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Leading by Example: A Call to Action for the New Committee “I.S.Mu.L.T. Sport and Disability” to Promote the Benefits of Sports for Individuals with Severe Disabilities

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The positive impact of sports on physical and mental well-being is widely acknowledged both in the general population and among individuals with disabilities (1). Sports participation provides physical, psychological, and social benefits for participants, creating the basis for personal empowerment of people with disabilities (2). Regular participation in sports offers benefits that extend beyond the physical realm, encompassing psychosocial aspects as well (3, 4). It promotes higher social interaction and independence, and increased self-confidence. Team sports, in particular, contribute significantly to character development, body awareness, group dynamics, and self-esteem, enhancing feelings of security and gratification (5). All of these features may positively influence the overall quality of life (QoL) (6). These people also recognize the disease as less cyclical and more chronic. This knowledge enables the development of strategies to keep people more involved in the community (7).

Individuals with severe degrees of disability face challenges such as poor health, limited community participation, and reduced quality of life (8). A poor QoL leads to emotional and mood disorders, while a high QoL is linked to a reduced perception of symptoms and an increased awareness of the course of the disease (9). Regardless of the disease, people with severe disabilities frequently perceive a negative body image. Physical differences resulting from impairments can lead to self-consciousness, negative self-perception, and dissatisfaction with their bodies (10). Low body image can harm their self-esteem, self-confidence, and overall psychological well-being (11). Generally, QoL results in significantly higher satisfaction in individuals with physical disabilities who practice any kind of sport respect than who do not participate (12). A similar finding was found for children and adolescents, including those with cerebral palsy (13), and for adults with neuromuscular diseases (2).

Medical treatments, even though necessary, may add constraints to these individuals’ lives, imposing several accesses to the hospital facilities (14). Unfortunately, most medical professionals and caregivers frequently perceive these subjects
as unfit to participate in sports, especially if strenuous muscular effort is required. Consequently, physicians arbitrarily recommend avoiding competitive physical activities, when they rarely suggest sports as a viable option for these subjects (8).

Sports participation can greatly contribute to disability empowerment (15). The Community Based Rehabilitation (CBR) Manual published by the World Health Organization focuses on empowerment as a primary goal (16). The integration of people with disabilities into the most common activities in the community is the most significant element in achieving this target. Organized sports give participants opportunities to gain independence and self-confidence (17). Empowerment is closely related to self-efficacy as the belief in one's own ability to perform a certain task or accomplish a certain goal. Empowered individuals tend to enhance their perceived competence in various domains such as academic, social, vocational, and behavior (18).

Social capital is another key benefit of sports involvement for people with disabilities. It is defined as “the relationships and the benefits derived from these relationships in conjunction with external variables (organizational characteristics, living situation, family involvement, personality characteristics, perceived level of disability)” (19). The benefits of participating in organized sports include social inclusion, which is closely linked to social capital (20). Disability communities also derive social capital through their direct participation in sports and through their extended involvement with others in their sports communities using social networking platforms. Through sport it is possible to bridge the gap between the disabled young person and the outside world, trying to reduce the distance created. Organizations can foster trust and cooperation among these individuals.

The specific assessment of the impact of sports activities in the life of individuals with different disabilities in terms of clinical and neuropsychological and functional status (even in terms of biomechanical aspects), the correct and adapted medical management in case of injuries and the analysis of the quality of life may help to overcome possible reservations about competitive sports activities of people suffering the different forms of disabilities, of their caregivers and of the healthcare professionals, too. This could also be the scientific basis for the formation of integrated social and health policies aimed at improving the management courses of these people, realizing the main rehabilitation objectives of integration into community life. The multifaceted complexity of these patients necessitates multidisciplinary management that involves a number of health professionals that interact with each other. Hence, adapted sports activities may help achieve total integration into the community and should be included in the multidisciplinary rehabilitative program (21).

Studying individuals with disabilities and the relationship between cognitive and motor abilities and sports holds the potential for developing evidence-based eligibility and classification systems that address the impact of impairment on athletic proficiency in Paralympic sports (22). By recognizing and supporting their participation in sports, we can empower individuals with severe disabilities to lead fulfilling and active lives, breaking barriers, and encouraging inclusivity. Sports benefits extend beyond the physical realm, positively impacting their overall quality of life and promoting a more inclusive and equitable society for all.

We invite the scientific community to take an active role in promoting sports benefits for individuals with disabilities with the newly established committee “I.S.Mu.L.T. Sport and Disability”. Our aims will be: 1) educate and inform, updating on the latest evidence regarding the benefits of sports for individuals with disabilities and taking the initiative to educate physicians, patients, and caregivers on adaptive sports programs, specialized equipment, and resources available in your community; 2) encourage sports as a health intervention, emphasizing the positive impact of sports on overall health and for mental well-being; 3) address concerns and barriers, examining adaptive sports options and modifications that can accommodate their specific needs and abilities, also collaborating among healthcare professionals; 4) collaborate with sports organizations, establishing successful relationships with sports organizations and disability sport programs with appropriate resources and support networks within the community; 5) advocate for inclusive sports, emphasizing the development of inclusive sports programs within the social community, actively participating in disability-inclusive sports events and supporting athletes with disabilities (leading by example).

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REFERENCES


Treatment with Conditioned Medium from Tenocytes Primary Cell Cultures Accelerates Histological and Functional Recovery of Achilles Tendon in Tenotomized Mice

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SUMMARY
Background. Tendon injuries are responsible for a crescent number of people retirement around the world. Recently, cell-based therapies has been shown to be effective for the treatment of tendon injuries.

Objective. We aimed to evaluate the effect of local treatment with conditioned medium produced by tenocytes cell cultures on the histological and functional recovery in tenotomized mice.

Methods. The tendon cells were cultured for conditioning culture medium that will be used as treatment. The animals were subjected to right Aquilles tenotomy and treated with saline solution (SAL), DMEM (DMEM) and DMEM conditioned in tenocytes cultures (CM) and compared to the control group (CTRL). For histological analysis, tissues were stained with HE. Tendon functionality was measured using the Achilles Functional Index (AFI) and mechanical sensitivity through the paw withdrawal threshold (PWT) using the Von Frey test. Analyzes were performed at 7-, 14- and 21-days post-injury (dpi). Statistics were performed by ANOVA-2 followed by Tukey’s post-test, p < 0.01.

Results. In the histological analysis, the CM group showed better tissue organization when compared to SAL and DMEM groups. The CM group showed improvement in the PWT at 7° and 14° dpi (2.24 ± 1.15; 2.66 ± 1.06) compared to the DMEM (0.15 ± 0.07; 0.45 ± 0.76 p < 0.01) and SAL (0.13 ± 1.15; 0.77 ± 0.95 p < 0.01). The CM group showed functional improvement at 7° and 14° dpi (-40.4 ± 12.6; -36.6 ± 10.4) compared to the DMEM (-76.5 ± 11.7; -71, 6 ± 7.9, p < 0.01) and SAL (-88.8 ± 15; -71.4 ± 12.6 p < 0.01).

Conclusions. We conclude that treatment with tenocytes conditioned medium accelerates tendon recovery, promoting improvement in mechanical sensitivity, functionality and tissue organization in the proposed injury model.

KEY WORDS
Cell therapy; tendinopathy; tenocytes; tendon; recovery.
INTRODUCTION
Tendon injuries are responsible for a crescent number of people retirement around the world (1). It is widely described in literature that tendon injuries represent more than fifty percent of all lesions in athletes, as well as that chronic pain and loss of muscle physiology represents very common symptoms observed in patients developing tendinopathies (2, 3). Histological reports describe tendon as a connective tissue with fibrous-elastic texture constituted by a large and dense collagen network (3, 4). Tendon tissue is formed by specialized fibroblast cells denominated tenocytes which are responsible by collagen synthesis and maintenance of biochemical components of tendons (4, 5). Although several studies demonstrated that tenocytes are able to produce different components for preservation of tendon tissue integrity, there are few studies demonstrating the potential effect of these cells on the recovery of injured tissue (6, 7).

Rupture of tendon triggers histological events initiating with an inflammatory period which is characterized by leucocytes migration to injured tissue and intense collagen degradation (8). This phase is followed by proliferation of tenocyte with consequent elevation of collagen synthesis. The last period of tissue repair is characterized by intense metabolism of extracellular matrix as well as by remodeling of collagen fibers into the injured tissue (9-11). The duration of these periods is determinant to assure the time of tendon recovery and to reestablishment of tendon functionality.

It is already evidenced that tenocytes present significant participation on tendon repair after tissue injury. In fact, tenocytes are responsible for maintenance of tissue repair during proliferation phase of tendon recovery (12-14). Previous reports also demonstrated that cellular therapy utilizing local insertion of tenocytes into injured tendons promotes significant acceleration in the tendon recovery (15, 16). However, there are no pre-clinical studies describing the effect of compounds released from tenocytes on the tendon Achilles in tenotomized subjects. In this way, the current work aimed to evaluate the effect of local treatment with conditioned medium produced by tenocytes cell cultures on the histological and functional recovery of Achilles tendon in tenotomized mice.

MATERIALS AND METHODS
Animals
Swiss mice were provided by animal facilities of Federal University of Pará (UFPa). Primary cell cultures of tenocytes were produced utilizing tendon tissue isolated from 2 days post-natal mice (PN) while adult mice (30-35 g) were utilized in experiments of Achilles tendon rupture. These animals were kept in polypropylene cages at 25 °C in controlled dark/light cycle (12:12) with food and water ad libitum. All experimental procedures were previously approved by the ethical committee for care and use of laboratory animals from UFPa (Protocol number: 4512080418 – Date of approval: June 21, 2018).

Tenocyte primary cell cultures and preparation of conditioned medium
Primary cell cultures of tenocytes were performed as previously described (17). Briefly, mice were deeply anesthetized with ketamine/xylasine solution (80 mg/kg and 12 mg/kg), quickly decapitated and their Achilles tendons were dissected. Tendon tissues were then transferred to Petri’s plate containing cold calcium media free (CMF) solution. After that, tendons were minced in 3mm pieces and placed in cell culture dish containing Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. Tenocytes cultures were maintained in CO2 stove at 37 °C until reach total cellular confluence.

As there are currently no protocols utilizing conditioned medium from tendon cells, the authors chose to use confluent cultures to produce the conditioned medium based on established protocols from previous tendon cell culture studies, that cultivated those cells until confluence (15, 18, 19). Besides that, previous analyses described that muscle and tendon-derived cells achieved higher cell numbers and full confluence more rapidly (20). This confluent could enhance the secretion of bioactive factors, contributing to a more robust conditioned medium. The conditioned medium of tenocyte cell cultures was based on adaptation of previously described protocols (21). After reaching confluence, the culture medium was removed, and then tenocyte cells were washed with a calcium-free medium. The decision to wash the cells with CMF before conditioning the medium aimed to eliminate any detached cells, ensuring the purity of the conditioned medium. This approach, rooted in well-established cell culture practices, enhances the reliability of experimental results by minimizing potential influences from floating cells on the composition of the conditioned medium (22). Afterward, cell cultures were incubated with 6 ml of DMEM without any supplementation was added for conditioning by the tenocyte cells themselves over a 6-hour period in CO2 stove to production of conditioned medium. After this period, conditioned medium was collected from tenocytes cell cultures and centrifuged to ensure the absence of cells. It was centrifuged at 1,500 rpm for five minutes. This approach was based on previous protocols for centrifugation of tendons cells cultures (23, 24), to enhance the reliability of the conditioned medium by minimizing the presence of cells. Supernatant was carefully collected and maintained at -80 °C until experimental utilization.
Achilles tendon tenotomy protocol

Animals were anesthetized by intraperitoneal injection of ketamine (80 mg/kg)/xylazine (12 mg/kg) solution. Tibia region of the right paw was tricotomized and a longitudinal skin incision was performed to access Achilles. After that, the right tendon was transected in axial fashion at 0.5 cm from calcaneal insertion followed by Kessler suture (25, 26). Afterwards, the tendon and skin were sutured with non-absorbable monofilament polyamide thread number 4.0 from TechFio® and subjected to local asepsis. After surgical treatment the animals were kept in cages without movement restriction or legs immobilization. The post-anesthetic recovery of the animals was monitored for 2h. Animals were subdivided in control group (CTRL, n = 9); saline group (SAL, n = 9), vehicle DMEM group (DMEM, n = 9) which were submitted to tendon rupture followed by surgical suture and post treated in loco with saline or DMEM, respectively and conditioned medium group (CM, n = 9) which was submitted to tendon rupture, surgical suture and posteriorly treated in loco with conditioned medium form primary cultures of tenocytes. All treatments were administered into the peritendinous region with a 26-gauge needle once every two days after injury until day 21.

Histological analysis

Achilles tendons of control and experimental groups were evaluated at 7th, 14th and 21st days post injury (dpi). Tendon tissues were quickly dissected and fixed in 4% paraformaldehyde during 12h as described by Moraes et al. (27). Briefly, tendons were washed tree times with 0.1M phosphate buffer pH 7.4 and cryoprotected by sequential immersion in gradient of sucrose solution (10%, 20% and 30%). Longitudinal sections (20 μm) of tendon tissues were stained with hematoxylin-eosin (HE) and coverslipped using Permount® (Fisher Scientific, New Jersey/U.S.A). The images were visualized and recorded utilizing a light microscopy (Nikon, Eclipse E800 Yokohama, Japan) and analyzed by ImageJ® software.

Von Frey behavioral test (Mechanical sensitivity)

The hind paw withdrawal threshold was determined using von Frey filaments (SORRI-BAURU, Brazil) ranging from 0.02 g to 10 g. Experimental procedures were realized as a blind test as previously described (28). The tests for control and ruptured groups began after 5 minutes of habituation. The series of von Frey filaments were applied from below customized platform as following. Ipsilateral hind paw of control or ruptured mice were pressed with one of a series of filaments gradually increasing stiffness (0.02-10 g) applied to the plantar surface for 5-6 s for each filament. Ipsilateral hind paw of control or ruptured mice were pressed with one of a series of filaments gradually increasing stiffness (0.02-10 g) applied to the plantar surface for 5-6 s for each filament. Each filament was applied 10 times and the minimum value that caused at least 3 responses which were recorded as paw withdrawal thresholds (PWT). Acute withdrawal, bite, licking or shaking the ipsilateral posterior limb and vocalization were considered positive signs of withdrawal. The average of these values was used for data analysis. The withdrawal threshold was determined to each animal before surgery at 0, 3-, 7-, 14- and 21-days post injury in independent groups.

Achilles functional index

Tendon functionality was measured utilizing Achilles functional index (AFI) as proposed by Murrell et al. (29). Control and experimental animals had their hind paws painted with nontoxic blue ink and then the animals were placed in the walkway apparatus (10 × 60 cm) leaving their footprints on a white paper. The animal footprints were recorded, digitalized and evaluated using the ImageJ software. The values of footprint length (FL), foot spreading (FS) and the intermediary test factor (IFT) were applied in the followed equation of Achilles functional index AFI = 74 (FL) + 161(FS) + 48(ITF) - 5.

Statistical analysis

All results were expressed as mean and standard derivation. The comparison among groups was evaluated using analysis of variance (ANOVA-2) followed by Tukey post-test. P-value < 0.01 was considered as significant and all statistical tests were performed using BioEstat 5.2 Software.

RESULTS

Data presented in figure 1 show primary cell cultures of tenocytes at 10 days of development. As observed in the photomicrographs, tenocyte in vitro present elongated morphology and a pattern of organization with rows of cells next to each other. We also have studied the histological alterations in tendon tissue of tenotomized animals. Our data have shown intense tissue disarrangement in tenotomized animals treated with saline or DMEM in the 7th, 14th and 21st days post injury (figure 2). However, tenotomized animals which received local injection of conditioned medium from primary cell cultures of tenocytes presented better tissue organization when compared with tenotomized groups (figure 2). Nociceptive evaluation demonstrated no differences in paw withdrawal thresholds (PWT) among the non tenotomized animals (figure 3). However, tendon ruptured animals presented significant decrease in the PWT values for ipsilateral paw at 7 (SAL = 0.13 ± 1.15; DMEM = 0.15 ± 0.07; CM = 2.24 ± 1.15), 14 (SAL = 0.77 ± 0.95; DMEM = 0.45 ± 0.76; CM = 2.66 ± 1.06) and 21 (SAL = 1. 40 ± 1.03; CM = 2.00 ± 1.0).
Conditioned Medium from Tenocytes Cultures Accelerates Recovery of Achilles Tendon

DMEM = 1.40 ± 1.03; CM = 2.06 ± 1.90) days post lesion when compared with control at 7 (6.0 ± 3.46) and 14 (5.33 ± 4.16) days post lesion (p < 0.01). Our data also showed that DMEM treatment was not able to prevent the decrease in PWT induced by tendon rupture. On the other side, treatment with conditioned medium from primary tenocytes cultures prevented the decrease in PWT induced by tendon rupture at 7 (CM = 2.24 ± 1.15) and 14 (2.66 ± 1.06) days post lesion when compared with ruptured groups and at 7 (SAL = 0.13 ± 1.15, p < 0.01; DMEM = 0.15 ± 0.07, p < 0.01) and 14 (SAL = 0.77 ± 0.95, p < 0.01; DMEM = 0.45 ± 0.76, p < 0.01) days post lesion.

Figure 1. Microscopy images of primary cell cultures from tenocytes obtained from the Achilles tendon of 1- or 2-day old mice in ten days of cultivation. Line shows pattern of organization and cell proliferation to spaces not yet filled. 20x objective, 50 μm scale bar.

Figure 2. Effects of treatment with conditioned medium from tenocytes primary cell cultures on tissue organization at 7-, 14- and 21-days post-injury. Tenotomized groups treated with saline (SAL), DMEM and Conditioned Medium from tenocytes (CM). HE marking. 20x objective, 50 μm scale bar, n = 9.

Figure 3. Effects of treatment with conditioned medium from tenocytes primary cell cultures on the mechanical sensitivity of mice. CTRL: Control group, SAL: Saline group, DMEM group and Conditioned Medium from tenocytes (CM). Analyzes performed at 0 before surgery, 3-, 7-, 14- and 21-days post injury. Data are presented as mean ± SD. *Significant difference (p < 0.01) vs CTRL and # (p < 0.01) vs DMEM, n = 9.

In concern of functional evaluation, our data have shown no statistical differences among the days in the Achilles functional index of non tenotomized control group. On the other way, tenotomized animals treated with saline presented significant reduction of Achilles functional index at 7 (CTRL = 0.33, ± 8.19 vs SAL = -88.82 ± 15, p < 0.01); 14 (CTRL = 2.86 ± 5.40 vs SAL = -71.43 ± 12.66, p < 0.01) and 21 (CTRL = -3.02 ± 5.46 vs SAL = -33.69 ± 6.49, p < 0.01) days post injury. Similar results were observed in tenotomized animals treated only with DMEM at 7 (CTRL = 0.33 ± 8.19 vs DMEM = -76.55 ± 11.72, p < 0.01); 14 (CTRL = 2.86 ± 5.40 vs DMEM = -71.62 ± 7.91, p < 0.01) and 21 (CTRL = -3.02 ± 5.46 vs DMEM = -37.16 ± 10.40, p < 0.01). However, animals tenotomized which were treated with conditioned medium of primary tenocytes cell cultures presented elevated values of Achilles functional index when...
compared with tenotomized animals treated with saline at 7 (SAL = -88.82 ± 15 vs CM = -40.46 ± 12.60, p < 0.01); 14 (SAL = 71.43 ± 12.66 vs CM = -36.66 ± 10.43, p < 0.01) or DMEM at 7 (DMEM = -76.55 ± 11.72 vs CM = -40.46 ± 12.60, p < 0.01) and 14 (DMEM = -71.62 ± 7.91 vs CM = -40.46 ± 12.60 p < 0.01) days post lesion.

Other relevant find in the present work was the anti-nociceptive effect exerted by treatment with conditioned medium from tenocytes in culture. As widely demonstrated in literature an important symptom of tendon rupture is the intense local pain (12, 37) which makes very hard the physiotherapeutic intervention in the patients. The molecular mechanism involved in the maintenance of prolonged pain in the injured tendon remains unclear, but increased inflammatory response, intense process of innervations, excessive production of nociceptive agents such as glutamate and substance P are associated with this phenomenon (38, 39).

Our data showed that treatment with conditioned medium have significantly attenuated the paw sensibility in tenotomized animals being this effect observed since the first days after tendon rupture. Although posterior experiments need to be performed to clarify this phenomenon, the capacity of tenocytes to produce and release anti-inflammatory cytokines, grown factors and substances able to inhibit substance P activity could explain the effect observed in animals treated with conditioned medium from tenocyte (7, 16, 40).

CONCLUSIONS
The beneficial effect exerted by the treatment with conditioned medium from tenocyte primary cell cultures on the paw sensibility of tenotomized animals was followed by significant attenuation of dysfunction in the march pattern induced by tendon rupture. Previous studies already demonstrated that better in the Achilles tendon functional index represents an important indicator of efficient treatments (41). In this context, the present pre-clinical study opens perspective to utilization of conditioned medium from tenocytes for treatment of different tedinopathies.

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

AM: experiments, data analysis and interpretation, writing - original draft. MLM: data analysis and interpretation. DRDP; MDF, LKRL: experiments, data analysis and interpretation. SASdM, RdSB, EdJOB, ACFP, KRHMO: data analysis and interpretation, resources, writing - original draft. AMH: conceptualization and design, experiments, data analysis and interpretation, writing - original draft, writing - review & editing.

CONFLICT OF INTERESTS

The author declare that they have no conflict of interests.

REFERENCES


Peripubertal Exposure to Glyphosate-Based Herbicides Promotes Histopathological Impairment in the Structure of the Diaphragm Muscle of C57BL/6 Mice

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SUMMARY

Introduction. Glyphosate is an organophosphate herbicide most used in Latin America, with multisystemic effects, including the respiratory system. In this sense, the objective of this research was to analyze the muscle fibers and neuromuscular junctions (NMJs) of the diaphragm muscle of adult mice exposed to the glyphosate-based herbicide in the peripubertal period.

Methods. Twelve male mice were used, divided into a control group (CTL, n = 6), which received water and a glyphosate-based herbicide group (GBH, n = 6), which received 50 mg/Kg/day of Roundup®, both by gavage from 30 to 60 days. At 150 days, the animals were euthanized, and the diaphragm was collected for analysis of the muscle fibers through hematoxylin-eosin, Masson’s trichrome and Picrossirius Red and the NMJs through the nonspecific esterases reaction.

Results. Rounded fibers, hypereosinophilic sarcoplasm and enlarged nuclei were found predominantly in GBH. A reduction in body weight, an increase in muscle fiber morphometry, an increase in type III collagen, a decrease in the overlapping of type I and III collagen, and an increase in the area and greater diameter of the NMJs in GBH were observed.

Conclusions. Peripubertal exposure to glyphosate-based herbicides showed morphological changes characteristic of muscle degeneration and altered the morphometry of muscle fibers and NMJs of the diaphragm of adult mice.

KEY WORDS
DOHaD; morphology; neuromuscular junction; pesticide; skeletal muscle.
INTRODUCTION

Glyphosate or N-(phosphonomethyl)glycine is an organophosphate pesticide, with systemic, non-selective and broad-spectrum action (1, 2), is the main active ingredient contained in Roundup® (3). Glyphosate-based herbicides are widely used worldwide (4), especially in South America, where it is the most commercialized herbicide in Brazil, reaching approximately 63% of the pesticide market in 2020 (5). In this sense, its exacerbated use in agriculture has left residues in soil, water and food (1).

Contact with pesticides can occur through mucous membranes, skin, breathing or ingestion (6) and this exposure can trigger different respiratory problems (7). The impairment of the respiratory muscles, caused by organophosphate intoxication, possibly contributes to the high morbidity rate in intoxications (8). The association of exposure to GBH, and other organophosphates, to respiratory changes has received attention due to the increased incidence of respiratory problems in regions of exposure to pesticides (7, 9).

GBH has a potential endocrine disruptor effect (10), with exogenous substances altering the functions of the endocrine system and, consequently, causing deleterious effects on the health of the organism or its offspring (11). Exposure to these xenobiotics during critical periods of development and maturation, such as fetal, perinatal and puberty, may be related to changes in gene expression patterns since it is during these periods that the organism establishes the main epigenetic patterns (12, 13). Since the consequences of this early exposure to glyphosate-based herbicides may only manifest in later stages of development and/or in adulthood (14).

Puberty is the period of childhood growth that precedes the onset of adolescence (15), it is a process of physical growth, emotional development and great brain plasticity (15). At this stage, there are structural and functional changes that can interfere with metabolism in adulthood (16), thus being a modulation window, where changes propitiated by the environment can lead to damage to normal development and affect the health of the individual, as is the case of exposure to pesticides. The diaphragm is considered the main muscle of the mammalian respiratory system (17). It is innervated by the phrenic nerves (18) and is composed of different types of fibers, therefore it is classified as a mixed muscle (19). It has some peculiarities such as greater resistance to fatigue, greater blood flow, greater oxidative capacity and greater capillary density. These properties allow for the necessary resistance to high-demand activities throughout life (20).

Due to its association with respiratory capacity, the diaphragm muscle was chosen for this study, where we sought to evaluate the structure of the muscle after chronic exposure to GBH. Thus, the objective of this research was to analyze the histomorphometric and histopathological characteristics of the muscle fibers and the structure of the neuromuscular junctions (NMJs) of the diaphragm of adult mice exposed to GBH during the peripubertal period.

MATERIALS AND METHODS

Ethical approval

The Ethics Committee for Animal Use from the #05/2018 (CEUA - UNIOESTE) approved all the procedures involving animals (date of approval May, 2018). The procedures were in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the Conselho Nacional de Controle de Experimentação Animal (CONCEA) and with the international guidelines for animal research (21).

Animals

For the present study, 12 male mice of the C57BL/6 lineage, 21 days old and with a body weight of 20 ± 2 g, were used. The animals were acquired from the central vivarium of the UNIOESTE and were kept in the sectoral vivarium of the Laboratory of Endocrine Physiology and Metabolism - LAFEM, under controlled conditions of temperature (28 ± 2 °C) and luminosity (12 hours light/dark). The animals were randomly divided into two groups, with only two offspring from the same mother allocated to the same group: the control group (CTL, n = 6) and the glyphosate-based herbicide group (GBH, n = 6). All mice received water ad libitum and were fed a Nuvilab® regular chow (Quintia S.A., Colombo, Paraná, Brazil) from weaning until 150 days of age.

Exposure to GBH

From 30 to 60 days of life, daily by gavage, the GBH group received 50 mg/Kg/day of (22) Roundup® Original DI solution (Monsanto, Brazil), containing 445 g/L of diammonium salt of N-phosphonomethylglycine (37.0% m/v), of the active component glyphosate and the CTL group received water. The GBH solution and the water had the pH corrected between 5 and 5.5 with hydrochloric acid.

Euthanasia and tissue collection

After 150 days of life, the animals were weighed and measured to obtain the nasoanal length. Then, the mice were anaesthetized with a mixture of 9 mg/Kg xylazine hydrochloride (Anasedan®, Vetbrands, Brazil) and 90 mg/Kg ketamine hydrochloride (Dopalen®, Vetbrands, Brazil). Then, laparotomy was performed to collect the organs. Retroperitoneal and peritoneal fat were collected and weighed and standardized by body weight.
For the collection of the diaphragm muscle, the animals were kept in a ventral position and an incision was made in the median region just below the thorax, with subsequent reflection of the skin and muscles. The removal of the diaphragm consisted only of its costal part, being divided into right and left hemicupules. Only the right hemicupula was weighed. Then, each hemicupula was fragmented into two segments, and in the right hemicupules, the upper and lower fragments were destined for histological processing in paraffin. In the left hemicupula, the upper and lower segments were destined for histochemical study of the NMJs. Then, the fragments were stored in specific fixatives to carry out the histological and histochemical study.

**Histological procedures**

For the histomorphological analysis of the diaphragm muscle, cross sections of the proximal portions of the right cupula were fixed in Methacarn (60% Methanol, 30% Chloroform and 10% Glacial Acetic Acid), and then destined for histological processing for embedding in paraffin. Subsequently, 7 μm-thick cross-sections of the muscles were obtained, which were collected on glass slides, and used for histomorphological and histopathological analysis. One slide from each animal was stained using Hematoxylin and Eosin and destined for histomorphometric analysis. The other two slides were used for the analysis of the connective tissue, one stained with the Masson’s Trichrome technique, and the other stained with the Picrosirius Red technique.

**Histochemical procedures**

For the morphological and morphometric analysis of the NMJs, the middle portions of the left antimeres were longitudinally sectioned with the aid of a stainless-steel blade to obtain several muscle fragments. These fragments were submitted to the Unspecific Esterase reaction and after the reaction mounted on a glass slide for analysis under light microscopy.

**Images obtention**

To acquire images for subsequent morphometric analysis, the slides stained by the picrosirius red technique were photographed in a polarized light microscope (Carl Zeiss™ AxioImager™). The slides obtained from the other techniques were photographed under a light microscope (Carl Zeiss™ Primo Star™). Both microscopes were coupled to the camera (Carl Zeiss™ AxioCam ERc 5s) and connected to the ZEN 3.1 program (Carl Zeiss™). According to the magnification used for the analyses, the following were used: 2 microscopic fields, for 40x magnification; 5 microscopic fields for 100x magnification; 10 microscopic fields for 200x magnification and 20 microscopic fields for 400x magnification. For image analysis, Image-Pro Plus 6.0® software (Media Cybernetics, MD, Rockville, USA) was used. For the analysis of connective tissue, collagen, lipid deposits and immunohistochemistry, pixel quantification was used, using the GNU - GIMP 2.10.30 program (GNU General Public License®, Berkeley, California, United States).

**Histomorphometric measurements**

For histomorphometric analysis, images containing whole muscle fibers were used and the relative area of each image was measured. Fibers, nuclei and capillaries were counted. From these data, it was calculated: fiber density (DENS = amount fibers per mm²), the ratio of capillaries per fiber (ratio CF = amount of capillaries/amount of fibers), the ratio of nucleus per fiber (ratio NF = amount of nucleus/amount of fibers) and percentage of the central nucleus (NC%). The cross-sectional areas (CSA) of muscle fibers (n = 150 events per animal) were also measured, as well as the largest (LD) and smallest (SD) diameters of muscle fibers (n = 150 per animal). These data were used to calculate the myonuclear domain (muscle fiber CSA/NF ratio).

The quantification of connective tissue was performed on slides stained with Masson and based on this total amount of connective tissue, the value for each envelope (epimysium, perimysium and endomysium) was calculated. Collagen quantification was performed similarly, quantifying the pixels referring to type I collagen (in red) and type III collagen (in green) and the overlapping areas of both (in yellow). To measure the NMJs, the CSA, LD and SD were measured in 150 neuromuscular junctions. The diameter ratio (LD/SD) was calculated to verify the possible roundness of the NMJs, and the Ratio of CSA Fiber to CSA NMJ.

**Histopathological Index Analysis**

For the characterization of the structural alterations of the muscular tissue, five patterns of reaction (23) were used. Based on the previously described reaction patterns, the slides were evaluated blindly, classifying the reaction patterns and their extensions in the sampling areas. The results of each evaluator were used to construct the index. For the quantitative evaluation of the histopathology, two values were used: 1) importance factor (w), previously tabulated and which takes into account the pathological importance of the alteration: 1 – Minimal importance, 2 – Moderate importance, 3 – Great importance; 2) score value (a), which varies according to the extent of the alteration, being: (0) none, (2) minimal, (4) moderate and (6) extensive occurrence. From these values, the mathematical calculation for the injury rates was developed, which can be used both for the quantification of the reaction patterns and for the total evaluation of the tissue.
Statistical analysis
Normality (Shapiro-Wilk test) and homoscedasticity tests (Barttlet’s test) were performed for all variables and those that were following these assumptions were analyzed by the Student’s t-test to examine the effect of glyphosate. When the premises were not in agreement, the Mann-Whitney U test was performed. All analyses were performed with alpha significance level $\alpha = 0.05$.

Then, the matrices of the variables were standardized and analyzed using the principal component analysis (PCA). With the PCA, factorial loads are established for each variable and analyzed in response components. The data provided by the PCA is reduced, the data overlays are removed, and the most representative linear units of the data are known. The factor loads of the main components were evaluated in terms of statistical significance using a student t-test. As the main components (PC) are ordered in decreasing order of importance for a structure of variance of the data set, the greater the retention of the total variance in a smaller number of linear formulas, the better the application of the procedure to the experimental data. All procedures were performed in software R version 4.3.0.

RESULTS

Body characteristics
Regarding body characteristics (table I), GBH animals showed a reduction of approximately 9% in body weight, when compared to CTL ($t = 2.9216$, df = 6.943, $P$-value = 0.0225). However, there was no difference between groups in nasaonal length ($t = 0.8613$, df = 6.7846, $P$-value = 0.4185), Lee’s index ($t = 1.5126$, df = 8.1398, $P$-value = 0.1682), retroperitoneal ($t = -0.43171$, df = 9.892, $P$-value = 0.6752) and peritoneal (W = 13, $P$-value = 0.4848) fat weight, body adiposity (W = 14, $P$-value = 0.5887) and diaphragm muscle ($t = -0.16552$, df = 9.2047, $P$-value = 0.8721) weight.

Table I. Body characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CTL</th>
<th>GBH</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>30.81 ± 1.04</td>
<td>27.75 ± 2.33$^A$</td>
<td>0.0225</td>
</tr>
<tr>
<td>Nasoanal Length (cm)</td>
<td>9.11 ± 0.43</td>
<td>8.95 ± 0.18</td>
<td>0.4185</td>
</tr>
<tr>
<td>Lee Index (g/cm³)</td>
<td>342.13 ± 12.61</td>
<td>333.08 ± 7.49</td>
<td>0.1682</td>
</tr>
<tr>
<td>Retroperitoneal (g/100 g)</td>
<td>0.24 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>0.6752</td>
</tr>
<tr>
<td>Perigonadal (g/100 g)</td>
<td>1.01 ± 0.18</td>
<td>1.25 ± 0.49</td>
<td>0.4848</td>
</tr>
<tr>
<td>Adiposity (%)</td>
<td>1.24 ± 0.20</td>
<td>1.51 ± 0.51</td>
<td>0.5887</td>
</tr>
<tr>
<td>Diaphragm (g/100 g)</td>
<td>0.15 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.8721</td>
</tr>
</tbody>
</table>

CTL: Control group (n = 6); GBH: Glyphosate group (n = 6); A: statistical difference versus control group ($p < 0.05$).

Diaphragm muscle morphology
The morphological characteristics of the diaphragm muscle were standard (figure 1). The morphology of the radial sections of the right hemicupule showed elongated and multinucleated polygonal fibers with nuclei displaced immediately below the sarcoplasmic membrane (figure 1A-H). The muscle fibers are individually enveloped by the connective tissue of the endomysium, organized into muscle fascicles delimited by the perimysium, with blood capillaries associated with these connective envelopes. However, fibers with increased nuclei and some with a basophilic halo were observed in greater numbers in the GBH group (figure 1D,F). Central nuclei were also found in both groups equally and fibers with a rounded shape and hypereosinophilic sarcoplasm were found in the CTL and GBH groups, but predominantly in the GBH group (figure 1G,H). It was also possible to observe an increase in type I

Figure 1. Photomicrographs of the diaphragm muscle of 150-day-old mice.

collagen deposition in GBH animals, with a greater predominance of reddish colouration (figure 1L). The NMJs found in the diaphragm muscle of the animals were polymorphic, with an oval, round or elliptical shape, however, in the GBH animals it was possible to notice an increase in junctions in elliptical and oval shapes (figure 1I, J).

**Diaphragm muscle fibers’ structure**

Regarding the structure of the muscle fibers (table II), it was possible to observe that the GBH animals presented a reduction of approximately 9% in fiber density (t = 5.8472, df = 9.4036, P-value = 0.0002), 25% in the ratio of nuclei per fiber (t = 5.9281, df = 5.8347, P-value = 0.0011), 37% in the percentage of central nuclei (t = 5.0659, df = 5.4176, P-value = 0.0031) and 32% in the ratio of capillaries to fiber (t = 5.1561, df = 7.4976, P-value = 0.0011). However, the GBH animals showed an increase of 15% in the cross-sectional area (t = -5.0021, df = 9.9462, P-value = 0.0005), 10% in the largest diameter (t = -6.0698, df = 8.5282, P-value = 0.0002) and 7% in the smallest diameter (t = -3.9581, df = 9.3541, P-value = 0.0031) of the muscle fibers when compared to the CTL, however, there was no difference between the groups in the ratio of these diameters (t = -2.1834, df = 5.7764, P-value = 0.0734). Finally, the GBH animals showed an increase of approximately 60% in the myonuclear domain (W = 0, P-value = 0.0021) when compared to the CTL animals.

**Extracellular matrix composition and neuromuscular junction**

Regarding the characteristics of the extracellular matrix (table III), it was possible to observe that the GBH animals presented a 43% increase in the proportion of type I collagen (W = 0, P-value = 0.0021) when compared to the CTL animals. Conversely, the GBH animals showed a 23% reduction in the overlapping of collagens I and III (W = 36, P-value = 0.0049), when compared to the CTL. However, there was no difference between the groups in the quantification of type III collagen (W = 17, P-value = 0.9372), nor the quantification of total connective tissue (W = 11, P-value = 0.2971).

### Table II. Diaphragm muscle fibers’ structure.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CTL</th>
<th>GBH</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber Density (mm²)</td>
<td>1338.81 ± 27.94</td>
<td>1229.74 ± 36.14^A</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fiber Cross-Sectional Area (µm²)</td>
<td>699.85 ± 44.78</td>
<td>824.67 ± 41.60^A</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fiber Largest Diameter (µm)</td>
<td>35.43 ± 1.41</td>
<td>39.61 ± 0.91^A</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fiber Smallest Diameter (µm)</td>
<td>22.94 ± 0.69</td>
<td>24.62 ± 0.75^A</td>
<td>0.0031</td>
</tr>
<tr>
<td>Diameters Ratio</td>
<td>1.54 ± 0.01</td>
<td>1.61 ± 0.04</td>
<td>0.0734</td>
</tr>
<tr>
<td>Nuclei per Fiber Ratio</td>
<td>1.38 ± 0.14</td>
<td>1.01 ± 0.04^A</td>
<td>0.0011</td>
</tr>
<tr>
<td>Central Nuclei (%)</td>
<td>3.08 ± 0.11</td>
<td>1.94 ± 0.54^A</td>
<td>0.0031</td>
</tr>
<tr>
<td>Mionuclear Domain (µm²) *</td>
<td>507.93 ± 32.79</td>
<td>817.63 ± 54.85^A*</td>
<td>0.0021</td>
</tr>
<tr>
<td>Capilar per Fiber Ratio</td>
<td>0.57 ± 0.08</td>
<td>0.38 ± 0.04^A</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

CTL: Control group (n = 6); GBH: Glyphosate group (n = 6); A: statistical difference versus control group (p < 0.05); *Mann-Whitney Test.

### Table III. Extracellular matrix composition and neuromuscular junction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CTL</th>
<th>GBH</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective Tissue (% pixels)*</td>
<td>2.98 ± 0.97</td>
<td>3.43 ± 0.66</td>
<td>0.2971</td>
</tr>
<tr>
<td>Collagen Type III (% pixels)*</td>
<td>16.53 ± 2.24</td>
<td>16.74 ± 1.25</td>
<td>0.9375</td>
</tr>
<tr>
<td>Collagen Type I (% pixels)*</td>
<td>28.66 ± 3.84</td>
<td>40.54 ± 4.88^A</td>
<td>0.0021</td>
</tr>
<tr>
<td>Collagen Merge (% pixels)*</td>
<td>54.82 ± 2.49</td>
<td>42.72 ± 6.05^A</td>
<td>0.0049</td>
</tr>
<tr>
<td>NMJ Cross-Sectional Area (µm²)</td>
<td>259.93 ± 13.86</td>
<td>346.25 ± 21.07^A</td>
<td>0.0000</td>
</tr>
<tr>
<td>NMJ Largest Diameter (µm)</td>
<td>24.05 ± 1.38</td>
<td>28.30 ± 0.57^A</td>
<td>0.0002</td>
</tr>
<tr>
<td>NMJ Smallest Diameter (µm)</td>
<td>12.35 ± 0.32</td>
<td>14.21 ± 0.68^A</td>
<td>0.0005</td>
</tr>
<tr>
<td>NMJ Diameters Ratio</td>
<td>1.95 ± 0.15</td>
<td>1.99 ± 0.07</td>
<td>0.5489</td>
</tr>
<tr>
<td>CSA Fiber / CSA NMJ Ratio</td>
<td>2.70 ± 0.25</td>
<td>2.39 ± 0.17^A</td>
<td>0.0345</td>
</tr>
</tbody>
</table>

CTL: Control group (n = 6); GBH: Glyphosate group (n = 6); A: statistical difference versus control group (p < 0.05); *Mann-Whitney Test.
Meanwhile, the neuromuscular junctions (table III) showed an increase of approximately 32% in the cross-sectional area (t = -8.3817, df = 8.6472, P-value = 0.0000), 20% in the largest diameter (t = -6.943, df = 6.7036, P-value = 0.0002) and 16% in the smallest diameter (t = -6.0155, df = 7.1036, P-value = 0.0005) in the GBH animals when compared to the CTL. However, there was no difference between groups in the ratio of diameters (t = -0.62626, df = 7.486, P-value = 0.5498), an important predictor of structural alteration of the joints. Finally, the ratio between the cross-sectional area of muscle fibers and neuromuscular junctions (t = 2.4861, df = 9.0216, P-value = 0.0345) showed a reduction of approximately 13% in GBH animals when compared to CTL.

Histopathological index

Regarding the histopathological characteristics (table IV), it was possible to notice that the GBH animals presented scores approximately 300% higher than the CTL animals in the total index of alterations (W = 0, P-value = 0.0048). Similarly, across subdivisions of the index, GBH animals showed a 360% increase in the circulatory and inflammatory disorders (CID) score (W = 0, P-value = 0.0045), a 300% increase in the muscle fiber alterations (MFA) score (W = 0, P-value = 0.0042), a 550% increase in the associated tissue alterations (ATA) score (W = 0.5, P-value = 0.0055), and 110% increase in progressive changes (PC) score (W = 5.5, P-value = 0.0455) when compared to CTL animals.

Principal components analysis

In the case of Principal Components Analysis (figure 2), the multivariate arrangement of the data in significance showed that in only 3 principal components, there was a retention of 93.06% of the variance of the model data. Regarding the First Component (data = Total Index, Muscle Fiber Alterations, Mionuclear Domain, NMJ Cross-Sectional Area and Largest Diameter, Circulatory and Inflammatory Disorders, Nuclei per Fiber Ratio, Fiber Density, Associated Tissues Alterations, Collagen Type I and Collagen Merge; Eigenvalue = 15.05; Retention Variance = 75.27%), the GBH animals showed a positive displacement concerning the CTL, showing that these animals presented greater alterations in the muscle fiber, nuclear alterations, alterations histopathological changes in the characteristics of the neuromuscular junctions and the composition of the extracellular matrix (t = -25.58, df = 9.8254, P-value = 0.0000). For the Second (data = Cross-Sectional Areas Ratio, Progressive Changes; Eigenvalue = 2.09; Retention Variance = 10.90%) and Third (data = NMJ Smallest Diameter, Capillary per Fiber Ratio, Table IV. Histopathological index.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CTL</th>
<th>GBH</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Index (max 320)*</td>
<td>18.00 ± 2.82</td>
<td>71.00 ± 3.03A</td>
<td>0.0048</td>
</tr>
<tr>
<td>CID (max 36)*</td>
<td>3.33 ± 1.63</td>
<td>15.33 ± 2.42A</td>
<td>0.0045</td>
</tr>
<tr>
<td>MFA (max 182)*</td>
<td>10.00 ± 2.19</td>
<td>40.00 ± 1.78A</td>
<td>0.0042</td>
</tr>
<tr>
<td>ATA (max 36)*</td>
<td>1.33 ± 1.63</td>
<td>8.67 ± 3.01A</td>
<td>0.0055</td>
</tr>
<tr>
<td>PC (max 36)*</td>
<td>3.33 ± 2.06</td>
<td>7.00 ± 2.75A</td>
<td>0.0455</td>
</tr>
</tbody>
</table>

CTL: Control group (n = 6); GBH: Glyphosate group (n = 6); circulatory and inflammatory disorders (CID); muscle fiber alterations (MFA); associated tissue alterations (ATA); progressive changes (PC); A: statistical difference versus the control group (p < 0.05). *Mann-Whitney Test.
Central Nuclei, Fiber Cross-Sectional Area, Fiber Largest Diameter, Diameters Ratio; Eigenvalue = 1.01; Retention Variance = 6.89% components, there was no difference between the CTL and GBH groups (t = -0.1642, df = 9.9892, P-value = 0.8728; t = 0.12797, df = 6.7539, P-value = 0.9019, respectively).

DISCUSSION
The present study sought to evaluate the histomorphometric and histopathological changes in the diaphragm muscle of adult animals chronically exposed during the peripubertal period to a dose of 50 mg/Kg/day of glyphosate-based herbicide (GBH). Thus, we observed that the animals exposed to GBH showed a reduction in body weight, without alteration in the other evaluated body characteristics (NAL, LEE, Adiposity, Diaphragm weight). However, in addition to the morphological changes found, the GBH animals showed an increase in the size of muscle fibers (Cross-Sectional Area, Largest and Smallest Diameters), in addition to an increase in the myonuclear domain. On the contrary, they presented a reduction in the density of muscle fibers, in the ratios of nucleus and capillaries per fiber and a reduction in the percentage of central nuclei. There was also an alteration in the components of the extracellular matrix, with a reduction in the overlapping of collagen I and III, due to the increase in the deposition of type I collagen), in addition to a change in its predominant format, contributing to the decrease in the ratio between the areas of muscle fibers and NMJ. Finally, the histopathological analysis showed an increase in total muscle damage in the animals exposed to GBH, due to the increased presence of circulatory and inflammatory disorders, muscle fiber alterations, associated tissue alterations and progressive changes.

Exposure to GBH is associated with changes in the development of animals, especially during critical periods of life, such as during pregnancy (24), perinatal (25) and peripubertal periods (26). Chronic and subchronic exposures to doses of 250 mg/kg/day and 500 mg/kg/day, regardless of the sex of the animals, have been reported to reduce weight gain in animals in the peripubertal period, such as those described in the present study (27, 28). Alterations in the weight gain of the animals may be related to several factors, in particular the reduction in the body's accumulation of lipids, the reduction in the glycogen reserve and the reduction in the synthesis of complex proteins, in this sense an effect on the organism's ability to reserve energy (26). This reduction in reserve energy supply may be related to the large consumption of ATP in the reactions involved in phase I and phase II of xenobiotic detoxification, pathways classically altered in chronic exposure to organophosphates (28, 29). Another factor that may justify the change in weight gain of the animals is the possible endocrine-disrupting effect of this herbicide (1), leading to lower synthesis of steroidal hormones, responsible for the positive modulation effect on the body growth rate during puberty (4, 30).

As one of the main routes of human contamination with GBH is the nasal-oral route, intoxication by this and other organophosphates may be related to respiratory problems, such as cases of respiratory failure (8, 31, 32). One of the main consequences of chronic respiratory failure is the progressive increase in tone and strength of muscle contraction, due to the workload exerted on the muscle (33, 34). In cases of alteration in the diaphragmatic workload, there is a significant variation in the recruitment of motor units, causing muscle hyperactivation (33). In this sense, it is common to find hypertrophy in muscle fibers (35) and in the junctional postsynaptic compartments (36), as found in the present study.

Also, exposure to GBH may be related to functional changes in the cardiovascular system (25, 37). In experimental models of heart failure, after biochemical evaluation of the diaphragm muscle, there was evidence of induction of oxidative distress and morphological and functional changes (38). In this sense, it is possible to associate the diaphragmatic alterations found in the present study as a result of pulmonary congestion, resulting from cardiac mechanical alteration and, consequently, reduction in cardiac output, caused by the use of glyphosate (37).

In addition to changes in fiber size, any change in functional demands can make muscle tissue respond differently, altering its structural, metabolic and functional aspects (39). The increase in myonuclear volume and domain found in animals exposed to GBH may be related to the toxic effect of organophosphates on the tissue, since, as a consequence of the cellular toxicity promoted by GBH, there may be a greater expression of proteins of the antioxidant defense system and repair (40). That way, nuclei with increased volume may indicate an increase in the protein synthesis rate (41), as well as related to the muscle fiber regeneration process (42). However, the reduction in nuclear density in animals exposed to glyphosate impairs this feature. In this sense, the reduction of central nuclei may signal a failure or reduction in the regenerative capacity of the muscle tissue of these animals, since this position of the nuclei is the determining factor for maintaining the health of the tissues since their presence in the muscle fibers indicates correct and coordinated muscle regeneration process (43, 44).

Finally, the connective envelopes are responsible for the structural maintenance of muscle fibers and for allowing the individually generated force of contraction to act on the
entire muscle. It is important to note that several pathological alterations in the muscle are also associated with some degree of thickening of the extracellular matrix, or the collagen composition of this matrix (45). Therefore, due to muscle damage caused by exposure to GBH, there may be an imbalance in the proliferation of growth factors involved in muscle regeneration and mesenchymal growth, including fibroblast growth factor (FGF), which acts by stimulating tissue synthesis. connective tissue, especially in the synthesis of collagen I, which determines the tensile strength and rigidity of the tissue (45). In this sense, a structural alteration, such as an increase in fibrotic tissue, can negatively impact the arrival of muscle growth signalling factors. Although the increase in connective tissue is an indicator of muscle regeneration, collagen overproduction often results in the formation of scar tissue that impairs muscle function (46).

According to the literature, the toxicity of glyphosate is low in subchronic or chronic exposure in adult individuals. However, this herbicide can induce metabolic and structural alterations in critical periods, such as the peripubertal period, a fact that can lead to the emergence of several associated comorbidities (47, 48). In the present study, we could observe that chronic exposure to lower doses than those studied in the literature in the peripubertal period was sufficient to promote changes in muscle fibers that may indicate a change in the mechanism of the functionality of the diaphragm muscle. We also found results that suggest an important histopathological alteration, which together with the nuclear alterations, can translate into a reduction in the muscle repair capacity, which, associated with the alteration of the neuromuscular junctions, can contribute to accentuate these damages throughout the life of these animals.

CONCLUSIONS

Because of the results, it is concluded that peripubertal exposure to GBH generates muscle adaptations that are dictated throughout development, with changes in weight and diaphragmatic muscle tissue being found in adduct mice. GBH led to histomorphological adaptations with hypertrophy of muscle fibers, an increase in the nuclear domain and type I collagen and NMJs, and changes involved in the process of muscle remodelling.

FUNDINGS

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DATA AVAILABILITY

Data from this study can be accessed at: Zazula M, Torrejais MM. GBH diaphragm DOHaD. Mendeley Data. 2023. doi: 10.17632/z77jdscnyc.1.

CONTRIBUTIONS

MFZ, LFCR, MMT: data collection, analysis and interpretation of results, draft manuscript preparation, review of results, final version of the manuscript. APM: data collection, analysis and interpretation of results, draft manuscript preparation, review of results. MCO, AB: data collection, analysis of results. MLB: data collection, analysis and interpretation of results, draft manuscript preparation, review of results.

ACKNOWLEDGEMENTS

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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INTRODUCTION

Subtalar dislocation (SD) is an uncommon type of ankle injury, approximately 1% of total dislocations (1), that involves concomitant loss of normal anatomic relations between talus, navicular and calcaneus bones. It was first described in 1811 by Dufarest and Judey (2) but it was not until 1853 that the first classification was performed (3). In 1856 Malgaigne and Buerguer (4) modified the classification which is used nowadays.

SD is characterized by a simultaneous dislocation of talocalcaneal and talonavicular joints while the tibiotalar and calcaneocuboid ones remain intact (3). Mechanism of subtalar dislocation is trauma to a plantar-flexed foot either in inversion, resulting in medial subtalar joint dislocation (80%), or eversion, resulting in lateral dislocation (17%); while anterior or posterior dislocations are rare (5, 6). The diagnosis of subtalar dislocation is usually made on anteroposterior (AP), lateral, and oblique radiographs of the foot or ankle (7). The nature of the deformity often limits radiographic positioning (7). After reduction, AP and lateral radiographs of the foot as well as AP and mortise views of the ankle are obtained to confirm optimal results (7). In the absence of deformity, post-reduction radiographs are usually of better quality than those obtained at the time of injury and associated fractures become more apparent (2).
The majority of subtalar dislocations are accompanied by fractures of the hindfoot, including osteochondral fractures, calcaneus fractures, and fractures of the posterior process and tubercles of the talus (7). Intra-articular osteochondral fractures are especially common, occurring in 50%-71% of all subtalar dislocations and up to 100% of lateral dislocations (2), the majority of which involve the subtalar joint (8). These fractures are less common in medial dislocations, occurring in only 12%-38% of injuries (2, 9). Associated intra-articular fractures are difficult to identify at plain radiography and their presence can hinder anatomic reduction and worsen the overall prognosis (10). Therefore, routine post-reduction CT has been recommended to detect these fractures more accurately to reduce the risk of disruption of normal bone articulation, arthritis or avascular necrosis (AVN) of talus (11, 12).

METHODS

Eligibility criteria
This study complies with ethical standards. All prospective and retrospective studies reporting subtalar dislocation were accessed. According to the authors’ language capabilities, articles in English, German, Italian, and Chinese were eligible. Only clinical articles of all levels of evidence, according to the Oxford Centre of Evidence-Based Medicine (13), were considered. Reviews, opinions, letters, and editorials were not considered. Animals, biomechanics, computational, and cadaveric studies were not eligible. Missing quantitative data under the outcomes of interests warranted the exclusion of the study.

Search strategy
This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the 2020 PRISMA statement (14). The PICOT algorithm was preliminarily pointed out:
- **P**: Problem;
- **I**: Intervention;
- **C**: Comparison;
- **O**: Outcomes;
- **T**: Timing.

In November 2023, the following databases were accessed: Pubmed, Web of Science, Google Scholar, and Embase. No time constraint was used for the search. The following keywords were used in combination: subtalar dislocation, subtalar joint, injuries, treatment and fractures.

Selection and data collection
Two authors (A.B., S.Z) independently performed the database search. All the resulting titles were screened and if suit-

able, the abstract was accessed. The full text of the abstracts which matched the topic of interest was accessed. The bibli-
ography of the full-text articles was also screened for inclu-
sion of further articles. Disagreements were debated and the final decision was made by a third author (F.O.).

Data items
Two authors (A.B., S.Z.) independently performed data extraction. The following data at baseline were extracted: author, year and journal of publication, length of the follow-up, female ratio, number of patients with related mean age, surgical technique, etiology of injury, and activities limitations. The primary outcome of interest was associated injuries to subtalar dislocation. The secondary outcome of interest was clinical outcomes. Data were extracted in Microsoft Office Excel version 16.72 (Microsoft Corporation, Redmond, USA).

Assessment of the risk of bias
Two authors (A.B., S.Z.) independently performed the methodological quality assessment using the Coleman Methodology Score (CMS) (15). Disagreements were discussed and resolved by consensus. The CMS is a 10-item scale designed to rate the methodological quality of the included studies. These items evaluated study size, mean follow-up, number of surgical approaches, type of study, diagnostic certainty, description of surgical procedure, postoperative rehabilitation, outcome measures, outcome assessment, and selection process. The final score ranges between 0 (poor) and 100 (excellent), with a score of 100 indicating the highest reported study methodological quality. The final score was categorized as excellent (85-100 points), good (70-84 points), fair (50-69 points), and poor (< 50 points).

Synthesis methods
The statistical analysis was performed by a single author (F.M.) following the recommendations highlighted in the Cochrane Handbook for Systematic Reviews of Interventions (16). The software IBM SPSS version 25 was used. For continuous data, the arithmetic mean and standard deviation were used. For dichotomous data, the number of events and observations was evaluated.

RESULTS
The initial literature search resulted in 194 studies. Of them, 70 duplicates were excluded. Another 97 articles were not eligible: not matching the topic (n = 84), focusing on surgical technique (n = 9), type of study (n = 2), and full text not accessible (n = 2). This left 27 articles for inclusion. Seven articles were excluded for lack of quantitative data. The arti-
Injuries Associated to Subtalar Dislocation

Articles included in the quantitative synthesis were 20: 12 retrospective and 8 prospective clinical investigations. The literature search results are shown in figure 1.

Methodological quality assessment
According to CMS, surgical technique, diagnosis, and rehabilitation protocols were generally well described. The study size, the retrospective design and the length of follow-up of the included studies represented the main limitations highlighted by the CMS. Outcome measures, assessment timing, and selection process were mostly clearly defined. The mean methodology score of 55.9 (range: 42 to 71) suggested an overall acceptable quality of the methodological assessment (table I). The values of each study are shown in table II.

Study characteristics and result of individual studies
Data from 178 patients were collected, 24.2% (43 of 178 patients) were female. The mean length of the follow-up was 30.2 ± 22.2 months. The mean age of the patients was 36.9 ± 18.4 years. An open surgical technique was reported in 39.4% (65 of 165). The right side was involved in 62.5% (95 of 152), and the dislocation of the subtalar joint was identified medial in 72.6% (122 of 168). The generalities and demographics of the included studies are shown in table II.

Synthesis of results
9.3% (4 of 43) patients were affected by a posterior and 17.4% (4 of 23) by an anterior dislocation of the subtalar joint. Talus fracture occurs in 18.9% (46 of 243) and fractures of the different os metatarsals were detected in 14.0% (19 of 136). Table III reports the overall results of the associated injuries to a subtalar dislocation.

DISCUSSION
Young, athletic men who have had high-energy trauma, such as a car accident or a fall from a height, are susceptible to subtalar dislocation, which develops early in life (20, 39). Significant clinical deformity can result from SD occur-

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Table I. Coleman Methodology Score (CMS).

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part A: Only one score to be given for each of the 7 sections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study size: number of patients</td>
<td>1.0</td>
<td>1.8</td>
<td>0-4</td>
</tr>
<tr>
<td>Mean follow-up</td>
<td>5.0</td>
<td>3.9</td>
<td>0-10</td>
</tr>
<tr>
<td>Surgical approach</td>
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<td></td>
</tr>
<tr>
<td>Type of study</td>
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<tr>
<td>Descriptions of surgical technique</td>
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<tr>
<td>Description of postoperative rehabilitation</td>
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</tr>
<tr>
<td><strong>Part B: Scores may be given for each option in each of the 3 sections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome criteria</td>
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<td>0.5</td>
<td>6-7</td>
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<tr>
<td>Procedure of assessing outcomes</td>
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<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Description of subject selection process</td>
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---
Table II. Generalities and characteristics of the included studies.

<table>
<thead>
<tr>
<th>Author et al., year</th>
<th>Journal name</th>
<th>Study design</th>
<th>CMS</th>
<th>Follow-up (months)</th>
<th>Open technique</th>
<th>Patients (n)</th>
<th>Mean age</th>
<th>Female (n)</th>
<th>Right</th>
<th>Left</th>
<th>Medial dislocation</th>
<th>Lateral dislocation</th>
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<td>Bak et al., 1991 (17)</td>
<td>Br J Sports Med</td>
<td>Case report</td>
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<td>1</td>
<td>20</td>
<td>0</td>
<td>1</td>
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<td>0</td>
<td>1</td>
<td>0</td>
</tr>
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<td>Benerjee et al., 2017 (18)</td>
<td>Case Rep Orthop</td>
<td>Case report</td>
<td>46</td>
<td>12</td>
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<td>1</td>
<td>52</td>
<td>0</td>
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<td>1</td>
<td>0</td>
<td>1</td>
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<td>Bibbo et al., 2001 (19)</td>
<td>Foot Ankle Int</td>
<td>Retrospective</td>
<td>46</td>
<td>14</td>
<td>1</td>
<td>9</td>
<td>27.8</td>
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<td>8</td>
<td>1</td>
<td>7</td>
<td>2</td>
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<td>Foot Ankle Int</td>
<td>Retrospective</td>
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<td>61.5</td>
<td>7</td>
<td>25</td>
<td>38</td>
<td>6</td>
<td>14</td>
<td>10</td>
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<td>Case report</td>
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<td>Case report</td>
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<td>Retrospective</td>
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<td>122.4</td>
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<td>18</td>
<td>34</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>13</td>
<td>5</td>
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<tr>
<td>Ghani et al., 2014 (26)</td>
<td>Ann R Coll Surg Engl</td>
<td>Case report</td>
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<td>24</td>
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<td>1</td>
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<td>Case report</td>
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<td>Case report</td>
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<td>75.6</td>
<td>2</td>
<td>20</td>
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<td>13</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
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<td>Foot Ankle Int</td>
<td>Case report</td>
<td>52</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>23</td>
<td>0</td>
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<td>0</td>
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<td>Case report</td>
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<td>Merchan et al., 1992 (32)</td>
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<td>66</td>
<td>16</td>
<td>39</td>
<td>35.5</td>
<td>12</td>
<td>31</td>
<td>8</td>
<td>29</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Milenkovic et al., 2006 (33)</td>
<td>Injury Prospective</td>
<td>57</td>
<td>23</td>
<td>11</td>
<td>11</td>
<td>30.4</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>0</td>
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</tr>
<tr>
<td>Ruiz Valdivieso et al., 1996 (34)</td>
<td>Int Orthop</td>
<td>Retrospective</td>
<td>71</td>
<td>94.8</td>
<td>7</td>
<td>19</td>
<td>31.7</td>
<td>7</td>
<td>11</td>
<td>8</td>
<td>16</td>
<td>3</td>
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<tr>
<td>Tucker et al., 1998 (35)</td>
<td>J Foot Ankle Surg</td>
<td>Case report</td>
<td>57</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>70</td>
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<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Veltman et al., 2016 (36)</td>
<td>World J Orthop</td>
<td>Case report</td>
<td>58</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Yglesias et al., 2018 (37)</td>
<td>J Surg Case Rep</td>
<td>Case report</td>
<td>48</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>30</td>
<td>0</td>
<td>1</td>
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<td>0</td>
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<tr>
<td>Zaraa et al., 2017 (38)</td>
<td>J Orthop Case Rep</td>
<td>Case report</td>
<td>62</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>46</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

CMS: Coleman Methodology Score.
Table III. Overall results of the associated injuries to a subtalar dislocation.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Frequency (events/observations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior dislocation</td>
<td>9.3% (4 of 43)</td>
</tr>
<tr>
<td>Anterior dislocation</td>
<td>17.4% (4 of 23)</td>
</tr>
<tr>
<td>Dorsal dislocation of the 4th and 5th</td>
<td>100.0% (1 of 1)</td>
</tr>
<tr>
<td>Talus fracture</td>
<td>18.9% (46 of 243)</td>
</tr>
<tr>
<td>Head of the talus fracture</td>
<td>13.6% (3 of 22)</td>
</tr>
<tr>
<td>Neck of the talus fracture</td>
<td>10.0% (3 of 30)</td>
</tr>
<tr>
<td>Lateral process of the talus fracture</td>
<td>100.0% (1 of 1)</td>
</tr>
<tr>
<td>Posterior process of the talus fracture</td>
<td>12.4% (11 of 89)</td>
</tr>
<tr>
<td>Tubercles of the talus fracture</td>
<td>20.0% (2 of 10)</td>
</tr>
<tr>
<td>Subtalar avulsion</td>
<td>100.0% (1 of 1)</td>
</tr>
<tr>
<td>Avulsion fracture of posteromedial aspect of talus</td>
<td>12.5% (3 of 24)</td>
</tr>
<tr>
<td>Calcaneus fracture</td>
<td>12.9% (12 of 93)</td>
</tr>
<tr>
<td>Os navicular fracture</td>
<td>14.0% (13 of 93)</td>
</tr>
<tr>
<td>Os cuneiform fracture</td>
<td>4.5% (2 of 44)</td>
</tr>
<tr>
<td>Medial os cuneiform fracture</td>
<td>10.0% (1 of 10)</td>
</tr>
<tr>
<td>Cuboid fracture</td>
<td>6.1% (8 of 131)</td>
</tr>
<tr>
<td>Os calcis fracture</td>
<td>10.3% (4 of 39)</td>
</tr>
<tr>
<td>Metatarsal fracture</td>
<td>14.0% (19 of 136)</td>
</tr>
<tr>
<td>Second metatarsal fracture</td>
<td>10.0% (1 of 10)</td>
</tr>
<tr>
<td>Third metatarsal fracture</td>
<td>100.0% (1 of 1)</td>
</tr>
<tr>
<td>Fourth metatarsal fracture</td>
<td>100.0% (1 of 1)</td>
</tr>
<tr>
<td>Fifth metatarsal fracture</td>
<td>12.1% (12 of 99)</td>
</tr>
<tr>
<td>Ankle fracture</td>
<td>60.9% (14 of 23)</td>
</tr>
<tr>
<td>Medial malleolus fracture</td>
<td>14.5% (10 of 69)</td>
</tr>
<tr>
<td>Lateral malleolus fracture</td>
<td>15.0% (6 of 40)</td>
</tr>
<tr>
<td>Tibia and fibula fracture</td>
<td>5.1% (2 of 39)</td>
</tr>
</tbody>
</table>

ring in any direction (21). According to the direction of the foot with respect to the talus, Broca classified this dislocation into three distinct patterns, namely medial, lateral, and posterior, which constitute the initial categorization of SD. In 1855, Malgaigne introduced anterior SD (21). The only factors that affected the result, according to Edmunds et al., are infection: the direction of the dislocation, the existence of fractures, or the length of post-reduction immobilization (23).

In the present systematic review 9.3% of patients were affected by posterior subtalar dislocation and 17.4% by anterior dislocation.

Subtalar dislocations are complicated injuries, and various patient- and injury-related variables frequently influence the course of treatment (18). Numerous bone and tendon injuries, many of which are seen in postreduction computed tomographic scans, have been reported in relation to subtalar dislocations. Camarda et al. stated that the occurrence of concomitant osseous injuries is what determines good clinical outcomes, not the direction of the dislocation (23).

In a retrospective review of nine instances, Bibbo et al. discovered that 100% of patients with extra injuries that were initially missed on plain radiographs were discovered by CT scans (19). The prognosis for open subtalar and total talar dislocations is often worse, with increased risk of osteomyelitis, posttraumatic arthrosis, avascular necrosis, and complicated regional pain syndrome (40). This injury might have resulted from a rapid eversion injury that caused the lateral subtalar dislocation, which was caused by prolonged longitudinal compression and plantar flexion of the foot’s lateral column (18). An inversion force applied to a plantarflexed foot causes a medial dislocation, which causes the tarsus to twist out of the subtalar joint after initially exiting the talonavicular joint. There is a displacement of the calcaneus medially and a dorsal appearance of the talus head. Additionally, Avascular necrosis of the talus occurs in 0-10% of closed subtalar dislocations; open dislocations have substantially higher occurrences, up to 50% (18). Talus necrosis is extremely rare because the two branches that supply the talus – the artery of the tarsal canal and the sinus tarsi arch – are unaffected by dislocation of the subtalar joint (41). Unless there is an irreversible lesion of the posterior tibial artery and the dislocation is quite severe, necrosis does not develop (34).

Significant ligamentous and bone injuries, such as tarsometatarsal dislocations, may be linked to low energy closed subtalar dislocations (18). Bibbo et al. reported that 32% of subtalar dislocations could not be reduced using closed methods. To be more precise, 50% of lateral dislocations and 27% of medial dislocations needed an open reduction (20). According to Bibbo et al., 50% of cases of an irreducible subtalar dislocation were caused by trapped soft tissue structures and bone blocks like the posterior tibial tendon, extensor digitorum muscle, extensor digitorum brevis muscle tendon, and interlocking osteochondral fracture (20). Thus, it is not advised to make repeated attempts to prevent iatrogenic talar fractures and neurovascular problems (21).

It is imperative to reduce an SD as soon as possible to avoid soft tissue problems and neurovascular lesions, as well as to lessen the likelihood of talus avascular necrosis (42). Major kinds of SD dislocation often respond well to closed reduction while under spinal or general anesthesia. Manual traction would be an easy way to accomplish it (42). Maintain-
ing a 90° flexion of the knee can help to release tension in the calf muscle and provide a longitudinal traction from the heel through leg countertraction (23).

The timing of postreduction immobilization should be determined by the patient’s age, as supported by the available literature: 4 weeks for young individuals with strong functional request and 3 weeks for those over 65 or with reduced functional request (21). According to Valdivieso et al., instability that may arise from an overly brief time of immobilization is far more significant than the loss of subtalar joint motion after lengthy treatment in plaster (34).

In the present systematic review, the rate of associated injuries is 14.3%. The most frequent associated injuries are talus and metatarsal fractures respectively 18.9% and 14.0%. This study has several limitations. First, the case report and retrospective design of the most included studies. Moreover, we excluded several studies because most of them did not report quantitative data of associated injuries.

CONCLUSIONS
Subtalar dislocations are rare, high mechanism injuries. SD are associated to numerous bone and tendon injuries. The imaging techniques are of primary importance in the study of SD, a standardized method for the study of SD would avoid unknown lesions. Better-quality future studies are needed to ascertain which associated injuries are more frequent.

FUNDINGS
None.

DATA AVAILABILITY
The datasets generated during and/or analysed during the current study are available throughout the manuscript.

CONTRIBUTIONS
AB, SZ: literature search, data extraction, risk of bias assessment, writing - original draft, writing - review & editing. FM: results interpretation. FO: conceptualization, design, risk of bias assessment, writing - original draft, writing - review & editing.

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

REFERENCES
Injuries Associated to Subtalar Dislocation


SUMMARY

Purpose. Plantar fasciitis (PF) is a degeneration of the plantar fascia. There are different forms of treatment for PF. Conservative treatment stands out as the main method of choice. The aim was to review in the literature the effectiveness of therapeutic exercise (TE) on pain and functionality in PF.

Methods. The search was carried out in the databases: PubMed, Web of Science, Embase and Lilacs, and in the gray literature: Google Scholar, Open Grey, LIVIVO and Brazilian Library of Theses and Dissertations. A Cochrane tool was used to assess the quality of the studies. The primary outcome was pain, and the secondary outcomes were range of motion (ROM) and functionality. The assessments were performed by two reviewers and a third reviewer resolved conflicts. Randomized controlled trials using therapeutic exercise compared with placebo, no treatment, phototherapy, thermotherapy, and electrotherapy. There was no restriction on time or language.

Results. The references of 2,984 studies were identified, 2,832 from major databases and 152 from gray literature. A total of 10 articles were included in this review. Nine studies assessed pain, and all had positive results in decreasing pain levels. All 10 studies used to stretch exercises, but only one used strengthening exercises.

Conclusions. It is concluded that TE, mainly through plantar fascia and triceps sural stretching, is effective in reducing pain and functionality when associated with other therapies in PF.

Study registration. The study is registered with the International Prospective Register of Systematic Review (PROSPERO) under protocol CRD42021296710.

KEY WORDS
Muscle strength; muscle stretching exercises; calcaneus; fascia.

INTRODUCTION

Plantar fasciitis (PF), considered the most common cause of sub-calcaneal pain, was first described in 1812, related to painful sensation in the calcaneus, resulting from degeneration of the plantar fascia. Some reported cases are the result of biomechanical failure that provides tension along the plantar fascia (1). It is a common dysfunction in runners, due to the high loads they exert on the plantar fascia, but other risk factors are also worth mentioning, such as increased plantiflexion, high body mass and high body mass index (2). There are different forms of PF treatments, in patients who do not respond adequately to conservative treatment after 6-12 months, surgical treatment is an option, with release of the plantar fascia, open, percutaneous, or endoscopic fasciotomy, neurectomy or neurolysis of the calcaneal nerve, and even calcaneal osteotomy (3). But conservative treatment stands out as the main method of choice, aiming to reduce the inflammatory process and pain (4, 5), as well as stimulating the remodeling of collagen tissue (6). The use of night splints (7), extracorporeal shock wave treatment (ESWT) (8), steroid injections and platelet-rich plasma (9) are all therapies that have been adopted, as well as modalities offered by physiotherapy, how manual therapy, electrostimulation, laser, ultrasound, cryotherapy, and kinesiotherapy. Kinesiotherapy,
based on the active stretching of the gastrocnemius muscle and plantar fascia, can improve painful symptoms. In addition, high-load strength training is performed to improve the foot functionality index, and thus patient satisfaction with kinesiotherapy (10). However, although kinesiotherapy has been shown to be positive in the treatment of PF, there are a variety of resources that can be used within it to achieve the proposed objectives, and generally research is concerned with using this resource as a control and not as the main form of therapy. Therefore, the aim of this study was to review the literature on the effectiveness of exercise therapy on pain and disability in patients with PF.

MATERIALS AND METHODS

Protocol
This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement.

Eligibility criteria
The acronym PICOS was used to formulate the question focused on this study:
- P: Population (individuals with chronic plantar fasciitis);
- I: Intervention (exercise therapy);
- C: Comparison (placebo, no treatment, phototherapy, thermotherapy, and electrotherapy);
- O: Outcome (pain and functionality);
- S: study design (randomized controlled trials).

Inclusion criteria included: individuals with chronic plantar fasciitis; humans; intervention using kinesiotherapy (stretching and strengthening, home and non-home) alone, compared to another conservative treatment such as phototherapy, thermotherapy and electrotherapy, or combination of more than one of these conservative treatments. Exclusion criteria were individuals who had undergone previous surgical treatment on the foot or lumbosacral spine; metabolic or connective tissue disorders; radiographic evidence of local pathology other than plantar fasciitis. Case studies, systematic reviews, case reports, cohort studies, literature reviews and editorials, as well as animal studies were also excluded.

Sources of information
The initial search was conducted using keywords in the PubMed database, with the Medical Subject Headings (MeSH) medical metadata system, descriptors defined in Health Sciences (DeCS), from the Virtual Health Library (VHL) website and free terms. Individual search strategies were developed for the databases: Pubmed, Embase, The Cochrane Library, Lilacs, Physiotherapy Evidence Database (PEDro) and Web of Science; and in the gray literature: Google scholar, Brazilian Catalog of Theses and Dissertations of CAPES and LIVIVO. In addition, citation searches were conducted. The reference manager software EndNote Web (Thomson Reuters) and Rayyan QCRI were used to collect references and exclude duplicates.

Study selection and data collection process
The study selection process was carried out by 2 reviewers in two phases. Articles were imported from the databases into the Endnote Web reference manager for duplicate removal, automatically and manually. They were then imported into Rayyan, and once again duplicates were removed manually by the first reviewer. In this way, the studies included in Phase 1 were defined for reading of titles and abstracts, according to the eligibility criteria by two blinded reviewers R1 (R.A.S) and R2 (B.T.). For studies with conflicts, a third blinded reviewer R3 (G.R.F.B.) performed the tiebreaker. Final selection, Phase 2 was based on the assessment of the full text of the studies by the two blinded reviewers.

Data collected
The main data collected were study characteristics (authors and year of publication), sample characteristics (sample size and mean age), intervention, outcomes and conclusion. The primary outcome was pain, and the secondary outcomes were functionality and range of motion (ROM).

Individual assessment of risk of bias in studies
Assessment of risk of bias was performed using the Cochrane tool, RoB 2, and carried out by the two blinded reviewers, and when necessary R3 was triggered. All included studies were assessed in the following domains: randomization process, timing of participant recruitment identification, deviations from intended interventions, lack of outcome data, outcome measurement, selection of reporting outcome, and overall bias. Each domain with an overall outcome: low risk, unclear or high risk.

Strategy for data synthesis
The results of clinically and statistically homogeneous studies were meta-analyzed using Review Manager (RevMan) software. Meta-analysis was performed using the inverse variance method for continuous outcomes. A random effects model was used.

Publication bias
To reduce the likelihood of publication bias, an exhaustive search was conducted without limitations on language, time period and with inclusion of gray literature (11). Therefore, the risk of publication bias could be mitigated but not eliminated.
RESULTS
This is followed by a narrative synthesis of the results of the included studies, structured around the reported outcomes. This is followed by a quantitative analysis.

Selection of studies
The references of 2,984 studies were identified, 2,832 from major databases and 152 from gray literature. All searches were conducted on a single day, November 22, 2021 (appendix 1) and were updated on August 12, 2023. After removing duplicates, 1,775 articles were left for Phase 1 (reading titles and abstracts) according to the eligibility criteria, and for Phase 2, 19 articles for reading the full texts. The articles included in Phase 2 that did not fit the eligibility criteria were then excluded due to: study design and interventions without isolated kinesiotherapy group. Ultimately, 10 studies were included in this review. Figure 1 summarizes the full selection process, and the summarization of studies is described in table I.

Characteristics of the studies
Ten randomized controlled trials (12-21) were included in this review, with publication date between 2009 and 2020.

Risk of bias analysis
In the individual assessment of risk of bias, it was observed that the high risks of bias were in the domains of outcome measurement (12, 13, 17, 21) and report outcome selection (13, 14, 17-19). Only three studies had overall bias at low risk (15, 16, 20). All others had high risk of overall bias (12-14, 17-19, 21) (figures 2,3).

![Figure 1](image1.png)
Figure 1. Flowchart of literature search and selection criteria, based on PRISMA 2020.

![Figure 2](image2.png)
Figure 2. Risk of bias summary: review authors’ judgements about each risk of bias item for each included study.

![Figure 3](image3.png)
Figure 3. Risk of bias graph: review authors’ judgements about each risk of bias item presented as percentages across all included studies.
Collection instruments
The following instruments were used to measure the main outcome (pain): visual analog scale (VAS), pain subscale-Foot Function Index (pain-FFI) and Numerical Rating Scale for pain (NRS-p) (12-21). The American Orthopedic Foot and Ankle Society Score (AOFAS) and the Activities of Daily Living subscale of the Foot and Ankle Ability Measure (ADL/FAAM) (7-11, 13-15) were used as secondary outcomes for functionality and ROM (13-17, 19-21).

Primary outcome: pain
A total of 9 included studies assessed pain level, all of which found results of decreased pain levels. The studies by Akinoglu et al. (17), Cinar et al. (15, 16), Chew et al. (19) and Mohamed et al. (12) found significant differences between the groups. Despite also reporting positive results on pain, Abdoli and Roohi (13), Akinoglu and Köse (18), Stratton et al. (21) and Rompe et al. (20) found no statistical differences in the intergroup analysis.

ESWT was used in 7 studies (12-14, 17-20) to evaluate its effects on pain compared to a stretching protocol (SP). The application of ESWT can be radial (ESWTr) or focal (ESWTf), the studies that used ESWTr were Akinoglu et al. (17), Akinoglu and Bose (18), Mohamed et al. (12) and Rompe et al. (20). ESWT therapy, regardless of the form of application, was shown to be effective in reducing pain in patients with PF, but did not provide an additional benefit beyond the benefits promoted by SP. In addition, Rompe et al. (20) and Mohamed et al. (12) compared the effects of ESWT and TE alone and in this case, TE was more effective in reducing pain than ESWT.

The use of low-level laser therapy (LLLT) was investigated in the study by Cinar et al. (16), and they observed that the association of LLLT with ET, the results in pain reduction were potentiated. In the case of Cinar et al. (15), it was also shown that the group receiving LLLT and SP had greater pain reduction compared to the groups of ESWT and SP, and SP alone. Regarding the use of low-frequency electrical stimulation (LFES), Stratton et al. (21) found no difference between the groups that associated SP with electrostimulation and those that were limited to SP alone.

Secondary outcomes: functionality and ROM
In the study by Abdoli & Roohi (13) both ESWT and SP were effective in improving ROM, although no significant differences were found between the groups. Additionally, Chew et al. (19) compared ESWTr combined with SP and SP alone and concluded that ESWTr associated with SP show superior functional improvement compared to home exercises alone. Cinar et al. (16) found no differences between the LLLT groups associated with SP and SP alone in the outcome of functionality. Similarly, Cinar et al. (14) found no additional benefit in functionality to the groups that received ESWT treatment in addition to SP. Finally, Stratton et al. (21) used LFES, also with the aim of assessing functionality, and both groups showed improvement, with no differences between them.

Meta-analysis
Therapeutic exercise vs ET + other therapy - VAS (pain)
Short term - 3 to 4 weeks
The studies by Akinoglu and Köse (18), Cinar et al. (16) and Stratton et al. (21) were included in the meta-analysis of the VAS pain endpoint, comparing ET with ET + other therapy in the short term (3 and 4 weeks) (figure 4), with a total sample of one hundred and eighty-five (n = 185) subjects (MD = 1.62; 95% CI

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Forest plot of comparison therapeutic exercise vs TE + other therapy (short and medium term).

Outcome: VAS (pain). (1) VAS - Morning pain (4 weeks) – US; (2) VAS - Pain before bedtime (4 weeks) – US; (3) VAS - Pain after 6MWT (4 weeks) – US; (4) VAS - Pain after experiment (3 weeks) – LLLTI; (5) VAS - Pain after experiment (4 weeks) – EEBF; (6) VAS - Pain after experiment (3 months) – LLLTI; (7) VAS - Pain after experiment (3 months) – EEBF.
0.80-2.44; p = 0.0001). The study by Akınoğlu and Köse (18) used as comparator TE + US in 4 weeks and at three different times, pain in the morning, pain before bedtime and pain after the 6-minute walk test. Cinar et al. (16) used as comparator TE + LLLT in 3 weeks. And Stratton et al. (21) used as comparator TE + EEBF in 4 weeks.

Medium term - 2 to 3 months
The studies by Cinar et al. (16) and Stratton et al. (21) were included in the meta-analysis of the VAS pain endpoint, comparing TE with TE + other therapy at mid-term (3 months) (figure 4), with a sample size of 77 subjects (MD = 1.28; 95%CI -0.14 to 2.70); p = 0.08).

The pooled effect estimates between the short- and medium-term studies (MD = 1.51; 95%CI 0.86-2.17); p < 0.00001) showed that TE versus TE combined with other therapy (US, LLLT and LFES) had a large effect (1.51) in decreasing pain in plantar fasciitis. Thus, being favorable to the TE group. When heterogeneity was analyzed by the degree of inconsistency between the results of the studies (I² = 0%), heterogeneity was not important (22, 23).

TE vs TE + ESWT - short term - VAS (pain)
The studies of Akınoğlu and Köse (18) and Mohamed et al. (12) were included in the meta-analysis of VAS-measured pain outcome comparing therapeutic exercise with TE + ESWT at short term (3 and 4 weeks) (figure 5), with a total sample of 138 subjects, pain was measured at different time points: morning pain, pain before bedtime, pain after 6MWT and pain after experiment.

The pooled estimate of effect across studies (MD = 1.62; 95% CI -0.17 to 3.41; p = 0.08) demonstrated that TE versus TE + ESWT had no advantageous effect on pain reduction. When heterogeneity was analyzed by the degree of inconsistency between study results (I² = 75%), heterogeneity was considerable (22, 23).

TE vs TE + Other therapy - FFI (pain) - short and medium term
The studies of Akınoğlu et al. (17), Cinar et al. (15) and Rompe et al. (20) were included in the meta-analysis of the pain outcome measured by FFI, comparing TE with TE + other therapy (ESWT, US or LLLT) in the short term (3 days to 3 weeks) and medium term (2 to 5 months), with a total sample of 544 subjects (figure 6).

Pooling the effect estimate across studies (MD = -3.20; 95% CI -8.97 to 2.56; p = 0.29) showed no advantage for TE vs TE + other therapy.

TE vs TE + other therapy - Functionality - short term
The studies by Akınoğlu et al. (17) and Cinar et al. (15, 16) were included in the meta-analysis of the functional endpoint comparing ET vs ET + other therapy in the short term (3 days and 3 weeks) (figure 7), with a total sample 129 subjects.

Pooling the effect estimate across studies (MD = -3.81; 95% CI -10.48 to 2.86); p = 0.26), showed that ET vs ET + other short-term therapy had a small effect (-3.81) on functionality in PF. When heterogeneity was analyzed by the degree of inconsistency between study results (I² =87%), heterogeneity was considerable (22, 23).

DISCUSSION
The initial hypothesis of this work was that kinesiotherapy, in the form of TE, is effective in the treatment of PF, and that different forms of application can promote relief of pain symptoms and disability. From this, the aim was to review the effectiveness of exercise therapy in plantar fasciitis showing some relevant results for clinical practice. All studies included in this review included stretching programs for plantar fascia and triceps suralis (12-21), however, only Mohamed et al. (12) used strengthening of intrinsic foot musculature in addition to the stretching program. Individuals with PF usually present with a history of progressive inferior and medial heel pain, but this may radiate proximally in more severe cases. Pain tends to be accentuated on passive dorsiflexion of the toes, and is worse when patients stand up after rest, usually in the early morning (18). In addition, pain may be exacerbated in activities requiring prolonged orthostasis (5, 24, 25). Plantar fasciitis can be a benign disease, but if not treated properly it can become disabling (5, 24, 25), which explains the choice of the secondary outcomes of this review, functionality, and ROM. It is known that the plantar fascia has the function of stabilizing the longitudinal arch, a passive mechanism that generates the ligament and bone stability of the foot. Most cases of PF are the result of a biomechanical failure that results in tension along the plantar fascia (26). Thus, it is believed that by strengthening the intrinsic musculature there may be a reduction in...
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<tr>
<td>Abdoli and Roohi, 2019</td>
<td>ECR</td>
<td>n = 30; G1: ESWT (n = 15); G2: TE (n = 15); Male athletes with PF. Average age 25 years.</td>
<td>ESWT: frequency 12 Hz to 15 Hz and 2500 pulses in 2 to 3 bar (once a week for 5 weeks). TE: stretching of the plantar fascia, Achilles tendon and triceps suralis (3 times a day, 10 stretches of 20 seconds each, for 5 weeks).</td>
<td>T0: baseline</td>
<td>VAS (0-10)</td>
<td>T0: VAS = 6.37 T1: VAS = 2.77 P-value between groups &gt; 0.05</td>
<td>It was concluded that both treatment by ESWT and TE are suitable methods to improve symptoms in PF, with no significant difference between them.</td>
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<td>Akinoglu et al., 2017</td>
<td>ECR</td>
<td>n = 54; G1: r-ESWT + TE (n = 18); G2: US + TE; (n = 18); G3: TE (n = 18); Women with unilateral PF. Mean age 48.44 years.</td>
<td>r-ESWT: 500 pulses. 3Hz. 0.2mJ/mm² in the heel area. and a dose of 1,500 pulses. 8Hz. 0.3mJ/mm² in the palpated tender point (1/week/for 3 weeks/total 3). US: technique with longitudinal movements through the plantar fascia. 3.0 MHz frequency. 1W/cm² intensity and ¼ pulsed wave duty cycle (2x/week/total 07 sessions). TE: stretches for triceps sural, plantar fascia and Achilles tendon (4 times per week. 10 times in the morning and 10 in the evening. 30 seconds each). All groups performed TE.</td>
<td>T0: baseline T1a: 1 week after r-ESWT treatment for the r-ESWT group. T1b: 3 days after US treatment for the US group. T1c: 4 weeks after onset in the TE group.</td>
<td>Pain-FFI (0-100)</td>
<td>T0 (mean ± SD, p-value): r-ESWT + TE = 62.94 ± 9.0 US + TE = 59.06 ± 11.86 TE = 54.61 ± 13.17 T1a (r-ESWT + TE = 43.28 ± 18.52 (0.001) US + TE = 28.56 ± 12.44 (0.001) TE = 38.89 ± 16.52 (0.004)</td>
<td>All interventions tested led to a decrease in symptoms, with US resulting in the greatest contribution.</td>
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<td>Disability-FFI (0-90)</td>
<td>T0: r-ESWT + TE = 75.61 ± 19.05 US + TE = 74.61 ± 18.78 TE = 63.06 ± 17.64 T1: r-ESWT + TE = 43.28 ± 18.52 (0.001) US + TE = 28.56 ± 12.44 (0.001) TE = 38.89 ± 16.52 (0.004)</td>
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<td>Activity limitation – FFI (0-50)</td>
<td>T0: r-ESWT + TE = 20.61 ± 6.48 US + TE = 20.61 ± 9.52 TE = 17.28 ± 8.57 T1: r-ESWT + TE = 8.83 ± 7.02 (0.001) US + TE = 4.28 ± 4.53 (0.001) TE = 11.89 ± 8.61 (0.049)</td>
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<td>AOFAS (0-100) Backfoot score</td>
<td>T0: r-ESWT + TE = 30.11 ± 12.49 (12.49) US + TE = 33.94 ± 14.02 TE = 37.50 ± 15.88 T1: r-ESWT + TE = 74.72 ± 13.55 (0.001) US + TE = 68.39 ± 12.91 (0.001) TE = 59.50 ± 9.34 (0.001)</td>
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<td>Akınoğlu and Köse, 2018</td>
<td>ECR</td>
<td>n = 54;  G1: r-ESWT + TE (n = 18); G2: US + TE (n = 18); G3 = TE (n = 18); Women with unilateral PF; Mean age 48.44 years.</td>
<td>r-ESWT: 1,500 pulses on the 5 most painful points, 0.3 mJ/mm², 8 Hz of 300 pulses were applied on the whole heel (3 times a week for one week); US: 3.0 MHz frequency and with 20% intermittently with 1 W/cm² power for 8 minute (7 sessions, 2 days per week); TE: stretching of Achilles tendon, sural triceps and plantar fascia. TE was applied in the 3 groups.</td>
<td>T0: baseline T1: 4 weeks</td>
<td>VAS - morning pain (0-10)</td>
<td>T0 (mean±SD, p-value): r-ESWT + TE = 9.33 ± 1.41 US + TE = 9.50 ± 1.29 TE = 8.50 ± 2.55 T1: r-ESWT + TE = 4.94 ± 4.12 (0.001) US + TE = 3.72 ± 2.47 (0.001) TE = 5.61 ± 5.38 (0.004)</td>
<td>It concluded that r-ESWT, US and TE are effective in reducing pain in patients with PF with no statistical difference between them.</td>
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<td>VAS – before bedtime (0-10)</td>
<td>T0: r-ESWT + TE = 9.00 ± 1.75 US + TE = 8.83 ± 1.86 TE = 9.06 ± 1.80 T1: r-ESWT + TE = 5.94 ± 3.64 (0.005) US + TE = 3.89 ± 2.35 (0.001) TE = 6.39 ± 2.75 (0.005)</td>
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<td>VAS – after 6MWT (0-10)</td>
<td>T0: r-ESWT + TE = 3.78 ± 3.44 US + TE = 8.17 ± 2.18 TE = 7.39 ± 1.97 T1: r-ESWT + TE = 3.78 ± 3.44 (0.001) US + TE = 3.17 ± 3.37 (0.001) TE = 4.83 ± 2.62 (0.002)</td>
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<td>Cinar; Saxena; Uygur, 2018a</td>
<td>ECR</td>
<td>n = 66; G1: ESWT + TE (n = 25); G2: LLLT + TE (n = 24); G3: TE (n = 17); Women (n = 56), Men (n = 19); Mean age 45.3 years.</td>
<td>ESWT: total pulse dose 1000mJ/mm² per attempt applied to the area of sensitivity; and 1000mJ/mm² over the plantar fascia (once a week, for 3 weeks). LLLT: 830nm with continuous output of 70mW, with dose from 0 to 56J/cm², each single exposure lasting 50 seconds was applied over the plantar fascia and at the points of sensitivity. (3 times per week totaling 10 sessions). TE: stretching of the gastrocnemius muscle and plantar fascia (3 times a day, for 30 seconds each, for 3 weeks. at home) and use of silicone insoles for 3 months.</td>
<td>T0: baseline T1: 3 weeks T2: 3 weeks</td>
<td>Pain-FFI (0-50)</td>
<td>T0 (mean / CI): ESWT + TE = 41.8/39.8 – 44.1 LLLT + TE = 38.3/35.1 – 41.5 P-value between groups (0.002) When LLLT and ESWT were combined with TE, LLLT was more effective than ESWT in reducing PF pain.</td>
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<td>T1: ESWT + TE = 33.2 / 28.0 – 38.3 LLLT + TE = 22.0 / 18.2 – 25.7 TE = 23.2 / 19.0 – 3.6</td>
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<td>NRS-p (0-10)</td>
<td>T0: ESWT + TE = 6.7 / 5.6 – 7.8 LLLT + TE = 6.3 / 5.7 – 6.9 TE = 6.2 / 5.1 – 7.3</td>
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<td>T1: ESWT + TE = 5.0 / 3.9 – 6.2 LLLT + TE = 2.5 / 1.6 – 3.5 TE = 4.2 / 2.6 – 5.7</td>
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<td>T2: ESWT + TE = 4.5 / 3.1 – 6.0 LLLT + TE = 1.9 / 0.9 – 2.9 TE = 3.5 / 1.9 – 5.1 P-value between groups (0.002)</td>
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Cinar; Saxena; Uygur, 2018b

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<tr>
<td>Cinar; Saxena; Uygur, 2018b</td>
<td>ECR</td>
<td>n = 51;</td>
<td>LLLT: 850nm with continuous output of 100mW, with a dose of 5.6J/cm² at 3 points, each exposure lasting 80 seconds was applied at the origin of the plantar fascia (3 times per week totaling 10 sessions).</td>
<td>T0: baseline</td>
<td>AOFAS – function subscale (0-50)</td>
<td>T0 (mean / CI): LLLT + TE = 44.16 / 42.58 – 45.4 TE = 44.55 / 43.75 – 47.34</td>
<td>The use of LLLT combined with TE and TE alone provides positive results on pain and functionality in patients with PF.</td>
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<td>G1: LLLT + TE (n = 27);</td>
<td>TE: stretching of the gastrocnemius muscle and plantar fascia (3 times a day, 10 repetitions, for 30 seconds each, for 3 months) and use of silicone insoles for 3 months.</td>
<td>T1: 3 weeks</td>
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<td>T1: (mean / CI): LLLT + TE = 48.15 / 46.87 – 49.42 TE = 48.32 / 46.87 – 49.77</td>
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<td>G2: TE (n = 22);</td>
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<td>T2: 3 months</td>
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<td>Women (n = 41); Men (n = 9)</td>
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<td></td>
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<td>Mean age 45.3 years.</td>
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<td>Both groups performed TE.</td>
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Cinar; Saxena; Uygur, 2020

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<th>Eligible studies</th>
<th>Type of study</th>
<th>Sample description</th>
<th>Intervention protocol</th>
<th>Period of assessment</th>
<th>Measurement instruments and outcomes</th>
<th>Results</th>
<th>Conclusions</th>
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<tr>
<td>Cinar; Saxena; Uygur, 2020</td>
<td>ECR</td>
<td>n = 44;</td>
<td>ESWT: 1000-1500 pulse was applied over the pain area and 1000 pulses over the plantar fascia (1 time per week, for 3 weeks).</td>
<td>T0: baseline</td>
<td>AOFAS – function subscale (0-50)</td>
<td>T0 (mean / CI): ESWT + TE = 6.13 / 5.41 – 6.85 TE = 5.49 / 4.67 – 6.31</td>
<td>The results revealed that ESWT did not have an added benefit over usual care for improving foot function and gait performance in patients with PF.</td>
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<td></td>
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<td>G1: ESWT + TE (n = 23);</td>
<td>TE: stretching of the gastrocnemius muscle and plantar fascia (3 times a day, 10 repetitions, for 30 seconds each, during 3 weeks) and use of silicone insoles for 3 months.</td>
<td>T1: 3 weeks</td>
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<td>T1 (mean / CI): ESWT + TE = 2.25 / 1.31 – 3.18 TE = 3.72 / 2.66 – 4.79</td>
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<td>G2: TE (n = 21);</td>
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<td>T2: 3 months</td>
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<td>Women (n = 40); Men (n = 4), with PF.</td>
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<td>Average age 45.</td>
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<td></td>
<td></td>
<td>Both groups performed the TE.</td>
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</tbody>
</table>
Therapeutic Exercise in Plantar Fasciitis

Chew et al., 2013

ECR: n = 54;
G1: ACP + TE (n = 19);
G2: ESWT + TE (n = 19);
G3: TE (n = 16);
Women (n = 25)
Men (n = 29)
Unilateral PF.

Mean age: 46.1 years.

ACP: 3ml of conditioned autologous plasma (extracted from the patient and injected into the thickening of the plantar fascia).
ESWT: 2 sessions of 2000 pulses with energy levels progressing from 0.02 mJ/mm³ to 0.42 mJ/mm³ (duration 10 min, 2 sessions 1 week apart).
TE: 1-2 sessions to learn triceps sural and plantar fascia stretching program (3 times per day, 3 times each stretch, for 30 seconds each). Guided to perform every day, including after study termination.

T0: baseline
T1: 1 month
T2: 3 months
T3: 6 months

AOFAS (0-100)

T0 (mean / amplitude):
ACP + TE = 65 / 38 – 77
ESWT + TE = 62 / 44 – 79
TE = 72 / 51 – 77

T1 (mean / amplitude):
ACP + TE = 75 / 35 – 84
ESWT + TE = 73 / 52 - 92
TE = 75 / 53 – 82

T2 (mean / amplitude):
ACP + TE = 86 / 4.6 – 7.9
ESWT + TE = 90 / 72 – 100
TE = 87 / 73 – 100

T3 (mean / amplitude):
ACP + TE = 90 / 4.6 – 7.9
ESWT + TE = 90 / 72 – 100
TE = 87 / 73 – 100

Treatment of PF with ESWT + TE resulted in improved pain and functional outcomes compared to TE alone.

VAS (0-10)

T0 (mean / amplitude):
ACP + TE = 7 / 4 – 10
ESWT + TE = 7 / 5 – 8.5
TE = 6 / 3 – 8

T1 (mean / amplitude):
ACP + TE = 4 / 1 – 10
ESWT + TE = 5 / 0 – 7
TE = 5 / 3 – 8

T2 (mean / amplitude):
ACP + TE = 4 / 0 – 8
ESWT + TE = 4 / 0 – 7
TE = 4 / 0 – 7

T3 (mean / amplitude):
ACP + TE = 2 / 0 – 6
ESWT + TE = 3 / 0 – 8
TE = 3 / 0 – 7

P-value between groups 0.045

P-value between groups 0.04

P-value between groups 0.061

P-value between groups 0.036

P-value between groups 0.053

P-value between groups 0.090

P-value between groups 0.036

P-value between groups 0.090
<table>
<thead>
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<th>Eligible studies</th>
<th>Type of study</th>
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<th>Intervention protocol</th>
<th>Period of assessment</th>
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<th>Results</th>
<th>Conclusions</th>
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<tbody>
<tr>
<td>Mohamed et al., 2019</td>
<td>ECR</td>
<td>n = 45; G1: r-ESWT (n = 15); G2: TE (n = 15); G3: r-ESWT + TE (n = 15); Women (n = 29) Men (n = 16) Unilateral PCF; Mean age 45.22 years.</td>
<td>r-ESWT: 2000 pulses, 3 bar energy. 15mm depth per session (for 3 weeks with one week break). TE = calf stretching 5 sets of 20 seconds; plantar fascia stretching 10 sets of 10 seconds + strengthening of intrinsic muscles of the feet; dorsiflexion with isometric contraction of the plantiflexors for 5 seconds 100 repetitions.</td>
<td>T0: baseline T1: 3 weeks</td>
<td>VAS (0-10)</td>
<td>T0 (mean±SD): r-ESWT = 8.98 ± 0.8 TE = 8.79 ± 1.05 r-ESWT + TE = 9.05 ± 0.85 T1 (mean±SD): r-ESWT = 3.34 ± 1.3 TE = 5.16 ± 1.2 (P-value 0.001) r-ESWT + TE = 1.82 ± 0.69 (P-value 0.001)</td>
<td>Therapy by r-ESWT alone or combined with TE is more effective than TE alone in improving pain in PF.</td>
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<tr>
<td>Stratton et al., 2009</td>
<td>ECR</td>
<td>n = 26; G1: LFES + TE (N = 13); G2: TE (N = 13); Individuals with PF; Average age 41 years.</td>
<td>LFES = low frequency electrical stimulation, in intensity until a moderate contraction is felt (10 pulses per second, for 20 min, for 4 weeks). TE = DiGiovanni fascia stretching protocol (10 times each stretch, for 10 sec, 3 times a day; for 4 weeks). Both groups wore a pair of prefabricated foot orthoses for a minimum of 1 month. Both groups performed TE.</td>
<td>T0: baseline T1: 4 weeks T2: 3 months</td>
<td>Daily Living Subscale of the Foot and Ankle Ability (ADL/FAAM) (0-100)</td>
<td>T0 (mean±SD): LFES + TE = 52.2 ± 13.8 TE = 52.3 ± 17.1 T1 (mean±SD): LFES + TE = 34.4 ± 13.3 TE = 37.3 ± 12.1 T2 (mean±SD): LFES + TE = 32.3 ± 13.4 TE = 32.3 ± 14.4</td>
<td>Based on these results, the efficacy of the use of low frequency electrical stimulation in the management of patients with PF is questioned.</td>
</tr>
</tbody>
</table>
Therapeutic Exercise in Plantar Fasciitis

the load imposed on the fascia and thereby improvement in symptoms (27). Kaur and Koley (28) compared the effectiveness of calf and Achilles tendon stretching on foot pain and functional disability in 50 patients with PF over four weeks. They observed better results in VAS and FFI, for individuals who received stretching in the Achilles tendon. However, calf and plantar fascia stretching also have possible effects on reducing pain and disability in individuals with PF (29). Ryan et al. (30) analyzed the efficacy of a program of stretching exercises, plantar fascia exercises, and balance training, compared with dexamethasone injection, in individuals with chronic PF. The sample consisted of 36 workers, who were orthostatic for more than 5 hours a day. Both groups had significant improvement in VAS pain score, as well as reductions in foot and ankle disability index compared to baseline scores. They mention that taking into account the side effects of corticosteroids, physiotherapy stands out in the choice of treatment for patients with PF. Muscle stretching has been used with advantages both in pain reduction and functional gains, for cases of subacromial impingement syndrome (31), knee osteoarthritis (32), chronic low back pain (33) and neck pain (34), aiming at improving dynamic balance (35), and even aiming at raising the pain threshold and muscle relaxation by stimulating the Golgi tendon organs (36).

In the present review, of the 10 studies included, only Mohamed et al. (12) associated strengthening in the home exercise program. A recent study of Gökçe et al. (37) compared a low-level laser therapy (LLLT) protocol associated with exercises, which included strengthening and stretching, with exercises alone, observing advantages when LLLT was used, but also positive effects of TE in individuals with plantar fasciitis, however, since it was a retrospective study, with no indication of randomization, it was not included in this review. Thong-On et al. (38) compared the effects of eight-week strengthening and stretching exercise programs on pain outcome in 84 patients with PF randomized to the strengthening or stretching exercise groups. As a result, both exercise programs were able to significantly

<table>
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<th>Eligible studies</th>
<th>Type of study</th>
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<th>Intervention protocol</th>
<th>Period of assessment</th>
<th>Measurement instruments and outcomes</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rompe et al., 2010</td>
<td>ECR</td>
<td>n = 102; G1: TE: (n = 54); G2: r-ESWT: (n = 48); Women (n = 66); Men (n = 36); Unilateral PF</td>
<td>TE = plantar fascia stretches (3 times a day, 10 times each exercise, for 10 sec each, for 8 weeks); r-ESWT: 2000 pulses with energy of 320 mJ/mm², 8 pulses per minute, applied at the site of pain (3 sessions, one week apart each).</td>
<td>T0: baseline; T1: 2 months; T2: 4 months; T3: 5 months</td>
<td>Pain subscale scores (PS-FFI) (0-50)</td>
<td>T1 (mean / CI): TE = -21.4 ± 10.6 / -24.3 a -18.5; r-ESWT = -6.6 ± 1.2 / -9.1 a -4.1; P-value between groups (&lt; 0.001)</td>
<td>TE for the plantar fascia is superior to repetitive r-ESWT therapy for the treatment of acute PF symptoms.</td>
</tr>
</tbody>
</table>
reduce VAS scores, although no superiority of one of the techniques was demonstrated.

Another widely used resource is ESWT, which is a therapeutic resource that uses high-energy sound waves, which cause an inflammatory response in the tissue and cause the body to respond to this condition with increased blood flow and metabolism (39, 40). In the study by Mohamed et al. (12) ESWT alone or associated with a TE program was more effective than performing TE alone. In addition, Greve et al. (41) pointed out that ESWT may be more effective for the treatment of PF pain than conventional physiotherapy. However, this therapy is still controversial, because in the study by Carlisi et al. (8), the volunteers had a high number of trigger points in the triceps sural, and when ESWT was performed on these points, despite being safe, there was no improvement in symptoms.

US is interesting for its thermal and non-thermal effects. It is characterized by kinetic oscillations produced by a vibrating transducer, which when applied to the skin, penetrates the body, and promotes therapeutic responses. Its mechanism is due to the agitation of the electrolytic medium of interstitial fluids, accelerating the inflammatory process and tissue repair, reducing pain and edema, assisting in tissue proliferation and protein synthesis, reducing joint stiffness and contractures, increasing the level of cellular activity and microcirculation (42, 43). In the studies of Akinoglu et al. (17) and Akinoglu and Köse (18) found reduction in pain level from this intervention, and in the first study US proved to be more effective than ESWT and TE. Cinar et al. (16) concluded that LLLT associated with TE was more effective than ESWT + TE in reducing pain. In this sense, the systematic review and meta-analysis by Wang et al. (44) aimed to investigate the clinical effectiveness of LLLT therapy in PF. In total, 6 randomized controlled trials were included, and the authors conclude that compared to the control group the VAS score decreased significantly at the end of treatment of patients treated with LLLT, an improvement that persisted for up to 3 months. Limitations of the present study include the absence of comparisons of the use of TE with placebo, but there are ethical implications that make this difficult.
Therapeutic Exercise in Plantar Fasciitis

CONCLUSIONS
It is concluded that TE, mainly through plantar fascia and triceps sural stretching, is effective in reducing pain and functionality alone, or combined with other therapies, such as ESWT, LLLT and US in PF, especially for the last two forms of association. In addition, new studies are suggested, with low risk of bias, which analyze the use of TE in the form of strengthening, because with the improvement of stabilization it is believed that functional improvement may follow.

FUNDINGS
The present study received funding from the State University of Western Paraná - Unioeste, in the form of a scientific initiation scholarship.

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

CONTRIBUTIONS
RAS, BT, FFC: research project, literature research, writing – original draft. DFL, CBD, GRFB: research project, literature research, critical review of the manuscripts. MRB: research project, literature research, critical review of the manuscript, coordination. FFC, DFL, CBD, GRFB, MRB: writing - review & editing.

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

Figure 7. Forest plot of comparison: therapeutic exercise vs TE + other therapy.
Outcome: functionality - short term.

Figure 8. Forest plot of comparison: TE vs TE + other therapy.
Outcome: functionality - medium term.
REFERENCES


5. Goff JD, Crawford R. Diagnosis and treatment of plantar fasciitis. Am Fam Physician 2011;84:676-82.


31. Tahran Ö, Yeşilyaprak SS. Effects of Modified Posterior Shoulder Stretching Exercises on Shoulder Mobility, Pain, and Dysfunction in Patients With Subacromial Impinge-
## ONLINE SUPPLEMENTS

### Appendix 1. Database searches.

<table>
<thead>
<tr>
<th>Database</th>
<th>Search</th>
<th>References found</th>
</tr>
</thead>
<tbody>
<tr>
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<td>((“fasciitis. plantar”(MeSH) OR “fasciitis. plantar”) OR (“fasciitis. plantar”(Title/Abstract)) OR (“fasciitis plantar” OR “Policeman’s Heel” OR “Policeman’s Heel” OR “Heel Spur Syndrome” OR “Chronic Plantar Fasciitis”)) AND ((“exercise therapy”(Mesh) OR “exercise therapy”) OR (“exercise therapy”(Title/Abstract)) OR (“exercise” OR “stretches” OR “strengths” OR “therapy” OR “exercise” OR “stretching” OR “muscle stretching exercises”(Mesh) OR “muscle stretching exercises” OR “strength” OR “strengthening” OR “resistance training” OR “intrinsic foot muscle” OR “static stretching” OR “passive stretching” OR “static passive stretching” OR “isometric stretching” OR “active stretching” OR “static active stretching” OR “ballistic stretching” OR “dynamic stretching”))</td>
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<td>Web of Science</td>
<td>(“fasciitis plantar” OR “Policeman’s Heel” OR “Policeman’s Heel” OR “Heel Spur Syndrome” OR “Chronic Plantar Fasciitis”) (Tópico) and (“exercise therapy” OR exercise OR stretches OR strengths OR therapy OR exercise OR stretching OR “muscle stretching exercises” OR strength OR strengthening OR “resistance training” OR “intrinsic foot muscle” OR “static stretching” OR “passive stretching” OR “static passive stretching” OR “isometric stretching” OR “active stretching” OR “static active stretching” OR “ballistic stretching” OR “dynamic stretching”))</td>
<td>186</td>
</tr>
<tr>
<td>Embase</td>
<td>(‘fasciitis plantar’/exp OR ‘fasciitis plantar’ OR ‘heel spur syndrome’/exp OR ‘heel spur syndrome’ OR ‘chronic plantar fasciitis’/exp OR ‘chronic plantar fasciitis’) AND (‘exercise therapy’/exp OR ‘exercise therapy’ OR stretches OR strengths OR ‘therapy’/exp OR therapy OR ‘exercise’/exp OR exercise OR stretching/OR ‘stretches’ OR ‘stretching’ OR ‘exercise therapy’ OR ‘muscle stretching exercises’/exp OR ‘muscle stretching exercises’ OR ‘strength’/exp OR strength OR strengthening/OR ‘resistance training’/exp OR ‘resistance training’ OR ‘intrinsic foot muscle’ OR ‘static stretching’/exp OR ‘static stretching’ OR ‘passive stretching’/exp OR ‘passive stretching’ OR ‘static passive stretching’ OR ‘isometric stretching’ OR ‘active stretching’ OR ‘static active stretching’ OR ‘ballistic stretching’ OR ‘dynamic stretching’)</td>
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<td>Lilacs</td>
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SUMMARY

Introduction. Despite trends to repair meniscal defects, meniscectomy continues to be a common procedure. Degenerative changes following these surgeries become candidates for meniscal allograft transplantation.

Purpose. The purpose of this meta-analysis was to review published articles that compared methods of meniscal allograft transplantation (bone plug, suture only, or bone bridge) for their biomechanical effects on tibiofemoral mean contact pressure and contact area in relation to intact meniscal states.


Results. The greatest contact area was found in the setting of an intact meniscal state, with a mean difference of 188.886 mm$^2$ (95%CI 148.059-229.672, p < 0.001) and 78.713 (95%CI 78.713-103.733, p < 0.001), when compared to meniscectomy and MAT respectively. Although MAT showed a lower contact area vs the intact knee, it did have a greater contact area than meniscectomy by 94.951 mm$^2$ (95%CI, 71.590-118.312, p < 0.001). The dovetail bone bridge technique was shown to have the highest difference in mean contact area of 207.676 mm$^2$ (95%CI 85.288-330.065, p < 0.001) when compared to bone plug technique.

Conclusions. Meniscal allograft transfer demonstrated significantly less contact area than a meniscus intact knee and significantly higher contact area than meniscectomy. In the systematic review, bone bridge meniscal allograft transplant techniques offered the closest reproduction of native knee biomechanics when compared to bone plug and suture only.

KEY WORDS
Meniscal allograft transplant techniques; biomechanics; contact area; meniscus; meniscectomy.
lenging meniscal deficiency. MAT success is dependent on numerous variables: graft size, knee stability, degree of arthrosis, axial alignment, rehabilitation protocol, and obesity. Recent studies have shown fair to excellent outcomes, second look arthroscopies have demonstrated healing to the peripheral rim, and some series have shown 70% clinical survivorship at 10 years postoperatively (2, 17-22). The most important surgeon controllable factor is the method of MAT fixation. To date there exist three methods of MAT: 1) dovetail bone bridge fixation - in which the posterior and anterior horns of the graft remain connected to a single piece of bone, 2) bone plug fixation – in which 2 separate osseous segments are attached to the posterior and anterior horns, 3) suture only – in which the meniscal allograft is tethered to the meniscocapsular rim via suture fixation. Various studies have demonstrated alteration in joint contact forces with these different fixation techniques; however there remains no literature directly comparing these methods across studies (18-20, 22-27).

The purpose of this meta-analysis was to review published articles that compared methods of meniscal allograft transplantation (bone plug, suture only, or bone bridge) for their biomechanical effects on tibiofemoral mean contact pressure and contact area in relation to intact meniscal states.

MATERIALS AND METHODS

There were no ethical concerns for this meta-analysis of biomechanical articles.

Article screening

We searched the PubMed database on July 15, 2020 for articles evaluating the knee kinematics of MAT in a biomechanical model. The search field was entered as: (“Meniscus allograft”[All Fields] OR “Meniscal allograft”[All Fields] OR “meniscus allograft transplant”[all fields] or “meniscal allograft transplant”[all fields]) AND (“Biomechanic”[All Fields] OR “Biomechanics”[All Fields] OR “Biomechanical”[All Fields] OR “cadaver”[all fields]). Two authors (BC and KP) independently screened the resulting 85 articles for inclusion or exclusion using Abstrackr software (Brown, Providence, RI). Disagreements were reviewed by the lead author (SK) who made the final decision whether to include or exclude the references. Inclusion criteria were biomechanical studies of MAT on human cadavers that reported mean contact area, mean contact pressure, mean peak contact pressure in native, total meniscectomy states as well as following MAT. Initial screening following a review of disagreements resulted in 15 articles for full review. The sources of these articles were reviewed and cross-referenced with the 85 original articles. Five additional articles were added to the full text review while seven were excluded for different reasons such as only reporting translation/strain forces, pull out strength, extrusion values and lack of mean and standard deviation values. Subsequently 12 articles were included in the study. These articles were then evaluated for quality using the Quality Appraisal for Cadaveric Studies (QUACS) scale, which is a validated means for assessing the quality of cadaveric studies.

Data collection and included studies

Data was collected from each including: sample size, mean contact pressure, mean peak contact pressure, mean contact area, MAT fixation type, force applied during testing, MAT location (medial versus lateral) and graft type. The included studies were Ambra, Brial, Dienst, Huang, Huang, Kim, McDermott, Paletta, Sekaren, Vrancken, Wang.

RESULTS

The results of the meta-analysis showed that the greatest contact area was found in the setting of an intact meniscal state, with a mean difference of 188.886 mm³ (95%CI 148.059-229.672, p < 0.001) and 78.713 (95%CI 78.713-103.733, p < 0.001), when compared to meniscectomy and MAT respectively. Although MAT showed a lower contact area vs the intact knee, it did have a greater contact area than meniscectomy by 94.951 mm² (95%CI, 71.590-118.312, p < 0.001) (table I).

Regarding mean contact pressure and mean peak contact pressure, meniscectomy had greater pressures vs MAT by 0.506 MPa (95%CI 0.351-0.660, p < 0.001) and intact states by 0.751 (95%CI 0.549-0.952, p < 0.001). MAT also was noted to have a greater mean contact and mean peak contact pressures than intact states by 0.239 Mpa (95%CI 0.145-0.332, p < 0.001) (table II).

Meta-regression showed that each of the MAT techniques chosen had differing effect on contact area and contact pressures (table III). The dovetail bone bridge technique was shown to have the highest difference in mean contact area of 207.676 mm² (95%CI 85.288-330.065, p < 0.001) when compared to bone plug technique. There was also a greater difference of 115.250 mm² (95% CI 40.761-189.740, p = 0.002) in the contact area of soft tissue grafts in comparison to bone plug technique. Additionally, the soft tissue graft had the lowest difference in mean contact pressure of 1.018 MPa (95%CI -1.688 to -0.347,
p = 0.003) less than the bone plug technique. Inconsequently, bone plug was shown to have the lowest peak contact pressure of 5.525 MPa (95%CI 4.923-6.126) with differences between dovetail and soft tissue graft being 2.074 (-0.027 to 4.175, p = 0.053) and 0.848 (-0.347 to 2.043, p = 0.164).

Table I. Characteristics of included studies.

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<th>Lead Author</th>
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<th>Technique</th>
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<th>Average Age Cadavers (Years)</th>
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<td>Vrancken</td>
<td>2014</td>
<td>Suture only (PCU vs allograft)</td>
<td>Medial</td>
<td>5</td>
<td>70-88</td>
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<tr>
<td>Wang</td>
<td>2014</td>
<td>Bone plug vs suture only</td>
<td>Medial</td>
<td>7</td>
<td>Not Reported</td>
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<tr>
<td>Alhalki</td>
<td>2000</td>
<td>Bone Plug (Allo vs Auto)</td>
<td>Medial</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>Alhalki</td>
<td>1999</td>
<td>Bone plug vs suture</td>
<td>Medial</td>
<td>10</td>
<td>70</td>
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</tbody>
</table>

Table II. Statistical analysis.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Difference in Mean Contact Area (mm²) (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact vs Meniscectomy</td>
<td>188.866 (148.059-229.672)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MAT vs Meniscectomy</td>
<td>94.951 (71.590-118.312)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intact vs MAT</td>
<td>78.713 (53.694-103.733)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

| Difference in Mean Contact Pressure (MPa) (95%CI) |
|-----------------------------------------------|-----------------------------------------------|---------|
| Meniscectomy vs Intact                       | 0.751 (0.549-0.952)                           | < 0.001 |
| Meniscectomy vs MAT                         | 0.506 (0.351-0.660)                           | < 0.001 |
| MAT vs Intact                               | 0.239 (0.145-0.332)                           | < 0.001 |

| Difference in Mean Peak Contact Pressure (MPa) (95%CI) |
|-----------------------------------------------|-----------------------------------------------|---------|
| Meniscectomy vs Intact                        | 2.730 (1.850-3.610)                           | < 0.001 |
| Meniscectomy vs MAT                          | 1.129 (0.538-1.720)                           | < 0.001 |
| MAT vs Intact                                | 1.676 (0.869-2.482)                           | < 0.001 |
DISCUSSION

Main findings of our paper

**Intact vs MAT vs meniscectomy and purpose of meta-analysis**

In agreement with prior literature on the topic, the meniscal allograft transplant does not restore knee biomechanics to a native state (2, 18, 20, 22, 24, 28). When compared to complete meniscectomy, meniscal allograft transplant in our review demonstrated improvement in contact area by 94.9 mm², which corresponded to a decrease in both mean contact pressures and mean peak pressures by 0.506 and 1.129 MPa – respectively. These findings reinforce that MAT improves knee biomechanics by increasing contact area. Although the contact area and pressures after MAT more closely approach those of an intact knee state, there remains a statistically significant difference when compared to an intact knee. These findings support the significant role of the meniscus as a load distributor within the knee and explains the correlation between degenerative changes and meniscal deficiency (13, 29-32). Additionally, Zaffagnini et al. found that knee laxity was reduced following MAT compared to the pre-operative state, indication a role for MAT in improving the biomechanics of the knee (33). As the body of evidence on meniscal allograft transplantation continues to grow, the gap between biomechanics of the meniscal transplanted knee and native knee may continue to shrink (1, 22, 24, 25).

The 12 high-quality biomechanical studies included in our systematic review determined that meniscal allograft transplantation through a dovetail bone bridge graft technique was shown to have a statistically significant higher contact area than bone plug and suture only techniques. Furthermore, the mean peak contact pressures encountered through bone plug techniques were lower than both dovetail and soft tissue techniques, although the difference between bone plug and soft tissue was not statistically significant. These are important variables to take into consideration when discussing surgical indications and techniques for meniscal allograft transplantation (2, 24). A pitfall of any MAT procedure is the size of the chondral defect, which is difficult to remedy no matter the technique. Steadman microfracture technique aims to fill the chondral defect with stable clot, thereby providing an ideal environment for repair. This technique has been shown to be effective in the management of high-grade chondral defects with great clinical outcomes at 11-year follow-up (34). No biomechanical studies have been performed to assess whether Steadman microfracture technique is able to restore knee biomechanics to a native state. The authors feel future research should target this question.

---

**Table III. Meta regression.**

<table>
<thead>
<tr>
<th></th>
<th>Contact Area (mm²)</th>
<th>Mean Contact Pressure</th>
<th>Peak Contact Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone plug</td>
<td>248.336 (209.345-287.328)</td>
<td>2.213 (1.876-2.550)</td>
<td>5.525 (4.923-6.126)</td>
</tr>
<tr>
<td>Dovetail</td>
<td>+207.676 (85.288-330.065) p &lt; 0.001</td>
<td>-0.923 (-2.662-0.816)</td>
<td>+2.074 (-0.027-4.175)</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>+115.250 (40.761-189.740) p = 0.002</td>
<td>-1.018 (-1.688 to -0.347) p = 0.003</td>
<td>+0.848 (-0.347-2.043) p = 0.164</td>
</tr>
<tr>
<td>Medial</td>
<td>377.123 (328.865-429.381)</td>
<td>1.297 (0.938-1.656)</td>
<td>5.125 (4.301-5.949)</td>
</tr>
<tr>
<td>Lateral</td>
<td>-146.367 (-210.082 to -82.651) p &lt; 0.001</td>
<td>1.239 (0.733-1.746)</td>
<td>+1.177 (0.132-2.222) p = 0.027</td>
</tr>
<tr>
<td>All MATs medial and lateral</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Contact area
Upon meta-regression, meniscal allograft transplantation through dovetail bone bridge technique had the greatest increase in compartment contact area compared to traditional bone plug by 207.676 mm² (95% CI 85.288-330.065) p < 0.001) and soft tissue grafts by 115.250 mm² (95% CI 40.761-189.740) p = 0.002. Although these values still significantly fell short of the native knee by an average of 78 mm².

In comparison to previous literature, our results corroborate the role of boney techniques in achieving rigid fixation of both the anterior and posterior horns – particularly the dovetail MAT, which was closest to restoring contact area in comparison to an intact knee. For example, Ambra et al. was not able to demonstrate significant difference in mean contact area between MAT with bone plug and native knee states, but suture only fixation was significantly associated with reduced contact area compared to native knee state within 0-30 degrees of flexion (28). Similarly, Wang et al. showed MAT via bone plugs resulted in increased contact area that more closely resembled native knee state than with MAT via suture only fixation (38). These differences in contact area from varying MAT techniques are likely the result of the degree of meniscal extrusion throughout dynamic knee activities. In fact, rigid fixation is an essential tenet to avoid meniscal extrusion under increasing axial loads (35). While technically easier, it remains controversial if soft-tissue-only fixation is comparable to boney techniques, as few biomechanical studies exist directly comparing the two (28). For example, one series citation found suture only to have a lower contact area than bone plug, but the difference was not statistically significant (36). Additionally, Alhalki et al. demonstrated no significant difference in medial knee contact area compared to native knee after MAT with bone plug or with suture (36).

Mean contact pressure/peak contact pressure
Our values for peak contact pressure correlate with established literature. Alhalki et al. showed that neither MAT with bone plug nor MAT with suture reconstituted normal medial knee peak contact pressures; however, normalized peak pressures were significantly higher when using a suture only technique. Furthermore, mean pressures were not significantly different from MAT with bone plug versus normal knee states, but were significantly greater with suture only technique (49-61% increased pressure throughout knee ROM 0-45 degrees) (36). McDermott et al. was not able to demonstrate a significant difference in peak contact pressures between MAT via bone block or MAT via suture compared to native knee states; however, direct comparison of peak pressures showed significant increase in suture only compared to bone block techniques (32). Similarly, Wang et al. and Ambra et al. demonstrated closer approximation of native knee contact and peak contact pressures with bone plug techniques as compared to soft tissue techniques.

Interestingly, meta regression revealed mean contact pressures were lowest with soft tissue techniques by 1.018 Mpa (p = 0.003) compared to bone plug and no significant difference between either of the boney techniques. These findings, however, are likely contributed to the great heterogeneity within studies. Of the 12 included studies for meta-analysis, only 4 directly compared boney to soft tissue procedures (28, 32, 36, 38), while 2 reported values exclusively for suture techniques (30, 39). Sub stratification of compartmental peak contact pressures by MAT technique demonstrated lowest pressures with bone plug (5.525 MPa) technique, which was not statistically different from dovetail (7.599, p = 0.053) or soft tissue techniques (Mpa = 6.373, p = 0.164).

Biomechanical comparisons between MAT techniques with consideration of medial vs lateral compartments
Direct comparison of medial versus lateral compartments after MAT did demonstrate that medial compartment more frequently had greater contact area, lower contact pressure, and lowest peak contact pressures. These results are consistent both with prior literature and native knee biomechanics. For example, Devaraj et al. discussed that the ability of the medial condyle to withstand greater forces is due to the more constrained medical meniscus as it is attached to the MCL (37). This necessitates a greater contact area as the medial condyle functions as the central point of pressure during the knee is in extension. Dynamically, when flexion is involved, the medial meniscus posteriorly carries loads during the early stance phase of walking. Because the asymmetric C-shape, the anterior portion of the medial meniscus is much thinner anteriorly and less readily absorbs the joint load compared with the posterior area (38). This inherently makes direct comparison of soft tissue techniques in the lateral meniscus more challenging.

The previous literature in consideration of the lateral compartment, has predominately focused on the biomechanical advantages that can be gained. It has been postulated that in order to gain biomechanical advantage with a MAT procedure, the circumferential distribution of axial loads by the menisci must be taken into account. This particularly applies to the allograft attachment to the anterior and posterior meniscal horns (40). Due to the proximity of the anterior and posterior horns of the lateral meniscus, some authors favor bone bridge fixation of the lateral compartment while more variability exists in medial compartment techniques (41).
Limitations
Anatomic meniscal allograft transplantation is a challenging procedure requiring technical precision. Indeed, it has been demonstrated in cadaveric studies that nonanatomic placement exceeding 5 mm has dramatic effects on contact pressures (42). This bears reason on clinicians frequently using size matched transplants when performing these procedures but was not the case for the studies mentioned. These cadaveric studies were frequently performed in a manner where external validity of accurate horn positioning was not always possible. This also brings into question the difference in protocols for each study and the lack of control groups used within.

The precise relationship between contact stress and area to articular cartilage longevity has not been determined (43). Numerous clinical studies have been published demonstrating a variety of surgical techniques and their clinical outcomes but still have not highlighted a particular advantage of boney to suture technique (26, 27, 44-47). Nonetheless it is generally regarded that biomechanical forces withstood by the tibiofemoral surface plays an important role in joint degradation (48-50). It should also be noted that despite any biomechanical limitations of MAT, graft survivorship at 10 years has been shown to be as high as 89% (51).

CONCLUSIONS
In conclusion, this meta-analysis showed that meniscal allograft transfer demonstrated a significantly higher contact area than meniscectomy. However, it still had significantly less contact area than a meniscus intact knee. Consequently, to reduce biomechanical burden through improving MAT techniques, we sought to better elucidate which is optimal among the three current methods of MAT: dovetail bone bridge, bone plug, and suture only. In this systematic review, dovetail bone bridge meniscal allograft transplant techniques offered the closest reproduction of native knee biomechanics with the significantly highest mean contact area. Although this cadaveric biomechanical evidence suggests an advantage in reproduction of native knee biomechanics with boney techniques in MAT, the clinical studies thus far do not demonstrate superiority with any technique. Further prospective study is necessary to understand the effects of MAT on native biomechanics.

FUNDINGS
None.

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

CONTRIBUTIONS
SK, KP, BC: data collection, writing - original draft, writing - review & editing. NR: writing - original draft, writing - review & editing. EH: data analysis, writing - original draft, writing - review & editing.

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

REFERENCES


Efficacy of Surgical Management versus Conservative Management to Decrease the Incidence of Re-Rupture in Adult Patients with Achilles Tendon Rupture: A Systematic Review and Meta-Analysis

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² Department of Surgery, Regional Hospital of Trujillo, Trujillo, Peru

INTRODUCTION
Achilles tendon rupture is defined as a break in continuity that partially or totally compromises its structure and that can occur at any anatomical level located between the musculo-tendinous junction and the insertion of the tendon at the level of the calcaneus, but the majority of ruptures occur where the blood supply to the gastrocnemius and soleus muscle is poor, i.e., 2 to 6 cm above the point of insertion (1-3). This pathology is attributed to the increase in the elderly and obese population, as well as the increase in sports activities among middle-aged individuals, showing a peak of increase between 30-39 years of age, suffering the risk of breaking...
when running, jumping, accelerating or decelerating. Suddenly, ruptures can cause significant pain and swelling. Further ruptures, such as those involving the gastrocnemius aponeurosis free flap, semitendinosus free graft, tendon transfers and, finally, allografts where end-to-end repair is possible if the gap is < 2.5 cm (8, 11). In the same way, studies recommend that in Achilles tendon re-ruptures between 6 and 8 cm, the V-Y descent tendon plasty, and the massive tendon-bone graft in larger ones, are good alternatives of treatment, achieving an adequate reconstruction, which allows early rehabilitation and recovery with satisfactory functional results (13).

The rest of the complications vary according to different factors such as the severity of the injury, the age and comorbidities of the patient, but mainly due to the treatment used. In addition to the high incidence of re-rupture with conservative management, it is important to mention the possibility of muscle atrophy, loss of ankle mobility, deep vein thrombosis, and chronic disability. Likewise, there are around 15.1% of complications, of which wound dehiscence, tendon exposure, surgical site infection and sural neuromas are associated with surgical management (3, 8, 9).

Finally, this systematic review and meta-analysis tries to determine if surgical management is more effective than conservative management in reducing the incidence of re-rupture in adult patients with Achilles tendon rupture. Likewise, to evaluate the functional result in the short and long term through the use of scores in adult patients with Achilles tendon rupture undergoing surgical management and conservative management; to identify the presence of chronic pain and compare the presence of adverse events (pulmonary embolism and deep vein thrombosis) in adult patients with Achilles tendon rupture undergoing surgical management and conservative management.

**MATERIALS AND METHODS**

A systematic review study was carried out based on previous studies that evaluated the efficacy of surgical management versus conservative management, evaluating the incidence of re-rupture in adult patients with Achilles tendon rupture. The efficacy of both treatments was evaluated through randomized clinical trials and relative risks based on: Achilles tendon re-rupture, functional outcome, chronic pain, and adverse effects (pulmonary embolism, deep vein thrombosis).

**Inclusion criteria**

All those studies of the type randomized clinical trials were included, where all those articles that included patients with Achilles tendon rupture, the intervention group to surgical management and the comparison group to conservative management were considered as population. Primary outcomes to Achilles tendon re-rupture were considered, while secondary outcomes to short- and long-term functional outcome through different SCORES, chronic pain, and adverse effects (pulmonary embolism, deep vein thrombosis).
Exclusion criteria
Observational, cross-sectional, descriptive and case report studies, studies in the pediatric population, studies that have not been completed or whose results have not been published.

Search method
An electronic search for related articles was performed, which were obtained from medical literature databases such as PubMed/Medline, Web of Science, Cochrane Library, Embase, Scopus, and Ovid/Medline. The search strategy included keywords (“Conservative” OR “Not surgical”) AND (“Surgical Treatment” OR “Conventional Open Surgery”) AND (“Acute Achilles’ Tendon Rupture” OR “Achilles Tendon re rupture”) AND (“Randomized Clinical Trial”) AND (“Adult patients”).

Procedure
The selected articles were stored in the Rayyan software to form a database and the citation of the bibliographic references. The next step was carried out among the members of the team, which consisted in making a comparison of the results, in case of finding differences, an additional author will intervene to discuss and reach an agreement. Using the Microsoft Excel 2021 software, the data that was related to the studies was recorded depending on the intervening variables. The systematic review was reported following the PRISMA 2020 recommendations.

Assessment of heterogeneity
Heterogeneity between each study was ascertained using the I² statistic, where values between 0-30% will be considered as low heterogeneity, 30-60% as moderate heterogeneity and values > 60% as high or significant heterogeneity.

Data extraction and management
For this step, a form created in the Cochrane Collaboration Revman 5.4.1 software was used. Data were extracted individually from studies that met the inclusion criteria.

Measures of effect
For dichotomous data, the relative risks with their respective confidence intervals were calculated. Continuous data were analyzed considering their arithmetic mean and standard deviation. A fixed effects model using the Mantel-Haenzel method was considered for analysis. The effect of the intervention is also presented by mean differences (MD), Odds ratios (OR), relative risk (RR) and/or Hazard Ratios (HR). These three components will have a 95% confidence interval.

Data synthesis
It was carried out by random effects, detecting the heterogeneity of the studies through the Chi² test and the I² statistic, likewise the variability between the studies will be evaluated with the Tau² test. When outcomes are reported in median and interquartile range (IQR), these units will be converted to mean and standard deviation (SD) for data analysis capability. For which, the following formula was used: \( x = \frac{(a + 2m + b)}{4} \), using the median values as the “m” value, and the P25 and P75 as “a” and “b” respectively.

RESULTS

Article selection
A total of 2,452 articles were identified in the following databases: PubMed, Web of Science, Scopus, Cochrane, Ovid Medline, and Embase, which were stored in the Rayyan tool. 315 duplicate articles were eliminated during the identification phase. After the elimination of duplicates, 2,137 articles were examined individually by two members of the team, then conflicts were resolved and an agreement was reached between the three members of the group, leaving a total of 28 articles to be analyzed in full text, of which 4 items failed to be recovered. Of the rest, 14 articles were excluded due to type of erroneous design, lack of results, erroneous variable or erroneous main outcome, leaving 10 randomized clinical trial type articles, from which relevant data and results could be extracted, so that this project can be carried out (figure 1).

Figure 1. PRISMA 2020 flow diagram.
Characteristics of the included articles

Data was extracted and stored in a qualitative and a quantitative table. The qualitative table was prepared taking into account the author, country where the study was conducted, year of publication of the study, total number of patients included, mean age, distribution by sex, and number of patients undergoing each type of management. Likewise, a quantitative table was made including data such as the number of patients who presented re-rupture for each type of management, frequencies in each of the groups according to scores in relation to the short and long-term functional result, number of patients who presented chronic pain and number of patients who presented adverse effects (pulmonary embolism and deep vein thrombosis) (tables I-VII).

Table I. Characteristics of the included studies, baseline characteristics of the included population.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>Sample</th>
<th>Sex</th>
<th>Mean age</th>
<th>Number of patients under CM</th>
<th>Number of patients under SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stale et al.</td>
<td>UK</td>
<td>2022</td>
<td>526</td>
<td>135</td>
<td>39.6</td>
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<td>Maempel et al.</td>
<td>UK</td>
<td>2020</td>
<td>64</td>
<td>-</td>
<td>57.6</td>
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<td>33</td>
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<td>Fischer et al.</td>
<td>Germany</td>
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<td>90</td>
<td>9</td>
<td>41.3</td>
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<td>Manent et al.</td>
<td>Spain</td>
<td>2019</td>
<td>34</td>
<td>3</td>
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<tr>
<td>Koslol et al.</td>
<td>Austria</td>
<td>2022</td>
<td>18</td>
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<td>17</td>
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<td>34</td>
<td>45.2</td>
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</table>

*CM: Conservative management; SM: Surgical management.

Table II. Re-rupture.

<table>
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<th>Author</th>
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<th>SM</th>
</tr>
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<tbody>
<tr>
<td>Events</td>
<td>Total</td>
<td></td>
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</tr>
<tr>
<td>Events</td>
<td>Total</td>
<td></td>
<td></td>
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<tr>
<td>Stale et al.</td>
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<td>30</td>
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<td>Olsson et al.</td>
<td>2013</td>
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<td>5</td>
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<tr>
<td>Miller et al.</td>
<td>2005</td>
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<td>32</td>
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</table>

CM: Conservative management; SM: Surgical management.

Table III. Short-term functional result.

<table>
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<th>ATRS</th>
<th>CM</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author</td>
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<td>SD</td>
</tr>
<tr>
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<td>5.46</td>
</tr>
<tr>
<td>Manent</td>
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<td>8.64</td>
</tr>
<tr>
<td>Koslol</td>
<td>78.7</td>
<td>8.60</td>
</tr>
<tr>
<td>Mihailo</td>
<td>87.5</td>
<td>1.72</td>
</tr>
<tr>
<td>Olsson</td>
<td>73</td>
<td>19</td>
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### Table IV. Long-term functional result.

<table>
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<tr>
<th>Author</th>
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<th>SF 36</th>
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<td>SM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Manent</td>
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</tr>
<tr>
<td>6</td>
<td>Mihailo</td>
<td>83.6</td>
<td>3.60</td>
</tr>
<tr>
<td>7</td>
<td>Olsson</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>Willits</td>
<td>76.3</td>
<td>9.12</td>
</tr>
<tr>
<td>2</td>
<td>Maempel</td>
<td>97.1</td>
<td>1.60</td>
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</tbody>
</table>

**CM**: Conservative management; **SM**: Surgical management; **SD**: Standard Deviation; **ATRS**: Achilles tendon total rupture score; **AFAOS**: American Orthopedics foot and ankle score; **SF-36**: 36 item short form survey.
Risk of bias of included articles
Overall bias for randomized clinical trial studies was graded as low risk, two within some concerns category, and one within the high-risk category, specifically in the domain of randomization process (figure 2).

**Figure 2.** Study bias from clinical trials included in the study
Statistical fragility

A systematic review of comparative studies on Achilles tendon rupture management found that the average number of outcome reversals needed to alter the significance of a given study was 2.90, indicating potential fragility in the statistical stability of studies comparing operative versus nonoperative management for Achilles tendon rupture. So, we evaluate additionally the sample size and enrollment, the baseline characteristics, the primary and secondary outcomes, sensitivity analyses, safety and time frame. The Fragility Index (FI) and Fragility Quotient (FQ) were calculated for various studies, revealing insights into the robustness of their findings. Stale et al. demonstrated a robust result with an FI of 10, indicating that a substantial number of events would need to change for the results to lose statistical significance. Maempel et al. (29) showed moderate robustness with an FI of 3 and an FQ of 75%, suggesting a moderate margin of safety. Fischer et al. (30) exhibited low robustness (FI: 0.33, FQ: 1.39%), indicating sensitivity to a small number of events. Manent et al. had a somewhat robust result (FI: 1.33, FQ: 11.95%), vulnerable to a moderate number of changes (18). Koslol et al. had no robustness (FI: 0, FQ: 0%), indicating high sensitivity to observed events. Mihailo et al. displayed moderate robustness (FI: 2, FQ: 4%), with a reasonable margin of safety. Olsson et al. (33) and Willits et al. (34) both demonstrated moderate robustness, with FIs of 3 and 2.5, and FQs of 6% and 3.47%, respectively. Metz et al. (35) showed moderately robust findings (FI: 4, FQ: 9.64%), suggesting a reasonable margin of safety. Miller et al. (36) displayed moderate robustness (FI: 1, FQ: 3.95%).

These indices provide valuable insights for assessing the reliability of study outcomes, considering the impact of potential changes in observed events on the statistical significance of the results (table VIII).

Table VIII. Certainty of the evidence (GRADE).

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Number of participants (studies)</th>
<th>Certainty of the evidence (GRADE)</th>
<th>Relative effect (95%CI)</th>
<th>Anticipated Absolute Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RR 0.35 (0.20 a 0.62)</td>
<td>Risk with CM: 69 per 1,000</td>
</tr>
<tr>
<td>Re-rupture</td>
<td>1259 (8 ECAs)</td>
<td>⬤⬤⬤ Highb,c</td>
<td>RR 0.35 (0.20 a 0.62)</td>
<td>45 less per 1,000 (55 less to 26 less)</td>
</tr>
<tr>
<td>Short-term functional result</td>
<td>718 (6 ECAs)</td>
<td>⬤⬤⬤⬤ Very lowd,e</td>
<td>-</td>
<td>The mean short-term functional outcome was 0</td>
</tr>
<tr>
<td>Long-term functional outcome</td>
<td>566 (5 ECAs)</td>
<td>⬤⬤⬤⬤ Very lowd,e</td>
<td>-</td>
<td>The mean long-term functional outcome was 0</td>
</tr>
<tr>
<td>Chronic pain</td>
<td>1293 (9 RCTs)</td>
<td>⬤⬤⬤ Highb,c</td>
<td>RR 1.68 (1.09 to 2.59)</td>
<td>48 per 1,000 33 more per 1,000 (4 more to 77 more)</td>
</tr>
<tr>
<td>Pulmonary Embolism</td>
<td>670 (2 RCTs)</td>
<td>⬤⬤⬤ Highb,c</td>
<td>RR 2.75 (0.31 to 24.75)</td>
<td>0 per 1,000 0 less per 1,000 (0 less to 0 less)</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>907 (5 RCTs)</td>
<td>⬤⬤⬤ Highb,c</td>
<td>RR 0.71 (0.23 to 2.23)</td>
<td>14 per 1,000 4 less per 1,000 (11 less to 17 more)</td>
</tr>
</tbody>
</table>


GRADE Working Group grades of evidence: High certainty: We are very confident that the true effect is close to that of the estimate of effect. Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the effect estimate, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited: the actual effect may be substantially different from the effect estimate. Very low certainty: We have very little confidence in the estimate of effect. The actual effect is likely to be substantially different from the estimate of effect.

The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% confidence interval). CI, confidence interval; RR, Risk ratio; Positivity: Homogeneity (I^2 = 0%; < 40%); “Wide confidence interval and the possibility of the point estimate crossing; “Significant sample size (n = 126); “Result of the significant statistical test (Test for overall effect p < 0.10); “Statistical heterogeneity between studies (F > 40%); and result of the statistical test not significant (Test for overall effect p > 0.10); “Wide confidence interval.
Primary outcome

**Incidence of Achilles tendon re-rupture**

This is the main result of the study, where eight studies presented data available to develop the meta-analysis. The results of the total synthesis of the eight studies show an RR of 0.35 with a 95% CI of 0.20-0.62, that is, the patients who underwent CM had a 65% lower incidence of re-rupture than those who underwent MC; therefore, the results are in favor of the intervention. In addition, they presented a low I² heterogeneity of 0% (figure 3).

Secondary results

**Short-term functional outcome**

Four scales were used to measure this outcome, the ATRS, AFAOS, LEPPILAHTI, and SF36 scales. Based on the mean and the standard deviation obtained from the data, a mean difference (MD) of 1.22 was obtained with a 95% CI of -1.62 to 4.05, that is, when comparing the results of the four scales, no found some significant difference, as well as a high I² heterogeneity of 93%. The first subgroup included five studies that measured short-term functional outcome using the ATRS scale, the results were an MD of -0.72 with 95% CI of -5.99 to 4.54 and I² heterogeneity of 90%. The second subgroup included three studies using the AFAOS scale, the results were an MD of 6.19 with a 95% CI of 3.90-8.48 and I² heterogeneity of 12%. The third subgroup included a study using the LEPPILAHTI scale, the results were an MD of 2.20 with a 95% CI of -0.36 to 4.76. Finally, the fourth subgroup included a study using the SF36 scale, where the results were an MD of -0.50 with a 95% CI of -1.13 to 0.13 (figure 4).

![Figure 3. Forest plot re-rupture.](image1)

![Figure 4. Forest plot short-term functional outcome.](image2)
Long-term functional outcome
The total synthesis of the studies gave us a mean difference of 0.72 with a 95%CI of 0.37-1.08, that is, the mean long-term functional outcome in patients undergoing MQ was 0.72 standard deviations more compared to the group of patients who underwent CM. In addition, they resulted in a high I2 heterogeneity of 82%. The first subgroup included three studies that measured long-term functional outcome using the ATRS scale, the results were an MD of -1.16 with 95%CI of -1.72 to 4.90 and F heterogeneity of 91%. The second subgroup included three studies using the AFAOS scale, the results were an MD of 1.50 with a 95%CI of 0.29-2.70 and an F heterogeneity of 71%. The third subgroup included a study using the LEPPILAHTI scale, the results were an MD of 0.40 with a 95%CI of -1.81 to 2.61. And the fourth subgroup included a study using the SF36 scale, where the results were an MD of 0.70 with a 95%CI of 0.32-1.08 (figure 5).

Presence of chronic pain
Nine studies were included for this variable, where the results of the total synthesis of the studies show an RR of 1.68 with a 95%CI of 1.09-2.59, that is, the probability of presenting chronic pain in the group subjected to MQ is 68% higher than that of the group subjected to CM, the results being in favor of the control. In addition, it presents a low F heterogeneity of 36% (figure 6).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Surgical</th>
<th>Conservative</th>
<th>Mean Difference</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Total</td>
<td>Mean</td>
<td>IV, Fixed, 95% CI</td>
</tr>
<tr>
<td>1.1 ATRS</td>
<td>Manent, 2019</td>
<td>77.5</td>
<td>14.2</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Olson, 2013</td>
<td>89</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Subtotal (95% CI)</td>
<td>72</td>
<td>62</td>
<td>0.3%</td>
</tr>
<tr>
<td>Heterogeneity: Chi² = 11.67, df = 1 (P = 0.0006); F = 91%</td>
<td>Test for overall effect: Z = 3.52 (P = 0.0004)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.2 AFAOS
Fischer, 2020 | 96.7 | 2.81 | 60 | 94.9 | 2.82 | 30 | 8.3% | 1.80 [0.57, 3.03] |
Manent, 2019 | 92 | 12.96 | 23 | 100 | 8.62 | 11 | 0.2% | -0.60 [-15.25, -0.65] |
Olson, 2013 | 90 | 20 | 49 | 90 | 23 | 51 | 0.2% | 0.00 [-8.04, 8.04] |
Subtotal (95% CI) | 132 | 92 | 8.7% | 1.50 [0.29, 2.70] |
Heterogeneity: Chi² = 6.78, df = 2 (P = 0.03); F = 71%
Test for overall effect: Z = 2.44 (P = 0.01)

1.3 LEPPILAHTI
Wiltens, 2010 | 82.6 | 6.41 | 72 | 82.2 | 7.1 | 72 | 2.6% | 0.40 [-1.81, 2.61] |
Heterogeneity: Not applicable
Test for overall effect: Z = 0.33 (P = 0.72)

1.4 SF36
Maampel, 2020 | 100 | 0.35 | 33 | 99.3 | 1.02 | 33 | 88.3% | 0.70 [0.32, 1.08] |
Subtotal (95% CI) | 33 | 33 | 88.3% | 0.70 [0.32, 1.08] |
Heterogeneity: Not applicable
Test for overall effect: Z = 3.63 (P = 0.0003)

Total (95% CI) | 309 | 257 | 100.0% | 0.72 [0.37, 1.08] |
Heterogeneity: Chi² = 14.26, df = 6 (P = 0.001); F = 82%
Test for overall effect: Z = 3.98 (P = 0.0001)
Test for subgroup differences: Chi² = 13.76, df = 3 (P = 0.001), F = 81.0%

Figure 5. Forest plot long-term functional outcome.

Figure 6. Forest plot chronic pain.
Presence of adverse effects

Pulmonary embolism

Two studies were included for this variable, where the results of the total synthesis of articles show an RR of 2.75 with a 95% CI of 0.31-2.47, non-statistically significant results, in addition to a low I² heterogeneity of 0% (figure 7).

Deep vein thrombosis

Five studies were included for this variable, where the results of the total synthesis of articles show an RR of 0.71 with a 95% CI of 0.23-2.23, non-statistically significant results, in addition to a low I² heterogeneity of 0% (figure 8).

DISCUSSION

Acute Achilles tendon ruptures can be treated with surgical management or conservative management. However, the optimal intervention for acute Achilles tendon rupture remains controversial.

The following systematic review, as well as the following meta-analyses, compared the effectiveness of conservative management (MC) versus surgical management (MQ) in the incidence of Achilles tendon re-rupture, in addition to other important variables such as short- and long-term functional outcome according to the use of scores, presence of chronic pain and presence of adverse effects such as pulmonary embolism and deep vein thrombosis.

The main result was the incidence of re-rupture of the Achilles tendon, where eight RCTs were included that when comparing MC with MQ, the result was favorable for MQ with a 65% lower incidence of re-rupture than those subjected to MC; therefore, the results are in favor of the intervention. (RR 0.35; 95%CI 0.20-0.62). A similar result was obtained in the meta-analysis by Ochen et al., where a 57% lower recurrence rate was reported in MQ compared to MC (RR 0.43; 95%CI 0.31-0.60; F: 22%) (37). Likewise, we found another study by Deng et al., where they included eight RCTs involving 762 patients in the meta-analysis. Overall, a new rupture occurred in 14 of 381 patients who underwent CM (3.7%) and in 37 of 377 patients who underwent MC (9.8%). The combined results showed that the total rate of re-rupture was significantly lower in the MQ than in the MC (RR 0.38; 95%CI 0.21-0.68) (38).

Another way to evaluate the efficacy is by measuring the functional result of the patient through different types of scores with high reliability, validity and sensitivity, it is an essential index to determine the best treatment. In this meta-analysis we decided to assess the short-term and long-term functional outcome. Regarding the short-term evaluation, no significant difference was reported between the groups (MD: 1.22; 95%CI -1.62 to 4.05). However, regarding the evaluation of long-term functionality, our analysis showed an MD of 0.72 (95% CI 0.37-1.08), that is, that the mean long-term functional result in patients undergoing MQ was 0.72 deviations. more standard compared to the group of patients undergoing CM. It can be compared with the results obtained from the meta-analysis by Soroceanu et al., which used four studies that reported functional results, expressed with the use of different scores (Musculoskeletal Functional Assessment Instrument (MFAI), Lower Leg and Ankle Functional Index (FIL), Leppilahti and modified Leppilahti) in each study. Fixed effect analysis showed that
the standardized mean difference did not differ significantly between the two groups (39). Another study that supports our results is that of She et al., where they also found no significant difference between the MQ and MC regarding the ATRS assessment (3 studies, 207 participants, RR 4.27; 95% CI 0.24 to -8.77) (40). However, the aforementioned studies do not evaluate in the short and long term, so they use a different range of recovery time when applying the scores, and this could lead to a possible bias since it does not allow a correct standardization of the variable.

Within the multiple complications observed in both procedures, it was decided to include the chronic pain variable, since it was present in nine of the ten included studies; our meta-analysis concluded that the probability of presenting chronic pain in the group undergoing CM is 68% more than that of the group undergoing CM, the results being in favor of control (RR 1.68; 95% CI 1.09-2.59). However, again the study by Ochen et al., concludes that the incidence of complications was 4.9% after MC compared to 1.6% after MC (risk difference of 3.3%), specifically 3 individuals out of 469 (0.03%) in the MQ group and 2 individuals out of 475 (0.02%) in the MC group (29). The aforementioned study is an example of the various meta-analyses found where the pain variable is found within the set of complications; however, it is not evaluated separately; more studies would be needed to evaluate the variable independently from the rest of the complications to get a more reliable conclusion.

For the pulmonary embolism variable, two studies were included, where the results of the total synthesis of articles show non-statistically significant results (RR 2.75; 95% CI 0.31-24.75). Unfortunately, the data found in various studies did not allow us to differentiate between pulmonary embolism and other complications.

Five studies were included to evaluate the incidence of deep vein thrombosis (DVT); however, we did not find statistically significant results (RR 0.71 95% CI 0.23-2.23). According to the results of Keating et al., DVT, as confirmed by Doppler ultrasound, did not occur in any of the 39 patients in the MQ, however, they reported a higher incidence of DVT in the MC group, presenting this complication in 2 patients of 41 (5%); despite this, it was not statistically significant (41). On the other hand, in the study by Nilsson-Helander et al., in which an evaluation of all patients in both management groups is also carried out through Doppler ultrasound, shows that it is a common complication in both management, since it appeared in 34% of cases after Achilles tendon rupture (34). In addition, this study adds the use of a routine thrombus prophylaxis protocol. This is important to highlight, since most of the analyzed studies are ambiguous when describing complications other than re-ruptures: they do not usually specify the use or not of thrombus prophylaxis. Finally, this shows us that DVT is a frequent complication in this pathology, so we must pay adequate attention to it.

One of the limitations to be highlighted is the high heterogeneity obtained in the functional outcome variable (42), which can be explained by the difference in the number of patients included in each group in the different articles; therefore, these results should be taken with caution.

Likewise, more research is required on the treatment of Achilles tendon rupture. For example, more RCTs are needed to compare percutaneous repair with MC to determine the difference in complications between the two management modalities, since, in some studies found, some groups of CM underwent open surgery and others percutaneous surgery, generating a possible risk of bias.

Finally, since not all complications are major, some patients and surgeons may find the increased rate of other complications after MQ an acceptable trade-off for the reduced rate of re-rupture. This information should be part of the informed consent discussion when CM is being considered, likewise, another area for future research would be the study of criteria that allow physicians to select patients who would be suitable for MC.

CONCLUSIONS
Surgical management is more effective in reducing the incidence of Achilles tendon re-rupture than conservative management. Surgical management presents a lower long-term functional result than conservative management. Surgical management reveals a greater probability of presenting chronic pain than conservative management.

FUNDINGS
None.

DATA AVAILABILITY
All the data used in this research are available at the referenced sources.

CONTRIBUTIONS
GC-F, CA-J: conceptualization, formal analysis, research, methodology, resources, software, validation, visualization, writing – original draft. ZC-C: methodology, validation, conceptualization, final redaction, writing – original draft, writing – review & editing.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.
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Managements to Decrease the Incidence of Re-Rupture in Adult Patients with Achilles Tendon Rupture


Postural Control Pre- and Post-Group Supervised Clinic Based Exercise or Home Based Exercise in Patients with Knee Osteoarthritis: A Randomized Clinical Trial

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Laboratório de Biomecânica, Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil

INTRODUCTION

Knee osteoarthritis (OA) is a chronic, degenerative, multifactorial, and progressively advancing disease (1) with 15% of the global population affected (2). This chronic condition is a primary cause of lower limb deficits in elderly with knee OA impacting around 40% of men and 47% of women (2). Numerous alterations occur in patients with knee OA, including reduced quadriceps strength (3, 4), decreased range of motion (4, 5), increased pain levels (3), and decreased proprioception (6). These changes contribute to walking difficulties, stair climbing challenges, general reduction in activities of daily living (5, 7), in addition to less postural control (8-11).

Postural control forms the foundation of the human motor control system, generating stability and enabling movement, encompassing the ability to achieve and maintain the desired body position during any activity, whether static or dynamic (12). Balance relies on the reception and integration of sensory stimuli for planning and subsequent execution of movements, governing the center of gravity over its support
base. This essential control is executed through the postural control system, with inputs received from the vestibular system, visual receptors, and somatosensory system (13, 14). In individuals with OA, postural control is diminished (8-11), along with other aspects like overall functional capacity (15), which directly influences quality of life and functional independence (16). Moreover, the prevalence of knee OA increases with age, and as age advances, alterations in the locomotor system, such as reduced step length and velocity, an enlarged base of support, and modification of the center of mass in pursuit of greater balance, become more pronounced (17, 18).

Considering that patients typically live with the disease for around 30 years (19) and that the physiological aging process leads to declines in motor recruitment, balance, and reaction time, therapeutic exercises can help mitigate these changes. Guidelines recommend exercise programs on land, weight control combined with exercises, mind-body exercises, and health education as the primary non-pharmacological treatment for OA patients (20, 21).

Physical exercise aims to slow the disease’s progression, in addition to being cost-effective and strongly recommended for maintaining and managing the signs and symptoms of knee OA (22, 23), leading to improved quality of life and functionality (22, 24). When conducted in a group setting, exercise practices reach a larger number of individuals compared to individual sessions and show enhanced pain reduction, quality of life, and patient function (25, 26). Although group strategies incur public health costs, this investment appears to outweigh standard patient care (26). On the other hand, prescribing exercises for home-based practice offers a simple, effective, and secure alternative for these patients (27-29), promoting autonomy and reducing expenses related to in-person visits. Home-based exercises with physiotherapeutic guidance demonstrated superiority over booklet instructions provided by orthopedic specialists (30).

Previous studies assessing postural control in OA patients have identified that these individuals tend to exhibit deficits in maintaining postural control and increased instability – conditions linked to functional limitations, advanced age, reduced quality of life, and decreased social interaction (4, 5, 16, 31, 32). The aim of this study was to compare postural control of patients with knee OA before and after group supervised clinic based or home-based exercises.

**MATERIALS AND METHODS**

**Study design**

This study was a randomized controlled trial, following the guidelines of the Consolidated Standards of Reporting Trials (CONSORT) and approved by the Ethics Committee on Research Involving Human Beings of a University Institution under number 2.009.624 – Date of approval: April 10, 2017. All participants were informed about the objectives and procedures of the research and signed the informed consent form in duplicate, one copy for themselves and the other for the researcher. The authors declare that the procedures were followed according to the regulations established by the Ethics Committee and to the Helsinki Declaration of the World Medical Association.

**Patients**

Individuals aged between 40 and 65 years, clinically diagnosed with knee OA classified as grades I, II, and III through the criteria of Kellgren Lawrence using anterior posterior knee radiographs (33-35) participated in the study. Individuals with prior lower limb surgeries, recent physiotherapy within the last six months, cardiac, respiratory, rheumatic, neurological, or vestibular disorders hinder participation in the proposed assessments and interventions, as well as those unable to attend treatment sessions were excluded from the study. Patients were recruited from the Physical Therapy Clinic at the University Hospital and through pamphlets distributed within the University.

**Measurements**

Participants completed a comprehensive assessment form, collecting identification data, sociodemographic characteristics, and current medical history to identify comorbidities and past medical history. Anthropometric measurements of body mass and height were performed, and body mass index (BMI) was calculated based on this measurement.

**Gait velocity assessment**

The participants’ gait velocity was assessed through the 40-meter walk test. This test involves marking a 10-meter distance on the floor, with cones positioned 1 meter before the start and end marks, requiring the participant to make a turn and eliminate acceleration and deceleration components. The participant performs four laps in the designated space, walking as fast and safely as possible, with the total time recorded using a chronometer. The velocity (m/s) is then estimated by dividing the distance by the time taken (36, 37).

**Postural control assessment**

Participants were instructed to stand barefoot with one foot on each force platform (AMTI, model OR6-6-2000), arms alongside the body, looking on a point positioned 3 meters away (figure 1). Participants with visual impairments were required to wear glasses during assessments. Foot position-
ing was marked on a sheet of paper to ensure consistent placement for all attempts. Participants were tested under four different conditions: 1) standing with eyes open; 2) standing with eyes closed; 3) standing with eyes open on a foam surface; and 4) standing with eyes closed on a foam surface. A non-deformable foam surface (Airex balance pad, Airex®) with dimensions of 49 cm length, 420 cm width, 6.35 cm height, and weighing 680 grams was used. This foam surface demonstrated high test-retest reliability in previous studies assessing postural control (38). Three attempts of 30-seconds were conducted under each test condition, with a 1-minute rest interval between them. Platform acquisition frequency was set at 100 Hz, and the raw force and moment data obtained from the platform were filtered using a fourth order low pass Butterworth filter with a cutoff frequency of 10 Hz. After filtering through a Matlab software routine, this data was used to calculate the center of pressure (COP) coordinates, from which the variables of interest were taken: amplitude of anteroposterior COP displacement (COPap) and mediolateral COP displacement (COPml). Larger values of these variables indicate greater postural oscillation.

Randomization

Participants were randomized through individual sealed opaque envelopes, each containing a treatment card, and one researcher was responsible for randomization and blinding. The supervised clinic-based exercise group (CBG) and the home-based exercise group (HBG) followed the same exercise protocol for 6 weeks. Each group was led by two blinded researchers in relation to the patient’s assessment data, with each researcher responsible for one of the treatment groups (CBG or HBG).

The CBG performed two supervised group exercise sessions per week, in groups of four patients. The HBG group had one weekly oriented exercise session and another session at home. The weekly oriented session lasted about 30 minutes, during which the patient received exercise instructions and adjustments in their activity diary. The second weekly session, the patient has to do the exercise at home. The selected exercises were based on previous studies that addressed pain and function management in individuals with OA (39-45) and included isometric quadriceps contraction, isotonic knee extension and flexion, sit-to-stand exercise, step-up and step-down exercise and quadriceps and hamstring stretching (figures 2,3).

According to patient evolution per patient progression and reports, exercise resistance was gradually increased according to the patient’s report.

Considering the minimum relevant difference of 13.3% in the Western Ontario and McMaster Universities Osteoar-
There were no statistically significant differences in COPap and COPml variables before and after exercise intervention, regardless of the group. An interaction (time and group) was observed for gait speed, demonstrating an increase in walking speed in patients of the CBG (table II).

**DISCUSSION**

This study investigated the postural control of patients with knee OA before and after an exercise protocol applied in a supervised clinic or home-based exercise group. Both groups did not show improvement in postural control variables, regardless of the mode of intervention. However, walking speed was superior in supervised clinic-based exercises.

Ghandali et al. (45) used a force platform to determine balance measurements in elderly knee OA patients after Tai Chi exercises. The balance assessments were similar to this study, and the groups were instructed to perform Tai Chi exercises for 8 weeks. They found a decrease in the area and speed of COP oscillation after Tai Chi exercises, both on rigid and unstable foam surfaces. The results of our study conflict with those of Ghandali et al. (45), as no significant differences were found in the COP area. Considering that Tai Chi exercises include relaxation, deep breathing, slow movements, and destabilization of the center of mass, promoting greater body awareness and flexibility (46), we believe that the type of exercise employed in the study explains this difference in results.

Costa et al. investigated the effect of using the undenatured collagen combined with a physiotherapy program on symptomatology, joint mobility, muscle strength, and knee joint function in patients with knee OA. Based on these findings, muscle strengthening exercises improved pain, mobility, strength, and function in knee OA patients. Pain reduction after strengthening exercises can be attributed to reversal of quadriceps weakness, which is one of the main conditions present in patients with knee OA (47).

**RESULTS**

Fifty-eight patients were enrolled to the study (figure 4), with 48 (35 females and 13 males) randomized into one of the exercise groups. The sample was homogeneous regarding anthropometric characteristics (table I), with no significant differences between the groups.

![Figure 4](image) Study flowchart for distribution of volunteers.

Table I. Characterization of the sample of knee osteoarthritis patients randomized into treatment groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CBG (n = 23)</th>
<th>HBG (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>17 (74 %)</td>
<td>18 (72 %)</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.22 ± 7.51</td>
<td>57.76 ± 5.46</td>
<td>0.42</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>85.60 ± 17.86</td>
<td>85.30 ± 15.43</td>
<td>0.95</td>
</tr>
<tr>
<td>Heigth (m)</td>
<td>1.59 ± 0.09</td>
<td>1.61 ± 0.08</td>
<td>0.59</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.61 ± 5.94</td>
<td>33.30 ± 7.28</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Data presented in % (percentage), mean ± standard deviation. CBG: supervised clinic-based exercise group; HBG: home based exercise group.
Although there are studies evaluating postural control in elderly individuals with knee OA (44-46), most of them use clinical tests to measure balance and compare healthy individuals with individuals with OA, being necessary to compare between the two groups of knee OA patients with different protocols (48). A recent meta-analysis investigating the influence of Tai Chi practice on walking speed and postural control in elderly individuals with knee OA (44) used indirect measures such as the 6-minute walk test and the WOMAC questionnaire to assess postural control ability. Authors claim that postures such as climbing and descending stairs, sitting and standing up, and maintaining both seated and standing positions and walking require balance. Thus, changes in these two indices may intuitively reflect the capacity for improving postural control. Even though clinical evaluations are important to assess balance, the gold standard measurement is force platform (12), leading to significant methodological differences between studies.

Regarding walking speed, patients showed better results after treatment, indicating post-exercise improvement in both groups. In other words, regardless of the intervention group, post-exercise results were better than pre-treatment. Although home-based exercise interventions for OA patients have reported good effectiveness in the literature (12, 27, 39-41) and offer benefits that do not significantly differ from individually supervised programs or clinic-based exercises (22), in countries like Brazil, this approach is not yet widely used (49). Cultural issues and concerns about treatment adherence could be reasons for the low implementation of this approach in our population. However, this study demonstrated that both approaches can be successfully implemented, reinforcing the possibility of using home-based exercises and weekly guidance, with the use of few materials and fewer visits to public rehabilitation clinics, thereby reducing expenses for patients and the healthcare system.

Some study limitations must be addressed. There was a lack of standardization in the elastic band resistance, with only patient-reported effort used to increase the load. Also, our sample was predominantly composed of females, which prevented the comparison of results between sexes. As strengths of the study, overall, both exercise programs were

### Table II. Comparison of postural control center of pressure (COP) in conditions of eyes open and eyes closed, with or without foam, in antero-posterior (AP) and medio-lateral (ML) directions (COPap and COPml) in patients with knee osteoarthritis who underwent clinic based exercise group (CBG) or home based exercise group (HBG).

<table>
<thead>
<tr>
<th></th>
<th>CBG (n = 23)</th>
<th>HBG (n = 25)</th>
<th>Anova (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time</td>
</tr>
<tr>
<td>COPap</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open Eyes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.23 ± 0.53</td>
<td>2.33 ± 0.75</td>
<td>0.59</td>
</tr>
<tr>
<td>Post</td>
<td>2.23 ± 0.52</td>
<td>2.28 ± 0.80</td>
<td>0.23</td>
</tr>
<tr>
<td>Close Eyes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.48 ± 0.76</td>
<td>2.53 ± 0.72</td>
<td>0.99</td>
</tr>
<tr>
<td>Post</td>
<td>2.35 ± 0.60</td>
<td>2.47 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>Open Eyes + Foam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.54 ± 0.77</td>
<td>4.31 ± 0.71</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>4.65 ± 1.09</td>
<td>4.20 ± 0.87</td>
<td></td>
</tr>
<tr>
<td>Close Eyes + Foam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>6.47 ± 1.45</td>
<td>6.05 ± 1.23</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>6.35 ± 1.40</td>
<td>6.17 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>COPml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open Eyes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.76 ± 0.63</td>
<td>1.95 ± 1.41</td>
<td>0.84</td>
</tr>
<tr>
<td>Post</td>
<td>1.73 ± 0.66</td>
<td>2.00 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>Close Eyes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.71 ± 0.64</td>
<td>2.07 ± 1.68</td>
<td>0.25</td>
</tr>
<tr>
<td>Post</td>
<td>1.85 ± 0.86</td>
<td>2.08 ± 1.65</td>
<td></td>
</tr>
<tr>
<td>Open Eyes + Foam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.95 ± 1.03</td>
<td>4.47 ± 1.15</td>
<td>0.11</td>
</tr>
<tr>
<td>Post</td>
<td>4.72 ± 1.09</td>
<td>4.20 ± 1.53</td>
<td></td>
</tr>
<tr>
<td>Close Eyes + Foam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>7.24 ± 2.19</td>
<td>6.78 ± 2.22</td>
<td>0.40</td>
</tr>
<tr>
<td>Post</td>
<td>7.09 ± 1.98</td>
<td>6.66 ± 2.24</td>
<td></td>
</tr>
<tr>
<td>Gait Velocity (m/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.60 ± 0.12</td>
<td>0.66 ± 0.19</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Post</td>
<td>0.68 ± 0.13</td>
<td>0.69 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation.
well tolerated, and the protocols were low-cost and easy to reproduce, making them more viable for the general public. Considering the impact that knee OA has on the general capacity of patients, both home-based and group exercises improve walking speed in this population, with no benefit in postural control. The possibility of easy practical application and the use of few materials to provide patient autonomy in their exercises are factors to be considered in implementing these exercises in the population. It is suggested that exercises to improve postural control be implemented in the protocols for these patients, as maintaining postural control in the aging population should be a health concern, in addition to addressing the clinical changes of OA.

CONCLUSIONS
Patients with knee OA undergoing either a group supervised clinic or home-based exercise intervention did not show improves in postural control after the treatment. Both groups demonstrated better gait speed after the stretching and lower limb strengthening exercise protocol.

REFERENCES

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

CONTRIBUTIONS
ESV: methodology, investigation, formal analysis, writing - original draft. JCS: methodology, investigation, formal analysis, writing - original draft. CBM: writing - original draft. MFS: conceptualization, methodology, supervision, writing-reviewing & editing. All authors: final approval.

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


SUMMARY

Objective. To investigate the effects of Manual Ischemic Compression (MIC) and Instrumental Ischemic Compression (ICG) and Pressure Algometer (ICA), in the treatment of women with MPS in descending trapezius.

Patients and Methods. This is a double-blinded, randomized, placebo-controlled trial. Patients were randomized into 3 groups: MIC, ICG, and ICA. Pain, pressure pain threshold (PPT), electromyography, disability (NDI), anxiety (GAD-7), and adverse treatment effects (AE) were assessed. The Shapiro-Wilk test was performed to verify the normality of the data, followed by consistent tests, being considered significant when p < 0.05.

Results. There was no intergroup difference for any analyzed variables. In the intra-group comparison, MIC group presented pain reduction (F: 7.70; p = 0.0002), between baseline and 1 week; and anxiety (p = 0.048), between baseline and 4 weeks. All groups showed increase in PPT (F: 37.62; p < 0.0001) and decrease in NDI score (F: 53.29; p < 0.0001). About AE, the MIC group reported the highest mean value of discomfort after the technique, 7.22.

Conclusions. An intragroup improvement was observed in the pain and anxiety variables for the MIC group, when compared to the baseline with one week and four weeks, respectively. There was an improvement in all groups in the PPT and NDI; however, with no differences between groups in the post-treatment.

Study registration. Brazilian Registry of Clinical Trials: ReBEC: RBR-2q24nb.

KEY WORDS
Neck pain; trigger point; manual therapy; IASTM; myofascial pain.
INTRODUCTION

Neck pain represents a significant portion of musculoskeletal disorders (1, 2). Among them, the Myofascial Pain Syndrome (MPS) stands out, a common clinical condition that occurs in about 46.1-55.4% of the population (3). Its etiology is associated with biomechanical (4) and emotional changes (5), repetitive movements (6), and excessive muscle contraction (5). The main feature is the emergence of trigger points (TP). Pain reduction and gain in ROM (19-21). Additionally, the pressure algometer is a similar instrument, although it does not belong to the IASTM group, used for pain assessment. Pain threshold, NDI score, GAD-7 score electromyography and adverse effects.

Participants and randomization

The study included 52 women at a mean age of 25.67 ± 5.71 years, 95% of the sample was composed of UFSC students. The volunteers were referred to the Locomotor Apparatus Evaluation and Rehabilitation Laboratory (LARAL) at UFSC, Araranguá campus, Mato Alto Unit (Santa Catarina, Brazil), the Free and Informed Consent Form was presented and signed, followed by the clinical evaluation. Data collection took place between October 2020 and February 2021. The inclusion criteria were: presence of active TP on descending trapezius; acute neck pain starting within 3 months; pain level equals to or greater than 3 (VAS) on the day of evaluation. Exclusion criteria were: presence of injury or self-reported limitation in the cervical or shoulder region; undergoing drug or physical therapy treatment for neck or shoulder pain; have used medications (painkillers, anti-inflammatories or muscle relaxants) within 48 hours before the evaluation. Following the Consolidated Standards of Reporting Trial (CONSORT) recommendations, the research team was composed of three evaluators: an independent evaluator who did the randomization, an evaluator who collected the variables, and a third evaluator, a physiotherapist with experience in the area, who performed all interventions. Finally, an external researcher monitored all stages of the research. The Research Randomizer website (www.randomizer.org) was used to randomize the volunteers into blocks and the result was allocated in sealed envelopes, which were opened by the physiotherapist at the time of each intervention. The volunteers were visually blinded by a dark blindfold, so they were unable to contact and identify their respective intervention groups. They also used a headset, playing music at high volume to prevent them from identifying noises coming especially from the pressure algometer. During the evaluations, the physiotherapist remained outside the laboratory. The same occurred with the evaluator during the interventions. Assessments were carried out by trained researchers, unaware of participants’ allocation.

Sample calculation

It was performed based on the main variable studied, subjective pain. The calculation was made using the G*Pow-
er version 3.1 software, for F tests, ANOVA with repeated measures, intra and intergroup interaction, effect size F 0.25, α 0.05, power (1-β) 0.95, for 3 groups and 5 measures, resulting in 13 volunteers per group, totaling a minimum of 39 subjects. However, 52 volunteers were evaluated, being: MIC = 18; ICG = 17; and ICA = 17.

Outcome measures
The main outcome variable was subjective pain, assessed using the Visual Analogue Scale (VAS). The other variables evaluated were: pressure pain threshold (PPT), assessed by pressure algometry; neck disability, assessed by neck disability index (NDI), generalized anxiety assessed by generalized anxiety disorder scale (GAD-7), and electromyographic parameters. In addition, adverse effects after intervention were investigated. The description of the outcomes measures are presented in Table I.

The data collection and intervention sequence was: a) Baseline (Evaluation form, VAS, TP location, NDI, GAD-7, PPT, EMG); b) Intervention (90 seconds of therapy); c) Adverse effects (AE) (Degree of discomfort); d) 10 minutes (VAS); e) 30 minutes (VAS, PPT, EMG); f) One week (VAS, NDI, GAD-7, AE); g) Four weeks (VAS, NDI, GAD-7, AE). The assessments were carried out by trained researchers, unaware of participants’ allocation.

Intervention
Initially, TP was located according to the main criteria (42): the presence of hypersensitive nodule, tense muscle band, limited range of motion, and when pressing the nodule, triggering familiar pain to the individual (43, 44). Palpation was performed medially to lateral and a dermographic pen was used to signal the TP location. In cases where there were more than one sensitized point or bilateral involvement, the intervention was performed on the most symptomatic TP (28).

During evaluation and intervention, the participant remained seated, in a neutral cervical position and the feet in total contact with the ground and the descending trapezoid totally exposed. During the intervention, the physiotherapist stood behind the volunteer. Each volunteer received only one session lasting 90 s, controlled by a chronometer, triggered when the pressure started to be performed (13). IC was applied using the dominant upper limb of the physiotherapist and the collection environment was air-conditioned to maintain a temperature of 20 °C (45).

Manual ischemic compression protocol
The physiotherapist applied pressure using the thumb directly on TP, slowly and gradually until reaching a sensation of moderate, but tolerable pain, VAS = 7, and maintained it during 90 s. When reaching this pressure, the volunteer should say “now” and, when the pressure was reduced by half, the volunteer should communicate it again so that the physiotherapist could increase the pressure (figure 1a) (13, 46).

Instrumental ischemic compression protocol with Gatilhex®
IC was performed with the Gatilhex® instrument (Reabilitech Mioblaster, São Paulo, Brazil - figure 1b), which was positioned directly on TP, perpendicularly. The pressure increased gradually, until reaching a moderate sensation of pain (VAS 7). When reaching this pressure, the volunteer should communicate it to the physiotherapist, by saying “now”, and when the pressure was reduced by half, she should report it back to the physiotherapist, so that the professional could increase the pressure, keeping the VAS score around 7 (13).

Instrumental ischemic compression protocol with pressure Algometer
IC was performed using an analog pressure algometer instrument, model NK-200 (Force Gauge, Elecall Electric, China, figure 1c), positioned perpendicularly to TP, and with a load referring to the average of the three values measured in the evaluation of LDP and maintained throughout the intervention (15).

Data analysis
The data were analyzed statistically with the GraphPad Prism® program, version 8.01 (GraphPad Software, La Jolla California, USA) by a researcher blinded to randomization and are presented as mean ± standard deviation. The normality of the data was verified with the Shapiro-Wilk test. For the analysis of electromyographic activity, the mean values of RMS of dynamic contraction and FMed of isometric contraction were used, and the 15 seconds of isometric contraction were subdivided into 5 windows of 3 s, to facilitate the analysis.

The outcomes pain, PPT and NDI were analyzed using repeated measures ANOVA and Tukey post-boc. GAD-7, RMS, and FMed were analyzed using Kruskal-Wallis test and Dunn post-boc.
Table I. Outcome measures.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Test/tool</th>
<th>Description</th>
<th>Evaluation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Visual Analogue Scale (VAS)</td>
<td>VAS is a unidirectional instrument that aims to measure the intensity of pain. It consists of a 10 cm line, in a continuum from 0 to 10, where 0 represents 'no pain' and 10 represents the 'worst pain'. Individuals were instructed to score the intensity of their pain at the time, by marking a number from 0 to 10 (25).</td>
<td>VAS was assessed in person, before the intervention, 10 min, and 30 min after the intervention; and electronically, through a follow-up form, one week and four weeks after the intervention.</td>
</tr>
<tr>
<td>Pressure Pain Threshold (PPT)</td>
<td>Pressure Algometry</td>
<td>PPT was measured using an analog pressure gauge, model NK-200 (Force Gauge, Elocall Electrical, China). The instrument was positioned perpendicularly to TP and increasing pressure was applied (26), in the proportion of 1 kg/cm²/s (Kilogram per square centimeter per second) (27, 28). The participant was instructed to remain relaxed and to signal when the sensation changed from pressure to pain, immediately interrupting the test (29).</td>
<td>Three measurements were performed, at an interval of 30 s between each one and, subsequently, the average of the values was calculated (29, 30). This procedure was performed at the pre-intervention and 30 minutes after.</td>
</tr>
<tr>
<td>Neck disability</td>
<td>Neck disability index (NDI)</td>
<td>NDI is a questionnaire developed and validated to investigate how pain in the neck region interferes with daily activities (31, 32). It has 10 sections with 6 alternatives each, and the participants are instructed to read and mark the one that most closely matches their symptoms. The scoring of the alternatives is given in ascending order from 0 to 5 and the questionnaire score ranges from 0 to 50 points. The score is 0-4 points, normal; 5-14, mild disability; 15-24, moderate disability; 25-34, severe disability; over 35, complete disability (31, 33).</td>
<td>It was applied in person, before the intervention, and then an electronic form was sent one week and four weeks after the evaluation.</td>
</tr>
<tr>
<td>Generalized anxiety</td>
<td>Generalized anxiety disorder scale (GAD-7)</td>
<td>GAD-7 is a simple questionnaire, reliable in diagnosing and monitoring the severity of generalized anxiety disorder. It features 7 sentences, each sentence has four alternatives: “none”, “several days”, “more than half the days” and “almost every day”, with the score given in ascending order, from 0 to 3, respectively (36). The questionnaire must be answered based on the 2 weeks preceding the evaluation date; nevertheless, in the evaluation, one week after the intervention, the volunteer was instructed to answer it based on the previously week. The final score ranges from 0 to 21, where 0-4 means no anxiety; 5-9, mild anxiety; 10-14, moderate anxiety; and above 15, severe anxiety (34).</td>
<td>The scale was applied in person, before the intervention, and after one week and four weeks via electronic form (37).</td>
</tr>
<tr>
<td>Outcome</td>
<td>Test/tool</td>
<td>Description</td>
<td>Evaluation time</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Electromyographic parameters</td>
<td>Surface electromyography (Miograph&lt;sup&gt;®&lt;/sup&gt; Software, Porto Alegre, Brazil) was used to evaluate the electrical signal of the descending trapezius muscle. The equipment has an analog to digital converter (A/D) with 16 bits of resolution, with 2000 Hz amplification, common rejection mode of 126 dB, input impedance 10 Ohm // 2pF, and bandpass filter of 20-500 Hz.</td>
<td>A line was drawn between the acromion and the 7&lt;sup&gt;th&lt;/sup&gt; cervical vertebra, and disposable electrodes (Kendall™, Mansfield, USA), made of polyethylene foam with hypoallergenic medical adhesive, adherent solid gel, and Ag/AgCl bipolar contact, (silver/silver chloride) were placed at the midpoint. The application followed the recommendation of SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles), with a distance of 2 cm between the poles, and the reference electrode was positioned over the styloid process ipsilateral limb ulna. Skin trichotomy was performed using a disposable razor, followed by asepsis with 70% alcohol (38). Initially, unilateral Isometric Maximum Voluntary Contraction (IMVC) was requested, with trunk control, on the side to be treated, for later data normalization. The participant was seated, in a neutral cervical position, with the arms along the body, and she was asked to perform a shoulder lift with a handle connected to the load cell. The movement was sustained in isometry for 5 s (39). For the assessment, the same position was maintained; however, without the shoulder strap. The volunteer repeated the shoulder lift dynamically 3 times, at an interval of 30 s between each measurement. Then, she repeated the movement, in an isometric way, 3 times for 15 s, at an interval of 30 s (39, 40). The analysis occurred in the time domain, using the Root Mean Square (RMS) percentage in relation to CVIM, and was presented in microvolts (µV). And in the frequency domain, through the median frequency (FMed), presented in Hertz. The mean value of dynamic and isometric contractions was used in the pre and post-treatment.</td>
<td>Measurement occurred pre-intervention and 30 minutes after. The signal processing was done in the MATLAB software, version R 2019 (MathWorks, Inc., Natick, USA), through a specific routine, developed by the laboratory.</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>Questionnaire</td>
<td>Each volunteer was asked, immediately after the intervention, about the degree of perceived discomfort, grading between 0, less discomfort, and 10, greater discomfort. A week later, an electronic form was sent with two questions, one to investigate if there were any symptoms in that period and the other to analyze whether these symptoms affected the activities of daily living (daily activities are understood as basic day-to-day activities, such as eating, personal hygiene and mobility) or work. Both were open questions, in which the participant was asked to describe details of the eventual symptom.</td>
<td>After intervention, a week later and four weeks later.</td>
</tr>
</tbody>
</table>
RESULTS

Participants
53 volunteers were recruited for face-to-face evaluation, with a sample loss of one volunteer for having VAS < 3. There was no sample loss after allocation or in the follow-up. The flowchart (figure 2) presents the research steps (CONSORT), and, at baseline, demographic and clinical characteristics were similar for all the groups (table II). All randomized participants were analyzed with an intention to treat approach.

Table II. Sample characteristics.

<table>
<thead>
<tr>
<th></th>
<th>MIC (n = 18)</th>
<th>ICG (n = 17)</th>
<th>ICA (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27 ± 6.05</td>
<td>25 ± 6.01</td>
<td>25 ± 4.47</td>
</tr>
<tr>
<td>Weight</td>
<td>63 ± 11.21</td>
<td>59.5 ± 15.04</td>
<td>62 ± 13.96</td>
</tr>
<tr>
<td>Height</td>
<td>1.64 ± 0.07</td>
<td>1.62 ± 0.09</td>
<td>1.64 ± 0.06</td>
</tr>
<tr>
<td>BMI</td>
<td>23.42</td>
<td>22.67</td>
<td>23.05</td>
</tr>
<tr>
<td>TP location (R/L)</td>
<td>14/4</td>
<td>12/5</td>
<td>9/8</td>
</tr>
<tr>
<td>VAS</td>
<td>4.18 ± 1.20</td>
<td>5.0 ± 1.44</td>
<td>4.5 ± 1.10</td>
</tr>
<tr>
<td>PPT</td>
<td>2.38 ± 0.56</td>
<td>2.46 ± 0.85</td>
<td>2.26 ± 1.31</td>
</tr>
<tr>
<td>NDI</td>
<td>14.5 ± 5.80</td>
<td>13.0 ± 6.14</td>
<td>11.0 ± 4.32</td>
</tr>
<tr>
<td>GAD-7</td>
<td>10.0 ± 4.87</td>
<td>9.0 ± 5.34</td>
<td>11.0 ± 5.05</td>
</tr>
</tbody>
</table>

Mean ± Standard deviation; MIC: Manual Ischemic Compression group; ICG: Ischemic Compression by Gatilhex group; ICA: Ischemic Compression by Pressure Algometer group; BMI: Body Mass Index; TP: trigger point; R: right; L: left; VAS: Visual Analogue Scale.

Effect of the intervention
After analyzing the data, it was found that there was no statistical difference in the intergroup comparison. However, in the intragroup comparison, the MIC group showed improvement in 4 of the 5 variables investigated, reduction of pain and of the scores of the applied questionnaires, NDI and GAD-7, and increase in PPT. It was also the group in which the average feeling of self-reported discomfort was higher, with no reported impairment in daily or work activities. While the ICG and ICA groups showed significant reductions for the variables PPT and NDI, they were also the ones that most reported painful symptoms or interference in Activities of the daily living (ADLS) and work activities after the procedure.

Pain
VAS was analyzed in the entire sample. The MIC group showed a reduction in pain between times (F: 7.70; p = 0.0002). The mean reduction was 2.17 cm between baseline and one week, going from the average value of 4.18 ± 1.20 to 2.61 ± 1.94 cm (p = 0.021; 95%CI = 0.21-4.13). There was no statistical difference between groups (F: 0.13; p = 0.87) (figure 3a).

Pressure pain threshold
All groups showed an increase, intragroup, in the comparison between baseline and 30 min (F: 37.62; p < 0.0001). The MIC group went from the mean value of 2.30 ± 0.56 kgf to 3.08 ± 1.12 (p = 0.002; 95%CI 0.32-1.23). The ICG group went from 2.44 ± 0.85 kgf to 2.97 ± 1.15 kgf (p = 0.015; 95%CI 0.11-0.93). And the ICA group went from 2.73 ± 1.30 kgf to 3.41 ± 1.50 kgf (p = 0.0002; 95%CI 0.38-0.96). In the intergroup comparison, there was no statistical difference (F: 0.71; p = 0.49) (figure 3b).

Generalized disability and anxiety index
There was an improvement in NDI between baseline and one week, and four weeks later (F: 53.29; p < 0.0001). The MIC group reduced the mean score in the comparison between base-
line and one week, from 14.50 ± 5.80 to 6.66 ± 4.27 (p < 0.0001; 95%CI -11.83 to -3.83), and baseline and four weeks (6.88 ± 4.71; p < 0.0001; 95%CI -11.61 to -3.61). The same happened in the ICG group, which went from the mean of 14.71 ± 6.14 in the baseline to 8.88 ± 6.34 after one week (p = 0.022; 95%CI -10.94 to -0.70), and to 8.47 ± 6.02 after four weeks (p = 0.013; 95%CI -11.35 to -1.11). The ICA group went from 12.47 ± 4.31 at baseline to 7.17 ± 5.73 after 4 weeks (p = 0.012; 95%CI -9.60 to -0.98). There was no intergroup difference (F: 0.45; p = 0.635, figure 3c).

As for GAD-7, in the MIC group, there was a reduction in the comparison between the baseline and 4 weeks after the intervention, going from the average value of 9.94 ± 4.86 to 6.16 ± 4.50 (F: 2.95; p = 0.048; 95%CI -7.54 to -0.01) (figure 3d). There was no intergroup difference (F: 0.25; p = 0.774).

Electromyography
There was no significant change in the electromyographic signal, for the RMS value (intergroup = F: 1.00; p = 0.37; intragroup = F: 0.01; p = 0.91). The same occurred with Fmed (intergroup = F: 0.915; p = 0.515, intragroup = F: 0.682; p = 0.841).

Adverse effects
For the adverse effects regarding the discomfort of the technique, the mean value was 7.22 points in the MIC group, 6.58 points ICG, and 6.23 in the ICA group. Sixteen volunteers reported some symptoms between 24h and one week after the intervention, and only 2 reported interference with their ADLS (according to these volunteers, there was an exacerbation of pain at the treatment site, within a period of up to 24 hours after, and this limited the movements of the ipsilateral upper limb and neck). Other symptoms are shown in table III.

Table III. Self-reported adverse effects.

<table>
<thead>
<tr>
<th></th>
<th>MIC (n = 18)</th>
<th>ICG (n = 17)</th>
<th>ICA (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain, n (%)</td>
<td>4 (22.2%)</td>
<td>5 (29.1%)</td>
<td>3 (17.3%)</td>
</tr>
<tr>
<td>Ipsilateral with remission within 24h</td>
<td>3 (16.6%)</td>
<td>1 (5.8%)</td>
<td>2 (11.4%)</td>
</tr>
<tr>
<td>Ipsilateral with remission up to 1 week</td>
<td>1 (5.5%)</td>
<td>2 (11.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Contralateral with remission within 24h</td>
<td>0</td>
<td>0</td>
<td>1 (5.8%)</td>
</tr>
<tr>
<td>Bilateral with remission within 24h</td>
<td>0</td>
<td>1 (5.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Irradiated</td>
<td>0</td>
<td>1 (5.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>-</td>
<td>1 (5.8%)</td>
<td>-</td>
</tr>
<tr>
<td>Numbness in the Upper Limb</td>
<td>0</td>
<td>1 (5.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Damage, n (%)</td>
<td>-</td>
<td>2 (11.7%)</td>
<td>1 (5.8%)</td>
</tr>
<tr>
<td>ADLS</td>
<td>0</td>
<td>2 (11.7%)</td>
<td>1 (5.8%)</td>
</tr>
<tr>
<td>Labor</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

n: Number of individuals; h: hours; ADLS: activities of daily living; MIC: Manual Ischemic Compression group; ICG: Ischemic Compression by Gatilhex group; ICA: Ischemic Compression by Pressure Algometer group.
DISCUSSION

The present study investigated the effects of manual and instrumental ischemic compression on pain, pressure pain threshold, disability level, generalized anxiety, and electromyographic activity in women with active descending trapezius TP, as well as the adverse effects of the techniques. Regarding pain, the reduction of 2.17 cm found in the MIC group is approximately 2.5 times greater than the 0.85 cm, which is the minimum clinically significant difference when measured by VAS (47, 48). The reduction in pain is thought to result from the improvement in local blood flow due to the mechanical stimulus applied to the muscle, which promotes sarcomere lengthening (7, 15, 49). The increase in tissue blood flow breaks the cycle of energy crisis, thereby interrupting pain signaling (50).

According to Bialosky et al. (51), after manual therapy, a cascade of physiological events occurs in both the central and peripheral nervous systems that control the response of inflammatory mediators and nociceptors. It is also known that the contact surface with the tissue, as well as the pressure applied, will stimulate different nociceptors responsible for the information input and consequent symptomatic effect (52, 53).

Thus, it is clear that 90 s of manual IC can reduce pain. In addition, this finding corroborates other studies that used a single IC intervention in the descending trapezius (27, 54, 55), as well as the systematic review by Cagnie et al. (13), which indicates the moderate effect of IC in the treatment of descending trapezius TP. The ICG group did not show a statistically significant reduction in pain, in contrast to studies investigating the effects of IASTM on pain (21, 57). However, most studies used larger instruments that treated the entire muscle or even a greater number of sessions to stimulate local circulation (16, 18).

The ICA group did not show much reduction in pain. It is possible that the fact of keeping the pressure constant for 90 s promotes tissue adaptation, but with a lower total pressure level to produce satisfactory hyperemia and break the actin-myosin bridge to desensitize the region (15). Studies using an algometer or similar device and gradually increasing pressure have shown a significant reduction in pain (22, 58, 59).

The increase in PPT corroborates the pain reduction obtained by VAS and indicates the local increase in blood supply produced by both friction on the tissue and sustained point compression, thus minimizing inflammatory responses and consequent sensitization of the region (60, 61). The ICA group showed a slightly higher gain than the others, confirming the findings of Abu Taleb et al. (22). The algometer is also used to measure this variable, suggesting that there may be tactile familiarization.

Furthermore, the results in the MIC group confirm the authors who obtained an increase in PPT after a single session (27, 62). While the increase in the ICG group confirms the results of studies using IASTM in the treatment of TP, both for the descending trapezius (16, 63) and for other muscle groups (57). When comparing manual therapy with IASTM, manual therapy is superior, confirming the results of the present study (16).

Another positive factor for both interventions was the reduction of the NDI score. The CIM group had an even greater reduction than the others, confirming other studies that used manual therapy in different protocols (14, 30, 58, 64). Nevertheless, studies using instruments also showed improvement in NDI scores and muscle function (16, 17, 24, 65), which is related to the reduction in pain found by both VAS and PPT, generating an increase in functionality (66). In addition, Simons (7) points out that the compressive and sustained stimulus on the TP restores the biomechanical properties of the muscle and its functionality.

Emotional factors can interfere and even trigger MPS. According to the present study, the groups presented an average score of 9.94 points on the GAD-7, representing mild anxiety (34), and after the intervention, the MIC group showed a reduction of 3.78 points, higher than the minimum clinically significant difference, which would be 3 points (67). Previous studies suggest that the relationship between pain and anxiety is directly proportional, such that individuals who report more pain tend to report more anxiety (68, 69).

In addition, women tend to have more anxiety in response to the painful experience (70, 71). Thus, it is reasonable to assume that the MIC group who experienced greater pain reduction may have also experienced a reduction in anxiety, supporting other studies that have used manual therapy in the cervical region and found a reduction in anxiety (72, 73).

Studies suggest muscle hyperactivity in the presence of active TP (12, 74) and changes in motor recruitment in the presence of musculoskeletal disorders (75-77). The present study found no changes in electromyographic activity after treatment, confirming the findings of Dibai-Filho et al. (78) who performed a similar study and found no changes in RMS and Fmed after a manual therapy session.

In terms of adverse effects, the group that received ICG was the most likely to report some type of discomfort that showed interference with ADLS, exceeding the 10% level that can be classified as a very common effect (79). Regarding the level of discomfort immediately after the intervention, the MIC group had the highest mean value and was the group with the greatest reduction in pain after treatment, suggesting that there is a relationship between the effect and the pressure exerted on the TP. However, further studies with larger samples should be conducted to investigate the effects of these therapies.
Finally, it is important to emphasize that the objective of the study was to compare three different modalities and the effects of manual compression were similar to instrumental in the sample and methodology used in the present study. Some limitations were identified in the study: protocol with only one intervention session, limited to the immediate effects; EMG and PPT were checked only on the affected side; no muscle strength test was performed to analyze the recruitment of a motor unit to produce the same strength before and after treatment.

CONCLUSIONS
In conclusion, an intragroup improvement was observed in the pain and anxiety variables for the MIC group, when comparing the baseline with one week and four weeks, respectively. However, no differences were observed between groups in the post-treatment. Additionally, the application of MIC showed fewer adverse effects than ICG and ICA.

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Effects of Ischemic Pre and Postconditioning on Indirect Markers of muscle damage: A Systematic Review and Meta-Analysis

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INTRODUCTION
Exercise-induced muscle damage (EIMD) results from following unaccustomed or eccentric exercise and is characterized by reduced strength and range of motion, delayed-onset muscle soreness (DOMS), swelling, increased creatine kinase (CK) activity and may lead to temporary functional impairments (1-3). EIMD generally leaves to transient ultrastructural myofibrillar disruption,
could generate mechanical alterations and metabolic stress (4). This kind of muscle damage stimulates the inflammatory system, that’s activate various cell types, like satellite cells, inflammatory cells (e.g., neutrophils, macrophages, T lymphocytes, mast cells), vascular cells and stromal cells (e.g., fibroblasts) (4). This inflammatory process is necessary to initiate tissue repair and remodeling, restoring the functional homeostasis of the damaged cell (5, 6). EIMD impairs muscle function, negatively affecting adherence to physical exercise programs (1-3).

Therapeutic methods including cryotherapy, massage, and photobiomodulation may accelerate the recovery from EIMD and help maintain the intensity and frequency of training (7-9). In addition, ischemic preconditioning (IPC) has also been used to mitigate EIMD consequences (10, 11). Ischemic conditioning consists of short periods (2 to 5 minutes) of intermittent vascular limb occlusion, followed by blood reperfusion cycles (10, 11). Ischemic conditioning may be applied before (ischemic preconditioning, IPCb) or after exercises (ischemic postconditioning, IPCa) (10, 11).

Recently, IPCb has been used to protect cardiac and skeletal muscles against ischemia-reperfusion (I/R) injury (12), defined as metabolic and contractile damage that occurs when blood supply returns to the tissue after a period of prolonged ischemia (13). Similarities, including increased intracellular calcium concentrations (14), blood muscle proteins (CK or lactate dehydrogenase (LDH)), and cytokine markers (interleukin-1, interleukin-2, interleukin-6, interleukin-7, tumor necrosis factor) and other factors, such as reactive oxygen species and c-reactive protein (12, 13, 15) have been observed between I/R injury and EIMD. IPCa could attenuates I/R injury and accelerate recovery after EIMD, because the technique mechanism involves the increase in post-ischemic blood flow (10), causing vasodilation (16, 17). Increased blood flow promotes changes in the sensitivity of ATP-sensitive K+ channels (KATP) (16), elevating adenosine levels (17) and reducing inflammatory process (18). Inflammation reduction is followed by decreased muscle swelling and intramuscular pressure, leading to reduced nociceptive stimulation and, consequently, attenuated DOMS (10, 19). Therefore, during the reperfusion period, vasodilation increases oxygen and nutrients supply to the muscles by improving substrate resynthesis, thus enhancing muscle function (17, 20).

IPCb could also be a helpful strategy in mitigating EIMD (21, 22). IPCb and eccentric exercise have similar effects regarding tissue damage (21, 22). For example, both lead to intramuscular acidosis, increased reactive oxygen species, and excessive immune response (21, 22). Franz et al. (21) verified that IPCb performed in eccentric exercise sessions of elbow flexors can attenuate EIMD and DOMS. According to this study, IPCb could attenuate EIMD through a down-regulation of pro-inflammatory signaling, reducing DOMS and CK activity (22).

Although some studies showed that IPCa effectively attenuates EIMD consequences and accelerate muscle recovery (10, 11, 21), others did not find differences between IPC and sham or control groups (20). Two recent systematic reviews investigated IPC effects on indirect markers of EIMD (23, 24). However, Ma et al. (23) did not perform meta-analysis in their review and included only one study about IPCb, limiting the analysis of this specific technique. In contrast, the results of a meta-analysis conducted by Arriel et al. (24) indicate that IPCa mitigates the increase in CK activity and DOMS. However, this meta-analysis grouped data extracted at 24, 48, and 72h after EIMD in a single time and omitted IPCb in their analyses.

The conduction of meta-analyses including IPCb and IPCa, considering subgroups analyses, would be essential to understand IPC effects on EIMD at different time points (i.e., 24, 48, and 72 h). Subgroup analyses are especially relevant for DOMS and CK activity outcomes because they tend to increase at 24h after EIMD and may last up to 72h (25, 26). Therefore, this systematic review and meta-analysis aimed to investigate the effects of IPCb and IPCa on indirect markers of EIMD. We hypothesize that IPCb and IPCa attenuate muscle damage and accelerate recovery. The attenuation of EIMD would occur by mitigating the DOMS response and CK activity, and a smaller reduction in performance in the IPC group than in the control.

**METHODS**

This systematic review was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (27).

**Eligibility criteria**

We considered randomized clinical trials published until June 2023 and evaluated the effects of IPCa or IPCb on indirect markers of EIMD. To be included, studies had to evaluate at least one indirect marker related to muscle damage, use some exercise modality to induce muscle damage (resistance or aerobic), and use sham IPC or no intervention as a control group. We did not include studies that used protocols without exercise, studies conducted on animals, pilots, protocols, expert opinion, book chapters, studies without a complete version or studies combining IPC with exercise.
Search strategy
We performed the searches in the National Library of Medicine (PubMed), Scopus, Web of Science, SPORTDiscus, Cochrane Central Register of Controlled Trials (CENTRAL), CINAH, PEDro, and Clinicaltrials.gov. Search terms derived from “ischemic preconditioning”, “muscle damage”, “blood occlusion”, “ischemic preconditioning”, “vascular occlusion”, and “recovery”. Searches occurred in two moments: one for IPCb and another for IPCa. The strategy used for IPCb was: (“Ischemic Preconditioning” AND “Muscle Damage”) and for BRFa was: (“Blood occlusion” OR “Ischemic preconditioning” OR “Vascular occlusion” AND “Recovery”). As filters we used complete studies, clinical trials, and human studies to guide the study selection process. We performed the last search in June 2023.

Screening of the studies
Two independent reviewers were responsible for screening the studies and a third reviewer resolved any disagreement regarding eligibility. Studies were stored on Mendeley® Desktop, and duplicates were removed. Subsequently, the studies were transferred to Rayyan QCRI® program (http://rayyan.qcri.org) (28). Titles and abstracts were read in Rayyan QCRI®, and only those meeting the inclusion criteria proceeded to the full-text screening. In case of unavailable full text or data or data that were not in mean and standard deviation, the corresponding authors were contacted via e-mail. A period of eight weeks was established for sending the information. If there was no response, the paper was excluded from the analysis.

Data extraction
We extracted the following data of the included studies: 1) characteristics of participants (sample size, training status, gender and age with mean and standard deviation); 2) indirect markers of EIMD (DOMS, CK, functional tests, muscle voluntary contraction, limb circumference); 3) intervention characteristics including cuff properties (cuff width, occlusion pressure, occlusion and reperfusion time, continuous or intermittent occlusion) and prescription mode (arbitrary or individualized); 4) comparison group including control (controlled time and conditions) or sham; 5) damaging protocol (segments involved, exercise modality, number of sets and repetitions, rest period, exercise load); 6) study design: randomized clinical trial (randomization between subjects, crossover, parallel); 7) time point measures (pre-exercise or post-exercise).

Assessment of risk of bias in included studies
Two independent reviewers assessed the risk of bias in included studies and a third reviewer was consulted in case of disagreement. We used the software RoB2 to assess risk of bias (29). Assessment was done using a series of signaling questions comprising five domains: randomization process, deviation from the intended interventions, absence of outcome data, outcome evaluation, and selection of reported results. Judgment was derived from algorithms based on the responses and is presented as “low risk”, “some concerns”, or “high risk”.

Data analysis
We used the Review Manager 5.4.1 software to conduct the statistical analyses and generate the forest plots. For included studies with data available in graph form only, the Image J (NIH, Maryland, USA) was used to calculate mean and standard deviation values. The random-effects model was used for analyses (30). To estimate heterogeneity between studies, the I² statistic was used, where I² = 25% was considered low, I² = 50% moderate, and I² = 75% high (26). For variables with the same measurement units and similar measurement methods, an analysis by Mean Difference (MD) was conducted. However, the Standardized Mean Difference (SMD) was used for discrepant measurements or those with distinct assessment methods. Subgroup analyses were conducted to assess different intervention time points (before, 24, 48, and 72h after EIMD). A delta (after - before) of the variables was used to calculate differences. For standard deviation, the formula used was: SD= √[(SDpre² / Npre) + (SDpost² / Npost)] (30).

RESULTS
Description of included studies
A total of 1055 scientific studies were retrieved in the databases (figure 1). After the removal of duplicates, 584 studies remained for title screening. Thirty-three studies proceeded to abstract screening, and the full-text version of 20 studies were screened for eligibility. Nine studies reporting data from 178 healthy participants (174 men and 4 women) were eligible for the systematic review (10, 11, 20, 21, 32-36). Studies were published between 2012 and 2021. The sample size of the included studies ranged from 12 to 30 participants, and participants were predominantly young adults, with a mean age ranging from 21.8 to 32 years. Four studies used a crossover/inter-subject design (10, 20, 33, 34), and five studies used a parallel design (11, 21, 32, 35, 36).
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Risk of bias

Only three studies detailed the randomization process (32, 34, 35), whereas six studies mentioned randomization but did not explain how it occurred (10, 11, 20, 21, 33, 36). Only one study detailed allocation concealment procedures and blinding (32). Three studies mentioned blinding the evaluators for some outcomes but not for others (32, 34, 35). Only one study reported adherence to a published protocol (32). All studies included appropriate measures for assessing the proposed outcomes, and eight performed statistical analyses consistent with the research objectives and registered measures (10, 20, 21, 32-36). The risk of bias of each study is represented in figure 2.

Moment of IPC application

Characteristics of the included studies are reported in two tables stratified by the moment of IPC application. Table I shows the summary of the three IPCb studies (21, 32, 36), and table II shows the summary of the six IPCa studies (10, 11, 20, 32-35).

Training status

Included studies reported data from healthy or recreationally active individuals (n = 4) (10, 11, 21, 23); individuals engaged in strength training programs (n = 2) (33, 36), or athletes including soccer players (n = 1) (34), rugby players (n = 1) (20), and trained cyclists (n = 1) (35).

Muscle-damaging protocol

Five studies used resistance exercise as the damaging protocol. Franz et al. (21) used maximum eccentric actions on the isokinetic dynamometer; while Northey et al. (33) used back squat and Page et al. (11) and Patterson et al. (36) used drop jumps. Three studies used aerobic exercise as the damaging protocol. Williams et al. (20) used 50 meters maximum sprint, Arriel et al. (35) used Maximal Incremental cycling test and Daab et al. (34) used the Loughborough Intermittent Shuttle Test. In turn, Beaven et al. (10) used resistance (squat jumps, countermovement jump, leg press) and aerobic exercises (maximal sprints of 40 meters) as muscle-damaging protocol.

Ischemic preconditioning before exercise (IPCb)

Three studies evaluated CK activity at baseline, 24, 48, and 72h post-EIMD (21, 32, 36). They also used a visual analog scale (VAS) to verify DOMS level and limb circumference as a parameter to measure muscle swelling at 24, 48, and 72h post-EIMD (21, 32, 36). Franz et al. (21) also measured tensiomyography to verify contraction speed and muscle stiffness.

Figure 1. Study flowchart.

Figure 2. Risk of bias graphs.
Table I. Summary of studies comparing the effects of IPCb on indirect markers of EIMD.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects and age; (M ± SD)</th>
<th>Markers</th>
<th>Damage protocol</th>
<th>Intervention</th>
<th>Control group</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franz et al. (2017)</td>
<td>19 (24.4 ± 4.2) healthy men without regular strength training</td>
<td>CK, Arm circumference, Muscle soreness (VAS) Tensiomyography</td>
<td>Eccentric exercise of bilateral elbow flexion with bar 3 × 10 of 80% of 1RM Rest: 1 min</td>
<td>3 × 5 min of I/R Width of cuff: 8 cm The proximal portion of the D and E arm RP: 200 mmHg</td>
<td>No intervention</td>
<td>CK is higher in control (24, 48 and 72 h); VAS is increased in control group (24 and 72 h). Tensiomyography was reduced in the control at all time intervals but did not change in IPCb. There was no intergroup difference for arm circumference</td>
</tr>
<tr>
<td>Cerqueira et al. (2021)</td>
<td>30 (21.9 ± 3.1) healthy men without regular strength training</td>
<td>CKR FFD Muscle soreness (VAS) pressure–pain threshold (Algometry) Knee flexion ROM Thigh circumference</td>
<td>120 maximal eccentric actions on isokinetic dynamometer 10 × 12 repetitions Rest: 30 s</td>
<td>I/R: 4 × 5 min Width of cuff: 20 cm Non-dominant lower limb Thigh proximal part Dorsal decubitus Individualized Doppler PRT</td>
<td>I/R: 4 × 5 min Width of cuff: 20 cm Non-dominant lower limb Thigh proximal part Dorsal decubitus RP: 20 mmHg</td>
<td>Muscle soreness reduced in the IPCb group in 48 h. The other variables had no significant difference between groups.</td>
</tr>
<tr>
<td>Patterson et al., (2021)</td>
<td>16 healthy trained men (23 ± 3)</td>
<td>CK Muscle soreness (VAS) MICV Thigh edema Countermovement jump</td>
<td>Drops jumps from a 0.6 m high box 5 × 20 reps with Rest: 2 min</td>
<td>3 × 5 min of I/R Width of cuff: 14.5 cm Thigh proximal part Bilateral RP: 220 mmHg</td>
<td>3 × 5 min of I/R Width of cuff: 14.5 cm Thigh proximal part Bilateral RP: 20 mmHg</td>
<td>IPCb group accelerated recovery in MICV and muscle swelling compared to sham. There was no difference between groups for countermovement jumping, muscle soreness, and CK.</td>
</tr>
</tbody>
</table>

CK: creatine kinase; RFD: rate of force development; VAS: visual analog scale; ROM: range of motion; IPCb: Ischemic preconditioning before exercise; TRP: total restriction pressure; RP: restriction pressure; MIVC: maximal isometric voluntary contraction; M: mean; SD: standard deviation.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Subjects and age (M ± SD)</th>
<th>Markers</th>
<th>Damage protocol</th>
<th>Intervention</th>
<th>Control group</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaven et al. (2012)</td>
<td>14 (32 ± 7) healthy subjects 10 men 4 women</td>
<td>3 squats jumps (90°) 3 countermovement jumps 6 repetitions on leg press 6 maximum sprints (40 m)</td>
<td>3 squats jumps (90°) 3 countermovement jumps 6 repetitions on leg press 6 maximum sprints (40 m)</td>
<td>I/R: 2 × 3 min Lower limb bilaterally Thigh proximal part RP: 220 mmHg</td>
<td>I/R: 2 × 3 min Lower limb bilaterally Thigh proximal part RP: 15 mmHg</td>
<td>IPCa showed beneficial effects in eccentric power and acceleration, squat jump, countermovement jump and jump height. There were small beneficial effects in sprint times (10 and 40 m).</td>
</tr>
<tr>
<td>Page et al. (2017)</td>
<td>16 (22.6 ± 2.8) recreationally active men</td>
<td>Pain (VAS) MICV Countermovement jump CK Recovery rate</td>
<td>Drops jumps from a 0.6 m high box 5 × 20 reps with 2 min rest between sets</td>
<td>I/R: 3 × 5 min I/R CW: 14,5 cm</td>
<td>Sham: 3 × 5 min I/R CW: 14,5 cm</td>
<td>CK and pain reduced in the IPCa compared to sham. There was no effect for thigh circumference and countermovement jump</td>
</tr>
<tr>
<td>Northey et al. (2016)</td>
<td>12 (24 ± 6.3) Men who perform strength Training</td>
<td>Recovery perception Muscle soreness PT quadriceps Squat jump Countermovement jump</td>
<td>10 ×10 back squats at 70% of 1 RM With 3 min rest between sets</td>
<td>I/R: 2 × 3 min Lower limbs unilaterally, alternating Thigh proximal part Dorsal decubitus RP: 220 mmHg 33 min lying in dorsal decubitus after BRFa</td>
<td>Control: Lie in dorsal decubitus for 45 min</td>
<td>There were no significant differences between conditions for any post-exercise measures</td>
</tr>
<tr>
<td>Williams et al. (2018)</td>
<td>24 (21.8 ± 3) Rugby players</td>
<td>Muscle soreness Recovery Perception Countermovement jumping CK</td>
<td>6 maximum sprints of 50 m 5 min rest between sprints</td>
<td>I/R: 2 × 3 min CW: 11 cm Lower limbs bilaterally Thigh proximal part RP: 60% of BP and thigh circumference</td>
<td>I/R: 2 × 3 min CW: 11 cm Lower limbs bilaterally Thigh proximal part RP: 15 mmHg</td>
<td>IPCa did not affect recovery in any of the variables</td>
</tr>
<tr>
<td>Arriel et al. (2018)</td>
<td>28 (27.1 ± 1.4) trained cyclists</td>
<td>CK Muscle soreness Power output</td>
<td>Maximal Incremental cycling Test (MICT) Starting at 40 W, increasing by 20 W/min to exhaustion I1: 2x 5 min 12:5x2 min CW: 21,5 cm Lower limbs bilaterally and alternately Thigh proximal part Dorsal decubitus RP: 50 mmHg above SBP</td>
<td>Sham1: 2 × 5 min Sham2: 5 × 2 min CW: 21,5 cm Lower limbs bilaterally and alternately Thigh proximal part Dorsal decubitus RP: 20 mmHg</td>
<td>There was no significant difference in power output or muscle soreness in 24 h compared with baseline values.</td>
<td></td>
</tr>
<tr>
<td>Daab et al. (2020)</td>
<td>12 (23.1 ± 1) semi-professional soccer players</td>
<td>Squat jump Countermovement jump MIVC Sprint 20 m CK Muscle soreness</td>
<td>Loughborough Intermittent Shuttle Test (LIST) 6 sets of 15 min (55 to 95% of estimated VO2max) Rest: 3 min</td>
<td>I/R: 3 × 5 min CW: 21.5 cm Lower limbs bilaterally Thigh proximal part Dorsal decubitus RP: 50 mmHg above SBP</td>
<td>I/R: 3 × 5 min CW: 21.5 cm Lower limbs bilaterally Thigh proximal part Dorsal decubitus RP: 20 mmHg</td>
<td>IPCa attenuated the decrease in Squat Jump, countermovement jump, MIVC and sprint. CK and LDH levels increased 24 h after in both conditions, but with a lower level in the IPC. Pain was significantly less in the IPCa 24 hours</td>
</tr>
</tbody>
</table>

CK: creatine kinase; VAS: visual analog scale; TRP: total restriction pressure; RP: restriction pressure; MVIC: maximum voluntary isometric contraction; PT: peak torque; CK: creatine kinase; IPCa: ischemic preconditioning after exercise. M: mean; SD: standard deviation; LDH: lactate dehydrogenase; CW: cuff width.
In contrast, Cerqueira et al. (32) measured pressure pain threshold using algometry and knee range of motion using a goniometer (Table I). Patterson et al. (36) evaluated DOMS, CK, swelling, countermovement jump, and maximal isometric voluntary contraction (MVIC). All studies showed beneficial effects in reducing DOMS in IPCb group compared with the control (21, 32, 36). Franz et al. (21) observed increased CK in control group compared with IPCb group. This study (17) also found that muscle stiffness measured by tensiomyography was reduced in control group despite the time point and did not show any changes in the IPCb group. Cerqueira et al. (32) found no statistically significant difference in CK, rate of force development, pressure pain threshold, and MVIC between sham and IPCb groups. Franz et al. (21) and Cerqueira et al. (32) showed no difference between groups regarding limb circumference. Patterson et al. (36) observed an attenuation of the loss of maximum voluntary contraction and muscle swelling in the IPCb group compared to sham group. Countermovement jump, DOMS, and CK activity were not different between groups (36).

**Ischemic preconditioning after exercise (IPCa)**

Five studies evaluated DOMS (11, 20, 33-35). Page et al. (11) and Williams et al. (20) evaluated thigh circumference to verify swelling. Five studies evaluated functional performance (10, 11, 20, 33, 34); Beaven et al. (10), Page et al. (11), Williams et al. (20), Northey et al. (33) and Daab et al. (34) evaluated countermovement jump, three evaluated squat jump (6, 29, 30), and two evaluated sprints (10, 34); four studies evaluated CK activity (11, 20, 34, 35); one study performed a neuromuscular evaluation measuring the knee extensors peak torque (33), and two used the maximum voluntary isometric contraction (MVIC) (11, 34). According to Page et al. (11) and Daab et al. (34), IPCa group showed decreased CK activity after EIMD (11, 34) and a significantly lower DOMS (7, 30). Similarly, IPCa group showed a faster recovery for MVIC (7), countermovement jump and squat jump (10, 34) than sham group. There were also beneficial effects on eccentric power and acceleration for the squat jump (10).

For sprinting, two studies showed a beneficial effect in IPCa group illustrated by less pronounced performance reduction (10, 34). Arriel et al. (35) showed that IPCa protocols maintained their performance in the maximum incremental test, performed 24h after EIMD.

Three studies reported no differences between IPCa and control groups concerning countermovement jump (11, 20, 33). The two studies evaluating thigh circumference did not notice differences between IPC and sham groups (11, 20). Concerning DOMS, three studies reported no significant difference between IPCa and control group for squat jump height and peak torque of knee extensors (33). Another study reported no difference between IPCa and sham groups for CK and LDH concentration (20). One study showed that IPC group demonstrated more fatigue than sham group (35) (Table II). According to Page et al. (11) and Daab et al. (34), IPCa group showed decreased CK activity after EIMD (11, 34) and a significantly lower DOMS (7, 30). Similarly, IPCa group showed a faster recovery for MVIC (7), countermovement jump and squat jump (10, 34) than sham group. There were also beneficial effects on eccentric power and acceleration for the squat jump (10).

**Summary of meta-analysis results**

**Descriptive results of IPCb studies**

Three studies evaluating IPCb were included in the meta-analysis. Forest plots were created using the variables: DOMS, CK activity, limb circumference and MVIC. Subgroup analyses were performed considering 24, 48, and 72h time points. The meta-analysis of MVIC was performed with data from Cerqueira et al. (32) and Patterson et al. (36), because these were the only studies analyzing MVIC. Results are reported in MD or SMD (95%CI) based on the methodological differences of each outcome.

**Delayed-onset muscle soreness (DOMS)**

IPCb showed a decrease in DOMS compared with the control group in 24h (SMD: -2.13; 95%CI -3.83 to -0.43; overall effect: p = 0.01), 48h (SMD: -3.62; 95%CI -4.47 to -2.77; overall effect: p < 0.00001) and 72h (SMD: -2.66; 95%CI -3.76 to -1.55; overall effect: p < 0.00001). The same is reflected for the overall effect meta-analysis (SMD: -2.8; 95%CI -3.67 to -1.92; overall effect: p < 0.00001). Howev-
er, heterogeneity was shown to be moderate to high ($I^2_{24h} = 83\%; I^2_{48h} = 0\%; I^2_{72h} = 54\%; I^2_{overall effect} = 77\%) (figure 3).

**Creatine Kinase (CK)**

IPCb showed no significant effects on CK activity compared with control in 24h (SMD: -1.17; 95%CI -3.06 to 0.71; overall effect: $p = 0.22$), 48h (SMD: -0.9; 95%CI -2.7 to 0.9; overall effect: $p = 0.33$) and 72h (SMD: -1.98; 95%CI -4.39 to 0.43; overall effect: $p = 0.11$). However, the overall effect meta-analysis favors the IPCb group (SMD: -1.27; 95%CI -2.22 to -0.31; overall effect: $p = <0.00001$). Heterogeneity was high ($I^2_{24h} = 90\%; I^2_{48h} = 90\%; I^2_{72h} = 92\%; I^2_{overall effect} = 88\%) (figure 4).

**Limb circumference**

Limb circumference was not different between groups in 24h (SMD: -0.37; 95%CI -0.86 to 0.13; overall effect: $p = 0.15$), 48h (SMD: -0.44; 95%CI -1.37 to 0.48; overall effect: $p = 0.35$), and 72h (SMD: -0.77; 95%CI -1.83 to 0.28; overall effect: $p = 0.15$). However, the overall effect meta-analysis showed that the IPCb group had circumference values of the lower limb compatible with less muscle swelling (SMD: -0.51; 95%CI -0.93 to -0.08; overall effect: $p = 0.08$). Heterogeneity was low to moderate ($I^2_{24h} = 0\%; I^2_{48h} = 68\%; I^2_{72h} = 73\%; I^2_{overall effect} = 51\%) (figure 5).

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<table>
<thead>
<tr>
<th>Outcome</th>
<th>Subgroup</th>
<th>Mean (SD) Control</th>
<th>Mean (SD) Sham</th>
<th>Mean Difference (95% CI)</th>
<th>$I^2$</th>
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<td></td>
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<tr>
<td>Conquesta et al., 2021</td>
<td>1.14 (0.8)</td>
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<td>Patterson et al., 2020</td>
<td>237.7 (4.7)</td>
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<td>452.5 (5.76)</td>
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<tr>
<td>Subtotal (95% CI)</td>
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<td>-2.13 (-3.83, -0.43)</td>
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<td><strong>Creatine Kinase (CK)</strong></td>
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<td>-2.86 (3.26, -5.55)</td>
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**Figure 3.** Forest plot for the comparison of ischemic preconditioning × control/sham. Outcome: muscle soreness (VAS cm); IPC: ischemic preconditioning; Mean: mean; SD: standard deviation; CI: Confidence Interval. $I^2$: heterogeneity.

**Figure 4.** Forest plot for the comparison of ischemic preconditioning × control/sham. Outcome: CK (U.L-1); IPC: ischemic preconditioning; Mean: mean; SD: standard deviation; Std. Mean difference: standardized mean difference; CI: Confidence Interval. F: heterogeneity.
Maximal Isometric Voluntary Contraction (MIVC)

There was no difference between groups at any time point: 24h (SMD: 19.4; 95%CI -48.18 to 86.45; overall effect: p = 0.58), 48h (SMD: 16.57; 95%CI -52.26 to 85.61; overall effect: p = 0.64), 72h (SMD: 21.82; 95%CI -36.72 to 100.36; overall effect: p = 0.59), and neither in the overall effect meta-analysis (SMD: -0.81; 95%CI -15.77 to 100.36; overall effect: p = 0.59). Heterogeneity was moderate to high (I²: 24h = 73%; 48h = 73%; 72h = 79%; overall effect = 60%) (figure 6).

Figure 5. Forest plot for the comparison of Ischemic preconditioning × control/sham.
Outcome: segment circumference (cm); IPC: ischemic preconditioning; Mean: mean; SD: standard deviation; Std. Mean difference: standardized mean difference; CI: Confidence Interval. I²: heterogeneity.

Figure 6. Forest plot for the comparison of Ischemic preconditioning × control/sham.
Outcome: maximal voluntary isometric contraction (N); IPC: ischemic preconditioning; Mean: mean; SD: standard deviation; Std. Mean difference: standardized mean difference; CI: Confidence Interval. I²: heterogeneity.
Ischemic preconditioning after exercise

Five studies evaluating IPCa were included in the meta-analysis. One study was excluded due to lack of data on mean and standard deviation. Analyses including DOMS, CK activity, squat, and countermovement jump were done separately. DOMS and countermovement jump were analyzed in three subgroups (24, 48, and 72h). The MVIC was not included in the meta-analysis due to the high heterogeneity among studies, and sprint could not be analyzed quantitatively due to data absence from one study (10). The study by Arriel et al. (35) was duplicated in the meta-analyses by using two different IPCa protocols. Outcomes including DOMS and CK were analyzed using SMD due to methodological differences (level of physical activity, instruments) and high heterogeneity among studies.

Delayed-onset muscle soreness (DOMS)

IPCa showed favorable results in time points of 24h (SMD: -1.96; 95%CI -3.56 to -0.36; overall effect: p = 0.02), 48h (SMD: -1.97; 95%CI -3.63 to -0.36; overall effect: p = 0.02), 72h (SMD: -2.99; 95%CI -3.94 to -2.03; overall effect: p < 0.00001) and in overall effect meta-analysis (SMD: -2.99; 95%CI -3.94 to -2.03; overall effect: p < 0.00001) (figure 7).

Creatine Kinase (CK)

IPCa group showed less CK concentration when compared to SHAM (SMD: -1.39; 95% CI; -2.26 to -0.51; overall effect: p = 0.002). Heterogeneity was moderate (I² = 63%) (figure 8).

**Figure 7.** Forest plot for the comparison ischemic postconditioning × control/sham.
Outcome: muscle soreness (VAS cm); IPC: ischemic postconditioning; F: heterogeneity; Mean: mean; SD: standard deviation; std. Mean difference: standardized mean difference; CI: Confidence Interval. F: heterogeneity; VAS: visual analogic scale.

**Figure 8.** Forest plot for the comparison ischemic postconditioning × control/sham.
Outcome: CK (U. L-1); IPC: ischemic postconditioning; F: heterogeneity; SD: standard deviation; std. Mean difference: standard mean difference; CI: Confidence Interval.
### Squat Jump Height

IPCa group was not different from the comparison group (MD: 1.49; 95% CI: -1.38 to 4.38; overall effect: p = 0.31). Heterogeneity was moderate ($I^2 = 55\%$) (figure 9).

### Countermovement Jump

The meta-analysis showed favorable results for IPCa at 24h (MD: 1.49; 95%CI 0.24 to 2.74; overall effect: p = 0.02), 48h (MD: 2.13; 95%CI 1.32 to 2.95; overall effect: p < 0.00001), 72h (MD: 1.55; 95%CI 0.59 to 2.52; overall effect: p = 0.002), and in the overall effect meta-analysis (MD: 1.72; 95%CI 1.13 to 2.30; overall effect: p < 0.00001). Heterogeneity remained moderate at 24h ($I^2 = 56\%$), and low in consecutive subgroups ($I^2_{24h} = 7\%$; $I^2_{48h} = 0\%$; $I^2_{72h} = 11\%$). Overall effect had a low heterogeneity ($I^2 = 33\%$) (figure 10).

### DISCUSSION

This systematic review aimed to analyze the evidence on IPC applied before (IPCb) or after (IPCa) exercise on indirect markers of EIMD. Our meta-analysis indicates that IPCb attenuates muscle soreness from EIMD. In addition, the reduction in countermovement jump height appeared to be less pronounced with IPCa, alongside its positive effects on reducing DOMS and CK activity compared with sham/control groups.

### Effects of IPCb on Indirect Markers of EIMD

We observed that IPCb attenuated DOMS compared with controls. There was no difference in limb circumference, CK activity, and MVIC for the 24, 48, and 72h. The positive effect of IPCb on muscle soreness may be related to the isch-
The authors describe the physiological adaptations as early and late (38). Early adaptations reflect significant increases in the antioxidant defense capacity (39), Ca\(^{2+}\) metabolism, and neural pathways regulation (40). Late adaptations lead to changes in gene expression and peripheral immune responses (18), which would cause a negative regulation of pro-inflammatory signaling with an associated decline in leukocyte-endothelial cell interaction via IPCb (41). Thus, IPCb may modify signaling pathways involved with DOMS and appear to attenuate muscle soreness that generally occurs following eccentric exercise (38, 40, 41).

Concerning CK activity and limb circumference, studies employed different methodologies, mainly differing in which limb served as a reference for measurement and comparison group (sham/control). Cerqueira et al. (32) and Patterson et al. (36) measured the lower limb circumference compared to a sham group. Franz et al. (22) performed EIMD in the upper limb and used no intervention as the control group. Furthermore, regarding MVIC, studies did not observe differences between groups at any time. Although CK activity is a valuable biomarker, it presents high variability among individuals (42), especially in athletes (43), and its measurement may not be sensitive for functional and eccentric activities (44). Furthermore, training level, muscle groups involved, and gender may influence CK activity more than differences in the volume of performed exercise (43). Due to the constant use of the eccentric action of knee extensors in daily living activities, generating significant damage in this region may be challenging (2). This challenge is demonstrated by Chen et al. (2) after inducing eccentric muscle damage in knee flexors and extensors. The authors observed reduced damage signs in knee extensors for the variables of strength (isometric and concentric isokinetic) and DOMS. In contrast, signs were much more evident in the upper limbs. There was no difference between groups for MVIC despite the time point assessed. This finding corroborates with the study conducted by Souza et al. (45), which did not observe differences in the number of repetitions performed or the MVIC between IPCb and sham groups followed by a knee extension protocol. Thus, IPCb acutely seems to have few local ergogenic effects on training volume variables, generalized peripheral fatigue or neuromuscular function for resistance exercise (45).

**Effects of IPCa on indirect markers of EIMD**

We observed that IPCa has positive effects in accelerating recovery for countermovement jump, DOMS, and CK. The squat jump height was not different from the comparison group. Due to data absence from one study, the sprint and power output variables could not be included in the meta-analysis (10). However, the two studies evaluating sprint demonstrated that IPCa positively affects recovery time, indicating accelerated muscular recovery (10, 34). Cunniffe et al. (46) compared different pressures (140, 160, and 180 mmHg) for ischemia in upper and lower limbs. They showed an increase in oxyhemoglobin and muscle oxygenation index above baseline with cuff deflation, which lasted up to 15 minutes in lower limb recovery, regardless of occlusion pressure. Thus, IPCa appears to increase muscle oxygenation in the reperfusion period following technique completion. In addition, IPCa may be related to the negative regulation of leukocyte circulation and increased nitric oxide (NO) (44), responsible for regulating intracellular and intercellular inflammation and muscle remodeling (47). NO also plays an essential role in reducing inflammation and promoting muscle repair (46, 47), further contributing with DOMS reduction. EIMD could also be attenuated by IPCa application through reactive vasodilation caused by cycles of blood restriction (17, 20). Increased blood flow would lead to more efficient and faster local responses, with increased oxygen and nutrient supply to the muscle (17, 20). Thus, it could reduce the inflammatory response and decrease CK activity and muscle swelling (17, 20). In turn, less pressure on involved tissues could reduce muscle-free nociceptor stimulation, reducing soreness (19).

Cellular effects might also be observed in IPCa, including changes in the sensitivity of KATP (16) and the elevation of adenosine levels (17). The increase in ADP/ATP ratio (*i.e.*, conditions where the oxygen supply is reduced and insufficient to meet the demands of metabolic tissues) leads to KATP channels opening and membrane hyperpolarization. Subsequently, vasodilation is a consequence of muscle cells relaxation in blood vessels (17). Moreover, when adenosine A3 receptors (17) are stimulated, serum concentrations of CK may decrease after an eccentric exercise protocol (23). In this way, a better activation of ATP in potassium channels and increased blood muscle volume (which would increase the availability and delivery of oxygen to the muscle) (17, 20). In turn, the reduction in height in countermovement jump could be less pronounced because the decreased serum concentration of CK, helping to create a favorable environment for muscle contraction.

No difference between IPCa and control groups was observed. The absence of differences between groups could be partially explained by differences between the studies, in which heterogeneity was moderate (I\(^2\) = 55%), especially concerning the type of damage protocol and participants characteristics. Daab et al. (34) used an aerobic protocol in a sample of soccer players, contrasting with Northey et al. (33) that used a resistance exercise protocol (back squat) in a sample of men practicing resistance training.
Another important aspect that may have interfered in results is the level of physical activity and the type of exercise used. According to Caru et al. (48) healthy and recreationally active individuals seem to benefit more from the effects of IPC. Due to mechanical and metabolic causes of EIMD, IPCa appears to have significant action on muscle metabolism and some activity on mechanical aspects (48). Meanwhile, aerobic exercises are similar to IPC in terms of having greater metabolic predominance (48). In this sense, aerobic exercises appear to be more beneficial to IPC than resistance exercise, which has a predominance of mechanical aspects (48). Therefore, individuals submitted to resistance training or to a high level of physical activity could benefit less from IPC (48).

Due to the similarities between the physiological aspects of the IPCb and the IPCa, information from the review by Caru et al. (48) can be used to try to explain the outcome for the squat jump. Because the difference in the sample and in the muscle damage protocol used between Northey et al. (33) (protocol: back squat, sample: trained men) and Daab et al. (34) (protocol: Loughborough Intermittent Shuttle Test, sample: football players), results may be contradictory, requiring attention to data interpretation.

In contrast with the qualitative analyses performed in the review conducted by Ma et al. (23) or the focus on IPCa presented in the review by Arriel et al. (24), our review adopted a quantitative approach with meta-analysis and stratified IPCb and IPCa effects. Our data analysis was divided into subgroups (24, 48, 72h) for DOMS and countermovement jump. Therefore, this review filled some existing gaps in literature by comparing IPCb and IPCa quantitatively.

Limitations

Differences were identified in intervention characteristics, damage protocols, and outcomes. Each author used a different muscle damage protocol with varied metabolic characteristics, intensity, duration, and rest time. The differences between the sample could explain the moderate to high heterogeneity found in most meta-analyses ($I^2 > 50\%$) (26). Due to the variation in our results and the fact that some studies evaluated up to 24h after the EIMD, some meta-analyses were conducted with only two studies, which compromises its robustness. Although this review included only clinical trials, the quality of the evidence was affected by the small sample sizes of included studies, absence of allocation concealment strategies, lack of explanation on randomization process, and a lack of description concerning blinding of participants and evaluators. Sample size calculation was absent in most studies (10, 11, 20, 21, 33, 35, 36). In addition, interventions were heterogeneous, especially regarding the restriction pressure, which was not individualized in most studies (10, 11, 21, 33, 36). I/R cycles ranged from 12 to 30 minutes in the IPCa studies and 30 to 40 minutes in the IPCb. Therefore, results should be interpreted with caution.

CONCLUSIONS

Current evidence supports that IPCb can attenuate DOMS. In turn, IPCa could contribute to recovery in countermovement jump and to attenuate CK activity. This systematic review emphasizes the need for clinical trials with adequate sample size, allocation concealment, and blinding of evaluators and participants. Future clinical trials should consider choosing specific and validated outcomes for assessing EIMD such as indirect markers (CK, DOMS, functional tests, neuromuscular evaluations), employing validated methods to find IPC restriction pressure, individualizing restriction pressures, using tables to display the results, and improving control over selection bias when adopting sampling strategies.

Practical applications

Ischemic preconditioning before (IPCb) or after (IPCa) exercise would be promising strategy to mitigate delayed-onset muscle soreness. Besides, IPCa appeared to accelerate recovery for countermovement jump and attenuate the rise in CK activity compared with control/sham. This technique could be more interesting for athletes, especially during competition periods, as they need to maintain performance and accelerate recovery. In addition, it is important to verify the most appropriate protocols for users and the modalities practiced, thus being able to use the IPC in the most suitable and specific way.

FUNDINGS

This systematic review was conducted with the authors’ own funding and no external sources.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

IMF, MSC: conceptualization. IMF, MSC, YMB: design, coordination. IMF: writing – original draft. SDP, WHBV: writing – review & editing.

ACKNOWLEDGMENTS

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


SUMMARY

Background. Recent narrative reviews suggest an association between lower extremity tendinopathies and metabolic and chronic diseases. This association might lead to early recognition and change in clinical management, but it has, however, never been assessed systematically.

Objective. To analyze the association between lower extremity tendinopathies and metabolic and chronic diseases in a systematic review. We searched studies in Embase, Medline Ovid, Web of Science, Cochrane library and Google Scholar. Articles were eligible if the association between clinically diagnosed lower extremity tendinopathies and a metabolic or chronic disease in adult patients was reported.

Results. From 4,287 eligible studies, we included 10 cohort studies and 10 case-control studies, involving 83,948 participants. Almost all (90%) included studies were assessed as having a high risk of bias. These studies had moderate evidence for an association between lower extremity tendinopathies and obesity, ankylosing spondylitis, psoriatic arthritis, and reactive arthritis. There was limited evidence for an association between lower extremity tendinopathies and heterozygous familial hypercholesterolemia, and Systemic Lupus Erythematosus.

Conclusions. We found multiple associations between lower extremity tendinopathies and metabolic and chronic diseases. These results suggest that medical professionals should screen for these specific metabolic and chronic diseases in patients with lower extremity tendinopathies.

Study registration. The review has been prospectively registered in the international PROSPERO database. Protocol details were submitted in June 2019 and registered in September 2019 (registration number: CRD42019140317).

KEY WORDS

Epidemiology; infection; inflammation; risk factors; tendon.
INTRODUCTION
Tendinopathies of the lower extremity and metabolic and chronic diseases, such as diabetes, occur frequently in the general population (1-5). Previous narrative reviews suggest a link between both conditions (6-8).

Lower extremity tendinopathies can be chronic, impact negatively on quality of life and have substantial socioeconomic consequences (9, 10). The etiology is mainly unknown, but degeneration and inflammation are hypothesized to play an important part in the pathogenesis (8, 11). There may be subgroups of lower extremity tendinopathies with variable underlying causes, in which metabolic or chronic diseases may play a role (6, 7).

Inflammation is suggested to be the key mechanism occurring both in tendinopathies as well as in metabolic and chronic diseases (6, 12, 13). In tendons, tenocytes and immune cells produce pro-inflammatory cytokines in response to loading (13). These pro-inflammatory cytokines affect several complex pathways and interactions, which may promote tendon healing and repair. On the other hand, persisting or recurring pro-inflammatory responses are thought to induce tendinopathy (7, 14-16). Metabolic and chronic diseases, such as obesity, diabetes and hypercholesterolemia, also lead to increased production of local or systemic low-grade pro-inflammatory cytokines (17). For instance, in diabetes, the increased blood glucose levels raise the production of Advanced Glycation End-products (AGE’s), which causes pro-inflammatory responses (18). The above-mentioned hypotheses imply that there is an association between tendinopathies and metabolic or chronic diseases. Awareness of this association could lead to early recognition and management of metabolic and chronic diseases in patients with lower extremity tendinopathies. To date, the association between tendinopathy and metabolic or chronic diseases has never been systematically examined. Therefore, we conducted this systematic review with the primary aim to analyze whether there is an association between lower extremity tendinopathies and metabolic and chronic diseases.

MATERIALS AND METHODS
Protocol
This systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) statement (19).

Search strategy
We conducted a search strategy in multiple databases with the assistance of a medical librarian (WM Bramer). Embase, Medline Ovid, Web of Science, Cochrane library and Google Scholar were searched up to the October 9, 2023. The search strategy is shown in appendix 1.

Eligibility criteria
The following lower extremity tendinopathies were included: adductor tendinopathy, hamstring tendinopathy (proximal and distal), quadriceps tendinopathy, patellar tendinopathy, tibialis posterior tendinopathy, peroneal tendinopathy, Achilles tendinopathy (insertional and midportion) and plantar fasciopathy. The diagnosis of tendinopathy should be based on clinical findings. Imaging was not deemed necessary for establishing the diagnosis and studies describing tendinopathy defined by imaging findings only were excluded. We did not pre-define specific metabolic conditions or chronic diseases as we aimed to provide an extensive overview of all possible associations with common tendinopathies. Examples of included metabolic and chronic diseases were obesity (body mass index (BMI) ≥ 30 kg/m²), metabolic syndrome, diabetes, dyslipidemia, cardiovascular disease, hypertension, thyroid dysfunction (hyper and hypothyroidism), rheumatic disease, renal failure, inflammatory bowel disease (such as Crohn’s and Colitis Ulcerosa), sarcoidosis, infectious diseases, polycystic ovarian syndrome (PCOS) and fibromyalgia. We did not pre-define specific criteria for metabolic disorders or chronic disease, as we did not pre-define specific conditions. Studies were excluded if 1) the population was aged younger than 18 years, 2) there was no adequate control group (e.g., contralateral tendon), 3) it was conducted in animals or 4) in the laboratory (preclinical in vitro studies), 5) the design was a case report or 6) the article was not available in English.

Study selection and data extraction
Titles and abstracts of all eligible articles were screened independently by two researchers (I.L. and B.N.). The same researchers read all included articles full-text. Disagreements were resolved by discussion, with the involvement of a third researcher (RV) if necessary. References of the included studies were screened for relevant studies that were not identified by the search strategy. In case of unpublished records, authors were contacted for availability of their data. We uploaded all selected studies to the Covidence platform (Melbourne, Australia). This not-for-profit management system facilitates an independent data selection, data extraction, and risk of bias assessment when performing systematic reviews.

Two researchers (I.L. and B.N.) performed data extraction and recorded study design, number of participants, study population, type of tendinopathy, type of metabolic or chronic diseases, outcome measures, duration of follow-up and conclusion(s) using standardized data extraction forms. We noted the diagnostic criteria used to establish tendinopathy, type of imaging used (if applicable), severity of pain (expressed by patient-reported outcome measure), duration of tendinopa-
thy, participation in sports activities, and whether the pain was unilateral or bilateral. For metabolic and chronic diseases, we noted the definition, the associated measurements (e.g., laboratory values, body mass index (BMI)), use of medication, and duration of the condition since the diagnosis.

Risk of bias assessment
Two reviewers (I.L. and B.N.) independently assessed risk of bias (ROB) of the included studies using a standardized form, the Newcastle-Ottawa quality assessment Scale (NOS) (appendix 2). Studies could receive a total of 4 stars in the selection domain (selection of cases), 2 stars in the comparability domain (whether the study corrects for variables) and up to 3 stars in the outcome/exposure domain (objectivity of the main outcome). The pre-defined thresholds for converting the NOS to good, fair and poor were as follows (20):
1. Good quality: ≥ 3 stars in selection domain AND ≥ 1 star(s) in comparability domain AND ≥ 2 stars in outcome/exposure domain.
2. Fair quality: 2 stars in selection domain AND ≥ 1 star(s) in comparability domain AND ≥ 2 stars in outcome/exposure domain.
3. Poor quality: 0-1 star(s) in selection domain OR 0 stars in comparability domain OR 0-1 star(s) in outcome/exposure domain.

Data synthesis
We considered pooling the data if studies were sufficiently homogeneous from both a statistical and clinical point of view. If data could not be pooled because of heterogeneity, we planned to perform a best evidence synthesis. If a best evidence synthesis was indicated, we dichotomized “good quality” to “high quality”, “Fair quality” and “poor quality” was deemed “low quality” in the best evidence synthesis. The best evidence synthesis holds five levels of evidence (21):
1. Strong evidence: ≥ 2 studies of high quality and generally consistent findings in all studies (≥ 75% of the studies report consistent findings).
2. Moderate evidence: 1 study of high quality and/or ≥ 2 studies of low quality and generally consistent findings in all studies (≥ 75% of the studies reporting consistent findings).
3. Limited evidence: 1 study of low quality.
4. Conflicting evidence: inconsistent findings in multiple studies (< 75% of the studies report consistent findings).
5. No evidence: no studies could be found.

Data analysis
Presented Odds Ratios (ORs) and their 95% confidence intervals (CIs) from cross-sectional and longitudinal case-control studies were used. In case the OR was not presented in the article, we chose to calculate the OR with the following formula: exposed cases × not exposed controls / exposed controls × not exposed cases. Results were considered statistically significant if the 95% confidence interval (CI) did not cross 1.

For the included single arm cohort studies, there was no control group with the presented outcome (tendinopathy or metabolic conditions and chronic diseases). In these cases, we decided to compare these data to existing data in the scientific literature about the prevalence of the outcome in the general population. Through this method, we were also able to estimate the OR in these single arm cohort studies. This study was identified by searching the MeSH term of the outcome in the missing study in combination with “/epidemiology” on PubMed on November 1, 2019.

RESULTS
Study selection
After database searching, we identified 6,469 records; 4,287 studies remained after duplicates were removed (figure 1). Of all articles, 4,186 were excluded after title and abstract screening. A total of 101 full-text articles were assessed for eligibility, of which 82 were excluded. One additional record was included through

![Figure 1. PRISMA flowchart of included articles.](image-url)
reference screening. The remaining 20 publications, involving 83,948 participants, were included for analysis (22-41).

**Description of included studies**

We included 10 case-control and 10 cohort studies. The mean age of the participants in these studies ranged from 37 to 69 years, holding 0 to 84% males (appendix 3). Reported tendinopathies were Achilles tendinopathy (n = 16), plantar fasciopathy (n = 8), patellar tendinopathy (n = 1) and gluteal tendinopathy (n = 1). Reported metabolic and chronic diseases were obesity (n = 4) (22, 32, 34, 36-38), diabetes (n = 4) (22, 23, 32, 35), hypertension (n = 3) (23, 32, 35), hypercholesterolemia (n = 2) (35, 41), heterozygous familial hypercholesterolemia (n = 2) (26, 39), ankylosing spondylitis (n = 3) (24, 25, 30), psoriatic arthritis (n = 3) (27-29), rheumatoid arthritis (n = 3) (30, 31), reactive arthritis (n = 2) (30, 40), and Systemic Lupus Erythematosus (n = 1) (33). In appendix 3, we present the data extraction table with the following items: year of publication, study design, baseline participant characteristics, primary aim, inclusion criteria disease, outcome disease and duration of follow-up. In appendix 4 we present the characteristics of lower extremity tendinopathies and metabolic and chronic diseases.

**Risk of bias assessment and best evidence synthesis**

Due to the clinical heterogeneity of the included participants, variability in reported associations and low methodological quality of the studies, it was not possible to perform statistical pooling of the data. We therefore carried out a best evidence synthesis. When dichotomizing the risk of bias assessment, all but two studies were of poor quality (table I; there was one study with good methodological quality and one study with fair quality). The poor risk of bias score was frequently related to lack of adjustment for confounders.

**Associations between metabolic or chronic diseases and lower extremity tendinopathies**

Eight case-control (22, 23, 32, 34-36, 38, 41) and two cohort studies (37, 39) investigated whether having lower extremity tendinopathies is associated with an increased risk of having

<table>
<thead>
<tr>
<th>Table I. Risk of bias (RoB) assessment of the included studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selection</strong></td>
</tr>
<tr>
<td>Abate et al., 2016 (22)</td>
</tr>
<tr>
<td>Abate et al., 2018 (23)</td>
</tr>
<tr>
<td>Aggarwal et al., 2009 (24)</td>
</tr>
<tr>
<td>Alam et al., 2017 (25)</td>
</tr>
<tr>
<td>Beeharry et al., 2006 (26)</td>
</tr>
<tr>
<td>Cantini et al., 2001 (27)</td>
</tr>
<tr>
<td>Çatal et al., 2021 (41)</td>
</tr>
<tr>
<td>Elkayam et al., 2000 (28)</td>
</tr>
<tr>
<td>Galluzo et al., 2000 (29)</td>
</tr>
<tr>
<td>Gerster et al., 1977 (30)</td>
</tr>
<tr>
<td>Hernandez-Diaz et al., 2019 (31)</td>
</tr>
<tr>
<td>Holmes et al., 2006 (32)</td>
</tr>
<tr>
<td>Jarrot et al., 2015 (33)</td>
</tr>
<tr>
<td>Klein et al., 2013 (34)</td>
</tr>
<tr>
<td>Kraemer et al., 2012 (35)</td>
</tr>
<tr>
<td>Owens et al., 2013 (36)</td>
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<tr>
<td>Plinsinga et al., 2018 (37)</td>
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<tr>
<td>Riddle et al., 2003 (38)</td>
</tr>
<tr>
<td>Singh, 2015 (39)</td>
</tr>
<tr>
<td>Smith et al., 1980 (40)</td>
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</tbody>
</table>

Good quality: > 3 stars in selection domain AND > 1 star(s) in comparability domain AND > 2 stars in outcome/exposure domain. Fair quality: 2 stars in selection domain AND ≥ 1 star(s) in comparability domain AND > 2 stars in outcome/exposure domain. Poor quality: 0-1 star(s) in selection domain OR 0 stars in comparability domain OR 0-1 star(s) in outcome/exposure domain.
Lower Extremity Tendinopathies are Associated with Metabolic and Chronic Diseases

Obesity

There is moderate evidence that patients with lower extremity tendinopathies have an increased risk of being obese (figure 2A, appendix 5). One case-control study in elderly patients (included when age > 65 years) reported no association (22). Four case-control studies and one cohort study showed that having lower extremity tendinopathies is associated with an increased risk of being obese (32, 34, 36-38). Obesity was defined by all studies as having a BMI of ≥ 30 kg/m². The prevalence of obesity in patients with lower extremity tendinopathies ranged from 11-62% (23, 32, 34, 36-38), while the prevalence in the general population is 12% (42).

No studies were conducted that had obesity as primary inclusion criterion and lower extremity tendinopathies as outcome.
Table II. The association between lower extremity tendinopathies and metabolic and chronic diseases.

<table>
<thead>
<tr>
<th>Metabolic or chronic disease</th>
<th>Study (first author and reference number)</th>
<th>Best evidence synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>Abate et al. (a) = (22), Holmes et al. ↑(32), Klein et al. ↑(34), Owens et al. ↑(36), Plinsinga et al. ↑(37), Riddle et al. ↑(38)</td>
<td>Moderate evidence for a positive association</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Abate et al. (b) = (23), Holmes et al. = (32), Kraemer et al. = (35) Abate et al. ↑(22)</td>
<td>Moderate evidence for no association</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Abate et al. (b) = (23), Kraemer et al. = (35), Holmes et al. ↑(32)</td>
<td>Conflicting evidence</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>Çatal et al. ↑(41), Kraemer et al. = (35)</td>
<td>Conflicting evidence</td>
</tr>
<tr>
<td>Heterozygous familial</td>
<td>Singh et al. = (39)</td>
<td>Limited evidence for no association</td>
</tr>
<tr>
<td>hypercholesterolaemia</td>
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<td></td>
</tr>
</tbody>
</table>

Studies with a metabolic and chronic disease as primary inclusion criteria and lower extremity tendinopathies as outcome

<table>
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<tr>
<th>Metabolic or chronic disease</th>
<th>Study (first author and reference number)</th>
<th>Best evidence synthesis</th>
</tr>
</thead>
<tbody>
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<td>Heterozygous familial</td>
<td>Beeharry et al. ↑(26)</td>
<td>Limited evidence for a positive association</td>
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<td>hypercholesterolaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>Aggarwal et al. ↑(24), Alam et al. ↑(25), Gerster et al. ↑(30)</td>
<td>Moderate evidence for a positive association</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>Cantini et al. ↑(27), Elkayam et al. ↑(28), Galluzzo et al. ↑(29)</td>
<td>Moderate evidence for a positive association</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Gerster et al. = (30), Hernandez-Diaz et al. = (31)</td>
<td>Moderate evidence for no association</td>
</tr>
<tr>
<td>Reactive arthritis</td>
<td>Gerster et al. ↑(30), Smith et al. ↑(40)</td>
<td>Moderate evidence for a positive association</td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>Jarrot et al. ↑(33)</td>
<td>Limited evidence for a positive association</td>
</tr>
</tbody>
</table>

**Diabetes**
There is moderate evidence that lower extremity tendinopathies are not associated with an increased risk of having diabetes (figure 2B, appendix 5). One case-control study showed that patients with lower extremity tendinopathies had an increased risk of having diabetes (22), while three case-control studies found no association (23, 32, 35). Diabetes was defined by the studies as self-reported (n = 1), confirmed diagnosis by an endocrinologist (n = 1), or receiving treatment for diabetes (n = 2). The reported prevalence of diabetes in patients with lower extremity tendinopathies was 1-42% (22, 23, 32, 35) versus 14% in the general population (44). No studies were conducted that had hypertension as primary inclusion criterion and lower extremity tendinopathies as outcome.

**Hypertension**
There is conflicting evidence on patients with lower extremity tendinopathies and the risk for hypertension (figure 2C, appendix 5). Two case-control studies with active patients found no association (23, 35), while one case-control study demonstrated that patients with lower extremity tendinopathies had an increased risk of having hypertension (32). Hypertension was defined by the studies as self-reported (n = 1), or a systolic blood pressure of > 140 mmHg and/or a diastolic blood pressure of > 90 mmHg (n = 2). The prevalence of hypertension in patients with lower extremity tendinopathies was 10-52% (23, 32, 35), and 32% in the general population (44). No studies were conducted that had hypertension as primary inclusion criterion and lower extremity tendinopathies as outcome.

**Hypercholesterolemia**
There is limited evidence that having lower extremity tendinopathies is not associated with an increased risk of having hypercholesterolemia (figure 2D, appendix 5). One case-control study found that patients with lower extremity tendinopathies had an increased risk of developing hypercholesterolemia (41), while one other study found no association (35). Hypercholesterolemia was defined by the studies as a cholesterol of ≥ 240 mg/dL or as self-reported (35, 41). The prevalence of hypercholesterolemia in patients with lower extremity tendinopathies ranged from 11% to 55%, and from 8% to 33% in the healthy control group (35, 41). There were no studies conducted where hypercholesterolemia was the primary inclusion criterion and lower extremity tendinopathies the outcome.
Heterozygous familial hypercholesterolemia
There is limited evidence that having lower extremity tendinopathies is not associated with an increased risk of having heterozygous familial hypercholesterolemia (figure 2E, appendix 5), as reported in one cohort study. (39) Heterozygous familial hypercholesterolemia was diagnosed in a lipid clinic, but the further criteria were not reported in this study. The prevalence of heterozygous familial hypercholesterolemia in patients with lower extremity tendinopathies was 1% (39) versus 0.4% in the general population (45).
There is limited evidence that heterozygous familial hypercholesterolemia is associated with an increased risk of developing lower extremity tendinopathies (figure 2F, appendix 5), as reported in one case-control study (26). The diagnosis was based on the following criteria: a) total cholesterol > 7.5 mmol/L or LDL-cholesterol > 4.9 mmol/L, AND either a) xanthomas in the patients or a first-degree relative or b) evidence of LDL-receptor or APO-B gene mutation. The prevalence of lower extremity tendinopathies in patients with heterozygous familial hypercholesterolemia was 47%, while healthy controls had a prevalence of 7% (26).

Ankylosing spondylitis
No studies were conducted that had lower extremity tendinopathies as primary inclusion criterion and ankylosing spondylitis as outcome.
There is moderate evidence that having ankylosing spondylitis is associated with an increased risk of developing lower extremity tendinopathies (figure 2F,H, appendix 5), as reported by 3 cohort studies (24, 25, 30). Ankylosing spondylitis was defined by different criteria (24, 25, 30). The prevalence of lower extremity tendinopathies ranged from 9-43% in patients with ankylosing spondylitis (24, 25, 30) versus 0.02% in the general population (2).

Psoriatic arthritis
No studies were conducted that researched the risk of developing psoriatic arthritis while having lower extremity tendinopathies.
There is moderate evidence that having psoriatic arthritis is associated with an increased risk of developing lower extremity tendinopathies (figure 2F,H, appendix 5), as reported by one case-control study (27) and two cohort studies (28, 29). Psoriatic arthritis was based on multiple criteria (seronegative for rheumatoid factors, and who presented with psoriasis and arthritis affecting the axial and/or peripheral joints) (27), defined as inflammatory arthritis, usually rheumatoid negative (28) or was based on the criteria of Vasey and Espinoza (29). The prevalence of lower extremity tendinopathies ranged from 3-26% in patients with psoriatic arthritis (27-29), and 0.02% in the general population (2).

Rheumatoid arthritis
There were no studies conducted that researched the risk of developing rheumatoid arthritis while having lower extremity tendinopathies.
There is moderate evidence that having rheumatoid arthritis is not associated with an increased risk of developing lower extremity tendinopathies (figure 2F,H, appendix 5), as reported by two cohort studies (30, 31). Rheumatoid arthritis was defined as fulfilling the criteria of the American Rheumatism Association (30), or fulfilling the 2010 American College of Rheumatology criteria (31). The prevalence of lower extremity tendinopathies was 1% in both cohorts of patients with rheumatoid arthritis (30, 31), and 0.02% in the general population (2).

Reactive arthritis
There were no studies conducted that researched the risk of developing reactive arthritis while having lower extremity tendinopathies.
There is moderate evidence that having reactive arthritis is associated with an increased risk of developing lower extremity tendinopathies (figure 2F,G, appendix 5), as reported by two cohort studies (30, 40). Reactive arthritis was defined as seronegative arthritis most compatible with Reiter’s disease (polyarthritis, urethritis and conjunctivitis) (40), or as non-gonococcal urethritis, arthritis and conjunctivitis (30). The prevalence of lower extremity tendinopathies ranged from 19.52% in patients with reactive arthritis (30, 40), versus 0.02% in the general population (2).

Systemic Lupus Erythematosus
There were no studies conducted that researched the risk of developing systemic lupus erythematosus while having lower extremity tendinopathies.
There is limited evidence that having systemic lupus erythematosus is associated with an increased risk of developing lower extremity tendinopathies (figure 2F, appendix 5), as reported by one cohort study (33). Systemic lupus erythematosus was defined as fulfilling the American College of Rheumatology criteria (33). The prevalence of lower extremity tendinopathies was 5% in patients with systemic lupus erythematosus (33) versus 0.02% in the general population (2).

DISCUSSION
This is the first prospectively registered, large and structurally designed systematic review assessing the association between lower extremity tendinopathies and metabolic and chronic diseases. We included 10 case-control and 10 cohort studies, involving 83,948 participants. Almost all (90%) included studies were assessed as having a high risk of bias. There is moderate evidence for an association...
between lower extremity tendinopathies and obesity, ankylosing spondylitis, psoriatic arthritis, and reactive arthritis. There is limited evidence for an association between lower extremity tendinopathies and heterozygous familial hypercholesterolemia, and Systemic Lupus Erythematosus. There was conflicting evidence for an association between lower extremity tendinopathies and hypertension, and lower extremity tendinopathies and hypercholesterolemia. We did not identify strong associations between lower extremity tendinopathies and metabolic and chronic diseases. This is partly due to the limited number of eligible studies and the high risk of bias of the included studies. Also, the way of measuring the outcome might have influenced the results of this systematic review (e.g., identifying hypercholesterolemia with a blood test versus a patient-reported questionnaire). Another reason might be that tendinopathy is considered a multifactorial disease. When we evaluate the association in a population of patients with tendinopathy, it is understandable that a strong association could not be detected. This is because other factors (e.g., suddenly increased tendon load) are more important for developing tendinopathies. We did observe a trend that studies including younger active patients did not detect an association or only a small association with metabolic and chronic diseases compared to older populations. These older individuals might be a subgroup where metabolic and chronic diseases are more strongly associated because overload is less likely to be a strong risk factor in this subgroup. When we evaluated the association in the group with metabolic and chronic diseases, we identified a tendency of stronger associations. This might have been caused by the fact that this was most frequently observed in the single-arm cohort studies, where we had to calculate the odds ratios based on prevalence data. This method is less robust than a case-control study design. Another reason could be that these are more homogeneous groups with respect to the metabolic factor. This enables researchers to find a more direct association between the metabolic disease and tendinopathy.

Obesity

Tendinopathy in load bearing tendons occurs more frequently in patients with obesity. This may be caused by two mechanisms: 1) tendon stress is increased due to the high body weight and 2) fat tissue releases low-grade detrimental systemic pro-inflammatory cytokines (17). A higher body weight leads to a higher local tendon stress; for example, during walking the load on the Achilles tendon reaches 2-5 times the body weight, which can increase to up to 12 times the body weight when sprinting (46). Furthermore, adipose tissue leads to a systemic state of low-grade inflammation by releasing adipokines (12). These specific proteins cause an increase in production of proteoglycans and pro-inflammatory molecules (12), causing disorganization of the collagen fibers and an increase in tendon stiffness, which leads to a decrease in maximum tendon load bearing capacity (47). The combination of a decreased maximum tendon load, an elevated tendon load caused by a high body weight and impaired tendon healing may eventually predispose obese patients for developing lower extremity tendinopathies (32, 34, 36-38).

This study is not the first systematic review that evaluates the association between lower extremity tendinopathies and obesity. Franchesci et al. (48) included all clinical studies that researched the association between obesity and tendinopathy. The authors decided to also include upper extremity tendinopathies. They concluded that obesity is a risk factor for tendinopathy, also for upper extremity tendinopathies, but that the association was stronger for Achilles tendinopathy and plantar fasciopathy (48). Since its publication in 2014 (48), more recent studies demonstrated an association between obesity and tendinopathy of the gluteal and patellar tendons (36, 37). With this additional information, we are able to draw more robust conclusions concerning the relation between obesity and lower extremity tendinopathies.

Diabetes

We found that lower extremity tendinopathies were not associated with an increased risk of having diabetes. A systematic review by De Oliveira et al. (49) included studies that analyzed the association between tendon disorders on imaging and diabetes. Although the included studies suggested an association, De Oliveira et al. (49) could not definitely draw this conclusion due to methodological limitations of the included studies. The difference between their tendency towards an association and our result of no association might be the way tendinopathy was diagnosed. The gold standard for diagnosing tendinopathy is currently based on diagnostic clinical criteria (50). As abnormal imaging results are also observed frequently in asymptomatic individuals, we chose to exclude studies that used imaging as only diagnostic tool (50).

Hypertension

We found conflicting evidence for the association between lower extremity tendinopathies and hypertension (23, 32, 35). This might be caused by the difference in study population between the three studies. Abate et al. (23) and Krammer et al. (35) found no association between Achilles tendinopathy and hypertension in a population of athletes, while Holmes et al. (32) detected an association in a general population not only consisting of athletes. This difference might
be explained by the lower prevalence of hypertension in a population of athletes (51). Hypertension damages the tunica intima of the artery, which eventually leads to restricted blood flow and hypoxia. Persisting hypoxia may lead to degenerative tendinopathy (52). More case-control studies in separate active and sedentary populations are needed to analyze hypertension as a risk factor for developing lower extremity tendinopathies.

Hypercholesterolemia and HeFH

We found conflicting evidence for the association between lower extremity tendinopathies and hypercholesterolemia (35, 41). This might be due to the criteria set to define hypercholesterolemia: Çatal et al. (41) defined hypercholesterolemia with a blood test (total cholesterol levels ≥ 240ml/dL), while hypercholesterolemia was self-reported in the study by Kraemer et al. (35).

We found limited evidence that patients with lower extremity tendinopathies did not have an increased risk of having HeFH (35, 39). Due to the methodological limitations of these studies and the limited evidence, definite conclusions of these risk factors should be drawn with caution.

Interestingly, we found that patients with HeFH have an increased risk of developing lower extremity tendinopathies (26). The hypothesis behind the association between these diseases is that the elevated blood lipid levels can lead to lipid accumulation in the tendon, so-called tendon xanthomas (53). These xanthomas may increase tendon stiffness and increase the synthesis of pro-inflammatory proteins, which may increase the risk of tendinopathy (17). We suggest that medical professionals offer a pharmacological intervention to decrease cholesterol, which may not only benefit the state of the tendon, but may also be life-saving (54).

Rheumatic diseases

There was limited to moderate evidence for an association between multiple rheumatic diseases and lower extremity tendinopathies (24, 25, 27-31, 33, 40). We did not find any studies that included patients with tendinopathy and researched whether the patients had an increased risk of having a rheumatic disease. It would be interesting for future studies to research whether an association is present in this patient population. In the meanwhile, we suggest that clinicians bear in mind that patients with tendinopathy might have an underlying rheumatic disease.

Clinical implications

Medical professionals should be aware of the associations of metabolic or chronic diseases with lower extremity tendinopathies. Screening for metabolic diseases during history taking and physical examination in patients with tendinopathy might identify factors that are part of the cause of tendinopathy. Treating metabolic diseases might not only improve the health of patients as a whole, but also their tendon health specifically. Future research should be performed to investigate whether influencing the metabolic diseases indeed results in improved outcomes for the lower extremity tendinopathy. Additionally, when medical professionals prescribe exercise to improve the patient’s metabolic profile or chronic disease, they should recommend seeking guidance to prevent development of lower extremity tendinopathies. This preventive intervention could be a very slow transition from non-weight bearing sports to weight bearing activities, although evidence for effectiveness of this intervention is currently lacking.

Strengths and limitations

A major strength of this systematic review is the use of the structured analysis according to the PRISMA guidelines (55). Based on this robust approach, we included 10 cohort and 10 case-control studies that analyzed the association between a clinically diagnosed lower extremity tendinopathies and a metabolic or chronic disease. By including clinically diagnosed lower extremity tendinopathies, we ensured that the data was not contaminated by asymptomatic patients with tendon imaging abnormalities, which are frequent in specific populations (56-59). By presenting the overview of the currently available research on the association between lower extremity tendinopathies and metabolic and chronic diseases, we were able to recommend implications for clinical care.

Despite its strengths, this systematic review also has limitations. First, due to heterogeneity of the articles, we were not able to pool the data. This hindered us from performing a meta-analysis, which is why we evaluated the associations with a best evidence synthesis. Second, many of the included articles were single-arm cohort studies. The OR's of these studies could not be calculated with their chosen study population, but were calculated with the prevalence of the lower extremity tendinopathies in the general population. The obtained OR's are therefore only an indication of the true OR and should be interpreted with caution. Third, tendinopathy has many synonyms and closely related pathologies which complicated the article selection. We strictly included articles that described clinical diagnosis of lower extremity tendinopathies. Fourth, it is debatable whether tendinopathy is the correct term for all included outcomes in this study. Terminology such as “enthesitis” and “xanthoma” is considered as other entities because they have a different pathogenesis. This is also supported by the findings of this systematic review. Last, many of the included studies did not correct for confounders. Subsequently,
the effect of the reported metabolic or chronic diseases in association with lower extremity tendinopathies might be influenced by other factors. For example, studies investigating multiple metabolic or chronic diseases (e.g., obesity, diabetes and hypercholesterolemia) did not assess whether these were independent factors.

**Future research**

To increase the evidence and to analyze further associations between lower extremity tendinopathies and metabolic or chronic diseases a large case-control study is needed. Patients should be diagnosed with metabolic or chronic diseases by valid criteria, such as blood tests. Healthy controls should have these criteria as exclusion criteria. In a follow-up study, all cases and controls should be screened for lower extremity tendinopathies. This study design should provide best evidence for the association between lower extremity tendinopathies and metabolic or chronic diseases.

We have included many metabolic or chronic diseases in this systematic review. There are probably more metabolic or chronic diseases possibly associated with lower extremity tendinopathies, such as hormonal disorders or thyroid disease (60, 61). To our knowledge, no clinical studies have been published that describe the association between tendinopathy and these conditions.

**CONCLUSIONS**

We found multiple associations between lower extremity tendinopathies and metabolic and chronic diseases. Lower extremity tendinopathies are moderately associated with obesity. There is also moderate evidence that patients with ankylosing spondylitis, psoriatic arthritis and reactive arthritis have an increased risk of developing lower extremity tendinopathies. There is limited evidence for an association between lower extremity tendinopathies and heterozygous familial hypercholesterolemia, and Systemic Lupus Erythematosus. Medical professionals should recognize these diseases in an early stage, which might improve personalized management.

**FUNDINGS**

None.

**DATA AVAILABILITY**

Data are available under reasonable request to the corresponding author.

**CONTRIBUTIONS**

IL, BN, FH, RB, MV, JV, RV: conceptualization, data performance, writing – original draft. IL, BN: data collection. IL, BN: data analysis.

**ACKNOWLEDGEMENTS**

We would like to thank WM Bramer, Biomedical Information Specialist at Erasmus MC University Medical Center Rotterdam, for designing a sensitive search strategy. We furthermore thank JH Waarsing, researcher at Erasmus MC University Medical Center Rotterdam, for their help with interpreting the data.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interests.

**REFERENCES**


Lower Extremity Tendinopathies are Associated with Metabolic and Chronic Diseases

Appendix 1. Search strategy.
09-10-2023

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</tbody>
</table>

*Science Citation Index Expanded (1975-present); Social Sciences Citation Index (1975-present); Arts & Humanities Citation Index (1975-present); Conference Proceedings Citation Index- Science (1990-present); Conference Proceedings Citation Index- Social Science & Humanities (1990-present); Emerging Sources Citation Index (2015-present); **Manually deleted abstracts from trial registries; ***Google Scholar was searched via “Publish or Perish” to download the results in EndNote. No other database limits were used than those specified in the search strategies.


ebase.com
(tendinitis)/exp OR (tendinitis OR tendinitid* OR tendinosis OR tendinoses OR tendonosis OR tendoneses OR tendonosis OR tendonopath* OR tenosynovitis OR tendonitis OR tendinopath* OR tenosynovit* OR (tendon* NEAR/6 (inflamm* OR irratit*))))/ti,kw) AND ('lower limb'/exp OR 'leg muscle'/exp OR 'achilles tendinitis'/de OR 'achilles tendon'/de OR 'hamstring tendon'/de OR 'quadriiceps tendon'/de OR 'leg disease'/exp OR ((low* NEXT/1 (limb OR limbs OR extremiti*)) OR hip OR leg OR foot OR heel OR heels OR hips OR legs OR feet OR thigh OR thighs OR ankle OR knee OR knees OR patella* OR Achilles OR plantar* OR peroneus* OR 'tibialis NEAR/3 posterior*') OR (triceps NEAR/3 surae) OR gastrocnemius OR soleus OR adductor OR hamstring OR quadriceps (OR quadratus NEAR/3 femoris))/ti,kw) AND ('cardiovascular disease'/exp OR Obesity/exp OR 'body mass'/de OR 'metabolic disorder'/exp OR 'glucose blood level'/exp OR 'thyroid disease'/exp OR 'rheumatic disease'/exp OR 'arthritis'/de OR 'chronic arthritis'/exp OR 'gout'/exp OR 'infectious arthritis'/exp OR 'monarthritis'/exp OR 'polyarthritis'/exp OR 'psuedogout'/exp OR 'psoriatic arthritis'/exp OR 'reactive arthritis'/exp OR 'rheumatoid arthritis'/exp OR 'uric acid blood level'/exp OR 'rheumatology'/de OR 'sarcoidosis'/exp OR 'infection'/exp OR 'inflammation'/de OR 'inflammatory disease'/de OR 'endocarditis'/exp OR 'carditis'/de OR 'pericarditis'/exp OR 'kidney disease'/exp OR 'dialysis'/exp OR 'hypercalcemia'/exp OR 'hyperparathyroidism'/exp OR 'inflammatory bowel disease'/exp OR 'ovary polycystic disease'/exp OR 'fibromyalgia'/exp OR (((cardiovascular* OR heart OR cerebrovascul* OR vascul* OR cardiac OR metabol* OR thyroid* OR inflamm* OR NEAR/3 (disease* OR syndrome* OR disorder*)) OR stroke OR eva OR hypertensi* OR (blood NEAR/3 pressure*) OR Obes* OR overweight OR body-mass* OR bmi OR adiposit* OR diabet* OR (insulin NEAR/3 resistan*) OR hyperinsulin* OR ((glucose OR lipid* OR cholesterol* OR uric-acid OR urate) OR dyslip* OR hypolip* OR hyperlip* OR dyscholesterol* OR hypercholesterol* OR hypertriglycerid* OR hyperthyroid* OR hyperuric* OR thrombosis* OR thromboembol* OR embolism* OR rheuma* OR arthritis OR gout OR sarcoid* OR Lofgren* OR mening* OR infecti* OR carditis* OR endocarditis* OR pericarditis* OR renal OR kidney OR dialysis* OR hemodialysis* OR haemodialysis* OR hypercalc* OR hyperparathyroid* OR (electrolyte* NEAR/3 (disturb* OR imbalan*)) OR (inflamm* NEAR/3 bowel* OR crohn* OR (ulcer* NEAR/3 colitis*) OR tuberculosis* OR (ovar* NEAR/3 polycyst*) OR PCOS OR fibromyalgia*))/ti,kw) NOT ((animals)/lim NOT (humans)/lim) NOT ((Conference Abstract)/lim) AND (english)/lim

Medline Ovid
(Tendinopathy/ OR Tenosynovitis/ OR (tendinitis OR tendinitid* OR tendinosis OR tendinoses OR tendonosis OR tendoneses OR tendonosis OR tendinitid* OR tendinopath* OR tendonopath* OR tenosynovitis OR (tendon*...
ADJ6 (inflammat* OR irritat*)\text{.} ab, ti, kf\text{.} AND (exp Lower Extremity/ OR Hamstring Muscles/ OR Fasciitis, Plantar/ OR Quadriceps Muscle/ OR Patellar Ligament/ OR Posterior Tibial Tendon Dysfunction/ OR Achilles Tendon/ OR Hamstring Tendons/ \text{ OR ((low* ADJ (limb or limbs or extremity)) \text{ OR hip or leg or foot or heel or heels or hips or legs or feet or thigh or thighs or ankle or knee or heels or patella* OR Achilles or plantar* OR peroneus* OR (tibialis ADJ3 posterior*)) OR (triceps ADJ3 surae) or gastrocnemius or soleus or adductor or hamstring or quadriceps or (quadratus ADJ3 femoris))\text{.} ab, ti, kf\text{.}) AND (exp Cardiovascular Diseases/ OR exp Overweight/ OR Body Mass Index/ OR exp Metabolic Diseases/ OR glucose/bl OR exp Thryoid Diseases/ OR exp Rheumatic Diseases/ OR Arthritis/ OR Gout/ OR Arthritis, Infectious/ OR Chondrocalcinosis/ OR Arthritis, Psoriatic/ OR Arthritis, Reactive/ OR Arthritis, Rheumatoid/ OR uric acid/bl OR Rheumatology/ OR Sarcoidosis/ OR exp Infection/ OR Inflammation/ OR Endocarditis/ OR Myocarditis/ OR Pericarditis/ OR exp Kidney Diseases/ OR Renal Dialysis/ OR Dialysis/ OR Hypercalcemia/ OR Hyperparathyroidism/ OR exp Inflammatory Bowel Diseases/ OR Polycystic Ovary Syndrome/ OR Fibromyalgia/ OR (((cardiovascular* OR heart or cerebrovascular* OR vascular* OR cardiac or metabol* OR thyroid* OR inflammat*) ADJ3 (disease* OR syndrome* OR disorder*)) OR stroke or cva or hypertension* OR (blood ADJ3 pressure*) OR Obes* or overweight or body-mass* or bmi or adipos- it* or diabet* or (insulin ADJ3 resistan*) or hyperinsulin- lin* or (glucose or lipid* or cholesterol* or uric-acid or urate) ADJ3 (blood or level* or plasma or concen- trate* or serum)) or dyslip* or hyperlip* or hyperlip* or hypercholesterol* or hypercholesterol* or hypercholesterol* or dystriglycerid* or hypertriglycerid* or hypertriglycerid* or dysthyroid* or hypothyroid* or hyper- thyroid* or dysuric* or hypouric* or hyperuric* or thrombosis* or thromboembol* or embolism* or rheuma-* or arthritis or gout or sarcoid* or Lofgren* or mening* or infecti* or carditis* or endocarditis* or pericarditis* or renal or kidney* or dialys* or hemo- dialys* or haemodialys* or hypercalc* OR hyperparathyroid* OR (electrolyte* NEAR/2 (disturb* or imbalan*)) OR (inflamma* NEAR/2/2 bowel*) or crohn* or (ulcer* NEAR/2 colitis*) or tuberculo* or (ovar* NEAR/2 poly- cyst*) or PCOS or fibromyalg*)\text{.} AND DT=(Article OR Review OR Letter OR Early Access) AND LA=(english).**

Web of Science

TS=(((((tendinitis OR tendinitid* OR tendinosis OR tendinosis OR tendonitis OR tendonopath* OR tendonopathy* OR tenosynovit* OR (tendon* NEAR/5 (inflammat* OR irritat*)) OR hip or leg or foot or heel or heels or hips or legs or feet or thigh or thighs or ankle or knee or heels or patella* OR Achilles or plantar* OR peroneus* OR (tibialis NEAR/2 posterior*)) OR (triceps NEAR/2 surae) or gastrocnemius or soleus or adductor or hamstring or quadriceps or (quadratus NEAR/2 femoris))\text{.} AND ((((cardiovascular* OR heart or cerebrovascular* OR vascular* OR cardiac or metabol* OR thyroid* OR inflammat*) NEAR/2 (disease* OR syndrome* OR disorder*)) OR stroke or cva or hypertension* OR (blood NEAR/2 pressure*) OR Obes* or overweight or body-mass* or bmi or adipos- it* or diabet* or (insulin NEAR/2 resistan*) or hyperinsulin- lin* or (glucose or lipid* or cholesterol* or uric-acid or urate) NEAR/2 (blood or level* or plasma or concentrate* or serum)) or dyslip* or hyperlip* or hyperlip* or dyscholesterol* or hypercholesterol* or hypercholesterol* or dystriglycerid* or hypertriglycerid* or dysthyroid* or hypothroid* or hyperthyroid* or dysuric* or hypouric* or hyperuric* or thrombosis* or thromboembol* or embolism* or rheuma-* or arthritis or gout or sarcoid* or Lofgren* or mening* or infecti* or carditis* or endocarditis* or pericarditis* or renal or kidney* or dialys* or hemo- dialys* or haemodialys* or hypercalc* OR hyperparathyroid* OR (electrolyte* NEAR/2 (disturb* or imbalan*)) OR (inflamma* NEAR/2/2 bowel*) or crohn* or (ulcer* NEAR/2 colitis*) or tuberculo* or (ovar* NEAR/2 poly- cyst*) or PCOS or fibromyalg*)\text{.} AND DT=(Article OR Review OR Letter OR Early Access) AND LA=(english).

Cochrane CENTRAL

TS=(((((tendinitis OR tendinitid* OR tendinosis OR tendinosis OR tendonitis OR tendonopath* OR tendonopathy* OR tenosynovit* OR (tendon* NEAR/6 (inflammat* OR irritat*))\text{.} ab, ti\text{.}) AND (((low* NEXT/1 (limb or limbs or extremity))) OR hip or leg or foot or heel or heels or hips or legs or feet or thigh or thighs or ankle or knee or heels or patella* OR Achilles or plantar* OR peroneus* OR (tibialis NEAR/3 posterior*)) OR (triceps NEAR/3 surae) or gastrocnemius or soleus or adductor or hamstring or quadriceps or (quadratus NEAR/3 femoris))\text{.} ab, ti\text{.}) AND (((cardiovascular* OR heart or cerebrovascular* OR vascular* OR cardiac or metabol* OR thyroid* OR inflammat*) NEAR/3 (disease* OR syndrome* OR disorder*)) OR stroke or cva or hypertension* OR (blood NEAR/3 pressure*) OR Obes* or overweight or body-mass* or bmi or adipos- it* or diabet* or (insulin NEAR/3 resistan*) or hyperinsulin- lin* or (glucose or lipid* or cholesterol* or uric-acid or urate) or NEAR/2 (blood or level* or plasma or concentrate* or serum)) or dyslip* or hyperlip* or hyperlip* or dyscholesterol* or hypercholesterol* or hypercholesterol* or dystriglycerid* or hypertriglycerid* or dysthyroid* or hypothroid* or hyperthyroid* or dysuric* or hypouric* or hyperuric* or thrombosis* or thromboembol* or embolism* or rheuma-* or arthritis or gout or sarcoid* or Lofgren* or mening* or infecti* or carditis* or endocarditis* or pericarditis* or renal or kidney* or dialys* or hemo- dialys* or haemodialys* or hypercalc* OR hyperparathyroid* OR (electrolyte* NEAR/2 (disturb* or imbalan*)) OR (inflamma* NEAR/2/2 bowel*) or crohn* or (ulcer* NEAR/2 colitis*) or tuberculo* or (ovar* NEAR/2 poly- cyst*) or PCOS or fibromyalg*)\text{.} AND DT=(Article OR Review OR Letter OR Early Access) AND LA=(english).

Web of Science

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Lower Extremity Tendinopathies are Associated with Metabolic and Chronic Diseases

OR ((glucose OR lipid* OR cholesterol* OR uric next acid OR urate) NEAR/3 (blood OR level* OR plasma OR concentrate* OR serum)) OR dyslip* OR hypolip* OR hyperlip* OR dyscholesterol* OR hypocholesterol* OR hypercholesterol* OR dystriglycerid* OR hypotriglycerid* OR hypertriglycerid* OR dysthyroid* OR hypothyroid* OR hyperthyroid* OR dysuric* OR hypouric* OR hyperuric* OR thrombosis* OR thromboembol* OR embolism* OR rheuma* OR arthritis OR gout OR sarcoid* OR Loefgren* OR mening* OR infecti* OR carditis* OR endocarditis* OR pericarditis* OR renal OR kidney* OR dialys* OR hemodialys* OR haemodialys* OR hypercalc* OR hyperparathyroid* OR (electrolyte* NEAR/3 (disturb* OR imbalan*)) OR (inflamma* NEAR/3 bowel*) OR crohn* OR (ulcer* NEAR/3 colitis*) OR tuberculo* OR (ovar* NEAR/3 polycyst*) OR PCOS OR fibromyalg*:ab,ti) NOT “conference abstract”:pt

Google scholar

tendinitis|tendinopathy “lower limb/extremity”|Achilles|plantar|peroneus “cardiovascular|heart|cerebrovascular|metabolism|thyroid|infection|infectious|syndrome”|hypertension|Obesity/overweight|’body-mass’|bmi|diabetes|hyperinsulinism|hypocholesterolism|thrombosis
tendinitis|tendinopathy ‘lower limb/extremity’|Achilles|plantar|peroneus ‘cardiovascular|heart|cerebrovascular|metabolism|thyroid|infection|infectious|syndrome’|hypertension|Obesity/overweight|’body-mass’|bmi|diabetes|hyperinsulinism|hypocholesterolism|thrombosis
**CASE-CONTROL STUDIES**

**Selection**

1. Is the case definition adequate?
   A. Yes, the case definition required independent validation (e.g. diagnosis was made by a medical professional based on clinical findings, laboratory measurements, imaging or surgery). 1 star
   B. Yes, but the diagnosis was based on records (e.g. ICD codes in database) or self-reported with no reference to primary record.
   C. No description

2. Representativeness of cases
   A. All eligible cases with outcome of interest over a pre-defined period of time, catchment area, hospital, clinic, or health maintenance organization, or an appropriate sample of those cases (e.g. random sample) were included. 1 star
   B. The cases do not meet the requirements in part A., or it is not described.

3. Selection of controls
   A. This item assesses whether the controls used in the study are derived from the same population of the cases and essentially would be cases if the outcome had been present.
   B. Healthy control group (i.e. control group is derived from the same population as cases and would be cases if the outcome had been present). 1 star
   C. Hospital control group, within same community as cases. The patients do not have the disease of interest, but they might have a disease that could influence the outcome.
   D. No description

4. Definition of controls
   A. Controls have the same inclusion criteria as the cases. If the cases have a first occurrence of the outcome, then it must explicitly be stated that controls have no history of this outcome. If cases have a new (not necessarily first) occurrence of the outcome, then controls with previous occurrence of the outcome of interest should not be excluded. 1 star
   B. No mention of history of outcome.

**Comparability**

Either cases and controls must be matched in the design and/or the analysis must be adjusted for confounders. Statements that no differences between groups was found are not sufficient. A maximum of 2 starts can be given for this category.

5. Comparability of cases and controls on the basis of the design or analysis
   A. The study corrects for age and sex. 1 star
   B. The study corrects for any other variable, like duration of the primary outcome. 1 star
   C. The study does not correct for any variable.

**Exposure**

6. Ascertainment of exposure
   A. A medical professional diagnosed the patient based on clinical findings, laboratory measurements, imaging or surgery. If the outcome is based on medical reports, inclusion criteria should be clearly stated and should mention the diagnosis by a medical professional and/or the diagnosis should be supported by clinical findings, laboratory measurements, imaging or surgery. 1 star
   B. A structured interview where the interviewer was blinded for case/control status. 1 star
   C. The interviewer was not blinded for case/control status.
   D. Self-reported or medical records only, which do not comply to 6A.
   E. No description.

7. Same method of ascertainment for cases and controls
   A. Yes, cases and controls were screened on same inclusion criteria and had the same outcome criteria. 1 star
   B. No.

8. Non-response rate
   A. Same rate of non-responders in both groups (in case of retrospective studies: similar percentage of missing data). 1 star
   B. Non-responders are only described.
   C. There is a difference in non-responders, or this is not described.

**COHORT STUDY**

**Selection**

1. Representativeness of the exposed cohort
   A. The cohort is a true representative of the average person with metabolic or general medical diseases or the average person with tendinopathy of the lower extremities. 1 star
B. The cohort is somewhat representative of the average person metabolic or general medical diseases or the average person with tendinopathy of the lower extremities. Think of patients with severe diabetes or only active patients. 1 star
C. The cohort is a select group, like nurses or volunteers. 1 star
D. There is no clear description of the derivation of the cohort.

2. Selection of the non-exposed cohort
   A. The group of patients without outcome are drawn from the same cohort as the group of patients with outcome. 1 star
   B. The group of patients without outcome is drawn from a different source. 1 star
   C. There is no description of the derivation of the non-exposed cohort.

3. Ascertainment of exposure
   A. A medical professional diagnosed the patient based on clinical findings, laboratory measurements, imaging or surgery. If the outcome is based on medical reports, inclusion criteria should be clearly stated and should mention the diagnosis by a medical professional and/or the diagnosis should be supported by clinical findings, laboratory measurements, imaging or surgery. 1 star
   B. A structured interview where the interviewer was blinded for case/control status. 1 star
   C. Self-reported or medical records only, which do not comply to 6A.
   D. No description.

4. Demonstration that outcome of interest was not present at the start of the study
   A. Yes, it is described that the outcome of interest was not present at the start of the study. 1 star
   B. No.

Comparability
Either cases and controls must be matched in the design and/or the analysis must be adjusted for confounders. Statements that no differences between groups was found are not sufficient. A maximum of 2 starts can be given for this category.

5. Comparability of cohorts on the basis of the design or analysis
   A. The study corrects for age and sex. 1 star
   B. The study corrects for any other additional variable, like duration of the primary outcome. 1 star
   C. The study does not correct for any variable.

Outcome

6. Assessment of outcome
   A. A medical professional diagnosed the patient based on clinical findings, laboratory measurements, imaging or surgery. If the outcome is based on medical reports, inclusion criteria should be clearly stated and should mention the diagnosis by a medical professional and/or the diagnosis should be supported by clinical findings, laboratory measurements, imaging or surgery. 1 star
   B. A structured interview where the interviewer was blinded for case/control status. 1 star
   C. The interviewer was not blinded for case/control status.
   D. Self-reported or medical records only, which do not comply to 6A.
   E. No description.

7. Was follow-up long enough for outcomes to occur?
   A. Yes. The diagnosis of metabolic or general medical disease or lower limb tendinopathy was made by a medical professional based on clinical findings, or based on laboratory measurements, imaging or surgery OR follow-up was longer than 1 year in case of self-reported outcome. 1 star
   B. Not described or does not comply with 7A.

8. Adequacy of follow-up of cohorts
   A. Complete follow-up. 1 star
   B. Less than 15% lost to follow-up. 1 star
   C. Follow-up rate is less than 85% or it is not described.
### Appendix 3. Baseline characteristics.

<table>
<thead>
<tr>
<th>First author (country, year of publication)</th>
<th>Type of study</th>
<th>Total group (cases) and population description</th>
<th>Age, mean (SD), years</th>
<th>Sex (% male)</th>
<th>Primary aim</th>
<th>Inclusion disease/disease cases</th>
<th>Outcome disease</th>
<th>Duration of follow-up (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abate, Italy, 2016 Case-control</td>
<td>76 (38) subjects aged &gt; 65 years with Achilles tendinopathy (case) or upper extremity musculoskeletal disorder (matched case)</td>
<td>69.30 (3.03)</td>
<td>84.21%</td>
<td>To assess the relationship between symptomatic Achilles tendinopathy and type II diabetes in elderly subjects</td>
<td>Achilles tendinopathy</td>
<td>Diabetes type II</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Abate, Italy, 2018 Case-control</td>
<td>64 (36) regular runners who started running because of overweight/obesity/abnormal metabolic parameters</td>
<td>39.21 (12.33)</td>
<td>62.50%</td>
<td>To evaluate whether overuse or metabolic pathologies were more responsible for midportion Achilles tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Obesity (BMI &gt;30), hypertension, diabetes, dyslipidemia</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Aggarwal, India, 2009 Prospective cohort</td>
<td>70 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>84.3%</td>
<td>To analyses the full spectrum of primary ankylosing spondylitis in Indian patients</td>
<td>Ankylosing spondylitis</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Alam, Qatar, 2016 Retrospective cohort</td>
<td>62 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>83.9%</td>
<td>To explore the characteristics of ankylosing spondylitis of patients living in Qatar</td>
<td>Ankylosing spondylitis</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Beeharry, United Kingdom, 2005 Case-control</td>
<td>220 (133) patients and their partners attending a lipid clinic</td>
<td>56.21 (range 42-64)</td>
<td>48.64%</td>
<td>To determine the prevalence of Achilles tendinopathy before diagnosis of heterozygous familial hypercholesterolaemia</td>
<td>Achilles tendinopathy</td>
<td>Heterozygous familial hypercholesterolaemia</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Cantini, Italy, 2001 Case-control</td>
<td>549 (183) patients with psoriatic arthritis or a different rheumatologic disease</td>
<td>51.27 (9.6)</td>
<td>49.18%</td>
<td>To evaluate the frequency of distal extremity swelling with pitting edema in patients with psoriatic arthritis</td>
<td>Psoriatic arthritis</td>
<td>Achilles tendinopathy</td>
<td>3 months</td>
<td></td>
</tr>
<tr>
<td>Çatal, Turkey, 2021 Case-control</td>
<td>478 (238) patients with plantar fasciopathy</td>
<td>Not reported</td>
<td>23.4%</td>
<td>To investigate the relationship between hypercholesterolaemia and plantar fasciopathy</td>
<td>Plantar fasciopathy</td>
<td>Hypercholesterolaemia</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Elkayam, Israel, 2000 Prospective cohort</td>
<td>70 patients with psoriatic arthritis</td>
<td>Not reported</td>
<td>55.71%</td>
<td>To investigate the relationship between clinical characteristics of the skin and joint manifestations in patients with psoriatic arthritis</td>
<td>Psoriatic arthritis</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Galluzzo, Italy, 2000 Prospective cohort</td>
<td>31 patients with psoriatic arthritis</td>
<td>48.1 (range 30-74)</td>
<td>58.06%</td>
<td>To evaluate ankle involvement in patients with psoriatic arthritis</td>
<td>Psoriatic arthritis</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Gerster, Switzerland, 1977 Prospective cohort</td>
<td>100 patients with rheumatoid arthritis, 35 with ankylosing spondylitis, 16 with Reiter's syndrome and 70 with osteoarthritis</td>
<td>59.58 (range 19-98)</td>
<td>42.99%</td>
<td>To determine the frequency of heel tenderness (talalgia) in rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome and generalized osteoarthritis</td>
<td>Rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome</td>
<td>Talalgia (Achilles tendinopathy or plantar fasciopathy)</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Total group (cases) and population description</td>
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</tr>
<tr>
<td>Hernandez-Diaz, Italy, 2019</td>
<td>Prospective cohort</td>
<td>112 patients with rheumatoid arthritis</td>
<td>51 (range 22-85)</td>
<td>10.71%</td>
<td>To describe the prevalence and distribution of clinical and ultrasound pathological findings at ankle level in patients with rheumatoid arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Plantar fasciopathy</td>
<td>Not reported</td>
</tr>
<tr>
<td>Holmes, USA, 2006</td>
<td>Case-control</td>
<td>282 (82) patients with Achilles tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>To determine the association between Achilles tendinopathy and obesity, diabetes mellitus and hypertension</td>
<td>Achilles tendinopathy</td>
<td>Obesity, diabetes mellitus, hypertension</td>
<td>Not reported</td>
</tr>
<tr>
<td>Jarrot, France, 2015</td>
<td>Retrospective cohort</td>
<td>158 patients with systemic lupus erythematosus</td>
<td>Not reported</td>
<td>9.49%</td>
<td>To describe the occurrence of Achilles tendinopathy in patients with systemic lupus erythematosus</td>
<td>Systemic lupus erythematosus</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
</tr>
<tr>
<td>Klein, USA, 2013</td>
<td>Case-control</td>
<td>944 (472) patients with Achilles tendinopathy or other foot pathology</td>
<td>51.60 (13.90)</td>
<td>51.91%</td>
<td>To elucidate the role of BMI in the development and treatment of Achilles tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Obesity</td>
<td>Not reported</td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>310 (161) sportsmen between 18-65 years old with Achilles tendinopathy or without any diseases</td>
<td>40.0 (11.0)</td>
<td>66.77%</td>
<td>To stratify risk factors for Achilles tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Diabetes mellitus, hypercholesterolaemia, arterial hypertension, cardiac diseases</td>
<td>Not reported</td>
</tr>
<tr>
<td>Owens, USA, 2013</td>
<td>Case-control</td>
<td>80106 (2262) military service members with patellar tendinopathy, Achilles tendinopathy, plantar fasciopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>To identify risk factors for developing lower extremity tendinopathy and plantar fasciopathy in military personnel</td>
<td>Patellar tendinopathy, Achilles tendinopathy, plantar fasciopathy</td>
<td>Obesity</td>
<td>Not reported</td>
</tr>
<tr>
<td>Plinsinga, Australia, 2018</td>
<td>Prospective cohort</td>
<td>204 patients with gluteal tendinopathy</td>
<td>35 (9)</td>
<td>18.14%</td>
<td>To examine the differences in physical and physiological factors in patients with gluteal tendinopathy</td>
<td>Gluteal tendinopathy</td>
<td>Obesity</td>
<td>Not reported</td>
</tr>
<tr>
<td>Riddle, USA, 2003</td>
<td>Case-Control</td>
<td>150 (50) patients with plantar fasciopathy or without any diseases</td>
<td>Not reported</td>
<td>51%</td>
<td>To identify risk factors for developing plantar fasciopathy</td>
<td>Plantar fasciopathy</td>
<td>Obesity</td>
<td>Not reported</td>
</tr>
<tr>
<td>Singh, United Kingdom, 2015</td>
<td>Prospective cohort</td>
<td>85 patients with midportion Achilles tendinopathy</td>
<td>Median 46 (range 25-79)</td>
<td>68.67%</td>
<td>To analyze the usefulness of serum cholesterol measurements in patients with midportion Achilles tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Dyslipidemia, heterozygous familial hypercholesterolaemia</td>
<td>Not reported</td>
</tr>
<tr>
<td>Smith, USA, 1980</td>
<td>Retrospective cohort</td>
<td>29 female patients with Reiter’s disease</td>
<td>36.7 (13.5)</td>
<td>0%</td>
<td>To report women with Reiter’s disease</td>
<td>Reiter’s disease</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

USA: United States of America; SD: Standard Deviation; BMI: Body Mass Index.
### Appendix 4. Additional study information.

<table>
<thead>
<tr>
<th>First author (country, year of publication)</th>
<th>Type of study</th>
<th>Type of tendinopathy</th>
<th>Unilateral or bilateral</th>
<th>Type of imaging used for confirming clinical diagnosis</th>
<th>Severity of pain</th>
<th>Duration of tendinopathy</th>
<th>Active/sedentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abate, Italy, 2016</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>Ultrasound</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Abate, Italy, 2018</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>Ultrasound, color Doppler</td>
<td>Not reported</td>
<td>Not reported</td>
<td>36 of 36 patients were active</td>
</tr>
<tr>
<td>Aggarwal, India, 2009</td>
<td>Prospective cohort</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td>None</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Alam, Qatar, 2016</td>
<td>Retrospective cohort</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td>None</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Beeharry, United Kingdom, 2005</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>None</td>
<td>Severe in 24/62 (37.7%) of patients with HeFH, and none of the controls exceeded moderate severity.</td>
<td>Described as a few days to several weeks with a median of 4 days</td>
<td>Not reported</td>
</tr>
<tr>
<td>Cantini, Italy, 2001</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Çatal, Turkey, 2021</td>
<td>Case-control</td>
<td>Plantar fasciopathy</td>
<td>Not reported</td>
<td>None</td>
<td>154 patients with symptoms &lt;1 year, 84 patients with symptoms &gt;1 year</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Elkayam, Israel, 2000</td>
<td>Prospective cohort</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Galluzo, Italy, 2000</td>
<td>Prospective cohort</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Unilateral</td>
<td>Radiography, ultrasound</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Gerster, Switzerland, 1977</td>
<td>Prospective cohort</td>
<td>Talalgia (Achilles tendinopathy or plantar fasciopathy)</td>
<td>Not reported</td>
<td>Radiography</td>
<td>Rheumatoid arthritis: 2% severe, 27% mild pain. Osteoarthritis: 1.4% severe, 15.7% mild pain. Reiter’s syndrome: 31% severe, 19% mild pain.</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Type of tendinopathy</td>
<td>Unilateral or bilateral</td>
<td>Type of imaging used for confirming clinical diagnosis</td>
<td>Severity of pain</td>
<td>Duration of tendinopathy</td>
<td>Active/sedentary</td>
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<tr>
<td>Hernandez-Diaz, Italy, 2019</td>
<td>Prospective cohort</td>
<td>Plantar fasciopathy</td>
<td>Not reported</td>
<td>Ultrasound</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Holmes, USA, 2006</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>Radiography and MRI</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Jarrot, France, 2015</td>
<td>Retrospective cohort</td>
<td>Achilles tendinopathy</td>
<td>9 unilateral, 1 bilateral</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Klein, USA, 2013</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>None</td>
<td>Not reported</td>
<td>22% 0-3 months, 15% 3-6 months, 13% 6-12 months, 25% 12-36 months, 25% &gt;36 months</td>
<td>Active</td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>Achilles tendinopathy</td>
<td>Not reported</td>
<td>None</td>
<td>Not reported</td>
<td>Median 24 months (IQR 8-48, range 3-192)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Owens, USA, 2013</td>
<td>Case-control</td>
<td>Patellar tendinopathy, Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>287 ± 550 days (median 123 days, range 14-3650 days)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Plinsinga, Australia, 2018</td>
<td>Prospective cohort</td>
<td>Gluteal tendinopathy</td>
<td>Not reported</td>
<td>MRI</td>
<td>VISA-G 59.5 (13.2)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Riddle, USA, 2003</td>
<td>Case-control</td>
<td>Plantar fasciopathy</td>
<td>Unilateral</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Singh, United Kingdom, 2015</td>
<td>Prospective cohort</td>
<td>Achilles tendinopathy</td>
<td>66 unilateral, 17 bilateral</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Smith, USA, 1980</td>
<td>Retrospective cohort</td>
<td>Achilles tendinopathy, plantar fasciopathy</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Type of metabolic or chronic disease</td>
<td>Definition</td>
<td>Associated measurements</td>
<td>Use of medication</td>
<td>Duration of condition since diagnosis</td>
<td></td>
</tr>
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<td>-------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Abate, Italy, 2016</td>
<td>Case-control</td>
<td>Diabetes</td>
<td>The diagnosis of diabetes based of history and current therapies</td>
<td>HbA1c</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Obesity (BMI &gt;30), hypertension (blood pressure 3 times at 4 minutes interval, systolic &gt;140 mmHg and diastolic &gt;90 mmHg), HbA1c &gt;5.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abate, Italy, 2018</td>
<td>Case-control</td>
<td>Obesity, hypertension, diabetes</td>
<td>Obesity (BMI &gt;30), hypertension (blood pressure 3 times at 4 minutes interval, systolic &gt;140 mmHg and diastolic &gt;90 mmHg), HbA1c &gt;5.7</td>
<td>Antidiabetic agents (4 in study group, 2 in control group), antihypertensive drugs (2 in study group, 1 in control group)</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Aggarwal, India, 2009</td>
<td>Prospective cohort</td>
<td>Ankylosing spondylitis</td>
<td>Modified New York criteria</td>
<td>HLA-B27</td>
<td>Not reported</td>
<td>9.3 (6.5) years</td>
<td></td>
</tr>
<tr>
<td>Alam, Qatar, 2016</td>
<td>Retrospective cohort</td>
<td>Ankylosing spondylitis</td>
<td>Assessment of SpondyloArthritis International Society (ASAS)</td>
<td>HLA-B27</td>
<td>Yes (NSAID monotherapy, NSAID + anti-TNFα, anti-TNFα monotheraphy, NSAID + DMARD)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Beeharry, United Kingdom, 2005</td>
<td>Case-control</td>
<td>Hypercholesterolaemia</td>
<td>Criteria of the Simon Broome Register Seronegative for rheumatoid factors and who presented psoriasis and arthritis affecting the axial and/or peripheral joints</td>
<td>Serum cholesterol</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Cantini, Italy, 2001</td>
<td>Case-control</td>
<td>Psoriatic arthritis</td>
<td>Anti-inflammatory arthritis, usually rheumatoid factor negative, associated with psoriasis</td>
<td>Rose-Waaler titer &lt;1:40 or nephelometric determination &lt;20 lu/ml on 2 or more occasions</td>
<td>Not reported</td>
<td>77.3 (70.5) months</td>
<td></td>
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<tr>
<td>Elkayam, Israel, 2000</td>
<td>Prospective cohort</td>
<td>Psoriatic arthritis</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
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<tr>
<td>Galluzo, Italy, 2000</td>
<td>Prospective cohort</td>
<td>Psoriatic arthritis</td>
<td>Criteria of Vasey and Espinoza</td>
<td>HLA-B27</td>
<td>Yes (NSAID’s)</td>
<td>5.3 years (range 6 months – 16 years)</td>
<td></td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Type of metabolic or chronic disease</td>
<td>Definition</td>
<td>Associated measurements</td>
<td>Use of medication</td>
<td>Duration of condition since diagnosis</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Gerster, Switzerland, 1977</td>
<td>Prospective cohort</td>
<td>Rheumatoid arthritis</td>
<td>American Rheumatism Association (1959) 2010 American College of Rheumatology (ACR) criteria Obesity (BMI &gt;30 kg/m²), hypertension (systolic blood pressure &gt;140 mmHg or diastolic blood pressure &gt;90mmHg or antihypertensive treatment), diabetes mellitus (treatment for diabetes or endocrinologist confirmed diagnosis)</td>
<td>Rheumatoid factor positive</td>
<td>Not reported</td>
<td>&gt;6 months</td>
<td></td>
</tr>
<tr>
<td>Hernandez-Diaz, Italy, 2019</td>
<td>Prospective cohort</td>
<td>Rheumatoid arthritis</td>
<td>Not reported</td>
<td>Not reported</td>
<td>72 months (range 2-456)</td>
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<td></td>
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<tr>
<td>Holmes, USA, 2006</td>
<td>Case-control</td>
<td>Obesity, hypertension, diabetes mellitus</td>
<td>Not reported</td>
<td>Yes (type not specified)</td>
<td>Not reported</td>
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<tr>
<td>Jarrot, France, 2015</td>
<td>Retrospective cohort</td>
<td>Systemic Lupus Erythematosus</td>
<td>American College of Rheumatology (ACR) criteria</td>
<td>ANA, dsDNA, complement fraction levels, antiphospholipid antibodies, rheumatoid factor, anti-CCP, HLA-B27 and serum uric-acid</td>
<td>Not reported</td>
<td>10.5 years (range 0.21 years)</td>
<td></td>
</tr>
<tr>
<td>Klein, USA, 2013</td>
<td>Case-control</td>
<td>Obesity</td>
<td>Obese (class I: 30.0-34.9 kg/m², class II: 35.0-39.9 kg/m²), morbidly obese (&gt;40 kg/m²)</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>Diabetes mellitus, hypercholesterolaemia, hypertension</td>
<td>Self-reported medical history</td>
<td>None</td>
<td>Not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owens, USA, 2013</td>
<td>Case-control</td>
<td>Obesity</td>
<td>BMI &gt;30 kg/m²</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Type of metabolic or chronic disease</td>
<td>Definition</td>
<td>Associated measurements</td>
<td>Use of medication</td>
<td>Duration of condition since diagnosis</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Plinsinga, Australia, 2018</td>
<td>Prospective cohort</td>
<td>Obesity</td>
<td>BMI &gt;30 kg/m²</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Riddle, USA, 2003</td>
<td>Case-control</td>
<td>Dyslipidemia</td>
<td>Not specified</td>
<td>Serum cholesterol</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Singh, United Kingdom, 2015</td>
<td>Prospective cohort</td>
<td>Dyslipidemia</td>
<td>Not specified</td>
<td>Serum cholesterol</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Smith, USA, 1980</td>
<td>Retrospective cohort</td>
<td>Reiter’s disease</td>
<td>Not specified</td>
<td>Rheumatoid factor, ANA, HLA-B27</td>
<td>Yes (NSAID, phenylbutazone, 6-mercaptopurine, intramuscular gold therapy, corticosteroids)</td>
<td>Not reported</td>
<td></td>
</tr>
</tbody>
</table>

**Appendix 5.** Baseline characteristics, and odds ratio of the association between metabolic or chronic disease and lower extremity tendinopathies.

<table>
<thead>
<tr>
<th>First author (country, year of publication)</th>
<th>Type of study</th>
<th>Baseline participant characteristics</th>
<th>Obesity</th>
<th>Gluteal tendinopathy</th>
<th>Patellar tendinopathy</th>
<th>Achilles tendinopathy</th>
<th>Plantar fasciopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abate, Italy, 2018 Case-control</td>
<td>64 (36) regular runners who started running because of overweight/obesity/abnormal metabolic parameters</td>
<td>39.21 (12.33)</td>
<td>62.50%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Holmes, USA, 2006 Case-control</td>
<td>282 (82) patients with Achilles tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Klein, USA, 2013 Case-control</td>
<td>944 (472) patients with Achilles tendinopathy or other foot pathology</td>
<td>51.60 (13.90)</td>
<td>51.91%</td>
<td>189 (40%)</td>
<td>189 (40%)</td>
<td>2.5 (1.9;3.4)</td>
<td>-</td>
</tr>
<tr>
<td>Owens, USA, 2013 Case-control</td>
<td>78486 (584) military service members with patellar tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>67 (12%)</td>
<td>61 (14%)</td>
<td>175 (14%)</td>
<td>1.7 (1.5;2.1)</td>
</tr>
<tr>
<td>Plinsinga, Australia, 2018 Prospective cohort</td>
<td>204 patients with gluteal tendinopathy</td>
<td>55 (9)</td>
<td>18.14%</td>
<td>57 (28%)</td>
<td>2.8 (1.4;5.6)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Riddle, USA, 2003 Case-control</td>
<td>150 (50) patients with Plantar fasciopathy or without any diseases</td>
<td>49 (11)</td>
<td>34%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29 (58%)</td>
</tr>
<tr>
<td>First author (country, year of publication)</td>
<td>Type of study</td>
<td>Baseline participant characteristics</td>
<td>Gluteal tendinopathy</td>
<td>Patellar tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Plantar fasciopathy</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td></td>
<td>Total group (cases) and population description</td>
<td>Age, mean (SD), years</td>
<td>Sex (% male)</td>
<td>Number (%)</td>
<td>OR (95% CI)</td>
<td>Number (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abate, Italy, 2016</td>
<td>Case-control</td>
<td>76 (38) subjects aged &gt; 65 years with Achilles tendinopathy (case) or upper extremity musculoskeletal disorder (matched case)</td>
<td>69.30 (3.05)</td>
<td>84.21%</td>
<td>16 (42%)</td>
<td>4.8 (2.5;9.3)</td>
<td></td>
</tr>
<tr>
<td>Abate, Italy, 2018</td>
<td>Case-control</td>
<td>64 (36) regular runners who started running because of overweight/obesity/abnormal metabolic parameters</td>
<td>39.21 (12.33)</td>
<td>62.50%</td>
<td>4 (15%)</td>
<td>0.7 (0.1;3.5)</td>
<td></td>
</tr>
<tr>
<td>Holmes, USA, 2006</td>
<td>Case-control</td>
<td>282 (82) patients with Achilles tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>6 (7%)</td>
<td>0.5 (0.2;1.1)</td>
<td></td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>310 (161) sportsmen between 18-65 years old with Achilles tendinopathy or without any diseases</td>
<td>40.0 (11.0)</td>
<td>66.77%</td>
<td>2 (1%)</td>
<td>0.5 (0.1;2.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abate, Italy, 2018</td>
<td>Case-control</td>
<td>64 (36) regular runners who started running because of overweight/obesity/abnormal metabolic parameters</td>
<td>39.21 (12.33)</td>
<td>62.50%</td>
<td>7 (27%)</td>
<td>0.5 (0.1;1.9)</td>
<td></td>
</tr>
<tr>
<td>Holmes, USA, 2006</td>
<td>Case-control</td>
<td>282 (82) patients with Achilles tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>Not reported</td>
<td>43 (52%)</td>
<td>2.4 (1.6;3.7)</td>
<td></td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>310 (161) sportsmen between 18-65 years old with Achilles tendinopathy or without any diseases</td>
<td>40.0 (11.0)</td>
<td>66.77%</td>
<td>16 (10%)</td>
<td>0.8 (0.3;1.8)</td>
<td></td>
</tr>
<tr>
<td>First author</td>
<td>Type of study</td>
<td>Total group (cases) and population description</td>
<td>Baseline participant characteristics</td>
<td>Gluteal tendinopathy</td>
<td>Patellar tendinopathy</td>
<td>Achilles tendinopathy</td>
<td>Plantar fasciopathy</td>
</tr>
<tr>
<td>------------------------------</td>
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</tr>
<tr>
<td>Çatal, Turkey, 2021</td>
<td>Case-control</td>
<td>478 (238) patients with plantar fasciopathy or without any diseases</td>
<td>Age, mean (SD), years</td>
<td>Sex (% male)</td>
<td>Number (%)</td>
<td>OR (95% CI)</td>
<td>Number (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>310 (161) sportsmen between 18-65 years old with Achilles tendinopathy or without any diseases</td>
<td>Not reported</td>
<td>23.43%</td>
<td>54 (23%)</td>
<td>2.8 (1.9;4.1)</td>
<td></td>
</tr>
<tr>
<td>Kraemer, Germany, 2012</td>
<td>Case-control</td>
<td>310 (161) sportsmen between 18-65 years old with Achilles tendinopathy or without any diseases</td>
<td>Age, mean (SD), years</td>
<td>Sex (% male)</td>
<td>Number (%)</td>
<td>OR (95% CI)</td>
<td>Number (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.0 (11.0)</td>
<td>66.77%</td>
<td>17 (11%)</td>
<td>1.4 (0.6;3.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh, United Kingdom, 2015</td>
<td>Prospective cohort</td>
<td>83 patients with midportion Achilles tendinopathy</td>
<td>Median 46 (range 25-79)</td>
<td>68.67%</td>
<td>1 (1%)</td>
<td>3.0 (0.3;27.5)</td>
<td>(4)</td>
</tr>
</tbody>
</table>
### Achilles tendinopathy

<table>
<thead>
<tr>
<th>First author (country, year of publication)</th>
<th>Type of study</th>
<th>Total group (cases) and population description</th>
<th>Baseline participant characteristics</th>
<th>Heterozygous familial hypercholesterolemia</th>
<th>Ankylosing spondylitis</th>
<th>Psoriatic arthritis</th>
<th>Rheumatoid arthritis</th>
<th>Reactive arthritis</th>
<th>Systemic Lupus Erythematosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggarwal, India, 2009</td>
<td>Prospective cohort</td>
<td>70 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>Age, mean (SD), years: 48.1 (range 42-64)</td>
<td>Sex (% male): 83.9%</td>
<td>Number (%): 17 (24%)</td>
<td>OR (95% CI): 61.4 (33.3;113.0)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Alam, Qatar, 2016</td>
<td>Retrospective cohort</td>
<td>62 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>Age, mean (SD), years: 56.21 (range 42-64)</td>
<td>Sex (% male): 83.9%</td>
<td>Number (%): 9 (15%)</td>
<td>OR (95% CI): 32.5 (15.2;69.3)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Beeharry, United Kingdom, 2005</td>
<td>Case-control</td>
<td>220 (133) patients and their partners attending a lipid clinic</td>
<td></td>
<td>Age, mean (SD), years: 51.27 (9.6)</td>
<td>Sex (% male): 48.18%</td>
<td>Number (%): 62 (47%)</td>
<td>OR (95% CI): 11.8 (4.8;28.9)</td>
<td>(5)</td>
<td></td>
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<tr>
<td>Cantini, Italy, 2001</td>
<td>Case-control</td>
<td>549 (183) patients with psoriatic arthritis or a different rheumatologic disease</td>
<td></td>
<td>Age, mean (SD), years: 51.27 (9.6)</td>
<td>Sex (% male): 49.18%</td>
<td>Number (%): 24 (13%)</td>
<td>OR (95% CI): 28.9 (17.4;48.0)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Elkayam, Israel, 2000</td>
<td>Prospective cohort</td>
<td>70 patients with psoriatic arthritis</td>
<td>Not reported</td>
<td>Age, mean (SD), years: 48.1 (range 30-74)</td>
<td>Sex (% male): 55.71%</td>
<td>Number (%): 18 (26%)</td>
<td>OR (95% CI): 66.2 (36.3;120.8)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Galluzzo, Italy, 2000</td>
<td>Prospective cohort</td>
<td>31 patients with psoriatic arthritis</td>
<td></td>
<td>Age, mean (SD), years: 48.1 (range 30-74)</td>
<td>Sex (% male): 58.06%</td>
<td>Number (%): 1 (3%)</td>
<td>OR (95% CI): 6.4 (0.9;47.6)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Gerster, Switzerland, 1977</td>
<td>Prospective cohort</td>
<td>35 patients with ankylosing spondylitis</td>
<td></td>
<td>Age, mean (SD), years: 59.58 (range 19-98)</td>
<td>Sex (% male): 42.99%</td>
<td>Number (%): 15 (43%)</td>
<td>OR (95% CI): 143.5 (69.6;295.6)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Jarrot, France, 2001</td>
<td>Retrospective cohort</td>
<td>158 patients with systemic lupus erythematosus</td>
<td>Not reported</td>
<td>Age, mean (SD), years:</td>
<td>Sex (% male): 9.49%</td>
<td>Number (%): 0 (0%)</td>
<td>OR (95% CI): 0</td>
<td>(5)</td>
<td>Number (%): 8 (5%)</td>
</tr>
</tbody>
</table>
## Achilles tendinopathy

<table>
<thead>
<tr>
<th>First author (country, year of publication)</th>
<th>Type of study</th>
<th>Baseline participant characteristics</th>
<th>Heterozygous familial hypercholesterolemia</th>
<th>Ankylosing spondylitis</th>
<th>Psoriatic arthritis</th>
<th>Rheumatoid arthritis</th>
<th>Reactive arthritis</th>
<th>Systemic Lupus Erythematosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggarwal, India, 2009</td>
<td>Prospective cohort</td>
<td>70 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>84.3%</td>
<td>14 (20%)</td>
<td>49.0 (27.0;89.2)</td>
<td>5 (95% CI)</td>
<td>29 (16%)</td>
</tr>
<tr>
<td>Alam, Qatar, 2016</td>
<td>Retrospective cohort</td>
<td>62 patients with ankylosing spondylitis</td>
<td>Not reported</td>
<td>83.9%</td>
<td>14 (23%)</td>
<td>44.6 (23.4;84.8)</td>
<td>5 (95% CI)</td>
<td>28 (15%)</td>
</tr>
<tr>
<td>Cantini, Italy, 2001</td>
<td>Case-control</td>
<td>549 (183) patients with psoriatic arthritis or a different rheumatologic disease</td>
<td></td>
<td>51.27 (9.6)</td>
<td>49.18%</td>
<td>5 (52% CI)</td>
<td>29 (95% CI)</td>
<td>35.3 (9.8;126.7)</td>
</tr>
<tr>
<td>Galluzzo, Italy, 2000</td>
<td>Prospective cohort</td>
<td>31 patients with psoriatic arthritis</td>
<td></td>
<td>48.1 (range 30-74)</td>
<td>58.06%</td>
<td>3 (10%)</td>
<td>16.4 (4.9;55.2)</td>
<td>5 (95% CI)</td>
</tr>
<tr>
<td>Gerster, Switzerland, 1977</td>
<td>Prospective cohort</td>
<td>35 patients with ankylosing spondylitis</td>
<td></td>
<td>59.58 (range 19-98)</td>
<td>42.99%</td>
<td>1 (1%)</td>
<td>1.5 (0.2;11.2)</td>
<td>5 (95% CI)</td>
</tr>
<tr>
<td>Hernandez-Diaz, Italy, 2019</td>
<td>Prospective cohort</td>
<td>112 patients with rheumatoid arthritis</td>
<td></td>
<td>51 (range 22-85)</td>
<td>10.71%</td>
<td>1 (1%)</td>
<td>1.4 (0.2;10.0)</td>
<td>5 (95% CI)</td>
</tr>
<tr>
<td>Smith, USA, 1980</td>
<td>Retrospective cohort</td>
<td>29 female patients with Reiter’s disease</td>
<td></td>
<td>36.7 (13.5)</td>
<td>0%</td>
<td>15 (52%)</td>
<td>973.0 (380.8;2486.1)</td>
<td>5 (95% CI)</td>
</tr>
</tbody>
</table>

SD: Standard Deviation; OR: Odds Ratio; BMI: Body Mass Index.

SUMMARY

Objective. Percutaneous electrolysis is a new treatment modality whose benefits have been described in recent years. The aim of this study was to analyze the articles published on percutaneous electrolysis and to compare bibliometric and altimetric analyses.

Methods. Using the keywords “percutaneous electrolysis”, all articles published in Web of Science were identified. For bibliometric analysis, article title, number and names of authors, h-index of authors, article citation count, citation index, journal names, impact factors, h- and q-indices, and types of articles were determined. For altimetric analysis, the “Altmetric Attention Score” (AAS) was recorded with a software automatically calculating the score. Correlation analyses between bibliometrics and altimetrics were performed.

Results. The total number of citations in the list was between 0 and 48. The country that produced the most articles was Spain and the most cited author was Fernandez-de-las-Penas. The journals that published the highest number of articles had each published four articles. The majority of the articles consisted of clinical trials. According to Pearson correlation coefficients, there was a moderate and significant correlation between the number of citations and the AASs (p < 0.005, r = 0.561).

Conclusions. This study provides a general overview of the level of interest shown in articles related to percutaneous electrolysis within the scientific community and on social media platforms. The findings can assist researchers in understanding the current state of research on percutaneous electrolysis and in identifying new directions for future research as promptly as possible. However, more research and collaboration are needed on a global scale.

KEY WORDS
Altmetric analysis; bibliometric analysis; citation; percutaneous electrolysis; galvanic current.

INTRODUCTION

Percutaneous Electrolysis is a medical technique that utilizes a cathode flow to generate a non-thermal electrochemical ablation, triggering a controlled and localized inflammatory response, enabling the activation of cellular mechanisms involved in the healing and regeneration of damaged soft tissue (1-3). It has been successfully applied, particularly in the treatment of chronic tendinopathy in recent years (3). Percutaneous electrolysis utilizes a combination of mechanical (needle) and electrical (galvanic current) stimulation to provide controlled microtrauma and non-thermal electrochemical ablation directly to the degenerated tendon area. This leads to the production of sodium hydroxide molecules, changes in pH, and an increase in oxygen in the treatment area, enabling cellular phagocytosis and activating tendon repair (3, 4). Scientific evidence regarding percutaneous electrolysis has increased in the last decade, and the method has become increasingly popular. Hence, there is a need for bibliometric and altimetric analyses that provide a comprehensive overview of percutaneous electrolysis.
Bibliometric analysis is an analytical method applied to assess the academic productivity of a field within a specific time frame (5). It is used to analyze influential articles, countries, journals, and authors or organizations within a field. Bibliometric analysis results can identify the deficiencies that need consideration in future research and provide guidance for further studies (6). Academics frequently use bibliometric analysis to identify the most valuable publications within their respective fields (7).

Due to the widespread use of the internet and the increased utilization of social media platforms in various aspects of life, alternative metrics have begun to be applied to measure the impact of research. Various internet-based tools allow for the rapid tracking of the social impact of a scientific publication by assessing social media shares, comments on platforms like YouTube, Facebook, Twitter, mentions in news outlets, and academic platforms (8). One of these tools, the “Altmetric Attention Score” (AAS), is a weighted score that assesses and measures the online impact of an article on various platforms, including social media, mainstream news, blogs, and forums (9, 10). AASs are calculated automatically using an algorithm based on the weighted count of all online attention received by a research paper.

The concept of “Altmetric Attention Score” gained prominence when Altmetric Explorer (Altmetric, London, UK) began conducting analyses in 2011. Although the concept is still in its infancy, there are articles using and analyzing AASs (11, 12).

The aim of the present study was to analyze all previously published articles related to percutaneous electrolysis using the aforementioned techniques and to assess the relationship between total citation count/citation index and the AAS. Hence, the aim is to contribute to the identification of hypotheses and methodologies for new studies through the data, such as popular topics or deficiencies in this field.

**MATERIALS AND METHODS**

**Study design and search strategy**

Similar studies in the literature were reviewed before the study, and the methodology was developed based on these studies (6). The Web of Science database (Clarivate, Analytics, Philadelphia, USA) was used to search for articles. On July 5, 2023, a search was conducted in the Web of Science database for articles published in English between 1975 and 2023 using the keyword “percutaneous electrolysis”. Then, using the “Number of Citations” option, the articles were ranked according to the number of citations from highest to lowest. Since the data used in this study were obtained from published articles, ethical approval was not required.

**Article selection**

The articles were identified through independent screening of abstracts/full texts by two reviewers (F.B. and B.A.) in the Web of Science database according to study type and subject. In case of any disagreement, a third researcher (M.Y.) assisted in reaching a consensus for the final decision. Only articles with a primary focus on percutaneous electrolysis were included in the study. Articles with a primary focus other than percutaneous electrolysis were excluded. Case report or case series, randomized controlled study, case-control study, retrospective study, cohort study, meta-analysis, editorial, prospective study, systematic review, and animal and laboratory study were included. Other types of publications were excluded.

**Data extraction**

All articles were read by the authors, and parameters such as article title, publication year, number of authors, author names (first and corresponding author), author’s country, author’s h-index, citation count, citation index, publication type, journal name, journal impact factor, journal’s h-index, and q-index were recorded for bibliometric analysis. For articles with authors from different countries, the country of the first author was selected as the country of publication. Citation index is a parameter obtained by calculating the number of citations on an annual basis. It is determined by the ratio of the total number of citations of the article to the number of years elapsed since its publication (13). The Altmetric Attention Score was obtained through the “Altmetric it” function available on the altmetric.com website. It evaluates the impact of an article by considering not only the citation count but also the views and downloads on social platforms. This includes social media visibility (such as newspapers, Twitter, and Facebook), mentions in research blogs, bookmarks in reference managers like Mendeley, news coverage, and more. The more an article is cited by different authors, the higher the AAS (14). Additionally, the characteristics of the citing authors are also influential in the calculation of the AAS. Details on the calculation method can be found on the aforementioned website.

**Statistical analysis**

To perform all statistical analyses, IBM SPSS Statistics v. 21.0 statistical software (Armonk, NY, USA) was used. Shapiro-Wilk test was used to determine the distribution of the variables. Descriptive statistics were expressed as “mean ± standard deviation” and “median, minimum-maximum” for quantitative variables and “frequency and percentage (n (%))” for categorical variables. Spearman’s rho test was used to evaluate the correlations between variables that did not follow a normal distribution. Accordingly, the correla-
tions were categorized as strong for rho score \( (r) \geq 0.60 \), moderate for \( r \) between 0.30 and 0.60, and weak for \( r \leq 0.30 \). \( p < 0.05 \) was considered statistically significant in all analyses (15).

RESULTS

A search of the Web of Science database between 1975 and 2023 using the keyword “percutaneous electrolysis” revealed a total of 56 articles published between 2010 and 2023. The articles, ranked from the most cited to the least cited, are presented in table I. The total number of citations varies between 0 and 48. The mean number of citations was \( 8.35 \pm 10.25 \) and the median number of citations was 6. For the citation index, the mean value was \( 1.59 \pm 1.49 \) and the median value was 1.35. The most cited article was “Clinical results after ultrasound-guided intratissue percutaneous electrolysis (EPI\textsuperscript{®}) and eccentric exercise in the treatment of patellar tendinopathy”. Published by Abat \textit{et al.} (3) in 2015, the article had 48 citations and a citation index of 5.33. The total number of citations and articles published by year are illustrated in figure 1.

AAS of the articles ranged from 1 to 45. For 7 articles, the AAS could not be calculated. The mean AAS was \( 7.67 \pm 9.38 \) and the median value was 4. The article with the highest AAS was published by Valtierra \textit{et al.} (16) in 2018 with the title “Ultrasound-Guided Application of Percutaneous Electrolysis as an Adjunct to Exercise and Manual Therapy for Subacromial Pain Syndrome: A Randomized Clinical Trial” (figure 2). The top three studies with the highest citation counts and AASs were studies examining treatment efficacy (3, 16, 17).

The most cited articles on percutaneous electrolysis were mostly published in 2022 \((n = 12)\), 2020 and 2021 \((n = 10)\) (figure 1). Articles on percutaneous electrolysis were published in a total of 39 journals. Two or more articles were published in 10 journals. The highest number of articles were published in Acupuncture in Medicine \((n = 4)\),

![Figure 1](image1.png)

\textbf{Figure 1.} The number of articles and citations are shown by year.

![Figure 2](image2.png)

\textbf{Figure 2.} Geographic distribution of the article with the highest altmetric score.
<table>
<thead>
<tr>
<th>No</th>
<th>Title</th>
<th>Authors</th>
<th>CA</th>
<th>Country</th>
<th>ST</th>
<th>PY</th>
<th>TC</th>
<th>CI</th>
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<th>NA</th>
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<tbody>
<tr>
<td>1</td>
<td>Clinical results after ultrasound-guided intratissue percutaneous</td>
<td>Abat et al.</td>
<td>Abat</td>
<td>Spain</td>
<td>Knee Surgery, Sports Traumatology, Arthroscopy</td>
<td>2015</td>
<td>48</td>
<td>5.33</td>
<td>22</td>
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<tr>
<td>2</td>
<td>Ultrasound-guided percutaneous needle electrolysis in chronic lateral</td>
<td>Valera-Garrido et al.</td>
<td>Valera-Garrido</td>
<td>Spain</td>
<td>Acupuncture in Medicine</td>
<td>2014</td>
<td>43</td>
<td>4.3</td>
<td>31</td>
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<tr>
<td>3</td>
<td>Randomized, double-blind study comparing percutaneous electrolysis</td>
<td>Lopez-Martos et al.</td>
<td>Gonzalez-Perez</td>
<td>Spain</td>
<td>Medicina Oral, Patología Oral y Cirugía Bucal</td>
<td>2018</td>
<td>33</td>
<td>5.5</td>
<td>13</td>
<td>6</td>
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<td>4</td>
<td>Ultrasound-Guided Percutaneous Electrolysis and Eccentric Exercises</td>
<td>Arias-Buria et al.</td>
<td>Fernandez-de-las-Penas</td>
<td>Spain</td>
<td>Evidence-Based Complementary and Alternative Medicine</td>
<td>2015</td>
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<td>2.89</td>
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<td>Autonomic responses to ultrasound-guided percutaneous needle</td>
<td>De-la-Cruz-Torres et al.</td>
<td>De-la-Cruz-Torres</td>
<td>Spain</td>
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<td>2016</td>
<td>23</td>
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<td>8</td>
<td>Effectiveness of Ultrasound-Guided Percutaneous Electrolysis for</td>
<td>Gomez-Chiguano et al.</td>
<td>Fernandez-de-las-Penas</td>
<td>Spain</td>
<td>Pain Medicine</td>
<td>2021</td>
<td>16</td>
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<tr>
<td>9</td>
<td>Intratissue percutaneous electrolysis combined with active physical</td>
<td>Moreno et al.</td>
<td>Moreno</td>
<td>Italy</td>
<td>Journal of Sports Medicine and Physical Fitness</td>
<td>2017</td>
<td>15</td>
<td>2.14</td>
<td>17</td>
<td>5</td>
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<tr>
<td>10</td>
<td>Effectiveness of Percutaneous Electrolysis in Supraspinatus</td>
<td>Rodriguez-Huguet et al.</td>
<td>Ibanez-Vera</td>
<td>Spain</td>
<td>Journal of Clinical Medicine</td>
<td>2020</td>
<td>14</td>
<td>3.5</td>
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<td>12</td>
<td>Changes in Gene Expression Associated with Collagen Regeneration</td>
<td>Sanchez-Sanchez et al.</td>
<td>Fernandez-de-las-Penas</td>
<td>Spain</td>
<td>Journal of Clinical Medicine</td>
<td>2020</td>
<td>12</td>
<td>3</td>
<td>28</td>
<td>6</td>
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<td>13</td>
<td>Autonomic Responses to Ultrasound-Guided Percutaneous Needle</td>
<td>Bermejo et al.</td>
<td>De-la-Cruz-Torres</td>
<td>Spain</td>
<td>Journal of Alternative and Complementary Medicine</td>
<td>2018</td>
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<td>15</td>
<td>Treatment of proximal hamstring tendinopathy-related sciatic nerve entrapment: presentation of an ultrasound-guided Intratissue Percutaneous Electrolysis application</td>
<td>Mattiussi <em>et al.</em></td>
<td>Moreno</td>
<td>Italy</td>
<td>Muscles, Ligaments and Tendons Journal</td>
<td>2016</td>
<td>11</td>
<td>1.38</td>
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<td>2</td>
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<td>16</td>
<td>A Comparative Study of Treatment Interventions for Patellar Tendinopathy: A Randomized Controlled Trial</td>
<td>Lopez-Royo <em>et al.</em></td>
<td>Herrero</td>
<td>Spain</td>
<td>Archives of Physical Medicine and Rehabilitation</td>
<td>2021</td>
<td>10</td>
<td>3.33</td>
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CA: Corresponding author; ST: Source title; PY: Publication year; TC: Total citations; AS: Altmetric score; CI: Citation index; NA: Number of authors.
Bibliometric and Altmetric Analysis of Percutaneous Electrolysis

Evidence-Based Complementary and Alternative Medicine (n = 4), Journal of Clinical Medicine (n = 4), and International Journal of Environmental Research and Public Health (n = 4) journals. Table II outlines the top 10 journals by the number of articles published, publishing companies, Q ranking of journals, h-index, and impact factor (IF) data. The majority of articles related to percutaneous electrolysis have originated from Spain (n = 49). Other contributing countries were Italy (n = 4), Qatar (n = 1), Kuwait (n = 1), and Saudi Arabia (n = 1). The number of authors for these articles varies between 1 and 14. In contrast, the majority of the articles (n = 38) were written by 5 or more authors. Authors with the most published articles were Fernandez-de-las-Penas (n = 9), Valera-Garrido (n = 9), and Herrero (n = 8). These three authors were the corresponding author in 20 articles and the first author in 4 articles (table III).

The majority of the articles were clinical studies (n = 45), the majority of which were randomized controlled trials. The remaining articles consisted of reviews (n = 8), case reports (n = 1), corrections (n = 1) and proceeding papers (n = 1) (figure 3). Nine articles were experimental studies on animals, cadavers or bacteria. A significant and moderate correlation was found between the total citation count and the AAS (p < 0.005, r = 0.561). There was a significant and moderate correlation between the citation index and the AAS (p < 0.005, r = 0.559) (figure 4).

DISCUSSION
Bibliometric analysis is a research method that employs quantitative methods such as mathematics and statistics to analyze relevant information about the number of articles, their distribution, changes, and quality within a specific time frame and region. Traditional bibliometrics is still recognized as a reliable tool. With the help of bibliometric analysis, it is possible to summarize key articles and general publication patterns on a precise scientific topic. However, there has been a growing interest in altimetric analysis in recent years. Due to the widespread use of social media, it is stated that citations from web-based journals cannot be accepted as the sole criterion for impact factor and that altimetric analysis can be used as an alternative to citation count, impact factor and similar evaluation methods.

The AAS is generated by calculating the amount

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*IF: Impact factor, 2022 Journal Citation Reports, Web of Science Group; **2022 SCImago Journal and Country Rank; NA: not available.
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<td>Gomez-Trullen</td>
<td>6</td>
<td>53</td>
<td>10</td>
<td>Spain</td>
<td>University of Zaragoza</td>
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<tr>
<td>8</td>
<td>Lopez-Royo</td>
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<td>46</td>
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<td>San Jorge University</td>
</tr>
<tr>
<td>9</td>
<td>Al-Boloushi</td>
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<td>4</td>
<td>Kuwait</td>
<td>Ministry of Health</td>
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<td>5</td>
<td>77</td>
<td>15</td>
<td>Spain</td>
<td>King Juan Carlos University</td>
</tr>
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</table>

Figure 3. Distribution of article type.

Figure 4. Scatter plot between total citation count and altmetric attention score (A); Scatter plot between citation index and altmetric attention score (B).
of interest in research studies with an automatic algorithm (19). Due to the increasing popularity of altimetric analysis, journals now offer AAS to researchers in addition to the traditional citation score of their articles. Similar to citation-based bibliometric measurements, altimetric measurements do not reflect the quality of a research; they solely measure the online interest in the research. It has been suggested that traditional bibliometric methods and altimetric analysis cannot be used interchangeably when assessing the online impact and dissemination of articles, but it can instead be used complementarily (20). To the best of our knowledge, there is no study in the literature on percutaneous electrolysis that includes both bibliometric and altimetric analyses. Therefore, a comprehensive, systematic, scientific, and objective research methodology has been employed to comprehensively understand the background and development trends of percutaneous electrolysis treatment, evaluate the current status of research, and explore the hotspots and limitations by combining bibliometric and altimetric analyses. Percutaneous electrolysis is a relatively new treatment modality. The first publication related to percutaneous electrolysis was made in 2010, and in the subsequent years, there has been a significant increasing trend in the number of publications regarding this topic. After the initial publication in 2010, the absence of publications in 2011, 2012, and 2013 is particularly noteworthy. It can be said that it took a certain amount of time for researchers to notice this study. In the following years, the topic attracted attention and the number of publications gradually increased. In 2022, it reached its peak. This trend indicates an increasing interest in percutaneous electrolysis treatment. We believe that the number of publications will continue to increase in the following years.

The highest contribution came from Spain with 49 articles, accounting for 87.5% of the articles published. This may be because percutaneous electrolysis was initially developed in Spain, attracting the interest of other researchers in the country. Usually, in previous bibliometric analyses, United States has been identified as the country with the most contribution (7, 21, 22), and this can be generalized for the entire medical literature. Although there are no previous bibliometric analyses on percutaneous electrolysis, the findings of this study are similar to previous bibliometric reviews on dry needling. In a bibliometric analysis of dry needling, it was observed that Spain was one of the two countries with the most contribution, similar to percutaneous electrolysis (23). Furthermore, similar to the present study, the author with the most publications was “Fernandez-de-las-Penas”. According to these results, both dry needling and percutaneous electrolysis topics hold a significant position in Spain. The journals publishing the most articles, with 4 articles each, were Acupuncture in Medicine, Evidence-Based Complementary and Alternative Medicine, Journal of Clinical Medicine, and International Journal of Environmental Research and Public Health. Two of the journals were of British origin, while the other two were from Switzerland. In general, researchers tend to publish their publications in their own countries (22). Interestingly, in the present study, the top 10 contributing journals were not of Spanish origin. We believe that one reason for this is researchers’ desire to publish their research in journals with higher publication value and accessibility to reach a wider audience.

The article titled “Clinical results after ultrasound-guided intratissue percutaneous electrolysis (EPI®) and eccentric exercise in the treatment of patellar tendinopathy”, published by Abat et al. in Knee Surgery, Sports Traumatology, Arthroscopy in 2015, was the most cited article with 48 citations (3). It had a citation index of 5.33. The article was a prospective study with a Level IV evidence that evaluated 40 patients with patellar tendinopathy over a 10-year follow-up period. The study emphasized that percutaneous electrolysis and eccentric exercises in patellar tendinopathy provided a rapid return to previous activity levels and highlighted their continued benefits during long-term follow-ups. No relapses or adverse events were reported during the 10-year follow-up.

The article titled “Ultrasound-guided percutaneous needle electrolysis in chronic lateral epicondylitis: short-term and long-term results” published by Valera-Garrido et al. in Acupuncture in Medicine in 2014 was the second most cited article with 43 citations (17). With an AAS of 31, it was also the study with the second highest AAS. This article had a citation index of 4.3. The effectiveness of percutaneous electrolysis was also emphasized in this prospective study reporting the efficacy of percutaneous electrolysis and eccentric exercise therapy in 36 patients with lateral epicondylitis. The study evaluated structural tendon changes and vascularity using ultrasonographic techniques. During the 52-week follow-up period, no relapse or adverse events were reported. It is noteworthy that the two most cited articles are not randomized controlled trials. In addition, eccentric exercise therapy was applied with percutaneous electrolysis treatment. It is not known how much treatment efficacy depended on either of the treatments. Due to the concurrent application of exercise therapy with percutaneous electrolysis, the efficacy of percutaneous electrolysis treatment could not be clearly established. Therefore, large randomized controlled trials comparing percutaneous electrolysis with control groups are needed.

The third most cited article was “Randomized, double-blind study comparing percutaneous electrolysis and dry needling
for the management of temporomandibular myofascial pain” by Lopez-Martos et al. published in Medicina Oral y Cirugia Bucal in 2018 (24). This article had 33 citations. It had a citation index of 5.5 and was the article with the highest citation index. Unlike the two most cited articles, this was a randomized controlled double-blind study. The study compared percutaneous electrolysis and dry needling in the treatment of temporomandibular myofascial pain. In this treatment applied on the trigger points of the lateral pterygoid muscle, 60 patients were divided into three groups and the control group underwent a sham needling procedure. Both dry needling and percutaneous electrolysis demonstrated benefits. Improvements were observed earlier in the percutaneous electrolysis group. Percutaneous electrolysis also uses acupuncture needles used in dry needling therapy. Therefore, the mechanical effect of both treatments in the area where they are applied is similar. The additional use of galvanic current may be the beneficial factor here. Top three articles with the highest AASs ranked 5th, 2nd, and 37th in terms of citation count. These findings also significantly emphasize the difference between bibliometric and altimetric analyses. According to AASs, the article with the highest AAS of 45 was published in The Journal of Pain titled “Ultrasound-Guided Application of Percutaneous Electrolysis as an Adjunct to Exercise and Manual Therapy for Subacromial Pain Syndrome: A Randomized Clinical Trial” (16). The article was published by Valtierra et al. in 2018. The findings of this randomized controlled study showed that adding percutaneous electrolysis treatment to exercise and manual therapy treatment was beneficial in subacromial pain syndrome. With 23 citations and a citation index of 3.83, this article was also the fifth article with the highest number of citations. The randomized controlled design was a strength of this study.

“Effectiveness of electrostimulation percutaneous intratissular (EPI®) in chronic insertional patellar tendinopathy”, the first published article on percutaneous electrolysis, had the third highest AAS with 29 points. It was published by Valera-Garrido et al. in the Trauma (Spain) journal in 2010 (25). The effectiveness of percutaneous electrolysis combined with eccentric exercises was evaluated in 32 patients with patellar tendinopathy, and the treatment’s efficacy was emphasized. Interestingly, this article has only three citations and ranks 37th in the citation count. This finding further emphasizes the difference between citation count and altimetric score. The article also has a citation index of 0.21. The most significant limitation of this study was that it was not a randomized controlled trial.

Interestingly, the fourth-highest article in terms of altimetric score, with an AAS of 28, was the one published in 2020 by Sanchez-Sanchez et al. titled “Changes in Gene Expression Associated with Collagen Regeneration and Remodeling of Extracellular Matrix after Percutaneous Electrolysis on Collagenase-Induced Achilles Tendinopathy in an Experimental Animal Model: A Pilot Study” (26). This animal study focused on histological and gene expression changes after percutaneous electrolysis in experimentally induced Achilles tendinopathy. The authors identified a significant increase in the molecular expression of COX2, MMP9 and VEGF genes in Achilles tendons treated with percutaneous electrolysis. They stated that these changes in gene expression could enhance collagen regeneration and remodeling of the extracellular matrix. These findings indicate that the effects of percutaneous electrolysis treatment at the tissue level have garnered attention in social media. The effects of galvanic current at the cellular and tissue level will be further investigated as percutaneous electrolysis therapy becomes more widespread and its benefits are demonstrated.

Articles related to percutaneous electrolysis were studies investigating its effectiveness, mechanisms of action, physiological effects, and reliability. Studies evaluating treatment efficacy were particularly prominent (n = 40). In the vast majority of these studies, the effectiveness of percutaneous electrolysis has been investigated in the treatment of chronic tendinopathy patients, both as a standalone treatment and in addition to exercise therapy. There are also publications investigating the effectiveness of percutaneous electrolysis in myofascial pain syndrome, breast fistula, and acute whip-lash syndrome (17, 27, 28). The general message conveyed by these studies is that percutaneous electrolysis treatment is beneficial. However, it has also been indicated that long-term randomized controlled trials are needed to demonstrate that this benefit persists over time (29). Additionally, it has been mentioned that applying percutaneous electrolysis under ultrasound guidance would provide maximum precision while minimizing damage to other structures (30). Although articles on this topic initially began to be published in 2010, the increasing number of studies resulted in the highest number of articles entering the list between 2020 and 2023. Upon evaluating the pool of articles included in the study based on the years, as expected, the largest number of citations was made to articles published in 2022. Upon examining other studies in the literature that combine bibliometric and altimetric analyses, although there are studies indicating a high correlation between AAS and citation count (5), most of the studies we reviewed show a moderate to weak correlation (6, 14, 22, 31, 32). Similarly, in the present study, the correlation between citation count and altimetric score was not completely consistent and the correlation level was moderate. Social media trends and traditional bibliometric data are often inconsistent. This is because AAS is calculated based on current interactions from social media trends and traditional bibliometric data.
media platforms, news, policy documents, blogs, and more. It therefore reflects the influence of individual users rather than professional researchers (6). Therefore, bibliometric and altimetric analyses should be considered complementary to each other.

From the articles included in this study, we found that 10 were published in journals focusing on Complementary and Integrative Medicine, 10 articles were published in general medical journals, 8 in orthopedic journals, and 7 in sports science journals. This is another finding showing the usage of percutaneous electrolysis treatment across various medical fields and pathologies.

Being the first study to assess the research productivity related to percutaneous electrolysis is a fundamental strength of this study. Bibliometric analysis provides researchers with precise information for constructing new research. In this regard, we believe that this analysis will serve as a reference point to attract researchers’ attention and shed light on new research interests related to percutaneous electrolysis. Among other strengths of our study, the analysis we conducted to provide a comprehensive perspective on percutaneous electrolysis was not solely based on social media and public platform interactions or solely on citation counts. Instead, these methods were used together to provide a clearer perspective, and the correlation between altimetric and bibliometric data was also evaluated. It is believed that such a measurement combination could provide the most robust insight into research productivity within a medical field.

This study also has some limitations. The research was conducted only within the Web of Science database, excluding other databases such as Google Scholar, Scopus, and PubMed, which might have affected the number of articles analyzed. However, Web of Science is one of the most popular databases and it covers a relatively comprehensive literature, has strong reliability and authority, and is widely cited in bibliometric analyses (33). Only English articles were included in the study. The inclusion of articles in other languages could provide a broader perspective. The citation analysis did not examine self-citations and interactions between citations. Parameters that may affect science, such as income levels of countries, were also excluded from the study. Some of the articles had no AAS. This is due to both AAS being a newer measurement in terms of research impact and potential reach, and the emergence and increasing popularity of percutaneous electrolysis in recent years. We believe that the popularity of percutaneous electrolysis will continue to increase on social media in the coming years. Daily changes in social media, fluctuations over time periods or with respect to certain events can alter the results. With the rapid changes in social media, the results also tend to be highly variable. This is one of the limitations of altimetric analysis. In addition, some social media outlets are not covered by Altmetric. Although the most popular and widely used social media tools are covered by Altmetric, this is also a limitation. There is a need for more comprehensive studies that analyze larger databases, include articles in other languages, identify links between authors and countries, examine financial support, and conduct self-citation analysis.

CONCLUSIONS
Using bibliometric and altimetric analysis methods, we revealed the global productivity characteristics of articles on percutaneous electrolysis. Bibliometric and altimetric analyses help uncover research trends in the respective field, aid in understanding current topics, highlight gaps in the field, and guide researchers for potential future research. In this regard, the present study may contribute to new developments in percutaneous electrolysis.

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None.

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

CONTRIBUTIONS
All authors contributed equally to this article.

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

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electrolysis and dry needle for the management of tempo-
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P. Randomized, double-blind study comparing percutaneous
electrolysis and dry needle for the management of tempo-


Effects of Subscapularis Muscle Dry Needling on clinical symptom improvement in People with Frozen Shoulder: A Randomized Controlled Trial Protocol

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INTRODUCTION

Frozen shoulder, a term that was introduced by Codman in 1934, referred to a condition with symptoms in the shoulder region, especially in the deltoid muscle insertion, with a slow onset that inability to sleep on the affected side is considered as the chief complaint. Frozen shoulder is a common musculoskeletal problem, affecting 2 to 5 percent of the population (1, 2). This condition is accompanied by a gradual and spontaneous increase in pain and a decrease in active and passive range of motion (ROM) (3) mainly because of the formation of fibrosis tissue and shortening of the joint capsule and ligaments surrounding the glenohumeral (GH) joint (4, 5). Considering that the GH joint is involved in many daily activities, any complication, such as a frozen shoulder or tendinitis, which reduces the ROM and causes pain in this area, leads to severe restrictions (6).
The role of rotator cuff muscles, especially the subscapularis muscle, is crucial in the dynamic stability of the GH joint (7). The subscapularis muscle is one of the major muscles between rotator cuff muscles, which primarily acts as an internal rotator of the humerus (8, 9), but it also has a function in the GH joint flexion, abduction, and adduction (10, 11).

Although the frozen shoulder, according to its definition, is a capsular problem, the development of myofascial trigger points (MTrPs) in the rotator cuff muscles may aggravate its symptoms (4). MTrPs are “hyper-irritable points in the taut band of skeletal muscles or fascia which cause local sensitivity and referred pain” (11). MTrPs are classified as active and latent MTrPs: “active MTrP has spontaneous pain or pain in response to movement, stretch or compression, while latent MTrP is a sensitive spot with pain or discomfort in response to just compression” (12). The subscapularis MTrPs are usually considered key MTrPs in the frozen shoulder syndrome, which can cause pain at rest and during motion (10, 12). The referral pain pattern of subscapularis MTrPs is felt at the posterior side of the shoulder, which may extend to the scapula and elbow, even more distant areas like the dorsal part of the wrist (11). In people with shoulder pain, the presence of subscapularis MTrP is often overlooked, which must be considered in the treatment plan (11). The initial symptoms of a frozen shoulder, including restricted shoulder movement, especially abduction, external rotation, and shoulder pain, are shared with the initial symptoms of active MTrPs of the subscapularis muscle. In many cases, they both can occur concurrently (11). After developing MTrPs in the subscapularis, other muscles in the shoulder region, like the head of the biceps brachii, deltoid (anterior portion), pectoralis major, teres major, and latissimus dorsi could develop MTrPs and the movement of the GH joint is severely limited in all planes (11).

Physiotherapy, as a conservative treatment, mainly suggests electrotherapy, hot packs, Laser, short-wave diathermy, ultrasound therapy, and manual mobilization. Dry needling (DN), as a new, effective, and minimally invasive method in the physiotherapy field is defined as “inserting the needle into the muscle to deactivate MTrPs via creating a response called the local twitch response (LTR)” (13). Besides of various interventions in treating patients with frozen shoulder, ultrasonography is a reliable method in assessing MTrP characteristics (14, 15) and ultrasound guided dry needling is used in addition to conventional physiotherapy in patients with musculoskeletal disorders (16, 17). In some studies, the effects of subscapularis MTrPs deactivation on clinical symptoms of people with frozen shoulder have been investigated. According to a study that was conducted in 2019, it was stated that possibly eliminating the MTrPs of the subscapularis muscle with manual techniques may reduce the pain intensity and increase the ROM of the external rotation of the shoulder joint in people with chronic frozen shoulder. In 2021, a study evaluated the impact of DN as a therapeutic intervention on the MTrPs in the rotator cuff muscles of individuals with frozen shoulder, and it was demonstrated that DN could be effective in alleviating the intensity of pain, increasing ROM, and increasing pain tolerance in people with frozen shoulder (4). There were some limitations in the study, such as the absence of a control group and performing the DN technique on a group of muscles, (not specific muscle), which should be considered in the design of future studies.

To the best of our knowledge, evidence of the impact of DN on a frozen shoulder is limited (4, 18, 19). On the other hand, in these studies based on the presence of MTrPs at different muscles, interventions have been done on various muscles, and evidence on deactivating subscapularis MTrPs as an isolated muscle is limited. For this, the study aims to investigate the impact of subscapularis MTrP-DN on improving pain, ROM of the GH joint, and intensity of upper limb functional disability in individuals with frozen shoulder.

**Study goal and its hypothesis**

The primary goal of the current study is to evaluate the impact of DN on MTrPs of the subscapularis muscle added to the conventional physiotherapy in comparison with conventional physiotherapy alone on pain intensity in the shoulder region and ROM in abduction, flexion, external rotation, and internal rotation, as well as pain pressure threshold (PPT) and upper limb functional disability. The study hypothesis is that the participants who receive MTrP-DN during their treatment procedure would experience better improvement in the level of pain, ROM, PPT, and upper limb functional disability compared to those who receive just conventional physiotherapy.

**METHODS**

**Study design**

This protocol is for a randomized, single-blinded trial in which the outcome assessor is blinded to the interventions and will be conducted in a physiotherapy clinic related to the Tabriz University of Medical Sciences. Different items in this paper have been written according to the principles defined at the SPIRIT 2013 statement.

**Ethical issues**

This study was registered in the ethics committee with IR.TBZMED.REC.1402.013 code on 2023-03-06 (date of approval: March 06, 2023).
Experimental procedures

Participants
Participants are adults who will be referred to the physiotherapy clinic with a diagnosis of frozen shoulder and the existence of the subscapularis muscle MTrPs of the involved side. The complete eligibility criteria (inclusion and exclusion criteria) are listed in Table I.

Preparation and treatment setting
Recruitment will begin by talking with people who will be referred to the physiotherapy clinic with a diagnosis of frozen shoulder; in case of eligibility confirmation, they will be informed about the study and its goals. Once they are acknowledged and still interested in participating, they will need to sign an informed consent form to be recruited in the study. Then, demographic information will be collected; there are six factors to consider: age, sex, height, weight, medication and physiotherapy history, and the affected shoulder.

Following the collection of the baseline data, 40 participants will be randomly recruited into intervention and control groups using the permuted block randomization method to avoid bias in the conclusion (20 patients for each group). Outcome measures include pain intensity, ROM of the glenohumeral joint (in flexion, abduction, internal and external rotation), upper limb functional disability, and pain pressure threshold. The outcome measures will be assessed twice by a blinded assessor, once at baseline and once after completion of the sessions. The Persian version of the Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire will be distributed and then collected during the 1st, 5th, and 10th sessions. Participants in both groups will receive ten treatment sessions which will be held on alternate days. The control group will receive conventional physiotherapy. The intervention group, simultaneously with the treatment of the control group, will receive three sessions of subscapularis MTrP-DN during the third, sixth, and ninth sessions. The trial schedule is shown in Figure 1.

Table I. Inclusion and exclusion criteria of the study.

<table>
<thead>
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<th>Inclusion criteria</th>
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<tr>
<td>Aged between 35 and 65 years</td>
<td>Skin problem at shoulder and scapula (6)</td>
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<tr>
<td>VAS at least 3 at subscapularis MTrPs while they compressed (25)</td>
<td>Antiplatelet therapy 3 days before starting of the study (6)</td>
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<td></td>
<td>Corticosteroid injections in the shoulder area 3 months before starting the study (6)</td>
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<td>History of cancer (6)</td>
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<td></td>
<td>Needle phobia (6)</td>
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<td></td>
<td>Positive subacromial entrapment tests (26)</td>
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<td>Shoulder joint surgery or arthroscopy (6)</td>
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<td>Cervical radiculopathy or any neurological damage in the upper limb (22)</td>
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<td>Rheumatoid disease (22)</td>
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<td>Pregnancy (27)</td>
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VAS: Visual Analogue Scale; MTrPs: Myofascial Trigger Points.
Performing assessment and treatment
The trial assessment is going to be performed by a blinded assessor, who is an expert physiotherapist (PT) with clinical experience. The treatment plan is going to be performed by another eligible PT who is certified in MTrP-DN and experienced in the treatment of musculoskeletal disorders.

Interventions

Trigger point diagnosis
The exact location of the subscapularis muscle MTrPs will be spotted by an expert PT according to Travel and Simons’ criteria (11): presence of 1) a taut band within the muscle, 2) a hyperirritable tender point in the taut band, 3) spontaneous pain, 4) local twitch response (LTR) after compression, and 5) a familiar referred pain in referral zone after irritating the tender point. For this purpose, the participant will be asked to lie in a supine position. The examiner moves the participant’s arm to 90 degrees of abduction. In the next step, the examiner takes the latissimus dorsi tendon with a pincer grip and marks the lateral border of the scapula with his/her fingertips. The examiner then slowly moves his/her fingertips downward to palpate the subscapularis muscle on the anterior side of the scapula. To locate the MTrP of the subscapularis muscle, which is placed in the upper portion of the lateral edge of the scapula, the examiner may go in an upward direction toward the coracoid process to palpate more fibers at the superior aspect and detect a taut band in the muscle (11). Sustained light to moderate pressure, In the presence of the MTrP, will reproduce pain at the posterior portion of the shoulder and scapula.

Control group
Participants in this group will receive conventional physiotherapy during 10 sessions on alternate days by an expert PT. Conventional physiotherapy consists of using continuous ultrasound with a frequency of 3 MHz for 6 minutes around the shoulder joint capsule (20), high frequency transcutaneous electrical nerve stimulation (high-TENS; 80 Hz, 50 µs) with a time duration of 20 minutes (21), and the use of hot pack simultaneously with the application of the electrical current. For the involved shoulder, posterior-anterior, anteroposterior, and caudal glides will be used at the rate of 2 to 3 glides per second and which continued for 30 seconds at each set (4). The number of sets for each glide is 5, and the rest interval between sets will be 30 seconds (4). To perform antro-posterior gliding techniques, the participant will be asked to lie in a supine position with the involved arm flexed about 90 degrees. After positioning, the therapist will perform antro-posterior and postro-anterior gliding techniques (22). For performing the caudal glide technique, the participant will be asked to lie in a supine position with the involved arm abducted about 90 degrees (22). Besides, the participant will be asked to stretch the subscapularis muscle as well at the same time; they stand in a door frame with the GH joint in the middle position and stretch the subscapularis with the external rotation motion. During this stretch, the individuals can activate the subscapularis muscle by softly applying pressure (a minimal contraction) to the door frame (internal rotation) and holding it for 5-10 seconds. After relaxing the muscle, they stretch the muscle more by rotating themselves away from the affected side (15). Stretch maneuver can be performed three–five times and repeated up to 3-4 times daily.

Participants will also be asked to perform active-assistive exercises using a towel for 5 minutes daily (4). For this, they raise the hand of the un-involved above the head with an elbow flexed and laterally rotate the shoulder and grip one head of the towel. The involved side will go back of the body internally rotated and grab the other head of the towel. With the help of the hand of the un-involved side, the involved side will be pulled upward (23).

Pulley exercises will be used to improve flexion and extension movements of the involved shoulder. The participant will be informed to sit on a chair holding a skipping rope that has passed over a pulley. Participants swing the rope alternatively up and down with the force generated by their un-involved side. They will also be taught to perform Codman exercises in such a way that they will bend forward with a non-affected forearm supported on a table or couch, shoulder relaxed, and then gently swing the affected side arms forwards and backward, side to side, clockwise and counterclockwise in pain-free range (24).

Finally, the participants will be instructed to exercise with a finger ladder to increase flexion ROM (table II).

Intervention group
Participants in this group will receive the exact treatment protocol of the control group, added 3 sessions of DN in the 3rd, 6th, and 9th sessions of the treatment. To do so, the MTrP will be determined and marked with a marker to remain constant during the treatment and study. The exact place of the subscapularis muscle MTrP should be sterilized using sanitary cotton and alcohol. For performing the DN technique, based on Dommerholt and Fernandez de-las-Penas approaches, participants will be asked to lie in a supine position on the bed, abduct the shoulder about 90 degrees, and externally rotate as much as possible. Bringing the scapula to the lateral side can make it easier to access the muscle. After determining and lifting the latissimus-dorsi tendon with a pincer grip, the
needle (Dong bang Acuprime Ltd, with dimensions: 25 mm × 50 mm, Korea) is inserted parallel to the chest and perpendicular to the anterior surface of the scapula to the MTrP (17). The exact place of MTrP must be confirmed with LTR. To release MTrP, the fast in-fast out technique will be performed, according to the recommendation of Hong (25). If LTR isn't felt after moving the needle 10 times, we can stop the technique. If there is more than one MTrP, the most painful point will be detected and treated (figure 2).

Table II. Therapeutic interventions for participants in the present study.

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Duration</th>
<th>Dose/ frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Passive intervention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antero-posterior glide</td>
<td>2-3 Hz for (30 seconds) × 5 sets</td>
<td>Once a day</td>
</tr>
<tr>
<td>Postero-anterior glide</td>
<td>2-3 Hz for (30 seconds) × 5 sets</td>
<td>Once a day</td>
</tr>
<tr>
<td>Caudal glide</td>
<td>2-3 Hz for (30 seconds) × 5 sets</td>
<td>Once a day</td>
</tr>
<tr>
<td><strong>Active-assistive interventions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretch the Subscapularis muscle</td>
<td>5-10 second × 3-5 times</td>
<td>3-4 times per day</td>
</tr>
<tr>
<td>Towel exercises</td>
<td>5 minutes</td>
<td>Once a day</td>
</tr>
<tr>
<td>Pulley exercises</td>
<td>5 minutes</td>
<td>Once a day</td>
</tr>
<tr>
<td>Codman exercises</td>
<td>5 minutes</td>
<td>Once a day</td>
</tr>
<tr>
<td>Finger ladder exercises</td>
<td>5 minutes</td>
<td>Once a day</td>
</tr>
<tr>
<td><strong>Modalities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High frequency TENS</td>
<td>20 minutes</td>
<td>80 Hz, 50 µs</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>6 minutes</td>
<td>3 MHz</td>
</tr>
</tbody>
</table>

µs: Microsecond; MHZ: Mega hertz; TENS: Transcutaneous electrical nerve stimulation; HZ: Hertz.

Figure 2. Study timeline according to the SPIRIT checklist recommandation.

T: Treatment session; ROM: Range of motion; VAS: Visual analogue scale; MTrP: Myofascial Trigger Point.
Choice of comparators
According to a reference (26), a combination of DN and conventional physiotherapy may have more positive effects than DN or conventional physiotherapy alone. Still, this hypothesis needs to be investigated in more musculoskeletal issues, including frozen shoulder.

Criteria for altering or stopping assigned treatments
If a participant would be absent for 2 successive sessions or wouldn’t be willing to complete the treatment procedure for any reason, the process would be dismissed and another eligible participant would be recruited.

Ways to enhance compliance with interventions
To motivate participants to complete their treatment procedure, all therapeutic intervention is free, and participants can be in contact with their therapist online or by phone to ask their clinical questions. Their personal information will be kept confidential and ensured that they won’t suffer any physical or emotional damage.

Relevant concomitant care permitted or prohibited during the trial
Participants will be allowed to consume analgesics like acetaminophen or ibuprofen if they can’t tolerate the pain caused by DN (27). In the case of using any special drug, they must consult with the doctor to prevent any drug interaction.

Sample size calculation
The sample size is determined with 3.1.2G power software with a power size of 0.8, \( \alpha \) error probability of 0.05, and an effect size of 0.5 with a dropout probability of 20%. For two groups, 40 subjects (20 subjects for each group) are calculated. It should be noted that the effect size was determined according to Kalia’s study with the mean and standard deviation of the pain intensity variable (4).

Recruitment and sequence generation
The Participants will be people referred to the physiotherapy clinic by orthopedists or rheumatologists. All participants in the study will be divided randomly into MTrP-DN of the subscapularis muscle plus conventional physiotherapy (intervention group) or conventional physiotherapy alone (control group) with an allocation ratio of 1:1. Participants will be randomly assigned to either group A (intervention) or group B (control) using a random allocation software. Randomization will be conducted using permuted blocks of 4 and 8. Then, the randomization schedule will be written on a paper with sequential numbers, and the papers will be placed in a sealed envelope. Someone outside the research team will do this procedure before the initiation of the trial.

Mechanism of allocation concealment
Each participant will receive a sealed envelope based on their assigned number after the initial assessments. After including each participant, the therapist will decide how to perform the treatment based on the group they allocated. To avoid bias, participants will be acknowledged to not reveal their allocated group to the outcome assessor.

Conduction
An individual who is not part of the study will conduct the allocation sequence, enrollment, and assignment of individuals to study groups.

Blinding
This is a single-blind study, and the outcome assessor is going to be blinded to the allocation sequence by the time the trial is conducted.

Permissible instances for unblinding
It is unacceptable for the outcome assessor to become unblinded during the process. After completing the participants assessment, they can be un-blind and informed about groups.

Primary outcome
The primary outcomes for this study are pain intensity and ROM, which would be felt in the shoulder region.

Pain intensity
Pain intensity of the shoulder region and subscapularis MTrPs will be evaluated by VAS which is a reliable and valid scale for chronic and experimental pain (28). VAS is once assessed at baseline and once after completing treatment sessions. For each time, the assessment will be conducted thrice, and the average outcome will be communicated. Participants will be asked to mark the intensity they feel in the affected shoulder region from zero (no pain) to 10 (the most severe pain that could be imagined). To measure subscapularis MTrP pain intensity, for 3 seconds, the pressure of 2.5 kg/cm² at MTrP will be applied with a rate of 1 kg/cm².s⁻¹ by digital algometer) FDX 50 force Gage, Wagner Instruments, United States (while the subjects are marked their pain intensity on the VAS to determine local pain evoked by applying pressure on the MTrP.

Range of motion of glenohumeral joint
The assessor would determine the GH joint ROM of the participants using a goniometer at baseline, and after completing the treatment sessions. Assessments will be performed three times and the mean will be reported.
Glenohumeral joint flexion
Participants will be asked to lie in a supine position with hands beside the body in a neutral position. The center of the goniometer will be matched with the lateral aspect of the greater tuberosity, the proximal arm aligned with the midaxillary line of the thorax, and the distal arm with the lateral midline of the humerus. Participants are asked to raise their affected arm in the sagittal plane (29).

Glenohumeral joint abduction
The participants will be asked to lie down in a supine position with hands on the supination position. The center of the goniometer will be matched to the anterior aspect of the acromial process; the proximal arm of the goniometer should be aligned with the midline of the sternum, while the distal arm should be aligned with the anterior midline of the humerus. After that, the participants will be asked to raise their affected arm in the frontal plane (29).

Glenohumeral joint internal/external rotation
For measuring internal rotation, the participant will be asked to lie down in the supine position, with shoulder and elbow at 90 degrees of abduction and hand in the pronated position. A rolled towel is placed under the distal of the humerus to keep the humerus parallel to the ground. The center of the goniometer will be considered over the olecranon process, the proximal arm will be perpendicular to the floor, and the distal arm will be aligned with the ulna. The olecranon process and the ulnar styloid process are considered reference points. To measure internal rotation, the participant will be asked to close the palmar surface of the affected hand to the ground (29). For measuring external rotation, the participant will be asked to close his/her dorsal surface of the affected-side hand to the ground (29).

Secondary outcome measures
The secondary outcome measures of this study will be upper limb functional disability and PPT.

Upper limb functional disability
To assess upper limb functional disability, participants will complete the DASH questionnaire (Persian version) in the 1st, 5th, and 10th sessions of their treatment in the control and intervention groups. The DASH questionnaire (Persian version) is a valid and reliable questionnaire that contains 30 items about the difficulty of performing daily routine tasks to evaluate the upper limb dysfunctions during performing the tasks (30). Higher DASH scores represent more severe functional disability of the upper limb. The participants can ignore 3 items during answering. Computing will be performed with formula beyond:
\[
\frac{\text{sum of } n \text{ responses}}{n} - 1 \times 25
\]
where n denotes the number of completed items (22).

Pain pressure threshold
PPT of subscapularis muscle MTrP will be assessed by an Algometer) FDX 50 force Gage, Wagner Instruments, United States, at baseline and the end of the treatment sessions. To measure PPT, the participants will be asked to lie in a supine position with 90 degrees of abduction, and elbow flexion with the dorsal of the hand on their forehead. The algometer will be placed on the subscapularis muscle MTrP, and the compression force applied by the assessor’s hand will gradually increase. The participant will be asked to report the moment when the pressure feeling turns into pain feeling. PPT will be assessed before initiation of treatment and after completing treatment sessions. For each time, PPT will be assessed three times with a resting interval time of 10 seconds, and the mean will be recorded.

Adverse events
Adverse events that may have occurred are classified into major and minor adverse events. Inserting a needle into the skin possibly causes complications like bruising, bleeding, post-dry needling soreness, and pain during or after performing the dry needling technique (13, 31, 32). If any adverse events that are shown in table III happen, it is the liability of the PT to report it.

Strategies to encourage participants to stay involved and follow through with the program completion
During the procedure period, the participants will be encouraged to be present regularly in the physiotherapy clinic by their PT. If they have any issues with how to perform their therapeutic exercises, they can ask PT in person or by phone. All treatment procedures would be free, and participants are not supposed to pay anything at different stages of the study.

<table>
<thead>
<tr>
<th>Minor</th>
<th>Major</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>Pneumothorax</td>
</tr>
<tr>
<td>Bleeding</td>
<td>Excessive bleeding</td>
</tr>
<tr>
<td>Feeling faint</td>
<td>Syncopal responses</td>
</tr>
<tr>
<td>Headache</td>
<td>Infection</td>
</tr>
<tr>
<td>Nausea</td>
<td>Forgotten needle</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>Fainting</td>
</tr>
<tr>
<td>Aggravated symptoms</td>
<td>Numbness</td>
</tr>
<tr>
<td>Pain during and after intervention</td>
<td>Prolonged symptoms aggravation</td>
</tr>
<tr>
<td>Lower limb weakness</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Potential adverse events.
Data management
The assessor will store participants’ personal information, pre-intervention, and post-intervention measures in a confidential Excel file for analysis.

Analyzing outcomes with statistical methods
SPSS software version 5 (SPSS Inc., Chicago, IL, USA) will be used. To compare the variables between the first and the tenth sessions, in the control and intervention groups and between the two groups, the Pair t-test and Independent t-test will be used respectively. In addition, a comparison of the changes in the severity of functional disability of the upper limb between the treatment sessions in both the intervention and control groups will be performed using Repeated Measures. The significance is set at p < 0.05. Shapiro-Wilk test will be used to evaluate the normal distribution of data, and if the distribution of measured data is expected, the parametric tests will be used. Additional analysis of results (e.g., subgroup analyses) is not predicted to be conducted.

Managing non-adherence and loss of follow-up data
Non-adhering or loss to follow-up data is going to be addressed using intention-to-treat analysis.

Monitoring the data collection
The trial will be supervised by faculty members of the Physiotherapy Department of Tabriz University of Medical Sciences. Interim analysis and auditing are not predicted in this trial.

Harms and medical care after potential harm
All participants will be informed about possible harms that may occur during MTrP-DN of the subscapularis muscle before they participate in the study. Possible harms will be handled by an expert PT, and the research team is liable for reporting any adverse events of the trial. The research team is responsible for compensating for any adverse events of MTrP-DN or any harm during the study.

Protocol amendments
If there were any amendments in the protocol, new changes will be uploaded to the registration organization website.

Consent or agreement and confidentiality
The informed consent form is prepared by the standards of the Ethics Committee of Tabriz University of Medical Sciences. The form is going to be collected by the clinic’s secretary from the participants. All eligible participants will receive information about the possible benefits and potential harms before signing the informed consent form. Any personal information from the participants will be stored confidentially during the trial timeline and also will be kept confidential after completing the treatment procedure.

DISCUSSION
“Frozen shoulder” which causes burning and throbbing pain sensation in the shoulder region and severe loss of motion, is one of the most common conditions encountered by orthopedic surgeons (5, 18). Different treatments have been introduced for this condition, which is usually accompanied by side effects. Alongside, physiotherapy is considered one of the effective methods in the treatment of frozen shoulder (15). Although the frozen shoulder is recognized as a capsular problem, the presence of MTrPs in the rotator cuff muscles may induce or exacerbate this condition, so it seems that releasing them may have positive effects on improving muscle activity and increasing ROM (4, 18, 19, 31, 33, 34). Among these muscles, the subscapularis muscle is one of the most common muscles prone to developing MTrPs, which causes restriction of abduction and lateral rotation of the GH joint (11). In contrast, lateral rotation is a prerequisite for complete elevation (abduction and flexion) of the humerus (11). Moreover, it is not possible to fully supinate the outstretched upper limb because of restricted lateral rotation of the shoulder. Thus, the subscapularis muscle MTrPs can impair shoulder and hand function (11). DN, as a new intervention in the field of physiotherapy, has been shown to have a positive effect on increasing the ROM and reduction of pain in people with chronic shoulder pain (35-37). DN with vasodilation in small vessels may improve blood circulation and oxygen levels (38-40). With LTR, DN may regulate spontaneous electrical activity levels in active MTrPs and may suppress substance P and calcitonin-generated peptide levels in active MTrPs (41). Furthermore, DN may influence the peripheral sensitization and pain processing centers in the central nervous system by releasing peripheral opioid analgesia (42) and indirectly activating dorsolateral tracts, respectively (12).
There is limited evidence for DN as an effective method in people with frozen shoulder (4, 43). Our study is the first study which is designed to evaluate the impact of DN in people with frozen shoulder. We expect that MTrP-DN of the subscapularis muscle plus conventional physiotherapy will have a more positive effect on frozen shoulder clinical symptoms compared to conventional physiotherapy alone (26), and DN could be considered as a complementary treatment in these people.

Limitations of this study are lack of follow-up and ignoring releasing possible MTrPs that could exist in other muscles in the shoulder region due to the interactions among muscles affecting the results of our study. Therefore, designing new studies with the approach of investigating the effect of dry needling on other muscles is suggested. Also, applying the sham dry needling technique for the control group is recommended in future studies. Furthermore, post-needling soreness, a frequent event after dry needling, should be considered as a factor that may have an effect on pain intensity and sensitivity. So, the assessment of post-needling soreness needs to be taken into account in future research.

**CONCLUSIONS**

In summary, this study is designed to investigate the impact of MTrP-DN of subscapularis muscle to conventional physiotherapy in patients with frozen shoulder. The final results may enhance the body of evidence of adding DN as a therapeutic intervention to conventional physiotherapy to improve clinical symptoms of various musculoskeletal disorders including frozen shoulder.

**FUNDINGS**

None.

**DATA AVAILABILITY**

Data are available under reasonable request to the corresponding author.

**CONTRIBUTIONS**

AM: conceptualization, methodology, writing - original draft. HA: conceptualization, methodology, supervision, writing - original draft, writing - review & editing. JA, AEO: writing - review & editing, project administration.

**ACKNOWLEDGMENTS**

The authors would like to thank the Tabriz University of Medical Sciences for providing the setting to perform this trial and funding support of this study.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interests.

**REFERENCES**

13. Martín-Pintado-Zugasti A, Del Moral OM, Gerwin RD, Fernández-Carnero J. Post-needling soreness after myofas-


40. Adigozali H, Shadmehr A, Ebrahimi E, Rezasoltani A, Naderi F. B mode, Doppler and ultrasound elastography imaging on active trigger point in women with myofascial pain syndrome
SUMMARY

Objective. The aim of this study was to systematically review current literature on the effectiveness of various exercises and modifications on thickness and electrical activity of abdominal muscles using ultrasonography and electromyography in healthy adults.

Methods. An electronic search was carried out on PubMed, Web of science, ProQuest, Google Scholar, Cochrane and Science Direct databases covering published studies from inception until December 2022. Fourteen papers were then included according to following inclusion criteria: 1) studies assessing abdominal exercises; 2) containing control and intervention groups; 3) conducted on young and healthy samples; 4) full texts being available in English; 5) containing the analysis of at least one of the four abdominal muscles through electromyography or ultrasonography.

Results. The main findings would suggest that stability challenges, rotatory exercise, combination of upper and lower limb movements, sensory cueing and grunting techniques such as Ki-hap make significant improvements in muscle structure and activity.

Conclusions. Despite the averagely fair quality of included papers and difficulty to reach a conclusion with inadequate data, our review suggests that new modifications of both traditional and core exercises can cause improvements in abdominal muscle thickness or activity with the aim of focusing on control and coordination of muscles.

Study registration. The protocol of the present systematic review is registered in the PROSPERO (CRD42022377864).

KEY WORDS
Abdominal muscle; electromyography; training; ultrasonography; exercise

INTRODUCTION

Abdominal muscles are considered as a structure that provide spinal support and stability for various daily functions (1). The spinal stability needed for activities is provided by the coordinated contraction of muscles such as transverse abdominis (TrA) and internal oblique (IO) which are attached to the thoracolumbar fascia and create lumbar spine stability. These muscles also increase intra-abdominal
pressure for the unstable spine along with the contraction of external oblique (EO) and rectus abdominis (RA) known as very important parts of the trunk due to their action of providing rotatory movements (2). Therefore, core muscles including the abdominals become important and crucial for stabilizing the spine and creating an effective support. Strengthening these muscles creates a powerful base along with different body movements so that the limbs can function properly relying on the provided trunk support (3). On the other hand, due to the fact that uncontrolled contraction and unorganized recruitment pattern of this muscular structure can lead to conditions such as low back pain (4), a proper exercise plan can prevent future problems. Traditional and core stability exercises such as sit-ups, planks and bridge exercises have been widely used by trainers and physiotherapists. Later, modified forms of exercises have been introduced using balls, devices or unstable surfaces for increasing the proprioceptive challenges and achieving higher activities (5-7). During the process of training, as therapists need to have a better understanding of the changes in muscles by the use of different forms of exercises, methods such as ultrasonography (US) and electromyography (EMG) are being used as reliable tools for the assessment of muscle thickness and electrical activity respectively (8-11). One review in 2013 that evaluated the effect of different exercises on EMG activation of core muscles including transverse abdominis, suggested free weight exercises over others forms of core exercises (11) and the latest review in 2020 carried out on all four abdominal muscles, evaluated the effect of more exercises including new modifications using EMG method (12). To our knowledge, as there is no review conducted on healthy population, using both EMG and US tools, there is a need for an updated systematic review in this field. The aim of this study was to systematically review the current studies on the effect of different exercises on either EMG or US activity of four abdominal muscles including rectus abdominis, external oblique, internal oblique, and transverse abdominis.

METHODS

Search strategy
The search strategy was based on the population intervention comparison outcome (PICO) method and included all relevant articles published from inception until December 2022. The procedure was then followed by the PRISMA methods (figure 1). An electronic data search was carried out using the online databases of PubMed, Science Direct, ProQuest, ISI web of science, Cochrane, and Google scholar (appendix 1). To cover the relevant RCTs for this systematic review, the following keywords were used in the search engines: (Abdominal muscle OR Rectus abdominis OR Internal oblique OR external oblique OR Transverse abdominis) AND (Exercise OR training OR activity OR growth OR mass OR thickness OR changes OR evaluation OR recovery OR quality OR performance) AND (Ultrasound imaging OR ultrasound OR ultrasonography OR Electromyography OR EMG OR thickness OR changes OR activity OR activation). Related studies suggested by the mentioned databases were also assessed.

Study selection
Only the articles meeting the following criteria were included: studies assessing abdominal exercises, containing control and intervention groups, conducted on young and healthy samples, the full texts being available in English and containing the analysis of at least one of the four abdominal muscles through EMG or ultrasonography. Articles including the following criteria were excluded: studies evaluating the effect of different postures, daily removal duplicates (n=4853), articles excluded with evaluation of title and keywords (n=4660), full text articles assessed for the eligibility (n=205), full text article/abstracts excluded with evaluation of method and study aim (n=131), added after examining the references (n=9), papers included (n=14).

Figure 1. Flowchart indicating the selection of articles through the PRISMA method.
tasks, carried out on unhealthy samples, and specific disorders (such as low back pain or any kinds of neurological deficits). Included articles were assessed by two reviewers, one of them assessing through the first steps of relevant titles and abstracts and full texts, and the other reviewer assessing the control and experimental groups in order to check and remove the irrelevant ones.

**Quality assessment**
The Physiotherapy Evidence Database (PEDro) scale is a reliable tool for the assessment of methodological quality of the studies and contains an 11-item list that checks both internal validity of the RCT and statistical information and finally reports a number based on the items (each item is reported as either 0 being absent or 1 being present). Based on the final reported score, the result is interpreted as poor if 0-3, fair if 4-5, good if 6-8, and excellent if 9-11. After the inclusion of studies, two reviewers examined the RCTs to come up with a PEDro score for each study in the process of quality assessment (table I).

**Data extraction**
The following data were extracted from each study: the author, year of publication, study method, control and intervention groups, conducted intervention, outcome measures, Pedro scale, mean and standard deviation before and after the intervention, and results (table II).

**Statistical analysis**
Because of the heterogeneous data and inadequate information of each outcome, we were unable to perform meta-analysis and therefore the extracted data have been expressed descriptively.

### RESULTS

**Description of studies**
Data of 14 papers were totally gathered and a descriptive analysis was carried out (figure 1). The quality of the studies ranged between 3 (poor) to 10 (excellent) and all the studies failed in blinding of subjects except one paper (17), failed in therapist blinding except 2 papers (17, 22) and failed in assessors blinding except 3 papers (17, 19, 23). According to the follow up time, 5 papers evaluated the immediate effect of the interventions (17, 20, 23-25), 8 papers were assessed a duration of less than 3 months (short term) (13-16, 18, 19, 22, 24), and only one contained a follow up of more than 3 months (long term) (21). In total, 60 exercises were included containing traditional exercises ($n = 11$), core stability

<table>
<thead>
<tr>
<th>Quality Items</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
<th>Q7</th>
<th>Q8</th>
<th>Q9</th>
<th>Q10</th>
<th>Q11</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligibility criteria</td>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td>Randomization into groups</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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</tr>
<tr>
<td>Concealing allocation</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Groups were similar at baseline</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7</td>
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<td>Subjects’ blinding</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td>Therapists’ blinding</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>5</td>
</tr>
<tr>
<td>Assessors’ blinding</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>4</td>
</tr>
<tr>
<td>At least one main outcome measure was significant</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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</tr>
<tr>
<td>Findings of between-group statistical comparisons</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>Providing both point measurements and variability measurements</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table I. PEDro score for each Item and total score.**
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year of publication</th>
<th>Participants</th>
<th>Control group</th>
<th>The Intervention</th>
<th>Study Type</th>
<th>Outcome Measure (tool)</th>
<th>Quality Status</th>
<th>Muscle tested</th>
<th>Follow-up time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wontae Gong et al.</td>
<td>2018</td>
<td>n = 32</td>
<td>CG: (F:15, M:1) Mean Age: 21.6 ± 0.2 BMI: 21.90 Non-Athletes</td>
<td>CG: no exercise</td>
<td>Continuous bridge exe at prone and side+ ADM</td>
<td>Single blinded RCT</td>
<td>Muscle thickness (US)</td>
<td>Fair</td>
<td>EO, IO, TrA</td>
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<td>TG: (F:15, M:1) Mean Age: 22.3 ± 2.1 BMI: 22.60 Non-Athletes</td>
<td>IG: continuous bridge exe</td>
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<tr>
<td>Misuk Cho et al.</td>
<td>2013</td>
<td>n = 30</td>
<td>CG (F:15, M:1) Mean Age: 20.8 ± 0.3 BMI: 21.33 Non-Athletes</td>
<td>CG: bridge exercise</td>
<td>Modified wall squat exe</td>
<td>No blinding RCT</td>
<td>Muscle thickness (US)</td>
<td>Poor</td>
<td>IO, TrA</td>
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<td>TG (F:15, M:1) Mean Age: 20.7 ± 0.3 BMI: 21.45 Non-Athletes</td>
<td>IG: modified wall squat exe</td>
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<td>Wontae Gong et al.</td>
<td>2015</td>
<td>n = 30</td>
<td>CG (F:15, M:15) Mean Age: 21.7 ± 0.8 BMI: 21.48 Non-Athletes</td>
<td>CG: no exercise</td>
<td>Dynamic exe utilizing PNF</td>
<td>Single blinded RCT</td>
<td>Muscle thickness (US)</td>
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<td>IO, EO, TrA</td>
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<td>TG (F:15, M:1) Mean Age: 22.4 ± 1.0 BMI: 22.39 Non-Athletes</td>
<td>IG: dynamic exe utilizing PNF</td>
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<td>Author(s)</td>
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<td>Control group</td>
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<td>Study Type</td>
<td>Outcome Measure (tool)</td>
<td>Quality Status</td>
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<td>Follow-up time</td>
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<tr>
<td>Misuk Cho et al.</td>
<td>2015</td>
<td>n = 30</td>
<td>CG (F:13, M:2)</td>
<td>CG Stable bridge exe</td>
<td>Bridge exe + ADM on balance pad</td>
<td>No blinding RCT</td>
<td>Muscle thickness (US)</td>
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<td>IO, TrA</td>
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<td>Mean Age: 21.7 ± 0.3</td>
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<td>IG: Unstable bridge exe</td>
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<td>BMI: 21.45</td>
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<td>TG (F:13 M:2)</td>
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<td>Mean Age: 22.4 ± 1.0</td>
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<td>BMI: 21.14</td>
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<tr>
<td>Brian Catania et al.</td>
<td>2021</td>
<td>n = 31</td>
<td>CG: Traditional core exe</td>
<td>Rotatory based exe</td>
<td>Triple blinded RCT</td>
<td>Muscle thickness (US)</td>
<td>excellent</td>
<td>EO, IO</td>
<td>Single session</td>
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<td>Mean Age: 20.8</td>
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<td>IG: rotary-based exe</td>
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<td>BMI: 24.50</td>
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<td>Duncan J. Critchley et al.</td>
<td>2011</td>
<td>n = 34 (F:28, M:6)</td>
<td>CG (n = 16)</td>
<td>CG: Conventional strength exe</td>
<td>Pilates</td>
<td>No blinding RCT</td>
<td>Muscle thickness (US)</td>
<td>Fair</td>
<td>IO, TrA</td>
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<td>Mean Age: 30 (8)</td>
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<td>IG: Pilates mat exe</td>
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<td>BMI: NS</td>
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<td>TG (n = 18)</td>
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<td>Mean Age: 31.5</td>
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<td>BMI: NS</td>
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<td>Non-Athletes</td>
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<tr>
<td>Seung-Chul Chon et al.</td>
<td>2010</td>
<td>n = 40 (F:22, M:18)</td>
<td>CG:ADM</td>
<td>Ankle dorsi flexion +ADM</td>
<td>Single blinded RCT</td>
<td>Muscle thickness (US)</td>
<td>Fair</td>
<td>EO, IO, TrA</td>
<td>2 weeks (5 times a week)</td>
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<td>Mean Age: 24 (1.5)</td>
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<td>IG: Ankle dorsi flexion +ADM</td>
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<td>Mean Age: 24 (1.6)</td>
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<td>Control group</td>
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<td>Study Type</td>
<td>Outcome Measure (tool)</td>
<td>Quality Status</td>
<td>Muscle tested</td>
<td>Follow-up time</td>
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<tr>
<td>Won-tae Gong et al.</td>
<td>2015</td>
<td>n = 32</td>
<td>CG (F:8, M:8)</td>
<td>Bridge exe with a sling</td>
<td>No blinding RCT</td>
<td>Muscle thickness (US)</td>
<td>Poor</td>
<td>IO, TrA</td>
<td>Single session</td>
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<tr>
<td></td>
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<td>Mean Age: 23.72 ± 1.4</td>
<td>Bridge exe with a sling and vibration</td>
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<td>BMI: 24.55</td>
<td>Non-Athletes</td>
<td>IG: Bridge exe with sling+vibration</td>
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<td>TG (F:8, M:8)</td>
<td>Mean Age: 23.17 ± 2.14</td>
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<td>BMI: 22.75</td>
<td>Non-Athletes</td>
<td>IG: Bridge exe with sling+vibration</td>
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<tr>
<td>Michia Tanimoto et al.</td>
<td>2008</td>
<td>n = 36</td>
<td>CG (n = 12)</td>
<td>Whole body low intensity resistance</td>
<td>No blinding RCT</td>
<td>Muscle thickness (US)</td>
<td>Fair</td>
<td>Abdomen</td>
<td>13 weeks (twice a week)</td>
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<td>Mean Age: 19.8 ± 0.7</td>
<td>IG 1: Low intensity-slow movement</td>
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<td>BMI: 21.70</td>
<td>Non-Athletes</td>
<td>IG 2: High intensity-normal movement</td>
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<td>HN (n = 12)</td>
<td>Mean Age: 19.9 ± 0.5</td>
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<td>BMI: 21.56</td>
<td>Non-Athletes</td>
<td>IG 2: High intensity-normal movement</td>
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<td>LST (n = 12)</td>
<td>Mean Age: 19 ± 0.6</td>
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<td>BMI: 20.96</td>
<td>Non-Athletes</td>
<td>IG 2: High intensity-normal movement</td>
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<tr>
<td>Pawel Niewiadomy et al.</td>
<td>2021</td>
<td>n = 73</td>
<td>CG (n = 33)</td>
<td>rotational movement exe</td>
<td>Single blinded RCT</td>
<td>Muscle thickness (US)</td>
<td>Good</td>
<td>EO, IO, TrA</td>
<td>4 weeks</td>
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<td>Mean Age: 24.94</td>
<td>IG: rotational movement exe</td>
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<td>BMI: 22.56</td>
<td>Non-Athletes</td>
<td>IG: rotational movement exe</td>
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<td>TG (n = 40)</td>
<td>Mean Age: 24.22</td>
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<td>BMI: 23.42</td>
<td>Non-Athletes</td>
<td>IG: rotational movement exe</td>
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<td>Year of publication</td>
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<td>Study Type</td>
<td>Outcome Measure (tool)</td>
<td>Quality Status</td>
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<td>Follow-up time</td>
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<tr>
<td>Chang-Yong Kim et al.</td>
<td>2017</td>
<td>n = 90</td>
<td>CG (F:15, M:15)</td>
<td>Crunch only</td>
<td>Single blinded RCT</td>
<td>Muscular electrical activity (EMG)</td>
<td>good</td>
<td>EO, IO, RA</td>
<td>1 session</td>
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<td>Mean Age: 24.37</td>
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<td>TG1: (F:15, M:15)</td>
<td>Crunch+Ki-hap</td>
<td>Ki-hap technique and verbal encouragement</td>
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<td>TG2: (F:15, M:15)</td>
<td>Crunch+Ki-hap+verbal encouragement</td>
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<td>Mean Age: 24.91</td>
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<td>BMI: 22.14</td>
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<tr>
<td>Ludmila M. Cosio-Lima et al.,</td>
<td>2003</td>
<td>n = 30 females</td>
<td>conventional</td>
<td>conventional</td>
<td>No blinding RCT</td>
<td>Muscular electrical activity (EMG)</td>
<td>Fair</td>
<td>RA</td>
<td>5 weeks</td>
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<td>CG (F:15)</td>
<td>exercise on floor</td>
<td>conventional exercise on physioball</td>
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<td>Mean Age: 22.87</td>
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<td>BMI: 21.49</td>
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<td>Non-Athletes</td>
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<td>TG (F:15)</td>
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<td>Mean Age: 19.47</td>
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<td>BMI: 23.14</td>
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<td>Non-Athletes</td>
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<tr>
<td>Marilyn I. Miller</td>
<td>1987</td>
<td>n = 40 males</td>
<td>back extension</td>
<td>back extension with multisensory cuing</td>
<td>No blinding RCT</td>
<td>Muscular electrical activity (EMG)</td>
<td>Fair</td>
<td>IO, TrA</td>
<td>1 session</td>
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<td>exercise</td>
<td>back extension +multisensory cuing</td>
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<td>Mean Age: 31.30</td>
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<td>Cheryl L. Hubley-Kozey et al.</td>
<td>2010</td>
<td>n = 33</td>
<td>Dynamic stability</td>
<td>Dynamic stability</td>
<td>No blinding RCT</td>
<td>Muscular electrical activity (EMG)</td>
<td>Poor</td>
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<td></td>
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<td>CG (n = 19)</td>
<td>exercise</td>
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ADM: abdominal draw-in maneuver; CG: control group; EMG: electromyography; EO: external oblique; IO: internal oblique; PNF: Proprioceptive Neuromuscular Facilitation; RA: rectus abdominis; RCT: randomized controlled trial; IG: intervention group; TrA: transverse abdominis; US: ultrasonography.
exercises (n = 27), free weight exercises (n = 12), ball/sling/pad/surface exercises (n = 3), and non-core exercises (n = 7) based on core exercise classifications (11). Four papers contained control groups with no types of exercises (13, 15, 21, 22).

Changes in the muscles along with the interventions were evaluated with muscular thickness via ultrasonography (13-22) and muscular electrical activation via electromyography (19, 23-26).

Summary of results

Rectus Abdominis (RA)
All included studies that assessed the changes of RA during exercises were carried out with the EMG tool. Two studies with score range 3-5 resulted in a significant improvement while doing unstable exercises (p = 0.04 for flexion and p = 0.01 for extension) or putting body in an unstable position (p < 0.005) (24, 26). Another article with good quality (score: 6) concluded that yelling or grunting (verbal activity) highly improved muscle activation (p = 0.024) (23).

External oblique (EO)

US findings
Five studies evaluated the effect of exercises on EO muscle thickness using the ultrasound tool. Three studies with scores 5-10 demonstrated statistically greater increase of muscle thickness in intervention groups receiving dynamic exercises utilizing Proprioceptive Neuromuscular Facilitation (PNF) and rotatory exercises (15, 17, 22). However, two studies with the score of 5 reported no significant change in the muscle thickness using continuous bridge exercise compared to no exercise, and abdominal draw-in maneuver (ADM) with ankle dorsiflexion compared to doing ADM alone (13, 19).

EMG findings
Two studies (scores: 4-6) evaluated EMG findings and resulted in significant increase in muscle activation using unstable dynamic exercise and crunch exercise with Ki-hap technique (23, 25).

Internal oblique (IO)

US findings
Nine studies were conducted with ultrasonography evaluations for IO. In six studies (scores: 3-10), interventions of continuous bridge exercise, wall squat, dynamic exercise utilizing PNF, bridge exercise on balance pad, and rotatory movements caused significant increase in muscle thickness (13-17, 22).

According to a study evaluating the effect of Pilates mat exercises (score: 4), the results demonstrated that not only the movements such as Hundreds or standing and supine exercise caused no effect on the thickness of IO, but also imprints movement caused decrease in this outcome (18).

In one study (score: 5), no significant difference was observed with doing ADM with ankle dorsiflexion compared to doing ADM alone (p = 0.092) (19). In another paper (score: 3), the thickness of IO was increased along with the increase of vibration (p < 0.05), but it was not much different with the control group (20).

EMG findings
Two studies with the scores of 4-6 evaluating the effects of Ki-hap technique and back extension with multisensory cueing resulted in significant increase in IO activity (23, 25).

Transverse abdominis

US findings
US findings contained 8 articles. Three studies with the scores of 5-10 concluded that the intervention groups with continuous bridge exercise, rotatory based movements, and ADM with ankle dorsiflexion demonstrated significant increase in thickness of TrA (13, 17, 19) and similarly, one study (score: 3) using vibration with sling bridging exercise concluded that there was a noticeable increase in the intervention group compared with control group (bridging with sling) especially with the pressure increase at 38, 42, 46 mmHg (p < 0.05) (20). Two studies (scores: 3) with wall squat and bridging on balance pad resulted in statistically great difference in TrA thickness, but no difference was seen between the control and experimental groups (p > 0.05) (14, 16). One paper evaluating the effects of Pilates (score: 4) represented that Hundreds. A exercise showed a great increase in TrA (p = 0.007) (18) and another study with good quality (score: 6) evaluating the efficacy of rotatory exercises indicated that the overall cross section of TrA+IO+EO was increased with bigger changes in IO and EO rather than TrA (p < 0.05) (24).

A paper (score: 5) examining the changes of the whole muscular size of abdomen concluded that both the high intensity-low speed and low intensity-slow movement groups displayed significant increase compared with the control group (21).

EMG findings
EMG findings contained 2 papers. In the study with doing back extension with multisensory cuing (score: 4), the experimental group had greater mean change than the control group that carried out the exercise without cueing (25) and similarly, the study with ankle dorsiflexion and
ADM (score: 5) displayed significant increase in muscle activation (19).

DISCUSSION
Since the abdominal muscles play an important role in supporting and increasing spinal stability, strengthening programs are effectively noticed and information about the various forms of exercises and how effective they are for each muscle becomes important for exercise prescription. This present study aimed to review the evidence on the impact of different abdominal exercises on muscular changes.

According to the findings, most interventions displayed an improvement in the changes of abdominal muscles measured by US and EMG. Modifications such as unstimulating the exercises by performing them on the balance pad, physioball, sling, and vibration or providing an unstable body position caused positive changes on outcome measures. This can be due to the co-contraction of muscles that try to provide balance (16). Also, adding multisensory cueing (by boosting the coordination and volunteer control) (25) or subjective verbal acting (by cerebral cortex stimulation affecting motor nerves) (23) are shown to be effective, although the result cannot easily be relied on in the study with the multisensory effects since factors such as instability are provided in the training group too. Similarly, rotational movements have positive impact on increasing muscle thickness, but majorly in EO and IO rather than TrA (22). However, a study evaluating Pilates movements demonstrated that with doing the Hundred A, TrA thickness was increased but IO thickness showed a decrease and this change happened only during the training and did not carry on to functional postures of daily activities (18).

However, according to the heterogeneous data and methodological diversity, it is difficult to make a final summation. For instance, while continuous bridge exercise is effective for the increase of IO and TrA thickness, no significant change was seen for EO muscle (13). Another study reported that during ADM with ankle dorsiflexion, the thickness of EO was decreased, but for TrA, there was an increase, and it was not effective for IO (19). The reason goes back to the spatial and temporal summation that stimulate only TrA as target (19). On the other hand, since exercises such as bridging and dynamic movements with PNF have been compared with control groups that received no exercises, it cannot be concluded whether these activities are more effective than the other types or not (13, 15).

The included RCTs considered a specific type of exercise without applying different frequencies or repetitions. Only one study considered the effect of intensity and speed of movement that changes in both criteria resulted in improvements on abdominal muscle thickness (21). Most papers were fairly qualified (13, 15, 18, 19, 21, 24, 25), and except 4 papers with poor quality and high risk of bias (14, 16, 20, 26), the other papers can almost be reliable. Except two studies with a high number of participants (22, 23), and one study with long-term follow-up (21), the sample sizes were small and follow ups were mostly immediate and short time. Besides, all studies were carried out on healthy population and these conclusions cannot be used the same for conditions such as low back pain. There is also a concern over the education of the movements in the studies about whether the exercises were properly conducted by the participants or not. Therefore, further studies need to be done with the assessments of frequency changes and number of repetitions of movements, duration and speed changes, larger sample sizes, long-term follow ups, and higher qualities.

Overall, despite the discrepancies and inadequate data, it is clear that how exercises and new modifications affect the outcomes and according to the findings, therapist and trainers can decide which movement to choose for each muscle. Therefore, with making small changes such as adding vibration, unstable surface, grunting, visual and sensory cueing, changing the speed, combining upper limb and lower limb movements and using free weight movements rather than isolated conventional exercises, significant improvements can be made.

CONCLUSIONS
This study systematically reviewed the present evidence on the effect of various exercises on muscular changes using ultrasonography and electromyography. Despite the averagely fair quality of the included papers and difficulty to reach a conclusion with inadequate data, our review suggests that new modifications of both traditional and core exercises such as stability challenges, adding vibration, sensory cueing or subjective verbal acting, rotational based activities and combination of upper and lower limbs’ movements can cause improvements in the abdominal muscle thickness or activity with the aim of focusing on the control and coordination of muscles. These strengthening techniques can contribute as a factor in preventing future problems such as low back pain and help to provide better abdominal and spinal performance for both stability and dynamic goals.

FUNDINGS
None.
DATA AVAILABILITY
Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS
NT: conceptualization. NT, HP: methodology. HP, AD: investigation. AD, MKZ: quality assessment; HP, MKZ: writing – original draft. AD, NT: writing – review & editing.

REFERENCES

ACKNOWLEDGMENTS
The authors would like to thank all the faculty members of the Physiotherapy Department of Shahid Beheshti, University of Medical Sciences, who assisted us in this research.

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


ONLINE SUPPLEMENTS

Appendix 1. Data search.

Search strategy

PubMed: 656
(((Abdominal muscle) OR (Rectus abdominis)) OR (Internal oblique)) OR (external oblique)) OR (Transverse abdominis) AND ((Exercise* OR training*)) AND (((Ultrasound imaging) OR (ultrasound)) OR (Electromyography* OR EMG)) OR (thickness OR activit*) OR (activation)) Filters: Clinical Trial, Randomized Controlled Trial

Web of science: 1127
1. (((ALL=(Abdominal muscle))) OR ALL=(Rectus abdominis)) OR ALL=(Internal oblique)) OR ALL=(external oblique)) OR ALL=(Transverse abdominis)
2. (ALL=(Exercise*)) OR ALL=(training*)
3. (((((Ultrasound imaging))) OR ALL=(ultrasound)) OR ALL=(Electromyography* OR EMG)) OR ALL=(thickness)) OR ALL=(activit*)) OR (activation))
Combined syntax: #1 AND #2 AND #3

ProQuest: 2283
(“ABdominal muscle” OR “Rectus abdominis” OR “Internal oblique” OR “external oblique” OR “Transverse abdominis”) AND (Exercise* OR training*) AND (“Ultrasound imaging” OR ultrasound OR Electromyography* OR EMG OR thickness OR activit* OR activation)

Google Scholar: 101
allintitle: (muscle) AND (Exercise OR training) AND (“Ultrasound imaging” OR ultrasonography OR sonography OR Electromyography)

Science Direct: 1514
(“Abdominal muscle” OR “Abdominal muscles”) AND (Exercise OR training) AND (Ultrasound imaging OR ultrasound OR sonography OR Electromyography OR EMG)

Cochrane: 791
(Abdominal muscle OR Rectus abdominis OR Internal oblique OR external oblique OR Transverse abdominis) AND (Exercise OR training) AND (Ultrasound imaging OR ultrasound OR Electromyography OR EMG OR thickness OR activity OR activation) in Title Abstract Keyword
Peculiarities of Adolescent, Qualified Female Volleyball Players’ Shoulder Girdle

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SUMMARY
Background and purpose. Many regularly performed spikes and serves lead to a shoulder girdle adaptation that could be sport-specific and result in traumatic events. The aim was to evaluate shoulder joints’ range of movements (ROM), peak isometric strength of arm muscles, submaximal force repetition error, and upper body dynamic stability in adolescent female volleyball players.

Materials and methods. 15 qualified, 17.7 ± 2.1-year-old, female volleyball players participated. The shoulder active ROM of internal rotation (IR), external rotation (ER), flexion, extension, abduction, adduction, horizontal abduction (HABD), and horizontal adduction (HADD) were measured. The peak force and the ability to repeat the same submaximal force of IR, ER, and extension were determined. Dynamic stability was detected by the Y-upper-body dynamic balance test (Y-UBDST).

Results. The ER ROM was increased but the IR and HADD ROMs were decreased on both shoulders. The extension and adduction ROMs were smaller in the dominant (D) shoulder (p < 0.05). The D arm’s IR, ER, and extension muscles developed greater peak forces (p < 0.05). ER/IR muscles’ peak forces ratio was 0.94 ± 0.18 in both shoulders. The ER and extension muscles submaximal force repetition error was smaller in the D arm (p < 0.05). The mean score of the normalized reaching distances in the Y-UBDST was symmetrical: 95 ± 6% in the D and 94 ± 7% in the non-dominant (ND) arm. The correlation between the rotators’ peak force and the Y-UBDST was not significant.

Conclusions. The shoulder girdle characteristics were optimal and adaptable for volleyball spike and serve movements in those adolescent qualified female volleyball players without pathological changes in pre-season.

KEY WORDS
Muscles strength; proprioception; range of movements; shoulder; volleyball.

INTRODUCTION
Shoulder pain syndrome represents the third of the most common injuries among female and male volleyball athletes and is the second most common overuse-related condition, accounting for 8-20% of all volleyball injuries (1, 2). There is body of evidence indicating that volleyball players’ performance depends on highly developed qualities like strength, power, speed, and agility. Thus, to be at their best level of performance, volleyball players need to spike the ball stronger and faster (3) by reaching an internal rotation velocity in the shoulder joint of about 7,000°/s (4). The main factor of the successful spiking technique in a competitive environment is the ability to produce a large range of motion (ROM) before the ball is hit (5). The considerable evidence shows that excessive external rotation movement may be a reason for overloading the surrounding muscles and ligaments, therefore causing the injury (6). Athletes with regular overhead movements of
the upper extremities have demonstrated altered shoulder internal (IR) and external rotation (ER) ranges of motion, thus indicating the reduction of IR and the increase of ER in the dominant (D) arm when compared with the non-dominant (ND) arm. These shoulder joint ROM changes contribute to alterations of the bones (humeral retroversion), joint capsule (posterior thickening), and increase of muscle passive stiffness (7). When the IR ROM is decreased for more than 18°-20° or the total arc of motion is decreased for 5°-8° in the D shoulder in comparison with the ND shoulder, a pathological glenohumeral IR deficit (GIRD) or total arc of motion deficit develops (8). This could cause alterations in the technique of sports movements with consequent risk of shoulder injury growth (8, 9). Schmalzl et al. (10) observed that GIRD larger than 10° led in nearly 75% of the male volleyball and handball players to a decrease in total range of motion and a high rate of postero-superior impingement. Controversially, Challoumas et al. (11) and Keller et al. (12) in their review articles did not determine a statistically significant increase of shoulder injury risk in those overhead movement athletes with the glenohumeral IR deficit and ER gain. Mizoguchi et al. (13) determined that there was no association between shoulder injury and GIRD among adolescent male and female volleyball players. Mizoguchi et al. (13) observed significantly larger ROM in shoulder ER-IR movement and a higher prevalence of shoulder injuries in adolescent volleyball female players (8.5% in the previous year) than in males (4.0%). The effect of volleyball players’ sex on shoulder joint adaptation to training and/or competitive conditions in volleyball has not been fully investigated. It has been established, that females’ joints possess lower stiffness during the co-contraction of muscles, and they have smaller muscle mass, especially in the upper extremities (14).

In comparison with the non-dominant shoulder, the D shoulder produces a higher strength within IR muscles, which leads to a relative shoulder ER/IR muscle torque ratio reduction. Thus, worsening the stability in comparison with the ND shoulder. This is especially observed in those elite male volleyball players (without shoulder injury), who play in the attacker and blocker positions (3, 15, 16, 17). Continuous overhead movements with eccentric contractions of the rotator cuff muscles can cause an overload on the glenohumeral joint, which, as follows, can lead to the formation of micro-damage to the muscles and limited joint ROM (18, 19). Many authors have suggested that the reduction of shoulder ER/IR muscle torque ratio is related to the increase of injury risk in athletes with regular overhead movements of the upper extremities (11, 16, 20). Based on the results, obtained by Hadzic et al. (21), there was significant preseason weakness of ER and decreased ER/IR strength ratio in the first and second national league male volleyball players, who experienced shoulder injuries during the previous season. Moreover, athletes with shoulder GIRD had a lower ER/IR peak torque ratio (0.56) than athletes without GIRD (0.83) (22). In sum, the shoulder girdle is exposed to tremendous cumulative load as the result of repetitive spiking and serving movements. The glenohumeral joint is capable of an exceptional range of motion but, unfortunately, possesses anatomical instability. The strength of muscles (dynamic stabilizers of the scapula and the humeral head) is one of the main factors in maintaining the functional integrity of the glenohumeral joint, and the ability to successfully spike and serve a ball (2).

Y- upper body dynamic balance test (Y-UBDBT) in volleyball is a popular functional movement indicator to measure dynamic stability of the upper extremity in a weight-bearing position, thus identifying factors for risks of injury (23-27). It is well known that proprioception plays a decisive role in shoulder stability and motor control of the upper limbs (28). Proprioception includes sub-modalities like joint position sense (the ability to reproduce joint angles actively or passively), kinesthesia (sense of movement), sense of force, and sense of change in velocity (29, 30). Proprioceptive deficit could alter the recruitment of the shoulder muscles, causing an over-activation or under-activation of the rotator cuff muscles, ultimately predisposing an athlete to injury (31). Mendez-Rebolledo et al. (28) evaluated possible correlations between shoulder position sense and the performance in the Y-UBDBT in 18-26-year-old male and female college volleyball players who trained ≥ 12 hours per week. They observed that better results of active joint position sense at 90° of shoulder IR (when 90° of the arm abduction is present) are associated with the better performance of the Y-UBDBT in superolateral, inferolateral direction, and composite score. Shoulder IR and ER force reproduction tests are found to be highly reliable, with interclass correlation coefficients of 0.849 in patients with rotator cuff tendinopathy and 0.909 in healthy subjects; the errors of ER were significantly larger than those of IR, and relative IR and ER force reproduction errors were similar in both groups of participants (32). Sense of force differentiation has not been investigated on a wide scale in athletes who perform regular overhead movements of the upper extremities. Hypothesis: volleyball training would lead to an increase of ER ROM and reduction of IR ROM, an increase of the arms
muscles’ strength and force differentiation ability, improvement of the upper body dynamic stability, and significant side-asymmetry of these characteristics in adolescent female volleyball players with high-performance levels. The aim was to evaluate shoulder joints’ range of movements (ROM), peak isometric strength of arm muscles, submaximal force repetition error, and upper body dynamic stability in adolescent female volleyball players.

MATERIALS AND METHODS
The present study used a cross-sectional research design. Data collection took place in Murjani Sports Gymnasium and occurred in the pre-season period.

Participants
The total number of participants was fifteen. They were female first-league volleyball players - students of the sports gymnasium.

Those adolescent female volleyball players who were playing in the first league (the highest league in Latvian volleyball) and participating in the training sessions and games at least five times per week were included in the research. Their mean age was 17.7 ± 2.1 years, and training experience 5.9 ± 2.6 years. They participated in training sessions during the last six months at least five times per week, a number of training hours from eight to 12 hours per week (mean 10.2 ± 2.0 hours/week), and additionally games at the weekends. Participants weekly training volume (10.2 ± 2.0 hours per week in five training sessions with one training session per day) coincided with the minimal training volume of the international performance-level volleyball players (33).

The exclusion criteria for all participants were acute or chronic shoulder or arm pain, present or recent (i.e., emerged in the last month) upper extremity or back injury, and previous surgery in the last 12 months. These exclusions were assessed by questionnaires.

The participants were informed of the possible risks of the tests, and all of them signed an informed consent form to take part in the investigation and voluntarily participated in the study. The study was proved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki (Protocol No.137/42722 - date of approval: May 28, 2021).

Assessment procedures
Anthropometry assessment
The height of athletes was measured in centimeters (cm) using an Ultrasound Height Measuring Unit MZ10020 (ADE, Hamburg). Body mass was measured in kilograms (kg) in athletes wearing briefs and brasseries using a Body Composition Analyzer BC-418 (Tanita Corporation, Japan). The same tester measured all 15 participants’ half-arm spans by tape in a standing vertical position with the shoulder abducted to 90°, the elbow fully extended, and the hand in a neutral position. The tester measured a distance from the processus spinosus of the seventh cervical vertebra C7 to the tip of the middle finger. The half-arm span was used in the Y – dynamic stability test to calculate the normalized reach distances of the arms and composite score (25). Then tester measured arm length from the posterolateral acromion process of the scapula to the distal ulnar styloid process and forearm length from the olecranon to the distal ulnar styloid process. These data were necessary to shoulder muscles exerted torque calculation (34).

Range of motion assessment
Before the shoulder flexibility, strength, and upper body dynamic stability measurements the participants performed general warm-up exercises, local warm-up, and active stretching for shoulders and arms for 20 minutes.

The active shoulder ROM was evaluated in IR-ER, flexion-extension, abduction-adduction, and horizontal abduction-adduction using a Jamar Plus Digital Goniometer (Performance Health, United Kingdom). The participants were placed in a standard position. They were asked to perform certain active motions as far as possible (35). A measurement of the movement was taken at the end of ROM. Shoulder IR-ER ranges were measured with the participants in a supine position with the shoulder abducted to 90°, the elbow flexed to 90°, and the forearm in a neutral pronation/supination position (36). To measure IR ROM, the forearm was placed perpendicular to the supporting surface, and the palm faced the feet. The full length of the humerus rested on the examining table. A pad was placed under the humerus to keep the humerus horizontal to the floor. The shoulder was active maximally internally rotated by moving the forearm anteriorly, bringing the palm toward the floor. The center of the goniometer was placed on the olecranon process of the humeral bone, the proximal arm of the goniometer was positioned perpendicularly to the floor, but the distal arm was placed parallel to the ulnar bone from the olecranon to the ulnar styloid process. ER ROM was measured in active rotation of the shoulder externally by moving the forearm posteriorly, bringing the dorsal surface of the palm toward the floor. Assistance was given to keep the shoulder on the table during the IR and ER motion, preventing compensatory use of the shoulder protractor muscles.
Active shoulder flexion was assessed with the participants in the supine position, with the palm facing upward and the elbow extended, with the arm over the side of the table (36). The active flexion was performed by lifting the humerus off the examining table and bringing the hand up over the individual’s head. The arm was maintained in neutral abduction and adduction during the motion. The center of the goniometer was placed on the acromion process, the proximal arm was positioned parallel to the floor, but the distal arm was placed parallel to the humeral bone from the acromion to the lateral epicondyle of the humeral bone.

Active shoulder extension was investigated in the prone position, with the face turned away from the shoulder being assessed. The shoulder was placed in 0 degrees of abduction, adduction, and rotation. The elbow was positioned in slight flexion. The forearm was placed in 0 degrees of supination and pronation so that the palm faced the body. The active shoulder extension was performed by maximally lifting the humerus off the examining table. The position of the goniometer was the same as in the shoulder flexion ROM measurement (36).

Active shoulder abduction was assessed with the participants in a supine position, with the shoulder in external rotation and 0 degrees of flexion and extension so that the palm was faced anteriorly. The elbow was extended. The active shoulder abduction was performed by maximally moving the humerus laterally away from the participant’s trunk. The arm was maintained in external rotation and neutral flexion and extension during the motion. The center of the goniometer was placed on the acromion process, the proximal arm was positioned parallel to the sternal bone, but the distal arm was placed parallel to the medial midline of the humeral bone from the acromion to the medial epicondyle of the humeral bone. Active shoulder adduction was assessed with the participants standing in a vertical position, with the elbow extended and the palm facing to the front. The endpoint was determined by the participant’s maximal active range, the arm was positioned posterior to the body. The center of the goniometer was placed on the acromion process, the proximal arm was positioned parallel to the sternal bone, but the distal arm was placed parallel to the medial midline of the humeral bone from the acromion to the medial epicondyle of the humeral bone (36).

Active shoulder horizontal abduction was measured with the participants in a supine position, with the shoulder in 90° of abduction and the elbow in 90° of flexion. The participant performed maximal active horizontal abduction by moving his arm downwards as far as possible. The center of the goniometer was placed on the acromion process, the proximal arm was positioned perpendicular to the floor, but the distal arm was placed parallel to the humeral bone from the acromion to the medial epicondyle of the humeral bone. To assess active horizontal adduction the participant performed maximal active horizontal adduction by moving his arm upwards as far as possible (37). Each active ROM measurement was performed twice, and the mean value was calculated.

**Force and peak torque assessment**

The peak force and the ability to repeat the submaximal force of IR, ER, and extension muscles were determined in isometric contractions using a handheld dynamometer (MicroFET2 Wireless; Hoggan Health Industries, West Jordan, USA) in newtons (N). The shoulder IR and ER or extension muscle force were measured in the vertical (sitting or standing) positions of each participant who pushed the dynamometer against the door frame with as much force as possible (34). The participant was sitting on the chair during measurements, and shoulder IR and ER peak forces were tested in the scapular plane or 30° from the frontal plane. The force assessment was performed with the shoulder positioned in 90° of abduction in the scapular plane and 90° of ER, the elbow in 90° of flexion, and the forearm in a neutral position to accommodate the handheld dynamometer force pad. Test position was selected to replicate the sport-specific position of a volleyball hit. Participants were asked to sit as erect as possible. The dynamometer was placed against the door frame for enhanced stability. Each participant fixed the dynamometer on the palmar surface of the tested hand with the curved surface of the device positioned against the distal heads of metatarsal bones to measure IR muscle force. To assess the ER muscle force, the dynamometer was fixed on the dorsal surface of the tested hand with the curved surface positioned against the distal heads of metatarsal bones.

To measure the extensor muscle force, the participant was standing near the door frame. The arm muscle extension force was evaluated with the shoulder positioned in 180° abduction, elbow extended, and forearm in a neutral position to accommodate the dynamometer force pad. The dynamometer was fixed on the palmar surface of the tested hand with the curved surface of the device positioned against the distal heads of metatarsal bones (38).

Each participant performed three repetitions of the peak shoulder extension, IR, and ER force production, pushing the dynamometer against the door frame with as much force as possible continuously for five seconds with each arm, a rest break with a duration of 60 seconds was given between the force measurement trials. The largest muscle-generated force depended on the degree of voluntary effort of the participant. Therefore, the value of the error of the peak force assessment could be underestimated due to the lack
of motivation of the player. To exclude this factor the peak force was measured in three trials, and the participant was encouraged to push the dynamometer as strongly as possible. The highest force of shoulder extension, IR, and ER muscle-developed force measurement in each shoulder was selected for further analysis. Two practice trials were allowed to familiarize the participants with testing procedures. The order of the isometric force testing in the D and ND shoulder was randomized. Shoulder IR and ER muscle peak torque values were calculated using the best repetition force and multiplied by the forearm length measurements (distance from olecranon to ulnar styloid process). Extensor muscle peak torque values were calculated using the best repetition force and multiplied by the arm length measurements (distance from acromion to ulnar styloid process). Then the torques were divided by body mass (Nm/kg) to obtain relative peak torques. Shoulder torque ratios were expressed as shoulder ER muscles’ peak torque divided by IR muscles’ peak torque (34).

Each participant performed three pairs of submaximal IR, ER, and extensor muscle force exertion trials in the same positions as the peak force assessment. They had to reproduce the same force (the same effort of the muscles) in approximately 50% of the maximal voluntary contraction continuously for five seconds twice, with a 30-second rest break between the two measurements in each trial. A timed rest break between the three different trials was 60 seconds. The mean force repetition error of the three measurement trials was calculated.

**Upper body dynamic stability assessment**

To evaluate participants’ upper body dynamic stability the Y-upper body dynamic stability test (Y-UBDST) for assessment modified kit elaborated by Cramer et al. (25) was used. Three cloth measuring tapes were fixed to the floor precisely matching the angles using a goniometer between the two tapes, one of 90° and two of 135°, an athletic tape was used to secure the measuring tapes and mark the starting point for the stationary hand, three wooden blocks measuring 2 × 4 × 8 cm were employed to determine the reach directions. Each participant was provided a demonstration of the procedures and allowed three practice trials of the Y-UBDST in each direction on their D and ND arm before data collection. Participants performed the testing procedures barefoot. Three trials for the test were recorded for the ND and the D stationary arm at each of the three reach directions: superolateral, medial, and inferolateral. While maintaining a push-up position with the feet at shoulder width, the participant performed maximal effort to reach with the free hand in three directions (medial, superolateral, and inferolateral). The distance reached in each direction was recorded. The mean of three trials was used for analysis (25). The mean reached distance in each of the three directions was divided by the half-arm span and multiplied by 100 (25), thus normalizing reaching distance in percent (%). Then the mean normalized reaching distance or composite score for all three directions was calculated in % from the half-arm span.

The data distribution normality of the anthropometrical characteristics, shoulder active ROM, maximal shoulder muscle force, and error of the submaximal force repetitions was established by the Shapiro-Wilk test from the RStudio program. The mean values and standard deviations were calculated for all characteristics. A t-test for paired samples was employed to determine differences between the mean characteristics of the dominant and non-dominant arms. The correlations between the shoulder ROM and muscle peak forces, between the muscle peak forces and the submaximal force repetition errors, and between the muscle peak force and dynamic stability test results were calculated. The differences were considered significant at p < 0.05. Microsoft Excel 2010 was applied to perform descriptive statistics, t-tests, and correlation analysis.

**RESULTS**

The anthropometrical characteristics of the participants are shown in [table I](#). A significant shoulder ROM side-asymmetry was observed in the arms’ extension and adduction of the female volleyball players with larger ROM in the ND shoulder (p = 0.04) ([figure 1](#)).

The ER ROM (97° ± 12° in the dominant D, 95° ± 10° in the nondominant ND shoulder) was increased (the cut-off value was 90° referred to by Loudon et al. (39)). The IR ROM (54° ± 12° D and 62° ± 8° ND) was decreased (the cut-off value was 70° referred to by Loudon et al. (39)), and HADD ROM (34° ± 8° D and 35° ± 10° ND) was decreased (the cut-off value was 40° referred to by Loudon et al. (39)). The mean total ER – IR ROM of the D shoulder was 151° ± 20°, and of the ND shoulder it was 157° ± 13°, the difference was not statistically significant (p > 0.05; p = 0.126).

IR, ER, and extension muscles developed significantly greater peak forces, peak torques, and relative peak torques, respectively, in the D arm in comparison with the ND arm of the volleyball players (p < 0.05) ([table II](#)). The mean ER/IR peak torque ratio was 0.94 ± 0.18 (the lowest ratio observed in one participant was 0.61, but the highest was 1.34) in the D and the same: 0.94 ± 0.18 (the lowest ratio observed in one participant was 0.61, but the highest was 1.23) in the ND shoulder (p > 0.05). ER/IR peak torque ratio of the D
shoulder exceeded 0.8 in 14 participants from 15 and was higher than one in three players. In the ND shoulder ER/IR peak torque ratio exceeded 0.8 in 13 participants out of 15 and was higher than one in five players.

A significant correlation was observed between the IR ROM and the peak force of IR muscles only for the D arm ($r = 0.59$; $p = 0.01$), this correlation was not significant for the ND arm.

Significant correlations were not detected between ER ROM and the peak force of ER muscles, extension ROM and the peak force of extensor muscles, ER ROM and the peak force of IR muscles, flexion ROM and the peak force of extensor muscles for both arms of the participants ($p > 0.05$).

The submaximal force reproduction error was significantly smaller in the extensor and ER muscles of the dominant arm compared to the ND arm ($p = 0.04$ or $< 0.05$).

### Table I. Mean anthropometric characteristics with standard deviation of qualified adolescent female volleyball players.

<table>
<thead>
<tr>
<th>Body mass, kg</th>
<th>Height, m</th>
<th>Body mass index, kg/m²</th>
<th>Half-arm span, m</th>
<th>Arm length, m</th>
<th>Forearm length, m</th>
</tr>
</thead>
<tbody>
<tr>
<td>66.5 ± 8.4</td>
<td>1.75 ± 0.08</td>
<td>21.7 ± 1.6</td>
<td>D 0.89 ± 0.05</td>
<td>D 0.58 ± 0.04</td>
<td>D 0.27 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ND 0.89 ± 0.05</td>
<td>ND 0.58 ± 0.04</td>
<td>ND 0.27 ± 0.03</td>
</tr>
</tbody>
</table>

D: dominant arm; ND: non-dominant arm.

### Table II. Mean strength characteristics with standard deviation of shoulder girdle muscles in adolescent, qualified female volleyball players.

<table>
<thead>
<tr>
<th>Muscle group</th>
<th>Arm</th>
<th>Peak force, N</th>
<th>Peak torque, N × m</th>
<th>Relative peak torque, N × m/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensors</td>
<td>D</td>
<td>50 ± 9</td>
<td>29 ± 6</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>46 ± 9</td>
<td>27 ± 6</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.036</td>
<td>0.040</td>
<td>0.041</td>
</tr>
<tr>
<td>IR</td>
<td>D</td>
<td>50 ± 8</td>
<td>14 ± 3</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>46 ± 9</td>
<td>13 ± 3</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.002</td>
<td>0.002</td>
<td>0.0008</td>
</tr>
<tr>
<td>ER</td>
<td>D</td>
<td>47 ± 11</td>
<td>13 ± 3</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>43 ± 12</td>
<td>12 ± 4</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0005</td>
<td>0.0006</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
Volleyball Players’ Shoulder

arm in comparison with the non-dominant arm (p ≤ 0.025) (figure 2). This error did not differ significantly in the IR muscles of both shoulders (p = 0.724).

The mean Y-UBDST composed (normalized) scores were 95 ± 6% in the D and 94 ± 7% for the ND arm, symmetrical on both sides (p = 0.045).

Correlations between the shoulder IR, ER muscles peak forces, and the mean composite score of the Y-dynamic balance test for the upper body were not detected (p > 0.05). A significant negative correlation was observed between the extensor muscles’ peak force and the composite score of this test only for the D arm (r = -0.59; p = 0.02). For the ND arm this correlation was not significant (p > 0.05).

**DISCUSSION**

The ER ROM (97° ± 12° in the D, 95° ± 10° in the ND shoulder) was increased (the cut-off value 90°). The IR ROM (54° ± 12°D and 62° ± 8°ND) was decreased (the cut-off value 70°), and HADD ROM (34° ± 8°D and 35° ± 10° ND) was decreased (the cut-off value 40°) in female volleyball players (the cut-off values referred to by Loudon et al. (39)). The mean total ER-IR ROM did not differ significantly in the D shoulder: 151° ± 20° and 157° ± 13° in the ND shoulder. The extension and adduction ROMs were smaller in the D than in the ND shoulder.

The D arm’s IR, ER, and extension muscles developed significantly greater peak forces, peak torques, and relative peak torques in the D arm in comparison with the ND arm. The mean ER/ IR peak torque ratio was the same on both shoulders: 0.94 ± 0.18, but this ratio varied on a large scale among the participants from 0.61 to 1.34.

The D arm’s submaximal force repetition error was smaller than the ND arm’s in the ER and extensor muscles, but this was not determined for IR muscles. A significant correlation was detected between the peak force of ER muscles and the submaximal force repetition error only in the D arm.

A significant correlation was detected between the peak force of ER muscles and the submaximal force repetition error in the D arm (r = 0.82; p < 0.0001). Correlations between the peak force of IR, extensor muscles, and their submaximal force repetition errors were not significant (p > 0.05).

In the Y-UBDST the largest reach distance was determined in the medial direction (104 ± 10 cm supported the body on the D arm and 102 ± 7 cm – on the ND arm), in the inferolateral direction the mean distances were the same for both arms (80 ± 11 cm), but the shortest distance was observed in the superolateral direction (70 ± 7 cm supported the body on the D arm and 68 ± 8 cm – on the ND arm) (figure 3). Any significant side-asymmetry between the reached distances of the D and ND was not detected in each direction (p < 0.05).

The ROM changes of volleyball players in the present study coincided with normal anatomical adaptation of the shoulder girdle in athletes who perform regular overhead movements of the arms: increased ER together with decreased IR or GIRD (8, 10, 11).

The ER ROM was increased for 16° in the D shoulder and 8° in the ND shoulder which was less than in the pathological GIRD: 18°-20°, but the total ER-IR ROM was not significantly lower in the D shoulder (6°) than in the ND shoulder (the border of cut-off values = 5°-8° referred by Manske et al. (8)).

The ER ROM of our adolescent qualified female volleyball players (97° ± 12° in the D, 95° ± 10° in the ND shoulder) was smaller in comparison with adolescent (15-17-year-old)
female high school league volleyball players from the study performed by Mizoguchi et al. (13) (118.1° ± 12.0° in the D and 106.2° ± 10.3° in the ND shoulder) and with adult female first division volleyball players from the data of Ness et al. (34) (105.0° ± 10.7° in the D shoulder). The IR ROM (54° ± 12° D and 62° ± 8° ND) in our participants was also smaller than in the volleyball players from the same investigation: 65.7° ± 10.8° in the D and 75.1° ± 12.9° in the ND (13), but larger than in the adult participants from the study of Ness et al. (34) respectively 47.4° ± 10.8° in the D shoulder. Therefore, the total ER-IR ROM in our players was smaller than in the data from Mizoguchi et al. (13): 183.8° ± 17.6° in the D and 181.3° ± 17.5° in the ND shoulder, but like the adult players from the data of Ness et al. (34) study, respectively 152.4° ± 11.6° D arm (34). The HADD ROM (34° ± 8° in the D and 35° ± 10° in the ND arm) was lower in our participants than in the players measured by Mizoguchi et al. (13): 50.8° ± 13.6° in the D and 55.3° ± 13.9° in the ND arm. The arm flexion ROM was the same in our and Mizoguchi et al. (13) studies: close to 178° in both arms of our players and close to 179° in both arms of the players in the Mizoguchi et al. (13) study. Shoulder IR and HADD ROMs were smaller in the D arm than in the ND arm in our and Mizoguchi et al. (13) study. Mizoguchi et al. (13) investigated not only females but also males and observed significantly larger ROM in shoulder ER-IR and a higher prevalence of shoulder injuries in adolescent females than males. The D arm’s IR, ER, and extension muscles developed significantly greater peak forces, peak torques, and relative peak torques in comparison with the ND arm of our volleyball players. The relative shoulder IR peak torque was 0.21 ± 0.03 N × m/kg in the D arm and 0.19 ± 0.03 N × m/kg in the ND arm. The relative shoulder ER peak torque was 0.19 ± 0.03 N × m/kg in the D arm and 0.17 ± 0.03 N × m/kg in the ND arm. These data were slightly lower in comparison with the results measured on adult female volleyball players by Ness et al. (34), who were using the same method of strength measurement: the relative isometric shoulder IR peak torque in their study was 0.22 ± 0.04 N × m/kg and the ER peak torque was 0.22 ± 0.04 N × m/kg in the D arm at the end of the preparation period. Later during the season IR peak torque increased to 0.33 ± 0.06 N × m/kg, but ER peak torque decreased to 0.19 ± 0.04 N × m/kg (34). We did not observe shoulder muscle strength changes during the competition season.

Hadjic et al. (17) observed significantly larger isokinetic peak torques of the shoulder IR in the D side than in the ND but did not detect significant side-asymmetry in the ER in adult female (first and second-division national-level volleyball players). Measurements were performed at the rotational velocity of 60°/s in the concentric contractions of the muscles. The mean isometric ER/IR peak torque ratio of our participants was the same on both shoulders: 0.94 ± 0.18 (varied among the participants from 0.61 to 1.33). These results were higher in comparison with the isokinetic ER/IR peak torque ratio of adult female volleyball players from the research performed by Hadzic et al. (17) where the ratio was 0.75 ± 0.15 in the D shoulder and 0.72 ± 0.15 in the ND. In the Ness et al. (34) study, the isometric ER/IR peak torque ratio was 0.97 ± 0.13 (varied among the participants from 0.81 to 1.13), which was close to our results. Furthermore, Ness et al. (34) observed a worsening of the ER/IR peak torque ratio during the competition season. The ratio decreased to 0.7 ± 0.1 in the post-season (varied from 0.57 to 0.82 among the players). Hadzic et al. (17) observed that female volleyball athletes playing at a higher level (first versus second national division) were 3.43 times more likely to have an abnormal strength ratio. This could be explained by the larger number of performed spikes and hours spent in training sessions in the athletes with higher performance levels. Based on the research of Ellenbecker and Davies (40), Wilk et al. (41), and Hurd et al., (42) the range of optimal ER/IR muscle strength ratio in the overhead athlete is 0.66 to 0.75, and the ratio is higher in the nondominant than in the dominant arm. Corrective exercises could be an effective method in reducing shoulder girdle and spine abnormalities, thus improving the performance of volleyball players. This was confirmed by Firouzjah et al. (43) who concluded that execution of correction exercises for 10 weeks (three sessions per week with a duration of each session 30-70 minutes) was an effective tool in shoulder girdle and spine abnormality reduction, thus improving performance in adolescent volleyball players with the upper cross syndrome.

The D arm’s submaximal force repetition error of the ER was smaller than in the ND arm (4 N in the D shoulder and 5 N in the ND shoulder) and in the extensor muscles (5 N in the D arm and 7 N in the ND arm), but this was not determined for IR muscles (5 N in both shoulders). Based on Maenhout et al. (52) data, the errors of shoulder ER were significantly larger than those of IR, and the relative IR and ER force reproduction errors were similar when compared to healthy participants with patients of rotator cuff tendinopathy. This was not determined in our volleyball players: the submaximal force reproduction error of IR and ER muscles was the same in the ND arm and even smaller in ER than IR in the D arm. If compared our study with other sports disciplines, it has been established, that the shoulder IR muscle’s submaximal force reproduction error is similar on both sides in male qualified swimmers, triathletes, and the controls (44), but larger (from 7 to 10.5 N) in comparison with volleyball players from the present research.
Nevertheless, the extensor muscles force reproduction error in the swimmers and triathletes has been determined larger (from 8 to 11.5 N) than in volleyball players (44), but similar (close to 7 N) in the control group and volleyball players. Therefore, we could suggest that volleyball training leads to improvement of the shoulder rotator muscles’ force differentiation ability when compared with the young physically active people who do not regularly perform overhead movements of their arms or swimmers who perform overhead movements of their arms in a water-denser environment.

A significant correlation was detected between the peak force of ER muscles and the submaximal force repetition error only in the D arm. This could be explained by a volleyball spike technique: the ER muscles of the D arm exert maximal force to stretch the IR muscles so that IR can achieve the maximal spike power. Therefore, the force differentiation ability of ER muscles is not that important. The aforementioned correlation was not significant in the ND arm (p > 0.05).

The composed score of Y-UBDST reaching distances was 95 ± 6% in the D and 94 ± 7% in the ND arm, which is symmetrical on both sides. The norm is > 85.2% ± 11.7% according to the data of Westrick et al. (27). The correlations between the shoulder rotators’ peak force and the Y-UBDBT results were not significant. Only a significant negative correlation was observed between the extensor muscles’ peak force and the composite score of this test for the D arm (r = -0.59; p = 0.02). For the ND arm this correlation was not significant (p > 0.05). Therefore, it can be assumed, that the strength of the shoulder rotator and extensor muscles does not substantially affect the dynamic stability of the upper body in volleyball players.

Limitations of the study
The number of participants was small. All the participants were from the same school and trained by the same coaches. The changes in shoulder ROM, muscle strength, proprioception, and dynamic stability during all training season stages were not detected.

Shoulder IR, ER, and extension muscle isometric peak force and the submaximal force reproduction error assessment in the static mode were not specific for volleyball spike-like highly dynamic motion. Research showed that there was a significant correlation between the shoulder muscles’ peak isometric and the peak dynamic strength characteristics in athletes who regularly performed overhead movements of the arms (45). Nevertheless, in the investigations performed in recent years, authors used more precise methods of Mathematical Statistics and proved that the real correlation between the peak isometric and dynamic strength did not exist (46). The peak dynamic strength depends on many variables, the most important is precise motor control of the sport-specific movement or sports technique: involvement of all muscles with the maximal amount of working motor units participating in this motion simultaneously to achieve greater power and faster speed of motion (spike, throw, passing a ball); types of muscles’ contractions in dynamic motions are different (concentric and eccentric). Therefore, there was a recommendation for coaches to measure not only the isometric but also the dynamic strength of the muscles in conditions that are like real sports situations not only in the laboratory but also in field tests (46). The peak isometric strength measurement alone does not provide objective athletes’ performance characteristics.

Practical application of the dynamometry method
Shoulder rotator muscles are the most important stabilizers of this joint during movements (47) which is especially important to volleyball players and other athletes who perform regular overhead movements of the arms in a large ROM. A useful tool in shoulder joint stability assessment is the ER/IR peak strength ratio because training with a large number of overhead movements of the arms leads to selective IR strength increase and reduction of the ER/IR peak strength ratio (3, 15-17). An overuse shoulder injury could occur because of weak ER strength (11, 16, 20). A gold standard and the most precise method to measure dynamic muscles’ strength and their ratios in a laboratory is isokinetic dynamometry (48) but this equipment is expensive and non-portable. Therefore, each physiotherapist and coach have no access to the isokinetic devices. On the other hand, the peak isometric strength measurement is a simple, time-efficient, safe (49), inexpensive method to assess muscles’ strength with a portable, handheld dynamometer in a training place. There is a good standardization of isometric test conditions with high test-retest reliability (50). The shoulder ER/IR peak strength ratio is possible to measure by handheld dynamometer in the isometric type of muscle contractions. The measurement is more reliable if the peak isometric strength is determined in the joint angles that are specific to certain sports movements, for example, a ball release during a volleyball spike (34). Therefore, the shoulder muscles’ peak isometric strength and submaximal strength differentiation ability measurement in sport movement-specific angles are recommended for the daily practice of physiotherapists to evaluate the shoulder joint stability, strength side-asymmetry, and acuity of the strength differentiation sense if the isokinetic dynamometry assessment is not available. The changes in these characteristics in different training periods could be observed on time to prevent shoulder overuse injuries in athletes. The normative data
of the adolescent qualified female volleyball players will be useful as reference data to estimate the rehabilitation process of shoulder injuries in volleyball players. The hypothesis of our research was confirmed: adolescent qualified female volleyball players possess their shoulder girdle adaptation typical for team sports athletes who perform regular overhead movements of the arms, increased ER ROM, decreased IR ROM, optimal upper body dynamic stability, and ER/IR muscles strength ratio but the significant side-asymmetry of these characteristics was not observed; shoulder IR, ER, and arm extensor muscles were significantly stronger in the D arm in comparison with the ND arm (p ≤ 0.041), and the error of submaximal force reproduction was significantly better in the shoulder ER and extensor muscles of the D arm than of the ND arm muscles (p ≤ 0.025).

CONCLUSIONS

Regular repetition of volleyball spikes led to an increase in ER, a decrease in IR and HADD ROMs, and muscle strength and its differentiation ability improvement, especially, in the D arm. The action of shoulder ER and IR muscles was balanced. The upper body dynamic stability was the norm and did not depend on the rotator and extensor muscles’ strength. Therefore, the shoulder girdle characteristics were optimal and adaptable for volleyball spike and serve movements in those adolescent qualified female volleyball players without pathological changes in pre-season. Regular check-ups of the shoulder joint are recommended in all volleyball players during all training season stages, especially in athletes with shoulder pain, injury, or surgery in anamnesis, to determine any deficits in the ROM, weaknesses of the muscles and proprioception, balance decrease, or side-asymmetries to correct these pathologies and to prevent worsening of the performance and diminish the risk of overuse injuries.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS


ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

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Dual Task Reduces Balance Score but not Joint Repositioning Error in Female Athletes with Dynamic Knee Valgus

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INTRODUCTION
Maintaining proper body posture has always been of interest to health professionals for the preservation of musculoskeletal health (1). Dynamic knee valgus (DKV) refers to an improper movement strategy of the lower limbs involving multiple planes. This pattern includes external rotation of the tibia, inward rotation of the femur, knee abduction, and inward movement of the hip joint (2). DKV is more prevalent in females compared to males, and the likelihood of developing it increases as individuals progress through childhood (3). The dynamic form of knee valgus is considered a risk factor for various lower extremity injuries, including anterior knee pain, anterior cruciate ligament (ACL) sprain, osteoarthritis, and runner’s knee (4-6). The main factors contributing to knee arthritis, in the elderly are the accumulated knee traumas experienced over their lifetime (7). Additionally, knee valgus can be a secondary condition resulting from various factors, such as weak hip abductor muscles, excessive femur antetorsion, internal tibial torsion, an increased quadriceps angle, and inadequate control of hip muscles (8). A dysfunctional sensorimotor system can disrupt the functioning of the neuromuscular system (9). This neuromuscular alteration can result in flawed movement patterns, like DKV, while performing functional tasks (10).

Disruption of joint position sense (JPS), which is a component of proprioception, can contribute to the development of...
inaccurate movement patterns. Proprioception is a comprehensive concept that involves perceiving motion in the body and limbs, as well as a sense of body movement and location (11). It constitutes a fundamental aspect of neuromuscular performance (12). Proprioception plays a paramount role in maintaining balance, joint stability, coordination, and preventing injuries (11). Proprioceptive dysfunction in the knee is linked to injuries such as anterior knee pain and ACL injuries (13). Excessive medial knee displacement during dynamic movements due to neuromuscular inefficiency has been identified as a central risk factor for ACL injuries in high-risk sports such as handball, volleyball, basketball, and soccer (14). It seems that DKV is controllable and preventable, and activation of core muscles, strength of hip external rotators and abductors, and neuromuscular control play a major role in this regard (15, 16).

A dual task often entails performing a primary activity while doing a secondary task (17). It includes activities including executive functioning, working memory, attention, and others that can all overtax the brain’s cognitive abilities (18). Adult cognitive function decreases with dual tasks (19). Therefore, dual tasks, especially single leg squat (SLS), are impressive for adults.

Previous research has indicated that dual task situations can impair the motor and cognitive function of younger adults and lead to a decline in working memory and executive performance among older adults (20-23). Various methods have been proposed to prevent and improve dynamic knee valgus. For example, research results show that the implementation of corrective exercises has a significant effect on improving the sense of proprioception of people with dynamic valgus (24, 25). Also, a study investigated the effects of a neuromuscular training session on the balance and position sense of the knee joint in female athletes with DKV. The results of this study indicated that these exercises could enhance proprioception, suggesting that both strength and proprioception are crucial factors in promoting joint stability and equilibrium (26). Moreover, alterations in position sense during dual task conditions have been studied in individuals with various musculoskeletal conditions, including anterior cruciate ligament injury, low back pain, and chronic ankle sprain (27, 28). It suggests that musculoskeletal injuries may increase the cognitive demands for joint position sense (29).

As mentioned, it’s worth noting that many female athletes experience a condition known as DKV. What is concerning is that this condition has been linked to a higher risk of suffering an ACL injury. Furthermore, studies have shown that changes in joint alignment, like DKV, might affect an athlete. These alterations in proprioception appear to influence a person’s balance, making them more susceptible to injuries. Additionally, it has been observed that when athletes have to handle cognitive tasks while performing a sports activity, it can affect their accuracy and precision. However, there hasn’t been any research paper that has closely examined how these cognitive tasks impact a person’s sense of joint positioning and dynamic balance during sport-specific tasks, especially in athletes dealing with DKV. Given the high prevalence of DKV and its potential adverse effects on performance of female athletes, the present research aimed to examine the impact of dual task conditions on joint repositioning sense of female athletes with DKV.

MATERIALS AND METHODS

Participants
In this controlled laboratory study, 31 volunteered female athletes who met specific criteria for inclusion and exclusion (17 with DKV, and 14 without DKV) were recruited. The participants were recruited based on the convenience sampling method. The inclusion criteria were female athletes with an age of 18 to 25 years, demonstrating DKV according to the single-leg squat test (SLS) (30), and athletes who had history of participating in regular exercises for at least 3 years and 3 days a week. Also, the exclusionary criteria were: a history of any type of fracture, surgery and joint diseases in the lower limb or spine in the last five years, having participated in a sports competition at least two days before the test, the presence of significant malalignment in the lower limb based on the New York test (31), the inability to implement the protocol, the presence of pain in any part of the body during the test so that the subject was unable to continue the test, and body mass index (BMI) less than eighteen or more than twenty five. The review board of the research ethics committee of Allameh Tabataba’i University (Code: IR.ATU.REC.1400.032 – Date of approval: August 15, 2021), approved the protocol of the present study. The participants provided written informed consent during a familiarization session and had their height, weight, and BMI measured. The participants were given the assurance that their personal information would be kept confidential and that they could opt-out of the study at any point.

Protocols
The entire data collection process took place in the corrective exercises laboratory at Allameh Tabataba’i University in Tehran, Iran. Participants were required to attend the laboratory on one day. On this day, kinematic data were initially recorded as pre-test measurements. After that, participants followed a predetermined protocol, and immediately after-
ward, photogrammetric data and dynamic balance were measured as post-test measurements.

DKV assessment
The assessment of DKV was carried out through the Single-Leg Stance (SLS) test. During this test, participants’ feet were in sagittal plane, and their arms were folded in front of the body, while standing. Each participant performed five repetitions of the SLS test using their dominant leg, which was determined as the leg used to kick the ball (32). Participants underwent training to execute a SLS with a sixty degrees of flexion in the knee joint and then return to the start position. The tempo of SLS was 2-0-2. The time allocated to eccentric and concentric phases was 2 seconds. Having more than three abnormal responses out of the five trials considered as positive SLS. To ensure proper technique, all participants performed the task two to three times. The validity and reliability of SLS test has been reported in a previous study (33). The SLS test has been suggested as a suitable method for evaluating DKV (34).

Knee proprioception measures
Before conducting the tests, each participant’s dominant leg was marked with a 10 mm diameter reflective adhesive tape. Then, they were asked to sit upright. To measure the frontal plane knee angle, reflective markers were attached to specific points, including the anterior superior iliac spine (ASIS), the midpoint between ASIS and the middle of the patella (mid-thigh), the midpoint between two malleoli (ankle), and the midpoint between the medial malleolus and the middle of the patella (mid shank). The position of markers was carefully chosen to minimize the potential impact of patellar movement on the valgus angle measurement. The valgus angle was calculated as an angle formed by the intersection of the line connecting the ASIS to the midpoint of the thigh with the line connecting the ankles and the midpoint of the shank markers.

The knee flexion angle in the sagittal plane was assessed using markers positioned on the greater trochanter, the lateral femoral epicondyle, and the lateral malleolus. The angle between two lines, the line connecting lateral femoral epicondyle to greater trochanter and the line connecting the lateral femoral epicondyle to lateral malleolus, is considered as knee angle. All of the reflective markers were attached to bony landmarks by the same assessor.

A test angle of 60 degrees of flexion in the knee joint was selected because muscles tend to operate most efficiently in the mid-range of movement. In the literature, test angles of 40 degrees and 80 degrees of knee flexion are also commonly used (35). Assessing the accuracy of reproducing a target angle and calculating the discrepancy between the target angle and estimated locations as the output is a valid approach for measuring knee position sensing (36). In this approach, JPS is characterized as the absolute error between the target position and the estimated position (35). Photogrammetric method was used for JPS assessment (figure 1). Closed chain assessment of knee JPS was executed, while the target angle was drawn on the wall. The participants executed a SLS on dominant leg with open eyes. When the participants estimated a knee flexion of sixty degrees, images were taken using cameras prepositioned in sagittal and frontal planes (Canon EOS 2000D, Canon, Ota, Tokyo, Japan). Both cameras situated one hundred and eighty-five centimeters away from the participants and at the height of their knees. The camera height for each participant was adjusted according to their own height, but the distance from the camera to the location of individuals was the same for everyone.

Figure 1. Calculation of knee angle in frontal (A) and sagittal (B) planes.

After completing three trials, the participant’s eyes were bound shut with a band to exclude visual feedback from the assessment. The next step was for each participant to imitate the 60 degrees of knee flexion, hold it for 5 seconds, and then recall this angle. The rest time between trials was 7 seconds. The repositioning error was then determined by subtracting the angles of closed-eye photographs from open-eye photos and determining the absolute values. The average repositioning error obtained from three measurements was utilized for subsequent analysis. The changes in repositioning error were calculated by deducting the repositioning error of the post-intervention from the pre-intervention in each session, and by this way the influence of a specific protocol on the repositioning error was assessed.
Dynamic balance measure
The Y-balance test (YBT) was used for dynamic balance assessment, as used in previous studies. The Y-balance test’s validity and reliability have been reported in a previous research, with an ICC (intraclass correlation coefficient) of 0.987 and a 95% confidence interval (37).

The YBT assesses the balance on a single foot and the dynamic neuromuscular control of the lower limbs. It involves a Y-shaped pattern marked on the ground by means of tape, and the participant stands at its center. During the test, the individual stands on one leg, with the fixed foot placed at the center of the Y, while attempting to reach as far as possible in three directions (front, back, and sides) using the tip of the other foot’s great toe. Lightly touching the floor during the reach is allowed, but the participant must return to the initial position without completely removing the weight from the fixed foot. A practice phase consisting of six repetitions is permitted to become familiar with the test. If the fixed foot is lifted from the start position, or if the non-supportive foot is used to regain balance, that particular trial is omitted. The YBT is conducted on both legs, starting with the dominant leg as the supportive foot. In each direction, three repetitions were completed, and the maximum distance reached in each direction was recorded as the final score.

To ensure a fair comparison among individuals, the true length of the limb is measured and used to normalize the values obtained during the test. Then for each direction the mean and standard deviation were calculated. Normalization is crucial to account for variations in limb length among athletes. The normalized value is obtained by summing the three range values and dividing the result by 3 times the lower extremity length, which is then multiplied by 100. A difference equal or less than 4 cm between the dominant and non-dominant lower extremity is considered as normal, but larger discrepancies are suggestive of a higher risk of injury.

Dual task
After conducting initial measurements of JPS and dynamic balance, the athletes were given a twenty-minute break. Following the break, they were asked to participate in two study tests involving a cognitive task, specifically a dual task that included backward digit counting. The examiner provided the participants with a verbal calculation just before the start of the tests. Each participant then carefully considered the answer and provided their response to the examiner after completing the study tests. For the addition task, the starting numbers ranged from 50 to 99, and they were presented in a random order using digits generated from a mobile calculator. Additionally, during the study tests, the participants were asked to perform 3-digit subtractions. They had to subtract three digits from the initial number and communicate the results audibly so that the examiner could hear their responses. All tests were instructed by the same examiner and data analyses was run by a statistician who was unaware about the grouping names and desired outcomes.

Statistical methods
The data were analyzed using SPSS (Version 19; IBM, Armonk, NY, USA). Descriptive statistics were used to calculate the mean and standard deviation of the study variables. The normality of the data was assessed using Shapiro-Wilk test. To compare the effect of dual task, the dependent t-test was used. All statistical analyses were done by considering the level of significance at 95% ($\alpha < 0.05$).

RESULTS
In table I, the demographic data of the participants are summarized. There were no significant differences between two groups regards to the demographics. The Shapiro-Wilk test was run to examine the data distribution. The data for all research variables exhibited a normal distribution ($p > 0.05$). Due to the reduction in knee repositioning error angles, the results of paired samples t-test showed that the knee JPS in the frontal ($p = 0.008$) planes improved by dual task. Moreover, the Y-balance scores in anterior ($p = 0.001$), posteromedial ($p = 0.003$), and composite score ($p = 0.003$) decreased significantly while perform dual task in female athletes with DKV, showing the ability to maintaining dynamic balance may decrease with cognitive loads. More information presented in the table II.

DISCUSSION
The purpose of the present study was to investigate the Effect of dual task on joint repositioning sense in female athletes with Dynamic Knee Valgus. According to results of the present study, in female athletes with the DKV the dual task program can improve the knee joint reposition sense in

Table I. Demographic data of participants with ($n = 17$) and without ($n = 14$) DKV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DKV</th>
<th>Without DKV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>166.59 ± 5.18</td>
<td>164.86 ± 6.55</td>
<td>0.24</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.00 ± 4.92</td>
<td>60.36 ± 4.78</td>
<td>0.54</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.24 ± 1.67</td>
<td>22.21 ± 1.67</td>
<td>0.972</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.32 ± 0.96</td>
<td>22.15 ± 1.12</td>
<td>0.67</td>
</tr>
</tbody>
</table>

DKV: Dynamic Knee Valgus; BMI: Body Mass Index.
the frontal plane, but it has no effect on the joint reposition sense of the knee in the sagittal plane. Similar research to the present study was not found, but several researches have been conducted in line with this study (21, 38).

Regards to this study results, some explanations may explain the better knee repositioning sense while performing dual task. Dual task activities could potentially enhance the sensitivity of the intrafusal muscle fibers, leading to an improvement in knee position sense (39). As a result, dual tasking reduces the level of error observed during the measurement of dual task performance. Apart from the peripheral changes in receptors, there might also be some central nervous system changes, such as the central facilitation of neural information and an expanded somatosensory field of proprioception in the sensory cortex, which could be a consequence of engaging in dual task activities (40). According to systems theory, the function of the sensory system in controlling balance depends on the purpose and environmental conditions, and each sensory system can be more important under certain conditions; That is, the superior sensory system at any moment outputs more accurate information about the current environmental situation (41). On the other hand, improper function of the muscles around the knee joint can affect its dynamic stability and put it at risk of injury. It seems that DKV with associated improper muscle function is a risk factor for acute and chronic lower limb injuries (42). But we should keep in mind that our results demonstrated that interestingly, the improved JPS did not associate with better ability of maintaining dynamic balance.

On the other hand, our results showed that the dual task resulted in decreased dynamic balance scores in athletes with DKV. Some explanations are exist to explain this finding. In Kahneman theory of attention, it is suggested that attention is limited and has a fixed central source that cannot be changed, and all activities compete for this source (43). According to this model, also known as the theory of the central source of attention, when performing two tasks simultaneously, attention demands exceed this central capacity (43). For this reason, it seems that the cognitive load may disrupt the performance of other concurrent tasks. The dual task method is developed using the notions of Kahneman’s theory, in which the attentional needs of an action or a part of a task are assessed (44). The level of interference in a special task (primary) is evaluated when the person simultaneously executes another task (secondary). The results often reveal that 1) the processing of different tasks is different and 2) the simultaneous execution of tasks can cause an overload on the limited capacity of the attention system (45). In a special type of dual task, the exploration method is also popular. If the exploring phase of the main task is perceived to require the total capacity for attention, the response to the secondary task will be longer than when the person has to only respond to the secondary task. This method has been long used to determine the attention needs of sports skills (46).

### Table II. The comparison of changes in mean scores using dependent t-test in both groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pretest mean ± SD</th>
<th>With dual task mean ± SD</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>JPS</td>
<td>DKV</td>
<td>2.98 ± 1.89</td>
<td>2.02 ± 1.25</td>
<td>3.06</td>
<td>0.008*</td>
</tr>
<tr>
<td>JPS</td>
<td>Without DKV</td>
<td>1.64 ± 0.94</td>
<td>2.10 ± 1.76</td>
<td>-1.21</td>
<td>0.248</td>
</tr>
<tr>
<td>JPS</td>
<td>DKV</td>
<td>2.38 ± 0.97</td>
<td>2.44 ± 1.13</td>
<td>-0.23</td>
<td>0.823</td>
</tr>
<tr>
<td>JPS</td>
<td>Without DKV</td>
<td>3.23 ± 1.74</td>
<td>2.67 ± 1.85</td>
<td>0.912</td>
<td>0.380</td>
</tr>
<tr>
<td>Knee flexion</td>
<td>DKV</td>
<td>2.98 ± 1.89</td>
<td>2.02 ± 1.25</td>
<td>3.06</td>
<td>0.008*</td>
</tr>
<tr>
<td>Knee flexion</td>
<td>Without DKV</td>
<td>1.64 ± 0.94</td>
<td>2.10 ± 1.76</td>
<td>-1.21</td>
<td>0.248</td>
</tr>
<tr>
<td>Y-balance</td>
<td>DKV</td>
<td>67.48 ± 6.86</td>
<td>65.95 ± 7.02</td>
<td>3.86</td>
<td>0.001*</td>
</tr>
<tr>
<td>Y-balance</td>
<td>Without DKV</td>
<td>68.31 ± 6.93</td>
<td>69.54 ± 7.38</td>
<td>-1.35</td>
<td>0.201</td>
</tr>
<tr>
<td>Y-balance</td>
<td>DKV</td>
<td>89.77 ± 7.34</td>
<td>88.21 ± 9.91</td>
<td>1.87</td>
<td>0.080</td>
</tr>
<tr>
<td>Y-balance</td>
<td>Without DKV</td>
<td>94.99 ± 11.44</td>
<td>95.85 ± 11.14</td>
<td>-0.70</td>
<td>0.495</td>
</tr>
<tr>
<td>Posterolateral</td>
<td>DKV</td>
<td>95.64 ± 10.89</td>
<td>93.10 ± 11.17</td>
<td>3.54</td>
<td>0.003*</td>
</tr>
<tr>
<td>Posterolateral</td>
<td>Without DKV</td>
<td>99.58 ± 11.15</td>
<td>100.40 ± 11.57</td>
<td>-0.78</td>
<td>0.452</td>
</tr>
<tr>
<td>Y-balance</td>
<td>DKV</td>
<td>84.29 ± 7.86</td>
<td>82.42 ± 8.53</td>
<td>3.55</td>
<td>0.003*</td>
</tr>
<tr>
<td>Y-balance</td>
<td>Without DKV</td>
<td>87.63 ± 8.91</td>
<td>88.60 ± 8.94</td>
<td>-1.04</td>
<td>0.318</td>
</tr>
</tbody>
</table>

DKV: Dynamic Knee Valgus; JPS: Joint Position Sense; *Statistically significant differences were observed.
For instance, Castiello et al. (46) employed dual tasking to identify the temporal differences in attention in several sports tasks, including receiving a volleyball serve, 100-meter run, 110-meter hurdle run, and receiving a tennis serve, the results of which revealed that in tennis and volleyball serve, all stages require attention, but not in equal proportions. Jafarzadeh et al. (47) investigated the effect of quadriceps muscle fatigue on knee joint position sense in healthy men. The participants in this study were 34 healthy young people who did not have any problems in muscle strength and joint flexibility at the knee joint. The subjects actively restored the 45-degree flexion angle (target angle) in the knee joint by concentric contraction of the quadriceps muscle. The results showed that fatigue of the quadriceps muscle decreases the accuracy and increases the absolute and constant error of knee joint angle reconstruction in healthy people (47). Also, Shahrjerdi and colleagues investigated the effect of proprioception training on the knee joint position sense of athletes with genu varum. The findings revealed that knee joint proprioception exercises effectively decreased the reconstruction error, indicating an enhancement in proprioception among athletes with genu varum (48). Mirzaee et al. reported improvement in balance and joint position sense in participants with DKV as a result of one session of reactive neuromuscular training. The outcomes of this study offer valuable insights for developing injury prevention and rehabilitation strategies targeted at athletes with DKV (26). A dual task condition is a technique used in neuropsychology, where a participant is required to accomplish two tasks concurrently to compare their function with single-task situations. If the scores on one or both tasks are lesser when done simultaneously compared to when done separately, it suggests that the two tasks interfere with each other. This interference implies that both tasks compete for the similar class of information processing resources in the central nervous system (49). Engaging in dual task activities can enhance knee position sense by promoting the restoration of muscle firing patterns’ synergy and synchrony needed for dynamic joint stability and precise motor control (50). Another reason for the beneficial impact of dual task exercises on knee position sense is their inherent capacity to enhance cognitive awareness of position and movement of the joint (51).

Therefore, it seems that paying attention to cognitive and sensory-motor characteristics, training with dual task in designing an effective course on the risk factors of dynamic valgus has a positive effect on improving balance and sense of joint position, and also reduces the injury rate. In other words, according to the results of this research, by taking advantage of the characteristic of exercise and consequently challenging the sensory-motor system involved in proprioception, balance, and most importantly, the low cost and availability of the cognitive task reduces the incidence of injuries caused by reducing the balance and sense of joint position, with the design of combined protocols, it has a positive effect on the sense of joint reconstruction and with the aim of improving the reduction of body dynamics and balance.

To investigate the generalizability of the data in this research, it is necessary to consider its limitations more closely. One limitation of this study is its case-study nature, thus leaving the long-term effects of cognitive loads on knee balance and proprioception largely unexplored. Moreover, it is essential to acknowledge that this study was conducted under laboratory conditions, and therefore, caution must be paid when extrapolating its findings to real-world sports settings. Furthermore, it should be noted that there is no consensus among different researchers regarding the likelihood of a relationship between changes in proprioception and the occurrence of lower limb injuries and sports performance. Consequently, it would be unwise to simply correlate alterations in proprioception with an increased likelihood of lower limb injuries in sports.

CONCLUSIONS
The present study aimed to examine the impact of dual task conditions on joint repositioning sense in female athletes with DKV. Our results showed that dual task training could improve knee joint sense of position in the frontal plane, but it does not have a clear effect on the joint position sense in the sagittal plane. Moreover, dual task may reduce dynamic balance in individuals with DKV. It appears that being in competitive conditions and dealing with cognitive loads may potentially impact both sports performance and the likelihood of sports-related injuries. Therefore, it is recommended to consider these findings when providing exercises to athletes with dynamic knee valgus.

FUNDINGS
None.

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

CONTRIBUTIONS
All authors: conceptualization, writing – original draft, writing – review & editing, project administration, data curation.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.
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Neuromuscular Control Training is Effective to Prevent Ankle Sprains in Athletes

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INTRODUCTION

The ankle joint is exposed to serious injuries due to its importance in bearing weight and maintaining posture control during jumping, leaping and sudden change of direction exercises. So that, ankle joint injuries are the most common body injuries reported and in sports, including 25% of all injuries (1, 2). The external ligaments of the ankle plays the most important role in maintaining the bone structure and condition of the ankle joint and it is one of the most common vulnerable structures in athletes (3, 4). The risk of injury can act as a deterrent to future participation in athletic activities (5, 6). Lateral ankle sprain is the most common musculoskeletal injury in the physically active population and is highly prevalent in the general population. This prevalence is significant and leads to the cost of health care for injuries (7). The cost of treating and diagnosing lateral ankle sprains was estimated at approximately $4.5 billion in 2015, up from $2 billion in 1984. It also has a high prevalence rate of over 2.15 per 1,000 people per year and more than half of all ankle sprain injuries that are reported in high school and college games in the United States (8).

In football, basketball, volleyball and handball, ankle sprains are reported between 10-30%, and the most common type of injury is plantarflexion with inversion and adduction (9). The external ankle ligament complex is one of the most commonly injured structures in athletes. In such a way that external sprains include 85% of ankle sprains (10). Ankle injury is a major medical and socioeconomic problem, because the prev-
Chronic ankle instability is one of the most common debilitating complications of acute ankle sprain, which usually leads to the athlete staying away from sports activities (12). In summary, factors like laxity of the ligaments, weak subtalar joints, poor proprioception and delayed muscle responses – especially due to past injuries – can compromise the ankle joint’s stability and proprioceptive functions, predisposing it to this type of injury. Early intervention to address these issues may help prevent recurrences (13). Recently, conservative treatments of chronic ankle instability have been accepted as the main means of managing the condition and preventing re-injury, so that the effect of exercise therapy in reducing the risk of recurrence of ankle sprains and chronic ankle instability has been shown (14, 15).

Damage to the mechanoreceptors near the ankle sprained ankle can lead to functional deficits, deficits in postural control and postural control, deficits in strength, range of motion, and chronic instability from the initial injury (16-18). In this regard, Ganesh et al. (2015) pointed out the negative impact of ankle sprain injury on performance (19). About 40-75% of people have experienced functional instability after ankle sprain. The high prevalence of functional instability after ankle sprain is alarming because instability is one of the known factors for secondary osteoarthritis. Repeated ankle sprains can also cause arterial damage (anterior tibia artery and posterior tibia artery). It has been observed that the defect in postural control and the reduction of performance following ankle sprains are the result of ankle functional instability. The role and importance of posture control is determinant in the daily life activities and performance of people, especially athletes, in such a way that the resulting instability causes disturbances in posture control and performance (20). Posture control and performance are not separate from each other, because postural control is the source of all conscious movement skills of people (21). Although posture disorder is not evident in athletes, the smallest change in their posture control results in a malfunction. Freeman et al. (1965) suggested that coordination exercises can effectively improve postural control and reduce the risk of recurrent ankle sprains in individuals with CAI. Therefore, incorporating coordination exercises into rehabilitation programs for individuals with CAI may be beneficial in improving their overall ankle stability and reducing the risk of future injuries (22). In another one, Shokouhi et al. (2015) compared the postural stability of soccer players with and without CAI (chronic ankle injury) in the functional test of landing jump in soccer players. They found that in the forward jump, the displacement variables of the center of pressure in the internal-external and anterior-posterior directions in soccer players with CAI were significantly higher than in healthy players, which reduces the efficiency and performance of athletes (23). Ankle injury is a major medical and socio-economic problem because the prevalence of ankle injuries is high and the cost of treatment and absence from work in this injury is high (4). Because ankle injury, in addition to the need for long breaks after the injury, which requires 2 to 4 months of rest for full recovery, also imposes a lot of costs on the subject. According to the mentioned cases, the sensitivity and importance of correct treatment and prevention of ankle injuries in athletes are more important and vital. Therefore, the goal of correctional and rehabilitation programs is to prevent and treat ankle injuries, correct or eliminate risk factors, and ultimately reduce injuries. Modifiable risk factors are neuro-muscular and biomechanical risk factors that can be modified with special sports exercises such as deep receptor exercises, neuromuscular exercises, stretching, plyometric and trunk and central muscle control exercise (24). Ankle mobility provides a buffer during landing and peroneus longus activation prevents ankle inversion. During the jump and landing, adaptation to the landing force and control of neuromuscular activation are very important in maintaining ankle stability. Together, they can effectively minimize the risk of ankle sprain injuries (25).

A set of plyometric exercises and a dynamic training method for the lower limb are hopping exercises which has the multiple nature of muscle strength, neuromuscular coordination, joint stability, balance and joint proprioception. Muscular strength and balance are two essential components of jumping performance. Hopping exercises cause neuromuscular adaptation and thus lead to increased performance and faster and more powerful execution of movements (26, 27). In a study Minoonejad et al. (2018) found that six weeks of hopping stabilization training was effective in improving neuromuscular control and self-reported performance in collegiate basketball players with ankle instability (28). Also, it seems that six weeks of jumping training reduces the time interval before starting the activity and increases the range of activity of the internal gastrocnemius and gluteus Maximus muscles in one-legged jump landing in athletes with functional ankle sprain (29). In general, neuromuscular control has a large effect on ankle sprains. For people with CAI, the quality of their joint movement patterns and muscle activity is somewhat compromised. However, they can still perform high-intensity exercise with satisfactory coordination and control strategies among their joints and muscles. Accordingly, in addition to facilitating the strengthening of the peroneus longus to train muscle recruitment and reduce the occurrence of inverted sprains, muscle groups around other joints should be strengthened to provide optimal landing patterns for individuals with CAI (25). Over the past decade, ankle exercises have been at the core of developing exercise programs aimed at preventing and recur-
Neuromuscular Control Training to Prevent Ankle Sprains

ring ankle sprains. Previous researchers have focused more on rehabilitating activities that do not replace functional tasks, such as open chain activities, static activities, and reaction time. This focus makes it difficult to compare results to weight-bearing activities that occur during most ankle sprains. Therefore, the present study was conducted with the aim of investigating the effect of a course of neuromuscular control exercises on reducing the risk factors of athletes prone to ankle sprains.

**MATERIALS AND METHODS**

This research has been conducted in the form of research plan No.140007065665 and ethics code is IR.UMSHA.REC.1400.487 (Hamadan University) – date of approval: November 09, 2021.

**Research method**

This interventional study on young athletes (n = 30), male members of volleyball, basketball, and handball teams, with an age range of 18-25 years, with ankle sprains (30). Athletes were randomly assigned to two intervention and control groups. To randomize the patients, the random block method with a block size of four was used. For this purpose, four sheets of paper were prepared. The letter I (test group) was written on two sheets and the letter C (control group) was written on the other two sheets. The sheets were mixed together and removed by the researcher consecutively without placing them. This process was repeated for the number of qualified people and the group of each subject was determined. The subjects of the test group performed hopping exercises for six weeks and three sessions a week. The inclusion criteria included being an athlete, having functional instability of the ankle, without any deformities affecting the research process (such as kyphosis, scoliosis, and sway back, uneven shoulder) and completing the consent form to participate in the research. The exclusion criteria for the participants were the age group above the purpose of the study, lack of consent to cooperate in the research process, and any pain during the work process.

**Data collection and evaluation method**

Ankle Joint Functional Assessment Tool (AJFAT) was used to identify people with ankle sprains. Participants selected responses that best described ankle use using the following scale: much less than the other ankle, slightly less than the other ankle, equally, slightly more than the other ankle, or slightly more than the other ankle. Each answer is assigned a point value between 0 and 4. The values of the scores were unknown to the participants. The maximum score in this evaluation tool is 48. Experimental data show that AJFAT has test-retest reliability (internal correlation coefficient 0.94) and precision (standard error of measurement 1.5) (31).

To evaluate the dynamic balance, the Y test was taken from the instability leg. In this way, the subject without shoes, stood in the center of a grid of lines measured by a Y tape measure. The lines extend from the center in three directions: anterior, posterior, internal, and posterior external, so that they form a 90-degree angle in the posterior direction and a 135-degree angle with the anterior direction. The subject’s foot was set in the center, so that the big toe of the subject’s foot was placed in the starting line of the center (figure 1). After setting the subject’s foot in the center, he was asked to stand on his foot and gently touch each of the lines on the floor with the tip of the big toe of his other foot as far as possible. The test was performed three times in each direction and the maximum reach distance was considered as the raw score of the reach distance in each direction. The total average of the maximum reaching distance of the subject in each direction was divided by the length of the lower limb and then multiplied by 100, and it was considered as the normalized composite score of the subject’s balance as a percentage.

**Figure 1.** How to perform the Y test in anterior, posterior, internal, and posterior-external directions.

A tape measure with an error of 1% was used to measure the length of the lower body. The actual length of the foot was measured from the upper anterior metatarsals to the medial ankle using a tape measure. For this purpose, the subject was placed in supine position, while the knees were in extension and the feet were 15 cm apart. The measurement on the left and right leg was repeated three times and the average of each was recorded as the length of the lower body (32). Then, the hopping test (single leg jump test, single leg cross jump, triple jump and single leg duration test) was taken from the subjects (figure 2).

**Hopping test (33)**

**Single Hop test**

The subject stands on the test leg and performs the hopping with one leg as far as possible and lands with the same leg, and the amount of distance achieved is recorded.
Crossover Hop test
The subject stands on the test leg and performs hopping on a 15 cm wide line in a crossover pattern as far as he can for 3 hops and finally hopping distance is recorded.

Triple Hop test
The subject performs three consecutive hopping as far as he can and finally the distance covered is recorded. Three tests recorded when the subject's attempt is successful and has stable landing. In order to achieve legitimate and correct efforts, one's landings must be successful and controlled. If the subject lands with the other foot, loses balance control, or has an extra hop after landing, the attempt must be repeated. The subject is asked to start the tests with the big toe on the floor just behind the starting line. The distance of hop tests was evaluated by a tape measure as the closest distance from the starting line to the back of the subject's heel.

6-min timed Hop test
The subject was stranded on a leg and performed the hopping in a 6-meter path that has been marked in advance. He was asked to travel this route as fast as possible and the time to travel this six-meter route is recorded for him using a stopwatch. The time was recorded from the time the subject's heel leaves the ground until the subject crosses the finish line. The training program includes 6 types of hopping exercises: hopping to the sides, hopping to the front and back, hopping in the form of four Latins, hopping moving forward, hopping in the form of eight Latins (figure 3), which is three sessions a week and it was done for six weeks. The intensity of the exercises increased from 80 to 160 feet contact with the ground during the weeks. Also, the exercises were performed on two legs and then on one leg, and the position of the hand was changed from the free position to the chest and behind the head to increase the difficulty and intensity of the exercises. Also, the level of training every week was changed from the floor of the hall to the surface of artificial grass and training mat. In the first three weeks, exercises were done in the form of hopping and stability (18) and from the third week onwards, hopping exercises were performed with a rhythm of 2 Hz using a metronome (34).

Data analysis
The effect of neuromuscular exercises on the scores of the Y test of the lower limb and the hop test between the pre-test and post-test stages was investigated separately for each of the test and control groups using the paired t-test. Also, analysis of covariance test was used to compare the Y test score between the test and control group by controlling the pre-test effect. Data analysis was done using SPSS version 19 software at a significance level of 0.05.

RESULTS
A total of 30 athletes were studied. These athletes were randomly divided into two groups of 15 people. The mean athletes’ age was 35.22 (standard deviation (SD) 55.3) years in the test group and 14.22 (SD 84.3) in the control group. The basic characteristics of the athletes, separated from the test and control groups, are presented in table 1. As can be seen, the basic characteristics in the two groups are almost similar.
The comparative pre and post-test results in each group using a paired t-test are presented in Table II. The results showed that significant differences were observed between the pre- and post-test in the test group for test score Y (p = 0.0001), Single Hop test (p = 0.038), Crossover Hop test (p = 0.0001), Triple Hop test (p = 0.003), and 6-min timed Hop test (p = 0.002). However, in the control group, there were no significant differences in all of the studied variables (p > 0.05).

The results of covariance analysis (Table III) showed that there is a significant difference between the test and control groups by controlling the pre-test effect (p < 0.005). So that the mean scores of Y test, single hop test, crossover hop test and triple hop test of athletes prone to ankle sprain in the test group increased compared to the control group. Also, the mean score of the 6-minute timed hop test of athletes prone to ankle sprains in the test group has decreased compared to the control group (p < 0.05).

**DISCUSSION**

The present study was conducted with the aim of investigating the effect of neuromuscular control exercises on reducing risk factors in athletes prone to ankle sprains. The results showed that neuromuscular exercises significantly improved the scores of the Y test and the hop test of athletes prone to ankle sprains between the pre-test and post-test stages (p < 0.05). Among the studies, in line with our results there is the Kordi Ashkezari et al. (2020) study, which aimed to compare the effect of 6 weeks of balancing and hopping strengthening training on the kinematics of the lower extremities of athletes with functional ankle instability while running. The study found that hopping training in dorsiflexion, ankle inversion, and knee flexion were significantly different from the control group. The results showed that both hopping and balance strengthening training improved the dorsiflexion, inversion, and knee flexion angles at the initial contact during running. However, hopping exercises were found to be more effective than balance strengthening in improving knee flexion angles. In addition, a significant difference was observed between the two groups of strengthening/balance training and hopping, increasing knee flexion. They found that the role and importance of posture control is very important in the daily life activities and performance of people, especially athletes, in

**Table I.** Average and standard deviation of age, height and weight of subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Number</th>
<th>Average</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Test</td>
<td>15</td>
<td>35.22</td>
<td>55.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>14.22</td>
<td>84.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Test</td>
<td>15</td>
<td>53.178</td>
<td>09.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>84.177</td>
<td>93.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Test</td>
<td>15</td>
<td>42.71</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>73.70</td>
<td>69.5</td>
</tr>
</tbody>
</table>

**Table II.** The results of the effect of neuromuscular exercises on the Y test score of the lower limb and the test score separately for each of the test and control groups.

<table>
<thead>
<tr>
<th>Hop test score</th>
<th>Group</th>
<th>Pre-exam mean ± SD</th>
<th>After the test mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test score Y</td>
<td>Test</td>
<td>21.11 ± 1.97</td>
<td>76.25 ± 1.99</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14.03 ± 1.98</td>
<td>14.51 ± 1.97</td>
<td>217.0</td>
</tr>
<tr>
<td>Single Hop test</td>
<td>Test</td>
<td>95.66 ± 14.106</td>
<td>13.06 ± 15.131</td>
<td>038.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>93.06 ± 13.107</td>
<td>73.06 ± 12.113</td>
<td>458.0</td>
</tr>
<tr>
<td>Crossover Hop test</td>
<td>Test</td>
<td>53.73 ± 24.361</td>
<td>99.80 ± 29.386</td>
<td>0001.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>94.60 ± 23.356</td>
<td>01.93 ± 22.363</td>
<td>434.0</td>
</tr>
<tr>
<td>Triple Hop test</td>
<td>Test</td>
<td>21.06 ± 28.470</td>
<td>78.33 ± 29.511</td>
<td>003.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>88.33 ± 36.474</td>
<td>16.06 ± 36.485</td>
<td>137.0</td>
</tr>
<tr>
<td>6-min timed Hop test</td>
<td>Test</td>
<td>24.12 ± 0.3</td>
<td>32.75 ± 0.2</td>
<td>002.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23.08 ± 0.3</td>
<td>27.99 ± 0.2</td>
<td>223.0</td>
</tr>
</tbody>
</table>

SD: Standard deviation.
such a way that the resulting instability causes disturbances in posture control and performance (35). Shahverdi et al. (2019) investigated the effect of neuromuscular exercises on balance symmetry in people with chronic ankle sprains. The results of their study, in accordance with our results, showed that ankle sprains cause balance asymmetry in both legs and that neuromuscular exercises improve this balance. Both legs and balance asymmetry compensation. After one month without training, balance symmetry was maintained in both legs (36).

In a study similar to ours, Hung (2015) investigated neuromuscular control and rehabilitation in people with chronic ankle injury, he stated that injury and lateral dislocation of the ankle is one of the common problems in athletes, and after decades of research, the main causes of sprains have not yet been fully explained. He concluded that which exercise method improves the effects and factors of re-injury in people with chronic ankle injury is still unclear. In their study, Fahim (2015) examined the effect of four weeks of neuromuscular training on the static balance of men and women with chronic ankle instability. Thirty subjects were divided into two experimental (7 women and 8 men) and control (7 women and 8 men) groups of 15 people. The results of this study showed that a course of neuromuscular exercises caused a significant improvement in the static balance of the experimental group. They concluded that neuromuscular exercises can be used in people with chronic ankle instability to improve posture control (38).

Many rehabilitation studies have been conducted to evaluate the effects of neuromuscular control, sometimes proprioceptive, as a method of increasing muscle and nervous system sensitivity because ankle sprains cause muscle weakness and loss of neuromuscular function, but the loss of neuromuscular function is more pronounced than the loss of strength (39). In a similar study, Tarang et al. (2014) investigated the effects of balance training for 4 weeks on patients with functional ankle instability. The results of their study indicated that after completing the training period, ankle instability is reduced and ankle joint proprioception is improved as a result of the effectiveness of exercises in patients. Therefore, the use of these exercises can be useful for people suffering from ankle functional instability (40).

The second main finding of this research was the results of covariance analysis between the two test and control groups, which showed a significant difference, so that by controlling the effect of the pre-test, a significant difference was observed between the two groups. The hop test is suitable for testing lower limb function in patients with ankle sprains and has been reported to be very reliable and valid (41, 42). This study also determined that the average score of the Y test, single hop, crossover hop and triple hop of athletes prone to ankle sprain increased significantly compared to the control group. Also, the average score of the 6-min timed hop test of athletes prone to ankle sprains has significantly decreased compared to the control group. In this regard, Karimizadeh et al. (2013) investigated the effect of six weeks of hopping exercises on the dynamic balance of athletes with functional ankle instability. The number of 30 male athletes, including two experimental and control groups, the experimental group underwent training for six weeks. Dynamic balance was evaluated by the star test. The results showed that hopping exercises caused a significant increase in the subject’s reaching distance in all eight directions of the star test. They recommended that due to the special need of people with ankle functional instability for dynamic balance in sports skills and also as an important rehabilitation factor in the design of exercise and rehabilitation programs, the benefits of the hopping training program should be used. Additionally, they recommended incorporating balance and proprioceptive exercises into a rehabilitation program can help reduce the risk of future ankle injuries and improve overall ankle function. Therefore, it is important for individuals with ankle functional instability to work with a physical therapist or sports medicine professional to develop a comprehensive exercise program that includes hopping and proprioceptive exercises to improve dynamic balance and stability (26, 30).

<table>
<thead>
<tr>
<th>Hop test score</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>Statistic f</th>
<th>P-value</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y test score</td>
<td>09.29</td>
<td>1</td>
<td>09.29</td>
<td>26.14</td>
<td>0.01</td>
<td>346.0</td>
</tr>
<tr>
<td>Single Hop test</td>
<td>362.412</td>
<td>1</td>
<td>362.412</td>
<td>22.12</td>
<td>0.002</td>
<td>312.0</td>
</tr>
<tr>
<td>Crossover Hop test</td>
<td>914.345</td>
<td>1</td>
<td>914.345</td>
<td>43.6</td>
<td>0.017</td>
<td>192.0</td>
</tr>
<tr>
<td>Triple Hop test</td>
<td>375.448</td>
<td>1</td>
<td>375.448</td>
<td>93.4</td>
<td>0.035</td>
<td>155.0</td>
</tr>
<tr>
<td>Triple Hop test</td>
<td>50.0</td>
<td>1</td>
<td>50.0</td>
<td>15.6</td>
<td>0.020</td>
<td>186.0</td>
</tr>
</tbody>
</table>
is difficult to determine which method is superior. Research directly comparing different treatment modalities is limited (43). Although this study and others have shown positive effects of neuromuscular training, the effectiveness of balance exercises along with neuromuscular exercises cannot be ignored. However, experts explain that it is possible to prevent injury through balance training, and more results are being published for injury prevention programs that incorporate the principles of balance training alongside neuromuscular training (44).

As with all studies, this study had limitations. The impossibility of investigating the daily life activities of participating athletes was one of the limitations of the research, and the researcher was confident about its results. Also, the researcher has conducted this research based on the available statistical population, which can be expanded in future studies. This study was conducted in order to investigate the effect of an effective training method to reduce the risk factors of ankle function following frequent ankle injuries in people who attend in jumping sports and to test the effectiveness of hopping exercises.

CONCLUSIONS
Chronic ankle instability is one of the most common disabling complications of acute ankle sprains. Which usually leads the athlete to stay away from sports activities. Based on these results, we recommend practical applications of hopping exercises to rehabilitation professionals. Rehabilitation experts may be able to provide both or one program (neuromuscular and balance) according to the needs and characteristics of the patient, especially in places where there is not enough equipment. Furthermore, much of the data on injury incidence and prevalence of musculoskeletal conditions among athlete populations from high-quality surveillance systems is focused on the most popular and highly populated team sports, such as basketball, football, and soccer. More research is needed on other similar activities and sports. It may be particularly important to evaluate the incidence of ankle sprains and the effect of neuromuscular exercises among athletes of other age groups.

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

CONTRIBUTIONS
The authors contributed equally to this work.

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

REFERENCES


Reliability and Sensitivity of Smartphone Accelerometer to Assess Postural Balance in Healthy Individuals

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INTRODUCTION

Human postural control is a complex process that encompasses several physiological systems, including the vestibular, visual, somatosensory, and musculoskeletal systems (1). Individuals with impairments in these systems may present a reduced capability to balance the center of mass after external perturbations, increasing the risk of adverse events, such as falls (2). Falls are particularly prevalent among the elderly population and are a leading cause of hospitalizations (3). In Brazil, between 2000 and 2020, there were 1,746,097 hospitalization cases attributed to falls, imposing substantial costs on the public healthcare system (4). Therefore, assessing postural balance is relevant to establishing interventions capable of mitigating the decay of this function.
or even increasing its performance over time, reducing the probability of falls and inherent health issues. In clinical practice, the assessment of postural balance often relies on measuring the maximum time a person can sustain a particular task (5) or from score-based instruments, such as Berg Balance Scale (6). Although these methods are easily accessible and widely used, they do not consider the postural adjustments made throughout the entire task, which can potentially introduce bias into the calculated results. The gold standard for assessing the biomechanical aspects of postural balance involves measuring the displacement of the center of pressure, a variable that has a strong correlation with the history of falls (7). This measurement is typically obtained through the use of force platforms or three-dimensional motion capture systems. However, these instruments are expensive and often located in laboratory settings, requiring professional expertise to collect, process, and interpret the data. Therefore, alternative solutions, such as inertial sensors, have been developed to circumvent these barriers.

In recent years, inertial sensors have gained significant attention, primarily due to their low cost and practicality, and are now extensively employed in the assessment of postural balance (8). Traditionally, inertial sensors include accelerometers, gyroscopes, and magnetometers, and these components may be embedded in mobile electronic devices, such as smartphones and tablets. By utilizing these sensors in such devices, it becomes feasible to acquire data for the evaluation of postural balance in a more accessible manner. However, despite this growing application, it is important to guarantee that the extracted parameter is indeed informative about postural control. Although the validity and within-session reliability of measurements acquired from smartphones have already been documented, particularly in tests involving scenarios with eyes open and closed (9), whether more challenging tasks can also provide reliable information remains an open question. For instance, it remains unclear whether tasks involving single-legged support can yield consistent results across different days, and whether this technique is sensitive enough to distinguish between activities of varying difficulty levels.

In this study, we aimed to explore the smartphone’s capability to distinguish between various levels of balance task difficulty by utilizing data from the accelerometer, while also evaluating the reliability of these measurements. Specifically, we assessed the concordance between the root mean square (RMS) value calculated from the accelerometer data acquired over a two-day period and compared the results across five static balance tasks, each graded in terms of difficulty level. If the smartphone is sensitive enough to differentiate between these tasks, we would expect to observe higher RMS values in tasks performed with eyes closed and single-legged support, as compared to tasks performed with eyes open and bipodal support. Moreover, if this technique is reliable between days, we expect to observe an intraclass correlation coefficient (ICC) > 0.6 and a coefficient of variation (CV) ≤ 10%.

**MATERIALS AND METHODS**

**Subjects**

Fifteen subjects (4 females) were recruited to participate in this study (mean ± SD; age: 30.73 ± 9.62 years; body mass: 75.27 ± 18.87 kg; height: 173 ± 10.54 cm). All participants were physically active and had not reported any lower limb musculoskeletal injuries within the six months prior to the study, nor did they exhibit any impairments in systems related to postural control. The study involved two separate laboratory visits. During the first visit, after the participants had read and signed the informed consent form, they familiarized themselves with the postural balance tasks until they felt comfortable and safe to perform the tests. Subsequently, data were collected. On the second visit, the same protocols were re-administered to assess the reliability of the measurements. The experimental procedures conformed to the Declaration of Helsinki and were approved by the local Ethics Committee (name: Instituto Nacional de Traumatología e Ortopedia; CAAE: 65807122.9.0000.5273 – date of approval: January 31, 2023).

**Tasks to assess the postural balance**

The smartphone was positioned on the lower back and fixed using an elastic belt (10). Five tasks were performed in random order to assess static balance: semi-tandem with 1) eyes open and 2) eyes closed; feet parallel with 3) eyes open and 4) eyes closed; 5) unipodal support with the dominant side. In the protocols where participants maintained a feet parallel stance, the hip was maintained in a neutral position, ensuring that the feet were directed forward and kept together (11). For the semi-tandem, the dominant limb was positioned behind, with the hallux placed next to the heel on the contralateral side (12). During tasks with eyes open, participants were instructed to maintain a fixed gaze on a target positioned at eye level and two meters away. Throughout all tasks, the hands remained resting on the waist (12). Each task was performed three times, with each trial lasting for 30 seconds. In the event that a participant could not maintain the designated task position, another attempt was made. In addition, a one-minute break was provided between each task. The average value between the
three measures was considered for analysis. This protocol was chosen because the tests have different levels of difficulty (semi-tandem, feet parallel, and unipodal support), as well as stimulating the sensorimotor system in different ways (open and closed eyes).

**Data analysis**

Acceleration data were acquired from a smartphone (iPhone 7, software version: 15.6.1) with built-in 3D accelerometer using the free MATLAB Mobile app (MathWorks, v.8.9.1). The sampling frequency was determined to be 100 Hz, and the raw data were transmitted via Bluetooth to a personal computer and subsequently analyzed using a custom MATLAB script (MathWorks, v. R2020b). The 3D acceleration signal was low-pass filtered at 5 Hz using a sixth-order Butterworth filter (13). The magnitude of acceleration was initially calculated using the Euclidean norm (12). Subsequently, the RMS value was computed from the magnitude of acceleration to obtain a measure capable of representing the body oscillations captured by the smartphone throughout each task. To minimize the effect of possible discrepant postural adjustments at the beginning and end of each task, for the calculation of the RMS value, first and last five seconds of each test were discarded. Consequently, a 20-second window for the subsequent analysis was considered.

**Statistical analysis**

Based on the effect size (Eta squared = 0.6) estimated from our data, acceleration data collected from 15 subjects during five postural balance tasks ensured high (99%) statistical power (post-hoc power analysis; G*Power V. 3.1.9.7). Parametric analysis was considered for inferential statistics after ensuring data normality (Shapiro-Wilk test; p > 0.05, for all cases) and homoscedasticity (Levene’s test; p = 0.33). The mean of two days was used to compare the RMS values across the postural balance tasks. One-way repeated-measures ANOVA was applied, and the Bonferroni post-hoc adjustment method was used for paired comparisons whenever the main effects were verified. ICC and CV were used to assess the inter-day reliability of the accelerometer data acquired during postural balance tasks. ICC values were calculated using the two-way mixed-effects model and absolute agreement definition (14) and interpreted using thresholds (poor: 0.00-0.39; fair: 0.40-0.59; good: 0.60-0.74; excellent: 0.75-1.00) (15). The averaged CV across participants was calculated for each task, and a one-sample t-test was applied to verify whether the CV is significantly different than a theoretical mean (10%). All statistical analyses were conducted using R programming language (version 4.2.0), and the significance level was set at 5%.

**RESULTS**

**Figure 1** illustrates the magnitude of acceleration during three performed tasks for a representative participant. Visual analysis clearly demonstrates an increase in the acceleration amplitude as the task difficulty level increases.

Regarding the group analyses, ANOVA revealed a significant effect (F = 26.03, p < 0.001, Eta squared = 0.6), with pairwise comparisons indicating a significant difference between the tasks (figure 2). The mean and ± SD across two days for tasks semi-tandem with eyes open, semi-tandem with eyes closed, parallel feet with eyes open, parallel feet with eyes closed, and unipodal support were 0.071 ± 0.019 m/s², 0.088 ± 0.025 m/s², 0.084 ± 0.019 m/s², 0.106 ± 0.030 m/s², and 0.157 ± 0.032 m/s², respectively.

**Figure 2.** Mean and standard deviation of RMS values for each postural task.

STEO: semi-tandem with eyes open; STEC: semi-tandem with eyes closed; PEO: parallel feet with eyes open; PEC: parallel feet with eyes closed; UNI: unipodal support; (a-d) significantly greater than STEO, STEC, PEO, and PEC, respectively (p < 0.01 for all cases).
As reported in table I, tasks semi-tandem with eyes open, feet parallel with eyes closed, and unipodal support showed excellent inter-day reliability (ICC > 0.75; CV < 12%, for all cases), while the task feet parallel with eyes open had good inter-day reliability. The task semi-tandem with eyes closed showed the lowest ICC and had a CV significantly greater than 10%, suggesting high inter-day variability.

**DISCUSSION**

The present study aimed to investigate whether different levels of difficulty in assessing static balance can be differentiated using measurements obtained from smartphone, and whether this measure is reliable across two days. The results indicate that the RMS value extracted from the smartphone accelerometer was sensitive to differentiating tasks with varying levels of difficulty. Additionally, our findings showed good-to-excellent between-day reliability of the RMS value obtained from each postural balance task.

Regarding the outcome used to assess postural balance, analysis of the magnitude of acceleration during tasks suggested that this method was sensitive enough to reveal balance adjustments induced by more challenging conditions. Specifically, tasks with greater difficulty levels, such as unipodal support and parallel feet with eyes closed, exhibited significantly higher RMS values than those with lower difficulty levels (figure 2). With respect to visual restricted feedback tasks, a greater RMS value observed in those tasks with eyes closed compared to eyes open with bipodal support can be explained by reduced visual input. Along with other sensory inputs, vision provides direct information about body position and orientation in relation to the environment, playing a crucial role in generating precise motor commands for postural adjustments (16). When visual input is transiently removed or restricted, balance performance during a quiet stance is altered because of its effect on the perception and representation of body positions and oscillations. Additionally, even performed with eyes open, unipodal support showed the greatest RMS value, indicating that it was indeed the most challenging task. Since during single leg stance the base of support in the mediolateral direction is smaller, restricting the control of postural stability (17), a higher magnitude of acceleration during all task is excepted (figure 1). These findings are in line with previous research using dedicated accelerometer devices to assess postural control in healthy subjects (18,19), and reinforce the utility of smartphones to assess balance during quiet stance tasks graded by difficulty level. Reliability analysis revealed that the semi-tandem with eyes open, feet parallel with eyes closed, and unipodal support tasks showed excellent inter-day reliability, whereas feet parallel with eyes open demonstrated good inter-day reliability. Except for semi-tandem with eyes closed, all other tasks exhibited a CV% close to 10%, implying a relatively low degree of variability across days (table I). Although the task semi-tandem with eyes closed showed a good ICC, the CV% was significantly greater than 10%, indicating a high relative variability between days. In general, these results align with previous research that demonstrated the reliability of measurements obtained from smartphone for assessing static postural balance (9). The authors reported moderate to high within-session reliability in tasks with eyes open and closed performed on firm and compliant superficies. The current study extends these findings by examining the reliability of smartphone measurements across different days and demonstrating their valuable utility in monitoring postural control over time.

A strength of the present study lies in the use of smartphones as a practical and accessible tool for postural balance assessment, which provides some advantages. Specifically, smartphones with built-in accelerometers are widely available and relatively affordable compared to traditional laboratory equipment, such as force plates (8). This accessibility makes smartphone-based assessments more feasible for widespread implementation in clinical settings, enabling the large-scale screening and monitoring of postural balance. Additionally, the portability of smartphones allows for convenient data collection in various environments, including home-based assessments and remote monitoring scenarios (20). In clin-

<table>
<thead>
<tr>
<th>Task</th>
<th>Mean ± SD (m/s²)</th>
<th>ICC</th>
<th>95%CI</th>
<th>P-value</th>
<th>CV%</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-tandem EO</td>
<td>0.073 ± 0.022</td>
<td>0.069 ± 0.020</td>
<td>0.841</td>
<td>0.525</td>
<td>0.946</td>
<td>&lt; 0.001</td>
<td>11.49</td>
</tr>
<tr>
<td>Semi-tandem EC</td>
<td>0.091 ± 0.034</td>
<td>0.084 ± 0.024</td>
<td>0.625</td>
<td>-0.116</td>
<td>0.874</td>
<td>0.038</td>
<td>18.72</td>
</tr>
<tr>
<td>Feet parallel EO</td>
<td>0.083 ± 0.018</td>
<td>0.084 ± 0.024</td>
<td>0.735</td>
<td>0.211</td>
<td>0.911</td>
<td>0.009</td>
<td>12.90</td>
</tr>
<tr>
<td>Feet parallel EC</td>
<td>0.102 ± 0.028</td>
<td>0.111 ± 0.035</td>
<td>0.873</td>
<td>0.624</td>
<td>0.958</td>
<td>&lt; 0.001</td>
<td>11.21</td>
</tr>
<tr>
<td>Unipodal support</td>
<td>0.150 ± 0.028</td>
<td>0.163 ± 0.039</td>
<td>0.866</td>
<td>0.601</td>
<td>0.955</td>
<td>&lt; 0.001</td>
<td>8.02</td>
</tr>
</tbody>
</table>

EO: eyes open; EC: eyes closed.

Table I. Absolute values and between-day reliability of RMS acceleration measurements.

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ical practice, smartphones have demonstrated their utility in several other domains. For instance, smartphone applications have been employed for measuring dorsiflexion range of motion (21, 22), tracking step counts and providing feedback to promote daily physical activity (23), as well as offering break reminders to encourage physical activity among office employees (24).

Limitations and future perspectives
The current study has some limitations. Firstly, our sample comprised young and healthy individuals, which does limit the extent to which we can extrapolate our findings to more diverse groups with varying health conditions. Individuals with lower functional status, such as the elderly, orthopedics, or with other clinical conditions, may exhibit different responses and outcomes; thus, caution should be taken when applying these findings to such populations. Second, the reliability and sensitivity of the device used in this study were specific to the five static balance conditions evaluated. The applicability of these results to a broader range of postural balance assessments such as dynamic tasks remain uncertain. Finally, because we rely on the magnitude of acceleration from the three axes to assess postural balance, it is not possible to discern whether certain tasks have a greater acceleration in a specific direction. Considering these limitations, future studies should aim to address these issues and expand our understanding of fall prevention and postural balance, particularly in diverse and clinically relevant populations, including the elderly and orthopedic patients. These future investigations could focus on optimizing the signal processing to obtain instantaneous results, adapting them for dynamic tasks, and exploring the aspects of postural balance for a more comprehensive perspective on fall prevention and rehabilitation in these specific groups.

CONCLUSIONS
In conclusion, this study demonstrated that smartphones may provide reliable and sensitive measurements when it comes to evaluating postural balance across tasks of differing complexity. These findings underscore the potential of smartphone as a cost-efficient and practical instrument for assessing postural balance, carrying significant implications for enhancing fall prevention strategies and monitoring of postural stability in clinical settings. Incorporating smartphone-based assessments into fall prevention programs could improve their effectiveness by offering a practical and accessible means of tracking postural balance, thereby enabling the identification of individuals at risk and implementing targeted interventions.

FUNDINGS
None.

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

CONTRIBUTIONS
JCSA: design, data collection, signal processing, analysis and interpretation of data, writing – original draft, writing – review & editing. CTL, SCS, TL: design, analysis and interpretation of data, writing – original draft, writing – review & editing.

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

REFERENCES


Combination of Training to Failure and Non-Failure: An Unexplored Approach of Number of Repetitions, Rate of Perceived Exertion, Time Under Tension, Volume-Load, and Muscle Swelling in Trained Individuals

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SUMMARY
Background. Previous studies suggest training to failure may not yield superior muscle responses compared to non-failure training, leaving the combined use unexplored.
Objective. To compare the number of repetitions, volume-load, time under tension (TUT), rate of perceived exertion (RPE), and muscle swelling between two protocols: one involving four sets to failure (TFAS) and the other involving only the final set reaching failure (TFLS).
Methods. Fourteen trained adults completed both conditions in four sessions, with the first two sessions dedicated to determining the one-repetition maximum (1RM) for the barbell preacher curl exercise. In the third session, participants completed TFAS, maxing repetitions across four sets at 70% of 1RM. In the fourth session (TFLS), they fixed repetition in the first three sets (mean achieved during TFAS), with the final set to failure. In both sessions, repetitions, volume-load, RPE, and TUT were recorded post-set. Before and immediately after both TFAS and TFLS, participants’ biceps brachii cross-sectional area at 50% of humerus length was measured using ultrasound imaging to assess muscle swelling. Comparisons used ANOVA and paired t-tests.
Results. TFAS had higher RPE across sets (TFLS = 32 ± 4; TFAL = 37 ± 4) and a greater total TUT compared to TFLS (TFLS = 168 ± 44s; TFAL = 178 ± 41s). TFAS achieved more repetitions (TFLS = 38.93 ± 6.51; TFAL = 37.36 ± 5.24) and volume-load (TFLS = 918.55 ± 235.38 kg; TFAL = 888.77 ± 241.46 kg). Both protocols showed similar muscle swelling (TFLS = 23.90 ± 5.27 cm²; TFAL = 20.73 ± 10.71 m²).
Conclusions. TFLS allowed for more repetitions with lower RPE, with no difference in swelling.

KEY WORDS
Failure; non-failure; muscle swelling; repetitions; rate of perceived exertion; repetitions.
INTRODUCTION

The prescription of a training program focused on enhancing strength and muscle hypertrophy requires careful consideration of various factors, including biological individuality, contractions mode, specificity, and the physiological effects (1, 2). Recently, there has been extensive debate on whether training to failure is more effective than training without reaching failure in terms of muscle hypertrophy (3-6). The results highlighted that both strategies of training could offer a similar stimulus to induce muscle growth (7). Following the reasoning that muscle hypertrophy is reflected by the difference between protein synthesis and degradation (8), and considering that training to failure can potentially result in a higher anabolic and catabolic milieu compared to non-failure training (9), the idea of finding a balance between the two training strategies for promoting hypertrophy is plausible.

In the study by Lacerda et al. (5), it was found that when one leg performed all sets until failure (TFAS: training to failure in all sets), the contralateral leg was able to match the average number of repetitions achieved in the sets to failure. This suggests that if the last set were performed to failure (TFLS: training to failure only at the last set), it could result in a higher number of repetitions than the average value, and, possibly, a greater total number of repetitions than training to failure. In this training perspective, perhaps the advantages of TFAS may complement the benefits of non-failure training in inducing muscle hypertrophy. However, further research is needed to determine the validity of incorporating a combination of training with and without failure across sets.

Exner et al. (3) demonstrated that TFAS could result in a higher number of repetitions, muscle swelling, and a higher rate of perceived exertion (RPE) compared to non-failure training. These findings support the notion that training to failure leads to increased physiological stress and physical discomfort compared to non-failure training. However, different results could be observed in a cross-sectional study if TFAS and TFLS protocols were compared using the same variables as Exner et al. (3). For instance, TFAS might result in a greater or similar number of repetitions compared to TFAS but with a lower RPE. This hypothesis is supported by Lacerda et al. (5) who found that TFAS elicited higher RPE throughout the sets compared to the condition where participants trained with the average number of repetitions performed by TFAS across sets. Therefore, when comparing RPE between TFAS and TFLS across sets, it is possible that during the initial sets, TFLS would exhibit a lower RPE value than TFAS. However, in the final set, the RPE could be similar between the conditions, as both would be training to failure. Comparing the RPE response between TFAS and TFLS could indicate the level of effort and stress in incorporating training to failure in the last set, enabling the analysis a variable relevant for chronic adaptations to exercise (10). Additionally, another variable that could reflect the difference between TFAS and TFLS would be the volume-load (number of repetitions x lifted weight), as this variable appears to influence muscular responses, such as hypertrophy and increased strength, given its association with mechanical stress (11).

When considering muscle swelling, previous studies have suggested that higher training volume could result in greater muscle swelling (12), which supports the findings of Exner et al. (3), where TFAS showed a greater number of repetitions and muscle swelling compared to non-failure training. However, a direct comparison of muscle swelling between TFAS and TFLS has not been conducted yet. If training volume indeed has an impact on muscle swelling, it is possible that both types of training could elicit a similar or potentially greater response if the latter condition performs a higher number of repetitions. Furthermore, it is worth noting that muscle swelling observed immediately after the first training session has been suggested as a potential predictor of muscle hypertrophy (13). In this context, comparing muscle swelling between TFAS and TFLS could provide support for the hypothesis of superiority of one condition over another, considering that both conditions would be trained for a period of time.

Nonetheless, as failure approaches, it is expected that the duration of each repetition will progressively increase, making it challenging to maintain a consistent pre-established duration for each muscular action (14). Consequently, the time under tension (TUT) (15) of a set or of a training session could be longer for TFAS compared to non-failure training, as TFAS would reach failure in all sets and the final repetitions at each set would be performed at a slower speed, resulting in an extended duration. Unfortunately, Lacerda et al. (5) did not present information regarding the TUT spent across the training sessions by TFAS and the training protocol that matched the number of repetitions without reaching the failure to elucidate this issue. Nevertheless, in comparing the TUT of a training session between TFAS and TFLS, it is possible to speculate that there would be an equivalence between these protocols. Given that the greater number of repetitions performed by the TFLS protocol could be compensated by the longer TUT of the TFAS protocol during the execution of the final repetitions in each set. Considering the impact of TUT on strength and muscle hypertrophy development (16), analyzing this variable between the TFAS and TFLS protocols would contribute to a better understanding of the effects of these protocols in relation to muscle adaptations in future studies. Furthermore, it could also lead to a deeper understanding of the muscle swelling response between the proto-
cols, taking into consideration the debate regarding a possible association of TUT with muscle swelling (17, 18). Taking these factors into account, the present study aims to compare the number of repetitions, RPE, TUT, volume-load, and muscle swelling following a training session between the TFAS and TFLS conditions. We hypothesize that TFLS will demonstrate higher NMR, volume-load, and muscle swelling compared to TFAS. Additionally, the latter condition will have a higher RPE, while both conditions will exhibit similar TST.

MATERIALS AND METHODS

Overview

Participants were assigned to two conditions: TFAS and TFLS, in crossover design. The first two experimental sessions aimed to familiarize the participants and perform the one-repetition maximum (1RM) test in the seated bar preacher curl exercise. In the third experimental session, participants performed repetitions to failure at 70% of their 1RM across four sets (TFAS condition). In the fourth experimental session, participants performed the average number of repetitions calculated from the previous session’s total repetitions during the first three sets, however, in the fourth set, participants performed repetitions until failure (TFLS condition). The TUT, RPE and volume-load were recorded in both conditions across sets and used for further comparison. Before and after the TFAS and TFLS, images of the cross-sectional area (CSA) of the right biceps brachii were obtained via b-mode ultrasound at 50% of the humerus length for analysis and comparison of muscle swelling. Figure 1 presents the study design.

Subjects

The sample size was calculated following the procedures suggested by Beck (2013) (19), by using the software G*Power 3.1 (Frauz Faur Universität Kiel, Germany). With reference to paired comparisons (t-test) of means, we calculated the minimum number of individuals needed to achieve a statistical power of at least 80% (p = 0.05). For this calculation, we used data on immediate muscle swelling following a strength training session from the study by Hirono et al. (2020) (13) (effect size = 0.967), which indicated a requirement of 14 volunteers. Therefore, the sample was composed of 14 individuals. Fourteen trained individuals (12 men and two women) with an age range of 18 to 30 years old (mean ± SD: age = 21.5 ± 2.7 years; body mass = 79.9 ± 9.4 kg; height = 1.79 ± 0.08 m; body fat percentage = 13.14 ± 4.08%; calculated with seven skinfold measurements, following established procedures (20); training experience greater than 6 months (30.4 ± 27.1 months) participated in the study). The Ethics committee of the Federal University of Santa Maria approved this study on March 23, 2023 by the number 69325723.2.20000.5346, which complied with international standards. In addition, each subject was instructed not to perform any physical activity on the testing session’s days.

Procedures

First experimental session: familiarization of 1RM test

Measurements of height, body mass, and fat percentage (skinfold thickness) were taken. Afterward, participants were positioned for the seated bar preacher curl exercise. The seat height was adjusted to achieve a 45° angle of shoulder flexion (between the humerus and trunk). The elbow was fully extended and reached the bar, which had fixed numbers to enable participants to place their hands at a standardized distance from the center of the bar to the grip location. The positions of the hand and seat were recorded for later replication. Next, the participants performed 10 repetitions using only the weight of the bar as resistance, with a 2s concentric (lifting) phase and a 2s eccentric (lowering) phase. These actions were controlled by a metronome and served as a warm-up. During each repetition, participants performed a 130-degree excursion of elbow flexion, with 0 degrees representing the elbow fully extended. This range of motion was measured and determined using a goniometer. Two minutes after the last repetition of the warm-up, participants initiated the familiarization of 1RM test in the same exercise and position of warm-up. The 1RM test familiarization was performed throughout a full ROM, with a 3 min recovery interval between attempts. A final value was obtained within 6 attempts. Each attempt started with the
elbow fully extended. The bar was handed to the participant in this initial position, who then performed a concentric muscle action until 130° of elbow flexion (forearm perpendicular to the ground). The bar load was progressively increased (minimum of 0.5 kg, where all the weights were previously measured using a three-digit scale) until the participant was unable to perform the concentric action with proper form. Hence, the 1RM value corresponded to the weight lifted in the previous successful attempt. Similar procedures for 1RM achievement have been previously described for the preacher curl exercise (21).

Second experimental session: 1RM test
This session occurred 48-72h after the last session. In this, a sole 1RM test was conducted, following the procedures previously established during the 1RM test familiarization. The data obtained from the 1RM test in this session was collected for subsequent statistical analysis. The Intra-class Correlation Coefficient (ICC3,1) value between the 1RM results of the familiarization session and the test session was calculated and were (r = 0.99). The average weight lifted during the familiarization phase was 33.42 ± 11.49 kg, while during the test session was 34.99 ± 11.44 kg.

Third experimental session: TFAS
This session took place 48-72 hours after the second session. Participants underwent ultrasound examination, during which two measurements of biceps brachii CSA were obtained at 50% of the distance from the acromion to the lateral epicondyle of each humerus using B-mode ultrasound (Siemens Healthcare, ACUSON S2000, Germany). Ultrasound images were captured at a frequency of 21 frames per second using a 10 MHz linear transducer with a depth of 6-8 cm and a gain of 13dB. The settings were adjusted individually to ensure a clear image of the entire muscle for an extended field of view and replication in subsequent moments. Two trained technicians performed the ultrasound scans, moving the transducer in a line perpendicular to the humeral at a relatively constant speed. The images were saved to a hard drive and coded for blinded CSA calculation using Horos® software (Annapolis, Maryland, USA). The mean CSA value from the two images was used for statistical analysis. Images of the CSA of the biceps brachii before and after the performance of the protocols are shown in [Figure 2].

After the ultrasound image collection, participants proceeded with a warm-up in the seated bar preacher curl exercise, following a similar protocol as the warm-up for the 1RM test. After a two-minute rest, participants performed repetitions (0°-130° of elbow flexion) to failure across four sets, with an intensity of 70% of their 1RM and 2 minutes interval between sets. The repetitions were performed with a controlled tempo of 2s for the concentric action and 2s for the eccentric action, guided by a metronome and the range of motion checked by one of the evaluators. A chronometer was started as each participant began lifting the weight and was stopped at the point of failure. The time spent from the timer’s initiation to its interruption is considered as the set’s TST. It was observed that as participants approached failure (~ last two repetitions), the duration of each repetition increased. Therefore, the last repetitions were performed with a longer duration than 4s. After each set, the participants indicated their RPE by selecting a number on a scale ranging from 0 to 10. The scale corresponds to the effort level to perform the whole set, with 0 representing minimal effort and 10 indicating maximum effort. The number of repetitions, TUT, and RPE were recorded for each set and were utilized for statistical analysis. In addition, immediately after the last set, participants underwent another round of ultrasound imaging following the same procedures as before. The purpose was to acquire two new images and measure the CSA value once again. The relative difference
Statistical analysis
Initially a descriptive analysis of the data was performed. The normality and homogeneity of variances were verified using Shapiro-Wilk and Mauchly’s tests, respectively. The biceps brachii CSAs at pre- and post-performance of TFAS and TFLS were transformed into relative values (%) as indicator of muscle swelling \(\frac{(post - pre)}{pre} \times 100\).

The ANOVA found an interaction effect between factors (\(p < 0.001; \eta^2 = 0.33\)). TFAS performed greater number of repetitions in the first set compared to TFLS, and this last condition performed greater number of repetitions at the other sets. Intra-condition analysis showed TFAS performed more repetitions at the first set compared to other sets, while TFLS did not show differences across sets. Additionally, paired t-test showed the TFLS performed more repetitions summed across sets than TFAS (\(p = 0.011\)). Considering the volume-load, ANOVA found an interaction effect between factors (\(p < 0.001; \text{ES} = 0.33\)). TFAS showed greater volume-load in the first set compared to TFLS, and this last condition exhibited greater volume-load in the other sets. Intra-condition analysis showed TFAS lifted more volume-load at the first set compared to other sets, while TFLS did not show differences across sets. Additionally, paired t-test showed the TFLS lifted more volume-load summed across sets than TFAS (\(p = 0.010\)). Figure 3 illustrates the comparison of number of repetitions and volume-load between conditions.

RESULTS
The normality of the distribution was confirmed by the Shapiro-Wilk test, and all volunteers who started the collection data were able to finish within the specified time. For most volunteers, a recovery period until 96 hours was needed after the TFAS condition execution. However, some volunteers needed longer time and two extra days were given. For note, volunteers reported greater pain in the biceps after TFAS compared to TFLS. The inferential comparisons of the investigated variables are presented in the following sequence.

Repetitions and volume load
The ANOVA found an interaction effect between factors (\(p < 0.001; \text{ES} = 0.33\)). TFAS performed greater number of repetitions in the first set compared to TFLS, and this last condition performed greater number of repetitions at the other sets. Intra-condition analysis showed TFAS performed more repetitions at the first set compared to other sets, while TFLS did not show differences across sets. Additionally, paired t-test showed the TFLS performed more repetitions summed across sets than TFAS (\(p = 0.011\)). Considering the volume-load, ANOVA found an interaction effect between factors (\(p < 0.001; \text{ES} = 0.33\)). TFAS showed greater volume-load in the first set compared to TFLS, and this last condition exhibited greater volume-load in the other sets. Intra-condition analysis showed TFAS lifted more volume-load at the first set compared to other sets, while TFLS did not show differences across sets. Additionally, paired t-test showed the TFLS lifted more volume-load summed across sets than TFAS (\(p = 0.010\)). Figure 3 illustrates the comparison of number of repetitions and volume-load between conditions.

Figure 3. Comparison of repetitions (A1, A2) and volume-load (B1, B2) between conditions.
*Greater than TFAS. #greater than TFLS; a1: greater than other sets within TFAS; a2: greater than 3rd and 4th set within TFAS; \(p < 0.05\); TFAS: training to failure in all sets; TFLS: training to failure only at the last set.
Time under tension (TUT) and Rate perceived exertion (RPE)

According to ANOVA there was an interaction effect between factors (p < 0.001; n² = 0.33). Compared to TFLS, TFAS presented greater TUT at the 1st set, similar at the 2nd set, and lower at the 3rd and 4th sets. Intra-condition analysis showed TFAS spent greater TUT at the 1st set than other sets, 2nd set was greater than the 4th set, and this last set presented similar TUT than 3rd set. TFLS presented greater TUT at the last set compared to other sets, in which presented similar values between them. Additionally, paired t-test showed the TFAS spend more TUT summed across sets than TFLS (p = 0.005).

Considering the RPE, ANOVA found an interaction effect between factors (p = 0.001; ES = 0.33). TFAS showed greater RPE in the first three sets compared to TFLS and a similar value at the last set. Intra-condition analysis showed the TFAS exhibited lower RPE at the first set compared to other sets, while in TFLS the RPE was increasing across sets. Additionally, paired t-test showed the TFAS exhibited greater RPE summed across sets than TFLS (p < 0.001). Figure 4 illustrates the comparison of TUT and RPE between conditions.

Muscle swelling

Considering the muscle swelling results, there was no difference in the mean pre-CSA values between conditions (11.67 ± 3.30 cm² × 11.46 ± 3.50 cm²) before TFAS and TFLS, respectively. This suggests that the recovery time was sufficient to reduce the swelling in the brachial biceps muscle in such a way that the CSA values were in similar conditions before the execution of each condition. Considering muscle swelling comparison results, the paired t-test did not find any differences between the conditions (p = 0.229). Figure 5 illustrates the muscle swelling comparison.

DISCUSSION

The present studied aimed to compare the number of repetitions, TUT, RPE, volume-load and muscle swelling between TFAS and TFLS across four sets. Compared to TFLS, TFAS showed greater TUT, RPE number of repetitions and volume-load only at the initial sets. At the final sets, TFLS showed greater number of repetitions, volume-load, TUT and similar RPE. Considering the amount of all TUT, RPE, volume-load and number of repetitions across sets, TFLS showed greater number of repetitions and volume-load, while TFAS showed greater values for TUT and RPE. In the intra-condition analysis, it was observed that TFAS exhibited a greater number of repetitions, volume-load, and TUT in the first set compared to the subsequent sets. Additionally, TFAS showed lower RPE values in the first set compared to the other sets. On the other hand, TFLS demonstrated similar values across sets concerning the number of repetitions, TUT, and volume-load. However, RPE increased as the sets progressed. Furthermore, there was no significant
difference in biceps swelling between the TFLS and TFAS conditions. The muscle swelling after a resistance training session has been recognized as a potent hypertrophy inducer (13). While the complete mechanism is still to be determined, it is believed that during exercises heavily reliant on anaerobic metabolism, muscle swelling occurs due to changes in intracellular and extracellular water balance caused by heightened vascular permeability (25). Prior studies utilizing bioimpedance spectroscopy have indicated associations between changes in intracellular and extracellular water balance and variations in ion concentration, with metabolic changes observed in skeletal muscle cells post-exercise (26). As a consequence, muscle swelling can be considered an indirect indicator of the accumulation of metabolic stress (27), and consequently, it may play an important role for the hypertrophy process (28). Corroborating with this argumentation, previous studies have found a positive association between acute muscle swelling in the middle of a training period and hypertrophy (29, 30). Furthermore, this association exists even when muscle swelling is measured on the first day training and linked to hypertrophy (13).

In this sense, our results allow us to speculate that TFAS and TFLS could promote a similar hypertrophic response, as there were no differences in the muscle swelling response between conditions. However, it should be noted that the TFLS group showed higher volume-load and number of repetitions and, furthermore, lower RPE compared to TFAS, suggesting a lower effort throughout the repetitions to achieve a higher total mechanical demand. This response advocates that there was a lower level of physiological stress (a lower catabolic environment) to achieve a higher mechanical effort. Considering the inhibitory effect of elevated catabolic markers (such as cortisol), on muscle hypertrophy (31, 32), as well as the importance of mechanical stress for this response (33), it is conceivable that TFLS could offer advantages in promoting hypertrophy over TFAS after a period of training. However, protein catabolism markers were not assessed in the current study, thus limiting our conclusions to speculative inferences. Future research endeavors are warranted to explore this aspect further and provide a more comprehensive understanding of the topic.

Furthermore, Exner et al. (2023) (3) demonstrated that training without reaching muscle failure at both 30% and 60% of 1RM, with a controlled tempo of 1s for concentric and 1s for eccentric phases, using the dumbbell elbow flexion exercise across three sets, resulted in less swelling in the elbow flexor muscles compared to TFAS, regardless of the intensity. However, in this study, TFAS performed greater number of repetitions and longer TUT; factors relevant for muscle swelling increase (CSA acute increase) (27, 34). Due to having completed a higher number of repetitions, it is reasonable to expect that TFAS would have shown a higher volume-load and anaerobic energy supply than training with no failure. This increased metabolite production may have contributed to a greater muscle swelling compared to the other condition. Additionally, the longer TUT of TFAS may have led to a greater reduction in blood flow than the other type of training, contributing to greater fluid retention by the muscle fibers. In the present study, despite TFAS having a longer TUT, TFLS showed a higher number of repetitions, potentially balancing the mechanisms that promote muscle swelling.

Considering the number of repetitions, and volume-load it becomes apparent that the time to recover from fatigue caused by the first set to failure was inadequate within the 2-minute rest period. Consequently, fatigue accumulates across the sets, resulting in a decline in the number of repetitions and volume-load in each subsequent set. This significant reduction in repetitions performed until failure aligns with findings from other studies employing a two or even three minutes rest period (35, 36). Notably, fatigue accumulation triggers various physiological changes, including reductions in pH and energetic substrate availability (37). On the other hand, incorporating sets with repetitions distant from failure in the initial sets and reserving failure only for the last set could facilitate consistent repetitions and volume-load across all sets. This strategic approach of avoiding failure until the final set may enable individuals to achieve higher total repetitions and volume-loads, potentially fostering advantageous effects on muscle hypertrophy and performance improvements. However, further chronic research is imperative to ascertain the most effective training approach between conditions.

Considering the TUT, TFAS exhibited greater values in the first set compared to TFLS, which is expected since the latter condition performed fewer repetitions. However, in the last two sets, TFLS showed greater TUT than TFAS due to a higher number of repetitions performed. Nonetheless, it is noteworthy that despite the higher overall number of repetitions in TFLS, it did not result in a greater overall TUT. This discrepancy suggests that factors other than the number of repetitions may influence the TUT in these training protocols. According to Vieira et al. (38), when repetitions are performed to failure, the muscle action speed is decreased due to fatigue, what leading to an increase in TUT. In support of this rationale, the present study found that despite there being no difference in the number of repetitions across sets for TFLS, the TUT to perform the last set was greater than in the previous sets. This finding supports the rationale that fatigue may slow down the repetitions performed near failure. Indeed, the slower duration of the repetitions near failure across the sets, likely influenced the overall greater TUT for TFAS compared to TFLS.
The present study has several limitations that should be taken into consideration. The sample consisted of young adults trained in strength, which means the results cannot be extrapolated to other populations and conditions. Additionally, only two women participated in the study, and it would have been more appropriate to have a more balanced gender representation to avoid gender bias. Blood collection and analysis were not conducted, which could have provided valuable insights into metabolic or hormonal responses. Additionally, the experimental design did not allow for randomization between conditions, which may have led to a potential learning effect or increased discomfort tolerance in the TFLS situation. Moreover, the study only analyzed one muscle and one muscular region, and other muscles and regions could potentially respond differently.

In summary, the results of TFAS corroborate with previous findings concerning a reduction in the number of repetitions and volume-load and an increasing of RPE and TUT across sets when compared to sets not performed to failure (38). The results of the present study open up an avenue for investigation and training focusing on muscle responses, such as muscle hypertrophy, through the utilization of TFLS. This condition demonstrated the ability to lift a higher amount of weight, perform a greater number of repetitions, and elicit a similar muscle swelling response as TFAS while being associated with lower perceived stress. Although a chronic study is essential for a better understanding of the responses using the TFLS protocol, the results of this study allow for the hypothesis of a superior or similar hypertrophic response to TFAS, with potentially less effort required, which could be applicable to other exercises or reduce the need for recovery between training sessions.

CONCLUSIONS
TFAS and TFLS induced a similar muscle swelling response, with greater number of repetitions and volume-load values for TFLS, while TFAS exhibited higher TUT and RPE. Our results indicate that TFLS in the barbell preacher curl exercise leads to significant muscle swelling and allows for achieving a higher number of repetitions and volume-load with lower discomfort compared to TFAS. This suggests that TFLS may promote an anabolic stimulus similar to TFAS but in a less catabolic environment, which could enhance the hypertrophy response. This is particularly interesting for individuals seeking this adaptation.

FUNDINGS
None.

DATA AVAILABILITY
Data are available as online supplement (appendix 1-7).

CONTRIBUTIONS
GFP, FLL: conceptualization, writing – original draft, data collection. ABGB: data collection and image analysis; AKL: conceptualization, data collection. MECR, DGDS, LRM, TDLN: data collection. LIL: study conception. All authors: writing - review & editing.

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

REFERENCES


### Appendix 1. Individual cross sectional area values at at 50% of biceps brachii.

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Two images were taken, and the mean value was used for statistical analysis. TFAS: Training to Failure at all Sets; TFLS: Training to Failure only at the Last Set; SD: standard deviation.
Appendix 2. Maximum number of repetitions performed across 4 sets using TFAS and TFLS in the preacher curl exercise.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>TFAS 1 SET</th>
<th>TFAS 2 SET</th>
<th>TFAS 3 SET</th>
<th>TFAS 4 SET</th>
<th>Summed</th>
<th>TFLS 1 SET</th>
<th>TFLS 2 SET</th>
<th>TFLS 3 SET</th>
<th>TFLS 4 SET</th>
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<td>12</td>
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<td>51</td>
</tr>
<tr>
<td>De</td>
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<td>13</td>
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<td>9</td>
<td>47</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>50</td>
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<tr>
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<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>28</td>
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<tr>
<td>Th</td>
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<td>6</td>
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<td>9</td>
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<td>9</td>
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<td>9</td>
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<td>37.36</td>
<td>9.50</td>
<td>9.50</td>
<td>9.29</td>
<td>10.64</td>
<td>38.93</td>
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<td>1.28</td>
<td>1.56</td>
<td>5.24</td>
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<td>1.34</td>
<td>1.64</td>
<td>2.92</td>
<td>6.51</td>
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</table>

TFAS: Training to Failure at All Sets; TFLS: Training to Failure only at the Last Set. SD: standard deviation.
Appendix 3. Rate of perceived exertion across 4 sets using TFAS and TFLS in the preacher curl exercise.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Rate Perceived Exertion</th>
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</tr>
<tr>
<td>De</td>
<td>7</td>
</tr>
<tr>
<td>Ug</td>
<td>10</td>
</tr>
<tr>
<td>Th</td>
<td>9</td>
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<tr>
<td>FJ</td>
<td>9</td>
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<tr>
<td>Sl</td>
<td>7</td>
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<tr>
<td>Vil</td>
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<td>Fm</td>
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<td>Hp</td>
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<tr>
<td>Rs</td>
<td>10</td>
</tr>
<tr>
<td>Mv</td>
<td>9</td>
</tr>
<tr>
<td>Jv</td>
<td>10</td>
</tr>
<tr>
<td>Mw</td>
<td>10</td>
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<td>MEAN</td>
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<tr>
<td>±</td>
<td>1.28</td>
</tr>
</tbody>
</table>

TFAS: Training to Failure at All Sets; TFLS: Training to Failure only at the Last Set. SD: standard deviation.
Appendix 4. Time under tension across 4 sets using TFAS and TFLS in the preacher curl exercise.

| Volunteer | TFAS | | | | | TFLS | | | | | Sum |
|-----------|------|---|---|---|---|---|---|---|---|---|---|---|
|           | 1 SET | 2 SET | 3 SET | 4 SET | Summed | 1 SET | 2 SET | 3 SET | 4 SET | Sum |
| Qa        | 99    | 43   | 44   | 20   | 206    | 45    | 47    | 47    | 65    | 204 |
| De        | 61    | 59   | 36   | 42   | 198    | 47    | 48    | 43    | 65    | 203 |
| Ug        | 51    | 30   | 24   | 21   | 126    | 26    | 26    | 29    | 34    | 115 |
| Th        | 73    | 46   | 33   | 27   | 179    | 35    | 41    | 28    | 58    | 162 |
| FJ        | 64    | 33   | 22   | 25   | 144    | 33    | 35    | 35    | 31    | 134 |
| SI        | 94    | 27   | 25   | 19   | 165    | 35    | 36    | 35    | 52    | 158 |
| VI        | 78    | 38   | 36   | 31   | 183    | 40    | 39    | 38    | 51    | 168 |
| Fm        | 57    | 39   | 26   | 27   | 149    | 31    | 35    | 34    | 43    | 143 |
| Bm        | 63    | 36   | 32   | 34   | 165    | 38    | 38    | 35    | 43    | 154 |
| Hp        | 63    | 41   | 32   | 24   | 160    | 29    | 32    | 34    | 34    | 129 |
| Rs        | 69    | 43   | 40   | 32   | 184    | 38    | 38    | 40    | 77    | 193 |
| Mv        | 94    | 33   | 32   | 26   | 185    | 36    | 36    | 40    | 47    | 159 |
| Jv        | 76    | 38   | 30   | 21   | 165    | 38    | 39    | 38    | 35    | 150 |
| Mw        | 75    | 32   | 32   | 39   | 178    | 32    | 35    | 35    | 76    | 178 |
| MEAN      | 72.64 | 38.43 | 31.71 | 27.71 | 170.50 | 35.93 | 37.50 | 36.50 | 50.79 | 160.71 |
| ±         | 14.61 | 8.03  | 6.16  | 7.08 | 21.55 | 5.76  | 5.57  | 5.05  | 15.49 | 26.82 |

TFAS: Training to Failure at All Sets; TFLS: Training to Failure only at the Last Set. SD: standard deviation.
Appendix 5. Volume load across 4 sets using TFAS and TFLS in the preacher curl exercise.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>TFAS 1 SET</th>
<th>TFAS 2 SET</th>
<th>TFAS 3 SET</th>
<th>TFAS 4 SET</th>
<th>Summed</th>
<th>TFLS 1 SET</th>
<th>TFLS 2 SET</th>
<th>TFLS 3 SET</th>
<th>TFLS 4 SET</th>
<th>Sum</th>
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<tbody>
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<td>88.01</td>
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<td>117.35</td>
<td>117.35</td>
<td>117.35</td>
<td>146.69</td>
<td>498.74</td>
</tr>
<tr>
<td>De</td>
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<td>305.12</td>
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<td>281.65</td>
<td>281.65</td>
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<td>256.86</td>
<td>256.86</td>
<td>256.86</td>
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<td>265.55</td>
<td>265.55</td>
<td>265.55</td>
<td>1003.18</td>
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<td>227.3</td>
<td>227.3</td>
<td>858.69</td>
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<td>90.55</td>
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<td>162.98</td>
<td>162.98</td>
<td>162.98</td>
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<td>136.37</td>
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<td>194.81</td>
<td>194.81</td>
<td>194.81</td>
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<td>225.4</td>
<td>225.4</td>
<td>225.4</td>
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<td>182.38</td>
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<td>234.49</td>
<td>234.49</td>
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<td>366.8</td>
<td>366.8</td>
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TFAS: Training to Failure at All Sets; TFLS: Training to Failure only at the Last Set. SD: standard deviation.
Appendix 6. Maximum repetition test result in the preacher curl exercise.

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<th>Volunteer</th>
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</table>

SD: Standard deviation.
**Appendix 7. Participants' profile.**

<table>
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<tr>
<th>Volunteer</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Time of experience (months)</th>
<th>Body fat (%)</th>
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SD: Standard deviation.
INTRODUCTION

Use of absorbable sutures in hand flexor tendon repair could not be better motivated than it was done by Boyes who, not long before the era of synthetic absorbable materials, analogized pull-out wire removal with disintegration of a hypothetical suture at three weeks after tendon repair (1). Boyes’ vision began to realize itself in the early 1970s with the emergence of the first synthetic absorbable suture composed of polyglycolic acid (PGA) (2-4).

Currently, both non-absorbable and slow absorbable (PDS and Maxon) sutures are used for flexor tenorrhaphies with varying prevalence (5-7). Traditionally, absorbable sutures have not been the surgeons’ first choice in tendon surgery, probably because of fear of progressive loss of suture strength that can endanger the repair during postoperative mobilization. This tendency, however, seems to have been changing in favor of slow-absorbable threads (5, 8). The available clinical reports indicate that flexor tendon repair with slow-absorbable PDS (polydioxanone (9, 10)) followed by both passive (11) and active (7, 12, 13) postoperative mobilization would not produce a significant increase in rupture rate. Similar results can be achieved with Maxon (copolymer of glycolic acid and trimethylene carbonate (7, 9, 10)), which has an intermediate rate of biodegradation but is close to PDS in retaining tensile strength (14).
Experimentally, suitability of absorbable materials for flexor tendon repair has been explored by a number of researchers (14-22). However, tenorrhaphy with fast-absorbable sutures has been little studied. The three available experimental reports on tensile strength of tendons sutured with fast-absorbable PGA-based threads present contradictory results. Minta (23) did not observe any difference in mechanical performance between silk and PGA (Dexon (9, 10)) in Achilles tenorrhaphies of rabbits at 1, 2, 3, and 6 weeks. In an unpublished study, Ketchum (24) detected that, at 4 weeks, tendons sutured with PGA were weaker by half than tendons joined with polyester. Greulich et al. (25) used 4-0 and 5-0 Vicryl (polyglactin 910, copolymer formed from nine parts of PGA and one part of polylactic acid (9, 10, 26, 27)) sutures that were employed for Tsuge repair of rabbit tendons. This study focused on a tenorrhaphy technique, rather than on suture performance. The article reported average repair gapping of 2-3 mm after 6 weeks of immobilization and did not mention any ruptures. The authors further described their clinical application of looped Vicryl suture and in about 60 cases of Tsuge repairs of hand flexor and extensor tendons. Unfortunately, no statistical details were given concerning several repair failures that were encountered. The only other available report on clinical use of Vicryl in hand tendon surgery involved extensor tenorrhaphies; no ruptures were observed (28). Vicryl has also been clinically used for Achilles tendon repairs (29-32); only few re-ruptures have been reported (29). Vicryl is one of the few available braided absorbable sutures. Breakdown of polyglactin 910 occurs in aqueous environment by hydrolysis and does not require enzymes (33), as is the case with all synthetic absorbable materials (9). Coated Vicryl sutures lose their strength in a linear fashion (34-36). They retain about 50% of their original strength at approximately three weeks and only about 5% at five weeks after incubation in rat soft tissue (36). Similarly to all multifilament sutures, Vicryl has a high elastic modulus and is resistant to elongation (37-39). Notably, initial load to failure of Vicryl has been found to be higher than that of other absorbable sutures and of non-absorbable Ethibond (35) that is recommended for flexor tendon repair (14, 40).

While, differently from Vicryl, PDS and Maxon retain their initial strength at three weeks after incubation in a Ringer-type solution (35), these sutures have a disadvantage of being monofilaments, which may be associated with their lesser anchoring capacity as compared with braided sutures (39, 41-43). Vicryl is soft and fits well in with the tissue; braided texture prevents sliding of the suture loops through tissue (25). Vicryl has a high knot security (44), probably due to relatively high friction between threads (45), which may be more important than contribution of this property to increase in tendon gliding resistance (46, 47).

The below report retrospectively analyses clinical results of flexor tendon repair with Vicryl sutures with a particular reference to postoperative rupture incidence in a limited series of patients.

**MATERIALS AND METHODS**

From 1999 to 2001, Vicryl sutures were used by the author to repair 122 flexor and 45 extensor tendons in 51 patients with predominantly complex hand injuries. Bioethics committees and relevant institutional regulations did not exist at the time in the country where the current study was performed. The author certifies that all other applicable institutional and governmental regulations concerning the ethical use of human subjects were followed during the course of this study. All cases of digital flexor tenorrhaphy ruptures and flexor repairs with a minimum follow-up of 10 weeks were included in the analysis. In regard to the possibility of progressive repair deterioration, this follow-up period was considered as sufficient, because ruptures mostly occur within the first five weeks (48), and the achieved range of active motion rarely decreases with time (49). Cases with articular fractures, multiple level amputations, and amputations in zones 1, 2, T1, and T2 were excluded. These injuries usually lead to joint stiffness and compromise functional recovery because of injury to delicate extensor mechanism.

Seventeen flexor digitorum profundus (FDP) and four flexor pollicis longus (FPL) tenorrhaphies in 12 patients were available for evaluation. A summary of the cases is presented in table I. Fifteen lacerations of the FDP tendons were associated with complete transection of the flexor digitorum superficialis (FDS) tendon, seven of which were repaired (cases 1, 5, and 8). Six flexor tenorrhaphies were accompanied by corresponding extensor tendon repairs (cases 1, 2, and 3). Depending on the tendon diameter, tenorrhaphy involved a two-strand Kirchmayr-Kessler-type suture with 4-0, 3-0 or 2-0 Vicryl and a circumferential running suture with the same or a separate absorbable thread. A 5-0 and 6-0 Monocryl (a fast-absorbable monofilament (9)) was used as a peripheral suture in cases 9 and 10, respectively. The extensor tendons were also sutured with Vicryl. The patients were taught to perform self-controlled early postoperative passive flexion and active extension. If extensor tendons were injured, only moderate passive motion was allowed. Active motion was begun at 3 weeks. In one patient with severed extensor tendons, active mobilization was initiated at four weeks (case 3). Most patients were from distant regions and frequent supervision of postoperative rehabilitation was not possible. Functional results were rated according to Strickland’s rating principles as excellent, good, fair, or poor (50). Ruptures yielded poor scores. A sudden decrease in the range of motion was rated as a rupture.
The obtained data were statistically compared with a corresponding hypothetical set of repair results consisting of only satisfactory (excellent and good) outcomes. One-tailed Fisher’s exact test was employed. The significance value was set at $p < 0.05$.

### RESULTS

All sharp lesions resulted in satisfactory outcomes, most of which were achieved after tenorrhaphies proximal to the metacarpophalangeal joints (tables I,II and figures 1,2). Unsatisfactory outcomes were observed only in patients with severe complex injuries involving fractures and nerve and artery lesions (table I). The unsatisfactory results included two ruptures (9.5%), both of which occurred in zone 2. In case 9, the FDP tendon repair failed during active flexion; the FDS tendon repair remained unaffected. In case 8, both the FDP and FDS tendon repairs ruptured during passive extension. Four patients underwent re-explorations because of a rupture (case 8) and adhesions (cases 2, 3, and 7).

A comparative increase in the rupture rate was statistically insignificant. The increase in both the proportion of unsatisfactory results after FDP tenorrhaphies and the overall proportion of unsatisfactory results was statistically significant (table II).

### DISCUSSION

The presented series of flexor tendon repairs resulted in a significant proportion of unsatisfactory results after the FDP tenorrhaphies (table II). The unsatisfactory results may be associated not only with suture material but also with complex injuries involving irregular wounds and fractures (table I). Such injuries are usually contaminated and do not allow intensive postoperative mobilization. Because of these factors, limited number of repairs, and varying levels of injury, it is not possible to compare the achieved functional results with those of standard flexor tendon repair studies, which mostly include sharp lesions. However, the proportion of ruptures, which appears to be statistically insignificant, carries a comparative value. This can be further demonstrated by the following

---

Table I. Summary of cases and outcomes.

<table>
<thead>
<tr>
<th>Case</th>
<th>Injury type/ Associated injuries</th>
<th>Tendon(^a)</th>
<th>Zone</th>
<th>Follow-up</th>
<th>Outcome(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crush-avulsion/ Incomplete amputation</td>
<td>FPL</td>
<td>5</td>
<td>12 mo</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Crush-avulsion/ Incomplete amputation</td>
<td>FDP 2</td>
<td>5</td>
<td>12 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td></td>
<td>Crush-avulsion/ Incomplete amputation</td>
<td>FDP 3</td>
<td>5</td>
<td>12 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>Ragged/ Complete amputation</td>
<td>FDP 4</td>
<td>3</td>
<td>11 mo</td>
<td>Poor</td>
</tr>
<tr>
<td>3</td>
<td>Ragged/ Complete amputation</td>
<td>FPL</td>
<td>T3</td>
<td>19 mo</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Ragged/ Complete amputation</td>
<td>FDP 2</td>
<td>3</td>
<td>19 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 4</td>
<td>8 mo</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FDP 5</td>
<td>3</td>
<td>18 mo</td>
<td>Poor</td>
</tr>
<tr>
<td>4</td>
<td>Ragged/ B, T, A, N</td>
<td>FPL</td>
<td>T3</td>
<td>11 mo</td>
<td>Fair</td>
</tr>
<tr>
<td>5</td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 2</td>
<td>4</td>
<td>11 mo</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 3</td>
<td>4</td>
<td>11 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td></td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 4</td>
<td>4</td>
<td>11 mo</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 5</td>
<td>4</td>
<td>11 mo</td>
<td>Fair</td>
</tr>
<tr>
<td>7</td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 3</td>
<td>2</td>
<td>8.5 mo</td>
<td>Poor</td>
</tr>
<tr>
<td>8</td>
<td>Ragged/ B, T, A, N</td>
<td>FDP 4</td>
<td>2</td>
<td>8.5 mo</td>
<td>Poor</td>
</tr>
<tr>
<td>9</td>
<td>Blunt/ T, A, N</td>
<td>FDP 3</td>
<td>2</td>
<td>3.5 wk</td>
<td>Rupture</td>
</tr>
<tr>
<td>10</td>
<td>Sharp/ T</td>
<td>FPL</td>
<td>4</td>
<td>2.5 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td>11</td>
<td>Sharp/ None</td>
<td>FDP 3</td>
<td>4</td>
<td>12 mo</td>
<td>Excellent</td>
</tr>
<tr>
<td>12</td>
<td>Sharp/ T</td>
<td>FDP 4</td>
<td>2</td>
<td>13 mo</td>
<td>Good</td>
</tr>
</tbody>
</table>

\(^a\)Numbers indicate digit; \(^b\)according to Strickland’s rating principals (49); B: bone; T: tendon other than the evaluated one; A: artery; N: nerve.
**Table II.** Distribution of the outcomes according to the location of injury and the results of a statistical comparison with ideal outcomes of a corresponding hypothetical series of repairs.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FPL (zones T3, 4, 5)</th>
<th>FDP (zone 2)</th>
<th>FDP (zones 3, 4, 5)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Good</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Fair</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>No ruptures / ruptures</td>
<td>4 / 0</td>
<td>3 / 2</td>
<td>12 / 0</td>
<td>19 / 21</td>
</tr>
<tr>
<td>Satisfactorya / unsatisfactoryb</td>
<td>3 / 1</td>
<td>1 / 4</td>
<td>8 / 4</td>
<td>12 / 9</td>
</tr>
<tr>
<td>Ideal ratio of satisfactory to unsatisfactory</td>
<td>4 / 0</td>
<td>5 / 0</td>
<td>12 / 0</td>
<td>21 / 0</td>
</tr>
</tbody>
</table>

*aSatisfactory = Excellent + Good; bUnsatisfactory = Fair + Poor + Ruptures; 1p = 0.22; 2p = 0.24; 3p = 0.5; 4p = 0.024; 5p = 0.047; 6p = 0.0007.*

Figure 1. A case of flexor tendon repair with a 4-0 Vicryl in a complex crush-avulsion injury (case 1).

Preoperative view (A); revision amputation of the little finger was performed. Active extension (B) and active flexion (C) of interphalangeal joints at 12 months. The lack of active flexion at the metacarpophalangeal joints (due to incomplete intrinsic muscle recovery) was not taken into account in accordance with Strickland’s rating principals (50).

Figure 2. A case of flexor tendon repair with a 4-0 Vicryl in a complex circular saw injury (case 3).

Preoperative view (A); the index finger was replanted in the middle finger position. The FDP tendon of the ring finger was repaired with a 3-cm interposition graft. Tenolysis of the grafted tendon was performed at 8 months because of a 6-cm flexion deficit. The graft was inadvertently divided in zone 2 during the procedure. Subsequent one stage grafting also failed. The patient refused further re-exploration. Active extension of the digits (B) and active flexion of the fingers (C) and of the thumb (D) at 19 months.

logic, which is designed to clarify whether weakening of the suture material might have caused the ruptures.

Strickland (40, 51) has crystallized data of a large number of studies into conservative working numbers in grams for the forces exerted on flexor tendons and for the strength of tendon repairs with non-absorbable sutures at various postoperative periods. To avoid underestimation of the strength of the two-strand repair in the present study, Strickland’s conservative numbers for six-strand repair could be used. These numbers (as shown in Strickland’s relevant table (40)) are approximately 5,400, 4,000, and 6,480 grams immediate-
ly, at three weeks, and at six weeks after repair, respectively. Thus, the approximate gain in strength due to healing at six weeks is 1,080 grams (6,480 minus 5,400). It follows that the estimated strength of four strand Vicryl 2-0 tenorrhaphies at six weeks is about 1,080 grams, because Vicryl 2-0 sutures lose all their holding strength at six weeks after incubation in rat soft tissue (36), as well as in synovial fluid (52). According to Strickland, light grip at six weeks generates a tensile stress of about 1,500 grams within a tendon repair (40, 51), which exceeds almost by one-third the estimated Vicryl repair strength (Figure 3). Obviously, this difference would be even larger if Strickland’s conservative number for two-strand repair were used. It follows that at six weeks all tendon repairs in the presented cases were destined for inevitable failure. In the light of this conclusion, the obtained incidence of ruptures can be regarded as low, and it is doubtful that the cause of the tenorrhaphy failures is due to the progressive weakening of the suture material. This echoes with the paradox of the pull-out wire repair: tendons usually do not rupture after the wire is removed at three weeks (1).

The following factors can account for this conundrum:

1. Muscle weakness after prolonged limited activity and patient’s caution in performing hand motion may prevent overload of the repair site. This may also be supported by the continuing successful use of absorbable sutures in Achilles tendon repairs (53), including absorbable bone anchors (54). Notably, most ruptures occur because of patient’s noncompliance (48). This accounts well for the rupture that occurred during passive extension (Table I, case 8), which is not allowed during early mobilization.

2. Adhesion formation may reduce the risk of rupture. Tenorrhaphies seem to fail after the adhesions are disrupted (55). Adhesions immobilize the tendon and thus protect the juncture from overload. However, adhesions may also have an opposite effect if they hold only the tendon part distal to the juncture and thus produce overload of the repair during active motion.

3. Hypotrophic tendon milieu may slow down degradation of sutures. Unfortunately, no studies could be found to explore dynamics of Vicryl degradation in tendons.

4. Polyglycolic acid may have properties that promote tendon regeneration. PGA-based scaffolds have successfully been used in tendon engineering (56-60). PGA degradation products (glycolic acid) may produce cellular activation, as has been shown with macrophages (61). Relevant tendon regeneration-targeted research could provide more definitive information.

5. Mobilization increases tendon repair strength. This phenomenon has been demonstrated by a number of researchers (40, 62) and has a substantial basic research support (63, 64). Most of this kind of studies appears to have involved non-absorbable suture material (65-68). Suture technique does not seem to have a considerable effect on the beneficial effects of flexor tendon mobilization (68). There is a lack of comparative studies on the effects of mobilization after tendon repair with non-absorbable and absorbable tendon suture. However, specifically relevant to the current report is an elegant study by Cao et al. (58) who demonstrated tensile strength increase in loaded engineered chicken tendons that were grown on scaffolds of unwoven PGA fibers seeded with tenocytes. Longitudinal load of such scaffolds also appeared to contribute to development of normal tendon structure (56-58). Furthermore, similar results have been achieved with PGA fibers seeded with human dermal fibroblasts (69) and adipose derived stem cells (70). Conjecturally, Vicryl within a tendon may be spontaneously seeded with tenocytes and thus eventually produce a neo-tendon and strengthen repair. Experimental confirmation of this supposition would imply that tendon repair with Vicryl may have a significant advantage over other synthetic sutures that lack a PGA component. It is noteworthy that rupture of suture material itself may occur not only because of biodegradation in the tissue but also because of possible suture weakening during storage. The author’s experience is that even non-absorbable sutures, before their expiry time, may be of inadequate strength. A good practice is to test the suture strength manually.

Table I

<table>
<thead>
<tr>
<th>Case</th>
<th>Suture Type</th>
<th>Repair Site</th>
<th>Rupture Time (weeks)</th>
<th>Rupture Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethibond</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Vicryl</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Ethibond</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Vicryl</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Ethibond</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Vicryl</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Ethibond</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Vicryl</td>
<td>Flexor</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 3. Approximate dynamics of six-strand flexor tendon repair strength versus grip strength.

The incremental loss of strength of the repair with Vicryl suture was arithmetically calculated by considering the gain in Ethibond repair strength as a tendon healing strength (see also the text). Ethibond is given as a representative of non-absorbable sutures. Adapted and modified from Strickland (40, 51).
An important advantage of absorbable sutures is that after dissolution they cease to incubate microbes (71), which may account for relatively high resistance to infection of absorbable threads (72). Notably, glycolic acid has been observed to exhibit bacteriostatic and bactericidal activity in vivo and in vitro (73). Fibrous structure of Vicryl allows its impregnation with therapeutic substances, which can be released in the course of suture degradation (59).

Inflammatory reaction to a absorbable sutures in tendons may be a matter of concern (14, 18, 20, 74). However, because of relatively quick degradation, inflammatory response to absorbable sutures is shorter than that to non-absorbable ones. This may be one of the reasons for varying inflammatory molecular-level effects within different absorbable (74) and non-absorbable sutures (75).

In general, PGA-based materials have a good biocompatibility (78, 79) because their degradation primarily occurs by hydrolysis rather than via cellular processes. Functional outcomes of tendon junctions may be more dependent on suture technique and postoperative rehabilitation than on suture material: silk, a natural material of poor biocompatibility (80), not long ago was regarded as a good suture, use which good results of tendon repair were achieved (81).

A considerable weakness of the current study is the limited number of cases, which has been attempted to overcome with a novel improvised statistical approach. Inclusion of anatomo-functionally different flexor tendons, different levels of injury, varying size of sutures, and purely controlled mobilization are additional drawbacks. Hopefully, these limitations will encourage further relevant well-designed studies.

CONCLUSIONS
Very few studies have explored tendon repair with fast-absorbable sutures. The current report indicates that Vicryl suture may withstand moderate early postoperative mobilization after hand flexor tendon repair. However, the high overall fraction of unsatisfactory results, even though this may be due to the complexity of injuries, suggests cautiousness in tenorrhaphies with fast-absorbable sutures. Although theoretical considerations suggest that fast-absorbable sutures may be beneficial in hand tendon surgery, more experimental research is needed to clarify the relationship between temporal dynamics of tendon repair and that of tensile strength of fast-absorbable sutures.

FUNDINGS
None.

DATA AVAILABILITY
Data are provided in the current study.

CONTRIBUTIONS
VM contributed entirely to this work.

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The presented cases were managed by the author from 1999 till 2001 at Vilnius University Hospital.

CONFLICT OF INTERESTS
The author declares that he has no conflict of interests.

REFERENCES


Absorbable Suture Tenorrhaphy


To the Editor,

biomechanically proven superiority of modern multistrand repairs has led to some distancing from the classical two-strand techniques (1). The essential requirements for the ideal flexor tendon repair include easy atraumatic suture placement and sufficient strength of the tendon anastomosis to allow early mobilization and to prevent gapping (2). In multistrand repairs, these prerequisites are somewhat contradictory, because repair complexity and traumatization increase with the number of the core sutures, i.e., with repair strength (3). Another drawback of multistrand repairs is that it is difficult to equalize tension between separate core sutures. Differential loading of individual strands weakens the repair (2) and may produce gapping. Furthermore, an excessive amount of foreign material within the tendon is undesirable.

The main advantage of the multistrand repairs is that they have a larger number of anchor points (3) compared to two-strand techniques. Betz et al. have shown that integration of the latter feature into Zechner two-strand repair (which includes locking loops of Kirchmayr-Kessler-type repairs (figure 1A)) by insertion of additional blocking loops significantly strengthens the tendon juncture (4). A problem with this modification may be that the suture loops are positioned on the same axis (figure 1B), which suggests that, differently from multistrand repairs, the same fiber bundles of the tendon are loaded, because tendons are comprised of longitudinally oriented collagen fibers (5, 6). This may contribute to damage of the anchored fiber bundles and to consequent repair gapping. This drawback of two-strand multilevel repairs could be eliminated by inserting the loop pairs in different transversal and/or sagittal planes. Possible designs of enhanced two-strand repairs are shown in figure 1C,D,E. Central (and/or volar) placement of the second pair of loops (figure 1C,E) may be technically simpler than outer (and/or dorsal) placement (figure 1D), because it is usually more difficult to manipulate the tendon at levels further from the juncture site.

Most of the available tendon repair techniques have been clinically used without preceding experimental testing, and biomechanical analyses of tendon repairs largely come without explanation by relevant physics. The laws of mechanics (7) suggest that any additional loop-strand pair results in corresponding spatial distribution of tension within the tendon (figure 2). However, this rule may not apply if additional loops are inserted on the same fiber bundle, because the loop that is closer to the tendon junction prevents transmission of force to the next loop. Why then did longitudinal arrangement of locking loops in the experiment by Betz et al. strengthen the two-strand tendon repairs (4)? The reason may be that there are multiple interconnections between tendon fibers (5, 6) and that during repair only a part of the same fibers is anchored along the same line of suture. Also, when
one of the loops fails, the other loop can prevent gapping. These considerations justify multidimensional placement of suture loops. Histological analysis of structure of tendon parts directly unaffected by suturing may be of value for definitive explanation of tendon repair physics and results of biomechanical testing. The mechanistic concept of multilevel multiplane two-strand repairs encourages relevant experimentation, which may result in a considerable clinical alternative to current multistrand techniques.

FUNDINGS
None.

DATA AVAILABILITY
N/A.

CONTRIBUTIONS
VM contributed entirely to this work.

CONFLICT OF INTERESTS
The author declares that he has no conflict of interests.

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