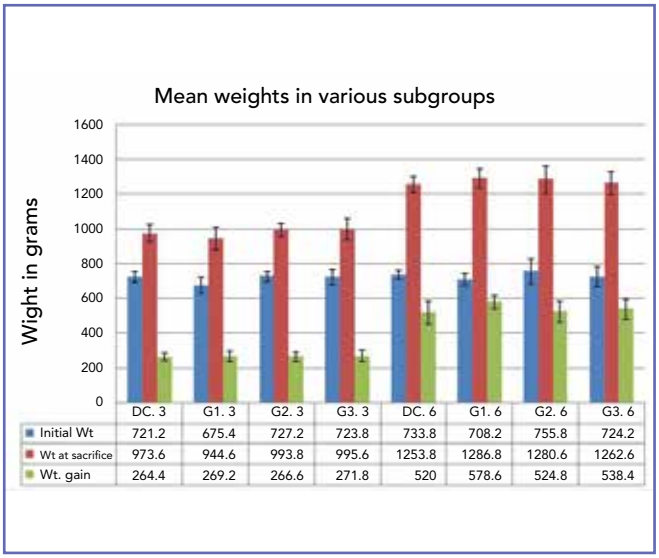

Figure 2. Graphical representation of initial weight, weight at sacrifice and weight gain for all the subgroups. There was no significant difference among the groups for any of the parameters at 3 or 6 months.(Standard deviation for initial weight DC.3-29.719, G1.3- 46.420, G2.3- 29.115, G3.3-43.234 DC.6- 24.356, G1.6- 34.215, G2.6-69.830 ,G3.6-.55.006 49.719. Standard deviation for final weight-DC.3-49.71G1.3-66.42, G2.3-39.115, G3.3-63.234, DC.6-44.356, G1.6-54.215, G2.6-81.83, G3.6- 65.006 S.D. for weight gain DC.3- 22.952, G1.3- 32.322, G2.3-27.162 ,G3.3-35.088 DC.6-63.887, G1.6- 39.310, G2.6-61.227 , G3.6-55.089).

Table III. Table representing p values for comparisons for initial weight, weight at sacrifice and weight gain, mean synovial scores, mean total articular cartilage scores and mean COMP concentrations in the INTERVENTION arm of various groups. (Significant p values have been marked in bold font and with an asterisk (*)).

Subgroup 1 for comparison	Subgroup 2 for comparison	p- value for comparison of initial weights.	p- value for comparison of weight at sacrifice.	p- value for comparison of weight gain.	P value for comparison of mean synovial scores	P value for comparison of mean articular cartilage scores	P value for mean synovial COMP levels
DC.3	G1.3	.510	.917	1.000	.013*	.442	.986
DC.3	G2.3	1.000	.981	1.000	.999	1.000	.997
DC.3	G3.3	1.000	.973	1.000	.005*	1.000	.861
G1.3	G2.3	.377	.555	1.000	.030*	.442	1.000
G1.3	G3.3	.451	.517	1.000	.999	.535	.996
G2.3	G3.3	1.000	1.000	1.000	.013*	1.000	.984
DC.6	G1.6	.947	.926	.461	.575	1.000	1.000
DC.6	G2.6	.972	.968	1.000	1.000	1.000	.966
DC.6	G3.6	.999	1.000	.992	.979	1.000	.968
G1.6	G2.6	.579	1.000	.552	.719	1.000	.988
G1.6	G3.6	.993	.980	.803	.932	1.000	.989
G2.6	G3.6	.881	.995	.998	.997	1.000	1.000

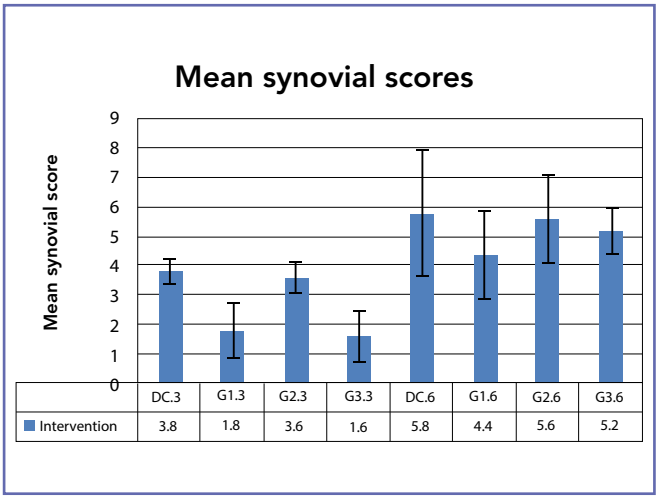

Figure 3. Graph representing the mean total synovial scores of intervention subgroup of various groups at 3 and 6 months. The scores were significantly better for the G1.3 and G3.3 compared to DC.3 and 2.3. (Standard deviation for intervention Knee DC.3-.447 , G1.3- 0.967, G2.3- .548, G3.3-.894, DC.6-2.168 , G1.6-1.517, G2.6-1.517, G3.6-.837).

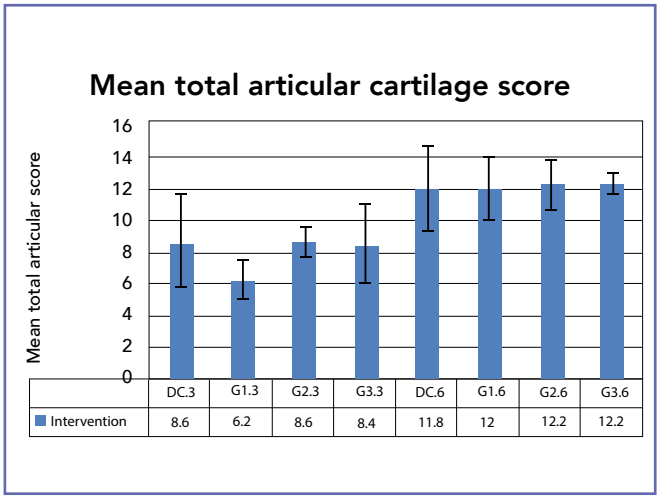

Figure 4. Graph representing mean total articular scores of intervention subgroups of various groups. No significant difference was noted among the groups at 3 or 6 months. (Standard deviation for intervention Knee DC.3- 3.050, G1.3-1.304, G2.3-.894 ,G3.3-2.608 DC.6- 2.864, G1.6-2.000, G2.6-1.643,G3.6.-837).

the PRP group and chitosan-PRP group were significantly better than the disease control group (p value = 0.013 & 0.005 respectively) and chitosan gel only group (p value = 0.03 & 0.013). However, there was no significant difference between the PRP and chitosan-PRP group in terms of mean synovitis score. There was no significant difference in the mean synovitis scores between all the subgroups at 6 months (**table III**). It was noted that although single injection of PRP and Chitosan-PRP resulted in better mean total synovial scores than Chitosan gel and disease control groups at 3 months, this effect was not sustained in the long term (6 months). It also appears that although PRP exerts an anti-inflammatory effect resulting in a decrease in synovial inflammation, the addition of chitosan gel did not improve its efficacy.

The mean total articular cartilage scores of the intervention arm are represented in **figure 4**. There was no significant difference among mean total articular scores of intervention subgroups of all groups at 3 months and 6 months. This implies that single injection of PRP and chitosan-PRP does not seem to offer any significant protective effect on the cartilage.

The mean synovial COMP concentrations for the intervention sub arms are represented in **figure 5**. There was no significant difference among mean COMP concentrations of intervention subgroups at 3 months (DC.3, G1.3, G2.3 and G3.3). Similarly, at 6 months, there was no significant difference among mean COMP concentrations of interven-

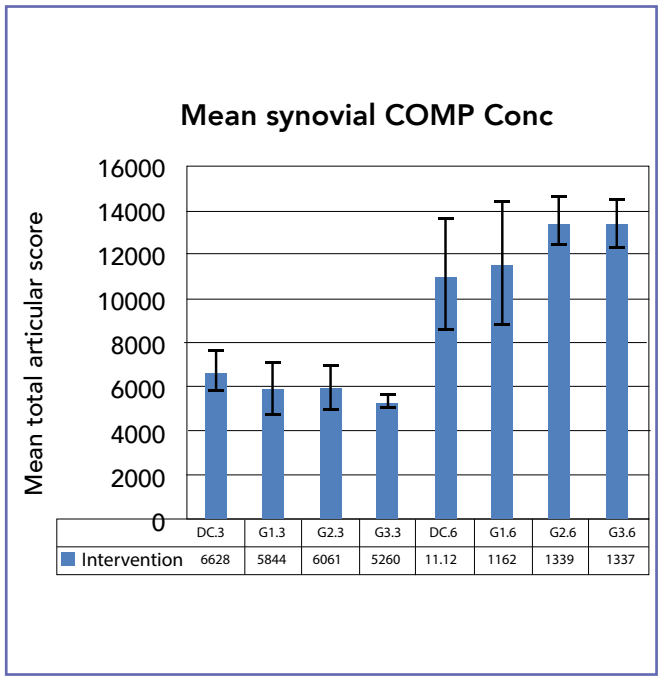

Figure 5. Graph representing mean COMP concentration of intervention subgroups of various groups. No significant difference was noted among the groups at 3 or 6 months (Standard deviation for intervention Knee DC.3- 1028.552, G1.3-1215.743, G2.3-872.709, G3.3-357.814, DC.6-2658.968, G1.6-2925.881, G2.6-1345.657, G3.6.- 1239.349).

tion arms (DC.6, G1.6, G2.6 and G3.6). Images of synovial and articular cartilage histological morphology are represented in **figure 6** and **figure 7** respectively.
No adverse events were noted in any of the groups.

DISCUSSION

As our understanding of the time limited efficacy of PRP evolved, attempts were made to use biomaterials with PRP, to see if the effect could be prolonged; this was started almost a decade ago. Since the initial work, there have been a variety of biomaterials which have been used along with PRP. Biomaterials like gelatin hydrogels (4), Chitosan (5), PLGA (poly lactic-co-glycolic acid) mesh and β-tricalcium phosphate scaffolds (6, 7) have been added to try and prolong and sustain the release of growth factors, with some success in specific scenarios.
Saito *et al.* (4) were the first to use biomaterials with PRP for intraarticular application in OA knee. They demonstrated the effects of PRP with gelatin biospheres in a surgically induced model of rabbit OA (ACL transection). 2 injections were given at an interval of 3 weeks. They evaluated the expression of proteoglycan core protein mRNA in the cartilage along with cartilage histology as a measure of outcome analysis. This study showed improved cartilage histology (as seen by 2 independent observers) and increased proteoglycan mRNA expression in the cartilage treated by PRP as compared to controls at 10 weeks.
Chitosan and PRP combinations have been studied in many in vitro and in vivo studies and is proven to be safe and nontoxic (11, 12). It has also been shown to enhance platelet aggregation, adhesion and increase glycoprotein III expression (10). Chitosan scaffolds with PRP have been shown to demonstrate a controlled and sustained release of the growth factors Transforming growth factor-β1 and platelet derived growth factor- AB compared to PRP alone (10, 11) and also cause significant growth of mesenchymal stem cells (13). Chitosan and PRP combination has also found application in wound healing (14), filling of bone defects (15) and treatment of cartilage defects (16, 17). Mohmadi *et al.* (14) showed that Chitosan film combined with PRP resulted in better wound healing than PRP or Chitosan alone, in iatrogenically created wounds in rat model. Bi *et al.* (15) used a composite scaffold made of PRP, Chitosan and tri-calcium phosphate and showed that this composite was better in healing bone defects in tibia of goat than control groups at 16 weeks.
Chitosan based products have been widely used in cartilage related pathologies. A chitosan-based scaffold "BST-CarGel®" in a randomized control trail has been shown to shown to be superior to microfractures alone for repair of cartilage defects. Patients treated with chitosan scaffolds showed significantly greater filling of lesion and better repair tissue than cases with micro fractures (30). The Chitosan and PRP combinations have also been encouraging in cartilage defects. Segundo *et al.* (16) in their study on 12 rabbits compared a combination of PRP, chitosan and hydroxyapatite with no treatment in surgically created cartilage defects. They noted that significantly greater amount of tissue at the bone cartilage interface in the test group. In another study, Dwivedi *et al.* (17) surgically created chronic defects in cartilage of 8 rabbits; these defects were treated by bone marrow stimulation (BMS) with PRP or freeze-dried Chitosan with PRP. They noted that animals treated with BMS + Chitosan PRP had better articular scores at 8 weeks compared to BMS +PRP. They hypothesized that Chitosan/PRP implants reside in vivo for several weeks and have significant bioactivity, in contrast to PRP implants alone that are quickly degraded in one day.

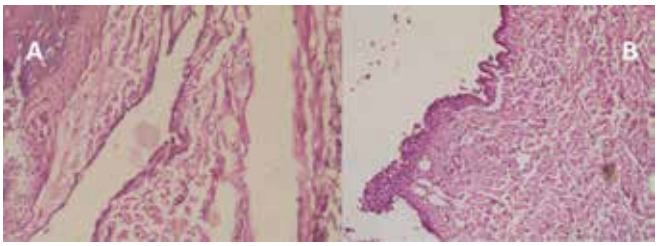

Figure 6. Hematoxylin and eosin stain showing synovial morphology. **A.** PRP treated knee (G1.3) showing no synovial hyperplasia (white arrow) and minimal inflammation at 3 months. **B.** Control (DC.3) knee showing 3 to 5 layered synovial hyperplasia (white arrow) and marked inflammation at 3 months.

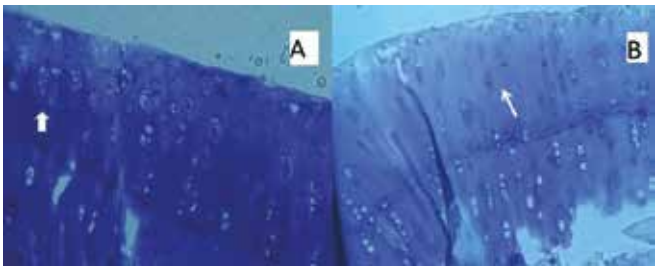

Figure 7. Toluidine Blue stain used for articular cartilage morphology. **A.** Chitosan PRP (G3.3) treated knee with a single superficial fissure, minimal loss of proteoglycan, normal cellularity (white arrow) and no duplication of tidemark. **B.** Knee from disease control group (DC.3) showing multiple superficial fissures, loss of proteoglycan, decreased cellularity (white arrow) and duplication of tidemark.

Though PRP has been used extensively for intraarticular use in osteoarthritis, there are no studies studying the effect of PRP in combination with Chitosan in a knee osteoarthritis model. In an attempt to replicate the positive effect of chitosan/PRP combination in other situations, we prepared an injectable combination of chitosan poloxamer gel with PRP, to see whether the positive effects seen in cartilage defect studies could be translated to knee osteoarthritis.

The Dunkin Hartley guinea pigs develop weight induced knee osteoarthritis. The progression of knee osteoarthritis in this model has been proven to be co-relating to the weight gain of these animals (20, 23, 31). We chose this model, as it is a naturally occurring model and its progression is proportionate and similar to humans (29, 31). The progression in surgically induced models is more rapid and in line with post traumatic arthritis than natural osteoarthritis. Moreover, the joint pathology in guinea pigs is affected by similar risk factors like age, weight gain and sedentary lifestyle (29, 31).

For uniformity of preparation we have used allogenic PRP prepared from guinea pig blood. Multiple studies have supported the use of allogeneic PRP in animals and have shown it to be safe, reliable and more consistent with respect to concentration of platelets (32, 33). The PRP prepared from guinea pig blood for injection in PRP group had an absolute platelet count of 1,500,000/µl. We prepared a double concentrate PRP for Chitosan PRP group and the platelet count was approximately 7 times the baseline. Due to dilution from Chitosan poloxamer gel in the ratio 3:1(Chitosan: PRP) and absolute platelet count was 1,090,625/µl. These values of platelet concentrations were within at least 2 to 3 times the baseline counts for guinea pigs (24).

Injectable thermosensitive Chitosan poloxamer gel has been used as a carrier of drugs in various fields. The gel offers the advantage of improved bioavailability, sustained and prolonged drug release (34). Chitosan poloxamer gels have also been established as a drug delivery option to cartilage, and have been proven to be safe and excellent in this role (35, 36). There have been no studies combining Chitosan poloxamer gels with PRP have been reported in the literature. The gel used in our study had a sol to gel transition temperature of 30-32 °C; it thus remained liquid at room temperature and turned to gel at body temperature. The gelling properties of Chitosan poloxamer gel remained intact after sterilization and even after dilution with PRP. The sol properties of gel at room temperature offer the advantage of easy injectability in joints of small animal like guinea pig with a 26 gauge needle. This is in contrast to Chitosan PRP scaffold preparations used by some of the previous authors where preparations had to be directly applied to cartilage or bone defects and could not be used as an injection (16, 37). The preparation by Diwidei *et al.* was injectable, but needed an 18 gauge needle, which may not be appropriate for small animals (17).

The synovitis scores were better in both treatments groups (PRP alone and Chitosan/PRP) than other groups (Disease control and Chitosan alone) at 3 months; no significant difference could be demonstrated between these. One issue could be the lower concentration of platelets in the Chitosan PRP group (due to dilution from Chitosan gel) and loss of intrinsic properties of Chitosan due to sterilization (26). Nevertheless, the gelling properties and Newtonian behavior of gel remained intact after sterilization of gel and addition of PRP. Although in our study, Chitosan poloxamer gel was not shown to enhance the anti-inflammatory effects of PRP, further studies are needed in different scenarios, with larger animal models to prove or disprove this point.

Mean articular cartilage score was measured by Mankin scoring and an increase in scores would have signified chondroprotective role of the treatment group. In the current study there was no significant difference between treatment and control groups in terms of mean articular cartilage score. It this may be due to the fact that we have collated our data at later periods in time (3 and 6 months) as compared to previous studies (maximum of 8 to 9 weeks), and the effect of PRP is known to wear out (16, 17).

We utilized synovial COMP level measurement as it provides an objective measure of the cartilage degradation and has been shown to correlate with the grade of OA in guinea pigs and humans (38, 39). Results of synovial fluid COMP levels were similar to that of mean articular cartilage score where in there was no significant difference in the COMP concentrations at 3 and 6 months between any of the groups.

We could not establish a beneficial role of Chitosan + PRP combination over PRP alone, and this may be due to the following limitations 1) lower concentration of platelets in the Chitosan PRP group (due to dilution from Chitosan gel); 2) inability to quantify any loss of intrinsic properties of Chitosan poloxamer gel due to sterilization (26); 3) inability to compensate lower concentration of platelets with more volume of Chitosan PRP due to small knee joints of the animal model used. A larger animal model may be more appropriate for such a study; 4) we were not able to shed light on the degradation profile of the Chitosan poloxamer gel preparation in vivo and in vitro. The degradation profile of our preparation may be different from freeze dried Chitosan glycerol phosphate preparation along with BMS used in some other studies (17).

CONCLUSIONS

Addition of chitosan poloxamer gel did not offer any additional advantage in prolonging efficacy of PRP in a Guin-

ea pig Knee OA model. Nevertheless, the efficacy of single dose PRP, with or without Chitosan, has been again demonstrated in the short term, due to decreased synovitis scores.

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AUTHOR CONTRIBUTIONS

TB was involved in the concept, design of study, performing the experiments, data collection and analysis. MSD was the senior guide and involved in study design, interpreting the result and editing the manuscript. SP gave the concept behind the study, the study design and edited the manuscript. DC was involved in the study design and edited manuscript. AB was involved in histological analysis and PRP preparation. BM was involved in performing the experiment, preparation of chitosan PRP and interpreting biomarker results. NK was involved in histological analysis.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interests.

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ORIGINAL ARTICLE

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Brown/Beige Fat Activation after Skeletal Muscle Ischemia-Reperfusion Injury

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LIST OF ABBREVIATIONS
IRI: Ischemia-Reperfusion Injury
BAT: Brown/Beige Adipose Tissue
KO: Knockout
UCP-1: Uncoupling Protein -1
WAT: White Adipose Tissue
WT: Wild Type
TA: Tibialis Anterior
PFA: Paraformaldehyde

SUMMARY
Objective. Skeletal muscle Ischemia-Reperfusion Injury (IRI) is a commonly seen orthopedic injury. However, the role of The Brown/Beige Adipose Tissue (BAT) in muscle regeneration after IRI remains unknown. In this study, we assessed the role of BAT in muscle regeneration using UCP-1 reporter and Knockout (KO) mice. We hypothesize that UCP-1 expression increases in BAT after muscle injury and UCP-1 KO mice have reduced muscle regeneration after IRI.
Methods. Unilateral hindlimb IRI was performed on mice by applying a rubber band on the thigh for three hours. Amibegron and antagonist, which activates/deactivates BAT, were also given to mice after IRI. DigiGait analysis was performed at 2 or 4 weeks after IR injury. White (epididymal) fat, brown (interscapular) fat, beige (inguinal) fat and gastrocnemius muscles on both injury and contralateral uninjured sides were harvested. Muscle regeneration index, RT-PCR were performed on brown, beige and white fat to evaluate promyogenic growth factor gene expression.
Results. After hindlimb IRI, UCP-1 expression increased in brown, beige and white fat, which was accompanied by increase of gene expression promyogenic growth factors of IGF1 and follistatin. IRI also induced UCP-1 expression in both injured and contralateral uninjured muscle. DigiGait analysis demonstrated significantly decreased ($p < 0.05$) hindlimb function at 2 weeks post-injury in UCP-1 KO mice compared to wildtype mice. Muscle histology showed significantly reduced muscle regeneration in UCP-1 KO mice compared to WT mice. Amibegron activates BAT and improves muscle regeneration, while SR-59230A deactivates BAT and reduced muscle regeneration after IRI.
Conclusions. BAT plays an important role in muscle regeneration of IRI. Pro-myogenic batokines from BAT may be the mediator for muscle regeneration after IRI. β3 adrenergic receptor agonists may be novel treatment for muscle IRI in the future.

KEY WORDS
Brown fat; beige fat; ischemia-reperfusion injury; muscle; regeneration; uncoupling protein-1.

INTRODUCTION

Skeletal muscle Ischemia-Reperfusion Injury (IRI) is a common and frequently serious complication of limb crush injuries, compartment syndrome, and vascular injuries (1). Deficient blood flow causes muscle lesions of structure disorganization and myocytes degeneration. Although prompt reperfusion restores the delivery of oxygen and substrates required for aerobic ATP generation and normalizes extracellular pH, reperfusion itself appears to have detrimental consequences. Reperfusion accelerates the development of necrosis of muscle. Reactive Oxygen Species (ROS), cytokines, and chemokines from re-perfused muscle, as well as macrophages and mast cells, neutrophils migrated to muscle are thought to be the underlying mechanism of muscle IRI (2).

Humans and many other mammals have two distinct types of fat: white and brown fat. Recent studies revealed a third interchangeable form of fat named beige fat. Though

morphologically similar to white fat, beige fat can differentiate into brown fat by expressing the hallmark molecular signature of brown fat, Uncoupling Protein 1 (UCP-1), under certain conditions (3). Beyond their metabolic role, Beige/Brown Adipose Tissue (BAT) has been identified as an endocrine organ, which secretes various growth factors, known as batokines, including myogenic trophic factors, that have a role in promoting muscle growth and SC population expansion (4). Both adipose tissue and skeletal muscle are the largest organs in the body. Fat-muscle interaction has been gaining increasing attention recently. Adipose tissue and skeletal muscle secrete a large range of bioactive proteins, namely adipokines and myokines. Adipokines and myokines are involved in autocrine/paracrine interactions within adipose tissue and muscle, as well as in an endocrine cross-talk to other tissues (5).

β3 adrenergic receptors are mainly found in brown adipose tissue, beige adipose tissue, white adipose tissue, myocardium, skeletal muscle and liver (6). β3 adrenoceptor agonists, Amibegron (SR 58611A) have been used as antidepressants in clinical trials. Amibegron can have effects on BAT mitochondrial multiplication along with energy expenditure. More interestingly, Amibegron can cause the fast changes occurring in Uncoupling Protein 1 (UCP-1) intrinsic action that is secondary to sympathetic stimulation and restore the brown adipocytes (7).

However, the role of BAT in muscle regeneration after IRI remains unknown. In this study, we assessed the role of brown, beige and white adipose tissue in muscle regeneration after IRI using UCP-1 reporter and Knockout (KO) mice. Moreover, Amibegron was also given to the mice to assess the role of UCP-1 in skeletal muscle recovery. We hypothesize that beige fat undergoes the "browning" process after IRI, and UCP-1 KO mice have impaired muscle regeneration after IRI compared to wildtype mice due to a lack of functional BAT.

MATERIAL AND METHODS

Unilateral hindlimb ischemia-reperfusion injury (IRI)

Unilateral hindlimb IRI was performed on 15 four months old male UCP-1$^{luc2\text{-}tdTomato}$ reporter mice (Jackson Laboratory Inc. Cat# 026690) using a calibrated 1/8 inch orthodontic latex elastic rubber band (6 ounces, Masel Orthodontics, Inc., Carlsbad, CA) as described previously (8). In brief, after general anesthesia by 1-5% isoflurane, a rubber band was applied and removed 3 hours later to allow reperfusion to the hindlimb. Mice were sacrificed at time points of 1, 2 and 4 weeks after injury. The number of animals required for this study was determined based upon power analysis using our approximation of true effect and anticipated sample variability using the assumption: α=0.05, ß=0.80 with the outcome of a 20% difference in gait and histology. With these calculations, we determined that 5 mice per group would be sufficient to demonstrate significant differences between the control and treatment groups (n=5 per time point per group). Five un-injured UCP-1 reporter mice were used as the controls. The same IRI procedure was also applied to UCP-1 WT (n=5) and UCP-1 KO (Jackson Laboratory Inc. Cat# 003124) mice (n=5). Mice were harvested at 2 weeks after IRI after gait analysis. For Amibegron test, β3 adrenergic receptor agonist (Amibegron), which activates BAT, was given at 10 mg/kg I.P. daily to wildtype mice (n=5) after IRI for 4 weeks. All animal care protocols were in compliance with our Institutional Animal Care and Use Committee (IACUC) (IACUC approved protocol# KIM17-017). Our study meets the ethical standards of the journal of MLTJ (9).

Gait Analysis

DigiGait™ (Mouse Specifics Inc., Quincy, Massachusetts) analysis was performed to measure hindlimb function at 2 weeks and 4 weeks after IRI as described previously (10). All mice walked at 10 cm/s for 10 s on the DigiGait system. Swing Stride, Stance Stride, Brake Stride and Stance/Swing ratio were recorded to assess hindlimb function.

Muscular and adipose tissue harvesting and wet muscle weight

After animals were sacrificed, white (epididymal) fat, brown (interscapular) fat, beige (inguinal) fat and Tibialis Anterior (TA) muscles on both injury and contralateral side were harvested. Wet weight of TA muscles was measured immediately after harvesting. Fat samples were fixed with 4% Paraformaldehyde (PFA), dehydrated with 30% sucrose and embedded in Paraffin (Leica Biosystems, IL). Muscle samples were flash-frozen in liquid nitrogen cooled isopentane. Both fat and muscles were cyrosectioned with a cyrotome.

Histology

TA muscles from the injury and contralateral sides were sectioned at 7 μm with Microm HM550 cyrosection (Thermo Fisher,) at the muscle belly. Sections were fixed with 4% PFA (Sigma, St. Louis, MO) for 20 minutes at room temperature and blocked with 5% BSA (Jackson Immuno Research Laboratories) in PBS. Sections were permeated

with 100% methanol (Lot # B0533756, ACROS Organic) at -20 °C for 6 min, rinsed with PBS and incubated with primary antibody laminin (L9393, Lot# 028M-4890V, Sigma, MO, USA) diluted 1:500 in 5% BSA at 4 °C overnight. After rinsing in PBS three times, sections were incubated at room temperature for 1 h with secondary antibody (donkey anti-rabbit conjugated to Alexa Fluor®-647 1/500, ab150075, Lot# GR3191436-2, Abcam) diluted in 5% BSA. After rinsing in PBS three times, coverslip was laid on and slides were sealed with Vectshield (H-1000, Vector Laboratories). Slides were then observed on an optical microscope (Zeiss, Oberkochen, Germany). Pictures were taken and analyzed using Bioquant (Nashville, TN, USA). All pictures were assessed by two blinded researchers. Muscle regeneration was evaluated by regeneration index (% of regenerating fibers with central nuclei among total fibers). Cross sections area was measured by for muscle fibers in five randomly selected areas on sections from mid-bellies of TA muscle. 600 to 2000 fibers in each sample were calculated using Bioquant (Nashville, TN, USA).

PFA fixed adipose tissue sections were processed with Benchtop Tissue Processor (Leica TP1020) and embedded in paraffin according to routine histologic techniques (Medite TES 99). Sections, 5-µm thick, were stained with DAPI and sealed with 10% glycerin till they were being ready to image.

Luciferase assay

UCP-1 gene expression level was also measured by Luc2 reporter gene with luciferase in fat tissue from UCP-1 reporter mice. Briefly, adipose tissue was fast frozen in liquid nitrogen and homogenized with 0.5 ml/100 mg lysis buffer using tissue grinder. The mixture was frozen and thawed in the -80 °C and 37 °C for 3 cycles. The solution was centrifuged at 4000 RPM for 5 minutes. Finally, the supernatant was collected and stored in -80° C until ready to analyze. Luciferase in fat lysate was measured with Luciferase assay System (E1501, Promega, Italy) as described previously (11). 100 µl of fat lysate supernatant were placed in 96 well plate and 200 µl of substrate and buffer were added into each well. Each sample was measured in triplicate. The plates were read by Bio-rad plate reader with luminometer for 10 seconds.

Reverse-Transcript Polymerase Chain Reaction (RT-PCR)

Total RNA for both brown (n=5 per group), beige (n=5 per group) and white (n=5 per group) was extracted using Trizol reagent (Fisher Scientific, CA, USA) according to the manufacture's instruction. Transcription First Strand cDNA Synthesis Kit (Roche Applied Bioscience Inc., Indianapolis, IN, USA) was applied to synthesize cDNA. RT-PCR was performed to quantify gene expression of using SYBR Green Detection and an Applied Biosystems Prism 7900HT detection system (Applied Biosystems, Inc., Foster City, CA). Brown fat markers UCP-1 and PRDM-16, promyogenic batokines of follistatin and IGF1 gene expression were analyzed via RT-PCR. Sequences of the primers for target genes were showed in **table I**. The expression level of each gene was normalized to that of the house-keeping gene of R26. Folds changes relative to naive control group were calculated by ΔΔCT.

Statistical analysis

A Student t-Test was performed to determine a significant difference between UCP-1 KO and wildtype mice (cross section area, regenerated fiber index) as well as between Amibegron and Dimethyl Sulfoxide (DMSO) treated group (cross section area, regenerated fiber index). Statistical significance was considered when $p < 0.05$.

RESULTS

UCP-1 were activated in adipose tissue and muscle after IRI

UCP-1 signal was detected in all adipose tissue after IRI. The UCP-1 signal of brown, beige and white adipose tissue

Table I. Primers used for qRT-PCR.

Gene	Forward (5'→3')	Reverse (5'→3')
Rps26	ACGGGAAACCCATCACCATC	CCCTTCCACGATGCCAAAGT
Fst-288	CTCTCTCTGCGATGAGCTGTGT	GGCTCAGGTTTTACAGGCAGAT
Fst-315	CTCTCTCTGCGATGAGCTGTGT	TCTTCCTCCTCCTCCTCTTCCT
IGF-1	AAAGCAGCCCCGCTCTATCC	CTTCTGAGTCTTGGGCATGTCA
UCP1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
PRDM16	CCCAGTTTAACCTGTTTGTAGGCA	ATCCGCCATTGTTAAGACC

were increased at both 1 week, 2 weeks and 4 weeks after IRI compared to control (**figure 1 a**). The signal intensity peaked at 2 weeks in adipose tissue and decreased at 4 weeks compared to the 2 weeks time point. Moreover, the UCP-1 signal was also detected in both contralateral and injured TA. The UCP-1 signal intensity peaked at 2 weeks and decreased at 4 weeks after IRI (**figure 1 a**). Interestingly, contralateral injured TA muscle had a higher percentage of UCP-1 expressing cells compared to the injured TA muscle (**figure 1 a**).
Luciferase assay confirmed findings from histology. UCP-1 driven Luc2 reporter gene expression significantly increased in brown, beige and white fat, with a peak at 2 weeks after IRI (**figure 1 b**).
RT-PCR showed that, UCP-1 and PRDM-16 expression significantly increased in Brown, Beige and white adipose tissue after IRI (**table II**, **figure 2**).

Promyogenic batokine gene expression increased in adipose tissue after IRI

RT-PCR showed that tissue-binding isoform follistatin (Follistatin 288), circulating isoform follistatin (Follistatin

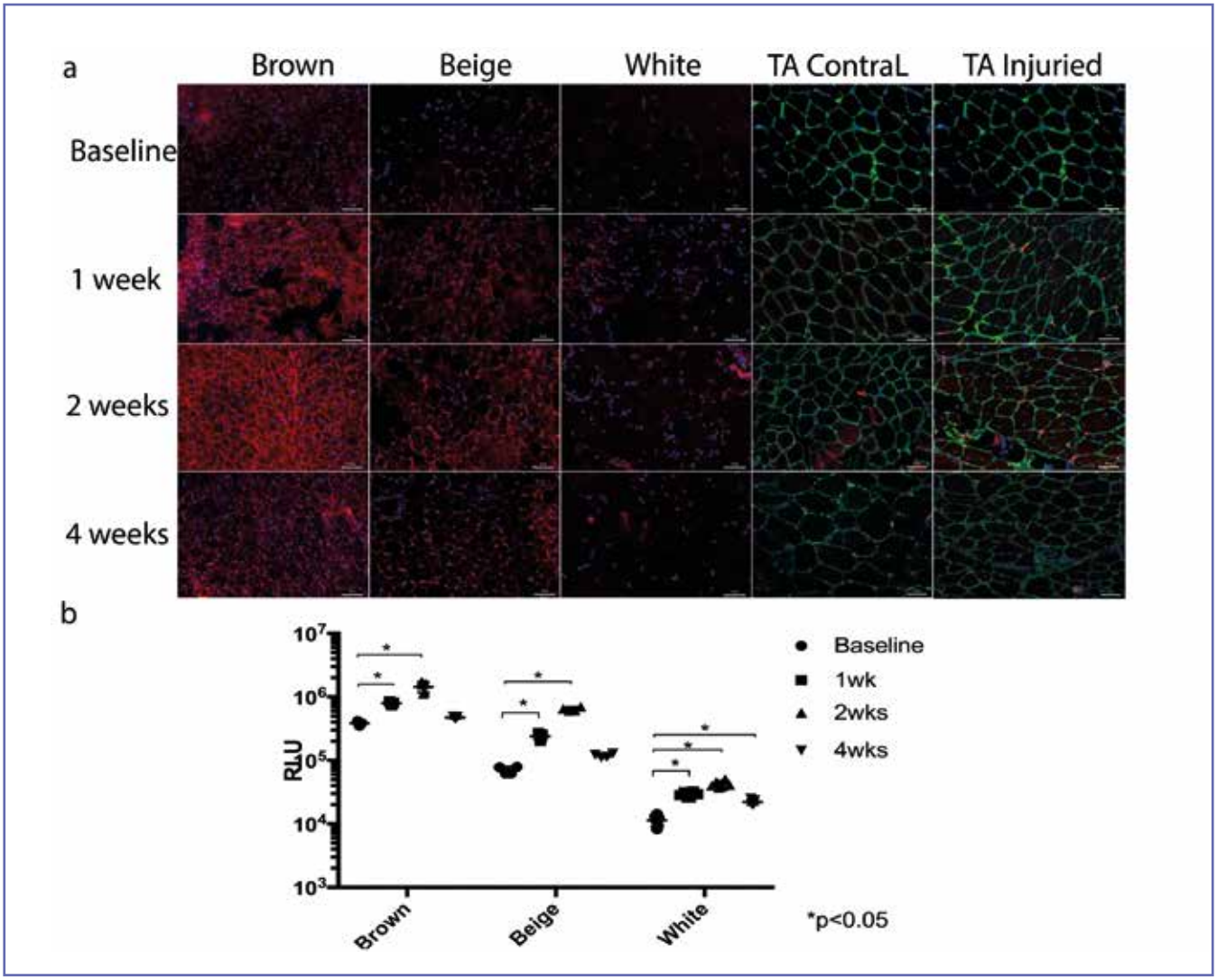

Figure 1. (a) Activation of BAT after IRI. Brown, beige, white fat, as well as TA muscle after IRI from UCP-1 reporter mice before (baseline) and at 1, 2, 4 weeks after IRI. Red signal-UCP-1 reporter gene UCP-1 expression increased at 1 and 2 weeks in all types of fat. It decreased to baseline level in brown and beige fat, but remain elevated in white fat at 4 weeks after IRI. (* $p < 0.05$).

Table II. RT-PCR results of mRNA expression in Brown, Beige and White adipose tissue at 1, 2 and 4 weeks after IR injury. (Mean ± SD, * indicates $p < 0.05$ compared to baseline control).

1 week	UCP-1	PRDM16	Follistatin 288	Follistatin 315	IGF-1
Brown	4.23 ± 1.95*	7.87 ± 1.15*	2.43 ± 0.31*	5.95 ± 1.22*	2.19 ± 0.35*
Beige	3.19 ± 1.81*	13.18 ± 2.86*	1.75 ± 0.19*	3.43 ± 0.57*	1.71 ± 0.46*
White	1.11 ± 0.48	7.95 ± 1.79*	1.05 ± 0.94*	2.32 ± 1.91	1.36 ± 0.27*
2 weeks	**UCP-1**	**PRDM16**	**Follistatin 288**	**Follistatin 315**	**IGF-1**
Brown	9.15 ± 2.26*	3.87 ± 0.46*	3.35 ± 0.37*	7.98 ± 1.27*	3.19 ± 0.53*
Beige	5.19 ± 1.10*	3.18 ± 0.86*	3.41 ± 0.17*	3.55 ± 0.68*	2.71 ± 0.24*
White	2.21 ± 0.32*	2.95 ± 1.97	2.06 ± 1.44	8.36 ± 2.03*	3.36 ± 0.38*
4 weeks	**UCP-1**	**PRDM16**	**Follistatin 288**	**Follistatin 315**	**IGF-1**
Brown	2.15 ± 1.29	1.87 ± 1.13	59.12 ± 7.14*	39.48 ± 1.38*	22.75 ± 3.13*
Beige	2.32 ± 0.28*	1.18 ± 0.56	74.38 ± 5.31*	10.47 ± 2.47*	5.96 ± 1.69*
White	1.16 ± 0.13	0.95 ± 0.48	59.68 ± 4.95*	49.29 ± 3.14*	18.65 ± 2.87*

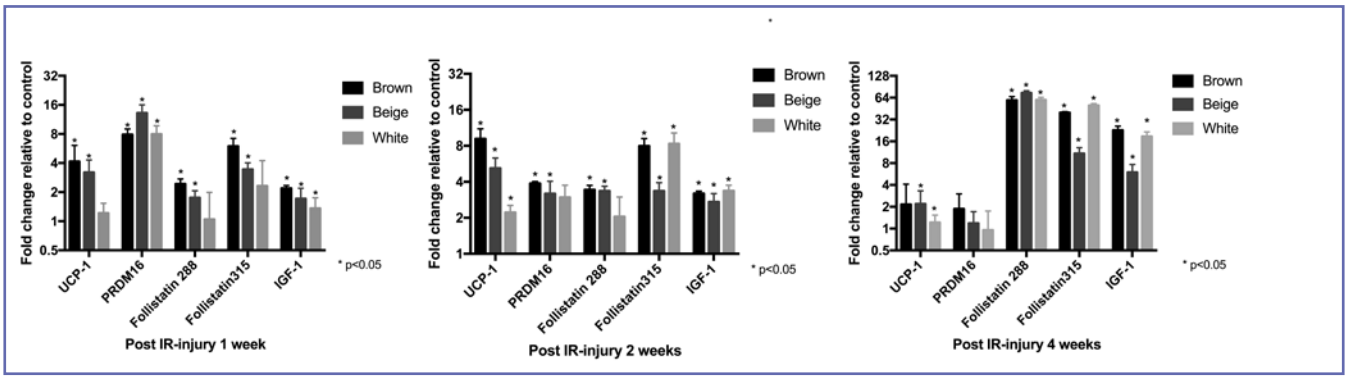

Figure 2. UCP-, PRDM-16, IGF1 and follistatin were significantly increased after 1 week, 2 weeks, and 4 weeks IR injury.

315) and IGF1 gene expression level increased in brown, beige and white adipose tissue after IRI (**table II**, **figure 2**).

UCP-1 (-/-) mice had reduced muscle regeneration compared to UCP-1 (+/+) WT mice after IRI

DigiGait analysis showed that the swing stride of hind limb increased by 18% ($p < 0.05$), the brake stride decreased by 28% ($p < 0.05$), the stance stride decreased by 26% ($p < 0.05$) and the stance/swing ratio decreased by 3 folds ($p < 0.05$) in the UCP-1 (-/-) mice compared to UCP-1 (+/+) wildtype mice at two weeks after IRI (**figure 3**).

Muscle histology analysis showed that the regenerating index of TA in UCP-1 (-/-) mice was significantly lower than UCP-1 (+/+) wildtype mice at two weeks after IRI (**figure 4**). The mean of cross section area of myofiber of TA on the injured side in UCP-1 (-/-) mice at two weeks

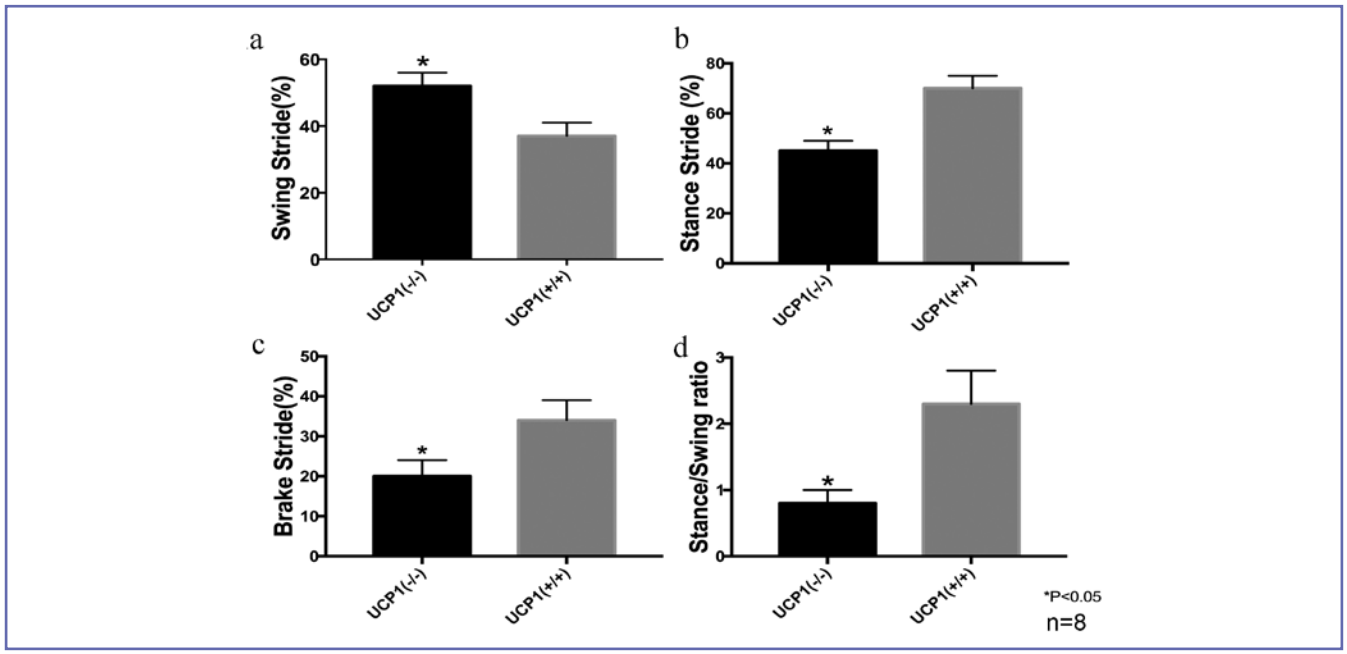

Figure 3. UCP-1 (-/-) mice have significantly increased swing stride and reduced stance stride, brake stride and stance/swing ratio compared to Wildtype (WT) mice at 2 weeks after IRI.

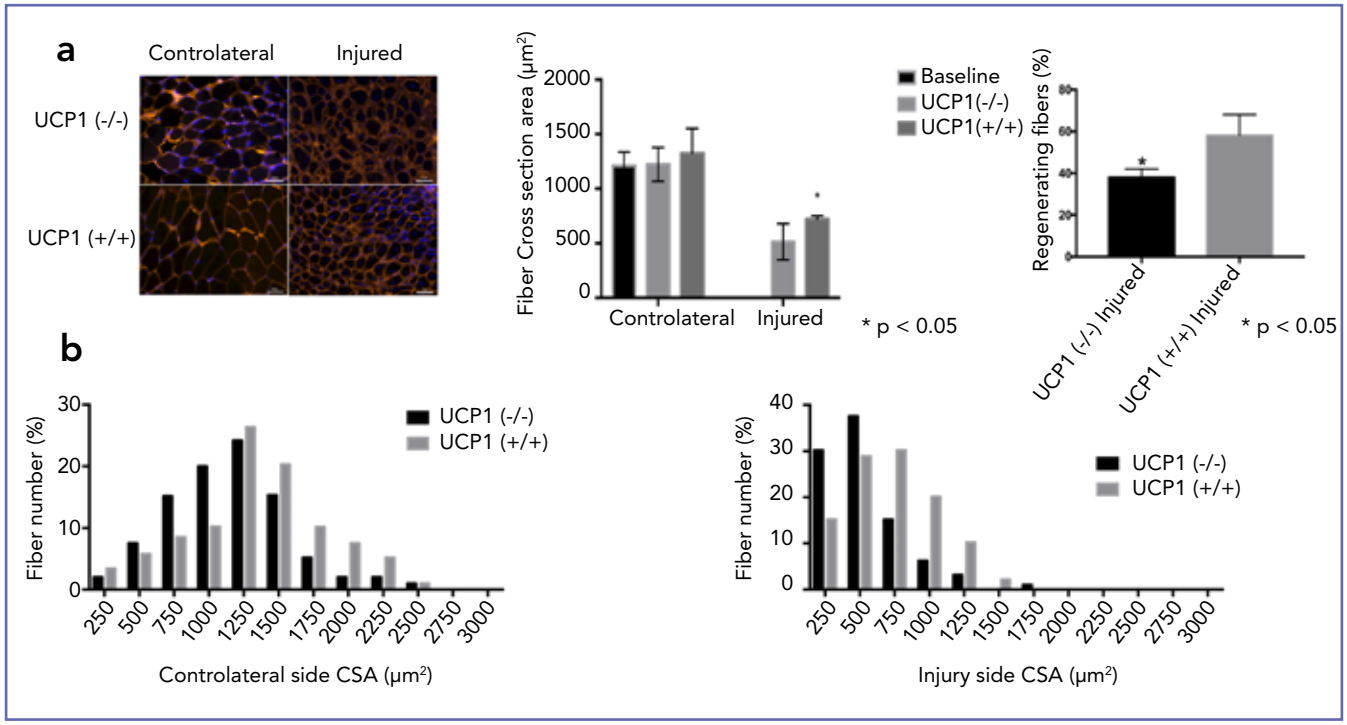

Figure 4. (a) UCP-1 (-/-) mice have significantly reduced regenerating fibers ratio (% of central nuclei regenerating fiber number/ total fiber number) and average muscle fiber cross section area at 2 weeks after IRI compared to UCP-1 (+/+) wildtype mice. (Scale bar stands for 50 μm). (* $p < 0.05$ compared to WT mice). (b) Histogram of muscle fiber cross-section area of TA muscles at contralateral and IRI sides of UCP-1 (-/-) and UCP-1 (+/+) mice at 2 weeks after IRI.

(513.15 μm²) was significantly smaller than that in UCP-1 (+/+) mice (720.625 μm²), though no significant difference was found at the contralateral uninjured TA (1324.5 μm² in UCP-1 (+/+) mice *vs.* 1222.75 μm² in UCP1 (-/-) mice (**figure 4**).

Amibegron improved hindlimb muscle regeneration after IRI

DigiGait analysis showed that, compared to DMSO vehicle-treated mice, mice treated with Amibegron had significantly increased stance width (p=0.03) and stride length (p=0.03) at 4 weeks after IRI (**figure 5**).

Muscle histology showed that the average regeneration index in TA of Amibegron treated mice was significantly higher than that in DMSO treated mice (p=0.04) (**figure 6 a**). Four weeks treatment of Amibegron also significantly increased mean cross section area of myofiber of TA after IRI (1113.15 μm² in Amibegron group compared to 892.20 μm² in DMSO group, p=0.01) (**figure 6 a, c**). Finally, the brown fat markers were increased in Amibegron treated group compared to DMSO in brown, beige, white adipose tissue. The batokines were increased significantly as well (**figure 6 b**).

DISCUSSION

Skeletal muscle and adipose tissue are made up large portion of human body. An average adult male has 42% as the skeletal muscle and an average adult female has 36% (a percentage of body mass) (12). The mean percentage of body fat ranges from 22.9% to 30.9% in adult males and from 32.0% to 42.4% in adult females. Despite increasing knowledge of cross-talk between muscle and fat, our understanding of interactions between these two biggest organs in our body remains limited. In this study, we have observed significant morphology and gene expression change in adipose tissue, including brown/beige fat activation and white fat "browning" after muscle Ischemia-Reperfusion Injury (IRI). Mice with non-functional BAT by knocking out UCP-1 have significantly reduced muscle regeneration. These new finding suggests a novel interaction between fat and muscle, that BAT promotes muscle regeneration after IRI.

Brown/Beige Adipose Tissue (BAT) is the main site of adaptive thermogenesis (13). Reduction of blood flow after IRI can lead to reduced temperature in the injured limb. Thus, activation of BAT after IRI may be a natural reaction to muscle IRI in response to increased need of thermogenesis, especially at early stage after injury. However, BAT possesses more functions other than thermogenesis. Recent studies

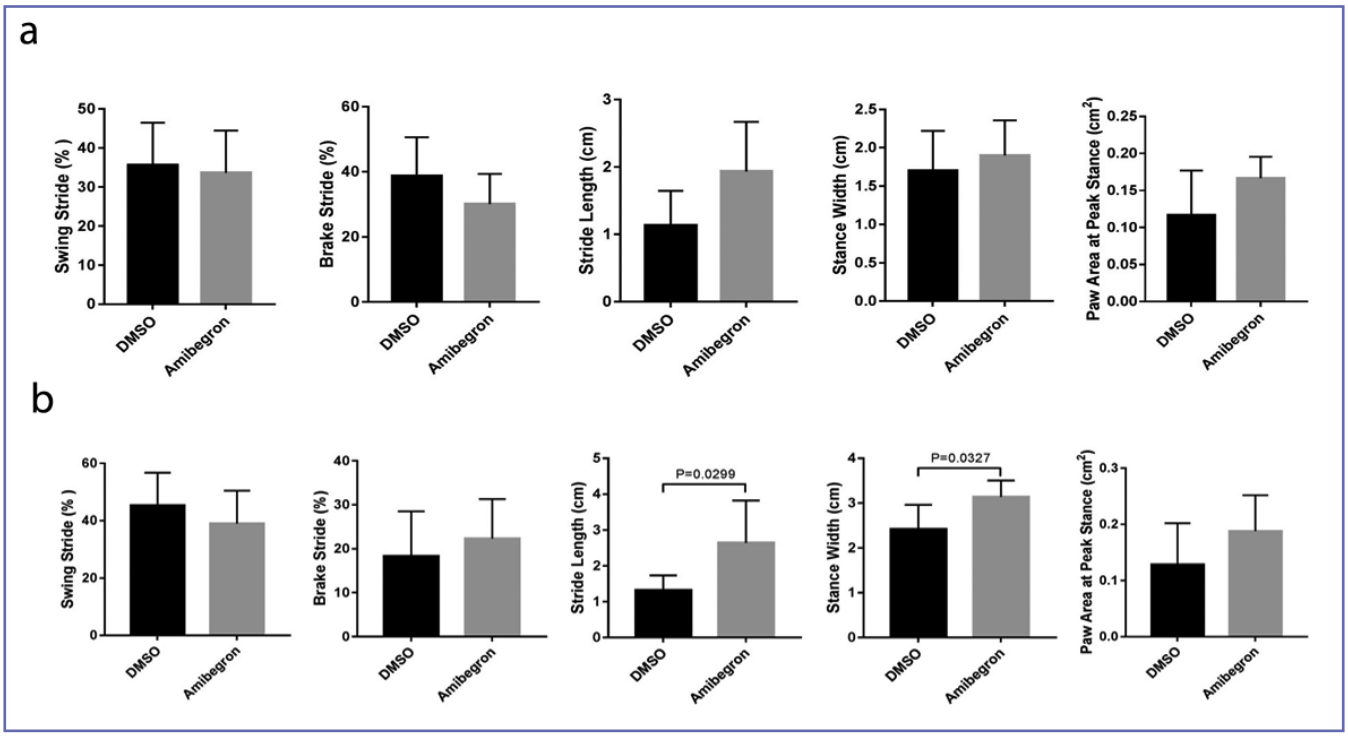

Figure 5. DigiGait analysis at 2 weeks (a) and 4 weeks (b) after IRI showed that Amibegron improves injured hindlimb limb function at 4 weeks after IRI.

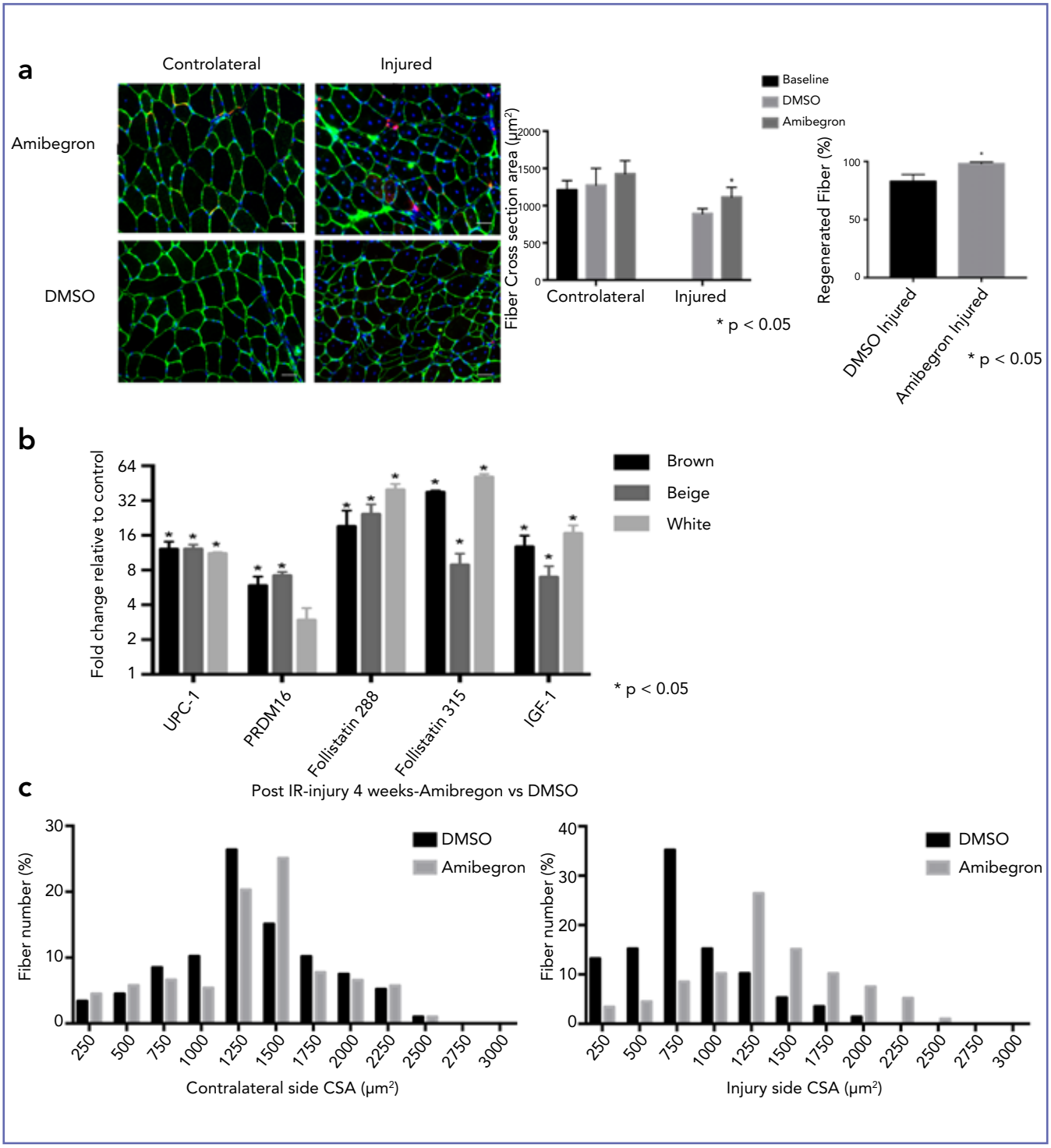

Figure 6. (a) Mice receiving Amibegron treatment for 4 weeks have significantly higher regenerating fiber % and average muscle fiber cross-section area after IRI compared those received DMSO (vehicle for Amibegron) (scale bar stands for 50 μm). (b) UCP1, PRDM-16, follistatin and IGF-1 expression significant increased compared to DMSO group. (c) Histogram of muscle fiber cross-section area of TA muscles at contralateral and IRI sides on mice with Amibegron and DMSO treatment at 4 weeks after IRI.

have been shown that BAT also has a secretory role, which could contribute to the systemic consequences of BAT activity. An increasing body of BAT-derived cytokines (namely batokine) have been identified as paracrine or endocrine factors (5, 14). In this study, we found significantly increased expression of IGF1 and follistatin, two important promyogenic batokines from BAT after muscle IRI, suggesting BAT may facilitate muscle regeneration after IRI through paracrine/endocrine function of promyogenic batokines.

It is interesting that we found thermogenesis regulated genes, including UCP-1 and PRDM-16 increased in BAT at relative early stage (1-2 weeks) after muscle IRI while promyogenic batokine (including follistatin and IGF1) gene expression were highly up-regulated at later stage (4 weeks) after IRI. This data suggests that BAT may facilitate mice recovery after IRI at two phases: thermogenesis in early stage and paracrine/endocrine of promyogenic batokines in late stage after IRI.

Epididymal fat pad in adult mice is considered a typical source of white fat. However, in this study, we saw remarkable UCP-1 reporter signal in epididymal fat pad after IRI. Though significantly lower than that in brown and beige fat, UCP-1 signal in epididymal fat remained increased even at 4 weeks after IRI. Expression of BAT markers, including UCP-1 in white fat is known as "white fat browning". Browning of white fat is accompanied with metabolism changes and energy consumption. Thus, white fat browning has been considered as new hope to treat obesity and type II diabetics. White fat browning is induced with drugs, exercise and cold inducement (15). In this study, we observed spontaneous white fat brown after muscle IRI. This suggests a dramatic change in metabolism after muscle IRI. Expression of promyogenic factors of follistatin and IGF1 also increased in the "browning" white fat, suggesting a possible endocrine role of browning white fat after muscle IRI (16).

Besides adipose tissue, we also found UCP-1 signaling in muscle after IRI. Interestingly, UCP-1 (+) BAT cells are not only found in injured muscle, but also within non-injured muscle at the contralateral side. The origin and function of intramuscular BAT after muscle IRI remains unknown at this time. Among all muscle progenitor cells, Fibro-Adipogenic Progenitors (FAPs) are most likely to be the cellular origin of the intramuscular BAT after muscle IRI, due to their adipogenic potential. A recent study showed that FAPs can express UCP-1 *in vitro* and *in vivo* (15, 17). Future work is needed to define the cellular source and function of intramuscular BAT after muscle IRI.

Follistatin (Fst) is a secreted glycoprotein that has a high affinity binding and neutralizing other proteins such as several members of the TGF-β superfamily including Activins and Myostatin (Mst). Follistatin has reported isoforms FST288, FST315, and FST300 (or FST303). FST288 and FST315 are known to be created by alternative splicing of the FST gene transcript, while FST300 is thought to be the product of posttranslational modification of C-terminal domain truncation. FST315 is primarily found in circulation, whereas FST288 displays greater cell surface affinity. Thus, FST288 is considered to function through an autocrine fashion while FST315 is more like to play an endocrine role.

Follistatin proteins were found to antagonize Myostatin mediated inhibition of myogenesis (18, 19). FST-transgenic mice produced a robust skeletal muscle hypertrophy phenotype, which proved its promyogenic role *in vivo* (20). Recent work suggests that Fst also promotes BAT differentiation and energy metabolism. Thus, Fst may play a dual role in both promoting muscle growth and inducing BAT activation after muscle IRI. In this study, we have observed significantly increased gene expression of both Fst288 (tissue binding isoform) and Fst315 (circulating isoform) from brown, beige and white fat. Considering their distinguished difference, Fst315 may be responsible for promoting muscle regeneration, while Fst288 may be responsible for BAT activation and white fat "browning". Future work is needed to define the exact function of Fst288 and Fst315 after muscle IRI.

BAT activity is highly regulated by sympathetic nerves system, mainly through β3 adrenergic receptor pathway. β3 adrenergic receptors are selectively expressed in adipose tissue and β3 adrenergic receptor agonists can effectively stimulate BAT activity in both animals and human (16). In this study, we found that Amibegron, a selective β3 adrenergic receptor agonist significantly improved muscle regeneration and limb function after IRI. This data suggests that selective β3 adrenergic receptor agonists may serve as a new treatment for muscle IRI. Mirabegron, a selective β3 adrenergic receptor agonist with a similar structure to Amibegron, has been approved by the FDA for treating over-reactive bladders (21). Mirabegron could be considered as a potential novel treatment to improve muscle regeneration after IRI.

Some limitations of this study should be noted. First of all, the effect of species differences on quantity of BAT between mouse and human is significant. Adult mice contain a significant amount of BAT, while the amount of BAT in adult humans is relatively small. Most BAT tissues in adult human remain inactive without appropriate physical or chemical stimulations. Second, we only used RT-PCR to investigate promyogenic batokine expression in adipose tissue in this study. To quantify the protein of batokines in both adipose tissue and circulation is needed in future works. Third, due to limited supply of knockout mice, we only employed

UCP-1 knockout mice at one time point (2 weeks) after IRI. Though the results are convincing, we will consider employing other time points when more knockout mice become available in the future. Last but not least, because the UCP-1 $^{luc2\text{-}dtTomato}$ reporter gene was inserted on to the Y chromosome in UCP-1 reporter mice, we predominantly used male mice in this study. Finding in this study needs to be verified on female mice in the future.

CONCLUSIONS

Results from this study suggest that BAT plays an important role in muscle regeneration of IRI. Pro-myogenic batokines from BAT may be the mediator for muscle regeneration after IRI. β3 adrenergic receptor agonists may be novel treatment for muscle IRI in the future.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Epidermal Growth Factor Stimulates Rabbit Achilles Tendon Histologically and Biomechanically Healing

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SUMMARY

Background. Epidermal Growth Factor (EGF) stimulates epidermis cell growth, proliferation and differentiation in skin regeneration. The aim of this study was to pre-clinically investigation of the role of EGF in tendon healing.

Methods. One cm defects were created at the right Achilles tendons of 30 New Zealand White rabbits. Ten rabbits were allocated to one of three groups: Group-1-(Sham) tendon defect with a gap that was splinted with a non-absorbable suture; Group-2-(EGF +) tendon defect with a gap that was splinted with a non-absorbable suture and a 25 µg/kg EGF injection into the defect; Group-3-(Scaffold + EGF) tendon defect was grafted with a biodegradable, porous Polycaprolactone (PCL) scaffold loaded with 25 µg/kg EGF and stabilized with a non-absorbable suture. Animals were sacrificed at 8 weeks post-surgery and Achilles tendon repair and healing status was investigated using histopathologic and biomechanical analysis methods.

Results. Group-2-(EGF +) had greater adipocyte development (moderate) than Group-1-(Sham) and Group-3-(Scaffold + EGF). Group-2-(EGF +) and Group-3-(Scaffold + EGF) had greater peripheral nerve development (weak) than Group-1-(Sham). Group-2-(EGF +) had greater vascularization (moderate) than Group-1-(Sham) and Group-3-(Scaffold + EGF). Group-2-(EGF +) had greater collagen Type-III development (moderate) than Group-1-(Sham) and Group-3-(Scaffold + EGF). Group-3-(Scaffold + EGF) had greater collagen Type-I development (moderate) than Group-1-(Sham) and Group-2-(EGF +). Groups did not display statistically significant differences for load to failure or elongation at failure. Group-2-(EGF +) and Group-3-(Scaffold + EGF) displayed less stiffness that the control (healthy contralateral Achilles tendon) ($p < 0.05$), however, experimental groups did not differ ($p > 0.05$).

Conclusions. The application of EGF and scaffold displayed superior histological tendon healing evidence, but there was no significant difference in terms of biomechanics.

KEY WORDS

Achilles tendon; tendon regeneration; epidermal growth factor; scaffold; rabbit.

INTRODUCTION

The Achilles tendon is the strongest tendon of the ankle joint, and one of the most important biomechanical structures in human gait (1). The Achilles tendon is frequently affected by ankle trauma and it is susceptible to acute or chronic injuries. As a result of these injuries, patients experience significant daily activity and sports performance impairments, functional limitations and disabilities.

Although many studies have been conducted to identify the causes of Achilles tendon rupture, its nature is still not clear-

ly understood. There is no consensus as to whether Achilles tendon rupture is the result of tendon disease, biomechanical loads, or some combination of both (2). Degenerative changes identified in biopsy specimens obtained during surgical repair suggest that acute Achilles tendon rupture may actually represent acute tears that occur in association with chronic tendon degenerative conditions (1, 3-5).

It has been suggested that biologic agents such as growth factors accelerate surgical tendon repair healing and reduce adhesion formation, particularly if they are administered within the first 10 days post-surgery (6, 7). Transforming Growth Factor-β (TGF-β) for example, has been shown to be particularly active near the proximal Achilles tendon defect, whereas Insulin-like Growth Factor (IGF) and Fibroblast Growth Factor (FGF) are more active throughout the entire defect area. Platelet-Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) activity was noted throughout the entire defect repair area (7-9).

Although bioactive agents such as Platelet-Rich Plasma (PRP), bone marrow aspiration, Mesenchymal Stem Cells (MSCs) and previously mentioned growth factors are being used as supplements to conventional treatment protocols, Epidermal Growth Factor (EGF) is not currently used for tendon healing. Rather, EGF is routinely used for skin healing, especially among patients with diabetic wounds to facilitate epidermal bridging either by topical infiltration or by intralesional or perilesional injections (10). Injection of EGF in these cases stimulates the growth and proliferation of vascular endothelial cells, keratinocytes and fibroblasts that have an important role in scar tissue formation. Clinical EGF application for subcutaneous wound healing is used worldwide (11). Based on the lack of information regarding the potential role of EGF for tendon healing, the objective of this study was to investigate its efficacy in EGF alone and EGF together with a Polycaprolactone (PCL) scaffold using a rabbit model. The study hypothesis was that use of EGF alone and with a polycaprolactone (PCL) scaffold would display superior Achilles tendon defect healing compared to a sham procedure.

METHODS

Preparation of the PCL scaffold as the EGF delivery system

Porous PCL scaffolds were prepared with freeze-drying as previously described (12). Briefly, PCL was dissolved in dichloromethane (4%, 10 mL), poured into petri dishes and lyophilized (Labconco Freezone 6, USA) after freezing at -20 °C overnight. Cylindrical porous scaffolds (D: 0.5 mm, L: 1 cm) were cut from the lyophilized foams. EGF (for a final concentration of 25 μg/kg when implanted) was loaded into the scaffolds by adsorption into the scaffold pores. For this, 85 μg EGF was suspended in 100 μL of 1%, w/v alginic acid solution and introduced to both sides of the scaffolds. After air- drying, the scaffolds were dipped into ethanol and then kept in 5% w/v $CaCl_2$ for 1 h to crosslink the alginic acid. Scaffolds were sterilized using ethylene oxide (Steri-Vac gas sterilizer 5XL) at 37°C for 4 h 45 min.

Animals

Thirty New Zealand White rabbits between 9-12 months old with an average body weight of 3350 ± 13 g were included in the study. In this randomized and controlled experimental study, 3 groups of 10 rabbits were created. All procedures were performed after obtaining approval from Animal Experiments Local Ethical Committee (13).

Surgical technique

A 1 cm long defect was created in the right Achilles tendon of each rabbit. All surgical procedures were performed by the same surgeon using the same suture material (3.0 Prolene, Ethicon, USA) for tendon repair. No operations were performed on the left Achilles tendons, which constituted the control group.

All animals were administered 20 mg/kg Cefazolin Sodium I.M. antibiotic prophylaxis and then were anesthetized using 35 mg/kg ketamine HCL and 5 mg/kg xylazine. A sufficient level of anesthetic depth was reached after corneal reflexes disappeared.

The surgery site was dyed using an antiseptic solution of 10% Batticon® and the rabbits were positioned in prone. After surgical site sterilization, the Achilles tendon was palpated and an approximately 4 cm long skin incision was made starting from the ankle posterior and extending proximally along the medial side of the Achilles tendon. Subcutaneous tissue was sharply dissected, and the Achilles tendon was reached. While the rabbit hind sole parallel to the ground, surgical pen and ruler were used to demarcate the region between 1.5 cm and 2.5 cm from the Achilles tendon calcaneal insertion (**figure 1 a**).

The marked tendon regions on the right Achilles tendons of all rabbits were excised to create a 1 cm long Achilles tendon defect (**figure 1 b**). After defect creation, surgical repair was performed using non-absorbable suture and the same suture method (modified Kessler technique) at the right Achilles tendon for each group of rabbits (**figure 1 c**). A different interventional procedure was then performed in each of the 3 experimental groups.

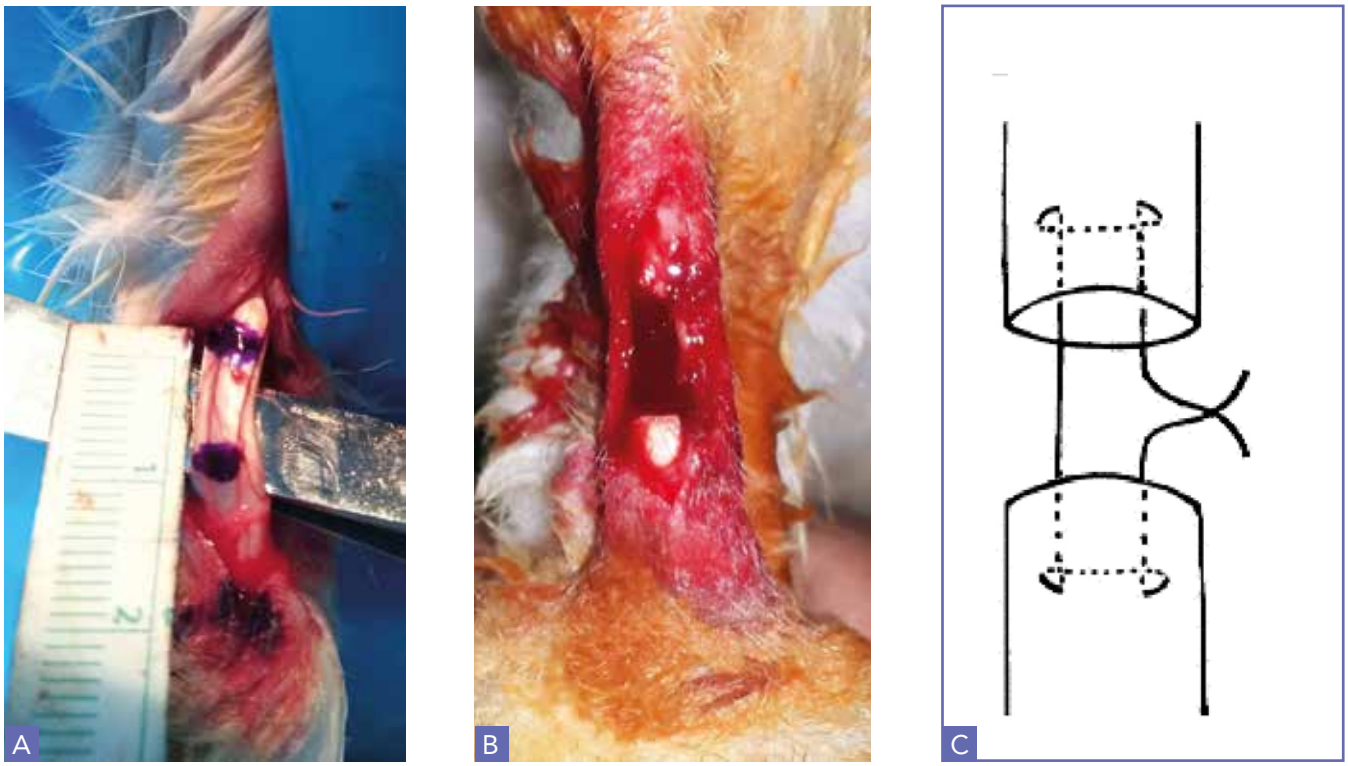

Figure 1. (a, b) Photographs showing 1 cm tendon defect formation in the rabbit Achilles tendon. **(c)** Modified Kessler Suture Method.

Group 1 (Sham): the 1 cm tendon defect was "splinted" leaving a 1 cm gap without tendon ends getting closer using non-absorbable Prolene® (3/0, polypropylene) suture, while the hind soles were positioned parallel to the ground (**figure 2 a**).
Group 2 (EGF +): the same as Group 1, but with the addition of an EGF injection (25 µg/kg) in the defect (**figure 2 b, c**).
Group 3 (Scaffold + EGF): the same as Group 1, but with the addition of a biodegradable, porous scaffold loaded with 25 µg/kg EGF (**figure 2 d**).
Postoperative 25 µg/kg EGF injections were made in the defect region for 10 days every other day in Group 2 and Group 3 (**figure 3**). Only ankle splint was applied in Group 1 as postoperative procedure.
The wounds of all rabbits were dressed on the second post-surgical day and changed each 2-day interval for 10 days. Three rabbits died during postoperative follow-up. One rabbit died in each group, leaving 9 rabbits in each group. At eight weeks post-surgery, a bloc excision was performed on the Achilles tendons of all sacrificed rabbits from the muscle-tendon junction proximal to the bone-tendon junction where the Achilles tendon adheres to the calcaneus. Repair and regeneration of sacrificed Achilles tendons were examined macroscopically, histologically, and biomechanically. Five of the 9 tendons in each group were allocated randomized (13) for biomechanical evaluation and the remaining 4 were used for histological evaluation.

Histological evaluation

Histological evaluation was performed by two independent university histology professors using an agreed upon criteria. Initial rater agreement was ≥ 80%. When disagreements existed, the raters consulted with each other to finalize their assessment.
Histological scoring was performed from healing site samples using the following criteria: 0=no development evidence; 1=weak development evidence; 2=moderate development evidence; 3=strong development evidence (13). Therefore, a score of 0 for adipocytes, for example, suggests that no adipocytes were observed the defect, while a score of 3 suggests a high adipocyte volume.
For this evaluation, 1 cm long tendon healing area sections and an equal length from the healthy contralateral Achilles tendon (control) were excised, and then rapidly fixed in 10% formalin. All tissue specimens were then processed

Figure 2. (a) Photograph showing surgical repair in Group 1 (Sham). **(b, c)** Photographs showing surgical repair and EGF injection on the Achilles tendon rupture site in Group 2 (EGF +). **(d)** Photograph showing surgical repair by grafting the Achilles tendon rupture defect with scaffold and impregnating the scaffold with EGF in Group 3 (Scaffold + EGF).

Figure 3. Photographs showing EGF injection, which were performed every other day for 10 days in Group 2 (EGF +) and Group 3 (Scaffold + EGF).

for routine light microscopy. All tissues were embedded in paraffin, and 10 µm thick sections were cut using a sliding microtome and stained with hematoxylin and eosin and Masson's trichrome. Sections were imaged digitally under a research microscope. Whole section imaging was performed using with MBF Bioscience Microlucida system (Williston USA) which consists of a motorized microscope (Leica DM4000) and an Optronics Microfire digital camera. Surgical side tissue samples were investigated comparatively in all groups for collagen fibril structure and pattern, changes in vascularity, and changes in adipocyte and inflammatory cell infiltration.

Biomechanical evaluation

Tendons that underwent biomechanical evaluation were immediately brought to the laboratory after harvesting for biomechanical testing. Specimens were placed in the soft tissue clamps of the test machine (Testometric, Rochdale, England) from both sides (**figure 4**). Tendons were strained at 10 mm/min. Stress-strain curves were obtained and the point where the force suddenly started to drop after the peak loading force was recorded (14). Moreover, elongation at failure (mm) and Young's modulus (N/mm^2) of the tendons (stiffness) were assessed.

Figure 4. Biomechanical evaluation with the Testometric device.

Statistical analysis

Statistical analysis was performed to compare the values obtained from the biomechanical tendon studies. In determining whether there were statistically significant differences between the 3 experimental groups and the control group for load at failure force, Young's modulus, and elongation at failure, the Shapiro-Wilks test was performed to confirm data normality. Following this, one-way Analysis of Variance (ANOVA) was applied to each of the 3 variables. Variance homogeneity assumption was evaluated using the Levene test. *Post-hoc* analysis was performed in order to better delineate which group created the difference for differences between the groups identified in Young's modulus variable, and the Bonferroni test was performed for paired comparisons at this stage. Fisher's exact tests were used to delineate group differences for histological scoring variables. All analyses were performed using the SPSS Version 22.0 statistical software package (IBM-SPSS, Armonk, NY, USA). An alpha level of $p < 0.05$ was selected to indicate statistical significance.

RESULTS

Macroscopic findings

Defect healing and bridging was macroscopically observed in all subject groups (**figure 5 a-c**).

Histological findings

Group 1 (Sham): new vessel formation, *i.e.* angiogenesis and Type III collagen were observed to be newly constructed tendon tissue. Rare adiposity was observed (**figure 6**).

New vessel formation was observed in the defect area, which was thought to originate from the pericytes adjacent to the endothelial cells present along the defect line. Therefore, growth factors and cells necessary for healing appeared in the defect area. Adipocytes and pericytes were also observed in association with new vessel formation.

Group 2 (EGF +): under light microscopy, tendon angiogenesis was observed. Group 2 displayed greater evidence of vascularization (moderate) than Group 1 and Group 3, ($p=0.006$). It was also observed that pericytes accumulated along the defect and healing continued. The concentration of adipocytes was greater than in Group 1. Group 2 displayed greater evidence of adipocyte development (moderate) than Group 1 and Group 3 ($p < 0.0001$) (**figure 7**). Increased adipocyte concentration suggests that tendon repair was more active in accelerated in Group 2 compared with Group 1 and Group 3. Group 2 displayed greater evidence of Collagen Type III development (moderate) than Group 1 and Group 3 ($p=0.006$). In contrast to Group 1, Group 2 also exhibited small quantities of Type I collagen fibers. The fact that Type I collagen was observed in this group, even in small quantities at 8 weeks post-repair indicates that tendon healing was at a more advanced stage and the healing progression was advanced compared with Group 1. Group 2 and Group 3 displayed greater evidence of peripheral nerve development (weak) than Group 1 ($p=0.006$).

Peripheral nerve buds that we considered differentiated from MSCs through the injection of EGF were also observed in Group 2 but not in Group 1 (**figure 7**).

Group 3 (Scaffold + EGF): under light microscopy, tendon angiogenesis was observed. It was also observed that pericytes with vascularization accumulated along the defect and healing continued. Vessel formation in the tendon, *i.e.*

Figure 5. (a) Group 1 (Sham), **(b)** Group 2 (EGF +) and **(c)** Group 3 (Scaffold + EGF).

angiogenesis was observed. Peripheral nerve buds that were considered to have differentiated from mesenchymal stem cells were also observed in this group.

Group 3 displayed greater evidence of Collagen Type I development (moderate) than Group 1 and Group 2 ($p < 0.0001$) (**table I**), suggesting more accelerated healing as Type III collagen was replaced by Type I collagen. The amount of Type III collagen was lower and Type I collagen was higher in Group 3 even compared to Group 2 (**table I**). The level of Achilles tendon healing that was observed in this group more closely resembled normal tendon structure. We perceive that with combined scaffold and EGF application, the necessity to produce Type III collagen to serve as a temporary healing scaffold was diminished so tissue plasticity was biased more toward Type I collagen transformation compared to the other experimental groups (**figure 8**). The amount of adipocytes was lower compared with Group 2 since this group was at a last phase of healing process where type I collagen making process is mostly accomplished (**table I**). This group, which was dominated by Type I collagen, appearing to have moved past the active proliferation phase by 8 weeks following defect repair, with lesser energy needs during the final phase of healing.

Biomechanical test findings

Peak load to failure forces were identified as 325.8 ± 101.7 N in the control group, 415.7 ± 111.5 N in Group 1, 314.7 ± 72 N in Group 2, and 311.1 ± 94.9 N in Group 3 group (**table II**). When Group 1, Group 2, and Group 3 were compared with each other and with the control group, significant group differences were not evident ($p > 0.05$) (**figure 9**).

The amount of elongation at failure (mm) was identified as 7.4 ± 2 mm in the control group, 10.4 ± 4.3 mm in Group 1, 12 ± 5 mm in Group 2 and 11.4 ± 5.3 mm in Group 3 (**table II**). There were no significant statistical differences between the groups ($p > 0.05$).

Among the three different biomechanical characteristics that were evaluated, a significant statistical difference was only identified for Young's modulus (stiffness) values ($p < 0.05$). Pairwise comparisons revealed that Group 2 and Group 3 displayed less stiffness compared to the control group ($p < 0.05$). Pairwise comparison between Group 1, Group 2 and Group 3 did not reveal statistically significant Young's modulus differences ($p > 0.05$) (**figure 10**).

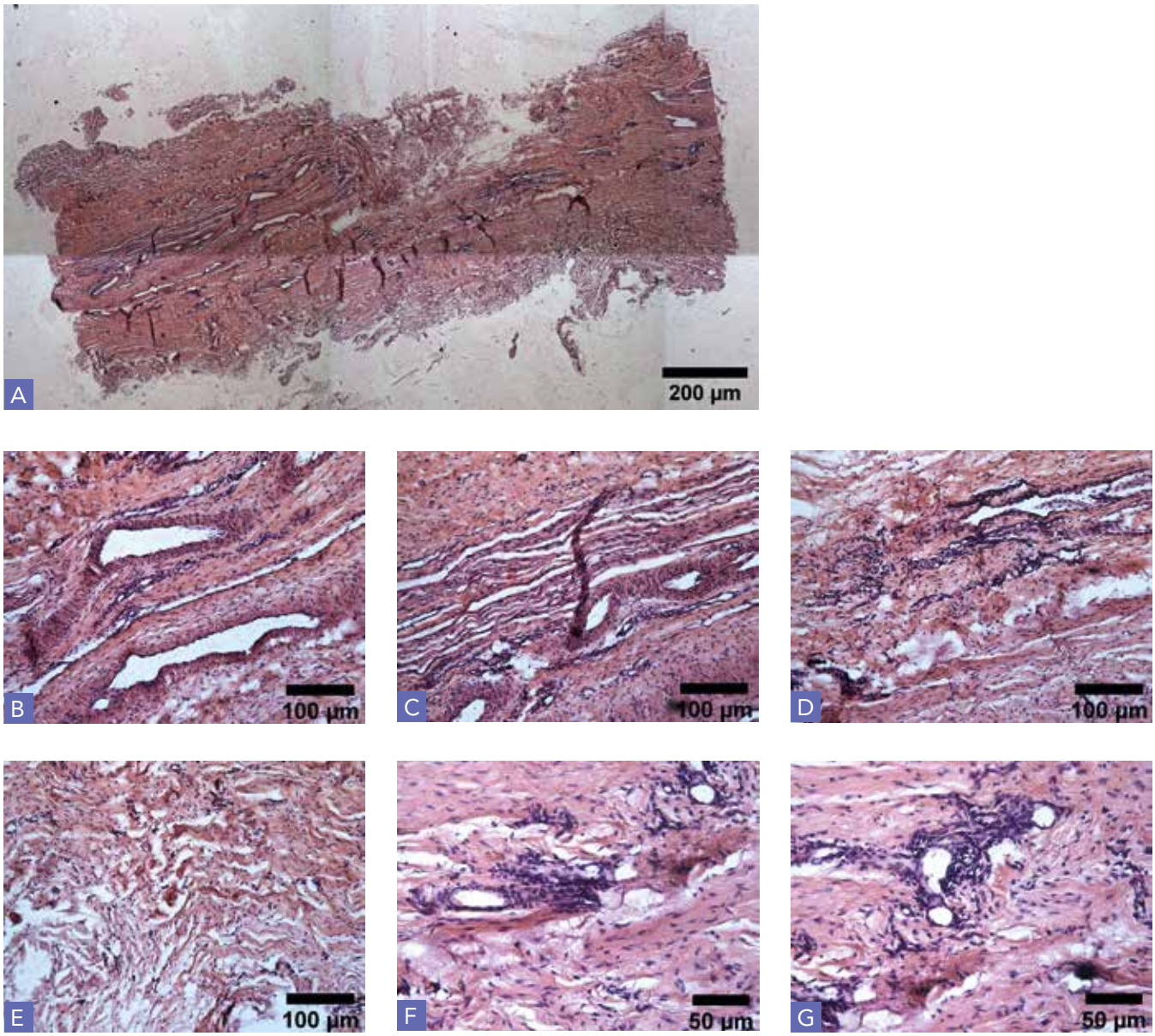

Figure 6. Histologic evaluation of tendon regeneration in Group 1 (Sham).

Table I. Histological group comparisons.

	SHAM	EGF	EGF + SCAFFOLD
Collagen type III	1	2	1
Collagen type I	0	1	2
Adipocyte	0	2	1
Vascularization	1	2	1
Peripheral nerve	0	1	1

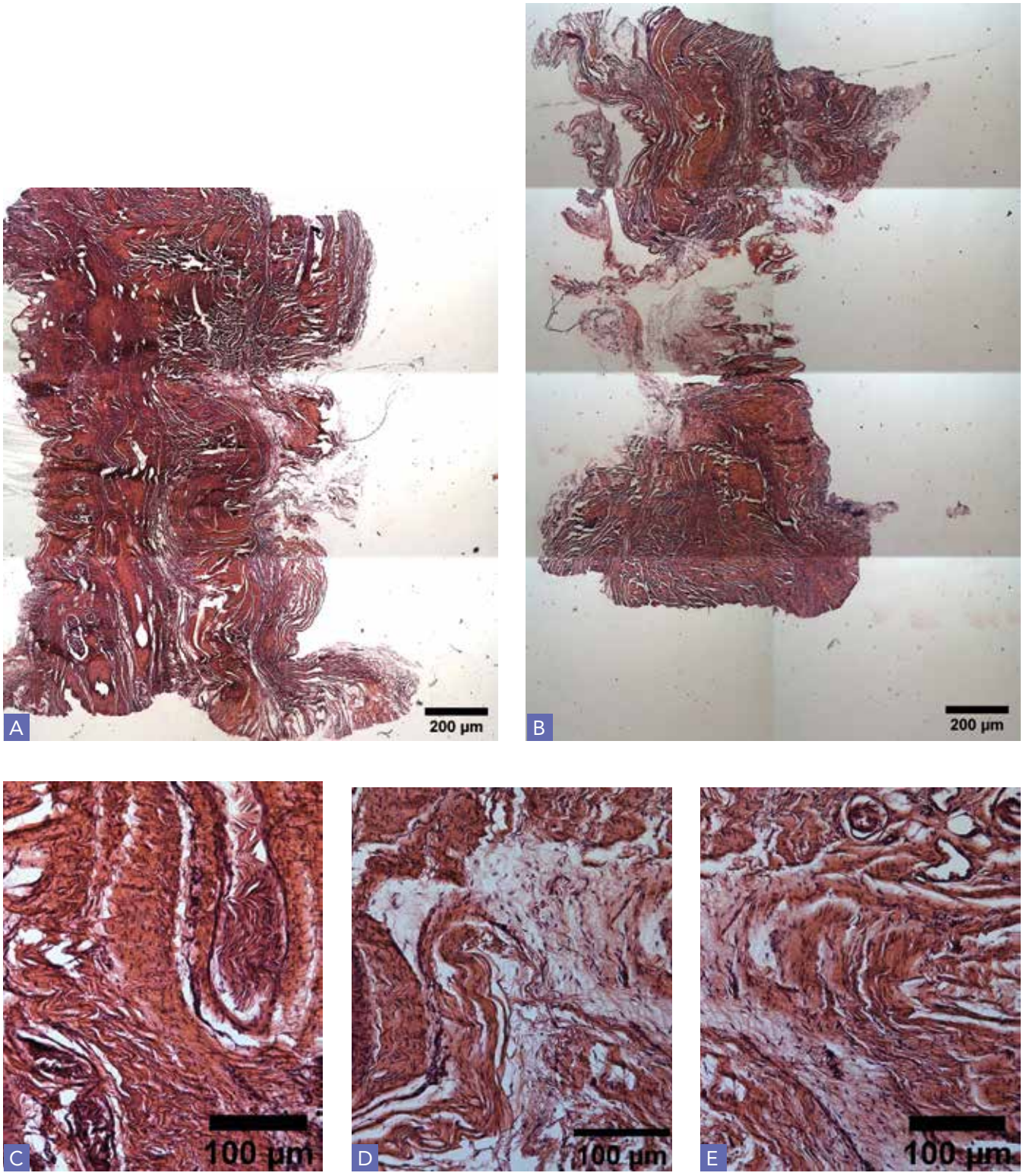

Figure 7. Type III > Type I collagen dominance and peripheral nerve buds were observed. Adipocytes were also observed. Group 2 (EGF +).

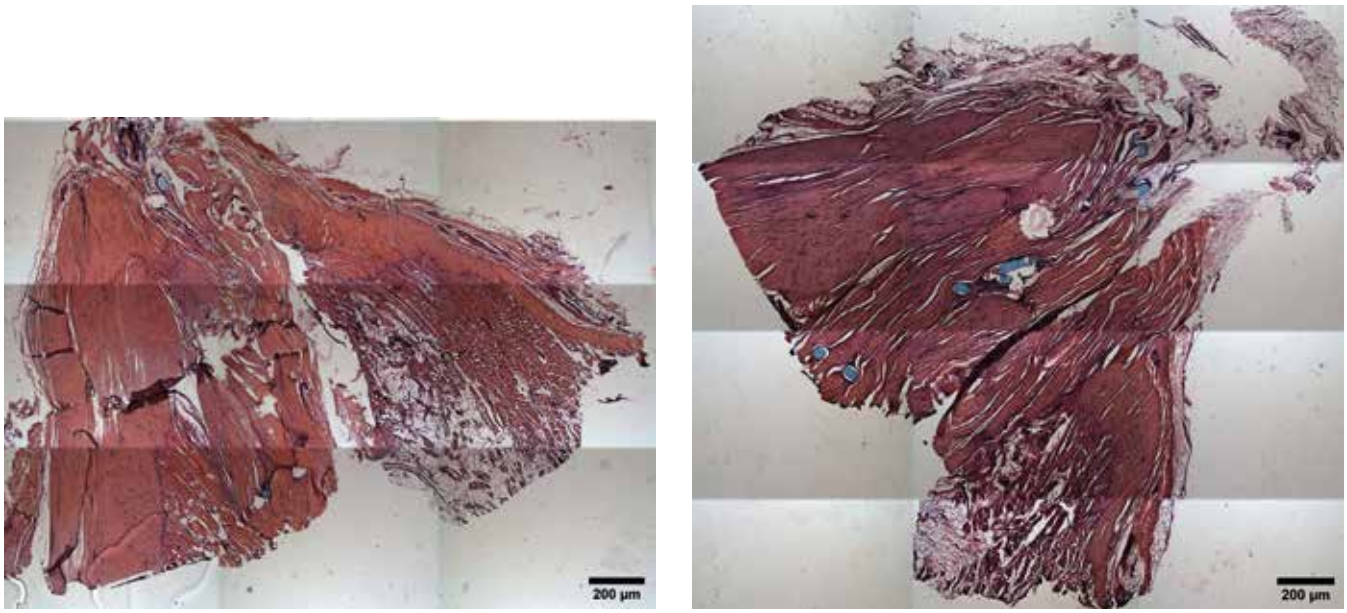

Figure 8. Type I collagen dominance was observed in Group 3 (Scaffold + EGF).

Table II. Biomechanical test group comparisons.

		N	Mean	Std. Deviation
Force Peak (N)	Control	5	325.8	101.7
	Group 1 (Sham)	5	415.8	111.6
	Group 2 (EGF +)	5	314.7	72.1
	Group 3 (Scaffold + EGF)	5	311.1	95.0
	Total	20	341.8	98.7
Elongation at Failure (mm)	Control	5	7.5	2,0
	Group 1 (Sham)	5	10.4	4.3
	Group 2 (EGF +)	5	12.1	5.0
	Group 3 (Scaffold + EGF)	5	11.4	5.3
	Total	20	10.4	4.4
Young modulus (N/mm²)	Control	5	190.6	61.4
	Group 1 (Sham)	5	127.9	34.1
	Group 2 (EGF +)	5	97.1	35.6
	Group 3 (Scaffold + EGF)	5	83.5	33.4
	Total	20	124.8	57.9

DISCUSSION

Current approaches to Achilles tendon rupture treatment involve surgical repair and early mobilization (15). The goal of surgical repair is to minimize the risk of tendon re-rupture and prolonged immobilization by creating a repair that has sufficient fixation strength, without negatively affecting natural tendon physiology (15-17). The more closely the healing tendon maintains natural anatomical and histological characteristics the lower the re-rupture and complication rates should be while still enabling earlier mobilization or therapeutic exercise performance.

There was no significant difference in strength between Krackow, Bunnell and Kessler suture techniques in Achilles tendon repairs in a human cadaver study (18). Karatekin *et*

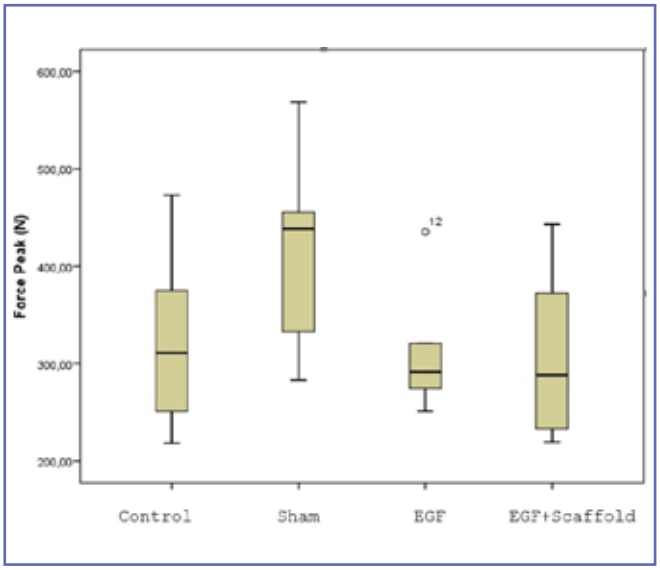

Figure 9. Peak load to failure.

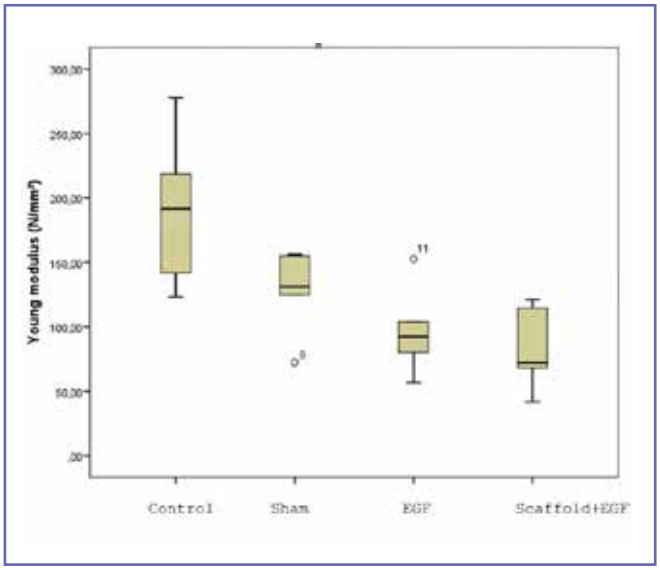

Figure 10. Young's modulus (construct stiffness).

al. reported similar functional and elastographic results of Krackow and modified Kessler suture methods in the long-term follow-up of the patients (19). Modified triple Kessler repair was stronger in this cadaveric biomechanical study compared with the traditionally used single Krackow technique. The modified Kessler technique was used because it was thought to be superior or not biomechanically different compared to other suture techniques.

Unfortunately, Achilles tendon healing following surgical repair often results in a fibrovascular scar that is mechanically weaker than the native tendon. This scar formation occurs in association with Type I/Type III collagen ratio changes, with greater Type III collagen fiber concentrations. Tendon healing strategies aim to bring this collagen type ratio as close as possible to that of healthy tendon (20). When applied within the initial 10 days following tendon rupture, growth factors accelerate tendon healing and reduce adhesions (7, 8). When growth factors and other biologic healing agents are concentrated in a given medium, the healing process can become accelerated (8).

Various biologic agents and growth factors are currently used in combination with surgical interventions (9, 21). However, there is no consensus or guidelines concerning the use of biologic agents and growth factors for Achilles tendon repair. Platelet Rich Plasma (PRP) releases various growth factors (*e.g.* TGF-β, PDGF, IGF, and VEGF) to enhance healing through accelerated revascularization (20, 22) However, a rabbit model study revealed that PRP injections led to decreased collagen fiber diameter (23). The use of PRP was also reported to be insufficient in the treatment of chronic Achilles tendinopathies (24-26). Additionally, PRP has not been shown to be better than placebo for human Achilles tendon repair (6, 27, 28).

Bone marrow aspirate is a biologic agent that releases MSC to the injury site. In a pre-clinical Achilles tendon rupture study Schepull *et al.* (29) reported that bone marrow aspirate applied to healing tendons provided poorer outcomes compared to tendons that received MSC and a non-treated control group. Achilles tendon repair can be improved by MSC use to increase the anti-apoptotic effect (30). Using a rabbit model study, Selek *et al.* (31) reported that MSC use increased collagen organization over the initial 3 weeks of healing, however, significant differences were not observed during the later healing periods. Although there have been major study findings indicating that MSC use enhances tendon repair, Kraus *et al.* (32) reported that rat Achilles tendon repairs supplemented with MSC use displayed poorer biomechanical test results than a control group at 28 days post-injury (32).

Various growth factors are also used in tendon rupture treatments. Increased FGF levels during the early healing phase display angiogenic and mitogenic effects, and increased Type I and Type III collagen fiber production. Fibroblast growth factor and platelet-derived growth factor were shown to stimulate proliferation of mature tendon fibroblasts in serum-free medium (33). Use of FGF has been reported to have a positive effect on rat rotator cuff rupture healing (34). Use of rhPDGF can accelerate tendon healing through several mechanisms. Small animal studies have shown that rhPDGF use enhances tendon mechani-

cal strength and movement range through matrix remodeling, collagen synthesis, improved blood clot formation and cell proliferation (35, 36). Use of TGF-β has also been shown to regulate cell differentiation and proliferation, and increase Type I and Type III collagen fiber production (37). Hou *et al.* (38) demonstrated that tendons treated with TGF-β displayed an increased resistance to mechanical forces (38). Animal model studies using IGF to induce tenocyte migration and collagen synthesis are ongoing. Use of VEGF increases angiogenesis and capillary permeability (39). Zhang *et al.* (37) reported that although there was a significant increase in Achilles tendon repair tensile strength at two weeks following VEGF application, there was no significant differences by the end of the 4th week (40). Each of the above-mentioned biological agents show varying levels of promise for Achilles tendon repair, however, only a few techniques have achieved clinical application. Therefore, at the current time there is no biologic agent available that constitutes a standard treatment protocol for Achilles tendon rupture with a consensus that healing will be obtained that more closely resembles that of native, healthy tendon tissue.

Although healthy tendons are dominated by Type I collagen fibers, a significant amount of Type III collagen fibers get synthesized in the repair site following rupture and repair which gradually transform into Type I collagen fibers during the later healing stages. Type III collagen has a very low resistance to tensile forces, therefore, the healing tendon is more susceptible to spontaneous rupture during this time period (41). The aim in tendon repair treatment is to achieve Type I collagen dominance by the end of the healing process. The higher the Type I/Type III ratio the lower the re-rupture and complication rate (42). Conceivably, earlier Type III to Type I collagen fiber transformation should help prevent re-rupture or repair site stretching during recovery while enabling earlier mobilization.

To date, to our knowledge, no pre-clinical or clinical studies regarding EGF use for tendon healing have been reported. Local EGF use has been clinically shown to improve chronic skin wound healing, closure, and epidermal bridging (11). Using a rat model, Brown *et al.* (43) reported increased collagen formation following EGF application at surgical incision sites, reporting a 200% increase in wound tensile strength at 7-14 days post-application. Clinical studies of EGF embedded in silver sulfadiazine has been reported to increase the epithelialization of chronic wounds, particularly when applied using intra- and peri-lesional injections (10). Various biologic agents have been used to facilitate tendon healing. Debates exists as to which agent(s) should be used and at what time they should be delivered during the healing process. To improve this understanding, we used a 1 cm defect Achilles tendon defect model to evaluate the efficacy of EGF applications over the initial 10 days post-injury. Histologic evaluation revealed that within the same 8-week time period Group 1 displayed less Type III collagen fiber formation than Group 2. Group 2 displayed significantly more Type III collagen fiber formation in combination with some evidence of earlier transformation to Type I collagen fibers. Therefore, the addition of EGF was found to accelerate Type III to Type I collagen fiber synthesis. Group 2 tendon healing was noted to be more advanced compared to Group 1.

Histologic examination revealed that Group 3 had the greatest level of Type I collagen fibers. Group 3 also had fewer adipocytes and Type III collagen fibers than Group 2. These findings suggest that Group 3 had moved past the active proliferation healing phase over the same period and the need for adipocytes decreased in proportion to the reduced active synthesis. The accelerated transformation of Type III to Type I collagen fibers was significantly more evident in this group. With the addition of a PCL scaffold of sufficient size to fill the defect, all tendon healing phases took place more quickly and more robustly. These positive findings using a PCL scaffold are in agreement with over reports (44).

Scaffolds are known to provide both mechanical support and guidance for migrating cells to grow during tendon healing (45). This study used biodegradable scaffolds made of PCL which is known to facilitate bone defect repair based on an animal model study (46). An optimal Achilles tendon repair scaffold should enable more natural healing and rapid tendon defect bridging (44). The scaffolds produced can be processed in a perfusion reactor, cultivating tenocytes or tendon precursor stem cells, to verify and to optimize the interaction between the tissue engineered structure and the cells (47). Growth factors are susceptible to degradation and their efficacy can be quickly reduced due to rapid elimination from the defect site following injection. When growth factors are encapsulated within scaffolds, they are maintained for a comparatively longer time period. Therefore, better tendon healing can be achieved by using scaffold and growth factors in combination. To sum up the histological findings, the addition of EGF accelerated healing, whereas scaffold and EGF use in combination led to the quickest histologic healing. Moreover, an unexpected outcome of this study was the peripheral nerve buds that were observed in the tendon following EGF injections.

Biomechanical testing revealed that load at failure and elongation at failure values revealed no differences between the 3 experimental groups. The only significant biomechanical characteristic difference was for Young's modulus (stiffness). Both Group 2 and Group 3 displayed lower Young's modu-

lus values compared to the control group (healthy contralateral Achilles tendon). Biomechanically, the difference between the groups showed that the addition of EGF and scaffold did not have a positive biomechanical contribution. Achilles tendon rupture treatment with lentiviral bFGF transduced mesenchymal stem cells did not show positive effects biomechanically in a long-term follow-up. Interestingly, in later stages stem cells had hardly any effects on biomechanical results (48). The PRGF did not have a major influence on cellular organization. It also had an undesirable effect on the biomechanical properties of repaired flexor tendons (49). In another PRGF study, the PRGF-treated tendons had higher force at 8 weeks compared with the placebo group biomechanically (50). Histologically, immediate injection of PRP for tendon injury improves tendon healing in rats. Nevertheless, PRP injection group in Achilles tendon injury had lower peak force biomechanically than the control group (51). Zhang *et al.* reported administration of exogenous VEGF can significantly improve tensile strength early in the course of the rat Achilles tendon healing but there was no significant difference in tensile strength among the groups at 4 weeks postoperatively (39). Application of the TGF-B1 and IGF-1 in a patellar tendon defect model resulted in a significant increase in force at failure, ultimate stress, stiffness, and energy uptake at 2 weeks, whereas none of the parameters revealed any significant difference between the two groups at 6 weeks (52). Local application of FGF-2 on tendon-to-bone remodeling in rats was reported FGF-treated specimens were stronger at 2 weeks but at 4 and 6 weeks, both specimens were exhibited similar strength biomechanically (53). In studies that added growth factors to tendon repair, there was no significant difference in long-term results with the control group, as in our study.

In terms of clinical practice, tendon biomechanics study is more important than histological study. Since there is no biomechanical positive effect with the addition of EGF and scaffold, it is not expected to reduce early joint movement start time, work turnaround time and risk of re-rupture. Although the addition of EGF is histologically significant, more studies are needed to use it in daily clinical practice.

Study limitations

This study is limited in that it represents the histological and biomechanical results of a small sample of rabbit Achilles tendons at only the 8th week post-injury. There are no mid- or long-term results. Additional studies are needed to determine if significant histological and biomechanical group differences exist at shorter or longer durations than 8 weeks. It may have affected the results since sufficient postoperative time has not been applied to the splint.

CONCLUSIONS

The combined addition of EGF and a scaffold to Achilles tendon defect treatment increased the Type I/Type III collagen ratio and provided better histological evidence for tendon healing compared to EGF alone or Sham conditions. Histological evaluation revealed that EGF use increased vascularization, pericyte concentration adjacent to vessel endothelial cells and adipocyte concentrations leading to accelerated and more robust tendon repair healing. There was no significant difference in terms of biomechanics with the addition of EGF and scaffold. Although addition of EGF is histologically significant, it has no advantage over control and sham groups biomechanically. Therefore, further studies with larger sample sizes and over longer study durations are needed prior to clinical use of EGF for tendon rupture treatment.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Rotator Cuff Repair with Different Suture Materials Results in Different Protein Expressions with Similar Histological Findings: An Experimental Study in Rabbits

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SUMMARY

Background. Previous studies have evaluated the biomechanical, histological, and clinical effects of rotator cuff repair with different suture materials. However, a histological and western blot analysis after repair comparing nonabsorbable polyester and polyethylene suture materials in a chronic rotator cuff tear model has not been conducted in any previous studies. The aim of this study is to evaluate the effect the two different suture materials, specifically Ethibond and Ultrabraid, have on repair based on a histological evaluation and protein expression by means of a western blot analysis.

Methods. Twenty-six rabbits were included in the study. Two rabbits served as normal controls (control group). A bilateral tenotomy of the subscapularis tendon was performed in the remaining 24 rabbits. Six weeks after the tenotomy, the animals were randomly divided into four repair groups (five rabbits in each group) according to the suture material used and the sacrifice time (Ultrabraid two weeks after repair, Ethibond two weeks after repair, Ultrabraid six weeks after repair, and Ethibond six weeks after repair). Two sham-operated groups with two rabbits each were sacrificed at eight and 12 weeks after the tenotomy without repair. Both shoulders of all the animals were used for a western blot (collagen type 1-3, MMP-2, TGF-*beta*-1, IGF-1, COX-2) analysis and a histological evaluation (fibrocartilage formation, inflammation, vascularisation, collagen density).

Results. All groups showed minimal-to-mild histological changes irrespective of the type of suture material used. There was no statistically significant difference in histological scores ($p > 0.05$). Fibrocartilage formation was greater at six weeks in the polyester suture repair groups without statistical significance ($p > 0.05$). Collagen type 1-3, MMP-2, TGF-*beta*-1, IGF-1, and COX-2 were significantly upregulated at two weeks compared to six weeks after repair ($p < 0.05$). Protein expression levels were statistically significantly higher in the Ultrabraid groups compared to the Ethibond groups ($p < 0.05$).

Conclusions. Changes in the expression of the markers related to rotator cuff healing were demonstrated when repairing with different suture materials, with these being in favour of polyester sutures; however, the histological findings are similar. This finding could influence the design of new suture materials.

KEY WORDS

Fibrocartilage formation; rabbit rotator cuff bone-tendon healing; suture material; western blot analysis; MMP-2; COX-2.

INTRODUCTION

The non-healing rate after rotator cuff repair has been reported as being between 20% and 70% (1, 2). Several factors, such as increased age, chronic diseases, quality of tendon, smoking, and obesity, have been demonstrated to be related to a failure to heal after rotator cuff repair (3-6). The use of different suture materials has also been thought to influence healing (7). In regard to nonabsorbable sutures, polyblend (Fiberwire, Herculine, Orthocord, and Ultrabraid) and conventional polyester (Ethibond) suture materials are used in rotator cuff surgery (8). In the last decade, polyblend sutures comprising Ultra-High-Molecular-Weight Polyethylene (UHMWPE) have become preferred because of their enhanced biomechanical properties (9). Polyblend sutures' biomechanical superiority to polyester suture materials has been previously demonstrated (10).

A recent *in vitro* study by Lock *et al.* showed changes in the synthesis of inflammatory markers at one, three, and five days after suture placement (11). In an acute tendon rupture model, Ergun *et al*. performed Achilles tendon repair with different suture materials. It was found that the biological properties of absorbable PDS sutures were superior compared to Vicrl and Ethibond sutures, and the study suggests using absorbable PDS sutures in Achilles tendon repairs (12). Su *et al*. investigated the effect of suture absorbability on enthesis regeneration and biomechanical properties in an acute rotator cuff healing model in rabbits (7). Repair with absorbable sutures rather than partially absorbable (Orthocord) or nonabsorbable (Ethibond) sutures was shown to evoke enthesis regeneration. Due to their possible stress-shielding effect, the healing properties of nonabsorbable sutures have been found to be inferior to those of absorbable and partially absorbable sutures. However, this study was performed on an acute rotator cuff tear model.

To date, biomechanical comparisons of suture cut-through (13), the influence of knot location (14), and the suture absorbability (7) of different suture materials in acute rotator cuff healing have been studied; however, whether the type of suture material used in surgery would affect chronic rotator cuff healing on histological and molecular levels remains unknown.

Therefore, this study aims to compare the healing effect of repair with two different suture materials, specifically Ethibond and Ultrabraid, at different intervals (at two and six weeks after repair) using histologic and western blot analysis. It was hypothesised that transosseous repair with different suture materials would influence the histologic findings and protein expressions, which are thought to have a role in catabolic, anabolic, and remodelling processes, during rotator cuff healing.

METHODS

This is a committee-approved study using an established rabbit rotator cuff repair model. A rabbit subscapularis chronic tear model was chosen because of comparable histologic and biomechanical similarities to human chronic rotator cuff pathology (15-18). All institutional and national guidelines for the care and use of laboratory animals were followed. Approval for this study was obtained from the Ethics Committee of Pendik Veterinary Control and Research Institute, Istanbul, Turkey. A priori power analysis indicated that a total of 26 animals were required. Twenty-six mature New Zealand White male rabbits with an average age of 36 weeks and a weight of 2.5 kg to 3.5 kg were included in the study.

Study design

Twenty-six rabbits were included in the study. Two rabbits in the control group did not undergo surgery. A bilateral tenotomy of the subscapularis tendon was performed in the remaining 24 rabbits. Six weeks after the tenotomy, secondary repair procedures were performed bilaterally. The rabbits were randomly divided into four groups of five rabbits each according to the suture type and euthanasia time as follows: Ethibond repair group (at two weeks after repair), Ethibond repair group (at six weeks after repair), Ultrabraid repair group (at two weeks after repair), and Ultrabraid repair group (at six weeks after repair). Six weeks after the tenotomy, the repair was made using either pure braided UHMWPE polyethylene (Ultrabraid) or braided polyester suture (Ethibond) in suture repair groups with five rabbits each. Two groups with two rabbits each were allocated as sham-operated groups without repair according to the after-sacrifice time, which was at eight weeks and 12 weeks after the tenotomy. A western blot analysis (collagen type I-III, Matrix Metalloproteinase-2 (MMP-2), Transforming Growth Factor (TGF)-*beta*-1, Insulin-like Growth Factor (IGF)-1, and Cyclooxygenase (COX)-2) and a histologic evaluation (inflammation, vascularisation, and collagen density) were conducted for all groups (16). For the repair groups, the fibrocartilage formation was evaluated using a quantitative histologic analysis.

Surgical technique

All of the surgical procedures were performed by the same surgeon. Anaesthesia was induced using an intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine hydrochloride. After skin preparation, an anterior transverse incision was made at the glenohumeral joint (**figure 1 A**). The deltopectoral interval and the coracobrachialis muscle were

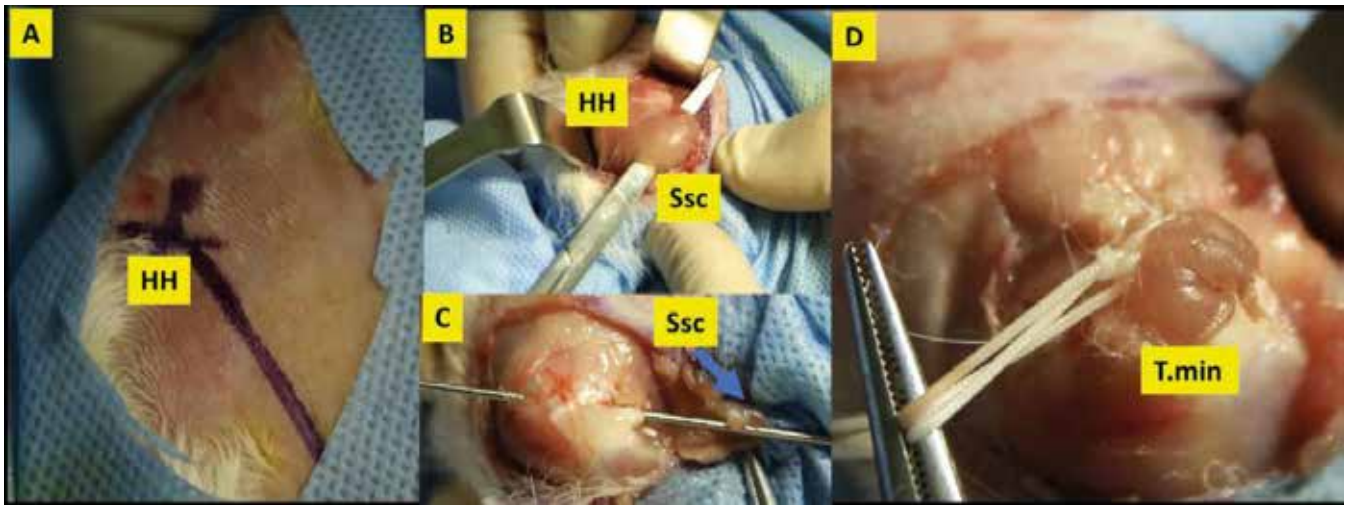

Figure 1. (A) Surgical planning, **(B)** identification of subscapularis tendon, **(C)** preparation of bony tunnel using 0.8 mm Kirschner wire, and **(D)** transosseous repair of the subscapularis tendon. Blue arrow indicates the subscapularis muscle.
HH: Humeral Head; Ssc: Subscapularis muscle; T.min: *Tuberculum minus*.

split. The subscapularis muscle under the spina scapula was identified using a right angle clamp (**figure 1 B**). The insertion of the subscapularis at the lesser tubercle of the humerus was sharply released with a scalpel. Six weeks after the tenotomy, the repair was performed using simple transosseous stitches in a Mason-Allen configuration and either No. 2 Ethibond Excel (Ethicon, Somerville, NJ) or No. 2 Ultrabraid Cobraid (Smith & Nephew, Memphis, Tennessee) sutures were used (**figure 1 C-D**). The control group was sacrificed without tenotomy or repair to provide a baseline evaluation. The other subjects in the sham-operated and suture repair groups were immobilised after surgery until euthanasia. This was accomplished by positioning a Vetflex elastic bandage (Kruuse group, Langeskov, Denmark) over the forelimb, shoulder, and torso, with both limbs placed at 90° of forward elevation 20° of abduction, which has been shown to have superior healing characteristics compared to postoperative mobilisation (19). During the first round of operations, three rabbits died, and one rabbit developed foot drop secondary to iatrogenic injury. Four new rabbits were added to the relevant groups. There was no change in the weight and health status of the entire study group.

Histologic evaluation

After euthanasia, five bone-tendon specimens from each group underwent histologic analysis. The specimens were fixed in 4% formic acid-sodium citrate solution for 45 days. The specimens were washed with flowing water for one day. Following dehydration in an alcohol series and clearing with xylene, the samples were embedded in paraffin blocks, which were cut through the longitudinal axis to the subscapularis tendon. Five-micrometre bone-subscapularis tendon sections were taken and placed onto slides. The macroscopic evaluation included the presence of a retear, scar formation, and mode of suture failure, either suture slippage or suture breakage, by observing the suture-tendon interface of each specimen. The sections were stained using Masson's trichrome for collagen fibril density and Safranin-O for inflammation, vascularisation, and collagen fibril density. The histologic criteria reported by Chung *et al*. were modified and used for the evaluation (20). The histologic examinations evaluated collagen fibre density, presence of inflammatory cells, and vascularity. The scores were graded as absent or minimally present (0 points), mildly present (1 point), moderately present (2 points), and severe or markedly present (3 points).

Regarding the repair groups, the fibrocartilage formation was evaluated using a previously reported technique (21). The areas of metachromasia were outlined on Safranin-O fast green slides at 100x magnification. The total area of fibrocartilage is provided with percentages as compared to the control groups. All slides were examined by two blinded histologists who were not included in the study. Regions of Interest (ROIs) were imaged at a magnification of 100x. To eliminate observer bias, one histologist performed a general histologic evaluation, and the other histologist performed the quantitative evaluation for fibrocartilage formation. Each slide was coded with randomly generated numbers. Images of three sequential sections were obtained from each

specimen under the same conditions to reduce sampling error. The results of these sections for each sample were averaged for histologic scoring and quantitative evaluation.

Western blot

After euthanasia, a western blot analysis of five samples from the musculotendinous junction for each group, including the suture-tendon samples, was performed. One hundred milligram of tissue for each specimen was lysed using 2 mL lysis buffer. After centrifugation at 15,000 rpm for 20 minutes, the proteins of the lysates were quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific, IL, USA). Equal amounts of total protein (30 µg) were run on 10% SDS polyacrylamide gels at a constant voltage of 200 V for 40 minutes and transferred onto a nitrocellulose membrane. After blocking the membranes with 5% milk for 10 min, they were incubated with specific primary antibodies of MMP-2, IGF-1, COX-2 , collagen type I *alpha* 1, collagen type III *alpha* 1, and TGF-*beta* 1 at 4 °C overnight and then washed with Tris-Buffered Saline (TBS) containing 0.1% Tween-20. After washing with TBS, the membranes were developed using an enhanced chemiluminescent detection kit (Santa Cruz Biotechnology, Texas, USA) against Horseradish Peroxidase (HRP) and then photographed using a ChemiDoc XRS system (Bio-Rad). The signal intensity of the digital blots was measured using processing and analysis software to generate semiquantitative data from the western blot images. An antibody against Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) (Novus Biologicals, USA) was used for normalisation of the protein loading on the blots.

Statistics and data analysis

The data on the histologic parameters and western blot analysis is provided as mean and standard deviations. Group differences were compared using the Kruskal-Wallis test, followed by post-hoc Mann-Whitney U testing with Bonferroni correction. A value of $p < 0.05$ was considered statistically significant. NCSS statistical software (version 2007, NCSS LLC, Kaysville, Utah, USA) was used to perform the analyses.

RESULTS

Histology

On macroscopic evaluation, all specimens were intact. Scar tissue was present in suture-tendon areas. None of the samples displayed bone-tendon dehiscence, suture pull-out, or suture breakage. An infected appearance was not observed. There was no gap formation in the bone-tendon areas (**figure 2**). Safranin-O staining showed the formation of four typical areas, namely tendon, demineralised fibrocartilage, mineralised fibrocartilage, and bone, in the enthesis sites (**figure 3**). Masson-trichrome staining showed increased collagen fibril density and parallel orientation of collagen over time for each group, with more density in the Ethibond repair groups (**figure 4**). The histologic healing scores are shown in **figure 5**. For both suture groups, the amount of fibrocartilage at six weeks was more than at two weeks. At each time point, more fibrocartilage had formed in the Ethibond suture groups compared to the Ultrabraid suture groups. The comparison of healing scores and fibrocartilage formation for Ultrabraid versus Ethibond in terms of repair period, *i.e.* two weeks versus six weeks, was not statistically significant ($p > 0.05$) (**figure 6**). At each time point, fibrocartilage formation (two weeks: p=0,137, six weeks: p=0,111; $p > 0.05$), inflammation (two weeks: p=0,118; six weeks: p=0,111; $p > 0.05$), vascularisation (two weeks and six weeks: 0,546; $p > 0.05$), and collagen density (two weeks: p=0,475, six weeks: p=0,319; $p > 0.05$) were similar in the Ultrabraid and Ethibond groups ($p > 0.05$) (**figure 6**).

Protein expression

A western blot analysis was performed for all groups. At each time point, collagen I expression was higher in the Ethibond groups than in the Ultrabraid groups (p=0.021; $p < 0.05$). At each time point, collagen I expression was higher in the Ethibond groups than in the Ultrabraid groups (p=0.020; $p < 0.05$). At each time point, IGF-1 (two weeks:

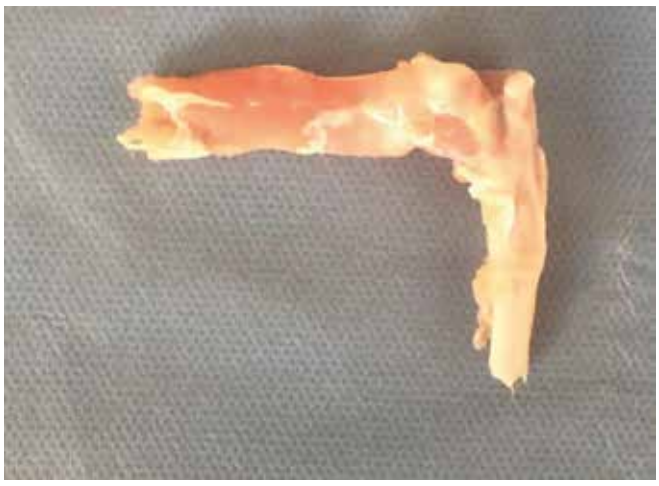

Figure 2. Macroscopic view of the subscapularis tendon-bone specimen.

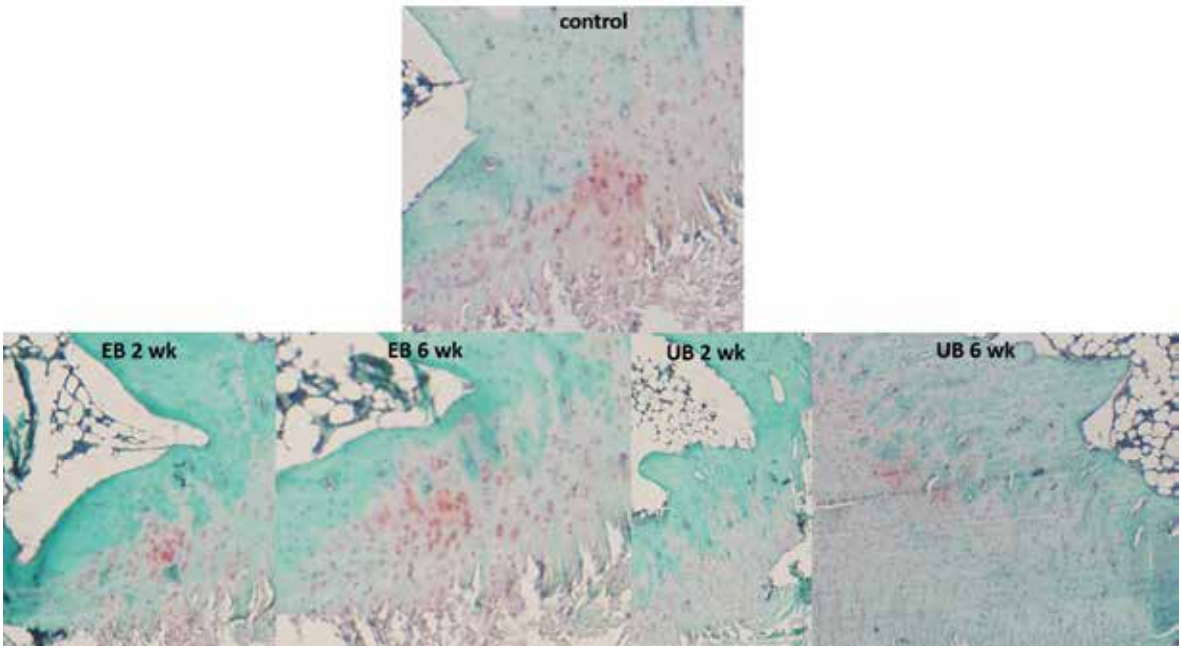

Figure 3. Safranin-O staining of the healing enthesis for the two suture materials at two weeks and six weeks after repair. Area of metachromasia is depicted in red (at an original magnification of 100x).
EB: Ethibond; UB: Ultrabraid.

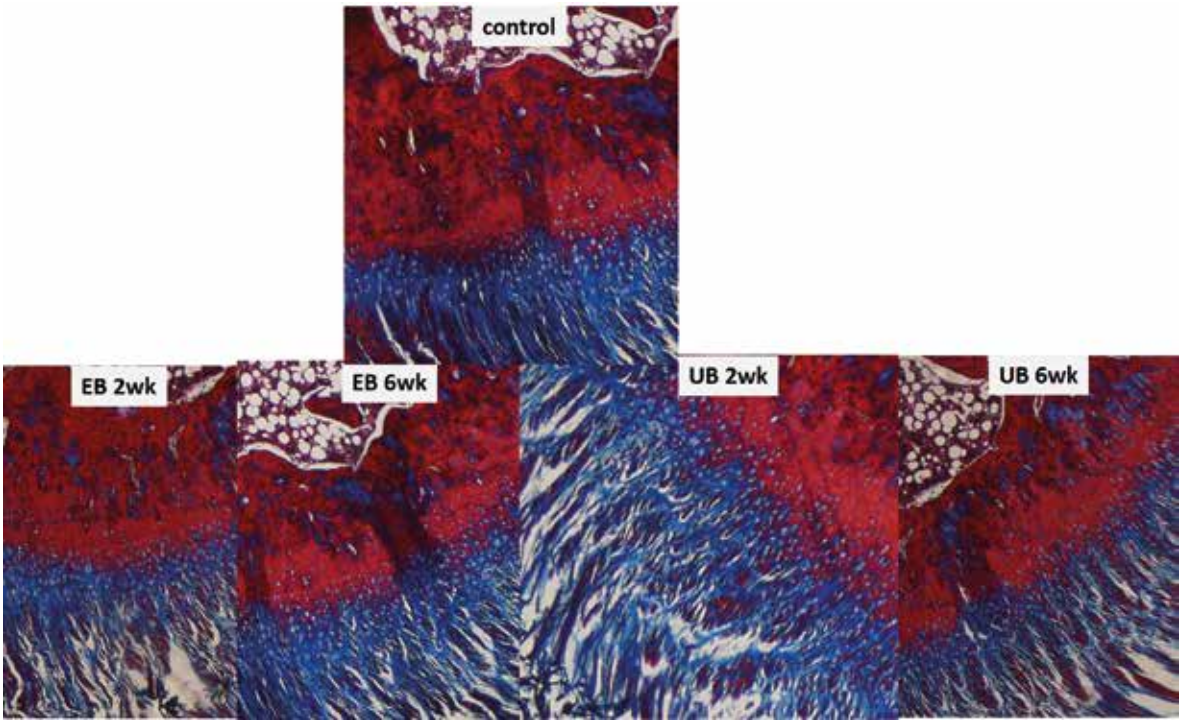

Figure 4. Masson trichrome staining for the evaluation of the collagen density at two weeks and six weeks after repair with the two suture materials (at an original magnification of 100x). At six weeks, more collagen fibres became evident at the tendon area of the newly formed enthesis.
EB: Ethibond; UB: Ultrabraid.

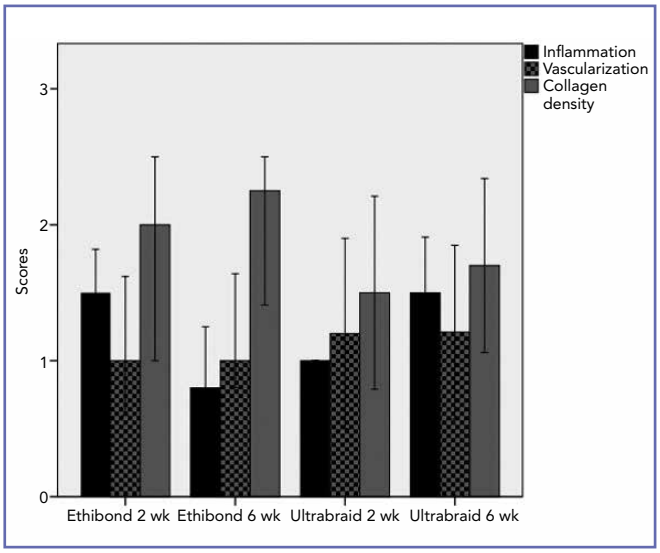

Figure 5. Comparison of the fibrocartilage formation in all the suture groups. Values are given as percentages relative to the control.

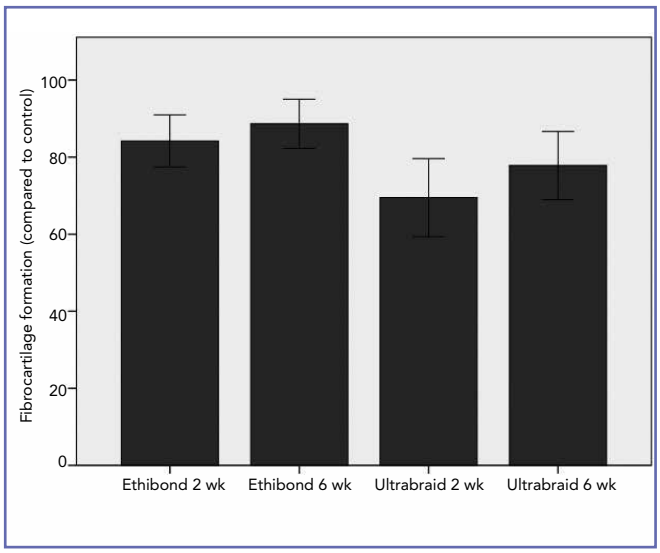

Figure 6. Semiquantitative histologic scoring of all the groups.

p=0.043, six weeks: p=0.021; $p < 0.05$), COX-2 (two weeks: p=0.021; six weeks: p=0.014; $p < 0.05$), MMP-2 (two weeks and six weeks: $p < 0.001$), and TGF-*beta* 1 (two weeks and six weeks: p=0.021; $p < 0.05$) expression were all higher in the Ultrabraid groups than in the Ethibond groups ($p < 0.05$) (**figure 7**).

DISCUSSION

This study shows that repair with Ethibond, a first-generation high-strength polyester suture, and Ultrabraid, a new type of high strength polyblend suture, demonstrates similar histologic changes at six weeks after repair; however, the protein expression levels in relation to rotator cuff healing were altered.

The histologic data is consistent with findings demonstrated by previous animal models of rotator cuff injury (14, 22). With various research purposes, several animal models of rotator cuff injury have evaluated healing and control subjects that had undergone rotator cuff repair with different suture materials (7, 14, 22). Friel *et al.* observed an intact bone-tendon junction at two weeks after acute rotator cuff repair with abundant scar formation on macroscopic evaluation (22). At eight weeks post-repair, parallel orientation of collagen fibrils, decreased cellularity, and an absence of vascularisation was noted. No difference was noted between two and eight weeks after repair with regard to the semiquantitative evaluation. Concerning the control groups with Ethibond only repair, all failures were at the suture-tendon repair site.

Su *et al.* investigated the effect of suture absorbability on acute rotator cuff healing and found that nonabsorbable sutures (PDS) have better biomechanical and histologic properties than partially absorbable (Orthocord) and nonabsorbable sutures (Ethibond) (7). This finding was attributed to the stress-shielding effect of nonabsorbable sutures; however, no data on repair using different suture materials in-vivo and protein expressions was provided (7). Contrarily, Sun *et al.* found no obvious inflammation at three weeks and nine weeks after repair with nonabsorbable Ethibond sutures and observed good tendon-bone healing (14). Placing knots on the bone, even with the use of Ethibond, results in a comparable improvement in histologic scores, regardless of the suture material used, which confirms the findings of the current study.

All studied structural proteins and enzymes that have an impact on rotator cuff healing have been shown to be elevated in each repair group. IGF-1, TGF-*beta* 1, MMP-2, and COX-2 levels are elevated at two weeks and six weeks after repair. This could be explained by the proliferation and remodelling phase, similar to the acute healing process, which can last up to eight weeks (23). Galatz *et al.* found that collagen I and III mRNA levels were high for 56 days in an acute rotator cuff tear model (24). Similarly, Würgler-Hauri *et al.* have demonstrated increased TGF-*beta* 1 synthesis at healing entheses at eight weeks and 16 weeks after a tenotomy in a rat model (25). Increased TGF-*beta* 1 synthesis at an earlier time point (two weeks) in suture repair groups refers to a fibrotic reaction against suture materials, which

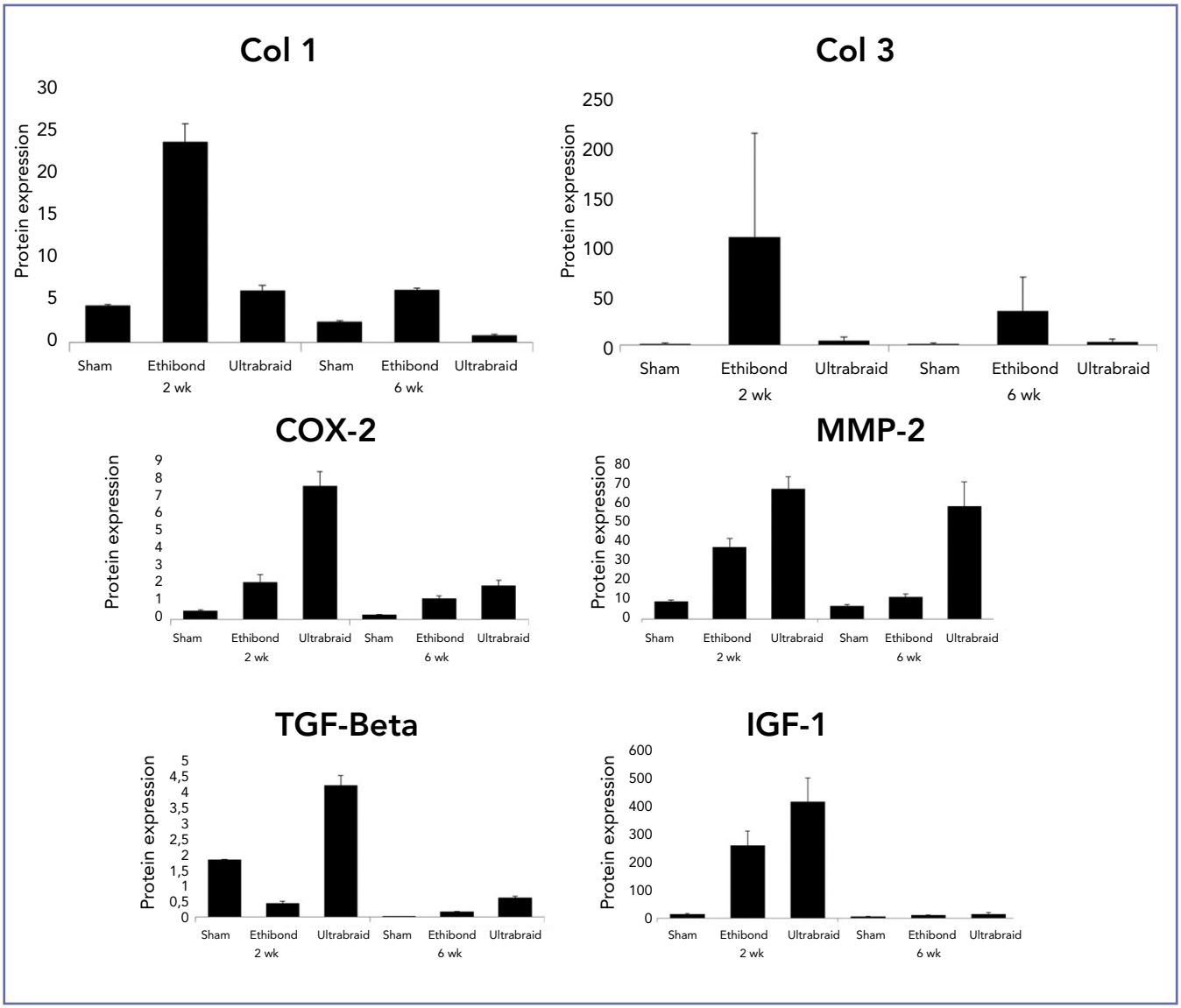

Figure 7. Protein fold expression of the different markers in relation to rotator cuff healing (compared to the controls). The western blot demonstrated a significant temporal decrease in protein expression following repair with both suture types. There is a significant difference between the two different suture groups at two weeks and six weeks after repair.
EB: Ethibond; UB: Ultrabraid.

is supported by the presence of the scar tissue detected in the suture-tendon interfaces of this study's suture repair groups. Although IGF-1 is an anabolic factor, it is elevated in the early phase after repair. Increased expression of IGF-1, along with MMP-2 and COX-2, implies its regulatory function in inflammation, which requires further investigation in prospective studies. Markers related to impaired rotator cuff healing, namely MMP-2 and COX-2, were obviously elevated in the Ultrabraid groups. This finding could explain the tendency for increased fibrocartilage formation in favour of the Ethibond suture at each time point. Finally, one clinical study found similar outcomes after transosseous repair with both nonabsorbable Ethibond and absorbable PDS sutures (26), confirming similar histologic findings after repair with different suture materials, as in the current study. Although recent literature has focused on the development of coatings for sutures with various growth factors to enhance tendon-to-bone healing (27), prospective clinical

and basic science studies investigating chronic rotator cuff healing could further confirm the findings of the current study by coating suture materials with COX-2 (ibuprofen) or MMP-2 inhibitors (doxycycline) (28, 29). This study could further direct new therapeutic targets as mentioned by Montiel Terrón *et al*. (30).

This study has several strengths. In addition to the effects of the suture materials, it provides insight into protein expressions in a chronic rotator cuff tear model. Temporal changes in collagen production, fibrotic (TGF-*beta* 1), anabolic (IGF-1), inflammatory, and catabolic markers (COX-2, MMP-2) were observed. Two different time points allowed the observation of time-dependent changes. Aged subjects were used to increase clinical adaptation. However, further studies are required, as there is insufficient evidence regarding the histopathological analysis of the rotator cuff healing (31).

This is a preliminary study. The limitations of this study include the use of an animal model; thus, any conclusions cannot be assumed to apply to humans or have any clinical significance. In addition, only two different time points were used, namely two weeks and six weeks, and rotator cuff tendon healing takes much longer in a clinical setting. Further, the activity of fibroblasts and adipocytes, which take part in rotator cuff muscle fatty infiltration, were not evaluated (32), and only one type of suture repair stitch, specifically the Mason-Allen, was tested; hence, this data may not apply to other stitch configurations or to repairs using suture anchors.

CONCLUSIONS

In this chronic rotator cuff model, repair with both Ultrabraid and Ethibond sutures resulted in a similar histologic response, but there were different levels of protein expression at two weeks and six weeks after repair according to the western blot analysis.

The study was conducted according to the journal's standards (33).

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Optimal Platelet Concentration for The Therapeutic Effect of Autologous Neutrophil-Reduced Platelet Rich Plasma in A Rat Model of Achilles Tendinopathy

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SUMMARY

Background. Autologous Platelet-Rich Plasma (PRP) therapy is used for treatment of tendinopathy. Efficacy, however, is variable, possibly caused by differences in PRP platelet concentrations. In an animal model of tendinopathy, we determined if there was an optimal PRP platelet concentration.

Methods. Tendinopathy was induced by collagenase injections into the Achilles tendons in rats. 10 days later, PRP at platelet concentrations of 50 (P50 group), 75 (P75 group), 100 (P100 group) and 150 × 10^4/μL (P150 group) or normal saline (control group) was injected into the tendons. To assess pain relief, spontaneous locomotor activity was measured for 12 hours at night. After 19 days, Achilles tendons were removed, and histological sections stained with hematoxylin-eosin or by TdT-mediated dUTP nick end labeling.

Results. Activities in the P75 and P100 groups were significantly greater than in the P50, P150 and control groups. The numbers of microtears, laminations and apoptotic cells in tendons in the P75 and P100 groups were significantly fewer than in the P50, P150 and control groups.

Conclusions. Pain relief and tendon repair were greatest in the P75 and P100 groups. In this rat model of tendinopathy, the optimal PRP platelet concentration in PRP was approximately a range of 75 to 100 × 10^4/μL.

KEY WORDS

Optimal concentration of platelets; platelets-rich plasma; tendinopathy; animal model; enthesopathy; Achilles tendinitis.

INTRODUCTION

Tendinopathy can occur in almost any tendon and is the most common tendon disorder. It is characterized by activity-related pain, focal tendon tenderness and decreased strength and movement, and can impair performance of workers in occupations that involve repetitive movements including various athletic pursuits (1).

Autologous Platelet-Rich Plasma (PRP) is now being used clinically for the treatment of several different tendinopathies, but effects have been variable. Several studies have shown PRP to be as effective or more effective than conventional therapies such as corticosteroid injections and focused shock-wave therapy for lateral or medial epicondylitis, jumper's knee and Achilles tendinopathy (2-12). In other studies, PRP had no significant therapeutic benefits in Achilles tendinopathy (13, 14). One possible factor contributing to different therapeutic outcomes is the difference in PRP platelet concentration in the different preparations. Since the PRP platelet concentration is dependent on the whole blood platelet concentration this is not unexpected, and has been noted in preparations from different institutions, and also from the same commercial preparation kit between different subjects. Platelets are the source of growth factors, cytokines and chemokines that regulate cellular anabolic metabolisms, and so different platelets concentrations might be expected to influence the tendon reparative reaction in the treatment of tendinopathies.

The optimal concentration of platelets in PRP for tendon healing is unclear. Several *in vitro* studies have shown that platelet concentrations up to approximately 1.0×10^6/μL had positive effects in tendons and tenocytes, including increasing growth factors and collagen synthesis, while higher concentrations had inhibitory effects including decreasing collagen synthesis and increasing inflammatory mediators (15-17). One animal study reported to date examined the therapeutic effects of neutrophil-reduced PRP at platelets concentrations of 5.0×10^5/μL and 10.0×10^5/μL in a model of patellar tendinopathy in rats (18), however the optimal concentration of platelets in the PRP was not determined.
The objective of the current study was to determine the optimal concentration of platelets in PRP for decreasing pain and improving tendon structure in a model of Achilles tendinopathy in rats. Tendinopathy was induced bilaterally by injections of collagenase, and starting 10 days later autologous neutrophil-reduced PRP at platelet concentrations from 5.0×10^5/μL to or 15.0×10^5/μL was administered. Efficacy was assessed by measuring a pain-relief by a locomotor activity score and the tendon tissue-repair by histology. There was also a different animal model of proximal patellar tendinopathy prepared using a repetitive running exercise on a rodent treadmill machine, in which Achilles tendinopathy was not induced, in rats (18).

MATERIALS AND METHODS

The study was conducted according to the journal's guidelines (19).

Reagents and materials

The autologous PRP preparation system, MyCells, was purchased from Kaylight, Tel Aviv, Israel. The normal saline for injections was purchased from Otsuka Pharmaceutical Factory, Inc., Naruto, Japan. The collagenase type I (185 IU/mg) derived from *Clostridium histolyticum* was purchased from Worthington Biochemical Corporation, Lakewood, NJ, USA. The Supermex system for measurement of spontaneous locomotor activity was purchased from Muromachi Kikai Company, Tokyo, Japan. The ApopTag Peroxidase *in Situ* Apoptosis Detection Kit was purchased from EMD Millipore Corporation, Billerica, MA, USA.

Animals

Sixty male Wistar rats (10 weeks of age, weighing 350-400 g) were purchased from Nippon SLC, Hamamatsu, Shizuoka, Japan. The rats were housed in an environmentally controlled animal facility on a 12:12 light/dark cycle. Food and water were available *ad libitum*.

Ten of these rats were untreated healthy controls. They were used for PRP preparation and for histological assessment of normal Achilles tendon structure. Fifty rats were used for the tendinopathy study. The general status of the animals was observed once a day from the day before collagenase injections to the end of the study. Body weight was measured every 5 days during the study period.

Collagenase-induced Achilles tendinopathy model

The study received Institutional approval (#29-003), and all experiments were conducted in accordance with the Institutional guidelines for the care and use of experimental animals. A rat model of collagenase-induced Achilles tendinopathy was prepared as previously described (20, 21). Collagenase type I powder was suspended in Phosphate Buffered Saline (PBS) at a concentration of 300 IU/mL and the solution was filtered through a sterile nylon syringe filter. Rats were anesthetized with 2.5% isoflurane. With the ankle flexed to 90 degrees to put tension the tendon, collagenase solution (50 μL) was injected into the distal insertional site of both Achilles tendons with 30-gauge needles. Spontaneous locomotor activity was measured with the Supermex system (see below) for 12 hours on the nights for 5 days before the collagenase injections and for 10 days after the collagenase injections. Rats with > 25% reduction in spontaneous locomotor activity 10 days after collagenase injections comparing with the average activities for 5 days before the collagenase injections were selected for the tendinopathy study. This decrease in activity was based on data from a preliminary study. To confirm the reproducibility and validity of the model, a preliminary study was performed in 30 rats. Consistent with reported observations, the values of locomotor activities or the reduction in spontaneous locomotor activity after collagenase injections was variable among rats (20, 21). In the preliminary study, locomotor activity decreased 21-31% 10 days post collagenase injections, with 26 rats (87%) having > 25% reduction. Histology of the Achilles tendons from 10 randomly selected rats with > 25% decrease in activity showed tendinopathy-related changes including microtears and laminations (*i.e.*, longitudinal disruptions between the fiber bundle layers at the insertion site of the Achilles tendon) (see later for details of the methods) (**figure 1**).

Treatment groups and injection of autologous platelet-rich plasma

The 40 rats with decreases in locomotor activity > 25% at 10 days post collagenase injections were randomized into five groups (n=8/group). The remaining three rats were

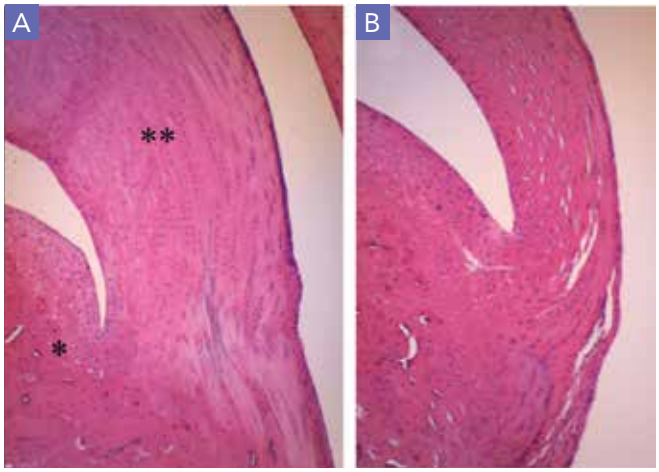

Figure 1. Histological sections of the insertion sites of Achilles tendons stained with Hematoxylin-Eosin (H & E) in a normal healthy rat **(a)** or in a collagenase-induced tendinopathy rat (a rat with > 25% decrease in locomotor activity 10 days after collagenase injections) **(b)** (×100). Two major pathological findings were found at the insertion site of Achilles tendons in the tendinopathy rats: 1) microtears in the body of the tendon and 2) several longitudinal disruptions between the layers of the fiber bundles of the tendon body, so-called laminations. An upper side is proximal. * Calcaneus bone, ** Achilles tendon.

reserved for late accidents. These groups had injections into the ankles bilaterally of normal saline (control group) or autologous neutrophil-reduced PRP at platelet concentrations of 50 × 10^4/μL (P50 group), 75 × 10^4/μL (P75 group), 100 × 10^4/μL (P100 group) and 150 × 10^4/μL (P150 group). For the injections, the rats were anesthetized with 2.5% isoflurane and the fur at the ankle joints was shaved. 50 μL of normal saline or PRP was injected into the distal insertional sites of the Achilles tendons bilaterally with a 0.5 mL injection syringe and a 30-gauge injection needle. All animals were sacrificed on day 19 after the PRP injections.

Preparation of autologous platelet-rich plasma

The autologous PRP preparation system, MyCells, was used to obtain a rat autologous neutrophil-reduced PRP. Twenty-two mL of whole blood was aspirated from the hearts of 10 normal healthy rats. From this whole blood, the PRP preparation system produced 4.0 mL of neutrophil-reduced PRP. Briefly, the whole blood was centrifuged for 7 minutes at 2000 × g. After aspiration of the supernatant plasma, the residual plasma of 4.0 mL was agitated to disperse the platelets, which were precipitated on the separation gel. This plasma was filtered to exclude the debris resulted in 4.0 mL of a filtered non-activated PRP (18). Analysis of a 100 μL sample of this rat autologous PRP showed a platelet concentration of 166 × 10^4/μL, and exclusion of approximately 90% of neutrophils and almost all erythrocytes. This PRP was classified as type 3 in Mishra's classification and as pure PRP in the PAW classification since the PRP was not activated before injection. This solution was diluted to produce the required platelet concentrations for the study.

Measurements of spontaneous locomotor activity

Spontaneous locomotor activity of each rat was measured with the Supermex system. Activity was measured for the 12 hours dark cycle the day before and after the collagenase injections, and then on alternate days through the end of the study (29 days after the collagenase injections and 19 days after the PRP injections). The locomotor activity has been shown to reflect the degree of the pain in limbs of rats, and so the degree of pain-relief in the ankle could be determined by the changes in locomotor activity. The Supermex system can quantify activity, and has been shown to be reliable compared to other methods. Details of spontaneous locomotor activity measurement have been described previously (18, 22, 23). Briefly, movement is detected from the infrared radiation emitted from the animal. Different lenses detect the radiation in different regions of the cage, and in this study, activity was measured as a single count when an animal moved from one region of the cage to another. The noise produced by grooming was filtered out. Total counts were calculated by summing up all counts of 10-min periods. Baseline locomotor activity is very variable among rats and so the post-PRP activity data are calculated and presented as % change in counts compared to the activity data at 10-day post-collagenase value. The absolute values for locomotor activities were different among rats, which were described in our previous literature (18).

Histological examinations

From the preliminary study, ankle tissue samples were obtained from 6 normal healthy rats and from 6 rats with > 25% decrease in activity 10 days post-collagenase injections. In the tendinopathy study, on day 19 post-PRP or normal saline injections, ankle tissue samples were obtained from all 40 rats in the P50, P75, P100, P150 and control groups. After anesthesia with 5% isoflurane, the rats were killed by an immediate blood draw. Whole ankle joints were dissected out and were fixed in 4% paraformaldehyde at pH 7.4 for 3 days, decalcified in 20% EDTA solution for 21 days at 4 °C, and then embedded in paraffin wax. Five μm thick

sections were taken in the sagittal plane and were stained with hematoxylin-eosin (H & E). A histopathological score was determined from the number of microtears and laminations, *i.e.*, several longitudinal disruptions between the fiber bundle layers seen at the insertion site of the Achilles tendon in a visual high-power (200×) field in each of five sections per rat. A score was doubled if the length of the tears or laminations were longer than half the diameter of the high-power field (18, 22). Histological sections were also prepared by TdT-mediated dUTP nick end labeling using the ApopTag Peroxidase *in Situ* Apoptosis Detection Kit according to the manufacturer's instructions. Apoptotic cells were quantified by counting the number of TUNEL-positive cells in high-power (400×) in each of five sections per rat. All H & E and TUNEL stained sections were analyzed by three researchers. The standard deviations of these scores or numbers among the three researchers were within 5%.

Statistical analysis

Comparative series were considered independent of each other. The % change in locomotor activity, the histopathological score, the number of apoptotic cells, and body weights are all presented as means and standard deviations for each group. The activity data, histopathological scores and apoptotic cell numbers were compared among groups with Mann-Whitney U Test. The body weight data was compared between groups with Student's t-Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

General status and body weight

During the experimental period, no abnormalities were observed in any of the groups. All groups showed a similar increases in body weight compared to the control group and no significant differences were observed in body weight among groups.

Collagenase-induced Achilles tendinopathy model

In 50 rats injected with collagenase solutions, 43 rats had decreases in spontaneous locomotor activity at 10 days after injections of > 25% (overall reduction rate was in the range of 20% to 33% in 50 rats) and 40 rats (an average reduction rate was 28.7%) were selected for the PRP/tendinopathy study.

Spontaneous locomotor activity

In the P75 group, the counts for spontaneous locomotor activity increased by approximately 20% at day 2 after PRP injections compared to the activity immediately pre-PRP. Activity gradually increased to 30% at day 12 and was maintained until the end of the study (**figure 2**). The activities in the P75 group after day 12 recovered to a level similar with the activities before the collagenase injections, since after collagenase injections the activities were reduced to be in the range of 70% to 75% and the increased activity values of 30% were in the range of 91% to 100%. Similarly, in the P100 group locomotor activity increased by approximately 20% at day 2 after PRP injections and was maintained around this level until the end of the study. On the other hand, the spontaneous locomotor activities in the P50 and P150 groups increased by approximately 10% at day 2, were maintained until day 8 or day 12 and then decreased to the baseline level until the end of the study. The activity in the control group was around baseline level throughout the study. The spontaneous locomotor activities in the P75 or the P100 groups were significantly greater than in the P50, P150 and control groups from day 2 to day 18 ($p < 0.05$), and there was significantly greater spontaneous locomotor activity in the P75 group than in the P100 group from day 8 to day 14 ($p < 0.05$). Spontaneous locomotor activity in the P50 group was significantly greater than in the control group ($p < 0.05$) from day 2 to day 8, and locomotor activity in the P150 group was significantly greater than in the control group ($p < 0.05$) from day 2 to day 12 (**figure 2**).

Histological findings

In rats that exhibited tendinopathy (> 25% decrease in locomotor activity after collagenase injections), two major pathological features were observed at the insertion sites of Achilles tendons (**figure 1 b**). These were microtears in the tendon body and laminations, *i.e.*, several longitudinal disruptions between the fiber bundle layers. No tendinopathy-specific histopathological changes were observed in the six normal healthy rats (**figure 1 a**). In the P75 and P100 groups, the histopathological scores determined by the number and length of the microtears and laminations were significantly lower than those in the P50, P150 and control groups ($p < 0.05$). This indicates that the tendon repair processes in the P75 or P100 groups had progressed compared to those in the P50, P150 and control groups. There were no significant differences in the histopathological scores between the P75 and P100 groups, between the P50 and P150 groups, between the P50 and control groups or between the P150 and control groups (**figure 3, 4**).

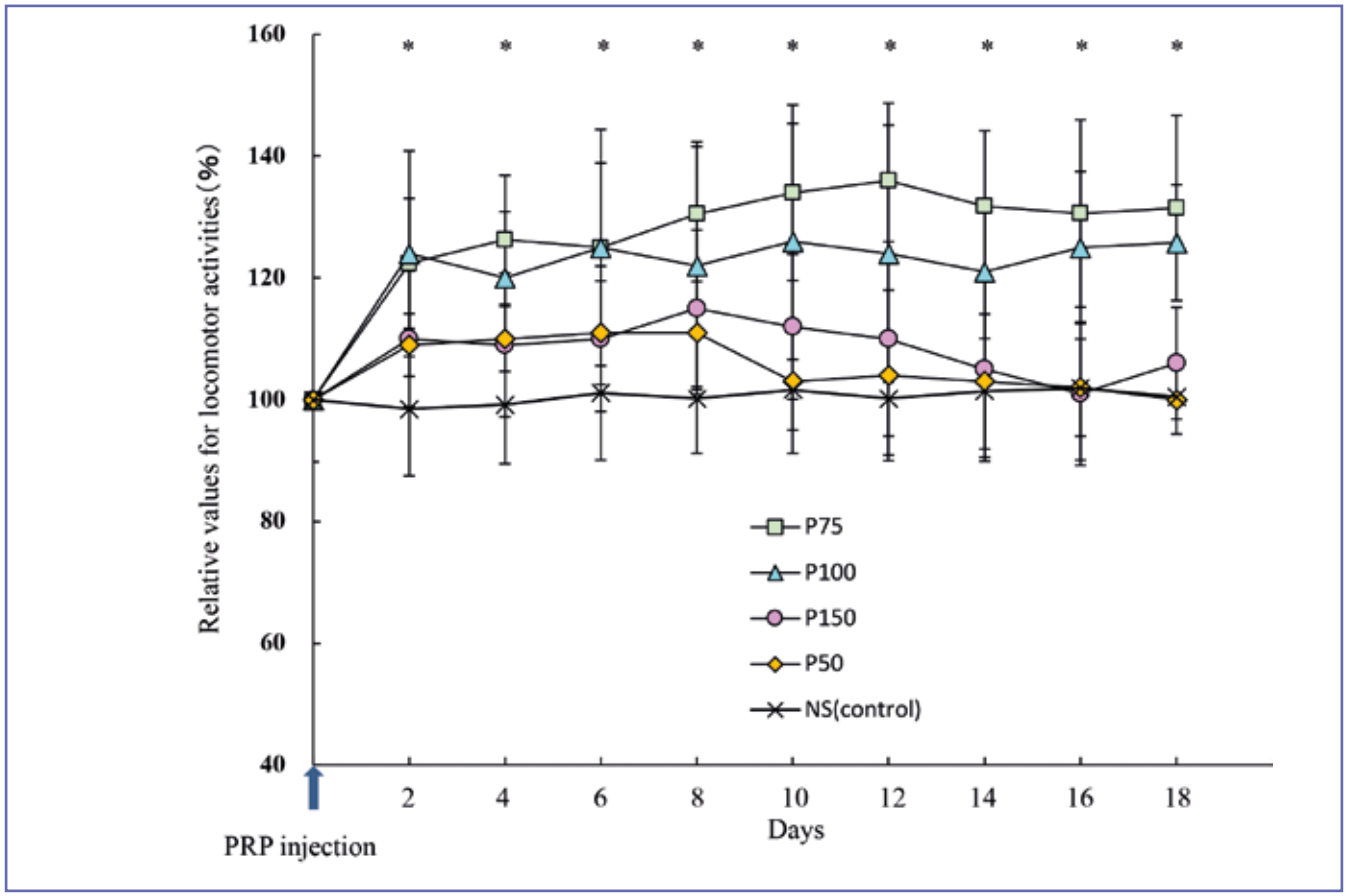

Figure 2. Relative values for spontaneous locomotor activities in the P50 (◊), P75 (□), P100 (O), P150 (Δ) and control groups (×). The spontaneous locomotor activities were measured using a Supermex system at night for 12 h on alternate days for 19 days after PRP injections. The relative counts of locomotor activities in each group were calculated from the post-collagenase pre-PRP baseline, and are shown as group means ± standard deviations.
*Significant differences between the P75 group and the P50, P150 and control groups ($p < 0.05$), and between the P100 group and the P50, P150 and control groups ($p < 0.05$).

In rats exhibiting tendinopathy, TUNEL-positive (apoptotic) cells were observed at the insertion site for Achilles tendon. In the P75 and P100 groups, the number of TUNEL-positive cells was significantly lower compared to the same tendon area in the P50, P150 and control groups ($p < 0.05$). There were no significant differences in the number of TUNEL-positive cells between the P75 and P100 groups, between the P50 and P150 groups, between the P50 and control groups or between the P150 and control groups (**figure 5, 6**).

DISCUSSION

In the present study, the following were the principal findings. In rats exhibiting collagenase-induced Achilles tendinopathy who were then treated with autologous neutrophil-reduced PRP at different platelet concentration or saline control: 1) the spontaneous locomotor activities in rats injected with PRP at platelet concentrations of $75 \times 10^4/\mu L$ and $100 \times 10^4/\mu L$ increased to pre-collagenase levels (similar to levels in normal, healthy rats) at day 2 after PRP injections and were then maintained for the following 17 days, the end of the study; 2) the spontaneous locomotor activities with PRP at platelet concentrations of $75 \times 10^4/\mu L$ and $100 \times 10^4/\mu L$ were significantly greater than those in the rats injected with PRP at platelet concentrations of $50 \times 10^4/\mu L$ and 150×10^4 µL or saline control at all times from day 2 until the end of the study; 3) the spontaneous locomotor activities in the rats injected with PRP at plate-

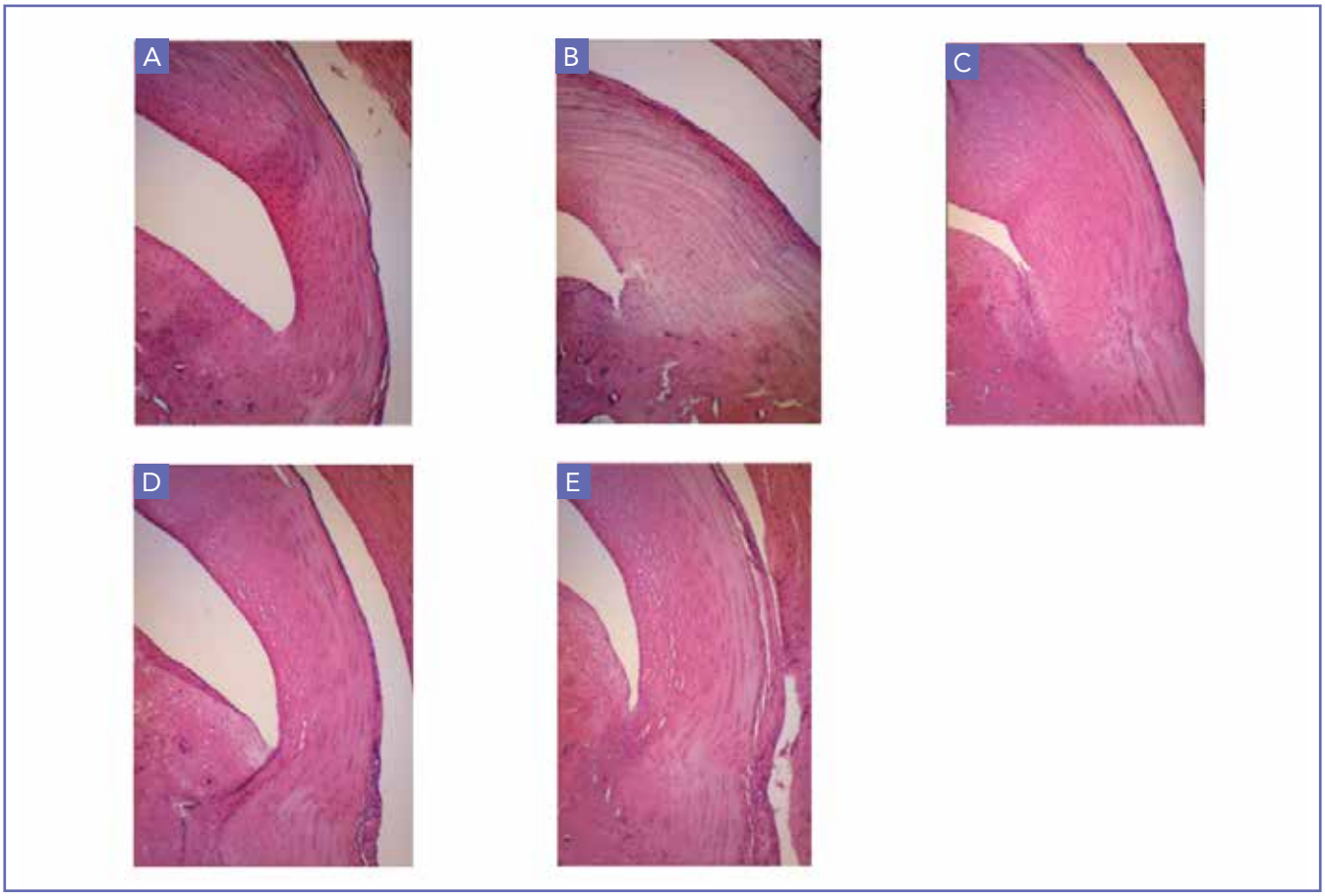

Figure 3. Histological sections of the insertion sites of Achilles tendons stained with Hematoxylin-Eosin (HE) in the P50 group **(a)**, P75 group **(b)**, P100 group **(c)**, P150 group **(d)** and control group **(e)** (×100). Ankle tissue samples were obtained from all 40 rats in all experimental groups on day 19 post-PRP or normal saline injections. Five μm thick sections were taken in the sagittal plane and were stained with Hematoxylin-Eosin (HE).

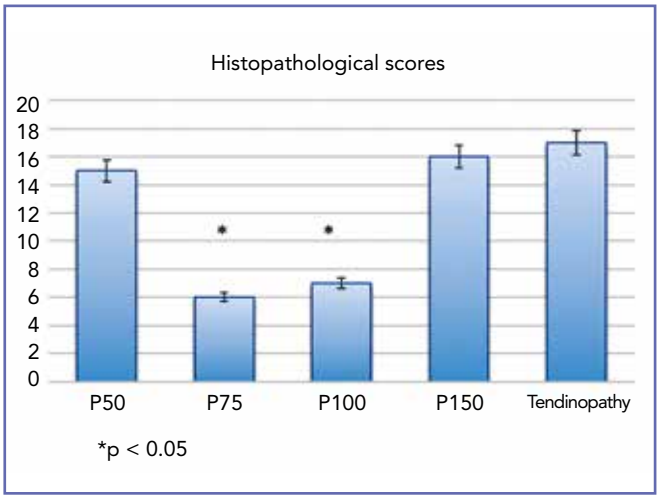

Figure 4. Histopathological scores for the HE-stained histological sections located at the insertion site of the Achilles tendon in the P50, P75, P100, P150 and control groups. A histopathological score was determined from the number of microtears and laminations, *i.e.*, several longitudinal disruptions between the fiber bundle layers seen at the insertion site of the Achilles tendon in a visual high-power (200×) field. A score was doubled if the length of the tears or laminations were longer than half the diameter of the high-power field. Scores are shown as group means ± standard deviations. The number of microtears and laminations counted in the P75 and P100 groups were significantly lower than those in the P50, P150 and control groups ($p < 0.05$). No significant difference was observed in the number of microtears or laminations between the P75 and P100 groups, between the P50 and P150 groups, between the P50 and control groups or between the P150 and control groups.

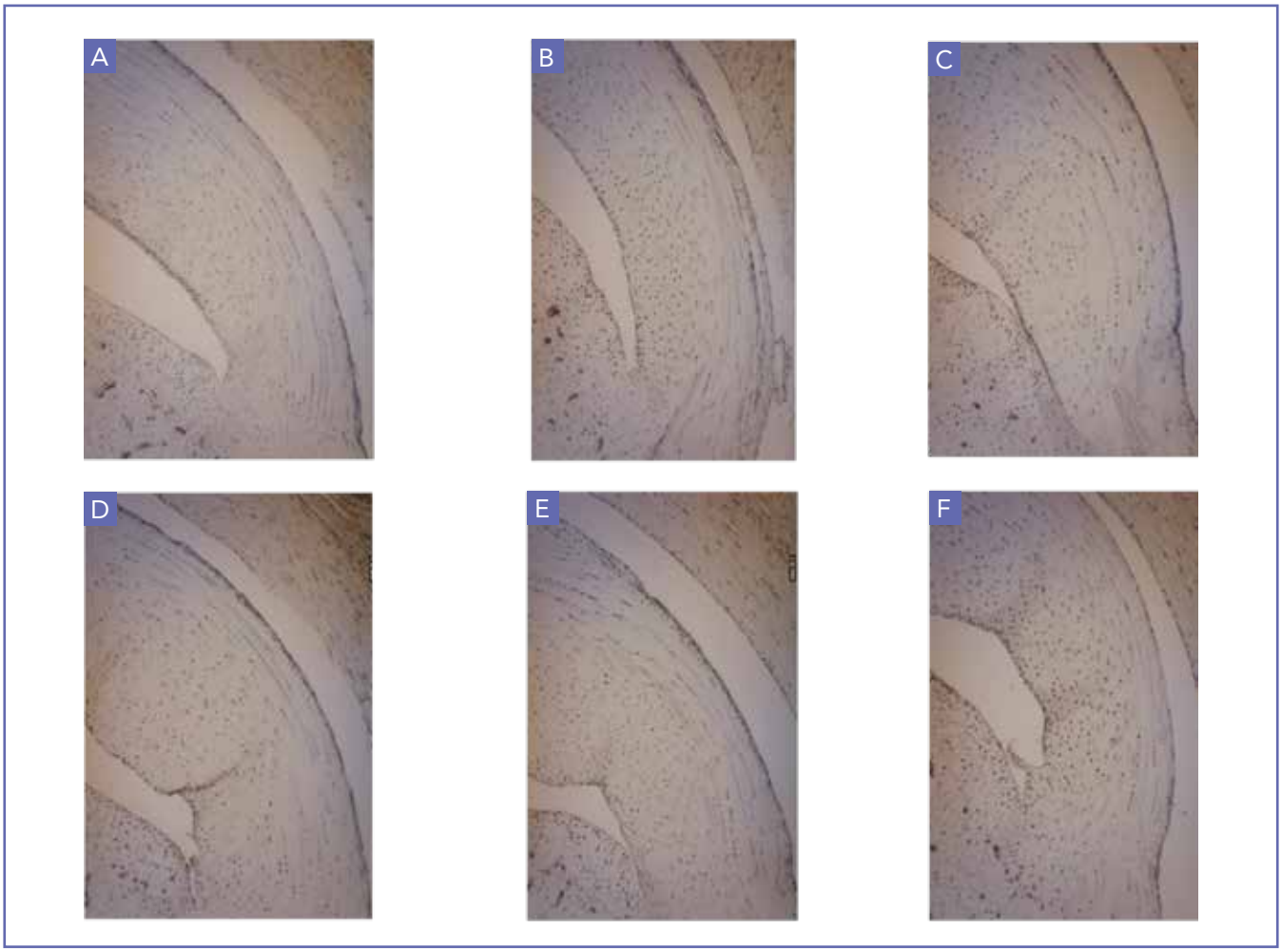

Figure 5. Histological sections of the insertion sites of Achilles tendons prepared by TdT-mediated dUTP nick end labeling (TUNEL) of healthy normal rats (**a**), and rats in the P50 group (**b**), P75 group (**c**), P100 group (**d**), P150 group (**e**) and control group (**f**) (×100). Ankle tissue samples were obtained from all 40 rats in all experimental groups on day 19 post-PRP or normal saline injections. Five µm thick sections were taken in the sagittal plane and were prepared by TUNEL using the ApopTag Peroxidase *in Situ* Apoptosis Detection Kit according to the manufacturer's instructions.

let concentrations of 50 × 10^4/µL and 150 × 10^4/µL were significantly greater than those in rats injected with saline control, although these activities were less than the pre-collagenase baseline; 4) in histological sections taken at the end of the study (day 19), the number of microtears and laminations, and the number of TUNEL-positive cells observed at the insertion site of the Achilles tendon in rats injected with PRP at platelet concentrations of 75 × 10^4/µL and 100 × 10^4/µL were significantly fewer than the numbers in rats injected with PRP at platelet concentration of 50 × 10^4/µL and 150 × 10^4/µL or with saline control; 5) there were no significant differences in the number of microtears and laminations, or in the number of TUNEL-positive cells between the P75 and P100 groups, or among the P50, P150 and saline control groups.

Taken together the findings indicate that in this model of Achilles tendinopathy the analgesic effect (from the increase in locomotor activity) and the reparation of the tendon (from the histological score and number of apoptotic cells) with PRP at platelet concentrations of 75 and 100 × 10^4/µL were greater than with PRP at platelet concentrations of 50 and 150 × 10^4/µL. The increase in locomotor activity to pre-collagenase levels suggest that the PRP at platelet concentration of 75 and 100 × 10^4/µL completely relieved the pain in these rats. From these data we propose that a platelet concentration of 75 to 100 × 10^4/µL in neutrophil-reduced PRP was

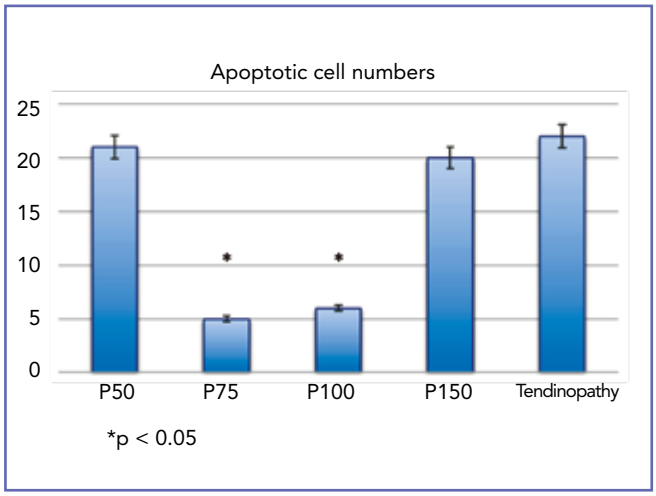

Figure 6. Cell number for apoptosis at the insertion site of the Achilles tendon of rats in the P50, P75, P100, P150 and control groups. Apoptotic cells were quantified by counting the number of TUNEL-positive cells in high-power (400×). The number of TUNEL-positive cells are shown as group means ± standard deviations. The number of TUNEL-positive cells in the P75 and P100 groups was significantly lower than those in the P50, P150 and control groups ($p < 0.05$). No significant difference was observed in the number of TUNEL-positive cells between the P75 and P100 groups, between the P50 and P150 groups, between the P50 and control groups or between the P150 and control groups.

the optimal range for effects of pain relief and tendon reparation in this rat model of Achilles tendinopathy. It is possible the optimal range extends to lower and higher concentrations, and this was not established. This is the first report to determine an optimal range of PRP platelet concentrations in an animal model (*i.e., in vivo*) of tendinopathy. The observation of platelet concentration dependent efficacy at low and medium concentrations and reduced efficacy at high concentrations is consistent with findings from *in vitro* cell culture studies on tendon metabolism (15-17).

Clinically, the platelet concentration in PRP isolated from commercial preparation kits is generally not adjusted before administration. It was suggested that different platelet concentrations in PRP might account for the different clinical outcomes of PRP therapy seen between institutions or between patients within an institution. Our data support this and suggest that for maximum therapeutic benefit in treatment of tendinopathy, the platelet concentration in PRP should be measured after isolation and be adjusted to an optimal concentration before administration. It is possible that the optimal concentration will be different for different tendinopathies, and this is an area for future study. It is hoped that eventually de novo PRP preparation kits will be available that can, automatically adjust the platelet concentration to the optimal range.

Locomotor activities in rats injected with PRP at platelet concentrations of 75 and 100 × 10^4/μL increased by approximately 20% at day 2 after PRP injections. Since the tendon tissue repair was unlikely to be complete at this stage, it was speculated that the mechanisms of PRP-induced pain relief and tissue restoration were different and separate. It has been suggested that pain generation in tendinopathy results from free nerve endings in abnormal capillary vessels with microshunts that form around the tendon (24-26), since focal embolization of these abnormal capillary vessels immediately relieved the tendinopathy pain (27). Therefore, we speculate that in this tendinopathy study a PRP-induced reaction degraded the abnormal capillary vessels and/or inhibited the free nerve endings to produce the initial pain relief. More detailed tendon histology is needed to confirm the presence of such capillary vessels and free nerve endings at the insertional sites of the Achilles tendons in this rat model of Achilles tendinopathy.

In general, an immobilization of the treatment sites after PRP therapy was recommended or ordered in order to escape the inhibition or the delay of the cellular reparative reactions in clinics for human (28). The present study demonstrated that tendinopathies recovered well in P75 or P100 groups without the immobilizations of the treatment sites after PRP therapy, suggesting that tendinopathies in human might also recover well by the PRP therapy under the condition that the concentrations of platelets in PRP were in the optimal range even though without immobilizations of the treatment sites.

It was found that locomotor activities of P50 or P150 groups increased up by approximately 10% for a week after PRP injections and then decreased down to baseline. Histopathological scores of these groups were significantly lower than those of P75 or 100 groups indicating the insufficient histological repair of the tendon tissues in P50 or P150 groups. For the reason of the finding it was suggested that cellular reparative reactions did not progress after the initial pain relieving affected in approximate half degree because of the non-optimal concentrations of platelets in PRP and no immobilizations for the ankles of rats in P50 or P150 groups.

Limitations of the present study were: small sample number, short experimental period and so reproducing early-stage but not late stage tendinopathy, lack of biomechanical evaluation, and different concentrations of leukocytes between experimental groups. In this study there was a maximal effect in terms of pain relief and tendon repair in the 75 to 100 × 10^4/μL platelet concentration range. A smaller

effect at a PRP platelet concentration of 50 × 10^4/μL can be explained by the lower number of platelets. A question is: why is the effect smaller at a platelet concentration of 150 × 10^4/μL? Neutrophils, a major source of inflammatory mediators that can impair healing and cause pain, were largely excluded from this PRP preparation. It has been suggested that the residual leukocytes other than neutrophils in PRP preparations have little or no effect on tendon metabolism. However, we cannot rule out that these residual leukocytes did contribute to the reduced efficacy in the P150 group. This requires further study.

CONCLUSIONS

In a rat model of Achilles tendinopathy, a single injection of autologous neutrophil-reduced PRP into the Achilles tendon reduced pain and initiated the tendon repair process. These effects of pain relief and tendon repair were greater at platelet concentrations of 75 and 100 × 10^4/μL than at platelet concentrations of 50 and 150 × 10^4/μL. These data suggest that there is an optimal PRP platelet concentration for maximal efficacy in the treatment of tendinopathy. Studies are needed to determine if there are similar optimal PRP platelet concentration ranges for maximal therapeutic efficacy in clinical tendinopathies.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT

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ORIGINAL ARTICLE

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Cross-Cultural Adaptation of The Visa-P Questionnaire for Danish-Speaking Patients with Patella Tendinopathy

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LEVEL OF EVIDENCE: 3B

SUMMARY

Introduction. Victorian Institute of Sports Assessment-Patella (VISA-P) questionnaire is a both valid and reliable Patient-Reported Outcome Measure (PROM) assessing the severity of Patella Tendinopathy (PT). The VISA-P questionnaire exists in several languages, but not in Danish.

Objective. To translate and cross-culturally adapt the original VISA-P questionnaire for a Danish-speaking PT population and verify its psychometric properties to produce a validated Danish version of the VISA-P questionnaire.

Methods. Translation and cross-cultural adaptation was performed according to international guidelines. Translation, synthesis, reverse translation, original authors' approval and pretesting were performed. Psychometric properties of VISA-P-DK were assessed on PT patients (n=86), healthy individuals (n=69) and on a PT patient group at 3-months follow-up.

Results. Mean VISA-P-DK score in PT patients was 54 (95% Confidence Interval (CI) 50-57), significantly lower than the healthy control score of 99 (95% CI 98-100). Test-retest reliability of the VISA-P-DK was good (r=0.87 and ICC=0.88). Internal consistency was also good (Crohnbach's *Alpha*=0.83). Correlations between the VISA-P-DK and scales measuring physical health by SF-36 (criterion validity) were in 2/3 cases moderate to good. No significant difference was found when comparing the PT group in the original study. Responsiveness showed a significant difference ($p < 0.05$) between baseline score and 3-months follow-up score.

Conclusions. VISA-P-DK is a valid and reliable tool for measuring and monitoring of Danish-speaking PT patients. VISA-P-DK is easily applicable in the clinical setting, and its comparability with other VISA-P questionnaires makes it a useful tool in research.

KEY WORDS

Tendon injury; exercise induced pain; joint; patella tendinopathy; sports medicine; jumpers knee.

INTRODUCTION

Patella Tendinopathy (PT) or jumper's knee is a common overuse injury in especially sports containing running and jumping (1). PT is present in both elite (14,2%) and non-elite athletes (8,5%), with elite volleyball players having the highest prevalence (44,6%) (2, 3). PT is invalidating and affects both athlete performance level and participation (4). Furthermore, the work life of nonprofessional athletes may be affected (5, 6). So far, no standardized patient-reported outcome measure to assess PT exists in Danish.

PT diagnosis is based on: 1) pain localized to the inferior pole of the patella, 2) load-related pain that increases as the demand on the knee extensors increases (2, 7-10). Risk factors for developing PT include male gender, hours of training, previous knee injury, as well as decreased hamstring flexibility (11). Ultrasound and MRI are often used when diagnosing PT (7, 9, 12, 13). However imaging does not correlate with reported PT symptoms (13).

Patella Tendinopathy (PT) is a both well-known and invalidating sports injury worldwide. Etiology of PT is still discussed, making treatment a challenge for clinicians (4, 14). Treatment options include: platelet-rich plasma injections, hyaluronic acid injections and shock wave therapy (14-16). PT diagnosis is primarily based on clinical examination. However, a patient-centered approach is considered more essential for diagnosing and monitoring. At present physicians only have one disease specific instrument (VISA-P) to determine PT severity and to monitor treatment systematically.

VISA-P17 is a disease specific PROM for measuring physical disability caused by PT. VISA-P does therefore measure experienced performance of the musculoskeletal system in the knee region. It is often used as an outcome measure when monitoring treatment or when studying PT (2, 7, 8). VISA-P is based on an inverted Numeric Rating Scale (NRS). It consists of 8 questions, resulting in a score ranging from 0 (total disability) to 100 points (no recorded problems). VISA-P is sensitive to changes in symptoms and is easily understood by patients, giving relevant answers to clinicians (18). In order to use VISA-P for non-English speaking patients, a translation and cultural adaptation of the questionnaire with a validity and reliability testing is needed. VISA-P has successfully been translated into at least 10 other languages. All translations are satisfactory cross-cultural adaptations (18-27). Other Victorian Institute of Sport Assessment questionnaires have previously been successfully translated into Danish, *e.g.* VISA-A (28). VISA-A-DK is now an international standardized tool for measuring Danish-speaking Achilles Tendinopathy (AT) patients. Clinicians are able to transfer severity of AT directly when comparing subjects and/or studies from different countries. Standardized examination makes it easier to produce international consensus on treatment strategies. However, a translation of an existing questionnaire leaves little room for improvement, and content validity will be consistent through all translations.

The aim of this study was to develop a Danish version of VISA-P, VISA-P-DK (see **Appendix 1**). Validation and reliability testing of the new questionnaire was carried out. VISA-P-DK has the advantage of being easily applicable in all clinical settings and has been long needed in the assessment of Danish-speaking PT patients, both in diagnostics and in follow-up.

METHODS

Translation

The cross-cultural adaptation was made in accordance with the guidelines set for self-reported questionnaires by Beaton *et al.* (2000) (29). The following 5 steps were performed (**figure 1**).

1. Translation: two independent Danish translations were made from the original English version of VISA-P questionnaire by two bilingual translators (JI + HL). Both translators had Danish as their first language and English as their second. Any challenges in translation were noted.
2. Synthesis: to produce one Danish version the two translations were then compared and synthesized. Any challeng-

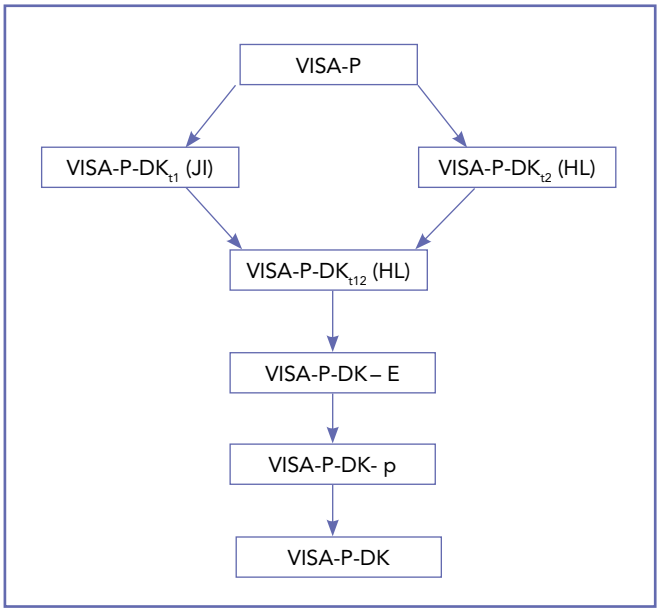

Figure 1. Synthesis of VISA-P-DK.

es in translation were discussed and agreed upon. The result was VISA-P-DKt (12).
3. Reverse translation: the synthesized Danish version VISA-P-DKt12 was then translated back into English. This was carried out by a certified Danish-English translator with no medical background, who was to be naive to the original VISA-P and the purpose of the study. Any challenges were noted by the translator. The result was VISA-P-DK-E.
4. Review: to ensure the new Danish questionnaire VISA-P-DKt12 was compatible to the original VISA-P (13), the translation of VISA-P-DK-E was reviewed and approved by the main authors of VISA-P (Visentini and Wark) (13). The resulting pilot version, VISA-P-DK-p, was used in the pretesting.
5. Pretesting: VISA-P-DK-p was tested on five healthy individuals aged 18-40 years, recruited from Hvidovre Hospital, Denmark. The translators interviewed all five individuals via e-mail. This test resulted in an inversion of question 2-6. The remodeling of the questionnaire was also sent to the main authors of the original article and accepted. The result was the Danish version of VISA-P: VISA-P-DK. VISA-P-DK was then validated in the following study.

When translating VISA-P, the time intervals given in question 8 were (in minutes) changed from (0, 1-5, 6-10, 11-15, > 15) to (0, 1-10, 11-20, 21-30, > 30). These changes were made because the authors believed that the difference of an athlete being able to participate 30 or 16 minutes, represented too big a difference in the severity of PT to be disregarded. The original authors approved these changes even though being different from the other language versions of the VISA-P questionnaire.

Patient recruitment and ethics

Patients were recruited through physicians at the University Hospital Aarhus, Denmark and through a private Physical Therapy Clinic in Dronninglund, Denmark. All participants gave informed oral consent to participate in the adaptation study.
The legislation of processing personal information was carried out in accordance with the guidelines of the Danish Data Protection Agency, and we provided a binding description stating the use of personal information prior to study start. The study is compliant to the ethical and scientific standard discussed in Padulo *et al.* (2018) (30).

Sample size and participants

To obtain a power of 90% (two-sided 5% level of significance) a minimum of 31 subjects in the two groups was required. VISA-P-DK was tested in 2 groups: 1) a group of athletes diagnosed with PT and 2) a control group consisting of healthy athletes (n=69). A PT subgroup also completed a SF-36 questionnaire (31, 32). PT patients were clinically diagnosed. The diagnosis was supported by ultrasound (US) (8). Currently or prior to injury, all subjects participated in sports at least three times a week, were > 16 years of age, understood the purpose of the study, and had given informed oral consent. If a participant was younger than 18 years, the parents were also asked for consent.

Statistics

Data processing was made in Excel and SPSS 25. Data were presented with a mean, standard deviation (SD) and a 95% Confidence Interval (CI 95%). Since the groups in this study were small, Central Limited Theorem (CLT) was applied (33). The Kolmogrov-Smirnov test was made to test all groups for normal distribution. Significance level was set to $p < 0.05$. Since VISA-P-DK is based on a numeric scale where symptoms are converted into a numerical value, all data in the study are considered non-parametric.

Reliability

To assess the reliability of VISA-P-DK, internal consistency, test-retest reliability and measurement error were tested (34, 35). To assess internal consistency Cronbach's *alpha* was calculated (35). Test-retest was performed on 33 PT patients who completed the questionnaire twice: 1) at their first visit to the physician, and 2) 2-5 days later. Agreement and correlation were measured using Intraclass Correlation Coefficient (ICC) model (2.1) (36) and Spearman's rank correlation (*rho*). To note any significant difference between the two days, Wilcoxon signed rank Test was performed. The Standard Error of Measurement (SEM) and Minimal Detectable Change (MDC) were calculated (37, 38). MDC was calculated based on a 95% confidence interval. To further demonstrate and visualize the reliability, a Bland & Altman plot was constructed.

Validity

To assess Validity, different aspects of validity were explored: Construct, Convergent, and Criterion validity (34, 35). Construct validity was measured comparing the control group with the PT group and the 3-months follow-up group (35). It was expected that the PT group would have the lowest score, the 3-month follow-up group a higher score, and the control group the highest. Since the groups were nonpaired, the Mann-Whitney U Test was applied (39).

Convergent validity was tested by a subgroup completing both VISA-P-DK and the validated SF-36. The correlation between the subscales examining physical health in SF-36 and VISA-P-DK score was calculated using Spearman's *rho*. As no golden standard for the assessment of PT in Denmark exists, criterion validity was tested comparing the scores of VISA-P-DK to the PT patient groups in the original article (17) (n=14) (34). Since only mean (55) and SD (12) were available, a two-sample t-Test was used to compare the two scores. Responsiveness was tested by having a subgroup complete the questionnaire after 3 months of treatment (35). To assess if there was a significant difference in the two completions of the questionnaire, the Wilcoxon signed rank test was applied. PT patients followed treatment decided by their physicians independently of their participation in the study.
The results were examined to see if floor or ceiling effects were present.

RESULTS

The Danish VISA-P questionnaire, VISA-P-DK is shown in **table I**. No problems understanding the questionnaire were reported. The re-translated version in English was approved by the original authors of VISA-P (13), and the cross-cultural adaptation of VISA-P for Danish-speaking PT patients was accepted.

VISA-P-DK Test population

86 PT Patients and 69 healthy controls were tested. The two groups were comparable regarding age, but not regarding gender. One subject received treatment in between testing and re-testing of the questionnaire. This subject was therefore removed from the re-test subgroup. Three PT patients only completed the VISA-P-DK after three months of treatment. These subjects were excluded when comparing the two groups. Applying Kolmogrov-Smirnov showed that both the PT patient group and subgroups followed a normal distribution. The control group did not follow a normal distribution.

Reliability

Test-retest reliability is summarized in **table II**. Total VISA-P-DK score showed good internal consistency and correlation with and ICC score of 0.88 and Spearman's *rho* 0.87, (an ICC score higher than 0,75 is considered good or excellent) (36). 5 of the questions showed moderate reliability ($0.5 < ICC < 0.75$). Total score MDC% was 12%. An MDC% of 30% is considered reasonable and 10% is considered excellent (37). When evaluating each question individually all had an MDC% higher than 30%. There was no significant difference in the data obtained on the two test days ($p > 0.05$).
Internal consistency of the 8 VISA-P-DK questions was 0.83 (Cronbachs *alpha* (40)). The Bland & Altman plot (**figure 2**) shows no indication of bias.

Validity

Construct validity

PT patients (n=86) had a mean VISA-P-DK score of 54 (95% CI 50,6-57,6). Healthy Controls (n=69) had a mean VISA-P-DK score of 99,1 (95% CI 98,2-100) (**table I**).

Table I. Descriptive data.

	n	Age (years) Mean, SD (95% CI)	Baseline VISA-P-DK score Mean, SD (95% CI)	Retest or follow-up VISA-P-DK score Mean, SD (95% CI)
PT patients	86 M 60 F 26	28.8 (26.6-31)	54.1, 16.5 (50.6-57.6) 54 (49,9-58,1) 54.2 (47,5-61)	
PT patients re-test group	33 M 23 F 10	26.5 (23.4-29.7)	52.8, 14.9 (47.8-57.9) 53.3 (48-58.6) 51.7 (39.7-63.7)	54.3, 15.2 (49.1-59.5) 54.6 (48.9-60.4) 53.5 (42.1-64.9)
PT patients 3 months follow up	33 M 21 F 12	26.7 (23.9-29.5)	54.1, 15.9 (48.7-59.5) 53.5 (47.1-59.7) 55.2 (44.6-65.7)	70.5, 17.4 (64.6-76.5) 68 (61.2-74.8) 75 (63.8-86.2)
Healthy controls	69 M 17 F 52	29.1 (25.9-32.2)	99.1 (98.2-100) 100 (100-100) 98.77 (98.6-99.9)	

M=Male subjects, F=Female subjects, CI=Confidence Interval, SD=Standard Deviation.

Table II. Test-retest reliability.

	Spearman's rho	ICC (95% CI)	Wilcoxon Z score (P)	SEM	MDC95	SEM%	MDC%
Q1	0.75	0.76 (0.57-0.88)	-0.04 (0.97)	1.8	3.8	28.5%	42.9%
Q2	0.56	0.64 (0.38-0.8)	-0.28 (0.78)	1.4	3.3	19.1%	75.5%
Q3	0.59	0.55 (0.25-0.75	-0.39 (0.7)	1.8	3.7	22.2%	66.9%
Q4	0.6	0.53 (0.23-0.73)	-1.9 (0.06)	2.1	4	35.9%	68.2%
Q5	0.8	0.79 (0.62-0.89)	-1.43 (0.15)	1.4	3.3	29%	68.8%
Q6	0.68	0.69 (0.46-0.83)	-0.77 (0.45)	1.7	3.6	31.2%	46.4%
Q7	0.69	0.7 (0.48-0,84)	-1.73 (0.084)	1.3	3.1	30.6%	44.3%
Q8	0.86	0.9 (0.81-0.95)	-1.12 (0.26)	2.8	4.7	26%	58.1%
Total	0.87	0.88 (0.77-0.94)	-0.85 (0.39)	5.4	6.4	10.1%	12.1%

ICC=Intraclass Corellation Coefficient. 95% CI =95% Confidence Interval.
SEM=Standard Error of Measurement. MDC =Minimal Detectable Change.
All ICC values had was statistically significant $p < 0.05$.

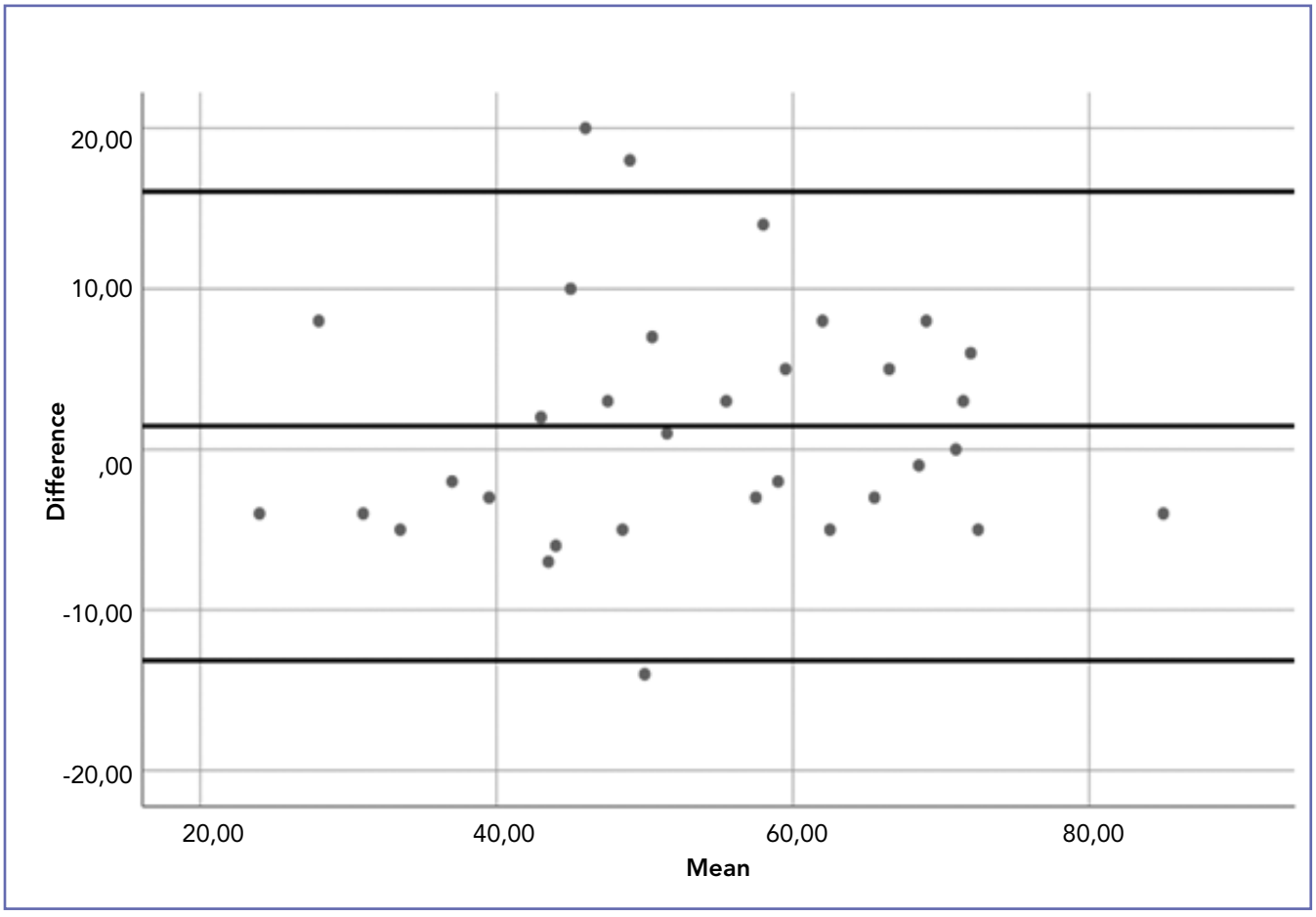

Figure 2. Bland & Altman plot from test-retest subgroup.
Mean: 1.5, 95% CI: -13.1-16.1.

The difference was tested with the Mann Whitney U Test and was statistically significant with a Z value of -11 ($p < 0.00001$).

Criterion validity

When comparing PT patients VISA-P-DK score (mean 54, SD 16.5) with the PT patients VISA-P score (mean 55, SD 12) from the original article (17) with a two sample t-Test, no significant difference was found ($p < 0.05$).

Convergent validity

When comparing the PT patient subgroup SF-36 scale scores with VISA-P-DK score Spearman's *rho* was: Physical Function (PF) 0.6 ($p < 0.05$), Role Function-physical (RF) 0.28 ($p > 0.05$) and Bodily Pain (BP) 0.5 ($p < 0.05$). Correlation based on the score is considered low < 0.3, moderate 0.3-0.6 and high < 0.6 (18).

Responsiveness

After 3 months of treatment a PT patient subgroup (n=33) completed the questionnaire once more. The subgroup showed at baseline a Mean VISA-P-DK score of 54,1 (95% CI 48,7-59,5) and a mean score of 70,6 (95% CI 64,6-76,5) after 3 months. This showed a significant rise in VISA-P-DK score following 3 months of treatment ($p < 0.05$).

Floor or ceiling effects

Floor and ceiling effects are present when 15% of the test population scores either the lowest or highest possible score (41). No subjects obtained a maximum or a minimum VISA-P-DK score at baseline. This also applied to the retest group. Only one patient in the 3-months follow-up subgroup obtained a maximum score after 3 months of treatment. In the control group 94% (65 subjects) obtained a maximum score. The maximum score found in the control group was expected, since they were chosen as healthy.

DISCUSSION

During pretesting of VISA-P-DK, problems understanding the inverted NRS score in question 2-6 resulted in a reversion of the scoring system regarding these 5 questions. The final VISA-P-DK questionnaire was intuitive, easily comprehended by our test subjects and accepted by the authors of the original article (17). The PT patient VISA-P-DK score was similar to the PT patient scores in the other versions of the VISA-P (**table III**) (18–27).

Test-retest reliability of the VISA-P-DK questionnaire was good (0.88 ICC) and consistent with ICC's of the other translations (**table III**). When looking at each question separately, poor reliability (ICC < 0.75) in at least 5 of the questions was found. In other translations of the VISA-P questionnaire, reliability of the individual questions was much higher. When comparing our version to the ones in the other VISA-P studies, the questionnaires remain consistent, but the setup were different. Therefore, a direct comparison cannot be made. The authors believe that a test-retest interval of 2-5 days and retesting only symptomatic patients is the best solution in reliability testing. Other studies have used shorter intervals (18, 25). This makes it easier for subjects to recall their previous response. Some studies only tested healthy subjects (19–22, 24), and some both symptomatic and healthy subjects (27). Healthy subjects are not expected to show any significant changes in VISA-P score as seen in symptomatic patients. In VISA-P-DK the MDC of each single question score was considered poor (MDC > 30%) (37). An MDC% of the total VISA-P-DK score of 12% indicates that the questionnaire is an acceptable measure of PT impact on patient life. When applying VISA-P-DK in the clinical setting the authors therefore suggest that only total VISA-P score is considered. Supporting this suggestion, internal consistency of 0.83 (Cronbachs *alpha*) indicated a good correlation between the questions. This shows that no question should be excluded, being in line with the other VISA-P translations (**table III**) (18–27).

When testing construct validity, a significant difference was shown between all three groups. The untreated PT group had the lowest, the 3-month follow-up the median and the control group the highest VISA-P-DK score. This indicates good construct validity. No significant difference was found when comparing PT-patients scores from VISA-P-DK to the scores from PT patients in the original VISA-P study. This indicated good criterion validity. The 3-month follow-up group had a significantly higher VISA-P-DK score after treatment. This shows that VISA-P-DK can detect a progress in PT severity, and responsiveness was considered good. Good responsiveness has been shown in only one other study (26).

The decision to use SF-36 to measure convergent validity, and not for example KOOS (42), was based on it being more widely used, as seen for the other adaptations of VISA-P. Only 3 of 8 subscales in SF-36 explores physical health. A guideline scoring and interpretation of each of the 8 scales individually was used, making the risk of bias small (43). The PF scale score and the BP scale score had a moderate and strong correlation to the VISA-P-DK score, indicating strong convergent validity. There was no significant correlation between the RF scale score and VISA-P-DK score. The explanation could be that the RF scale score had a high floor or ceiling effect with 50% scoring 0 or 100 points on the transformed scale score.

Table III. Comparison of VISA-P adaptations.

	PT patients VISA-P score	Healthy group VISA-P score	ICC	Cronbachs α
Italian version	44 (n=25)			0.78 (kappa)
Swedish version	48 ± 20 (n=17)	83 ± 13 (n=17)	0.97	0.83
French version	53 ± 17 (n=28)	99 ± 2 (n=22)	0.99	0.9
Danish version	54 ± 17 (n=86)	99 ± 4 (n=69)	0.88	0.83
Original-English version	55 ± 12 (n=14)	95 ± 8 (n=26)	0.99 (Pearsons)	
Spanish version	56 ±13 (n=40)	96 ± 2 (n=40)	0.99	0.83
Dutch version	58 ± 19 (n=20)	95 ± 9 (n=18)	0.74	0.73
Brazilian-Portuguese version	59 ± 18 (n=52)		0.91	0.76
Turkish version	59 ± 12 (n=34)	94 ± 9 (n=29)	0.96	0.79
German version	62 ± 13 (n=23)	95 ±6 (n=52)	0.88	0.88
Korean version	68 ± 16 (n=23)	93 ± 9 (n=5)	0.97	0.8

Mean score ± SD.
ICC=Intraclass Correlation Coefficient.

All in all, the translation, adaptation, validation and psychometric properties of this study show that VISA-P-DK is a useful and reliable tool when assessing severity and monitoring PT in Danish-speaking PT patients.
The development of the Danish version of the Patient Reported Outcome Measure VISA-P will in future be able to help Danish physicians when treating PT and when carrying out further research on PT. The validation of the Danish version of VISA-P will also allow for comparing VISA-P data from Danish populations with populations form other language groups where VISA-P is validated as a tool.

Future considerations

PROMs provide a more patient-centered assessment by not only assessing disease specific activity but also life impact of the disease. As a result, the role of PROM's in the measurement of health outcomes has become increasingly important. PROMs have developed from being generic (*e.g.* SF-36) to disease specific (*e.g.* VISA-P (17), VISA-A-DK (28)). PROMs are expected to play a role in patient-centered health care. Now VISA-P-DK can contribute as a disease specific questionnaire. For a better comparison to other versions, a more consistent setup is asked for when reliability testing.
To further develop VISA-P-DK a Rasch analysis would give an even better perspective of the use of this tool. This would require a larger PT patient group. A Rasch analysis would add a weighing of the questions and could therefore lead to an even more precise VISA-P-DK.

Perspective

VISA-P-DK is a valid and reliable tool for assessing PT and should preferably be completed by the patient alone and prior to discussing patient condition with the physician. VISA-P now exists in 11 different languages including Danish and is therefore useful in cross-cultural comparison of PT.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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APPENDIX 1. The VISA-P-DK questionnaire.

Navn:	
CPR:	
Dato:	

VISA-P-DK Spørgeskemaet: Vurdering af sværhedsgrad af 'springerknæ' (patellar tendinopati)

I DETTE SPØRGESKEMA HENVISER ORDET SMERTE SPECIFIKT TIL SMERTE I KNÆSENEREGIONEN

1. Hvor mange minutter kan du sidde uden at få smerter?

POINT

0 min | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 100 min
0 1 2 3 4 5 6 7 8 9 10

2. Får du smerter ved at gå almindeligt ned ad trapper?

POINT

Ingen smerte | | | | | | | | | | | | Stærk/svær smerte
10 9 8 7 6 5 4 3 2 1 0

3. Får du knæsmerter ved at strække knæet uden vægtbæring?

POINT

Ingen smerte | | | | | | | | | | | | Stærk/svær smerte
10 9 8 7 6 5 4 3 2 1 0

4. Får du smerter når du lægger vægten på forreste bøjede knæ i fremfald i gangstående stilling?

POINT

Ingen smerte ☐☐☐☐☐☐☐☐☐☐☐ Stærk/svær smerte
10 9 8 7 6 5 4 3 2 1 0

5. Får du problemer når du sidder på hug?

POINT

Ingen problemer ☐☐☐☐☐☐☐☐☐☐☐ Kan ikke
10 9 8 7 6 5 4 3 2 1 0

6. Får du smerter under eller umiddelbart efter udførelse af 10 hop på ét ben?

POINT

Ingen smerte ☐☐☐☐☐☐☐☐☐☐☐ Stærk/svær smerte/kan ikke
10 9 8 7 6 5 4 3 2 1 0

7. Deltager du i øjeblikket i sport eller anden fysisk aktivitet?

0 ☐ Overhovedet ikke

4 ☐ Moderat træning ± modificeret konkurrence

7 ☐ Fuld træning ± konkurrence, men ikke på samme niveau, som da symptomerne startede

POINT

10 ☐ Konkurrerer på samme eller højere niveau, end da symptomerne startede

8. Udfyld venligst A, B eller C i dette spørgsmål.

- Hvis du ikke har smerter under sport der belaster knæsenen, udfyld da venligst kun spørgsmål 8A.
- Hvis du har smerter under sport der belaster knæsenen, men det ikke stopper dig i at fuldføre aktiviteten, udfyld venligst kun 8B.
- Hvis du har smerter der hindrer dig i at fuldføre sport der belaster knæsenen, udfyld venligst kun 8C.

A. Hvis du ikke har smerter under sport der belaster knæsenen, i hvor lang tid kan du så træne/fortsætte?

0 min	0-10 min	11-20 min	21-30 min	> 30 min	POINT
0	7	14	21	30	

ELLER

B. Hvis du har nogen smerte under sport der belaster knæsenen, men det ikke hindrer dig i at færdiggøre aktiviteten, hvor lang tid kan du så træne/fortsætte?

0 min	0-10 min	11-20 min	21-30 min	> 30 min	POINT
0	4	10	14	20	

ELLER

C. Hvis du har smerter der hindrer dig i at færdiggøre sport der belaster knæsenen, hvor lang tid kan du så træne/fortsætte?

0 min	1-10 min	11-20 min	21-30 min	> 30 min	POINT
0	2	5	7	10	

TOTAL SCORE (/100) %

ORIGINAL ARTICLE

Nr 2020;10 (4):633-639

Female Cadaveric Study of Neurovascular Pedicle in *Latissimus Dorsi* Muscle Flap

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LEVEL OF EVIDENCE: 4

SUMMARY

Background. The goal of this investigation was to analyse the branching pattern of the arterial supply and innervation of *Latissimus Dorsi* (LD). The aim was also to determine the distance of origin of its neurovascular pedicle from the inferior scapular angle.

Methods. The present study included 9 adult female embalmed cadavers, which were available in the department of anatomy. Neurovascular pedicle of the *latissimus dorsi* was studied over the right and left sides (n=18). The long thoracic artery origin was also observed. The distance of the neurovascular pedicle from the scapular inferior angle was measured by using a digital Vernier calliper.

Results. It was observed that, in 44.4% cases, type 1a pattern of the thoracodorsal artery was observed. Type A pattern of thoracodorsal nerve was observed in 55.5% cases. It was observed that the thoracodorsal nerve was laterally related to the artery to LD in 94.5% cases. The distance of origin of neurovascular pedicle of LD from the inferior scapular angle was 60.8 ± 6.2 mm over the right side and the same measurement was 59.8 ± 6.4 mm over the left side. The comparison of this data didn't show statistical significance (Paired t Test, p=0.49).

Conclusions. It was observed that the arterial branching pattern of the LD muscle was extremely variable. The findings will assist the plastic surgery during the breast reconstruction procedure.

KEY WORDS

Breast reconstruction; latissimus dorsi; pedicled flap; surgical flap; breast tumors; cosmetic surgery.

BACKGROUND

Latissimus Dorsi (LD) is a back muscle, which is sometimes known as "climbers muscle". The LD is used in flap surgeries by the plastic surgeons particularly during the breast reconstruction. The LD flap is preferred in mammary gland reconstruction because of its lesser perioperative and long term postoperative morbidity (1). LD is innervated by thoraco dorsal nerve and its arterial supply is by the thoraco dorsal artery. In the posterior wall of axilla, the subscapular artery is inferiorly related to the LD and gives a branch, the circumflex scapular artery. After this branch, the thoracodorsal artery is the continuation of subscapular artery. The long thoracic artery supplies the serratus anterior muscle, which is a branch of thoracodorsal artery (2). Thoracodorsal artery provides two branches inside the LD, descending and transverse branches (3). LD also gets nourishment by the lumbar and posterior intercostal arteries. Thoracodorsal artery has a wider diameter and with least anatomical variations, becomes a highly reliable source (3, 4).

A pedicle of size, 50-100 millimetres can be safely harvested from the subscapular system. Since the arteries branches inside the *latissimus dorsi* muscle, the muscle can be cut longitudinally and bilobed flap can be harvested. These pedicled flaps could be used to cover the posterior defects (2). LD is also used for the reconstruction of face, scalp and cranium defects, pedicle transplant rotator cuff repairs, tendon graft surgeries and reconstruction flap for the extremities (2). LD flap is used to cover the extremely larger and deeper wounds. Because of the larger size of the LD, there are no postharvest motor deficits (2). The anatomical reports about the variations in the arterial supply branching pattern and innervation are scarce in the scientific literature. This was the stimulus to perform the present study in the female cadaveric specimens of south Indian origin. The goal

of the present investigation was to analyse the branching pattern of the arterial tree and innervation of LD muscle. The morphology of various branching patterns was noted and analysed. The aim was also to determine the distance of origin of its neurovascular pedicle from the inferior scapular angle.

MATERIALS AND METHODS

The present study included 9 adult female embalmed cadavers, which were available in the department of anatomy. The neurovascular pedicle of LD was studied over the right and left sides (n=18). The sample size was calculated after consultation with the statistician. This study has been approved by the ethics committee of our institution. Male cadavers were not included because this study was aimed to help the breast construction procedures. This was a posterior exposure just like the surgical approach and the muscular flap was elevated from the origin of LD. This is not routinely done in regular anatomical dissections, where we perform it from the anterior approach.

The incision was put at T3 level both horizontally and vertically. After reflecting the superficial and deep fascia, the LD muscle was defined. The part of LD below the scapular inferior angle was released. The attachments of LD at the spine in the back was also released. The dissection was continued below the LD toward the axillary region. The arteries to LD and serratus anterior became obvious as we approached towards the axilla. Once the flap was raised from proximal to distal, the neurovascular pedicle was dissected near the posterior wall of axilla. The distance of the pedicle near the lateral border of scapula from the scapular inferior angle was noted as this may give an idea to the operating surgeon where exactly he or she can locate the pedicle. This measurement was performed by using a digital Vernier calliper. The upper extremity of the cadavers was abducted to 90º position with respect to the thorax, both during the dissection procedure and while performing the measurement.

The branching pattern of the nerve to LD muscle (thoracodorsal nerve) and arterial tree of LD were analysed. The origin of artery to serratus anterior (long thoracic artery) was also studied. The observations were analysed and tabulated. The branching pattern of the artery to LD, was classified based on its branching pattern into types 1a, 1b, 2a, 2b, 2c, 3a, 3b and 3c, which are as below (**figure 1**):

Type 1a: artery to LD (4 in the picture) was branching from SCA and later divided into two branches (4a and 4b in the pictures).

Type 1b: artery to LD (4 in the picture) was branching from SCA and later divided into three branches (4a, 4b and 4c in the pictures).

Type 2a: artery to LD (4 in the picture) was branching from SCA and later divided into two branches (4a and 4b in the pictures). These two branches again branched into two more branches each (4a and 4b again branched into 4a1, 4a2, 4b1 and 4b2 branches).

Type 2b: artery to LD (4 in the picture) was branching from SCA and later divided into two branches (4a and 4b in the pictures). The first branch 4a, again branched into 4a1 and 4a2 branches. The second branch 4b, there was no further branching.

Type 2c: artery to LD (4 in the picture) was branching from SCA and later divided into two branches (4a and 4b in the pictures). The second branch 4b, again branched into 4b1 and 4b2 branches. The first branch 4a, there was no further branching.

Type 3a: there was no single trunk (4), instead there were two arteries to LD directly arising from the SCA (4a and 4b).

Type 3b: there was no single trunk (4), instead there were three arteries to LD directly arising from the SCA (4a, 4b and 4c).

Type 3c: there was no single trunk (4), instead there were two arteries to LD directly arising from the SCA (4a and 4b). These two branches again branched into two more branches each (4a and 4b again branched into 4a1, 4a2, 4b1 and 4b2 branches).

The branching pattern of the thoracodorsal nerve was classified based on its level of branching a type A, type B and type C, which are as below (**figure 1**):

Type A: thoracodorsal nerve dividing at the level of origin of circumflex scapular artery.

Type B: thoracodorsal nerve dividing above the level of origin of circumflex scapular artery.

Type C: thoracodorsal nerve dividing below the level of origin of circumflex scapular artery.

The dimension measured was analyzed statistically by using "EZR software, version 1.38, 2019". The statistical analysis of right and left side dimensions were done by using the paired t Test. The comparison was considered as significant statistically only if the p value is smaller than 0.05.

We state that the present anatomical investigation was performed as per the international ethical standards, which are required by the international scientific indexed journal as per the opinion of Padulo *et al.* (5).

RESULTS

In all the cadavers, artery to LD was branching from the subscapular artery. But the branching was extremely variable after its origin from the subscapular artery. The various morphological branching patterns of the vascular pedicles are represented along with their frequency in **figures 2-8**.

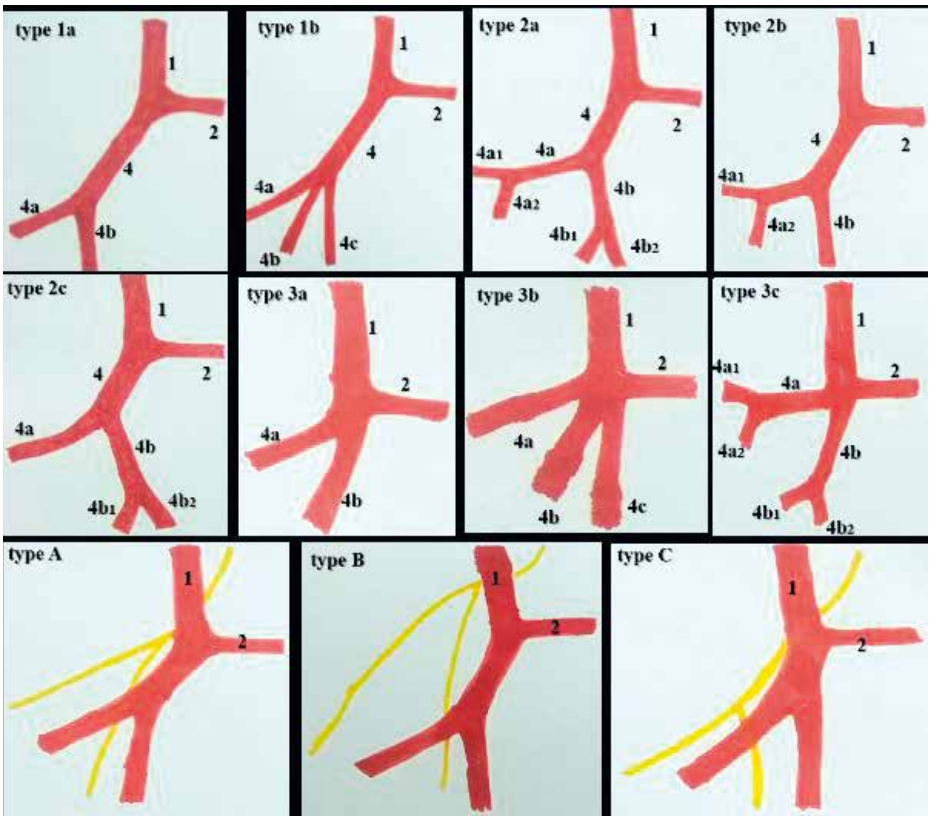

Figure 1. Schematic diagrams representing the various branching pattern of the artery to LD (type 1a, type 1b, type 2a, type 2b, type 2c, type 3a, type 3b and type 3c) and thoracodorsal nerve (type A, type B and type C), which are observed in this study.

Their frequency over the right and left sides are represented in **table I**. It was observed that, in 44.4% cases, type 1a (**figure 2**) pattern was observed, this was the most common pattern. In this pattern, artery to LD was branching from the SCA and later divided into two branches. It was also found that the branching pattern is not the same over the right and left side (77.8% cases) of the same person. The branching pattern was same bilaterally in only 22.2% cases. It was observed that in 17 cases (94.4%), the artery to serratus anterior (long thoracic artery) was branching from the circumflex scapular artery. However, in 1 case (5.6%), the long thoracic artery was branching from the artery to LD.

Table II represents the frequency of various morphological types of branching pattern of the thoracodorsal nerve. The type A variety is seen in **figure 4**, type B in **figure 8** and type C in **figure 6**, respectively. The branching pattern of thoracodorsal nerve was similar in 5 cadavers (55.5% cases) and it was different in 44.5% cases. It was observed that the thoracodorsal nerve was laterally related to the artery

Table I. Various morphological patterns of vascular pedicles of LD and their frequency over right (n=9) and left (n=9) sides.

Branching pattern	Right side	Left side
Type 1a	4 (22.2%)	4 (22.2%)
Type 1b	2 (11.2%)	1 (5.5%)
Type 2a	NUL (0%)	NUL (0%)
Type 2b	1 (5.5%)	1 (5.5%)
Type 2c	NUL (0%)	1 (5.5%)
Type 3a	1 (5.5%)	NUL (0%)
Type 3b	NUL (0%)	2 (11.2%)
Type 3c	1 (5.5%)	NUL (0%)

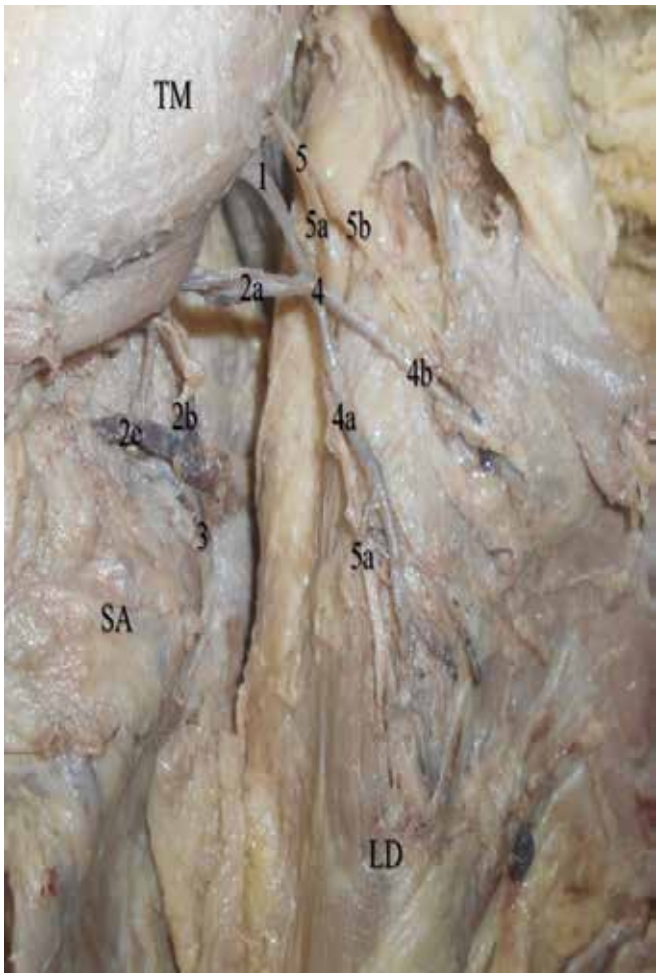

Figure 2. *Latissimus dorsi* muscle having type 1a branching pattern of the vascular pedicle (8 cases, 44.4%).

to LD (94.5% cases), except in only one side (5.5%), this was found on the medial side of the artery. The distance of the origin of neurovascular pedicle of the LD from the inferior angle of scapula was 60.8 ± 6.2 mm over the right side and the same measurement was 59.8 ± 6.4 mm over the left side. The measurements of all the cadavers over the right and left sides with respect to the distance of pedicle is given in **table III**. The comparison of this data didn't show statistical significance (paired t Test, p=0.49).

DISCUSSION

The prevalence rate of carcinoma of breast has increased in the recent years. It is fortunate that there are good treatment modalities available like surgery, chemotherapy and radiotherapy. Radical mastectomy is the best surgical proce-

Table II. Morphological patterns of the thoracodorsal nerve and their frequency (n=18).

Morphological pattern	Frequency
Type A	10 (55.5%)
Type B	5 (27.8%)
Type C	3 (16.7%)

Table III. Distance of the neurovascular pedicle from the inferior angle of scapula (n=18).

Cadaver number	Right side	Left side
1	61 mm	61 mm
2	56 mm	56 mm
3	57 mm	51 mm
4	72 mm	66 mm
5	65 mm	65 mm
6	63 mm	61 mm
7	50 mm	55 mm
8	66 mm	71 mm
9	57 mm	52 mm

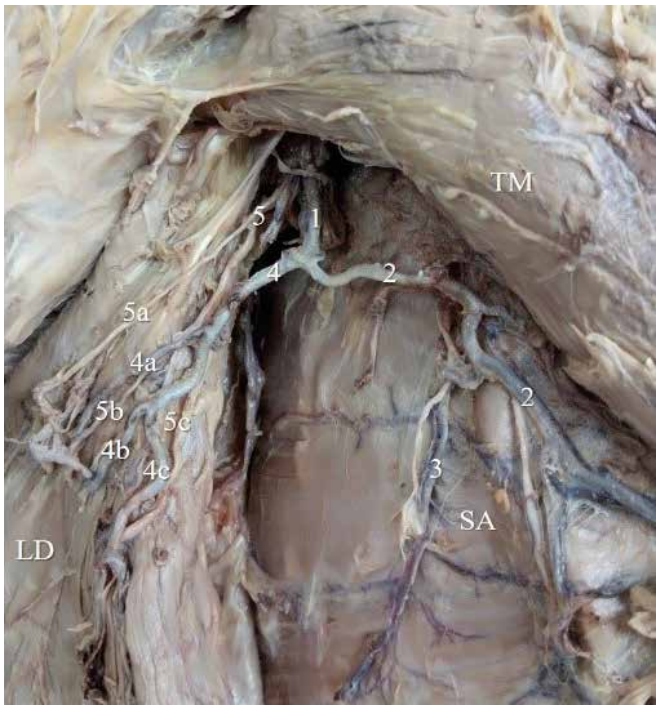

Figure 3. *Latissimus dorsi* muscle having type 1b branching pattern of the vascular pedicle (3 cases, 16.7%).

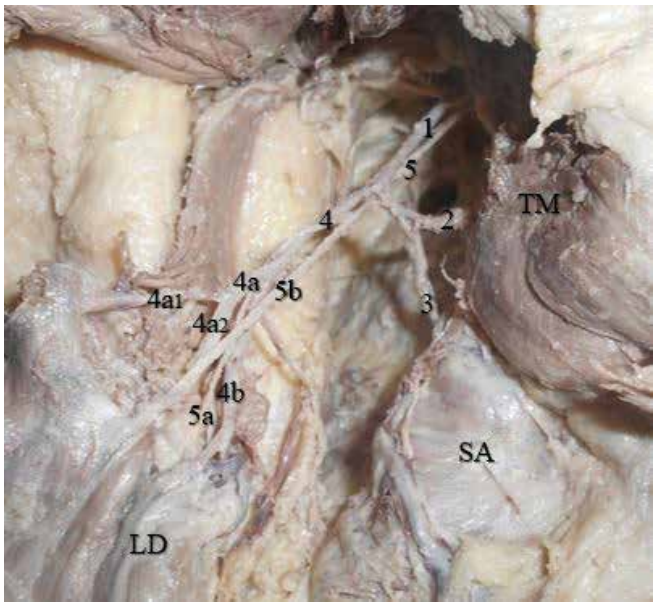

Figure 4. *Latissimus dorsi* muscle having type 2b branching pattern of the vascular pedicle (2 cases, 11.2%). The thoracodorsal nerve branching pattern in this picture is type A (n=10, 55.5% cases).

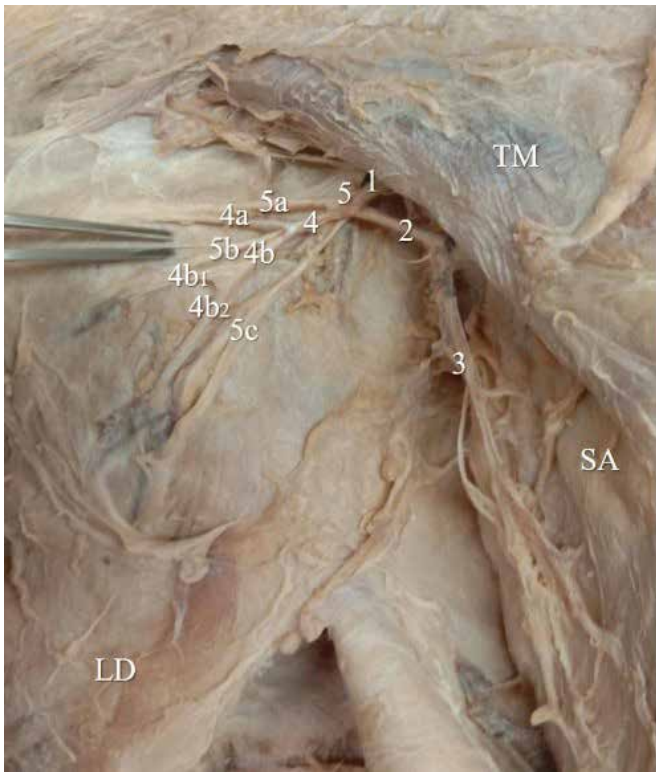

Figure 5. *Latissimus dorsi* muscle having type 2c branching pattern of the vascular pedicle (1 case, 5.5%).

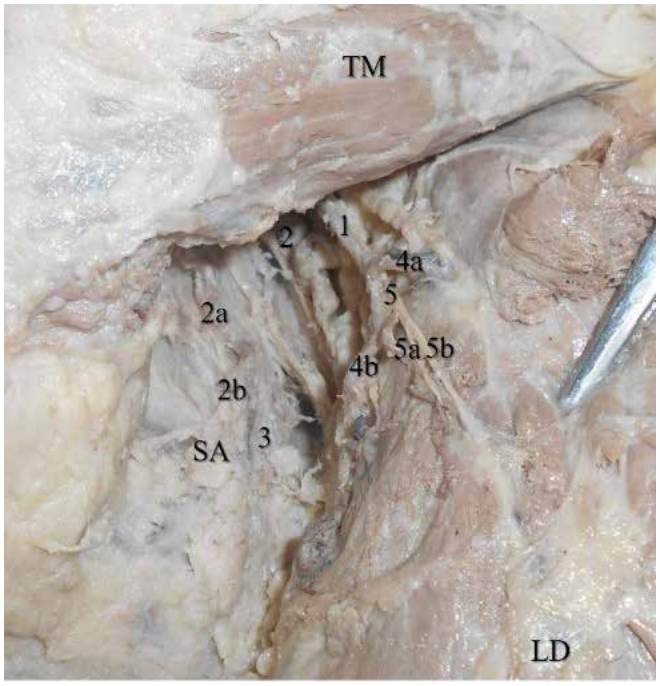

Figure 6. *Latissimus dorsi* muscle having type 3a branching pattern of the vascular pedicle (1 case, 5.5%). The thoracodorsal nerve branching pattern in this picture is type C (n=3, 16.7% cases).

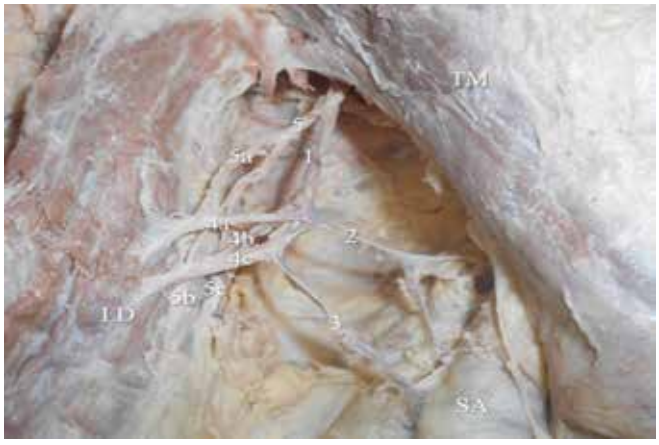

Figure 7. *Latissimus dorsi* muscle having type 3b branching pattern of the vascular pedicle (2 cases, 11.2%).

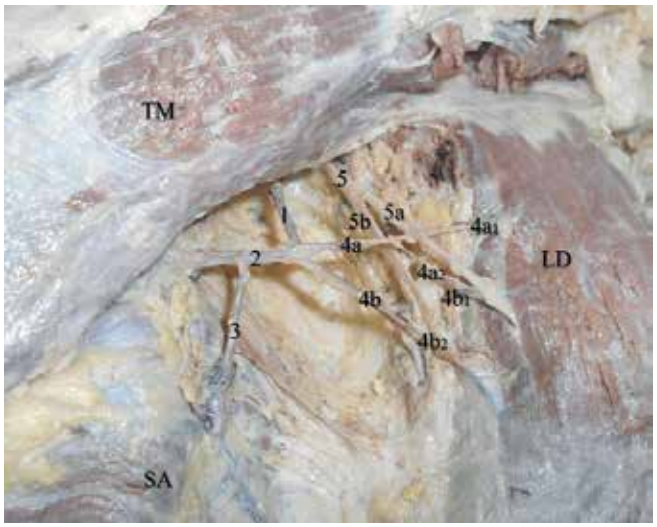

Figure 8. *Latissimus dorsi* muscle having type 3c (n=1, 5.5% cases) branching pattern of the vascular pedicle. The thoracodorsal nerve branching pattern in this case is type B (n=5, 27.8% cases).

dure, which is followed by the breast reconstruction. This is performed by the plastic surgeon for the cosmetic purpose. The graft can be harvested from the same patient during the mastectomy procedure. The LD muscle flap technique was described in 1906 by Iginio Tansini, but it became popular only in 1970s for the breast reconstruction (6). William Halsted included LD flap during the radical mastectomy (3) and Schneider *et al.* (7) reported the use of LDF in implant-based mammary gland reconstruction. LD flap maintained the shape and function of the silicone implant by forming the muscle coverage. Bostwick *et al.* (8) made a skin island over the LD to replace the skin loss during the post mastectomy breast reconstruction. Papp and McCraw (9) found a LDF which is de-epithelialized and it provided the volume replacement. The muscle flap with its nerve intact can be used for the reconstruction of the paralysed face and in Poland syndrome. With the nerve inside the flap, the muscle function is maintained (10). LD with its nerve and artery can be used as a flap to reconstruct the paralysed deltoid and triceps muscles (11). The osteo-musculo-cutaneous flaps with the LD can be used to reconstruct the mandible (12).

LD tendon transfer is among the best treatment options for the massive posterior-superior rotator cuff tears, especially in the younger patients (13). The LD is best suited for the transfer procedures, because of its strength, vascularization, and larger surface area. These things are essential to the successful healing (14). In the arthroscopic surgeries performed with the lateral decubitus position for the rotator cuff tears, the traction is given to the LD muscle. This requires the topographical location of the thoracodorsal nerve and knowledge about its variability. It was described that the LD pedicle with supply by the long thoracic artery and nerve can be mobilized without tension up to 80 mm (14).

During the surgical mobilization of the LD muscle, all the perforating arteries from the posterior intercostal and lumbar will be divided and the only arterial supply from the thoracodorsal artery remains (15). Hence it was essential to study the branching pattern of thoracodorsal artery inside the LD. The LD is supplied by a single motor nerve, thoracodorsal nerve which divides after entering the LD (16). The nerve passes posterior to the axillary artery, joins the vascular pedicle, and enters the LD along with the artery. The artery and nerve to LD bifurcate after their entry into the LD muscle. It was reported that, based on this branching pattern, one can use one or both units of the muscle for the transfer as required by the individual case (17, 18).

In the literature, there are few clinical reports available, there are not enough anatomical studies which explain the neurovascular distribution of the LD. Anatomical reports are very much essential as they serve as the basic in the medical research. The present study has examined the detailed distribution of thoracodorsal nerve and branching of the thoracodorsal artery in female cadavers. This type of study is not reported earlier from the female cadavers of the south Indian population. The data of the present study were compared with the few available previous reports. Bartlett *et al* (17) observed the subscapular-thoracodorsal artery vascular pedicles of 11 cm mean length. The long thoracic artery branches from the thoracodorsal artery were observed previously by Bartlett *et al.* (17). In the present study, this pattern of origin of long thoracic artery was observed only in 5.6% cases. It was observed that, in 94.4% of our cases the long thoracic artery was branching from the circumflex scapular artery. Bartlett *et al.* (17) reported that the neurovascular structures in the *latissimus dorsi* were bifurcated into superior and lateral intramuscular bundles in 86% of their specimens. This pattern is labelled as type 1a in this study and it was observed in only 44.4% cases. Kwon *et al.* (10) opined that the thoracodorsal nerve always branched before the thoracodorsal artery, and the distance from the first branch of the nerve to the first branch of the artery was 20.4 ± 8.9 mm. In the present study, the thoracodorsal nerve branching before the artery was labelled as type B and observed in 27.8% cases.

It is described that the surgeon should have adequate knowledge about the arterial tree and divisions of thoracodorsal nerve of the LD muscle. This LD muscle is commonly used in the pedicle grafts in the plastic surgical practice

following the mastectomy. It was observed that the arterial branching pattern of the LD muscle was extremely variable. The finding of the present study is useful to the reconstructive surgery, since the distance of origin of neurovascular pedicle of LD from the inferior scapular angle was constant. There was no statistically significant difference between the right *versus* left sides, in all the female embalmed cadavers. So, the data of the present study can be used as anatomical guide for the surgical dissection with respect to the distance of the neurovascular pedicle from the inferior angle of scapula.

However, this study has certain limitations like the sample size, which was very small. We could be able to dissect only 18 muscles, from the 9 female cadavers. The study can be more accurate with a large sample size of female cadavers. This study also focused on evidence gathered using cadavers from Indian region, the results may be influenced by the ethnic predispositions in the Indian sample. Further studies, based on larger and ethnically diverse samples, may determine whether outcomes vary for different ethnic groups. The high variability in the pattern of the vascular tree between the two sides of the same subject has modest clinical relevance. The surgeon, in fact, operates just one out of the two sides. But the remarkable variability of the vascular and nervous pattern, on the other hand, has practical relevance to the surgeon. The upper extremities were positioned at 90º abduction and this can affect the distance of the lower angle of the scapula to the pedicle. But the patients can be positioned with 90º abduction of arm in the surgical setting as well.

CONCLUSIONS

The present study has provided information about the various branching pattern of the arterial supply and innervation of the LD muscle. This study was performed exclusively by using the female cadavers from the sample south Indian population. The data of the present study may help the operating surgeons while harvesting the neurovascular pedicle flaps during the breast reconstruction procedure.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

Nr 2020;10 (4):640-644

Exploring Correlation between Extent of Tumour Necrosis on MRI And Histological Grade of Soft Tissue Sarcoma – An Initial Experience

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SUMMARY

Objective. Soft Tissue Sarcoma (STS) is a rare form of cancer which includes a variety of malignant connective tissue neoplasms with high morbidity and mortality. Amongst these, undifferentiated pleomorphic sarcoma – accounting for up to 20% of all sarcoma – lacks a specifically identified line of cellular differentiation. Magnetic Resonance imaging (MR) have been used for local staging of STS, determining tumour extent, tumour characteristics, and neurovascular bundle infiltration. In addition to histologic evaluation following percutaneous biopsy, MR can possibly be used as a non-invasive investigation to determine tumour grade as it has been successfully used as a surrogate marker for other malignant tumours, for example: brain tumours. We evaluated similar MRI utility for undifferentiated pleomorphic sarcoma to assess necrosis as a possible predictor of histological grading.

Methods. We evaluated 47 cases of histologically proven undifferentiated pleomorphic sarcoma. We hypothesized that there was a direct correlation between the degree of tumour necrosis on MR and histologic grade. Two experienced consultant musculoskeletal radiologists blinded to histological grade of tumour evaluated MR images retrospectively. The extent of tumour necrosis was categorised as < 25% – MR grade I, 26-50% – MR grade II, 51-75% – MR grade III and > 76% – MR grade IV. Final pathologic grading using Fédération Nationale des Centres de Lutte le Cancer (FNCLCC) classification were obtained for all patients which divide tumour grades into low (grade I) and high (grade II and III) category and correlated with MRI results.

Results. 17 (36.1%) patients with MR Necrosis grade I, 8 (17.02%) patients with MR necrosis grade II and 10 (21.27%) patients with MR necrosis grade III, were high-grade STS (grade III significantly higher than grade II) histologically. Whereas, out of 12 (25.53%) patients with MR necrosis grade IV demonstrating more than 75% tumour necrosis, 1 (2.12%) patient was low-grade (FNCLCC grade I) and rest were high-grade STS including 1 (2.12%) patient of grade II and 10 (21.27%) patients of grade III.

Conclusions. We were unable to establish positive or negative correlation between the degree of necrosis on MR and histological grade of STS on this cohort study.

KEY WORDS

Soft tissue sarcoma; necrosis; grade; undifferentiated pleomorphic sarcoma; histological grade; tumour necrosis; MRI grading.

INTRODUCTION

Soft Tissue Sarcoma (STS) is a rare form of cancer which includes a variety of malignant connective tissue neoplasms with high morbidity and mortality. In the UK, in 2010, 3272, new cases of STS were diagnosed. Once diagnosed with these inherently aggressive neoplasms, the survival rate in STS patients steadily declines with time showing 10 years age-standardised relative survival rate for STS is 45% in the UK (1, 2).

Soft tissue sarcoma are further classified into numerous categories depending upon predominant tissue. These include leiomyosarcoma, liposarcoma, fibroblastic sarcoma including undifferentiated pleomorphic sarcoma (previously known as Malignant Fibrous Histiocytoma (MFH)), rhabdomyosarcoma, soft tissue Ewing's sarcoma, synovial sarcoma, vascular sarcoma, malignant peripheral nerve sheath tumours and other rarer forms of sarcoma which are not otherwise specified (sarcoma NOS) (1).

MRI is usually the imaging investigation of choice for sarcoma, determining tumour extent, tumour characteristics (**figure 1**) and neurovascular bundle infiltration, characterising lesions and prognosticating them. Tumour necrosis has been successfully used as a surrogate marker for different malignant tumours, for example, brain tumours. Our effort was to evaluate similar MRI utility for undifferentiated pleomorphic sarcoma to assess necrosis as a possible predictor of pathological grading.

MATERIALS AND METHODS

After institutional board review approval, we created a retrospective search of prospectively maintained radiology database at our institution and found 47 cases of histologically proven undifferentiated pleomorphic sarcoma (3). We hypothesised that there was a correct direct relation between the degree of tumour necrosis on MR and histologic grade. Two experienced consultant musculoskeletal radiologists who were blinded to histological grade of tumour evaluated MR images retrospectively. The extent of tumour necrosis was categorised as < 25% – MR grade I, 26-50% – MR grade II, 51-75% – MR grade III and > 75% – MR grade IV. Final pathologic grading using Fédération Nationale des Centres de Lutte le Cancer (FNCLCC) classification (4) modified initially from Trojani classification, were obtained for all patients which divide tumour grades into low (grade I) and high (grade II and III) category.

RESULTS

We have compiled a dataset for 47 patients comparing necrosis on MR imaging with histological grading (**table I**). Amongst 17 (36.1%) patients with MR Necrosis grade I, 8 (17.02%) patients with MR necrosis grade II and 10 (21.27%) patients with MR necrosis grade III, all turned out to be high-grade STS (grade III significantly higher than grade II) on histologic grading. Whereas, in 12

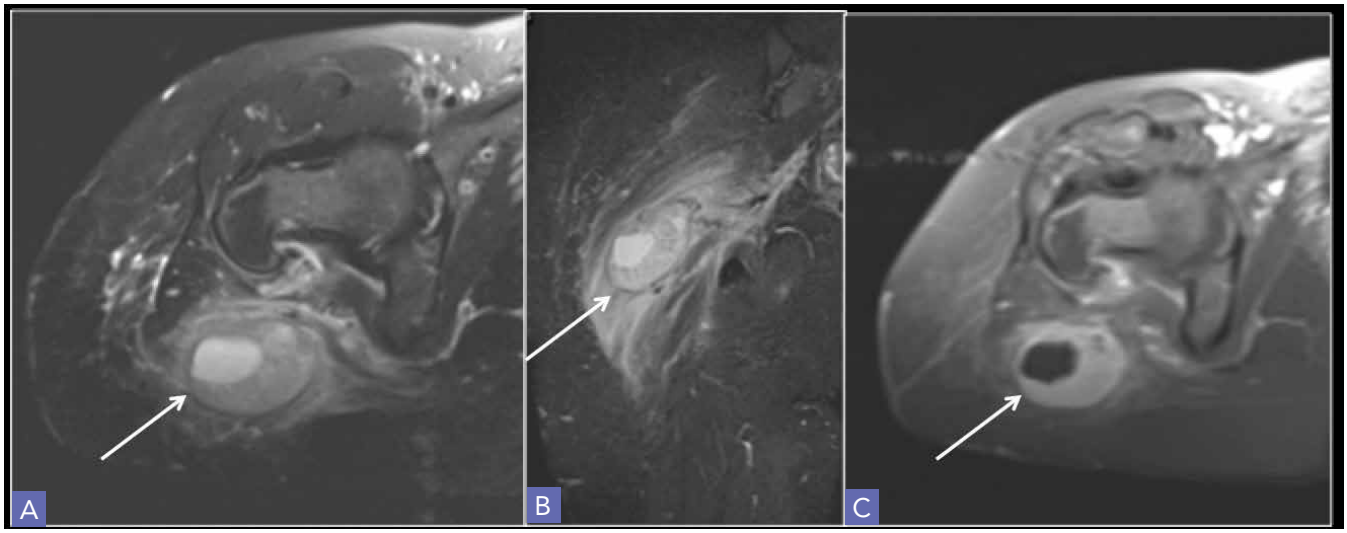

Figure 1. STIR axial (a) and coronal (b) and T1 fat suppressed post contrast axial (c) images of the right hip demonstrates necrotic tumour with MR necrosis grade 1 which was histologically grade III.

Table I. Demonstrates the grade of tumour and degree of necrosis. (# Tumour necrosis on MR imaging evaluated by two blinded musculoskeletal radiologists, percentage of necrosis is calculated against whole-tumour volume in a semi-quantitative manner; * Histologic grading using Fédération Nationale des Centres de Lutte le Cancer (FNCLCC) classification).

MR necrosis grade# ↓ / Histologic grade* →	Grade I (Low-grade)	Grade II (High-grade)	Grade III (High-grade)	Total
MR grade I (Necrosis <25%)	0 (0.00%)	3 (6.38%)	14 (29.78%)	17 (36.1%)
MR grade II (Necrosis 26-50%)	0 (0.00%)	2 (4.25%)	6 (12.76%)	8 (17.02%)
MR grade III (Necrosis 51-75%)	0 (0.00%)	3 (6.38%)	7 (14.89%)	10 (21.27%)
MR grade IV (Necrosis >75%)	1 (2.12%)	1 (2.12%)	10 (21.27%)	12 (25.53%)
Total	1 (2.12%)	9 (19.15%)	37 (78.72%)	47 (100%)

(25.53%) patients with MR necrosis grade IV demonstrating more than 75% tumour necrosis, 1 (2.12%) patient was low-grade (FNCLCC grade I) and rest were high-grade STS including 1 (2.12%) patient of grade II and 10 (21.27%) patients of grade III (**figure 1-3**). We concluded that percentage of MR necrosis does not follow histological grading and in turn aggressiveness of the soft tissue sarcoma and we were unable to find correlation between percentage of MR tumour necrosis and histological grading, whether positive or negative.

DISCUSSION

STS tumours are inherently heterogeneous consisting of multiple tissue components that include cellular compartments, fat, cystic changes, and necrosis. MRI is an imaging investigation of choice for prognostication of STS tumours whereas histological tumour grading remains the most widely accepted prognostic biomarker, as it serves as the proxy marker of the relative risk of tumour metastasis and survival. Additionally, the histological grade of the tumour is used as a key factor to decide whether the

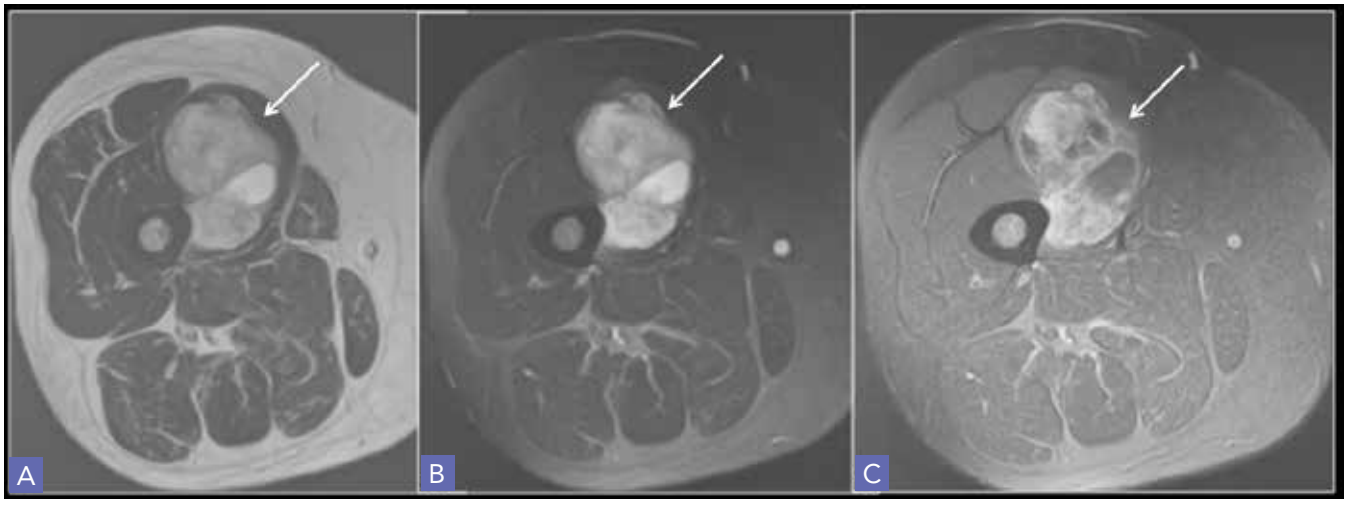

Figure 2. (a) T2, (b) STIR and (c) post-contrast T1 fat-suppressed axial images demonstrating irregular medial thigh compartment intramuscular neoplastic lesion showing less than 25% of the tumour necrosis in keeping with MR necrosis grade I. Histologically, the lesion was grade III demonstrating no direct positive correlation between MR necrosis and histological grading.

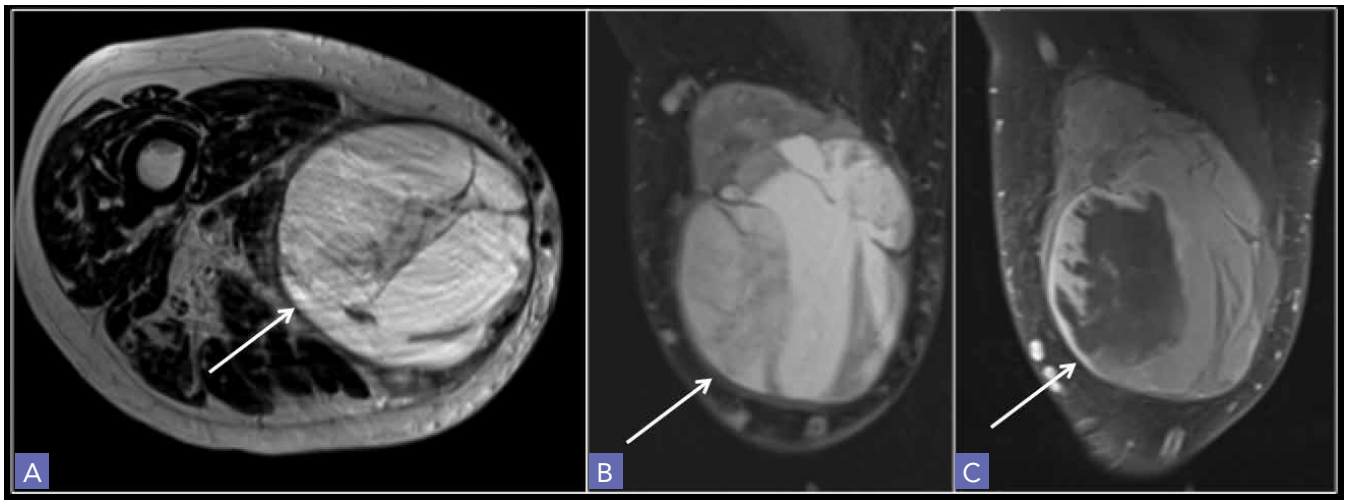

Figure 3. (a) T2 axial, (b) STIR coronal and (c) post contrast T1 fat-suppressed coronal images demonstrating irregular medial thigh compartment intramuscular neoplastic lesion showing approximately 75% of the tumour necrosis in keeping with MR necrosis grade III. Histologically, the lesion was grade III.

patient will need neoadjuvant chemotherapy in addition to surgery (5, 6).

Amongst these, undifferentiated pleomorphic sarcoma – accounting for up to 20% of all sarcoma – is a particular category of STS, which lacks a specifically identified line of cellular differentiation when analysed by histological examination and immunohistochemical staining. Analysis of 70 cases of undifferentiated pleomorphic sarcoma showed highly complex with no specific recurrent aberration, making them impossible to categorise into more known sarcoma even at a genetic level (7). Similarly, it is not possible to characterise undifferentiated pleomorphic sarcoma solely on imaging as they do not demonstrate pathognomonic imaging characteristics.

Magnetic Resonance (MR) imaging, owing to its excellent contrast resolution, is the cornerstone imaging method for local staging (8). Several papers are looking at various MR imaging features and histological grading of the STS, but none is specifically looking at the extent of necrosis (9). According to Zhao *et al.*, high-grade STS differs significantly from low-grade STS in terms of tumour size, tumour margin, heterogeneous signal intensity on T2-weighted images, and peritumoural high signal intensity. Presence of peritumoural contrast enhancement may act as a sole predictor of high-grade STS. Overall, in addition to histologic evaluation following percutaneous biopsy, MRI can be used as a supportive non-invasive investigation to determine tumour grade (10).

For brain tumours, tumour necrosis on MRI is poor prognostic factor and demonstrates direct positive correlation with histological grading and aggressiveness of the tumour. We used to exploit possibility of similar correlation for undifferentiated pleomorphic sarcoma. Contrary to the brain tumours, STS with less than 25% tumour necrosis could be high-grade histologically (**figure 2**) whereas it is also possible for STS with more than 75% tumour necrosis to be histologically low-grade or high-grade (**figure 3**). Hence, we were unable to establish correlation between MRI and histology, whether positive or negative.

We have searched multiple databases for similar studies and our study is, by far, the largest study evaluating correlation between MR necrosis and histological grading for proven cases of undifferentiated pleomorphic sarcoma. Possible limitations of this study may include small sample size, single centre experience and including only undifferentiated pleomorphic sarcoma. It is also possible that soft tissue sarcomas demonstrate completely different cellular lineage than brain tumours which may alter its growth characteristics including neoangiogenesis and their effect on tumour necrosis. However, we recommend multicentric study with a large sample size and involvement of more histological subtypes of soft tissue sarcomas to provide more robust statistical analysis.

CONCLUSIONS

In conclusion, we could not establish positive or negative correlation between the degree of necrosis on MR and histological grade of STS. Limitations of study were single centre pilot study, inclusion of only undifferentiated pleomorphic

sarcoma and small sample size. We recommend further multicentric study with a larger sample size and other variety of soft tissue sarcoma.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Hamstring Structural Injury in Futsal Players: The Effect of Active Range of Motion (AROM) Deficit on Rehabilitation Period

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SUMMARY

Background. Hamstring muscle injuries are among the most common traumas occurring in athletes and football players. Thus, the rehabilitation time is crucial for their return to full athlete activity.

Objective. Knee active range of motion deficit after the hamstring injury in futsal players correlates with rehabilitation time. The objective is to examine cases of hamstring structural injury in futsal players and find association between the Active Range of Motion (AROM) deficit and rehabilitation time.

Methods. For this study, 200 futsal players with acute, first-time, unilateral posterior hamstring injuries were recruited, all men. All patients underwent clinical examination and ultrasonography. In 74 of 200 patients, sonography revealed no abnormalities in the musculoligamentary structures. Thereby, they were excluded from further investigation. Only 126 futsal players underwent the active range of motion testing. Injured areas were compared with the normal hamstrings in all athletes and with the control group. The AROM deficit was evaluated. The association between time to full recovery and the AROM deficit was identified. A control group (100 men) underwent examination in the same series of steps as the study group.

Results. Biceps femoris was the most commonly injured muscle, making up 80% of injuries. The musculotendinous junction, proximal and distal, was involved in 91% of injury cases. Twelve athletes had an AROM deficit of 26 to 35 degrees. For them, the average length of the recovery period was 2.5 months at its minimum. Seventy or 55.5% of athletes had an AROM deficit less than 15 degrees. These athletes felt fully recovered after 3-4 weeks of rehabilitation.

Conclusions: The active range of knee movement deficiency is an indicator that allows an objective prediction of rehabilitation time in futsal players.

KEY WORDS

Hamstrings; futsal players; knee active range of motion; ultrasound; classification; injury classification.

INTRODUCTION

Active sports, in particular football, are characterized by an increased traumatic risk. The most common type of sustained injuries occurring in sports are the muscle injuries, their frequency varying from 25 to 50% of all cases (1-5).

Almost all muscle injuries (95% according to Hallen and Ekstrand (4)) can be divided in four groups: hamstring injury, adductor injury, quadriceps injury, and the calf muscle injury. Among the above groups, hamstring injuries are the most common type, presenting a third of all cases. Overall, the number of hamstring injuries varies depending on the

competition. In football, for example, their frequency is 52 per 1000 players per year (1, 2, 6).

The choice of treatment and rehabilitation program for muscle injuries depends on the severity of the injury. Studies classify muscle injuries differently depending on its location, size, and cause. Traditional muscle injury classification systems were devised by O'Donoghue (7), Ryan (8), and Askling (from van der Made *et al.* (9)). As is the case with other muscle injuries, hamstring strains are classified depending on the degree of damage to the muscular-tendon junction.

Ultrasound and Magnetic Resonance Imaging (MRI) can provide detailed information about the nature and extent of damage to the hamstring muscles (10, 11). To evaluate the hamstring muscle damage based on imaging findings, the Peetrons classification (12) for ultrasound and its modification for MRI are used (13). In order to provide clear terminology and classification of muscle injuries in sport, Mueller-Wohlfahrt *et al.* (14) published an important consensus statement. Their classification divides indirect muscle disorder/injury into functional muscle disorder (type 1 – overexertion-related muscle disorder, type 2 – neuromuscular muscle disorder) and structural muscle injury (type 3 – partial muscle tear, type 4 – subtotal or total tear). This classification system concerns symptoms, clinical signs, and location of damage as well as ultrasound and MRI data. Maffulli *et al.* (15) offered a similar grading system. Sant'Anna *et al.* (16) provide a review of other classification systems. The above grading frameworks failed to associate various characteristics of muscle injury with the rehabilitation time. The forecast of complete recovery from muscle injuries and return to play has significant financial and strategic implications. For this reason, it is important to optimize the diagnostic, therapeutic and rehabilitation processes. Approximately 30% of professional football players experience a re-injury within a year after the treatment (17). A premature return to full activity imposes a high risk of reoccurring injury. A solution to avoid this implication involves establishing a reliable set of injury severity assessment criteria. The size and location of the injury on MRI or ultrasound may be indicative of the time required to complete recovery (18). Clinical examination such as AROM deficiency assessment can be as useful as visualizing the rehabilitation time. Malliaropoulos *et al.* (19) investigated the correlation of AROM deficiency with the time to full recovery in Greek elite athletes. The authors showed that the active range of motion deficiency 48 hours after the unilateral damage to the hamstring correlates with the time to complete recovery. Furthermore, this indicator served as an objective and accurate measure. Other studies examined the effect of initial injury severity on the subsequent risk of repeated injuries (20). According to authors, the low-grade hamstring muscle lesions inflicted a higher risk of re-injury as compared to high-grade hamstring muscle lesions. Furthermore, the Active Range of Motion (AROM) in elite Greek athletes was assessed (21). Unfortunately, the number of studies on the correlation between AROM deficiency and time to full recovery is insufficient.

The Range of Motion (ROM) variable can reflect alterations in the injured muscle flexibility. The results, however, may be inaccurate due to pain. The proper treatment of the injury, a more careful study and evidence on the most effective decisions are important to predict rehabilitation time and provide full, effective and quick return to sport activities.

The research hypothesis is that the knee active range of motion deficit after hamstring injury correlates with rehabilitation time in futsal players. The purpose of this study was to investigate cases of hamstring injury in futsal players and find association between the active ROM deficit and the rehabilitation period.

METHODS

Participants and acceptance criteria

A total of 200 male futsal players with first-time hamstring injuries were admitted into the study (age range, 18–23 years) between January 2010 and December 2016. No patients required surgical intervention. Exclusion criteria were concomitant bilateral or asynchronous hamstring strain (with chronic tendonitis); confirmed or suspected previous hamstring injury; extrinsic injury to the posterior thigh; pain at the palpation of the proximal hamstring tendon-bon junction; non-structural hamstring injury; proximal hamstring tendon tear, and grade IV injury according to Maffulli *et al.* (15).

The ultrasound showed no anatomical lesions in 74 patients (grade 0 according to Peetrons (12)). Thereby, only 126 futsal players were included for further research. The control group included 100 men of the same age who had never experienced a hamstring muscle injury.

Research protocol was approved by the Ministry of Sport of the Russian Federation and all athletes signed an informed consent to participate in the study. The study follows international ethical guidelines and recommendations for the clinical and field science research (22).

Clinical assessment

All athletes underwent an examination by a sport medicine doctor. A traumatologist was only involved in the most severe cases. All athletes had the following: a) local pain

on palpation, and b) pain with resisted movements (*e.g.* hip extension, knee flexion). Athletes were managed with the PRICE protocol (Protect, Rest, Ice, Compression, and Elevation). Ice was applied for 15 minutes every hour for the first 6 hours after the injury and initial evaluation, and then every 3 hours. The thigh was protected and compressed using a compressive elastic bandage and was kept elevated. No motion was allowed for the first 6 hours and isometric exercises were encouraged for all the periarticular muscles of the hip and knee thereafter.

Clinical evaluation conducted 2 days after the injury included the following: a) inspection for bruising, b) ability to walk on level ground without pain, c) palpation of the hamstring with the athlete prone and knee extended (for presence or absence of tenderness), d) provocation of pain on isometric hamstring contraction, e) provocation of pain on passive movements (hip flexion with the knee extended and athletes supine), and f) AROM testing under the Askling protocol (23, 24). These parameters are important for both obtaining accurate data on injury severity and predicting the length of the recovery period.

Athletes enrolled in the study underwent clinical and ultrasound examinations. The study and control groups were exposed to the same AROM assessment procedures.

Protocol processing

The athlete was positioned supine with both hip and knee flexed to 90 degrees (**figure 1 A**). The unaffected leg was placed flat on the couch with the knee fully extended and maintained in this position throughout the test. The athlete was then instructed to actively extend the knee through the full available ROM until firm resistance was felt (**figure 1 B**). Meanwhile the hip was maintained at 90 degrees of flexion. The degree angle was measured by a double-arm 30 cm clear plastic inclinometer. The inclinometer was aligned along the femur with the reference point at the greater trochanter of the femur. All measurements were done in triplicate by the same physician in order to reduce examiner bias. The rehabilitation time is expressed as an interval from the trauma event to pre-injury sports activity (return to play). The difference in AROM data between the injured and uninjured leg was expressed as an AROM deficit.

Rehabilitation protocol

Injured athletes were supervised by experienced physiotherapists and traumatologists. The rehabilitation process was divided into 4 phases:

- traumatic or acute phase. Normalization of gait, involved the use of strapping and/or crutches;
- rehabilitation or strength phase. Regaining of pain-free ROM, starting with concentric training and progressing to eccentric training;
- functional phase. Application of limited loading and return to full activities under the supervision of a doctor or according to recommendations;
- full recovery phase. Return to full sport activities.

Follow-up

The athletes were followed weekly in the clinic during the rehabilitation program. The clinical follow-up period lasted until the athlete returned to pain-free full sports activity. All

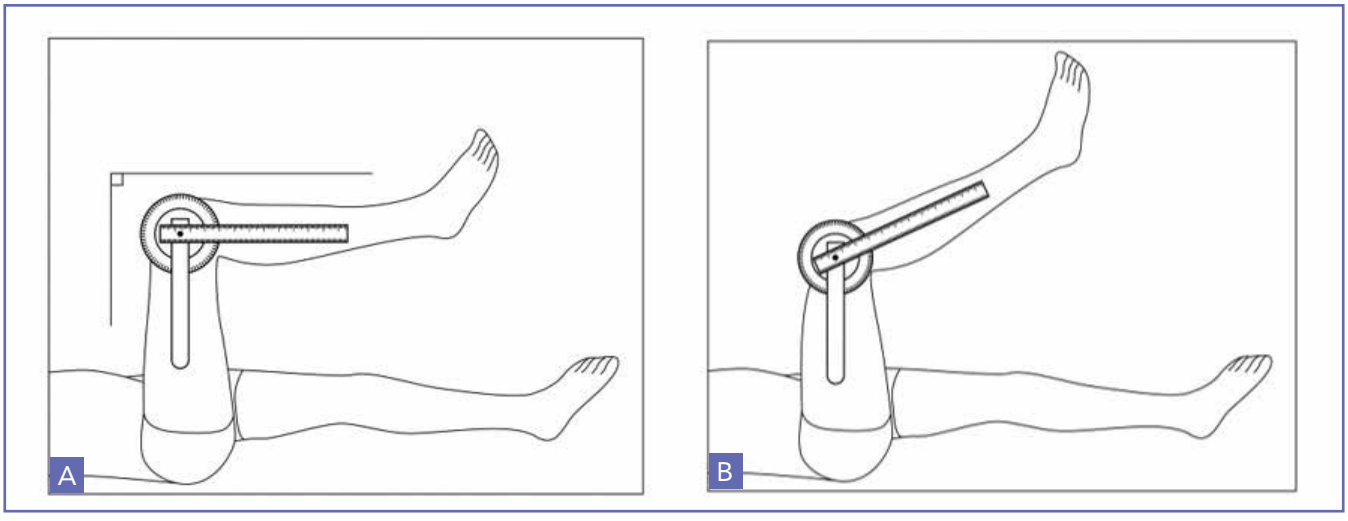

Figure 1. (A) Positioning of the inclinometer; **(B)** Active knee extension testing technique.

athletes were exposed to additional remote monitoring by telephone. Telephone contacts with the athletes and their coaches were held at 3, 6, 12, 18 and 24 months after injury.

Statistical analysis

Pearson correlation was used to find correlation between the return to full athletic activity (4th phase) and AROM findings. Statistical data were processed using the 1-way analysis of variance, the Chi-Square (χ^2) test and regression analysis. The significance level (t-test) was set at $p < 0.05$. Statistical processing was performed with the Past v. 3.0 software.

RESULTS

Among 126 (63%) athletes with sonographic abnormalities, 101 or 80% had injuries to the biceps femoris muscle. Musculotendinous Junction (MTJ), proximal or distal, was involved in 91% of injuries or in 115 athletes. None of the athletes had more than one injured muscle. Characteristics of muscle injuries in 126 athletes with abnormal ultrasound findings are presented in **table I**.

Ultrasound scans revealed grade I injuries (according to Peetrons (12)) in almost half of the participants (62 or 49.2%), and grade II injuries in 64 (50.8%) athletes.

Table I. Muscle injury characteristics in 126 athletes with abnormal findings documented by Ultrasound Imaging.

Injured area, muscle and ligaments	Number of athletes (%)
Biceps femoris	80.0
Semimembranosus	9.0
Semitendinosus	11.0
Proximal musculotendinous junction (MTJ)	38.7
Distal musculotendinous junction (MTJ)	52.3
Intramuscular tendon	40.1
Myofascial injury	9.8
Hematoma	17.8

The mean AROM of the injured leg in the study group was 56.12 ± 6.9 degrees (range, 11-90 degrees; $p < 0.05$). For asymptomatic side, it was 68.9 ± 5.4 degrees (range, 40-91 degrees; $p < 0.05$). The mean AROM deficit in the study group was 12.8 ± 6.8 degrees. In the control group, the mean AROM indicated similarly to uninjured side of examined athletes, 67.9 ± 6.5 degrees at the range 42 to 93 degrees. There were no significant differences in AROM values between the uninjured side of the athletes and the control individuals ($p < 0.697$). However, there was a significant difference ($p < 0.001$) between the injured legs of athletes in the study groups and controls.

The majority of athletes in the study group (70 or 55.5%) had an AROM deficit of less than 15 degrees. Their average recovery period ranged from three weeks to a month. Forty-four athletes, or 35%, had an AROM deficit of 15 to 25 degrees. For them, this took slightly more than a month. Twelve of 126 athletes, or 9.5%, experienced an AROM deficit of 25 to 35 degrees and it took them more than 2.5 months to recover (**table II**). The average number of days lost from futsal training was 29 days ± 3.9, ranging from seven up to 80 days. Hamstring strains were categorized into five grades based on the AROM deficit: grade 0 – AROM deficiency is absent, grade I – less than 15°, grade II – 16° to 25°, grade III – 26° to 35°, grade IV – over 35°. AROM deficit grades I and II correlate with grade I ultrasound according to Peetrons (12) (r=0.86), with first degree injuries according to Askling (9) (r=0.82), and with type 3A injuries according to the comprehensive muscle injury classification (14, 15) (r=0.88). AROM deficit grade III correlates with grade II ultrasound, second degree injuries, and type 3B injuries (r=0.92, r=0.84, and r=0.85, respectively).

DISCUSSION

The study shows a connection between AROM deficit and time to full recovery. The larger the AROM deficit, the longer the rehabilitation. With an AROM deficit of less than 15 degrees (grade I according to the present grading system), the recovery period was 3 to 4 weeks. For athletes with the AROM deficit of 15 to 25 degrees, this took slightly longer than a month. Athletes with the AROM deficit of > 25 degrees reached full recovery by the end of

Table II. Hamstring stains in 126 athletes by clinical grade.

Clinical grade	AROM deficit	Rehab. days	Number of athletes	%
I	less 15°	25.9	70	55.5
II	16° to 25°	30.7	44	35.0
III	26° to 35°	75.0	12	9.5

2 months. AROM deficit also correlated with the percentage of muscles involved in the injury. The results of the hamstring injury classification based on ultrasound imaging (12) largely coincided with the AROM deficit-based classification, excluding cases with the boundary percentage of muscles involved.

Clinical and ultrasound examinations were performed 48 hours after injury. In the acute setting, immediately after the injury, significant pain and disability are present. For this reason, attempts to accurately determine the athlete's ROM on the injured side would be unreliable (25).

In this study, the majority of athletes with hamstring injuries under consideration recovered their active ROM and returned to full activity in the span of 3 to 5 weeks. Rarely (1 in 10 cases), the recovery time exceeded 10 weeks. Athletes with the worst recovery prognosis can be identified early, as their ROM deficit is more than 25 degrees.

Studies using MRI (26) or ultrasound scanning (18, 24) showed that athletes with normal imaging returned to competition significantly faster, but there was no correlation between the presence of hematoma and the length of the rehabilitation period (18).

Other authors, however, found that fluid or hemorrhagic collections, cross-sectional involvement more than 50%, and distal musculotendinous injury were associated with longer rehabilitation (27).

The predominance of biceps femoris injuries in the study group is consistent with other reports (1, 18, 28-31). The incidence of semitendinosus and semimembranosus muscles in this study was 9% and 11%, respectively. These indicators vary in different studies (2, 18, 28, 32-34), which suggests the existence of differences in the injury patterns between sports (35-37).

This and several other studies show that injuries occur mainly at the musculotendinous junctions (35-40). Recent studies tend to differentiate injuries at the proximal and distal MTJ, as the proximal case takes more time to heal (39).

This study has several limitations. First, the research only addressed structural hamstring injuries, whereas unclear and severe cases requiring surgical treatment were excluded. The reason behind this decision is that unclear and severe situations require a different rehabilitation program. Furthermore, these cases are less informative in respect of the AROM deficit. Second, this study did not use the MRI method. Third, the study population included only the local-level futsal players and thus highlighted no injury characteristics in different sporting populations. Fourth, the athlete's behavior was unknown and could be the subject of future research.

CONCLUSIONS

This study sheds light upon the relationship between AROM deficit and time to full recovery. According the study sample, the prognosis of patients with the AROM deficit < 15 degrees was 3 to 4 weeks. Patients the AROM deficit between 15 and 25 degrees were projected to return to play in the span of one month. The average recovery time for athletes with the AROM deficit between 26 and 35 degrees was 2 months at its minimum. The AROM deficit may be considered an indicator that permits an objective prediction of time to full recovery for futsal-related hamstring injuries. Clinical evaluation has proven to be an adequate tool in recovery prognosis. Ultrasound imaging may be good for athletes having an excessive reduction in AROM, a hematoma or complete rupture of the muscle.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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No difference in Achilles Tendon Resting Angle, Patient-reported outcome or Heel-rise height Index between Non- and Early-weightbearing the First Year after an Achilles Tendon Rupture

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SUMMARY

Background. Patient-reported outcome scores and comparable re-rupture rates in randomized controlled trials have not shown a definitive benefit for operative treatment after acute Achilles tendon rupture. This, together with the increasing rupture rates in the older age group has led to non-operative treatment being increasingly used.

Objective. This study aimed to determine the variation in Achilles Tendon Resting Angle (ATRA) together with patient reported and functional outcome, with non-operative management of the ruptured Achilles tendon using two different regimes, which have been shown to offer low re-rupture rates.

Methods. This is a non-randomised cohort comparison of Achilles tendon rupture patients managed with Non-Weight-Bearing (NWB) for 6 weeks *vs*. Early Weight-Bearing (EWB). The NWB-group received a cast in plantar flexion for 2 weeks followed by 6 weeks in a controlled ankle motion boot with incremental diminishing plantar flexion. The EWB-group received an initial anterior protective plaster slab in plantar flexion followed by 6 weeks of weight-bearing on the meta-tarsal heads, with an anterior shell restricting dorsiflexion.

Results. At 12 months after the injury there were no differences in any of the variables between the two treatment groups. The NWB-group compared to the EWB-group reported at mean (SD) for ATRA -9.8° (4.6°) *versus* -11.4° (5°), p=0.32, for Achilles tendon Total Rupture Score (ATRS) 87 (10) *versus* 79 (19), p=0.43 and for Heel-Rise Height Index (HRHI) 71% (19%) *versus* 59% (13%), p=0.13.

Conclusions. The two methods of non-operative treatment studied lead to increased relative ATRA following injury, however, patients report only minor limitation in terms of outcome. Patients had almost a third less heel-rise height compared with the non-injured ankle.

KEY WORDS

Achilles tendon rupture; non-operative management; patient choice; Achilles tendon resting angle; heel-rise height; weight bearing.

BACKGROUND

Following an Achilles tendon rupture, patients suffer up to 10-30% of calf weakness (1-3). This is manifest as reduced heel-rise height (3), decreased ankle plantar flexion strength 3 and push off during gait (4).

Despite the reports about operative treatment is leading to less strength deficits (5-8) and tendon elongation (8), non-operative treatment after an Achilles tendon rupture has increased (9). A reason for this may be the lack of superiority in terms of Patient Reported Outcome Measures (PROMS) for operative treatment in randomized controlled

trials. Non-operative treatment may be considered to be the current evidence-based guideline following acute Achilles tendon rupture (9) although in mainland Europe there is a trend towards individualized patient treatment as per the ISMuLT Achilles tendon guidelines (10).

A wide variety of non-operative treatments have been used, with different duration, cast or bracing techniques, weight-bearing and early functional movement (10, 11). Weight-bearing compared with non-weight-bearing during rehabilitation leads to higher health-related quality of life without reduction in Achilles specific outcome scores (12).

Low re-rupture rates of 1.1%-2.9% have in some studies been reported for Achilles tendon rupture managed non-operatively (13, 14) with protected weight-bearing in a brace for up to 4 months. Using a full below knee weight-bearing plantar flexion cast for 6 weeks followed by 6 weeks in a boot with reducing wedges showed satisfactory functional outcomes and low re-rupture rate (15). As early functional rehabilitation is adopted more commonly, it is important to avoid tendon elongation to optimize functional outcome (3). However, the 12-month functional outcome of commonly adopted non-operative rehabilitation regimes is not known (13, 14).

Recent, biomechanical studies of ankle position and tendon end apposition using casts and functional braces have shown that the frequently used walker boot with wedges does not provide plantar flexion at the ankle but at the mid-foot instead (16). The use of a cast in maximal *equinus* has been recommended to appose tendon ends of a simulated ruptured tendon (16). Post-operative regimes have used an anterior shell to restrict dorsiflexion together with heel wedges have been extended to non-operative regimes giving excellent or good outcome. Nevertheless, patients were noted to have increased passive dorsiflexion, which correlated with reduced vertical force output during gait analysis.

The Achilles Tendon Resting Angle (ATRA) has been shown to be a valid measure of ankle position (17, 18); it correlates with Achilles tendon length (19) and independently found to have excellent reliability (ICC ≥ 0.75) (20). The ATRA is increased following rupture, is decreased by operative repair and then increases again to approximately that of the non-injured side at 6 weeks after weight-bearing using a functional brace to prevent dorsiflexion. After the brace is removed the ATRA increases into dorsiflexion (17).

This study aimed to determine the variation in ATRA together with patient reported and functional outcomes between two different non-operative regimes for patients at different time points during the first 12 months after their Achilles tendon rupture. It was hypothesised there would be no difference in any variable between the two treatment groups.

MATERIALS AND METHODS

This is a non-randomized cohort comparison study between two non-operative rehabilitation regimes. Observational analysis of the outcome of patients who declined enrolment has also been performed. All patients consented for inclusion in the study and National Research Ethics Service The study received Research and Ethical Committee Approval (IRAS Number 15-WA-0058). The study meets the ethical standards of the journal (21).

Non-Weight Bearing (NWB) group

Between 2013 and 2018, 29 patients were approached for inclusion in the study (**figure 1**). All patients demonstrated the triad of a palpable gap, the absence of plantar flexion with the calf squeeze test and increased dorsiflexion of the ankle on resting.

This left a study group of 24 patients who were managed similarly to a non-operative protocol described by Wallace *et al*. (13) (**figure 2**). Following diagnosis in the Emergency department, the patient was immobilised in a plaster back slab in full plantar flexion. The diagnosis was confirmed by clinical examination in fracture clinic, within 1-2 days, and the back slab was changed to a full cast in full plantar flexion and the patient was referred to a Specialist Achilles tendon clinic. At 2-4 weeks following rupture the cast was changed to a functional brace, with a Controlled Ankle Motion (CAM) hinge brace (**figure 3 a**).

The CAM brace application was performed using a standard method as per the user instructions. The liner was applied around the calf so that the heel pad was directly beneath the heel. The lateral and medial malleoli were palpated through the liner to determine the axis of the ankle joint. The graduated hinge of the CAM brace was centred at the tip of the lateral malleolus. The leg arm of the brace was placed along the shaft of the fibula aiming for the head of the fibula proximally. The liner was then wrapped and secured around the ankle and the two straps tightened and secured to plantar flex the ankle to the pre-determined hinge angle.

The brace, worn 24 hours a day, was initially positioned at 30° of plantar flexion for 2 weeks, then adjusted to 15° plantar flexion for 2 weeks and finally at plantigrade/neutral for a final two weeks. The patient was non-weight-bearing for 6 weeks. At the 6-week time-point, when the ankle was plantigrade in the brace weight-bearing was permitted. Low molecular heparin thromboprophylaxis was prescribed for the first 6 weeks. The brace was discontinued after 8 weeks and the patient referred to the physical therapist.

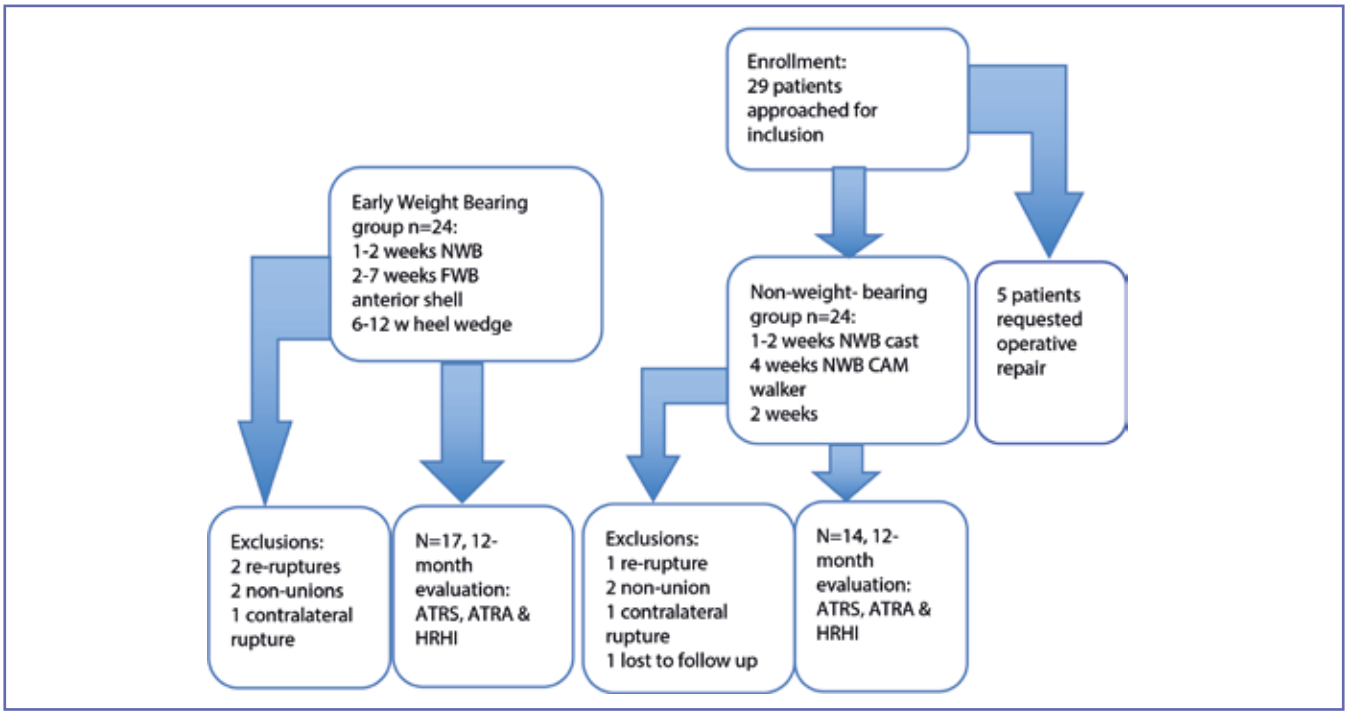

Figure 1. Flow chart for the study.

Early Weight-Bearing (EWB) Regime

0-2 weeks- Emergency Department-placed NWB & below knee anterior plaster slab in full plantar flexion applied.
Fracture clinic-NWB and below knee anterior plaster slab in maximal plantar flexion, referral to Specialist Achilles clinic

2 weeks- diagnosis confirmed and full synthetic reinforced below knee cast, applied, split & supported with velcro straps, WB as tolerated using crutches on meta-tarsal heads only

4 weeks- back shell removed, continue FWB with crutches protected by anterior shell & velcro straps

7 weeks- anterior shell removed, physio referral and 1.5cm in shoe heel wedge to be worn for all WB until 3 months

Non-Weight-Bearing (NWB) Regime

0-2 weeks- Emergency Department-placed NWB & below knee anterior plaster slab in full plantar flexion applied, Fracture clinic- full NWB maximal plantar flexion cast

2 weeks- Controlled Ankle Motion (CAM) walker blocked at 30˚, NWB

4 weeks- CAM walker blocked at 15˚, NWB

6 weeks- CAM walker adjusted to neutral & WB

8 weeks- CAM walker removed and physio referral

Figure 2. The rehabilitation protocols used in the NWB and EWB groups.

Figure 3. Figure 3 a (left) and 3 b (right) showing the Non-Weight-Bearing and Early-Weight-Bearing regimes.

Early weight-bearing (EWB) group

Twenty patients, who were included in the EWB group after discussion of the benefits and risk of operative treatment, chose non-operative treatment with accelerated rehabilitation (**figures 1, 2**). Following diagnosis at the Emergency Department, the lower leg was placed into a back slab in full plantar flexion and referred to fracture clinic. In the fracture clinic the ankle placed into a synthetic cast in full plantar flexion. The cast was split and secured with 4 circumferential elasticated velcro straps. Weight-bearing was permitted as tolerated on the meta-tarsal heads only and the patients advised to use crutches for all mobilisation. After 2 weeks, the posterior half of the cast was removed and the plantar flexed anterior shell held in position using the straps (**figure 3 b**). Early active movement exercises consisting of plantar flexion, inversion and eversion contractions of 10 s duration performed for 10 repetitions, 3 times per day were commenced. This regime was similar to the post-operative rehabilitation following percutaneous and minimally-invasive repair (17).

The anterior shell was discontinued after 7 weeks of management and patients were permitted to load on the heel whilst using a 1.5 cm in-shoe heel wedge until 3 months. Referral for formal physiotherapy occurred at the 7-8-week time point consisting of gait retraining and strengthening with double heel rises progressing to single heel rises. Stretching and plyometric exercises were avoided until the 3-month time-point.

Outcome evaluation

Patients were reviewed at 6 weeks, 8 weeks and 3, 6, 9 and 12 months after the injury. Symptoms and function were evaluated using the Achilles tendon Total Rupture Score (ATRS) (17).

Patients were examined for palpable tendon gaps and tendon continuity using a calf squeeze test. For calculations the relative ATRA was used. This is the difference between the ATRA of the injured and the non-injured sides. A relative dorsiflexed angle is expressed as being negative and plantar flexion as positive. A maximal single-leg Heel Rise Height (HRH), (17) was compared with the non-affected side at 6, 9, and 12 months respectively. Fingertip contact with the wall was permitted for balance. Limb Symmetry producing a Heel Rise Height Index (HRHI) was calculated as the maximal height of a single heel rise on the injured side/the maximal height of a single heel rise on the uninjured side x 100, presented as percent.

Statistical analysis

All data were analysed using IBM SPSS Statistics Version 26 (IBM Corp, Armonk NY). All patients who met the inclusion criteria at the two different hospitals between 2013 and 2018 were offered to participate in the study. Therefore, no sample size calculation was performed. Descriptive statistics for ATRS were reported using mean ± Standard Devia-

tion (SD) and median (range). A patient-reported outcome of > 80 points using the ATRS was considered to be good. Normality was tested by Shapiro Wilks test. For comparison between groups, Mann Whitney-U test was used. A level of significance was set at $p < 0.05$.

Patients who suffered a major complication, which may influence the ATRS, ATRA and HRHI evaluations, *e.g.* those sustaining a re-rupture and/or required reconstruction surgery for non-union or healing with gaping were excluded from 3- to 12-month evaluation. Patients were also excluded if they had previously sustained a contra-lateral Achilles tendon rupture.

RESULTS

Patients were enrolled between 2013 and 2018 from Princess Royal Hospital and Royal Shrewsbury Hospital, illustrated in flow chart (**figure 1**). The overall number of patients studied was 44, with 20 in the NWB and 19 in the EWB groups. The demographic details of these patients are shown in **table I**.

Complications of management sustained by the patients are shown in **table II**. Two patients sustained neuropraxia of the deep peroneal nerve relating to cast and brace compression. Both recovered with time. One patient sustained soft tissue infection consisting of cellulitis following a cast sore requiring oral antibiotic treatment. Elongation was considered to be a relative ATRA of more than ≥ 12° at 12-month evaluation, an angle considered to be consistent with an acutely ruptured Achilles tendon (17).

There were no differences in the relative ATRA at 12 months between the groups (mean (SD)) ATRA NWB -9.8° (4.6°) and EWB -11.4° (5°) (p=0.3). There was a difference in relative ATRA at the 6-week time-point (p=0.03) between the NWB and EWB groups although no difference in any other outcome measures between the non-operatively managed groups (**figure 4-6** and **table III**).

At 12 months following rupture, NWB and EWB patients reported an ATRS of mean (SD) 87.4 (10) and 79.2 (19), (p=0.43) (**figure 5**) and the HRHI was at mean (SD) 71 (19.4)% and 59 (13)% (p=0.13) for the groups respectively (**figure 6**).

Table I. Group Demographics.

	NWB (n=20)	EWB (n=19)
Age Mean (SD) Min-max	 55 (15) (29-77)	 55(14) (29-81)
Left:Right	9:11	6:13
Male:Female	3:1	2.1:1
Tegner	6 (6-8)	7 (3-7)

Table II. Complications of management.

Complication N (%)	NWB (n=20)	EWB (n=19)
Re-rupture	1 (5%)	2 (11%)
Non-union	2 (10%)	1 (5%)
Nerve injury	1 (5%)	1 (5%)
DVT	1 (5%)	1 (5%)
Infection	0 (0%)	1 (5%)
Elongation	4 (20%)	4 (21%)

NWB = Non-Weight-Bearing, EWB = Early-Weight-Bearing, DVT = Deep Venous Thrombosis.

Table III. Differences in ATRA, ATRS and HRHI 6 and 12 months after the injury between the NWB- and EWB-groups.

	NWB mean (SD)	EWB Mean (SD)	Mean difference	95% CI	P value
ATRA 6 m (°) (n=16/19)	-12.5 (6.1)	-10.7 (8)	1.8	-2-7	0.30
ATRA 12 m (°) (n=12/14)	-9.8 (4.6)	-11.4 (5.0)	-1.6	-5-1	0.32
ATRS 6 m (points) (n=16/19)	70.3 (17)	64.6 (23)	-5.7	-22-12	0.43
ATRS 12 m (points) (n=17/14)	87.4 (10)	79.2 (19)	-8.2	-21-5	0.43
HRHI 6 m (%) (n=11/17)	54 (21)	51 (27)	-3	-22-17	0.80
HRHI 12 m (%) (n=11/13)	71 (19)	59 (13)	-12	-27-2	0.13

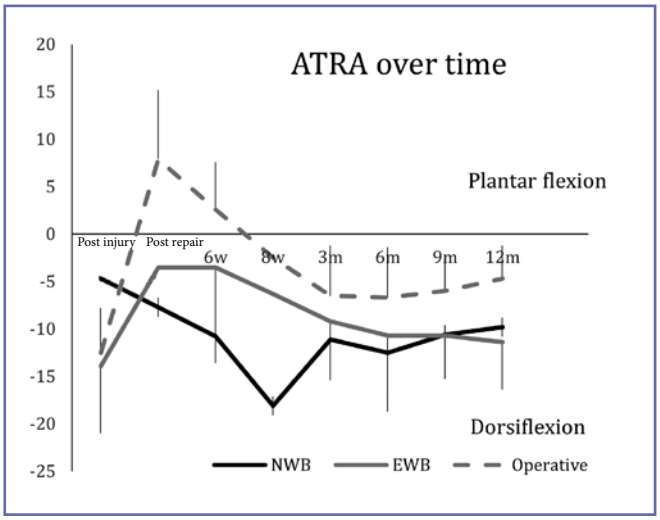

Figure 4. The change in Relative Achilles Tendon Resting Angle over time for NWB and EWB groups with negative error bars. The operative data in this figure is included for visual comparison and shows the post-operative variation in ATRA from a historical cohort of patients managed using minimally-invasive repair and a post-operative regime identical to the EWB cohort (17).

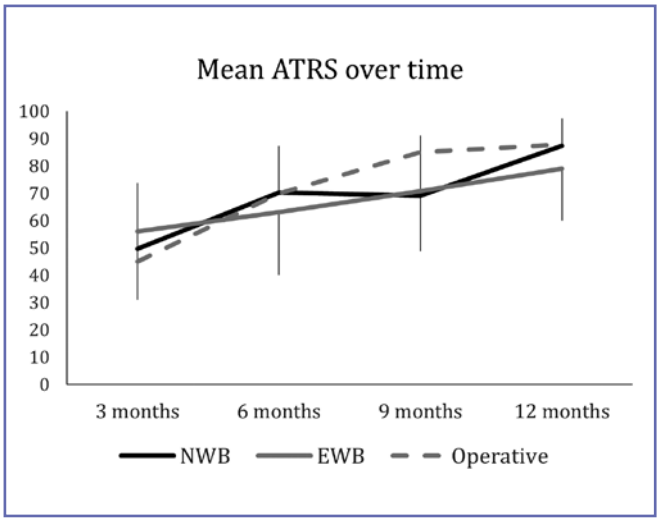

Figure 5. The Achilles tendon Total Rupture Score increasing (ATRS) over time in the patients evaluated. The Operative data in this figure is included for visual comparison (17). Positive error bars are shown for NWB and a comparison operative regime, negative error bars for the EWB regime.

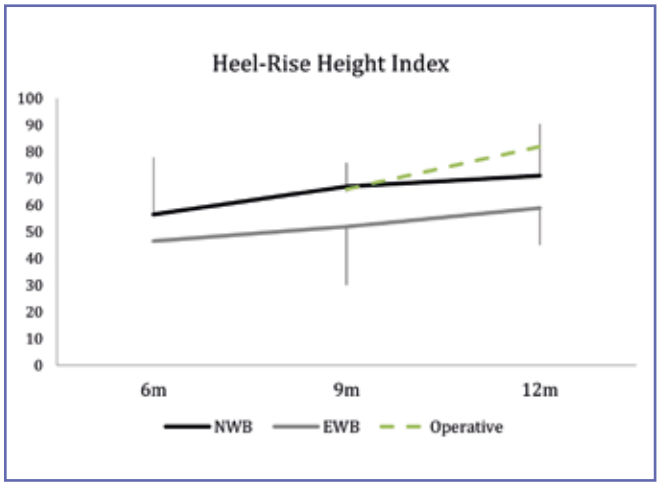

Figure 6. HRHI over time over time in the patients evaluated. The Operative data in this figure is included for visual comparison (17). Positive error bars are shown for NWB and a comparison operative regime, negative error bars for the EWB regime.

DISCUSSION

The most important finding of this study of non-operative management of Achilles tendon rupture was that there was no difference in NWB and EWB for any of the outcome measures studied. In these cohorts managed non-operatively, there was considerable reduction in heel-rise height compared with the non-injured leg although both groups reported an ATRS of ≥ 79 points at 12 months following injury.

At 3 months following rupture in patients managed using both non-operative regimes the relative ATRA had increased to approximately -12°, a value similar to the relative ATRA immediately after the injury. In the NWB group, the ATRA in the injured limb decreased over the period of non-weight bearing, and increased when the plantar flexion angle of the controlled ankle motion brace was changed in the brace and increased further during the subsequent two weeks when weight-bearing was permitted. In the EWB group, the ATRA increased despite restriction of dorsiflexion with the anterior shell, and increased further during heel weight-bearing in the 1.5 cm heel wedge. In the 6 weeks of non-operative management, neither brace nor rehabilitation program prevented a subsequent increase in ATRA with weight-bearing and patients had a considerable reduction in heel-rise height at the 12-month time-point.

The observed changes in ATRA in patients managed non-operatively with the EWB regime were different compared with the historical cohort receiving minimally-invasive repair with similar rehabilitation (17). This historical group had a similar elongation pattern as previous series of patients following percutaneous and minimally invasive repair using the same rehabilitation regime (17). Following

the tightening of the tendon during surgical repair, elongation occurred with immediate weight-bearing in the functional brace, but to only that of the non-injured side. During this time the ATRA increased, but not to the extent of the ATRA following injury.

In the NWB- EWB- and historical, surgically treated, cohort groups, once initial elongation had occurred, the relative ATRA tended to decrease over time, however, this was not significant, similar to other studies on tendon length (22). The elongation findings are similar to Eliasson *et al.*'s study (22) with changes in tendon length with elongation occurring during the period of strengthening during the first 6 months following rupture. In their study there was no effect of weight-bearing nor movement during the first 7 weeks following operative repair (22).

Recent series of patients with Achilles tendon rupture managed non-operatively have used periods of brace protection for much longer than those used in this series (13, 14). In Hutchison *et al.*'s study, patients were immobilised in a cast for 2 weeks followed by an additional 7 weeks using a Vacoped boot. At 9 months, 43 out of 273 patients were evaluated and had a mean (SD) ATRS of 72.4 (14) together with an Achilles tendon repair score of mean (SD) 72.3 (13) indicating that heel-rise height was less than the non-injured side at this time point (13). In Ecker *et al.*'s non weight-bearing protocol using full weight-bearing and cast immobilization over a 3-month time period, 76% of patients performed ≥ 75% repetitive single heel rises compared with the uninjured side after 27 months of follow up (15). Ninety-five percent of patients had ≤ 10° difference in resting plantar flexor angle. The Leicester Achilles rupture Management Protocol (24) involves immediate weight-bearing with graduated dynamic plantar flexion using a Vacoped boot for 8 weeks, and a mean ATRS of 75.5 with a functional outcome in terms of HRHI of 77.2% (Heel-Rise Height (HRH) of 8.5 *vs.* 10.1 cm) at 12 months or more follow-up 50. Taken together, there is still no consensus neither for the optimal time being immobilized after an Achilles tendon rupture nor when it is the best time to start to weight bear in order to receive the best possible functional outcome.

Maffulli *et al.* (25) placed patients in a synthetic plantar flexed cast or brace with wedges for a combined duration of 11 weeks following the minimally-invasive repair of acute (< 14 days) and delayed Achilles tendon rupture (14-30 days) (n=21 per group). The repair consisted of a modified Bunnell and Kessler suture using an absorbable suture, with immediate post-operative weight bearing on the metatarsal heads similar to the method previously described by Carmont *et al.* (17) represented in **figure 4-6**. Despite the longer period of immobilization in the brace, the ATRA at 12 months following repair was similar in both acute (-3.9°(2)) and delayed (-3.7 (1.9)) groups to the historical group (-4.7° (6.5)) and re-rupture did not occur in either group (25). This may indicate the importance of brace protection to reduce the re-rupture rate.

Strengths of the present study were that all patients were assessed by the same observer leading to no inter-assessor bias. Limitations of this study include the small number of patients per group although the number is similar to other studies looking at ATRA over time. In common with other studies of regimes including non-weight-bearing without weight sensors it can never be known how compliant patients have been. Additionally, there was a loss to follow-up over time for patients in both groups. Other limitations include the inability to determine ATRA within the first two weeks of injury in the NWB patients, which was due to the time limitations of referrals. The ATRA at initial referral was negative indicating that the patients had ankles that were in relative dorsiflexion (-5.4°) at this time point. This was, however, within ≤ 10° of the non-injured side, the criteria used for non-operative treatment in the study by Ecker *et al.* (15). Another limitation of this study is that ultrasonography was not available to determine the location of tendon tears or to assess tendon continuity or tendon length during follow-up.

Beyond the 8-week time-point patients received departmental physiotherapy with advice to restore gait, and strengthening exercises to the calf in the form of double heel rises to single heel rises. Stretching and plyometric exercises were to be avoided until beyond the 3-month time point. The physiotherapy received by each individual patient was not standardized and will be gauged upon individual progress. In the literature, a number of rehabilitation programs have been presented, but there is no consensus.

Taken together, this study has shown that these two methods of non-operative treatment lead to increased ATRA compared with that of the un-injured ankle. However, patients report little limitation on outcome but there was more calf weakness compared with other studies (17, 22). One possible explanation can be that functional braces that restrict dorsiflexion and maintain the resting angle of the ankle more effectively were used in these studies.

The two methods of non-operative treatment studied lead to increased ATRA following injury. However, patients report little limitation in terms of outcome. Patients had almost a third less calf muscle performance compared with the non-injured ankle one year after the injury. This considerable reduced calf muscle performance should be discussed with patients when counseling between non-operative and operative treatment options.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Prevalence of Self-Reported Psychological Characteristics of Adults with Lower Limb Tendinopathy

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SUMMARY

Purpose. There is an emerging body of literature describing psychological associations with lower limb tendinopathies. The literature suggests that those experiencing a lower limb tendinopathy are likely to experience varying degrees of kinesiophobia, depression and catastrophisation. These studies have typically been confined to one lower limb tendinopathy. The current study sought to explore whether these psychological influences were experienced across a range of lower limb tendinopathies in a clinical practice setting.

Materials and methods. The current study utilised a cross-sectional cohort design to explore associations between those presenting with any lower limb tendinopathy and psychological factors. Consecutive patients attending a private physiotherapy practice in Melbourne (Australia) were invited to participate. Those who chose to participate were invited to complete a health questionnaire along with the Hospital Anxiety & Depression Scale (HADS), Tampa Scale of Kinesiophobia and the Life Orientation Test – Revised.

Results. Ninety-one patients were recruited, with just over half identifying as male, and more than half experiencing a tendinopathy for more than twelve months. Nearly two-thirds (63.7%) of the cohort demonstrated kinesiophobia (n=58, 63.7%). Patients were classified as depressed in 13% of cases while 21% were classified as anxious.

Conclusions. Routine screening for kinesiophobia may be valuable for patients presenting with any lower limb tendinopathy. The results also support the potential value of screening patients for the presence of anxiety and/or depression. The extent to which these psychological influences are associated with individual patient's experience of lower limb tendinopathy, requires further exploration, as does the development of these influences over the duration of the tendinopathy.

KEY WORDS

Kinesiophobia; tendinopathy; psychological; anxiety; depression; optimism.

BACKGROUND

Tendinopathy is characterised clinically by tendon pain and loss of function (1, 2). The aetiology of tendinopathy is multifactorial and imbalance between load demands placed on the tendon and its ability to remodel is considered a major factor (3). Other factors that influence the capacity of the tendon to remodel and increase the risk of developing tendinopathy include older age, genetic profile, and metabolic factors such as elevated cholesterol or diabetes (3, 4). Once established, the tissue changes in tendinopathy include matrix degradation, characterised by inferior quality and disorganised collagen, accumulation of hydrophil-

ic proteoglycan molecules that increase bound water and swelling, as well as ingrowth of blood vessels and nerves (2). Breakdown in the endotendon, including degradation of the interfasicular matrix, has also been shown to limit the fatigue resistance of energy storage tendons, which may explain why aging tendons are more susceptible to injury (5). A major challenge in the management of tendinopathy is an incomplete understanding of pain mechanisms and factors that influence tendon pain (6), including psychological determinants that effect chronicity and recovery.

The role of psychological disorders such as depression, anxiety, catastrophisation and kinesiophobia is well established in chronic musculoskeletal pain states, (7, 8) and their influence on, or association with, lower limb tendinopathies is beginning to emerge in the literature. A recent systematic review of cross-sectional and prognostic studies suggests that kinesiophobia, depression, stress and catastrophisation are positively associated with plantar heel pain (9). Kinesiophobia, catastrophisation and depression have also been associated with increased symptom severity in gluteal tendinopathy (10), with kinesiophobia contributing to suboptimal outcomes in Achilles tendinopathy (11). These associations have led to the suggestion that sensitisation of the nervous system, and impaired pain processing, may explain persistent tendinopathy pain states, and ongoing loss of function that can occur following tissue-based intervention in tendinopathy (12).

Although there have been a number of recent studies investigating the role of psychological factors in people with lower limb tendinopathy (9-11), to our knowledge, no current study has assessed its prevalence in a consecutive cohort of patients. The aim of this study was to explore the prevalence of psychological factors in a cohort of consecutive patients seeking physiotherapy care for a lower limb tendinopathy. This work also sought to describe the characteristics of the patient cohort and how these psychological factors correlated with these characteristics.

METHODS

In this cross-sectional study, data was collected on a cohort of consecutive patients presenting for treatment for lower limb tendinopathy at a single physiotherapy clinic in Melbourne, Australia. All patients were managed by a single physiotherapist (PM) at this centre, who has specialised in tendinopathy management for 15 years. Patients were either self-referred or referred by other health professionals (general practitioners, sports and exercise medicine physicians, orthopaedic surgeons, physiotherapists, osteopaths, chiropractors). Data was collected over 18 months between July 2016 and December 2017. The study was approved by the Victoria University Human Research Ethics Committee (HRE16-079), consistent with the journal recommendations (13). All participants provided informed consent.

Participants

Participants were either referred to the specialist physiotherapist or elected to attend themselves, for a possible tendon-related complaint. Patients were required to be over the age of 18 years and able to read English at a year 7 (12 years of age) level. The clinic administrative staff recruited participants for the study, the practitioner was not aware if the patient they were treating was a participant or not. Non-participation in the study did not influence the opportunity to receive care.

Questionnaires and measures

Potential participants were provided with a tablet that contained an online version (Qualtrics, Utah, USA) of the survey. The first part of the survey included the information sheet and consent process. Participants who agreed to participate were asked to complete the questionnaires prior to their consultation with the physiotherapist.

Participants completed a range of demographic and health behaviour questions, in addition to single item screening questions for general health ("Please rate your general health"), and life satisfaction ("Overall, how satisfied are you with your life?") (14). Participants were also asked to complete questionnaires exploring psychological factors outlined below.

Hospital Anxiety and Depression Scale (HADS)

The HADS was originally developed by Zigmond and Snaith as a self-report tool to detect and measure the severity of depression and anxiety (15). The HADS-D was originally developed based on the symptoms of anhedonia, whilst the HADS-A was based on the developer's research on anxiety and the Hamilton Anxiety Scale (15, 16). It has two separate subscales for each emotional disorder and was originally intended for use in a hospital outpatient setting (15). It is extensively used with psychiatric, medical, rheumatological and chronic pain patients (16). The HADS (15) comprises 14 items rated from 0-3 divided into two subscales: anxiety (7 items) and depression (7 items), scores range from 0-21 for each subscale. A total score is generated for each of the anxiety and depression subscales. The HADS subscales are analysed separately, scores from 8-10 indicate a possible clinical disorder and scores 11-21 a probable clinical disorder (17). Scores greater than 11 are

used to identify patients with anxiety or depression. The HADS has been found to be an effective tool in the detection of anxiety and depression (18, 19) with a sensitivity and specificity of approximately 0.8 (17) and more than acceptable internal consistency with Cronbach alpha ranges from 0.78-0.93 for the HADS-A and 0.82-0.90 for the HADS-D (16, 17). It has been concluded that the HADS has both high clinical and research usability to identify the cognitive symptoms of anxiety and depression, in addition to differentiating between the two disorders (16, 17). Johnston, Wright and Weinman (20) have proposed that four score ranges can be used to classify the presence and severity of anxiety or depression: 0-7 normal, 8-10 mild, 11-14 moderate and 15-21 severe.

Tampa Scale for Kinesiophobia (TSK)

The Tampa Scale for Kinesiophobia (TSK) was originally developed to measure fear of movement and its current use has retained its original scoring format. The TSK is a seventeen-item scale used to subjectively measure fear of movement (21) and unhelpful beliefs about pain. The scale is based on the model of fear avoidance, fear of work-related injury and fear of reinjury (22). The TSK has 17 items rated on a 4-point Likert-type scale. Total score ranges from 17 to 68 with a cut off score of 37 or over being considered a high score (22). Four items on the questionnaire are inversely worded and thus negatively scored. Several studies have shown the TSK to be a valid and reliable psychometric measure (21, 23, 24) with high internal consistency (Cronbachs alpha=0.84) (25). The TSK has been found to be significantly correlated with other scales that measure pain catastrophization and fear of movement which suggests that it is a valid measure of these constructs (25). The TSK was scored according to published cut-off scores (22).

Life Orientation Test-Revised (LOT-R)

The Life Orientation Test-Revised (LOT-R) (26) measures dispositional optimism or pessimism. There are ten items: three that measure optimism, three pessimism and four fillers which are ranked by the participant but are not included in the scoring. Agreement or otherwise with a statement is rated on a 5-point Likert-type scale, with a higher score being reflective of greater optimism (27). The LOT-R scoring is a continuous dimension and there is no defined cut-off. The original Life Orientation Test was first published in 1985 as a measure of dispositional optimism, which is characterized as an expectation in people that good things will happen (28, 29). The revised version (LOT-R) was later developed to provide a more realistic representation of optimism, taking into account the effect of optimism on other health outcomes (28). In the revised version, two items from the original LOT were removed (28). It is primarily a research instrument rather than a clinical measure of the positive trait. Psychometric properties of the revised scale have been shown to be satisfactory (Cronbachs alpha: optimism 0.70, pessimism 0.74, total score 0.68) and its use supported to measure pessimism and optimism as independent constructs (28).

Inclusion criteria

Tendinopathies of five different lower limb tendons were accepted for inclusion in the study: the gluteal tendon, proximal hamstring tendon, patella tendon, Achilles tendon and plantar fascia. Although the plantar fascia transcends the typical definition of a tendon as it does not connect muscle to bone (rather fascia to bone), it does still display characteristics that are consistent with tendinopathy on ultrasound (hypechogenicity and thickening) and in response to loading programs (30). It is for this reason that plantar fasciopathy was considered a tendinopathy in this study. Participants were retained if they had comorbidity or secondary musculoskeletal diagnoses, provided that the lower limb tendinopathy was the primary complaint for which they sought care. Potential participants were only excluded following data collection if clinical examination revealed that their pain was not tendon related or if questionnaires were incomplete.

Tendinopathy diagnosis

Tendinopathy diagnosis was based on a combination of clinical presentation and tests, as is recommended by expert opinion and consensus (**Supplementary file 1**) (31-35). A single physiotherapist undertook all diagnoses for each participant. Participants were asked to report the location of pain they experienced during the loading tests undertaken during the diagnostic process. Diagnostic imaging was not a prerequisite of diagnostic classification and this is consistent with recommended practice (36). Differential diagnoses for each tendinopathy site were considered using validated tests where possible (**Supplementary file 2**). Participants were excluded if an alternative diagnosis (not tendinopathy) was their main pain complaint. Participants were retained in the cohort if they had a comorbid pain state (*e.g.* sacroiliac joint-related pain), but this was not their primary pain complaint.

Pain descriptors

Self-reported duration of tendon pain was reported on a 4-point scale: 0-4 months, 5-8 months, 9-12 months and

greater than 12 months. Patients were asked several questions about their symptom behaviour and pain severity. The percentage of the day that participants experienced pain was rated (0-25%, 26-50%, 51-75%, 76-100%). Participants rated the average pain severity over the last 7 days on a visual analogue scale (0-100, 100=worst pain imaginable) during rest as well as activities that are commonly associated with pain for each tendinopathy (**table I**). Scores for these questions were then averaged to provide a total score for pain severity that was used in analyses. These activities and questions were adapted from validated pain and function questionnaires where possible (37-40). We did not use validated pain and function questionnaires as they were not available for every tendinopathy included in the current study.

Statistical analysis

Data were exported from Qualtrics to SPSS (IBM Corp USA, version 24) for analysis. Each completed patient response was screened and the tendon diagnosis added. Descriptive statistics were generated for each of the demographic and health information variables and reported for each tendinopathy diagnosis. Each of the HADS, LOT-R and Tampa were scored according their respective instructions. Results of each questionnaire were coded to reflect the classifications for the HADS (anxious or depressed), LOT-R (optimism or pessimism) and Tampa (kinesiophobia) questionnaires. Inferential statistics (Spearman's *rho*) were used to investigate the relationship between psychological questionnaires and pain dimensions (duration, activity pain severity, rest pain severity). Descriptive statistics (mean, median, standard deviation, percentage) were generated for each questionnaire and the internal structure evaluated using Cronbach's alpha. Alpha was set at $p < 0.05$ and effect sizes were calculated where relevant.

Table I. Questions about pain severity.

Site of tendinopathy	Aggravating activity
Gluteal	Arising after prolonged sitting Walking Sleep
Proximal hamstring	Sitting Lunging Start of run/walk Running/walking faster
Patellar	Going downstairs Sitting Jumping
Achilles	Stiffness in the morning Start of run/walk
Plantar fascia	Stiffness in the morning Start of run/walk Prolonged standing

RESULTS

Demographics and health behaviours

One hundred and thirty-eight (n=138) consecutive patients were invited to participate with ninety-one (n=91) agreeing (65.9% response rate). The median age range of the cohort was 45-49 and comprised 50 men (55%) and 41 women (45%). More than half of the participants reported tendon pain for greater than 12 months (**figure 1**). Eighty-six (95%) participants spoke English at home, while only 8% (n=7) lived alone. Eighty (88%) participants had private health insurance, 26% (n=24) had a health care card, and 70% (n=64) had university education or higher. The median amount of sleep per night was between 7 and 8 hours and the median exercises per day was between 30 and 59 minutes. Fifty-seven participants (63%) self-assessed their general health as very good or excellent. **Table II** demonstrates the descriptive and internal consistency statistics for the psychological measures.

Table III shows demographic and pain related data for the tendinopathy presentations. Achilles tendinopathy accounted for nearly half of the patient cohort (n=43, 47.3%), and over half of the patient cohort had experienced their tendon complaint for greater than 12 months (n=48, 52.7%, **figure 1**). Plantar fasciopathy and gluteal tendinopathy were most prevalent in the 50 and over age group, whilst proximal hamstring, patellar and Achilles tendinopathy were

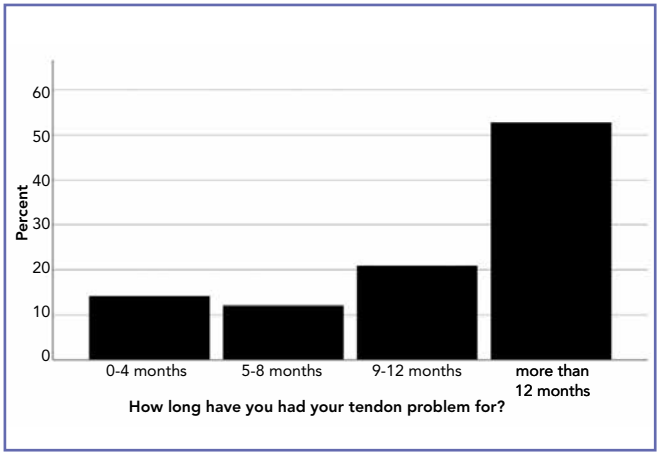

Figure 1. Duration of tendon pain across the entire cohort.

Table II. Descriptive statistics and internal consistency for each of the psychological measures.

Psychological measure	Mean (SD)	Range	Cronbach's alpha
Tampa Scale for Kinesiophobia (TSK)			
Somatic Focus	11.2 (3.0)	5-18	0.76
Activity Avoidance	12.5 (3.0)	6-22	0.83
Life Orientation Test -Revised (LOT-R)			
Optimism	11.5 (1.8)	7-15	0.70
Pessimism	6.6 (1.9)	3-12	0.71
Hospital Anxiety and Depression Scale (HADS)			
Depression	3.9 (2.8)	0-16	0.74
Anxiety	5.5 (2.6)	0-13	0.65

Table III. Demographic and symptom data for each tendinopathy.

Site of tendinopathy	Cases (%)	Most common age range (yrs)	Percentage of men with this tendinopathy (%)	Presence of secondary diagnosis (%)
Gluteal	11 (12.1)	50 or over	9	27
Proximal hamstring	17 (18.7)	35-39	56	41
Patella	13 (14.3)	30-34	86	46
Achilles	43 (47.3)	35-39	60	35
Plantar fascia	7 (7.7)	50 or over	43	29

more common in adults in their thirties (**table III**). Gluteal tendinopathy was more prevalent in women (91% *vs* 9%, $p < 0.05$), whilst patellar tendinopathy was more prevalent among men (86% *vs* 14%, $p < 0.05$).

Patellar tendinopathy was the lower limb tendinopathy most likely to co-exist with a secondary condition (**figure 2**), while gluteal and plantar fasciopathy were most likely to exist in isolation. Musculoskeletal secondary diagnoses included: hip osteoarthritis, knee osteoarthritis, patellofemoral pain syndrome, sacroiliac joint dysfunction, lower back pain and multiple tendinopathy sites. Systemic concomitant diagnoses included: hypertension, nephropathy, fibromyalgia and psoriatic arthritis. Neurological secondary diagnoses included: sciatic neuropathy and sural neuropathy.

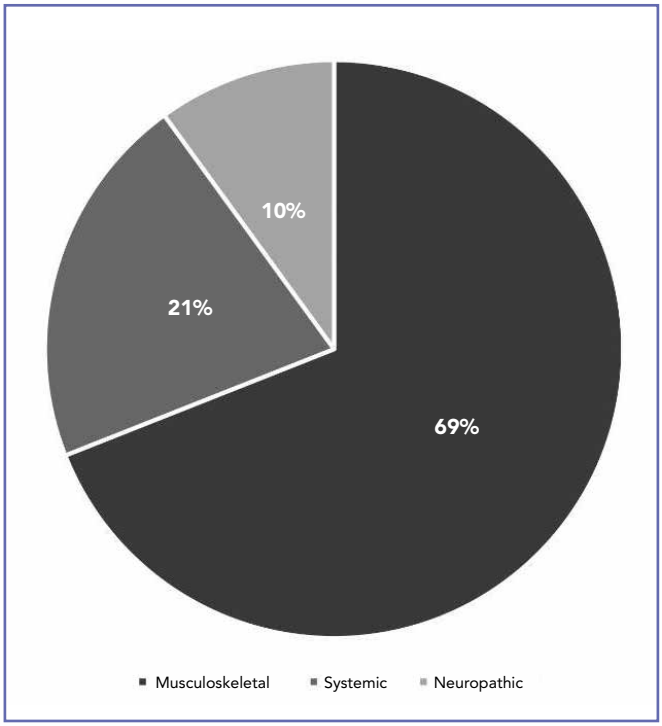

Figure 2. Co-existing diagnoses associated with tendinopathy presentations.

Symptoms

Participants rated their average pain with activity and with rest (**figure 3**). In addition, duration of tendinopathy-related pain was reported (**figure 4**). Achilles tendinopathy was on average the most painful tendon with activity and generated the highest average pain scores at rest. Patellar tendinopathy and hamstring tendinopathies were the least painful tendons at rest. Patellar tendinopathy was also on average

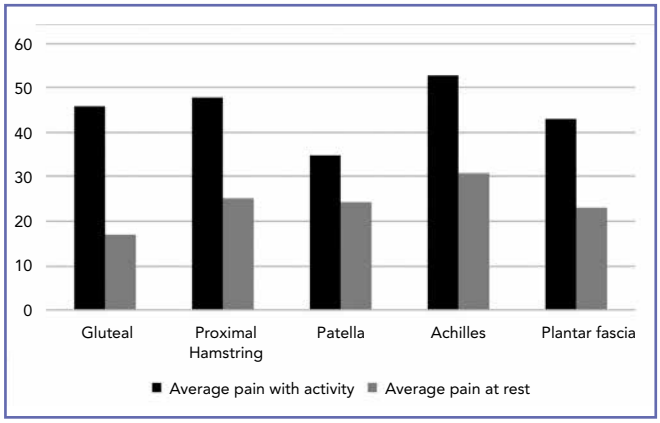

Figure 3. Average pain severity for each tendon subgroup.

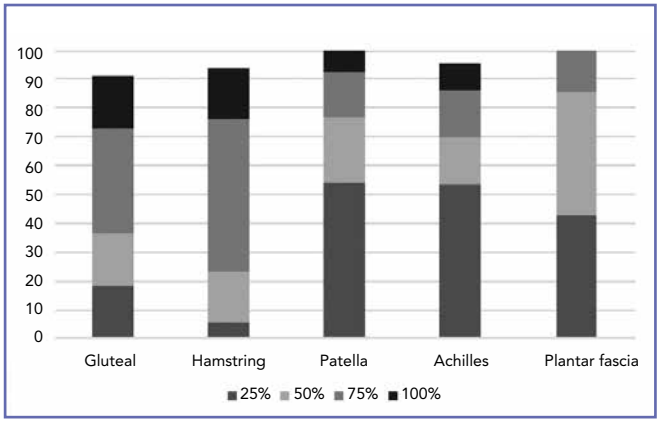

Figure 4. Pain experienced as a percentage of the day for each tendon subgroup.

the least painful condition during activity. Gluteal tendinopathy displayed the greatest discrepancy between pain with activity and rest pain.

The gluteal tendinopathy subgroup demonstrated the highest proportion of participants that experienced pain 100% of the day (18.2%). Over half of the hamstring tendinopathy subgroup experienced pain for three quarters on an average day (52.2%). The patellar tendinopathy and Achilles tendinopathy subgroups had the highest proportion of participants that experienced pain for 25% of the day. Participants with plantar fasciopathy were the only subgroup to not have participants that experienced pain for 100% of the day.

There was a negative association between self-rated general health and the LOT-R pessimism subscale (*rho*=-0.40, $p < 0.05$, medium) and a positive association with the LOT-R optimism subscale (*rho*=0.23, $p < 0.05$, small). Small negative correlations were observed between general health and both TSK subscales (*rho*=-0.-25, $p < 0.05$, small), and the HADS depression subscale (*rho*=-0.23, $p < 0.05$, small). Life satisfaction was positively associated with the LOT-R optimism subscale (*rho*=0.36, $p < 0.05$, small). The HADS subscales were negatively associated with satisfaction with life (depression, *rho*=-0.39, $p < 0.05$, medium; anxiety, *rho*= -0.25, $p < 0.05$, small), however trivial associations were observed between kinesiophobia and life satisfaction.

Tendinopathy and psychological variables

Nearly two-thirds of the entire cohort scored above the TSK cut-off score (n=58, 63.7%) with 76.9% (n=10) of those with patellar tendinopathy classified as having a fear of movement (**figure 5**). Further, 13% of participants appear to be affected by depression, while 21% were classified as anxious. Those patients with gluteal and hamstring tendinopathy exhibited the highest rates of anxiety across the cohort.

Small negative relationships were observed between rest pain severity and the HADS depression subscale (*rho*=-0.22, $p < 0.01$, small) and TSK activity avoidance subscale (*rho*=-0.19, $p < 0.05$, small) scores. The TSK activity subscale also demonstrated a small relationship to the severity of pain with activity (*rho*=-0.21, $p < 0.05$, small). Symptom duration demonstrated trivial correlations with the subscales on all three psychological questionnaires (*rho* < 0.11) and LOT-R subscales demonstrated trivial correlations with rest pain and activity pain intensity (*rho* < 0.10).

DISCUSSION

This study sought to evaluate the presence of a range of psychological factors that may be associated with the presence of a tendinopathy affecting the lower limb, in a cohort of patients attending for care at a private physiotherapy clinic. Patients in our cohort who were seeking treatment by a physiotherapist with tendinopathy expertise, had undertaken previous treatment (including exercise, injections, and surgery), and 50% reported they had experienced lower limb tendon pain for longer than 12 months. Further, our cohort was from a high sociodemographic population with 88% having private health insurance and 70% having a tertiary education.

Kinesiophobia

The main finding of our study is that kinesiophobia was highly prevalent across the current cohort regardless of tendinopathy location. Sixty-three percent of all participants displayed beliefs suggestive of fear of movement. This was based on published cut-off scores for the TSK (22) with

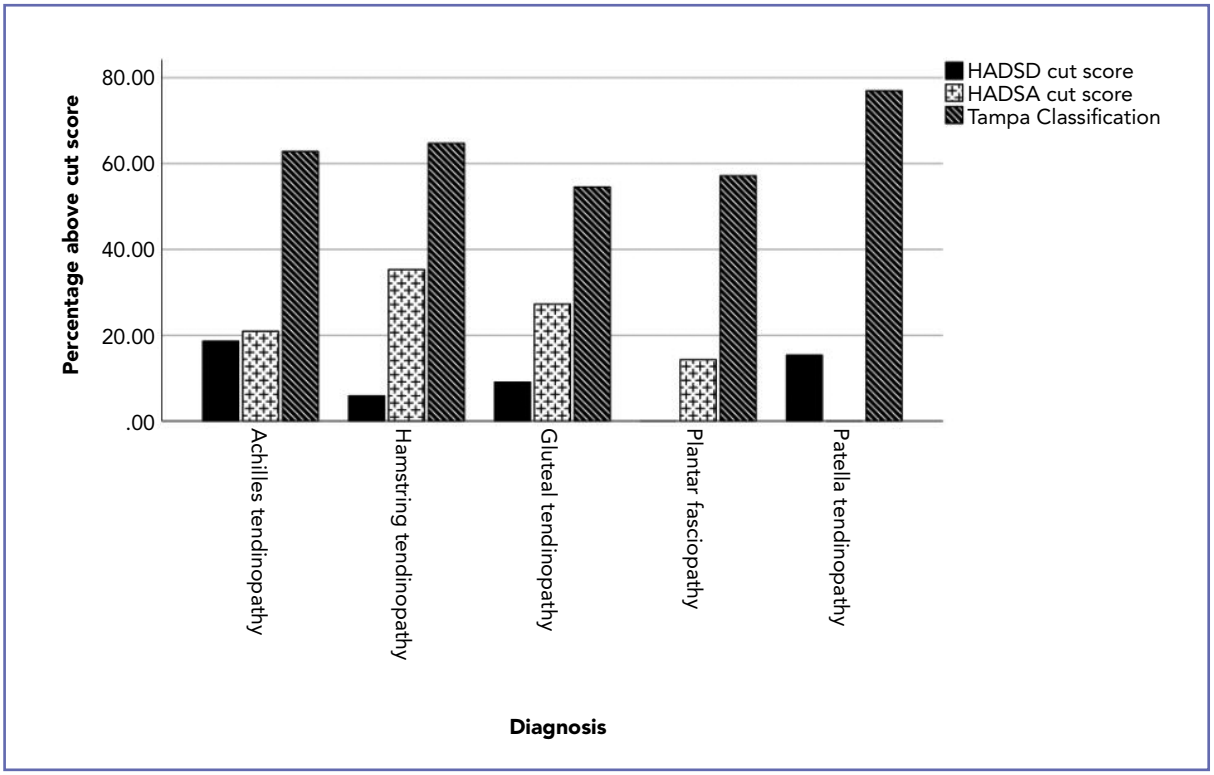

Figure 5. Participants classified with kinesiophobia, depression and anxiety.

prevalence ranging from 54.5% for gluteal tendinopathy to 76.9% for patellar tendinopathy.

Results of the current study suggest that those patients who displayed fear of movement according to the TSK, are more likely to hold the belief that pain is proportionate to damage, and that avoidance of physical activity or exercise may be necessary to prevent exacerbation of their tendinopathy condition (41). These patient cognitions are at odds with the current understanding of tendinopathy pathogenesis and management. Although the pain source in tendinopathy is not clear (42), the accepted hypothesis is that pain is a product of peripheral sensitisation that may be caused by one or a combination of multiple biochemical changes that occur in the local tissues (43). Pain is not always reflective of the state of the tissues and there is no evidence that tendon pain is an indication of structural tendon damage (3). There is also a substantial body of evidence that demonstrates structured exercise improves tendon pain and function, and exercise is the first-line recommended treatment for upper and lower limb tendinopathy (1, 4, 44, 45). Future studies should explore potential associations between kinesiophobia and treatment outcomes in lower limb tendinopathy.

Given the cross-sectional design of our study, we are unable to draw conclusions on the mechanistic relationship between kinesiophobia and tendon pain – whether fear of movement precedes or is a product of tendon pain. Our results also suggest there is a small negative association between pain severity (both at rest and with activity) and kinesophobia in patients with lower limb tendinopathies. The presence of kinesiophobia might form part of a multifaceted risk factor profile for the development of a chronic lower limb tendinopathy given this small association. One explanation for this could be that there is an underlying tendency towards kinesiophobia in some people and not in others, in a similar way that some people have a genetic or situational predisposition towards psychological conditions such as anxiety or depression (46). There is a body of evidence which demonstrates that fear-avoidance beliefs play a significant role in the transition from acute to chronic back pain (47) and is correlated to disability and quality of life measures (48). With respect to low back pain, high levels of kinesiophobia at baseline predicted duration, severity and disability at follow up. Further, for those without low back pain at baseline, a high level of kinesiophobia could predict back

pain and disability at follow up (49). These results suggest a propensity to develop fear with movement could exist within a genotype of an individual, and that pain as an environmental trigger could lead to its phenotypic expression. This assertion is possibly supported by the negligible association between kinesiophobia and complaint duration in the current study suggesting other factors may be contributing the development of fear of movement. How these results translate to lower limb tendinopathies requires further exploration, however.

Alternatively, kinesiophobia could be thought of as a secondary sequalae to longstanding pain in some individuals. This could be explained as a maladaptive movement behavioural response to threatening pain, whereby the central nervous system interprets repeated nociceptive input from peripheral tissues, upregulates output pain and alters motor patterns to de-load the injured tissues (50-52). Whilst the temporal relationship between pain and fear of movement is unclear, our study suggests that identifying and managing these cognitions may have a role in the management of lower limb tendinopathies.

Depression and anxiety

Among our cohort, 13.2% (n=12) would be classified as experiencing depression and 20.9% (n=19) experiencing anxiety, with the prevalence of both in the current cohort being relatively consistent with Australian population data (53). Pain at rest demonstrated a small association with the HADS depression subscale score, with depression being most prevalent in Achilles tendinopathy. The latter findings are consistent with the psychological burden among people with Achilles tendinopathy, as identified by McAuliffe *et al.* (54). Depression has also been identified as a significant component of the patient profile of those with severe gluteal tendinopathy (10), suggesting that clinicians should screen for psychological distress, or the presence of possible depression in patients with lower limb tendinopathies, to better manage these complaints (54). Anxiety appeared to be most prevalent in those with hamstring tendinopathy in the current study, but was not identified in those with patellar tendinopathy. The reason for these associations is not clear and further exploration in larger samples is warranted, particularly as these psychopathologies do not appear to be present to the same degree across all lower limb tendinopathies. As discussed earlier, whether psychological distress precedes, develops in conjunction with, or is exacerbated by, a lower limb tendinopathy requires further research.

Life orientation

The current study is the first to explore the construct of life orientation (dispositional optimism) in the context of lower limb tendinopathies. Optimism and pessimism comprise this construct with higher levels of pessimism being associated with lower physical health outcomes (55). Pessimism demonstrated a trivial negative correlation relationship and optimism a trivial positive relationship with duration of symptoms. Likewise, pain with either rest or activity demonstrated trivial correlations with dispositional optimism. These results suggest that life orientation may not be a factor in lower limb tendinopathies broadly. However, there may be a relationship between life orientation and individual tendinopathies that was not able to be identified given the sample size in the current study. Further work to evaluate the association of life orientation and specific lower limb tendinopathies to confirm or refute the current findings is required.

Limitations

There are several limitations of this study, the first being inclusion of people with concomitant musculoskeletal pathology such as hip osteoarthritis, patellofemoral pain syndrome or lower back pain as this may have influenced our findings. The presence of a secondary diagnoses may have resulted in over reporting of prevalence of psychological factors such as kinesiophobia. Another limitation of our study is that we did not use validated measures of tendinopathy pain and function (37-40). This is because there is no one measure that incorporates all lower limb tendinopathy diagnoses that we included in the study. This may have limited our ability to identify a relationship between severity of pain and psychological factors, which consequently may be under reported. In addition, our consecutive cohort was a chronic cohort presenting to a tertiary referral specialist clinic and may not represent patients with shorter term pain, presenting to primary and secondary care centres, or patients with these conditions who do not actively seek specialist treatment. Generalising our findings to other populations such as those who have never sought treatment for their tendinopathy, or those with acute symptom durations is not recommended.

We urge caution generalizing our findings, given the relatively small subgroups of some of the tendinopathies represented in our cohort. Given that ours is a pragmatic exploratory study we did not consider power calculations *a-priori*. Finally, there were very few cases of some tendinopathy diagnoses included in the study. Plantar fasciopathy was

the lowest (n=7). For this reason, we did not analyse relationships between tendinopathy types and associated pain measures. Consequently, there is a limit on generalisability of our results to individual tendinopathy types included in the study. Despite these limitations, our results demonstrate the high prevalence of kinesiophobia across all tendinopathy diagnoses among our consecutive cohort of patients. The authors therefore advocate that this psychological factor warrants further investigation in future research in the field of lower limb tendinopathy.

CONCLUSIONS

The current work explored a range of demographic, health behaviour, psychological and psychosocial variables in a cohort of patients presenting to physiotherapy for treatment of a lower limb tendinopathy. Regardless of the tendinopathy location, kinesophobia appeared to be prevalent in the majority of participants in the study. This finding would suggest that routinely screening for kinesophobia may be indicated, as it may provide valuable clinical information to incorporate into patient management strategies. This result also provides an opportunity to evaluate the impact of kinesophobia on treatment outcomes. Some of the current patient cohort also demonstrated possible depression and anxiety – again, screening and co-management of these psychopathologies may be required to effectively manage patients with lower limb tendinopathy. Of note is that these psychopathologies appeared to be independent of other demographic variables in this cohort. The current study adds to the understanding of the prevalence and associations between lower limb tendinopathies and a range of psychological and psychosocial variables. These results could provide the basis for further work to evaluate the impact of addressing psychological and psychosocial variables in the management of lower limb tendinopathies. Further research could be directed towards tracking psychological changes during the management of a tendinopathy through to resolution, including the use of other measures that observe coping, self-management behaviours and self-efficacy. These additional measures may assist in the identification of factors that predict chronicity and poor treatment outcomes. Whilst quality of life and functional measures were not included in this study, they should be included in future studies to enable a more complete exploration of the relationship between tendinopathy and psychological factors.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTARY FILE 1

Table I Suppl. Clinical diagnosis criteria for tendinopathy.

Site of tendinopathy	Clinical diagnosis criteria
Gluteal	Primary complaint of lateral hip pain, pain on palpation of the greater trochanter, and pain reproduced with either passive flexion abduction external rotation (FADER), muscle isometric contraction in FADER or single leg stance on the affected leg for 30 seconds (1, 2)
Proximal hamstring	Primary complaint of ischial tuberosity pain, pain on single leg bridge, single leg long lever bridge or single leg deadlift loading tests (3)
Patella	Primary complaint of localised pain at the inferior pole of the patellar, corresponding tenderness on palpation, pain on single leg decline squat or submaximal hop loading tests (4)
Achilles	Primary complaint of Achilles insertion or midportion pain, corresponding tenderness on palpation, pain on calf raise or submaximal hop loading tests (5)
Plantar fascia	Primary complaint of pain at the proximal plantar fascia insertion, corresponding pain on palpation, pain on calf raise or submaximal hop loading tests (6)

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SUPPLEMENTARY FILE 2

Table II Suppl. Differential diagnoses considered (*systemic & sinister pathologies considered and screened for in all cases).

Site of tendinopathy	Differential diagnosis
Gluteal	Lumbar stenosis, radiculopathy or discogenic pathology (1, 2) Sacroiliac joint dysfunction (3) Hip joint pathology and osteoarthritis (4) Ischiofemoral impingement (4) Neck of femur fracture (2) Sciatic neuropathy (2)
Proximal hamstring	Lumbar stenosis, radiculopathy, or discogenic pathology (1, 5) Sacroiliac joint dysfunction (6) Sciatic neuropathy (5) Ischiofemoral impingement, deep gluteal tear, apophysitis or avulsion (5) Tear or rupture of proximal hamstring tendon (5) Pubic or ischial ramus bone stress injury, apophysitis or avulsion (5) Slipped Capital epiphysis (7)
Patellar	Patellofemoral joint dysfunction (8) and osteoarthritis (9) Patellar inferior pole bone stress injury and osteochondroses of the knee (10) Infrapatellar fat pad (11) Plica and chondral surface pathology (12, 13)
Achilles	Sural neuropathy (14) Paratenon (15) Tendon partial tear, rupture (16) Plantaris tendinopathy (17)
Plantar fascia	Tibial neuropathy (18, 19) Calcaneum bone stress injury (18, 19) Fat pad contusion (18, 19)

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ORIGINAL ARTICLE

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Morphometric Profile of Distal Biceps Tendon: A Cadaveric Approach

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SUMMARY

Background. The biceps muscle has proximal tendons that attach the muscle to the shoulder and one distal tendon that attaches at the elbow, called the distal biceps tendon. Normal morphometric measurements of the distal biceps tendon serve as an important landmark in restoring its biomechanical characteristics and anthropometric evaluation during surgical tendon repairs. Hence the objective of this study was to provide a detailed morphometric profile of the distal biceps tendon.

Methods. A total of 50 dissected adult limbs were studied. Out of the 50 limbs, 25 belonged to the right side while 25 were of the left side. The insertion pattern of the distal tendon was dissected and recorded. Total length, post aponeurotic length, the proximal, and distal thickness of the tendon was measured. Results were tabulated and correlated using SPSS.

Results. The mean length of the tendon was 8 cm. The proximal thickness varied from 2 to 4 cm, distal thickness from 1 to 3 cm. Tendon length and thickness showed no statistically significant differences between the right and left sides. However, significant correlation ($p < 0.001$) was observed between length and thickness, proximal and distal thickness of the tendon.

Conclusions. The variation in length, width of distal biceps footprints and radial tuberosities may be significant in the anatomical restoration of the distal biceps tendon. This morphometric study of distal biceps tendon would be beneficial to sports medicine physicians for diagnosis and treatment of biceps tendon tear and tendinopathy.

KEY WORDS

Anatomic biceps repair; biceps rupture; distal biceps tendon; radial tuberosity; tendinopathy; graft.

BACKGROUND

The biceps brachii is one of the key muscles in the flexor compartment of the arm. It has three attachments: long and short heads proximally and a distal tendon at insertion. The long head of the muscle has intracapsular origin and arises from the supraglenoid tubercle of the scapula, whereas the short head is attached to the coracoid process of the scapula. The distal tendon is attached to the posterior rough area on the radial tuberosity. From the medial border of the biceps tendon, a fibrous expansion known as bicipital aponeurosis is given out, which expands and gets attached to the posterior subcutaneous border of ulna (1). Biceps is the chief supinator of the forearm and contributes to flexion at the elbow joint along with the brachialis muscle.

Biceps brachii has been described as one of the muscles with the most frequent anatomic variations mainly concerning its proximal attachment. However, there is a little description regarding the normal and surgical anatomy of the Distal Biceps Tendon (DBT) or its anatomic variations in the literature. Anatomy textbooks provide an ambiguous description regarding the tendon's morphology and its insertion onto the radial tuberosity. Hence, the DBT anatomy is poorly understood. Despite the increase in clinical knowledge, the prevalence of complications following DBT repairs remains high (2). A precise understanding of the anatomy of the DBT and its relationship to the radial tuberosity is crucial to the successful surgical repair of a ruptured tendon (3). Thorough knowledge is essential to optimize

the treatment outcome and to avoid complications when repairing a DBT.

During our literature review, we found that there is a paucity of data on the morphometric measurements of the DBT anatomy. Hence, the purpose of the present study was to determine the key morphology of the DBT (length, width, and thickness) in preserved cadaveric elbows from a surgical perspective.

MATERIALS AND METHODS

A total of 50 dissected upper limbs from formalin fixed, adult human cadavers was studied. Out of the 50 limbs, 25 belonged to the right side while 25 were of the left side. These limbs were obtained from the Department of Anatomy of our Medical College. The study meets the ethical standards of the journal (4). Diseased, fractured limbs were excluded from the study. After removal of skin, superficial and deep fascia, the muscles of the front of the arm were exposed, from origin to insertion. The long and short heads of biceps were dissected, and their relationships with each other and the proportions of each relating to the formation of the distal tendon were observed and recorded. The bicipital aponeurosis and the insertion pattern of the distal tendon were dissected and recorded. The various morphometric measurements of the tendon were measured using a ruler (accuracy, 0.5 mm). Following parameters were measured for each tendon: total length (from the musculotendinous junction of the muscle till its attachment to the radial tuberosity), post aponeurotic length (length of the tendon from bicipital aponeurosis till its insertion) (**figure 1**), proximal thickness (after bicipital aponeurosis), distal thickness (near its insertion) were measured and documented.

The distal thickness of the tendon was noted at the closest point of insertion to the radial tuberosity. These values were then tabulated statistically using SPSS v 16.0. Mann-Whitney U test was used for comparison of the parameters between two independent variables (right and left limb) to find whether there is a statistically significant difference in the morphometric measurements between the two groups. Spearman's correlation analysis was done to determine the level of positive correlation between the measured parameters of the DBT. P value less than 0.05 was considered statistically significant.

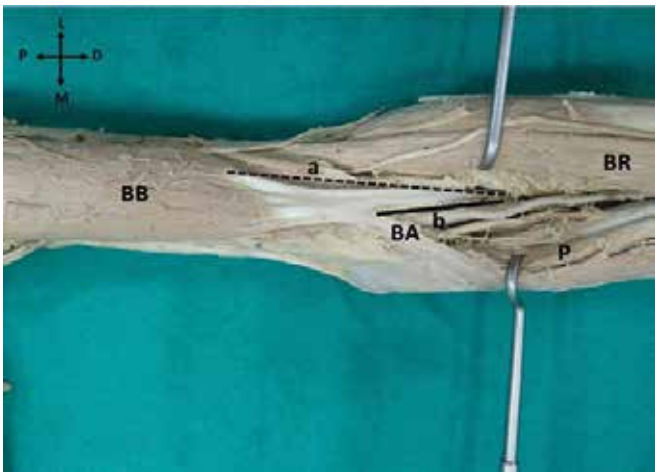

Figure 1. Figure showing the various measurements of the distal biceps tendon. a: total length of tendon, b: post aponeurotic length of tendon. BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

RESULTS

The mean values of the various morphometric measurements of the DBT are shown in **table I**.

DBT length and thickness did not show a statistically significant difference between the right and left side tendons. The average length of the tendon was 8 cm. However, the longest tendon was observed which measured 11.5 cm (**figure 2**) and the shortest tendon being 5.5 cm (**figure 3**). The proximal thickness of the tendon varied from 2 to 4 cm and the distal thickness from 1 to 3 cm. No statistically significant difference was observed in these measurements between the right and left limbs. In all elbows, the insertion of the DBT was situated on the posterior rim of the radial tuberosity of the radius. Further, we could not compare these measurements with other body measurements because all the limbs studied were isolated limbs.

Table I. Table showing the various morphometric measurements of the Distal Biceps Tendon (DBT). Values are expressed as mean ± SD.

	Total length (cm)	Post aponeurotic length (cm)	Proximal thickness (cm)	Distal thickness (cm)
Right limb (n=25)	8.1 ± 1.5	1.9 ± 0.8	3.1 ± 0.6	2.1 ± 0.5
Left limb (n=25)	8.6 ± 1.4	2.4 ± 0.7	3.0 ± 0.4	2.0 ± 0.3

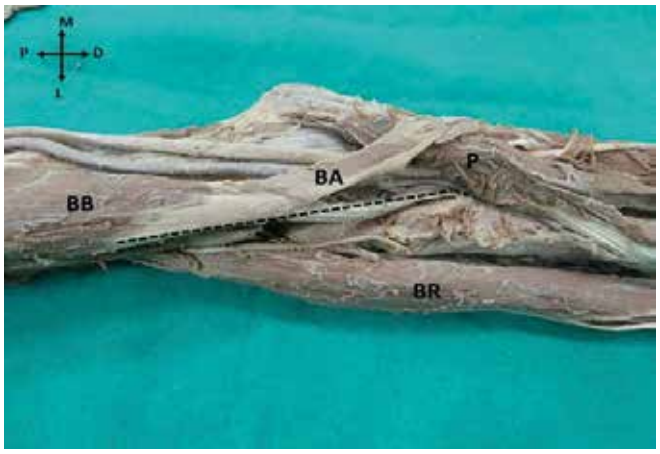

Figure 2. Figure showing a long distal biceps tendon (dotted line). BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

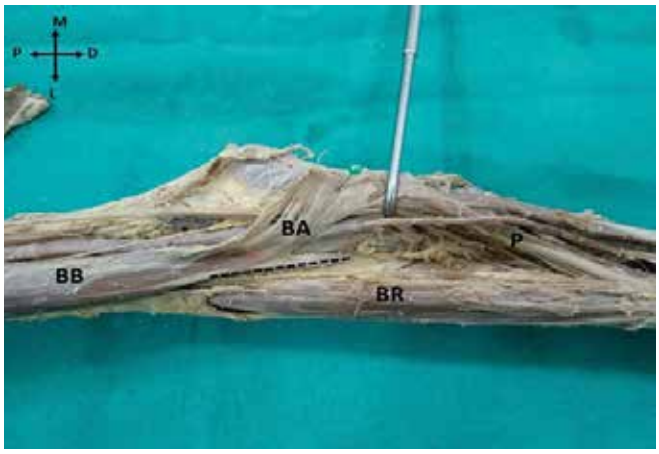

Figure 3. Figure showing a very short distal biceps tendon (dotted line). BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

Spearman's correlation analysis showed significant correlation between total length and post aponeurotic length (r=0.30, p=0.01). Slight correlation existed between the length of the tendon and its thickness (r=0.20) but was not statistically significant. Significant correlation was observed between the proximal and distal thickness of the tendon (r=0.5, $p < 0.001$).

Regarding the formation of the tendon, it was observed that in most of the specimens, the DBT was mainly formed from the elongation of the tendon of the long head. Medial fibers of short head contributed to the formation of bicipital aponeurosis, while lateral fibers contributed to the formation of DBT. However, in few specimens, the DBT was formed equally from both long and short heads (6 specimens). A Uniform pattern was observed in most of the specimens at the Musculotendinous Junction (MTJ). There were few specimens with straight (**figure 4**), 'U' shaped (**figure 5**), and 'V' shaped MTJ patterns (**figure 6**).

DISCUSSION

The purpose of this anatomic study was to focus on the morphometric profile of the distal biceps tendon. Variations in biceps brachii muscle have been observed mainly

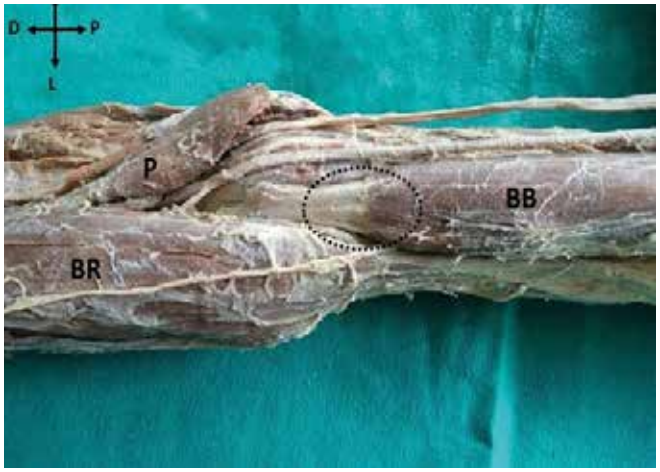

Figure 4. Figure showing a 'straight' musculotendinous junction (circle). BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

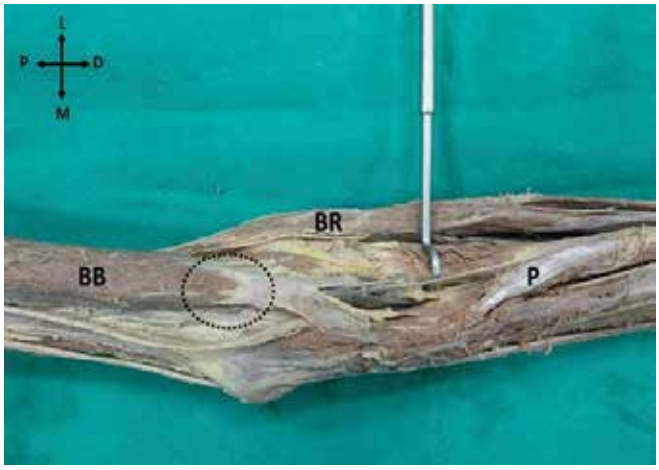

Figure 5. Figure showing a 'U shaped' musculotendinous junction (circle). BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

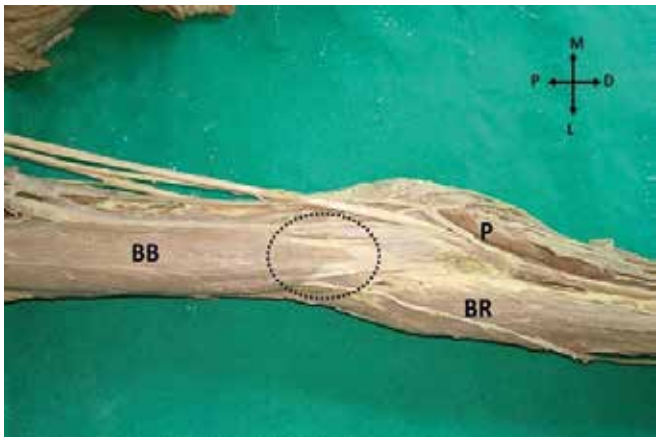

Figure 6. Figure showing a 'V shaped' musculotendinous junction (circle). BB: biceps brachii, BA: bicipital aponeurosis, P: pronator teres, BR: brachioradialis.

in the number of heads of origin (5). Variations or study on the biceps distal tendon at insertion are comparatively rare. Bryce stated that morphological variations associated with the biceps muscle could be due to its characters being attained late in the human phylum. According to him, phylogenetically, biceps was a single-headed muscle arising from the coracoid bone, which merged with brachialis at its insertion point at ulna and radius. Later, the second head of the biceps developed at the base of coracoid bone. This represents the long head of biceps muscle. As the development advanced, the radial head of insertion became the main insertion of the muscle, while the bicipital aponeurosis denoted the ulnar part of the muscle (6). The DBT is of clinical interest as it can show partial or complete tears that need to be distinguished from other naturally occurring anatomical variations (5). A clear understanding of these anatomic variations and its relationship with radial tuberosity is vital in planning and performing a distal biceps tendon repair and also in determining if a graft is required to supplement or replace the native tendon. There are studies done previously which describe the gross morphological anatomy of the distal biceps with main focus on distal footprint types and characteristics (7, 8). But these studies do not give the normal morphometric measurements of the tendon. There are very limited studies which focus on the cadaveric anatomy of the tendon. Cucca *et al*., in their study, have reported the mean length of DBT as 5.7 cm (9), and Joshi *et al*., have mentioned the length of the tendon as 7.5 cm in their study on North Indian population (10). The observed dimension of Joshi *et al*., relates to the present study where the mean length of the tendon was measured as 8 cm. The authors opine that the difference in the findings is due to observational difference. Cucca *et al*., considered the length of the tendon as visible length, wherein, they measured the tendon from the point of muscle fiber termination to its insertion. In contrast, Joshi *et al*., have considered the length as the maximum visible length of the tendon, in which, the tendon was measured from the point of its beginning to its insertion on the radial tuberosity. In the present study, the technique used in the latter study was followed and the maximum visible length of the tendon was measured. We also noted the post aponeurotic length (length of DBT from bicipital aponeurosis to its insertion) and thickness of the tendon at two points (before insertion, at insertion) which would be beneficial for graft selection as a more accurate representation could be obtained by taking the average of multiple points of the tendon. Kulshreshtha *et al*., examined the width and thickness of the DBT at the antecubital fossa in 74 cadaveric arms but did not measure the tendon length (11). The values of proximal and distal thickness of the tendon (3.1 cm and 2.1 cm, respectively) noted in the present study was in range with the previous studies (11, 12). In the present study, significant correlation between the lengths and thickness of the tendon was observed, which indicates that the morphometric measurements are interrelated. Thinner tendons are more susceptible to the risk of tendinopathy and exhibit a higher tendency of rupture when compared to thicker tendons.

Injury to the DBT at its insertion at radial tuberosity is the most common tendinous injury at the elbow (13). Other injuries of DBT include, tearing of the musculotendinous junction and rupture of the tendon itself. Precise understanding of the DBT anatomy and biomechanics in the past years has greatly enhanced a surgeons ability to advise treatment options and treat patients with ruptured DBT (14). Gilcreest has done a comprehensive study regarding the injuries of the entire biceps brachii from origin to insertion. His study of over 100 cases shows that 6 of the cases were of the DBT injuries. Of those, 3 were complete avulsions of the tendon at the point of insertion, 2 were at the musculotendinous junction, and 1 was complete tendon rupture (15). Several authors have expressed their concern over significant loss of range of supination and muscle strength after DBT repair. They have opined that the variation in length, width, area, and shape of the DBT is important in anatomical restoration of the DBT. The radial tuberosity and distal biceps insertion footprint are critical structures which affect the forearm supination mechanics, and anatomical repair of a ruptured tendon is necessary for the restoration of power, endurance, and terminal forearm rotation (16). If the biceps tendon is not repaired to its anatomic location and is merely inserted into the radial tuberosity, the power of supination will never be restored to preinjury levels (17). During our

literature review, we found an incidence of bifurcated distal biceps tendon insertion (18). However, in all the specimens studied by us, the distal biceps tendon was united. The distal biceps tendon was inserted on the posterior rim of the radial tuberosity, which is in accordance with previous study (19). The anatomy of the DBT and radial tuberosity is important to understand the pathophysiology of tendon rupture. It is postulated that the distance crossed by the tendon over the raised ridge on the radial tuberosity, functions as a pulley and increases the mechanical advantage of the musculotendinous unit (20). Nevertheless, in the present work we did not study the measurements of radial tuberosity as our focus was mainly on DBT anatomy. Eames *et al*., in their study on the insertional anatomy of DBT have observed that the posterior attachment of the long head tendon on the radius, potentially increases its supination power, while, the more distal attachment of the short head, generates greater flexion power for the muscle (21). Previously, the anatomy of the DBT has received little attention. Detailed data on the insertion footprint of the tendon and its relationship to radial tuberosity have been reported. Most of the reported studies are computed tomography studies or cadaveric studies on frozen limbs which have focused mainly on the insertional footprint of the DBT, biomechanical studies and surgical reconstruction articles. Very little focus has been given to basic morphometric measurents of the DBT.

A variety of grafts (autografts, allografts, and synthetics) have been reported in the literature to reconstruct the DBT. The decision to proceed with graft and also the choice of the graft should be guided by the understanding of normal DBT morphology. Also, knowledge of the orientation of the tendon fibers near the musculotendinous junction (MTJ) can help guide graft-weaving orientation and suture placement, which improves the proximal fixation (12). In the present study different patterns of MTJ were observed. However, the relationship between the MTJ patterns and the DBT rupture currently lacks in the literature.

Limitations of this present study include small sample size, the use of embalmed cadaveric specimens, and the lack of consideration of demographic information, such as height, weight, and hand dominance. Although it was observed that the distal biceps tendons were slightly thicker in the right limbs than the left limbs at the elbow level, it might reflect the hand dominance of the population. However, we did not find any relevant studies in the literature supporting this assumption.

CONCLUSIONS

The present study attempts to describe the various morphometric measurements of the DBT that might be of relevance to the surgical inventions in distal biceps tendon repair. The variations in MTJ patterns, length and thickness of the tendon at various points and its insertion at radial tuberosity has received renewed interest as these factors manily contribute in tendon reattachment. Surgeons can use these cadaveric morphometric dimensions of the DBT when deciding whether reconstruction is necessary, as well as for graft selection of similar morphology. Nonanatomic reconstruction of a ruptured biceps tendon can lead to some amount of rotational weakness and loss of supination strength. Hence, it is rational to assume that surgical repair should replicate the normal anatomy as close as possible to ensure an excellent clinical outcome. Thus such studies are essential in the anatomical restoration of the DBT and for sports medicine physicians for diagnosis of tendinopathy.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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REVIEW

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Operative *Versus* Non-Operative Management of Distal Biceps *Brachii* Tendon Rupture: A Systematic Review

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SUMMARY

Background. Distal biceps *brachii* tendon ruptures occur rarely accounting for 3% of all biceps tendon injuries. This systematic review aims to assess the outcomes of both operative and non-operative management of distal biceps *brachii* tendon rupture for adult patients. The literature has mostly advocated that surgical intervention to be the best management of such injuries however in recent times studies have shown acceptable outcomes of non-operative treatment in those affected and therefore offer an alternative to current practise.

Methods. The NICE Healthcare Databases Advanced Search (HDAS) tool was used to identify articles in the PubMed, Medline, EMBASE, and the Cochrane Central Register of Controlled Trials databases to identify articles relating to distal biceps *brachii* tendon rupture management. The search strategy was formed by using the set key words. Studies published from 1998-2008 were included. Functional outcome questionnaires and isometric strength tests were used. An inclusion criterion identified articles to be critically appraised.

Results. Initially 427 papers were identified with the key words in the search approach from the use of the electronic databases. 294 papers were excluded because they were either duplicates or unrelated to distal rupture of the tendon biceps *brachii*. It recognized 17 papers and abstracts of which 11 were omitted because they were either in a different language or were case reports.

Conclusions. This review highlights that the current evidence for surgical management of distal biceps *brachii* tendon rupture is weak. Studies in recent times have mostly focused on the different methods of surgical fixation. We noted that surgical fixation resulted in a large number of complications, which if communicated to the patient may result in them opting for conservative management.

However equally important to highlight is that patients who are middle-aged, with a modest physical demand should be made aware that if treated non-operatively they may likely have good to excellent functional outcome as well as having approximately 50% strength in the flexed and supinated position of the injured arm, whilst avoiding the potential complications of surgery. This review also highlights that more higher quality evidence is required in future.

KEY WORDS

Distal biceps brachii tendon rupture; distal biceps tendon rupture; surgical treatment; conservative treatment; systematic review.

INTRODUCTION

In 1843 Starks reported the first distal biceps *brachii* tendon rupture. The first repair of this injury was reported fifty years later in 1897 by Johnson (1, 2). Distal biceps *brachii* tendon ruptures occur rarely accounting for 3% of all biceps tendon injuries (3), with the majority of ruptures occurring in the tendon of the long head of biceps or more frequently in the short head of biceps. It occurs when the flexed and supinated elbow has an abrupt eccentric load applied and most often involves middle aged men who are active and in their

4th to 6th decades of life (4), with incidence reported to be 1.2 per 100,000 per year. Smoking and steroid use have been identified as two independent risk factors (5). The literature typically recommends that operative treatment offers the best outcome, with several retrospective case series concluding that strength is best restored with surgical intervention (6). Operative treatment has a reported complication rate ranging from 23% to 27% (7, 8). This is due to the close proximity to surrounding structures such as the posterior interosseous nerve, the median nerve, the lateral cutaneous nerve to the distal biceps *brachii* tendon, to common interosseous artery and the radial recurrent branch of the radial artery (9). There are a range of surgical approaches that have been used: a single anterior incision approach (10, 11), a two incision approach developed by Boyd and Anderson (12), a modified two incision approach known as the "Solento Technique" (13), the distal biceps being attached to the brachialis (14), as well techniques which employ a suture anchor, bone tunnel, interference screws or cortical buttons (15). The most common complication with the anterior approach is that of nerve injury, with the posterior interosseous nerve being injured in 5% of cases and the lateral antebrachial cutaneous nerve being injured in up to 7% of cases. In the two incision approach the most common complication is that of heterotopic ossification in 10% to 15% of cases (16). There are other reported complications such as re-rupture, infection, persistent pain, reduced range of motion, complex regional pain syndrome (7, 8). The risks of operative management lead to the question of whether the risks of surgery are greater than the benefits.

Carroll *et al*. reported that at the time of one year follow up, there was no loss of flexion and supination in twenty patients when treated conservatively for distal biceps *brachii* tendon rupture (17). Morrey *et al*. reported that 3 patients who had been treated conservatively had 31% flexion strength and 40% supination strength when compared to normal individuals (18). Baker and Bierwagen compared the injured arm with the contralateral arm when treated conservatively and reported that the average differences in flexion and supination strength of 21% and 27% respectively, in addition to reduced endurance (19).

More recently there have been studies comparing operative treatment to non-operative management of distal biceps tendon rupture. Chillemi *et al.* reported in those who had anatomical repair had superior European Society for Surgery of the Shoulder and the Elbow scores (SECEC), in comparison to those who were treated conservatively (20). Hetsroni *et al*. concluded that even though surgical repair resulted in higher satisfaction, non-operative management resulted in good to excellent outcomes as well and therefore should be a considered treatment plan given the potential complications of surgery (21). Freeman *et al.* concludes that nonoperative treatment can produce satisfactory outcomes with modestly reduced strength (4). In their study, Pavelka *et al.* reported that the loss of supination and flexion strength was not as severe as reported in the literature and that non-operative management was an acceptable alternative (22).

The aim of this systematic review is to assess the outcomes of both operative and non-operative management of distal biceps *brachii* tendon rupture for adult patients. The literature has mostly advocated that surgical intervention to be the best management of such injuries however in recent times studies have shown acceptable outcomes of non-operative treatment in those affected and therefore offer an alternative to current practise, avoiding the potential serious complications associated with repairing a ruptured distal biceps *brachii* tendon.

METHODS

To guide the systematic review the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used and adhered to (23). Studies comparing non-operative management to surgical management of distal biceps *brachii* tendon rupture as well as studies assessing the effectiveness of non-operative management alone were evaluated against the set inclusion criteria. To formulate the inclusion and exclusion criteria (**table I**), the PICOT criteria was used (24). This focused the search and reduced ambiguity. Surgical intervention included all types of anatomic re-insertion of the distal biceps *brachii* tendon to the radial tuberosity or any other area such as the brachialis. No restrictions were placed on the way patients were treated conservatively. Studies that used the Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire, shortened Disabilities of the Arm, Shoulder, and Hand (QuickDASH) questionnaire, Single Assessment Numerical Evaluation (SANE), Biceps Disability Questionnaire (BDQ), Oxford Elbow Score (OES), Mayo Elbow Performance Index (MEPI), Visual Analogue Scale (VAS), Broberg and Morrey Functional Rating Index (BMFI), European Society for Surgery of the Shoulder and the Elbow score (SECEC), the Constant Score (CS), American Shoulder and Elbow Surgeons score (ASES) and modified Neer Scale as a way to measure the functional outcome were eligible. Publication time was limited to the years 1998 and 2018 and to English-language papers.

To search for papers related to distal biceps *brachii* tendon rupture management, the NICE Healthcare Databases Advanced Search (HDAS) tool was used to define articles in the PubMed, Medline, EMBASE and Cochrane Central

Table I. Criteria for inclusion and exclusion.

	Inclusion Criteria	Exclusion Criteria
Population	-Age > 16	-Age < 16 -Bilateral distal biceps tendon *brachii* rupture
Intervention	-Operative management of distal biceps *brachii* tendon rupture -Non-operative management of distal biceps *brachii*	Not Applicable
Comparison Group	-Studies comparing operative with non-operative management	Not Applicable
Outcome	-Studies measuring an outcome of the intervention using standardised scores	-Studies not measuring functional outcome or isokinetic strength
Time	-Studies between the years 1998 to 2018	-Studies published prior to 1998
Study type	-Randomised Control Trials -Clinical Trials -Cohort Studies -Observational Studies	-Letters -Case reports
Language	-English only	-Studies published in languages other than English

Register of Controlled Trials databases. Two independent co-authors (M.Q, A.E.) undertook the search process. All relevant articles and reviews were screened for suitable papers. To formulate the search approach, the following main terms were used:

- "Distal Biceps Tendon Rupture" OR "Distal biceps *brachii* rupture" AND "Non-operative treatment" OR "Non-operative management" OR "Conservative management" OR "Conservative treatment".
- "Distal Biceps Tendon Rupture" OR "Distal biceps *brachii* rupture" AND "Operative treatment" OR "Operative management" OR "Surgical management" OR "Surgical treatment".
- "Distal Biceps Tendon Rupture" OR "Distal biceps *brachii* rupture" AND "Non-operative management *versus* operative management" OR "Non-operative treatment *versus* operative treatment" OR "Conservative management *versus* surgical management" OR "Conservative treatment *versus* surgical treatment".

Articles that matched all elements of the requirements for inclusion were then critically evaluated using the Critical Appraisal Skills Program (CASP) tool (25) and The Cochrane Handbook for Systematic Reviews of Interventions (26) (**figure 1**).

This review meets the ethical standards of the Muscles, Ligaments and Tendon Journal (27).

RESULTS

Initially 427 papers were identified with the key words used in the search approach from the use of the electronic databases. 294 papers were excluded because they were either duplicates or unrelated to distal rupture of the tendon biceps

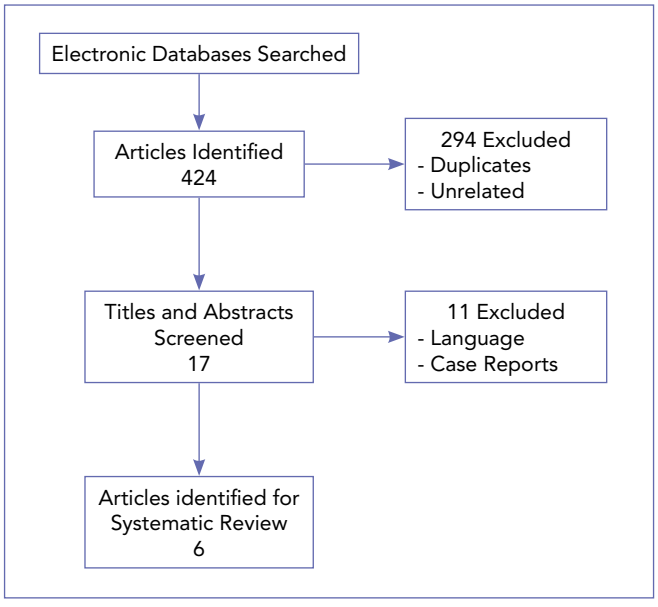

Figure 1. Flow diagram of search process.

brachii. It recognized 17 papers and abstracts of which 11 were omitted because 3 were in a different language and 8 were case reports.

DISCUSSION

Surgical treatment of distal biceps *brachii* tendon repair has been the mainstay of treatment since the 1980's after Baker *et al.* (19) and Morrey *et al.* (18) published their research, stating that the power of supination decreased by 40% and endurance decreased by 47%, with patients reported having trouble with tasks requiring supination. Since then the focus in the literature has been to look at the optimal surgical intervention for the best outcomes, until recently in 2007 where Chillemi *et al.* (20) compared surgical intervention to non-operative intervention. Even though they concluded that surgical intervention was superior it stimulated the question of what the outcomes were in those treated non-operatively. Since 2007 there have been numerous studies published looking into this. The majority have been retrospective in nature and with low numbers, which is expected given that surgical intervention has been the convention for decades (**table II**).

Chillemi *et al.* conducted a retrospective study looking at nine patients who had sustained traumatic total distal biceps *brachii* tendon ruptures, five of which underwent surgery, and four patients were treated conservatively. All the patients were male and had a median age of 49. Patients were followed up after median 24 months. The SECEC elbow score was the only outcome measure used. Otherwise the patients were clinically and radiologically examined. They discovered a minimal deficit of flexion and extension in two patients, decreased supination in six patients and decreased pronation in four patients. Two patients experienced a post-operative paralysis of the deep branch of the radial nerve, which recovered after two and six months. However, they did not specify from which group these findings were found in. They noted that on those who had surgical intervention, two patients were reported to have radial tuberosity heterotopic bone formation at the site of tendon re-insertion, as well as ectopic ossification more proximally in the biceps muscle region. Looking at their SECEC results, each item tested was in favour of surgery and therefore they simply found that surgical management was superior to conservative management. Even though the paper did not explore the outcomes of the non-operative group in great detail, it is significant as it was one of the first papers in recent times to look into both management options (20).

Hetsroni *et al.* conducted a retrospective study which looked at 29 middle-aged men with a mean age of 47 who had been diagnosed with total distal biceps *brachii* tendon rupture. Twelve of the patients were treated non-operatively, whereas seventeen were treated with surgical management. Patients functional outcome was evaluated using both the DASH and the modified Neer scale questionnaires. Isokinetic evaluation was also undertaken. In those operated on, the range of forearm rotation declined by a mean of 11 degrees in supination and 17 degrees in pronation compared to no deficit in both supination and pronation in the conservatively managed group. Functional outcome evaluation using both the DASH and Neer scale questionnaires demonstrated superior functional outcome and increased satisfaction in the surgically managed group compared to the conservatively managed group. A mean loss of 20% of the maximum strength of elbow flexors and a minimal flexor endurance deficit were observed for those treated conservatively when isokinetic testing was carried out. The operated group had regained full strength when tested. When testing supination, maximal strength was reduced by 35% and endurance reduced by 40% in those treated conservatively. Whereas in the operated group there was 15% loss in supination maximal strength and 10% supination endurance loss. Two patients treated with surgery suffered complications. One patient gradually developed median nerve paraesthesia and was noted to have heterotopic ossification of the proximal volar forearm after six months of the operation date. Symptoms were not spontaneously resolved, and the calcified mass was removed surgically, followed by full resolution of symptoms. The second case involved impaired posterior interosseous neuropathy, immediately after surgery and resolved four weeks later. This study made a point to exclude athletes and only included middle aged men as they believed this to be representative of the majority of patients who suffer this injury. They concluded that even though surgical repair of a completely avulsed distal biceps *brachii* tendon achieves a greater subjective functional outcome and greater strength and endurance. Patients should be made aware that non-operative management is likely to lead to good to excellent functional outcomes, avoiding possible surgical complications (21). The weakness of this paper was that it had a low number of patients.

Freeman *et al.* conducted a retrospective study comparing patients who had been treated non-operatively in their institute for total distal biceps *brachii* tendon rupture with a historical control using past studies in which patient's had been treated surgically. Twenty men and two women were identified in their institute who had been treated conservatively, of which four were excluded from the study. The median age was fifty years and the median follow up was thirty-eight months. Functional outcomes were measured using the MEPI, BMFI and DASH questionnaires. Isokinetic strength testing was also undertaken. The affected arm

Table II. Summary of articles identified for review.

Author	Type of Study	Country of study	Patient Cohort	Treatment	Follow up	Primary Outcome	Conclusion
Chillemi *et al.* (2007)	Retrospective	Italy	-9 male patients -Median age 49 (39-53) years	-5 treated operatively -4 treated conservatively	-24 months median follow up	-SECEC	Patients who had surgical intervention scored better in the 4 assessed categories of pain, activities of daily living, painless activities movement and strength
Hetsroni *et al.* (2008)	Retrospective	Israel	-29 male patients -Mean age 47 (37-63) years	-12 treated operatively -10 treated operatively -7 excluded	-2 years mean follow up (1-5 years)	-DASH -Modified Neer Scale -Isokinetic evaluation	Surgical repair results in superior outcome in subjective and objective measurements. However conservative treatment results in good to excellent outcome as well
Freeman *et al.* (2009)	Retrospective	USA	-24 patients -22 male -4 female -Median age 50 (35-74) years	-All had non-operative treatment -4 excluded	-38 months median follow up; mean, 59 months; range, 11 to 146 months	-DASH -MEPI -BMFI -Isometric strength test	Conservative treatment can yield acceptable outcomes with modestly reduced strength, particularly supination
Pavelka *et al.* (2015)	Retrospective	Austria	-206 patients -Mean age 52 (25-79) years	-176 treated operatively -30 treated conservatively -Mean age of those treated conservatively 51.6 (25-77) -4 excluded	-80 months mean follow up (18-138)	-MEPI -Isometric strength test	Conservative management resulted in high patient satisfaction. Given that there is a loss of strength and a potential risk of complications, conservative management is an adequate alternative option
Legg *et al.* (2016)	Retrospective	UK	-65 male patients -3 patients had bilateral ruptures	-55 treated operatively -13 treated conservatively -Mean age 42 (30-60) years in operated group -Mean age 54 (42-57) years	-3 years median follow up in operated group -2 years median follow up in conservative group	-QuickDASH -OES -Isometric strength test	Surgical repair results in superior functional outcome scores and significantly weaker isometric flexion and supination
Schmidt *et al.* (2018)	Prospective	USA	-32 patients	-14 treated conservatively -18 patients from matched control group -Mean age 54 ± 15 in nonoperative group -Mean age 51 ± 7 in control group	-3.1 ± 2.0 years	-DASH -SANE -BDQ	Patients treated non-operatively will have a varying degree of functional loss. Resulting in a change of supination kinematics during repetitive activities, leading to a loss of 47% of supination power

was found to have a median supination strength of 63% and a median flexion strength of 93% compared to the uninjured arm. In those who had suffered the injury in the non-dominant arm had good results when strength was compared to the uninjured dominant contralateral arm. The non-dominant arms had median flexion and supination strengths of 80% and 71%, respectively, compared to the uninjured arm. The median flexion and supination strengths of those with injuries to the dominant arm were 63% and 100%, respectively, compared to the uninjured arm. Every patient had a full range of motion, with the exception of one, who was noted to have an extension flexion arc of 10 degrees to 115 degrees, 80 degrees of supination and 70 degrees of supination. Looking at the functional outcome scores patients returned satisfactory scores overall. Eight patients described subjective weakness when attempting to lift weighty items and six patients described subjective weakness when supinating the affected arm. A significant difference in mean strength between the dominant affected arms and the non-dominant affected arms in terms of flexion were noted when statistically analysing these patients, but not in terms of supination. When comparing their patients to the historical control group the authors concluded that patients managed non-operatively achieved satisfactory results in both strength testing and functional outcome scores. Given the advantage of avoiding operational risk, their research suggested that those who are cautious about getting surgery, who present late or who are too unwell to undergo surgery, conservative management of distal biceps *brachii* tendon rupture is likely to accomplish acceptable results with modestly decreased supination strength (4). The patients in this study were compared to a historical control group derived from the literature and therefore leading to inaccuracies in comparing strength scores as there was a difference in equipment used when testing isokinetic strength. However, they did compare the injured arm to the uninjured arm and stated whether it was dominant or non-dominant hand when testing strength.

Pavelka *et al.* conducted a retrospective study looking at two hundred and six patients who had suffered from partial or total distal biceps *brachii* tendon rupture in their institute between 1999 and 2010. Of These patients, thirty were treated non-operatively. Functional outcomes were measure using MEPI and isokinetic strength measurements of supination and flexion were tested. Twenty-four patients were followed up, of which the mean age was 51 years. The group consisted of twenty-two men and two women. Of these patients, four were noted to have a partial rupture. In this study, it was shown that the mean Range of Motion (ROM) for flexion to be 139.1 degrees, for pronation to be 85 degrees and for supination to be 83.75 degrees. Elbow extension was normal in all patients except one who had a 20-degree deficit. All patients apart from two had normal supination. When examining the strength of the injured arm and comparing it with the un-injured arm, the loss of strength in the flexed position was 16.1%, 17.3% in the neutral position and 18.1% in the supinated position. A minor decrease in flexion strength was observed in the group of partial ruptures and no difference in supination strength was discovered. The authors found that their research indicates that we should also reconsider always opting for surgical management as the suggested treatment option can also produce excellent outcomes. Especially in the elderly and those who have low physical demands, non-operative management may be a suitable option. This study had a small number of patients treated non-operatively as well as including patients with partial ruptures of the distal biceps *brachii* tendon repair. They did not compare the group to those treated surgically and stated they were unable to perform statistical analysis due to the small numbers (22).

Legg *et al.* conducted a retrospective cohort study of patients who had suffered total biceps *brachii* tendon rupture and who had either surgical treatment or treated conservatively. 65 patients were identified from 2002 until 2013 and all were male. 55 patients underwent surgical treatment and 13 were treated conservatively. Of these patients 50 were available for follow up. The mean age of the operated group was 42 years compared to 54 years in the non-operated group. The average follow-up was 3 years in the operated group and 2 years in the non-operated group. Isokinetic strength was tested, and functional outcomes were measured using the OES, MEPI and QuickDASH questionnaires. 75% of patients had ruptured their distal biceps *brachii* tendon in their dominant hand. Functional outcome questionnaires revealed significantly better mean scores in the operated group. In either cohort, the range of motion was not significantly reduced compared to the uninjured arm. The isometric flexion and supination strength differed significantly when the operated group was compared with the conservatively managed group, the latter being weaker. Two patient's developed posterior interosseous neuropath in the surgically treated group, which recovered completely within 3 months. Incidental heterotopic ossification and tunnel lysis was noted in these patients but no impact on function was found. The authors concluded that surgical management restored normal flexion and supination as well as near normal strength in these planes of motion. In doing so, they did not focus on the fact that their results showed acceptable outcomes of those treated conservatively.

Schmidt *et al.* undertook a prospective cross-sectional study in their institution comparing nonoperatively managed patients who had unilateral complete distal biceps *brachii*

tendon rupture with a matched unaffected control group. The functional outcomes were measured using the DASH, SANE and BDQ questionnaires. Isometric testing was undertaken using a custom device designed to re-produce high intensity forearm supination motions. 14 patients who were all male, had an average age of 54 and had sustained a distal biceps *brachii* tendon rupture participated in the study. The time between injury and testing was 3.1 years. 12 of the men injured their dominant arm. The non-operative patients reported having difficulty in light or intense tasks or both. The functional outcome scores in the control group were significantly better compared to the non-operated group. It was observed that non-operatively managed patients replaced the lost biceps function by increasing the adduction of the shoulder to perform supination activities. On average, these patients reduced their arc of supination by 27%, transferring the centre of the rotation arc to 45 degrees in a more pronated position and using 27% more shoulder adduction to perform the tests. The study also was able to show a power reduction of up to 47% in the injured arm. The authors found that the non-operated patients perceive some degree of impairment and that the patients compensated by locking their wrist and adducting their shoulder to perform supination tasks. This study is the first to state that there is a greater loss in elbow supination rather than elbow flexion as noted in all the other studies. The comparison in this study was made with a control group made up of uninjured patients and therefore potentially exaggerating the differences noted between the two groups. Surgical management has been the mainstay of treatment for decades and has been considered to result in considerably greater outcomes, resulting in the lack of high-level studies to evaluate this treatment method. This has led to the majority of studies comparing surgical methods without considering the outcome of those treated non-operatively.

Limitations

This systematic review focused on 5 retrospective studies and 1 prospective study. There were no randomised controlled trials conducted in this area. Therefore, high quality evidence is lacking from this review.

An extensive search was performed, however there is a possibility that studies eligible for review were not identified due to a possible lack of robustness in the search terms or due to publication bias. A language bias was also introduced as studies written in languages other than English were excluded. Study selection and data extraction was carried out by one reviewer, introducing a selection bias. The literature says that at least two assessors are needed to evaluate data and conduct analyses to prevent selection bias (26).
Regarding the studies identified there was range of questionnaires used to assess functional outcome. When undertaking isokinetic tests, there was a range of devices used to measure strength. The studies all had very low patient numbers.
One study included both total and partial ruptures. Two studies grouped male and female patients together.

CONCLUSIONS

This review highlights that the current evidence for surgical management of distal biceps *brachii* tendon rupture is weak. Studies in recent times have mostly focused on the different methods of surgical fixation. We noted that surgical fixation resulted in a large number of complications, which if communicated to the patient may result in them opting for conservative management.
However equally important to highlight is that patients who are middle-aged, with a modest physical demand should be made aware that if treated non-operatively they may likely have good to excellent functional outcome as well as having approximately 50% flexion and supination strength in the affected arm after injury, whilst avoiding the potential complications of surgery.
This review highlights the need for higher level evidence. Given that the outcomes of the non-operatively treated patients are satisfactory, this review challenges the *status quo* and advocates that higher-level studies should be conducted. Non-operative treatment can be a good alternative in those who are less active and of low demand.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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A description of Physical Therapists' Knowledge in Basic Competence Examination of Musculoskeletal Conditions: an Italian National Cross-Sectional Survey

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SUMMARY

Background. According to the World Health Organization, musculoskeletal conditions (MSC) are a major contributor of disability worldwide. The prevalence and impact of MSC has created the need for more careful reflection on how all health professionals are trained about the appropriateness and cost-effectiveness of treatments for these complaints.

Aim. To evaluate basic musculoskeletal knowledge in a population of Italian students and physiotherapists.

Methods. Adaptation in Italian context and administration of 25-items quantitative cross-sectional paper-based survey was conducted. 460 physiotherapy students and graduates were involved in the survey. The survey focuses on the most common diagnoses seen in the musculoskeletal field and in the context of primary care. Data was analyzed by descriptive and inferential statistics.

Results. Four-hundred and sixty respondents completed this paper-based survey. Overall respondents' average score was 45.03/100 (pass rate of 7.8%). Physiotherapists with degrees (n=318) obtained an average score of 49.28/100 (SD +/- 18.08), with an overall pass rate of 10.7%. Physiotherapists with degrees and specialized in Manual Therapy obtained higher scores than non-specialized colleagues (62.40/100, SD +/- 16.63 and 39.83/100, SD +/- 15.90, respectively). Moreover, physiotherapists specialized in Manual Therapy obtained a significantly different sufficiency pass rate and did better than their non-specialized colleagues (28.3% *versus* 1.7%, $p < 0.01$) and even better than their colleagues specialized in Sports physiotherapy (average score 52.89/100, SD +/- 17.50, pass rate 11.1%). Physiotherapists with a Master of Science averaged a score of 61.37/100 (SD +/- 17.94) and a pass rate of 37.5%. Second year physiotherapy students scored 15.83/100 (SD +/- 10.57), while third year students scored 39.53/100 (SD +/- 14.26); however, students achieved a very low pass rate (0% and 1.7% respectively for the 2nd and the 3rd year students).

Discussion and conclusions. This study provokes deep reflection on the structure and contents of the physiotherapy degree course: the results of this survey could lead to a radical restructuring of the academic syllabus so as to allow this scientific discipline to reach its full potential.

KEY WORDS

Italian survey; musculoskeletal conditions; physiotherapy; professional competence; screening.

BACKGROUND

According to the World Health Organization, Musculoskeletal Conditions (MSC) are: a) a major contributor of disability worldwide, with lower back pain being the single leading cause of disability globally; b) highly prevalent throughout a person's lifespan, occurring at all decades over the course of adulthood; and c) a particular economic burden in developed countries, where more than half of chronic medical conditions in patients around the age of fifty are bone and joint disorders (1). The United States Bone and Joint Initiative (USBJI) noted that MSC strikes half of the adult population of the United States of America (USA), around 126 million individuals, far outstripping circulatory (31%) and respiratory (28%) diseases (2). Furthermore, recent economic analyses of spending estimates stratified by condition, age and sex group, and type of care in the USA (3), indicate that lower back and neck pain accounted for the third-highest amount, with estimated health care spending of $ 87.6 billion (4). Due to this, the prevalence and impact of MSC has created the need for a more careful reflection on how all health professionals are being taught about the appropriateness and cost-effectiveness of treatments for MSC.

As physiotherapists are health professionals associated with treating patients with MSC (5), it follows that each professional should demonstrate proficiency in MSC management. The medical profession requires a more critical review of the MSC content in training clinicians and students (6).

Surveys regarding basic screening skills relating to the musculoskeletal area began with the development and validation of the Basic Competency Examination in Musculoskeletal Medicine (BCEMM) in 1998 by Freedman and Bernstein, a 25-items open answer questionnaire which evaluated competences concerning the knowledge of MSC (6). The prevalence and costs of MSC increase and access to physiotherapy so physiotherapist have to provide guideline-consistent non-pharmacological care. However, so far, there are no published studies that have investigated the levels of musculoskeletal knowledge of Italian physiotherapists. For such reason, the main aim of this study is to evaluate the musculoskeletal basic knowledge in a population of Italian students and physiotherapists by means of a cross-cultural adaptation and administration of the BCEMM; the secondary aim of this survey is to compare the results of Italian respondents with American ones.

MATERIALS AND METHODS

A quantitative paper-based cross-sectional survey was used to evaluate knowledge in the management of MSC. The survey was administered to Italian students and physiotherapists following the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) guidelines (7) and STrengthening the Reporting of OBservational Studies in Epidemiology (STROBE) (8) between June and December 2016.

This survey was approved by Ethical Committee of ASL Lecce (Italy) (report number 36, of the 13th of May 2016).

Participants and setting

This survey was administered to 2nd and 3rd year Italian students of Physiotherapy and to Italian physiotherapy graduates.

The Italian Association of Physiotherapists (AIFI) promoted the survey and sent a mail to its regional offices in order to invite participants to the study. A further reminder was sent three months after the first contact in case of missing response. Furthermore, AIFI requested the collaboration of the coordinators of three-year degree courses and Masters' degree courses of specialization in Manual Therapy and in Sport Rehabilitation. Masters' degree course of specialization in Manual Therapy is an advanced specialization training that represents the academic post-graduate program in Manual Therapy in Italy, based on the standards established by the International Federation of Orthopaedic Manipulative Physical Therapists (9). The post-graduate study courses (Specialized Masters) in Sports Physiotherapy aim to develop advanced skills referring to the standards dictated by the International Federation of Sports Physiotherapy (IFSPT) official WCPT subgroup, for the recognition of the title of Italian Sports Physical therapist (10).

The following groups were included within the target population; a) physiotherapists (three years "standard" University course) that answered to the AIFI recall and participated to the on-topic meeting for administration of the survey, b) physiotherapist students that were registered on the University Graduation Courses that comply to participate in the survey, c) physiotherapists specialized in Manual Therapy (OMPts) and Sport Rehabilitation that agreed to complete the survey and d) physiotherapists with Master of Science (three years of basic graduation plus two years of "advanced" University course) (11). All participants completed the informative consent form before taking part in the survey. In order to allow comparisons between competences based on the current Italian University system, samples were divided according to different educational levels of the respondents: students in their second and third year, physiotherapists (graduates, three years), physiotherapists with first level graduation plus Specialization in Manual Therapy or Sport Rehabilitation (specializations courses had an average of 16 months), physiotherapists with first level graduation plus Master of Science (two years after basic 3-years Physiotherapy degree).

Questionnaire development and pre-testing

The questionnaire administered was the Italian Version of the BCEMM. To improve the consistency of the content and face validity, the authors of this paper made a trans-cultural adaptation process following Beaton's Guidelines, 2000 (12) (detailed steps for cultural adaptation of the BCEMM into Italian are showed in **Appendix 1**).

Questionnaire implementation

Questions of BCEMM were based around the most common diagnoses seen in the musculoskeletal field and in the context of primary care (6). Pathologies which require immediate referral to a specialist or pathologies not requiring physiotherapist expertise, or pathologies that require emergency room treatment were also included (6). The whole administered questionnaire was reported in **Appendix 2**.

The socio-demographic variables were implemented from Friedman's study questionnaire and were investigated by 6 open questions (*e.g.*, age, graduation year) and 5 closed multiple-choice questions (*e.g.*, gender, educational qualification).

For clinical questions, authors in this paper and experts form the Expert Committee decided not to improve, modify or make any changes to the questions because they judged them to be complete and exhaustive as far as the content of the questionnaire was concerned. This also makes it possible for the authors to compare BCEMM-I with preceding administrations of the questionnaire (6, 13-15).

Questionnaire administration

The different regional Committees contacted by AIFI, organized some local meetings in which the survey was administered. Firstly, the authors explained the aims of the study. The participants expressed their willingness to participate in the voluntary study signing the consent forms. The forms with the demographic questions were handed out. When all the demographic questions forms were completed and handed in, the main questionnaire was then handed out and the participants were given 30 minutes to complete it. In the past administrations of the questionnaire (6, 13-15), no time limit for completion was ever decided. However, the authors of this study opted for a 30 minutes time limit due to administrative reasons (*i.e.* availability of co-workers to administer the questionnaire).

The questionnaire was completed under supervision (1 supervisor for about 10 respondents).

Data collection procedure

The survey was paper based, so every time that the questionnaire was administrated to a group of respondents, all the documentation was put into an envelope by a referring collaborator, sealed and signed, and then delivered to the first author of this study (GG). After the demographic information had been collected, the contents of the test and all the study data was archived in the AIFI National Headquarters, stored in a safe to guarantee the privacy of the participants' data as declared to the Ethics committee.

ANALYSIS

The privacy of the subjects was maintained by using study ID numbers assigned by a secretary who was unaware of the study. ID numbers were obtained from the chronological number of the survey's completion. In addition, unidentified demographic data was obtained, including details regarding the participant's training background, their qualifications (*i.e.* students, three years degree, Specializations in Manual Therapy or Sport Rehabilitation, Master of Science) and work experience as free-lance professionals or as employees.

The chief investigator (GG) trained 4 of the judges (FB, MP, AS, FBo) on how to award points to the participants. The judges and the principle investigator are all physiotherapists specialized in MSC and work as university lecturers in Post-graduated Master for Rehabilitation in Musculoskeletal fields. Therefore, the judges conducted a blind correction of the questionnaires for the calculation of the overall score, following the procedures described by Freedman and Bernstein (6): a) a maximum point of 1 was awarded for each question; b) zero points for unanswered question; c) the validation process used a partial credit-weighted system to award a mark to partially correct answers; d) spelling mistakes were not to be taken into account; e) the overall score was obtained by adding up the scores for each individual question and the result was then multiplied by 4 to obtain the score as a percentage (6). The entire process was supervised by the chief investigator. The results of the questionnaires and the demographic data were recorded on an excel file that was sent by e-mail to a statistician (MM), who performed a blind statistical analysis.

Participants whose score was above the previously established threshold of 73.1% would be deemed successful in passing the test (6).

Frequency distribution for categorical variables and measures of central tendency with standard deviation for continuous variables were calculated using STATA for Windows 11.0.1

(Calcago, IL). T-test for independent samples and Kruskal Wallis test were both used to compare the knowledge among the different levels of education (*i.e.* students, graduates of three years courses, respondents specialized in Manual therapy, respondents specialized in Sport Rehabilitation, respondents with Master of Science). Alternative analysis of variance (ANOVA) is used to compare the means between more than two groups. The difference in pass rates among the sub groups of physiotherapists divided by level of education was tested using Pearson's chi-squared test. The significance level was established beforehand as being < 0.05.

RESULTS

All participants (n=460, 278 males and 182 females) Mean sample age was 27.7 years, SD +/- 7.3; this parameter was calculated on 447/460 subjects as 13 participants did not declare their age. All respondents completed the informed consent form before taking part. No student or graduate physiotherapist reported having any knowledge of the content of the questionnaire used in the study. Mean scores on the questionnaire and pass rates are reported in **table I** and **table II**, respectively. Average time for completing survey was 18 minutes.

One hundred and forty-two students of Physiotherapy (118 of the third year, 24 of the second year) completed the survey. The average age of the Physiotherapy students participating was 23.31 (SD +/- 2.98 for students from third year) and 21.75 (SD +/- 3.12 for students from second year). All of the student respondents completed the questionnaire under supervision.

Three-hundred and eighteen physiotherapists who were qualified to practice their profession in Italy, completed the survey, 79/460 of them (17.17%) worked as employees, the remaining worked in private practice (n=381/460, 82.83%). The average age of the participating physiotherapists graduated was 29.7 (SD +/- 7.6). One hundred and thirty-one of the graduated physiotherapists (28.48% of

Table I. Field of work, provenience and mean score of respondents.

		N	Mean (Std Dev)	p-value for comparison of means
Sample		460	45.03 (18.66)	
Gender	Male	278	45.86 (19.27)	0.24
	Female	182	43.77 (17.66)	
High School	Scientific high school	349	44.61 (19.20)	0.51
	Classical high school	46	48.65 (17.20)	
	Professional school	13	50.08 (21.96)	
	Magistral institute	11	44.36 (21.54)	
	Others	41	43.15 (12.89)	
University degree	Degree	318	49.28 (18.08)	<0.001
	Student 2 year	24	15.83 (10.57)	
	Student 3 year	118	39.53 (14.26)	
Master of Science	No	444	44.44 (18.43)	<0.001
	Yes	16	61.37 (17.94)	
Master Manual Therapy	No	354	39.83 (15.89)	<0.001
	Yes	106	62.40 (16.63)	
Master Sport Physical Therapy	No	451	44.88 (18.66)	0.202
	Yes	9	52.89 (17.50)	
Sample provenance	North	177	53.05 (17.26)	<0.001
	Center	184	39.50 (18.43)	
	South	98	41.13 (16.48)	
Work	Self employed	381	44.88 (19.20)	0.709
	Employee	79	45.75 (15.87)	

Table II. Pass rates of respondents.

		N	Pass rate	Chi squared test for indipendence
Sample		460	0.078	
Gender	Male	278	0.086	0.43
	Female	182	0.066	
High school	Scientific high school	349	0.083	0.08
	Classical high school	46	0.087	
	Professional school	13	0.077	
	Magistral institute	11	0.091	
	Others	41	0.024	
University degree	Degree	318	0.107	0.003
	Student 2 year	24	0	
	Student 3 year	118	0.017	
Master of Science	No	444	0.067	<0.01
	Yes	16	0.375	
Master Manual Tharapy	No	354	0.017	<0.01
	Yes	106	0.283	
Master Sport Physical Therapy	No	451	0.077	0.711
	Yes	9	0.111	
Sample Provenence	North	177	0.147	<0.01
	Center	184	0.027	
	South	98	0.051	
	Estero	1	0	
work	Self-employed	381	0.076	
	Employee	79	0.088	0.707

the whole sampling recruited) were certified as specialized: in particular 106 respondents (23.04%) were University post-graduated specialized in Manual Therapy, 9 respondents (1.96%) were specialized in University post-graduated training in Sports Physiotherapy and 16 respondents (3.48%) had a Master of Science for Health-professions training graduation.

Both students and graduate physiotherapists coming from the North of Italy (n=177/460) obtained significantly higher scores compared to their counterparts from South (n=98/460) and Central (n=185/460) Italy (t-test and Kruskal-Wallys test p-value < 0.01). Furthermore, overall pass rates were 14.7%, 5.1% and 2.7% respectively, and chi-square test of independence between sufficiency at the test and area of origin results p < 0.01. Employment status (private practice or employed) had no significant statistical effect on scores (p=0.71).

Physiotherapists possessing a degree (n=318/460) obtained an average score of 49.40/100 (SD +/- 18.07), with an overall pass rate of 10.7%, while Physiotherapy third year and second year students obtained a score of 39.53/100 (SD +/- 14.26) and 15.83/100 (SD +/- 10.57) respectively. The mean score of graduated therapists is significantly different from the mean score of undergraduate ones (t-test has a p-value < 0.01, confirmed with Kruskal-Wallys).

Physiotherapy students obtained an overall pass rate of 1.7% and 0% respectively for third and second year (chi-square test of independence between graduation and sufficiency at the test results p < 0.01). Physiotherapists specialized in Manual Therapy (n=106/460) obtained an average score of 62.40/100 (SD +/- 16.63) and a significative different sufficiency pass rate than their non-specialized colleagues (28.3% *versus* 1.7% respectively, p < 0.01). The mean score between respondents with specialization in Manual Therapy is significantly different (p < 0.001) from the mean of respondents without specialization. Physiotherapists specialized in Sports Physiotherapy obtained a pass rate of 11.1% and collected a mean score of 52.89/100 (SD +/- 17.50). T-test for the mean score between respondents with Sport Physiotherapy specialization and without showed

no significant result (p=0.202). There were not significant differences in the overall pass rate between professionals in possession of Sport Physiotherapy specialization and those without (p=0.71).
Physiotherapists with Master of Science (n=16/460) obtained a mean score of 61.37/100 (SD +/- 17.94) and had a significantly different overall pass rate compared to colleagues without Master of Science (37.5% *versus* 6.7% respectively, $p < 0.01$). Furthermore, T-test for the mean score between respondents with Master of Science and respondents without showed a significant difference ($p < 0.001$)
The overall scores of the Italian physiotherapy students and Italian physiotherapists are reported in **figure 1** (blue columns). Furthermore, in **figure 1**, the results of this study were compared to those of the American physiotherapists and health professionals reported in the study of Child *et al.* (13) and with the medical categories questioned in the earlier work of Matzkin (14) (red columns).

DISCUSSION

The main aim of this survey is to assess the competence of Italian physiotherapists and physiotherapy students in MSC by the administration of the BCEMM. In addition to this, the survey also aims to compare the results from Italian samples with those from the American one.
In 2005, the BCEMM questionnaire was used to test American physiotherapists and verify the adequacy of their basic and specialist training concerning their evaluation and management of MSC (13). Physiotherapist graduates who had not specialized through specific postgraduate programs demonstrated sufficient knowledge passing the exam with a mean score of 74/100, just one point above the level considered to be satisfactory (73.1/100), and above any other category of medical speciality apart from orthopaedic surgeons who attained the highest score of all, 94/100 (13).
American physiotherapists specialized in Musculoskeletal physiotherapy or Sport physiotherapy registered the test score of 81/100, making a notable gain in the direction of the orthopaedists and widening the gap between them and all the other medical specialists questioned (13). American students' average scores were above 60/100 but did not reach the minimum estimated to be satisfactory. However, American students attained a higher level of preparation than that of the components of all the other medical specialties questioned (13).
Indeed, the scores of Italian physiotherapy students are far below the sufficiency threshold, below those of their Amer-

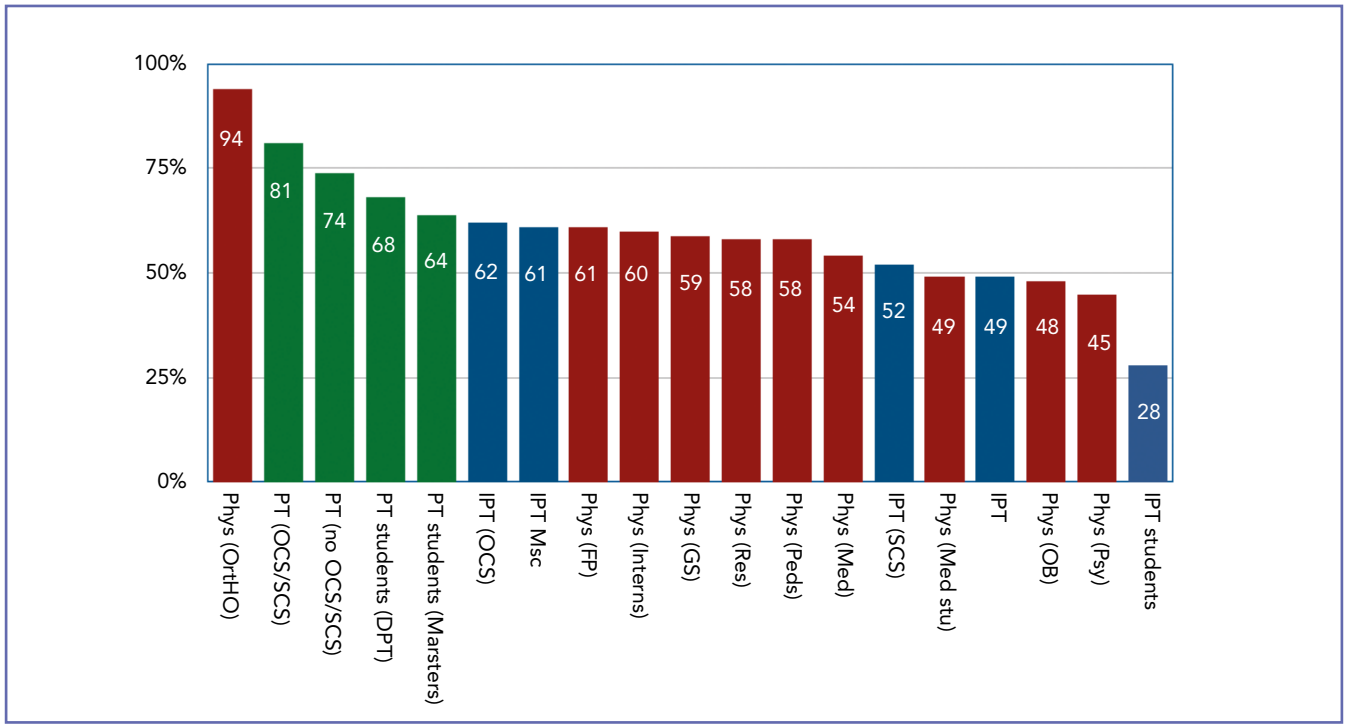

Figure 1. Overall score of musculoskeletal knowledge in Italian physiotherapy students, physiotherapists and specialized physiotherapists (blue columns) and comparison with previous studies by Child *et al.* (13) and by Matzkin (14) (red columns).

ican counterparts, and considerably lower also than those of American medical students. Such poor results might be justified by the differences in University programs in America and Italy and the greater importance attributed to manual issues (16). Therefore, knowledge of MSC and serious pathologies tends not to be analyzed in depth.

In fact, Italian physiotherapy students and physiotherapy graduates, especially the latter, not in possession of a post graduate specialization, showed poor knowledge of MSC that can be found in patients in direct access and this may consequently translate into a poor management of patients presenting these conditions. Only professionals specialized in Manual Therapy and professionals with a Master of Science collect a mean score greater than 60/100. Overall, Italian physiotherapy professionals result in having lower pass rates compared to American professionals.

One of the primary syllabus areas most emphasized in university physiotherapy programs around the world is the differential diagnosis of MSC, a necessary competence in the context of private clinical practice as well as in primary care (17). This data provides preliminary evidence that greater attention to the screening of the more common MSCs is present in physiotherapy programs in the United States but not in Italy. A threshold of 73.1 points has been established as satisfactory by directors of American schools of specialization and therefore as the minimum level of knowledge necessary to guarantee for someone's competence in MSC medicine (6). Italian three-year degree courses in physiotherapy appear to be far behind that threshold. This suggests, in the light of the growing availability of direct access treatment for patients with MSC in Italy, that a considerable quantity of content regarding these competences should be introduced into Italian physiotherapy University courses, especially in University courses from Center and South of Italy.

In fact, from our results, it seems that physiotherapists with a wider background in MSC screenings could have attained higher scores than those with only slight exposure to an orthopedic expertise and this is a consideration evidenced by the statistical significance expressed in the difference in scores obtained by Italian physiotherapists in different geographical regions: physiotherapists from the North had considerably higher scores than their colleagues from central and southern Italy. This can be explained because training programs in physiotherapy do not adopt standardized curricula in Italy and therefore exposure to didactic and clinical experience relative to the management of MSC differs.

Our sample size numerosity (n=460; 142 students; 318 physiotherapists) compares favorably to that of Childs and colleagues (13) (a priori sample size=174 students and 182 physiotherapists). According to the latest European Commission Estimates there are over 61000 Italian physiotherapists, whereas is America there are three times as many; however, our sample size was almost double the size of the sample of American physiotherapists while the number of sample size for students was the same.

Given the wide spectrum of degrees of experience of doctors and physiotherapists and specialties represented in previous studies (13), this data offers a convincing standard of reference for at least a preliminary discussion on the on-topic knowledge of Italian physiotherapists compared to American physiotherapists and doctors regarding the management of MSC since an identical test has been used and administered using similar procedures to those used in the previous studies (6, 13, 14).

Taken as a whole, the results of this survey proved that graduates and specialized physiotherapists in Italy tend to have lower levels of knowledge of the management of MSC than their American counterparts (13). However, Italian specialized physiotherapists (*i.e.* Master of Science and Manual Therapy specialized professionals) tend to have scores which overlap or are at least comparable to American medical students, doctors and all medical specialists, aside from orthopedists. A possible explanation may be due to the degree to which management of MSC is emphasized in Italian physiotherapy degree courses as opposed to postgraduate specialization courses, which would require independent investigation.

At the moment, in Italy, there is a total of 85 first level degree courses in physiotherapy, 16 Masters' degree programs, 5 postgraduate courses for Manual Therapy specialization and 1 for specialization in Sport Rehabilitation with international recognition. In the last 25 years the figure and the role on the physiotherapist in Italy has changed considerably. The decree law number 502 form 1992, in line with community policies and in an attempt to meet the challenges connected with the sustainability of the healthcare system, has reformed the role of the physiotherapist's professional profile, conferring competences and responsibilities to all health workers involved in the Italian health system. Since 1994, the physiotherapist's professional profile has undergone a profound transformation, and what was once regard as an auxiliary non-autonomous professional figure has now turned into an intellectual autonomous and thus competent and responsible professional.

Here lies the paradox as the profession was legitimized by law but not by its own evolutionary process based on the continuous acquisition of specific theoretical and practical competences during its practice. In the year 2000, the law number 251, instituted a five-year degree course dividing it into a 3-year clinical degree course, which allowed the holder of such degree to practice his profession, and a two-year

course aimed at providing a qualification in management, research and teaching (Second cycle degree - Master of Science). However the law did not edit, reform or improved the study programs or syllabus. In our Knowledge only some studies have assessed the core competencies among physiotherapist students, trying to formulate an uniform educational program (18).

Overall, data from this survey shows the importance of Italian university specialization courses in the MSC area for physiotherapists, and also reveals the necessity to improve basic university programs, particularly those related to MSC screening, assessment and treatment.

The physiotherapist is an health professional that can work both autonomously and/or in strict collaboration with others medical health professionals such as physiatrist, orthopaedic, neurologist (19).

To date, physiotherapists workplace in Italy can be divided in employee into National Health System with filtered-access patients (the patient came to the physiotherapist with a prescription by another clinician -medical doctor) or private practice with direct-access patients (the patient came to the physiotherapist without any previous assessment) (5). Therefore, is mandatory that Universities programmes must be implemented in reference to the processes of screening for referral in physiotherapy (20, 21). Italian physiotherapist need to upgrade the knowledge and the detection abilities for screening extra-physiotherapy expertise pathologies that need of a deeper investigations by another health professionist (22, 23). However physiotherapy screening clearly differ from medical diagnosis. Physiotherapists, in fact, could not medically diagnose patients but must be well-prepared to identify signs and symptoms outside the scope of physiotherapy practice and refer to a physiatrist or specialist as appropriate (*e.g.* ortopaedic, neurologist) (24). Because of this, and to prepare the graduated physiotherapist to properly assess the patient coming in direct access, Universities must improve their programmes about the knowledge on the extra professional pathologies, their existence and specific clinical features in order to incrementing the suspect and guide a proper referral (25).

Authors in this paper suggest to a) review the musculoskeletal curricula and assure that the content is relevant and up to date, b) ensure that standardized assessment of students are implemented to guarantee that competencies are achieved, and c) implement reassessment of musculoskeletal knowledge during students university lessons, based on the evidence available in the literature (20, 22).

Another point of discussion is that medical training and training programs in physiotherapy do not adopt standardized curricula, neither in Italy or in the United States and therefore the amount of teaching and clinical experience related to the management of patients with MSC differs. This consideration was highlighted by the statistical significance expressed in the different scores obtained by Italian physiotherapists in different geographical regions (26).

Papers who consider the role of the qualification in professional skills concluded that acquiring further specialization could update the knowledge and change the professional clinical practice in managing people with shoulder pain (27), could reduce prevalence of injury in amateur athletes (28) and give to the professional more qualification, improved manual skills and increased the confidence (29).

Strength and limits of the study

The fact that respondents to this survey come from a wide variety of degree courses and from all areas around the country increases the generalizability of its results.

One of the strengths of this survey is the blind correction of the questionnaires by the four judges and the blind statistical analysis: this way of managing a test promotes unbiased correction of the answers and helps to acquire trustworthy data.

In this study, the questionnaire was administered under supervision; this might exclude any type of contamination of the data by use of external sources: this aspect is a point of strength of this survey as it reinforces the assessing of the real knowledge of respondents. Furthermore this study obliged respondents to answer the questions they were given with time limit of 30 minutes: this aspect could also limit the possibility of the use of external sources (*i.e.* telematic assistance, smart phones, access to the internet, remote resources, books of notes or anything which could create an advantage over each participant's knowledge) and thus makes the collection of the real knowledge and data of the respondents much more reliable. A careful analysis however is necessary and requires that various limitations be taken into consideration.

In fact, in this study a generalization of the whole population of Italian physiotherapist is not possible due to:

a) the exiguous sample size recruited;

b) the respondents coming mostly from events organized by AIFI. Furthermore, in Italy, not all the physiotherapists are subscribed to AIFI.

In addition, the content of the test was mainly focused on the differential diagnosis of MSC commonly encountered in the context of primary care (for example, fractures and dislocations, low back pain, sciatica and arthritis) and emergency orthopedics which require immediate referral to an orthopedic surgeon or to the emergency room (for example compartment syndrome, dislocation of the hip, *etc.*) (13). This data, therefore, might not be generalizable to other theoretical or practical subjects specific to physiotherapy.

CONCLUSIONS

The authors of this paper hope that this study will trigger, within Italian academic and ministerial institutions, deep reflection on the structure and contents of the physiotherapy degree courses, which could lead to a radical restructuring of these academic paths so as to allow this scientific discipline to reach its full potential. A profession such as physiotherapy, which based on clinical science, needs high quality scientific research to guarantee effective treatments that can improve not only the state of health of patients, but also cost-effectiveness, which in turn could optimize the resources of the healthcare service.

Italian physiotherapists specialized in Manual Therapy and physiotherapists with a Master of Science scored higher than their colleagues with the basic graduation and the specialization in Sport Rehabilitation. Furthermore, their score was similar to that from the American physiotherapy student in Master and doctorate in Manual Therapy, American family practice and interns.

AUTHORS' CONTRIBUTIONS

GG: Conceptualization, Roles/Writing – original draft, Project administration; MP: Investigations, Project administration, Roles/Writing – original draft; AS: Investigations, Project administration, Roles/Writing – original draft; MT: Data curations, Project administration. FBo: Methodology, Supervision, Writing- Reviewing and Editing; FB: Methodology, Supervision, Writing- Reviewing and Editing; MM: Statistics, Reviewing and Editing; AD: Supervision, Validation, Writing- Reviewing and Editing

LIST OF ABBREVIATIONS

MSC= Musculoskeletal conditions.
USBJI= The United States Bone and Joint Initiative.
BCEMM= Basic Competency Examination in Musculoskeletal Medicine.
AIFI= Italian Physiotherapy Association.
OMPT= Orthoapedic Manipulative Physical Therapy.
IFOMPT= International Federation of Orthopaedic Physical Therapists.
MSc= Master of Science.

ACRONYMS

PHYS (ortho)= Physicians (orthopeadic); PT(OCS/SCS)= Physiotherapists (Orthopedic clinical specialists/ Sports clinical specialists); PT(NO ocs/scs)= Physiotherapists (no Orthopedic clinical specialists/ Sports clinical special); PT students= Physiotherapists students (doctorate in manual therapy); IPT(ocs)= Italian Physiotherapists (specialized in Manual Therapy); IPTMsc= Italian Physiotherapists (specalized Master of Science); PHYS(FP)= Physicians (Family practice); PHYS(interns)= Physicians (Interns);PHYS(gs)= Physicians (General surgery);PHYS(res)= Physicians (Resident); PHYS(ped)= Physicians (Paediatrics); PHYS(med)= Physicians (Internal medicine); IPT(scs)= Italian Physiotherapists (specialized in Sports Rehabilitation); PHYS(med stu)= Physicians (medical student); IPT= Italian physiotherapists; PHYS(ob)= Physicians (obstetrics-gynecology); PHYS(psy)= Physicians (psychiatry); IPT students= Italian physiotherapists students

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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APPENDIX 1

Translation and Italian cross-cultural adaptation of the BCEMM

Step 1: translation into Italian. Two mother tongue Italian translators developed two Italian versions of the BCEMM independently. One translator (GG) was a physiotherapist, with 16 years clinical experience and lecturer in physiotherapist's University courses for physiotherapists. The other translator was a professional translator with 5-years of, but with no medical background.

Step 2: synthesis. The two independent translations were compared and no discrepancies were noted. Both translators and an observer (FB) worked together on a final draft of the translation.

Step 3: backward translation. Working from that final draw, two English mother-tongue translators, without medical background, translated the questionnaire back into English.

Step 4: expert committee. The translators, a linguistic expert, an orthopaedic surgeon, and three physiotherapists (FBo, PM, AS) constituted a committee of experts. The linguistic expert was a MSc with 20 years of experience as an English teacher. The orthopaedic surgeon had 13 years of experience, and the physiotherapists on the Committee of Experts had clinical expertise in back and neck diseases and had attended training courses in pathology screening for physiotherapists. The committee analysed the translated document and developed the pre-final Italian version of BCEMM (BCEMM-I)

Step 5: pretesting. The BCEMM-I was administered to 50 subjects (26 physiotherapists, 15 physiotherapy students and 9 physiotherapists specialized in Manual Therapy and Sport Rehabilitation). Furthermore, a face-to-face interview between the respondents and the expert committee components was conducted to explore the possible difficulties encountered while filling in the questionnaire (*e.g.*, unclear or confusing questions, unknown or uncommon words and expressions) to ask about the experience in responding to the questions and to further clarify some of the answers given by the respondents.
In conclusion, the outcome of the pretesting stage was satisfactory and it was determined that no changes to the questionnaire were necessary. After that, the final BCEMM-I was developed.

APPENDIX 2

The BCEMM paper-based survey

Section A - demographic

1. Age (years)
2. Gender
   - Male
   - Female
3. High school attended
   - Classical high school
   - Scientific high school
   - Magistral Institute
   - Professional school
   - Others
4. University degree
   - Student 2° year
   - Student 3° year
   - Degree
5. Additional qualification
   - Master of Science
   - Master In Manual Therapy
   - Master in Sport physical therapy
6. Work
   - Employee
   - Self-employeed
7. Sample provenance
   - North
   - Center
   - South

Section B – Technical questions

1. What common problem must all newborns be examined for?
2. What is a compartment syndrome?
3. Acute septic arthritis of the knee may be differentiated from inflammatory arthritis by which laboratory test?
4. A patient dislocates his knee in a car accident. What structure(s) is/are at risk for injury and therefore must be evaluated?
5. A patient punches his companion in the face and sustains a fracture of the 5th metacarpal and a 3-mm break in the skin over the fracture. What is the correct treatment, and why?
6. A patient comes to the office complaining of low-back pain that wakes him up from sleep. What two diagnoses are you concerned about?

7. How is compartment syndrome treated?
8. A patient lands on his hand and is tender to palpation in the "snuff box" (the space between the thumb extensor and abductor tendons). Initial radiographs do not show a fracture. What diagnosis must be considered?
9. A 25-year-old male is involved in a motor vehicle accident. His left limb is in a position of flexion at the knee and hip, with internal rotation and adduction of the hip. What is the most likely diagnosis?
10. What nerve is compressed in carpal tunnel syndrome?
11. A patient has a disc herniation pressing on the 5th lumbar nerve root. How is motor function of the 5th lumbar nerve root tested?
12. How is motor function of the median nerve tested in the hand?
13. A 12-year-old boy severely twists his ankle. Radiographs show only soft-tissue swelling. He is tender at the distal aspect of the fibula. What are 2 possible diagnoses?
14. A patient presents with new-onset low back pain. Under what conditions are plain radiographs indicated? Please name 5 (example: history of trauma).
15. A patient has a displaced fracture near the fibular neck. What structure is at risk for injury?
16. A 20-year-old injured his knee while playing football. You see him on the same day, and he has a knee effusion. An aspiration shows frank blood. What are the three most common diagnoses?
17. What are the five most common sources of cancer metastatic to bone?
18. Name two differences between rheumatoid arthritis and osteoarthrosis
19. Which malignancy may be present in bone yet typically is not detected with a bone scan
20. What is the function of the normal anterior cruciate ligament at the knee?
21. What is the difference between osteoporosis and osteomalacia?
22. In elderly patients, displaced fractures of the femoral neck are typically treated with joint replacement, whereas fractures near the trochanter are treated with plates and screws. Why?
23. What muscle(s) is/are involved in lateral epicondylitis (tennis elbow)?
24. Rupture of the biceps at the elbow results in weakness of both elbow flexion and ?
25. What muscle(s) control(s) external rotation of the humerus with the arm at the side?

ORIGINAL ARTICLE

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Assessment and Management of Lateral Elbow Pain in Physiotherapy Clinical Practice: an Italian National Survey

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SUMMARY

Introduction. Lateral Elbow Pain (LEP) represents a disabling condition with a 1-3% prevalence in general population and a low spontaneous resolution rate within a year. This study investigated the methods of assessment and management of LEP in a sample of physiotherapists in relation to their level of experience.

Methods. We conducted a cross sectional survey consisting of 12 questions. The investigation lasted 2 months and was disseminated through the informative channels of the Italian Physiotherapists Association and by e-mail. The percentage of responses was expressed for different levels of experience in terms of years of work and number of patients with LEP seen in 1 month. Data were analyzed with the SPSS software.

Results. 1405 responses were collected. For LEP management, Psycho-Social Factors (PSFs) are more considered by the interviewees with few years of clinical practice than those who have worked for several years ($p < 0.001$; $\chi^2=36.795$); surgery is less considered as a therapeutic option by professionals who see 0-1 patients per month than those who see much more patients ($p < 0.001$; $\chi^2=20.521$). The percentage of use of manual therapy ($p=0.017$; $\chi^2=32.901$) and the use of the Maudsley and SALT tests ($p=0.012$; $\chi^2=65.360$) result higher in the group that sees more than 3 patients per month; corticosteroid infiltrations are not considered useful by those who see more than 3 patients per month compared to those who see 1 patient or none ($p < 0.001$; $\chi^2=43.632$).

Conclusions. Despite being aware of the new evidence on the etiology of LEP, only a small percentage of interviewed physiotherapists resort to adequate and specific assessment tools. Clinicians should update their patient management options to better act on the PSFs and mostly consider a multidisciplinary approach to LEP disorders.

KEY WORDS

Lateral Epicondylitis; assessment; cross sectional survey; elbow; lateral elbow pain; physiotherapy.

INTRODUCTION

Lateral Elbow Pain (LEP), a condition also known as tennis elbow, epicondylalgia, lateral epicondylitis or tendinosis, is a very common disabling condition (1): 40% of the general population experiences this problem at least once in lifetime. The most affected category seems to be manual workers with high demands for strength, repetitive movements, vibrations and uncomfortable postures (1), in an age group between 35-50 years (2, 3).

This condition was described for the first time in medical literature by Runge in 1873 (4) as a chronic symptomatic degeneration of the extensor tendons of the wrist (3) and

the most involved component is the origin of the common tendon itself, on the lateral epicondyle of the humerus. LEP can also be considered a "tendinosis", which means a chronic symptomatic degeneration of the common tendon of the extensor muscles of the forearm at the level of the lateral epicondyle of the humerus (5). To date, in literature, the term LEP underlies two different components as the origin of pain: extra-articular and intra-articular of the elbow (5, 6). Clinically, LEP presents itself as pain projected on the dorsal surface of the forearm and reduction of the gripping force, mainly present in the dominant limb (1). It often causes severe limitation of daily and sport activities (1, 7). Generally, spontaneous resolution rate within a year is low: 50% of symptoms persist for up to 18 months and 20% recur in the 3-5 years following the onset (8). This entails significant socio-economic costs (9): the resulting absenteeism from work was estimated to cost $ 27 million in 2012 in the UK alone (10), indeed.

In the last twenty years, numerous studies have considered an integrated model for the assessment of musculoskeletal pathologies (Bio-Psycho-Social-BPS model) to explain the dichotomy between linear and gradual healing of the injured tissue and the non-linear resolution of painful symptoms (11, 12). By linear healing is meant the foreseen period for tissue healing which includes (13):

- an inflammatory phase lasting 2-4 days;
- a proliferative phase lasting about 6 weeks for the tendons (13), in which occurs collagen synthesis and deposition, the removal of inflammation mediators and the growth of the capillary vessel in the area, with a significative increase in fibroblast activity and the development of granulation tissue (13).

In particular, in LEP, to explain the persistence of pain beyond these expected times, Coombes and colleagues in 2009 (14) developed a new model, which took into account the tendon structural pathology, the consequent mechanisms that alter motor control and the biochemical justification which leads patients to persistent pain in absence of tendon pathology (14).

As far as clinical evaluation is concerned, numerous tests are cited in literature, although there is a low presence of articles with accurate descriptions of them (15, 16). The most cited are: the Maudsley test (Hsu, Moen, Levine, and Ahmad, 2012) (resisted extension of the third finger with elbow flexed at 90° and pronated forearm; it results positive for pain appearance on the lateral aspect of the joint), the Cozen test (17) (fully extended elbow and forearm in pronation; the patient is asked to resist an opposing force manually applied on the hand, closed in a punch with extended wrist and radial deviation; the test is considered positive if it reproduces pain or other symptoms in the lateral epicondyle area). Pain-Free Grip test is also described (PFG) (18): it reproduces patient's pain (18) and measures the reduction of the gripping force (18). The Supination and Antero-Lateral pain Test (SALT) (5) and the Posterior Elbow Pain by Palpation-Extension of the Radio-capitellar joint (PEPPER) (5) are two recently described diagnostic tests, instead: they evoke the pain generated, with higher probability, from intra-articular structures. **Table I** summarizes the diagnostic accuracy values of the aforementioned tests.

During the evaluation phase, in addition to the clinical tests, it is useful to use rating scale and questionnaires to quantify entities of changes following a possible treatment. In literature, described tools for LEP are: Disability of the Arm, Shoulder and Hand (DASH) (19) for the upper limb, in general, and the Patient Rated Tennis Elbow Evaluation (PRTEE) (20), which is much more specific for the elbow; the Center for Epidemiological Studies Depression Scale (CES-D) and the Hospital Anxiety and Depression Scale (HADS) are two scales which investigate patient's psychological state (21, 22).

Over the past decade, over 40 different techniques used in physiotherapy for the treatment of LEP have been described in over 250 clinical studies; all concluded that there is no ideal or unique treatment (23).

Given these uncertainties and due to the absence of consistent data relating to the Italian territory, the aim of this study was to investigate the methods and approaches used by Italian physiotherapists, both in the management and

Table I. Diagnostic accuracy of elbow assessment test.

Test	Sensibility %	Specificity %	PPV %	NPV %
Maudsley test	88	0	85	0
Cozen test	84	0	84	0
SALT	87.50	50	87.50	50
PEPPER	37.50	100	100	28.57

NPV: Negative Predictive Value; PEPPER: Posterior Elbow Pain by Palpation Extention of the Radio-capitellar joint; PPV: positive predictive value; SALT: Supination and Antero-Lateral Pain Test; %: percentage.

in the assessment of LEP, in relation to the level of their professional experience, in terms of years of work and the number of patients with LEP seen in 1 month. Moreover, we assessed their adherence to the most recent scientific evidence-based literature.
Considering LEP as a disabling condition with high recurrence rates, it could be helpful to understand and observe the management activities of Italian physiotherapists in evaluating and treating such clinical condition in such a way to fill any gaps in the spectrum of Italian physiotherapist's competence.
On the other hand, concrete and favorable answers could be given in relation to the patients' requests, for example in the context of reduction of painful symptoms, dejection of direct and indirect costs related to this condition, restoration of better quality of life. To the authors' knowledge, this is the first study to investigate this field.

METHODS

Study design

The study was designed as a cross-sectional observational study based on the administration of questionnaires both directly on paper and in web mode, based on the guidelines of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (for the paper questionnaire) (24) and the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) (for the online questionnaire) (25). The questionnaire was designed with reference to similar questionnaires (26, 27) and with the consent of 10 experts OMPT physiotherapists (Orthopedic Manipulative Physical Therapist) with at least 5 years after specialization experience.

Survey structuring

The questionnaire consisted of 12 questions (**Appendix 1**- Questionnaires), all designed with multiple answers, with a completion time estimated by the software of 3 minutes and distributed in 1 single page. Authors chose to structure the survey in this way since it has been shown that, although the "ideal" duration of a survey varies according to the topic, population and context, a positive impact on completion rates of the survey was found if minimum length is guaranteed (one or two pages, at most) (28, 29). Questions identified with Q5, Q8 and Q9 had, as additional answer field the choice "Other", with the obligation to specify alternative answers to those present in the text box. The first three questions (Q1-Q3) collected information on the professional sphere and were used to understand the level of work experience of our target population (years of work, work setting, number of patients with LEP assessed in a month). The other nine questions (Q4-Q12) were specific for the assessment and management of LEP, in particular regarding to which anatomical structures were considered during patient's evaluation, which were the most commonly used clinical tests, which considerations the physiotherapists have towards psychosocial factors, the weight of the instrumental diagnostic investigations and the role of surgical and/or infiltrative treatment.
The survey was analyzed by a panel of ten experts physiotherapist with extensive experience in survey design in order to increase its internal consistency and technical functionality of the electronic questionnaire (30); all physiotherapists were professionals with more than 5 years of experience in the treatment of upper limb pathologies and, in particular, of the elbow district. The survey could be checked and modified at the end of the compilation before sending it. Experts worked independently on the questionnaire and agreed on the final version of the questionnaire with respect to the terms, feedback, questions and structure of the survey, which was gradually enriched with their suggestions.
When agreement was reached, a preliminary version of the survey was administered to a sample of 20 physiotherapists to increase the content validity of the survey itself. When the pilot internship was completed, experts conducted a one-to-one interview with the 20 physiotherapists about the possible difficulties encountered during the survey (for example, identifying questions which required further explanation, too difficult question formulation, possibility of respondents seemed to be confused in answers). During the pilot stage, no ambiguous questions emerged, the words used were simple and easy to understand, therefore no change was necessary.

Ethical approving

This study was approved by the Ethics Committee of the Local Health Authority of Lecce on March 7, 2019 with protocol number 30/2019. The complete version of the survey, in Italian and English language, is available as supplementary material (**Appendix 1**- Questionnaires).

Sampling and recruitment

The questionnaire was addressed to all professionals with a degree in physiotherapy and practitioners in Italy. The questionnaire was spread through:

- informative channels of the Italian Physiotherapists Association (A.I.F.I);

- social media (Facebook and WhatsApp);
- direct e-mail contacts of physiotherapists.

An electronic invitation form and an information letter were provided to all participants (**Appendix 2** - Information letter). The questionnaire could be only completed once per email address. Before starting the compilation of the questionnaire, written informed consent was requested.

Data collection

The survey was available on the SurveyMonkey® platform (www.surveymonkey.com) for 2 months from March 20th to May 20th. Two months are considered a reasonable time for data acquisition (31), also because no further request for completing the questionnaire has been registered after this date. The estimated sample size was 1050 respondents, calculated with a dedicated software within the Sample Size Calculator platform (https://www.surveymonkey.com/mp/sample-size-calculator), considering a 95% Confidence Level, a margin of error of 3%. Target population has been identified in all the Italian physiotherapists surveyed by AIFI, with a number equal to 65000 (https://aifi.net/censimento-aifi-italia-65mila-fisioterapisti/). The collected data were stored in the software, protected by an access password.

Analytical procedures

The SurveyMonkey® software allowed us to extrapolate the raw data and export it into Microsoft Excel. The data were analyzed with SPSS Statistical Software (Version 25.0. Armonk, NY: IBM Corp).

The demographic information was entirely analyzed and used to define the characteristics of the studied population, the level of experience and the number of patients seen in 1 month. Each question relating to the assessment and management of the patient with LEP (Q4-Q12) was evaluated in relation to the level of experience of the subject interviewed (Q1-Q3). The percentage of responses for different levels of experience was expressed through proportions.

Based on the type of data being analyzed (dichotomous and categorical variables), the Chi-Square Test (χ^2) was chosen to highlight the presence of significant differences in the comparison between the various proportions. For this test, a p-value < 0.05 was assumed as significant.

Subsequently, to identify the responsible couples for this existing difference, the z-test was used with a p-value adjusted according to the "Bonferroni correction for multiple comparisons".

RESULTS

A total of 1405 responses were collected with a 100% completion rate and an average time taken of 3 minutes and 25 seconds to fill the question form. Most of the responses to the survey were collected during the first week, the number drastically reduced in the following 3 weeks and then peaked again in the fifth week (when the questionnaire was disseminated by the A.I.Fi. informative channels).

The data collected showed that the majority of respondents (32.23%) have been working as physiotherapists for less than 5 years, in a private study (63.46%) and visit less than 2 LEP patients per month (**table II**).

Most of respondent (78.23%) considered both the insertion tendon of the extensor muscles of the carpus and fingers as well as the myo-tendon junction of the extensor muscles of the carpus and fingers and the humerus-radial and radio-ulnar joint responsible for pain in a patient with LEP.

The most used clinical tests during the evaluation, in order of frequency, were: the Cozen Test (63.81%), the Maudsley Test (46.82%), SALT (23.79%) and PEPPER (21.50%) Test.

Almost all the respondents (88.95%) considered that Psychosocial Factors (PSFs) can significantly influence the natural history and management of the patient with LEP. Of these, most adopt multiple strategies for managing psychosocial factors (patient education, cognitive behavioral approach).

The rating scales and questionnaires mainly most used with this type of patient were the DASH (57.81%) and the PRTEE (38.12%). Other response have been entered (19.54%), using option box "Other".

Table II. Information on the professional dimension.

Q1-Q3	**n=1405**
Year of clinical practice	%
0-5	32.23
5-10	28.94
10-20	23.93
>20	19.90
Setting	%
Private study	63.46
Nursing home	5.89
Hospital	12.49
Affiliated clinica	18.16
Number of patients/month	%
0-1	46.59
1-2	29.53
2-3	14.48
>3	9.39

LEP: Lateral Elbow Pain; n: respondents; %: percentage.

Significant 63.96% of respondents considered instrumental diagnostic investigations (Magnetic Resonance Imaging, Computed Tomography, Ultrasonography) useful in the presence of signs and symptoms that suggest a pathology of non-physiotherapeutic competence. A reduced percentage expressed specific cases for which the use of diagnostic investigations is relevant or consider these investigation tools "always" or "useful".

Surgery intervention is considered a therapeutic alternative in case of persistent LEP only by 29.54% of physiotherapists. Most of the physiotherapists (38.31%) didn't consider corticosteroid infiltrations to be useful in a patient with LEP, the others considered them useful only in a certain phase of the pathology.

Most respondents (37.61%) used Therapeutic Exercise (TE) combined with Manual Therapy (MT) as proper conservative approach; smaller percentages used a unimodal approach. All the results to these answers were summarized in **table III**.

Analyzing the methods of assessment and management of LEP (Q4-Q12) in relation to the level of experience of physiotherapists, it emerged that:

- who has more years of clinical practice (> 20), did not consider that psychosocial factors may influence the natural history and management of the patient with LEP with a significantly higher percentage than all the others ($p < 0.001$; $\chi^2=36.795$) (**figure 1**);
- there was no significant difference between the groups with several years of experience and the consideration of surgery as a therapeutic alternative in persistent LEP ($p=0.258$; $\chi^2=4.031$) (**figure 2**);
- the use of the Maudsley and SALT clinical tests was significantly greater in the group that saw more than 3 patients per month compared to "0-1" and "1-2" ($p=0.012$; $\chi^2=65.360$) (**table IV**);
- those who saw 0-1 patients in 1 month considered surgery less useful than those who saw more patients ($p < 0.001$; $\chi^2=20.521$) (**figure 3**);
- in relation to the question *"Do you consider corticosteroid infiltrations useful in a patient with LEP?"*, the answer "No" was significantly greater in the group "over 3" compared to "0-1"; the answer "I don't know" was significantly greater in the group "0-1" than in "2-3" and "beyond 3"; the answer "Yes, in chronic patient" was significantly greater in the group "2-3" compared to "0-1" and "1-2" ($p < 0.001$; $\chi^2= 43.632$) (**figure 4**);
- the percentage of TM use was significantly higher in the group that saw more than 3 patients in 1 month compared to the group that saw 0-1 and 1-2 patients in 1 month ($p=0.017$; $\chi^2=32.901$) (**figure 5**).

Table III. Assessment and management of the patient with LEP.

Q4-Q12	
Anatomical structure that cause pain	**%**
CED and EC tendon	10.63
CED and EC myo-tendinous junction	6.75
Joint and surroundings tissues	4.38
All mentioned structures	78.23
Clinical test used	%
Cozen	63.81
Maudsley	46.82
SALT	23.79
PEPPER	21.50
Others	16.91
Considerations on PSFs	%
Yes	88.95
No	11.05
Used strategies in the management of PSFs	%
Pain neurophysiology education	28.69
Cognitive-behavioral approach	9.43
Encouragmente on active life style	28.62
All mentioned	60.22
Evaluation scale and questionnaires	%
PRTEE	38.12
DASH	57.81
CES-D	2.55
HADS	5.02
Others	19.54
Usefulness of diagnostic investigation (CT, MRI, USI)	%
Never	1.51
Always	5.74
Instability	8.97
Visible acute signs of inflammation	5.81
Non-physiotherapic competence pathology	63.96
Non-feasible conservative treatmente	12.28
Others	1.72
Consideration on surgery in LEP › 6 months	%
Yes	29.54
No	70.46
Usefulness of corticosteroids injections	%
No	38.31
Always	0.65
Acute phase	16.71
LEP › 6 months	23.39

Q4-Q12	
Anatomical structure that cause pain	**%**
I don't know	20.95
Conservative treatment	%
TE	7.78
MT	12.75
B	1.08
TE+MT	37.61
TE+B	2.81
MT+B	4.90
TE+MT+B	33.07
Q4-Q12	
Anatomical structure that cause pain	%
CED and EC tendon	10.63
CED and EC myo-tendinous junction	6.75
Joint and surroundings tissues	4.38
All mentioned structures	78.23
Clinical test used	%
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SALT	23.79
PEPPER	21.50
Others	16.91
Considerations on PSFs	%
Yes	88.95
No	11.05
Used strategies in the management of PSFs	%
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Encouragmente on active life style	28.62
All mentioned	60.22
Evaluation scale and questionnaires	%
PRTEE	38.12
DASH	57.81
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Others	19.54
Usefulness of diagnostic investigation (CT, MRI, USI)	%
Never	1.51
Always	5.74
Instability	8.97
Visible acute signs of inflammation	5.81
Non-physiotherapic competence pathology	63.96

Q4-Q12	
Anatomical structure that cause pain	**%**
Non-feasible conservative treatmente	12.28
Others	1.72
Consideration on surgery in LEP › 6 months	%
Yes	29.54
No	70.46
Usefulness of corticosteroids injections	%
No	38.31
Always	0.65
Acute phase	16.71
LEP › 6 months	23.39
I don't know	20.95
Conservative treatment	%
TE	7.78
MT	12.75
B	1.08
TE + MT	37.61
TE + B	2.81
MT + B	4.90
TE + MT + B	33.07

B: bracing; CES-D: Center for Epidemiological Studies Depression Scale; CT: Computed Tomography; DASH: Disabilities of the Arm, Shoulder and Hand; EC: Extensor of the carpus; ECD: Extensor common digits; TE: Therapeutic Exercises; PSFs: Psycho-social factors; HADS: Hospital Anxiety and Depression Scale; LEP: Lateral Elbow Pain; MRI: Magnetic Resonance Imaging; MT: Manual Therapy; PEPPER: Posterior Elbow Pain by Palpation Extension of the Radio-capitellar joint; PRTEE: Patient-Rated Tennis Elbow Evaluation; SALT: Supination and Antero-Lateral Pain Test; TE: Therapeutic Exercise; USI: Ultrasound Imaging; %: percentage.

DISCUSSIONS

The purpose of this study was to find out which LEP assessment and management strategies are in relation to the level of experience of the Italian physiotherapists. In 2010, McDermid and his team (32) conducted a similar study in Canada concluding that their clinical practice was aligned with literature despite the lacking an optimal definition of education and exercise as tools rehabilitation (32). From the analysis of the results of the present study, it emerged that about half of the sample treats none or at most one patient per month with this problem: this may be defined as a general poor experience regarding the LEP by the respondents. However, most of the respondent, updated with the most recent evidence-based literature on the etiology of LEP, believe that the main cause of the problem is multifactorial (5, 6, 14), recognizing as pathogenic "noxa" not only the

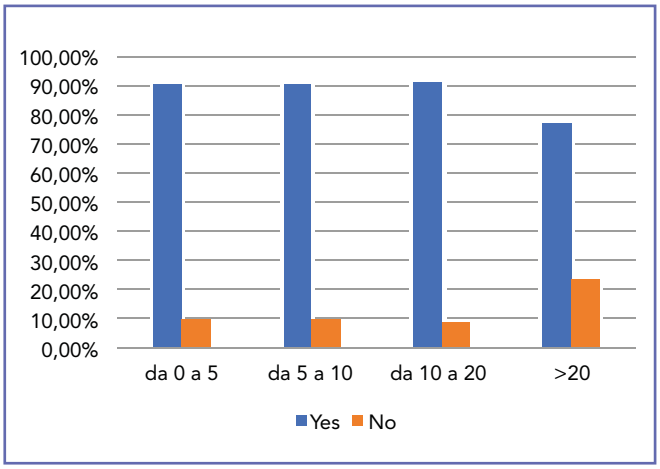

Figure 1. Comparison of proportions between considerations on PSFs and numbers of years of clinical practice. ($p < 0.001$; $\chi^2=36.795$).
*: "No" significantly different between "> 20" and "0-5"; ^: No significantly different between "> 20" and "10-20"; †: No significantly different between "> 20" and "5-10".

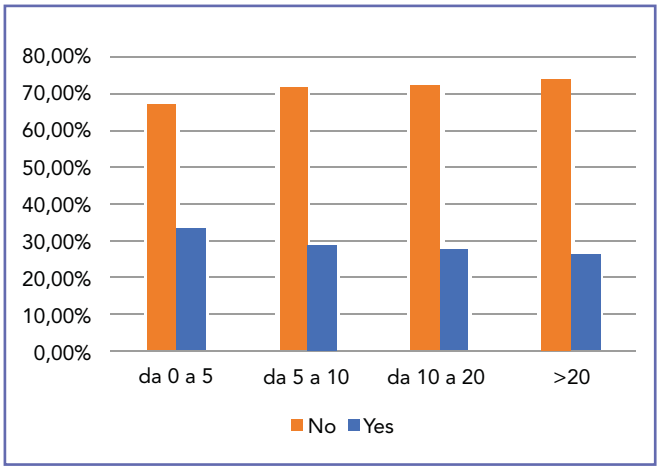

Figure 2. Comparison between proportions of surgery considerations and years of clinical practice. Do you consider corticosteroid infiltrations useful in a patient with LEP? $p=0.258$; $\chi^2=4.031$.

Table IV. Comparison between proportions of clinical tests and patients/months.

		0-1	1- 2	2-3	> 3
Q5	**None**	14.6%	15.5%	16.3%	15.1%
	C	22.9%	23.5%	17.8%	13.7%
	M	11.5%	11.4%	11.9%	16%
	S	4.2%	5.6%	3.5%	4.6%
	P	3.7%	4.6%	3.5%	5.3%
	C + M + S + P	7.1%	4.9%	6.4%	4.6%
	C + M + S	1.4%	1%	2%	0.8%
	C + M	21.7%	20.9%	24.8%	20.6%
	M + S	0.8% ^	0.2% *	2%	6.1%
	M + S + P	0.3%	0.5%	1%	0%
	S + P	4.2%	1.7%	3%	5.3%
	C + S + P	1,5%	1.2%	1%	0%
	C + S	3.1%	5.1%	5%	4.6%
	C + P	2.5%	2.4%	1.5%	3.8%
	C + M + P	0.6%	1.5%	0.5%	0%

$p=0.012$; $\chi^2=65.360$

*: "M + S" significantly different between "1-2" and "> 3"; ^: "M + S" significantly different between "0-1" and "> 3"; C=Cozen's Test; M=Maudsley Test; P=PEPPER Test; S=SALT Test.

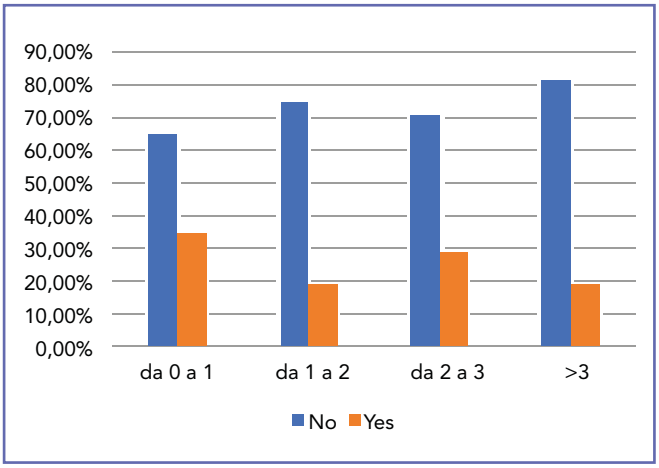

Figure 3. Comparison between proportions of surgery and patients/months. Do you consider corticosteroid infiltrations useful in a patient with LEP? p <0 .001; χ^2=20.521.
*: "No" significantly different between "0-1" and "1-2"; ^: No significantly different between "0-1" and "> 3".

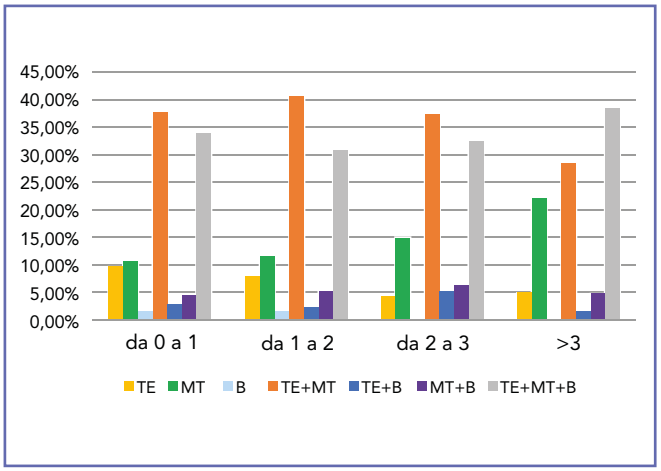

Figure 5. Comparison between proportions of use on corticosteroid injections and number of patients/months. What kind of conservative approach do you use? p=0.017; χ^2=32.901.
*: "MT" significantly different between "0-1" and "> 3"; ^: "MT" significantly different between "1-2" and "> 3"; B: Bracing; MT: Manual Therapy; TE: Therapeutic Exercise.

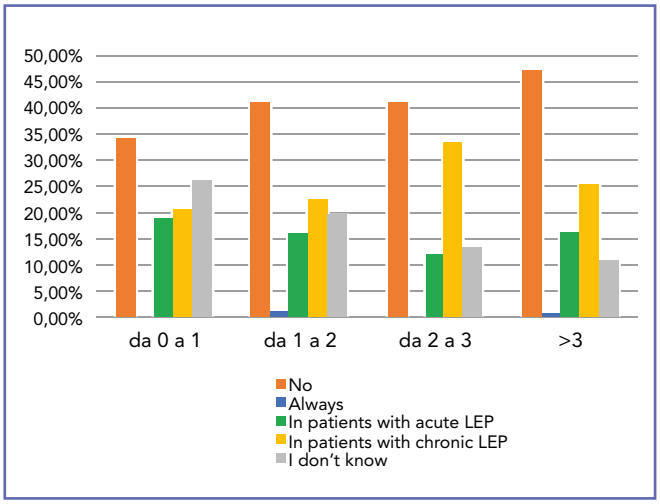

Figure 4. Comparison between proportions of use on corticosteroid injections and patients/months. Do you think corticosteroid infiltrations are useful in a patient with LEP? P < 0.001; χ^2=43.632.
*: "No" significantly different between "0-1" and "> 3"; ^: "I don't know" significantly different between "0-1" and "> 3"; +: "I don't know" significantly different between "0-1" and "2-3"; α: "Yes, in a chronic patient" significantly different between "0-1" and "2-3"; #: £: "In a chronic patient" significantly different between "0-1" and "1-2".

tendon component (33-36). In 2017, the study conducted by Arrigoni *et al.* (6) hypothesized the existence of a specific sub-category of patients, in which the joint complex could play a predominant role in LEP.
Moreover, most of the respondents declared to consider PSFs (1, 37-39) to have a significant influence on natural history and in the management of the patient with LEP; they also declared to adopt specific strategies in this regard. Therefore, it was possible to state that the paradigm of the person as a Biopsychosocial entity (BPS) found its clinical application in LEP (40-42). Nevertheless, only very small percentage of professionals resort to evaluation scales like CES-D and HADS. Strategies used for the management of PSFs were often considered and individually used by the respondents, thus not maximizing the effect of a treatment based on the BPS model, which instead promoted the use of multiple treatment strategies and techniques (12).
Regarding clinical tests, SALT and PEPPER tests was proposed in battery only by 11% of our respondents. It was important to remember that the scientific evidence which demonstrates the usefulness of these two tests was rather recent (5) and this could explain their scarce diffusion.
Saroja in 2014 (43) verified the diagnostic accuracy of the Cozen, Maudsley and Mills tests (**table I**). Through this questionnaire, it emerged that Cozen and Maudsley tests

were used more frequently during the evaluation of a patient with a suspected LEP (**table III**). In a very recent systematic review (44), it emerged that a lot of clinical tests for elbow pathologies were described in literature, but none can provide adequate certainty to rule in or rule out a disease based on sufficient diagnostic accuracy.

Still with regards to assessment procedures, more than half of the respondents declared to mainly use the DASH questionnaire, which is not specific for elbow diseases such as PRTEE. DASH questionnaire was validated in Italian on 108 patients with upper limb problems, of which only 25 affected by epicondylitis (19). It was highly probable that this small number of patients was due to sampling procedure and a low prevalence of LEP disorders compared to other pathologies of the upper limb (45) (**table III).**

More than half of the respondents considered imaging studies (46-48) as an useful option in the presence of signs and symptoms that suggest a specific pathology of non-physiotherapeutic competence, like cases in which a multidisciplinary assessment would be more appropriate (**table II**). This could be due to the fact that, in Italy, physiotherapists could not prescribe diagnostic imaging investigations. Therefore, Italian physiotherapist were not used to supplement imaging independently as support for clinical practice.

For the management of LEP, we can distinguish between conservative treatment (exercise, manual therapy, brace), injections and surgical treatments. In literature it was recommended to use a conservative approach in the first few months (49) but it would be also appropriate to know how to recognize when conservative treatment was no more effective. This was possible through an accurate medical history and a greater use of dedicated clinical tests (5, 17, 18, 26) in order to evaluate alternative strategies for the resolution of symptoms, such as surgery (50-52) and corticosteroid infiltrations (53).

Surgery

Most respondents (70.46%) did not consider surgery as a therapeutic alternative. Regarding this treatment option, a Cochrane systematic review published in 2011 (54) concluded that there is insufficient evidence to support or reject its efficacy due to a small number of studies, a large heterogeneity in the interventions, samples of small size and poor recording of results (54). Common surgical procedures may be performed via percutaneous, arthroscopic, or open approaches (55). A systematic review conducted in 2018 (56) highlights no difference between open surgery and arthroscopy, in terms of efficacy, while it suggests that arthroscopic procedures are associated with less complications (56). Recently, a review of Stover *et al.* highlighted how Ultrasound-Guided Tenotomy (USGT) represented a minimally invasive treatment option for recalcitrant cases to conservative management (57).

Injections

Corticosteroid injections showed to produce substantial short-term pain relief (53, 58, 59), unexpected with the lack of inflammatory biomarkers in tendinopathy (60, 61).

A plausible explanation was that placebo and contextual effects (62-64) may be associated. In the long term, no evidence of efficacy was found (53, 58, 65), due to the high recurrence rates, the risks of fibrosis, adiponecrosis and calcification, as well as the worsening of the clinical course (62, 66). In a randomized controlled trial with 1-year follow-up (53), recurrence of symptoms was evident in 72% of patients treated with corticosteroid injection compared to 8% after physiotherapy treatment (elbow manipulation, therapeutic exercise and education). Coombes *et al.* in 2009 (67) conducted a clinical study that highlighted how the combined approach (physiotherapy + corticosteroid injections) is preferred to infiltration alone (67).

Although still with few supporting studies, collagen infiltrations were also used and should be considered for future research to enhance available tools to clinicians (68). Several studies have investigated also the use of topically-applied steroids for tendon pain treatment, demonstrating a positive effect on pain in patients with LEP: most advantages reside in their safety application and being well tolerated (69).

Conservative Treatment

As far as the conservative treatment was concerned, the majority of respondents declared to use a multimodal approach (37.61% TE + MT, 33.07% TE + B + MT) (**table III**).

Appreciating the therapeutic success (7), in 2003 Vincenzino and colleagues (70) considered therapeutic exercise the main strategy for the management of LEP together with additional procedures such as manipulative therapy (71, 72) and sports taping (73) techniques. These showed to provide substantial relief from initial pain, to speed up recovery processes and to motivate the patient to adhere to the proposed exercise program. Indeed, a recent systematic review (74) concluded that the majority of consistent findings supported the inclusion of eccentric exercise as part of a multimodal therapy programme due to its effect on improved outcomes in patients with LEP (74).

The protective brace had also proven to be effective for short-term pain reduction (75, 76), but no more effective than other strategies such as shock-wave therapy (77, 78).

Therefore, it was not possible to define which is the best treatment for LEP, which must be researched taking into consideration: the experience of the team, the availability of the specific equipment, the clinician experience and expertise and the patient's responses (79).

CONCLUSIONS

Most of the respondents are updated with the most recent scientific evidence on the etiology of LEP.
It is plausible that, for musculoskeletal pathologies management, in addition to focusing on improving manual skills, clinicians should also update their diagnostic framing skills and their ability of taking in charge patients. It is important that clinicians also take into account that they can, and should, consider and act on the PSFs. In addition, a multidisciplinary type of assessment and management should be increasingly considered, which is hoped for the given complexity of this disorder. The main goal is to avoid mistakes or diagnosis delays, to avoid the development of chronic symptoms in presence of because worst results, to ethically inform patients of the heterogeneity of the treatment and the natural history of LEP. Such behavior can come true only with a consistent improvement of the medical and health culture.

Perspectives

We have noticed that heterogeneous clinical labelling of lateral elbow pain is found in the literature, also taking into consideration some manuscripts that investigate the psychosocial aspects (40-42).
Therefore, we believe that:

1. a common agreement must be reached soon in scientific community toward the most appropriate terminology used to identify this clinical condition;
2. inclusion criteria used so far in literature could represent a gap to be filled, as probably factors involved in the genesis of the pathology are manifold and could be indicators of specific subgroups with probable different response to the proposed therapeutic pathways;
3. two different subgroups of patient with lateral elbow pain could be identified, where the intra- (5, 6, 80) or extra-articular (5) component is responsible for most of the symptoms referred by patients, being aware that more likely there is never a single and exclusive involvement of a single structure.

For this reason, we decided to identify a unique terminology to define, respectively, the pain in the elbow with an etiological share of the myo-tendon compartment or of the functional component (non-Specific Lateral Elbow Pain – nSLEP) and the pain most likely to be sustained by problems affecting the osteo-articular component, or true structural component (Specific Lateral Elbow Pain – SLEPs) (81).
The terminology nSLEP and SLEPs refers to a classification proposal of lateral elbow pain, with its relative decision-making algorithm, inherent to the research strand of this work. Moreover, we decided to follow principles and recommendations in clinical and field Science Research (82).

LIST OF ABBREVIATIONS

nSLEP: non-Specific Lateral Elbow Pain
B: Bracing
BPS: Bio-Psycho-Social
CES-D: Center for Epidemiological Studies Depression Scale
CHERRIES: Checklist for Reporting Results of Internet E-Survey
CT: Computed Tomography
DASH: Disabilities of the Arm, Shoulder and Hand
HADS: Hospital Anxiety and Depression Scale
LEP: Lateral Elbow Pain
MRI: Magnetic Resonance Imaging
MT: Manual Therapy
OMPT: Orthopedic Manipulative Physical Therapist
PEPPER: Posterior Elbow Pain by Palpation-Extension of the Radio-capitellar Joint
PGF: Pain-Free Grip
PRTEE: Patient Rated Tennis Elbow Evaluation
PSFs: Psychosocial Factors
Q: Question
SALT: Supination and Anterolateral Pain Test
SLEPs: Specific Lateral Elbow Pain
SPSS: Statistical Package for Social Science
STROBE: Strengthening the Reporting of Observational Studies in Epidemiology
TE: Therapeutic Exercise
USI: Ultrasound Imaging

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ETHICS STATEMENT

This study was approved by the Ethics Committee of Lecce (Italy) on March 7, 2019 with protocol number 30/2019.

Written informed consent was requested by the used software before starting the questionnaire.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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APPENDIX 1

QUESTIONNAIRES

Q1 - How many years of experience do you have as a physiotherapist?
- 0-5
- 5-10
- 10-20
- > 20

Q2 - In which job setting do you work?
- Public health system (hospital)
- Private Practice
- Nursing home (private or in convection with the national health system)
- Retirement home

Q3 - How many patients with lateral elbow pain (LEP), a condition also known as tennis elbow or epicondylalgia or lateral epicondylitis, do you visit each month?
- From 0 to 1
- From 1 to 2
- From 2 to 3
- Over 3

Q4 - Which anatomical structures do you think may be the cause of pain in a patient with LEP?
- Common insertion tendon of the extensor muscles of carpus and fingers
- Myotendinous junction of the extensor muscles of carpus and fingers
- Humero-radial and radio-ulnar joint, including surrounding tissues (capsule and ligaments)
- All the structures mentioned above

Q5 - Which clinical tests do you use most often when evaluating a patient with probable LEP? (it is possible to mark more than one box)
- Cozen's Test
- Maudsley Test
- Salt Test
- Pepper Test
- Other (specify)

Q6 - Do you consider that psycho-social factors can significantly influence the natural history and management of the patient with LEP and do you possibly adopt specific strategies in this regard?
- Yes
- No

Q7 - If you answered 'YES' to question 6, which strategies do you use? (it is possible to mark more than one box)
- Pain neurophysiology education
- Cognitive-behavioral approach
- Counseling: encouraging the patient to maintain an active lifestyle
- All the above mentioned

Q8 - In daily clinical practice, which assessment scales and questionnaires do you use with this kind of patient? (it is possible to mark more than one box)
- Patient-Rated Tennis Elbow Evaluation (PRTEE)
- Disabilities of the Arm, Shoulder and Hand (DASH)
- Center for Epidemiological Studies Depression Scale (CES-D)
- Hospital Anxiety and Depression Scale (HADS)
- Other (specify)

Q9 - In your clinical practice, when do you think instrumental diagnostic investigations (MRI, CT, Ultrasound etc.) are useful in some cases of LEP?
- Never
- Always
- In case of significant disability
- In case of visible acute inflammatory signs
- In presence of signs and symptoms that make one suspect of non-physiotherapy competence pathology
- In the case the patient can not be subjected to conservative treatment
- Other (specify)

Q10 - Do you consider surgery a therapeutic alternative in case of LEP lasting for over 6 months?
- Yes
- No

Q11 - Do you consider corticosteroid injections useful in a patient with LEP?
- NO
- Yes, always
- Yes, in patient with LEP in acute phase
- Yes, in chronic patient (over 6 months)
- I do not know

Q12 - Which kind of conservative approach to you mostly adopt?
- Therapeutic exercise (TE)
- Manual therapy (MT)
- Protection bracing
- TE + MT
- TE + bracing
- MT + bracing
- TE + MT + bracing

APPENDIX 2

INFORMATION LETTER

Dear colleague,

I ask for your collaboration for the achievement of a prevalence observational study which aims to investigate the methods of approach used by Italian physiotherapists in the assessment and management of lateral elbow pain (LEP).
This study has already obtained the approval of the Ethics Committee of Lecce (ASL Lecce, Minutes n.30, 7 March 2019 - Elbow Lateral Pain Survey) and is entirely conducted by the University of Rome "Tor Vergata" - Master's Degree in Applied Manual Therapy to Physiotherapy.
Therefore, we submit a simple questionnaire of 12 questions, made on the basis of the most recent scientific literature in collaboration with the following colleagues: Brindisino Fabrizio, Di Filippo Luigi, Maselli Filippo, Pennella Denis and Salomon Mattia.
Reading and completing the questionnaire anonymously takes only three minutes.
(link: https://it.surveymonkey.com/r/5P8SRLW)

Trusting in your collaboration, thank you and kindly best regards.

ORIGINAL ARTICLE

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Effectiveness of A Smartphone Directed Physical Activity Program on Cardiometabolic Disease Risk in Desk-Based Office Employees – A Pragmatic, Two-Arm, Parallel, Cluster Randomised Trial

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SUMMARY

Background. Excessive uninterrupted sitting is found to be associated with increased cardiometabolic disease risk in desk-based employees. Point-of-choice prompts using a smartphone application (SmPh-app) may be a feasible method of promoting physical activity and negating cardiometabolic disease risk. The study aims to investigate the effectiveness of SmPh-app directed walk reminders on cardiometabolic disease risk (Fasting Blood Glucose (FBG), aerobic capacity (VO2 max) and Heart Rate Variability (HRV) in desk-based office employees.

Methods. In this pragmatic, two-arm, parallel, cluster randomised trial, desk-based employees (n=53) of five administrative offices (clusters) of a university were randomised to two groups: SMART group in which employees were provided with SmPh-app based (six) walk reminders during working hours and CONTROL group in which employees continued their usual work. As FBG, VO2 max and HRV data were skewed, nonparametric statistical analyses were used for intention-to-treat analysis.

Results. Of 53 desk-based employees initially included in the trial, 47 completed the four weeks trial. Employees in SMART group showed a statistically significant difference in FBG ($p < 0.001$), VO_2 max ($p < 0.05$) and nearly all domains (time, frequency and non-linear) of HRV (pre- *vs*. post-intervention) compared to CONTROL group. SMART group employees were found to reduce FBG by 8 mmol/dL (interquartile range (IQR): -12.25 to -3.75 mmol/dL), improve VO2 max by 2.23 ml/kg/min (IQR: 0.12 to 4.34 ml/kg/min) and improve HRV in all the domains compared to CONTROL group.

Conclusions. SmPh-app based walk reminders improved FBG, functional capacity and HRV in desk-based office employees.

KEY WORDS

Smartphone; break reminders; walk; blood glucose; aerobic capacity; heart rate.

BACKGROUND

Due to automation and excessive use of computers for most occupational tasks, a modern man spends around two-thirds of waking hours in a seated position. "Sedentary behaviour" in office characterised by an energy expenditure ≤ 1.5 Metabolic Equivalents (METs) in sitting or a reclining posture has become inevitable and socially acceptable for a contemporary man (1).

Desk-based employees spend 60–75% of their routine work time in the sitting position and sometimes they

tend to accumulate several bouts of uninterrupted sitting (for more than 30 minutes) in a day (2). Several studies have shown that chronic cardio-metabolic diseases such as diabetes, dyslipidaemia, ischemic coronary disorders, obesity, and hypertension are associated with sedentary behaviour (3). New evidence claims that accumulated bouts of uninterrupted sitting are associated with an increased cardiometabolic disease risk owing to altered glucose and lipid metabolism, reduced heart rate variability, and reduced functional capacity (4). Moreover, prolonged sitting is negatively associated with time and frequency domain measures of Heart Rate Variability (HRV); however, there is a dearth of studies that substantiate the impact of breaking sitting on HRV (5).

Having said that, interrupting prolonged sitting behaviour with light to moderate physical activity or even standing may reduce cardiometabolic disease risk (4). Point-of-choice prompting software installed on electronic devices (*e.g.* work computer, smartphone (SmPh), *etc.*), standing or height-adjustable desks, treadmill and bike work stations, and walking meetings are some interventions employed to break prolonged sitting (6). Moreover, computer or web-based prompts and smartphone (SmPh) based applications (apps) have been the focus of most research studies in the past decade, as a means of interrupting occupational sitting and thus negating its adverse health effects (6). Light physical activities such as walking during break times have been found to improve fasting and postprandial glucose and insulin levels in a customised office setting (7). Further, breaking prolonged sitting behaviour and indulging in light-moderate activities during office hours have been reported to improve energy expenditure and aerobic capacity have been associated with light to moderate activities rather than standing alone during office hours (8).

Nowadays SmPhs are ubiquitous in use and may be used to influence human behaviour based on the principles of the social cognitive approach and ecological model (9). Smph-based applications (app) embedded with accelerometers, magnetometers and gyroscopes could be used to monitor and influence human physical activity patterns. There have been studies on Smph-guided break reminders that have shown a positive effect on lowering the sedentary time and postprandial glucose levels in people with chronic diseases (10). However, the influence of SmPh-based break reminders of physical activity on office employees and their aerobic capacity and heart rate variability remains largely unknown. We thus investigated the effects of a SmPh-app to provide break reminders to "take a walk" on cardiometabolic disease risk, especially Fasting Blood Glucose (FBG), functional capacity (VO_2 max) and Heart Rate Variability (HRV) in desk-based office employees.

METHODS

Study design and ethical approval

This was a pragmantic, two-arm, parallel, cluster randomised controlled trial conducted at administrative offices of Manipal Academy of Higher Education (MAHE) in India from February 2019 to June 2019. Kasturba Medical College and Hospitals (KMC & KH) Institutional Ethics Committee (676/2018) approved the study which was then registered with the clinical trial registry of India (CTRI/2019/01/017117). The research was conducted ethically according to international standards and as described by Padulo *et al.* (11).

Study setting and participants

Only seven of 23 administrative offices of respective constituent institutions of MAHE were contacted and necessary permissions were sought from them for participant recruitment. Desk-based employees, aged 25-60 years, of either gender, with a sitting time > 6 hours per day and a self-reported physical activity < 150 minutes/week), who agreed to undergo fasting glucose testing (both pre- and post-intervention period) and be available for four weeks of study/intervention routine were recruited. Employees who were pre-diagnosed with cardiometabolic disorders including uncontrolled hypertension, diabetes and heart diseases, and neuromusculoskeletal disorders that would affect their participation in the study were excluded. Desk-based employees were eligible in the intervention cluster only if they had smartphones (Android version above 4.0). The desk-based employees who were pregnant, planning to go on leave or serving a notice period following resignation were also excluded from the study. As we did not get the necessary permission from two administrative offices out of seven approached, only five clusters (n=76) were included and randomised to either arm (intervention or control). Employees in the clusters signed written informed consent before their participation in the study. All the study participants were advised to follow a standard dietary intake of 2300 Kcal/day from a dietary chart given during the period of familiarisation.

Sample size and power

To establish a between-arm difference of at least a 3 mmol/dl (12) difference of fasting glucose (effect size of 0.5) with an intra-cluster correlation coefficient (ρ) of 0.5 and clusters having a small sample size of 14 ± 5, we required 42 desk-based employees with 21 in each arm at a 95% significance level and a power of 50%. The formula used was:

Effective sample size = $^{mk}/_{1 + \rho (m - 1)}$ where:

- m is the population size in each group;
- k is the number of clusters;
- ρ is the inter cluster coefficient.

SmPh-app based walk reminders

"Take a walk" (TW) (Happy Lives, Pune 2018) is a simple Android based app that runs on a Java platform and is freely available on the Google Play store. The TW app reminds the employees to walk at regular intervals during the day where the timings and dates can be set according to their individual needs. Necessary permissions through e-mail were sought from the developer for administering the TW app to desk-based employees.

Randomization and blinding

Random allocation of clusters to intervention (SMART) and usual work (CONTROL) groups (**figure 1**) was done (by TCZ) to minimise the contamination of the interventions among the participants. An experienced exercise physiologist, who was blinded to the randomisation and intervention assignment, measured the outcome variables of interest at the baseline and follow-up period. The measurements were entered in a coded sheet; data entry was done in a password-protected computer.

Baseline screening for eligibility

Participants belonging to the clusters of each group were instructed to abstain from caffeinated beverages and vigorous exercise for 24 hours and should have had eight hours of adequate sleep before the day of the baseline measurement. The participants underwent FBG test by the finger-stick method and maximal aerobic capacity (VO_2 max) was quantified through a submaximal exercise test. The sedentary healthy desk-based office employees from the clusters were included if they had an FBG < 100 mg/dL and a reduced aerobic capacity with VO2 max < 26.5 ml/kg/min.

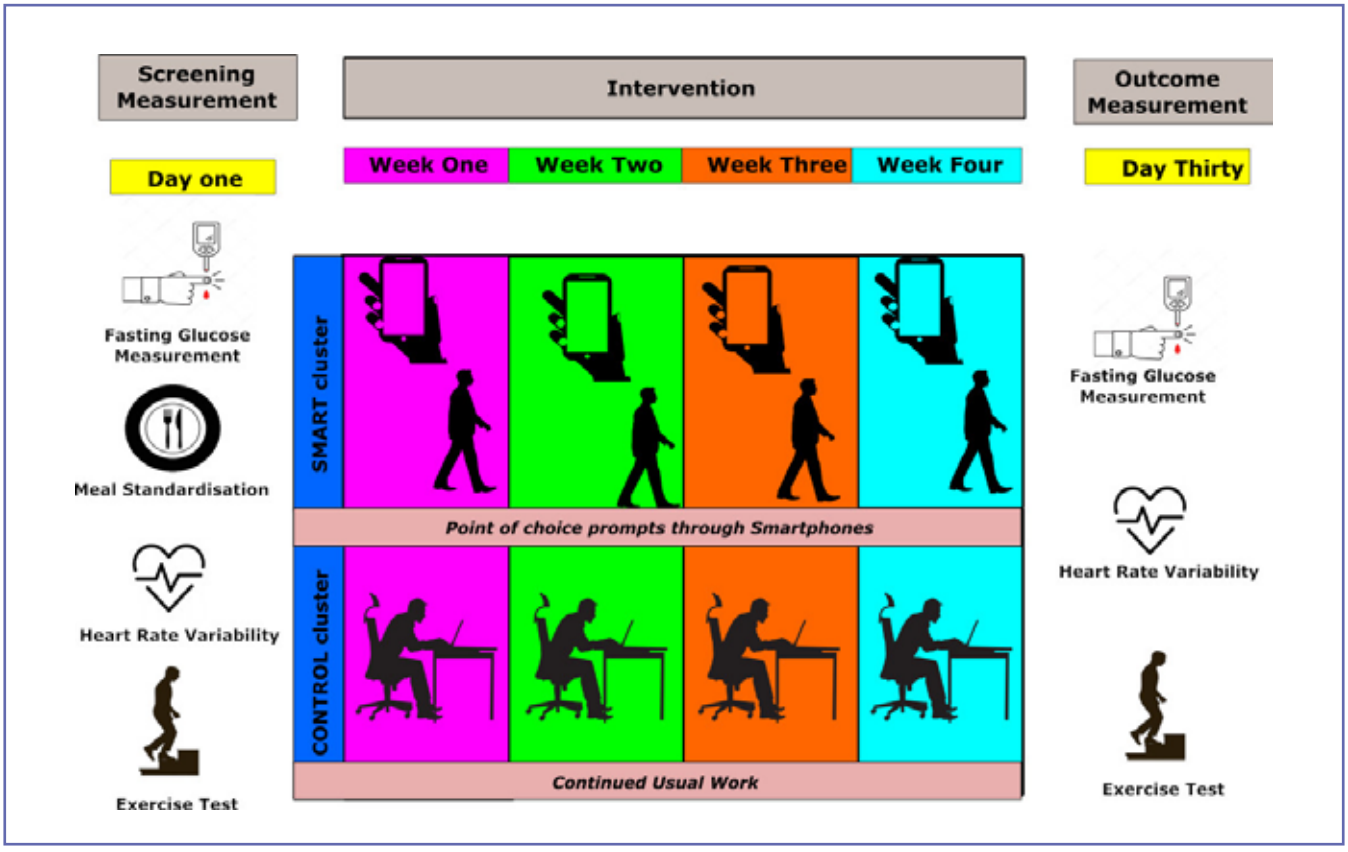

Figure 1. Infographic showing the randomisation process and the trial procedure.

Procedure

After the baseline measurements of FBG, VO2 max and HRV, the clusters were randomised, respectively, for four weeks of intervention. The employees of the SMART group were then requested to download and install the TW app in their SmPh. Explanation of the procedure and familiarization of log entry were given to the participants on the initial day of the intervention. The break reminders were set for two minutes of walk every one hour during work time (9:00 AM, 10:00 AM, 11:00 AM, 12:00 AM, 3:00 PM, and 4:00 PM). Participants manually logged into the TW app at the end of every 2 minutes of "walk break". The self-paced walk was administered for two minutes during the break reminders, and the compliance to the break reminders was logged both in the TW app and activity manual log, to cross validate the break timings with the application. Every week adherence to the walk breaks was collected from the employees SmPhs. At least 70% of walk attendance in four weeks was deemed necessary for inclusion in the final analysis. The employees recruited in the CONTROL group continued their routine occupational and leisure activities for the next four weeks of the trial period. Both the clusters were assessed for VO2 max, HRV and FBS at the end of four weeks by the blinded assessor. The whole procedure is presented as an infographic (**figure 1**).

Outcome data

FBG

Finger-stick Glucose Testing (FGT) was used for testing the FBG in the employees at 7:15 AM after 8 hrs of fasting. The Accu-Chek Active™ blood glucometer and test strips (Roche Diagnostics, Mannheim, Germany) were used for the FBG measurements. The glucometer was calibrated daily using the control solution as per the manufacturer's standards. The coefficient of variation for FBS through finger stick measurement was found to be 1.9-6.4% (13). The FGT has been proven to be an accurate measure compared to laboratory blood glucose monitoring by enzyme-linked immunoassay method for diagnostic purposes (sensitivity of 81% and specificity of 65%) (14).

VO_2 max

VO_2 max was estimated by a submaximal exercise test - the Queens College Step (QCS) test. The QCS was performed using a box with a height of 41.3 cm. A cadence of 24 steps/minute and 22 steps/minute, for males and females respectively, was performed with a metronome for three minutes. After cessation of the test, the radial pulse rate was measured from 5 to 20 s during the recovery period. The pulse count was multiplied by four (beats per minute) to obtain the Recovery Heart Rate (RHR), which was then employed in predicting the VO_2 max (111.3 – (0.42 × RHR)) (15). An Indian study found that the estimated VO_2 max by the QCS test is reliable and valid ($r=0.95$; $p < 0.001$) as compared to directly measured VO_2 max by the Douglas bag method (15).

HRV

HRV was measured as per earlier guidelines outlined by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (16) The employees were provided with a Polar H7 chest strap (Polar Electro Oy, Kempele, Finland) and instructed to secure it snuggly around the chest at the level of fifth intercostal space. Resting heart rate was recorded in a lying position for 10 minutes through a Bluetooth paired Smph-app (Elite HRV, NC, USA) with a sampling frequency of 1100 Hz. The participants were instructed to relax and bre athe at a natural rate. The R-R files were exported from Elite HRV SmPh application by email to a personal computer for analysis using Kubios HRV 3.3.1 (Kubios, Finland). Each file was corrected for artefacts as per manufacturer guidelines of Kubios HRV (cubic spline interpolation method). The validity of the Polar sensors has been reported to be higher (ICC > 0.99) compared to an electrocardiogram in measuring HRV (16).

The time domain HRV indices calculated were the Root Mean Squared Successive Differences (RMSSD) of Inter-Beat Intervals (IBIs) and the standard deviation of IBIs (SDNN). The frequency domain HRV indices calculated were very low frequency power (VLF, 0.003-0.04 Hz), low frequency power (LF, 0.04-0.15 Hz), high frequency power (HF, 0.15-0.4 Hz), and the ratio between LF and HF components (LF/HF). The nonlinear domains (SD1, SD2, SD1/SD2 index) were measured using a cubic spline interpolation method.

Adherence

Walking adherence was calculated by counting the number of sessions performed as mentioned in the activity diary (log) (17) with self-reported break time and duration of the walks. A threshold of 70% adherence was deemed necessary for inclusion in the data analysis; *i.e.*, a minimum of 100/144 break reminders should have been utilized for walking during the 4-week intervention by the SMART group employees.

Statistical Analysis

The continuous variables of FBG, VO_2 max, and time and frequency domain measures of HRV before and after intervention were not normally distributed as noted in the Shap-

iro-Wilk tests and histograms. Data are expressed as median and interquartile range. Since follow-up of six employees were lost during the four weeks intervention, the data of these participants were also included in the intention-to-treat analysis. We used baseline observation carried forward (BOCF) for including the missing data in analysis.

The Mann-Whitney U test (between-group baseline comparisons), Wilcoxon Signed-Rank test (within-group comparisons) and the Kruskal Wallis test (between-group comparisons) were used as appropriate. *Post-hoc* comparisons were done using the Wilcoxon Signed-Rank test and Holm-Bonferroni method with the adjusted p value for multiple comparisons. Adjusted p value= Target p value/ (Rank number of the pairs that are significant (n) + 1). To be statistically significant, the post-hoc pairwise comparisons of FBG, VO_2 max and time, frequency and non-linear domains of HRV should have a p value less than 0.025. The effect sizes were calculated by Cramer V imputations of Chi-square statistics, $V=\sqrt{\frac{\bar{x}}{n(\alpha_f)}}$

where:

- is the Chi-square value;
- n is the sample;
- df is the degrees of freedom.

The effect sizes of < 0.3, > 0.3 to < 0.5 and > 0.5 are considered as low, medium and high respectively (18). All the statistical analyses were done using the Statistical Package for Social Sciences Software (SPSS IBM® v. 23, IBM, USA). The study results are reported based on the recommendations of Padulo *et al.* (19).

RESULTS

Flow of participants through the study

Out of fifty-three desk-based workers from five clusters included initially into the study, 47 participants (SMART group, n=28 (18 females) with ≥ 70% walk attendance; CONTROL group, n=19 (11 females)) completed the four weeks of the study (**figure 2**). On average, the total walk time during the scheduled breaks was 10.2 ± 1.8 min for the SMART group employees as calculated from their self-reported activity log. The baseline characteristics were not significantly different between groups (**table I**).

FBG

A statistically significant difference in FBG was found between the SMART group compared to the CONTROL group (X^2=24.10; p <0.001) (**table II**).

The SMART group demonstrated a reduction in FBG with an effect size of 0.674 (moderate) compared to the CONTROL group post-intervention. Within-group comparisons revealed that the SMART group showed a significant reduction in FBG by 8 mmol/dL (IQR -12.25 to -3.75 mmol/dL); Z=-4.37; p < 0.001, compared to the CONTROL group (Z=-0.41; p=0.682) (**table III**).

VO_2 max

VO_2 max scores were significantly different between the SMART and CONTROL groups (X^2=13.22; p < 0.05). The SMART group employees were found to improve VO2 max by 2.23 ml/kg/min (IQR 0.12 TO 4.34 ml/kg/min) post-intervention than the CONTROL group (**table II**) which may not be clinically significant (effect size=0.499, p > 0.025). Within-group comparisons revealed that the SMART group demonstrated a significant difference in VO_2 max (Z=-3.95; p=0.000) compared to the CONTROL group (Z=-0.41; p=0.685) (**table III**).

HRV

Between SMART and control groups, all the variables of time, frequency and non-linear domains showed a significant difference except SDNN (X^2=2.24; p=0.135) (**table II**). A statistically significant difference in time, frequency and nonlinear domains were found within the SMART group compared to CONTROL (**table III**). On *post-hoc* pair-wise comparisons using the Holm–Bonferroni correction revealed statistically significant improvement in all the variables of time, frequency and nonlinear domains for the SMART (p < 0.025). On the contrary, though some HRV variables turned statistically significant in the CONTROL group they did not seem to clinically improve at the end of study. There were no adverse events during the study.

DISCUSSION

The purpose of this study was to investigate whether SmPhapp (TW) directed physical activity reminders would facilitate walking and improve FBG, VO_2 max, and time and frequency domain measures of HRV in desk-based office employees. This study found significant differences in fasting glucose, functional capacity and heart rate variability after a 4-week smartphone mediated physical activity program for desk-based office employees than the usual workgroup in real-time office settings. The key findings in this study are: a) compared to the control group, statistically significant reductions in FBS, VO2 max and HRV in the SMART group; b) SMART group showed significant reduction in FBG, and improvement in functional capacity and HRV compared to baseline values, with no clinical-

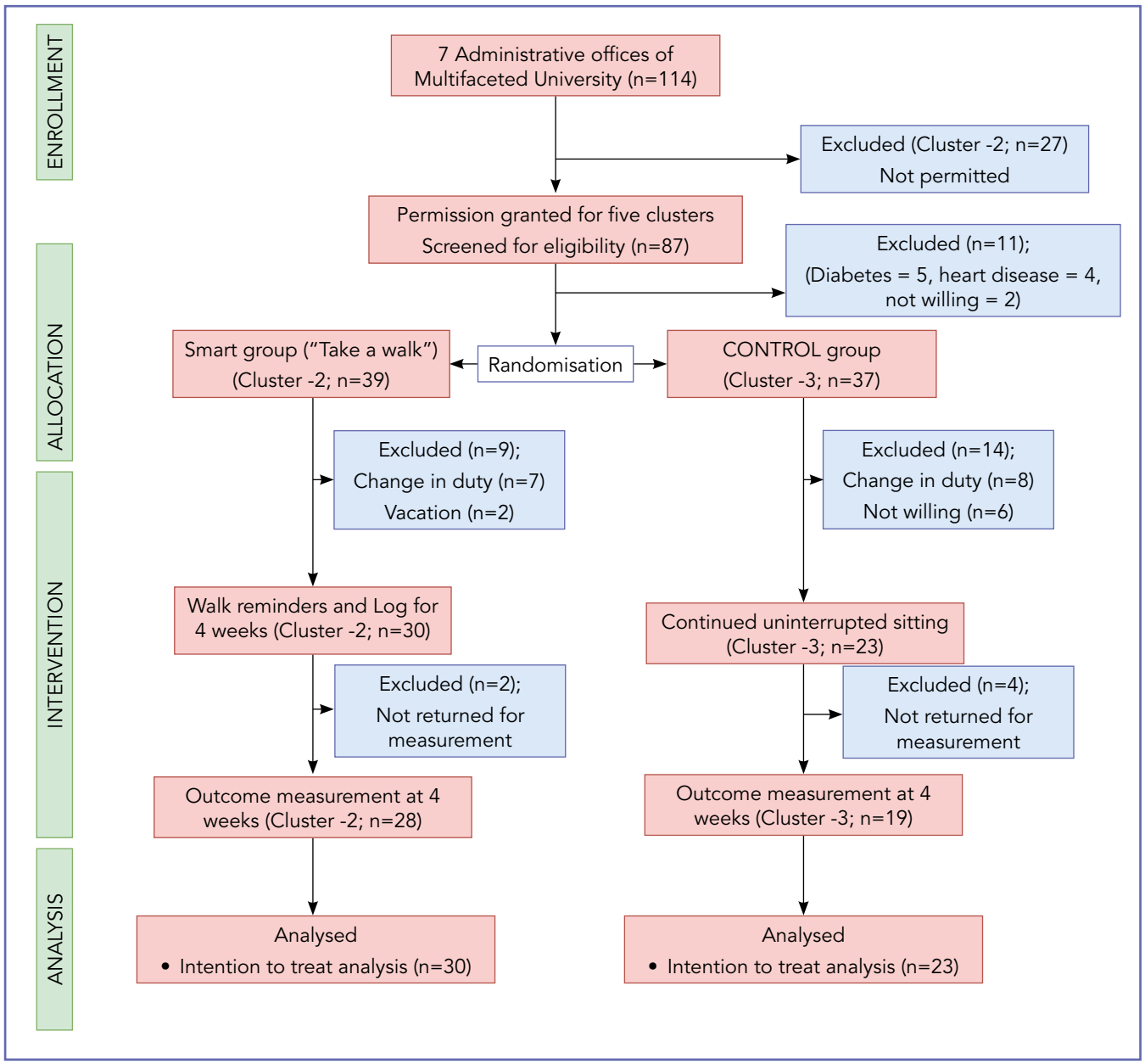

Figure 2. CONSORT flow diagram showing the flow of participants through the study.

ly evident improvement in the control group. These observations suggest that SmPh-app directed physical activity reminders may improve cardiometabolic risk markers such as FBS, VO2 max and heart rate variability. Smartphones based physical activity reminders may positively impact on attitude and perceived behavioural control by the theoretical framework of reasoned action, operant conditioning and planned behaviour (20).

FBG

Our study found a significant reduction of 8 mmol/dL in the FBG levels within the SMART group. Our study results agree with the earlier lab-based studies that have demonstrated a significant reduction in FBG levels after interrupting prolonged-sitting (21). The proposed mechanisms for this significant drop (11% from the baseline) in the FBG may be postural muscles contraction mediated transloca-

Table I. Baseline characteristics of the SMART and CONTROL groups involved in the study.

Baseline Characteristics			SMART group (n = 30) *Median (IQR)*	CONTROL group (n = 23) *Median (IQR)*	Z	p*
Age (years)			46 (45 – 48)	46 (44 – 54)	-2.832	0.825
BMI			22.85 (20.18 – 24.43)	23.15 (19.86 – 24.62)	-1.524	0.526
Fasting Blood Glucose (mmol/dL)			89.00 (78.00 – 93.25)	87.00 (81.00 – 88.00)	-0.054	0.257
Maximal Aerobic Capacity VO_{2max} (ml/kg/min)			45.31 (39.01 – 52.32)	36.00 (36.00 – 41.16)	-4.723	0.062
Heart Rate Variability	**Time Domain**	SDNN (ms)	52.68 (51.36 – 53.36)	49.12 (44.76 – 52.12)	-3.267	0.092
		RMSSD (ms)	52.39 (48.24 – 54.20)	54.23 (52.43 – 55.67)	-2.541	0.924
		NN50 (%)	129.43 (129.07 – 129.67)	129.67 (128.86 – 129.18)	-2.620	0.845
		pNN50 (%)	37.43 (37.32 – 37.60)	37.16 (37.02 – 37.24)	-3.446	0.762
	Frequency Domain	VLF (ms^2/Hz)	103.00 (101 – 105)	103.00 (90 – 103)	-2.883	0.164
		LF (ms^2/Hz)	981.00 (972 – 988.25)	984.28 (983.58 – 985.68)	-2.335	0.187
		HF (ms^2/Hz)	970.00 (958 – 984)	981.55 (965 – 984)	-0.953	0.340
		LF/HF (%)	1.02 (0.98 – 1.04)	1.01 (0.96 – 1.04)	-0.363	0.717
	Non-Linear Indices	SD_1 (ms)	37.20 (36.12 – 38.33)	22.10 (22.00 – 25.10)	-6.990	0.067
		SD_2 (ms)	55.00 (51.83 – 59.68)	33.50 (30.7 – 33.5)	-6.990	0.246
		SD_1/SD_2 (%)	1.48 (1.20 – 1.80)	1.52 (1.23 – 1.52)	-3.343	0.922

BMI – Body Mass Index; HF power – Absolute power of the high-frequency band (0.15 – 0.4 Hz); LF power – Absolute power of the low-frequency band (0.04 – 0.15 Hz); pNN50 – percentage of successive RR intervals that differ by more than 50 ms; RMSSD – Root Mean Square of successive RR interval differences; SDNN interval (ms) – Standard Deviation of NN intervals; *singnifcance as specified by Mann Whitney U test.

tion of GLUT4 transporters expression and insulin receptor sensitivity similar to the moderate-vigorous physical activities as a physical activity intervention (22). Our findings are in concordance with a recent metanalysis that established a moderate effect on post-prandial glucose levels (SEM=0.54; p=0.00001) with frequent breaks in sitting compared continuous/uninterrupted sitting (23).

VO_2 max

We found a statistically significant improvement of maximal aerobic capacity (VO_2 max) by 1.4 ml/kg/min (1.33%) which is not a clinically significant improvement. This may be an important finding of our study stating that simple walk breaks during office hours may not improve functional capacity. The clinically meaningful difference of VO2 max is claimed to be a 6% increase from the baseline based on previous study (24). Though it could be speculated that the moderate-vigorous walk may improve the functional capacity through improved energy expenditure and cardiovascular stress, it is not evident in our study. We hypothesized that simple low-moderate walking may not improve aerobic capacity whereas moderate-vigorous walking may improve VO2 max (Cohen's d=5.28).

HRV

Our study found a significant improvement of almost all the variables across all the domains of HRV. Though poor HRV is recognized an indicator of cardiometabolic disease risk, its relation with prolonged sitting is established recently (5). By breaking sitting, we could reverse altered autonomic function (enhanced sympathetic and reduced parasympathetic reflexes) which may reduce long-term cardiometabolic risk in sedentary desk-based office employees. In our study, we did not find statistical significance only in SDNN component of time domain. This finding warrants further studies to find the insignificant effect of sitting breaks on time domain, SDNN. We hypothesize that the insignificant results on SDNN may be due to inherent short-term variability of successive RR intervals itself (25). Among other time domain variables, SDNN may be volatile due to circadian rhythm when recorded in short term but it could be a gold standard for assessing cardiometabolic disease risk when recorded long-term (25).

Strengths and limitations of the study

Studies using SmPh-based physical activity measurement and interventions are less common in developing countries

Table II. Comparison of the median change scores between the SMART and CONTROL groups.

Cardiometabolic disease risk variables		SMART Group (n = 30) Change score ($\Delta t_4 - \Delta t_0$) Median (IQR)	CONTROL Group (n = 23) Change score ($\Delta t_4 - \Delta t_0$) Median (IQR)	Between-group median change scores Median (IQR)	Chi-square (X^2)	Kruskal Wallis Significance P < 0.05	Effect Size
Fasting blood glucose (mmol/dl)		-9.00 *(-11.25 – -6.00)*	-1.00 *(-2.52 – 0.86)*	-8 (-12.25 – -3.75)	24.10	0.000**	0.674#
VO_{2max} (ml/kg/min)		1.31 *(0.19 – 3.14)*	0.5*(-0.84 – 0.00)*	2.23 (0.12 – 4.34)	13.22	0.000**	0.499$
Heart Rate Variability measures	Time Domain	SDNN interval (ms)	1.59 *(-0.30 – 9.56)*	0.00 *(-0.24 – -3.65)*	1.59 (-4.37 – 7.55)	2.73	0.098	0.227
		RMSSD interval (ms)	4.9 *(3.53 – 10.23)*	-0.10 *(-1.69 – 2.27)*	5.03 (2.3 – 7.76)	24.39	0.000**	0.678#
		NN50 (%)	0.96 *(0.38 – 2.14)*	-2.78 *(-5.09 – -1.93)*	3.74 (2.34 – 5.14)	36.63	0.000**	0.831#
		pNN50 (%)	0.96 *(0.38 – 2.14)*	-2.78 *(-5.09 – -1.93)*	3.74 (2.34 – 5.14)	36.63	0.000**	0.831#
	Frequency Domain	VLF (ms^2/Hz)	4 *(3.91 – 4.11)*	-1.98 *(-13.00 – 0.00)*	5.98 (-6.82 – 18.78)	20.84	0.000**	0.627#
		LF (ms^2/Hz)	8.13 *(2.06 – 13.25)*	-6.89 *(-21.81 – 0.00)*	15.61 (4.39 – 25.63)	23.00	0.000**	0.659#
		HF (ms^2/Hz)	12.63 *(0.00 – 24.5)*	-5.55 *(-13.00 – 0.00)*	18.18 (6.68 – 29.68)	15.69	0.000**	0.544$
		LF/HF index (%)	-0.04 *(-0.07 – 0.02)*	0.00 *(-0.03 – 0.02)*	-0.04 (-0.49 – 0.41)	14.54	0.000**	0.524$
	Non-linear indices	SD1(ms)	3.9 *(3.28 – 4.51)*	13.8 *(10.7 – 13.80)*	-9.9 (-13 – 6.8)	21.81	0.000**	0.641$
		SD2 (ms)	33.6 *(16.96 – 33.6)*	25.9 *(21.82 – 29.26)*	-7.7 (-8.94 – 24.34)	7.94	0.005**	0.387*
		SD1/SD2 (%)	0.67 *(0.28 – 1.04)*	0.13 *(0.05 – 0.21)*	-0.55 (-0.54 – 0.56)	29.10	0.000**	0.741#

**p < 0.001; * - Low effect; $ - medium effect; # - High effect.

HR peak – Peak heart rate attained at the end of the submaximal exercise test; HF – Absolute power of the high-frequency band (0.15 – 0.4 Hz); IQR – Inter Quartile Range; LF– Absolute power of the low-frequency band (0.04 – 0.15 Hz); LF/HF – ratio of LF – HF power; pNN50 – percentage of successive RR intervals that differ by more than 50 ms; RMSSD – Root Mean Square of successive RR interval differences; SDNN interval (ms) – Standard Deviation of NN intervals; SD1 & 2 – Poincare plots perpendicular to line of identity; VO2max – Maximal Aerobic Capacity; Δt_0 – Mean before start of the intervention; Δt_4 – Mean at the end of four weeks.

Table III. Within-group comparisons (pre- *vs.* post-intervention) for cardiometabolic disease risk variables.

Cardiometabolic disease risk variables			SMART Group (n = 30)				CONTROL group (n = 23)			
			Pre (t_0) Median (IQR)	Post (t_4) Median (IQR)	Z	p	Pre (t_0) Median (IQR)	Post (t_4) Median (IQR)	Z	p
Fasting blood glucose (mmol/dl)			89 *(78 – 93.25)*	83 (72 – 86)	-4.37	0.000**	87.00 *(81.00 – 88.00)*	87.00 *(80.96 – 92.12)*	-0.41	0.682
VO_{2max} (ml/kg/min)			45.31 *(39.01 – 52.32)*	47.62 (39.57 – 55.86)	-3.95	0.000**	36.00 *(36.00 – 41.16)*	37.54 *(35 – 40.32)*	-0.41	0.685
Heart Rate Variability measures	**Time Domain**	SDNN interval (ms)	52.68 *(51.36 – 53.36)*	54.49 (52.53 -60.23)	-2.87	0.004**	49.12 *(44.76 – 52.12)*	49.38 *(48.12 – 50.62)*	-0.77	0.444
		RMSSD interval (ms)	52.39 *(48.24 – 54.20)*	58.12 (57.37 – 58.56)	-4.62	0.000**	54.23 *(52.43 – 55.67)*	55.14 *(53.24 – 55.67)*	0.04	0.968
		NN50 (beats)	129.43 *(129.07 – 129.67)*	130.35 (129.63 – 132.14)	-4.51	0.000**	129.67 *(128.86 – 129.18)*	126.20 *(123.82 – 127.88)*	3.82	0.000**
		pNN50 (%)	37.43 *(37.32 – 37.60)*	39.03 (38.24 – 39.22)	-4.63	0.000**	37.16 *(37.02 – 37.24)*	35.26 *(34.25 – 37.12)*	3.54	0.000**
	Frequency Domains	VLF (ms^2/Hz)	103 *(101 – 105)*	107 (102 – 112)	-4.70	0.000**	103.00 *(90 – 103)*	90.12 *(84.68 – 103.12)*	2.77	0.006**
		LF (ms^2/Hz)	981 *(972 – 988.25)*	986.5 (978 – 996)	-3.21	0.001**	984.28 *(983.58 – 985.68)*	976.00 *(962 – 984)*	3.22	0.001**
		HF (ms^2/Hz)	970 *(958 – 984)*	986 (973 – 994.5)	-3.29	0.001**	981.55 *(965 – 984)*	975.00 *(962 – 982)*	-2.50	0.013*
		LF/HF (%)	1.02 (0.98 – 1.04)	0.98 (0.94 – 1.01)	-4.55	0.001**	1.01 *(0.96 – 1.04)*	1.01 *(0.98 – 1.04)*	-0.31	0.760
	Non-linear indices	SD1(ms)	37.2 (36.12 – 38.33)	41.10 (38.18 – 44.12)	-4.77	0.000**	22.10 *(22 – 25.10)*	35.8(34.52 – 37.67)	-4.02	0.000**
		SD2 (ms)	55 (51.83 – 59.68)	88.6 (71.96 – 88.60)	-4.84	0.000**	33.50 *(30.7 – 33.5)*	59.4(57.62 – 61.08)	-2.61	0.000**
		SD1/SD2 (%)	1.48 (1.20 – 1.80)	2.15 (1.86 – 2.25)	-5.01	0.000**	1.52 *(1.23 – 1.52)*	1.66 *(1.28 – 2.01)*	-1.08	0.000**

*p < 0.05; **p < 0.005; p as significance from Wilcoxon Sign Rank Test.

HF – Absolute power of the high-frequency band (0.15 – 0.4 Hz); IQR – Inter Quartile Range; LF – Absolute power of the low-frequency band (0.04 – 0.15 Hz); LF/HF – ratio of LF – HF power; pNN50 – percentage of successive RR intervals that differ by more than 50 ms; RMSSD – Root Mean Square of successive RR interval differences; SDNN interval (ms) – Standard Deviation of NN intervals; SD1 & 2 – Poincare plots perpendicular to line of identity; VO2max – Maximal Aerobic Capacity

and our study is the first of its kind in India to demonstrate a significant improvement of HRV through breaking sitting through walk reminders using a freely available SmPh-app (TW) among desk-based employees. Being a pragmatic trial, we just used the TW app to provide break reminders and asked employees to maintain a log of walk attendance; however, we did not monitor the frequency, intensity and/or duration of walking intervention using accelerometers, pedometers etc owing to lack of availability of these devices at our study setting. We emphasize the need for future studies to monitor walking parameters during such interventions. Though a diet chart (recommending 2300 Kcal/day intake) was provided to the employees, their dietary intake and adherence to the recommended diet was not followed during the intervention. If the 6 participants (11.32%) who missed the final measurements would have been retested, it might have added to the strength of the study. However, the retesting was not possible due to change in their work time during the intervention period. Moreover, we estimated the functional capacity through a submaximal test (Queens college step test) whose validity in healthy population is questioned. Future trials should administer maximal exercise test with indirect calorimeter to directly measure VO2 max for precise clinical change.

CONCLUSIONS

In conclusion, our study results augment the fact that SmPh-app based physical activity intervention may reduce cardiometabolic disease risk through behaviour change in short-term in sedentary desk-based employees. Further clinical trials are warranted to use robust SmPh-apps with in-built motion sensors and accelerometers that monitor walking parameters (frequency, intensity, and duration) along with walk reminders.

ETHICS STATEMENT

The study proposal was reviewed and approved by the Kasturba Medical College and Hospitals (KMC & KH) Institutional Ethics Committee [Reference: 676/2018].

ACKNOWLEDGEMENTS

The authors wish to thank Dr Fiddy Davis, Head of the Department of Exercise and Sports Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India for his continuous support and motivation for this research project.

HUMAN RIGHTS

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

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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Treadmill Low-Intensity Training Mediates Fibrosis and Maintains the Function of Dystrophic Muscle: Understanding the Effects Over Time

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SUMMARY

Background. Dystrophic skeletal muscle undergoes fast degeneration and therapeutic exercise is controversial in this condition. The aim of this study was investigate the effects of a low-intensity training protocol on muscle fibrosis and function of the *mdx* model.

Methods. *Mdx* male animals with 8 weeks of age were (T0; n=8) underwent a protocol of eight weeks at a horizontal treadmill (9 m/min, 3x/week, 30 min/day). *Mdx* animals were randomly allocated at the Trained group (*mdx*-T, n=8) or sedentary (*mdx*-NT, n=8). In vivo functional measurements of strength and performance, immunolocalization of TGF-β1 and histomorphometry of intramuscular collagen fibers were assessed over time of protocol (T0, T4, T8) on Tibial Anterior (TA) and Soleus (SOL).

Results. Strength decreased after 4 weeks of training (T4) and was regained at T8 at *mdx*-T. The percentage of intramuscular collagen fibers area decreased at SOL muscle of *mdx*-T at T4 when compared to T0 (p=0,025) and TA of *mdx*-T had a lesser area at T8 when compared to TA of *mdx*-NT group (p=0,002). TGF-β1 was observed at the sarcoplasm of TA and SOL muscles of *mdx*-NT group, with an age-dependent change.

Conclusions. Treadmill low-intensity training provoked dystrophic skeletal muscle adaptation of fibrosis and maintained grip strength of the *mdx* model.

KEY WORDS

Duchenne muscular dystrophy; mdx model; low-intensity training; treadmill; fibrosis; TGF-β1.

BACKGROUND

Duchenne Muscular Dystrophy (DMD) is the most common muscular dystrophy that affects 1:3500 boys due to an x linked disorder with genetic inheritance. Mutations in the dystrophin gene lead to protein expression deficiency resulting in muscular degeneration, necrosis and atrophy (1). Dystrophin is typically expressed on skeletal, smooth and cardiac muscles and on the brain but its absence on DMD is particularly observed on skeletal and cardiac muscles (2).

The muscle damage is constant and outpaces the muscles normal ability for self-regeneration through its resident population of adult stem cells (satellite cells). In dystrophin's absence, there is increased cellular membrane damage (even under light muscle tension), constant necrotic inflammation, and satellite cell exhaustion. Eventually, muscle cells are replaced with nonfunctional fibrotic collagen build up and adipose tissue accumulation (3) inflammatory cells that infiltrate at the necrosis place are a source of transforming growth factor beta (TGF-β). TGF-β is a multifunctional cytokine that acts in different cellular types. Its role on the regulation of skeletal muscle inflammatory response, inhibition of regeneration, regulation of the extra-

cellular matrix remodeling and fibrosis promotion is well established on literature (3), and also is positively regulated on *mdx* muscle (4).

Skeletal muscle fibrosis increases the area of Extracellular Matrix (ECM) on muscle sections and, as a result of the high deposition of collagen fibers, increases of muscle stiffness and contractibility; it limits muscle function and mobility. Muscle fibrosis also limits the amount of available muscle tissue to therapy and repair (4).

Important events of DMD natural history were clarified by preclinical studies using the *mdx* model. They include the sarcolemma mechanical fragility (5), aberrant calcium homeostasis (6), mitochondrial distress, imbalanced oxidative stress (7) and chronic inflammation (8).

The endurance training was proposed to enhance muscle strength and avoid contractures on boys with DMD, but the impact and beneficial mechanisms due to the endurance exercise regimen need more research (9, 10) once the studies with *mdx* model produced inconsistent results.

High intensity training, as a horizontal treadmill running at high speeds of 12 m/min, has been suggested to exacerbate muscular dystrophy (11). In the other hand, voluntary exercise as swimming and voluntary wheel running seems to delay the disease progression (10, 12, 13). However, an exactly dose of these therapeutic exercise considered as of low intensity has not been determined yet. According to Kostek and Gordon (12) it is unknown how much stress is necessary to induce the positive effects of exercise without exacerbate the disease.

This study investigated the effects of chronic low-intensity training on function and regeneration of skeletal muscle of the *mdx* model over time.

MATERIALS AND METHODS

Forty males dystrophic *mdx* mice (C57BL/10ScSn- Dmd*mdx*/J) acquired from UniABC, São Paulo, Brazil (CQB-172/02) were studied. Animals were maintained in cages on a 12h day/12h dark inverted cycle with ambient temperature controlled at 22 °C and supplied with food and water ad libitum. Thirty-two *mdx* animals were randomly assigned into two groups: Trained (*mdx*-T, n=16) or No-Trained (*mdx*-NT, n=16) group. The other eight animals were control (n=8) of the initial time T0. This research was approved by the Ethics Committee on Animal Use of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (CEUA/UFVJM), protocol nº 025/15 and meets the ethical standards of the Muscles, Ligaments and Tendons Journal (14).

Design

Training protocol began after the first assessment (T0). One group had trained for 4 weeks – T4: Short protocol (*mdx*-T=8 and *mdx*-NT=8) and another one had trained for 8 weeks – T8: Long protocol (*mdx*-T=8 and *mdx*-NT=8) (**figure 1**). T0 was determined at the time dystrophic animals had 8 weeks of age. The age of 8 to 12 weeks is considered a morphological stable phase of the disease to *mdx* model

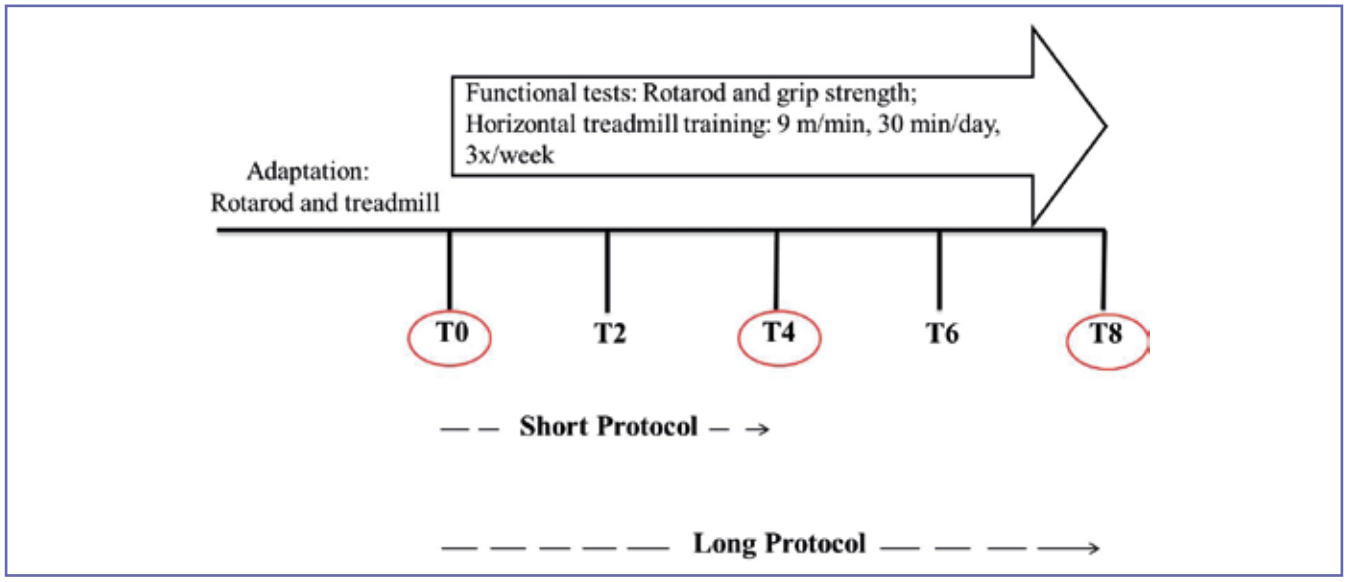

Figure 1. Experimental design: Times of assessment and collection of biological collection. Functional measurements of mdx-T and mdx-NT: Rotarod and Grip Strength at T0, T2, T4, T6 e T8; Biological collection at T0, T4 e T8.

once animals have already suffered an important cycle of degeneration/regeneration (15).

Training protocol

After an adaptation protocol (**table I**), *mdx*-T group were stimulated to run at a motorized treadmill (EP 131; Insight®, Brazil) at 9 m/min. The speed was controlled at 9 m/min so the protocol was considered of low intensity according to previously described protocols specific to this animal model (16). The protocol consisted of 30 min/day, 3x/week during 4 weeks (Short protocol -T4) or 8 weeks (Long protocol – T8). Animals of *mdx*-NT group were placed at the horizontal treadmill with speed at 0m/, 3x/week during 4 weeks aiming to be exposed to the same environmental conditions.

Functional tests

Grip Strength and Rotarod were used as functional measurements. They were assessed each 2 weeks of protocol over time (T0, T2, T4, T6 and T8) (**figure 1**). Every moment of functional measurements animals were weighted at a semi-analytic balance (UX-420H 0,001g precision) to follow animals well-being and also to further use to normalization the values of grip strength avoiding the influence of weight on this variable as described by Van Putten *et al.* (17).

Rotarod

Performance at Rotarod was used to assess coordination, balance, muscle strength and condition over time of protocol (17). An automated device was used (MP13977, Insight®, Brazil). After adaptation at the device (**table II**), the mice were placed in the rotarod tube as it rotated at a slow and steady speed of 5 rpm. The running started as soon as all the mice were in position. The tube speed accelerated from 5 to 37 rpm and maintained that speed. The running time was stopped automatically when mice dropped from the tube, as this activated the time bar positioned below the tube, but the animals were repositioned immediately. The test session was terminated for mice capable of running for 500 seconds. The mice were given a maximum of two more attempts, which allowed them to improve their execution time when they fell earlier. The maximum execution time (*i.e.* the longest of the tests) was used for further analysis (17).

Table I. Adaptation protocol of mdx-T group at the treadmill.

Time	Speed
4 min	2 m/min
15 min	4 m/min
30 min	6 m/min
15 min	9 m/min
30 min	9 m/min

*Adaptation protocol started at the week before the training protocol where mdx animals were 7 weeks of age and lasted 5 days long.

Table II. Adaptation protocol to Rotarod.

Time	Rotarod speed
250 sec	16 rpm
250 sec	25 rpm
500 sec	37 rpm

*Rotarod adaptation started at the week before the training protocol where mdx animals were 7 weeks of age and lasted 3 days long.

Grip Strength test

The forelimb grip strength was performed with the Grip Strength Meter (PanLAb®, Brazil). Animals were suspended by the tail above the grid and after they grasped, they were pulled backwards. The value of maximal force was registered by the Grip Strength Meter. Each animal was tested five times with one minute interval between trials. The three highest values were averaged to calculate the absolute strength, which was divided by the corporal mass in grams. All measurements were made by the same blinded investigator to avoid bias.

Muscle tissue analysis

Animals were euthanized during the protocol period: n=8 at T0, n=16 at T4 and n=16 at T8 according to CEUA protocol nº 025/15 seventy-two hours after the final exercise session. All animals were subjected to an overdose of ketamine hydrochloride (200 mg/kg) and xylazine hydrochloride (20 mg/kg), via intraperitoneal injection. Tibial Anterior (TA) and Soleus (SOL) muscle were collected and fixed in paraformaldehyde solution at 4% during 24h and further tranfered to phosphate buffer. They were treated with increasing ethanol concentrations (70 to 100%) to dehydrate and with xylene to clear. The samples were then embedded in paraffin (Ervplast®) and sections of 5 mm in thickness were obtained. The cross-sections were oven-dried (60 °C) at a horizontal position for better adhesion of the cuts. After deparaffinization protocol, the sections were stained using Hematoxylin-Eosin (HE) according to conventional histological procedures to identify histopathological features. Histochemical reaction using Picrossirius red, a combination of Sirius red F3BA (Sigma-Aldrich, Color Index 35780) dissolved in a saturated picric acid solution, was used in order to distinguish collagen from the skeletal muscle fibers.

Intramuscular collagen fiber quantification

Slides reacted with picrossirius red were analyzed under polarized light in 400x. Photomicrographs of ~20 sequential images of each animal studied were performed to carry out the analysis of the whole section transverse muscle (18) totalizing an analysis of an area of 2000 to 3000 fibers/animal. The amount of deposition of the collagen fibers was calculated by the percentage of the area of collagen fibers in relation to the total area (57248.52 μm^2) of each image, through binary analysis (black/white) and expressed in micrometers using ImageJ® software: "Process" > "Binary" > "Make Binary" > "Analyze" > "Set Measurements" > selected "Area' + Area fraction" > "Analyze" > "Measure".

Immunohistochemical analysis (IHC)

Primary polyclonal antibodies against TGFβ-1 (anti-human) (StressMarq Biosciences®), 1:750 were applied on TA and SOL muscle sections. Sections were immersed in citric acid solution at 0.01M, pH 6.0 and submitted to 95 °C for 30 min to antigenic recovery. Next, the blockade of endogenous peroxidase with hydrogen peroxide at 3% for 40 min was performed. Primary antibodies were applied and incubated for 20 h in a damp chamber at 4 °C. After three more rinses in Buffered Saline Solution (PBS), secondary antibody (N-Histofine®) was applied and incubated for 30 min at room temperature (24 °C). IHC reaction was revealed with DAB (Chromogen/Substrate Bulk Pack, ScyTek Laboratories) for 2 min. In the negative control, the primary antibody was omitted and all slides were counterstained with hematoxylin.

All tissue photomicrographs for histological, histomorphometrical and IHC analyses were made under an optical microscope (LABOMED® LxPol) equipped with an Axio CAM HRc camera and Software Capture Pro 2.9.0.1.

Data analysis

Qualitative assessments of histological, histochemical and IHC of muscle samples were analyzed by observing three sections from each one of the animals (n=8)/per group=5: T0, T4 *mdx*-T and *mdx*-NT, T8 *mdx*-T an *mdx*-NT. The descriptive statistical analysis was performed via mean and standard error calculations (grip strength, Rotarod and intramuscular collagen fiber quantification). Analysis of the normality of the data was performed by the Shapiro-Wilk test and considering normal distribution the p value > 0.05. To detect difference between groups the Student t test analysis was conducted and to detect difference intra groups an Analysis of Variance (ANOVA) was performed and the Tukey test was used as *post-hoc*. The effect size (Cohen's d) was measured by Cohen's D Test. The IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA) was used with the level of significance set at $p < 0.05$.

RESULTS

The *mdx*-T and *mdx*-NT groups presented an age dependent increased in corporal mass, without difference between groups. All *mdx*-T animals from Short (4 weeks) and Long Protocol (8 weeks) have finished the established treadmill protocol completing the 30 min/session at 9 m/min. The mean values of the length of stay at Rotarod also have not shown difference between or intra groups (**figure 2 a**).

The normalized grip strength data are shown at **figure 2 b**-**d**. There was a difference in T2 between groups (p=0.028), with the *mdx*-T group presenting higher values at this time (**figure 2 b**). There was a decrease of the grip strength values at T4 related to the protocol training. The *mdx*-T showed difference between T2 to T4 (p=0.009), T4 to T6 (p=0.022) and T4 to T8 (p=0.002) (figure 2D). There was an increase of grip strength of the trained animals after the 4th week (T4) of training until the T8.

The *mdx*-NT group showed a gradual decreased on grip strength values until T6 and a slightly increase at T8 not superior to T0. There was significant difference between T0 to T2 (p=0.006), T0 to T4 (p=0.049), T0 to T6 (p=0.002), T0 to T8 (p=0.043), T4 to T8 (p=0.047), and T6 to T8 (p=0.047) times of assessment (**figure 2 c**).

The area of intramuscular collagen fibers of SOL muscle was different between groups (p=0.025). At T4 (0.31 ± 0.04) it was lower than T0 to *mdx*-T animals (0.48 ± 0.05) (p=0.04) (**figure 3 a**). The TA muscle showed different percentage of area between groups (p=0.002). The *mdx*-NT group at T8 (0.31 ± 0.03) was different from the *mdx*-T group at T4 (0.29 ± 0.01) (p=0.021) and T8 (0.31 ± 0.04) (p=0.001) (**figure 3 b**).

For more details of Grip Strength, Rotarod and Intramuscular collagen deposition morphometry analysis, see the Supplementary material.

Typical histopathological features of dystrophic skeletal muscle were observed at SOL and TA muscle of both groups. Collagen fibers were distributed between muscular fibers with thicker tracts at perimysium than at endomysium. The *mdx*-T group presented innumerous thinner tracts at endomysium, mainly at T4 with thicker tracts observed at T0 than at T8, time were the collagen fibers had covered a higher area. Tracts of *mdx*-NT group were thicker at perimysium at T0 when compared to T4 (**figure 4**).

TA muscle showed thinner collagen fibers at perimysium of *mdx*-T group with the thicker ones at the perimysium of *mdx*-NT group on all assessed moments, especially at T8 (**figure 5**).

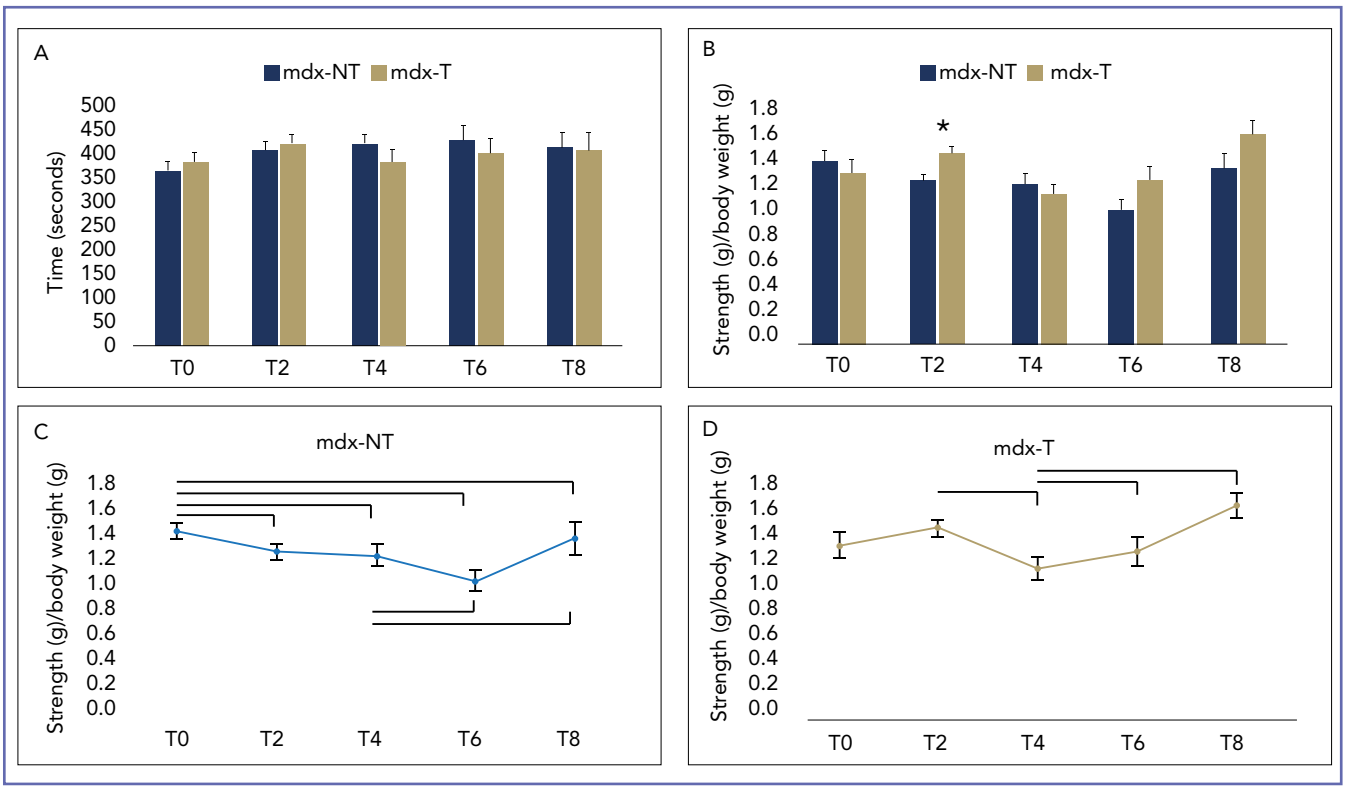

Figure 2. (**A**) Lenght of stay (seconds) at Rotarod over time of protocol training. (**B**) Normalized Grip Strength over time comparing *mdx-T* and *mdx*-NT groups. (**C**, **D**) Normalized Grip Strength of *mdx*-NT and *mdx*-T. *Difference between groups, independent *t* Test; horizontal bars show difference intra-group, paired *t* test with $p < 0.05$.

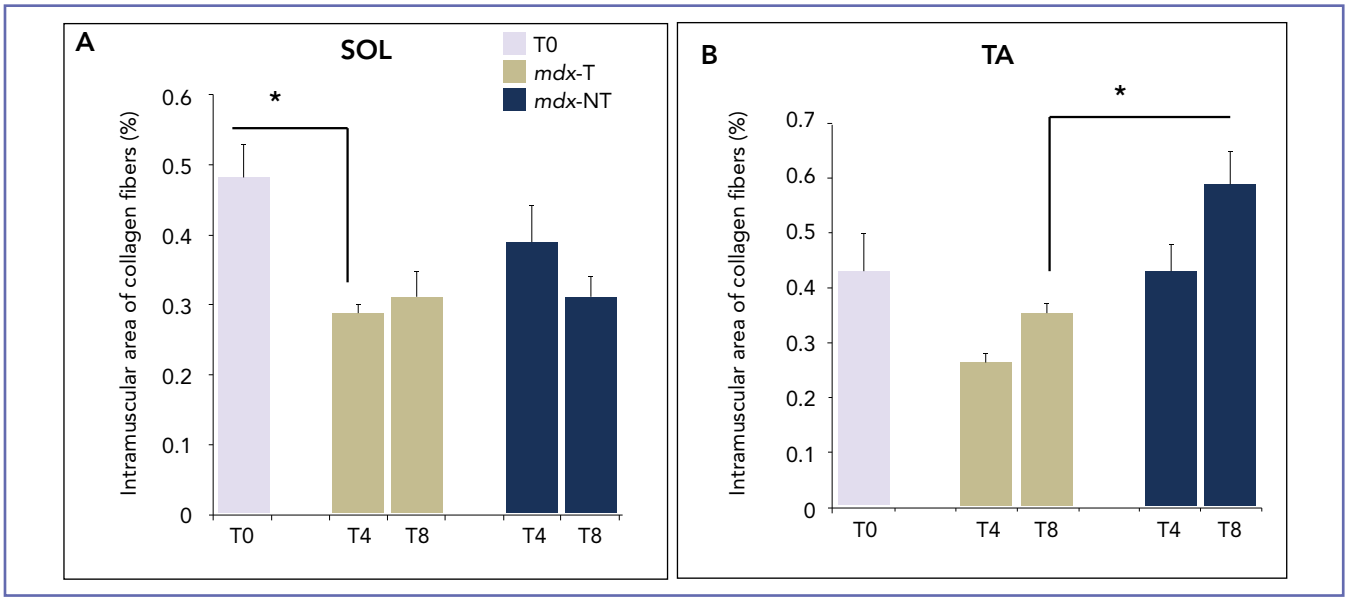

Figure 3. Effect of low-intensity training on the percentage of intramuscular collagen fibers deposition. Morphometric analysis of the percentage of intramuscular collagen fiber deposition in soleus (**A**) and tibial anterior (**B**). *Significant difference between groups (ANOVA with *post-hoc* Tukey).

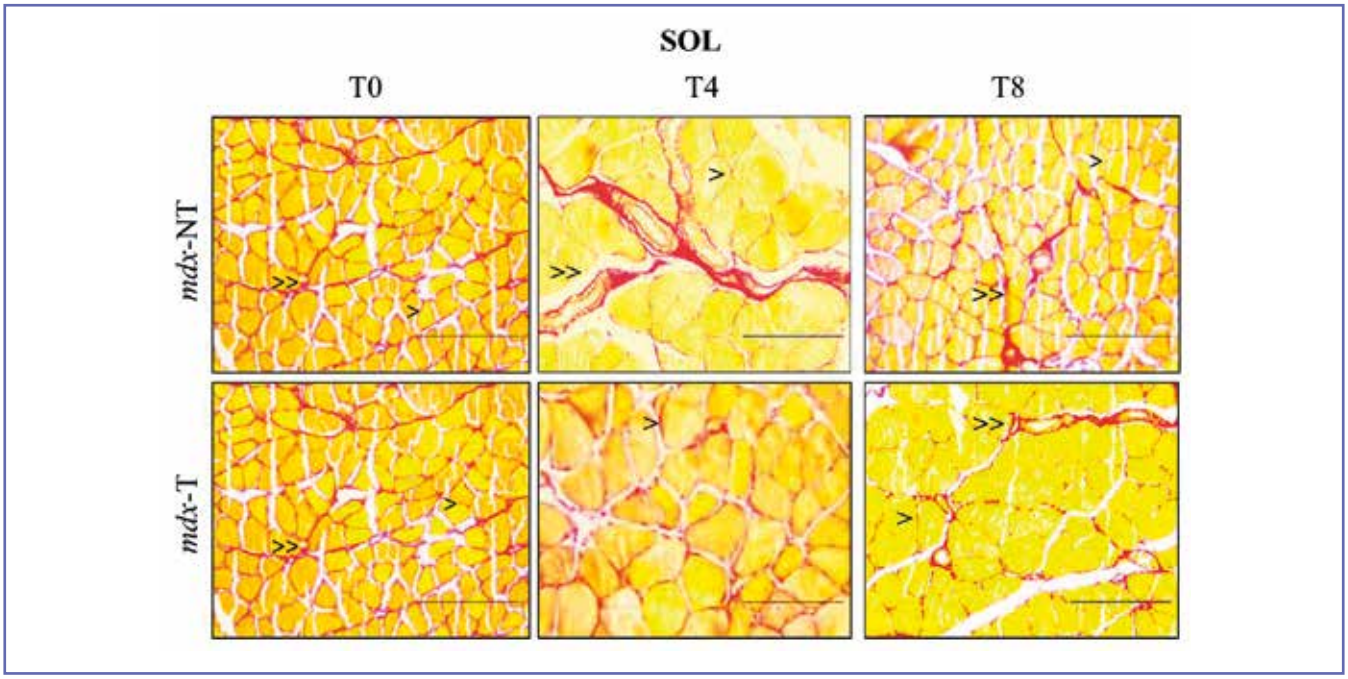

Figure 4. Morphological analysis of the deposition of collagen fibers on soleus muscle of *mdx*-NT and *mdx*-T. Picrossirius red reaction. >> Deposition of collagen fibers in the perimysium. > Deposition of collagen fibers in endomysium. Scale bar=100 µm.

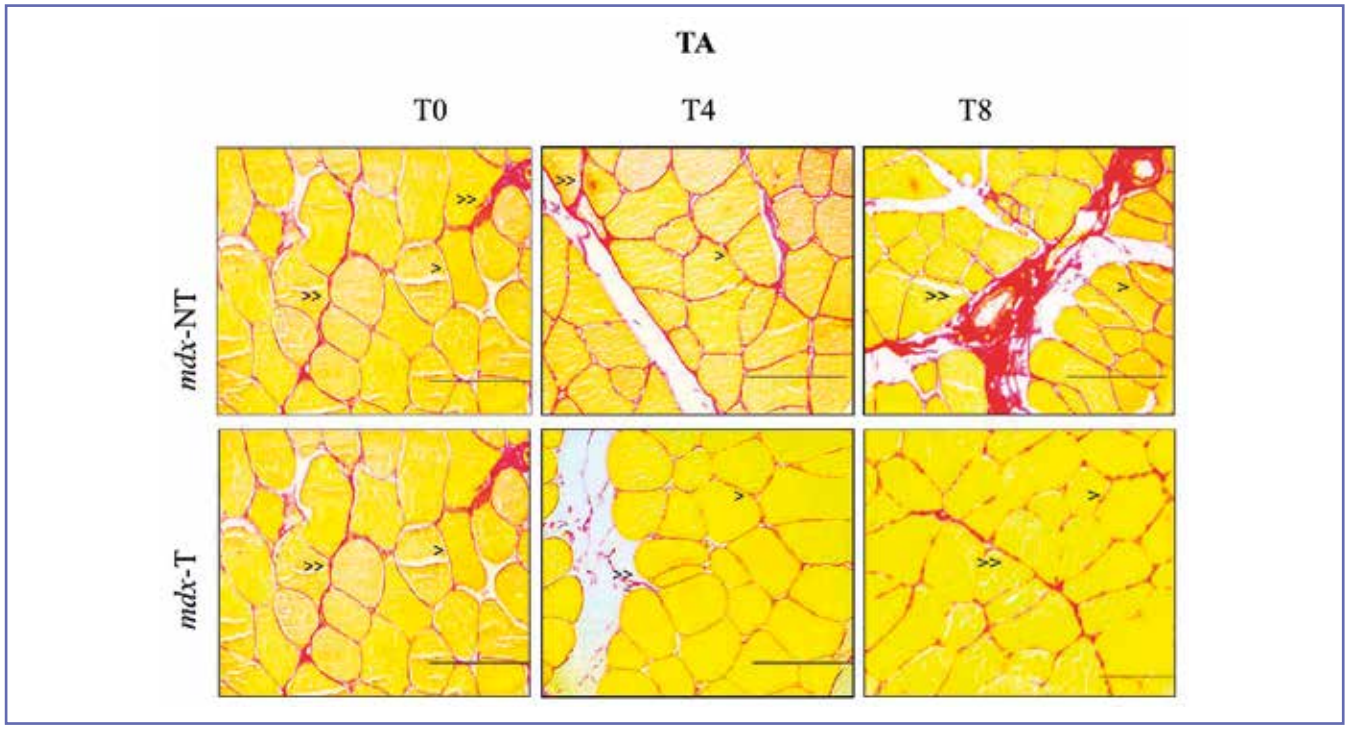

Figure 5. Morphological analysis of the deposition of collagen fibers on tibial anterior muscle of *mdx*-NT and *mdx*-T. Picrossirius red reaction. >> Deposition of collagen fibers in the perimysium. > Deposition of collagen fibers in endomysium. Scale bar=100 µm.

Immunolocalization of TGF-β1 has shown the presence of this pro fibrotic marker in the endomysium and perimysium of the TA and SOL muscles at T0, T4 and T8. It was also observed in sarcoplasm at T0 and *mdx*-NT at T4 and T8 (**figure 6 , 7**).

DISCUSSION

The low-intensity protocol training had maintained grip strength, performance on rotarod and favored a decline on the intramuscular collagen fibers area of tibial anterior muscle over time.

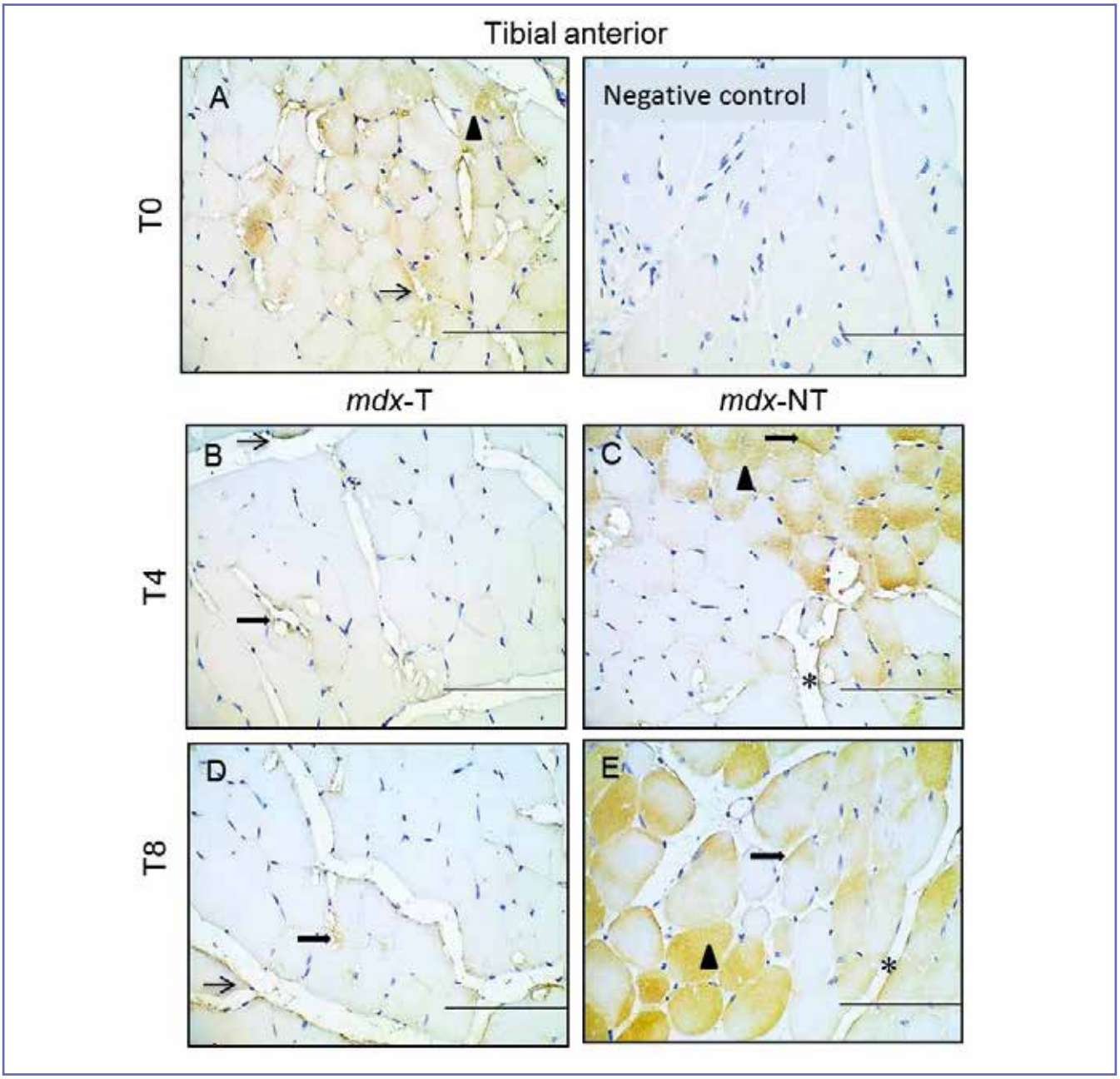

Figure 6. Morphological analysis of tibial anterior muscle of *mdx*-T and *mdx*-NT groups at T0, T4 and T8, 400x, IHC. Immunolocalization of TGF-β1 and negative control of immunohistochemical reaction. On **A**: Tibial anterior muscle at T0; **B** (*mdx*-T) and **C** (*mdx*-NT) at T4; **D** (*mdx*-T) and **E** (*mdx*-NT) at T8. *Perimysium → endomysium, → ▲ sarcoplasm. Bar: 100 μm.

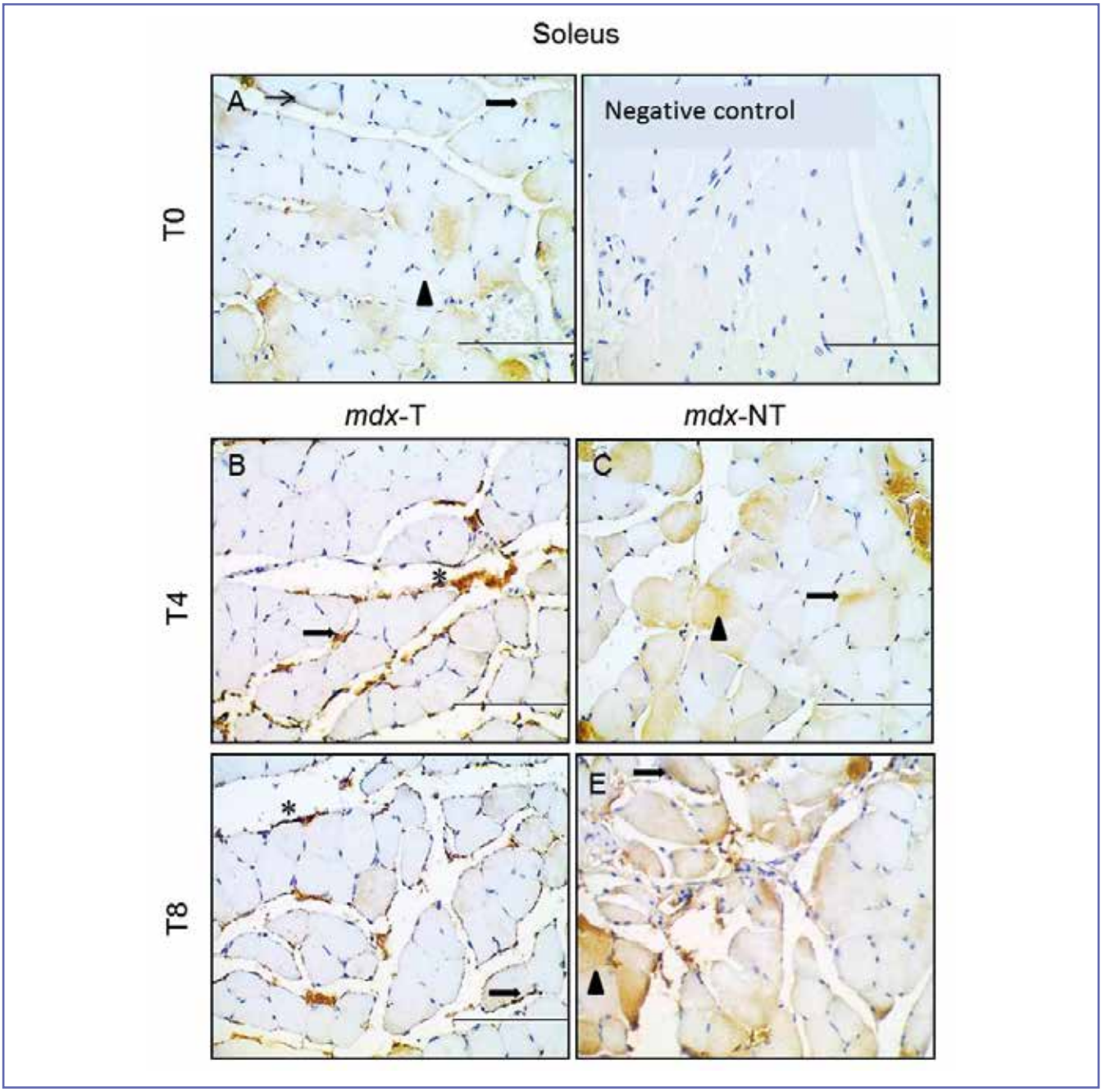

Figure 7. Morphological analysis of Soleus muscle of *mdx*-T and *mdx*-NT groups at T0, T4 and T8, 400x, IHC. Immunolocalization of TGF-β1 and negative control of immunohistochemical reaction. On A: Soleus muscle at T0; B (*mdx*-T) and C (*mdx*-NT) at T4; D (*mdx*-T) and E (*mdx*-NT) at T8. *Perimysium → endomysium, → ▲ sarcoplasm. Bar: 100 μm.

TGF-β1 stained at endomysium and perimysium tracts of muscle fibers, as expected to dystrophic muscle. Sarcoplasm of sedentary animals was also stained to TGF-β1 at T8. According to Ismaeel *et al.* (4) the expression of TGF-β1 increase progressively with the progression of the disease and is positively correlated to the collagen density.

The effect of the intensity and of the time of treadmill training protocol on forelimb strength in vivo and on corporal

mass of *mdx* mice has been already studied (11). Our results attest that a training of low-intensity did not worsened muscle function of *mdx* mice. It is interesting to note that at the moment were animals were 12 weeks old (at T4), the length of stay at Rotarod was lower than at the beginning or end of the 8 weeks of protocol. The same was observed to grip strength which suffered a decline at T4.

Some studies suggest that the strength of *mdx* mice decrease after 3 months of age (15). It is related to degeneration/regeneration cycles that are present between 4 and 15 weeks of age of these animals. This result is in accordance with the forelimb strength decrease that was found in *mdx* mice of 5 to 12 weeks age that have not made other functional tests besides grip strength or treadmill running (18). Capogrosso *et al.* (19) submitted animals of 4 and 5 weeks age to a protocol of 12 weeks of treadmill training, at 12 m/min and also found a decline on grip strength after 4 weeks of protocol.

Even that trained and not trained mice have not shown difference between their grip strength (inter group), there was a considered increase of grip strength of trained mice between T6 to T8 that attest an adaptation to trained that could not be observed on sedentary ones.

Exercise regulates function, morphology and metabolism of skeletal muscle through modulation of different signaling pathways (11). Also, exercise decrease dysfunctional adipose tissue and improve oxygenation (20).

Physical exercise has been proposed as an adjuvant therapy to humans DMD aiming to maintain muscle strength and prevent contractures as long as possible. However, exercise practice remains controversial once dystrophic muscle can be damage due to excessive use (8). According to recent systematic reviews, besides some studies have demonstrate the benefice of exercise in slow up motor function damage in young people with DMD, more research must be conducted to understand cardiac and respiratory function to exercise, the effect of long-term training protocol on dystrophic muscle and the effects of running and swimming on DMD (8, 12).

The intensity of training and age of dystrophic animals influence the effects of exercise on dystrophic muscle. High intensity training using forced treadmill or downhill running causes loss of muscle strength, myofibres necrosis (21) and fibrosis (22).

Moreover, damage-related genes as the transforming growth factor-β1 (TGF-β1), tumor necrosis factor (TNF-α) and tyrosine kinase c-src are severely increased, which reinforce lesion signs and muscle dysfunction (11).

Muscles are susceptible different to damage induced by exercise. Hindlimb muscles are directly involved in running exercises (19). Soleus and tibial anterior muscle are hindlimb muscles and are constituted by different fibers types which explain their different answer to training. In general, tibial anterior muscle is more affected to treadmill training because it has predominantly type II fibers (23). The fibrosis of tibial anterior muscle was influenced by low-intensity training protocol demonstrating reduce values at the end of the protocol in TA muscles of the animals that were submitted to exercise. We also observed a reduction of collagen fibers on soleus muscle after 4 weeks of protocol. Even in the dystrophic genotype, a muscle of slow type fibers is more resistant to mechanical challenge (11).

When fibrosis occurs, collagen not only increases its amount but also suffers post transduction dysfunction that alters its organization and contribute to tissue stiffness (18). Picrossirius red staining is one of the most known histochemical techniques able to selectively detect tracts of collagen and it becomes more specific when associated to the detection under polarized light (24).

On *mdx* model the infiltration of immune cells reaches a peak between 4 and 8 weeks of age (9) and fibrosis can be seen after 10 weeks age (8), which is in accordance to our finding on the muscle of *mdx* mice at T0 (8 weeks of age). In this way, we suggest that training had positively mediated collagen deposition in both studied muscle at T4 and had also kept smaller areas of fibrosis on trained mice in relation to sedentary *mdx* mice.

Fibrosis was here studied by intramuscular collagen fibers morphometry using picrossirius red under polarized light and the immunolocalization of TGF-β1. It is possible to observe that TGF-β1 on *mdx* mice skeletal muscle is age dependent and is located at epimysium and perimysium. Also, at sarcoplasm and around blood vessels of sedentary animals, features very close to intramuscular collagen fibers. Sedentary *mdx* mice showed TGF-β1 localized at sarcoplasm of muscle fibers, mainly at T8, time where they were 16 weeks old. TGF-β1 is generally present at epimysium and perimysium of dystrophic muscle while on healthy muscle no stain can be seen to this pro fibrotic marker (25).

Song *et al.* (1) attested that the expression of TGF-β1 is correlated to the degree of pathology and clinic progression of DMD. Its expression is regulated specifically at sarcoplasm of muscle cells and at myenteric plexus of human with DMD. Diverse animals' studies observed answers of TGF-β to muscle damage by eccentric muscle contraction, a modality of contraction proven harmful to the dystrophic muscle (15). Studies show that levels of TGF-β are increased after an eccentric muscle contraction (3).

CONCLUSIONS

As fibrosis reflects the final phase of a chronic inflammatory process typical of the dystrophic muscle, we suggest that low-intensity training can mediate the regeneration of

muscle fibers of the hindlimb maintaining muscle strength during a chronic protocol of exercise. The limitation of this study is that cardiac and diaphragm muscles were not analyzed and the profibrotic marker was not quantified. The clinical contribution of these results is that DMD boys in the ambulatory phase of the disease can benefit of training considered of low-intensity. Further clinical studies are necessary to attest modalities of low-intensity training in humans at this phase and its real effects not only on clinical parameters but also on cardiac and respiratory muscle and limb muscles involved on functional activities.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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REVIEW

Nr 2020;10 (4):734-739

Rehabilitation in Subjects with Chronic non-specific Low Back Pain with Sacroiliac Joint Origin: Protocol for a Systematic Review

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SUMMARY

Introduction. This systematic review protocol aims to evaluate the effect of rehabilitation for chronic non-specific low back pain with sacroiliac joint origin.

Methods. Search will be done in Pubmed, ISI Web of Science, Scopus, Clinical Key, Science Direct, Medline, Embase, PEDro, ProQuest, the Cochrane Library, PROSPERO, the MOH Thesis, MOH Articles, Magiran, and SID. Google Scholar search engine will also be used. All types of Clinical Trials, Cohort, Case-controls, Cross-sectionals, Observational Descriptive, Case Report, Case Series, Ecological Studies, Systematic Reviews, thesis and dissertation in English and Persian published prior to September 2019 will be included. The articles recruiting 18 to 60 years old will be included. Considering PICO, the finally retrieved articles will be assessed qualitatively by CONSORT, STROBE, PEDro, NIH and CASP checklists. Changes in pain and function will be favorable.

Dissemination. The protocol presented in present paper will be used to summarize and qualify present literatures on conservative therapy for chronic non-specific low back pain with sacroiliac joint origin.

KEY WORDS

Rehabilitation; phisycal therapy; pain; function; non-specific chronic low back pain; sacroiliac joint dysfunction.

INTRODUCTION

Today, Low Back Pain (LBP) has become one the most common musculoskeletal disorder in the societies with average global prevalence of 38.5% (1, 2). It is hypothesized that the 15-30% of Chronic Non-specific Low Back Pain (CNLBP) have sacroiliac joint origin (3-5). They are two main approaches dealing with sacroiliac joint impairment (6, 7). Non-invasive treatments such as physiotherapy (8-11) are the forefront of treatment in these cases (12-14). If not helpful, invasive approaches including fixation or fusion may be prescribed (15).

Since physiotherapy interventions as a non-invasive technique seems to be effective in treating chronic LBP (16), the aim of this study is to design a systematic review study seeking and comparing the effects of various interventions in this category on the pain and function of subjects that suffer from CNLBP with sacroiliac joint origin; the results will be of clinical value to determine the effectiveness of each method for this particular subgroup of LBP. If enough paper retrieved, the comparison can be made and a comprehensive therapeutic physical therapy plan may be suggested.

OBJECTIVES

To determine the effect of rehabilitation interventions on the pain and function in individuals suffering from CNLBP with sacroiliac joint origin.

MATERIALS AND METHODS

Trial eligibility criteria

Strict inclusion/exclusion criteria have been introduced in order to precisely protect search strategies and PICOs. The criteria are summarized below.

Study types

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 and thesis and dissertation will be included.

Participants

People between 18-60 with no regard to gender and ethnicity who suffer from non-specific CLBP of sacroiliac joint origin and received conservative/rehabilitative interventions. The study will be approved for more detailed analysis if the participants suffered from back pain not less than three months with the signs of SIJ involvement. Studies targeting nonhuman samples, professional athletes, subjects with acute LBP, symptoms persisted for less than three months will be excluded. LBPs of specified origin like inflammatory diseases, spondylo-arthropathies, disk hernia, spinal canal/foraminal stenosis, visceral pains, fractures and trauma, those with referral or radicular symptoms, studies on pregnant women, children (under 18 years) and elderly (over 60 years) will be excluded.

Interventions

At least one of the study groups has to undertake a rehabilitative or conservative intervention including:

- electrotherapy modalities: electrical stimulation currents (Transcutaneous Electrical Nerve Stimulation (TENS), Interferential (IF), Diadynamic, High-voltage, Russian currents, Faradic…), LASERs, ultrasound, shockwave, tecar, magnet, shortwave and microwave diathermy, infra-red radiation, hot packs, cold packs…;
- manual techniques: mobilization, manipulation, Muscle Energy Techniques (MET), soft tissue release, massage techniques, Instrumented Assisted Soft Tissue Manipulation (IASTM), visceral manipulation…;
- exercise therapy: any type of exercise including the proprioceptive neuromuscular facilitation (PNF) approaches;
- taping: Kinesio Taping®, McConnell & Mulligan, elastic bandages, Prophylactic athletic taping, non-medicated taping;
- needling: dry needling, acupuncture, electro-acupuncture;
- orthosis.

Comparators

Studies that compare the effect of aforementioned interventions with a control group (without treatment), sham group (placebo treatment), healthy group (of matched healthy subjects) or those comparing two or more interventions will be included.

Outcome measures

Studies will be included that the experimental (case) group and the control group were established, and the related monitoring data were introduced. Pain and function will be the primary outcome measures if are reported by valid scale or devices. Pain will be assessed by the Numerical Rating Scale (NRS), Visual Analogue Scale (VAS), Pressure Pain Threshold (PPT), McGill Pain Questionnaire, pain provocation tests. Function will be measured by Roland-Morris Disability Questionnaire, Oswestry Disability Index, or clinical/functional tests like active single leg raise, reverse single leg raise. Other tools may be also considered according to the included studies.

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 may 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

Articles will be accessed from international (Pubmed, ISI Web of Science, Scopus, Clinical Key, Science Direct, Medline, Embase, PEDro, ProQuest, the Cochrane Library, PROSPERO) and national (MOH Thesis, MOH Articles, Magiran, and SID) databases. Google Scholar search engine will also be searched.

Although narrative reviews and qualitative studies will not be targeted in current project, their references will be checked through Cross Reference. The main key words will be rehabilitation, conservative, physical therapy, pain, func-

tion, non-specific chronic low back pain, sacroiliac that will be updated with study progression. The search strategy for accessing articles will be like the following search query and covers PICO.

Nonspecific AND chronic AND ("low back pain" OR (low AND back AND pain) OR "back ache") AND ("sacroiliac joint*" OR (sacroiliac AND joint) OR "sacroiliac*") 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 OR LASERs OR ultrasound OR shockwave OR tecar OR magnet OR shortwave OR microwave OR diathermy OR infra-red OR hot pack OR cold packs OR ("manual techniques*" OR (manual AND technique)) OR mobilization OR manipulation OR ("muscle energy*" OR (muscle AND energy)) OR ("soft tissue release*" OR ("soft tissue*" AND release) OR ("visceral manipulation" OR (visceral AND manipulation)) OR ("instrumented assisted soft tissue manipulation*" OR ("instrumented assisted*" AND "soft tissue manipulation*") OR exercise OR ("proprioceptive neuromuscular facilitation*" OR (proprioceptive AND neuromuscular AND facilitation) OR taping OR needling OR acupuncture OR orthosis) 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 OR LASERs OR ultrasound OR shockwave OR tecar OR magnet OR shortwave OR microwave OR diathermy OR infra-red OR hot pack OR cold packs OR ("manual techniques*" OR (manual AND technique)) OR mobilization OR manipulation OR ("muscle energy*" OR (muscle AND energy)) OR ("soft tissue release*" OR ("soft tissue*" AND release) OR ("visceral manipulation" OR (visceral AND manipulation)) OR ("instrumented assisted soft tissue manipulation*" OR ("instrumented assisted*" AND "soft tissue manipulation*") OR exercise OR ("proprioceptive neuromuscular facilitation*" OR (proprioceptive AND neuromuscular AND facilitation) OR taping OR needling OR acupuncture OR orthosis).

C: "control group*" OR ((placebo or unrealistic) AND (treatment OR therapy)).

O: Therapy OR Treatment OR function*.

The research process will be conducted independently by two researchers (SIL and TSM) and their results will be compared each week. For all included articles, the search in the reference list (Hand Search) will also be performed. If the full text of the article is not found, the researchers will email the authors or the editor of the journal three times. If the reply was not satisfying, that article will be excluded. To find grey sources, special search in their related databases including registries of clinical trials (*i.e.* http://www.irct.ir/), http://www.trialscentral.com/, http://www.proquest.com/, http://www.gateway.com/worldwide/ will be done.

Study selection

Any article published before the end of September 2019 (Shahrivar 9th, 1398 Persian Calendar) will be potentially suitable. The search will be extended three years before the publication of the first article in the field of each intervention type. Search results and Reference lists will be transferred into the citation manager software. Duplicates and those marked as irrelevant will be ignored by screening titles and summaries. The full text of the remaining articles will be reviewed in detail. Under supervision of peers, *i.e.* ZSR, FB and AR, the whole procedure will run by two researchers (SIL and TSM) who are blind to each other's work. Any ambiguity or controversy will be discussed in consensus.

Figure 1 summarizes the study selection flow according to the PRISMA flow diagram.

Data extraction

The databases mentioned previously will be searched. To determine the inter-rater agreement, screening of PubMed title/summaries will be done independently by two researchers (SIL and TSM) as supervised by ZSR, AR and FB. The reference list in excluded articles types (qualitative studies and narrative reviews) will be checked by Cross Referencing. The search line will be revised and key words will be updated as the project progresses. Key words from included studies will be merged in the search line. The references list of all accepted studies will be checked using Hand Search strategy. If the researchers did not access the full text of any article, the corresponding/first author or the editorial board of the publishing journal will be emailed thrice. If not effective, the article will be excluded.

The screening of the title/summaries will be performed independently by SIL and TSM and duplicated articles or unrelated ones will be excluded. They also will report the number of the articles retrieved from each database in a flowchart. The whole search results will be transferred into a citation manager. The screening will be start over every three months for validating fast exclusions.

The research team (SIL, ZSR, FB, AR and TSM) will criticize the included studies according to their full-text inde-

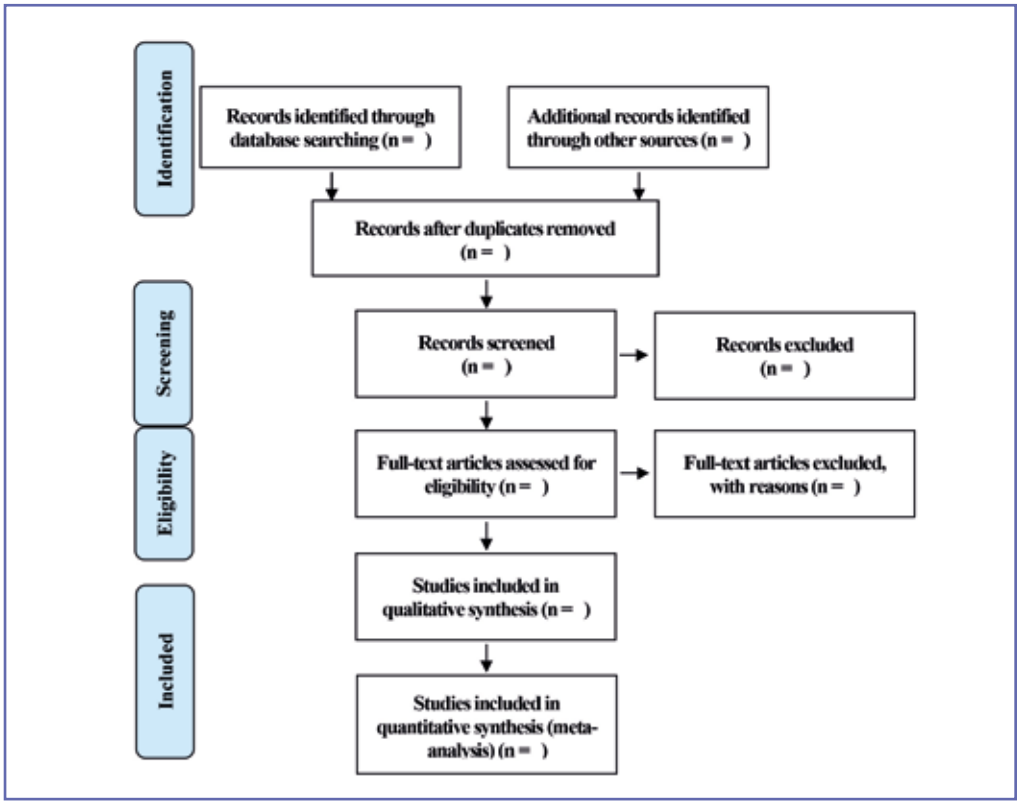

Figure 1. PRISMA 2009 Flow diagram (17) of the articles selection process.

pendently. Final decision on whether a study should be accepted for quality and quantity analysis will be made according to inclusion/exclusion criteria in a consensus. Reasons for the exclusion will be reported.

Data from the approved full-texts will be categorized in an Excel sheet (data extraction) according to publication indices (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. If needed, more information will be requested from original authors. PI (ZSR) supervises the procedure. Any ambiguity or controversy in any phase will be illuminated in the consensus.

Quality assessment

Supervised by PI (ZSR), each approved article will be qualified in expert consensus with regard to its design by Consort, STROBE, PEDro, CASP and NHLBI checklists. Every checklist will be scored. Articles that get at least 50% of total score of one checklist will be approved quantitative analysis. The articles' quality will be considered as high (75%), medium (50-75%), low (25-50%), poor (< 25%) based on the scores they gained by each single checklist. Any ranking dissimilarities will be discussed in expert consensus.

PEDro is an 11-item scale for clinical trials with "plus" (well addressed items) or "minus" (not-localized item) marks (18). In addition, as the identical, internationally accepted standard for qualification of clinical trials (19), the CONSORT checklist will also be used in present work. For more detailed assessment, the appropriate CONSORT extension may also be used (20).

The STROBE designed separate evaluation checklists to qualify case-control studies, cohort and other designs of studies (21, 22). On the other hand, some researchers believe that the STROBE checklists are not for formal quality assessment like the procedure required in a systematic review and recommend Study Quality Assessment Tools proposed by National Heart, Lung, and Blood Institute (NHLBI) instead (23). Both STROBE and NHLBI checklists were be administered in present study.

CASP also introduced specific checklist for each study designs (24) and will be used for determining the evidence ranking. For inclusive qualification of all article types, TIDieR checklist will be administered beside the main checklist.

Measure of Treatment Effects
Mean Difference (MD) with a 95% CI will be the format of choice for reporting continuous outcomes (like pain scales). If other forms of reports are present in articles, they will be covert into MD. For dichotomous or binary data (*i.e.* adverse events), a risk ratio (RR) with a 95% CI will be calculated.

Missing data
If there was a potential of missing data, the original research teams will be contacted. If they do not reply properly, only available data will be analyzed.

Statistical Methods

Data synthesis
If the number of homogenous studies was sufficient, data synthesis will be conducted using Stata software 8.0 (Stata Corporation, College Station, TX). If possible, meta-analysis will be considered using RevMan (Review Manager Software, Version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, The Cochrane Collaboration, Oxford, England). The RR and MD with the 95% CI will be determined for dichotomous and continuous data respectively. Heterogeneity assessment will be carried out using the Q test and the I^2 index. If $I^2 \leq 50\%$, fixed-effects model will be used for calculating the RR and MD; the Mantel-Haenszel random-effects model will be applied, and aggregate participant data will be used. Nonetheless, the random-effects model will be of choice. If quantitative synthesis is not applicable, the results will be discussed descriptively. The procedure will be performed by two researchers (SIL and TSM) independently. Again, any disagreements will be resolved through consensus.

Assessment of heterogeneity
For calculating the heterogeneity, the I^2 and χ^2 tests will be used. In I^2 analysis the cut off will be set as 50%. If $I^2 > 50\%$, subgroup analysis will be run to highlight the potential factors.

Subgroup and sensitivity analysis
In order to determine the heterogeneity among included studies, subgroup analysis that categorizes each intervention mode according to its frequency and/or timing, type of control, countries and different outcomes may be considered. Then, if the heterogeneity persists or if studies with incomplete results were included, the sensitivity analysis will be done with omitting low quality articles. The meta-analysis will be developed again and the results of these two meta-analyses will be matched and discussed in terms of the sample size, strength of evidence and influence on the pooled effect size.

Assessment of reporting biases
Risk of bias will be assessed by two reviewers (SIL and TSM) using the Cochrane Collaboration's tool. Disagreements will be resolved in consensus. Reaching appropriate number of studies for qualitative analysis (at least 10 per intervention), funnel plots will be developed for analyzing the publication bias. Besides, the effect of possible selective reporting, reporting deviations from the original protocols, effect of protocol compliance and adherence will be tracked.

DISCUSSION
This systematic review will provide a comprehensive search to retrieve all existing evidences concerning the effects of physiotherapy interventions in CNLBP with sacroiliac joint origin. The inclusion and exclusion criteria provide a reasonable base to assure that these effects will be discussable in temporal spectrum from immediate to very long term effects upon the time intervals of follow ups in the included studies. The main reason for conducting this review was to summarize clinical value of physiotherapy in treatment of these subjects with regard to evidence hierarchy and indicate their strengths. For best internal validity, various checklists will be administered for each study design and articles scoring will be scheduled in a peers' consensus.

The study will provide data for developing rehabilitative guidelines, apprise insurance coverage and standard protocols of physical therapy planning. According to the retrieved articles, cost-effectiveness and best practice of various physiotherapy intervention may be judged. However, it should be kept in mind that the main challenge in rehabilitation of CNLBP with sacroiliac origin is the accuracy of diagnosis that is not easily confirmed based on clinical examination alone. This fact needed to be appropriately dealt with in the original studies included in the review. We will try to collect all existing studies in this field covering all study designs and all physiotherapy interventions subheadings. The review results will also highlight the existing research and clinical gaps to conduct future researches.

As the search has been started right now, it seems that there is not any study available concerning some interventions. In addition, meta-analysis will be applicable only if the retrieved articles were not heterogeneous.

ETHICS
The study has been funded and ethically approved by Isfahan University of Medical Sciences (Ethics Code: IR.MUI.REC.1397.335) as a part of a thesis for Master's Degree in Physical Therapy by Taraneh Shahmahmoodi (Registration

code: 297122). 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 (25).

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This study will be developed with the financial support and ethically approval by Isfahan University of Medical Sciences (Ethics Code: IR.MUI.REC.1397.335) as a part of a thesis for Master's Degree in Physical Therapy by Taraneh Shahmahmoodi (Registration code: 297122). The protocol has been registered in International Prospective Register of Systematic Reviews (CRD42020121383). 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.

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ORIGINAL ARTICLE

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Lumbopelvic Stabilization Exercises and McKenzie Method in Low Back Pain Due to Disc Protrusion: A Blind Randomized Clinical Trial

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SUMMARY

Introduction. Disc Protrusion (DP) is a degenerative spinal disorder. Lumbar intervertebral disc protrusion is one of the most common orthopedic injuries, leading to low back pain that radiates to the lower limbs. Physical exercise is the main element in the conservative treatment of low back pain.

Objective. Comparing the effects of the McKenzie method and core stabilization exercises on the rehabilitation of patients with lumbar DP.

Methods. We allocated sixty-nine patients with low back pain due to disc protrusion to the Core Group (CG), the McKenzie Group (MG), or the Core + McKenzie Group (CMG). All groups underwent three interventions per week for four weeks, totaling 12 sessions. We assessed pain intensity, hamstring flexibility, functional capacity, muscle strength, and lumbopelvic stability.

Results. All intervention groups significantly reduced pain intensity and functional disability, besides improving posterior muscle chain flexibility, quality of life, trunk muscle strength, and lumbopelvic stability ($p < 0.05$). For functional capacity, assessed through the SF-26 Questionnaire, as well as for posture holding time in the lumbopelvic stability tests, the CG and CMG significantly improved the results compared to the MG ($p < 0.05$).

Conclusions. The results suggest that both exercise methods were efficient in reducing pain and improving function in patients with low back pain due to disc protrusion. However, the groups that used stabilization exercises showed better results in activating lumbopelvic stabilizing muscles.

KEY WORDS

Low back pain; intervertebral disc displacement; physical therapy modalities; exercise therapy; spine osteoarthritis.

INTRODUCTION

Low back pain is a major health problem, with enormous economic and social costs (1). Disc Protrusion (DP) is a degenerative spinal disorder (1). The degenerated disc has focal or localized abnormalities on the disc margin (2, 3). This disorder relates to age, gender, prolonged workload, trauma, and pregnancy (4). Lumbar intervertebral disc protrusion is one of the most common orthopedic conditions, producing, in many cases, low back pain that radiates to the lower limbs (5, 6). Lumbar radiculopathy may be the result of a lumbar disc protrusion or hernia that can irritate a sacral plexus trunk, leading to intraneural inflammation (6). A protruding or herniated degenerated disc can cause paresthesia and weakness in the lower limbs, in addition to pain (6). In the worst cases, lumbar radiculopathy can be chronic, resulting in pain, disability, and substantial burden in the long run (6). This condition leads to health-related socioeconomic consequences that directly affect well-being and quality of life (5, 7).

Degenerated protruding discs and narrow spinal canals are common conditions in the elderly (8). However, most subjects adapt perfectly well to the degenerative findings of imaging tests, keeping in shape, and avoiding poor posture (8). Physical activity is the main element in the prevention and treatment of low back pain (9). It does not require expensive materials, therefore providing an accessible intervention in which the patient directly controls pain (9).

Spinal stabilization and the McKenzie approach are two different interventions for the treatment and prevention of low back pain (9). The core stabilization program emphasizes the training of specific deep or local stabilizing muscles of the spine, keeping the lumbar region in a neutral position during occupational and daily life activities (10). The McKenzie method, also called Mechanical Diagnosis and Therapy (MDT) (8), emphasizes an examination/treatment that incorporates repeated spinal movements (10, 11). The patient's response to these repeated movements determines a classification and a preferred direction for therapeutic exercise (11-14). Postural correction and maintenance of normal lordosis are an integral part of the McKenzie program, and treatment programs generally emphasize the performance of repeated lumbar movements for the effective management of specific syndromes (13). This method considers the patient's symptomatic and mechanical response to repeated movements and sustained postures for evaluation and treatment (8, 14). Therefore, it uses a directional preference to reduce or increase the patient's symptoms (12, 14).

The spinal stabilization system, also called core system, is composed of the spinal column, which provides intrinsic stability, muscles that provide dynamic stability, and a neural control unit that evaluates and determines the stability and coordination requirements of the muscle response (8). The central stabilizing muscles, also called core muscles, are categorized, according to their function, as global muscles (*rectus abdominis*, spinal erectors, and external *obliquus abdominis*) and local muscles (*transversus abdominis* and lumbar multifidus) (15). Global muscles participate in trunk movements, while local muscles play an important role in stabilizing the core (15). The *transversus abdominis* and the lumbar multifidus play important roles in the functional activities of the spine (15). Core stabilization exercises aim to activate central muscles, including the *transversus abdominis*, lumbar multifidus, and paraspinal, abdominal, diaphragmatic, and pelvic muscles. This provides spinal stability and prevents lumbopelvic injuries by controlling joint tension, strengthening muscles, increasing resistance, and correcting posture (7, 16-20).

The present study compares the effects of the McKenzie method and core stabilization exercises, as well as their association, on the rehabilitation of patients with low back pain due to disc protrusion.

METHODS

Blind randomized clinical trial conducted from August 2017 to June 2019. The study was registered in the Brazilian Clinical Trials Registry (REBEC) under the identification number RBR-5mcvt6. The study was approved by the Committee of Ethics on Human Research of Universidade Luterana do Brasil under the number 2, 152, 592 (21).

Sample calculation

We used pain intensity, measured by VAS, as the primary outcome of the study. Based on the study by Hasanpour-Dehkordi, Dehghani and Solati (11), we used a study power of 80%, a 95% Confidence Interval, and a sample size ratio of 1:1:1 (Core; McKenzie; Core + McKenzie) to estimate the number of participants for each intervention group. Believing that losses and refusals would be around 20%, we reached the final number of 23 participants for each study group, totaling 69 participants.

Sample randomization

After the initial assessment, we randomized the eligible participants by using a list of random numbers provided by the EPI-Info® software. We allocated a total of 69 patients to the following groups: McKenzie Group (MG), which performed only one McKenzie exercise protocol, Core Group (CG), to which we applied lumbopelvic stabilization exercises, and Core + McKenzie Group (CMG), which performed both protocols (**figure 1**).

Elegibility criteria

The study included 69 patients of both genders, aged between 18 and 65 years, who had low back pain due to disc protrusion. The participants should have complementary exams (magnetic resonance or computed tomography) showing DP and/or herniated disc, and should not be performing any other type of physiotherapeutic approach for low back pain at the time of the study. All participants signed the Informed Consent Form (ICF).

We excluded from the study patients who had severe spinal pathologies (fractures, tumors, and inflammatory pathologies such as ankylosing spondylitis), who had previously undergone lumbar surgery, patients with severe cardiovascular and cardiorespiratory disease, pregnant women, patients who did not attend three consecutive or intermit-

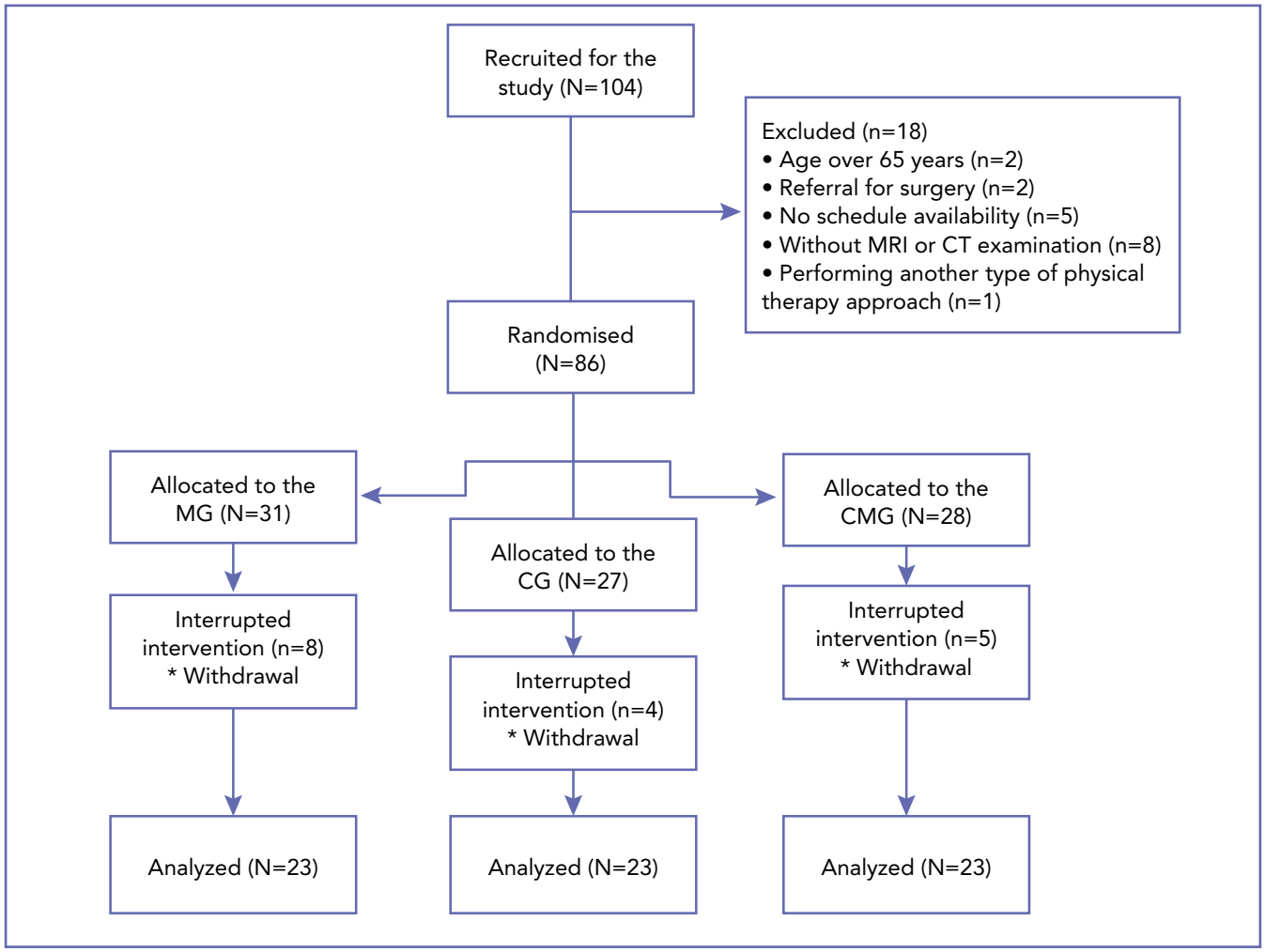

Figure 1. Flowchart – Recruitment and evaluation of patients with disc protrusion.
RM: MRI; CT: Computed Tomography; CG: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.

tent consultations, and those that presented some neurological disorder that prevented them from performing the proposed exercises.

Data collection

The evaluation protocol was conducted by a blind evaluator, that is who did not know which group the participant belonged to. The evaluation took place in two stages of the study: initially – prior to the randomization of the subjects, and at the end of the intervention protocol.

Anthropometric assessment

We calculated the Body Mass Index (BMI) by measuring body height and weight.

Pain intensity assessment

We assessed pain intensity by using the Visual Analogue Scale (VAS). In this scale, the evaluator asks the patient 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.

Quality of life assessment

We assessed quality of life by using the SF-36 Questionnaire. This instrument consists of 11 questions and 36 items that encompass eight domains represented by functional capacity, physical aspects, pain, general health status, vitality, social aspects, emotional aspects, and mental health. The application of this questionnaire takes place through the attribution of a score for each question, later trans-

formed into a scale from "0" to "100" per domain, where "0" corresponds to the worst quality of life and "100" the best quality of life.

Functional capacity assessment

To quantify disability, we used the Roland-Morris Disability Questionnaire. This instrument consists of 24 items that describe daily tasks that patients have difficulty performing due to low back pain. The questions have a dichotomous answer (yes or no), and the result corresponds to the sum of the yes answers. This result can vary between 0 and 24, with zero corresponding to a person without complaints, and the maximum value corresponding to a patient with very severe limitations. The greater the number of alternatives filled in, the greater the functional impairment. We instructed the individuals to complete the items that better described them on the day of application.

Hamstring flexibility assessment

We measured hamstring flexibility by using the Wells bench. For performing this test, the patient seated with legs extended and feet supported on the device. Then, the individual should flex his/her trunk and hips the maximum possible, with hands superimposed and supported on the measuring tape installed on the upper part of the Wells bench. The evaluator asked the patient to stretch his/her hands as far as he/she could reach without bending the knees, thus obtaining the maximum reach point.

Trunk extensor muscle strength assessment

We assessed trunk extensor muscle strength by using the Crown® dorsal dynamometer. In this test, the patient remained standing, with feet supported and parallel, knees extended, and the spine initially flexed. At the command of the examiner, the patient extended the spine by holding the lever bar with his/her upper limbs for dynamometer measurement.

Lumbopelvic stability assessment

To analyze lumbopelvic stability, we applied the Single Leg Squat Test (unilateral squat), the Runner Pose Test, the Prone Instability Test, the Right and Left Lateral Bridge Test, and the Sorenson Test (extensor endurance test).

Intervention protocol

The intervention protocol consisted of three interventions per week for four weeks, with all study groups performing a total of 12 sessions. The service took place individually. Each session lasted between 40 and 60 minutes. Initially, all participants performed a 10-minute stationary bicycle warm-up. Afterwards, we performed the intervention according to the randomized group. The intervention protocol was applied by a researcher previously trained and familiar with the protocol. The interventor controlled during the sessions the technique of performing each exercise, as well as its intensity and duration in both intervention groups.

Intervention protocol for the McKenzie Group

The intervention protocol for the MG consisted of three sets of exercises with 10 repetitions each, with a 30-second interval between sets. We maintained the exercises for five seconds initially, subsequently maintaining them for 10 seconds according to the patient's performance. We defined the treatment from the result of the evaluation performed prior to the randomization stage, and guided it according to the preferred direction of movement, that is flexion, extension, or lateral displacement of the spine. We applied the McKenzie protocol according to Garcia *et al.* (13) (**table I**).

Intervention protocol for the Core Group

In the CG protocol, we developed the exercises according to the studies of Moon *et al.* (21), who used eight exercises based on static postures, initially maintained for five seconds. As the program evolved, the degree of difficulty

Table I. McKenzie group exercise protocol.

Exercise	Number of repetitions
Exercise 1. Flexion of the lower limbs on the bench press	3 sets with 10 repetitions
Exercise 2. Bending the trunk while sitting on the chair	3 sets with 10 repetitions
Exercise 3. Standing trunk flexion	3 sets with 10 repetitions
Exercise 4. Prone trunk extension	3 sets with 10 repetitions
Exercise 5. Standing trunk extension	3 sets with 10 repetitions
Exercise 6. Lateralization of the standing pelvis	3 sets with 10 repetitions for each side
Exercise 7. Lateralization of the pelvis against the wall	3 sets with 10 repetitions for each side

in controlling the posture increased, with the posture holding time changing to 10 seconds. Participants of this group repeated each exercise 10 times, with a 30-second interval between them. The researcher commanded continuous contraction of stabilizers ("abdominal press") in all exercises (**table II**).

Intervention protocol for the Core + McKenzie group

In the CMG protocol, patients performed both exercise methods in association. The patients started with McKenzie exercises and performed core stabilization exercises after a five-minute rest according to the defined protocol.

Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS) version 23.0 for data and statistical analyses. We entered the data twice so as to avoid typing errors, and expressed them as mean and standard deviation. Afterwards, we statistically analyzed the data by parametric tests: Analysis of Variance (ANOVA) for repeated measures within each group from baseline to endpoint, and unpaired Student's t Test for intergroup analysis at each time point. For nonparametric variables, we used the Wilcoxon and Chi-Square Tests, respectively. We established the level of significance of $p < 0.05$ for the statistical test.

RESULTS

The study initially included 104 participants. Of these, 18 were excluded and 86 were randomized to the study groups. Among the randomized participants, 17 discontinued intervention due to withdrawal. Thus, the present study analyzed a total of 69 participants (**figure 1**). Of these, 25 (36.23%) were men, with an average age of 46 years. The groups were homogeneous for the variables skin color, occupation, time of pain, and anthropometric aspects (**table III**).

All intervention groups decreased pain intensity after the intervention protocol, with no differences between them ($p < 0.001$) (**figure 2**).

Hamstring flexibility increased significantly in all intervention groups, with no differences between them (**figure 3**).

All intervention groups decreased the Roland-Morris Disability Questionnaire (RMDQ) score ($p < 0.001$). The scores did not differ between the intervention groups (**figure 4**).

All study groups also improved quality of life according to the SF-36 Questionnaire, with no differences between them (**figure 5**). The three intervention groups improved all domains of the questionnaire. However, for the Functional Capacity domain, the CG showed a more significant variation in the score than the MG and CMG ($p=0.018$) (**table IV**).

Regarding isometric trunk muscle strength (MVIC), all groups increased isometric strength after the intervention, with no differences between them (**figure 6**).

Regarding lumbopelvic stability tests, all study groups significantly improved the scores of the Single Leg Squat Test, Runner Pose Test, and Prone Instability Test ($p < 0.05$). Notwithstanding, when analyzing the results between the groups, the CG and CMG scored better than the MG ($p < 0.05$) (**table V**).

All intervention groups improved the scores of the Lateral Bridge and Sorenson tests after the intervention ($p < 0.05$). The CG and CMG showed a significantly higher variation in posture holding time than the MG in the Left and Right Lateral Bridge Test ($p=0.009$ and $p=0.012$, respectively) (**table VI**).

Table II. Core stabilization Group exercise protocol.

Exercise	Number of repetitions
Exercise 1. Bench press	10 times from 5 to 10 sec., 10 sec. rest
Exercise 2. Bird dog	10 times from 5 to 10 sec. for each side
Exercise 3. Lateral bridge	10 times from 5 to 10 sec. for each side
Exercise 4. Prone bridge	10 times from 5 to 10 sec., 10 sec. rest
Exercise 5. Isometry (lower portion of the rectus abdominis)	10 times from 5 to 10 sec., 10 sec. rest
Exercise 6. Supine bridge with extended lower limbs	10 times from 5 to 10 sec., 10 sec. rest
Exercise 7. Minisquat on the wall with a Swiss ball	10 times from 5 to 10 sec., 10 sec. rest
Exercise 8. Isometry (bench press flexion)	10 times from 5 to 10 sec., 10 sec. rest

Table III. Characterization of the study sample (n=69).

	Intervention group			
Variable	**CG (n=23)**	**MG (n=23)**	**CMG (n=23)**	**p**
Age, years (mean ± SD)	45.43 ± 10.40	51.00 ± 6.81	43.96 ± 10.56	0.034[#]
Gender, M/F	9/14	8/15	8/15	0.757[$]
Skin color, n (%)				1.000[$]
White	23 (100.0)	23 (100.0)	23 (100.0)	
Black	0 (0.0)	0 (0.0)	0 (0.0)	
Occupation, n (%)				0.454[$]
Farmer	1 (4.3)	5 (21.7)	6 (26.0)	
Homekeeper	2 (8.7)	7 (30.4)	3 (13.0)	
Joiner	1 (4.3)	1 (4.3)	0 (0.0)	
Teacher	1 (4.3)	0 (0.0)	2 (8.7)	
Physiotherapist	1 (4.3)	0 (0.0)	1 (4.3)	
General Services	1 (4.3)	1 (4.3)	0 (0.0)	
Other	16 (69.8)	9 (39.3)	11 (48.0)	
Time of pain, months (mean ± SD)	72.70 ± 66.44	112.83 ± 80.88	104.74 ± 117.63	0.135[&]
Directional preference				0.129[$]
Flexion	13 (56.5)	16 (69.6)	10 (43.5)	
Extension	6 (26.1)	7 (30.4)	11 (47.8)	
Side slope	4 (17.4)	0 (0.0)	2 (8.7)	
Weight, Kg (mean ± SD)	77.42 ± 11.15	76.70 ± 14.26	72.96 ± 13.50	0.465[#]
Height, cm (mean ± SD)	167.22 ± 9.47	164.39 ± 7.52	157.90 ± 35.37	0.330[#]
BMI, Kg/cm^2 (mean ± SD)	27.69 ± 3.52	28.21 ± 3.79	26.80 ± 4.08	0.448[#]

Legend: CG=Core Group; MG=McKenzie Group; CMG=Core + McKenzie Group; Kg: kilogram; cm: centimeters; M: male; F: female.
[#] One-way ANOVA.
[$] Chi-square.
[&] Kruskal-Wallis test.

DISCUSSION

This study compares the effectiveness of two exercise programs for low back pain due to DP, also analyzing the association of these two programs. The results do not support the initial hypothesis that the core stabilization exercise program is more effective than the McKenzie exercise program in relieving pain and improving posterior muscle chain flexibility and functional disability scores in individuals with low back pain secondary to disc protrusion/degeneration. Both programs, alone and in association, led to positive results.

The analyses did not reveal statistical differences in pain intensity, isometric trunk extensor strength, and function. These results corroborate those found in other previously published studies (10, 22, 24, 25). However, specific tests for lumbopelvic-hip complex stabilization showed that the groups that performed core stabilization exercises had greater postural control than the group that performed only the McKenzie exercise program.

Spinal stabilization exercises and the McKenzie approach are two different interventions generally used to treat low back pain. The stabilization program applied in this study addressed specific muscle abnormalities associated with low back pain due or related to degenerative discopathy and DP. The program emphasized the training of deep and local spinal stabilizing muscles, keeping the lumbar region in a neutral position (10, 22, 23). The McKenzie approach emphasizes an examination/treatment that incorporates repeated spinal movements. The patient's response to these repeated movements determines a classification and a preferred direction for therapeutic exercise (10, 22, 23).

O'Sullivan *et al.* (26) observed that several participants in their study needed four to five weeks to learn how to coactivate Transverse Abdominis (TrA) and multifidus muscles.

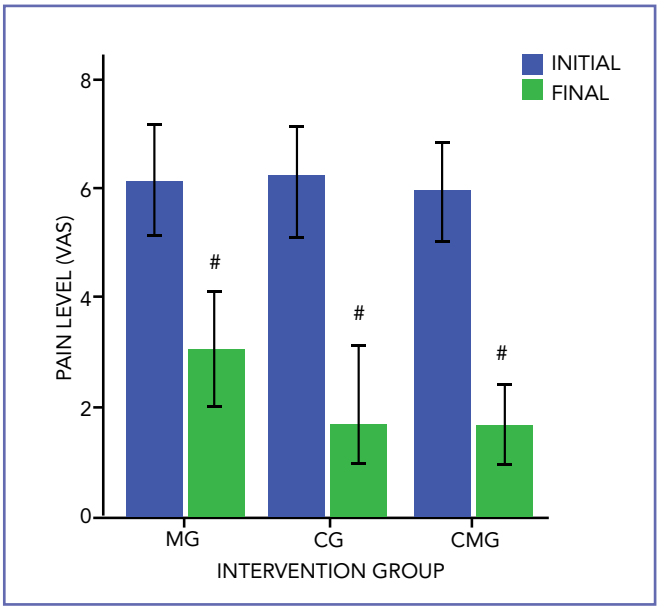

Figure 2. Pain intensity assessment in the study groups before and after intervention.
CG: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group; VAS: Visual Analogue Scale.
# p < 0.001 compared to baseline in the same group. Student's t test.

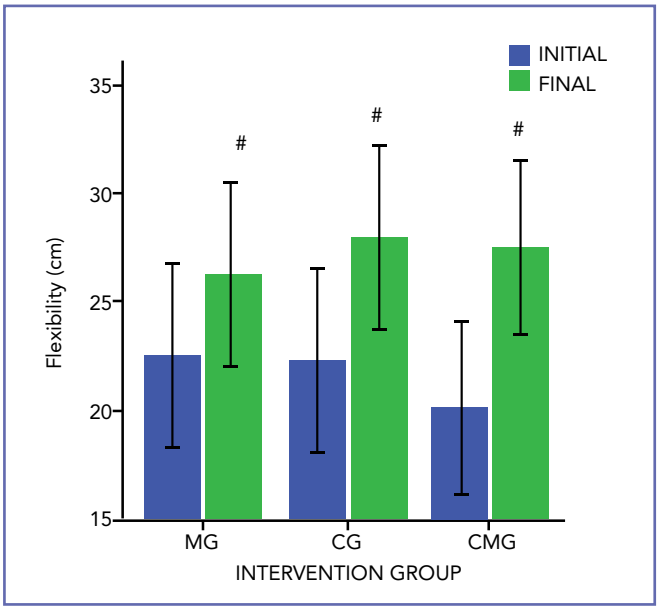

Figure 3. Hamstring flexibility assessment through the Wells bench in the study groups before and after intervention.
GC: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.
# p < 0.001 compared to baseline in the same group. Student's t test.

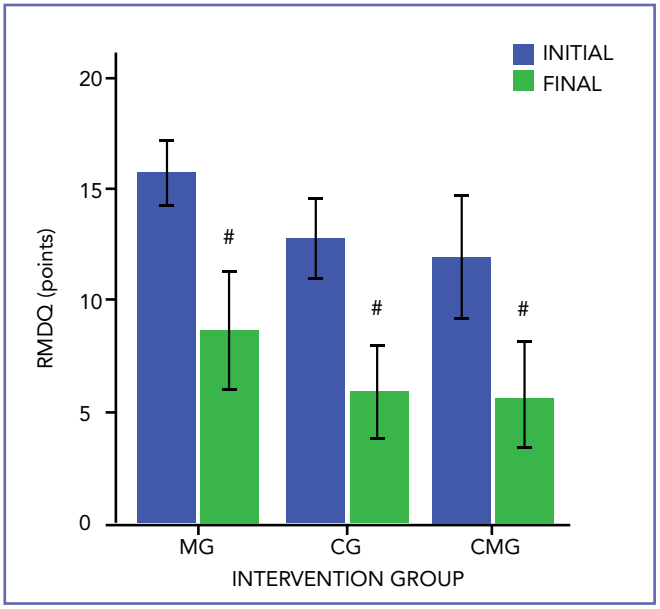

Figure 4. Roland-Morris Disability Questionnaire (RMDQ) score in the study groups before and after intervention.
GC: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.
# p < 0.001 compared to baseline in the same group. Student's t test.

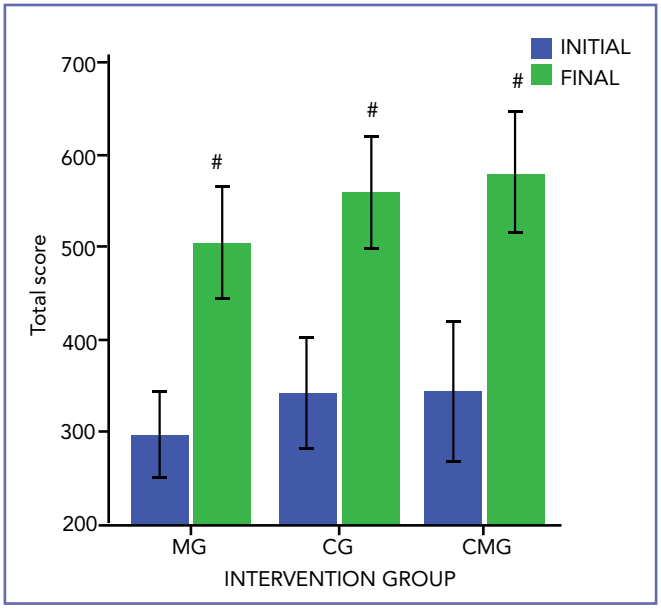

Figure 5. Total SF-36 score in the study groups before and after intervention.
GC: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.
# p < 0.001 compared to baseline in the same group. Student's t test.

Table IV. Evaluation of the SF-36 questionnaire subitems (n=69).

SF-36 items	Group	Baseline, mean ± SD	Postintervention, mean ± SD	pa	Effect Size	Score variation within the group (95% CI)	F	pb
Functional capacity	CG	40.44 ± 22.20	73.04 ± 19.99	**0.000**	-7.07	-32.61 (-42.16 to -23.05)	4.272	**0.018#**
	MG	29.35 ± 14.73	57.61 ± 21.47	**0.000**	-6.19	-28.26 (-37.72 to -18.80)		
	CMG	45.21 ± 23.86	74.57 ± 23.69	**0.000**	-5.19	-29.34 (-39.64 to -19.05)		
Physical Limitation	CG	19.57 ± 34.51	73.91 ± 35.74	**0.000**	-5.56	-54.34 (-74.61 to -34.08)	1.577	0.214
	MG	7.61 ± 13.97	56.52 ± 43.33	**0.000**	-5.93	-48.91 (-66.00 to -31.82)		
	CMG	21.74 ± 37.16	73.26 ± 32.98	**0.000**	-6.66	-51.52 (-67.57 to -35.47)		
Pain	CG	31.61 ± 19.26	64.00 ± 23.58	**0.000**	-5.20	-13.61 (-45.30 to -19.48)	2.163	0.123
	MG	34.91 ± 15.73	55.48 ± 15.97	**0.000**	-5.17	-20.56 (-32.56 to -9.35)		
	CMG	32.52 ± 22.99	67.48 ± 20.11	**0.000**	-7.60	-34.96 (-44.49 to -25.42)		
Health Condition	CG	53.26 ± 22.81	66.87 ± 22.82	**0.001**	-3.83	-13.61 (-20.97 to -6.24)	0.800	0.454
	MG	38.18 ± 20.28	59.13 ± 20.79	**0.001**	-3.74	-20.96 (-32.56 to -12.31)		
	CMG	46.61 ± 24.79	64.96 ± 21.18	**0.000**	-4.24	-18.35 (-27.33 to -9.37)		
Vitality	CG	41.52 ± 19.51	60.65 ± 22.92	**0.001**	-3.85	-19.13 (-29.43 to -8.83)	2.498	0.090
	MG	41.08 ± 21.74	56.96 ± 20.77	**0.001**	-3.98	-15.86 (-24.13 to -7.60)		
	CMG	49.57 ± 25.49	70.35 ± 19.08	**0.000**	-4.66	-20.78 (-30.04 to -11.52)		
Social Aspect	CG	48.80 ± 22.88	77.63 ± 21.97	**0.000**	-5.39	-28.83 (-39.93 to -17.73)	0.472	0.626
	MG	52.09 ± 23.74	75.52 ± 21.49	**0.001**	-3.89	-23.43 (-35.91 to -10.95)		
	CMG	44.02 ± 28.71	82.09 ± 26.41	**0.000**	-6.61	-30.06 (-50.01 to -26.12)		
Emotional Aspect	CG	52.12 ± 45.86	76.76 ± 40.78	**0.037**	-2.21	-24.66 (-47.74 to -1.57)	0.073	0.930
	MG	40.47 ± 38.81	78.37 ± 31.14	**0.000**	-4.64	-37.90 (-54.82 to -20.97)		
	CMG	40.56 ± 47.09	74.33 ± 36.00	**0.001**	-3.88	-33.77 (-51.81 to -15.72)		
Mental Health	CG	56.35 ± 24.39	71.13 ± 18.84	**0.011**	-2.78	-14.78 (-25.79 to -3.78)	0.890	0.445
	MG	54.26 ± 23.00	65.74 ± 22.10	**0.034**	-2.26	-11.48 (-22.00 to -0.95)		
	CMG	62.78 ± 22.45	73.39 ± 21.43	**0.003**	-3.36	-10.61 (-17.15 to -4.07)		

Legend: CG: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group; CI: Confidence Interval; SD: Standard Deviation.
Bold values are statistically significant.
[a] Intragroup Student's t-test.
[b] One-way ANOVA.
[#] Significant difference between the MG, CG, and CMG.

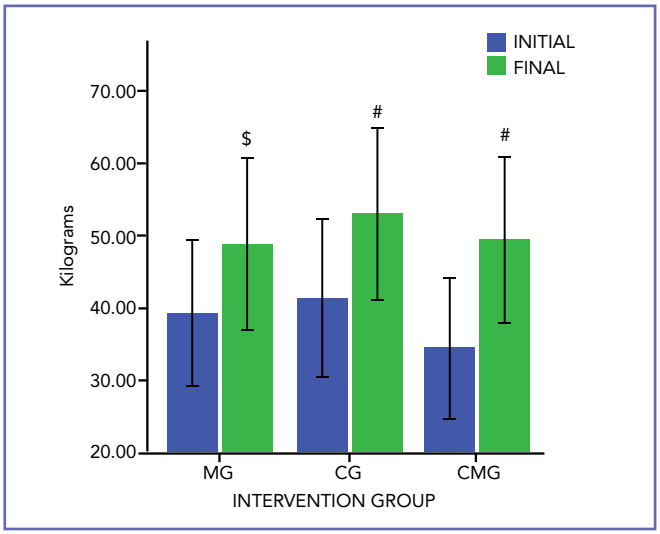

Figure 6. Trunk muscle strength (Maximum Voluntary Isometric Contraction) assessment in the study groups before and after intervention.
GC: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.
# p < 0.001 compared to baseline in the same group.
$ p < 0.005 compared to baseline in the same group. Student's t test.

The ability to perform an isolated contraction of these muscles is the basis of the specific stabilization approach. Inability to master this exercise component prevents patients from progressing on their exercise program (26). The stabilization exercise program applied in this study lasted four weeks. Perhaps many of these patients who participated in the study could have responded more favorably to the program with a longer intervention period. The exercise dosage can significantly affect the treatment of low back pain (25). Miller *et al*. approached 30 subjects with chronic low back pain using the McKenzie exercises and stabilization exercises for six weeks (9). Both groups improved pain and function after the intervention (10).
Both exercise programs significantly reduced pain intensity after four weeks of intervention. Notwithstanding, it seems to us that the reduction in pain intensity occurs through different mechanisms in the two proposed programs. The positive results associated with the McKenzie approach may relate to the activation of pain gate control mechanisms or to relaxation and/or decompression of neural tissues (10, 16). The application of controlled forces to the spine through active exercises or techniques can temporarily reduce pain intensity by altering the fluid dynamics of the injured tissue (10, 16). Another theory that may justify this analgesic

Table V. Results of lumbopelvic stability tests in the study groups before and after intervention (n=69).

Variable	CG (n=23)		MG (n=23)		CMG (n=23)		
	Initial	Final	Initial	Final	Initial	Final	p$
Single Leg Squat Test							**0.043**
Positive	23 (100.0)	9 (39.1)	23 (100.0)	16 (69.6)	20 (87.0)	11 (47.8)	
Negative	0 (0.0)	14 (60.9)	0 (0.0)	7 (30.4)	3 (13.0)	12 (52.2)	
p#	**0.000**		**0.006**		**0.007**		
Runner Pose Test							**0.003**
Positive	15 (65.2)	5	16 (69.6)	11	5 (21.7)	1	
Negative	8 (34.8)	18	7 (30.4)	12	18 (78.3)	22	
p#	**0.002**		**0.025**		**0.046**		
Prone Instability Test							**0.019**
Positive	20	8	21	15	17	6	
Negative	3	15	2	8	6	17	
p#	**0.001**		**0.014**		**0.002**		

Legend: CG: Core Group; MG: McKenzie Group; CMG: Core + McKenzie Group.
Bold values are statistically significant.
[$] Chi-square test.
[#] Wilcoxon test.

Table VI. Results of the posture holding time in the core stabilization tests of the intervention groups (n=69).

Functional Test	Group	Baseline, mean ± SD	Postintervention, mean ± SD	p[a]	Effect Size	Score variation within the group (95% CI)	F	p[b]
Right side bridge	CG	17.13 ± 17.42	42.30 ± 35.05	**0.000**	-5.33	-25.17 (-34.97 to -15.38)	5.093	**0.009**[#]
	MG	14.83 ± 15.04	22.17 ± 20.25	**0.001**	-6.19	-7.35 (-37.72 to -18.80)		
	CMG	22.17 ± 15.98	42.08 ± 25.41	**0.001**	-3.83	-19.91 (-11.32 to -3.37)		
Left side bridge	CG	20.34 ± 16.84	44.30 ± 24.98	**0.000**	-6.59	-23.96 (-31.50 to -16.41)	4.757	**0.012**[#]
	MG	15.78 ± 16.44	23.87 ± 24.49	**0.003**	-5.93	-8.08 (-13.14 to -3.04)		
	CMG	22.48 ± 15.48	43.17 ± 26.26	**0.000**	-4.09	-20.69 (-31.20 to -10.19)		
Sorenson Test	CG	37.70 ± 29.55	62.96 ± 37.82	**0.001**	-3.90	-25.26 (-38.69 to -11.83)	2.860	0.069
	MG	21.35 ± 16.84	41.57 ± 35.04	**0.005**	-5.17	-20.56 (-32.56 to -9.35)		
	CMG	30.22 ± 32.58	68.17 ± 46.25	**0.000**	-6.21	-37.95 (-50.64 to -25.27)		

Legend: CG: Core Group; GM: McKenzie Group; CMG: Core + McKenzie Group; CI: Confidence Interval; SD: Standard Deviation.
Bold values are statistically significant
[a] Intragroup Student's t-test.
[b] One-Way ANOVA.
[#] Significant difference between the MG, CG, and CMG.

effect in the McKenzie method is that stimulation of arterial, venous, and lymphatic drainage or mechanoreceptive stimulation with subsequent increase in the afferent pathway to the central nervous system can result in pain modulation and inhibition of the hypertonic muscle (10). Trunk extension programs have improved some of the main stability measures with activation of the local and global muscle system, stabilizing the region around the spine and reducing pain (22).

The literature describes the effects of core stabilization exercises on pain through several mechanisms. These mechanisms include reducing the load and improving the quality of movements after improving trunk muscle coordination (16). Furthermore, stabilization exercises aim to activate mainly the deep muscles, commonly affected by low back pain (16).

Regarding spinal instability, Panjabi (27, 28) describes a neutral zone where there is a synergistic interaction between passive and active elements and the neural control of the spine to control the movement between intervertebral segments (10, 29). If one of these elements is compromised, for example, by disc degeneration, spinal injury, or muscle dysfunction, an imbalance occurs within the neutral zone resulting in instability of this complex and, consequently, pain (10, 29).

Hence, lumbosacral spine instability in the studied population may relate to the presence of an advanced discogenic pathology that was most affected by the extension program. Although McKenzie's conceptual model of pulpal nucleus displacement with repeated movements in the final range so as to reduce abnormal disc pressures remains controversial, several studies have demonstrated anterior or posterior nuclear migration away from the compressive forces of flexion or extension movements in the sagittal plane.

Some studies describe core muscle imbalance in patients with low back pain compared to healthy individuals (23). In comparison with general exercises or spinal manipulation, motor control exercises for patients with low back pain improve the size and recruitment of deep spinal muscles, including TrA, improving pain and short-term function (24). De Georgio, Padulo and Kuvacic (30) reported that people who develop chronic pain in the absence of other associated pathologies, as in the case of chronic non-specific low back pain, may be predisposed to avoid some gestures, movements, daily routines and/or sports activities with the intention to decrease the pain. This can lead to a significant reduction in the level of physical activities, that is, a "disuse syndrome", which increases the perception of stiffness and back pain. All of these aspects can lead to the development of a vicious circle that results in the worsening of the patient's quality of life.

The proposed mechanism of action for motor control exercises involves better coordination of deep spinal and trunk muscles, such as TrA and Internal Oblique (IO) muscles (24). There is a better ratio between TrA and IO muscle activation and rectus abdominis improvement in individuals treated with core stabilization exercises (30). These exercises increase the activation of segmental muscles, reducing pain in individuals with chronic nonspecific low back pain (30).

Hosseinifar *et al.* demonstrated that stabilization exercises were more effective than McKenzie exercises in increas-

ing the resting thickness of the left and right TrA muscles and the multifidus muscle (15). These findings corroborate those of the present study, where the CG had a greater activation of these muscle groups, as shown by the Lateral Bridge, Single Leg Squat, and Prone Instability tests.

One of the most widely validated outcome measures for low back pain is the Roland-Morris Disability Questionnaire (RMDQ) (23). The present study showed a significant reduction in the RMDQ score in all intervention groups, with no differences between them. The possible explanations for these findings are the improvement of trunk muscle activity and the increase in the range of lumbar motion, leading to less disability and functional recovery. According to the fear-avoidance model, people's fear of low back pain aggravates pain, leading to chronic pain and, ultimately, permanent functional disability (22).

In the treatment of low back pain using McKenzie and, especially, stabilization exercises, focusing on the preferred direction of the TrA and multifidus muscles, respectively, relieves pain, which helps patients to recover physically and psychologically (22). Exercises combat chronic pain and functional disability by conquering fear and, subsequently, preventing pain in patients (22). Thus, patients report reduced pain and disability scores after interventions (22). The specificity of the exercises helps in managing pain that stems from tense muscles and therefore facilitates healing (22). In other words, exercises help patients to cope with their pain, leading to recovery (22). Chopani *et al.* (32) approached 24 subjects with spondylolisthesis, randomly divided into a group of stabilization exercises or a group of general exercises. The authors observed that pain and disability improved in both groups, but the variables of postural stability were not changed in different directions in the stabilization group. This result can demonstrate that several exercise methods can offer benefits on low back pain and disability. Notarnicola *et al.* (33) approached 60 people with chronic non-specific low back pain divided into a group receiving the Pilates method and a control group. Participants in the Pilates group received an hour of daily exercise, five times a week for six months. The Pilates group demonstrated an important improvement in pain, disability, and physical and psychological health perception.

Results suggest that both exercise programs improve pain and functional disability through their own and distinct mechanisms. The choice of one or the other seems to be an important contributor to the previously considered clinical outcomes (26). Patients and clinicians may use either approach to improve long-term clinical outcomes. However, this choice must consider both the clinical experience with the technique and the response of each patient. In addition, it is important to emphasize the importance of exercise as a routine physical activity program in the prevention of low back pain originating from a degenerative process of the intervertebral disc.

Study limitations

This research has limitations such as the lack of evaluation and control of analgesic intake by patients during the study period. Another limitation is the lack of a follow-up to observe the long-term effects of the exercise programs administered. The results are further limited by a small sample size that limits statistical power. In addition, the study was pragmatic, without a true control group (non-intervention). Future studies are necessary to include such variables so as to correlate and enhance the results of this research.

CONCLUSIONS

The results of this study suggest that McKenzie and core stabilization exercises, either alone or in association, are effective in treating low back pain due to Disc Protrusion (DP). In particular, core exercises enhanced lumbopelvic complex stabilization and functional capacity according to the domains of the SF-36 quality of life questionnaire.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Evaluation of Trunk Muscle Endurance in People with And Without forward Head Posture: a Cross-Sectional Study

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SUMMARY

Background. Muscle endurance is an important factor to maintain the proper posture. Improper posture in head and neck may alter muscle endurance in other segments of the vertebral column. This cross-sectional study was first aimed to compare the trunk muscle endurance in two groups of people with and without forward head posture. The second aim was to explore the relationships between forward head posture and trunk muscles endurance.

Methods. Ninety seven participants (70 males and 27 females; mean age = 23.30 yrs.; mean body mass index = 22.76 Kg/m^2) were assigned to two groups of people with forward head posture and people without forward head posture based on the amount of craniovertebral angle measured by photogrammetry technique. The endurance of trunk flexor, extensor and side-flexor muscles was measured in seconds and compared between groups.

Results. The endurance of trunk muscles was significantly lower in the forward head posture group compared to control group ($P < 0.05$). There were also negative relationships between the severity of forward head posture and the endurance of trunk muscles ($P < 0.05$).

Conclusions. The low endurance of trunk muscles in the presence of forward head posture may lead to poor muscular control of the spine and may predispose people to pain and disability in the future not only in the neck but also in the other spinal segments.

KEY WORDS

Head; neck; torso; muscle; posture; photogrammetry; physical endurance.

BACKGROUND

Forward head posture (FHP) is one of the more common habitual and/or structural poor postures resulted from various postural or occupational demands such as excessive computer and smartphone usage (1, 2). FHP causes numerous complaints such as neck pain and disability (1-4). It is worth noting that FHP not only affects the function of the head and neck musculoskeletal structures, but also affects the whole body postural control (5). Previous studies revealed that the head position has effects on the lower spine posture and the activity of the trunk muscles during lifting and prone bridging tasks (6, 7). Hlavenka (6), *et al* compared the effects of retracting neck posture with free style neck posture on the activation of the neck and trunk muscles and also trunk posture in people performing moderate intensity lifting task. The retracted neck position led to less flexion in the lumbar spine. This position also altered the activation of the neck, thoracic, lumbar and abdominal muscles (6). Yu (7), *et al.* also examined the effects of three head positions (head in neutral, in flexion, and in extension) on abdominal and lumbar muscle activities during prone bridging exercise. The activity of the rectus femoris and multifidus muscles was varied in head flexion and extension postures compared to the neutral head posture (7). These findings revealed that head posture may have effects on the trunk

and lumbar vertebral alignment and consequently alters the muscular performance not only in the cervical spine but also in other spinal levels (6, 7). On the other hand, the thoracic and lumbar posture can alter the head/neck position and the activity of cervical and thoracic muscles (8). In this regard, the amount of head translation is higher in the slump sitting position compared to the lumbo-pelvic and thoracic upright sitting postures (8). Cervical erector spine muscle activity is also related to the type of the acquired sitting posture (8). Furthermore, the ability of trunk muscles to maintain enough activity level over a long period of time is necessary to maintain the neutral spine posture (9, 10). It seems that inadequate muscle endurance may lead to postural mal-alignments, especially in the young population (4, 11, 2). This may predispose people to pain and disability in the future (7).

As mentioned earlier, FHP is a common poor posture in people suffering neck pain (4) and interestingly a national survey in the US disclosed that low back pain and neck pain is the most common disabling complaints among adults which often occur concurrently (13). Therefore, the importance of assessing trunk muscle endurance in the presence of the habitual head postures such as FHP is highlighted. Furthermore, muscle endurance tests of lower trunk can be used to predict low back pain (14). Based on the authors' knowledge, the endurance of trunk muscles in people with FHP is not clarified yet. Evaluating the endurance of trunk muscles in people with FHP may have implications in designing preventive strategies in the clinical settings. Therefore, the first hypothesis was that the trunk muscle endurance of people with FHP was different from people without FHP. The second hypothesis was that the FHP severity could be related to the amount trunk muscle endurance. Accordingly, the present study was conducted to evaluate the trunk muscle endurance in peoples with and without FHP and to determine the relationships between FHP severity and trunk muscle endurance.

MATERIALS AND METHODS

Participants

Ninety seven recreationally active participants (70 males and 27 females) aged 23.30 ± 2.24 years (mean ± SD), and with body mass index (BMI) ranged 22.76 ± 2.63 kg/m^2 volunteered to participate in this cross-sectional study using convenient sampling. The amount of power was calculated by G Power software version 3.1.9.2 and was equal to 0.88. The inclusion criteria were having no complaints of neck and/or back pain during the last 6 months leading treatments (15, 16). The exclusion criteria were as follows: subjects with the neurological deficits, history of trunk or neck surgery, doing professional sport, the presence of obvious spinal deformities, or obvious medical conditions that contraindicated vigorous exercise (15, 16). Written informed consent was obtained from participants and the study protocol was approved by the local ethics committee (Ethic's code: …1395.555). Also, written informed consent was obtained from the participant who volunteered in preparing illustrations of this study. The study meets the ethical standards of the journal (17). This study was conducted in the posture research laboratory of Tabriz University of Medical Sciences since 2016 until 2018.

Experimental procedure

Initially in a pilot study, the intra-rater reliability of the trunk muscle endurance tests was evaluated in 30 participants with FHP revealing ICC values were ranged 0.93-0.98 for photogrammetry method and endurance testing. Two qualified physiotherapist with 15 years' experience in manual therapy field were performed examinations. One experienced physiotherapist evaluated FHP through measuring craniovertebral angle (CVA) in the standing position using photogrammetry technique. People with CVA values of lower than 48° were considered to have FHP (18). Another qualified physiotherapist assessed the endurance of trunk flexor, extensor, and lateral flexor muscles. The endurance tests were applied in a random order. The endurance scores were measured in seconds and the tests were terminated if the subject cannot maintain the tests' defined position based on special considerations for each test or experiences discomfort or pain (19). The rest period between each trial was 5 minutes (3 trials for all tests) and the rest interval between separated was 10 minutes. Detailed explanations of the procedures were given to all participants. Verbal and tactile feedbacks were also provided to the subjects to maintain the endurance test positions accurately (20). The endurance examiner was not aware of the results of photogrammetry to minimize the risk of bias. Prior to the data collection, the examiners practiced test procedures to ensure accurate protocols were applied.

Measurement of craniovertebral angle (CVA)

Photogrammetry technique was used to measure the CVA with a digital camera (Fuji Film JX700, Japan) which was placed laterally at the shoulder height about 1.5 meters from the subjects in a standing position on a fixed base without tilt and rotation. The CVA demonstrates the angle between the spinous process of the seventh neck vertebrae (C7) and the ear tragus with the horizontal line through neck seventh vertebrae. Self-balanced positioning was instructed to the

participants to standardize head and neck posture based on the previous recommendations (16, 21). The importance of maintaining the neutral head posture was explained to participants. CVA was calculated using AutoCAD software (**figure 1**).

Endurance tests

Biering-Sorenson test

Biering-Sorenson test is the most widely used procedure to evaluate the isometric endurance of trunk extensor muscles (20). The subject was asked to lie prone with the lower body fixed to the table in all joints by three straps. Upper body was out of the table and extended over a stool. The participant was asked to release the table while upper extremities were held in the crossed position on the chest. It was instructed to lift off the upper body from the floor and maintain the position horizontally as long as possible (8). As long as the position was maintained the examiner calculated the endurance time (**figure 2**).

Trunk flexor endurance test

The participant was sat on a table against a wedge with an angle of 60° from the table. Hips and knees were held at 90° flexed position and feet were fixed to the table. The arms were folded across the chest and toes were held fixed by another examiner. The subject was asked to maintain the body position while the supporting wedge was pulled back about 10 cm to start the test (19). The examiner calculated the endurance time in seconds. The test continued as long as the subject maintain the position (**figure 3**).

Side bridge test

The subject was requested to lie on an exercise mat on one side with legs positioned at the extension. The upper leg was placed in front of the lower leg on the mat. The participant was instructed to lift off his hips and maintain the full body in straight line with support on the elbow and feet. The upper arm was maintained on the opposite shoulder. The test terminated if the hips returned to the table (19). The examiner calculated the time as long as the subject tolerating the position. The identical procedure was conducted to the other side (**figure 4**).

Figure 1. Measurement of the craniovertebral angle. One landmark was placed on the tragus of the ear and the other landmark was placed on the spinous process of the seventh cervical vertebrae.

Figure 2. Muscle endurance evaluation of the trunk extensors using Biering-Sorenson test.

Figure 3. Muscle endurance examination of the trunk flexor muscles.

Figure 4. Muscle endurance examination of the trunk side flexor muscles using side bridge test.

Figure 5. Muscle endurance examination of the trunk extensors using prone double straight-leg raise test.

Prone double straight–leg raise test

The participant was asked to lie in a prone-lying position with the hips extended. The hands were held underneath the forehead. The arms were kept vertical to the body. Then the subject was instructed to lift off both legs until the knee clearance was achieved (20). The test continued as long as the participant kept the position. The endurance time was calculated in seconds (**figure 5**).

Statistical analysis

The Kolmogorov-Smirnov test was applied to check the data normality. Analysis of covariance (ANCOVA) was conducted to compare the trunk muscles' endurance differences between two groups (with and without FHP) controlling the effects of gender, age and body mass index. Pairwise comparisons were conducted after Bonferroni adjustment. The Pearson correlation coefficient was determined using bivariate correlation to explore the relationships between CVA and endurance tests. The magnitudes of these correlations were considered as negligible (0.0 - 0.1), small (0.1 - 0.3), moderate (0.3 - 0.5), large (0.5 - 0.7), very large (0.7 - 0.9), or extremely large (0.9 - 1.0) (22). The significance level was set at $P < 0.05$. There were no missing data. All statistical analysis was performed using SPSS statistics version 17.

RESULTS

All of the eligible participants fulfilled the procedures. Participants' characteristics are presented in **table I**.

The endurance tests showed significant differences between two groups (Wilk's Lambda = 0.803; F (7, 86 = 3.021); P= 0.007; Eta- squared = 0.197). Pairwise comparisons revealed that trunk muscle endurance of extensors, flexors and lateral flexors were lower in the presence of FHP ($P < 0.05$) (**table II**).

There were positive correlations between FHP severity and trunk muscle endurance. The fair to moderate direct correlation was found between CVA and Sorenson score. The amount of correlation between CVA and trunk flex-

Table I. Demographic characteristics of participants in two groups with and without FHP.

	With FHP (n= 36)	Without FHP (n=61)
Age (y), mean ± SD	23.25 ± 1.70	23.33 ± 2.52
Height (cm), mean ± SD	171.57 ± 7.36	169.49 ± 7.64
Weight (Kg), mean ± SD	67.28 ± 8.82	65.47 ± 9.43
BMI (Kg/m2), mean ± SD	22.80 ± 2.66	22.73 ± 2.64
Craniovertebral Angle, Mean ± SD	45.64 ± 2.16	53.05 ± 3.14

* denotes significant differences between groups ($P < 0.05$); FHP: Forward head posture

Table II. Between-group differences for trunk muscle endurance in people with and without forward head posture.

	With FHP (n= 36) mean ± SD	Without FHP (n=61) mean ± SD	P-value
Biering-Sorenson test (second)	44.05 ± 4.58	67.65 ± 3.51	0.000*
Trunk flexor endurance test (second)	36.36 ± 3.66	48.95 ± 2.80	0.008*
Right Side bridge test (second)	35.49 ± 3.07	45.87 ± 2.36	0.009*
Left Side bridge test (second)	35.83 ± 3.03	44.08 ± 2.32	0.034*
Prone Double straight-leg raise test (second)	42.74 ± 3.15	54.49 ± 2.42	0.004*

* denotes significant differences between groups (P < 0.05); FHP: Forward head posture

Table III. The relationship between the results of muscle endurance tests and craniovertebral angle expressed as correlation coefficients.

Relationship	P-value	RP
Craniovertebral angle and trunk flexor endurance test score	0.010*	0.260
Craniovertebral angle and Biering-Sorenson test score	0.001*	0.334
Craniovertebral angle and prone double straight-leg raise test score	0.015*	0.247
Craniovertebral angle and right side bridge test score	0.020*	0.235
Craniovertebral angle and left side bridge score	0.045*	0.234

* denotes significant correlations (P < 0.05); n = 97

or and lateral flexor muscle endurance scores was almost fair and direct (**table III**). The score of the prone double straight leg raise test is also fairly correlated to the amount of the CVA.

DISCUSSION

In the current study, the trunk endurance tests demonstrated significant differences between two groups of people with and without FHP. The trunk muscle endurance was lower in subjects with FHP compared to subjects without FHP. There were also negative relationships between the FHP severity and trunk muscle endurance.

The abnormal posture may be a predisposing factor in disability (23). FHP as a common poor posture is defined as the anterior position of the head in relation to the base of the neck and characterized by hyper extension of the upper cervical spine and flexion of lower cervical spine (24). Previous studies reported that FHP has effects on pain and disability, especially in the head, neck and shoulder region (3, 4, 23). The results of the present study are in accordance with others who examining the effects of adopting different head postures on trunk and lumbar muscle function (6). The activation of the sternocleidomastoid, external obliques and lumbar erector spinae muscles during moderate intensity lifting was higher when adopting retracted neck posture (*e.g.* chin tucks) compared to the freestyle neck posture in 7 participants with no history of low back pain. Decreased activity in the neck and trunk dorsal muscles was also associated with retracted neck posture (6). Therefore, the hyperextension of the upper cervical spine seen in FHP may contribute to the lower endurance of trunk and back muscles and eventually may predispose people to the back injury in the future when doing high demanding tasks such as lifting. Compared with our results, Yu (7), *et al.* reported that maintaining head flexion in prone bridging exercise can facilitate the abdominal muscle activity and enhances the treatment effect of prone bridging exercise. On the other hand, the activity of the lumbar multifidus muscles is higher during head extension compared to the neutral head position (7). Dejanovic (25), *et al.* also showed that adding cervical extension to the Biering-Sorenson test in children resulted in higher back endurance scores compared to the cervical flexion (25).These findings reveal that FHP may have effects on the lumbar and trunk muscle performance in different ways and depends on functional situations. Conversely, acquiring a slump sitting position imposes an anterior translation to the head and increases the activity of cervical extensors compared to the upright sitting (9). In this situation, the activation of superficial lumbar multifidus and internal oblique muscles is also diminished (26).

Therefore, there is a linkage between the alignment of the spine and muscular performance in different spinal levels. Furthermore, a randomized clinical trial demonstrated that adding corrective exercises to reduce FHP causes a decrease in symptoms of people suffering from chronic lumbosacral radiculopathy who had FHP concurred (27). It is also worthy to consider that FHP severity is related to the balance disturbances in healthy computer users (5) and the FHP is commonly present in patients with acute low back pain (23). Therefore, it can be concluded that habitual head posture is an important factor affecting the trunk and lumbar muscle performance maintaining neutral spine (28). We suggest examining the effects of FHP on trunk and lumbar muscle performance in various de-stabilizing conditions of the lumbar spine.

The prone double straight-leg raise test and the Sorenson test are the two most common procedures to assess the back extensor muscle endurance (20). According to McIntosh (20) *et al.*, the endurance of lower back extensor muscles can be assessed by prone double straight-leg raise test while the upper back extensors can be assessed by the Sorenson test (20). Decreased scores of the Sorenson endurance test is considered as a risk factor for low back pain (LBP) episodes (29). While, the prone double straight-leg raise test has the highest sensitivity, specificity, and predictive value of low back pain among Iranian people (30). Based on the results of the current study, the negative relationship between Sorenson test and FHP severity reveals that having abnormal head posture may be a contributing factor to the development of low back pain in the future. However, the amount of this relationship is fair to moderate. It may be due to low severity of the FHP in our participants. Evaluating these relationships based on the wider ranges of FHP severity is recommended (24).

The quadratus lumborum muscle function can be assessed by lateral bridge test (31). Cholewicki (25) *et al.* stated that the quadratus lumborum muscle is a major stabilizer of the lumbar spine while applying minimal loads on the lumbar spine (28). As shown before, young male elite golfers with low endurance scores on the side-bridge test are more likely to report future episodes of moderate and severe low back pain (32). The negative relationship between the FHP and trunk flexor muscle endurance can also be explained by the results reported by Su (33) *et al.* They found the higher activity level of abdominal muscles in crook-lying position adopting a craniocervical flexion position (33). Overall, Consistent with the findings of the current study, Hlavenka (6) *et al.* reported that adopting retracted head posture leads to increase in the activity of the lumbar erector spinae and external oblique muscles. This posture is considered as a safe strategy to perform lifting tasks (6). Altogether, it is recommended to identify the long-term effects of habitual abnormal postures on spinal muscle function because there are linkages between different parts of the spine. This information can give us better insight into the understanding of possible causes of postural pain disorders of the spine. Also, the significant difference in trunk muscles endurance between groups with and without FHP highlights the importance of screening postural imbalance in populations without obvious clinical manifestations to be enabled in designing preventive strategies to minimize injury risks in the spine. Finally, more research in this concern is needed to explore the cause-and-effect relationships between spinal muscular performance and postural mal-alignments.

Our study has some limitations. First, relatively young participants were included in this study. Of course, the possible effects of gender and age were controlled. In this regard, the severity of FHP is age dependent (11). Therefore, the age effect should be considered in the future studies. Second, the physical activity level is also an important factor influencing the muscular performance including endurance tests. We excluded people doing regular sport-specific exercises. Investigating the effect of FHP and other postural impairments on athletes' muscular performance may have implications for reducing athletic injuries in the future.

In conclusion, the lower endurance of trunk muscles in people with FHP compared to people without FHP predicates the importance of assessing the muscular performance at different levels of the spine in the presence of FHP. The negative relationships between FHP severity and trunk muscles endurance, demonstrate that the muscle endurance is related to the alignment of the spinal column. These findings may have implications to design preventive strategies for people with poor postures and reduce the risk of injury in the future.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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ORIGINAL ARTICLE

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The Relationship between Nordic Hamstring Test and Isokinetic Dynamometry in Football and Track and Field Student Athletes: a Cross-Sectional Study

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SUMMARY

Background. Nordic hamstring test is a field-based test to estimate the hamstrings' injury risk. The aim of this study was to investigate the relationship between Nordic hamstring test and isokinetic dynamometry in football and track and field student athletes.

Methods. Knee flexor eccentric peak torque of hamstring, optimum length, and muscle balance indices were obtained in two seated and lying positions in 22 footballs and 22 track and field student athletes. The Nordic break point angle was also measured.

Results. There were significant correlations between isokinetic dynamometry indices and breakpoint angles (P=0.000). Best predictors of break point angle were muscle balance indices and eccentric hamstring peak torques in lying position ($P < 0.05$).

Conclusions. It seems that Nordic hamstring test is related to isokinetic dynamometry. This relationship is also dependent on the hip joint positioning. These findings may have implications in the assessment of athletic performance.

KEY WORDS

Hamstring muscles performance; athletic; muscle strength; track and field; football.

BACKGROUND

Hamstring strain (HS) is one of the most prevalent injuries in sprint-related sports like football, rugby and track and field that cause high financial costs for teams and athletes (1). In Australian football league, the cost of HS increased to 40021 AUD for every single injury in 2012 (1). The Injury rate is between 6-29% in different sports (2). In football, 8%- 25% of all injuries and 26% of all injuries in track and field athletes are related to the HS (3). Overstretch and explosive eccentric contractions are two main mechanisms for HS occurrence (2).

Isokinetic dynamometry is commonly used as a golden standard to assess the hamstring eccentric ability (4). The hamstring eccentric ability is one of the most important dynamometry indices to estimate the risk of HS; that means decreased eccentric hamstring muscle strength can lead to injury (4-7). Another index is reciprocal knee joint muscle balance that is commonly expressed as hamstring to quadriceps muscle ratios (H/Q ratios) (4, 8). H/Q ratios including hamstring $_{Concentric}$ to Quadriceps $_{Concentric}$ ratio and hamstring $_{Eccentric}$ to Quadriceps $_{Concentric}$ ratio (4). Previous studies have shown that a decrease in H/Q ratios will increase the risk of HS among athletes (4, 9, 10). On the other hand, some authors claimed that these ratios cannot predict the injury occurrence (11). Therefore, recently a new muscle balance index is introduced as the angle of crossover (ACO). ACO represents the angle at which the net joint torque around the knee joint crosses zero (12). It reveals hamstring capability to eccentrically overcome the quadriceps concentric action (12). ACO is proposed to be a better index than H/Q ratios to estimate the HS risk (12). Shortened hamstring optimum length (OL) is considered as another HS risk factor (13). OL is the length that muscle demonstrates peak torque (14). Shorter the OL shifts, more susceptibility to the HS (15). Overall, isokinetic dynamometry needs technical expertise and is difficultly accessible in athletic centers because

of high cost and time consumption (16). In addition, the occurrence of exercise-induced muscle soreness after isokinetic dynamometry is high (17). Ultimately, dynamometry procedure cannot be applicable on athletic field. Therefore, developing field based tests with low cost and easy access seems to be necessary.

Nordic hamstring exercise was introduced in 2001 as a hamstring eccentric strengthening exercise (18). It is shown that Nordic hamstring exercise can improve eccentric strength of hamstrings and probably decrease the rate and recurrence of HS in athletes (19, 20). Recently, some authors claimed that this exercise defined as Nordic hamstring test (NHT) can also be used as a field test for assessing the hamstring eccentric capability (17, 21, 22). Breakpoint angle (BPA) is defined as an angle that an individual cannot overcome increasing gravitational moment and falls to the floor when performing NHT. BPA is considered as an outcome measure for the athletic performance (21). Correlations between BPA and peak torques of hamstrings were demonstrated in football players (17, 21, 22). As said before, HS is also prevalent in track and field (3, 23). The applicability of NHT as a field test in track and field has not known yet.

These relationships between NHT and isokinetic dynamometry were explored in the seated position only (17, 21, 22). Whereas, the athletic performances are commonly carried out in standing position, and the amount of knee joint torques depends on the change in the hip and knee joint positions (24, 25). Hence, the position of the hip joint should be considered when evaluating the relationship between NHT and isokinetic dynamometry. Therefore, the purpose of this study was to investigate the relationships between NHT as a field test and isokinetic dynamometry as a gold standard laboratory test (dynamometry indices including eccentric hamstring peak torques, H/Q ratios, ACO, and OL) performed in seated and lying positions in two groups of football and track and field athletes.

MATERIALS AND METHODS

Study design

A cross sectional study was designed to determine the relationship between Nordic hamstring test and isokinetic dynamometry in two groups of football and track and field athletes.

Participants

Forty four healthy male student athletes (26) were purposefully divided into two groups including 22 football players and 22 track and field athletes. Convenient sample from the available student athletes were participated voluntarily in both groups and were matched based on the demographic variables mentioned above. Participants included based on performing specific sport drills as the training programs designed by university coaches and received scheduled training programs on the weekly basis in university teams. They aged between 18 to 24 years and were classified as physically active based on the Tegner physical activity questionnaire (gained score 6 or more on the questionnaire). Participants with the history of hamstring strain and/or other lower limb problems like fractures and soft tissue injuries at last 6 months ago, presences of hyper lordosis in the lumbar spine (evaluated by an experienced physiotherapist as postural screening), and the history of epilepsy or other neurologic disorders were excluded. Another physiotherapist conducted the remained procedure including dynamometry and NHT. The sample size was calculated based on the expected correlation coefficient reported in the study of sconce (21). The total number of participants in each group was equal to 22 persons.

The present study was approved by the … ethical committee (….1396.979) and signed informed consent was obtained from each participant. Also, participants have given written informed consent to publish these case details. The study meets the ethical standards of the journal (27). This study was conducted in Biomechanics laboratory of … since 2016 to 2018.

Experimental procedures

After familiarization with the procedure, each subject filled the basic information form. Subjects were asked to avoid eating and performing high intensity training exercises two and 48 hours before the tests respectively. Warm up including lower extremity stretching exercises and walking for three minutes was performed at first. Then, isokinetic dynamometry and Nordic hamstring tests were done.

Isokinetic dynamometry

Participants were familiarized with Cybex HUMAC NORM Isokinetic Extremity System [CSMI, Stoughton, MA] through performing four sub-maximal contractions. The dominant leg was tested in all subjects. Each subject was examined in two seated and lying positions using dynamometer to extract hamstring and quadriceps eccentric and concentric peak torques at the constant velocity 60°/s (21). In each position, subject's trunk and thighs were fixed with straps to avoid extra and uncontrolled movements and axis of rotation of the dynamometer was aligned to the knee joint axis of rotation. In seated position, hip joint angle was

adjusted at 90° of knee joint flexion. Range of motion was 0 to 90°. Full extension was considered as zero (**figure 1**).
In the lying position, hip joint was held in full extension and range of motion was adjusted from 0° to 110° (**figure 2**).
After that, participants were asked to do three maximal and reciprocal flexion and extension movements (repetitive cycles that were started with flexion movement and ended to extension movement) eccentrically and concentrically for knee joint flexors and extensors with 15 seconds rest between every repetitions and two minutes rest between trials (21, 22). The resistance provided by the weight of the lower leg was recorded at 30° of flexion for gravity correction.
Data were recorded HUMAC NORM Software [HUMAC 2009, v.9.7.1] and Microsoft Excel files were extracted for the subsequent analysis. Peak torque values were normalized to body weight. H/Q ratios and the ACO were calculated using equations below:

Equation 1:
Conventional H/Q ratio = knee flexor concentric peak torque/knee extensor concentric peak torque×100.

Equation 2 (4, 28):
Functional H/Q ratio = knee flexor eccentric peak torque/knee extensor concentric peak torque×100

Angle of cross over is an angle in which the difference between knee extensor concentric torque and knee flexor eccentric torque reaches zero (22).
OL as the angle that hamstrings demonstrate the eccentric peak torque in each testing position was also measured.

Nordic hamstring test

Subjects performed three repetitions of Nordic hamstring test on the mat. In a kneeling position, four LED markers attached to the lateral of hip, shoulder, knee joints and the lateral malleolus by an expert examiner. Subjects were instructed to keep shoulder, hip and knee joints in a straight line and were asked to try to keep this position steadily entire the movement. Ankles were stabilized by the examiner. Then, the subjects were instructed to do forward falling until they could no longer resist the gravity force and start to fall (**figure 3**). This Procedure (in three trials with two minutes rest between trials) was recorded by the Apple iPhone 7+ camera [Slo-mo video support for 720 pixels at 240 frames per second] at the rate of 240 frames per second at the distance of two-meters away from the subjects. The best of three repetitions (close to ground) was considered as the test trial and used for the further analysis. After the videos were recorded, data sent to the Kinovea Software. The validity and reliability of the Kinovea program [Beta 0.8.27] in obtaining angles and distances was studied in some studies recently (29). This program was detecting the LED markers on the video file and extract joints coordinates. Using coordinates, joint angle and speed diagrams

Figure 1. Isokinetic dynamometry of knee extensors and flexors was done in seated position.

Figure 2. Isokinetic dynamometry of knee extensors and flexors was done in lying position.

Figure 3. Nordic hamstring test was carried out using a camera. Figure a showing starting position and figure b showing mid-pint position and c the end position to indicate the break point angle. The break point angle is defined as the angle between the line passing the shoulder through the lateral aspect of the knee joint and the horizontal line when the subject cannot overcome the gravitational moment and falls to the floor.

were plotted. The point that speed was greater than 10°/second was considered as the BPA (17, 21, 22).

Statistical analysis

Data collected from subjects were analyzed by SPSS version 13.0 [Statistical Package for Social Sciences, Chicago, IL]. Normality of the data was confirmed by Shapiro-Wilk test. The ICC values were interpreted according to the Landis and Koch criteria (0-0.02 as poor, 0.21-0.40 as fair, 0.41-0.6 as moderate, 0.71-0.80 as substantial and 0.81-1 as almost perfect (30)). Correlation between BPA and isokinetic dynamometry indices was calculated using Bivariate Pearson product-moment correlation coefficient (r). The magnitudes of these correlations were considered as negligible (0.0-0.1), small (0.1-0.3), moderate (0.3-0.5), large (0.5-0.7), very large (0.7-0.9), or extremely large (0.9-1.0). Linear regression was also conducted to explore the relationships between isokinetic dynamometry indicesand BPA. $P < 0.05$ was assumed as significant level (31, 32).

RESULTS

Demographic variables and descriptive statistics for isokinetic dynamometry indices and BPA are presented in **table I**. Two groups of athletes were matched based on the demographic variables (**table I**). The results of test-retest analysis show that all variables have almost perfect reliability (**table II**).

There were strong correlations between isokinetic dynamometry indices (hamstring eccentric peak torques and ACO) and Nordic break point angle in football ($P = 0.000$). In track and field, the correlation between isokinetic dynamometry indices (hamstring eccentric peak torque and ACO) and Nordic break point angle was also observed ($P = 0.000$). The amounts of Pearson product-moment correlation coefficient (r) were higher in the lying position of dynamometry compared to the seated position. The results of correlation between isokinetic dynamometry indices and BPA are presented in **table III**.

Based on the regression analysis, dynamometry indices in lying position including knee flexor eccentric peak torque, ACO, and H/Qs were predictors of BPA in football. In track and field, only two indices including ACO and conventional H/Q were related to the BPA (**table III**).

DISCUSSION

The results revealed that in both groups, the correlations between BPA and hamstring eccentric peak torque and ACO were very strong. The amounts of correlation were higher in the lying position compared to the seated position. There were moderate correlations between the OL and BPA in two groups although higher correlations were obtained for lying position. These results may be revealing that the NHT as a field test is related to the isokinetic dynamometry as a laboratory gold standard test. Therefore, the Nordic hamstring test can be applied as an alternate screening tool to evaluate athletic performance. Although, there were no correlations between H/Q ratios and BPA, the conventional H/Q ratios have predicted BPA. Other predictors of BPA were as follows: hamstring eccentric peak torque, and ACO respectively in football, and ACO in track and field. It means that muscle balance ratios are important laboratory

Table I. Mean ± SD for demographic variables and descriptive for isokinetic dynamometry indices and Nordic hamstring test index (break point angle).

Variable	Football (N=22)	Track and field (N=22)	P-value
Age (years)	21.41 ± 1.86	21.55 ± 1.62	0.797
Body weight (Kg)	69.32 ± 9.70	65.21 ± 10.51	0.503
Height (meter)	1.79 ± 0.09	1.77 ± 0.10	0.185
Body mass index (Kg/ m2)	21.53 ± 1.04	20.67 ± 1.10	0.112
Seated Knee-flexor eccentric peak torque (Nm)	118.93 ± 26.43	131.88 ± 32.08	0.151
Lying knee-flexor eccentric peak torque (Nm)	99.09 ± 17.92	99.79 ± 29.16	0.924
Seated angle of Crossover (°)	33.27 ± 3.81	34.73 ± 5.36	0.305
Lying angle of Crossover (°)	30.09 ± 3.39	32.35 ± 4.48	0.066
Seated Knee-flexor eccentric optimum length (°)	41.04 ± 6.56	37.96 ± 7.22	0.146
Lying Knee-flexor eccentric optimum length (°)	47.15 ± 5.40	46.06 ± 5.31	0.504
Seated conventional hamstring to quadriceps ratio	0.68 ± 5.19	0.68 ± 6.00	0.730
Lying conventional hamstring to quadriceps ratio	0.63 ± 6.78	0.61 ± 6.70	0.404
Seated functional hamstring to quadriceps ratio	0.77 ± 6.17	0.76 ± 5.31	0.846
Lying functional hamstring to quadriceps ratio	0.76 ± 5.94	0.75 ± 4.98	0.226
Nordic break point angle (°)	41.67 ± 3.47	38.04 ± 5.39	*0.011

*P-value less than 0.05 was considered as significant

Table II. Test-retest analysis.

Variable	Football ICC(1, 3) 95% CI [a]	Track and field ICC(1, 3) 95% CI
Seated Knee-flexor eccentric peak torque (Nm)	0.990 (0.9750.996)	0.997 (0.993-0.999)
Lying knee-flexor eccentric peak torque (Nm)	0.984 (0.9610.993)	0.994 (0.9880.998)
Optimum length (Seated) (°)	0.986 (0.9670.994)	0.997 (0.993-0.999)
Optimum length (Lying) (°)	0.977 (0.9450.990)	0.973 (0.9360.989)
Angle of crossover (Seated) (°)	0.983 (0.9610.993)	0.986 (0.9670.994)
Angle of crossover (Lying) (°)	0.976 (0.9450.990)	0.994 (0.9870.998)
Seated conventional H/Q[c]	0.885 (0.7230.952)	0.871 (0.686-0.946)
Lying conventional H/Q	0.929 (0.8280.970)	0.934 (0.8420.973)
Seated functional H/Q	0.899 (0.7570.958)	0.890 (0.7360.954)
Lying functional H/Q	0.931 (0.8340.971)	0.831 (0.5930.930)
Nordic break point angle (°)	0.931 (0.8420.971)	0.981 (0.9550.992)

[a] Confidence Interval

indices to predict athletic performance on field. The results of reliability analysis also reveal that isokinetic dynamometry and NHT indices have almost perfect reliability which is in agreement with previous reports (17).

Although isokinetic dynamometry is introduced as a golden standard test for muscle strength assessment (33), as mentioned earlier, it has various practical limitations. Therefore, it is recommended in the previous literature to use more functional and easily applicable tests on field for athletes (21,34). The results of this study support the findings of the previous studies with regard to NHT (17, 21, 22). The strong correlation between eccentric hamstring peak torque and BPA indicates that NHT can be used as a field test to demonstrate eccentric hamstring capabilities in football (17, 21, 22). Generally, in the present study the amounts of correlation between eccentric hamstrings peak torques and BPA in football were higher compared to other reports in this area (17, 21, 22). Differences in physical activity level

Table III. Correlations between isokinetic dynamometry indices and Nordic Break point angle.

Variable	Football (N=22)	Track and field (N=22)
Seated Knee-flexor eccentric peak torque (Nm)	(r = -0.816, P < 0.001)*	(r = -0.795, P < 0.001)*
Lying knee-flexor eccentric peak torque (Nm)	(r = -0.860, P < 0.001)*	(r = -0.816, P < 0.001)*
Seated ACO[a](°)	(r = -0.797, P < 0.001)*	(r = -0.877, P < 0.001)*
Lying ACO (°)	(r = -0.817, P < 0.001)*	(r = -0.902, P < 0.001)*
Seated OL[b](°)	(r = 0.448, P = 0.037)*	(r = 0.466, P = 0.029)*
Lying OL (°)	(r = 0.499, P = 0.018)*	(r = 0.486, P = 0.022)*
Seated conventional H/Q[c]	(r = -0.194, P = 0.386)	(r = -0.258, P = 0.247)
Lying conventional H/Q	(r = -0.411, P = 0.058)	(r = -0.496, P = 0.019)*
Seated functional H/Q	(r = -0.304, P = 0.170)	(r = -0.183, P = 0.414)
Lying functional H/Q	(r = 0.010, P = 0.964)	(r = -0.275, P = 0.215)

* Denotes significant.
[a] Knee-flexor eccentric Optimum length.
[b] Hamstring to Quadriceps Ratio.

Table IV. The results of regression analysis to explore the relationships between isokinetic dynamometry indices and Nordic Break point angle.

Variable	Football (N=22)	Track and field (N=22)
Seated Knee-flexor eccentric peak torque (Nm)	(Beta= -0.010, P=0.979)	(Beta= -0.316, P=0.309)
Lying knee-flexor eccentric peak torque (Nm)	(Beta= -0.851, P=0.031)*	(Beta= -0.529, P=0.97)
Seated ACO[a](°)	(Beta= -0.401, P=0.057)	(Beta= -0.168, P=0.620)
Lying ACO (°)	(Beta= -0.498, P=0.021)*	(Beta= -0.742, P=0.038)*
Seated OL[b](°)	(Beta= 0.177, P=0.547)	(Beta= 0.260, P=0.326)
Lying OL (°)	(Beta= 0.369, P=0.217)	(Beta= 0.316, P=0.235)
Seated conventional H/Q[c]	(Beta= 0.006, P=0.983)	(Beta= -0.202, P=0.589)
Lying conventional H/Q	(Beta= -1.02, P=0.002)*	(Beta= -0.901, P=0.031)*
Seated functional H/Q	(Beta= -0.285, P=0.352)	(Beta= 0.134, P=0.718)
Lying functional H/Q	(Beta= -0.946, P=0.004)*	(Beta= 0.506, P=0.195)

* Denotes significant.
[a] Angle of cross over.
[b] Knee flexor eccentric optimum length.
[c] Hamstring to Quadriceps ratio.

of athletes included in the studies may explain this discrepancy to some extent (17, 21). In the present study student athletes were included based on the Tegner physical activity questionnaire score. In the study of sconce *et al.*, the physical activity level of participating athletes was not clarified (21). In the study of Lee *et al.*, semi-professional athletes were participated (22) and in another study by Lee et, al, professional football players were selected (17). It is worth noting that the physical activity level is one of the factors attributable to the HS risk in athletes (6) and previous literature claimed that the level of athletic performance could affect the results of dynamometry (35). Therefore, it is not possible to generalize the results of NHT without considering the skill and physical activity level of athletes. Also, the time interval between dynamometry and NHT in the Lee study was about 7 to 10 days (22). Accordingly, methodological differences may also account to obtain different relationships.

As mentioned earlier, NHT characteristics were only surveyed in football (17, 21, 22). Thus, the applicability of NHT for other sports remains questionable. Since the HS is defined as the most common muscle injury in track and field athletes (36), we assessed the relationship between NHT and isokinetic dynamometry indices in both football and

track and field. Interestingly, the strong correlation between eccentric hamstring peak torque and NHT was seen in track and field. It means NHT can also be used to assess athletic performance in track and field. Of course, the present study is the first study that reports the results of NHT in track and field sport. More studies are warranted in this area.

Eccentric hamstring peak torque is categorized as one of the most important risk factors for the HS (5, 6, 37-39). ACO as an indicator of knee joint muscle balance is another isokinetic dynamometry index that was also considered to assess the HS risk among athletes (4, 12). In accordance to the other reports (21) the results of the present study demonstrated that there are strong relationships between ACO and NHT in both groups and in two test positions. Moreover, the relationship of eccentric hamstring peak torque and NHT was the largest in football while, the relationship of ACO and NHT was largest in track and field. These findings indicate that relationships between laboratory and on-field test to estimate athletic performance are sport specific. Therefore, these considerations should be taken into account when estimating athletic performance based on the laboratory measures. Although, the role of H/Q ratios to predict HS is remaining controversial (10, 40). Lee, *et al.* reported that conventional H/Q ratio obtained below 50.5% increases the HS risk to almost threefold in soccer (37). However, Bennell showed that these ratios were unable to predict the HS in Australian football (41). As mentioned before, in the current study there are almost no correlations between H/Q ratios and NHT in both groups. However, regression analysis showed that best predictors of BPA in football were lying conventional H/Q ratio, hamstring eccentric peak torque, and lying ACO respectively and also best predictors of BPA in track and field were lying conventional H/Q ratio and lying ACO. Altogether, muscle balance indices seem to be an important index alongside the other laboratory measures. These indices possibly can explore different aspects of hamstring muscle eccentric performance in various sports. A moderate correlation between OL and BPA was found in the current study which is similar to the findings of Sconce study (21).

Commonly the relationship between seated isokinetic dynamometry indices and NHT were determined (17, 21, 22). The hamstring muscle is a bi-articular muscle. Therefore, the hip joint position affects the amount of torque produced by hamstrings. Previous studies revealed that the hamstrings'eccentric peak torque was higher in more flexed hip joint angles (24, 25, 42). NHT is designated to evaluate the hamstring eccentric capability while maintaining hip joint in nearly extended position (17, 21, 22). Therefore, knee joint dynamometry in hip extension is better resembling the athletic performance. The correlations between isokinetic dynamometry obtained in the lying position and BPA indices were higher compared to dynamometry in the seated position. Furthermore, only isokinetic indices obtained in the lying position could predict the NHT results. Finally, it is recommended to consider the role of mono-and bi-articular muscles when estimating athletic performance based on the laboratory results.

Our study has some limitations. Side to side difference is not achievable by the results of NHT. It is recommended to assess the amounts of hamstring muscle activity level and onset latencies with respect to the BPA in the future studies. Only male athletes were allocated in this study. Since, HS may occur in other sports such as rugby and Australian football; we suggest NHT survey with respect to sport type, activity level and playing position in different playing seasons.

Based on the results, Nordic hamstring test is correlated with isokinetic dynamometry as a gold standard. Therefore, NHT can be used on field to explore the athletic performance in football and track and field. Muscle balance about knee joint is also an important issue considering hamstring injury risk factors in football and track and field. When using isokinetic devices, the dynamometry position should be taken into account.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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