Improving how Orthopedic Journals Report Research Outcomes Based on Sex and Gender*

Seth S. Leopold¹, Robert N. Hensinger², Andrew J. Schoenfeld³, Marc Swiontkowski⁴, Michael J. Rossi⁵, Kimberly J. Templeton⁶-⁹

¹ Editor-in-Chief, Clinical Orthopaedics and Related Research
² Editor-in-Chief, Journal of Pediatric Orthopaedics
³ Editor-in-Chief, Spine
⁴ Editor-in-Chief, The Journal of Bone and Joint Surgery
⁵ Assistant Editor-in-Chief, Arthroscopy: The Journal of Arthroscopic & Related Surgery
⁶ Associate Editor, JBJS Case Connector
⁷ Past President, Ruth Jackson Orthopaedic Society
⁸ Past President, American Medical Women’s Association
⁹ Sex and Gender Research in Orthopaedic Journals Group

* The authors of this editorial are the Editors-in-Chief of Clinical Orthopaedics and Related Research, Journal of Pediatric Orthopaedics, Spine, and The Journal of Bone and Joint Surgery; the Assistant Editor-in-Chief of Arthroscopy: The Journal of Arthroscopic & Related Surgery; an Associate Editor of JBJS Case Connector; and the members of the Sex and Gender Research in Orthopaedic Journals Group. This editorial is being published concurrently in the first 5 journals listed above. The articles are identical except for minor stylistic and spelling differences in keeping with each journal’s style. Citation of any of the 5 journals can be used when citing this article. The members of the Sex and Gender Research in Orthopaedic Journals Group are provided in a NOTE at the end of the article.

DOI:
10.32098/mltj.02.2024.00

LEVEL OF EVIDENCE: N/A

Sex-based differences in cell biology, tissue function, and anatomy impact disease risk, presentation, and treatment outcomes (1), including in musculoskeletal care (2-4). As such, these differences should influence how orthopedic surgeons and other healthcare professionals conduct research and provide care for patients who have musculoskeletal disease and injury. In addition, gender roles influence interactions with people who conduct research and with healthcare professionals as well as the likelihood that patients will seek care and how they will respond to treatment (1, 5, 6).

Musculoskeletal research, similar to research in other areas of healthcare, does not always disaggregate results based on a patient’s sex or gender (7). Although some orthopedic surgery journals have explicit editorial standards on the topic of sex and gender in scientific reporting, and although international entities have published sensible guidelines about it (8), we have observed that these standards are inconsistently applied (7).

Inattention to high-quality standards of scientific reporting can harm patients (9, 10). Women have been underrepresented in medical research (11), and this trend continues.
Improving how Orthopedic Journals Report Research Outcomes Based on Sex and Gender

We recognize that while sex is generally considered to be biologic and assigned at birth in the overwhelming majority of people, gender is more challenging to address because 1) the concept of gender is a complex social role designation, 2) gender can be fluid, and 3) the methods of most retrospective studies will be insufficient to characterize it in ways that seem important to us now. When this limitation impacts the interpretation and application of a study’s main findings, we will ask authors to justify it in the Methods section (that is, to explain why incomplete characterization of sex and/or gender is not a disqualifying problem) and to discuss it in the limitations section of the Discussion so as to help readers interpret the findings in light of this methodological shortcoming.

We recommend that, going forward, clinical researchers seek to suitably characterize participants by sex and/or gender, and laboratory scientists (for research involving animals or cell lines or tissues derived from humans or animals) characterize the sex of the animals or the cell line/tissue source(s). In addition, researchers should plan to analyze and report data disaggregated by these factors when appropriate to their work, so that the influence of these important factors can be better ascertained in orthopaedic research.

We hope that by sharing these resolutions with readers, many of whom are also researchers and representatives on institutional review boards (IRBs), institutional animal care and use committees (IACUCs), and/or funding agencies and organizations, the orthopedic research of the future will be both better designed and better reported.
apy; Jon Karlsson, MD, PhD, Editor-in-Chief, Knee Surgery, Sports Traumatology, Arthroscopy; Mauro Alini, PhD, Robert L. Mauck, PhD, and Daisuke Sakai, MD, PhD, Editors-in-Chief, JOR Spine; David Hunter, MBBS (Hons), MSc (Clin Epi), M SpMed, PhD, FRACP (Rheum) and Anne-Marie Malfait, MD, PhD, co-Editors-in-Chief, Osteoarthritis and Cartilage; Henri Migaud, MD, Editor-in-Chief, Orthopaedics & Traumatology: Surgery & Research; William J. Mallon, MD, Editor-in-Chief, Journal of Shoulder and Elbow Surgery; Clare L. Arden, PT, PhD, Editor-in-Chief, Journal of Orthopaedic & Sports Physical Therapy, and Christopher Bono, MD, Editor-in-Chief, The Spine Journal.

FUNDINGS

Funding for this conference was made possible (in part) by (1R13AR082710-01) from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services, nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

DATA AVAILABILITY

All ICMJE Disclosure of Potential Conflicts of Interest forms for the Editors-in-Chief of Clinical Orthopaedics and Related Research, Journal of Pediatric Orthopaedics, and Spine; the Assistant Editor-in-Chief of Arthroscopy: The Journal of Arthroscopic & Related Surgery; and the Associate Editor of JBJS Case Connector are on file with the individual publications and can be viewed on request.

CONTRIBUTIONS

All authors contributed equally to this work.

CONFLICT OF INTERESTS

The disclosure of potential conflicts of interest form for the Editor-in-Chief of The Journal of Bone and Joint Surgery is provided with the online version of the article (http://links.lww.com/JBJS/H974).

REFERENCES

8. SAGER Guidelines. Available at: https://ease.org.uk/communities/gender-policy-committee/the-sager-guidelines/. Last access date: 03/12/2024.
Improving how Orthopedic Journals Report Research Outcomes Based on Sex and Gender


To the Editor,

we would like to thank Serra et al. for the published article *The effect of hydrokinetic therapy on patients with low back pain: a systematic review and meta-analysis* (doi: 10.32098/mltj.01.2023.10), because we believe how difficult it is to carry out a systematic review. By the way, systematic reviews with meta-analyses aim to resolve controversies arising from conflicting results and answer questions not clarified by individual studies. However, they can lead to errors when there is variation between the studies and when some biases are not considered. The Cochrane Collaboration provides a guide for correctly carrying out this type of study, with the intention of reducing possible errors and uncertainties, as well as proposing a reliable study in terms of internal and external validity. Therefore, the recommendations of the Cochrane Collaboration and the PRISMA statement should be followed.

According to the recommendations of the Cochrane Handbook, a meta-analysis should only be carried out when the pooled studies are sufficiently homogeneous in the criteria of participants (P), interventions (I), comparisons (C), and outcomes (O) (1). However, in the analyses presented in this review, studies with different interventions and comparisons were pooled together, invalidating the answers obtained by each one. For example, in meta-analysis 1, regarding the results of the visual analogue scale for the four-week follow-up, in the comparison between aquatic therapy and conventional therapy, the following studies were included:

- Balogh et al. (2005) (2), which evaluated the therapeutic efficacy of reduced sulfur water in comparison with reduced sulfur mineral water and modified tap water with corresponding odor in patients with low back pain in a three-month follow-up.
- Dilekci et al. (2020) (3), which compared conventional on land physiotherapy with the association of conventional on land physiotherapy and balneotherapy in a three-week follow-up.

Eduarda Hirle dos Santos, Jefferson Rosa Cardoso

Laboratory of Biomechanics and Clinical Epidemiology, PAIFIT Research Group, Universidade Estadual de Londrina, Londrina (PR), Brazil

**CORRESPONDING AUTHOR:**
Jefferson Rosa Cardoso
Laboratory of Biomechanics and Clinical Epidemiology
PAIFIT Research Group
Universidade Estadual de Londrina
Av. Robert Kock 60
Londrina (PR), Brazil 86038-440
E-mail: jeffcar@uel.br

**DOI:**
10.32098/mltj.02.2024.01

**LEVEL OF EVIDENCE:** N/A

---


**Key words:** Aquatic therapy; musculoskeletal disorders; low back pain; aquatic exercise; hydrokinetic therapy.
Bias 2 is prioritized, referring to the most recent tool available of bias extremely important. To this end, the use of Risk of Methodological Errors, which makes assessing the risk of primary studies that need to be included are not completely free of methodological errors, with aquatic exercises, or the combination of both, that is, the studies are not comparable. This contradiction can be observed in the results of the study, which presented -1.22 (-1.45; -0.99); p < 0.0001, with a heterogeneity of 91%. In addition to not using the standardized mean difference, the analysis was based on the fixed effect, which ignores the heterogeneity between studies and incorporates a very narrow confidence interval that is far from reality. In this case, to carry out any meta-analysis, the random effect is recommended, as it incorporates the hypothesis that there is diversity in the conduction of the studies, even if they are sufficiently homogeneous. Furthermore, the standard error adjustment proposed by Hartung and Knapp, that widens the confidence interval to reflect the uncertainty in the heterogeneity estimate and the estimation method by Sidik and Jonkman, should be used.

When conducting a systematic review, it is expected that the primary studies that need to be included are not completely free of methodological errors, which makes assessing the risk of bias extremely important. To this end, the use of Risk of Bias 2 is prioritized, referring to the most recent tool available, which systematically classifies studies as low risk of bias, some concerns, or high risk of bias. The Cochrane handbook discourages the use of scales, as they include topics that are not consistent with the true assessment of internal validity. As an example, the PEDro scale includes an assessment based on masking the patient and therapist in physiotherapy studies, where it is only possible to mask the evaluator. Furthermore, scales like the Jadad are outdated and have been out of use for years. Therefore, the GRADE system should be incorporated in order to ensure that factors such as imprecision, heterogeneity, risk of bias, and publication bias are considered when interpreting the review results. After all, what is the point of studies with degree of evidence number 1?

**FUNDINGS**
The authors wish to thank the CNPq for the productivity scholarship and CAPES for the MSc scholarship (Funding Code: 001).

**DATA AVAILABILITY**
N/A.

**CONTRIBUTIONS**
All authors contributed equally to this work.

**CONFLICT OF INTERESTS**
The authors declare that they have no conflict of interests.

**REFERENCES**
Reply to: “Ref: Serra et al. The effect of hydrokinetic therapy on patients with low back pain: a systematic review and meta-analysis”

Giovanni Galeoto¹,²

¹ Department of Human Neurosciences, Sapienza University of Rome, Rome, Italy
² IRCSS Neuromed, Isernia, Italy

As corresponding author, I am grateful to the author of the letter received regarding the publication entitled The Effect of Hydrokinetic Therapy on Patients with Low Back Pain: A Systematic Review and Meta-Analysis (doi: 10.32098/mltj.01.2023.10).

The interest aroused by this contribution makes me proud and highlights the desire for an intellectual and constructive exchange on the methodology of systematic review without any personal interest.

With this letter I would like to respond to all comments where appropriate.

Regarding the objective of systematic reviews, as reported by Uman 2011 it is difficult, if not impossible, for busy clinicians and researchers to keep up with the literature. Systematic reviews that summarize the results of various intervention studies are therefore a method extremely efficient at getting the bottom line on what works and what doesn’t (1). Another definition from Devis et al. (2014) states that these techniques are used to synthesize research findings to determine an estimate of the overall effect for a population of studies. A systematic review refers to the process of systematically identifying and collecting all available information about an effect. Meta-analysis refers to the statistical techniques used to combine this information to provide an overall estimate of the effect in the population (2). Therefore, the purpose of a systematic review is not only to resolve controversies, but sometimes also to bring them to light.

As regards Bias Risk within the rehabilitation research, in this study all three of the most useful tools for assessing Bias Risk were rightly used. The JADAD is the scale created for the evaluation of the risk of bias of pharmacological RCTs, subsequently the PEDRO scale was developed to solve the problem of the floor effects of the JADAD for the evaluation of rehabilitation studies. As the author of Letter to the Editor, rightly knows, my research group published a work in 2022 in which it was highlighted that although the scale has excellent psychometric properties with regards to test-retest reliability and construct validity, the scale presents ambiguous levels of internal validity due to a significant ceiling effect of some items (4).

In the study by Serra et al. (2023), it is possible to notice that the Risk of Bias 1, related to the scores of the individual studies, is reported in the meta-analysis graphs. The total risk assessment graph highlighted that all studies...
presented present a risk due to lack of blinding, for this reason we did not stratify by risk score. Furthermore, regarding the use of Risk of Bias 2, I guess that the author of the letter did not notice that, as reported in the methods section of the document under discussion, the study was conducted in 2021 when the guidelines for the use of Risk of Bias 2 were not published. The author of the letter to the Director itself cites the document published only in 2023. Finally, regarding the last comment regarding the use of the GRADE method in the systematic review, I would like to underline that this method is often used to evaluate different studies within a guideline. In the Prisma Check list that the author rightly mentioned, it is not required to report the GRADE method.

The reason why the scientific community is required to carry out systematic reviews is that this research is useful in a clinical setting and allows healthcare professionals to carry out scientifically valid work. Hoping to have answered all the doubts raised, I will update the review including, as suggested by the author of the letter, the use of the most updated version of the Risk of Bias.

REFERENCES

Muscle and tendon injuries encompass a wide range of conditions, including both acute and chronic ailments, and are common among athletes, representing a significant cause of injuries in various sports. Frequently affected areas include the hamstrings, quadriceps, gastrocnemius, and hip flexors. Concurrently, tendinopathy presents a complex clinical challenge, affecting a majority of athletes due to high load demands and repetitive movements, with its incidence in sports practice increasing over the last decades to account for up to 30% of all injuries. The substantial costs, both in terms of time away from competition and financial loss, have spurred growing scientific interest in therapies that can enhance the healing process of these injuries. In this context, innovative orthobiologic approaches, particularly platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs), have been explored for their potential to facilitate the return-to-play phase and reduce the risk of reinjury by modulating inflammation and promoting tissue regeneration. This narrative review aims to summarize the current evidence regarding the role of orthobiologics in the management of sports-related muscle and tendon injuries.

**SUMMARY**

Muscle and tendon injuries encompass a wide range of conditions, including both acute and chronic ailments, and are common among athletes, representing a significant cause of injuries in various sports. Frequently affected areas include the hamstrings, quadriceps, gastrocnemius, and hip flexors. Concurrently, tendinopathy presents a complex clinical challenge, affecting a majority of athletes due to high load demands and repetitive movements, with its incidence in sports practice increasing over the last decades to account for up to 30% of all injuries. The substantial costs, both in terms of time away from competition and financial loss, have spurred growing scientific interest in therapies that can enhance the healing process of these injuries. In this context, innovative orthobiologic approaches, particularly platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs), have been explored for their potential to facilitate the return-to-play phase and reduce the risk of reinjury by modulating inflammation and promoting tissue regeneration. This narrative review aims to summarize the current evidence regarding the role of orthobiologics in the management of sports-related muscle and tendon injuries.

**KEY WORDS**

Orthobiologics; mesenchymal stem cell; PRP; athletes; tendinopathy; muscle injuries; sport medicine.
INTRODUCTION

Muscle and tendon injuries are common causes of morbidity in athletes, manifesting as acute traumatic episodes and chronic conditions (1). Such injuries are prominent among athletes in sports like soccer and basketball (2). According to the classification by the Italian Society of Muscles, Ligaments, and Tendons (ISMuLT) based on the mechanism of onset, muscle injuries can be divided into direct and indirect categories, which are further classified as non-structural and structural. Direct injuries result from the application of an external force, such as a direct trauma, while indirect injuries are caused by overstretching beyond the viscoelastic limits of the muscle. Indirect structural muscle injuries, commonly referred to as “muscle tears”, are frequently encountered in clinical practice (3). Among professional soccer players, muscle injuries account for more than 30% of all injuries, leading to significant time away from matches (4). Certain muscle groups, including the hamstrings, quadriceps, gastrocnemius, and adductors, are more susceptible to strains, often during eccentric contractions. In cases of re-injury, the return-to-sport time is typically longer than after the first episode. Generally, the incidence of muscle injuries increases with age, especially in the case of calf muscle injuries (5), and with certain health conditions, such as COVID-19 (6). Tendinopathy represents a heterogeneous clinical condition affecting athletes due to high load demands and repetitive mechanical exposure, leading to a persistently failed healing response. This failure results in the progressive accumulation of matrix damage and micro-ruptures of collagen fibrils in tendons. The incidence of tendinopathies in sports has been rising over the last decades, representing up to 30% of all sports-related injuries, with a specific anatomical distribution. Achilles tendinopathy, for instance, affects up to 30% of runners, predominantly those who are middle-aged, often untrained, and engage in activities sporadically (7, 8), while sudden ruptures are more common in younger individuals (9). Overuse injuries in the pelvis and hip tendon structures, such as greater trochanter pain syndrome, proximal hamstring tendinopathy, or groin pain, are prevalent; patellar tendinopathy, or jumper’s knee, primarily affects volleyball and basketball players (10, 11). Lateral elbow tendinopathies and plantar fasciopathy are significantly more common in sports like tennis and running (12).

The considerable time out of competition and financial losses associated with muscle and tendon lesions have spurred interest in therapies that can aid the healing process. Methods aimed at improving the biological aspects of tissue healing, particularly the use of “orthobiologic” agents like platelet-rich plasma (PRP) and bone marrow-derived cells, have gained popularity in the treatment of tendinopathies and soft tissue pathologies in athletes (13, 14). Despite the increasing use of these regenerative therapies, evidence supporting orthobiologics remains mixed, and reports on their formulations are highly variable. This highlights the necessity for standardized production methods and more rigorous studies. Therefore, this narrative review seeks to summarize the current scientific evidence on the management of muscle injuries and tendinopathies using various emerging orthobiologic approaches, with a special focus on cell therapy and PRP (figure 1).

FORMULATION OF ORTHOBIOLOGICS INJECTION THERAPIES

Cell therapies offer a broad range of strategies for tissue healing, with stem cells playing a pivotal role due to their ability to self-renew and differentiate into various cell types depending on their biological environment (15). Mesenchymal stem cells (MSCs), for example, can differentiate into adipocytes, chondrocytes, and osteoblasts. Furthermore, MSCs are capable of producing numerous molecules, including growth factors, cytokines, and chemokines, which are essential in healing processes such as immunomodulation, anti-apoptosis, and neo-angiogenesis (16). MSCs are primarily derived from bone marrow (with the distal femur and proximal humerus being common sites), but they can also be isolated from other tissues such as adipose, skin, and synovial fluid (17, 18). Cell therapies can be categorized into culture-expanded undifferentiated and differentiated cells, culture-expanded differentiated cells, and minimally manipulated heterogeneous native cells. Populations of culture-expanded MSCs and laboratory-purified cell lines may offer higher potency (19). However, the complexity of cell expansion and the regulatory and legal considerations may limit their practical application in clinical settings. Conversely, minimally manipulated cells do not require expansion, allowing physicians to prepare and utilize them directly for tendon tissue regeneration. Ongoing research aims to optimize the harvesting, processing, and delivery techniques of stem cells for treating athletes’ injuries (20).

Figure 1. Orthobiologics’ most frequent clinical targets in sports medicine.
Platelet-rich plasma (PRP) is among the most utilized orthobiologic therapies in athletes, playing a crucial role in the healing and regenerative processes of injuries. PRP, containing platelets, inflammatory cells, and a rich array of proteins such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), and adhesion molecules (21), promotes cell recruitment, proliferation, and neo-angiogenesis at the injury site (figure 2). PRP is derived from the patient’s own blood, which is centrifuged to separate platelets and leukocytes from erythrocytes (22), and then concentrated (almost 4 times). However, significant variability exists in preparation methods, including the absolute number of platelets, the presence of leukocytes, and the activation techniques. This variability allows PRP to be further categorized based on its cellular composition, most notably into leukocyte-rich PRP and leukocyte-poor PRP. The biology of the individual patient also plays a crucial role in these differences. PRP has gained increasing interest in recent decades due to its relatively low cost and minimally invasive application (23). Nonetheless, the lack of standardization challenges the generation of robust scientific data, thus hindering the development of evidence-based treatment protocols (24).

**ORTHOBIOLOGICS IN MUSCLE INJURY: RATIONALE AND CLINICAL EVIDENCE**

The absence of high-quality studies makes it challenging to draw definitive conclusions about the efficacy and safety of MSC therapy for human muscle injuries. However, preclinical evaluations suggesting that the local injection of autologous PRP might reduce the recovery time for muscle injuries have led to its increased use in clinical practice, primarily for sports-related injuries (25). Theoretically, PRP’s effectiveness in promoting muscle injury healing is attributed to its high concentration of paracrine healing and growth factors, which enhance muscle regeneration and myogenesis, besides regulating inflammation response and pain control. Moreover, several clinical studies have demonstrated the benefits of PRP applications in the muscle healing process, achieving a significantly faster return to play (25-28). PRP reduced the time to return to sports after acute muscle injuries in athletes compared with a control group in the study by Rossi *et al.* (25). Delos *et al.* (26) reported that injured athletes could recover in half the usual time with ultrasound-guided PRP injections. Nonetheless, some recent high-quality studies have cast doubt on these benefits (27, 28). A meta-analysis by Grassi *et al.* (29), which included six randomized clinical trials involving 374 patients, investigated the effects of PRP compared to placebo injections or physical therapy in acute muscle injuries. The generalization of the results was challenging due to the variability in PRP protocols and the heterogeneity of muscle injury types, showing no clear advantage of PRP injections in terms of clinical outcomes, time to return to sport, and recurrence rates. There were also controversies regarding subjective pain evaluation and muscle strength.

In 2017, Sheth *et al.* (30) conducted a meta-analysis comparing PRP injection, physiotherapy, and placebo injection in athletes with acute grade I or II muscle strains. The primary outcome was the time to return to sport; the secondary outcome was the reinjury rate with a minimum follow-up of 6 months. A specific subgroup analysis was also performed to assess the efficacy of PRP in treating hamstring muscle strains (grade I/II) alone. This analysis included five randomized controlled trials and analyzed 268 patients. The outcomes indicated a significant reduction in the time to return to sport for the PRP group compared to the control group. However, the subgroup analysis showed no difference between PRP and the control group in the time to return to activity. Moreover, no significant difference was observed in the secondary outcome at 6 months of follow-up. The authors concluded that PRP could offer an earlier return to sport for patients

---

**Figure 2.** Blood sample manipulation through centrifugation to extract PRP.
with grade I/II muscle strains without an increased risk of reinjury at follow-up. Nonetheless, specifically for patients with grade I/II hamstring muscle strains alone, no significant difference was noted. The high heterogeneity of the results does not provide clear evidence for the use of PRP in muscle injuries (31).

**ORTHOBIOLOGICS IN TENDINOPATHIES: RATIONALE AND CLINICAL EVIDENCE**

The pathogenesis of tendinopathy is multifactorial and complex, beginning with repetitive tendon overload leading to structural microscopic damage. The tendon’s poor intrinsic healing ability, insufficient blood supply, and lack of adequate recovery time may result in a gradual accumulation of matrix damage (32). Consequently, tendon tissue cannot efficiently repair itself, leading to the formation of fibrous and scar tissues, which can cause adhesions. To treat chronic tendinopathy in its degenerative phase, it’s crucial to restore the tendon’s reparative capacity by modulating inflammation, thereby curbing degeneration and reinforcing the pro-resolving system.

MSC therapy, administered through cell injection, is suggested to exert an immunomodulatory effect that can steer the inflammatory environment towards healing the injured tendon. The ability of stem cells to reduce inflammatory status and promote cellular response proliferation can be harnessed to modulate the degenerative tendon environment, making this cell therapy a promising approach to treating these injuries (33). Recent data have clearly shown that stem cells present in tendon connective tissue are capable of self-regeneration and multipotential differentiation. It is, therefore, critical to thoroughly investigate their potential to develop new biological therapeutic strategies for tendinopathies. Human tendon-derived stem cells (hTDSCs) have potential future applications in various scientific fields, such as sports medicine and rehabilitation, because these cells can be enhanced in vitro and then injected into the patient’s tendon. Moreover, TDSCs can be directly stimulated in the tendon using specific supplements/drugs and cytokines/chemokines (34).

Studies evaluating MSC therapy for tendon healing have demonstrated a promising significant effect size for pain and functional scores, as well as structural healing (35). Some studies have reported superior radiological and clinical outcomes for cell therapy in tendon disease, while others have noted faster healing rates (36). Specifically, Hernigou et al. (37) showed that patients who received a bone marrow adipose cell injection into their rotator cuff tears experienced faster healing, improved repair quality, and fewer relapses than those in the control group (38).

PRP is a therapeutic option for chronic tendon injuries in athletes, with many studies showing positive outcomes on tendon healing (39). One preclinical study demonstrated that PRP, when locally injected into the Achilles tendon of rats, increased tendon stiffness and strength by about 30% after just seven days, compared to controls (40). Additional findings have highlighted enhanced growth factor release and increased immunoreactivity of collagen I/III when PRP was percutaneously injected into the rat patellar tendon during the early phases of tendon healing (41). Furthermore, the osteoinductive efficacy of PRP in tendon-to-bone healing was analyzed in a sheep infraspinatus repair model through histology and MRI scans, reporting fibrocartilage and new bone formation at the lesion site (42). Scientific data on the use of PRP in human Achilles, patellar, wrist, and supraspinatus tendons have been published but are largely limited to case reports, lacking high methodological quality. The most beneficial results have been observed in chronic patellar tendinopathy, gluteus medius tendinopathy (43), and lateral epicondylitis (44), with promising outcomes for the rotator cuff, while no benefit has been reported for Achilles tendinopathy (45-47).

**WHAT’S THE EVIDENCE?**

Sports injuries involving tendons and muscles are common, and their conservative treatment often fails to produce lasting and decisive outcomes (47, 48). In this context, orthobiologic injection therapies emerge as a highly innovative and compelling treatment option. Injecting MSC and PRP into muscles and tendons has the dual effect of modulating the inflammatory cascade underlying tissue damage through the secretion of neoangiogenic and anti-inflammatory factors and promoting tissue repair through the release of specific growth factors. These injections are now considered relatively safe, with serious adverse events being rare. However, the results reported in this review are sparse and conflicting, suggesting that sports physicians should carefully weigh the existing evidence against the clinical situation and patient preferences, especially when considering orthobiologics with limited clinical evidence (49).

Regarding muscle injuries, PRP injections have not shown a protective effect against reinjury risk, and no significant differences have been found between PRP and placebo injections or rehabilitation in speeding up the return to sport after injury. As for the use of MSCs in muscle injuries, the literature currently lacks sufficient data to provide scientific evidence and recommendations, despite their proven efficacy in promoting immunomodulation and cell regeneration. In the case of tendinopathies related to sports injuries, *in vitro* and *in vivo* data suggest that tendon MSCs contribute
to tendon-regenerative processes. This capacity is crucial in redirecting tendon repair from profibrotic degeneration to tissue regeneration. However, future research on cellular therapies must implement standardized methods in clinical practice to explore tenogenic differentiation phases more comprehensively.

Moreover, less is known about the potential benefits of combining MSCs with biomaterials to improve muscle and tendon tissue regeneration while optimizing functional tissue activity. Regarding tendon injuries, the lack of sufficient scientific evidence precludes the provision of clinical recommendations.

Table I summarizes the 2020 National Basketball Association (NBA) consensus statement recommendations on the use of orthobiologics (50). Similarly, the National Football Association (NFA) has expressed concerns over the lack of scientific support for these therapies, cautioning against their routine recommendation due to potential health risks for athletes from indiscriminate use of orthobiologics (51).

### Table I. NBA Orthobiologics Consensus Statement Recommendations

<table>
<thead>
<tr>
<th>Injury</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle injuries</td>
<td>Not routinely recommended</td>
</tr>
<tr>
<td>Patellar tendinopathy</td>
<td>Leukocyte-poor PRP injection as an adjunct to first-line conservative treatment and/or when conservative treatment fail</td>
</tr>
<tr>
<td>Cell-based therapy not recommended</td>
<td></td>
</tr>
<tr>
<td>Achilles tendinopathy</td>
<td>Not routinely recommended</td>
</tr>
<tr>
<td>Plantar fasciopathy</td>
<td>PRP injection as an adjunct to first-line conservative treatment and/or when conservative treatment fail</td>
</tr>
<tr>
<td>Cell-based therapy not recommended</td>
<td></td>
</tr>
</tbody>
</table>

PRP: platelet-rich plasma.

### Table II. Important variables to be considered when preparing orthobiologics.

<table>
<thead>
<tr>
<th>Variables for consideration</th>
<th>PRP</th>
<th>MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting volume</td>
<td>Whole blood sample</td>
<td>60 mL bone marrow</td>
</tr>
<tr>
<td>Final volume injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of anticoagulant used</td>
<td>Acid citrate dextrose solution A, calcium citrate, citric acid, citrate phosphate dextrose, sodium citrate</td>
<td>Iliac crest</td>
</tr>
<tr>
<td>Method of separation and processing machine used</td>
<td>Centrifugation</td>
<td></td>
</tr>
<tr>
<td>Setting of the machine used</td>
<td>Number of spin cycles, duration of spin cycles, spin rate and/or G force</td>
<td></td>
</tr>
<tr>
<td>Desired concentration of platelets for clinical indication</td>
<td>Leukocyte rich vs leukocyte poor</td>
<td></td>
</tr>
<tr>
<td>Platelet-activation method</td>
<td>CaCl₂, thrombin, dry needling, calcium gluconate, tissue factor</td>
<td>NaHCO₃</td>
</tr>
<tr>
<td>Use of buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial nucleated cell count</td>
<td></td>
<td>4.7 × 10⁶ cells/mL</td>
</tr>
<tr>
<td>Final nucleated cell count</td>
<td></td>
<td>24.9 × 10⁶ cells/mL</td>
</tr>
<tr>
<td>Final composition</td>
<td>Leukocyte, erythrocyte and platelets</td>
<td></td>
</tr>
<tr>
<td>Final volume</td>
<td>3.5 mL</td>
<td></td>
</tr>
<tr>
<td>Time for preparation to use</td>
<td>1 hour</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

At present, there is limited scientific evidence to support the routine use of orthobiologic injections in sports medicine. Clinical trials need to be designed, conducted, and reported with higher quality to substantiate the use of MSCs and PRP in managing muscle and tendon injuries. Further research, particularly on human subjects, is required to develop more data and perform risk/benefit analyses, especially concerning athletic sports injuries. Given the high functional demands of this population, it is prudent to exercise caution when employing MSCs/PRP in rehabilitation pathways, adhering to rigorous guidelines both in preparation (table II) (52) and clinical practice (table III) (44, 53).
Table III. The rigorous and structured approach to follow when using orthobiologics in clinical practice.

<table>
<thead>
<tr>
<th>Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Always consider firstly therapies less invasive, safer, with higher scientific evidence and more cost-effective</td>
</tr>
<tr>
<td>Avoid patients’ motivation as a therapy choice</td>
</tr>
<tr>
<td>Never be influenced by commercial motivations</td>
</tr>
<tr>
<td>The regenerative medicine therapy should be delivered following research and manufacturer recommendation</td>
</tr>
<tr>
<td>Prefer image-guided procedures</td>
</tr>
<tr>
<td>Keep always continuing to study and to update about injection procedures</td>
</tr>
<tr>
<td>The patient’s informed consent must be always obtained</td>
</tr>
</tbody>
</table>

Muscloskeletal-specific Patient-Reported Outcome Measures are useful in the clinical management of patients

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

ACKNOWLEDGMENTS
Figure 1 is an adapted image by master1305 freely downloadable from Freepik.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES


Muscles, Ligaments and Tendons Journal 2024;14 (2)
Effect of Mechanical Traction and Therapeutic Exercises in Treatment of Primary Knee Osteoarthritis

Moaaz Ragab Riyad, Ibrahim Magdy Elnaggar, Karima Abdelaty Hassan

Department of Physical Therapy for Musculoskeletal Disorders, Faculty of Physical Therapy, Cairo University, Cairo, Egypt

INTRODUCTION

Knee osteoarthritis is a degenerative disorder causing pain and disability due to joint wear and tear, ageing and obesity (1). It is distinguishable by cartilage degeneration, joint space loss, bone sclerosis and osteophytes (2). In Egypt, knee osteoarthritis prevalence rate ranges from 7.8 to 9.3% (3). Patients with knee osteoarthritis present clinically with pain, crepitus, morning stiffness, abnormal joint loading and functional impairment (4, 5).

For knee osteoarthritis, therapeutic exercises are considered as an effective and safe intervention (6). Therapeutic exercises of low to moderate intensity does not accelerate the progression of knee osteoarthritis (7, 8). Therapeutic exercises are an established treatment option and upcoming studies are hardly going to change confidence in this fact (9, 10). Therapeutic exercises mainly improve aerobic fitness, hip and knee muscle strength and flexibility (11, 12). Therapeutic exercises were reported to
reduce knee pain, disability, improve functional performance, quality of life and spatiotemporal gait parameters (11, 12).

Traction was primarily introduced as surgical joint traction (13). Limitations during and after the application of the skeletal traction (14) lead to a research interest in non-surgical traction (15-20). Recent studies (21, 22) reported observations of suppression of articular cartilage degeneration after the application of non-surgical unloading.

Mechanical traction is a conservative technique based on the same concept of skeletal knee joint traction; it provides transient joint unloading. The application of mechanical traction is clinically promising with positive observations related to pain, disability, range of motion, functional performance and quality of life (16-20). The addition of mechanical traction to therapeutic exercises was proposed to help gain the beneficial effects of both exercises (11, 12) and unloading techniques (21-23). There is a need for better description and reporting of these treatment methods to confirm the positive effects of the combined use of both techniques. Moreover, the effect of mechanical traction on quadriceps and hamstring muscle strength was not studied in the literature. Combined application of mechanical traction with therapeutic exercises was assumed to optimize muscle function by reversing the arthrogenic muscle inhibition (24-27). Hence making exercise performance easier and exercise gains faster (26).

The current study may provide a more conclusive insight in treatment of patients with primary knee osteoarthritis. This combined program might be beneficial within a short period of time and may be more efficient than therapeutic exercises alone (28). Therefore, the purpose of this current study was to compare between therapeutic exercises and therapeutic exercises preceded by mechanical traction in treatment of primary knee osteoarthritis.

Patients and methods

Patients

Forty male and female patients with the diagnosis of primary knee osteoarthritis participated in this study. Patients were included if they had grade II or III primary knee osteoarthritis, their age ranged from 45 to 65 years with duration of illness ranged from 3 to 12 months. In patients with bilateral primary knee osteoarthritis, the more painful knee was chosen for the study. Patients were excluded if they had skin lesions or infections at the treatment site. All patients were assessed and treated in the outpatient clinic of the faculty of physical therapy in Cairo University.

Patients were randomly distributed into two equal experimental groups. The first experimental group consisted of 20 patients (18 females and 2 males) who received stretching and strengthening exercises of selected muscles of the thigh and leg. The second experimental group consisted of 20 patients (18 females and 2 males) who received continuous mechanical knee joint traction followed by the same exercise program of the first group. All patients were treated for 12 sessions, 3 times per week each other day for 4 weeks. An informed consent was obtained from each patient prior to the study. The ethics committee of the faculty of physical therapy in Cairo university gave its approval to the study (P.T.REC/012/002876 - date of approval: September 09, 2020). The study was conducted from May 2022 to December 2022.

Assessment procedures

Clinical assessment included knee pain severity, functional disability, isometric quadriceps and hamstring muscle strength and functional performance. Assessment was done pre-treatment (2-3 days before the first treatment session) and post-treatment (2-3 days after the last treatment session).

Knee pain severity was measured by using the Arabic numerical pain rating scale (29) where 0 equals no pain and 10 equals the worst possible pain. The patient was instructed to choose only one number on this scale.

Knee functional disability was measured by using the Arabic version of the Western Ontario and McMaster Universities Osteoarthritis Index (30). It is a valid, reliable and specific patient reported index which consists of 24 items measuring pain, stiffness and physical function. Every item has five possible answers graded from 0 (none) to 4 (extreme). Total score is the sum of the 24 items, and it ranges from 0 (best) to 96 (worst) points. The patient was instructed to choose the best answer which describes the level of difficulty he faces during daily activities in each of the 24 items.

Isometric muscle strength was measured for knee muscles by using a hand-held dynamometer (model 12-0393 baseline push/pull dynamometer, USA). It is both a valid and reliable tool for measuring of isometric muscle strength (31). The isometric muscle force was shown in pounds and kilograms. Force was later converted to Newtons according to the work of Jaric (32).

Based on the work of Bohannon (31), isometric quadriceps muscle strength was measured from the sitting position with both hips and knees flexed 90°. The end
A piece of the dynamometer was applied to the anterior surface of the distal leg just above the ankle joint, then the patient was instructed to extend his knee, contract quadriceps maximally and hold for 5 seconds before relaxation. Isometric hamstring muscle strength was measured from the same position with the end piece of the dynamometer applied to the posterior surface of the distal leg just above the ankle joint. The patient was instructed to flex his knee, contract hamstring maximally and hold for 5 seconds before relaxation. The patient performed three trials of 5 seconds each and had 30 seconds of rest between trials. In each trial, the registered force on the dynamometer was recorded and the dynamometer was readjusted to zero before the next trial. The mean of the three trials of each test was chosen for the purpose of data analysis.

Functional performance was measured by using the 40 meter fast paced walk test and the 12-step stair test based on the work of Stratford et al. (33). Both tests have high reliability with low measurement error. The first test measures the time needed to walk a 40 meters distance. The patient stood behind a predetermined start mark, then was instructed to walk as quickly as possible on the command “GO”, but not to run. The patient walked a 20 meters distance along a leveled unobstructed indoor course departing at the start mark and then walked another 20 meters returning to it. The second test measures the time needed to ascend and descend a 12-step stair where step height equals 17 centimeters and step depth equals 30 centimeters. The patient stood behind a predetermined start mark, and then was instructed to ascend and descend the stair as quickly as possible on the command “GO”. Safety was assured by holding the handrail during the task. Scores were calculated using a stopwatch mobile application as the time needed to complete each test. Three trials were done for each test with 5 minutes rest between trials and 10 minutes rest between tests. The mean of the three trials of each test was chosen for the purpose of data analysis.

**Treatment procedures**

All patients were treated individually under the direct supervision of the main investigator. The exercise group received therapeutic exercises in the form of stretching and strengthening exercises of selected muscles of the thigh and leg based on the recommendations of Rausch Osthoff et al. (34). Passive stretching exercises were done for the rectus femoris, hamstring, and calf muscles based on the work of Reid and McNair (35). Each exercise was done for three repetitions, with a hold of 30-60 seconds and a rest of 30-60 seconds between repetitions. Each repetition was performed slowly until a feeling of tightness was noticed. This group also received strengthening exercises of the quadriceps (terminal knee extension, knee extension and straight leg raise from supine) and hamstring muscles (hamstring curl from prone and standing positions and straight leg raise from prone) according to the work of Kus and Yeldan (36). These exercises are shown in Table 1. Each exercise was done for 3 sets, each set consisted of 10 repetitions with a rest of 2-3 minutes between sets. The given resistance was 50% of 1 repetition maximum of the patient. Each repetition was performed slowly with a hold for 5 seconds at the end position. Exercises were carried out within a tolerable level of pain. If joint swelling or unacceptable pain occurs, the exercise repetition or resistance was reduced.

The traction and exercise group received mechanical knee joint traction followed by the same exercises of the first group. Mechanical traction of the knee involved the use of a mechanical traction system that included pulleys, weights and a specially designed greave to apply traction to the knee joint. The protocol of using mechanical traction was based on the work of Khademi-Kalantari et al. (17). Mechanical traction was applied for 20 minutes continuously in the supine lying position with the affected knee flexed at 25-30° by a wedge placed under the thigh. The thigh was stabilized by a strap and the leg was held by the specially designed greave with the weight of traction hanging throughout the pulley system, as shown in Figure 1. The initial traction force was set to 10% of the body weight. Traction force was increased each week by 1% of body weight if the patient could tolerate the duration of 20 minutes with the preset force. Traction force was maintained or reduced if intense pain or swelling occurred.

![Figure 1. Application of continuous mechanical traction of the knee joint.](image-url)
RESULTS

For data analysis SPSS version 21.0 for windows was used. The level of significance was set as \( p < 0.05 \). Age, weight and height in addition to all the dependent variables of the study were shown to be normally distributed by using the Kolomogrov-Smirnov test of normality \( (p > 0.05) \). Unpaired t-test showed non-significant difference between groups for age, weight and height \( (p > 0.05) \), as shown in Table II. Unpaired t-test also showed non-significant difference between the pretreatment means of both groups regarding functional disability, isometric quadriceps strength, walking time and stairs time \( (p > 0.05) \). However, a significant difference was found between the pre-treatment means of both groups regarding knee pain severity and isometric hamstring strength \( (p < 0.05) \), as shown in Table III.

Post-treatment within groups difference

Paired t-test showed significant difference between the pre-treatment and post-treatment means of all the dependent variables in the exercise group as well as in the traction and exercise group \( (p < 0.05) \), as shown in Table IV.

Post-treatment between groups difference

Unpaired t-test showed significant difference between the post-treatment means of both groups regarding functional

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamstring muscle stretching</td>
<td>Done with the patient lying down on the back with the investigator holding the affected leg, keeping the affected knee fully extended and stabilizing the opposite extremity. The investigator flexed the hip slowly till a feeling of tightness was felt.</td>
</tr>
<tr>
<td>Rectus femoris muscle stretching</td>
<td>Done with the patient lying down on the back close to the edge of the treatment table with the non-affected leg flexed toward the patient’s chest to stabilize the pelvis and spine while the affected leg was hanging outside the treatment table. The investigator pushed the affected limb downwards into hip hyperextension and then flexed the affected knee slowly till a feeling of tightness was felt.</td>
</tr>
<tr>
<td>Calf muscle stretching</td>
<td>Done with the patient lying down on the back. The investigator grasped the patient’s heel and placed his forearm along the plantar surface of the foot while stabilizing the lower leg just above the ankle joint with his other hand. Then the investigator dorsiflexed the ankle slowly till a feeling of tightness was felt.</td>
</tr>
<tr>
<td>Terminal knee extension</td>
<td>Done with the patient in back lying position with the affected leg supported over some rolled-up towels and bent to about 30° of knee flexion. The sand weight was placed just above the ankle joint. The patient was instructed to straighten the affected knee by slowly lifting the heel off and hold for 5 seconds.</td>
</tr>
<tr>
<td>Knee extension</td>
<td>Done with the patient sitting over the edge of the treatment table with both legs off the ground and knees at 90° flexion. The sand weight was placed just above the ankle joint. The patient was instructed to straighten the affected knee by slowly lifting the foot as high as possible and hold for 5 seconds.</td>
</tr>
<tr>
<td>Straight leg raises from supine</td>
<td>Done with the patient in a back lying position with the affected leg straight. The sand weight was placed just above the ankle joint. The patient was instructed to raise the affected limb slowly off the treatment table while keeping the affected knee extended and hold for 5 seconds.</td>
</tr>
<tr>
<td>Hamstring curl from prone</td>
<td>Done with the patient in the prone lying position with the affected leg straight. The sand weight was placed just above the ankle joint. The patient was instructed to bend the affected knee slowly as much as he could and hold for 5 seconds.</td>
</tr>
<tr>
<td>Hamstring curl from standing</td>
<td>Done with the patient in a standing position with the thigh pressed against the treatment table to prevent hip flexion. The sand weight was placed just above the ankle joint. The patient was instructed to bend the affected knee slowly as much as he could and hold for 5 seconds.</td>
</tr>
<tr>
<td>Straight leg raises from prone</td>
<td>Done with the patient in the prone lying position with the affected leg straight. The sand weight was placed just above the ankle joint. The patient was instructed to raise the affected limb slowly off the treatment table while keeping the affected knee extended and hold for 5 seconds.</td>
</tr>
</tbody>
</table>

Table II. Comparison between groups for age, weight, and height.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group Mean ± SD</th>
<th>Traction and exercise group Mean ± SD</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>55.30 ± 5.95</td>
<td>56.90 ± 5.78</td>
<td>0.86</td>
<td>0.39</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>91.40 ± 9.28</td>
<td>87.40 ± 10.96</td>
<td>1.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 ± 5.60</td>
<td>165 ± 7.80</td>
<td>1.77</td>
<td>0.09</td>
</tr>
</tbody>
</table>
disability and isometric quadriceps strength in favor of the traction and exercise group (p < 0.05). However, this test showed non-significant difference between post-treatment means regarding walking time and stairs ascending and descending time (p > 0.05), as shown in table V. Unpaired t-test also showed significant difference between the mean differences of both groups regarding knee pain severity and isometric hamstring strength in favor of the traction and exercise group (p < 0.05), as shown in table VI.

Table III. Pretreatment between groups difference.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group Mean ± SD</th>
<th>Traction and exercise group Mean ± SD</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee pain severity</td>
<td>8.35 ± 1.09</td>
<td>9.05 ± 0.89</td>
<td>2.23</td>
<td>0.03†</td>
</tr>
<tr>
<td>Functional disability</td>
<td>75.10 ± 9.85</td>
<td>79.80 ± 11.09</td>
<td>1.43</td>
<td>0.16</td>
</tr>
<tr>
<td>Isometric quadriceps strength</td>
<td>93.45 ± 31.50</td>
<td>77.88 ± 35.79</td>
<td>1.46</td>
<td>0.15</td>
</tr>
<tr>
<td>Isometric hamstring strength</td>
<td>86.11 ± 25.96</td>
<td>64.30 ± 29.50</td>
<td>2.48</td>
<td>0.02†</td>
</tr>
<tr>
<td>Walking time</td>
<td>52.60 ± 12.28</td>
<td>56.10 ± 14.31</td>
<td>0.83</td>
<td>0.41</td>
</tr>
<tr>
<td>Stairs time</td>
<td>31.80 ± 12.00</td>
<td>35.40 ± 15.61</td>
<td>0.82</td>
<td>0.42</td>
</tr>
</tbody>
</table>

†Significant difference.

Table IV. Within groups difference.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-treatment Mean ± SD</th>
<th>Post-treatment Mean ± SD</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee pain severity</td>
<td>8.35 ± 1.09</td>
<td>6.75 ± 1.41</td>
<td>7.61</td>
<td>0.001†</td>
</tr>
<tr>
<td>Functional disability</td>
<td>75.10 ± 9.85</td>
<td>58.30 ± 10.24</td>
<td>12.52</td>
<td>0.001†</td>
</tr>
<tr>
<td>Isometric quadriceps strength</td>
<td>93.45 ± 31.50</td>
<td>110.36 ± 33.16</td>
<td>9.63</td>
<td>0.001†</td>
</tr>
<tr>
<td>Isometric hamstring strength</td>
<td>86.11 ± 25.96</td>
<td>101.90 ± 26.02</td>
<td>11.09</td>
<td>0.001†</td>
</tr>
<tr>
<td>Walking time</td>
<td>52.60 ± 12.28</td>
<td>44.90 ± 10.35</td>
<td>8.47</td>
<td>0.001†</td>
</tr>
<tr>
<td>Stairs time</td>
<td>31.80 ± 12.00</td>
<td>26.25 ± 9.28</td>
<td>6.46</td>
<td>0.001†</td>
</tr>
<tr>
<td>Traction and exercise group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee pain severity</td>
<td>9.05 ± 0.89</td>
<td>4.05 ± 1.64</td>
<td>15.41</td>
<td>0.001†</td>
</tr>
<tr>
<td>Functional disability</td>
<td>79.80 ± 11.09</td>
<td>40.10 ± 11.32</td>
<td>14.64</td>
<td>0.001†</td>
</tr>
<tr>
<td>Isometric quadriceps strength</td>
<td>77.88 ± 35.79</td>
<td>135.06 ± 37.96</td>
<td>10.51</td>
<td>0.001†</td>
</tr>
<tr>
<td>Isometric hamstring strength</td>
<td>64.30 ± 29.50</td>
<td>106.80 ± 21.17</td>
<td>8.56</td>
<td>0.001†</td>
</tr>
<tr>
<td>Walking time</td>
<td>56.10 ± 14.31</td>
<td>39.35 ± 6.76</td>
<td>8.11</td>
<td>0.001†</td>
</tr>
<tr>
<td>Stairs time</td>
<td>35.40 ± 15.61</td>
<td>21.25 ± 10.47</td>
<td>7.90</td>
<td>0.001†</td>
</tr>
</tbody>
</table>

†Significant difference.
In our study there was a significant reduction of knee pain severity, functional disability and improvement of functional performance in the exercise group. These findings are in agreement with some previous investigations (11, 12, 36-40). Pain reduction through therapeutic exercises might be attributed to the reduction of external stress falling on the articular surface of the joint through the improvement of aerobic fitness, hip and knee muscles strength and flexibility (11, 12). This might be also related to stimulation of mechanoreceptors located in the joint capsule and ligaments, blocking the activation of pain pathways (41).

The reduction of functional disability and improvement of functional performance by therapeutic exercises might be related to an increase of thigh strength, decrease of knee extension impairment, and improvement in proprioception (12). Strengthening of the quadriceps and hamstring muscles increases joint stability, reduces excessive joint stress and increases physical activity (37). Stronger muscles are associated with lower knee pain, easier performance of activities of daily living and greater levels of mobility (42). Furthermore, stretching exercises increase the flexibility of the hip flexors, quadriceps, hamstring and calf muscles (38). More flexible muscles increase range of motion, peak passive torque and force generating capacity (35). Moreover, more flexible muscles allow for better functional performance and improvement in spatiotemporal gait parameters such as step length and speed (38, 39).

There was also a significant increase of isometric quadriceps and hamstring muscle strength in the exercise group. This result coincides with the findings of earlier studies (35-37). The increase of isometric quadriceps and hamstring muscle strength through therapeutic exercises might be related to improvement in different muscles contractile properties, e.g., force-generating capacity, shortening velocity, force-velocity relationship, peak power, muscle mass, maximum fiber recruitment, fiber number and size (43).

In the traction and exercise group, there was also a significant reduction of knee pain severity, functional disability and improvement of functional performance. These findings are in agreement with earlier studies (16-20). Pain reduction through mechanical traction might be attributed to the reduction of intraarticular stress falling on the knee through increasing knee joint space and unloading of articular cartilage (44-46). This in turn increases microcirculation and synovial fluid movement (44), increases the delivery of nutrients and facilitates cartilage regeneration (47), reduces secondary inflammation and halts cartilage degeneration (21, 22). Pain reduction might also be related to the reduction of the subchondral and intraarticular joint pressure which in turn may reduce the pressure on the damaged tissues, pain receptors and free nerve endings (41). Pain reduction might also be attributed to increased soft tissue extensibility through breaking of intraarticular adhesions and stretching of the periarticular soft tissues (15).

The reduction of functional disability and improvement of functional performance through mechanical traction is based primarily on the reduction of pain, stiffness and the improvement of physical function (30). Traction was reported to be clinically significant in the reduction of pain both at rest and during movement (16-20). Moreover, mechanical traction was reported to ease the sensation of morning stiffness and restore joint resiliency (15). Furthermore, mechanical traction was reported to improve physical function as reported in the six-minute walking test (17) and the timed up and go test (20).

There was also a significant increase of isometric quadriceps and hamstring muscle strength in the traction and exercise group. The increase of isometric quadriceps and hamstring muscle strength through mechanical traction is primarily attributed to the reduction of knee pain (24-27). Quadriceps muscle is considered a very important muscle among patients with knee osteoarthritis, knee pain is mainly linked to quadriceps weakness and reduced quadriceps cross-sectional area (48, 49). It was reported that experimental pain induction to the knee joint was correlated with immediate reduction of quadriceps muscle activation (25) and impaired quadriceps force steadiness (26). Thus, a reduction of pain would allow for a reduction of arthrogenic muscle inhibition (24), normalization of activation patterns (25) and force control ability of quadriceps and hamstring muscles (27). Between groups comparison showed that traction and therapeutic exercises were more effective than therapeutic exercises alone in reduction of knee pain severity and functional disability and in increasing isometric quadriceps and hamstring muscle strength. This may be attributed to

### DISCUSSION

**Table VI.** Between groups difference for knee pain severity and isometric hamstring strength.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise group Mean difference ± SD</th>
<th>Traction &amp; exercise group Mean difference ± SD</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee pain severity</td>
<td>1.60 ± 0.94</td>
<td>5.00 ± 1.45</td>
<td>8.79</td>
<td>0.001*</td>
</tr>
<tr>
<td>Isometric hamstring strength</td>
<td>15.79 ± 6.37</td>
<td>42.49 ± 22.20</td>
<td>5.17</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significant difference.
the combined positive effects of both therapeutic exercises (11, 12, 35-43) and mechanical traction (16-22, 44-47). Moreover, the application of traction before exercises may have provided an opportunity for greater pain reduction, greater normalization of quadriceps and hamstring activation patterns before exercise performance (25) opening a window for easier performance (26) and a greater force control during exercises (27). This may be related to greater distractive forces imposed on the joint during traction (46) opposite to greater compressive and shear forces falling on the joint during exercises (50). Furthermore, the application of traction before exercises may have provided greater improvement in patient’s motivation, confidence and self-efficacy during exercise performance.

On the other hand, there was no significant difference between groups in functional performance (walking time and stairs ascending and descending time). This means that the use of mechanical traction did not provide any significant additional effect on functional performance when combined with therapeutic exercises. This may be attributed to inability of the used mechanical traction parameters (force, duration and total number of sessions) to induce a significant difference between groups.

This study has three main limitations. The first limitation is the small number of participants. The second limitation is the absence of a control group while the third is the lack of follow up of patient’s improvement after the end of the study.

CONCLUSIONS
Therapeutic exercises preceded by mechanical traction are more effective than therapeutic exercises alone in the reduction of both knee pain severity and functional disability and in increasing isometric quadriceps and hamstring muscle strength. This is mainly attributed to the combined positive effects of both types of treatment encompassing joint gapping, unloading of articular cartilage, reduction of intraarticular stress, decreased pressure on free nerve endings, blocking of pain pathways, reduction of morning stiffness, reduction of arthrogenic muscle inhibition, normalization of muscle activation patterns, increased soft tissue extensibility and increased muscle strength. However, both treatments are equally effective in the reduction of walking time and stairs ascending and descending time. This may be related to inability of the used mechanical traction parameters to induce a significant difference between groups.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
MRR: project administration, investigation, writing – original draft. IME, KAH: writing – review & editing.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES


Effects of Long and Sprint High-Intensity Interval Training on Body Mass Composition, Aerobic Capacity, and Biochemical Markers of Metabolic Syndrome and Liver Damage in Physical Activity Practitioners Adults

Luciano Lima dos Santos¹,², Aline Tito Barbosa Silva⁴, Marcos Alexandre de Souza Peçanha da Cruz⁴, Samir Ezequiel da Rosa⁴, Marcos de Sá Rego Fortes⁴, Rodolfo de Alkmim Moreira Nunes¹,², Juliana Brandão Pinto de Castro¹,², Diego Gama Linhares¹,², Andressa Oliveira Barros dos Santos¹,², Lilliany de Souza Cordeiro¹,², Claudio Joaquim Borba-Pinheiro³, Rodrigo Gomes de Souza Vale¹,²

¹ Postgraduate Program in Exercise and Sport Sciences, Rio de Janeiro State University, Rio de Janeiro, Brazil
² Laboratory of Exercise and Sport, Institute of Physical Education and Sports, Rio de Janeiro State University, Rio de Janeiro, Brazil
³ Federal Institute of Pará, Pará State University, Pará, Brazil
⁴ Army Physical Training Research Institute, Rio de Janeiro, Brazil

SUMMARY

Background. Highlights High-Intensity Long Interval Training (HILIT) and Sprint Interval Training (SIT) to morpho functional improvement and reduce effects of Metabolic Associated Fatty Liver Disease (MAFLD) and the Metabolic Syndrome (MS).

Objective. This study aims to verify the effects of HILIT and SIT on physiological and pathological markers of MS and liver health in adults submitted to 12 weeks of training.

Methods. A randomized clinical trial was carried out with a design for two groups, HILIT and SIT Groups. The sample consisted of 38 physical activity practitioners male adults aged between 30 and 55 years (42.75 ± 8.26). Body composition assessments, cardiac stress tests, measurements of blood pressure (BP), and blood samples were analyzed: triglycerides (TRIG), high-density lipoprotein (HDL-C) and glucose (GLU) and liver damage: Albumin (ALB), Bilirubin (BIL), Aspartate Aminotransferase (AST), Alamine Aminotransferase (ALT), Gamma Glutamyl Transferase (GGT).

Results. For HILIT there was a significant intragroup improvement in the parameters of fat mass, lean mass, body mass index (BMI), visceral adipose fat (VAT), and oxygen consumption (VO2). For SIT there was a significant intragroup improvement in the parameters of VAT, GLU, ALB, DB, GGT, and distance run. There was a significant difference in the intergroup comparison only for BP in favor of the SIT group.

Conclusions. We conclude that 12 weeks of HILIT and SIT interval training were able to produce positive effects on body composition variables, aerobic capacity, Metabolic Syndrome, and Liver Health in physical activity practitioners adult men, with better results for HILIT in this population.

KEY WORDS

Metabolic syndrome; metabolic associated fatty liver disease; high-intensity long interval training; sprint interval training; training impulse.
INTRODUCTION
High-Intensity Interval Training (HIIT) is characterized by short, intermittent exercises of vigorous activity interspersed with periods of passive or active recovery (1, 2). It is considered an efficient alternative to improve conditioning and reduce overweight and obesity (3). Due to the excellent adherence of HIIT in society, the experiment focuses on High-Intensity Long Interval Training (HILIT) and Sprint Interval Training (SIT) (4, 5).
HILIT is proposed in the running modality on flat ground with sprints over 1 minute considered long duration with power or speed between the second ventilatory threshold and maximum oxygen consumption (VO$_{2max}$) (4,5). About SIT method is also in the running modality on flat terrain. It is performed with sprints lasting less than 1 minute and with a power or speed above those associated with VO$_{2max}$ (4, 5). Several studies indicate that running appears to be more effective in reducing total fat mass, including visceral adipose tissue (VAT). However, there are not many studies carried out outdoors using an athletics track, which would encourage the applicability of the method (6, 7).
In the context of prescribing the intervention, based on the variables obtained in the cardiac stress test, it was decided to use Banister’s Training Impulse (TRIMP) formula, dimensioning the training in the intervention sessions in a balanced way (8).
Le Jemtel et al. (9) showed HIIT as a method to reverse visceral adipose tissue, reducing cardiovascular risk and improving body composition. In the same direction, other training methods studied such as resistance training, aerobic training and combined exercises aimed Metabolic Syndrome (MS). Liang et al. (10) suggested that combined exercise is the most effective choice in improving the MS and cardiovascular risk parameters, whereas aerobic exercise reveals the minimum effect. These studies suggest health beneficial effects as body composition, aerobic capacity, lean mass, and blood biomarkers.
Zhou et al. (11) suggest positive effects on the liver health using aerobic training, resistance training, and aerobic training with resistance training. Therefore, the HILIT and SIT methods will be investigated to improve body composition, improve VO$_2$ capacity and reduce MS and liver markers. Through HILIT and SIT, the aim is to reduce risk factors for MS and Metabolic Associated Fatty Liver Disease (MAFLD).
MS is a major challenge for global public health, with important risk factors for cardiovascular diseases (CVD) and type 2 diabetes (DMT2) (12). Diagnosed in the presence of three of the five metabolic measurements: waist circumference (WC) > 90 cm, triglycerides (TRIG) > 100 mg/dL, HDL-C < 40 mg/dL, systolic blood pressure (SBP) > 130 and/or diastolic blood pressure (DBP) > 85 mmHg and glucose (GLU) > 150 mg/dL (13). In Brazil the prevalence of MS is 38.4% with emphasis on increased WC (65.5%) and low HDL cholesterol (49.4%) as the most prevalent components, including in young individuals (14).
MAFLD is the hepatic expression of MS with a diagnosis of fatty liver by histology (biopsy), imaging or blood biomarker, in addition to at least one of the three dangerous criteria of overweight or obesity, the presence of DMT2 or evidence of metabolic dysregulation (15, 16).
This study is justified by the gap in the literature in knowing the effects of High-intensity interval training on long and sprint variations in individuals at risk of developing metabolic syndrome and liver disease. This type of intervention can reduce or even regress this pathological condition. The general hypothesis of this study is that HILIT and SIT can improve the health of physical activity practitioners adults. Therefore, this study aimed to verify the effects of long and sprint high-intensity interval training on body mass composition, aerobic capacity, and biochemical markers of metabolic syndrome and liver damage in physical activity practitioners adults undergoing 12 weeks of training.

MATERIALS AND METHODS

Design
This is experimental research with a design for two groups that will be evaluated pre-intervention and post-intervention period (17).

Participants
The sample was made up of 38 physical activity practitioners adult men Army soldiers from a military music band, between 30 and 55 years old. The sample size calculation indicated by the GPower program was estimated at 36 individuals (18), using the following information: ANOVA with repeated measures, effect size of 0.25, alpha error of 0.05, power of the experiment of 0.80 and correlation between measurements of 0.5 for two groups at two measurement times (19). The following inclusion criteria were adopted: 1) male, 2) military personnel belonging to military music bands, 3) submitted to a dietary recall and meals in the headquarters barracks (20). The exclusion criteria involved: 1) military personnel who underwent any type of abdominal surgery, with some type of osteoarticular or musculoskeletal injury and other clinical conditions that prevented them from carrying out training or any of the assessments, which limited movements or undergoing restrictive medical treatments, 2) military personnel who were using any substance or drug.
capable of altering test results, 3) who were unable to attend all stages of data collection or considered physically unfit by a prior medical assessment, mainly assessment of cardiopulmonary health. After the sampling process the participants were randomly distributed into two groups HILIT and SIT (figure 1) using the random function of the Excel software. All 38 individuals agreed to participate in the present study and signed the Informed Consent Form (ICF), respecting the ethical aspects of research involving human beings in resolution 466/12 of the National Health Council, at which time it was clarified to them that their identities would be preserved, in accordance with the ethical standards provided. The study was also submitted to an ethics and research committee (ERC) through the Brazil platform with approval from the ERC of the Army Physical Training Center under registration CAAE: 52772121.3.0000.9433 – Date of approval: May 27, 2022.

Study organization
Participants were evaluated at two different moments with a 12-week interval between them (figure 2). At first, each participant underwent body composition assessment using the dual-emission X-ray absorptiometry device (DXA) and a biochemical blood test, followed by the cardiac exercise test (CET) to assess aerobic capacity. In the second moment, the volunteers were randomized and divided into two groups, a HILIT group and a SIT group, carried out a planned 12-week intervention, being prescribed in an equalized way according to the TRIMP equation proposed by Banister, the groups carried out running training in athletics track or football field and it was common in the training session, for both groups, the warm-up and the 10 exercises for neuromuscular strengthening of the CORE, being the differentiating factor to the specific prescription of HILIT and SIT. All tests, pre-experiment and post-experiment, were carried out at the Army Physical Training Center and the training sessions were carried out in a guided and assisted manner in the respective military organizations where the soldiers were based.

Figure 1. Study random process

Data collection procedure
On the first visit, the study procedures were explained to everyone. The informed consent form (ICF) was completed, and the PAR-Q pre-exercise screening and anamnesis were carried out (21). The PAR-Q is a questionnaire composed of 7 questions with the aim of detecting possible risks when carrying out any physical activity. If the response was positive, the individual was advised not to carry out the activity and was suggested to undergo a medical evaluation (21). Participants then underwent 12 weeks of specific HILIT and SIT intervention for each group and finally a new data collection of all study variables.

Nutrition
Before the start of the experiment, lectures were given to volunteers about nutritional guidance and healthy eating. There was no proposed diet for the experiment and nutritional control was carried out through dietary recall, where three meals were eaten in the headquarters barracks (20). The procedures for planning and conducting the nutritional guidance lectures and their respective analyzes were conducted pre-intervention by the nutritionist from the Army Physical Training Research Institute (APTRI) in Rio de Janeiro, RJ.

Body composition
Body composition is assessed using a DXA (GE Healthcare, Madison, WI, USA) in measurements of total mass, lean mass, fat mass, visceral adipose tissue (VAT) (22).
The DXA evaluation procedures and their respective analyzies were carried out during an 8-hour fast, conducted by a team of qualified military professionals with laboratory experience, radiology technicians and doctors belonging to APTRI in Rio de Janeiro, RJ.

**Aerobic capacity**

VO$_{2\text{max}}$ aerobic fitness was indirectly assessed using a CET assisted by a cardiologist. The CET protocol was carried out on an Inbramed treadmill model ATL Super (Cascavel, Paraná, Brazil) using Heartware’s ErgoMet13v1 0.3.6 software until the participant was exhausted, in progressive phases lasting 120 seconds and an initial speed of 4 km/h with an increment of 2 km/h and a constant inclination of 1%. The recovery phase was performed at a speed of 40% of the maximum speed reached for one minute to observe cardiopulmonary behavior returning to rest (23).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) variables, heart rate during effort, maximum distance covered and estimated VO$_2$ achieved by each participant were measured (24).

The aerobic capacity assessment procedures and their respective analyzies were carried out by a cardiologist and an auxiliary team belonging to APTRI in Rio de Janeiro, RJ.

**Blood collection**

Blood collections were conducted to evaluate the biochemical markers of MS (25) of liver damage (26): GLU, HDL-C, LDL-C, TRIG, AST, ALT, GGT, ALB and BIL. Blood samples (14 ml) from the individuals were collected via the antecubital vein, with the individuals remaining in a sitting position. Immediately after collection, the blood was centrifuged, and the plasma or serum was frozen and stored at -80 °C for subsequent analysis of clinical biomarkers in the APTRI Exercise Biochemistry laboratory. After the pre-analytical procedures, the BT 3000 automated biochemical analyzer, manufactured by the Wiener Lab Company (Wiener Lab, Rosário, BA, Argentina) was used. All biochemical marker tests were duplicated, and the coefficient of variation (CV) was less than 3%.

The blood sample collection procedures and their respective analyzies were carried out by a team of pharmaceutical or biochemical professionals or a biochemistry technician with experience in the area at the APTRI Biochemistry Laboratory, Rio de Janeiro, RJ. All materials used in blood collection was disposable and followed the criteria for disposal of biological waste and sharps. After completing all blood sample analysis procedures, leftover laboratory samples containing blood were discarded in accordance with National Health Surveillance Agency (ANVISA) Legislation – RDC 306 of December 7th, 2004, which provides for technical regulations for waste management and health services.

**Intervention**

A 12-week longitudinal design was chosen to prescribe the HILIT and SIT intervention with two to three running sessions were conducted per week and total training session time ranged from 27 to 50 minutes (table I) (27), using the running modality, which was based on individualized aerobic capacity data obtained by the CET.

The exercise sessions were conducted by a physical education professional, holder of the Army Physical Education School Course or a degree in Physical Education. The first two weeks of intervention, to minimize musculoskeletal injuries and consequent sample loss, involved adaptation to high-intensity training, where the volume was reduced to 50% based on the calculation of the prescribed

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic warm up</td>
<td>2 - 3 min light running; and 9 (nine) localized effects exercises in movement.</td>
<td>6-8</td>
</tr>
<tr>
<td>Pentagon</td>
<td>10 (ten) exercises, being dynamic and calisthenics with their load based on number of repetitions and execution time; and 5 (five) exercises for CORE region.</td>
<td>8-10</td>
</tr>
<tr>
<td>HILIT or SIT</td>
<td>HILIT: 2 - 12 shots of 80 to 90% HRmax lasting 60 to 120 seconds, with active interval at 50% HRmax lasting 60 to 120 seconds; and SIT: 1 to 8 sprints shots at 160% vVO$_2\text{max}$ with an effort time of 10 to 20 seconds and also a 4 minutes active interval at 50% HRmax between each shot.</td>
<td>8-27</td>
</tr>
<tr>
<td>Calm down</td>
<td>Stretching and relaxation exercises involving the joints and muscles involved in the proposed intervention.</td>
<td>5</td>
</tr>
</tbody>
</table>

**Total time of training session** 27-50

HILIT: High-Intensity Long Interval Training; SIT: Sprint Interval Training; min: minute; CORE: body center; HRmax: Maximum Heart Rate; vVO$_{2\text{max}}$: maximum speed reached at the moment of maximum oxygen consumption.
Effects of Long and Sprint High-Intensity Interval Training

training, aiming for better neuromuscular adaptation. From the 3rd week until the 12th week, once adaptation to training has been made, we follow the proposal of 100% prescription volume of the individualized intervention.

The training session was composed based on the Brazilian Army (EB) doctrine prescription, for both groups and in a centralized way, consisting of a dynamic warm-up, a series of calisthenic exercises and exercises from the CORE called Pentagon, and of HILIT or SIT session (randomized groups) and after that the calm down session (28).

The dynamic warm-up aimed to improve performance, increase body temperature, muscle extensibility and increase heart rate, consisting of a light intensity run of 2 to 3 minutes and subsequently with 9 (nine) exercises with localized effects in movement, like: running with rotation of the arms, abduction and adduction of the arms horizontally, alternating extension of the arms vertically, jumping jacks, running with trunk twisting, lateral running, running with extending the leg forward, running with elevating the heels and running with knee elevation (28).

After the end of the warm-up, with the purpose of complementing and maintaining the common physical standards required of Brazilian Army personnel, Pentagon calisthenics training was carried out, consisting of 10 exercises that include exercises for the upper and lower limbs and the CORE region (oblique abdominal, rectus abdominis and lumbar region), being dynamic and calisthenics with their load based on number of repetitions and execution time: 30 (thirty) jumping jacks, 5 (five) vertical jump lunges, 10 (ten) lunge squats, stationary running with raising the knees and heels (coordination), and 15 (fifteen) seconds of 5 (five) exercises for the CORE region: isometric bipedal bridge, front plank, triangle with arm support (side plank), single-leg reverse support, abdominal supra, ankle proprioception (28, 29).

HILIT was prescribed in the zone of 80 to 90% of HRmax with shots lasting 60 to 120 seconds, in an active interval at 50% HRmax lasting 60 to 120 seconds on athletics track with monthly training monitoring using First Beat software for load monitoring and load adjustment (4, 5).

In the same way, SIT prescribed the intervention based on individualized VO2max data from the CET, to calculate the maximum speed of maximum oxygen uptake (vVO2max). SIT protocol ranged from 1 to 8 short sprints at 160% vVO2max with a shots time of 10 to 20 seconds in duration and a 4-minute active interval at 50% HRmax between sprints, in running mode on athletics track with monthly training monitoring with First Beat software for monitoring and adjusting the load (4, 5, 30).

Previously, the experimental phase of training, with the aim of better controlling and adjusting workloads during the experiment, was calculated based on the participant’s individual CET result, accounting for data on HRmax, exertional heart rate (EHR), frequency resting heart rate (RHR) and the execution time of high-intensity training and its intervals that resulted in a number of arbitrary units (AU) of Training Impulse measurement (TRIMP) proposed by Banister with the formula (figure 3) (31).

After the randomized division of groups into HILIT and SIT, applying the TRIMP formula, the average TRIMP UA of each group was calculated for comparison and equalization of training, resulting in HILIT and SIT groups with balanced TRIMP UA providing a comparison more isonomic of the effort expended in the training session between the intervention groups (32).

**Figure 3.** TRIMP formula proposed by Banister.

\[
TRIMP = T \times \left[ \frac{(HR_{ex} - HR_{rest})}{(HR_{max} - HR_{rest})} \right] \\
1.672 \times 0.86e \quad \text{Females} \\
1.92 \times 0.64e \quad \text{Males}
\]

T: exercise duration (minutes); HRex: average heart rate during exercise; HRrest: resting heart rate; and HRmax: maximal heart rate.
Data analysis
The data were analyzed using the IBM SPSS Statistics 25 for Windows program and presented as mean and standard deviation. The normality and sphericity of the sample data were analyzed using the Shapiro-Wilk and Bartlett tests, respectively. To determine the effect of the independent variables (HILIT and SIT training) on the outcome variables (Biochemical tests, body composition and TEC), an ANOVA with repeated measures (2 x 2) was performed, followed by the adjusted Bonferroni post-hoc, for the comparison of the study's dependent variables and between the assessments of the independent variables arising from the HILIT and SIT groups (33). Cohen's effect size (d) was calculated to analyze the clinical impact of the different interventions on the study variables and interpreted according to the following correlation standardization: 0 to 0.2 weak; 0.3 to 0.7 moderate; and equal or above 0.8 strong. The value of p < 0.05 was adopted for statistical significance (34).

RESULTS
Table II presents the characterization of the sample by groups, in mean and standard deviation values regarding

Table II. Basic characteristics of participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HILIT (n = 23)</th>
<th>SIT (n = 15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.72 ± 6.39</td>
<td>1.74 ± 7.63</td>
<td>0.986</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.75 ± 8.26</td>
<td>38.88 ± 6.35</td>
<td>0.100</td>
</tr>
<tr>
<td>TBM (kg)</td>
<td>84.15 ± 15.56</td>
<td>83.89 ± 14.10</td>
<td>0.236</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.46 ± 4.25</td>
<td>27.48 ± 3.67</td>
<td>0.154</td>
</tr>
</tbody>
</table>

TBM: Total Body Mass; BMI: Body Mass Index.

Table III. Analysis of body composition between groups HILIT e SIT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean pre ± SD</th>
<th>Mean post ± SD</th>
<th>Δ%</th>
<th>d</th>
<th>P-value</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat Mass (kg)</td>
<td>HILIT</td>
<td>25.74 ± 8.81</td>
<td>24.36 ± 8.40</td>
<td>-5.38</td>
<td>-0.16</td>
<td>0.002</td>
<td>0.610</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>HILIT</td>
<td>55.45 ± 8.42</td>
<td>56.04 ± 8.43</td>
<td>1.06</td>
<td>0.07</td>
<td>0.002</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>57.15 ± 7.31</td>
<td>57.59 ± 7.42</td>
<td>0.77</td>
<td>0.06</td>
<td>0.052</td>
<td>0.701</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>HILIT</td>
<td>93.33 ± 10.59</td>
<td>92.55 ± 9.84</td>
<td>-0.83</td>
<td>-0.07</td>
<td>0.251</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>92.19 ± 8.73</td>
<td>91.35 ± 8.41</td>
<td>-0.91</td>
<td>-0.10</td>
<td>0.316</td>
<td>0.548</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>HILIT</td>
<td>28.47 ± 4.25</td>
<td>28.14 ± 4.03</td>
<td>-1.16</td>
<td>-0.08</td>
<td>0.046</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>27.48 ± 3.66</td>
<td>27.36 ± 3.62</td>
<td>-0.44</td>
<td>-0.03</td>
<td>0.548</td>
<td>0.120</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>HILIT</td>
<td>1,358.00 ± 684.53</td>
<td>1,251.61 ± 653.65</td>
<td>-7.83</td>
<td>-0.16</td>
<td>0.013</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>1,044.13 ± 566.53</td>
<td>941.60 ± 462.42</td>
<td>-9.82</td>
<td>-0.18</td>
<td>0.048</td>
<td>0.048</td>
</tr>
</tbody>
</table>

*p < 0.05; pre vs post; †p < 0.05; HILIT post vs post SIT; d: effect size (Cohen); SD: Standard Deviation; BMI: Body Mass Index; WC: Waist Circumference; VAT: Visceral Adipose Tissue.
### Table IV. Analysis of aerobic capacity between groups HILIT e SIT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean pre ± SD</th>
<th>Mean post ± SD</th>
<th>Δ%</th>
<th>d</th>
<th>P-value*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (m)</td>
<td>HILIT</td>
<td>1321.78 ± 415.98</td>
<td>1424.00 ± 417.56</td>
<td>7.73</td>
<td>0.25</td>
<td>0.005</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>1491.33 ± 378.24</td>
<td>1607.87 ± 376.08</td>
<td>7.81</td>
<td>0.31</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; (mL/kg·min)</td>
<td>HILIT</td>
<td>44.59 ± 6.78</td>
<td>47.02 ± 6.66</td>
<td>5.46</td>
<td>0.36</td>
<td>0.002</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>49.23 ± 5.20</td>
<td>50.17 ± 6.30</td>
<td>1.90</td>
<td>0.18</td>
<td>0.307</td>
<td></td>
</tr>
<tr>
<td>SBP Rest (mmHg)</td>
<td>HILIT</td>
<td>122.00 ± 15.79</td>
<td>116.00 ± 11.52</td>
<td>-4.92</td>
<td>-0.38</td>
<td>0.051</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>115.73 ± 16.21</td>
<td>112.40 ± 11.72</td>
<td>-2.88</td>
<td>-0.21</td>
<td>0.371</td>
<td></td>
</tr>
<tr>
<td>DBP Rest (mmHg)</td>
<td>HILIT</td>
<td>78.35 ± 10.73</td>
<td>78.87 ± 10.73</td>
<td>0.66</td>
<td>0.05</td>
<td>0.804</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>70.40 ± 7.53</td>
<td>67.47 ± 7.07</td>
<td>-4.16</td>
<td>-0.39</td>
<td>0.265</td>
<td></td>
</tr>
</tbody>
</table>

*<i>p < 0.05, pre vs post</i>; †<i>p < 0.05; HILIT post vs post SIT</i>; d: effect size (Cohen); SD: Standard Deviation; VO<sub>2</sub>: Oxygen Consumed; SBP Rest: Resting Systolic Blood Pressure; DBP Rest: Resting Diastolic Blood Pressure.

### Table V. Biochemical blood analysis between groups HILIT e SIT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean pre ± SD</th>
<th>Mean post ± SD</th>
<th>Δ%</th>
<th>d</th>
<th>P-value*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIG (mg/dl)</td>
<td>HILIT</td>
<td>135.96 ± 74.91</td>
<td>126.30 ± 87.60</td>
<td>-7.10</td>
<td>-0.13</td>
<td>0.431</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>95.73 ± 30.61</td>
<td>98.13 ± 51.76</td>
<td>2.51</td>
<td>0.08</td>
<td>0.874</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>HILIT</td>
<td>51.43 ± 8.40</td>
<td>53.87 ± 11.67</td>
<td>4.73</td>
<td>0.29</td>
<td>0.193</td>
<td>0.957</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>52.60 ± 8.02</td>
<td>54.07 ± 9.32</td>
<td>2.79</td>
<td>0.18</td>
<td>0.523</td>
<td></td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>HILIT</td>
<td>99.52 ± 15.71</td>
<td>95.00 ± 17.54</td>
<td>-4.54</td>
<td>-0.29</td>
<td>0.011</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>94.00 ± 8.12</td>
<td>88.20 ± 6.43</td>
<td>-6.17</td>
<td>-0.71</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>ALB (g/dL)</td>
<td>HILIT</td>
<td>4.27 ± 0.15</td>
<td>3.89 ± 0.42</td>
<td>-9.05</td>
<td>-2.51</td>
<td>&lt;0.001</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>4.23 ± 0.14</td>
<td>3.94 ± 0.41</td>
<td>-6.93</td>
<td>-2.10</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>DB (mg/dl)</td>
<td>HILIT</td>
<td>0.13 ± 0.07</td>
<td>0.20 ± 0.10</td>
<td>56.16</td>
<td>0.95</td>
<td>0.002</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>0.11 ± 0.09</td>
<td>0.20 ± 0.08</td>
<td>81.07</td>
<td>1.02</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>HILIT</td>
<td>0.50 ± 0.31</td>
<td>0.57 ± 0.33</td>
<td>13.41</td>
<td>0.22</td>
<td>0.376</td>
<td>0.579</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>0.58 ± 0.52</td>
<td>0.63 ± 0.29</td>
<td>7.94</td>
<td>0.09</td>
<td>0.622</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>HILIT</td>
<td>25.35 ± 7.68</td>
<td>24.26 ± 9.15</td>
<td>-4.29</td>
<td>-0.14</td>
<td>0.624</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>26.73 ± 13.13</td>
<td>22.00 ± 9.89</td>
<td>-17.71</td>
<td>-0.36</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>HILIT</td>
<td>30.83 ± 11.87</td>
<td>31.57 ± 14.57</td>
<td>2.40</td>
<td>0.06</td>
<td>0.740</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>32.80 ± 31.95</td>
<td>28.33 ± 21.90</td>
<td>-13.62</td>
<td>-0.14</td>
<td>0.112</td>
<td></td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>HILIT</td>
<td>35.35 ± 18.09</td>
<td>35.96 ± 15.61</td>
<td>1.72</td>
<td>0.03</td>
<td>0.807</td>
<td>0.512</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>39.67 ± 35.73</td>
<td>31.73 ± 23.76</td>
<td>-20.00</td>
<td>-0.22</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

*<i>p < 0.05; pre vs post</i>; †<i>p < 0.05; HILIT post vs post SIT</i>; d: effect size (Cohen); SD: Standard Deviation; TRIG: triglycerides; HDL: high-density lipoprotein; GLU: glucose; ALB: Albumin; DB: Direct Bilirubin; TB: Total Bilirubin; AST: Aspartate Aminotransferase; ALT: Alamine Aminotransferase; GGT: Gamma Glutamyl Transferase.
DISCUSSION

This study, a randomized clinical trial, aimed to analyze the effects of long and sprint high-intensity interval training on body mass composition, aerobic capacity, and biochemical markers of metabolic syndrome and liver damage in physical activity practitioners adults undergoing 12 weeks of training. The data were discussed resulting from the statistical analysis of the crossing between the pre-test and post-test in the intragroup and intergroup conditions.

It was observed (table II) that there was a normal behavior of the statistical curve of the participants’ basic characteristics, where the data on Total Body Mass (TBM) and the resulting mean BMI of the HILIT and SIT groups describe a mean of overweight sample (30 > BMI > 25). The groups were similar at the beginning of the study, with no difference between the variables analyzed (34).

Body composition was investigated in this study through DXA (35). In the intragroup comparison (table III) in the HILIT group there was a reduction in TBM, VAT and an increase in lean mass. Corroborating the present study, the work of systematic reviews and meta-analyses carried out by Bellicha et al. (36) analyzed 12 systematic reviews with meta-analysis that encompassed 149 evaluated studies, with adults over 18 years old, in a situation of obesity or overweight, which used HIIT exercise as an intervention, finding differences in VAT, with improvements in cardiometabolic health. Similarly, Taylor et al. (37), in a randomized controlled study with 38 adults performing a HIIT intervention and Continuous Moderate Intensity Training (CMIT), obtained significant results in reducing VAT.

In this way, the study by Irving et al. (38) investigated the effects of 16-week physical training intensity on abdominal VAT and body composition in 27 (51 ± 9 years) obese women with MS and reported that HIIT was more effective in reducing total abdominal fat, subcutaneous abdominal fat and VAT. Still about the body assessment, the reduction in VAT was identified (table III) in intragroup comparisons in both HILIT and SIT groups, with a mean difference of 1,358.00 ± 684.53 g to 1,251.61 ± 653.65 g for the HILIT group and 1,044.13 ± 566.53 g to 941.60 ± 462.42 g for the SIT group.

Table VI. Analysis of MS between groups HILIT e SIT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean pre ± SD</th>
<th>Mean post ± SD</th>
<th>Δ%</th>
<th>d</th>
<th>P-value*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIG (mg/dl)</td>
<td>HILIT</td>
<td>135.96 ± 74.91</td>
<td>126.30 ± 87.60</td>
<td>-7.10</td>
<td>-0.13</td>
<td>0.043</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>95.73 ± 30.61</td>
<td>98.13 ± 51.76</td>
<td>2.51</td>
<td>0.08</td>
<td>0.874</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>HILIT</td>
<td>51.43 ± 8.40</td>
<td>53.87 ± 11.67</td>
<td>4.73</td>
<td>0.29</td>
<td>0.193</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>52.60 ± 8.02</td>
<td>54.07 ± 9.32</td>
<td>2.79</td>
<td>0.18</td>
<td>0.523</td>
<td></td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>HILIT</td>
<td>99.52 ± 15.71</td>
<td>95.00 ± 17.54</td>
<td>-4.54</td>
<td>-0.29</td>
<td>0.011</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>94.00 ± 8.12</td>
<td>88.20 ± 6.43</td>
<td>-6.17</td>
<td>-0.71</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>HILIT</td>
<td>93.33 ± 10.59</td>
<td>92.55 ± 9.84</td>
<td>-0.83</td>
<td>-0.07</td>
<td>0.251</td>
<td>0.701</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>92.19 ± 8.73</td>
<td>91.35 ± 8.41</td>
<td>-0.91</td>
<td>-0.10</td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>SBP Rest (mmHg)</td>
<td>HILIT</td>
<td>122.00 ± 15.79</td>
<td>116.00 ± 11.52</td>
<td>-4.92</td>
<td>-0.38</td>
<td>0.051</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>115.73 ± 16.21</td>
<td>112.40 ± 11.72</td>
<td>-2.88</td>
<td>-0.21</td>
<td>0.371</td>
<td></td>
</tr>
<tr>
<td>DBP Rest (mmHg)</td>
<td>HILIT</td>
<td>78.35 ± 10.73</td>
<td>78.87 ± 10.73</td>
<td>0.66</td>
<td>0.05</td>
<td>0.804</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>SIT</td>
<td>70.40 ± 7.53</td>
<td>67.47 ± 7.07</td>
<td>-4.16</td>
<td>-0.39</td>
<td>0.265</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; pre vs post; † p < 0.05; HILIT post vs post SIT; d: effect size (Cohen); SD: Standard Deviation; triglycerides (TRIG); high-density lipoprotein (HDL); glucose (GLU); WC: Waist Circumference; SBP Rest: Resting Systolic Blood Pressure; DBP Rest: Resting Diastolic Blood Pressure.
which shows a significant reduction in body VAT behavior through training. Corroborating the present study, Khalafi et al. (39) in a systematic review with meta-analysis, where 10 studies investigated the effect of HIIT training on liver fat content in overweight or obese adults with metabolic disorders, observing VAT decrease which is directly correlated with the decrease in content fatty liver.

In addition, regarding the analysis of lean mass (table III), it was understood that in the HILIT group there was an increase that reflected in the difference in the average from 55.45 ± 8.41 kg to 56.04 ± 8.43 kg, indicating that the behavior of lean mass improved with training, reaching one of the most frequent objectives in exercise programs focused on health and aesthetics (40).

Especially, in the assessment of fat mass, there was a decrease (table III) in the HILIT group from 25.74 ± 8.81 kg to 24.36 ± 8.40 kg. This shows that body fat mass reduced with the proposed training, achieving another important objective of the study. It is important to highlight that the findings of reduction in fat mass (6, 7) and the increase in lean mass represented the definition of body recomposition resulting from a 12-week experiment without diet (40).

Comparatively, in the meta-analysis study by Maillard et al. (41), which evaluated 39 studies with 617 individuals with a mean age of 38.8 years ± 14.4, HIIT reduced total and abdominal fat mass and VAT, with no differences between the sexes. They also revealed that running was effective in reducing visceral and total fat mass and that HIIT above 90% of maximum heart rate was more successful than other intensities in reducing total body adiposity.

As a point to be highlighted, the equalization is strategic and combines elements of intensity and duration of training in the unique concept of training load, which in the present study was carried out using the Banister equation, demonstrating that TRIMP brought more equality of effort between groups for results achieved, which at first sight could mean a scientific result more coherent and definitive (4, 5, 32). In this sense, the systematic review study with meta-analysis by Andreato et al. (42) corroborates the need for equality between interventions who meta-analyzed 48 studies aiming to evaluate the influence of high-intensity interval training (HIIT) on the anthropometric variables of 1,222 adults of both sexes, aged between 18 and 65 years, overweight or obese. This study showed that HIIT was effective in reducing body mass, BMI, waist circumference, Waist-to-Hip Ratio (WHR), body fat percentage and VAT area, however, when considering studies with training equalization, the only difference that remained was for body mass reduction.

In turn, the study by Andreato et al. (42) presented interesting data regarding the different choices of exercise modalities, showing that 30 studies adopted cycling and 18 studies chose running/walking, 12 of which were on a treadmill, 4 on an indoor sports court, 1 study performed exercise outdoors, and 1 in a court, where a reduction in VAT was observed in none of them for equalized studies. As another point to be highlighted, liver markers are used because they are cheaper, less invasive, easy to manage and have moderate diagnostic accuracy, providing a relevant perspective on liver disease, such as cell damage or inflammation (43). Santos et al. (44) pointed out that physical exercise with resistance training, aerobic training and HIIT interventions favored the reduction of biochemical markers (AST, ALT, GGT, ferritin, indirect bilirubin, and ALP), and showed a reduction in ALT in the exercise groups.

In the same direction, the ALB behavior (table V) consolidates the statement that there was no diet or ALB supplementation, as a marker for evaluating the nutritional status important for the body, serum albumin serves as a test of liver synthetic function. However, low serum albumin is not specific for liver disease and may occur in other conditions such as malnutrition, infections, nephrotic syndrome, or protein-losing enteropathy (45).

Specially about our findings regarding ALB, the averages were within the reference limits between 3.9 and 4.9 g/L, with results (table V) demonstrating that the HILIT exercise reduced the average by 4.27 ± 0.15 g/L to 3.89 ± 0.42 g/L and in the SIT group it decreased from 4.23±0.14 g/L to 3.94±0.41 g/L, depicting a decrease in their values, leaving a central average trend and approaching the lower value of the reference (46).

In addition, about GGT result were observed a significant reduction (p < 0.05) in SIT (table V), in the mean from 39.67 ± 35.73 U/L to 31.73 ± 23.76 U/L, although the means were within normal limits between 11 and 50 U/L (46), this demonstrates that exercise in the SIT modality produced an effect on improving hepatic metabolism of GGT, with beneficial effects on health. Increased serum GGT is a sensitive indicator of the presence of damage to the bile ducts or liver (45).

Subsequently in the analysis of TB, the stability of Indirect Bilirubin (IB) was verified, its increase refers to the increase in the function of DB, for both groups, which translates into an increase in the conjugation function of Bilirubin to its more soluble form, which correlates with the bile formation activities and intestinal function (45). Furthermore, our study showed an increase in DB, being a specific phase of bilirubin in a more soluble format to later be transported to the bile to aid intestinal functions of absorption of fat and other substances. In the HILIT group it ranged from 0.13 ± 0.07 mg/dL to 0.20 ± 0.10 mg/dL and in the SIT group from 0.11 ± 0.09 mg/dL to 0.20 ± 0.13 mg/dL.
0.08 mg/dL which is still within the normal limits established by hepatology (46). Such findings can be explained by the increase in physiological stress caused by exercise in the hemolysis process, leading to an increase in the conjugation function of Bilirubin (45).

As an important point to be highlighted, the study by Bellicha et al. (36) emphasized, in the same way as the present study, the use of CET in cardiovascular safety for prescription and initiation of high-intensity interval training. Also in the same direction as the present study and valuing cardiopulmonary assessment, the study by Mayorga et al. (47) meta-analyzed 123 studies examining the criterion validity of walking or running tests based on distance and time to estimate cardiorespiratory fitness among apparently healthy children and adults, providing strong evidence that cardiorespiratory fitness constitutes an important predictor of morbidity and mortality, being considered one of the most powerful health markers, with relevance over other traditional indicators, such as weight status, blood pressure or cholesterol level.

In another perspective, Taylor et al. (48), in a 12-month longitudinal study with one hundred adults with heart disease, corroborated the safety of using HIIT and the results of improvements in VO₂ peak due to the powerful exercise stimulus provided during high-intensity interval periods, believing that higher intensities invoke greater aerobic and cardiovascular adaptation than low to moderate intensities. There was no record of accidents during the pre, and post CET performed, and all participants completed the CET on a treadmill until exhaustion, with no cardiac anomaly that would contraindicate the participant.

In addition, the protocol used tested in a pilot study, estimated VO₂ (mL/kg x min) through an indirect test with good association with the Rating Perceived Exertion (RPE) presented by the study participants. Moreover, recent studies suggest the establishment an additional verification phase in the CET to obtain true VO₂ (49).

In particular about the distance reached assessment (table IV), there was an increase in the difference in the mean distance achieved in the CET protocol from 1,321.7 ± 415.98 m to 1,424.0 ± 417.56 m for HILIT group and for the SIT group, the increase was from 1,491.3 ± 378.24 m to 1,607.8 ± 376.07 m, which reveals that the behavior of the distance achieved in the CET improved the conditioning and running performance in both exercise groups. The improvement in the VO₂ variable the HILIT group 44.59 ± 6.78 to 47.02 ± 6.66 demonstrates a specific development of the running modality. In the same direction, Oliveira-Nunes et al. (50) in the meta-analysis study compared HIIT and SIT methodologies, they showed similar gains in cardiorespiratory fitness.

In the case of BMI result (table III), the HILIT group the average went from 28.46 ± 4.25 kg/m² to 28.14±4.03 kg/m², being an important factor that contributes to improved health. Correspondingly, the study by Fortes et al. (51) focusing on the average BMI 25.1 ± 3.4 kg/m², proved to be a good predictor of change in physiological markers of MS. A decrease in BMI is strongly associated with physiological markers of health.

Another important topic: according to Kumari et al. (52) the decrease of hypertension is identified as the main risk factor for mortality, and which ranks third as a cause of reduced years of life due to disability, also being a common manifestation of metabolic disorders associated with insulin resistance and hyperinsulinemia. In the study by Fortes et al. (51) it was found cross-sectionally in 2,719 Brazilian Army soldiers that the mean SBP 120.3 ± 10.1 was quantitatively like the means found in this study.

Specifically, about DBP was found that the means were within the reference limits of 85.0 mmHg (26), but there was a significant improve intergroups. In the same direction, Fortes et al. (51) found similar DBP means 77.2 ± 8.8. The present study filled an important gap in the scientific literature by carrying out the running intervention outdoors (53). According to Andreato et al. (42) who verified the influence of HIIT on the anthropometric variables of overweight or obese adults, evaluating 48 studies with 1,222 people, showed that in relation to the HIIT modality there were around 30 studies that adopted ergometric cycling, 18 that adopted running or walking, 12 used the treadmill, 3 used the indoor sports court, 1 used in the court and 1 single outdoor study.

According to Foster et al. (32) about use of technology to monitor load, it contributes to safety in carrying out training for experiment participants and controlling important training variables. During the training sessions of the present study, physical activity practitioners participants had their heart rates monitored by the First Beat software, which contributed during the training execution to adjust the load and check the HILIT and SIT target zones, and it was observed that the SIT monitoring is more complex due to the late heart rate response recorded on the devices and the difficulty in monitoring maximal or submaximal efforts in sprints (4, 5, 32).

Singularly enriching our discussion, studies indicate a better use of SIT when the individual has a profile of physical qualities more focused on explosion than endurance (4, 5). The active interval at 50% HRmax between high-intensity interval bursts was used as a common aspect for both the HILIT and SIT groups. In the same direction, Mello et al. (54) verified in an experimental study with 15 military men from the Brazilian Army with excellent physical
conditioning, the contribution of the active interval as a choice capable of promoting less muscle damage and that for the present study there was an improvement in VO$_2$ and a good adaptation of participants to the HILIT and SIT methods. The studies by Germano et al. (55) corroborate the active recovery intervals that have greater potential for reaching and remaining at a high percentage of V'O$_{2max}$ and HRonax. In contrast, other studies have suggested that the passive recovery interval can induce higher VO$_2$ (4).

In the context of choosing the SIT method, it appears that the sprint and the gain in aerobic capacity were important gains from this study with excellent potential to be developed, if stratified within the age ranges of interest, and for both sexes, which reflect a trend (56). Furthermore, they also have engagement and applicability in team sports such as football and others (57).

In the comparison between the proposed methods HILIT and SIT, the present study indicates a better result for HILIT. In the same way, Rosenblat et al. (58), in a meta-analysis, systematically reviewed six randomized studies, with moderately trained adults between 18 and 45 years old, determining which mode of interval training, HIIT versus SIT, showed HILIT as an ideal form of interval training to improve performance.

The strengths of the present study were a more complete approach with load equalization using different participant assessment methods: body composition, physical load test, and blood biochemical markers. As mentioned before, regarding the intergroup comparison arising from exercise, the discussion reveals that there was an improvement in important parameters of MS, liver health and aerobic capacity. However, there were limitations in the study that must be taken into consideration, such as the impossibility of controlling the feeding variable and the use of the ramp CET. In the future, CET studies may establish a verification phase to obtain true VO$_2$, which was also not part of this study due to logistical circumstances to enable tests for the experimental sample and ergospirometric material.

CONCLUSIONS
Considering the results found, the present study showed the positive effects of HILIT and SIT on physiological and pathological markers of MS and liver health in in activity practitioners adults. In the SIT method, there was an improvement in the parameters VAT, GLU, ALB, DB, GGT, and distance running. HILIT proved to be more efficient than SIT, and provided improvements in liver health, body composition and aerobic capacity in the health parameters fat mass, lean mass, BMI, VAT, GLU, ALB, DB, distance covered and VO$_2$.

Thus, the study has applicability to society in general, specifically to the military population and physical activity practitioners adults. Future studies involving the use of a gold standard non-invasive liver imaging instrument such as elastography are recommended. It would also be interesting to investigate the relations between HILIT and SIT, MS and liver health correlated with circadian cycle behavior, and epigenetics.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
SLL: writing – original draft, project administration, conceptualization. B-PCJ, SATB, CMASP, RSE, FMSR, NRAM, CJBP, LDG, SAOB, CLS: project administration. VRGS: conceptualization.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES


Effects of Long and Sprint High-Intensity Interval Training


Analysis of Muscle Damage Through Urine Staining and Density as Prevention of Rhabdomyolysis During Physical Training

Guilherme Henrique Mattos Dantas¹,², Raman Alves dos Reis³,⁴, Rodolfo de Alkmim Moreira Nunes¹,², Eduardo Borba Neves⁵, Giullio César Pereira Salustiano Mallen da Silva¹,², Gustavo Casimiro Lopes²,⁴, Rodrigo Gomes de Souza Vale¹,²,⁶

¹ Laboratory of Exercise and Sport (LABEES), Institute of Physical Education and Sports, Rio de Janeiro State University, Rio de Janeiro, RJ, Brazil
² Postgraduate Program in Exercise and Sport Sciences (PPGCEE), Rio de Janeiro State University, Rio de Janeiro, RJ, Brazil
³ School of Physical Education and Sports, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
⁴ Laboratory of Exercise Pathophysiology (LAFE), Institute of Physical Education and Sports, Rio de Janeiro State University, Rio de Janeiro, RJ, Brazil
⁵ Postgraduate Program on Biomedical Engineering, Federal Technological University of Paraná (PPGEB/UTFPR), Curitiba, PR, Brazil
⁶ Laboratory of Exercise Physiology, Estácio de Sá University, Cabo Frio, RJ, Brazil

INTRODUCTION
Rhabdomyolysis is a syndrome characterized by skeletal muscle cell injury and muscle necrosis, which can be defined by unscheduled cell death derived from external factors. With this content in the bloodstream, the organs are affected and eliminate these toxic components, through the urine (1–3).

Urine color can vary, ranging from colorless to black. These color variations can be caused by changes in hydra-
tion, diseases (metabolic, genetic, muscle injuries, stones, tumors), physical activity, and sample storage conditions. However, only the change in urine color does not indicate abnormal functioning of the body and rhabdomyolysis (4). The measurement of myoglobin or myoglobinuria is less sensitive than the measurement of plasma creatine kinase (CK) for diagnosing rhabdomyolysis (5). However, CK is the first substance of muscular origin to increase and the first to contribute to kidney damage, which can trigger nephrotoxic acute kidney injury (5-7). Controlling these indicators is important for intensive care and maintenance of renal health, as the mortality rate can reach 59% in critically ill patients with acute kidney injury (8).

Difficulty in detection may be a factor related to high mortality rates since non-invasive tests do not provide diagnostic accuracy. The most sensitive test for detecting rhabdomyolysis is plasma CK (3). This makes individuals who undergo intense physical exertion, such as athletes, recreational practitioners, and military personnel, more susceptible to this syndrome because part of their training routine consists of long walks and tests and strenuous physical exercises (9).

According to the latest data from the U.S. Armed Forces Health Surveillance Center, among active-duty service members in 2021, there were 513 cases of exertional rhabdomyolysis, resulting in an unadjusted incidence rate of 38.6 cases per 100,000 person-years, a slight decrease of 0.2 compared to the previous year (10). Although physical exercise, namely resistance training, reduces the risks of metabolic diseases and improves fitness levels, it is essential to understand the physiological responses of practitioners based on the prescribed dosage (11, 12). Thus, there is a concern with the military operational routine, since maximum levels of physical conditioning and perfection in the execution of activities are required for participation in military operational courses, which may include, among other skills and functions, patrolling violent urban areas with the driving of a police dog in the midst of weather adversities and geographically rugged for a long period, almost strenuously (13).

In this context, prevention becomes a crucial strategic action to avoid cases of rhabdomyolysis during a military course or operational activity. The emergence and development of the syndrome are intricately linked to the practice of high-intensity physical activity. Hence, the present study aimed to analyze the variables that could serve as evidence of rhabdomyolysis, including room temperature, overall temperature, rating of perceived exertion (RPE), urine color (myoglobinuria), and urine density, as well as clinical and physical symptoms in a military police operational course conducted in the city of Rio de Janeiro.

MATERIALS AND METHODS

Experimental design

This study is characterized as a quantitative and descriptive field research (14). The participants were recruited from a pool of 500 service members aged between 20 and 40 after undergoing a selection process, which included specific ability tests, physical fitness tests and health inspections. This made them able to start the course without any physiological marker of non-standard assessment of general health status. There were no health complications that could interfere with the results of this research. Thus, no pre-course data were collected to be used as baseline in this research.

Forty-nine male service members were approved to start the Dog Handlers Course for Police Employment of the Dog Action Battalion of the Rio de Janeiro State Military Police Secretariat. Military personnel who were discharged during the course for any reason other than related to clinical complications, those who requested dismissal of their own volition, and those who presented technical insufficiency in the tasks performed during the course were excluded from the study. The study was approved by the Research Ethics Committee N° 95856318.0.0000.5259 – date of approval: October 4, 2018 –, with opinion N° 2.940.992 and the participants signed the free and informed consent form in accordance with resolution 466/12 of the National Health Council.

Procedures

Eight days of data collection were carried out, spaced at least three days apart, throughout the 38-day duration of the course. The first collection took place on the second day after the beginning of the course, and the last one was two days before the end of the course. The collections were always carried out in the morning, after the practice of physical education, according to the schedule of technical and operational activities to be carried out.

In the military operational course, the activities were carried out with military personnel in uniform and with overloaded equipment. One of the few exceptions to this condition is physical education, which was conducted in the morning with appropriate clothing for exercise (shorts, shirt, sneakers, and socks). The specific activities of the course are essential for training and cause high physical stress. Among them are patrol conduct in a hostile environment, rappelling and navigating geographically rugged terrains, as well as long walks, jumps, and runs. During physical education, long-distance running activities were carried out on different terrains (average of 12 km), along with resistance exercises and military physical training consisting of physi-
physical fitness tests (PFT) which included pull-up exercises on the fixed bar, sit-ups and running.

Urine and perceived exertion scale data collection
The urine collectors were coded according to the numerical identity acquired by everyone during the course and were arranged on a white poster board divided into numbered quadrants. The collectors were removed one by one by each soldier according to their corresponding number. When returning the collected material, the service members, individually, classified their perceived exertion using the Borg Perceived Exertion Scale (CR-10) (15). All collectors with at least 25 ml of urine were arranged in such a way that two instructors selected the three collectors with the darkest urine and abnormal color. These collectors were separated for later analysis and comparison with numerical values. In the event of a disagreement, a third instructor was consulted.

Urine data analysis
The sedimentation level of urine samples was analyzed immediately after the collection, using a manual refractometer (model RHC-200/ATC, Megabrix, China), to verify the density values in Urine Specific Gravity (USG). On the other hand, a spectrophotometer for ELISA was used to measure urine color, using the Skanalt software. The measurement is measured by absorbance, with a wavelength of 350 nm to 500 nm (nanometer) in 1 nm steps without changing the filter. An object or substance absorbs all incident light except that of the wavelength range observed by vision (350 nm-750 nm). Thus, the color of a solution is complementary to the absorbed light (16). The longer the wavelength, the more quantity and value attributes there are, since color perception is determined by the frequency range received by the visual system (17). The wave value of 350 nm was used for the analyses of the present study, because it presents better performance for the high sample quantity and less comprehensive color results, which facilitates visualization, comparison, and analysis (17).

Wet globe temperature
The wet globe temperature (WBGT) was measured on all days of collection in the morning using a Wet Dry Bulb Globe Thermometer (model TGM-200, Homis, Brazil). WBGT is a type of temperature used to estimate the effect of heat stress related to humidity, in addition to heat through radiation and ambient temperature (18, 19). The risk value was calculated and classified using the risk of heat stroke due to exertion, as follows: WBGT < 18.3 °C = Low risk of heat illness; WBGT from 18.3 to 22.8 °C = Moderate risk of heat illness; WBGT from 22.9 to 27.8 °C = High risk of heat illness; WBGT ≥ 27.9 °C = Extreme risk of heat illness (18).

Statistical analysis
Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 25, and the results were presented as mean, standard deviation, as well as minimum and maximum values. The normality of the data was assessed using the Shapiro-Wilk test. For intra-group comparisons of RPE, Friedman’s test was employed, followed by Dunn’s post-hoc test. Spearman’s correlation test was utilized to analyze the associations between the research variables. The study considered a significance level of p < 0.05 for statistical significance.

RESULTS
By the end of the intervention, 37 service members completed the course, while 12 participants dropped out and a female military member excluded from this study to make the sample homogeneous. A total of 311 urine samples and 311 perceived exertion values were collected. The ambient temperature had an average of 24.75 ± 4.46 °C, with a low peak of 18 °C and a high of 33 °C, during the mornings, in which the collections were carried out during the mornings when the samples were collected. The WBGT consistently fell within the 10 to 18.3 °C range, categorizing it as “low risk of heat illness” based on the risk value calculated and classified per the Risk of Heat Stroke due to Stress (18).

Borg’s rating of perceived exertion (RPE) (CR-10) (2000) indicated a significant increase between the first and last days of data collection. Except for collection 3, the scale exhibited values of evolution of the mean RPE during the course period (table I) (15).

The urine samples showed a mean density value of 1,024 ± 6.45 USG, with a minimum value of 1,005 USG and a maxi-

Table I. Evolution of the rating of perceived exertion (RPE) of military personnel completing the course.

<table>
<thead>
<tr>
<th></th>
<th>Cll - 1</th>
<th>Cll - 2</th>
<th>Cll - 3</th>
<th>Cll - 4</th>
<th>Cll - 5</th>
<th>Cll - 6</th>
<th>Cll - 7</th>
<th>Cll - 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>5.45</td>
<td>8.69</td>
<td>6.08</td>
<td>8.62</td>
<td>8.49</td>
<td>8.08</td>
<td>8.26</td>
<td>9.10</td>
</tr>
<tr>
<td>SP</td>
<td>1.29</td>
<td>1.51</td>
<td>1.22</td>
<td>1.13</td>
<td>1.06</td>
<td>0.98</td>
<td>1.01</td>
<td>0.62</td>
</tr>
<tr>
<td>CV</td>
<td>0.24</td>
<td>0.17</td>
<td>0.20</td>
<td>0.13</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Cll: Collection; SP: standard deviation; CV: coefficient of variation; *P-value < 0.05; Collection 8 vs Collection 1.
maximum of 1,040 USG, a variation of 3.48%. They presented a mean color value, in absorbance, of 1.287 ± 0.41 nm with a minimum value of 0.286 nm and a maximum of 2.379 nm, a variation of 731.81%.

**Figure 1** illustrates the mean numerical values of density (USG) and color by absorbance in nanometers (nm) of the selected urine samples among the three with the greatest color changes on each collection day. Additionally, it includes the mean values of all collections that exhibited altered coloration. Of the 311 urine samples collected in this study, 12 exhibited an orange coloration (distinct from the usual yellow hue), 1 showed a reddish tint (Figure 2), and 298 appeared yellow (ranging from colorless to light yellow, medium yellow, and dark yellow). The densities ranged from 1,001 to 1,012 USG (n = 17; 5.46%), 1,013 to 1,029 USG (n = 194; 62.37%), and equal to or greater than 1,030 USG (n = 100; 32.15%), with two samples having a value of 1,040 USG. The variation in numerical color values for urine samples with a density ≥ 1,030 USG was 309.46%. The collector indicated by the arrow (A) has lighter urine color with a density of 1,015 USG and an absorbance of 1.587 nm, while the collector marked by arrow (B) has darker urine with a density of 1,030 USG and absorbance of 1.812 nm.

**Figure 2** visually represents the relationship between urine density and color, highlighting the collectors with discrepant numerical values. It displays the correlation observed between

**Figure 1.** Urine density (A) and color (B) values by perception.

Mean urine densities: measured by refractometer that showed altered color through perception with the human naked eye - values in USG. Mean Urine Color: measured by a spectrophotometer that showed altered urine color by the naked eye - values and nm. The decimal place of the Urine Color has been subtracted for better visualization. AVG Overall mean values of urine density (USG) and coloration (nm).

**Figure 2.** Visual relationship between urine color and density.
urine color (measured in absorbance in nanometers - nm) and urine density (measured in relative density - USG). A positive and statistically significant correlation was found between absorbance and density in the collected urine samples. Therefore, as absorbance values increase, urine density also increases.

**DISCUSSION**

The current study aimed to analyze the variables of ambient temperature, global temperature, and the rating of perceived exertion (RPE) in relation to muscle damage, as indicated by urine color (myoglobinuria) and density. Extreme temperatures are often regarded as one of the primary contributing factors to the development of rhabdomyolysis (19). However, in the current study, ambient temperature and wet bulb globe temperature did not appear to impact the outcomes, as the recorded values during the collection period were deemed to fall within the normal range (18).

As a potential consequence of this external factor combined with intense physical exertion, the typical clinical presentation of rhabdomyolysis may manifest, characterized by the classic triad of myalgia, asthenia, and dark urine (20). Given this risk and the significant physical demands imposed throughout the operational course, the study measured the control of overall perceived exertion and muscle discomfort using the Borg Rating of Perceived Exertion (RPE) scale (CR-10) (15). The findings revealed a consistent increase during the course, which was considered a normal response to the escalating physical, psychological, and technical requirements of the training. A similar measurement was employed in a study by Jameson and Ring (21) to assess overall perceived exertion, leg muscle discomfort, knee pain, breathlessness, and heart rate intensity in cyclists. However, when the urine samples were analyzed, changes were observed in the color of the urine seen with the naked eye when the density was compared. The spectrophotometer-based analysis of urine color provided indicators that raised concerns about dehydration, muscle damage, and the persistence of these conditions despite attempts at rehydration with water.

Military personnel are at an elevated risk of developing rhabdomyolysis during their training routines (9) because the symptoms overlap with those of heat-related illnesses during physical activities (22). Environmental temperature and dark urine are among the causes of rhabdomyolysis, with the latter being influenced by an individual’s hydration status and detected through urine density (23, 24). Urine density, as determined by refractometers, offers initial insights into the kidney’s capacity to selectively reabsorb essential substances and water from the glomerular filtrate. This quantification is impacted by the quantity and size of particles present in the sample (25). Normal urine typically exhibits characteristics such as a citrus yellow color, a distinct odor, clear appearance, a density ranging from 1.005 USG to 1.030 USG, and a pH between 4.5 and 7.8 (26). Consequently, these variables may be linked to the causes of rhabdomyolysis and the development of more severe cases of acute kidney injury (AKI).

Urinary hydration status can be categorized based on density values as follows: Hypohydration = 1,030 USG; Euvhydrated = 1,013 to 1,029 USG; Hyperhydration = 1,001 to 1,012 USG (24). Savvides et al. (27) evaluated the effects of dehydration on archery athletes in a simulated competition and classified urine with a density of 1.032 ± 0.005 USG and euhydrates with a density of 1.015 ± 0.004 USG as dehydrated. Thus, if the classification used by Savvides et al. (27) is applied to the urine collected in the present study, 32.15% of the urine was in the condition of dehydration and 10.61% of the urine collected in the condition of euhydration, without variation and 51.76% lower than the classification of Casa et al. (24), respectively.

The most common aspect of urine alteration is its color, which can indicate potential causes of harm to the body. Here is a color guide: light yellow to yellow = normal; colorless or very diluted; dark yellow = highly concentrated, indicating bilirubinuria; red to brownish red = may suggest hematuria, hemoglobinuria, or myoglobinuria; greenish = could indicate bilirubinuria (26). Costa et al. (28) reported that ultra-endurance athletes often adjust their fluid intake to maintain urine concentration within normal parameters in terms of color and osmolarity. However, in the present study, this strategy proved ineffective in controlling hydration during the operational course, as there was a significant variety of distinct shades of yellow observed in the collected samples.

Figure 1 shows the mean values of urine density and color in absorbance (number) of the participants who had their urine selected, by the instructors, with the darkest color on each day of collection during the course. All urine colors showed values above 1,600 nm. This can demonstrate the homogeneity of the sample, or a cut-off range in which color perception visually altered to the naked eye, begins to be confirmed by color in absolute values. The difficulty in standardizing colors was reported by Armstrong et al. (29) when they created a scale of eight colors, which vary in scale from noticeably light yellow to brownish green. However, this scale does not evaluate other colors that may be important for identifying abnormalities in the functioning of the body. Thus, the analysis of urine color by absorbance seems
to provide more accurate information to be used in the prevention of rhabdomyolysis.
In the present study, the collected urine samples exhibited a significant variation in density, even though constrained within the normal reference range of 25 units (1,005 USG-1,030 USG) (24). However, the average of the samples, more influenced by color perception on each collection day (figure 1), indicates that none of the urine was in a hydrated state (24).
Electrolyte imbalances, including dehydration, are significant contributors to muscle damage and, subsequently, the development of rhabdomyolysis (30, 31). Highly concentrated and excessively alkaline urine can lead to false positive results concerning altered renal function. These false results may be attributed to a range of factors, from simple dehydration to chronic kidney disease (32). The findings of this study demonstrated that all urine samples with a density falling within the range of 1,005 USG to 1,015 USG appeared diluted and exhibited a color outside the typical yellow hue, confirming the lack of precision in diagnosing electrolyte disturbances, dehydration, and muscle structure damage.

Spectrophotometric urine analysis has demonstrated its potential as a method for preventing and managing muscle damage, and consequently, rhabdomyolysis. In the urine samples depicted in figure 2, the urine marked as (A) was classified as normal based on color and euhydrated based on density. Conversely, the urine labeled as (B) was classified as highly concentrated in terms of color and hydrophydrated based on density (24, 26). However, the absorbance values for color were quite similar.

This study revealed that not all dark or altered urine had high density, and conversely, not all light-colored urine had low density. Some sources Knochel (33) argue that myoglobinuria only occurs within the context of rhabdomyolysis. However, Khan (34) reported that myoglobinuria does not manifest without eventually leading to rhabdomyolysis. It is worth noting that visible myoglobinuria may not occur until the body has already been affected by rhabdomyolysis. This underscores the importance of effective prevention measures, as the color of urine can be influenced by the myoglobinuria process, even if it is subtle or imperceptible to the naked eye.

The relationship between the density and the color of urine in absorbance (number) is characterized by a positive correlation between these variables, because when the density increases, the color also suffers an increase in its values, but in a more detailed way. When the density increases, there is a corresponding increase in color values, albeit in a more nuanced manner. For urine samples with a borderline density indicating hypohydration at 1,030 USG, according to the classification by Casa et al. (24), there was a significant percentage of variation. Thus, it can be suggested that color analysis may be a more accurate, sensitive, and reliable marker. Typically, myoglobin is detected in urine when serum levels exceed values of 1,500 to 3,000 mg/ml (35). The measurement of myoglobin in serum is challenging to determine in the laboratory and is not always readily available.

No studies investigating rhabdomyolysis and its antecedent processes, such as muscle damage and myoglobinuria, through urine color analysis alone were identified. However, Mansor et al. (36) explored the relationship between color and dehydration, and Armstrong et al. (37) examined the interplay between density and color. In all these studies, color measurements were conducted using a composite color strip with eight distinct colors, which was initially introduced by Armstrong et al. (29) and is known as the “Urine Color Chart.” Relying solely on this color strip for urine analysis may not offer a highly dependable parameter for assessing physiological changes. This limitation arises from the fact that this strip summarizes colors into just eight categories, potentially overlooking intermediate shades of color between adjacent bands on the strip. Consequently, these intermediary shades may obscure indicators of normality when values are borderline, leading to a misinterpretation of potential renal system overload.

In this study, a single instance of urine was identified with a reddish color (distinct from the typical yellowish hue) and a satisfactory density value. This occurred after a physical activity involving a 14 km run in an environment with an ambient temperature of 18 °C. The soldier who produced this urine sample reported not using any substances to enhance performance or recovery, nor was he taking any medications. Furthermore, he did not experience muscle pain or severe fatigue. The reddish or brownish discoloration of urine (indicating myoglobinuria) results from the release of myoglobin into the bloodstream and subsequent excretion through the urinary system. This pathophysiological process is initiated by physical activity and can potentially lead to rhabdomyolysis and renal failure. This cycle of renal system damage persists until interrupted by rehydration with a saline solution (38, 39).

As noted by Yu et al. (4), the guidelines from the Brazilian Society of Nephrology for acute renal failure advise the use of saline solution, sodium bicarbonate, and mannitol in cases involving myoglobinuria and hemoglobinuria. These interventions are aimed at reducing the occurrence and severity of kidney damage. This suggests that while increased water intake can help hydrate the body and potentially alter the color of urine, the results from this study indicate that it may not effectively interrupt the pathophysiological damage caused by insufficient or inadequate fluid intake.
The randomized study conducted by Johnson et al. (40) concluded that consuming approximately 1,500 mL of additional water, in addition to the average daily intake of 2,990 and 3,515 mL, was necessary to restore urine to an appropriate color within 24 hours, even after three days of restricted intake. Research by Perrier et al. (41) involved experiments where total daily water intake was adjusted and demonstrated that an increase of 1,110 mL was required to reduce urine color by two units on an eight-point scale strip. Both Perrier et al. (42) and Kavouras et al. (43) emphasized that in adults and children, urine has the potential to indicate hydration status through color alone. Throughout the course, the soldiers were hydrated with plenty of oral water, as a result, the light-colored urine samples should have low numerical values of density and staining through spectrophotometry. However, this did not happen with several of the urine samples collected.

A limitation of this study is the lack of an association between these urine samples and a direct marker of muscle damage or renal system overload, as hyperhydration during the rest period can alter urine color.

CONCLUSIONS

The positive correlation between urine absorbance and density suggests signs of muscle fiber destruction, which may indicate the onset of rhabdomyolysis and renal system overload in participants of the operational course, as the collected samples showed high coloration values. On the other hand, the rating of perceived exertion did not directly justify an influence on the result of urinary analysis, but it did register high values on the scale, which may support the physical discomfort perceived by the military personnel. This underscores the high physical and psychological demands imposed on military personnel during an operational course.

The analysis of urine color by spectrophotometry and urine properties has emerged as a method with potential efficiency for detecting myoglobinuria. The absolute cutoff value obtained through urine coloration can indicate massive destruction of muscle structures during the execution of strenuous exercises. However, caution is needed when assessing the association between urine color and physiological damage, as other factors such as medication use, bacterial contamination, and overhydration may influence changes in urine color, masking potential pre-existing damage that could lead to rhabdomyolysis and renal failure.

For future studies, it is suggested to investigate the association between urine color, physical symptoms, and specific markers of renal system damage, such as Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Gamma-Glutamyl Transpeptidase (GGT).

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GHMD: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. RAR: investigation, methodology, formal analysis. RAMN: investigation, methodology, formal analysis, investigation. EBN: Investigation, methodology, formal analysis. GCPSMS: formal analysis, writing – review & editing, visualization. GCL: investigation, methodology, formal analysis, investigation. RGSV: conceptualization, methodology, formal analysis, investigation, writing – review & editing, visualization.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

Analysis of Muscle Damage through Urine as Prevention of Rhabdomyolysis


Effect of Therapeutic Ultrasound on the Recovery of the Tibialis Anterior Muscle in Remobilized Wistar Rats after a Period of Immobilization: An Analysis Using the Pathological Index

Ana Julia Kufner Meassi, Caroline de Souza Oliveira, Giulia Pedroso de Oliveira, Juliana Mayumi Novais, Rafaela Sotana Rodrigues, Lucinéia de Fátima Chasko Ribeiro, Dérick Patrick Artioli, Gladson Ricardo Flor Bertolini

1 Universidade Estadual do Oeste do Paraná (Unioeste), Cascavel, Paraná, Brazil
2 Centro Universitário Lusíada (Unilus), Santos, São Paulo, Brazil

SUMMARY
Background. Prolonged joint immobilization causes muscle hypotrophy and joint stiffness, which worsen as the immobilization time increases. Muscle remobilization is essential to restore muscle mass and activity as soon as possible. There are various treatments for muscle remobilization, but therapeutic ultrasound has proved to be a promising technique, with thermal and athermic actions, generating possibilities for increasing tissue extensibility and cell metabolism, helping with the anabolic effects of static stretching.

Objective. To evaluate the histopathological changes in the tibialis anterior muscle of Wistar rats after remobilization using therapeutic ultrasound, using the histopathological index.

Methods. 21 male Wistar rats were used, divided into 3 groups (n = 7): immobilization group, immobilization and free remobilization group, immobilization and remobilization with ultrasound group. Immobilization was carried out with the knee flexed at 120° and total ankle plantiflexion for three weeks. Ultrasound was used at a frequency of 1.0 MHz with an intensity of 0.5 w/cm², lasting 2 minutes on the knee and a further 2 minutes on the ankle. Analyses were carried out using the histopathological index to measure the level of alterations and lesions in the muscle tissue, classifying them as severe, moderate or mild.

Conclusions. It was concluded that the anabolic stimulus of ultrasound was favorable to the trophic recovery of muscle tissue.

KEY WORDS
Immobilization; ultrasonic therapy; pathology; skeletal muscle; physical therapy modalities.

INTRODUCTION
The reduction in muscle activity, especially when there is a lack of mechanical loads, such as in cases of joint immobilization, leads to various changes and adaptations in muscle tissue, including a reduction in physical performance, with loss of mass, endurance and muscle strength, with adaptation in neural activity generating a reduction in motor control. In addition to these effects, there is also an increase in the amount of intramuscular fibrous and adipose tissue, an angiotrophic effect and a reduction in the pain threshold, such characteristics being observed in experimental studies in both humans and animals (1-4).

Another effect that immobilization generates is inflammatory infiltration, which can be seen in degenerative changes...
in a joint immobilization model and joint contractures associated with fibrosis (1, 5-7). During remobilization, there are doubts about the behavior of the inflammatory process. It is argued that it tends to be less important than during immobilization (8), or that there is an increase in it due to microlesions and the involvement of nitric oxide (9). Remobilization is essential for joint and muscle patterns to be restored (9-11). There are several techniques for the remobilization period, however, there is no unanimity as to the best form, nor regarding the necessary period of stimulation (4, 10, 12-17). One of these techniques is therapeutic ultrasound, which among its effects generates analgesic, anti-inflammatory, pro-repair and tissue regeneration action, in addition to improving vascularization, stimulating cell activity and protein synthesis, contributing to tissue reorganization (18-20).

With a view to the striated skeletal muscle, evaluations of infiltrates and hypercellularity have been analyzed in the literature (21). The changes caused by both immobilization and remobilization can be assessed quantitatively by a histopathological index proposed by Zazula et al. (22) which analyses different degrees of tissue damage, with good replicability and parsimony. In view of the gap in this type of analysis in remobilization aided by the effects of therapeutic ultrasound, the aim of this study was to evaluate the histopathological changes in the tibialis anterior muscle of Wistar rats after remobilization using therapeutic ultrasound.

**Materials and Methods**

We used 21 male Wistar rats, aged 10 weeks, obtained from the Central Bioterium of the State University of Western Paraná. They were kept in polypropylene boxes, with access to water and food at will, room temperature maintained at 22 °C and a 12-hour light/dark photoperiod, grouped into three animals per box. The study was conducted in accordance with International Standards and approved by Unioeste’s Animal Use Ethics Committee (approval number - 09-21 – date of approval December 07, 2021). The animals were randomly separated into 3 experimental groups with 7 animals in each group:

- **Immobilization group (IG):** they were immobilized and euthanized after the 3rd week of immobilization.
- **Immobilization and Free Remobilization Group (FRG):** they underwent the immobilization protocol and remained for 3 weeks without receiving any type of treatment.
- **Immobilization and remobilization with ultrasound group (USG):** they underwent the immobilization protocol and received ultrasound treatment for 3 weeks during the remobilization period.

**Immobilization protocol**

To perform the immobilization, the animals were anesthetized and immobilized with a plaster bandage according to a model adapted by Wutzke et al. (4). The tibialis anterior muscle was immobilized in an elongated position for 3 weeks. The orthosis was molded from the abdominal region, just below the last ribs, and then to the right pelvic limb of each animal, with the knee joint in 120° flexion and the ankle in total plantiflexion.

**Therapeutic Ultrasound Protocol**

The Sonoplus Ibramed® device, which was certified for calibration during the research period, was used to carry out the ultrasound therapy. The parameters used were: effective radiation area (ERA) of 1 cm², continuous emission, frequency of 1.0 MHz, power density of 0.5 W/cm², for 2 minutes on the knee (1 min on the lateral side and 1 min on the medial side) and 2 minutes on the ankle (1 min on the lateral side and 1 min on the medial side) of the right pelvic limb of the animals belonging to G3, three times a week for three weeks, totaling 9 sessions.

**Euthanasia of the animals, preparation of slides and histopathological analysis**

The animals in group G1 were euthanized after 3 weeks of immobilization, while the other groups (G2 and G3) were euthanized after 3 weeks of remobilization. For euthanasia, the animals were previously anesthetized with an intraperitoneal injection of ketamine hydrochloride and xylazine and, after checking their state of consciousness by clamping the interdigital folds, the animals were decapitated using a guillotine. The tibialis anterior muscle of the right pelvic limb was collected and fixed in Metacarn (70% methanol + 20% chloroform + 10% glacial acetic acid) for 2 hours and stored in 70% alcohol, then processed for inclusion in histological paraffin. The muscles were cut transversely at a thickness of 7 μm using an Olympus CUT 4055 microtome, and the slides were stained in hematoxylin and eosin to measure the histopathological parameters.

**Histopathological index**

The histopathological index classifies lesions into three categories: severe, moderate or mild. The formula used to calculate the histopathological index was $X = a \times w$. In this formula, “$X$” represents the total sum of the damage observed in the muscle tissue. The “$a$” parameter corresponds to the score that measures the extent of the lesion, where 0 indicates no lesion, 2 represents minimal lesion, 4 indicates moderate lesion and 6 represents severe lesion. The “$w$” parameter refers to the pathological importance.
factor attributed to each lesion, which can be minimal, moderate or major.
The score obtained using the formula determines the injury index, and the higher the score, the greater the extent of the tissue damage. The injury index can vary, reaching a maximum of 320 points, depending on the characteristics and severity of the injuries present in the muscle tissue analyzed. The slides were analyzed using light microscopy and the changes observed were recorded in a Microsoft Excel® table. The structures found were measured and classified according to the index into: inflammatory and circulatory disorders - hemorrhage, edema, exudate and inflammatory infiltrate; regressive changes - rounded, angulated, divided fibers, with degeneration, vacuolization, atrophy, necrosis, myonuclei, increased nuclei, adipose tissue, altered nerve tissue and muscle spindles; and progressive changes - hypertrophy or hyperplasia of muscle tissue, hypertrophy or hyperplasia of connective tissue and neoplasms (figure 1).

Statistical analysis
Statistical analysis was carried out using the SPSS 20.0® program. Comparisons were made using Generalized Linear Models, with Fisher’s post-test (LSD) applied. The significance value adopted was p < 0.05 and the results were presented as mean and standard deviation.

RESULTS
When analyzing the histopathological index, significant differences were observed between all the groups. IG had the highest score compared to the others, with an average of 106 points. On the other hand, FRG had an average score 65 points higher than USG, which had an average of 20 points. These results indicate important variations in the lesions and alterations observed in the muscle tissues between the groups analyzed.
A number of tissue alterations were observed in the IG, with the following standing out: circulatory disorders, a centralized nucleus, a basophilic halo, connective tissue hypertrophy and tissue disorganization in both connective and muscle tissue. These alterations indicate a significant compromise in the structure and integrity of the tissues analyzed in the group.
When comparing the groups, important differences were observed in the alterations. Group FRG showed less tissue disorganization and a basophilic halo compared to group IG, suggesting a reduction in the inflammatory process in this group. On the other hand, group USG showed a decrease in connective tissue hypertrophy and a major circulatory disorder, although without the presence of edema. In addition, a significant decrease in the number of cells undergoing tissue necrosis was identified in the USG. These results indicate that USG showed an improvement in the inflammatory response compared to groups IG and FRG. These findings highlight the importance of considering the different responses and developments observed in the different groups during histopathological analysis.

DISCUSSION
This study evaluated the histopathological changes in the tibialis anterior muscle of Wistar rats after remobilization using therapeutic ultrasound for 3 weeks, in order to confirm its effectiveness. The histopathological index presented by Zazula et al. (22), was used for this purpose. This approach allows a detailed assessment of changes and lesions in muscle tissue, providing relevant information for understanding the damage that occurred in the context of this study, and an index with a significantly lower value was observed in the ultrasound-treated group, indicating a better restoration of tissue patterns within normality, i.e. less indicative of tissue damage.
The use of ultrasound is important in the musculoskeletal system, both diagnostically (23) and therapeutically (18). In this experiment, continuous therapeutic ultrasound was used, as this can provide a thermal effect, acting directly on tissue extensibility, reducing contractures and increasing local circulation (18, 24-27). In addition to these, ultrasound is also an important anabolic stimulus, acting on changes in cellular ion flow (mainly related to calcium, which in this case has anabolic rather than catabolic effects, which is also one of its functions in skeletal muscle) (28-33), resulting in, among other effects, a reduction in interleukin activity, an increase in the concentration of growth factors and protein synthesis, neovascularization and analgesia (27, 34-40).

All the effects mentioned above are extremely important during the remobilization period, as immobilization leads to a decrease in protein synthesis and an increase in protein degradation, with consequent muscle atrophy (1, 41), meaning that the anabolic stimulus of ultrasound is favorable to the trophic recovery of muscle tissue. Furthermore, since immobilization generates pro-inflammatory tissue characteristics, a reduction in angiogenic stimulus, as well as the replacement of contractile tissue by fibrous and fatty tissue (2, 3, 41), ultrasound may have an important action against these characteristics (37, 42). Also to be taken into account is the nociceptive alteration that immobilization generates (4), which in turn can affect the dorsal root reflex and the axonal reflex, promoters of neurogenic inflammation (43, 44), or be precisely caused by them. Regardless of the initial cause, the analgesic effects that ultrasound promotes (37) can prevent this configuration and thus produce effects such as those observed in the study of reducing the pathological tissue level, which was observed for GI and GRL that showed muscle fiber necrosis, hypertrophy and connective tissue disorganization. These findings show the effect of therapeutic ultrasound, which reduced connective tissue hypertrophy, muscle remodeling and the number of cells undergoing necrosis.

The absence of biochemical analysis which could indicate changes in interleukins and other inflammatory factors (45) is a limitation of the study, and suggestions for future studies which will be crucial to deepen the understanding of therapeutic ultrasound, including dosimetric curves, and its application in muscle rehabilitation, thus contributing to the development of more effective rehabilitation strategies to promote the recovery and adaptation of muscle tissue in situations of injury or functional incapacity. These suggestions are also aimed at advancing research towards clinical practice in humans. However, it is worth highlighting the novelty of this study, in which for the first time the histopathological index was used to analyze the muscle remobilization process.

CONCLUSIONS
It is concluded that therapeutic ultrasound has the potential to reduce the cellular inflammatory process, as evidenced by the reduction in connective tissue hypertrophy and the number of cells in the necrotic process in the tibialis anterior muscle.

FUNDINGS
Araucária Foundation through a call for proposals for basic and applied research.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
AJKF, CSO, GPO, JMN, RSR, LFCR, DPA, GRFB: conceptualization. AJKF, CSO, GPO, JMN, RSR: data collection. LFCR, DPA, GRFB: results analysis and interpretation. AJKF, CSO, GPO, JMN, RSR: writing – original draft. LFCR, DPA, GRFB: writing – review & editing.

REFERENCES
5. Lu W, Wang L, Wo C, Yao J. Ketamine attenuates osteoarthritis of the knee via modulation of inflammatory respons-
Ultrasound in Tibialis Anterior Remobilization

Clinical Assessment of Ankle Instability: An Update on Which, When, Why

Raffaele Vitiello¹,²,³, Andrea De Fazio¹,², Angelo Carosini¹,², Roberto Rossi³, Matteo Turchetta⁴, Giulio Maccuro¹,², Fabrizio Forconi³

¹ Fondazione Policlinico Universitario A. Gemelli IRCSS, Rome, Italy
² Università Cattolica Del Sacro Cuore, Rome, Italy
³ Clinic Villa Stuart, Rome, Italy
⁴ University Hospital Policlinico Vittorio Emanuele, University of Catania, Catania, Italy

INTRODUCTION
Defining chronic ankle instability (CAI) is not standardized yet. Chronic ankle instability (CAI) has been defined as “a condition characterized by repetitive episodes or perceptions of the ankle giving way; ongoing symptoms such as pain, weakness, or reduced ankle range of motion (ROM); diminished self-reported function; and recurrent ankle sprains that persist for more than 1 year after the initial injury” (1). The most commonly reported symptoms of patients with CAI are ankle failure, pain and swelling, mechanical instability and loss of strength. In addition, patients with CAI are often susceptible to recurrent sprains and functional instability (2). Symptoms can persist for years and up to 72% of people fail to return to previous levels of physical activity (2, 3).

Ankle instability results as one of the complications following an ankle sprain. Ankle sprain is the most common injury in sports medicine (4, 5), accounting for 10% to 15% of all injuries (5, 6). CAI patients are more frequently young and female (7).
Ankle instability should not be confused with ankle joint laxity. Joint laxity is the ability of a synovial joint to exceed the normal range of motion (8). Joint laxity plays an important role in recurrent postoperative knee and shoulder instabilities (9).

Some authors have demonstrated that generalized joint laxity (GJL) is an independent risk factor for poor outcomes and recurrence after surgical procedures in lateral CAI (10). However, there appears to be no association between GJL and the risk of injury in sporting activity, and its role in the CAI is still controversial (11). To evaluate joint laxity the most used and validated score is the Brighten score (BS) (8). However, a recent study has shown that it cannot be used in place of clinical tests and that it should be used with caution for the evaluation of CAI (12).

Ankle sprains account for 7% to 10% of all visits to the emergency room (6, 8-13). Most of these injuries happen to people under the age of 35 (13, 14). Instability is one of the most common complications resulting from an ankle sprain, especially after re-injury. The risk of re-injury or recurrent ankle sprain is between 12% and 47%. Some of these patients with acute or recurrent sprains may develop chronic ankle instability (CAI), making this condition relatively common (6, 13).

Having a dysfunctional ligament complex results in an unstable ankle. Ankle stability is provided by a ligament complex consisting of a medial compartment, a lateral compartment, and the ligaments of the syndesmosis. The lateral compartment is composed of 3 bundles: anterior talofibular ligament (ATFL), posterior talofibular ligament (PTFL), and calcaneofibular ligament (CFL). ATFL and PTFL give the ankle sagittal and rotational stability while the CFL gives stability in the coronal plane. The most frequent injury of the ankle is the rupture of the ligaments of the lateral compartment secondary to a supination trauma (14, 15). This will result in instability in several planes with a tendency for varus tilt, and the talus will tend to translate anteriorly.

It appears that ATFL is composed of two bundles, one more proximal intra-articular, and one more distal extra-articular; the distal one not only shares a fibular footprint with the CFL but is connected to it via some arciform fibers, forming the Later FibuloTaloCalcaneal Ligament (6, 8-13).

This is the anatomic support for the idea that the repair of the ATFL will produce an indirect re-insertion of the CFL. In addition to this further connection exist between the proximal fiber of ATFL and the Basset ligament. The superior intra-articular fascicle of ATFL is the first and the most often damaged ligament during an ankle inversion sprain and can be responsible for a subtle clinical condition called “microinstability”. When the lesion progress through the ATFL inferior fascicle and CFL a major instability occurs.

Medial instability can result from poorly treated isolated deltoid ligament injuries, combined ligament injuries, or inerterate lateral instability (16). The medial ligament complex is composed of 3 main structures: the deltoid ligament, the calcaneonavicular ligament, and the talocalcaneal ligament (6). The deltoid ligament plays a primary role in anteroposterior and rotational stability. It is composed of two main bundles: superficial and deep. The superficial one is composed of an additional four bundles, while the deep one is composed of two. The two deep bundles are composed of posterior and anterior tibiotalar ligaments. The superficial ligaments are composed of the tibiospring, tibionavicular, superficial posterior tibiotalar and tibiocalcaneal ligaments (16). Two clinical pictures may emerge after MLC ruptures: medial instability and rotational instability (17).

Several definitions and models have been proposed over the years to try to include all clinical conditions related to ankle instability. Initially, patients affected were divided by Hertel into two main groups: mechanical instability and functional instability (16, 18). This distinction was made based on the presence or absence of ankle laxity in clinical or radiological tests. Later, Hiller proposed an evolution of Hertel’s model, with three different types of patients with CAI:

- Mechanical instability.
- Perceived instability.
- Recurrent sprains (2, 3).

There is an additional group of patients, as we mentioned before, it is the micro unstable. Indeed, micro instability of the ankle can be defined as an occult source of mechanical instability resulting from infra-articular injuries (the superior intraarticular fascicle of ATFL is injured) that can be clearly identified with an arthroscopic examination and can be easily missed un MRI (18).

Another classification regards how to divide conditions into acute and chronic. It is not easy to define a chronic pathology because we do not have the resources to define when the body has stopped trying to repair the acute injury. For this reason, instability lasting more than 3-6 months is usually anecdotally defined as chronic.

Valid diagnostic support comes from radiology. The tests that can help clinicians are radiography, ultrasound and MRI. In stress radiography instability is revealed by the separation of bony structures which reveals a ligamentous inability. An absolute anterior translation > 9 mm or a translation > 5 mm concerning the uninjured side indicates a significant laxity of the ATFL, while an inclination of the talus angle > 10° in total or more than 5° concerning the contralateral indicates pathological laxity of the CF
It is important to start the clinical evaluation of a patient with CAI starting with a careful inspection and looking for anatomical alterations and signs of misalignment. It is important to recognize and diagnose an underlying former fracture. The typical symptoms of CAI are characterized by widespread pain (which is accentuated by the pressure of the ATFL), edema, swelling, reduced range of motion, and sometimes difficulty in walking (6). It is also important to evaluate the tenderness of the muscles and tendons which can be contracted or stretched (31). Bilateral evaluation is fundamental, always comparing the healthy side with the pathological one. This bilateral evaluation should be performed both in subsequent checks.

RANGE OF MOTION AND STRENGTH
During a clinical evaluation of a patient with CAI is important to evaluate the Range of Motion (ROM) of the ankle joint. One of the risk factors contributing to the onset of CAI is the reduction in dorsiflexion ROM (1, 2). Even though patients have this limitation of ankle range of motion they are still able to perform all daily activities, walking and sports activities but will tend to assume a more injury-prone position (3, 4). During the clinical evaluation, ankle strength is also examined using manual muscle testing or with instrumented dynamometry. Specific deficiencies in ankle strength have been identified as risk factors for Lateral Ankle Sprain (LAS) (8) as well as a characteristic of CAI (32).

ANTERIOR DRAWER TEST
The optimal time to test clinical stability for ligamentous rupture is between 4 and 7 days following the last injury when the acute pain and swelling have subsided, and the patient can relax (31). The anterior drawer test (ADT) is the most clinically important test for identifying ankle instability and is often the first test performed in the patient’s physical examination (31). This is used to assess the integrity of the Deltoid ligament, the major contributor to medial stability, and ATFL, the ligament of the lateral compartment of the ankle most frequently involved in LAS. According to Kovaleski et al. (23), the most anterior translation of the ATFL ligament occurred with the knee flexed to 90° and the ankle at 10° plantar flexion. The test is performed with the patient supine, the knee flexed, the ankle joint in 10-20° flexion, and the heel resting on the palm of the examiner’s hand resting on the table, thus stabilizing the heel. The examiner then stabilizes the lower limb while pulling the calcaneus forward, observing the amount of anterior translation. In ATFL rupture an anterior translation of the talus concerning the tibia could be observed, especially if the translation is markedly different from the opposite side. To date, there are still no definite measures in the literature for attributing positivity or non-positivity to the ADT, ranging from 2 mm.

PHYSICAL EXAMINATION
It is important to start the clinical evaluation of a patient with CAI starting with a careful inspection and looking for anatomical alterations and signs of misalignment. It is important to recognize and diagnose an underlying former fracture. The typical symptoms of CAI are characterized by widespread pain (which is accentuated by the pressure of the ATFL), edema, swelling, reduced range of motion, and sometimes difficulty in walking (6). It is also important to evaluate the tenderness of the muscles and tendons which can be contracted or stretched (31). Bilateral evaluation is fundamental, always comparing the healthy side with the pathological one. This bilateral evaluation should be performed both in subsequent checks.
HOP TEST (SIDE HOP, TIMED HOPPING, MULTIPLE-HOP, FIGURE-8-HOP, SQUARE HOP, ETC.)

Hop tests are a group of functional performance tests that compare the ankle with CAI with the unaffected side. Several variants can be administered to the patient, the main ones are:

1. Side-hop test requires the individual to jump over a 30-cm distance medially and laterally on a single limb. The fastest feasible time is used to complete ten repetitions. The execution time is the outcome.

2. Multiple-hop test: the subjects, in monopodial stance, were asked to jump and land with the same lower limb at 10 different points (grids 20 × 20 cm). During the execution of the test, the subjects were asked to try to balance the landings and avoid postural corrections. The test was assessed using 3 outcome measures: time to complete the test, perceived difficulty performing, and several balance errors.

3. Figure-of-8 hop: two cones are positioned 5 meters apart. The participant was told to hop as quickly as they could twice in a figure-of-eight manner around cones. The result is the turnaround time.

4. Square hop test: hops five times as quickly as they can in and out of a square on the ground that is 40 × 40 cm in size. The result is the execution time.

5. Six-meter timed hop test: a participant hops as quickly as they can on a 6 m long line. The result is the execution time.

6. Six-meter crossover hop test: a participant hops as quickly as they can while changing sides on a 15 cm width by 6 m long line. The result is the execution time.

Few studies in the literature show a difference between the functional tests of CAI and uninjured ankles (43, 44). Individually, the hop tests showed a low sensitivity in detecting patients with CAI, this ranged from 44 to 58%. However, it is also evident that the combination of various hop tests can increase the sensitivity, which on average goes from 50% to 62%, respectively, for one or two tests, but that the difference between two and four tests is negligible (45).

The side hop and timed hop test provided the best ability to discriminate patients with CAI. In particular, side hop was found to be the best, probably because this test puts the lateral ligament structures under greater stress (43). Some studies have also evaluated these tests in individual patients by comparing the result obtained from the injured side and the uninjured side. These studies demonstrated worse performance on the injured side with a difference in the time of execution of the tests. This difference is 0.37 seconds for the Figure 8 test, 0.57 seconds for the side hop test, 0.42 seconds for the 6-meter crossover hop test and 2.22 seconds for the square hop test (46).

FOOT-LIFT

The foot-lift test involves a single-legged stance on a firm surface and is used to assess static balance. The test involves counting how many times the foot raises off the ground in 30 seconds. One mistake was committed for every rise of the grounded foot counted on each side (47). Using a higher frequency of test-foot lifts throughout the course of a 30-second trial, this test was able to distinguish between people with and without CAI.

STAR EXCURSION BALANCE TEST (SEBT)

The Star Excursion Balance Test (SEBT) is a dynamic test described by Gray (48), as a rehabilitation tool consisting of...
a series of squats with only one limb to reach 8 points placed on 8 lines placed at 45° from each other. The directions are called anterior, anteromedial, anterolateral, medial, lateral, posterior, posteromedial and posterolateral. The measurement or result of the SEBT performance is the distance the participant can reach. A test was invalid if the balance was lost, the foot was lifted or moved from the center, the hands leave the hip, or the reach leg provided support upon touching down. A standardized protocol of 4 practice trials followed by 3 test trials was performed in each of the eight directions to minimize the learning effect. The average of the three test trials normalized for the length of the stance leg was used for analysis.

Not all directions of SEBT for CAI evaluation have a similar prognostic value, in the studies there is consensus in attributing the greatest diagnostic capacity to the anteromedial, medial, and posteromedial direction (48, 49). Some authors compared the injured and uninjured sides of participants with unilateral CAI and showed worse results in the unstable side, especially in the anterior, medial, anteromedial, and posterolateral directions (49, 50). Other authors have shown that healthy patients have a better total score when compared with patients with CAI (51).

Y-BALANCE TEST
Y-balance test is nothing more than a simplification of SEBT. It includes anterior, posteromedial, and posterolateral direction (52, 54). It has been shown that posteromedial distance achieved are those with higher prognostic value for CAI and therefore more clinically useful (53).

TALAR TILT TEST AND EVERSION TALAR TILT TEST
The talar tilt test was first described by Dehme in 1993 (54). This is a clinical maneuver to identify lateral ligament insufficiency of the ankle. The test is performed by holding the hindfoot by the calcaneus and applying varus stress while performing counterpressure with the other hand on the medial distal tibia (55).

Rosen et al. showed that the talar tilt test has good specificity (72-94%) but poor sensitivity (23-52%). The positive likelihood ratio (+LR) values for the talar tilt test are 2.23-4.14, the negative LRs are 0.58-0.66. The diagnostic odds ratios ranged from 1.43 to 8.96. It would thus appear that this test alone has little clinical utility, but may be useful when combined with a test with high sensitivity. It may be useful in the exclusion of a CAI (56).

The Eversion Stress test allows assessment of the stability of the deltoid ligament complex and thus the medial compartment. It is also known as Eversion Talar tilt Test. The patient is positioned in sitting or supine lying with the knee in full extension. The physician lifts and abducts the heel while stabilizing the distal tibia simultaneously. The test should be performed on each side. Greater laxity and pain on the injured side than on the uninjured side indicate a positive test (57).

NEUROLOGICAL EVALUATION OF FUNCTIONAL INSTABILITY
Superficial electromyography (EMG): the rationale behind this test is the fact that functional instability could result from some lesions in proprioception and the muscular response to nerve activation. Some authors have instead associated with CAI a delayed response of activation of the ankle muscles (58, 59). In particular, the Peroneus Longus (PL) and the short Peroneus Brevis (PB) seem to have a longer reaction time (RT) of about 1 ms in patients with CAI when the ankle is subjected to an inversion of about 30° (60), while if subjected to an inversion greater than 50° there would not seem to be a significant difference (61). There would not even seem to be any differences in the RTs of gluteus medius (GM) and erector spine (ES) thus excluding involvement of the proximal muscles (58).

As part of the neurological evaluation of the ankle in functional CAI, a very simple test that evaluates the proprioceptive capacity is the Romberg test. To perform the test, the patient must remain in an upright position leaning only on the limb to be examined, this position must first be maintained with the eyes open and then with the eyes closed. The test is positive if the position becomes unstable when the eyes are closed (61).

DISCUSSION
CAI is an extremely common post-traumatic condition that has a negative impact, especially on athletic patients’ outcomes. Most of the CAI can be attributed to the lateral compartment (62). Medial ankle instability is rarer than lateral instability, in fact isolated deltoid ligament injury accounts for approximately 3-4% of ankle ligament injuries and is often associated with fractures (6).

Today, there is no accredited diagnostic protocol to follow to identify and classify CAI patients and assess their eventual return to sports activity. Each test considered in this study has a consensus on the method of performance and efficacy, but there are no cutoffs to identify the CAI-affected population from the non-injured population. Based on our experience, we believe
that in a suspect of CAI, the diagnostic algorithm should begin with a clinical and anamnestic evaluation of the ankle involved. Asking about the traumatic mechanism of later injuries to identify the structures involved, which should be given priority during the clinical evaluation. We also need to investigate the time passed since the first episode of an ankle sprain. Previous episodes of lateral ankle sprains increase the risk of recurrence (63).

Having to formulate a diagnosis we can rely on clinical and radiological tests. Radiological exams have high specificity but not complete sensitivity. They could miss to diagnose the most suspect forms of CAI like the micro or functional instability. That’s why is mandatory to spend time and attention on clinical and functional examination. There are many clinical trials. Certainly, the most used, even in the acute phase, is the anterior drawer test which, however, only shows us the dislocation on the anterior plane and does not reproduce the classic traumatic movement in pronation. The ADLT variant overcomes this problem by neutralizing the effect of the medial ligaments. Besides, those tests are dependent on the skill of the examiner and the compliance of the patient. The subjective nature of this kind of approach needs to be integrated by standardized functional examination able to gain results as a sharp numerical score in a reproducible execution.

Hop tests appear to be a promising functional test suite for the identification of CAI. The difference in the time taken to perform the exercise between the healthy side and the injured side is significant. Using them individually, they do not have a high sensitivity. However, when used in combination with each other and when used to compare the two ankles of an individual with suspected CAI they appear to have a good diagnostic capability. However, their standardization in terms of outcome and cutoff is missing. The SEBT is a very useful functional test, especially in some particular trajectories. As for the hop tests, also for the SEBT, the main problem consists in not having cutoffs that distinguish the healthy patient from the patient with CAI. The test with the greatest sensitivity is the ALDT, and so it would seem logical to start with that in any diagnostic protocol. Later tests with higher specificity such as hope tests and the SEBT can be used as confirmation of CAI. Talar tilt test may be useful in ruling out CAI. It should also be kept in mind that these tests can be used in the follow-up of patients with ankle ligament injuries treated conservatively or surgically (64).

CONCLUSIONS
From our research, it could be hypothesized that the most promising tests for a correct diagnosis of CAI are the ALDT, two hop tests chosen by the clinician, the foot lift, and the SEBT also in the simplified Y-Balance version. In the patient with functional instability, a correct classification can derive from neurological tests such as the Romberg and EMG of the PB and PL. Patient with negative radiological exams but with symptoms and positive clinical and functional tests, it would be advisable to investigate further with ankle arthroscopy.

However, the major difficulty for a clinician in diagnosing CAI, is represented by the total absence of cut-offs in the various functional tests and by a standardized diagnostic protocol. In our opinion, further studies need to be directed toward this goal.

FUNDINGS
N/A.

DATA AVAILABILITY
N/A.

CONTRIBUTIONS
RV, ADF, AC: conceptualization, methodology, writing – original draft. GM, FF: validation. RV, ADF, AC, MT: investigation. MT: writing – review & editing. FF: supervision.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES
Clinical Assessment of Ankle Instability


Prevalence and Radiological Features of Fabella in a Saudi Arabian Population

Vasanthakumar Packirisamy¹, Khaled Aljarrah², Sameer E. Tasslaq³, Jayakumar Saikarthik⁴, Satheesha B. Nayak⁵

¹ St. Matthew’s University School of Medicine, Grand Cayman, Cayman Islands
² Jordan University of Science and Technology, Irbid, Jordan
³ College of Applied Medical Sciences, King Saud Bin Abdulaziz University for Health Sciences, Al Ahsa, Saudi Arabia
⁴ Department of Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Majmaah University, Al Majmaah, Saudi Arabia
⁵ Division of Anatomy, Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal, India

CORRESPONDING AUTHOR:
Vasanthakumar Pakirisamy
St. Matthew’s University School of Medicine
303 A, Leeward 4
Regatta Office Park
Grand Cayman, KY1-1204, Cayman Islands
E-mail: vasan_ana@yahoo.co.in;
vkumar@stmatthews.edu

DOI:
10.32098/mltj.02.2024.08

LEVEL OF EVIDENCE: 4

INTRODUCTION
Fabella is a small bean-shaped sesamoid bone located in the anatomically complex posterolateral region of the knee joint. Topographically, fabella lodges within the tendinous lateral head of the gastrocnemius (1). It develops by endochondral ossification from a combination of genetic regulation and compressive load on the gastrocnemius lateral head, which provides a biomechanical advantage (1, 2). As a genetically controlled structure, fabella originates from Sox9⁺/Scx⁺ progenitors under the regulation of transforming growth factor b (TGFb), while differentiation is mediated by BMP4 and BMP2 (3). The biomechanics of fabella are to alleviate friction-induced tendon injury, enhance the gastrocnemius muscle efficiency, and stabilize the posterolateral joint. Considering its ethnic variations and surgical importance, we measured the dimensions and recorded the prevalence of fabella in a Saudi population. This study aims to compare the features of fabella in Saudis with other populations and to test whether its incidence is associated with gender, side, laterality, and age.

SUMMARY
Background. The fabella, a sesamoid bone in the posterolateral aspect of the knee, induces clinical conditions. Considering its ethnic variations and surgical importance, we measured the dimensions and recorded the prevalence of fabella in a Saudi population. This study aims to compare the features of fabella in Saudis with other populations and to test whether its incidence is associated with gender, side, laterality, and age.

Methods. In this retrospective cross-sectional study, we reviewed knee radiographs and magnetic resonance imaging films of 820 patients aged 20-90 years. Descriptive statistics were performed to estimate the prevalence of fabella. The chi-square test was used to evaluate the associations of sex, age, and laterality with the prevalence of fabella. The size of the fabella was measured and compared between the sexes and with other studies using independent t-tests. The level of significance was set at p < 0.05.

Results. The overall prevalence of bony fabellae was 20.73 %, but no cartilaginous fabella was identified. Its prevalence was significantly associated with age (p = 0.009), right side (p = 0.003) and laterality (p = 0.002). The mean length, thickness, and width were 6.4 mm, 6.07 mm, and 4.74 mm, respectively. The size was significantly larger in males than in females (p < 0.001). The size of the fabella in Saudis is significantly larger than in Turks and smaller than in Asians (p < 0.001).

Conclusions. The anatomical features and prevalence of fabella in Saudis vary from those of other ethnic populations. It is clinically crucial to comprehend its variations and features for health professionals, aiding in the differential diagnosis of pathological loose bodies and preventing complications during knee arthroplasty.

KEY WORDS
Fabella; gastrocnemius; prevalence; radiological; Saudi; knee pain.
lateral part of the knee by reinforcing the fabellofibular liga-
ment. Additionally, the articulating groove on the back of
the lateral femoral condyle stabilizes the fabella (4, 5).
Medical professionals must know its normal variations,
prevalence, anatomy, and biomechanics to understand
the pathological conditions associated with the knee joint
and in the selection of treatment options (1, 6). Accord-
ing to ontogenetic theory, cartilaginous fabellae develop
in fetuses as early as 15 weeks, and once they ossify, they
are often evident on radiographs of the knee’s lateral aspect
(7). However, from a clinical point of view, it is crucial to
categorize its anatomical characteristics from osteophytes,
 intra-articular loose bodies, and intra-meniscal calcifications
in osteoarthritis (8). Although the fabella functions as a
knee joint stabilizer, its presence can lead to knee disorders.
These include chondromalacia, osteoarthritis, and Fabel-
la pain syndrome. Additionally, fabella hypertrophy causes
entrapment syndrome associated with the popliteal artery
and common fibular nerve (1, 9).
It is common in non-human mammals, but observation-
al epidemiological studies in humans have found that the
prevalence rate varies widely from 3 to 87% across different
populations (10, 11). Its prevalence is population depen-
dent, 3.1% to 31.3% in Caucasians and 30.6% to 85.8%
in Asians, with no significant difference between sexes (4,
10, 12, 13). As a result of this variation, studies on different
populations are warranted. To the best of our knowledge,
there are no baseline reports in the literature on the preva-
ience of fabella in the Saudi population. Against this back-
ground, in this study, we used radiographs and MRI imag-
es to describe the size and prevalence of fabella in a Saudi
population. The secondary aim was to compare the features
of fabella in Saudis with other populations and also to test
the hypothesis of whether its prevalence is associated with
gender, side of the limb, and age.

MATERIALS AND METHODS

Study design and subjects
This retrospective cross-sectional study was designed with
820 (410 males and 410 females, aged from 20 to 90 years,
1,640 knee MRI images) patients referred to the radiological
department for radiological investigation between 2016 and
2022. This study protocol (SP21A/289/06) was approved by
the Institutional Review Board of King Saud Bin Abdulaziz
University for Health Sciences, Al Ahsa, Saudi Arabia. The
inclusion criteria were patients aged more than 20 years.
The exclusion criteria were patients with advanced osteo-
arthritis, knee fractures and injuries, ligament injuries, and
if the posterior osteophytes and the fabella were not distin-
guished. The present study was conducted according to the
principles of the Declaration of Helsinki.

Data collection method
The patient data was collected from the hospital’s data
management system (BESTCare 2.0) from the Radiolo-
gy Department, King Abdullah Military City Hospital, Al
Ahsa, Saudi Arabia. The digital bilateral radiographs and
MRI scans of the knee were reviewed using the Picture
Archiving Communication System (PACS). In general,
radiographs of the knee were used to identify bony fabel-
lae, and MRI scans to detect both bony and cartilaginous
fabellae. All the identified fabella were osseous structures
located in the lateral heads of gastrocnemius. The incidence
of bony fabella on the MRI scans and radiographs of both
sides of the knees was documented according to age and
sex. The whole study group subjects were classified into
five age groups at an interval of ten years. The following
parameters, maximum length (figure 1), maximum thick-
ness of the fabella (figure 2), and maximum width (figure
3), were measured using different MRI views (sagittal, and
axial views). One researcher independently measured all the
fabella measurements using the software-integrated ruler
functionality. The measurements were repeated twice, and
the average value was calculated.

Figure 1. Sagittal section MR image of the knee demonstrates
the measurement of the maximum length of the fabella.
Statistical analyses

The IBM SPSS software package (ver. 23; IBM Corp., Armonk, NY, USA) performed the statistical analysis for Windows. Descriptive statistics (mean, standard deviation, frequency, minimum, and maximum) were used to evaluate the demographic and radiological features (size prevalence, side, and laterality). The chi-square test was used to determine the association of gender, age, and laterality with the prevalence of fabella. The fabella size was measured and compared between the sexes and with other studies using independent t-tests. The level of significance was set at $p < 0.05$.

RESULTS

The overall prevalence rate of fabella was found in 170 (20.73%) out of 820 subjects. The distribution of the fabella among the sexes is presented in Table I. The prevalence of fabella was greater in males [93 (22.7%)] than in females [77 (18.8%)], with no significant differences. It was found bilaterally in 114 (14%) subjects [62 (15.1%) males and 52 (12.7%) females] and unilaterally in 56 (6.85%) subjects [31 (7.56%) males and 25 (6.1%) females] with significant difference ($p = 0.002$). Among unilateral cases (56 subjects), the prevalence of fabella was significantly greater ($p = 0.003$) on the right side (39 subjects) than on the left side (17 subjects). The distribution of the fabella according to different age groups is presented in Table II. The prevalence rate of the fabella between the age category groups differed significantly ($p = 0.009$). Its prevalence was highest in the age category 41 to 50 years (28.6%), followed by the age categories 51 to 60 years (26.1%) and 61 to 70 years (27%), and decreased in the age categories over 70 years.

The overall mean length of the fabella was 6.4 ± 1.32 (4.1-9.1) mm, with 7.01 mm in males and 5.78 mm in females. The overall mean thickness of the fabella was 4.74 ± 1.29 (3.1-7.7) mm, with 4.99 mm in males and 4.49 mm in females. The mean width of the fabella in the overall population was 6.07 ± 1.30 (3.9-9.4) mm with 6.53 mm in males and 5.61 mm in females. The fabella was significantly ($p < 0.001$) longer, wider, and thicker in males compared to females (Table III).

The fabella dimensions of the Saudis differ significantly from those in previous studies in terms of ethnicity. On average the Saudi fabella is significantly shorter than those of Singaporeans and Romanians, but significantly wider and narrower than the Chinese. In contrast, the Saudi fabella is significantly longer, wider, and thicker than the Turks fabella (Table IV).
Table I. Crosstabulation of fabella laterality with sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Fabella absent, n (%)</th>
<th>Fabella present</th>
<th>Fabella present</th>
<th>Bilateral, n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Right n (%)</td>
<td>Left n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>317 (77.32)</td>
<td>23 (5.61)</td>
<td>8 (1.95)</td>
<td>62 (15.12)</td>
<td>410</td>
</tr>
<tr>
<td>Female</td>
<td>333 (81.22)</td>
<td>16 (3.9)</td>
<td>9 (2.2)</td>
<td>52 (12.68)</td>
<td>410</td>
</tr>
<tr>
<td>Total</td>
<td>650 (79.27)</td>
<td>39 (4.76)</td>
<td>17 (2.07)</td>
<td>114 (13.9)</td>
<td>820</td>
</tr>
</tbody>
</table>

Table II. Crosstabulation of age and presence of fabella.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Fabella Absent n (%)</th>
<th>Fabella Present n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>88 (89.80)</td>
<td>10 (10.20)</td>
<td>98</td>
</tr>
<tr>
<td>31-40</td>
<td>92 (76.67)</td>
<td>28 (23.33)</td>
<td>120</td>
</tr>
<tr>
<td>41-50</td>
<td>108 (72)</td>
<td>42 (28)</td>
<td>150</td>
</tr>
<tr>
<td>51-60</td>
<td>96 (73.85)</td>
<td>34 (26.15)</td>
<td>130</td>
</tr>
<tr>
<td>61-70</td>
<td>89 (72.95)</td>
<td>33 (27.05)</td>
<td>122</td>
</tr>
<tr>
<td>71-80</td>
<td>87 (87)</td>
<td>13 (13)</td>
<td>100</td>
</tr>
<tr>
<td>81-90</td>
<td>90 (90)</td>
<td>10 (10)</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>650 (79.27)</td>
<td>170 (20.73)</td>
<td>820</td>
</tr>
</tbody>
</table>

Table III. Comparison of parameters of the fabellae between Saudi males and females.

<table>
<thead>
<tr>
<th>Fabella parameters</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm)</td>
<td>7.01 ± 1.52 (6.89-9.1)</td>
<td>5.78 ± 1.33 (4.1-6.87)</td>
<td>6.4 ± 1.32 (4.1-9.1)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>6.53 ± 1.54 (3.9-9.4)</td>
<td>5.61 ± 1.18 (3.91-7.43)</td>
<td>6.07 ± 1.30 (3.9-9.4)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>4.99 ± 1.34 (3.1-7.7)</td>
<td>4.49 ± 1.21 (3.41-5.99)</td>
<td>4.74 ± 1.29 (3.1-7.7)</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

Table IV. Comparison of the fabella dimension (mm) of the present study results with other studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Ethnic group</th>
<th>Length</th>
<th>Width</th>
<th>Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeng et al. (11)</td>
<td>Asian (China)</td>
<td>-</td>
<td>11.67 ± 2.68'</td>
<td>5.76 ± 1.28'</td>
</tr>
<tr>
<td>Chew et al. (17)</td>
<td>Asian (Singapore)</td>
<td>7.06 ± 1.90'</td>
<td>6.12 ± 3.26</td>
<td>4.89 ± 1.94</td>
</tr>
<tr>
<td>Ortega and Olave (12)</td>
<td>Caucasian (Chile)</td>
<td>7.32</td>
<td>-</td>
<td>3.22</td>
</tr>
<tr>
<td>Pop et al. (14)</td>
<td>Caucasian (Romanian)</td>
<td>8.71±0.41'</td>
<td>4.81 ± 0.56'</td>
<td>-</td>
</tr>
<tr>
<td>Adedigba et al. (18)</td>
<td>African (Nigeria)</td>
<td>4.04</td>
<td>5.24</td>
<td>-</td>
</tr>
<tr>
<td>Özbay et al. (20)</td>
<td>Caucasian (Turkey)</td>
<td>6.05 ± 1.4'</td>
<td>5.92 ± 1.2'</td>
<td>4.63 ± 1.09'</td>
</tr>
<tr>
<td>Present study</td>
<td>Asian (Saudi Arabia)</td>
<td>6.4 ± 1.32</td>
<td>6.07 ± 1.31</td>
<td>4.74 ± 1.27</td>
</tr>
</tbody>
</table>

*Indicates the fabella dimensions of other studies that are significantly different from the present study.
DISCUSSION
Several epidemiological studies reported the prevalence of fabella using observational methods like radiography, computed tomography, MRI, or cadaver studies (table V). However, the reported prevalence rate varied among the methods, populations, sexes, and ages (4, 10-14). In light of its wide variations between the ethnic groups, we believe that general statistics on the presence of fabella cannot be extrapolated against other ethnic groups. As a result, in this study, we investigated the incidence of fabella in a Saudi population using a combination of MRI and radiography.

Asghar et al. (15) reported in a systemic review that the average prevalence rate of fabella worldwide from 34,733 knee joints is 25%. In the Saudi population, the prevalence of fabella is greater than the global average rate. As the literature review shows, fabella occurs more frequently in the East than in the West. Our study prevalence rate of fabella (20.73%) is on the higher end of the range reported (3.1%-31.3%) in Western populations, and it is much lower than the prevalence rates reported in East Asian countries: China (86.9%) and Japan (85.8%) (4, 10-12). The highest prevalence rate of fabella was reported in the Oceanians, followed by the East Asian population (1, 15). In contrast, another Southeast Asian study from Singapore reported a lower incidence rate (31.25%) (17). Our findings were comparable to the lowest prevalence rates in South Africa (11.94%) and Nepal (12.3%) (18, 19). Recently conducted MRI studies on the Middle Eastern population include Turkish and Omanis (24.1%). In addition, our research on Saudis found a prevalence rate of 20.73%, which is identical to Turks (20.63%) and lesser than Omanis (24.1%) (20, 21). A variation in fabella prevalence among Middle Eastern populations lends credence to the notion that the prevalence of fabella differs among ethnic groups.

The type of method also has an impact on the prevalence rate of fabella. For instance, Asian studies conducted using cadavers reported a higher prevalence of fabella than radiological studies because both cartilaginous and bony fabella can easily be identified and described in cadavers, which might have contributed to the high prevalence of fabella in cadaver studies.

Table V. Comparison of the prevalence of fabella in the present study with other studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Ethnic group</th>
<th>Method</th>
<th>Number of knee samples (n)</th>
<th>Prevalence of fabella (%)</th>
<th>M (%)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minowa et al. (4)</td>
<td>2004</td>
<td>Asian (Japan)</td>
<td>Cadaver</td>
<td>212</td>
<td>85.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kawashima et al. (23)</td>
<td>2007</td>
<td>Asian (Japan)</td>
<td>Cadaver</td>
<td>75</td>
<td>92</td>
<td>40.3</td>
<td>50</td>
</tr>
<tr>
<td>Silva et al. (10)</td>
<td>2010</td>
<td>Caucasian (Brazil)</td>
<td>Cadaver</td>
<td>64</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phukubye and Oyedele (22)</td>
<td>2011</td>
<td>South African</td>
<td>Cadaver</td>
<td>102</td>
<td>23.5</td>
<td>21.2</td>
<td>27.8</td>
</tr>
<tr>
<td>Piyawinijwong et al. (24)</td>
<td>2012</td>
<td>Asian (Thailand)</td>
<td>Cadaver; Radiography</td>
<td>372</td>
<td>50.53</td>
<td>20.16</td>
<td>18.54</td>
</tr>
<tr>
<td>Zeng et al. (11)</td>
<td>2012</td>
<td>Asian (China)</td>
<td>Cadaver; Radiography</td>
<td>61</td>
<td>86.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tabira et al. (25)</td>
<td>2013</td>
<td>Asian (Japan)</td>
<td>Cadaver</td>
<td>102</td>
<td>68.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ehara et al. (13)</td>
<td>2014</td>
<td>Asian (Japan)</td>
<td>MRI</td>
<td>653</td>
<td>30.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chew et al. (17)</td>
<td>2014</td>
<td>Asian (Singapore)</td>
<td>Radiography; MRI</td>
<td>80</td>
<td>31.25</td>
<td>31.94</td>
<td>25</td>
</tr>
<tr>
<td>Hauser et al. (5)</td>
<td>2015</td>
<td>European (Switzerland)</td>
<td>CT</td>
<td>400</td>
<td>26.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Egerci et al. (31)</td>
<td>2016</td>
<td>Caucasian (Turkey)</td>
<td>Radiography</td>
<td>1000</td>
<td>22.8</td>
<td>21.6</td>
<td>24</td>
</tr>
<tr>
<td>Ghimire et al. (19)</td>
<td>2017</td>
<td>Asian (Nepal)</td>
<td>Radiography</td>
<td>155</td>
<td>12.3</td>
<td>8.2</td>
<td>14.7</td>
</tr>
<tr>
<td>Ortega and Olave (12)</td>
<td>2018</td>
<td>Caucasian (Chile)</td>
<td>Radiography</td>
<td>400</td>
<td>31.25</td>
<td>20.45</td>
<td>42.3</td>
</tr>
<tr>
<td>Pop et al. (14)</td>
<td>2018</td>
<td>Caucasian (Romanian)</td>
<td>MRI</td>
<td>431</td>
<td>16.93</td>
<td>15.76</td>
<td>17.98</td>
</tr>
<tr>
<td>Calsikan et al. (30)</td>
<td>2020</td>
<td>Caucasian (Turkey)</td>
<td>MRI</td>
<td>500</td>
<td>28</td>
<td>31.88</td>
<td>23.21</td>
</tr>
<tr>
<td>Al Matroushi et al. (21)</td>
<td>2021</td>
<td>Asian (Oman)</td>
<td>Radiography; MRI</td>
<td>813;119</td>
<td>24.1</td>
<td>28.6</td>
<td>20</td>
</tr>
<tr>
<td>Adedigba et al. (18)</td>
<td>2021</td>
<td>African (Nigeria)</td>
<td>Radiography</td>
<td>754</td>
<td>11.94</td>
<td>14.55</td>
<td>10.04</td>
</tr>
<tr>
<td>Özbay et al. (20)</td>
<td>2022</td>
<td>Caucasian (Turkey)</td>
<td>MRI</td>
<td>1008</td>
<td>20.63</td>
<td>21.21</td>
<td>20.27</td>
</tr>
<tr>
<td>Present study</td>
<td>2024</td>
<td>Asian (Saudi Arabia)</td>
<td>Radiography MRI</td>
<td>870</td>
<td>28</td>
<td>31.88</td>
<td>23.21</td>
</tr>
</tbody>
</table>

M: male; F: female.
ence rate of fabella (4, 11, 22-25). Another reason for the high prevalence of fabella in the Asian population has been hypothesized to be a result of the daily activities that Japanese and Chinese engage in, such as kneeling, squatting, and tailor sitting. However, this plea alone does not explain the high prevalence (15). Although the Saudis and Omani also have the habit of flexing the knee while offering ritual prayers, the incidence of fabella was minimally lower than in the Asian mongoloids (15, 21). The difference in knee joint alignment among ethnic groups may also explain the higher prevalence of fabella. The Oceanians, Chinese, and Japanese have a valgus alignment of the femoral angle as opposed to the varus alignment seen in Middle Eastern populations (26, 27). The valgus alignment shifts weight-bearing from the medial to the lateral compartment. The pressure of the fabella against the posterior aspect of the lateral femoral condyle might spur the development and ossification of the fabella (5, 28).

In this study on the Saudi population, the overall mean length, thickness and width of the fabella were 6.4 mm, 4.74 mm, and 6.07 mm, respectively. Fabella lengths in the range of 4 to 22 mm with a diameter of about 10 mm have been reported in symptomatic patients (12, 35). Overall, the dimensions of fabella in Saudis were significantly larger than those of African and Turkish populations (18, 20). On the other hand, the fabella size in the Asians was larger than in the Saudis (11, 17). Despite the ethnic differences with Saudis, the fabella size was significantly larger in males than in females among Turks and Romanians (14, 20). These variations in fabella size and prevalence across populations and genders may be due to biomechanical, genetic, and geographical factors (17, 18).

Apart from the emphasis on ethnic differences in fabella prevalence, it has been widely debated in the existing literature if gender, laterality, and side are associated with fabella prevalence. Lamentably, discussion of this point is difficult because only a few studies established a significant association, but most authors failed to demonstrate any significant association. The overall prevalence of fabella prevalence in Saudis was much higher in males (16.33%) than in females (14.33%), with no significant difference. Similar to our findings, different studies reported a higher frequency rate of fabella in males, whereas few other studies found it in females with no significant sex difference (5, 12, 17, 19, 22, 23, 29). In contrast, Hauser et al. (5) and Adedigba et al. (18) found approximately a 1:1 prevalence of fabella in males and females. Furthermore, Ortega and Olave (12) and Akdeniz et al. (29) study’s found that the females outnumber males in the Chilean and Turkish populations by 1.5:1 and 2:1, respectively, with no significant differences. In contrast, another study from Turkey by Caliskan et al. (30) found that male predominance was statistically significant.

In terms of fabella laterality, the bilateral versus unilateral case ratio was 2:1, with a significant difference. A similar ratio was found in studies conducted by Adedigba et al. (18), Phukubye and Oyedele (22), Piyawinijwong et al. (24), and Egerci et al. (31), but no significant difference was observed. In line with our study, Akkoc et al. (32) found a significant difference in the ratio. This pattern reveals that the bilateral presence of fabella was higher than unilateral, regardless of ethnicity. Regarding the symmetry of fabella incidence in Saudis, the unilateral incidence of fabella is significantly more common only on the right side. However, no significant sex difference was found on both sides of the knee. In contrast, Phukubye and Oyedele (22), Caliskan et al. (30), and Egerci et al. (31) found no statistically significant difference between the sides. Matroushi et al. (21) observed a significant sex difference in the occurrence of fabella in the left knee.

Although the exact age of fabellar ossification is ambiguous, ossified fabellae have been found as young as 12-14 years old. Several studies have examined the relationship between prevalence rates and ages (13). In line with our findings, Matroushi et al. (21), Phukubye and Oyedele (22), and Akdeniz et al. (29) reported that the ossification of the fabella increases with age. In this study, fabella prevalence increased approximately up to age 70 and decreased gradually after the age 70s. In contrast, Berthame and Bull (1), Hauser et al. (5), Tabira et al. (25), and Egerci et al. (31) demonstrated no association between age and the presence of fabella. The structure of fabella has been characterized as either bony or nonbony in various anatomical investigations. Kawashima et al. (23) observed that the majority of the fabellae in the gastrocnemius’s lateral head were cartilaginous (57.3%) than the bony (34.7%) in a Japanese population. According to histological studies, the nonbony fabella is composed of dense connective tissue with a cartilage matrix. The bony fabellae had a cartilage matrix, compact bone with bone marrow, filled by adipocytes (11, 22). Minowa et al. (4) categorized the fabella based on texture as hard and elastic. On the other hand, Chew et al. (17) and Matuouishi et al. (21) reported that all the identified fabella were osseous structures in their investigation utilizing MRI scans. Similarly, all the observed fabellae were bony structures in the Saudis.

The strength of our study is that this is the first study to explore the prevalence and characteristics of fabella in a Saudi population using both plain radiographs and MRI films. Secondly, the medical images of the patients were enrolled from the archive system, allowing us to investigate big data sets with an accurate distribution of age.
and gender across the population so the results would be more representative. However, this observational retrospective study has some limitations. Since this study is from a single region of Saudi Arabia, the outcomes do not apply to the entire Saudi population. Furthermore, it was a retrospective study for which selection bias may be concerned. In light of the current study, further multicenter clinical research with larger sample sizes emphasizing the radiological and histological characteristics of fabella would be beneficial in the clinical diagnosis and treatment of patients with knee pain.

CONCLUSIONS
In conclusion, the prevalence of bony fabella among Saudis is 20.73%, with males typically having larger fabella size than females. The prevalence rate correlated positively with age but was not influenced by sex or laterality. Compared to Turks, Saudis have a similar prevalence rate but larger fabella size, while Asians have a higher prevalence rate and larger fabella. Given variations across populations, understanding the fabella prevalence rate and characteristics in Saudis is crucial for accurate diagnosis and preventing complications during knee arthroplasty.

REFERENCES
15. Asghar A, Naaz S, Chaudhary B. The Ethnic and Geographical Distribution of Fabella: A Systematic Review and Meta-Analy-


Comparison of Radiological Parameters to Predict the Adult Acquired Flatfoot Deformity (AAFD) in Indian population: A Retrospective Study

Yuvraj Gowtham Krishna¹, Hitesh Shah¹, Monappa Aroor Naik¹, Peruvaje Ramakrishna Krishnaprasad¹, Karthikeya Damodar Hebbar¹, Yelluru Lakshmisha Rao², Bukkambudhi Virupakshamurthy Murlimanju²

¹ Department of Orthopaedics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India
² Department of Anatomy, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

SUMMARY
Background. The purpose of the study was to describe the most sensitive and specific radiological parameter to diagnose symptomatic adult acquired flatfoot deformity (AAFD) with weight bearing radiographs in Indian population.

Methods. A retrospective study was conducted in which 50 consecutive patients with 100 flat feet were included at department of orthopedics from August 2021 to October 2022. Amongst them, 32 were bilateral (64 feet) and 18 were unilateral (18 flat feet and 18 normal feet) flat feet.

Results. Out of these 50 subjects, 32 (64%) had bilateral flat feet, 18 (36%) were unilateral (11 left foot and 7 right foot). Out of 50 patients (100 feet), 24 had symptoms on left foot, whereas 26 had symptoms on right foot (total number of symptomatic patients were 50). Amongst our study population (50 subjects), 23 (46%) patients did not have any co-morbidities. 18 (36%) patients had type 2 diabetes, 5 (10%) patients had hypertension, 3 (6%) patients were associated with hypothyroidism and one individual (2%) showed psychiatric disorder. The talar 1st metatarsal angle in lateral view, talar uncoverage angle and the talar 1st metatarsal angle in anteroposterior view, were found to be statistically significant.

Conclusions. Three radiologic parameters-talar 1st metatarsal angle in lateral view and talar uncoverage angle and talar-1st metatarsal angle in anteroposterior (AP) view, can be the most relevant radiological parameters in the assessment of AAFD in Indian population. We also conclude that the talar uncoverage angle has a higher sensitivity of 82% to predict symptomatic flat foot in adults.

Keywords. Flatfeet, Indian population; evaluation; radiological parameter; radiographs.

BACKGROUND
Painful, AAFD is a common condition, which is also known as pes plano-valgus, pes planus, or lateral peri-talar subluxation. The deformity involves “shortening” of the lateral column, plantar inclination of the talar head, and lateral peri-talar subluxation of the navicular bone (1). Dysfunction of the posterior tibial tendon is a most common pathology to cause AAFD, associated with collapse of the medial longitudinal arch, subtalar joint eversion, forefoot abduction at the talonavicular joint, and hindfoot valgus (1, 2). AAFD can be diagnosed by multiple modalities and one of the most common method is clinical evaluation supported by plain
weight bearing radiographs, which is reliable and affordable (3). Younger et al. (4), in his study, the ‘radiographic assessment of adult flatfoot’ states that a few selective radiographic parameters can be used as a diagnostic tool for flat foot deformity in adults. Among the several radiographic parameters, few parameters like calcaneal pitch angle, arch angle, talar first metatarsal angle, lateral talar 1st metatarsal angle, talar inclination angle and the navicular index are of significance as described by Khan et al. (2). MRI can be considered as the gold standard for detecting posterior tibial tendon dysfunction and can be used to get a three-dimensional image of the foot and thereby accurately diagnose AAFD. But the advantages of using simple lateral radiograph of foot to diagnose flatfoot cannot be discredited as it is fast, low cost and simple to perform (5). The present study is relevant, as there is no study till date to our knowledge, which describes most sensitive and specific radiological parameter to diagnose symptomatic AAFD in Indian population. The present study aims to identify and define the most relevant radiological parameter for assessment of AAFD.

MATERIALS AND METHODS

Patients, presented with AAFD to outpatient department of orthopedics of our institution were chosen for the study. The inclusion criteria were adult patients with AAFD between the age of 18 to 70 years. This research included fifty patients, among them, 33 (66%) were female and 17 (44%) were male. Database, including demography, clinical symptoms were identified. Patients presented with pain, commonly had posterior tibial tendon dysfunction which was diagnosed clinically and confirmed by ultrasonogram. The patients with previous surgeries of foot and ankle, with pre-existing congenital/developmental/metabolic deformities of foot and ankle, and patients with rheumatoid arthritis or osteoarthritis and Charcot arthropathy involving the lower limb joints were excluded. Weight bearing plain radiographic views, antero-posterior (AP) and lateral were taken, with patient standing on straight knee, with the hands resting on railings and the opposite foot non-weight bearing for both the views (4).

After obtaining the radiographs in digital format with picture archiving and communication system version 4 (PACS), the patients’ identity information was cropped, the contrast and brightness were optimized, and the measurements were performed as per the description by Younger et al. (4) and Chadha et al. (6). The standard normal radiological values were considered from the study done by Flores et al. (7). After blinding and assigning the random numbers, intra and interobserver error was determined using 10 radiographs viewed on two occasions by two observers (fellowship trained foot and ankle surgeon and musculoskeletal radiologist).

The 82 flatfeet from our subjects were compared with the 18 normal feet. These 18 normal feet were from the unilateral flatfoot patients of our study group. This means that, these 18 patients were having flat foot only one side and on the other side, there was no flat foot (Table I).

Table I. Patient details.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>44.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deformity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral flatfoot</td>
<td>32</td>
<td>64.0</td>
</tr>
<tr>
<td>Left flatfoot only</td>
<td>11</td>
<td>22.0</td>
</tr>
<tr>
<td>Right flatfoot only</td>
<td>7</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Data analysis

Data obtained was stored and analyzed using the statistical package for Social Sciences Software (SPSS) version v 21.0 IBM corporation (SPSS Inc., Chicago, Illinois). Descriptive statistics were used to describe epidemiological variables such as age, gender, comorbidities (like hypertension, diabetes), side of symptomatic foot. Independent t-test was used to compare independent variables including, the radiological parameters for the assessment of flatfoot. A P-value of < 0.05 was considered significant. For the correlation of the radiological parameters between symptomatic and asymptomatic flatfeet, Spearman’s correlation coefficient was used. P-value of < 0.05 and area under the ROC curve of more than 0.6 was considered statistically significant.

This present study has the approval of our Institutional Ethics Committee, and it is in accordance with the international ethical standards, as per the opinion of Padulo et al. (8). Before starting the present study, the approval was obtained from Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee (Registration number ECR/146/Inst/KA2013/RR-19; DHR Registration No.EC/NEW/INST/2019/374; reference number IEC:399/2021; date of approval: April 14, 2021). Since, the present study is a retrospective analysis of the data, signed informed consent to participate and consent to publish from the patients are not applicable.

RESULTS

The total number of symptomatic patients in this study were 50. Among them, 32 (64%) had bilateral pes planus, 11 (22%) had left side flatfoot and 7 (14%) had right side flat foot (Table I). Overall 100 feet, 24 had left side symptoms, and 26 had right side symptoms. Out of 50 subjects, 23 (46%) of the subjects did not have any co-morbidities. Type 2 diabetes 18 (36%) was the most common co-mor-
bidity followed by hypertension 5 (10%), hypothyroidism 3 (6%) and psychiatric disorder 1 (2%) (table II).

Table II. Distribution of subjects based on the symptomatic side and co-morbidities.

<table>
<thead>
<tr>
<th>Symptomatic side</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>24</td>
</tr>
<tr>
<td>Right</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Co-morbidities</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 DM</td>
<td>18</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>5</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>3</td>
</tr>
<tr>
<td>Psychiatric disorder</td>
<td>1</td>
</tr>
</tbody>
</table>

Comparing different angles and heights, between flatfoot and normal foot in our study population

After the assessment of all the radiological parameters, it was observed, that the lateral talar 1st metatarsal angle (figure 1), the talar 1st metatarsal angle (figure 2) and talar uncoverage angle (figure 3) AP view were found to be statistically significant (p < 0.05) (table III).

Sensitivity, and specificity amongst the statistically significant three radiological parameters

With respect to lateral talar-1st metatarsal angle, at a cut-off value of 13.2250°, the sensitivity and specificity was 58% and 70%, respectively. With respect to talar-uncoverage angle, at a cut-off value of 10.055°, the sensitivity and specificity was 82% and 60%, respectively. With respect to talar 1st metatarsal angle in AP view, at a cut-off value of 8.64°, the sensitivity and specificity was 76% and 62% respectively (table IV).

Figure 1. Lateral view, weight bearing radiograph, showing lateral talar 1st metatarsal angle (Meary angle). It is the angle created by drawing lines along the axis of the talus bone and the first metatarsal. These axes are normally parallel, but in this subject with AAFD radiograph it is showing an angle of 9.32°.

Figure 2. Anteroposterior (AP) view, weight bearing radiograph, showing AP talar 1st metatarsal angle. It is the angle created by drawing lines along the axis of the talus bone and the first metatarsal. Normally talar axis is angled slightly lateral to the axis of the 1st metatarsal shaft (13), but here talar axis is making an angle of 22.06° to that of 1st metatarsal axis.

Figure 3. Anteroposterior (AP) view, weight bearing radiograph, showing talar-uncoverage angle. It is the angle created by articular surface of talar head and articular surface of proximal navicular bone. Normally they are parallel. In the present radiograph it is making an angle of 6.83°.

The comparison of relevant radiological parameters of foot alignment for the assessment of AAFD in our study with the standard is represented in table V.
### Table V. Comparison of relevant radiological parameters of foot alignment for the assessment of AAFD in our study with the standard.

<table>
<thead>
<tr>
<th>Radiological view</th>
<th>Radiological metric</th>
<th>Construction</th>
<th>Normal (7)</th>
<th>Abnormal</th>
<th>Our study (mean)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>Talar-first metatarsal angle</td>
<td>Angle between the long axis of the talus and the long axis of the first metatarsal</td>
<td>0° (parallel)</td>
<td>Mild: &gt; 4 Moderate: &gt; 15 Severe: &gt; 30 Pes planus: &lt; 18</td>
<td>15.16°</td>
<td>0.001</td>
</tr>
<tr>
<td>Antero-posterior</td>
<td>Talar un-coverage angle</td>
<td>Angle between the articular surface of the talus head and the articular surface of the proximal navicular bone</td>
<td>0° (parallel)</td>
<td>Pes planus: &gt; 7</td>
<td>15.77°</td>
<td>0.000</td>
</tr>
<tr>
<td>Antero-posterior</td>
<td>AP talar 1\textsuperscript{st} metatarsal angle</td>
<td>Line drawn along the long axis of the talus, extended into the forefoot, its orientation compared with that of the first metatarsal shaft</td>
<td>0° (parallel)</td>
<td>Mild: &gt; 4 Moderate: &gt; 15 Severe: &gt; 30</td>
<td>12.61°</td>
<td>0.000</td>
</tr>
</tbody>
</table>
**DISCUSSION**

The present study aimed to identify the most common radiological parameter to define AAFD and also the most sensitive and specific radiological parameter to diagnose symptomatic flatfoot with plain radiographs. Though multiple radiological parameters can be measured, there is no specific radiographical parameter to define and diagnose AAFD amongst Indian population. In a study done by Vulcano et al. (9), on approach to treatment of AAFD, states that the peak age of presentation was 55 years and women were more commonly affected than men. In our study it was observed that the mean age of presentation is 49 years, which is lesser than the mean age reported by Vulcano et al (9). However, in our study too, females were more commonly affected than males (66 and 44 percent respectively). In a study done by Goss et al. (10), on natural history of flatfoot reconstructions in AAFD, over a period of 14-year, it was found that among 321 patients, 13.7% had diabetes and 3.7% had rheumatoid arthritis associated with AAFD. In our study it was found that, 36% of patients had type 2 diabetes mellitus and 10 percent had hypertension. In the present study, we found the mean value of calcaneal pitch angle in the AAFD was higher (14.05 ± 4.80), which was statistically non-significant (p = 0.5). In a study done by Lo et al. (11), among young Taiwanese male military recruits, states that, the calcaneal pitch angle with a cut off value of 12.06° was the best predictor of AAFD. In contrast to the above study, we found, the lateral-talar 1st metatarsal angle, talar uncoverage angle and the talar-1st metatarsal angle in AP view were more specific in diagnosing AAFD. However, our study includes a wider age group and both genders.

In our study, we found that the lateral talar 1st-metatarsal angle (Meary’s angle), with a P-value of 0.001 with the mean value of (15.16 ± 6.56) on AAFD compared to normal feet (10.68 ± 5.16), which was correlating with a study done by Lebel and Karasik (12), who had also evaluated weight bearing lateral radiographs in 30 patients with surgically proven posterior tibial tendon tear induced AAFD with tenosynovitis, found that the lateral talar 1st-metatarsal angle was increased in 47% of patients. Lalevée et al. (13), also reported that Meary’s angle was significantly higher (p = 0.037) in case of AAFD with posterior tibial tendon deficit. In our study, the medial column height with a P-value of 0.159, and the mean value (1.59 ± 0.25) was higher in the normal side, in comparison to side with AAFD. However, this had no significance in diagnosing AAFD. This result was comparable to study done by Kang et al. (14), who had studied the lateral and medial column lengths in patients with AAFD and found that there was no statistical significance with the medial column height when comparing patients with flatfoot and normal foot with a mean value of 1.08 cm. In our study, we found that the radiographic parameter, 5th metatarsal to medial cuneiform height was higher in the side with flatfoot (0.44 ± 0.21) than the normal side (0.41 ± 0.16). However, this measurement was found with no statistical significance. This was comparable to the study done Younger et al. (4), who also found that the 5th metatarsal to medial cuneiform height with a (P-value of < 0.0001) was significant to diagnose AAFD. The lateral column height, in our study with a P-value of 0.16 on the right side and 0.22 on the left side had no significance in diagnosing AAFD or predicting the symptoms. These results were correlating with an observation by Kang et al. (14), who did a study amongst 85 feet having AAFD and they concluded that the lateral column height was not a statistically significant parameter. This conclusion was similar to our study. Based on these findings it is evident that, the lateral column height is not an accurate predictor of AAFD.

The talar uncoverage angle also known as the talo-navicular coverage angle. In our study, we found that this radiographic measurement was statistically significant parameter and the mean talar uncoverage angle was higher in AAFD (15.77 ± 7.58) compared to normal foot (10.23 ± 7.47). The difference in talar uncoverage angle was also found to be significant statistically (P-value < 0.05). The talar uncoverage angle had a sensitivity of 82% which was higher amongst all the radiological parameters measured. This shows that the talar uncoverage angle can be used as a screening tool in outpatients, to predict AAFD due to its high sensitivity. Filardi et al. (15) states that talonavicular uncoverage angle of greater than 7° indicates, lateral talar subluxation and forefoot abduction. This parameter can be a reliable diagnostic tool in patients with AAFD. Moreover, this observation is correlating with our study, as even our mean value of talar uncoverage angle in flatfoot patients was more than 7°. Knutson et al. (16) identified the relevant radiographic measures which were correlating with the articular coverage areas of clinical interest, possibly aiding to the better quantification of progressive collapsing foot deformity.

In our study, the mean value of calcaneo-fifth metatarsal angle was lower in flat foot (160.094 ± 6.53) in comparison to normal foot (161.93 ± 6.53), which was not significant statistically (P-value > 0.05). This result was in accordance with the study by Younger et al. (4), who also found that the calcaneo fifth metatarsal angle is not significant. Hence, we can conclude that the calcaneo-fifth metatarsal angle is not a reliable parameter in diagnosing the AAFD. In our study, the mean value of talar 1st metatarsal angle, measured in AP view, was higher in flatfoot (12.61 ± 5.26) compared to normal foot (9.00 ± 4.25). The difference in talar 1st meta-
tarsal angle was found to be significant statistically (P-value < 0.05). This observation was similar to the study done by Younger et al. (4).

A cross-sectional study conducted in Spanish population, estimated the prevalence of flatfoot as 26.62% in the patients over the age of 40, with a higher incidence in older patients (17). In another Japanese study (18), there was identical results in comparison to the Spanish study as there was a prevalence of 26.5% in patients aged 60 and above. However, these patients were neither hospitalized nor disabled and were performing their regular activities (18). Almaawi et al. (19) used three different footprint parameters, Clark’s angle, Chippaux-Smirak index, and Staheli index in Saudi Arabian population to define the foot arch. They were able to observe wide variations between the results of each parameter in their study. However, the Chippaux-Smirak and Staheli indices exhibited a significant concordance in diagnosing the flatfoot with kappa value > 0.8 and P-value < 0.05. In Indian population, a cross-sectional study (20) was done with 500 healthy participants, who were aged between 18-21 years and the flatfoot prevalence were determined as 13.6%. In males, it was 12.8% and 14.4% in females (20). The difference in frequency of prevalence in these studies with respect to Indian population is due to the fact that the authors used different methods.

There are few clinical studies (20-23), which are available in the literature about the flatfoot in Indian population. Rao and Joseph (23) observed a higher prevalence (8.6%) of flatfoot in Indian children, who used closed shoe footwear. This was higher with respect to the children who were predominantly using the sandals or barefooted. Sachithanandam and Joseph (22) observed an association between the duration of footwear usage and the flatfootness. However, the literature search did not reveal radiological studies with respect to the clinical diagnosis of the AAFD in Indian population. Hence, this aspect is being highlighted in the CONCLUSIONS.

From our study we can conclude that, three radiological parameters, the talar 1st metatarsal angle (lateral view), talar uncoverage angle (AP view) and the talar-1st metatarsal angle (AP view) can be used as a reliable diagnostic tool to diagnose AAFD in Indian population. We also conclude that the talar uncoverage angle certainly has a higher sensitivity of 82% to predict the symptomatic AAFD.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES


The Screening Value of Single Leg Squat and Vertical Drop Jump for Predicting Lower Limb Injuries in Professional Male Football Players

Haniyeh Mohammadi\textsuperscript{1}, Raheleh Ghaffari\textsuperscript{2}, Mahdi Hosseinzadeh\textsuperscript{3}

\textsuperscript{1} Sport Injuries and Corrective Exercises, Faculty of Physical Education and Sports Sciences, Shomal University, Amol, Iran
\textsuperscript{2} Sport Injuries & Corrective Exercises, Faculty of Sport Science, Shomal University, Amol, Iran
\textsuperscript{3} Department of Sport Injuries and Corrective Exercises, Sport Sciences Research Institute, Tehran, Iran

CORRESPONDING AUTHOR:
Mahdi Hosseinzadeh
Department of Sport Injuries and Corrective Exercises
Sport Sciences Research Institute
5\textsuperscript{th} Alley No. 3, Miremad Street, Motahhari Street
Tehran, Iran
E-mail: Metti@ssrc.ac.ir
DOI:
10.32098/mltj.02.2024.10
LEVEL OF EVIDENCE: 2

INTRODUCTION
Complications caused by football injuries, including damage to the player’s individual health and the economic, social and health system (1), have led researchers to injury prevention programs and identification of risk factors (2, 3). In elite football, 30.3 to 47.9\% of injuries that lead to loss of time for training and competition are related to the lower limbs (4). Since biomechanical and neuromuscular factors are internal variables that can be developed, these injuries can be reduced as much as possible by following an organized prevention program. Neuromuscular defects cause disturbances in balance, strength, power and create patterns that increase joint loads and compensatory movements in the lower limbs (5, 6). One of the critical movements that cause ACL injury in players is the landing phase after jumping (heading in football) (7). Knee loading and knee motion during landing predicts the risk of anterior cruciate ligament injury in female athletes (8). Among the injury prevention programs are functional movement screening tests that are challenging \textit{per se} (9-11). These functional tests are suitable tools for evaluating the functional capacity of the lower limb and analyzing injury risk factors (12). The Single Leg Squat (SLS) and Vertical Drop Jump (VDJ) tests are among the functional tests that are used to identify core strength and biomechanics after landing (13, 14). To date, conflicting evidence has been found regarding the effectiveness of these tests in predicting injury risk. Petushek \textit{et al.} used SLS and VDJ tests in order

SUMMARY
Objective. To evaluate the ability of Single Leg Squat (SLS) and Vertical Drop Jump (VDJ) tests to predict lower limb injuries in professional footballers.

Methods. In a prospective cohort study SLS and VDJ scores were recorded before the start of the season and the lower limb injuries were recorded as followed up during a 9-month season (2021-2022). Logistic regression analysis was used to determine the accuracy of the prognosis of SLS and VDJ tests.

Results. During the nine months of follow up, a total of twenty-five lower limb injuries (in twenty-three footballers) were recorded for 121 professional male footballers. The model explained 22\% (Nagelkerke R\textsuperscript{2}) of the variance in lower limb injury and correctly predicted 85.1\% of cases. ROC analysis showed significant accuracy of the VDJ performance score of the footballer (AUC 0.659, 95\%CI 3.639-63.434, p = 0.001, OR = 0.204) in discriminating between injured and uninjured players. The optimum cut-off level of the VDJ performance score of the football players was 0.5.

Conclusions. VDJ test is a suitable tool for predicting lower limb injuries in professional male footballers, but SLS cannot be used as a reliable screening tool.

KEY WORDS
Single leg squat test; vertical drop jump test; screening test; football player; sports medicine; soccer.
to screen athletes prone to anterior cruciate ligament injury. According to their findings, there was no relationship between these two tests and the risk of future injury in athletes (15). In another study, the SLS test, however, was introduced as a suitable tool for use in sports examinations to evaluate dynamic knee valgus and the risk of lower limb injury (16). Redler et al. also demonstrated the validity of a field-based observational vertical drop jump test as a screening tool to identify athletes at risk for ACL injury (17). To the best knowledge of the authors the findings regarding the screening value of SLS and VDJ is not clear enough and contradictory findings has been reported. Furthermore, such research has not been conducted on a professional population of Iranian football players. The purpose of the present study was therefore to investigate the screening value of SLS and VDJ scores for predicting lower limb injuries in professional Iranian male football players.

**MATERIALS AND METHODS**

**Participants**

The participants included 121 professional male football players of the National League One (Azadegan League) – age: 22.78 ± 4.60 years, height: 180.02 ± 5.09 cm, weight: 72.45 ± 6.26 Kg, BMI: 22.29 ± 1.34 Kg/m²) – who were expected to participate in the 2021-2022 season. Registration of player injuries was prospectively followed for one football season (about 9 months).

**Procedures**

This study was conducted based on the principles of the Declaration of Helsinki and its latest amendments, and the study protocol was approved by the research ethics committee of Sport Science Research Institute of Iran (IR.SSRC. REC.1401.084 – date of approval: December 21, 2022). All players signed an informed consent form to participate in the study. Participants had the right to withdraw from the study at any stage without further notice.

In a briefing session, the football players were taught the correct procedures and procedures for performing SLS and VDJ tests. A physical therapist with three years' sport experience checked the health of the participants before entering the test. All the functional tests were conducted on the football field in order to standardize the application of tests, and to simulate football matches.

**Functional screening tests**

Before performing the tests, the athletes performed a standard warm-up procedure including (5 minutes of running, dynamic stretching, small skips, open and close the gate, heel kicks, low shuffle and carioca) (18). An experienced athletic trainer (more than 3 years of physical therapy practice, expert in evaluating individuals’ sport performance through SLS and VDJ in their daily clinical practice) participated as the evaluator.

**Single leg squat**

This test was performed similar to the single leg squat test described by Sciaccia and Kibler (19). The athletes were asked to put their hands on their waists and bend 90 degrees on one leg while looking forward. If the athletes keep the opposite leg in front of the body during the squat, the opposite leg touches the standing leg, the opposite leg touches the ground, the hands are removed from both sides of the body, or if the athlete loses his balance, the test was considered invalid. The performance of the right and left leg was recorded separately. All the participants started the test with the right foot (20). Athletes' ability to control their knee in the frontal plane was evaluated using a graded scoring scale from 0 to 2. A score of zero equaled good control. In fact, the participant demonstrates proper alignment with a straight line from the knee to the toes, no obvious valgus movement in either knee, and no mediolateral knee sway during performance. A score of one corresponded to decreased control and indicated poor alignment, with one or both knees moving into a mild valgus position and some knee sway during performance. Score 2 corresponded to poor control and this score was considered when the knee alignment was weak and at least one knee was clearly and significantly in valgus or there was significant mediolateral sway during performance. The scoring criteria for the hip was the same as for the knee, but the focus was on hip motion/tilt. Specifically, scores of 0, 1, and 2 was given for no, some, and significant pelvic lateral tilt, respectively. After separate scores, the hip and knee asymmetry score was calculated with the absolute difference between the two sides, where 0 indicates symmetry, 1 is moderate asymmetry, and 2 is high asymmetry (15). Ordinal scale measures displayed intrarater reliability ranging from 0.38 to 0.94 and interrater reliability of 0.68 (0.46-0.87). Intrarater reliability of frontal plane knee measures ranged from 0.88 to 0.98 and interrater reliability of 0.99 (0.97-1.00) (21).

**Vertical drop jump**

To evaluate knee control in the frontal plane, athletes performed the VDJ test from a 20 cm box. Starting the exercise from the top of the box, the athletes were instructed to land from the box using a symmetrical landing strategy and immediately after to perform a maximal vertical jump.
To normalize the test with soccer conditions and to ensure that the athletes perform the jump with maximum effort and concentration, a soccer ball was thrown by the evaluator towards the footballers, and the footballers blocked the ball by heading and immediately landed on the ground. If the athletes did not perform the landing strategy correctly or did not perform the vertical jump with maximum effort after the first landing, the test was considered invalid. The athletes were allowed to do 2 practice trials before the test, then they did the valid and main test. Athletes’ ability to control their knees in the frontal plane during the jump landing phase was evaluated using a scoring scale from 0 to 2. A total score for both legs was used for scoring. The scoring criteria for the knee were the same as the SLS criteria (15). Intrarater reliability for VDJ was reported as substantial to almost perfect (kappa 0.72 rater 1; 0.85 rater 2). Inter-rater reliability was reported as substantial to almost perfect (kappa from 0.68 to 0.83) (22).

Injury record
The lower limb injury status of each athlete was recorded until the end of the competition season through monthly contact or sending an online form to the player. At the end of each month, if a player had not completed his injury form, the evaluator, H.M., contacted the sports coach or the team doctor to follow up on the completion of the injury registration form. All lower limb injuries (contact and non-contact) that kept the individual out of training or competition for 24 hours, causing a visit to the doctor were recorded and considered an injury (23).

Statistical analysis
Data analysis was analyzed in two parts, descriptive and inferential statistics using SPSS for Windows, version 26.0, (SPSS Inc., Chicago, perness, USA software). The normality of the data was evaluated using the Kolmogorov-Smirnov statistical test. The comparison of two groups of footballers (injured footballers and non-injured footballers) was investigated with Student’s t test. The predictive value of SLS and VDJ was investigated using logistic regression. A ROC curve was conducted to determine a cutoff point for VDJ whereby injury risk was identified to increase. The statistical significance level was 0.05 and effect sizes with 95% CI are presented for all outcomes. Where appropriate, effect sizes were quantified using odds ratios (OR) and considered trivial (0.77-1.00 or 1.00-1.29), small (0.51-0.78 or 1.30-1.99), moderate (0.25-0.50 or 2.00-3.99), and large (≤ 0.24 or ≥ 4.00) (24).

RESULTS
Twenty-five lower limb sport injuries were recorded for 121 male footballers during the 9-month follow-ups. Age, height, weight, BMI, and score of the screening tests of all the players, and also injured and uninjured players are showed in Table I. Comparisons of the score of the screening tests were statistically different between the injured vs uninjured footballers (all p ≤ 0.05) (table I). The separate model created by the logistic regression analysis for score of the screening tests is provided in Table II. This model for SLS was not statistically significant (χ²(1) = 0.967, p > 0.05). However, this model for VDJ was statistically significant (χ²(1) = 17.794, p < 0.001). The model explained 22% (Nagelkerke R²) of the variance in lower limb injury and correctly predicted 85.1% of cases. Finally, ROC analysis (Figure 1) showed significant accuracy of the curve for the VDJ performance score of the footballers (AUC 0.659, AUC 95%CI 0.639-0.634, p = 0.001) in discriminating between injured and uninjured players. The optimum cut-off level of the VDJ performance score of the footballers was 0.50 (where sensitivity was 0.969, and specificity was 0.652). The frequency of the injuries per location is provided in Table III.

Table I. Characterization of the total sample study population and the results of the statistical comparisons between footballers with or without MSI.

<table>
<thead>
<tr>
<th></th>
<th>Participants enrolled total (n = 121)</th>
<th>Uninjured players (n = 98)</th>
<th>Injured players (n = 23)</th>
<th>P-value</th>
<th>Effect size d (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.78 ± 4.60</td>
<td>23.03 ± 4.65</td>
<td>21.70 ± 4.27</td>
<td>0.29</td>
<td>-</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180.02 ± 5.09</td>
<td>180.32 ± 4.82</td>
<td>178.78 ± 6.10</td>
<td>0.22</td>
<td>-</td>
</tr>
<tr>
<td>Weight, Kg</td>
<td>72.45 ± 6.26</td>
<td>72.38 ± 6.17</td>
<td>72.74 ± 6.77</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>22.29 ± 1.34</td>
<td>22.23 ± 1.32</td>
<td>22.59 ± 1.41</td>
<td>0.492</td>
<td>-</td>
</tr>
<tr>
<td>SLS (n)</td>
<td>0.37 ± .565</td>
<td>0.35 ± 0.558</td>
<td>0.48 ± 0.593</td>
<td>0.341</td>
<td>-</td>
</tr>
<tr>
<td>VDJ (n)</td>
<td>0.10 ± .327</td>
<td>0.03 ± 0.173</td>
<td>0.39 ± 0.583</td>
<td>0.001</td>
<td>1.22 (0.76-1.68)</td>
</tr>
</tbody>
</table>

SLS: single leg squat; VDJ: vertical drop jump; n: number.
DISCUSSION

The present study investigated the ability of SLS and VDJ tests in predicting lower limb injuries in Iranian professional male football players. Our findings showed that the VDJ test can be used as a suitable tool for pre-season screening of professional football players. Field evaluations of biomechanics are associated with injury risk in football players (25). Considering that a suitable screening tool should have high sensitivity, it was shown that the sensitivity of VDJ = 96%, which means that most football players are screened by this test. Knee valgus is considered an important part of football injuries (26) so the scoring method in these tests was based on knee biomechanics. Since good inter- and intra-rater reliability and high sensitivity of VDJ test has been shown (27), this test was used in this research to identify football players prone to injury. Previous research has shown that increased valgus motion and valgus moments at the knee joint during landing jump tasks are key predictors of ACL injury in athletes (8). Contrary to the study of Tron Krosshaug and colleagues, who found in a prospective study on 710 elite female athletes that the VDJ test is not able to predict ACL injury in elite female athletes (28), considering that one of the situations that cause ACL injury in footballs heading (26), in the current study, the VDJ test was simulated with this situation, and when performing the test with the player’s jump, the examiner threw a ball at his head and the player had to perform the act of heading. Tests commonly used to assess dynamic knee valgus, such as squats or jump-landing tasks, may show different results. Although the same movement pattern is used in both tests, which includes bending and extension of the trunk, knees, wrists, and hips in the sagittal plane, the landing task bears more external load than the squat (29). The present study showed that SLS is not able to predict lower limb injuries in professional male football players, which is contrary to the findings of Ugalde et al., who introduced this test as a suitable screening tool (16). One of the reasons for this inconsistency can be the examiner’s inaccuracy in the visual evaluation, and we also declared the squat depth to be 90 degrees, which caused variation in the amount of effort and sitting of the players.

Table II. Significance of risk factors for MSI in the logistic regression creating a separate model with VDJ and SLS.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>P-value</th>
<th>R2 (Nagelkerke)</th>
<th>Exp(B)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLS (n)</td>
<td>0.387</td>
<td>0.317</td>
<td>0.01</td>
<td>1.473</td>
</tr>
<tr>
<td>VDJ (n)</td>
<td>2.721</td>
<td>0.001</td>
<td>0.22</td>
<td>15.193</td>
</tr>
</tbody>
</table>

SLS: single leg squat; VDJ: vertical drop jump; n: number.

Table III. Injury locations.

<table>
<thead>
<tr>
<th>Injury locations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligament and knee injuries</td>
<td>32%</td>
</tr>
<tr>
<td>Muscle injuries</td>
<td>36%</td>
</tr>
<tr>
<td>Leg injuries</td>
<td>4%</td>
</tr>
<tr>
<td>Ankle sprain</td>
<td>28%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure 1. Receiver operating characteristic (ROC) curve for the VDJ score of the footballers participating in this study. The straight line shows the reference line, which was approximated by the ROC curve plotted on sensitivity (true positive rate) over 1-specificity (false positive rate) for the VDJ.
Study limitations
In the previous studies, video analysis was used to check the visual tests, but in the present study, due to cost and time saving, these functional tests were performed visually. One of the limitations of the study is the lack of examination of previous injuries and exposure time, which the players and coaches did not provide to us due to personal reasons. Also, in this study, we did not compare side-by-side differences and used a general score for both sides. Future studies should also be conducted for women soccer players.

CONCLUSIONS
Identifying athletes prone to injury is an important step towards the health of athletes and increasing the quality of sports. The results of the present study showed that, unlike the SLS test, which did not have the ability to predict lower limb injuries in professional male soccer players, the VDJ test is a suitable tool for assessing injury-prone football players. Sports team coaches and team medical staff can use VDJ as a screening tool to predict lower limb injuries in professional football players and to identify people prone to injury during the pre-season and before signing contracts with players. In this way, this screening tool reduces the medical and health costs incurred by the player and the team during the injury.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
HM, MH: writing – original draft, formal analysis. HM: data curation, investigation. RG: writing – review & editing.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES


The Effect of Short-Set High-Load and Long-Set Low-Load Isometric Exercises Based on Stress-Strain Isometric Time-Dependent Strength Trial on Weightlifters Performing Resistance Training Periodization

Yeliz Kahraman, İsmail Varol

Laboratory of Movement and Training, Department of Health Science, Faculty of Sport Science, Akdeniz University, Akdeniz, Antalya, Turkey

SUMMARY
Introduction. Isometric deload resistance training is both short set high load and long set low load, combining the implementation so-called “recovery” or “light” weeks to expose stress load strain energy generation, enabling to isometric load time set curve – indeed controversial and previously uncleared – on undulation, different set configuration and repetition zone working.

Objective. Our study aimed to investigate available stress-strain relationship on different set configuration “deload training regimes” in Olympic and professional weightlifters.

Materials and methods. Four Olympic and professional weightlifters (1 women and 3 men) were included, mean age: 21.50 ± 7.85 years, height: 1.68 ± 0.03 m, weight: 70.75 ± 13.69 kg, deload resistance training over 3 week and per week 2 days.

Results. Isometric modeling deload resistance training periodization was generated, stress load strain energy generation promoting low load vs high load repetition session into light weeks, different set configuration and rest interval. Each isometric resistance exercise session showed dynamic strength increased by undulation sets and repetition zone ranges, isolated isometric load time decreased by training volume change, and endurance localization increased by deload loading working principle on maximize performance.

Conclusions. Specific undulation short term periodization properly enhanced maximize performance of weightlifting resistance training athletes, in this case bi-weekly non-linear different set configuration can be supported to optimize stress load strain energy generation.

KEY WORDS
Deload resistance training; maximize performance; weightlifters; isometric load; low-high set.

INTRODUCTION
Current isometric stress load strain energy generation – controversial one of sustained-contraction strength training model – enable to maximum repetition strategy associated with load time set curve (1, 2). Generally, isometric loading condition is high sustained contraction to enhance periodic single vs multiple set session, intensity and rest interval, uncleared different resistance training periodization (2). Furthermore, isometric modeling deload resistance training previously unexplained on sustained contraction related to early and late isometric load time set curve performing maximize micro strength performance (3, 4). Periodic isometric load time set sessions form stress-strain energy generation by constant training hyperbola, with sustained early and late
contraction repetition session to adopt isometric resistance training properly periodization (1, 3, 5). In this resistance loading training, the shape performed maximum repetition zone, set change and isolated isometric load time on non-linearly bi-weekly periodization (4). Indeed, isometric modeling deload resistance training periodization linearly enhances low load and high load set configuration related to normal stress “σ1, σ2, σ3” load factors (2, 3). Isometric stress load therefore (1), one of resistance period is strain energy (ε) generation, able to one periodic single set session (2); however, strain energies gradually increase on sustained contraction force potential, while cleared isometric speed phase session (4). In this case, isometric early and late force development were demonstrated to perform load time set curve on dynamic strength, isolated isometric load time to load increase one set finish resistance training processes (3, 4). Commonly, isometric training period was explained on load time set curve accordingly, stress load strain energy generation completed on time under sustained and non-sustained contraction single set session (4). In this case, potential isometric strain force energy generation based load time set change as a constant training hyperbola (Fxε = σ/ε), where “Fx” is strain energy, “σ” is isolated load time stress, “ε” is time-dependent trial and totally “Δε” so-called difference energy generation. Currently, isometric training period may be clarify isolated isometric load time on single set load session both at 90% of 1RM and 120% of 1RM time-dependent strength trials (4). However, dynamic strength reach was determined on maximum repetition zone working principle based on constant training volume equally endurance localization, both with 70% of 1RM and 75% of 1RM moderate load stress set configuration (4). High load set and low load set non-linearly bi-weekly periodization conduct stress-strain energy generation associated with different load time set curve and strategic deload strategy (3, 4). For this common resistance training hyperbola approach to weightlifters, stress-tension strength trials contribute to personal customization of training programs in multiple sets and low-high loads (5). In addition, isometric strain energy generally worked on high load stress used to high force potential production by self-selected time speed (4). Accordingly, isometric sustained contraction potential force phases progress load time set relationship on reaching high strength effort and variable strain force enhancement (6, 7). Therefore, isometric modeling deload resistance training regimes were including both low load high set or high load low set within light weeks and recovery weeks previously non-planned on short time resistance training periodization. Thus, this study aimed to deload training regimes enhance stress load strain energy generation by performing different set configuration, repetition zone range and rest interval in Olympic and professional weightlifters.

MATERIALS AND METHODS

The study formed on 2 Olympic and 2 professional weightlifters: 1 woman and 3 men included in mean age 21.50 ± 7.85 years, height 1.68 ± 0.03 m, weight 70.75 ± 13.69 kg, participated in isometric modeling deload resistance training periodization. Olympic and professional weightlifting resistance training woman and men had 6.50 ± 9.11 years of weightlifting and resistance training experience. We obtained the permission from Akdeniz University Clinic Ethic Committee Research Protocol (No: 2023/629 – date of approval: September 21, 2023).

Procedure

The isometric modeling deload resistance training regimes is one of the current weightlifting training performances preferred to short time to maximize micro strength performance over 3 week and 2 days per week, enabling to detect dynamic strength, isolated isometric load time and endurance localization.

Experimental conditions were respectively: 1) day dynamic strength, 2) day isolated isometric load time, 3) day endurance localization performed on sport area weightlifting room (in Turkey).

The experimental condition of isometric modeling deload resistance training periodization was conducted on loaded isom. jump squat on barbell free, bench press on bench machine, isom. deadlift on weightlifting rack platform and back squat on weightlifting rack platform – preferred to non-periodically maximum repetition zone range working, principle non-sustained day to day performance (figure 1). The experimental procedure started on dynamic strength day performing with an initial load at 40% of 1RM (10 rep) warm-up to each training performance and test baseline session; then, gradually maximal strength sessions were applied at 70% of 1RM (5 rep) and 90% of 1RM (2 rep), by adding 10-20 kg load (Eleiko, Sweden and Werk- san, Turkei). Then after, specific relative strength was used to body mass strength with load intensity calculator. After, their in isolated isometric load time day by load time-dependent strength trial was performed on high load at 80% of 1RM and 90% of 1RM isometric load 1 trial by using time calculator lab. Again, endurance localization (max. rep) was executed to high load training session detection by absolute local endurance at 70% of 1RM conducting maximum high repetition to one set finish.

After the experimental procedure, isometric modeling deload resistance training regimes were determined on 60%
of 1RM low load endurance zone volumes, 75% of 1RM moderate load hypertrophy zone volumes, and 90% of 1RM high load strength zone volumes completed weekly (table I). In this condition, total training volumes were provided on weekly deload resistance training regimes, changing detecting light or recovery weeks (8) (figure 2).

Statistical analysis
Primary analyses were resolved on pre-test and post-test comparisons using descriptive mean, standard deviation and confidence interval detection. Pre-test and post-test measurements were investigated in one paired-t test to weightlifters. Isometric modeling deload resistance training periodization outcomes were conducted on descriptor effect sizes: 0.00 < 0.20 - very weak, 0.20 < 0.50 - weak, 0.50 < 0.80 - strong, 0.80 < 1.20 - very strong, 2 or > 2 - extremely strong; alpha level was significant when p < 0.05 (9).

RESULTS
Isometric modeling deload resistance training outcomes resulted from non-sustained non-linearily daily zone and low-high load set configuration performance on total training volume for dynamic strength, isolated isometric load time and endurance localization (figure 3). Currently, maximize micro strength performance outcomes were concluded on dynamic strength for loaded isom. jump squat (p = 0.038; t = -5.000), bench press (p = 0.000; t = -6.000), isom. deadlift (p = 0.073; t = -3.500), back squat (p = 0.300; t = -1.387), endurance localization was resulted on 80% of 1RM for loaded isom.
Deload Resistance Training

Jump squat (p = 0.368; t = 1.555), bench press (p = 0.185; t = -1.987), isom. deadlift (p = 0.433; t = 0.974), back squat (p = 0.057; t = -4.000), and isometric strength at 80% of 1RM for loaded isom. jump squat (p = 0.183; t = -2.001), bench press (p = 0.790; t = 0.303), isom. deadlift (p = 0.142; t = -2.336), back squat (p = 0.149; t = 2.293), and 90% of 1RM was determined on loaded isom. jump squat (p = 0.026; t = -6.058), bench press (p = 0.032; t = -5.450), isom. deadlift (p = 0.174; t = 2.069), back squat (p = 0.739; t = 0.382) (table II).

DISCUSSION

Isometric modeling deload resistance training non-linearly light weeks periodization has been enhanced maximizing micro strength performance; additional short and high differently set configurations indeed were executed on load time set curve by performing deload repetition training regimes. Thus, low and high set non-linear bi-weekly short time recovery periodization to accurate maximum repetition zone working was explored on maximize micro strength perfor-

Table II. Maximize strength performance outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>t</th>
<th>p</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loaded isom jump squat (kg)</td>
<td>76.66 ± 25.16</td>
<td>110.00 ± 26.45</td>
<td>-5.000</td>
<td>0.038</td>
<td>1.29</td>
</tr>
<tr>
<td>Relative (kg/kg)</td>
<td>1.08 ± 0.26</td>
<td>1.55 ± 0.10</td>
<td>-5.198</td>
<td>0.035</td>
<td>2.38</td>
</tr>
<tr>
<td>70%-1RM (kg of rep)</td>
<td>10.33 ± 2.86</td>
<td>8.33 ± 1.52</td>
<td>1.155</td>
<td>0.368</td>
<td>Trival</td>
</tr>
<tr>
<td>80%-1RM (kg/t)</td>
<td>36.23 ± 11.53</td>
<td>54.51 ± 19.79</td>
<td>-2.001</td>
<td>0.183</td>
<td>Trival</td>
</tr>
<tr>
<td>90%-1RM (kg/t)</td>
<td>35.91 ± 14.46</td>
<td>53.31 ± 17.07</td>
<td>-6.058</td>
<td>0.026</td>
<td>1.09</td>
</tr>
<tr>
<td>Bench press (kg)</td>
<td>63.33 ± 32.14</td>
<td>73.50 ± 31.96</td>
<td>-6.000</td>
<td>0.000</td>
<td>0.31</td>
</tr>
<tr>
<td>Relative (kg/kg)</td>
<td>0.86 ± 0.23</td>
<td>1.01 ± 0.20</td>
<td>-7.852</td>
<td>0.016</td>
<td>0.69</td>
</tr>
<tr>
<td>70%-1RM (kg of rep)</td>
<td>7.66 ± 4.50</td>
<td>12.66 ± 6.80</td>
<td>-1.987</td>
<td>0.185</td>
<td>Trival</td>
</tr>
<tr>
<td>80%-1RM (kg/t)</td>
<td>34.54 ± 21.74</td>
<td>32.37 ± 9.44</td>
<td>0.303</td>
<td>0.790</td>
<td>Trival</td>
</tr>
<tr>
<td>90%-1RM (kg/t)</td>
<td>36.82 ± 16.66</td>
<td>47.97 ± 13.77</td>
<td>-5.450</td>
<td>0.032</td>
<td>0.72</td>
</tr>
<tr>
<td>Isom deadlift (kg)</td>
<td>116.66 ± 40.41</td>
<td>140.00 ± 43.58</td>
<td>-3.500</td>
<td>0.073</td>
<td>Trival</td>
</tr>
<tr>
<td>Relative (kg/kg)</td>
<td>1.63 ± 0.29</td>
<td>1.91 ± 0.17</td>
<td>-3.580</td>
<td>0.070</td>
<td>Trival</td>
</tr>
<tr>
<td>70%-1RM (kg of rep)</td>
<td>14.33 ± 10.06</td>
<td>9.33 ± 2.08</td>
<td>0.974</td>
<td>0.433</td>
<td>Trival</td>
</tr>
<tr>
<td>80%-1RM (kg/t)</td>
<td>41.10 ± 7.59</td>
<td>60.31 ± 21.55</td>
<td>-2.336</td>
<td>0.142</td>
<td>Trival</td>
</tr>
<tr>
<td>90%-1RM (kg/t)</td>
<td>89.32 ± 39.24</td>
<td>59.73 ± 25.39</td>
<td>2.069</td>
<td>0.174</td>
<td>Trival</td>
</tr>
<tr>
<td>Back squat (kg)</td>
<td>123.33 ± 41.63</td>
<td>140.00 ± 60.82</td>
<td>-1.387</td>
<td>0.300</td>
<td>Trival</td>
</tr>
<tr>
<td>Relative (kg/kg)</td>
<td>1.72 ± 0.23</td>
<td>1.92 ± 0.36</td>
<td>-1.554</td>
<td>0.260</td>
<td>Trival</td>
</tr>
<tr>
<td>70%-1RM (kg of rep)</td>
<td>7.66 ± 1.52</td>
<td>10.33 ± 0.57</td>
<td>-4.000</td>
<td>0.057</td>
<td>Trival</td>
</tr>
<tr>
<td>80%-1RM (kg/t)</td>
<td>50.12 ± 22.80</td>
<td>44.94 ± 23.22</td>
<td>2.293</td>
<td>0.149</td>
<td>Trival</td>
</tr>
<tr>
<td>90%-1RM (kg/t)</td>
<td>58.93 ± 45.18</td>
<td>55.93 ± 31.71</td>
<td>0.382</td>
<td>0.739</td>
<td>Trival</td>
</tr>
</tbody>
</table>
mance of weightlifters. Isometric modeling deload repetition resistance exercises are popular sustained contraction resistance training performance for one of repetition maximum method and available high load single session achieved on high strength effort (10). Similar resistance training condition as traditionally low load back squat barbell training session was selected on 10-20% load lifting range one set session maximal strength effort, indeed normally load incremental set session condition may be planned hypertrophy zone and strength zone to long time high load non-linear periodization (11). In this case, maximum repetition zone periodic resistance training strategies are specific training volume planning at 80% of 1RM – 4 set on bench press 8% and squat 19% incremental intense for increase maximize micro strength effort (11). In other condition, endurance localization was determined on moderate load 70% of 1RM and 75% of 1RM to develop maximum repetition deload repetition increasing (4). In this case, endurance localization based maximal strength reach increase to high repetition maximum single set training period session within stress load strain energy factors (12, 13). Additionally, endurance localization zone ranges may be planned on low load 60% of 1RM, indeed military press 4% and bench press 8% were increased in long time periodization (12). However, optimize micro and macro strength performances were estimated both at low load 30% of 1RM and 60% of 1RM periodic deload sessions are similar for maximize strength performance (13). In contrast, resistance training incremental strength approach – so-called zone repetition range – was estimated one repetition loaded jump squat at low load 30% of 1RM and high load 80% of 1RM for detect maximal strength periodization (14). In this condition, the current study reported that resistance training high strength zone effort performed on 90% of 1RM by upper and lower body working (3, 4). Therefore, maximal speed-based strength reach to develop isometric time dependent strength trial resulted in constant strain time on high load 90% of 1RM and 120% of 1RM low repetition (1 s) and high load high repetition (3 s) isolated isometric load (4). This maximal strength phases were determined on sustained and non-sustained deload periodization to execute isolated isometric load time performance (4, 8). Therefore, stress load to deload planning was explained on dynamic strength phases, thus similar results may be other working repetition load maximal zone ranges (15). Isometric modeling deload repetition resistance training, one of resistance training regimes used to clarify maximum repetition zone working principle, based on load time set curve and different undulation set configuration to enhance stress load strain energy generation – both low and high load volume implementation. Thus, current study limited on light or recovery weeks of weightlifters.

Limitations
In this research, stress-strain time-dependent strength trials based on isometric exercises were carried out as a deload strategy in resistance training periodization. Limited to short-term load-reducing training regimes. In the study, the isometric method was performed with low load high set and high load low set, but the changing set configuration was limited to light weeks.

CONCLUSIONS
High effective isometric modeling deload resistance training periodization depends on isometric time-dependent strength trial performing reach to develop maximal micro strength performance. It is a periodic development strength detection. Indeed current resistance training periodization is load increase and time decrease performance related non-sustained contraction stress-strain potential energy generation. Specialized training methods need to be continued, as it supports optimized stress load strain energy formation under the influence of isometric deload resistance training, which improves short-term maximum performance in weightlifters, and supports short set high load and long set low load performances. Current stress load strain energy generation showed in short time periodization on light or recovery weeks of weightlifters, however, literature was unclear about stress load development dependent on strain energy with load and time session, different set configuration and deload repetition loading zone performance on different sport modalities, athletic branches and coaches.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
The authors contributed equally to this work.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.
REFERENCES
Acute Effect of Static Stretching Duration for Paravertebral Muscles on Lumbar Multifidus Myoelectric Activity: A Crossover Clinical Trial

Camila Amaral Coracini¹, Polyana Bortoletto¹, Érica Provensi¹, Mateus Vieira Furtuoso¹, Gladson Ricardo Flor Bertolini¹, Fábio Micolis de Azevedo², Alberito Rodrigo de Carvalho¹

¹ Integrative Biodynamics Evaluation Laboratory, Universidade Estadual do Oeste do Paraná (Unioeste), Cascavel, Paraná, Brazil
² Universidade Estadual Paulista (Unesp), Presidente Prudente (SP), Brazil

SUMMARY
Introduction. Considering that stretching can reduce neural impulses, this study aimed to verify whether stretching to paravertebral muscles changes the electromyographic activity characteristics of the lumbar multifidus.

Methods. The volunteers (n = 46) were randomly allocated to four experimental conditions performed in 4 visits: control and three paravertebral stretching interventions sustained for 10, 30, and 60 s. At each visit, the electromyographic activity of the multifidus was collected in the following sequence: a) pre-intervention in the neutral orthostatic position; b) during the intervention targeted by the visit; c) post intervention in the neutral orthostatic position. Outcomes were analyzed by MatLab codes using wavelet methodology. In addition to the inferential statistics generated by the generalized estimation equation model, it presented reproducibility and responsiveness metrics.

Results. Only the median frequency difference between the sustained stretches for 10 and 60 s was found. However, responsiveness measures indicated that such a difference was due to measurement variability rather than the actual change caused by the intervention.

Conclusions. Stretching duration did not influence the electrical activity of the lumbar multifidus.

Study registration. This study was registered at Brazilian Registry of Clinical Trials - REBEC (number: RBR-7bvg7p): http://www.ensaiosclinicos.gov.br/.

KEY WORDS
Electromyography; paraspinal muscles; skeletal muscle.

INTRODUCTION
Static stretching is often used to reverse adaptive muscle shortening (1, 2), and even to prevent lower back pain (3). However, it is suggested that prolonged maintenance of stretching promotes changes in muscle function, such as a decrease in the ability to produce strength and power in the period after immediate stretching (1, 4).

Although the strength or power production and muscle activity relationship may be controversial, studies suggest that one effect of stretching is a reduction in the neural impulse to the muscle, which can produce changes in muscle stiffness (5, 6). It is believed that, once the neural impulse can be reduced by stretching (1), the recruitment of motor units (MU) will be reduced, with a consequent reduc-
tion in myoelectric activity. This reduction is because the neural activation directed to the muscle comes from a moto-
neuron pool, and this neural signal is the sum of the activi-
ties of the action potentials of the motoneurons, generated
by the transformation of synaptic inputs from the motoneu-
rons into output pulse trains (7). Corroborating, passive
stretching induced the suppression of the excitability of
the monosynaptic spinal reflex and that afferent informa-
tion produced by the stretching, probably coming from the
muscle spindles and the Golgi tendon organ, inhibited the
spinal reflex motor response (8). It is assumed, then, that
the input produced by stretching modifies the neural drive.
Surface electromyography (EMG) is a tool to estimate the
level of muscle activation (9, 10), as it carries amplitude and
frequency contents related to depolarization. Signal’s ampli-
tude information works as an indirect measure of muscle
activation level, while the signal’s frequency spectrum is
related to the depolarization frequencies of the MU (11).
The EMG analysis allows us to quantify the level and dura-
tion of muscle activity and fatigue rate (12, 13), including
in response to interventions such as lengthening. However,
observations on electromyographic activity in poststretch-
ing are not conclusive (1).
The Williams series exercises are used when restoring
lumbar spinal mobility is desired (14-16). However, little
is known if stretching aimed at paravertebral muscles by
these exercises can generate neurophysiological respons-
es in them. Only one study found that performing flexion
exercises in this series, with maintenance of the posterior
tilt, minimizes electromyographic activity at time domain, in
the lumbosacral region (17). Even less is known if changes
in the frequency domain occur in the paravertebral muscles
because of stretching induced by the Williams series exer-
cises, and if there is a dose-response effect for the exercise
sustaining time.
It is relevant to investigate whether stretching durations
routinely used in the clinical context for lumbar paraverte-
bral muscles, modify the myoelectric activity characteristics
of the multifidus since this muscle plays a relevant role in
stabilizing the lumbopelvic region (18, 19), including chan-
ges in lumbago sufferers (20). Therefore, the objective of
this study was to verify whether stretching to paravertebral
muscles changes the electromyographic activity characteris-
tics of the lumbar multifidus.

METHODS

Trial design
This cross-over clinical trial was approved by the Research
Ethics Committee Involving Human Beings at the State
University of Western Paraná (protocol number: 2748177
– date of approval: February 07, 2018). All volunteers gave
their free and informed consent.

Participants
Young adults of both sexes were recruited through personal
invitations and also publicity actions at the university center.
Volunteers with no history of spinal or lower limb disorders
(acute or chronic) in the past 12 months were included in
the study, and who did not practice regular and systematic
physical activity. Volunteers with abdominal, hip, and spine
surgery history or neurological diseases, pregnant women,
and make use of muscle relaxants were excluded.

For the sample calculation, the percentage magnitude
relative to the maximum electromyographic activity of
the paravertebral muscles was used during the push-ups
of the Williams series (17), which generated an effect size
in the order of 0.26. Using the G.Power 3.16 software,
the sample size was obtained from the following input
data: effect size: 0.25; alpha: 0.05; power: 0.85; number of
groups: 4; number of measures: 3. The minimum sample
size required was 44 volunteers.

Collection instruments
Myoelectric activity of the lumbar multifidus muscle, bilater-
ally, was quantified by a signal conditioning module (model
BIO EMG 1000- 8-4l, brand LYNX® Tecnologia Eletrônica
Ltda, São Paulo-SP, Brazil) with a sampling frequency of
2,000 Hz. For the signal acquisition, disposable, self-adhe-
sive, bipolar electrodes with Ag/AgCl uptake sites and 10
mm diameter were used. The volunteer’s skin was properly
shaved and cleaned to fix the electrodes.
The electrodes were positioned on the lumbar multifidus
muscles following the recommendation of the Surface Elec-
 tromyography for the Non-Invasive Assessment of Muscles
(SENIAM). The reference electrode was positioned in the
lateral malleolus of the left lower limb, as shown in figure 1.
Methodological procedures

Each volunteer participated in a battery of evaluations, familiarization, and stretching protocols, totaling five visits with a minimum interval of seven days and a maximum of 10 days between them. The stretching protocols were based on the lift time of 60 s or less, which are recommended for clinical use (4, 21), they being 10 (Stretch_10), 30 (Stretch_30) and 60 (Stretch_60) s, and a control condition in which the volunteer only remained lying on the supine stretcher, with the legs extended, for 30 s.

Visit 1

First, a brief clinical evaluation was carried out, containing personal data, history of injury, and use of medications. Then, the volunteer was familiarized with the intervention procedure. The stretch for the lumbar paravertebral musculature proposed was the exercise “Knees in the Chest”, number three of the Williams Series (17), shown in figure 2. In this familiarization, the volunteer was asked to make repetitions of the stretching gesture, to the maximum extent possible, with the assistance of the evaluator if necessary, until the volunteer learned the correct execution of the stretching. After familiarization, the order of interventions was established by a lot carried out by the main researcher.

Visits 2 to 5

In each of these visits, the skin was initially prepared and the electrodes were subsequently placed to acquire the myoelectric signal. To ensure reproducibility in the placement of electrodes for recording myoelectric activity on different days, on visit 2, positioning maps were drawn on transparent slides that took into account signs on the skin such as blood vessels, stains, scars and anatomical references. In visits 3 to 5, the placement of the electrodes was based on these positioning maps. All methodological procedures were the same in all visits, except for the length of time for which the stretch was sustained, following the order drawn for each volunteer. In the pre-intervention assessment, the electromyographic activity of the multifidus was measured in a neutral orthostatic position. Then, the target intervention of the visit was applied, during which the recording of electromyographic activity was also performed. After the intervention, myoelectric activity was reevaluated in the orthostatic position. The procedure can be seen in figure 2.

Data processing

The mathematical processing of the data occurred on MatLab (R2015b; Natick, MA, USA).

Time domain analysis

For the time domain treatment, a wavelet filter was initially applied to the raw signal and, subsequently, the Matlab “envelope” function that provided the RMS value. The RMS was normalized by the peak value of the signal (22).

Frequency domain analysis

For the time-frequency analysis, the central frequency was determined. A MatLab Wavelet code was used to decompose the signal into its frequency spectrum: Wavelet Packet Decomposition 1-D (“wpdec” function, level 6, and wavelet mother symlet14). By this processing, the frequencies that constitute the signal were stored in their corresponding scales, the latter being converted to frequency values by mathematical treatment. The amount of scales generated is dependent on the characteristics of the selected mother wavelet. As it is a time-scale analysis, the temporal information corresponded to the length of the vector originating from the signal corresponding to the duration of the stretching maintenance. This decomposition generated a matrix with 64 columns, corresponding to the scales, and the number of lines corresponding to the length of the vector that contained the raw signal. In sequence, the MatLab function “wpspectrum” was applied, which returned a matrix of Wavelet Packet Power Spectrum, based on Wavelet Packet Transform, and identified the frequency values, expressed in Hz, contained in each scale, at each instant of collection, which corresponds to the period. Then, for each column of the matrix, which corresponded to the scales and whose lines represented the frequencies that made up the signal stored in the scale over time, we calculated the median of these frequencies which resulted in a 1 × 64 line vector. Finally, we place the medians of frequencies in this vector line in an ascending order (from scale 1 to 64 scale) and divide it into three points: the median of frequencies in scale 1, which are the lowest frequencies in the spectrum, the median of frequencies on the 57
Table I. Median of frequencies observed on scales 1, 57, and 64 of the line vector resulting from the process of decomposition of the signal from the lumbar multifidus muscles.

<table>
<thead>
<tr>
<th>Scale 1</th>
<th>Scale 57</th>
<th>Scale 64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median ± standard deviation (Hz)</td>
<td>2.3 ± 5.7</td>
<td>56.9 ± 86.5</td>
</tr>
</tbody>
</table>

The values represent all evaluations carried out, in all interventions and moments of all volunteers.

scale, which represents 90% of the signal spectrum, and the median of frequencies on the 64 scale, which correspond to the highest frequencies in the spectrum. The values corresponding to each of these points can be seen in table I. Considering that 90% of the signal frequency spectrum was between scales 1 to 57, for the statistical analysis of the present study, we assumed two frequency ranges: the low frequency range, obtained by the median of frequencies between scales 1 and 57, and the range of high frequencies, obtained by the median of frequencies between 58 and 64 scales.

Statistical methods

For statistical analysis, the software SPSS 20 was used. The significance level adopted was 5% (α = 0.05). As a first step in the data analysis, the mean values and the 95% confidence interval (IC-95) of all dependent variables were determined for each intervention at each time of collection (control / pre; control / during; control / post; Strech_10 / pre; Strech_10 / during; Strech_10 / post; Strech_30 / pre; Strech_30 / during; Strech_30 / post; Strech_60 / pre; Strech_60 / during; Strech_60 / post). It was assumed for the present study that data that are below the lower limit of the 95% CI subtracted from the value corresponding to 80% of that value (lower limit - 80% of the lower limit) or above the upper limit of the 95% CI added to the value corresponding to 80% of that value (upper limit + 80% of the upper limit) were considered as measurement error and excluded from the analysis. However, the missing data were imputed by the statistical model employed (23, 24).

For comparison, the statistical test used was the generalized estimation equation (GEE) model with data analysis based on the intention to treat using the maximum likelihood principle to extrapolate the missing data. The best fit of the data was tested by two distribution models: Linear and Gamma (23). The model that obtained the lowest quasi-likelihood value under independence model criterion (QIC) was chosen as the model with the best fit. The Sidak test was used as a post-hoc test.

The factors used in the analysis of the frequency domain were the same used in the time domain. The dependent variables in the model were the low and high frequency ranges. The Gamma model presented the lowest QIC value, both for low and high frequencies, and was used in the analyzes: range of low frequencies / Linear (QIC = 3,908.173) and Gamma (QIC = 173.158); high frequency range / Linear (QIC = 1,582,814.637) and Gamma (QIC = 261.2).

To present metrics complementary to inferential statistics, to better understand changes in outcomes or absence of changes, measures of reproducibility, responsiveness, and effect size were calculated.

To determine reproducibility and responsiveness, we used data from the control group at the pre and post collection times. The effect size was calculated by Hedges’ g with the following interpretation (25): insignificant < 0.19; small 0.20-0.49; medium 0.50-0.79; large 0.80-1.29; very large > 1.30. The relative reproducibility was tested by the intraclass correlation coefficient (ICC) and the absolute reproducibility by the standard error of measurement (SEM). We apply ICC2, k (bidirectional random model) (26), with the strength of reliability being described as: 0-0.50 poor; moderate 0.50-0.75; 0.75-0.90 good; greater than 0.90 excellent (27). The SEM was determined by the square root of the error variance. Responsiveness was assessed by the minimum detectable change (MDC) determined by the equation: $MDC = SEM \times 1.96 \times \sqrt{2}$ (26, 28).

Aiming comparisons between inferential and complementary metrics, we calculated the difference average between comparison pairs (Diff) for all outcomes, being:

\[
Diff = \left(\overline{x} - \overline{GC}\right) + \left(\overline{x} - \overline{G10}\right) + \left(\overline{x} - \overline{G30}\right) + \left(\overline{x} - \overline{G60}\right)
\]

where: $x - GC =$ mean of the control group; $x - G10 =$ average of the Strech_10 group; $x - G30 =$ average of the Strech_30 group; $x - G60 =$ average of the Strech_60 group; $11 =$ indicate that the value is absolute.

RESULTS

Forty-six volunteers participated in the study who were recruited from August 2018 to July 2019. The characterization of the sample and the flow of volunteers can be seen in figure 3.
Figure 3. Flowchart of study participants in all interventions (control, elongation sustained for 10, 30, and 60 s) and sample characterization data (mean and standard deviation).

For time domain analyzes, no effects were observed for the interventions (Wald $\chi^2 (3) = 0.110; p = 0.991$), for the moments of collection (Wald $\chi^2 (2) = 2.983; p = 0.225$) and neither for the interaction interventions/moments (Wald $\chi^2 (6) = 10.276; p = 0.114$) in the RMS.

For the analyzes of the frequency domain, no effects were observed for the moments (Wald $\chi^2 (2) = 0.366; p = 0.833$) or for the interaction interventions/moments (Wald $\chi^2 (6) = 5.013; p = 0.542$) in the range low frequencies, but there was a significant effect for the interventions (Wald $\chi^2 (3) = 15.383; p = 0.002$) and the Stretch_10 group had a higher average than the Stretch_60 group.

Still in the analyzes of the frequency domain, there were no effects for the interventions (Wald $\chi^2 (3) = 5.701; p = 0.127$), for the moments of collection (Wald $\chi^2 (2) = 2.867; p = 0.238$) and neither for the interaction interventions/moments (Wald $\chi^2 (6) = 9.254; p = 0.160$) in the high frequency range.

The average values of RMS, low frequencies, high frequencies and their respective 95% confidence intervals, both for interventions and for moments, can be seen in figure 4.

The ICC showed moderate reproducibility for the RMS measurements (ICC = 0.59 [0.25 to 0.77]; $p = 0.002$), good reproducibility for the low frequency range (ICC = 0.78 [0.61 to 0.88]; $p < 0.001$), and poor reproducibility for the high frequency range (ICC = 0.47 [0.05 to 0.70]; $p = 0.016$), all with an insignificant effect size (ES RMS = 0.004; ES low frequency = 0.04; ES high frequency = 0.17). For all outcomes, SEM and MDC showed important variability in measurements. However, it can be seen in figure 5 that the average value of the differences between the averages of the interventions (Diff).
DISCUSSION

In the present study, no statistical differences were found for most comparisons. For the median of low frequencies, comparing the interventions, a significant difference was found between Stretch_10 and Stretch_60. However, when considering the MDC value, it was noted that the difference between the groups was less than this measure. Thus, despite the statistical difference, it was not a real change, but a consequence of the variability in the measure.

Anatomically, the multifidus has superficial and deep fibers; a study that evaluated the fiber type distribution in the multifidus found that surface fibers have 57.4% type I fibers, while deep fibers at the level of L4-L5 have 62.6% and at the level of L5-S1 have 61.7% (29). Based on the rationale that whereas low frequencies in the EMG signal reflect the recruitment of slow and oxidative MUs, high frequencies indicate the recruitment of MU associated with fast fibers (30, 31), the spectral composition observed in the present study seems consistent with the literature. It was observed that the median of low frequencies, which corresponded to 90% of the spectral content, was around 4 Hz, suggesting that predominantly type I fibers were active.

However, about the relation between EMG spectral properties and the fiber-type composition of a muscle, we must be cautious. There is a wide range of confounding factors that permeate this relationship, which is based on the average conduction velocity of muscle fiber action potentials. Among these confounding factors, we can mention that both fiber types do not have distinct conduction velocities in humans, but the average conduction velocity of muscle fiber action potentials can differ among populations of motor units due to differences in fiber diameter and it is independent of changes in fiber-type proportions. We can also add that the number of muscle fibers innervated by a motor unit has a skewed distribution (32). Thus, the correspondence between the spectral content of our findings and the histological characteristics of the multifidus described in other studies regarding to the type of fiber can be considered only as a speculative coincidence.

Theories about the action of stretching on motor control suggest that stretching modifies muscle spindle activity (6, 33-37). Gamma intrafusal motor neurons act as facilitators for alpha extrafusal motor neurons; and this control is essential for maintaining muscle stiffness (38), which would be sharply reduced as an effect of stretching.

Since the results of the present study did not show a change in the activity of the multifidus by exercising the Williams series, it is speculated that the cause of the absence may be the amount of tension produced in the tissue by the stretching technique or consequence of the type of muscle studied. Some studies point to a relationship between intensity and the time for maintaining tension in the various outcomes produced by stretching. Investigations that inversely manipulated the stretching intensity and duration variables to gain knee amplitude, observed that the various compositions between intensity and duration tested generated different effects on the passive torque - angle curve (39).

In a study that compared the effect of stretching at a constant angle and that at constant torque in the range of motion, it was found that, although both improved stretching with constant torque was more effective in increasing the amplitude and the feeling of discomfort in the maximum amplitude, and to reduce the passive stiffness of the tendon muscle unit (40). Although these authors explain the results by changes in the mechanical components of the tissue, it is possible to speculate that stretches that impose greater torques are also more likely to affect the neural drive.

It is believed that the knee-to-chest technique did not produce enough tension to modify the EMG parameters, and this can be supported by the fact that the positioning of the trunk or pelvis during stretching affects muscle stiffness. Masaki and collaborators (41) evaluated the repercussion of the position of the trunk on the muscular stiffness of the lumbar multifidus in an elongated position by means of elastography by ultrasonic shear waves. They concluded that the multifidus are effectively stretched when the back is flexed between 40 and 45 degrees in a sitting position with the hips and knees fully flexed. The Blackburn and Portney study (17) also showed that pelvic tilt, whether anterior or posterior, affects differently electromyographic activity. However, in the present study, it was decided to perform the exercise as it is used in the clinical environment.

The muscle spindle is the only proprioceptor that receives motor innervation in addition to its sensorial innervation, and it can be subdivided into primary and secondary spindles. Although both are sensitive to changes in muscle length and velocity, the primary has greater dynamic sensitivity. Muscles with dynamic and postural functions have predominantly different types of muscle spindles (42) which may characterize different responses to stretching. The limitations of this study are that the sample consisted of young, healthy volunteers, so the results cannot be extrapolated. In addition, physical stress was subjectively individualized, and no method was used to control the force used.

CONCLUSIONS

As a conclusion, the stretching of the paravertebral muscles using the “Knees in the Chest” technique of the Williams series, sustained for 10, 30, and 60 s, does not produce acute changes in the activity of the multifidus, neither in
the domain of time nor frequency. In other words, in the population in question, there are no problems with loss of muscular activity for exercises that require muscular endurance and power to be performed after stretching.

**FUNDINGS**

None.

**DATA AVAILABILITY**

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

**REFERENCES**


**CONTRIBUTIONS**


**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interests.


Effect of Kinesio Tape Applied to Supraspinatus Muscle on Shoulder Scaption Strength and Upper Extremity Stability in Healthy Women Aged 20 to 30 Years: A Double Blind Randomized Controlled Trial

Dilay Kurt¹,², Havvanur Albayrak², Mahir Topaloglu², Sami Sokucu³, Ozden Ozyemisci Taskiran²

¹ Department of Physiotherapy and Rehabilitation, Istanbul Aydin University, Istanbul, Turkey
² Department of Physical Medicine and Rehabilitation, Koç University Faculty of Medicine, Istanbul, Turkey
³ Department of Orthopedics and Traumatology, Istanbul Aydin University Faculty of Medicine, Istanbul, Turkey

SUMMARY
Objective. To investigate the effect of kinesio tape applied to the supraspinatus muscle on shoulder scaption muscle strength and upper extremity stability in healthy women.

Methods. 26 healthy female volunteers aged 20-30 years were included in the study. Individuals were randomized into two groups, kinesio taping (n = 13) and sham taping (n = 13). In the kinesio taping group a Y-shaped tape was applied to the supraspinatus muscle in the direction of facilitation while in the sham group an I tape was applied on the scapula. Before taping and 20 minutes after taping the shoulder scaption strength was measured with a dynamometer and functional evaluations were performed by the closed kinetic chain upper extremity stability test.

Results. In the kinesio tape group, median scaption strength decreased after taping (p = 0.016). The median contact, normalized, and power scores increased significantly (respectively, p = 0.001, p = 0.001, and p = 0.001). In the sham group there was no significant difference in the median scaption strength after taping; the median contact, normalized and power scores increased significantly (p = 0.004, p = 0.004, and p = 0.006, respectively). There was no significant difference in scaption strength and upper extremity stability test scores between groups. After taping the changes were categorized as “no change”, “increase” and “decrease” according to the minimal clinically significant difference value, the number of individuals with increased scaption strength was higher in the kinesio tape group compared to the sham group, although not statistically significant.

Conclusions. The significance of kinesio taping applied to healthy women was not observed compared to sham taping on shoulder scaption muscle strength and upper extremity stability.

KEY WORDS
Muscle strength; kinesio taping; shoulder; stability; supraspinatus.
INTRODUCTION

Glennohumeral joint is the most mobile joint in the human body. Its stability is provided mainly by muscles, which can be divided into movers and stabilizers. The stabilizing muscles are defined as the rotator cuff muscles and they maintain glennohumeral joint alignment which is essential in daily activities. Supraspinatus muscle, one of the rotator cuff muscles functions both as a mover and as a stabilizer during arm abduction (1). Shoulder complaints are frequently encountered (2) and the supraspinatus muscle is a common source of pain (3). Understanding the mechanical effects of the supraspinatus muscle on strength and stability will help in the prevention and management of shoulder injuries and complaints. Guidelines recommend therapeutic exercises during the rehabilitation of rotator cuff tears (4). In daily life, especially during overhead activities, both the rotator cuff and periscapular musculature provide shoulder stability and painless mobility. Scaption is shoulder elevation in the scapular plane and is defined parallel to the scapular plane, and 30° anterior to the coronal plane. There are studies showing that scaption strength is beneficial for preventing injury, reducing pain, and promoting good posture (5). It has been shown that scapion strengthening exercises form the core of the shoulder muscle strengthening program (6). Kinesio taping is widely used in the treatment of many musculoskeletal problems, especially sports injuries (7-10). The tape used is similar to the structural feature and flexibility of human skin. It does not limit joint range of motion but allows movement. Kinesio taping is supposed to have several effects such as supporting muscle and fascia, improving muscle strength (11), function (12), and proprioception (13), reducing pain (14), and improving lymphatic circulation (15).

Studies show conflicting results regarding the effects of kinesio taping in healthy individuals: kinesio taping increased quadriceps muscle strength in one study (16), while it had no effect on hand grip strength in another study (17). Inconsistencies between studies may be due to the differences in methodology such as study design, sample size, or outcome measures used. Similar conflicting results are seen in shoulder kinesio taping studies. Studies investigating the effect of kinesio taping applied to the muscles around the shoulder such as infraspinatus and pectoralis major muscles demonstrated strength improvement in healthy individuals (18). On the other hand, another study reported that kinesio taping of infraspinatus and teres minor muscles did not make a significant difference in shoulder external rotation peak torque in healthy individuals (19). To our knowledge, there is no study regarding kinesio taping applied solely to the supraspinatus muscle in healthy individuals.

The aim of this study is to investigate the effect of kinesio taping applied to the supraspinatus muscle with the facilitation technique on strength and stability in healthy individuals. The hypothesis of the study is that kinesiotaping will increase strength and improve function compared to sham application.

METHODS

Permission and approval number 2021.431.IRB.123 was received for this study by the Koç University Hospital Clinical Research Ethics Committee on November 24, 2021. This study was written according to the Consolidated Standards of Reporting Trials “CONSORT” statements.

Participants

Twenty-six healthy volunteers were included in this study. Inclusion criteria were being 20-30 years old, female, and having a body mass index below 35 kg/m². Those with shoulder and neck pain, body mass index over 35 kg/m², professional athletes, pregnant women, and individuals with chronic muscle and/or neurological diseases were excluded from the study.

Study design

This is a randomized controlled and double-blind study. Individuals were randomized 1:1 into two groups; kinesio taping (n = 13) and sham (n = 13) using a computer program (https://www.randomizer.org/). The flow chart diagram of the study design is shown in figure 1.
Blinding
Kinesio taping was applied by the Physical Medicine and Rehabilitation Physician who did not participate in the assessment of the patients. Taping was applied to all of the individuals who did not know which application was made to them. After kinesio tape application, the participants were asked to wear t-shirts in a way that would prevent the tapes from being seen by the assessor. The assessor performed muscle strength and stability tests without knowing which band was applied to the patients.

Interventions
KinesioTex Gold (Kinesio Holding Corporation, 2017, Albuquerque, NM – GKT15024) with a single color was used in this study. The dominant extremity of the individuals was determined by asking which hand they wrote with. Taping was applied to the dominant extremity of the individuals. Applications were made on hairless, clean skin. After the individuals were asked to take off their clothes, the application was made while sitting comfortably. All taping procedures were performed by the same investigator, based on methods used in previous research, with the technique to stimulate the supraspinatus muscle by facilitation from the origin to the insertion of the muscle group (20). The distance from the supraspinous fossa to the tuberculum major of the humerus was measured with a tape measure (approximately 20 centimeter) and a Y band of this length was prepared. While the individual was in the resting position, the anchor part of the tape was adhered to the end point of the muscle without tension. Then the individual was placed in the position where the supraspinatus muscle was in its longest size with the shoulder in protraction and internal rotation. By applying 50% tension to the area except the first and last 5 centimeters of the tape, the tape was adhered so that the ends were at the origin and the anchor part was at the insertion point of the muscle. Thence the supraspinatus muscle was kept between the arms of the Y band (20-22) (figure 2). In the sham group, the kinesio taping was applied vertically over the scapula not coinciding with the supraspinatus muscle. A single 8 centimeters I tape was adhered to the scapula without stretching, in a way that it would not cross any joint and would not cause activation or inhibition, similar to a previous study (14, 23-25) (figure 3).

Outcome measurements
Demographic information of individuals was recorded before the evaluation. Before and 20 minutes after taping, individuals were evaluated with a dynamometer (Mecmesin Myometer Test, UK) and closed kinetic chain upper extremity stability test. A-20-minute interval was left for the activation of tape (20). Scaption muscle strength was measured with a dynamometer (Mecmesin Myometer Test, UK) and upper extremity stability was evaluated with a closed kinetic chain upper extremity stability test. The Mecmesin Myometer Test is an adjustable belt electronic dynamometer designed to measure shoulder muscle strength. Assessing shoulder strength with a hand-held dynamometer has been shown to be more reliable and objective than manual muscle testing (26, 27). Measurements were recorded on a computer connected to the dynamometer. Visual feedback was provided to the individual as a graphic output on the computer screen (28). While the individual was standing, the arm was positioned in 90 degrees scapular abduction, the elbow in extension and the forearm in neutral (29, 30) (figure 4a). Three measurements were performed with one-minute break. During the measurements, standard verbal instructions (ready 5, 4, 3, 2, 1, push, push, push) were given to the individuals. Each
measurement lasted approximately 5 seconds, the average value at the 2nd and 3rd seconds of the measurements was taken into account and analyzed (31) (figure 4b). The minimal clinically significant difference was accepted as 3 Newtons for the scaption muscle strength (28, 32). The closed kinetic chain upper extremity stability test was used to assess shoulder stability (33, 34). The participant was placed in the push-up position with their knees touching the ground (figure 5). The distance between the two hands was set to 90 cm (35). Three different scores were recorded in this test:
1. Contact Score: for 15 seconds the participant was asked to touch her right hand with her left hand and touch her left hand with her right hand consecutively. Number of hand contacts were counted. This test was repeated three times. Between trials, the individual was rested for 45 seconds. The average score of 3 repetitions was used for the test score (35, 36).
2. Normalized Score: it was calculated by dividing the contact score of the individual by her body height.
3. Power Score: it was calculated by multiplying 68% of the individuals contact score and dividing her weight by 15. The minimal clinically significant difference was accepted as 3.43 units for the contact score, 0.05 units for the normalized score and 18.30 units for the power score (36, 37).

Statistical analysis
A pilot study was conducted by the authors on 10 women before this study. Considering the scaption strength difference values obtained from the pilot study, sample size was calculated as 26 women with 0.05 type 1 error and 0.80 study power. Data were analyzed using SPSS 26.0 for Windows (IBM Corp., Armonk, NY, USA). Data were presented using, median, interquartile range (IQR), minimum and maximum values. 95% confidence intervals were provided. Mann Whitney U test was used to compare two independent continuous variables. Wilcoxon Signed Rank test was used to compare two dependent continuous variables. Chi-square test was used to compare the categorical variables of the groups. The significance level was determined as 0.05 in all analyzes.
RESULTS
Twenty-six healthy women volunteered to participate in the study. There was no statistically significant difference between the age, height, body weight and dominant extremities of the individuals in the kinesio taping and sham groups (table I). In the kinesio tape group, the median scaption strength decreased after taping, whereas there was no significant difference in the median scaption strength after sham taping. The median contact, normalized and power scores increased significantly in both groups (table II). There was no statistically significant difference in terms of scaption strength or upper extremity stability test scores between kinesio tape and sham groups (table II).

Table I. Age, height, weight, body mass index and dominant extremity of participants.

<table>
<thead>
<tr>
<th></th>
<th>Kinesiotape Group Median (IQR)</th>
<th>Sham Group Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>25.0 (4.5)</td>
<td>25.0 (7.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.68 (0.06)</td>
<td>1.65 (0.08)</td>
<td>0.695</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>58.0 (6.5)</td>
<td>65.0 (22.5)</td>
<td>0.695</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.05 (1.44)</td>
<td>22.72 (5.06)</td>
<td>0.434</td>
</tr>
<tr>
<td>Dominant extremity, right, %</td>
<td>92</td>
<td>69</td>
<td>0.322</td>
</tr>
</tbody>
</table>

IQR: interquartile range (75%-25%); BMI: body mass index.

Table II. Kinesiotape and sham groups pre-taping and post-taping strength measurements and upper extremity stability test scores.

<table>
<thead>
<tr>
<th></th>
<th>Kinesiotape Group Median (IQR)</th>
<th>Sham Group Median (IQR)</th>
<th>95%CI*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaption Strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>37.5 (22.8)</td>
<td>41.6 (17.9)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Post-</td>
<td>36.8 (24.0)</td>
<td>42.1 (13.5)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Difference</td>
<td>4.1 (9.0)</td>
<td>0.5 (5.1)</td>
<td>2.80-4.97</td>
<td>0.115</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.016</td>
<td>0.507</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95%CI**</td>
<td>0.2-10.7</td>
<td>-2.5 to 4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>17.3 (4.8)</td>
<td>16.7 (4.0)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Post-</td>
<td>20.7 (3.3)</td>
<td>18.3 (4.8)</td>
<td></td>
<td>0.434</td>
</tr>
<tr>
<td>Difference</td>
<td>3.0 (2.5)</td>
<td>1.7 (2.9)</td>
<td>0.34-1.66</td>
<td>0.115</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.001</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95%CI**</td>
<td>1.3-4.3</td>
<td>0.3-4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>10.2 (3.2)</td>
<td>9.8 (2.7)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Post-</td>
<td>12.7 (2.3)</td>
<td>10.7 (2.1)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Difference</td>
<td>1.9 (1.4)</td>
<td>1.0 (1.8)</td>
<td>0.35-0.96</td>
<td>0.115</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.001</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95%CI**</td>
<td>0.8-2.6</td>
<td>0.2-2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>44.7 (8.3)</td>
<td>51.0 (15.8)</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Post-</td>
<td>54.3 (2.7)</td>
<td>52.0 (21.4)</td>
<td></td>
<td>0.434</td>
</tr>
<tr>
<td>Difference</td>
<td>7.6 (7.0)</td>
<td>5.4 (7.5)</td>
<td>1.19-3.85</td>
<td>1.000</td>
</tr>
<tr>
<td>P-value**</td>
<td>0.001</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95%CI**</td>
<td>3.7-12.2</td>
<td>1.1-9.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IQR: interquartile range (75%-25%); CI: confidence interval; *Mann-Whitney U test, **Wilcoxon signed rank test.


Table III. Comparison of the number of individuals who did no change, increase or decrease according to the minimum clinical significant difference value of the scaption strength and upper extremity stability test scores after taping.

<table>
<thead>
<tr>
<th></th>
<th>Number of individuals with increased value</th>
<th>Number of individuals with decreased value</th>
<th>Number of individuals with no change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kinesiotape</td>
<td>Sham</td>
<td>Kinesiotape</td>
<td>Sham</td>
</tr>
<tr>
<td>Scaption Strength Change</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Contact Score Change</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normalized Score Change</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Power Score Change</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

When the differences in the measurements before and after taping were categorized as “no change”, “increase” and “decrease” according to the minimal clinically significant difference value, the number of individuals with increased scaption strength was higher in the kinesio tape group compared to the sham group, however it did not reach to the level of statistical significance (p = 0.055). The number of individuals with an increase in upper extremity stability test scores was found to be similar between the two groups (table III).

DISCUSSION

This study demonstrated that the number of individuals who observed an increase greater than the minimum clinically significant difference in muscle strength and upper extremity stability test scores with kinesio taping was higher, however it was not statistically significant when compared with the sham group. There are many studies examining the effects of kinesio taping. Significant improvements were obtained in some of these studies (16, 38-40), whereas statistically significant changes were not observed in others (17, 41, 42). There are not sufficient data to explain the reasons for these discrepancies.

In 20 healthy students, taping the deltoid muscle (a Y tape and an I tape perpendicular to it at the coracoid process level) had no significant effect on shoulder stability or functional performance (42). In another study, the immediate effect of kinesio taping applied with the facilitation technique to the infraspinatus and teres minor muscles in the form of two I tapes demonstrated no significant difference regarding peak torque of the shoulder external rotators and shoulder internal-external range of motion in 39 healthy individuals compared to the placebo taping (19). Similarly, in male athletes with rounded shoulders, rotational kinesio tape application did not have immediate effect on shoulder dynamic balance and proprioception, compared to group without tape (43). The abovementioned studies performed the assessments immediately after taping, as in our study. The short time interval might prevent the possible potential beneficial effects of taping on the muscle functions. Hand grip strength measurements performed immediately after kinesio taping applied to the dominant forearm extensor muscles were higher than that of inelastic taping in healthy adults (39). In another study consisting of 150 people, kinesio taping group had greater hand grip strength than that of control group, whereas similar to the group of the soft tissue mobilization with graston (44). Regular users of kinesio tape to the forearm extensor muscles demonstrated a significant increase in hand grip strength compared to those who were not regular users. However, electromyographic activity and self-perceived performance did not differ between the two groups (45).

In studies investigating the long-term effects of kinesio tape, 2 weeks of kinesio tape applied to the dominant forearm in combination to exercise of both extremities, a significant increase was observed in the hand grip strength of the dominant extremity compared to the non-dominant extremity in 32 healthy individuals (46). In another study, taping to the forearm extensor muscles increased maximum hand grip strength significantly as time progressed in the measurements performed at the 30th, 60th, 90th and 120th minutes in 40 healthy individuals (47). Considering the results of these studies, it can be interpreted that the effects of kinesio tape on muscle strength become more evident as the duration of stay of kinesio tape in the body in healthy individuals is extended.

Regarding the direction of kinesio tape application either in a facilitatory or inhibitory direction, studies did not support one’s superiority over another in improving muscle function. Hand grip strength, electromyographic activity or self-perceived performance did not differ when kinesio taping applied to the forearm extensor muscles either in the facilitatory or inhibitory direction in healthy subjects (43). Kine-
Kinesio taping applied to the biceps brachii muscle from proximal to distal or distal to proximal in healthy individuals did not differ, however the application of two horizontal stripes provided the highest peak force (48). Although studies did not suggest any relationship between the direction of kinesio taping and its effect as it was thought, we preferred to apply kinesio taping in the direction of facilitation in our study. The results of kinesio tape applications on the lower extremities were conflicting. Electromyographic activation and torque value increased 24 hours after the application of kinesio tape to the vastus medialis muscle in healthy subjects and the increase lasted 72 hours after the removal of tape (40). In another study, both concentric and eccentric torque of the muscle increased after taping applied to the quadriceps muscle together with isokinetic exercise in healthy women (16). Kinesio tape application to quadriceps muscle improved strength and functional performance greater than that of kinesio taping in combination with brace or only brace in healthy individuals (38). Immediate effect of kinesio taping applied to the quadriceps muscle did not differ regarding bioelectrical activity in electromyography, postural balance or lower extremity functions compared to inelastic adhesive tape in 60 healthy women (49).

In studies investigating athletes, kinesio taping applied to the forearm muscles was not superior to the placebo in the immediately measured hand grip strength (17). With Y-shaped kinesio tape applied to the quadriceps muscle of the athletes; no significant difference was observed in muscle strength measurements performed immediately or 12 hours after taping (41). It can be concluded that the high fitness level of the healthy athletes and their regular training would reduce the possibility of the additional effect of kinesio taping.

In 39 patients with partial rotator cuff tendinopathy, 4 weeks of kinesio tape applied over 3 regions (the supraspinatus and infraspinatus, deltoid muscle belly and an I-shaped tape anchoring the others from the coracoid process towards the scapular spine) found that kinesio taping increased muscle strength of flexors and internal rotators while kinesio taping and exercise increased muscle strength in all directions (50). In our study, kinesio taping was applied only to healthy people and in direction of facilitation, whereas Martins de Silva et al. applied it with the muscle inhibition method. Facilitation of muscle activity due to pain inhibition may have played a role in the increase in muscle strength they found. In our study, we may not have seen an increase in strength because the people were already healthy. The effect of kinesio tape application on shoulder joint position sense, strength, function and return to sports can be investigated in new studies to be planned in athletes such as swimmers, where shoulder pathologies such as scapular dyskinesia are common (51), and overhead athletes following arthroscopic rotator cuff repair (52).

**Limitations of the study**

Absence of long-term follow up is a limitation of our study, we only investigated the acute effects of kinesio taping on muscle strength and upper extremity stability. The results of our study cannot be generalized to individuals and athletes with musculoskeletal problems, since only healthy individuals were included. Sham tape application may be another limitation of the study. Although sham was applied to the posterior surface of the scapula not aligned with the direction of muscle fibers and without tension, we cannot be completely sure that it had no effect on the infraspinatus or trapezius muscle.

**CONCLUSIONS**

Kinesio taping applied to healthy women was not superior to sham taping on shoulder scaption muscle strength and upper extremity stability. In order to better understand the effects of kinesio taping in future studies, it is recommended to make measurements at different times after the application and to plan studies that will examine the effects on athletes and patients.

**FUNDINGS**

None.

**DATA AVAILABILITY**

Data are available under reasonable request to the corresponding author.

**CONTRIBUTIONS**

DK, HA, OOT: data collection, data analysis and interpretation, writing - original draft, writing - review & editing. All authors: study design.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interests.
Effect of Kinesio Tape Applied to Supraspinatus Muscle on Shoulder Scaption Strength and Upper Extremity Stability

REFERENCES


SUMMARY

Background. Kyphosis is a prevalent condition in older women that can significantly impact their mobility. Various therapeutic approaches and modalities can be used to treat hyperkyphosis. Cervicothoracic fascia stretching reduces kyphotic angle, decreases cervical pain, and improves various dimensions of thoracic mobility.

Purpose of the study. The aim of this study is to evaluate the effect of the cervicothoracic fascia stretch on the thoracic mobility of older women.

Subjects and methods. Sixty older women with non-specific neck discomfort, whose ages ranged from 60 to 70, were recruited from the outpatient clinics at Heliopolis University. The recruited patients were randomly divided into two groups of equal numbers of subjects: Group A included 30 patients who underwent cervicothoracic fascia stretching, and 30 patients were included in Group B who underwent diaphragmatic breathing exercises three times a week for four weeks. The neck pain was assessed utilizing the Neck Pain and Disability Scale (NPAD), while thoracic kyphosis was assessed utilizing a flexion curve ruler. Moreover, thoracic circumference excursion was measured using tape, while transverse and latero-lateral dimensions of the thorax were assessed using a chest depth caliper.

Results. The comparison between pre- and post-study results showed a significant enhancement in all measured variables, including neck pain, thoracic circumference, chest width dimension, chest depth dimension, and kyphotic angle in both groups. Our findings indicated a statistically significant decrease in neck pain and kyphotic angle. Conversely, there was a statistically significant increase in thoracic circumference and chest width dimension for the cervicothoracic stretch group after treatment.

Conclusions. Both interventions, such as intrathoracic fascia stretch and diaphragmatic breathing exercises exhibited extraordinary effectiveness in the management of reduced thoracic mobility and neck pain in older women with better results in favor of cervicothoracic stretch.

KEY WORDS
Cervicothoracic fascia; elderly; chest dimensions; thoracic mobility; fascia properties.

INTRODUCTION

The aging process is associated with gradual alterations in the physiology of the musculoskeletal system, resulting in excessive bone destruction, vertebral fractures, muscle weakness, and degenerative disorders that result in thoracic hyperkyphosis (1). Hyperkyphosis is defined as excessive concavity of the thoracic spine (> 40 degrees) and affects 20-40% of the elderly population worldwide (2).
Limited mobility in the thoracic spine has been recognized as a predictor of neck pain due to the close anatomical link between the cervical and thoracic spines (3). Fascia generates network connections all over the body parts and also connects the muscular and non-muscular parts (4). In the event that the fascia is subjected to tension from a particular area, it will result in the displacement of other interconnected anatomical structures in relation to this tension (5).

Endothoracic fascia originates in the deep and median cervical fascia (6). It runs from the occipital pharyngeal tubercle to the inner surface of the thoracic region; strongly connected to the pleural, the middle cervical fascia stretched into the front thorax to cover the pleural dome (7). The endothoracic fascia is also connected to the subclavian artery sheath in the frontal region, establishing a link with the cervical fascia (8). The coastal insertions of the diaphragm act as a barrier between the base of the lower endothoracic, which covers the diaphragm, and the abdominal wall, which is connected to it by the fascia transversalis (9). This fascia has a direct relationship to the pleura, pericardium, and diaphragm (10). The fascia also has a solid connection to the spine vertebra, affecting the alignment of the spine (5). The interior of the fascia is firmly fused to the parietal pleura (11). Around the mediastinum, it is thick and velvety (6). The typical locations between the endothoracic fascia and the pleura membrane are directly connected to the vertebral column, the thoracic wall anteriorly in the sternum, and posteriorly between the posterior angle of the ribs the vertebral column (12).

Many different techniques have been used to manage thoracic mobility and chest expansion, including rib mobilization, thoracic vertebral manipulation, thoracic muscle stretching, breathing exercises, and recoil manipulation of the sternum (3). The technique of stretching the intrathoracic fascia has been found to be an effective means of enhancing motor function, restoring mobility, and alleviating discomfort (13). Restoring the correct tension between the various linked anatomical places helps increase thoracic mobility and chest expansion, enhances motion range and flexibility, and improves optimal spine alignment and tension balance in chest muscles (14).

To date, only a limited number of studies have explored the correlation between fascia and the kyphotic curve of the thoracic neck, as well as its width, depth, neck discomfort, and thoracic excursion (15). The study was hypothesized that there is no significant effect of cervicothoracic fascia stretch on thoracic mobility in elderly women. Consequently, the comparability of our findings with those of prior studies on all pertinent aspects was limited.

MATERIALS AND METHODS

Study design
A six-month randomized controlled trial was conducted in orthopedic outpatient clinics of Orthopedic Helioptolis University (February 2021 to July 2021). The patients received a detailed explanation of the treatment program and measurement devices and provided their written informed consent prior to the start of our trial. The study was approved by the ethics committee of the physical therapy faculty at Cairo University (P.T.REC/012/003586 – date of approval: January 04, 2022).

Participants
The patients were included according to the following criteria: 60 older women with non-specific neck pain, their age between 60 and 70 years, body mass index (BMI) was 25-29.9 kg/m², osteopenia level was between -1.0 and -2.5. The patients were included only if their vital signs were controlled and deemed medically and clinically stable during the study. To avoid type II error, sample size calculation was performed using G*POWER statistical software (version 3.1.9.2; Franz Faul, Universitat Kiel, Germany) [F tests- MANOVA-repeated measures, within and between interaction, α = 0.05, power = 80%, effect size = 0.19] and revealed that the appropriate sample size for this study was n = 60 (figure 1).

To date, only a limited number of studies have explored the correlation between fascia and the kyphotic curve of the thoracic neck, as well as its width, depth, neck discomfort, and thoracic excursion (15). The study was hypothesized that there is no significant effect of cervicothoracic fascia stretch on thoracic mobility in elderly women. Consequently, the comparability of our findings with those of prior studies on all pertinent aspects was limited.

Figure 1. Power analysis.

Patients were excluded from the study if they met any of the following criteria
Acute cervical disc problem, spinal cord indentation, acute spinal fracture, acute infection, recent trauma, local acute inflammation, and osteoporosis T-score below -2.5. All participants were randomly divided into two groups, group A included 30 female patients who received a cervicothoracic fascia stretch; physiotherapy treatment was...
administered to all patients three days a week for one month; group B included 30 female patients who received diaphragmatic breathing exercises and physical therapy treatment was administered to all patients three days a week for one month.

Measurement procedures
Measurement and assessment were performed before and four weeks after the initiation of the treatment protocol for all patients in both groups. The experimental protocol was thoroughly explained to each patient before the initial assessment, and written consent was obtained from all recruited subjects. The treated patients were advised to disclose any side effects experienced during treatment sessions.

The chest depth caliper is a flexible opening tool to measure different chest dimensions in depth and width in centimeters; it was used to measure the different chest expansions, including anteroposterior and transverse dimensions which is considered as good reliability tool (16). The kyphotic angle is measured by the flexion curve ruler, which is made of plastic material and a flexible tool that can be molded on the thoracic spine curve from C7 to T12 (17). The Flexion curve method is as reliable and valid method to measure thoracic kyphosis, which is a technique that can be easily applied in both clinical and human posture research contexts (17). Chest circumference is measured by tap measurement; it measures the circumference of the chest during the thoracic excursion which is reliable method for healthy, asthma and COPD patients (17). The Neck Pain and Disability Scale (NPAD) was used to measure the intensity of neck pain, limitations in activity, and restricted motion during usual daily living and working times (18). Each patient’s measurements were obtained three times in order to ensure accuracy and attain intrarater reliability.

Treatment procedures
This study was conducted to evaluate the efficacy of cervicothoracic fascia stretch versus diaphragmatic breathing on thoracic mobility among elderly women using multiple assessments, including chest depth Caliper, flexion curve ruler, neck pain disability scale, and tape measurement to measure chest width and depth dimension, kyphosis curve, pain level, as well as chest excursion, respectively.

Group A

Patient preparation
The patients were positioned in a supine position on the T2-3 level, legs straight, both shoulders held against the table, and arms straight beside the patient’s body. Each patient in this group received an intrathoracic fascia stretching technique on the cervicothoracic fascia for three weekly sessions for four weeks (19). Prior to beginning treatment, patients were evaluated for previous medical histories such as cardiovascular, renal, hepatic, and musculoskeletal diseases.

The intra-thoracic fascia stretch technique
The intrathoracic fascia stretch technique was for the central and two lateral fascia chains. For central fascia stretch, patients were placed supine with the patient’s head out of the plinth and held under the occiput bone to start little traction. The patients were asked to take deep breaths, and then traction was performed from the occiput bone in a straight upward direction while maintaining deep breathing. During abdominal inhalation, downward pressure of the diaphragm was observed, the ribs displaced laterally, and the sternum moved in ventrally/cranial motion. The head was positioned on the opposite side of the lateral fascia, bending with ipsilateral-lateral rotation to the body side. Traction was administered under the occiput, and the patient was asked to take a deep inhalation through the abdomen; then, the traction was performed by pulling gradually with the maintenance of the head position. Repeated the gradual occiput traction on both fascia chains (20).

Group B

Patient preparation
The participants were given instructions to remove any form-fitting shirts and replace them with looser clothes. Prior to beginning treatment, patients were evaluated for previous medical histories such as cardiovascular, renal, hepatic, and musculoskeletal diseases.

Signs should be observed during diaphragmatic breathing
The patient’s abdomen should rise and fall while breathing, but the upper chest should be relatively still. Patients should not use accessory breathing muscles to concentrate on diaphragm muscle training. The regular breathing was observed to prevent irregular patterns. Patients should not eat for two hours prior to treatment (21).

Diaphragmatic breathing technique
The recruited subjects were seated in a comfortable position and instructed to take slow and deep breaths, inhale through the nose and exhale through the mouth. Before the beginning of the therapeutic intervention, patients were instructed to “place one hand on their abdomen to
expand it during inhalation and the other hand on the upper chest to control thoracic motion”. The hands under the ribs gave light resistance. All patients started with a set breathing cycle: natural breathing for 5 min, followed by the deep breathing phase for 5 min, and then more than 5 min of natural breathing. Each patient took four sets, and the rest time was 1-2 minutes between sets for three weekly sessions for four weeks (22).

Statistical analysis
Unpaired t-tests were utilized to compare subject characteristics between groups. The Shapiro-Wilk test was utilized to confirm the normal distribution of data, while Levene’s test was conducted to assess the homogeneity of variances between groups. Mixed MANOVA was used to examine the impact of treatment on the thoracic circumference, chest depth, chest width, NPAD, and kyphotic angle. Subsequent multiple comparisons were conducted utilizing post hoc tests with Bonferroni correction. The significance level for all statistical tests was set at p < 0.05. All statistical analyses were done utilizing IBM SPSS V.25 for Windows.

RESULTS

Subject characteristics
Our findings revealed that there were no significant differences between the two groups in relation to age, weight, height, and BMI (p > 0.05) (table I).

Effect of treatment on thoracic circumference excursion, chest depth, chest width, NPAD, and kyphotic angle
There was a significant increase in thoracic circumference excursion, chest depth, and width post-treatment in both groups compared to pre-treatment (p > 0.001). Group A exhibited a percentage increase of 123.72%, 29.33%, and 36.38% in the thoracic circumference, chest depth, and width excursion, respectively. In contrast, Group B demonstrated a percentage increase of 73.53%, 25.81%, and Both study groups demonstrated a statistically significant reduction in NPAD and kyphotic angle following the intervention in comparison to their respective baseline measurements (p > 0.001). The findings indicate that the percentage of decrease between NPAD and kyphotic angle for group A was 29.89% and 16.93%, respectively. For group B, the corresponding percentages were 6.76% and 7.9%, accordingly (table II).

Between groups
Before the initiation of the intervention, no statistically significant distinctions were observed among the cohorts (p > 0.05). However, post-treatment comparison indicated a significant increase in thoracic circumference excursion and chest width for group A compared to group B (p < 0.01). Conversely, there was no significant difference in chest depth (p > 0.05). Moreover, the NPAD and kyphotic angle of group A exhibited a significant decrease in post-treatment as compared to group B (p < 0.001).

DISCUSSION
The aim of the present investigation was to evaluate the impact of intra-thoracic fascia stretching on thoracic mobility among elderly females. The present investigation revealed that there were no significant dissimilarities in weight or height among the two groups that were matched for age. The present study’s results pertaining to the influence of intrathoracic fascia stretching on chest dimensions indicate that a 4-week stretching intervention resulted in a statistically significant enhancement in chest width, exhibiting a percentage change of 36.38%, and an improvement in depth, demonstrating a percentage change of 29.33%.

The present findings align with the research conducted by Kevin Coulier et al. (23), which documented an improvement in lung function and thoracic mobility among adult patients with chronic respiratory diseases who underwent...
upper thoracic and rib therapy alongside intrathoracic fascia stretching. However, the present research contradicts the results of Heneghan et al. (24), as they observed that rib mobilization was more effective in enhancing thoracic mobility in patients with respiratory issues than the myofascial release of increased modulation of joint structure and more efficient enhancement of mobility function. The Heneghan study’s sample size and health conditions differed from the current study’s, which resulted in the conclusion that rib mobilization is preferred to fascial release. Therefore, the primary factors elucidating the varied effects of cervicothoracic fascia stretching on thoracic mobility are the sample size, sex, and health condition of the patients (24). Moreover, the present study indicates a significant improvement in chest circumference due to the stretching of intrathoracic fascia, with a percentage change of 123.72%.

Bordoni et al. (25) stated that although the study did not explore the impact of stretching the endothoracic (cervicothoracic) fascia on individuals with respiratory limitations, their outcomes revealed that the structure of the fascia could be a contributing factor to the ongoing restriction of thoracic excursion. Furthermore, Bordoni et al. (25) suggests that when the fascia is stretched, it triggers the activation of mechanical and nociceptive receptors, which subsequently impacts the pain threshold and mobility of the fascia; additional, they have reported that the cervicothoracic (endo thoracic) can be stretched by targeting the deep fascial layer.

### Table II. Statistical analysis of data between Groups A and B.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>MD</th>
<th>% of change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic circumference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>excursion (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>2.53 ± 0.67</td>
<td>5.66 ± 1.71</td>
<td>-3.13</td>
<td>123.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>2.72 ± 0.52</td>
<td>4.72 ± 1.1</td>
<td>-2</td>
<td>73.53</td>
<td>0.001</td>
</tr>
<tr>
<td>MD</td>
<td>-0.19</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.21</td>
<td></td>
<td>p = 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest depth (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>18.14 ± 1.67</td>
<td>23.46 ± 1.59</td>
<td>-5.32</td>
<td>29.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>18.6 ± 1.22</td>
<td>23.4 ± 1.24</td>
<td>-4.8</td>
<td>25.81</td>
<td>0.001</td>
</tr>
<tr>
<td>MD</td>
<td>-0.46</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.23</td>
<td></td>
<td>p = 0.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest width (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>16.63 ± 1.86</td>
<td>22.68 ± 1.69</td>
<td>-6.05</td>
<td>36.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>16.6 ± 1.54</td>
<td>21.53 ± 1.47</td>
<td>-4.93</td>
<td>29.70</td>
<td>0.001</td>
</tr>
<tr>
<td>MD</td>
<td>0.03</td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.94</td>
<td></td>
<td>p = 0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>26.26 ± 3.89</td>
<td>19.46 ± 4.47</td>
<td>6.8</td>
<td>25.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>27.2 ± 3.49</td>
<td>25.36 ± 4.11</td>
<td>1.84</td>
<td>6.76</td>
<td>0.001</td>
</tr>
<tr>
<td>MD</td>
<td>-0.94</td>
<td>-5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.33</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyphotic angle (degrees)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>15.48 ± 1.51</td>
<td>12.86 ± 1.34</td>
<td>2.62</td>
<td>16.93</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>15.56 ± 1.43</td>
<td>14.33 ± 1.42</td>
<td>1.23</td>
<td>7.90</td>
<td>0.001</td>
</tr>
<tr>
<td>MD</td>
<td>-0.08</td>
<td>-1.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.83</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation; MD: mean difference.
The current findings were in conflict with those of Swapna et al. (26), who found no appreciable improvement in dyspnea and expansion of chest circumference in restricted respiratory patients after the myofascial release of the structure of the soft tissue of the chest. Herein, the intrathoracic fascia stretches also showed statistically significant improvements in the kyphotic angle curve with percentage changes of 16.93% and 25.89%, as well as cervical pain. This result is in agreement with Mousavi et al. (27), who found that corrective exercise and stretching of the soft myofascial pectoral region combined had a more significant impact than exercise alone in reducing the kyphotic curve and neck pain. Moreover, Rodriguez-Huguet et al. (28) reported that patients with neck pain who underwent cervical myofascial release experienced a significant improvement in their neck pain. However, Kim et al. (29) discovered that cervical myofascial release had no statistically significant impact on neck discomfort and head forward posture. The diaphragmatic breathing exercise led to statistically significant improvements in chest depth and width motions, with a percentage change of 25.81% and 29.7%, respectively. Furthermore, the diaphragmatic breathing exercises positively impacted asthmatic patients’ chest dimensions, according to the findings reported by Shaw & Shaw (30). Our conclusions indicated that diaphragmatic breathing exercises significantly increased intraabdominal pressure, which in turn caused the lower ribs to migrate laterally upward and outward, thus widening the chest, the upper ribs, and the sternum to shift anteriorly and posteriorly, consequently deepening the chest. Herein, the diaphragmatic breathing exercise resulted in a post-treatment statistically significant improvement in thoracic circumference excursion, with a percent change of 73.53%. Consistently, Kim et al. (22) found that the utilization of diaphragmatic breathing exercises and mobilization of the rib cage led to the activation of parasympathetic nerves and suppression of sympathetic nerves. Diaphragmatic breathing exercises showed a statistically significant post-treatment reduction in neck discomfort in the current trial, with a percent change of 6.76% and 7.9% in the kyphotic angle. Studies have indicated that practicing diaphragmatic breathing techniques can improve posture by decreasing spinal kyphosis and promoting relaxation of the inflexible rib cage. Therefore, there is a belief that they may be a viable treatment option for individuals who suffer from chronic non-specific neck pain (31).

The current investigation utilized identical variables to conduct a comparative analysis between the two groups. The research findings concluded no statistically significant variation in chest depth between the two groups. However, it is significant that the intrathoracic stretch group exhibited superior performance in all other measures compared to the diaphragmatic breathing group. The observed phenomenon could potentially be attributed to the activation of fascial mechanoreceptors resulting from the stretching of the intrathoracic fascia, leading to a modification in the distribution of fascial forces across the chest region (32). The augmentation of blood circulation and elongation of the fascia spanning from the cervical to the thoracic region resulted in an enhancement of thoracic mobility (33). The present study centers on the utilization of intrathoracic fascia stretching in combination with other therapeutic modalities to enhance the rehabilitative outcomes of elderly patients (34). One of the study’s limitations was the small sample size. Another limitation of the study was its restricted emphasis on women and a certain age range. Our study aimed to assess the efficacy of intrathoracic fascia stretching in comparison to various intervention techniques, as well as to identify the most successful treatment ways for various medical diseases. It was also recommended to include people of all ages and a large sample size in order to find the correlation between the cervicothoracic fascia stretch with other modalities and how the combination will affect different samples.

CONCLUSIONS

The study’s findings suggest incorporating intrathoracic fascia stretching into treatment plans can effectively address thoracic mobility restriction, chest expansion of varying dimensions, and thoracic kyphotic angle in elderly female patients. The implementation of intrathoracic fascia as a therapeutic option may be considered a safe and effective approach to address complications in older women within a treatment program.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

NGE, SAFE, AME: conceptualization, methodology, writing - review & editing, supervision. RAHY: resources, investigation, writing – original draft.

CONFICT OF INTERESTS

The authors declare that they have no conflict of interests.
REFERENCES


33. Lesondak D, Akey AM. Fascia, function, and medical applications. 2020. CRC Press.
Effect of Pilates Exercise Combined with Balance Training on Lumbopelvic Stability and Shooting Accuracy in National Level Archers

Sadra Khan¹, Deepak Malhotra², Meenu Dhingra³, Mohammad Ahsan⁴, Shibili Nuhmani⁴, Mohd Arshad Bari⁵

1 Department of Rehabilitation Sciences SNSAH, Jamia Hamdard, New Delhi, India
2 Department of Physiotherapy, Jamia Hamdard, New Delhi, India
3 Human Performance Lab, Sports Authority of India, Jawaharlal Nehru Stadium Complex, New Delhi, India
4 Department of Physical Therapy, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
5 Department of Physical Education, Aligarh Muslim University, Aligarh, India

SUMMARY
Objective. Lumbopelvic stability and shooting accuracy are some of the key determinants in the performance of archers. This study aimed to investigate the effect of Pilates exercise intervention combined with balance training on lumbopelvic stability and shooting accuracy in national level archers.

Methods. A randomized control trial with pre-test and post-test control group design was conducted with thirty male professional archers between the ages of 18-30 years. The participants were randomly allocated into three groups (ten in each group: PB = Balance and Pilates exercises; B = Balance Exercises; C = No exercises). The lumbopelvic stability was determined using the Knee Lift Abdominal Test (KLAT) and Bent Knee Fall-Out Test (BKFOT) for both legs by using a pressure biofeedback unit. Shooting accuracy was measured by taking the score of two ends with 12 arrows. Baseline measurements were taken before given intervention. The training program was given for 20 minutes a day and three times per week for six weeks.

Results. One-way ANOVA showed significant difference in shooting accuracy (\(F = 5.11, \eta^2 = 0.159, p = 0.03\)), KLAT (Right) (\(F = 8.51, \eta^2 = 0.240, p = 0.00\)), KLAT (Left) (\(F = 6.52, \eta^2 = 0.187, p = 0.01\)) and BKFOT (Left) (\(F = 6.31, \eta^2 = 0.195, p = 0.01\)). Whereas no significant difference is shown in BKFOT (Right) (\(F = 0.99, \eta^2 = 0.035, p = 0.33\)) among B, PB, and Control group for baseline measurement and after intervention.

Conclusions. Although balance training showed improvements in the post-test values, the most significant improvements were recorded in the Pilates plus balance training group. This information can be used to plan the training protocols for archers.

KEY WORDS
Accuracy lumbopelvic; stability; Pilates exercises; balance exercises; pre-test-post-test.

INTRODUCTION
Archery is a non-contact, static sport that demands precision, control, focus, physical ability, and determination. Archers must have a high level of physical fitness and motor competence to win national and international level competitions. Improving physical fitness and motor skill factors like core body strength, upper body strength, handgrip, leg power, and static balance can positively impact performance of the
archers (1, 2). In archery, upper limb and shoulder muscles’ endurance and shooting accuracy are the most important factors to achieve the best performance in the sport. The endurance component of the upper limb muscles is much warranted, reflecting the quantity of pull and push forces that these muscles exert continuously. The major muscles of the upper limbs are activated during the shooting process (3). Accuracy is a principal skill that the archer should acquire. A good shot accuracy will be produced if the technique is good and consistent. It will be difficult for an archer to win a competition if the shooting accuracy is poor (4).

Pilates are low impact exercises that concentrate on the smaller and deeper muscles, which help in improving flexibility, abdominal, lumbopelvic stability and muscular activity. Pilates method emphasizes on precision of movement with synchronization of the breathing. It’s a well-structured exercise program with engagement of body core while implementing the fluidic movements. The principles of Pilates exercises include centering, focus, control, breath, precision, and fluidity (5, 6). Pilates exercises facilitate activation of transversus abdominis, diaphragm, multifidus and pelvic floor muscles and these muscles contribute to the lumbopelvic stability (7, 8). Pilates exercises can be considered an exercise intervention that increases muscle strength and improves static and dynamic balance. Emery et al. reported that Pilates exercises improve poor posture by strengthening the muscles and improving the balance (9). A study on Pilates exercise and balance indicated that 16-week mat Pilates exercises improved static and dynamic balance in university students (10). Bird et al. demonstrated that a 5-weeks Pilates training program resulted in larger benefits in static and dynamic balance (11). Jang also indicated that balance ability was improved through Pilates exercises (12).

Balance is the process of maintaining the body’s center of gravity (COG) vertically over the base of support. Balance training has been used to promote balance and sports-related skills and prevent and rehabilitate lower extremity sports injuries (13). It has been proven as an effective intervention to improve static postural sway and dynamic balance in athletes and non-athletes (8). It is also seen that an improved balance is linked to good lumbopelvic stability (14). Kim found that 12 weeks of balance training in the Sprinter/Skater pattern affects a high school archer’s static and dynamic stability (15). According to Brill, posture stability and static balance skills increased after the Pilates exercise program (16).

Previous research has shown the significance of postural stability in archery performance, especially in the last second before string release and during the release itself (17-19). Limited postural sway and postural consistency across shots are other important predictors of archery efficiency (20, 21). The performance characteristics are needed in the pelvic region, trunk, shoulder girdle, and arms to ensure shooting accuracy. In addition to strength and endurance, postural stability is an important factor in determining the success of each shot.

In order to have better performance, archers require proper strength, balanced pose and stability of respiratory function (22). Consistency and stability are vital in archery; quite often archers have to maintain an asymmetric posture, which could be a reason for their poor performance (23). Hence, their shooting accuracy and stability need to be improved to increase their performance, which is thought to be done through balance training and Pilates exercises. Therefore, this study aimed to investigate the effect of Pilates exercise intervention combined with balance training on lumbopelvic stability and shooting accuracy in national level archers.

**MATERIALS AND METHODS**

**Study design and setting**

A randomized control trial with pre-test and post-test control group design was selected to conduct this study. The participants were selected from an International Stadium in, New Delhi and the study was conducted in the shooting range of JLN stadium.

**Ethical approval**

The Institutional ethics committee of Jamia Hamdard approved the study (reference no. 02/19 – date of approval: February 13, 2019). Written informed consent was taken from every participant. This study was conducted as per the principles of Helsinki.

**Sample size calculation**

The sample size was calculated by G*Power software (3.1.94) with the combination as statistical test-means: differences between matched pairs, types of power analysis – *A priori*: compute require sample size – given α = 0.05, Power (1-β err prob) = 0.80, and effect size dz = 0.5. The calculated sample size is 27. The 10% samples were increased to conduct this study.

**Participant**

Thirty male archers with an average age of 20.33 years and an average participation experience of 2.9 years at the national level, who were in regular practice, were recruited for this study. The participants were randomly allocated into three groups (ten in each group: PB = Balance and Pilates exercises; B = Balance Exercises; C = No exercises) by an independent researcher who was not part of the current study. The consort diagram of participant flow is available.

Sadra Khan, Deepak Malhotra, Meenu Dhingra, et al.
as figure 1. The participants who had a recent history of musculoskeletal injury or neurological impairment, biomechanical abnormalities, and history of any medication or vertigo that directly or indirectly impairs the performance were excluded.

**Outcome measures**

**Lumbopelvic stability**

The lumbopelvic stability was determined using the Knee Lift Abdominal Test (KLAT) (24) and Bent Knee Fall-Out Test (BKFOT) (25) (figures 2,3) for both legs by using a pressure bio-feedback unit (Stabilizer®, Chattanooga Group Inc., Hixson, TN, USA., SKU-9296-5338276 USA). This device provides a measuring range of 0-200 mmHg pressure with an accuracy of ± 3 mmHg. This unit consists of a combined gauge and inflation bulb connected to a pressure cell. The highest absolute mmHg variation from the pre-set baseline pressure (40 mmHg) was recorded.

**Shooting accuracy**

Shooting accuracy was measured by taking the score of two ends with 12 arrows (each end containing 6 arrows). The position of the shaft on the target determines the scores. Mostly the targets have ten concentric circles with different color codes. The whole target is divided into 10 scoring zones ranging from 10 to 1 depending upon the proximity from the pinhole in the center. Hitting the innermost circle will fetch an archer 10 points and hitting the outermost circle will fetch 1 point. For an end of 6 arrows, the archer gets a time limit of 4 minutes. Arrows shot outside the time limit are not counted. If an arrow’s
shaft crosses two colors or any separating lines between two scoring zones, the arrow will score the greater value of the two zones (2).

Interventions

Pilates exercise training
Pilates exercise training was given to the participants as per the Park et al. protocol (23). The warmup included breathing, rolling back, coccyx curl and hundred breathing. The main workout included exercises like single leg stretch, straight leg raises, basic bridge, bridging variation, quadruped, clap with seal motion, mermaid twist, swimming, double leg stretch, shoulder bridge, swan dive and leg full front. Rest position, cat with arm/leg extension and breathing were given as cool down activities. The whole protocol took around 50 minutes in administration. The above article may be referred for the details of the protocol (23).

Balance training
Balance training was given to the participants as per the recommendation of the present literature. The training was done in double limb stance, single leg stance and tandem stance for a period of six weeks with three training sessions per week. The progression was done in terms of progression from double limb stance to single limb stance to tandem stance; from eyes open to eyes closed and from a firm stable surface to an unstable surface like foam mat. The double limb eyes open stance on the firm surface was skipped for all the subjects. Each session lasted for a period of 20 minutes (26, 27).

Procedure
Upon their arrival at the shooting range, all the necessary information, relative risk, and study benefits were imparted to all the participants. The participants were screened according to the inclusion and exclusion criteria, and demographic and anthropometric measurements were taken. A baseline measurement of lumbopelvic stability and shooting accuracy was taken for all the three groups. Lumbopelvic stability was tested with Pressure Biofeedback Unit with the help of two tests, i.e., KLAT and BKFOT as follows. The participants were in a crook lying position for the KLAT. The pressure biofeedback unit was inserted vertically under the lumbar spine and the lower edge two centimeters caudal of the posterior superior iliac spines on the lower edge the opposing side of the flexed knee. In addition, a folded towel was placed near the pressure biofeedback unit to maintain the same height on both sides of the lumbar spine. The pressure biofeedback unit’s basal pressure was increased to 40 mmHg (baseline pressure). After that, one hip was flexed, and the knee was also flexed to 120 degrees, with the foot lying on the examination couch’s surface. Participants were instructed to return to the beginning position by carefully bending their hip to about 45 degrees of abduction/lateral rotation while keeping their foot supported alongside their straight knee. The maximum pressure deviation was measured and analyzed further (26). A simple long arm goniometer with a 360° angle was used to control the starting positions of the hips and knees during KLAT and BKFO. The participants were tested for their shooting accuracy as well. Accuracy was tested by taking the scores of 2 ends, i.e., 12 arrows. The position of the shaft on the surface of the target determines the score.

After taking the baseline (pre-test) measurements, the PB group (Pilates + Balance training) was given a Pilates exercise program of 50 minutes with 10 minutes warm-up and 10 minutes cool down period for six weeks with a frequency of three times per week. Also, balance training was given in this group for 20 minutes 3 times per week for six weeks. The exercises were breathing, rolling back, coccyx curl, and a hundred for warm-up. In the workout session, the exercises were single leg stretch, straight leg raise, basic bridge, bridging variation, quadruped, clap with seal motion, mermaid twist, swimming, double leg stretch, shoulder bridge, swan dive, leg full front. The cooldown period consisted of rest, cat with arm/leg extension and breathing.

The balance training (B) group received a balance training program for 20 minutes and three times per week for six weeks. The training was adjusted and progressed according to the capability of the athletes. At first, the training was performed on the floor and then the same exercises were performed on foam. Exercises started with double limb eyes open progressing to double limb eyes close progressing to single-limb eyes open to single-limb eyes close and then progressing to tandem stance with eyes open and then closed. Group C (control) did not receive any type of training. Post-test measurements for lumbopelvic stability and shooting accuracy were taken of all the three groups after completion of the six weeks training program.

Statistical analysis
Statistical analysis was computed using IBM SPSS software version 23. Data were screened for missing data, outliers, typo
error, and normality of data was confirmed with the Shapire-Wilk test. The descriptive statistics of the anthropometric data were used to calculate the mean and standard deviation. The inferential statistics were used to determine the variance in the anthropometric data. One way analysis of variance (ANOVA) was used to determine the variance between groups for lumbopelvic stability and shooting accuracy of national-level archers. The least significant differences (LSD) post-hoc test was used to find the significant difference within groups. Partial Eta squared ($\eta^2$) was used to determine the effect size as the $\eta^2 = 0.01$ considered as small effect; $\eta^2 = 0.06$ considered as medium effect; and $\eta^2 = 0.14$ considered as large effect (28). The statistical results were considered statistically significant if the P-value was less than 0.05.

**RESULTS**

One-way ANOVA showed significant difference in shooting accuracy ($F = 5.11, \eta^2 = 0.159, p = 0.03$), KLAT (Right) ($F = 8.51, \eta^2 = 0.240, p = 0.00$) KLAT (Left) ($F = 6.62, \eta^2 = 0.187, p = 0.01$) and BKFOT (Left) ($F = 6.31, \eta^2 = 0.195, p = 0.01$). Whereas no significant difference showed in BKFOT (Right) ($F = 0.99, \eta^2 = 0.035, p = 0.33$) among B, PB, and Control group for baseline measurement and after intervention. Post-hoc (LSD) test showed a significant difference in shooting accuracy ($p = 0.03$) for Pilates plus balance training group only when compared to the control and the balance training group. For KLAT (Left and Right) there were statistically significant differences between the balance only and the control group ($p = 0.03$ and 0.02). Pilates plus

<table>
<thead>
<tr>
<th>Anthropometric Characteristics</th>
<th>Balance training (Mean ± SD)</th>
<th>Pilates+Balance training (Mean ± SD)</th>
<th>Control (Mean ± SD)</th>
<th>F-value</th>
<th>Partial Eta Squared</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.40 ± 2.06</td>
<td>19.40 ± 2.11</td>
<td>22.20 ± 4.46</td>
<td>1.73</td>
<td>0.159</td>
<td>0.032</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>64.74 ± 9.54</td>
<td>65.54 ± 11.64</td>
<td>62.89 ± 10.37</td>
<td>2.34</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>164.76 ± 5.40</td>
<td>168.57 ± 4.37</td>
<td>165.28 ± 6.21</td>
<td>1.63</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.78 ± 2.07</td>
<td>22.70 ± 2.45</td>
<td>22.30 ± 1.64</td>
<td>2.36</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 0.05 level; BMI: Body Mass Index.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline Mean ± SD</th>
<th>Post-training Mean ± SD</th>
<th>F-value</th>
<th>Partial Eta Squared</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shooting Accuracy (Score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>108.50 ± 9.05</td>
<td>111.40 ± 6.31</td>
<td>5.11</td>
<td>0.159</td>
<td>0.032</td>
</tr>
<tr>
<td>PB</td>
<td>96.10 ± 12.65</td>
<td>103.30 ± 12.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>107.60 ± 6.43</td>
<td>106.20 ± 10.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLAT (Right) mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>60.70 ± 7.67</td>
<td>55.10 ± 9.98</td>
<td>8.51</td>
<td>0.240</td>
<td>0.007</td>
</tr>
<tr>
<td>PB</td>
<td>50.80 ± 6.39</td>
<td>45.30 ± 3.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56.80 ± 8.40</td>
<td>51.60 ± 10.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLAT (Left) mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>63.60 ± 12.24</td>
<td>54.80 ± 6.87</td>
<td>6.62</td>
<td>0.187</td>
<td>0.016</td>
</tr>
<tr>
<td>PB</td>
<td>51.80 ± 8.85</td>
<td>44.40 ± 4.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56.00 ± 12.68</td>
<td>61.00 ± 10.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKFOT (Right) mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>50.10 ± 5.60</td>
<td>48.20 ± 6.44</td>
<td>0.99</td>
<td>0.035</td>
<td>0.328</td>
</tr>
<tr>
<td>PB</td>
<td>47.20 ± 2.57</td>
<td>46.60 ± 3.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>49.20 ± 5.69</td>
<td>48.70 ± 6.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKFOT (Left) mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>44.50 ± 4.40</td>
<td>46.50 ± 3.40</td>
<td>6.31</td>
<td>0.195</td>
<td>0.018</td>
</tr>
<tr>
<td>PB</td>
<td>46.90 ± 4.67</td>
<td>43.30 ± 2.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>46.30 ± 3.80</td>
<td>49.50 ± 5.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 0.05 level.
balance training demonstrated a close to significant difference compared to control (p = 0.05). For BKFOT (Left), only the PB group demonstrated any significant difference (p = 0.02) compared to the other two groups.

**DISCUSSION**

This study was aimed to determine the effect of Pilates exercise combined with a balanced training program on lumbopelvic stability and shooting accuracy in national level archers. After six-week training, it was found that KLAT (Right) showed improvements in all the three groups, but the between-group comparison showed no significant difference between PB vs Control and PB vs B. Both PB and B groups improved their scores for KLAT (Left). A significant difference was also found between the B and C groups; also, there was a very close to the significant difference between PB and C groups. For BKFOT (Right), it was seen that all the groups showed improvement in their values, but there was no significant difference between any of the three groups. For BKFOT (Left), only the PB group showed improvement, and there was a significant change between the group PB and group C only. On comparing the results of shooting accuracy, it was found that although there was an improvement in both the groups (PB and B), a significant difference in the shooting accuracy was only present between the PB and C groups. It is important to see if static or dynamic balance training may help archers improve their shooting ability. Mason and Pelgrim investigated the relationship between balance ability and shooting accuracy in junior and adult archers. They found that junior archers have a significant relationship between balance ability and shooting accuracy – the adult archers showed a higher level of balance ability than junior archers (18). Mononen et al. reported that shooters who control their postural balance have a steadier platform in aiming, enhancing the shooting accuracy (29). Norton et al. revealed that balance training enhances stability and minimizes the body’s shaking, which can help in improvement in scores (30). Conversely, Kim concluded that balance training helped improve balance ability for archers, but it did not have any direct effect on shooting accuracy (15).

The Pilates exercises focus on core engagement and breath control; activation of transversus abdominis, diaphragm, multifidus and pelvic floor muscles is facilitated. These muscles contribute to the stability of the lumbopelvic region (7). This is an important finding when considering the training design for archers, as incorporating Pilates exercises in their program can significantly change their stability and performance. The specificity of Pilates exercises and the principles on which it is based might have resulted in improved accuracy of performance and stability. In addition to this, there are some obvious relationships between some of the measured variables found in previous studies (11, 12, 23, 24). Park et al. conducted a study on 20 high school archers and showed attentive improvements in the static balance of the right and left side on comparing with the baseline score. Some of the components of the dynamic balance score improved in the exercise group compared with the control group (23). Panhan et al. reported that Pilates exercises are effective in training the internal oblique muscle to improve the neuromuscular efficiency in women engaged in Pilates exercises 2 times a week (31). Phrompaet et al. conducted a single-blinded controlled design study on 40 subjects to determine the effects of Pilates exercises on flexibility and lumbopelvic control. The exercise group participants showed improvements in flexibility and lumbopelvic movement control (32). Hyun et al. also found significant effects on the static and dynamic balance of older women suggesting that these exercises effectively enhance the balance ability of older women (33). Perrott et al. conducted a study to determine the relationship between balance and lumbopelvic stability and reported that athletes with poor lumbopelvic stability have poorer balance than athletes with more optimal lumbopelvic stability (14). These studies offer an insight to everyone that can take such information to the level of practical application. The Pilates exercise program can and should be given as an adjunct to the training protocols of archers.

There are a few limitations to this study. Firstly, the volunteers were chosen by draw – due to that, some of the participants did not participate happily. Secondly, the
time interval, i.e., six weeks, was sufficient but not fair enough to bring about large improvements. However, more improvements could have been observed if the time interval had been longer. Last, it is a single-blinded study; a double-blinded study would have increased the validity of the research.

CONCLUSIONS

Pilates exercises appear to be an effective way to improve archers’ lumbo-pelvic stability and shooting accuracy when combined with balance exercises. However, balance training also showed improvements in comparing the pre-test and post-test values of the balance training group, but the most significant improvements were recorded in only the Pilates plus balance training group. Archery coaches could use this information to plan a training protocol for archers.

REFERENCES


FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS


CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.


Exercise is Effective for Disease Activity Control with Various Effects on Entheses: An Ultrasound-Based Study

Maroua Slouma\(^1,2\), Siwar Ben Dhia\(^1,2\), Lobna Kharrat\(^1,2\), Celia Bellagha\(^1,2\), Hedia Bellali\(^2,3\), Imen Gharsallah\(^1,2\)

\(^1\) Department of Rheumatology, Military Hospital, Tunis, Tunisia
\(^2\) University of Tunis El Manar, Tunis, Tunisia
\(^3\) Department of Epidemiology, Habib Thameur Hospital, Tunis, Tunisia

**INTRODUCTION**

Radiographic axial Spondyloarthritis (SpA), also called ankylosing spondylitis, is a chronic rheumatic disease affecting typically young men. Its prevalence ranges between 0.2% and 1.6% (1). Sacroiliac joints and spine involvement are the main characteristic features of SpA (2). It can be responsible for pain, stiffness, and disability.

The inflammation of enthesis, also called enthesitis, is believed to be the central lesion in SpA (3). Entheses are skeletal anchorage points of the tendon, ligament, and joint capsule (4).

The pathophysiology of enthesis is not fully elucidated, pro-inflammatory cytokines (IL-17, IL-22, and IL-23) and metalloproteinases seem to be involved in this disease (5-8).
Enthesitis can be asymptomatic or responsible for inflammatory pain (9). Ultrasonography (US) can detect subclinical enthesitis (10-13) and reveal early enthesal abnormalities (14, 15). The therapeutic management of axial and enthesal manifestations is based on non-steroidal anti-inflammatory drugs (NSAIDs) and biological Disease-Modifying Anti-Rheumatic drugs (bDMARDs). The treatment goal is to achieve remission or at least low disease activity (16).

Several scores are used to assess axial disease activity and clinical and US entheses involvement. Data regarding the relationship between physical activity and disease activity and entheses scores in SpA patients are scarce. The effect of physical activity on SpA disease activity and enthesitis is unknown. The aim of this study was to assess the association between physical activity, disease activity, and ultrasonographic enthesal abnormalities in SpA patients.

**METHODS**

**Patients**

We conducted a cross-sectional study including consecutive patients followed for SpA and recruited from the outpatient clinic of the rheumatology department.

**Inclusion criteria**

We included patients fulfilling the Assessment of SpondyloArthritis International Society (ASAS) 2009 criteria (17).

**Non-inclusion criteria**

Non-inclusion criteria were SAPHO (Synovitis-Acne-Pustulosis-Hyperostosis-Osteitis) syndrome and crystal-induced arthritis.

**Clinical assessment**

The following patients’ and SpA characteristics were collected: age, gender, body mass index (BMI), age of SpA onset, disease duration, extra-articular manifestations (uveitis, psoriasis, inflammatory bowel disease (IBD), pulmonary involvement, cardiac involvement, renal involvement, and osteoporosis), comorbidities according to the European League Against Rheumatism (EULAR) (18), and therapeutic management (NSAIDs, conventional synthetic DMARDs (csDMARDs), and bDMARDs).

Disease activity was assessed using Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (19) and the Ankylosing Spondylitis Disease Activity Score (ASDAS<sub>CRP</sub>) (20). Active disease corresponds to an ASDAS<sub>CRP</sub> higher than 2.1 or BASDAI higher than 4.

Clinical assessment of enthesitis was performed using the Maastricht Ankylosing Spondylitis Enthesitis Score (MASES), Spondyloarthritis Research Consortium of Canada Enthesitis Index (SPARCC), and Leeds Enthesitis Index (LEI).

**Physical activity and exercise evaluation**

Physical activity is any bodily movement that increases energy expenditure above resting levels. However, exercise, is a subset of physical activity that is planned, structured and repetitive: jogging, biking, playing football or tennis, weightlifting, etc. (21).

Physical activity was assessed using the University of California and Los Angeles (UCLA) and the Tegner activity scales. UCLA activity scale is a 10-item scale including 10 descriptive activity levels ranging from wholly inactive and dependent on others (level 1), to regular participation in impact sports such as jogging or tennis (level 10) (22, 23). Tegner activity scale is an 11-item scale with activity levels ranging from 0 (sick leave or disability) to 10 (competitive sports (national elite)) (24).

The patients were also asked to estimate the weekly duration of exercise and of physical activity.

**Biological assessment**

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were measured.

**Ultrasonography assessment**

A board-certified rheumatologist trained in ultrasonography performed the US examination. The rheumatologist was blinded for clinical data. The US evaluation was performed using a Mindaay DC-70 device equipped with a 6-16 MHz linear transducer. The US was carried out after 15 minutes of rest at a room temperature maintained at 20 °C. We explored the entheses of the quadricipital tendon (QT), proximal patellar tendon (PPT), distal patellar tendon (DPT), calcaneal tendon (CT), plantar aponeurosis (PA), lateral epicondyles (LET), and triceps tendon (TT).

For each patient, we calculated the following ultrasonographic enthesitis assessment scores: Spanish Enthesitis Index (SEI) (25), Glasgow Ultrasound Enthesitis Scoring System (GUESS) (12), and Madrid Sonographic Enthesitis Index (MASEI) (11).

The enthesis was assessed according to the Outcome Measures Rheumatoid Arthritis Clinical (OMERACT) recommendations (26), which means 2 mm of the insertion of the bony cortex on both longitudinal and transversal axes. For each enthesis, we specified the presence or not of enthesophyte, hypoechoogenicity, calcification, or bony erosion. Evaluated US abnormalities were:
Exercise is Effective for Disease Activity Control with Various Effects on Entheses

- US tendon insertion damage: visualized as a thickened tendon or structural changes of the tendon insertion (hypoechoic or loss of its fibrillated appearance). The following cut-offs were used to define a thickened tendon: 5.29 mm for the CT, 4.4 mm for the PA, 6.1 mm for QT, 4 mm for both PPT and DPT (12), 4.3 for TT (11), and 3 mm for LET (27).
- Structural damage corresponds to enthesophytes, calcifications, or erosions.
- A power Doppler (PD) was evaluated at the bony insertion of the enthesis and was scored as a binary item (negative if absent and positive if any signal was present).

Statistical analyses
Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 23. Quantitative variables were tested for normal distribution using the Shapiro-Wilk test. We compared means of independent series using the independent samples Student T test for normally distributed variables. We performed the Mann-Whitney test to compare continuous variables with non-normal distribution. The Chi-square test was performed to assess the association between two categorical variables. As the number of patients was higher than 30, correlations between the tendon thickening and clinical or biological parameters were tested by Pearson’s correlation coefficient. The prevalence ratio (PR) was used to quantify the link between independent variables and physical activity or high disease activity. We performed a stepwise backward binary logistic regression to identify risk factors independently associated with high disease activity (25) and US abnormalities associated with physical activity. For this study, we included parameters that were significantly associated within the univariate analysis and those having a P-value < 0.20. All statistical tests were two-sided, and the level of statistical significance was set up at (P-value < 0.05).

Ethical consideration
This study was approved by the ethics committee of the Military Hospital of Tunis (Local Person Protection Committee, Military Hospital of Tunis. Number protocol: 81/2020/CLPP - date of approval: November 10, 2020). It was conducted according to the Declaration of Helsinki. Each participant signed consent after explaining the aims and methods of the study.

RESULTS
Patients’ characteristics
A total number of 518 entheses was assessed. We included 37 patients (29 men (78%) and 8 women (22%)). The mean age was 44.6 ± 12.3 years. The mean BMI was 26.5 ± 4.1 kg/m². Using BASDAI, 23 patients had active disease. Clinical and biological characteristics are summarized in table I.

Physical activity characteristics
Fifty-one percent of patients practiced exercises. Aerobic exercise was performed by 49% of patients (n = 19) (walking: 27%, jogging: 8%, football: 5%, biking: 3%, handball: 3%, and fitness exercise: 3%). Anaerobic exercise (weightlifting) was performed by only one patient. Variations of UCLA physical activity scale and Tegner activity scale are represented in the figure 1. Physical activity characteristics are summarized in table I.

Figure 1. Variation of UCLA physical activity scale and Tegner activity scale.
Clinical and US entheses characteristics

Fourteen patients had > 3 US enthesophytes. As shown in Table II, US entheses abnormalities were frequent. The CT enthesis was the most affected, followed by the PA in the lower limbs. Enthesophytes affecting LET and TT were found in 57% of patients.

Structural damages were found mainly in QT, CT, and PA. The median number of painful entheses was 5 (IQR: 2-12) entheses. The median (IQR 25%-75%) MASES, LEI, and SPARCC were 2 (0.5-5), 1 (0-2), and 2 (0-4.5), respectively. The mean SEI, GUESS, and MASEI were 12.4 ± 3.7, 8.2 ± 3.8, and 25.7 ± 9.7, respectively.

Table I. SpA patients' characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Age at onset, mean ± SD years</td>
<td>38.1 ± 11.3</td>
</tr>
<tr>
<td>Disease duration, median (IQR 25%-75%), years</td>
<td>5.3 (3 – 14)</td>
</tr>
<tr>
<td>BASDAI, mean ± SD</td>
<td>4.5 ±2.2</td>
</tr>
<tr>
<td>ASDAS$_{ESR}$ mean ± SD</td>
<td>3.2 ±1.3</td>
</tr>
<tr>
<td>ASDAS$_{CRP}$ mean ± SD</td>
<td>3 ± 1.2</td>
</tr>
<tr>
<td>Extra-articular manifestations, n (%)</td>
<td>15 (41)</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td>19 (51)</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>NSAIDs n (%)</td>
<td>23 (92)</td>
</tr>
<tr>
<td>Continuous NSAIDs n (%)</td>
<td>12 (32)</td>
</tr>
<tr>
<td>bDMARDs n (%)</td>
<td>10 (27)</td>
</tr>
<tr>
<td><strong>Biological characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>CRP, median (IQR 25%-75%), mg/L</td>
<td>8 (4 -33.5)</td>
</tr>
<tr>
<td>ESR, median (IQR 25%-75%), mm</td>
<td>28 (11 - 66.5)</td>
</tr>
<tr>
<td><strong>Clinical assessment of enthesitis</strong></td>
<td></td>
</tr>
<tr>
<td>Painful entheses, median (IQR 25%-75%)</td>
<td>5 (2-12)</td>
</tr>
<tr>
<td>MASES, median (IQR 25%-75%)</td>
<td>2 (0.5-5)</td>
</tr>
<tr>
<td>LEI, median (IQR 25%-75%)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>SPARCC, median (IQR 25%-75%)</td>
<td>2 (0-4.5)</td>
</tr>
<tr>
<td><strong>Ultrasound assessment of enthesitis</strong></td>
<td></td>
</tr>
<tr>
<td>SEI, mean ± SD</td>
<td>12.46±3.79</td>
</tr>
<tr>
<td>GUESS, mean ± SD</td>
<td>9.62±3.86</td>
</tr>
<tr>
<td>MASEI, mean ± SD</td>
<td>25.76±9.79</td>
</tr>
<tr>
<td><strong>Physical activity characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Patients doing exercises, n (%)</td>
<td>19 (51)</td>
</tr>
<tr>
<td>Weekly duration of exercise, median (IQR 25%-75%), hours</td>
<td>0.75 (0-3.5)</td>
</tr>
<tr>
<td>Weekly duration of physical activity (IQR 25%-75%), hours</td>
<td>3 (1.9 - 7)</td>
</tr>
<tr>
<td>UCLA activity scale, median (IQR 25%-75%)</td>
<td>4 (4-5)</td>
</tr>
<tr>
<td>Tegner physical activity scale, median (IQR 25%-75%)</td>
<td>3 (3-5)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (standard deviation); n: subjects number; BMI: Body mass index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; CRP: c-reactive protein; ESR: erythrocyte sedimentation rate; NSAIDs: Non-steroidal anti-inflammatory drugs; bDMARDs: biological disease-modifying anti-rheumatic drugs; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; SPARCC: Spondyloarthritis Research Consortium of Canada Enthesitis Index; LEI: Leeds Enthesitis Index; SEI: Spanish Enthesitis Index; GUESS: Glasgow Ultrasound Enthesitis Scoring System; MASEI: Madrid Sonographic Enthesitis Index UCLA: University of California and Los Angeles activity scale; IQR: interquartile range.
Exercise is Effective for Disease Activity Control with Various Effects on Entheses

Relationship between physical activity and clinical characteristics of the disease
As shown in table III, patients who performed exercise had a significantly lower disease activity, mean number of painful entheses, and lower clinical enthesis scores.

Table III. Association between disease activity, enthesis scores, and exercise.

<table>
<thead>
<tr>
<th>Exercise</th>
<th>No (n = 18)</th>
<th>Yes (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASDAI</td>
<td>5.4 ± 1.9</td>
<td>3.5 ± 2</td>
<td>0.004</td>
</tr>
<tr>
<td>ASDAS_CRP</td>
<td>3.4 ± 1.1</td>
<td>2.6 ± 1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>ASDAS_ESR</td>
<td>3.7 ± 1.1</td>
<td>2.8 ± 1.3</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP</td>
<td>39.6 ± 58.5</td>
<td>16.1 ± 20.1</td>
<td>0.107</td>
</tr>
<tr>
<td>ESR</td>
<td>48.7 ± 37.4</td>
<td>34.7 ± 30</td>
<td>0.217</td>
</tr>
<tr>
<td>Painful entheses</td>
<td>9.1 ± 6.4</td>
<td>3.9 ± 4.5</td>
<td>0.008</td>
</tr>
<tr>
<td>MASES</td>
<td>4.2 ± 3.2</td>
<td>1.7 ± 1.8</td>
<td>0.016</td>
</tr>
<tr>
<td>LEI</td>
<td>2.1 ± 1.9</td>
<td>0.9 ± 1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>SPARCC</td>
<td>4.2 ± 3.5</td>
<td>1.7 ± 2.9</td>
<td>0.011</td>
</tr>
<tr>
<td>SEI</td>
<td>12.3 ± 4</td>
<td>12.6 ± 3.6</td>
<td>0.781</td>
</tr>
<tr>
<td>GUESS</td>
<td>10.1 ± 4</td>
<td>9.2 ± 3.8</td>
<td>0.513</td>
</tr>
<tr>
<td>MASEI</td>
<td>26.7 ± 10.8</td>
<td>24.9 ± 8.9</td>
<td>0.590</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (standard deviation); n: subjects' number; p: significance value; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; CRP: C-reactive protein; ESR: Erythrocyte Sedimentation Rate; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; SPARCC: Spondyloarthritis Research Consortium of Canada Enthesitis Index; LEI: Leeds Enthesitis Index; SEI: Spanish Enthesitis Index; GUESS: Glasgow Ultrasound Enthesitis Scoring System; MASEI: Madrid Sonographic Enthesitis Index; NS: not significant.
SPARCC, and MASES. A negative correlation was also found between the weekly duration of physical activity and MASES. No correlations were found between these parameters and UCLA or Tegner activity scales.

In the multivariate study, we found that high disease activity was associated with increased inflammatory biomarkers (OR 6.565, p = 0.04, 95%CI 1.092-39.459). Nevertheless, exercise was associated with a lower risk of disease activity (OR 0.123, p = 0.022, 95%CI 0.020 -0.742).

**Relationship between physical activity and ultrasonographic characteristics**

Triceps tendon thickening, the SEI, and thickening of the distal insertion of the patellar tendon were significantly higher in patients performing physical activity (triceps tendon’s thickening: 4.20 ± 0.62 vs 3.7 ± 0.3 mm, p = 0.044; SEI of the distal insertion of the patellar tendon: 2.5 ± 1.4 vs 1.1 ± 1.1, p = 0.03, and thickening of the distal insertion of the patellar tendon: 4.6 ± 0.7 vs 3.9 ± 0.3, p = 0.02). The thickness of the lateral epicondyle enthesis was also positively correlated with weekly duration of physical activity as shown in table IV.

Patients doing exercise had a significantly lower risk of having ≥ 3 US enthesophytes (PR: 0.22; 95%CI 0.3-0.9, p = 0.031) and PA enthesophyte (PR: 0.18; 95%CI 0.04-0.87, p = 0.026). Patients doing physical activity had a higher risk of hypoechogenicity in the distal insertion of the patellar tendon (PR: 2.88; 95%CI 1.75-4.5, p = 0.004). A higher UCLA activity scale was found in patients with loss of the fibrillar aspect of the DPT (6 ± 2.73 vs 4.56 ± 1.18, p = 0.047).

Lower UCLA was found in patients with structural damage lesions of the QT (4.42 ± 1.13 vs 5.54 ± 2.01, p = 0.038) and TT enthesitis (4.34 ± 1.09 vs 5.72 ± 1.95, p = 0.09).

Besides, a negative correlation was found between MASEI and UCLA activity index (r = -0.326, p = 0.049). However, Tegner activity scale was positively correlated with the thickness of the PPT (r = 0.349, p = 0.034).

**Table IV. Correlations between clinical and ultrasonographic characteristics and physical activity characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Weekly duration of exercise</th>
<th>Weekly duration of physical activity</th>
<th>UCLA activity scale</th>
<th>Tegner Physical Activity scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASDAI</td>
<td>0.313</td>
<td>-0.363*</td>
<td>-0.121</td>
<td>-0.199</td>
<td>-0.107</td>
</tr>
<tr>
<td>ASDAS ESR</td>
<td>0.247</td>
<td>-0.196</td>
<td>-0.009</td>
<td>-0.167</td>
<td>-0.105</td>
</tr>
<tr>
<td>ASDAS CRP</td>
<td>0.267</td>
<td>-0.155</td>
<td>0.140</td>
<td>-0.084</td>
<td>0.032</td>
</tr>
<tr>
<td>number of painful entheses</td>
<td>0.284</td>
<td>-0.425*</td>
<td>-0.321</td>
<td>-0.281</td>
<td>-0.274</td>
</tr>
<tr>
<td>SPARCC</td>
<td>0.373*</td>
<td>-0.414*</td>
<td>-0.292</td>
<td>-0.242</td>
<td>-0.270</td>
</tr>
<tr>
<td>MASES</td>
<td>0.121</td>
<td>-0.401*</td>
<td>-0.326*</td>
<td>-0.291</td>
<td>-0.183</td>
</tr>
<tr>
<td>LEI</td>
<td>0.329*</td>
<td>-0.323</td>
<td>-0.189</td>
<td>-0.206</td>
<td>-0.128</td>
</tr>
<tr>
<td>SEI</td>
<td>0.247</td>
<td>0.295</td>
<td>0.286</td>
<td>-0.183</td>
<td>0.126</td>
</tr>
<tr>
<td>GUESS</td>
<td>0.379*</td>
<td>-0.097</td>
<td>0.058</td>
<td>-0.196</td>
<td>0.080</td>
</tr>
<tr>
<td>MASEI</td>
<td>0.450*</td>
<td>0.032</td>
<td>0.159</td>
<td>-0.326*</td>
<td>-0.010</td>
</tr>
</tbody>
</table>

Tendon thickness

<table>
<thead>
<tr>
<th></th>
<th>Weekly duration of exercise</th>
<th>Weekly duration of physical activity</th>
<th>UCLA activity scale</th>
<th>Tegner Physical Activity scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT</td>
<td>0.332*</td>
<td>0.258</td>
<td>0.045</td>
<td>0.169</td>
</tr>
<tr>
<td>PPT</td>
<td>0</td>
<td>0.147</td>
<td>-0.093</td>
<td>0.019</td>
</tr>
<tr>
<td>DPT</td>
<td>-0.015</td>
<td>0.039</td>
<td>0.061</td>
<td>-0.048</td>
</tr>
<tr>
<td>CT</td>
<td>0.345*</td>
<td>-0.029</td>
<td>0.131</td>
<td>-0.158</td>
</tr>
<tr>
<td>PA</td>
<td>0.209</td>
<td>0.006</td>
<td>0.246</td>
<td>0.050</td>
</tr>
<tr>
<td>LET</td>
<td>0.124</td>
<td>0.378*</td>
<td>0.306</td>
<td>0.119</td>
</tr>
<tr>
<td>TT</td>
<td>0.213</td>
<td>0.114</td>
<td>0.172</td>
<td>-0.146</td>
</tr>
</tbody>
</table>

Data are presented as r of Pearson correlations; *p < 0.05; **p < 0.01; ***p < 0.001; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; ESR: Erythrocyte Sedimentation Rate; CRP: C-reactive protein; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; SPARCC: Spondyloarthritis Research Consortium of Canada Enthesitis Index; LEI: Leeds Enthesitis Index; SEI: Spanish Enthesitis Index; GUESS: Glasgow Ultrasound Enthesitis Scoring System; MASEI: Madrid Sonographic Enthesitis Index; QT: Quadriceps tendon enthesis; PPT: Proximal insertion of the patellar tendon; DPT: Distal insertion of the patellar tendon; CT: Calcaneal tendon; PA: Plantar aponeurosis; LET: Lateral epicondyle tendon; TT: triceps tendon; UCLA: University of California and Los Angeles activity scale.
In the multivariate study, we found that exercise was associated with a lower risk of having ≥ 3 enthesophytes (OR 0.213, p = 0.036, 95%CI 0.05-0.90).

DISCUSSION

We attempted to assess the association between physical activity, disease activity, and ultrasonographic enthesal abnormalities in SpA patients. Our study showed that patients who performed regular exercise had significantly lower disease activity. However, UCLA and Tegner activity scales were not correlated with disease activity.

There are controversial results regarding the effect of physical activity on disease activity and entheses. Two clinical trials assessed the effect of physical activity on psoriatic arthritis patients. No adverse events related to exercise or inflammatory flares of disease had been reported (28, 29). However, in Häkkinen’s study, inflammatory flare occurred in six psoriatic arthritis patients in the exercise group and five patients in the control group leading to a temporary stoppage of the protocol (30). In their systematic review, Kessler et al. emphasize the widespread benefits of physical activity on psoriatic arthritis patients regarding functional capacity, general well-being, fatigue, and quality of life (31).

In a study including SpA patients, the authors demonstrated that a 3-month combined home exercise associating range-of-motion, strengthening, and aerobic exercise was more effective than range-of-motion home exercise alone in terms of aerobic capacity and functional ability assessed using Bath Ankylosing Spondylitis Functional Index (BASFI) (32). Jennings et al. found that aerobic exercise associated with stretching or stretching alone could improve the functional capacity of SpA patients (33).

In a metaanalysis including twenty-six trials with a total of 1,286 patients followed for inflammatory rheumatic diseases, the authors demonstrated a beneficial effect of physical activity on disease activity scores (Standardized Mean Difference (SMD): 0.19, 95%CI 0.05-0.33, p < 0.01), joint damage (SMD: 0.27, 95%CI 0.07-0.46, p < 0.01), and ESR (SMD: 0.20, 95%CI 0.00-0.39, p = 0.04) (34). We did not find a significant difference in ESR and CRP between patients with or without physical activity. Nevertheless, patients doing exercise had a significantly lower disease activity.

This finding highlights that physical activity can be considered a non-pharmacological treatment for the management of spondyloarthritis. Therefore, regular physical activity, including cardiorespiratory fitness, muscle strength, flexibility, and neuromotor performance, is highly recommended for patients with inflammatory diseases (35). Data regarding the effects of physical activity on entheses involvement in patients with SpA are scarce. Our study showed that physical activity was associated with a lower clinical enthesitis score. Besides, negative correlations were found between the weekly duration of exercise and the following parameters: number of painful entheses, SPARCC, and MASES. These correlations were not found with UCLA and Tegner activity scales.

A negative correlation was also found between the weekly duration of physical activity and MASES. These results highlight the beneficial effect of physical activity on clinical entheses scores.

To our knowledge, the link between physical activity and US enthesis score has not been studied in axial spondyloarthritis patients. We did not find a significant difference in US scores between patients doing or not doing exercise.

In a study including 84 patients followed for psoriatic arthritis, patients reporting avoidance of activity had lower MASEI (β: -1.71, 95%CI -3.1 to -0.32) than those who did not (36). Triceps tendon thickening, SEI, and thickening of the distal insertion of the patellar tendon were higher in patients performing physical activity. Patients doing physical activity had also a higher risk of hypoechogenicity in the lateral epicondyle tendons.

Bakirci et al. showed that US changes within the entheses of healthy subjects were associated with physical activity (β: 4.41, 95%CI 1.25-7.58, p = 0.007). Thickening was the common lesion, affecting mostly the patellar tendon insertions (37).

This result suggests that biomechanical forces on the enthesis can be responsible for US entheses abnormalities without any signs and symptoms. The effect of physical activity on entheses depends on the type and amount of exercise.

The onset of sports-related enthesopathy seemed to be due to muscle contraction rather than the amount of exercise (38). Ozone et al. showed that, unlike concentric contraction-dominant exercises, eccentric contraction-dominant exercise can be responsible for sports-related enthesopathy-like morphological changes via the activation of transforming growth factor-β superfamily pathway in enthesis (39).

In our study, patients with loss of the fibrillar aspect of the DPT had significantly higher UCLA activity scale. This score was, however, negatively correlated with MASEI. A lower Tegner activity scale was found in patients with loss of the fibrillar aspect of EPL and with TT calcification.

Interestingly, we found that patients doing exercise had a lower risk of having ≥ 3 US enthesophytes and planter aponeurosis enthesophyte.

These results suggest that physical activity can be responsible for US tendon insertion damage due to biomechanical forces.
Conclusions

Our study showed that patients who exercised had significantly lower disease activity. These findings suggest that physical activity could be considered a non-pharmacological treatment for the management of spondyloarthritis and can be recommended for these patients. We also found that physical activity was associated with a lower clinical enthesitis score. It can be responsible for ultrasonographic tendon insertion damage, but it doesn’t accentuate the structural damage. The promotion of physical activity could be considered a healthcare priority.

FUNDINGS
None.

CONTRIBUTIONS
MS: ultrasonography performance, writing – original draft. SBD, LK: writing – original draft, data collection. HB, CB: data analysis. IG: conceptualization.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

REFERENCES
15. Wiel C, Szkułdarek M, Hasselquist M, et al. Power Doppler ultrasonography of painful Achilles tendons and entheses in...


IS SARM - Selective Androgen Receptor Modulator: A Chance for Therapeutic Approach in Muscle-Wasting Chronic Conditions?

Alessandro Bartoli¹², Nicola Maffulli³⁴⁵, Gabriella Oliva⁶, Salvatore Ziello¹², Francesco Oliva⁷

¹ Department of Musculoskeletal Disorders, Faculty of Medicine and Surgery, University of Salerno, Baronissi, Italy
² Orthopedic Clinic, “San Giovanni di Dio e Ruggi d’Aragona” Hospital, Salerno, Italy
³ Queen Mary University of London, Barts and the London School of Medicine and Dentistry, Centre for Sports and Exercise Medicine, Mile End Hospital, London, U.K.
⁴ Faculty of Medicine, School of Pharmacy and Bioengineering, Guy Hilton Research Centre, Keele University, Stoke-on-Trent, U.K
⁵ Department of Trauma and Orthopaedic Surgery, Faculty of Medicine and Psychology, University La Sapienza, Rome, Italy
⁶ Department of Internal Medicine, Ospedale del Mare, ASL NA1 Centro, Naples, Italy
⁷ Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, Rome, Italy

SUMMARY
Purpose of review. The purpose of this paper is to review the current concepts regarding a new-old pharmacological class, SARM (Selective Androgen Receptor Modulator). Especially how SARM can be beneficial in treatment of orthopedic disease.

Materials and methods. Androgens (testosterone and dihydrotestosterone (DHT)) are the male sex hormones required for development of the male reproductive system and secondary sexual characteristics. SARMs due to ability to bind androgen receptor are excellent in treatment of various diseases.

Discussion. Based on the published literature, a review has been carried out on SARM and its fields of application in the treatment of muscle wasting pathologies. Starting from the history of the SARMs, its role in prevention of muscle wasting, anabolic effects and side effects were addressed.

Conclusions. Nonsteroidal SARMs are unique in their ability to bind the androgen receptor with high affinity and exhibit tissue selective activity. SARMs can be used to treat muscle wasting conditions, osteoporosis, frailty syndrome without unwanted side effects associated with androgens.

KEY WORDS
SARM; SERM; muscle-wasting; cachexia; sarcopenia.

INTRODUCTION
Androgens (testosterone and dihydrotestosterone (DHT)) are the male sex hormones required for development of the male reproductive system and secondary sexual characteristics (1). Androgens actions are mediated via the androgen receptor (AR), modulating functions as a transcription factor (2) (figure 1). AR is a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family. AR is expressed in multiple reproductive tissues, with important effects on multiple organ...
systems (3, 4). These play critical roles in the regulation of many male, and female sexual, somatic and behavioral functions critical to lifelong health, like bone density, strength muscle mass, coagulations, cognition, metabolism (5, 6) (Table I). Low endogenous testosterone concentrations are associated with sarcopenia and frailty due to decreased muscle mass, and bone mineral density. Testosterone replacement therapy is potential benefit, but is often curtailed of side effects like erythrocytosis, hepatotoxicity, testicular atrophy, prostate hypertrophy, aromatization to estrogen (7, 8). To avoid these side effects, molecules capable of selectively binding and modulating the androgen receptor have been developed since the 2000s (9). This is how SARMs (Selective Androgen Receptor Modulator) were born, to bypass the pharmacological and pharmacokinetic limitations of steroid (10). SARMs have been chemically engineered to target AR function more specifically in certain tissues because of tissue specificity and not being substrates for aromatase or 5-α reductase (4).

**MATERIALS AND METHODS**

**Search strategy**

The literature search of the present narrative review was conducted according to this protocol:
- SARM.
- Linkage about SARM and muscle wasting.
- AR, SARM, muscle wasting, cachexia, sarcopenia.

**Literature search**

In March 2023 the following databases were accessed: PubMed, Embase, Scopus, Web of Science, Google Scholar. The following keywords were used in combination: AR, SARM, muscle wasting, cachexia, sarcopenia. If title and abstract matched the topic, the full text was accessed. The bibliographies of the full-text articles were also screened for inclusion. Disagreements were solved by a third author. All the articles that investigate possible use of SARMs for muscle wasting pathologies. According to the authors language capabilities, articles in English, French, German, Italian, and Spanish were considered.

**Table I. Testosterone and synthetic steroid.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabolic effects</td>
<td>Increase of bone mass; muscle mass</td>
</tr>
<tr>
<td>Androgenic effects</td>
<td>Virilization, impaired fertility</td>
</tr>
</tbody>
</table>
DISCUSSION

Based on the published literature, a review has been carried out on SARM and its fields of application in the treatment of muscle wasting pathologies. Starting from the history of the SARMs, its role in prevention of muscle wasting, anabolic effects and side effects were addressed.

History of Selective Androgen Receptor Modulator (SARM)

The history of SARMs begins in 1990 with the development of a drug, tamoxifen, a so-called SERM (selective estrogen receptor modulator), used in the treatment of breast cancer, which stimulated interest in analogous drugs to modulate the androgen receptor (AR) (11, 12). As demonstrated by the development and clinical use of SERMs like tamoxifen, the key characteristic underlying the therapeutic potential of SARMs is their tissue specificity. Several labs began working on identifying lead candidates and specific pharmacophores from 2000 to today, with from 2000 to now, with the design of 4 categories of SARMs pharmacophores (table II).

The first preclinical evidence for tissue-selective activation of the AR was that arylpropionamide SARMs increased levator ani muscle weight in castrated rats to the level of sham-operated rats, but only partially increased the prostate and seminal vesicles weight (16, 17). Over the next decade structure-activity relationship studies were conducted on the arylpropionamide class of SARMs that culminated in two clinical candidates, with enobosarm being the most advanced in clinical development (17, 18). In addition to their effects on muscle, enobosarm and other arylpropionamide SARMs also demonstrated beneficial effects on bone (19). Enobosarm has been evaluated in several phase II and phase III clinical trials for multiple indications (5, 6, 20).

Ligand Pharmaceuticals developed tricyclic quinolinones that coincided with the discovery of arylpropionamide SARMs (21, 22). Similar to the arylpropionamide SARMs, these quinolinones also bind to and activate the AR in low nanomolar concentrations while eliciting tissue-selective activation of the AR in muscle.

Based on structure activity relationship of several SARM templates, Ligand Pharmaceuticals synthetized LGD2226 as their first clinical candidate (23). LGD2226 demonstrated myo- and osteo-anabolic activity and maintenance of sexual function in various preclinical models.

Muscle wasting chronic conditions

Weight loss can be considered as a warning sign in the progression of various diseases, acute or chronic. “Muscle wasting disease” (MWD) means an integrative association of age-related sarcopenia and chronic disease-related cachexia (24), this condition is observed in a variety of pathologies such as cancer, chronic kidney disease as well as after prolonged inactivity or during aging (table III). Cachexia is a multi-organ syndrome associated with cancer and other chronic diseases, characterized by body weight loss, muscle and adipose tissue wasting and inflammation, being often associated with anorexia (25, 26). Sarcopenia is characterized by a progressive loss of muscle mass, function, and physical performance during aging (27). The incidence of sarcopenia reaches up to 5-13% in 60-70 years old population and 11-50% in those at 80 years or above (28). Muscle wasting is the result of a combination of an imbalance between synthetic and degradative protein pathways together with increased myocyte apoptosis and decreased regenerative capacity (29).

Table II. SARMs pharmacophores.

<table>
<thead>
<tr>
<th>Categories SARMs Pharmacophores</th>
<th>Aryl-Propionamide</th>
<th>Ostarine, Andarine</th>
<th>Crawford et al. (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Byciclic Hydantoin</td>
<td>BMS 564929</td>
<td>Thevis et al. (13)</td>
</tr>
<tr>
<td></td>
<td>Quinoline</td>
<td>LGD2226 (Ligandrol)</td>
<td>van Oeveren et al. (14)</td>
</tr>
<tr>
<td></td>
<td>Tetrahydroquinoline</td>
<td>S-40503</td>
<td>Goli et al. (15)</td>
</tr>
</tbody>
</table>

Table III. Pathways of Modalities for Muscle Wasting Disease (MWD).

<table>
<thead>
<tr>
<th>Cachexia</th>
<th>Sarcopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Inflammation</td>
<td>Chronic Inflammation</td>
</tr>
<tr>
<td>Reduction in muscle mass and strength</td>
<td>Reduction in muscle contractibility</td>
</tr>
<tr>
<td>Mitochondrial Disfunction</td>
<td>Alterations in protein turnover</td>
</tr>
<tr>
<td>Anabolic Resistance</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Impaired regeneration</td>
</tr>
</tbody>
</table>
Loss of muscle mass is associated with higher morbidity and mortality which translate in reduced quality of life (30). Over the years many efforts have been made to treat these conditions while avoiding further adverse effects on the already precarious health of these people. Physical activity and diet, through changes in microbiota are the main activities for the management of these conditions (31). However, it is understood that in some cases it is difficult to implement these principles (32). Currently no products approved for treatment of muscle wasting secondary to age or chronic disease states, including cancer. Most of the current treatment options have focused on stimulating nutritional intake or reversing some of the inflammatory processes involved in cachexia.

Most studied SARMS are Ostarine, Andarine, Enobosarm developed by GTx and Merck & Co. Inc. which arrived in Enobosarm case also in Phase III of pharmaceutical development (38).

Enobosarm might offer a promising option for the prevention and treatment of muscle wasting due to cancer by increasing muscle mass without the side effects associated with non-selective anabolic androgenic steroids or with growth hormone or growth hormone-releasing hormone therapies. Enobosarm resulted also in larger improvements in lean body mass and physical function in healthy older men and postmenopausal women (20, 38). Ostarine although is a non-steroidal SARM, tested in Phase I and II clinical trials, demonstrating a safety profile, an high tissue selectivity with beneficial effects on lean body mass and improving physical performance.

CONCLUSIONS
Nonsteroidal SARMS are unique in their ability to bind the androgen receptor with high affinity and exhibit tissue selective activity. SARMS can be used to treat muscle wasting conditions, osteoporosis, frailty syndrome without unwanted side effects associated with androgens. Last clinical trials for SARMS were in 2013, so 10 years have gone and appear to be no studies to explore the extraordinary capabilities of these drugs. SARMS are not FDA (Food and Drug Administration) approved, but recently they are being used by gym goers, with an high risk for health, because of fake product. A return in clinical trials is required to assess the role of nonsteroidal SARMS and if they can be used in near future.

FUNDINGS
None.

DATA AVAILABILITY
Data are available in the review.

Table IV. Therapeutic Modalities for MWD.

<table>
<thead>
<tr>
<th>Modalities</th>
<th>Function</th>
<th>Limitation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite Stimulants (Corticosteroids and Megestrol acetate)</td>
<td>- Increase food intake and produce weight gain</td>
<td>- Limited long-term benefits on quality life</td>
<td>Behl et al. (33)</td>
</tr>
<tr>
<td>Thalidomide, Cox-2 inhibitor</td>
<td>- Targets the inflammation associated with MWD</td>
<td>- Chest pain</td>
<td>Tazi et al. (34)</td>
</tr>
<tr>
<td>COLECOXIB, anti IL-6</td>
<td></td>
<td>- Confusion</td>
<td>Mantovani, et al. (35)</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>- Act on GH receptors to increase appetite,</td>
<td>- Short half-life</td>
<td>Garcia et al. (36)</td>
</tr>
<tr>
<td></td>
<td>- GH secretion and lean body mass</td>
<td>- Administration via inj.</td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>- Anabolic qualities</td>
<td>- Cardiovascular events</td>
<td>Giovanelli et al. (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Hepatotoxicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Increase hematocrit</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES


CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.
Comparison of the Effect of Sportsmetrics Soccer Training on Movement Performance in Soccer Players with and without Anterior Cruciate Ligament Reconstruction

Amir Shamlou Kazemi¹, Hassan Daneshmandi¹, Parisa Sedaghati¹, Yasin Hoseini²

¹ Department of Sports Injuries and Corrective Exercise, Faculty of Sport Sciences, University of Guilan, Rasht, Iran
² Department of Physical Education and Sport Science, Faculty of Human Science, Malayer University, Hamedan, Iran

INTRODUCTION
Soccer is one of the most popular and attractive sports in the world (1). With the increasing popularity of this sport and the rapid growth in the number of participants in this field, the prevalence of sports injuries in this field is also increasing (2). On the other hand, because soccer involves physical encounters, there is a possibility of injury regardless of age and gender. 66% of soccer injuries occur in the lower limbs, with the ankle and knee being the most common injury sites (3). ACL tear is one of the most severe and complicated knee injuries. This injury often leads to reduced performance, extended absence from training sessions, laxity of the knee joint, proprioception disorder, and premature osteoarthritis (4). Post-traumatic financial issues such as the cost of surgery, rehabilitation, and psychological factors have highlighted the need to use injury prevention programs (5).

Prevention of injuries has been one of the major soccer issues in recent years (6). Overall, prevention protocols
can be divided into two main groups: neuromuscular training and special warm-up (7). Neuromuscular training is considered the best and most effective way to prevent injury and improve performance techniques (8). Alentorn-Geli et al. (9) concluded that multi-component prevention programs have better results in ACL injury prevention compared to single-component programs. In this context, the SMST protocol has specialized in preventing injuries and enhancing the performance of soccer players (10). SMST offers a variety of training methods that have been used with preventive goals and improving the performance of athletes in previous research (4). For example, Noyes et al. (4) observed a significant increase in the performance of female soccer players by combining the general warm-up program and specialized SMST training.

On the other hand, movement assessment allows health and physical fitness professionals to develop an injury prevention model by observing movement defects and muscle imbalances, as well as using deformed muscles and the scores of functional movement screening (FMS) tests to predict lower limb injuries in athletes (11). Screening is done to prevent injuries and improve implementation strategies. FMS tests include seven movement tests that can detect limitations and changes in normal movement patterns. These tests are designed to assess the interaction between the mobility of the movement chain and the stability required to execute functional and essential movement patterns (12). The maximum score in this test is 21, and a score below 14, according to research, indicates a person’s susceptibility to injury (13). FMS tests can assess the quality of performance patterns and identify individuals at risk of injury, making them a useful method for injury identification and prevention (14).

Many studies have reported the positive effect of SMST training on injury reduction. However, no studies have been found that measured the effect of this training on the FMS tests in soccer players with ACLR. Additionally, very limited research has been done on the effect of injury prevention training on the FMS test items in order to overcome the discrepancies of previous research. Hence, it seems essential to examine different items of the FMS test using the training that has already been proven effective. Therefore, the aim of the present study was to compare the evaluation of the FMS test items using a period of SMST training in soccer players with and without ACLR.

**MATERIALS AND METHODS**

This quasi-experimental study was conducted with a pre-test-post-test design. The statistical population consisted of soccer players from the Premier League and First Division in two countries. These players had at least three years of team training experience in soccer and were between the ages of 18 and 30. This age range was chosen to remove the initial effects of training. Based on previous papers and the use of G Power software, it was determined that a minimum of 42 subjects were needed to achieve a statistical power of 0.8, a reliability coefficient of 0.8, and a significance level of 0.05. From the statistical population, 42 cases were selected as a statistical sample using purposive and accessible sampling. These cases were then randomly divided into two groups: experimental (n = 21 with ACLR) and control (n = 21 without ACLR). Inclusion criteria included 3-5 years of experience in teamwork and training at the club level of soccer. The subjects in the ACLR groups were undergoing ACL reconstruction. They should have no history of diseases associated with poor balance, a body mass index in the normal range, and no injuries in the lower limb other than ACL in the last 6 months. They should also have no obvious abnormalities in the lower limbs (anteversion, genu valgum, genu varum, tibial torsion, and flat foot) as determined by the New York test. Additionally, no less than 6 and no more than 24 months should have passed since ACL reconstruction. Exclusion criteria included non-participation in more than three sessions or two consecutive sessions of the SMST knee injury prevention program. Subjects who experienced pain or discomfort during training, suffered an injury during training, or withdrew from the study were also excluded (4). Taking into consideration the ethical considerations of the study, such as the confidentiality of the subjects’ information, the use of an experienced instructor and examiner to prevent any possible injury, giving full authority to the subjects to leave the research at any stage, and explaining the purpose of the research to all the subjects before starting the exercises and signing the consent form to participate in the research. The present study was approved by the ethics committee of the Iranian Research Institute of Physical Education and Sport Sciences with IR.SSRC.REC.1402.093 code – date of approval: August 07, 2023).

**Functional Movement Screen (FMS) test**

FMS was used to test the values of pre- and post-test, before and after the training period, according to Cook’s et al. instructions. The FMS tests are composed of seven performance tests, which include deep squat, hurdle step, in-line lunge, shoulder mobility, trunk stability, active straight leg rise, and rotary stability (figure 1). The scoring for each test ranges from 0 to 3. The scoring for the FMS™ consists of four discrete possibilities (13, 15). The scores range from zero to three, with three being the best possible score. An individual is given a score of zero if they
experience pain anywhere in the body during the testing. If pain occurs, a score of zero is given and the painful area is noted. If the patient does not score a zero, a score of one is given if they are unable to complete the movement pattern or assume the position to perform the movement. A score of two is given if the person is able to complete the movement but must compensate in some way to perform the fundamental movement. A score of three is given if the person performs the movement correctly without any compensation, complying with standard movement expectations associated with each test. After completing all seven tests, the scores are added together and considered as the individual’s overall FMS score. Subjects can get a grade between zero and 21, which, according to previous reports, a score less than 14 can be indicative of the probability of injury (16). FMS test scores were determined by observation of videos recorded by two cameras from the front and side views during the implementation of FMS tests.

**Table I**

<table>
<thead>
<tr>
<th>Test</th>
<th>Intragroup Comparison</th>
<th>Between Groups Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overhead Squat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurdle Step</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-Line Lunge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder Mobility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotatory Stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active Straight Leg Rise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk Stability</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Seven tests from the Functional Movement Screen.

**Sportsmetrics Soccer Training**

The SMST protocol has several basic parts that are essential for the performance of soccer players. It consists of four training components: agility and reaction, speed and endurance, plyometric, and strength. This protocol is designed in a way that soccer players engage in new exercises for each section every week, while the nature of the training sections remains the same. It is tailored to the specific requirements of each training section. The necessary tools for performing the exercises include cones and training funnels, resistance therabands, ladders, and Pilates bands. The training program varied each week, and at the beginning of each week, the entire weekly program was taught. Both groups received theoretical instruction on the entire weekly program at the start of each week. The duration of each session in the protocol ranged from 60 to 90 minutes. Both groups underwent sportsmetrics training (table I). The training took place during the bodybuilding season and before the competition.

**Statistical analyses**

After collecting data, descriptive statistics were used to calculate the mean and standard deviation of height, weight, age, and body mass index (BMI). The normality of data distribution was evaluated using the Shapiro-Wilk test. A dependent t-test was used for intragroup comparison, and an analysis of One-Way ANOVA test was used for comparison between two groups with and without ACLR in pre-test and post-test. Statistical calculations were performed using SPSS ver.27 software with a significance level of p ≤ 0.05.

**RESULTS**

The mean and standard deviation of the demographic characteristics of the subjects are reported in table II. For intra-group comparisons, a dependent t-test was used. The results of the ACLR group in table III and the results of the group without ACLR were reported in table IV.

The results of table III show that there is a significant difference between the values of all items and the overall score of the FMS test after 6 weeks of SMST training in the pre-test and post-test in the group with ACLR. We observe an improvement in the average of these items and the overall score.

The results of table IV show that there is a significant difference between the values of all items and the overall score of the FMS test after 6 weeks of SMST training in the pre-test and post-test in the group without ACLR. We observe an improvement in the average of these items and the overall score.

The results of table V indicated that there was a significant difference between the records of the Deep Squat (p = 0.03), Hurdle Step (p = 0.03), In-Line Lunge (p = 0.01), Shoulder Mobility (p = 0.04), Rotatory Stability (p = 0.01), Active Straight Leg Rise (p = 0.03), Trunk Stability (p = 0.03), and Total (p = 0.01) in the pre-test of two groups with and without ACLR after 6 weeks of SMST training.

The results of table VI indicated that there was no significant difference between the record of the Deep Squat (p = 0.31), Hurdle Step (p = 0.54), In-Line Lunge (p = 0.76), Shoulder Mobility (p = 0.56), Rotatory Stability (p = 0.47), Active Straight Leg Rise (p = 0.80), Trunk Stability (p = 0.78), and Total (p = 0.78) in the post-test of two groups with and without ACLR after 6 weeks of SMST training.
### Table I. Sportsmetrics soccer training program (4).

<table>
<thead>
<tr>
<th>Week (Sessions)</th>
<th>Jump Training</th>
<th>Agility, Reaction</th>
<th>Acceleration, Aerobic, Endurance</th>
<th>Ladders-Quick Feet, Dot Jump Drills</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1 (1-3)</td>
<td>Wall jump (20 s); tuck jump (20 s); squat jump (10 s); barrier jumps (20 s); side-to-side; forward-backward; 180 jump (20 s); broad jump (5 repetitions); bounding in place (20 s)</td>
<td>Serpentine run ¼ field (3 repetitions); wheel drill: listen to the instructor, 30 s, 2 repetitions</td>
<td>Partner push offs, hold 5 s; 5 repetitions (sprint to 10-yd line and back); sprint-backpedal, ½ field or 50 yd, 5 repetitions; 4 laps around the field (1280 yd)</td>
<td>Ladder: up-up and back-back, 2 repetitions; dot drill: double leg jumps, 5 repetitions × 3</td>
</tr>
<tr>
<td>Week 2 (4-6)</td>
<td>Same as sessions 1–3; add 5 s to each jump; add 5 repetitions to broad jump</td>
<td>Modified shuttle ¼ field, 3 repetitions; sprint-stop feet listen, 30 s, 2 repetitions</td>
<td>Acceleration with a band (to 10-yd line); sprint with ground touches backpedal, ½ field or 50 yd, 5 repetitions; 100-yd shuttle: 3 × 100 (300 yd), 4 repetitions</td>
<td>Ladders: toe touches, 2 repetitions; dot drills: add split leg jumps, 5 repetitions × 3</td>
</tr>
<tr>
<td>Week 3 (7-9)</td>
<td>Wall jump (25 s); tuck jump (25 s); triple broad into vertical jump (5 repetitions); squat jump (15 s); barrier hops (25 s each); side-to-side; forward-backward; single-leg hop (5 repetitions); scissors jump (25 s); bounding for distance (1 run)</td>
<td>Square drill, 30’ × 30’ box, 2 repetitions; sprint quick feet-listen, 45 s, 2 repetitions</td>
<td>Partner push offs, hold 10 s; 5 repetitions (sprint to 10-yd line and back); ¼ eagle, instructor cued, into sprint, jog back, ½ field or 50 yd, 6 repetitions; 50-yd shuttle: up and back 3 × 100 (300 yd), 4 repetitions</td>
<td>Ladders: outside foot in, 2 repetitions; dot drills: add 180 split leg jumps, 5 repetitions × 3</td>
</tr>
<tr>
<td>Week 4 (10-12)</td>
<td>Same as sessions 7-9; add 5 s to each jump; add 3 repetitions to triple broad into vertical jump</td>
<td>Nebraska drill, 30’ long, 4 repetitions; reaction drill-watch instructor point, 45 s, 2 repetitions</td>
<td>Acceleration with band (to 20-yd line); box drill, sprint-90-backpedal, ½ field, 3 repetitions; 50-yd cone drill: 10 y-back, 20 y-back, 30 y-back, 40 y-back, 50 y-back; 4 repetitions</td>
<td>Ladders: in-in, out-out, 2 repetitions; dot drills: add single-leg hops, 5 repetitions</td>
</tr>
<tr>
<td>Week 5 (13-15)</td>
<td>Wall jump (20 s); step, jump up, down, vertical (30 s); squat jump (25 s); mattress jumps (30 s each); side-to-side; forward-backward; triple single-leg hop, stick (5 repetitions each leg) jump into bounding (3 runs)</td>
<td>Illinois drill, 15’ × 10’, 4 repetitions; reaction mirror drill pressing, 60 s, 2 repetitions</td>
<td>Partner push offs, hold 15 s; 5 repetitions (sprint to 10-yd line and back); sprint-180-backpedal, jog back, ½ field or 50 yd, 7 repetitions; jingle jangle 20 yd, up and back × 5 (200 yd), 5 repetitions</td>
<td>Ladder: up-up and back-back, 2 repetitions; dot drills: combo all jumps, 5 repetitions × 3</td>
</tr>
<tr>
<td>Week 6 (16-18)</td>
<td>Same as sessions 13-15; add 5 repetitions to step, jump up, down, vertical; add 1 run to jump into bounding</td>
<td>T-drill: 5-10-5, 4 repetitions; advanced wheel drill: listen to the instructor, 60 s, 2 repetition</td>
<td>Acceleration with a band (to 30-yd line); sprint-360-sprint (jog back), ½ field or 50 yd, 7 repetitions; jingle jangle 10 yd, up and back × 5 (100 yd), 6 repetitions</td>
<td>Ladder: 1 foot forward, 1 foot backward (scissors), 2 repetitions; dot drills: combo all jumps, 5 repetitions</td>
</tr>
</tbody>
</table>

### Table II. Demographic characteristics of the subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Experimental 1’</td>
<td>21</td>
<td>23.23 ± 2.30</td>
</tr>
<tr>
<td></td>
<td>Experimental 2’</td>
<td>21</td>
<td>23.19 ± 2.24</td>
</tr>
<tr>
<td></td>
<td>Experimental 1</td>
<td>21</td>
<td>179.66 ± 7.26</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>180.14 ± 4.43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Experimental 1</td>
<td>21</td>
<td>71.90 ± 8.33</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>71.52 ± 4.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Experimental 1</td>
<td>21</td>
<td>22.13 ± 2.39</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>21.98 ± 1.04</td>
</tr>
</tbody>
</table>

*Experimental 1: with ACLR; Experimental 2: without ACLR.
Table III. The results of the dependent t-test in the group with ACLR.

<table>
<thead>
<tr>
<th>FMS TEST</th>
<th>Mean ± SD Pre-test</th>
<th>Mean ± SD Post-test</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Squat</td>
<td>1.38 ± 0.49</td>
<td>2.23 ± 0.62</td>
<td>-6.85</td>
<td>0.01</td>
</tr>
<tr>
<td>Hurdle Step</td>
<td>1.71 ± 0.46</td>
<td>2.42 ± 0.50</td>
<td>-5.08</td>
<td>0.01</td>
</tr>
<tr>
<td>In-Line Lunge</td>
<td>1.23 ± 0.43</td>
<td>2.47 ± 0.51</td>
<td>-10.52</td>
<td>0.01</td>
</tr>
<tr>
<td>Shoulder Mobility</td>
<td>1.71 ± 0.56</td>
<td>2.61 ± 0.58</td>
<td>-6.63</td>
<td>0.01</td>
</tr>
<tr>
<td>Rotatory Stability</td>
<td>1.14 ± 0.35</td>
<td>2.09 ± 0.70</td>
<td>-6.52</td>
<td>0.01</td>
</tr>
<tr>
<td>Active Straight leg rise</td>
<td>1.33 ± 0.48</td>
<td>2.18 ± 0.49</td>
<td>-6.85</td>
<td>0.01</td>
</tr>
<tr>
<td>Trunk Stability</td>
<td>1.71 ± 0.56</td>
<td>2.47 ± 0.60</td>
<td>-6.47</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10.23 ± 1.67</strong></td>
<td><strong>16.51 ± 2.88</strong></td>
<td><strong>-17.48</strong></td>
<td><strong>0.01</strong></td>
</tr>
</tbody>
</table>

Table IV. The results of the dependent t-test in the group without ACLR.

<table>
<thead>
<tr>
<th>FMS TEST</th>
<th>Mean ± SD Pre-test</th>
<th>Mean ± SD Post-test</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Squat</td>
<td>1.95 ± 0.58</td>
<td>2.44 ± 0.56</td>
<td>-4.26</td>
<td>0.01</td>
</tr>
<tr>
<td>Hurdle Step</td>
<td>2.09 ± 0.30</td>
<td>2.52 ± 0.49</td>
<td>-3.87</td>
<td>0.01</td>
</tr>
<tr>
<td>In-Line Lunge</td>
<td>1.66 ± 0.57</td>
<td>2.42 ± 0.50</td>
<td>-8.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Shoulder Mobility</td>
<td>2.23 ± 0.53</td>
<td>2.71 ± 0.26</td>
<td>-4.26</td>
<td>0.01</td>
</tr>
<tr>
<td>Rotatory Stability</td>
<td>1.47 ± 0.51</td>
<td>1.95 ± 0.74</td>
<td>-4.26</td>
<td>0.01</td>
</tr>
<tr>
<td>Active Straight leg rise</td>
<td>1.85 ± 0.57</td>
<td>2.23 ± 0.62</td>
<td>-3.50</td>
<td>0.02</td>
</tr>
<tr>
<td>Trunk Stability</td>
<td>2.19 ± 0.40</td>
<td>2.52 ± 0.51</td>
<td>-3.16</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13.58 ± 2.10</strong></td>
<td><strong>16.82 ± 2.48</strong></td>
<td><strong>-12.20</strong></td>
<td><strong>0.01</strong></td>
</tr>
</tbody>
</table>

Table V. The results of the One-Way ANOVA analysis test for comparison between groups in the pre-test.

<table>
<thead>
<tr>
<th>FMS TEST</th>
<th>Group</th>
<th>n</th>
<th>Mean ± SD Pre-test</th>
<th>p</th>
<th>F</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Squat</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.38 ± 0.49</td>
<td>0.03</td>
<td>9.86</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>1.95 ± 0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurdle Step</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.71 ± 0.46</td>
<td>0.03</td>
<td>10.00</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>2.09 ± 0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-Line Lunge</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.23 ± 0.43</td>
<td>0.01</td>
<td>7.36</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>1.66 ± 0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder Mobility</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.71 ± 0.56</td>
<td>0.04</td>
<td>9.52</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>2.23 ± 0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotatory Stability</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.14 ± 0.35</td>
<td>0.01</td>
<td>5.97</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>1.47 ± 0.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active Straight leg rise</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.33 ± 0.48</td>
<td>0.03</td>
<td>10.25</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>1.85 ± 0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk Stability</td>
<td>Experimental 1</td>
<td>21</td>
<td>1.71 ± 0.56</td>
<td>0.03</td>
<td>10.00</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td>2.19 ± 0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Experimental 1</td>
<td>21</td>
<td><strong>10.23 ± 1.67</strong></td>
<td><strong>0.01</strong></td>
<td><strong>32.13</strong></td>
<td><strong>0.60</strong></td>
</tr>
<tr>
<td></td>
<td>Experimental 2</td>
<td>21</td>
<td><strong>13.58 ± 2.10</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION
The results show the effect of the SMST protocol on all items and the overall score of the FMS test in both groups of soccer players with and without ACLR. In the review of individual items, the results of the research showed that in all items in both groups with and without ACLR, we have seen an increase in the score, even to a very small amount. Although in the pre-test we saw a significant difference between the items and the overall score of the FMS test in the comparison between the two groups, there was no significant difference in the post-test after 6 weeks of SMST protocol exercises. This result indicates that the players who had a history of ACLR, although in the pre-test they had a weaker performance in the scores of the FMS test on average than the group without ACLR, but in the post-test this difference was not significant. This shows the positive effect of the SMST protocol in players with ACLR. In fact, according to the results of the current research, the SMST protocol can guarantee the return to sports with a high level of performance in players with a history of ACL injury. While regular muscle strength programs usually work on the sagittal or coronal plane, functional training also works on the transverse plane, where ACL injuries usually occur (18). One of the objectives of our protocol was to prevent ACL injury mechanisms: adduction and internal rotation of the hip, knee valgus, external rotation and anterior translation of the tibia, and eversion of the ankle (19, 20). The goal of functional training is to create a balance between agonist and antagonist muscles. Therefore, quadriceps dominance, which can increase the stress levels on the ACL and make it prone to injury, is modified by dynamic neuromuscular training (21, 22).

FMS test is one of the most widely used performance assessment methods used both in the medical and sport world (23, 24). FMS test is considered as a screening tool to determine the ineffectiveness of an individual’s movement, an additional assessment to determine the dynamic and functional capacity, as well as to prepare for a return to play after rehabilitation from injury or surgery (25). It is believed that the FMS, alongside isokinetic muscle testing and injury risk assessment questionnaires, is one of the most popular systems for detecting injury predisposing factors among professional soccer players (26). McCall et al. analyzed the practice of medical staff of some of the world’s top soccer clubs, showing that almost 66% of them use the above three methods to assess injury risk (27). In soccer, three of the most important risk factors for non-contact injuries are previous injury, fatigue,
and muscle imbalance. FMS is the most common screening tool used to identify these factors and predict the risk of injury in Premier League teams (27). In this regard, Letafatkar *et al.*, based on the relationship between the injury history and FMS scores, reported that the FMS tests provided positive information in predicting the injury (14).

In our research, we selected FMS scores to assess study groups because it analyzes the whole body together. This test helps to identify defects in mobility, stability, and neuromuscular coordination. Muscle strength, flexibility, range of motion, coordination, balance, and proprioception are required to successfully complete the seven basic movement patterns. Kiesel *et al.* (28) noted that a low FMS score is a proven risk factor for injury in professional soccer players. Bonazza *et al.* (25) concluded that individuals with a score ≤ 14 on the FMS score are more than twice as likely to be at risk for musculoskeletal injury as those with a score ≥ 14. Therefore, according to our results of FMS scores, the SMST protocol can be a new tool to support the promotion of a safe return to sports activities after ACLR.

In the literature review of research, we can find a number of studies that have evaluated the effect of a particular type of training on FMS items. Papiez *et al.* (29) conducted a study of a similar nature to the research we presented. They evaluated the impact of corrective activities on the FMS test items and compared a group of soccer players to players who were only engaging in recreational physical activity. Similar to our findings, they also reported improved scores after performing functional rehabilitation exercises. Therefore, they were able to show that providing FMS-based reforms in regular soccer training could significantly improve athletes’ performance and reduce the risk of injury. Campa *et al.* (30) also reached similar results in a study on a group of soccer players belonging to the top four Italian youth clubs. After a 20-week corrective exercise cycle, soccer players significantly improved their overall FMS test score. In fact, researchers were able to prove that the measures taken will definitely affect the results of the experiments, thus reducing asymmetry and movement pattern impairment. Baron *et al.* (31) also conducted a study on young soccer players competing in the junior league. The purpose of their research was to evaluate the functional and physical parameters in order to choose a suitable process to improve these parameters. However, unlike our study, they only focused on three items of the FMS test (deep squat, hurdle step, and lunge). Additionally, they evaluated the speed tests. In the assessment done before the training program, the athletes had weaker performance in the above three items of the FMS test compared to our own research. However, after the completion of the functional training cycle, significant changes in the FMS test scores were observed among the evaluated players, which were similar to the results of our study. However, one can also find research in literature that does not support FMS test-based improvement. For example, Dossa *et al.* (32) stated that the FMS test could not be used in the preseason as a method for assessing the risk of injury in an ice hockey team. In addition, a study by Dorrell *et al.* confirmed low diagnostic reliability of this test to assess the risk of injury (21). However, in the present study, the effect of SMST on improving FMS items in both groups was significant.

Overall, the investigation had previously examined the impact of SMST on athletes without defects and concluded that SMST was a successful protocol in this category (33). However, studies of the impact of SMST on athletes with neuromuscular defects and those who had ACLR were unclear and extremely limited. Furthermore, it has been shown that the previous experience of an ACL injury has a negative effect on performance (34). Therefore, the effect of SMST on soccer players with ACLR, as well as its effect on the scores of FMS tests, was essential. Based on this, the present study was performed. Based on the results of the present study, coaches can use the SMST protocol to improve and correct movement restrictions and patterns identified by FMS in order to prevent re-injury. They can also identify movement restrictions and muscle imbalances using FMS and evaluate the athletes’ readiness to return to play. Because returning to sports after an ACL injury is a complex and subjective process (35).

**Limitations and research suggestions**

Our research had limitations. Since the SMSTS training is difficult and time-consuming, many soccer players did not insist on participating in this prevention protocol. Additionally, FMS assessments were done by only one person, which can increase the chance of personal bias and influence. However, Bonazza *et al.* and Teyhen *et al.* reported that the FMS scoring system has an interrater reliability ranging between moderate to good evaluators, with an acceptable level of measurement error (29, 36). Another limitation was that impairments in rehabilitation following injury of the ACL were not known, and their impact on FMS results could not be removed. In future research, the role of FMS should be clarified as an indicator for identifying the risk of non-contact injuries in soccer players.

**CONCLUSIONS**

According to our results, SMST can be effective in improving FMS test item scores in soccer players with ACLR. Performing functional training appropriate to individu-
al and test characteristics can have a positive effect on the overall score and individual items of the FMS test. The FMS test is an effective diagnostic tool for identifying previous injuries among soccer players.

FUNDINGS
None.

DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

REFERENCES

CONTRIBUTIONS
ASK: design. YH: data collection. ASK: writing – original draft. HD, PS: writing – review & editing, results analysis.

ACKNOWLEDGMENTS
This manuscript is drafted from the PhD. thesis of the Faculty of Physical Education and Sport Sciences, Guilan University, Rasht, Iran.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.
SUMMARY
Tendons are connective tissues with limited healing potential which results in permanently impaired function. Although direct mechanical testing of tendon remains the gold standard for functional analyses, this assay is terminal and tracking healing over time requires the use of many animals. An alternative method for quantifying tendon function is gait analysis, which is non-terminal and enables longitudinal tracking of the same animal. To date, commercial systems used to analyze gait are mostly applied to study neurobehavior, and applications for tendon research has been limited. Since these systems typically output many parameters, it is challenging to know which parameter is most relevant for a specific injury model. To address this challenge, we used a well-established rodent locomotion system (CatWalk XT) to measure longitudinal gait parameters in sham and Achilles tendon-injured mice (from PREOP to 56 days post-injury) and identified relevant and reproducible parameters specifically associated with Achilles tendon injury and healing. Micro computed tomography (micro-CT) and mechanical testing also confirmed persistent tendon impairment in the same animals at the terminal timepoint. Collectively, our results provide a useful reference and recommendation of CatWalk gait parameters and the control comparisons that both conserve the use of animals while maintaining high reproducibility standards for Achilles tendon injury studies.

KEY WORDS
Mouse gait; tendon injury; tendon healing; gait biomechanics; Catwalk XT.

INTRODUCTION
Tendons are dense, fibrous connective tissues that attach muscles to bones to transduce force and allow bodily movements. Although tendon injuries are extremely common (making up 30-50% of sport-related injuries), tendons heal by disorganized, fibrotic scar tissue that does not restore full function. For the Achilles tendon, patients at 12 months post-treatment still display functional deficits compared to the uninjured limb, regardless of surgical or non-surgical intervention. This poor innate healing is often accompanied by chronic inflammation and pain which significantly decreases quality of life (1, 2).

The mouse has emerged as an important model to investigate basic cell and molecular mechanisms of mammalian tendon healing, as genetic tools are widely available for mouse and the anatomic structure and patterning of tendons closely resemble that of humans (3). The gold standard metric of functional tendon healing remains tensile testing of dissected tendon tissues, however other assessments such as limb gait can also serve as useful indicators for functional restoration. Key advantages of gait function include its non-invasive and non-terminal nature and the ability to repeatedly measure parameters in the same animal over time, thus improving reproducibility since pre-operative baseline measurements can be acquired and fewer animals are needed. Gait function may also be more relevant clinically, as evaluation of tendon healing in human patients frequently relies on general limb functional measures, like self-reported assessments of physical activity, as well as physical performance tests that evaluate jumping, strength and muscular endurance (1), since direct tensile testing of tendons is not possible in humans.
In rodents, gait outcome parameters are typically obtained by collecting and analyzing pawprints using paint (4), running wheels, or custom gait arenas. Commercially available systems such as Digigait, MouseWalker, Treadscan and Catwalk XT (5, 6) are also now widely used, particularly in the field of neurobehavior. Commercial systems are advantageous from a rigor and reproducibility standpoint in that studies can be readily compared across different labs. While the Digigait System has been used to test tendon healing outcomes in both mouse and rat and parameters associated with specific tendon injuries have been reported (1, 7-12), there is little information on the other systems, particularly in the context of tendon healing. One widely used system originally developed for spinal injury is CatWalk XT (Noldus Information Technologies). Unlike Digigait which relies on treadmill running, CatWalk is based on free movement of rodents. Paw placement is captured by LED lights emitted in a glass walkway such that pawprints are illuminated by light refraction where the paw touches the glass. These illuminated footprints are captured by a high-speed digital camera and the information is processed by an algorithm that generates gait parameters for each limb. The Catwalk XT system provides several advantages compared to its counterparts; for instance, animal stress is minimized by allowing the animal to ambulate freely and a red ceiling light is used. Furthermore, the data output better recapitulates natural walking habits without potential artifacts resulting from forced movement and algorithmic calculations. Although Catwalk XT is one of the most widely used commercial rodent gait systems, there are few studies reporting its use for tendon research. As of December 2023, a PubMed literature search for “Catwalk XT AND tendon” yielded only 8 research articles. The Achilles tendon was the focus of 5 out of 8 articles, and partial transection injury was the most commonly studied (13-16). Other tendons examined in the remaining 3 articles included rotator cuff and flexor digitorum longus (17-19). In general, most of these studies reported a limited number of parameters (in some cases only a single parameter was reported), and how these parameters were selected was unclear. Since there is evidence that presenting one or a few parameters does not provide a reliable, accurate description of animal gait (5), we focused on comprehensively defining functional gait parameters specifically associated with Achilles tendon injury and healing using the CatWalk XT system. Since full transection of the Achilles tendon is one of the most commonly applied injury models in the field and is widely accessible as no special equipment and limited surgical expertise is required (20-25), we applied these injuries to mice and identified all of the Catwalk XT hindlimb paw statistics indicating either transiently or persistently impaired function and identified the most robust controls for Achilles tendon gait analyses using this system. Collectively, these results form the basis of our recommendation to the tendon injury field for Achilles tendon-relevant gait analysis using CatWalk XT.

MATERIALS AND METHODS

Mouse Achilles tendon injury model
20 wild-type C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA), housed in a pathogen-free barrier facility at the Institute of Comparative Medicine at Columbia University Medical Center and aged to 4 months old when musculoskeletal development is largely complete (26). To account for sex and weight differences that are known to affect gait studies (27), equal numbers of male and female mice were distributed to two different groups undergoing different surgeries: sham injury (10 mice) and Achilles tendon transection injury (10 mice). For these mice, we found that while males were significantly heavier than females, the combined weights of sham vs injured mice were not different (appendix 1). Before surgery, animals were injected intra-peritoneally with extended-release buprenorphine for post-operative pain management, according to the manufacturer’s instructions (Ethiqa XR, Fidelis Pharmaceutical’s LLC). Sham injuries were carried out on the right hindlimb by transecting only the skin adjacent to the Achilles tendon which was then closed by one simple continuous suture (6-0 non-absorbable polypropylene). Achilles tendon transection surgeries were carried out on a separate cohort of animals on the right hindlimb, without repair. Following tendon transection, skin was closed as in the sham animals. All animal procedures were carried out in accordance with the Institutional Animal Care and Use Committee guidelines at Columbia University (AC-ABN0552 – approval date: April 2023).

CatWalk XT Gait Analyses
Mouse gait was captured pre- and post-operatively using the CatWalk XT system (Noldus Information Technologies). Briefly, mice were allowed to freely ambulate in a corridor on a glass plate and illuminated paw prints were captured with a high-speed digital camera. Mice were gaited before the surgery (pre-operative, PREOP), and at 3-, 14-, 28-, and 56-days post-injury (3-56 DPI) to capture baseline gait characteristics before tendon injury and during various stages of tendon healing and remodeling. These timepoints were selected based on our prior research in this model characterizing the phases of inflammation, cell recruitment, and matrix deposition (8). The following
settings were used for all animals at all timepoints: 99% allowed maximum speed variation, 1 s minimum run duration, 20 s maximum run duration, 3-5 minimum compliant runs to acquire, 12.4 dB camera gain, 0.1 green intensity threshold, 17.7 V ceiling light and 18 V walkway light. After image acquisition, each video was analyzed individually to classify any paw placement that wasn’t automatically identified previously through the system algorithm. After exporting the data, all 3-5 compliant run parameters were averaged. For the purposes of these studies, we only focused our analyses on the right (injured) and left (uninjured) hindlimbs of each mouse. The parameters listed by the Catwalk XT software as “base of support”, “step sequence” and “other statistics” were not analyzed. However, all the parameters listed as “paw statistics” were examined, except for the toe spread values, which were not consistently recorded. Representative images of sham and injured mouse hindlimbs at 3 DPI collected by CatWalk is shown in figure 1. Analysis of average speeds across timepoints for sham and injured mice showed almost no change in average speed (appendix 2).

Micro-CT

At 56 DPI, mice were sacrificed, and the Achilles tendons dissected for microcomputed tomography (micro-CT) imaging, keeping the calcaneus bone and most of the gastrocnemius muscle intact. Specimens were maintained in PBS at 4 °C until ready for testing the same day. Micro-CT scans were acquired as previously described (27), using the Bruker micro-CT instrument at 55 kVp, with an A1 0.25 filter and 6.4 uM resolution with a 0.6° rotation step. The images were processed and reconstructed with the following softwares: SkyScan NRecon, SkyScan Data-Viewer, Micro-CT CT-Analyser (Ctan) to obtain the minimum cross-sectional tendon area.

Tensile testing

After micro-CT imaging, the gastrocnemius muscle was removed to perform tensile testing as previously described, using a custom 3D printed fixture for gripping the calcaneus bone which was mounted onto a ElectroForce 3200 mechanical tester (TA Instruments) (28). Tendons were pre-conditioned for 5 cycles between 0.05 N and 2 N, held for 120 s followed by a ramp to failure at 1% strain/s. Load-deformation curves were generated with Microsoft Excel (version 16.59) to determine the maximum force achieved and stiffness (linear region). Then, stress-strain curves were generated to obtain maximum stress, as well as the Young’s modulus as previously described (28).

Statistics

Gait

For all gait parameters, we completed ROUT outlier tests (Q = 1%) and Shapiro-Wilks normality tests to remove prominent outliers and ensure a normal distribution. Significant differences were detected using ANOVA/mixed effect analyses followed by Sidak’s or Dunnett post-hoc testing with correction to compare groups. For all comparisons (except sham vs injured hindlimb measurements), repeated and paired comparisons were used.
Micro-CT and tensile testing

After performing ROUT outlier tests and Shapiro-Wilks normality tests as described above, one way ANOVA tests with Tukey’s *post-hoc* comparisons were used to compare uninjured, sham, and injured tendons. Uninjured tendons were collected equally from sham and injured animals. For all analyses, significance was set at $p < 0.05$. All tests were completed using the GraphPad Prism software (version 9.5.1).

RESULTS

Identification of relevant gait parameters for Achilles Tendon injury

To identify relevant gait parameters, we initially focused on parameters that were consistently changed in a single direction (increased or decreased) for injured hindlimbs over time relative to pre-operative controls (PREOP) (figure 2). Parameters that displayed increased values at one timepoint and decreased values at another timepoint were not assessed further even if differences were statistically significant. Out of the six parameters identified meeting these criteria, three showed only transient changes at 3 DPI (print area, swing, and swing speed) before returning to baseline PREOP values from 14 DPI onward (figure 2). The remaining three parameters (mean intensity, max intensity, and duty cycle) showed consistent and persistent deficits compared to PREOP at almost every timepoint post-injury (figure 2). Analysis of the same parameters in sham-injured animals showed either no change (swing, swing speed, duty cycle) or inconsistent changes (print area, mean intensity, and max intensity) compared to PREOP. The inconsistent changes observed were typically in the opposite direction observed in tendon-injured hindlimbs and were inconsistent across timepoints (figure 3).

We next compared tendon-injured hindlimbs to their contralateral uninjured hindlimbs over time. As expected, no differences were observed between hindlimbs at PREOP for any parameter (figure 4). While some parameters only showed transient changes at early stages (max contact, swing, swing speed, and duty cycle), the parameters mean and max intensity both showed consistently impaired values at all post-injury timepoints (figure 4). Importantly, there were no differences observed for sham-injured hindlimbs and their contralateral uninjured hindlimbs for any of these parameters (figure 5). Finally, we directly compared sham-injured and tendon-injured hindlimbs and again found no differences in PREOP values. We also identified a subset of transiently affected parameters (print area, swing, single stance, duty cycle) and more consistently impaired parameters (mean and max intensity) in tendon-injured hindlimbs compared to sham hindlimbs for all timepoints post-injury (figure 6).

Figure 2. Gait parameters associated with Achilles tendon transection injury compared to pre-operative values. Analysis of the injured hindlimb compared to its own pre-operative values identified several gait parameters that were significantly changed with injury. $n = 9-10$ mice; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure 3. Analysis of injury gait parameters for sham hindlimbs compared to pre-operative values. Analysis of the gait parameters identified in figure 2 for sham hindlimbs compared to its own pre-operative values showed some significant changes but in variable direction (higher or lower depending on timepoint). $n = 9-10$ mice; *$p < 0.05$; **$p < 0.01$. 

Muscles, Ligaments and Tendons Journal 2024;14 (2)
However, significant differences were not detected at 28 DPI (figure 6). A full list of parameters identified (beyond those represented in the figures) is listed in table I.

**Tendon mechanical properties are not recovered after injury**

To confirm that tendon mechanical properties remained impaired at the end of the study duration (56 DPI), we carried out tensile testing and determined structural and material mechanical properties. Micro-CT imaging showed the presence of heterotopic ossification within both Achilles tendon stubs, as we and others have previously reported (figure 7A). Consistent with prior literature (28), stiffness and modulus were also significantly decreased for injured tendons relative to both sham and uninjured tendons (figure 7B,C). Cross-sectional area
Figure 6. Gait parameters associated with Achilles tendon transection injury compared to sham injured hindlimbs. Analysis of the injured hindlimb compared to sham injuries performed on a separate cohort of mice identified several gait parameters that were significantly changed with injury.

\( n = 9-10 \) mice; \(^* \) \( p < 0.05 \); \(^{**} \) \( p < 0.01 \); \(^{***} \) \( p < 0.001 \).

Table I. Relevant Catwalk XT parameters to observe functional outcomes of tendon healing following complete Achilles transection without repair.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PREOP RH compared to POST-OP RH</th>
<th>Injured/sham RH compared to contralateral LH</th>
<th>Injured RH compared to sham RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print area</td>
<td>-</td>
<td>Print area</td>
<td>-</td>
</tr>
<tr>
<td>Max contact (% stand)</td>
<td>-</td>
<td>Max contact (% stand)</td>
<td>-</td>
</tr>
<tr>
<td>Max intensity at max contact</td>
<td>-</td>
<td>Max intensity at max contact</td>
<td>-</td>
</tr>
<tr>
<td>Mean intensity at max contact</td>
<td>-</td>
<td>Mean intensity at max contact</td>
<td>-</td>
</tr>
<tr>
<td>Max intensity</td>
<td>Mean intensity</td>
<td>Max intensity</td>
<td>Mean intensity</td>
</tr>
<tr>
<td>Mean intensity</td>
<td>Mean intensity</td>
<td>Mean intensity</td>
<td>Mean intensity</td>
</tr>
<tr>
<td>Mean intensity of the 15 most intense pixels</td>
<td>Mean intensity of the 15 most intense pixels</td>
<td>Mean intensity of the 15 most intense pixels</td>
<td>Mean intensity of the 15 most intense pixels</td>
</tr>
<tr>
<td>Swing</td>
<td>Swing</td>
<td>Swing</td>
<td>Swing</td>
</tr>
<tr>
<td>Swing speed</td>
<td>Swing speed</td>
<td>Duty cycle</td>
<td>Duty cycle</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>-</td>
<td>Max contact area</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Max contact area</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>Min intensity</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>Single stance</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

was also significantly increased in injured tendons, indicative of fibrotic scar formation. No differences in failure properties (max force and max stress) were observed.

**DISCUSSION**

In this study, we identified useful and reproducible gait parameters associated specifically with Achilles tendon injury and healing using the CatWalk XT system. Overall, performing paired comparisons between the injured and contralateral uninjured hindlimbs yielded the greatest number of detectable and reproducible differences. Importantly, differences in these parameters using the contralateral control limb were highly specific to Achilles tendon injury group and the sham group was largely not affected. Although we found that comparisons to baseline also yielded consistent results in the Achilles tendon injured limb, the sham limbs in this comparison yielded varying and inconsi-
tent results, which can challenge interpretation. Since direct comparisons to sham injury did not produce additional parameters of interest and mechanical properties were also not altered compared to uninjured, we recommend that functional studies focused on Achilles tendon injury and healing can reasonably omit a separate sham control group in the interest of conserving animal use. Comparing different animals also might require more stringent weight and speed normalization to obtain reliable differences (27). Note that our recommendation is limited to the gait and relatively limited mechanical analyses we carried out here, as some biological considerations (related to immune responses for example) might require separate sham rather than uninjured controls. This should be determined in future studies. In general, we found that differences in intensity measurements (such as max and mean intensity, and other related intensity measurements listed in table I), were the most consistently associated with Achilles tendon injury in skeletally mature adult mice. Since intensity measurements depend on animal weight, it is possible that these parameters would not be useful in younger animals below a certain weight threshold, although neurological studies have successfully performed gait analysis in young mice (29, 30). Whether tendon injury-induced gait changes can be detected in young animals must be determined in future studies. In adults, male mice are also typically heavier than female mice (we observed ~5 g difference in C57/Bl6 mice at this age). We intentionally combined male and female mice in our analyses to determine whether statistically significant parameters could still be detected using minimum animals. Although the study was not powered to detect sex differences, when we analyzed the parameters separated by sex (n = 5 per group), we did find that females generally showed greater variation, which could be due to their lower weight and variation in estrus cycle stage, which has been shown to affect mouse physical performance (31). Ongoing studies will increase sample size for both sexes

Table I. Relevant Catwalk XT parameters to observe functional outcomes of tendon healing following complete Achilles transection without repair.

<table>
<thead>
<tr>
<th>PREOP RH compared to POST-OP RH</th>
<th>Injured/sham RH compared to contralateral LH</th>
<th>Injured RH compared to sham RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print area</td>
<td>Max contact (%) stand</td>
<td>Max contact (%) stand</td>
</tr>
<tr>
<td>Max intensity at max contact</td>
<td>Max intensity at max contact</td>
<td>Max intensity at max contact</td>
</tr>
<tr>
<td>Mean intensity at max contact</td>
<td>Mean intensity at max contact</td>
<td>Mean intensity at max contact</td>
</tr>
<tr>
<td>Max intensity</td>
<td>Max intensity</td>
<td>Max intensity</td>
</tr>
<tr>
<td>Mean intensity</td>
<td>Mean intensity</td>
<td>Mean intensity</td>
</tr>
<tr>
<td>Mean intensity of the 15 most intense pixels</td>
<td>Mean intensity of the 15 most intense pixels</td>
<td>Mean intensity of the 15 most intense pixels</td>
</tr>
<tr>
<td>Swing</td>
<td>Swing</td>
<td>Swing</td>
</tr>
<tr>
<td>Swing speed</td>
<td>Swing Speed</td>
<td>Swing Speed</td>
</tr>
<tr>
<td>Duty cycle</td>
<td>Duty cycle</td>
<td>Duty cycle</td>
</tr>
<tr>
<td>-</td>
<td>Max contact area</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Min intensity</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Single stance</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 7. Tensile mechanical properties are impaired at 56 days post-injury.

(A) MicroCT imaging showed the presence of heterotopic ossification in the original Achilles tendon stubs at 56 DPI; (B) Tensile structural properties; (C) material properties show persistent deficits at 56 DPI. n = 9-10 mice; *p < 0.05; **p < 0.01.
to determine whether any of the parameters may have sex-dependent differences (especially since previous studies showed baseline sex differences in material mechanical properties for the Achilles tendon) (28). While permanent deficits in tendon function were best represented by the intensity parameters, we also identified several parameters that showed transient differences only at early timepoints (typically 3 DPI). These parameters may be useful in studies where scar-mediated healing is impaired (for example in the presence of genetic mutations or experimental interventions such as prolonged inflammatory challenge). Under those scenarios we could anticipate that deficits in these typically transient parameters would be prolonged into extended timepoints post-injury.

In addition to limitations related to sex, our study also focused solely on hindlimb statistics and did not determine changes in the forelimbs; there may be additional useful parameters associated with compensatory forelimb gait that were missed. We also did not include analyses of “base of support” and “step sequence” which are associated with inter-paw coordination. It is also challenging to separate the effects of pain from the injury vs pure functional considerations. It is possible that some of the changes we identified are more closely associated with painful behaviors, especially in the early stages of healing when inflammation is at its height. Moreover, another limitation of our studies is the lack of habituation to the gait testing facility, and lack of training on the runway prior to our longitudinal gait testing. This creates greater variability within the data since mice are less likely to walk uniformly as they tend to explore their surroundings. To only record uniform walking trajectories (without standing, stopping etc.), lower maximum allowed speed variation settings will be tested in future studies. While gait results were confirmed by mechanical function results, structure was not evaluated despite the importance of structural assessment in tendon healing (32). This will be determined in future studies. Finally, it is important to note that the current study focused on tendon transection injury; other disease conditions such as more subtle tendinopathy models due overuse or fatigue loading (33) will need to be separately validated in terms of gait. Despite these limitations however, these results provide researchers using CatWalk XT a reference to decide which parameters are worth examining to investigate functional Achilles tendon injury outcomes and which controls may be most appropriate.

CONCLUSIONS

In conclusion, these results demonstrate reproducible CatWalk gait parameters that are significantly associated with adult Achilles tendon injury and impaired functional healing.

FUNDINGS

This work was supported by NIH/NIAMS funding (R56 AR076984, R01 AR081674) to AH and T32 AR080744 fellowship to EK.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GC, AHH: conceptualization, design. GC, EK: data collection and analyses. All authors: data interpretation, writing – original draft, writing – review & editing.

ACKNOWLEDGMENTS

We thank the Mouse NeuroBehavior Core at the Institute for Comparative Medicine at Columbia University Medical Center for assisting with training and usage of the Catwalk XT. We also thank Dr. Iden Kurtalaij and Dr. Steve Thomopoulos for training and assistance with micro-CT and mechanical testing.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES


SUPPLEMENTS

Appendix 1. (A) Average weights of sham and injured groups at PREOP; (B) Average weights of males and females at PREOP. 
n = 10 mice; """" p < 0.0001.

Appendix 2. (A) Average speeds for sham and injured groups overtime; (B) Average speeds of injured males and females overtime. 
n = 10 mice; * p < 0.05.