The morpho-mechanical and neuromuscular properties of the Triceps surae muscle-tendon unit in individuals with Achilles tendinopathy and the effects of exercise

By

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ABBREVIATIONS

Acronym	Definition
AT	Achilles tendinopathy
AP	Antero-posterior
BNBRL	British National Bibliography for Report Literature
CMEP	Cervicomedullary Magnetic Stimulation
CNS	Central nervous system
CON	Concentric
CONSORT	Consolidate Standards of Reporting Trials
COV torque	Coefficient of variation of torque
COVisi	Coefficient of variation of the interspike interval
CSA	Cross-sectional area
CST	Cumulative spike train
ECC	Eccentric
EMD	Electromechanical delay
EMG	Electromyography
FAAM	Foot and Ankle Ability Measure
GRADE	Grading of Recommendation, Assessment, Development, and Evaluation
H/M	H-reflex/ M-wave
HD-sEMG	High-density surface electromyography
IAT	Insertional Achilles tendinopathy
ICC	Intraclass correlation coefficient
IPAQ	International Physical Activity Questionnaire
IPAQ-SF	International Physical Activity Questionnaire short-form
LG	Lateral gastrocnemius

MEP	Motor Evoked Potential
MESH	Medical Subject Headings
MG	Medial gastrocnemius
MRI	Magnetic Resonance Imaging
MTJ	Myotendinous junction
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
MVIC	Maximal voluntary isometric contraction
MVC	Maximal voluntary contraction
NIAT	Non-insertional Achilles tendinopathy
NMD	Neuromechanical delay
NOS	Newcastle-Ottawa Scale
NRIS	Non-Randomised Interventional Study
NRS	Numerical Rating Scale
PCS	Pain Catastrophising Scale
PICOS	Population, Intervention, Comparison, Outcomes and Study
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
RCT	Randomised Controlled Trial
ROI	Region of interest
SO	Soleus
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SWE	Shear-wave elastography
TSK	Tampa Scale of Kinesiophobia
TMS	Transcranial Magnetic Stimulation
V/M	V-wave/ M-wave

VAS	Visual Analog Scale
VISA-A	Victorian Institute of Sport Assessment-Achilles

ABSTRACT

Achilles tendinopathy (AT), whether non-insertional (NIAT) or insertional (IAT), is a debilitating overuse injury causing significant functional impairments in both athletic and general populations. However, these are two distinct disorders with different underlying pathophysiology and treatments. NIAT is the most prevalent tendinopathy in the lower limb. Nevertheless, its aetiology remains unclear, and it is likely to be multifactorial. This thesis consists of 6 Chapters. *Chapter 1* provides an overview of tendon structure and function, with a particular emphasis on the Achilles tendon. Subsequently, the chapter explores relevant topics about Achilles tendinopathy and the assessment of the morpho-mechanical and neuromuscular properties of the muscle-tendon unit. *Chapter 2* presents the results of a systematic review showing that individuals with NIAT and IAT exhibit neuromechanical changes indicated by differences in the triceps surae muscle's neuromuscular properties and the Achilles tendon's morpho-mechanical properties. Additionally, exercise-induced mechanical tendon loading showed inconsistent results in modifying the morpho-mechanical properties of the Achilles tendon in individuals with NIAT. *Chapter 3* revealed the results of an observational study showing a contraction-intensity dependent relationship between motor unit firing rate properties of the triceps surae muscles and the morpho-mechanical parameters of the Achilles tendon in asymptomatic individuals. One of the most relevant findings is that individuals with increased tendon stiffness showed a lower triceps surae muscle discharge rate at low target forces. *Chapter 4* exhibited the outcomes of an observational study demonstrating that individuals with NIAT have load-dependent changes in triceps surae motor unit firing rate properties. Specifically, an increase in the discharge rate and a decrease in the derecruitment threshold of the lateral gastrocnemius muscle were observed in individuals with NIAT compared to controls during isometric plantarflexion contractions at high forces. Furthermore, it was observed that the contribution of the lateral gastrocnemius to the net plantarflexion force is altered in individuals with NIAT. *Chapter 5* presents the results of a randomised controlled trial showing that a 6-week torque feedback intervention based on eccentric or concentric exercises induces load-dependent changes in the triceps surae motor unit firing properties in individuals with NIAT. Additionally, these exercise

interventions effectively decreased pain, improved function, and increased tendon stiffness in individuals with NIAT. Moreover, our findings indicate that both exercise interventions produced similar triceps surae motor unit firing rate adaptations compared to controls, suggesting a normalisation of these properties after the intervention in individuals with NIAT. Finally, *Chapter 6* highlights the most relevant findings of this thesis, explores the limitations of the studies and suggests possible future investigations.

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LIST OF PAPERS AND CONFERENCE ABSTRACTS

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Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Load-dependent changes in triceps surae motor unit firing properties in individuals with non-insertional Achilles tendinopathy.

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Eccentric and concentric torque feedback training induces similar improvements in pain, function, and adjustments in triceps surae motor unit firing rate properties in individuals with non-insertional Achilles tendinopathy. A randomised controlled trial.

Conference Presentations:

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Morphological and mechanical properties of the Achilles tendon and their relationship with triceps surae motor unit firing properties during isometric contractions. International Society of Electrophysiology and Kinesiology (ISEK). Quebec, Canada, June 22-25, 2022.

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Future Presentations:

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CHAPTER 1

General Introduction

1.1 Overview of tendons

Tendons are a distinctive type of connective tissue that play a critical role in the musculoskeletal system by primarily connecting muscles to bone (Nourissat et al., 2015); however, occasionally can extend into the muscles or connect muscle bellies (Benjamin et al., 2008). Tendons allow the transmission of muscle forces to the skeletal system, a process mediated by the extracellular matrix components within their hierarchical structure (Zhang et al., 2022, Chen et al., 2015) **(Figure 1)**. This structure includes several levels: the first level comprise fibrils, which are rod-like collagen molecules (Wang, 2006); the second level consists of fibres, which are a formed by several fibrils; the third level includes the fascicle, which are bundles of fibers enclosed by a layer of connective tissue referred as endotenon (Kastelic et al., 1978); and the fourth level correspond to a group of fascicles surrounded by a connective tissue sheath known as epitenon, which is particularly important since contains the lymphatic, vascular and nerve supply to the tendon (Zhang et al., 2022, Kastelic et al., 1978). Most tendons are also enclosed by a loose alveolar connective tissue known as paratenon or synovial sheath, which facilitates the tendon's movement by reducing the friction with the adjacent tissues (Amiel et al., 1984, Kastelic et al., 1978).

The macrostructure varies depending on its location in the body, with tendon geometries ranging from long and cylindrical to short and flat (Diamant et al., 1972, Yahia and Drouin, 1989). Typically, tendons that primarily transmit uniaxial tensile loads are more cylindrical, with the collagen fibers aligned parallel to the direction of the applied forces (Gomes et al., 2015). In contrast, tendons exposed to more complex patterns of loads exhibit variations in collagen fiber alignment (Gomes et al., 2015). Overall, three different regions have been identified in tendons: the tendon-bone junction, the muscle-tendon junction and the main body. The tendon-bone junction, known as the enthesis, can withstand tensile, compressive, and shear forces (Wang, 2006). The muscle-tendon junction, also called myotendinous junction (MTJ), enables the transmission of the tensile forces produced by contractile proteins (actin and myosin) of muscles fibres to the tendon collagen fibers (Michna, 1983, Tidball, 1984, Tidball, 1991). The main body of the tendon, as previously described, provides the primary structure for force transmission. Studies have shown that the tensile forces applied to the enthesis can be fourfold greater than those applied to the main body (McGonagle et al., 2003) and that the MTJ represents the most vulnerable point of the tendon (Garrett, 1990).

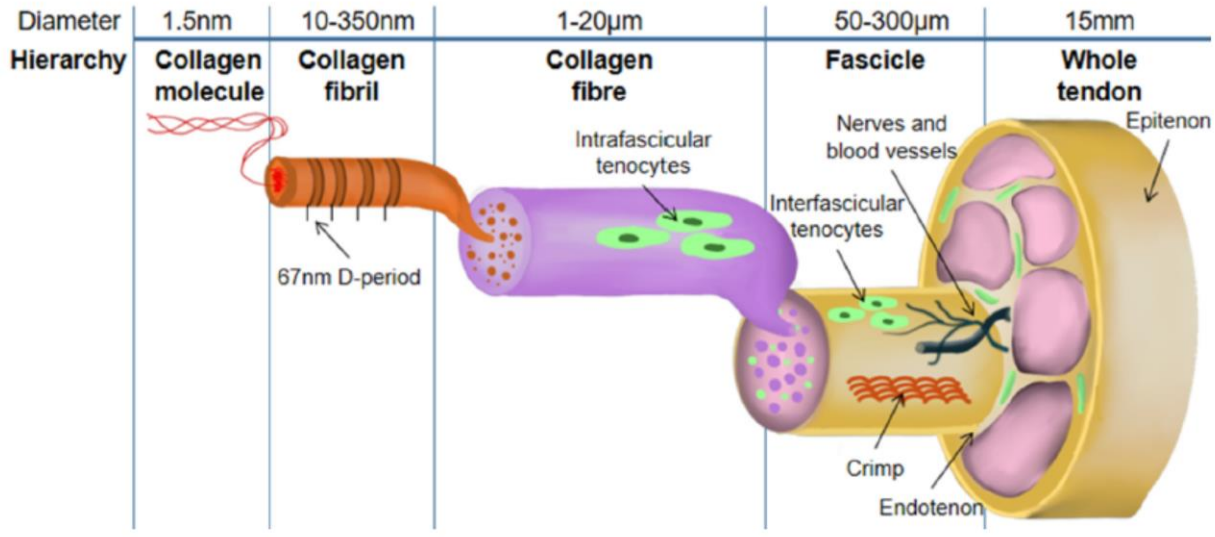

Figure 1. Schematic diagram of the structural hierarchy of tendons.
Adapted from Zhang et al. 2021.

Tendons are composed of collagens, proteoglycans, glycoproteins, water, and cells, with collagen type I constituting about 60% of the tendon's dry weight and approximately 95% of the total collagen (Evans and Barbenel, 1975, Riley et al., 1994a). Collagen III and V represents the remaining 5%, with traces of other types of collagen (Fukuta et al., 1998). Tendons also contain proteoglycans in small portions, including aggrecan and decorin (Vogel and Heinegård, 1985), among others. It has been determined that the proteoglycan content differs depending on the tendon's region and is mostly influenced by the mechanical load conditions applied (e.g., tension vs compression) (Berenson et al., 1996, Riley et al., 1994b). Furthermore, tendons contain several glycoproteins, such as fibronectin, laminin, thrombospondin, tenasin-C, and elastin (Wang, 2006, Kannus, 2000). The primary cell type in tendons is the fibroblast, which includes both tenoblasts and tenocytes, and constitutes 90-95% of tendon cells (Winnicki et al., 2020). In addition, synovial, chondrocytes, and vascular cells are also present (Doral et al., 2010, Kannus, 2000, Tresoldi et al., 2013). Fibroblasts play a fundamental role in generating extracellular matrix proteins, orchestrating the formation of an organised collagen matrix (Wang, 2006).

This connective tissue is considered mechanosensitive, responding and adapting to the mechanical load transmitted by the muscle fibers (Nourissat et al., 2015). However, the mechanisms involved in the detection of these mechanical forces by the tendon's cells, and how these signals are converted into biochemical signals to produce adaptations in the morphological and mechanical properties of tendons, are not yet fully understood (Wang, 2006). These mechanical signals comprise tension, compression, hydrostatic pressure, and fluid shear stress (Banes et al., 1995, Docking et al., 2013), which depending on the magnitude, frequency, direction and duration of the stimuli (Banes et al., 1995, Lavagnino et al., 2003, Screen et al., 2005) may activate or inhibit specific biochemical pathways involved in processes such as differentiation, proliferation, and tissue development (Banes et al., 1995, Docking et al., 2013). Various components of the cell surface such as cilia, focal adhesions, gap junctions, and cytoskeleton, which are connected to the cellular nucleus, allow tendon cells to elicit a biological response (Lavagnino et al., 2015, Chatterjee et al., 2022). Within this framework, physiological

loading is essential for maintaining tendon homeostasis (Hannafin et al., 1995, Galloway et al., 2013). It has been observed that understimulation of tendon cells triggers a catabolic response, leading to a degenerative cascade of events. Conversely, overstimulation can cause sub-rupture damage, impairing the fibrils' capacity to transmit load and consequently, disrupting cell-matrix interactions (Chatterjee et al., 2022).

1.1.1 Mechanical properties of tendons

Tendons experience varying mechanical forces in vivo, which results in fiber patterns and viscoelastic properties that define their distinct mechanical behavior (Wang, 2006). The elastic component refers to the capacity to return to their original shape following deformation, while the viscous component refers to their tendency to remain in a deformed state, depending on the strain rate (Towers et al., 2003). Traditionally, the study of tendon mechanical properties has employed techniques that include stretching isolated tendon samples until failure, while measuring the force applied and the resulting elongation of the tendon (Maganaris et al., 2008). Consequently, the tendon's response to stretching can be characterised by four distinct phases on the force-elongation curve produced. Region I, known as the "toe" region of the tendon, involves forces that change the collagen fibre's initial crimped configuration without inducing further stretching. Region II, or "linear region", where the mechanical loading stretches the already aligned fibres, and some fibres start to break at the endpoint of this region. Region III, where further tendon elongation produces additional fibre failure in an unpredictable manner. Finally, Region IV is charaterised by further tendon elongation, where complete failure occurs (Viidik, 1973, Butler et al., 1978, Partington and Wood, 1963, Elliott, 1965, Diamant et al., 1972) **(Figure 2)**.

The parameters obtained from the force-elongation curve include stiffness, ultimate load, ultimate elongation, and energy absorbed at failure (Jung et al., 2009). Since the specific shape of the force-elongation curve varies depending on the size and dimension of the tendon, tendon forces and elongation are converted into stress and strain values by normalising them against the tendon's cross-sectional area (CSA) and

length, respectively (Maganaris et al., 2008). The mechanical parameters that can be extracted from the stress-strain curve include Young's modulus, ultimate tensile stress, and ultimate strain (Jung et al., 2009). Young's modulus corresponds to the slope of the stress-strain curve during "linear phase" (Butler et al., 1978, Bennett et al., 1986, Shadwick, 1990, Pollock and Shadwick, 1994). The ultimate stress, which is the maximum tensile stress the tendon can withstand before failure, is around 100 MPa, and the ultimate strain, which is the maximum strain the tendon can endure before failure, varies from 4 to 10% in humans (Butler et al., 1978, Partington and Wood, 1963, Elliott, 1965, Bennett et al., 1986, Shadwick, 1990). Furthermore, tendons present viscoelastic properties such as hysteresis (i.e., internal energy dissipation during loading and unloading cycles), creep (i.e., gradual increase in deformation under a constant load), and stress relaxation (i.e., reduction in stress over time under constant deformation) (Jung et al., 2009). These properties ultimately reflect the time-dependent interaction between the collagen fibres and the inter-fibre matrix (Cohen et al., 1976, Hooley et al., 1980).

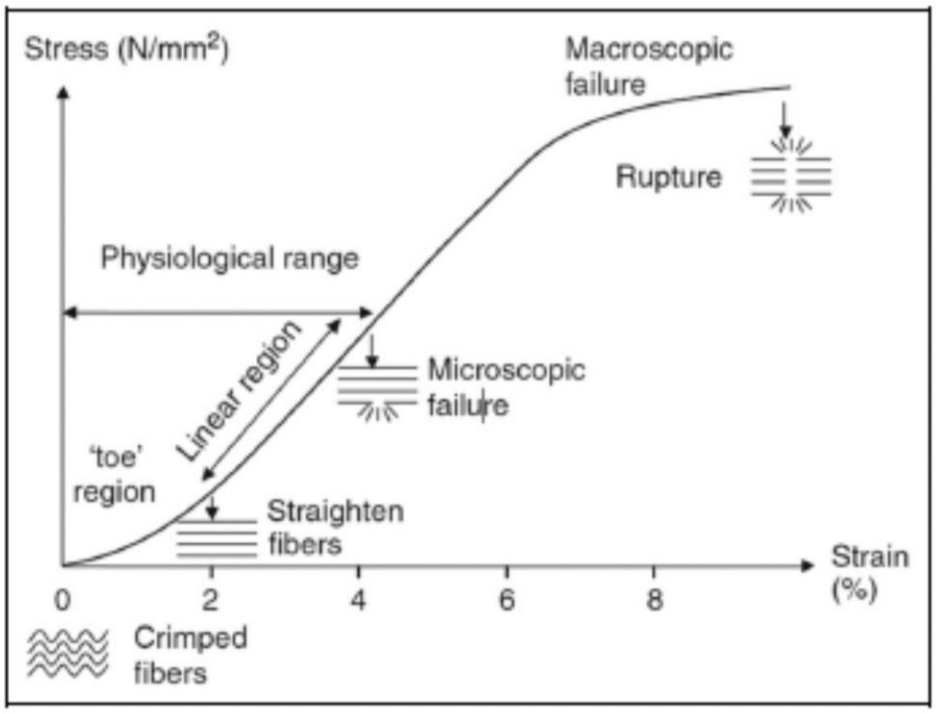

Figure 2. Tendon-strain curve.
Adapted from Wang 2006.

In the last decades, new methodologies based on real-time examination have been used to determine the mechanical properties of human tendons in vivo. These methodologies include the use of high-resolution ultrasonography, 3D ultrasonography, panoramic ultrasound, Magnetic Resonance Imaging (MRI), dynamometry and shear-wave elastography (SWE) (Winnicki et al., 2020). The most relevant advantage of these in vivo methodologies is the possibility to assess the mechanical behavior of tendons during various activities (Arampatzis et al., 2005, Kubo et al., 2000b, Kubo et al., 2004, Maganaris and Paul, 2002). In this context, real-time ultrasonography has enabled the in vivo assessment of the changes in the muscle-tendon length unit during voluntary or electrically induced contractions in humans (Fukunaga et al., 1996). This methodology includes the concurrent assessment of the MTJ using real-time ultrasonography and the torque exerted by the participants using an isokinetic dynamometer during cycles of contraction and relaxation (Maganaris et al., 2008). Additionally, the real-time ultrasonography allows the assessment of the tendon's dimensions, enabling the conversion of force-elongation curves obtained during contraction and relaxation cycles into stress-strain curves (Maganaris et al., 2008). Despite the advantages of assessing tendon mechanical properties in its physiological environment, in vivo methods have some limitations such as incorporating heat loss by the interfaces muscle-tendon and tendon-bone and the friction with other nearby tissues, and the uneven stress distribution across the tendon with increasing muscle forces (Maganaris et al., 2008).

1.2 Achilles tendon and the triceps surae muscle

The Achilles tendon is the strongest, thickest, and largest tendon in the human body (Freedman et al., 2014, Joseph et al., 2012, Ying et al., 2003). It is formed by the tendons of the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SO) muscles (O'Brien, 2005), with a minor contribution of the plantaris muscle (Dalmau-Pastor et al., 2014). Each of these tendons is considered a subtendon. The Achilles tendon has a distinctive twisted structure, with the subtendon of the MG rotating laterally and the subtendons of the LG and SO rotating medially (Pierre-Jerome et al., 2010) **(Figure 3).**

This rotation between the subtendons allows for elongation and elastic recoil within the tendon possible (Alexander and Bennet-Clark, 1977). It has been observed that the rotation of the subtendons peaks 2 to 5 cm proximal to the tendon insertion, producing high stress in this area (Nickisch, 2009). This high stress has been linked to the poor vascularity, which may explain the susceptibility to tendinopathy and rupture of this region (Nickisch, 2009).

The Achilles tendon length is approximately 10 to 15 cm (Cummins et al., 1946), with a thickness of 5-7 mm, and a width of around 20 mm (Nickisch, 2009). It is broad and flat at the MTJ and become rounded approximately 4 cm from its insertion; however, it flattens again near the enthesis (Cummins et al., 1946). At this level, the tendon attaches to the middle third of the calcaneal tuberosity posterior surface (Chao et al., 1997). Typically, the insertion extends further on the medial side, and the more distal fibres merge into the fibrous tissue of the calcaneus, eventually joining the plantar fascia distally (O'Brien, 2005). The SO and gastrocnemius subtendons differ in their orientation, extent of fusion, and contribution to the Achilles tendon (Cummins et al., 1946). Studies have shown that, on average, the soleus subtendon contributes 52%, while the gastrocnemius subtendons contributes 48% to the Achilles tendon in 52% of subjects (O'Brien, 2005).

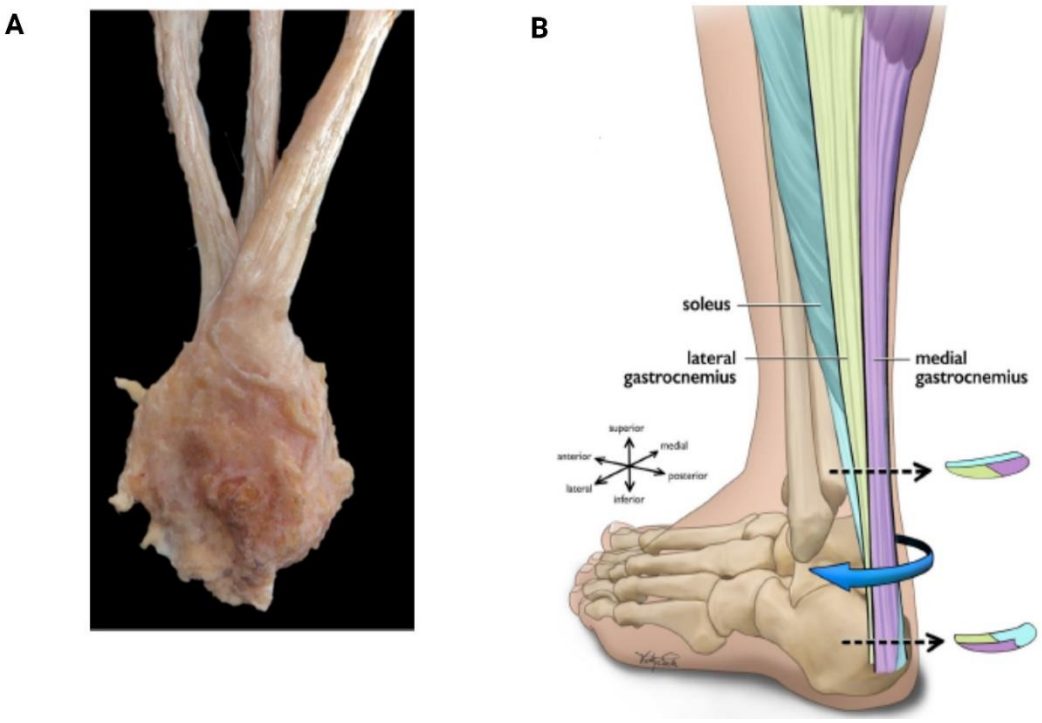

Figure 3. Anatomy of the Achilles tendon.
A. Cadaveric model of the Achiles subtendons (originating from the lateral and medial heads of the gastrocnemius muscle and the soleus muscle). Adapted from Winnicki et al. 2020. B. Representation of the left Achilles tendon and the three subtendons rotating clockwise, traveling distally down the tendon. Adapted from Merry et al. 2022.

The gastrocnemius muscles are involve in knee flexion, ankle plantarflexion, and subtalar inversion (Nickisch, 2009). The gastrocnemius involves two heads, medial and lateral. The medial head originates on the popliteal aspect of the distal femur (Dalmau-Pastor et al., 2014) and is larger and longer compared to the lateral head (O'Brien, 2005). Conversely, the lateral head originates from the posterior lateral supracondylar ridge (Dalmau-Pastor et al., 2014). The SO is a broad, bulky and pennate muscle located deeper than the gastrocnemius muscles, and is considered the most important ankle plantarflexor (Kvist, 1994). The SO has an origin at the head and upper part of the fibula, and a tibial origin at the inferior part of the soleal line and the midsection of the tibia's medial border (Dalmau-Pastor et al., 2014). The subtendons of the MG, LG, and SO fuse to become one tendon about 5-6 cm proximal to the calcaneal insertion (Pierre-Jerome et al., 2010).

1.2.1 Mechanical properties of the Achilles tendon

The Achilles tendon is exposed to high forces during daily life activities and sports. It has been estimated that the Achilles tendon is exposed to approximately 3 kN during maximal isometric contractions, 5 kN during unilateral hopping, and 9 kN while running (Joseph et al., 2014). The magnitude of the applied load during daily life activities results in a positive adaptation of the Achilles tendon; however, excessive and repetitive mechanical forces can lead to tendon degeneration (Järvinen et al., 1997, Wang et al., 2006). Regarding the mechanical properties, the results vary greatly, with studies showing maximal tendon force of 200-3800 N, elongation of 2-24 mm, stress of 20-42 MPa, strain of 5-8%, stiffness of 17-760 N/m, Young's modulus of 0.3-1.4 GPa and hysteresis of 11-19% in young sedentary adults (Maganaris and Paul, 2002, Muramatsu et al., 2001, Magnusson et al., 2001, Bojsen-Møller et al., 2004, Hansen et al., 2003, Arampatzis et al., 2005, Kubo et al., 2000a). The variability of the mechanical properties may be due to differences in the methodology employed such as the incorporation of the synergistic or antagonist muscles in the calculation of force or the location of the ultrasound probe during the assessment (Maganaris et al., 2008).

1.3 Achilles tendinopathy

Achilles tendinopathy (AT) is a medical condition characterised by pain, impaired function and swelling in and around the tendon (Longo et al., 2009, Maffulli et al., 2020). AT can be categorised into insertional (IAT) and non-insertional (NIAT), each with distinct pathophysiological characteristics and treatment approaches (Maffulli et al., 2020). However, it has been observed that symptoms in the insertion and midportion are relatively frequent, and that approximately 30% of patients report pain bilaterally (Silbernagel et al., 2007a). Regarding Achilles tendon disorders, the most common diagnosed condition is NIAT, which accounts for 55 to 66% of cases, followed by insertional complaints, such as IAT or retrocalcaneal bursitis, representing 20 to 25% of cases (Kvist, 1994). AT is prevalent in individuals involved in competitive sports, such as

middle and long-distance running, badminton, tennis, volleyball and soccer (Paavola et al., 2002). Nonetheless, AT is not exclusively an athletic injury since 65% of injuries diagnosed in clinical practice are unrelated to sports activities (de Jonge et al., 2011).

1.3.1 Aetiology and pathophysiology

The aetiology of NIAT is influenced by multiple intrinsic and extrinsic factors. These factors can either reduce tendon's load tolerance or produce movement patterns that place excessive stress on the tendon (Martin et al., 2018, Silbernagel et al., 2020). Intrinsic factors include decreased plantarflexion strength, loss of ankle dorsiflexion and subtalar joint motion, increased foot pronation, altered neuromuscular control of the hip muscles, increased body weight (Martin et al., 2018), systemic disease, genetic variants, and family history of tendinopathy (Magnan et al., 2014). Extrinsic factors include the type of footwear, the surface of training, training-load errors (such as a decrease in recovering time or sudden increase in the training duration/intensity), asymmetric training, poor technique and fatigue; however, there is limited evidence associating these factors with the development of AT (Martin et al., 2018, Magnan et al., 2014, Vlist et al., 2019, Paavola et al., 2002). The aetiology of NIAT is still a subject of debate (Maffulli et al., 2020); however, excessive loading is widely regarded as the primary contributing factor. Recent studies have suggested that the triceps surae muscle may influence the magnitude and distribution of the load, stress and strain within the Achilles tendon (Debenham et al., 2017, O'Neill et al., 2015); consequently, it has been proposed that an alteration of the force produced by the triceps surae muscles could lead to uneven loading across the tendon, contributing to the development of NIAT (Bojsen-Møller and Magnusson, 2015, Hug and Tucker, 2017).

Tendinopathy fundamentally involved an altered healing response, characterised by degeneration and disorganised growth of tenocytes, disruption of collagen fibres and increase in collagen matrix (Maffulli et al., 2020). On a cellular level, it has been observed uneven and irregular crimping, as well as loosening and increased waviness in the collagen fibres, associated with an increase in type 3 collagen (Longo et al., 2009).

Additionally, excessive and repetitive mechanical forces on the tendon may produce tenocyte apoptosis, chondroid metaplasia, production of matrix metalloproteinases and increased expression of insulin-like growth factor 1 and substance P (Ackermann and Renström, 2012, Riley, 2008). At the tissue level, tendinopathy manifests as either localised or widespread tendon thickening (tendinosis), a disruption in normal collagen structure, an increased amount of proteoglycans and deterioration of tissue organisation (Silbernagel et al., 2020). Nevertheless, the physiopathological mechanisms responsible for the morpho-mechanical alterations observed in individuals with AT are not well understood (Hess et al., 1989, Kvist, 1994, Fenwick et al., 2001).

1.3.2 Morpho-mechanical properties in individuals with NIAT

NIAT has been shown to result in altered tendon composition, structure, and morphology, including increased thickness, CSA, and volume (Shalabi et al., 2004, Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Docking and Cook, 2016) **(Figure 4)**. Additionally, the tendinopathic Achilles tendon also exhibits changes in the mechanical properties, including lower stiffness and Young's modulus, alongside increased strains in both longitudinal and transverse directions and increased hysteresis compared to healthy tendons under the same tensile load (Arya and Kulig, 2010, Child et al., 2010, Wang et al., 2012, Chang and Kulig, 2015). These changes in the mechanical properties have relevant functional implications for the triceps surae muscle. Firstly, when the triceps surae muscle is attached to a compliant tendon, it affects the muscle's ability to control the position of the joint that the tendon spans (Maganaris et al., 2008). Secondly, for a given contractive force, a compliant tendon increases the shortening of the triceps surae muscle, which affects the sarcomere force-length relationship and thereby reduces its force production (Maganaris et al., 2008).

1.3.3 Diagnosis and physical examination

The diagnosis of AT should primarily be based on the patient's medical history and clinical examination (Robinson et al., 2001, Schepsis et al., 2002). Relevant information includes the duration between the onset of symptoms and the initial consultation with the physician, as well as the injury mechanisms in cases of acute onset and previous Achilles tendon problems and their treatment (Schepsis et al., 2002). Common symptoms are morning stiffness or stiffness after a period of inactivity, and progressive onset of pain during activity (Maffulli et al., 2020). The sequence of events following the onset of symptoms, particularly focusing on activities that seem to exacerbate the pain and the interventions that appear to alleviate it, provide valuable additional information. In athletes, pain usually occurs at the beginning and end of the training session, with a period of reduced discomfort in between (Maffulli et al., 2020). As the condition progresses, pain can manifest during activities of daily living and even at rest (Maffulli et al., 2020). The physical examination should include inspection and palpation to identify possible regions of swelling and crepitation, increased erythema, local heat, and palpable tendon nodules or defects (Fredericson, 1996). During the early stages of AT, the tendon appears diffusely swollen; tenderness is most pronounced in the middle third of the tendon, and crepitation may be palpated (Kvist, 1994). Additionally, the area of swelling and tenderness does not move when the ankle is dorsiflexed. Conversely, in more chronic stages of AT, crepitation and swelling tend to decrease (Paavola et al., 2002); however, a tender nodular swelling is usually present (Kvist, 1994, Galloway et al., 1992). Furthermore, the focal tender nodules tend to move when the ankle is dorsiflexed. It is important to rule out several differential diagnoses that could produce posterior ankle pain, such as posterior ankle impingement, Achilles tendon rupture, sural nerve irritation, fat-pad irritation, and systemic inflammatory disease (Martin et al., 2018).

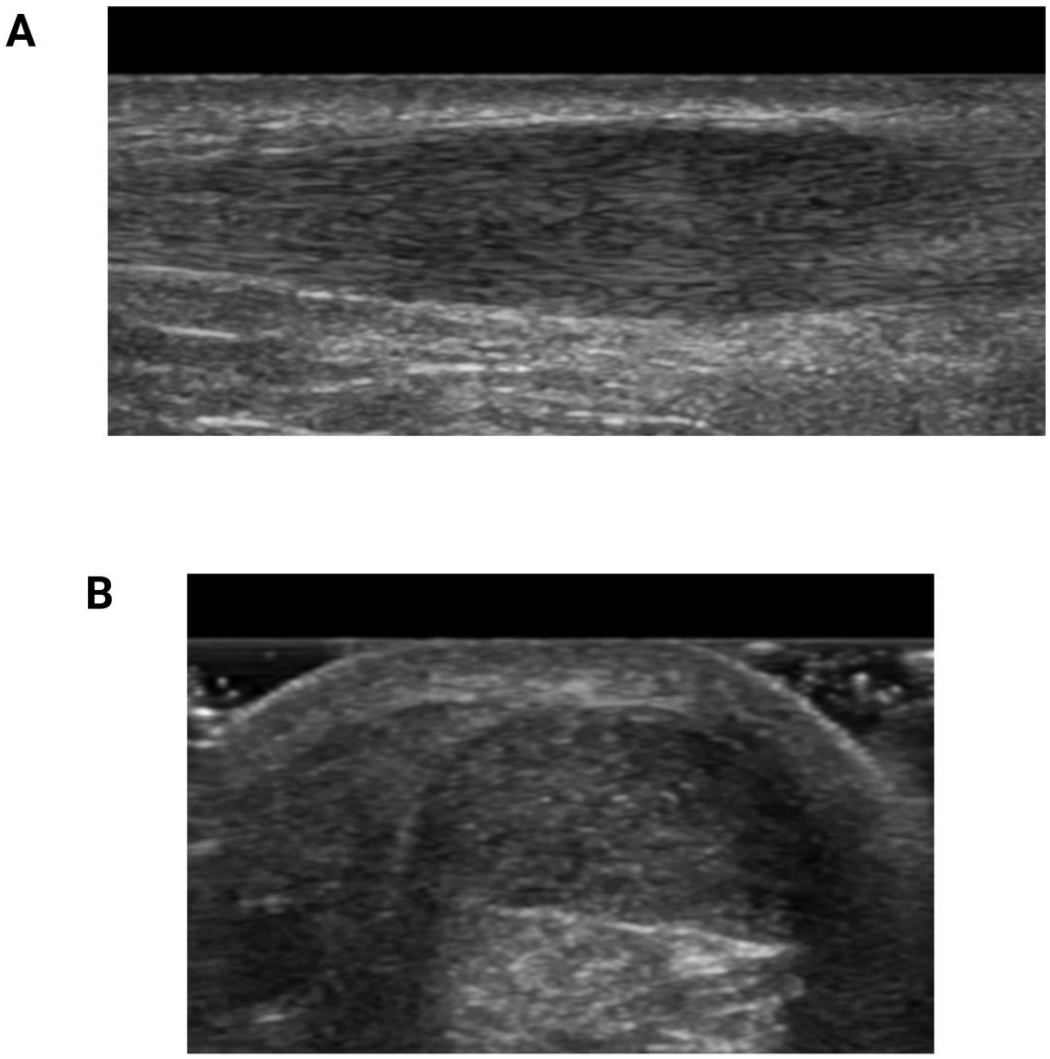

Figure 4. Ultrasound images of an individual with non-insertional Achilles tendinopathy (NIAT).
A. Longitudinal ultrasound image showing increased Achilles tendon thickness. B. Transverse ultrasound image showing increased Achilles tendon cross-sectional area (CSA). Images correspond to one of the participants in our study with NIAT.

1.3.4 Imaging methods

The imaging modalities most frequently used for diagnosing Achilles tendon problems are conventional radiography, ultrasonography, and MRI. These imaging methods play a critical role in evaluating individuals with clinical symptoms, in differential assessment of disease and in determining the severity of the condition. Furthermore, imaging can reveal changes in the tendon's condition after treatment and providing

prognostic information regarding the tendon function (Bleakney and White, 2005). Although conventional radiography is foundational for imaging bones and joints, particularly in trauma, they do not provide precise details of soft tissues due to its limited contrast capabilities. Consequently, imaging techniques such as ultrasonography and MRI, known for their excellent soft tissue contrast, have emerged as preferred methods for examining the Achilles tendon (Bleakney and White, 2005).

Ultrasonography presents several advantages over MRI: it is readily available, has a quick scan time, better patient tolerability, allows comparisons with the asymptomatic tendon (Paavola et al., 2002) and helps reproduce the symptoms by transducer compression (Gibbon, 1996). Nevertheless, it is operator dependent, requiring proper training and experience for accurate diagnosis. Additionally, despite the use of high-frequency transducers that provide a better spatial resolution, ultrasonography is not the imaging method recommended to assess deeper structures due to the poor return of echoes (Bleakney and White, 2005). MRI is widely used for identifying tendon abnormalities because it offers a high spatial resolution, allowing for the clear identification of detailed anatomical structures and the ability to capture images in the longitudinal, transverse, and oblique planes (Kerr et al., 1990). However, MRI disadvantages include relatively high cost, time-consuming scanning, and limited accessibility (Sandmeier and Renström, 1997). Both imaging methods have demonstrated high specificity and sensitivity but also present a relatively high incidence of false positive findings (Khan and Maffulli, 1998).

Ultrasonographic assessments of the Achilles tendon should be conducted in both longitudinal and transverse planes (Fornage and Rifkin, 1988, Lin et al., 2000). To obtain optimal images, it is important to locate the ultrasound probe in a position that facilitates the striking of the ultrasound waves perpendicular to the tendon (Lin et al., 2000). If the alignment is not perpendicular, the transducer will not receive most of the ultrasound waves reflected and the Achilles tendon will appear hypoechoic or anechoic (Bleakney and White, 2005). This angle-dependent property is known as acoustic fibre anisotropy (Dussik et al., 1958). In the acute phase of NIAT, ultrasonography usually shows fluid

around the tendon (Paavola et al., 2002); however, in the chronic phase, focal or diffuse thickening of the Achilles tendon of approximately 7 mm to 16 mm is commonly observed (Bertolotto et al., 1995). Additionally, focal hypoechoic intratendinous areas, thickening of the paratenon and discontinuity of tendon fibres can also be observed (Paavola et al., 1998, Kälebo et al., 1992). Although ultrasonography can precisely display structural abnormalities in the Achilles tendon, it has been observed in individuals with chronic NIAT that there is only a moderate correlation between ultrasound findings and symptoms, showing inaccuracy as a predictor of subsequent clinical outcomes (Khan et al., 2003).

1.3.5 Shear-wave elastography

Continual improvement in ultrasonography technologies such as higher frequency transducers, smaller footprint probes, power Doppler, extended field view capabilities, three-dimensional imaging, tissue harmonics and elastography techniques have generated an extended use of ultrasonography for research and clinical purposes (Bleakney and White, 2005). Recently developed and rapidly evolving ultrasound elastography provides relevant information regarding tissue properties by assessing tissue elasticity, contributing to the diagnosis (Klauser et al., 2014, Drakonaki et al., 2012). There are four main types of ultrasound elastography techniques: compression sonoelastography, transient elastography, tension elastography, and SWE (Taljanovic et al., 2017). Specifically, SWE has shown promising results assessing tendons, muscles, nerves, and ligaments (Taljanovic et al., 2017). SWE has the advantage of assessing the propagation speed of shear stress waves, allowing the calculation of Young's modulus, which is a measure of tissue stiffness (Gennisson et al., 2007). This technique is based on the principle that, for a quasi-incompressible elastic solid, the shear wave speed (VS) is related to the shear modulus (μ) and to the Young's modulus (E) according to the formula: $E = 3\mu = 3\rho VS^2$, where ρ is the density of biological tissues (~1.100 kg/m^3) (Ngo et al., 2022).

During the assessment, quantitative shear modulus maps are presented in a colour-coded elastogram showing shear-wave velocities in meters per second or tissue

elasticity in kilopascals. SWE is more objective and reproducible than other ultrasound elastography methods, allowing direct assessment of tissue elasticity without the need for manual compression (Taljanovic et al., 2017). Nevertheless, SWE has some limitations, including a delay of a few seconds before starting a new acquisition, making it unsuitable for assessing structures in motion, sensitivity to transducer pressure and angle, and decreased depth of penetration (Taljanovic et al., 2015). In general, current evidence suggests that shear waves propagate faster in healthy tendons compared to tendinopathic ones, faster in contracted tendons than those that are relaxed, and faster in the long axis compared to the short axis (Taljanovic et al., 2017).

1.3.6 Strength assessments in NIAT

Persistent strength deficits resulting from NIAT have been related to difficulties performing tendon-loading activities, such as jumping and running. Currently, there is no widespread agreement on whether strength deficits are a consistent feature of NIAT (McAuliffe et al., 2019). In clinical settings, the calf-raise or heel-raise test has been the main valid approach to assessing plantarflexion function in individuals with NIAT (Hébert-Losier et al., 2009). Nevertheless, depending exclusively on unidimensional measures to assess plantarflexion function may affect the detection of functional deficits in individuals with this condition (McAuliffe et al., 2019). Due to this limitation, other methods have been used to determine muscle strength, such as isokinetic dynamometry, which allows the evaluation of the muscle strength by measuring the joint torque produced during movements at a constant angular velocity **(Figure 5)**. The isokinetic dynamometer has numerous applications in sports, exercise, and clinical settings. These include assessing the outcomes of training interventions or rehabilitation programs, establishing parameters for the return of athletes to competition, conducting basic research on the mechanics of musculoskeletal tissues, among others.

Human movement is generated by the rotation of body segments around the joint's instantaneous axis of rotation. If we consider plantarflexion movement occurring in the sagittal plane around an instantaneous axis of rotation that is perpendicular to the plane

of motion, the moment that causes the rotation can be calculated as the muscle force multiplied by the shortest distance to the rotation axis, known as moment arm (Payton and Bartlett, 2007). Muscle strength is usually described as the highest joint moment produced across various conditions of muscle length, speed, and type of contraction (such as concentric (CON), eccentric (ECC), or isometric). Therefore, assessing the net joint moment at various joint angles, speeds and type of contractions is crucial for determining muscle strength and the dynamic capabilities of the muscular system (Payton and Bartlett, 2007). Regarding individuals with NIAT, a recent systematic review has shown deficits of 18% to 44% in maximal strength during ECC and CON isokinetic assessments and 5% to 12% for maximal isometric strength (McAuliffe et al., 2019). Nevertheless, maximal strength variables represent one dimension of the strength profile and might not adequately assess the explosive strength that is essential for sports-related activities (McAuliffe et al., 2019).

Figure 5. Experimental setup used in our study.
On the right side, Biodex isokinetic dynamometer can be seen.

1.3.7 Effect of exercise in individuals with NIAT

Exercise rehabilitation is the most effective treatment for AT (Silbernagel et al., 2007a, Martin et al., 2018, Silbernagel et al., 2006). The purpose of these interventions is to apply controlled mechanical load to the tendon to induce a decrease in the level of pain, promote tendon remodelling, improve triceps surae muscle strength and endurance, and optimise lower limb functionality (Silbernagel et al., 2007a, Silbernagel and Crossley, 2015). In the last decades, ECC exercises have emerged as the primary non-surgical treatment option for AT (Alfredson et al., 1998). However, evidence supporting the superior effectiveness of this type of exercise in the management of this condition remains limited. It is believed that ECC exercises may enhance tendon remodelling by promoting changes in collagen fibre cross-link formation (Maffulli and Longo, 2008). Nevertheless,

evidence is lacking supporting histological tendon changes after applying an ECC exercise intervention. Although numerous studies have examined the effect of ECC exercise interventions on individuals with NIAT, the precise mechanism by which this type of exercise may alleviate tendon pain and symptoms remains elusive (Maffulli et al., 2020). Additionally, studies applying exercise interventions, including isolated CON or a combination of CON and ECC contractions, have reported positive results (Silbernagel et al., 2007a, Beyer et al., 2015). Furthermore, no significant differences have been reported in peak tendon forces during ECC and CON contractions (Ackermann et al., 2018). Together, these findings question the idea that tendon response differs based on the type of exercise intervention applied (Silbernagel et al., 2020). Within this framework, the optimal loading parameters such as load progression, load magnitude, frequency and time between sessions have not been established; however, it seems that exercise interventions with progressively controlled higher loads and more prolonged contractions times may be the best option to increase tendon strength and size (Silbernagel et al., 2020).

1.4 Electromyography and muscle activity

Successful human movement relies on the integrity of each component within the hierarchical control system, comprising the central nervous system (CNS), muscles and tendons (Chang and Kulig, 2015). Basically, the CNS plans, initiates, and sends motor commands to the muscles, and muscles execute these commands, generating force that is transmitted to the tendon (Chang and Kulig, 2015). Electromyography (EMG) is a technique that allows the identification of muscle activity, assessing the magnitude, timing, and distribution of muscle activation (Merletti and Farina, 2016). EMG enables the quantification of electrical signals that trigger muscle contractions, representing the endpoint of complex neuromuscular activities that underpin volitional movement. The EMG signals represent the electrical potential field produced by the depolarization of the outer membrane of the muscle fibres, known as sarcolemma (Merletti and Farina, 2016). The electromyogram is a compound signal that reflects the activity of dozens to hundreds of motor units (Sherrington, 1997). Each motor unit consists of an alpha-motor neuron

located in the ventral horn of the spinal cord, its axon, and the muscle fibres that the axon innervates (Sherrington, 1997). These motor units are activated asynchronously, depending on the contraction force, and their electrical contribution to the electromyogram is represented by the motor unit action potentials (MUAP) (Merletti et al., 2010). In general, there are two methods to detect these signals: intramuscular EMG, and surface EMG. These two methods use different types of electrodes (intramuscular or surface electrodes). For intramuscular EMG, the impact of the tissues between the electrodes and the muscle fibres is relatively low because the recording electrodes are placed close to the source of the electrical signals; however, in surface EMG, the tissue acting as a volume conductor can significantly affect the recordings (Merletti and Farina, 2016).

Traditional bipolar surface EMG recordings are acquired using a pair of electrodes placed over the skin of the muscle of interest, along with a reference electrode positioned on a bony prominence. The reference electrode assesses the conductance of the skin in baseline conditions; therefore, it allows comparisons between non-contractile and contractile regions (Merletti and Farina, 2016). The pair of electrodes are usually placed 20-30 mm apart (inter-electrode distance) and are aligned with the orientation of the muscle fibres (Falla and Gallina, 2020). After the electrode placement, the electrical potential is recorded at a specific sample frequency, amplified and filtered (Merletti and Farina, 2016). Conventional EMG can effectively describe the global activation of muscles. Yet, its relatively large detection volume often limits its ability to discern variations in activation patterns within specific muscle regions or among several muscles located closely (Falla and Gallina, 2020). Alternatively, intramuscular EMG recordings represent a highly selective method to obtain the electrical activity of the muscle fibre in proximity to the wire electrode (Falla and Gallina, 2020). Currently, this is the only approach to assess the EMG activity of deeper muscles. Nevertheless, intramuscular EMG has some limitations, including the identification of a small number of motor units, the time required for data processing and analysis, and the potential discomfort caused by electrode insertion (Hu et al., 2013).

1.4.1 High-density surface EMG

In recent years, the detection of surface EMG has undergone considerable improvements due to the development of two-dimensional electrode arrays (Merletti et al., 2010). High-density surface EMG (HD-sEMG) employs a large number of electrodes arranged in a two-dimensional grid that is placed over the muscle of interest. The small inter-electrode distance (often 10 mm or less) and small electrode size (usually less than 3 mm) enhance the sensitivity of the recordings; therefore, each electrode is able to record the activation of the surrounding muscle fibres (Falla and Gallina, 2020). These features provide a time-evolving spatial sample of the instantaneous image of the surface potential distribution and describe the spatial distribution of the surface EMG amplitude or spectral variables estimated in a period of time (Merletti et al., 2010). HD-sEMG has been used to determine motor unit firing rate properties with a method called surface EMG motor unit decomposition (Merletti et al., 2008, Holobar et al., 2009). This method required that the number of observation sites from the surface EMG should be high enough to discriminate different MUAPs (Farina et al., 2008). Thus, the extraction of different motor units from the EMG signal can be assessed with algorithms based on blind source separation, which can recognise and differentiate individual MUAP shapes and later determine the exact time that each motor unit was active during the contraction (Holobar and Zazula, 2004).

HD-sEMG offers significant advantages compared with other methods since allows the detection of an increased number of motor units (Merletti et al., 2008), enables the assessment of a broader spectrum of force levels (Holobar et al., 2014), can detect the dynamic changes in force and the variations in the common synaptic input to the motor neuron pool (Enoka and Farina, 2021), allows the study of muscle fibre membrane properties (conduction velocity) (Farina et al., 2001) and permit the identification of the same motor unit during multiple sessions (Martinez-Valdes et al., 2017b).

1.4.2 Factors influencing surface EMG recordings

The characteristics of the surface EMG signals depend on several nonphysiologic and physiologic factors. Non-physiological factors usually include the thickness of the cutaneous tissue, distribution and size of motor unit territories, muscle fibres' length, skin-electrode contact, electrode size, shape and interelectrode distance, location of the electrodes over the muscle belly, and crosstalk coming from nearby muscles, among others (Farina et al., 2004). Physiological factors include the muscle fibre conduction velocity, distribution of motor unit conduction velocities, shape of intracellular action potentials, number of recruited motor units, distribution of motor unit discharge rate and motor unit synchronisation (Farina et al., 2004). These influences can be unpredictable and change with the conditions of the experiment; however, when the experiment design considers the reduction of these factors, valuable insights can be obtained from surface EMG recordings.

1.5 Motor unit

1.5.1 Motor unit properties

On average, each motor neuron innervates approximately 300 muscle fibres; however, this relationship is influenced by the muscle size fluctuating from tens to thousands (Enoka and Fuglevand, 2001). The group of muscle fibres innervated by the same motor units is called muscle unit (Heckman and Enoka, 2012). These fibres are distributed throughout the muscle and intermixed with muscle fibers from different muscle units (Bodine et al., 1988, Wang and Kernell, 2001, Weijs et al., 1993).

Due to a high safety margin for synaptic transmission at the neuromuscular junction, action potentials discharged by a motor neuron invariably result in the production of action potentials that propagate along the fibers of the muscle unit (Farina et al., 2010, Heckman and Enoka, 2012). Consequently, the action potentials generated during the contraction are strongly related to the magnitude of the motor command discharged by

the motor neuron pool (Farina et al., 2010, Heckman and Enoka, 2012). The force produced by the single stimulation of a motor unit is called “twitch”, and it is considered the basic contractile property of a motor unit (Enoka and Duchateau, 2015). Action potentials produced by the motor neuron generate a rapid fluctuation in the voltage, which is known as MUAP or spike.

During sustained contractions, several MUAPs are sent to the muscles to generate a series of overlapping twitches or spike trains (Enoka, 2015). From these spike trains, motor unit firing rate properties, such as discharge rate and discharge rate variability, can be estimated **(Figure 6)**.

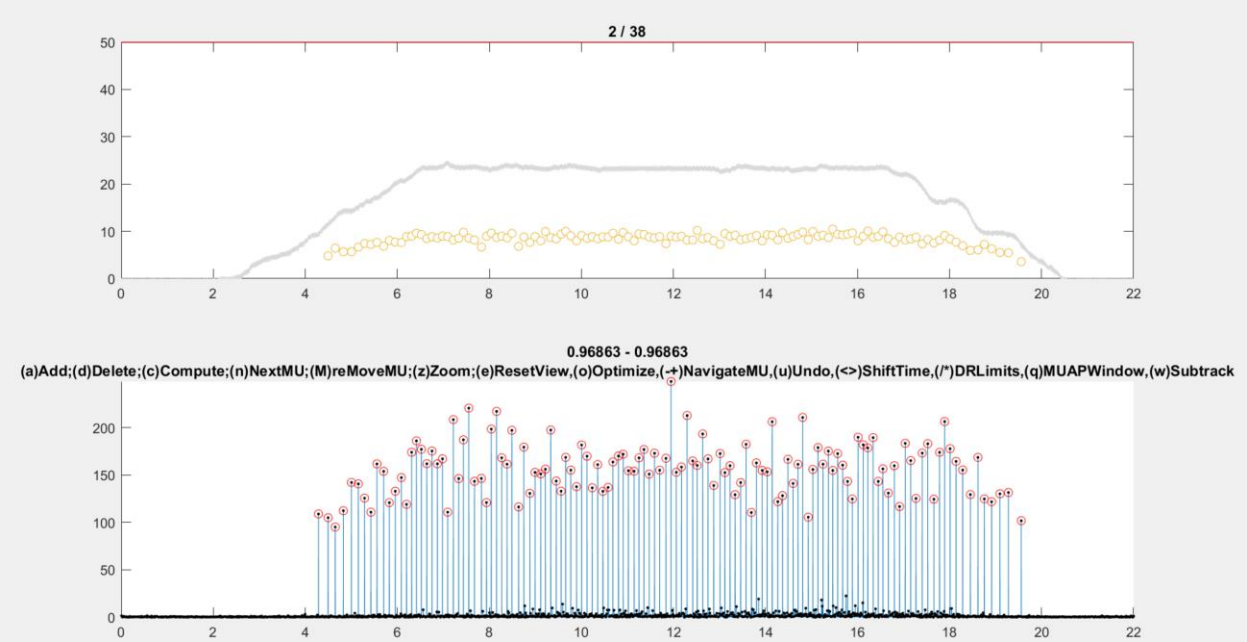

Figure 6. Motor unit spike trains.
The motor unit decomposition algorithm shows the spike trains of the motor unit identified in the medial gastrocnemius muscle (MG) during the plantarflexion contractions at 40% maximal voluntary contraction (MVC). Spike trains correspond to one of the asymptomatic individuals assessed in our study.

1.5.2 Motor units and force

The net force generated by a muscle is influenced by different factors including the number of active motor units and the frequency at which motor neurons fire action potentials, the contractile and morphological characteristics of the muscle fiber, and the mechanical properties of the connective tissues responsible for the transmission of muscle forces (Enoka and Duchateau, 2015). Motor units are recruited according to the size principle, which states that smaller motor units are recruited first at low force levels while larger motor units are recruited last at high force levels, representing two different populations of motor units (low-threshold and high-threshold) (Enoka, 2015). Larger motor units typically generate more twitch than smaller ones, indicating a higher force capacity (Milner-Brown et al., 1973). It has been hypothesised that the size principle may have functional advantages in force production (Heckman and Enoka, 2012); however, this perspective has been the subject of recent scrutiny.

During a submaximal contraction, when an individual aims to sustain a constant force, the applied force tends to vary around an average value instead of remaining constant (Enoka and Farina, 2021). At low forces, the normalised amplitude of these force fluctuations is high and decreases during moderate to high forces (Barry et al., 2007, Jones et al., 2002). This pattern of force fluctuations, or the coefficient of variation, provides a measure of force steadiness (Galganski et al., 1993). It has been shown that force fluctuations correlate with summed discharge times (cumulative spike trains (CST)), which represent the neural drive to the muscle, rather than the oscillation in the absolute EMG amplitude (Thompson et al., 2018). Nevertheless, force steadiness can also be affected by the contractile properties of the muscle units, the total number of motor unit, and the maximal motor unit recruitment threshold (Castronovo et al., 2018, Dideriksen et al., 2012).

1.5.3 Adaptations in motor unit properties with training

There is a body of evidence investigating changes in motor unit firing parameters following strength training. These studies usually include outcomes such as motor unit recruitment threshold, discharge rate, and discharge rate variability (Elgueta-Cancino et al., 2022). Unfortunately, these studies have shown inconclusive results. For example, Del Vecchio et al. 2019 reported a significant increase in motor unit discharge rate in the tibialis anterior muscle after 4 weeks of resistance training; similarly, Vila-Chã et al. 2016 found an increase in discharge rate in the vastus lateralis and vastus medialis obliquus after 3 (vastus medialis obliquus only) and 6 weeks of resistance training (Del Vecchio et al., 2019, Vila-Chã and Falla, 2016). Conversely, Rich and Cafarelli 2000 reported no differences in motor unit discharge rate in the vastus lateralis after 8 weeks of resistance training, and Pucci et al. 2006 found no differences in motor unit discharge rate in the vastus lateralis after 3 weeks of resistance training (Rich and Cafarelli, 2000, Pucci et al., 2006). Regarding recruitment threshold, Del Vecchio et al. 2019 found a decrease in the motor unit recruitment threshold of the anterior tibialis muscle after 4 weeks of resistance training (Del Vecchio et al., 2019). However, Sterczala et al. 2020 reported no differences in the motor unit recruitment threshold of the vastus lateralis muscle after 8 weeks of resistance training (Sterczala et al., 2020). Only one study assessed the motor unit discharge rate variability, which showed a decrease after 6 weeks of resistance training (Vila-Chã and Falla, 2016). These differences may be explained by the characteristics of the training protocol applied (duration, single-joint vs multi-joint exercises, and determination of the load applied) or the muscle assessed (Elgueta-Cancino et al., 2022). Nevertheless, to the best of our knowledge there are no studies investigating the effects of strength training on the motor unit firing rate parameters in musculoskeletal conditions.

1.6 THESIS AIMS

This thesis presents a series of studies that examine the morpho-mechanical parameters of the Achilles tendon and the neuromuscular properties of the triceps surae muscles in individuals with NIAT and the effect of eccentric and concentric exercises on these parameters.

1.7 OBJECTIVES

The objectives of this thesis have been separated by Chapter.

Chapter 2. Synthesise the current literature regarding 1) Triceps surae-Achilles tendon neuromechanical changes in individuals with non-insertional Achilles tendinopathy and insertional Achilles tendinopathy and 2) The effect of exercise-induced mechanical tendon loading on neuromechanical changes in individuals with non-insertional Achilles tendinopathy and insertional Achilles tendinopathy

Chapter 3. Determine the relationship between triceps surae motor unit firing properties and the morpho-mechanical parameters of the Achilles tendon in asymptomatic individuals.

Chapter 4. Investigate triceps surae motor unit firing parameters in individuals with non-insertional Achilles tendinopathy compared to controls at low, intermediate, and high force levels during isometric plantarflexion contractions.

Chapter 5. Investigate the changes in triceps surae motor unit firing properties, pain and function, and Achilles tendon morpho-mechanical properties after applying a 6-week intervention protocol based on either controlled eccentric or concentric contractions in individuals with non-insertional Achilles tendinopathy. Furthermore, we aimed to examine whether exercise-induced changes in triceps surae motor unit firing properties differed from asymptomatic controls.

CHAPTER 2

Neuromechanical changes in Achilles tendinopathy and the effects of exercise-induced mechanical tendon loading. A systematic review.

This chapter reports in full the contents of a manuscript that has been prepared for publication along with changes made in the text specifically for the context of this thesis. Additionally, the methodology of this chapter has been previously published in a protocol manuscript before conducting the systematic review (Appendix 1).

Publication:

Ignacio Contreras-Hernandez, Deborah Falla, Alessandro Schneebeli, Eduardo Martinez-Valdes. Neuromechanical changes in Achilles tendinopathy and the effects of exercise-induced mechanical tendon loading: a protocol for a systematic review, BMJ Open 2022;12: e050186

Manuscript in preparation:

Ignacio Contreras-Hernandez, Deborah Falla, Alessandro Schneebeli, Eduardo Martinez-Valdes. Neuromechanical changes in Achilles tendinopathy and the effects of exercise-induced mechanical tendon loading. A systematic review.

Authors Contributions:

IC-H, EM-V, and DF conceived and designed research. IC-H and AS performed data curation and formal analysis. IC-H prepared figures and tables. IC-H drafted manuscript. EM-V, and DF edited and revised manuscript.

2.1 ABSTRACT

AT is commonly classified as non-insertional and insertional; however, they are two distinct disorders with different underlying pathophysiology and management options. This study aimed to synthesise the current literature regarding 1) Triceps surae-Achilles tendon neuromechanical changes in individuals with NIAT and IAT and 2) The effect of exercise-induced mechanical tendon loading on neuromechanical changes in individuals with NIAT and IAT. The systematic review was performed in a two-step process to address each aim. Systematic electronic searches were performed in Pubmed, MEDLINE, EMBASE, CINAHL Plus, Web of Science and SPORTDiscus from inception to August 2023. Additionally, grey literature and key journals were searched. Eligible studies must investigate the neuromuscular properties of the triceps surae muscle or the morpho-mechanical properties of the Achilles tendon in individuals with NIAT or IAT, or the effect of exercise-induced mechanical tendon loading on the neuromuscular properties of the triceps surae muscle or the morpho-mechanical properties of the Achilles tendon in individuals with NIAT or IAT. Two reviewers independently conducted screening, data extraction, and quality assessment for Steps 1 and 2. Data synthesis was conducted for each step. Thirty-nine studies were included in Step 1, and thirteen in Step 2. To facilitate the interpretation of the results the outcomes of interest were grouped in morpho-mechanical and neuromuscular properties for individuals with NIAT and IAT separately. There was consistent evidence showing that individuals with NIAT and IAT exhibit changes in the triceps surae muscle's neuromuscular properties and Achilles tendon morpho-mechanical parameters; however, exercise-induced mechanical tendon loading showed inconsistent results in modifying the morpho-mechanical properties of the Achilles tendon in individuals with NIAT, with no studies assessing these properties in individuals with IAT. Furthermore, no studies have investigated the effect of exercise-induced mechanical tendon loading on the neuromuscular properties of the triceps surae muscle in individuals with NIAT and IAT.

2.2 INTRODUCTION

AT, whether non-insertional or insertional, is a debilitating overuse injury causing significant functional impairment in both athletic and general populations (Kvist, 1994, Rolf and Movin, 1997). Common complaints are pain during and after physical activity, tenderness to palpation and morning stiffness (Alfredson, 2003, Paavola et al., 2002, Kader et al., 2002). Patients may also experience pain during daily activities such as walking or driving (Kader et al., 2002, Paavola et al., 2002), or during more challenging functional tasks such as running and hopping (Sancho et al., 2019). NIAT and IAT are two distinct disorders with different underlying pathophysiology and management options (Maffulli et al., 2020). IAT differs from NIAT, not only in the location of symptoms at the tendon's calcaneal insertion but also in the bony-deformity in the posterior region of the calcaneus commonly observed in individuals with IAT (Chimenti et al., 2014).

Several studies have investigated the morpho-mechanical properties of the Achilles tendon in individuals with NIAT, showing increases in tendon thickness, CSA, volume (Shalabi et al., 2004, Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Docking and Cook, 2016), hysteresis, and longitudinal and transverse strain. Furthermore, studies have reported reductions in stiffness and Young's modulus (Arya and Kulig, 2010, Child et al., 2010, Wang et al., 2012, Chang and Kulig, 2015). However, a limited number of studies have assessed these properties in individuals with IAT. It has been observed in individuals with IAT that the symptomatic leg have higher tendon diameter, strain and lower stiffness (whole tendon) compared to the asymptomatic leg and controls (Chimenti et al., 2014). Though, it also been reported low levels of compressive strain in the deep and superficial tendon regions compared to controls (Chimenti et al., 2017), supporting the theory that repeated compression of the Achilles tendon insertion during functional activities could produce an adaptation of the tendon insertion, resulting in a stiffer tissue (Chimenti et al., 2017).

The muscle-tendon unit functions as a dynamic element in human movement, capable of working as a motor, damper, and spring for exerting, absorbing, and releasing

energy (Alexander, 1991, Roberts and Azizi, 2011). These complex functions are possible through the interconnected activation of force-generating tissues and the transmission of force by passive tissues, employing the capacity to shift energy between active and passive components (Bojsen-Møller and Magnusson, 2019). However, this adaptability requires that the amount of elastic energy stored should be modulated by the muscular contraction (Lichtwark and Wilson, 2006). Therefore, changes in the morpho-mechanical properties of the Achilles tendon may influence the behavior of muscle fascicles and triceps surae motor unit firing properties. Within this framework, a decrease in tendon stiffness will potentially increase both motor unit rate coding and muscle fascicles shortening length to account for the increased tendon compliance, which would ultimately restrict the muscle's capacity to function within the force-length curve's optimal region (Arya and Kulig, 2010).

Several neuromechanical changes have been reported in individuals with NIAT (Chang and Kulig, 2015). Neuromechanical adaptations include how the mechanical system may offload a task required by the CNS (Nishikawa et al., 2007), a process possible only through a close interaction between the sensory and motor systems. In particular, it has been shown that individuals with NIAT have longer electromechanical delay (EMD) (Chang and Kulig, 2015), lower contribution of the gastrocnemius lateralis (LG) for the production of plantar flexor torque at low forces (Crouzier et al., 2020), greater levels of intra-cortical inhibition associated with lower plantarflexion endurance (Fernandes et al., 2022), higher spinal and supraspinal responses (V-wave and H-reflex, respectively) (Chang and Kulig, 2015), longer preactivation of the triceps surae muscles (Chang and Kulig, 2015) and higher co-contraction ratio (ratio of activation between tibialis anterior muscle and plantar flexor muscles) (Chang and Kulig, 2015).

Despite its prevalence, the management of AT lacks evidence-based support (Maffulli et al., 2020). Over the last decade, tendon-loading rehabilitation programs, particularly interventions that include eccentric exercises for the triceps surae muscle, have emerged as the main non-surgical treatment option for the management of AT (Alfredson et al., 1998). However, evidence of histological changes after a program of

eccentric exercise are lacking, and the mechanisms by which eccentric exercises may help to resolve the symptoms of tendinopathy remain unclear (Maffulli et al., 2020). Consistent with this notion, a recent systematic review concluded that there is little clinical or mechanistic evidence supporting the use of isolated eccentric exercises alone (Malliaras et al., 2013). Well-conducted studies comparing different loading programs are still scarce (Malliaras et al., 2013). The purpose of exercise-based rehabilitation programs is to apply controlled mechanical loads to the tendon to promote remodeling, decrease pain, and improve triceps surae muscle endurance and strength (Silbernagel et al., 2007a, Silbernagel and Crossley, 2015). Nonetheless, the successful management of the AT remains challenging, possible due to an incomplete understanding of the effect of loading parameters (i.e., load progression, load magnitude, frequency (sets and repetitions), and interval of recovery between treatment sessions (Beyer et al., 2015).

There are several systematic reviews about the effects of exercise on individuals with AT. However, most are focused on pain and function, relying predominantly on self-reported outcome measures. Given the substantial variability in self-reported outcomes among the population, the conclusions drawn from these studies should be interpreted cautiously. Although two recent systematic reviews presented the effect of exercise in the morphological properties of the Achilles tendon in individuals with AT (Merza et al., 2021, Färnqvist et al., 2020). To date, no systematic review has examined the neuromechanical changes that occur in individuals with AT or the effects of exercise on these properties. Thus, the aim of this systematic review is to synthesise the current literature regarding 1) Triceps surae-Achilles tendon neuromechanical changes in individuals with NIAT and IAT and 2) The effect of exercise-induced mechanical tendon loading on neuromechanical changes in individuals with NIAT and IAT.

2.3 METHODS

This systematic review was planned according to the Cochrane Handbook for Systematic Reviews (Higgins JPT TJ, 2022.) and the review is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 26-01-2021 (CDR42021231933) and published in advance (Contreras-Hernandez et al., 2022b).

2.3.1 Eligibility criteria

The PICOS framework (Population, Intervention, Comparison, Outcomes, and Study Design) was used to define the eligibility criteria for the inclusion and exclusion of studies (Shamseer et al., 2015, Smith et al., 2011). Due to the characteristics of this systematic review, we used the “Indicator” in the same category of “Intervention” as it has been used previously (Arvanitidis et al., 2021, Devecchi et al., 2019).

2.3.2 Population

The population of interest were adults (aged 18 to 65 years) with NIAT or IAT and pain-free adults as a control group. Included studies must have stated the criteria for the identification of individuals with NIAT or IAT. Due to the prevalence of bilateral Achilles tendinopathy and to avoid excluding relevant articles identified during the scoping search, individuals with bilateral symptoms were also included. Studies that assessed the asymptomatic lower limb as a control were also included. Studies that included participants with underlying medical conditions (e.g. neurological disorders, systemic inflammatory conditions, cardiovascular diseases) and/or history of Achilles tendon surgery were excluded.

2.3.3 Intervention/Indicator

To accomplish the first aim of this systematic review, eligible studies were those which included the use of any electrophysiological technique (e.g. transcranial magnetic stimulation, cervicomedullary magnetic stimulation, peripheral nerve stimulation, surface electromyography, intramuscular electromyography, or high-density surface electromyography) to assess neuromuscular, spinal, subcortical or supraspinal changes induced by AT. Moreover, eligible studies were those which have used ultrasonography or MRI to measure the morphological and/or mechanical properties of the Achilles tendon. Likewise, to address the second aim of this systematic review eligible studies were those which included the use of any electrophysiological technique to assess the neuromuscular, spinal, subcortical or supraspinal changes produced by any exercise-induced mechanical tendon loading protocol (e.g. rehabilitation protocols, plyometric exercises, stretching, eccentric, isometric or concentric contractions). Furthermore, eligible studies were those which included the use of ultrasonography or MRI to determine the changes in the morphological and/or mechanical properties of the Achilles tendon produced by any exercise-induced mechanical loading.

2.3.4 Comparison

Included studies involved a comparison of the morpho-mechanical properties of the Achilles tendon or neuromuscular properties of the triceps surae muscle between individuals with AT and controls or between symptomatic and asymptomatic lower limbs. Similarly, studies assessing the effects of exercise-induced mechanical tendon loading included a comparison of the morpho-mechanical properties of the Achilles tendon or the neuromuscular features of the triceps surae muscle in individuals with AT and controls, between symptomatic and asymptomatic lower limbs or before and after intervention.

2.3.5 Outcomes

Primary outcomes included the morpho-mechanical properties of the Achilles tendon and the neuromuscular features of the triceps surae muscle. The morpho-mechanical properties of the Achilles tendon included thickness (mm), CSA (mm^2), length (cm), volume (cm^3), stiffness (N/mm or kPa), strain (%), stress (kPa), modulus (kPa), creep, and elasticity (kPa). The neuromuscular features of the triceps surae muscle included the amplitude and timing of EMG activity of the gastrocnemius-soleus (millivolts and milliseconds, respectively), tibialis anterior and gastrocnemius-soleus co-activation (co-contraction ratio, %), H-reflex (peak-to-peak amplitude, microvolts), F-wave or V-wave obtained by peripheral nerve stimulation (tibial nerve), motor evoked potentials (MEPs, microvolts) obtained from transcranial and cervicomedullary magnetic stimulation (TMS and CMEPs) and motor unit data (motor unit discharge rate, Hz; variability of discharge rate, i.e., coefficient of variation of the inter-spike interval (COVisi)) obtained from intramuscular and/or high-density surface EMG recordings (millivolts). Secondary outcomes included type of tendinopathy (mid-portion or insertional), severity of symptoms (Victorian Institute of Sports Assessment-Achilles questionnaire (VISA-A) or Visual Analog Scale (VAS) or Numerical Rating Scale (NRS)), diagnostic confirmation (clinical assessment and/or ultrasound evaluation), and physical activity level (International Physical Activity Questionnaire (IPAQ), hours of physical activity per week, etc.).

2.3.6 Study design

Randomised controlled trials (RCT) and non-randomised controlled trials (i.e., cohort, cross-sectional studies and observational studies) were considered to address both objectives of this systematic review adequately. The following study types were excluded: non-original literature (e.g., systematic, and narrative reviews), single-case studies, study protocols, cadaveric or animal studies as well as studies not written in English.

2.3.7 Information sources

The following electronic databases were searched initially from inception to February 2021 by one reviewer (IC); updated up to August 2023 by the same reviewer (IC): Pubmed, MEDLINE (Ovid Interface), EMBASE (Ovid Interface), CINAHL Plus (EBSCO Interface), Web of Science (WOS; Clarivate Analytics) and SPORTDiscus (EBSCO Interface). Additionally, hand searching of key journals was conducted, including the Journal of Physiology, Journal of Electromyography and Kinesiology, Journal of Biomechanics, Journal of Applied Physiology, British Journal of Sports Medicine, Journal of Orthopaedic & Sports Physical Therapy, Journal of Science and Medicine in Sports, Isokinetics and Exercise Science, Clinical Biomechanics and Medicine and Science in Sports and Exercise. The eligibility of the studies identified in hand searching was defined using the PICOS framework. Grey literature was included in the search using the British national bibliography for report literature (BNBRL), ProQuest Dissertations & These Global, OpenGrey database and EThOs to reduce the risk of publication bias. Reference lists of included studies and relevant systematic reviews were checked for any further studies, accordingly with the MECIR standards (Higgins JPT, 2016).

2.3.8 Search strategy

This search was conducted in a two steps process:

Step1. Initial search to identify studies with morpho-mechanical properties of the Achilles tendon or neuromuscular properties of the triceps surae muscle in individuals with NIAT or IAT (Appendix 3).

Step 2. Secondary search identifying studies assessing the effects of exercise-induced tendon mechanical loading on the morpho-mechanical properties of the Achilles tendon or the neuromuscular features of the triceps surae muscle in individuals with NIAT or IAT (Appendix 4).

Two independent reviewers (IC and AS) completed the search and identified potential studies to be included in each step. No restrictions in terms of date, design, or language were applied, to ensure the inclusion of all relevant articles. The search strategy was developed considering medical subject heading (MESH) terms to improve search results. A search strategy example for MEDLINE (Ovid Interface) database of each step was provided in the published protocol (Contreras-Hernandez et al., 2022b) and includes MESH terms, keywords and search strings to ensure maximal retrieval (Jenuwine and Floyd, 2004). Specific search terms were modified to reflect differences in keywords and syntax between databases, but search strategy consistency was guaranteed.

2.3.9 Data management

Literature search results for each step, including citation and abstract of potentially eligible studies, were imported into EndNote X9 (Clarivate Analytics PCL) reference manage software by one reviewer (IC), allowing the identification and removal of any duplicates before the screening process. Abstracts and full texts of potentially eligible studies were saved in an individual folder for each reviewer (IC, AS) and eligible studies were retrieved and stored in EndNote X9.

2.3.10 Study selection

For both steps, two independent reviewers (IC, AS) assessed titles and abstracts of identified studies and classified them into definitely eligible, definitely ineligible, or doubtful (Lefebvre C, 2019.) based on pre-defined eligibility criteria. Then, the reviewers performed full-text screening of potentially eligible studies independently. Full text was sought for any articles which could not be excluded based on the information in the abstract. Articles were included if both reviewers agreed on eligibility. Any disagreements between the reviewers in the study selection process were resolved by discussion. PRISMA flow diagram was used to summarise the study selection process (Shamseer et al., 2015).

2.3.11 Data collection process and data items

One reviewer (IC) extracted the data from the eligible studies using a customised data extraction form and then the accuracy was confirmed by a second reviewer (AS). A third reviewer (EMV) was available to mediate any disagreements. Authors of the primary studies were contacted if any critical information that needed to be extracted was missing. In the case that authors did not reply with the requested information, WebPlotDigitizer software was used to extract data from figures. The following items were extracted: Population (inclusion/exclusion criteria, sample size, demographics (i.e., sex, age, height, weight, and physical activity level) and clinical characteristics, such as severity of symptoms, duration of symptoms, diagnostic confirmation, and type of tendinopathy; Intervention/Indicator (measurement instrument, type of task performed or type of intervention exercise applied); Outcomes (morpho-mechanical properties of the Achilles tendon or neuromuscular features of the triceps surae muscle/metrics); and Results (mean and SD of outcome measures and their statistical significance). If any eligible studies included more than two groups, data was extracted only from those who met the eligibility criteria.

2.3.12 Risk of bias

The risk of bias was determined independently by two reviewers (IC, AS) using Rob 2, ROBINS-I and modified version of the Newcastle-Ottawa Scale (NOS). RoB 2 is a tool used to assess the risk of bias in randomised trials and includes the domains: bias arising from the randomisation process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, bias in selection of the reported result, and overall bias (Sterne et al., 2019). The options for a domain-level risk-of-bias judgement were “Low”, “Some concerns”, and “High” risk of bias (Sterne et al., 2019).

ROBINS-I is a tool used to determine the risk of bias in non-randomised studies of interventions and include the domains: bias due to confounding, bias in selection of participants into the study, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, and bias in the selection of the reported result (Sterne et al., 2016). The options for a domain-level risk-of-bias judgement were “Low”, “Moderate”, “Serious” or “Critical” risk of bias, with an additional option of “No information” (Sterne et al., 2016). Disagreements between the reviewers regarding the risk of bias were resolved by discussion.

NOS is the most used tool to assess the risk of bias in observational studies. It has been reported that NOS has moderate to good inter-rater reliability with a short scoring time (Losilla et al., 2018). An adapted version of the NOS for cross-sectional studies was used since the original version of the scale was developed to assess the risk of bias in cohort and case-control studies. Studies were rated in three main domains by using eight items overall: selection (four items), comparability (one item) and exposure (three items) (Wells et al., 2014). The same star ranking system was used as in the published version; however, the “exposure” domain of the original scale, was modified to “outcome”(Herzog et al., 2013), with the number of stars in each item of the studies classified as “good”, “fair” and “poor” quality (Wells et al., 2014).

2.3.13 Data synthesis

Due to the heterogeneity of the studies in Step 1 (i.e., region of the tendon assessed, the difference in the control group (control or asymptomatic leg as a control), and task performed) and Step 2 (i.e., tendon region assessed, exercise intervention, and timepoints of measurements), meta-analysis was not possible; therefore, a narrative synthesis was conducted. Synthesis without meta-analysis was conducted in accordance with recent guidelines to facilitate the interpretation of findings and the identification of patterns in data (Campbell et al., 2020). Results for Step 1 and Step 2 were reported independently. Additionally, outcome measures of interest were grouped in morpho-

mechanical and neuromuscular properties. For each outcome measure of interest, results were reported for individuals with NIAT and IAT separately.

2.3.14 Quality of evidence

Overall certainty of evidence was determined using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE), following guidance from the GRADE handbook (Schünemann et al., 2020). Studies were categorised into the outcomes they measured. Outcomes were assessed using six criteria: study design, study limitations, inconsistency, indirectness, imprecision, and publication bias. Outcomes from randomised trials and non-randomised trials were assessed separately. Overall, the level of evidence was identified as “High”, “Moderate”, “Low”, or “Very Low” (Balshem et al., 2011).

2.4 RESULTS

2.4.1 STEP 1

2.4.1.1 Study selection

The database search retrieved a total of 8879 records. After the removal of duplicates, the titles and abstracts of 5069 records were screened by the two reviewers and, the full text of 117 studies were screened. 39 studies fulfilled the inclusion criteria and were included in the quantitative narrative synthesis (**Figure 7**). Study characteristics can be found for the 39 included studies in **Table 1**.

2.4.1.2 Characteristics of included studies

Of the 39 included studies, 2 were RCT(Malmgaard-Clausen et al., 2021, Petersen et al., 2007), 5 were NRSI (Resteghini and Yeoh, 2012, Shalabi et al., 2004, Tsehaie et al., 2017, Grigg et al., 2012, Pingel et al., 2013), and 32 were observational studies (Aggouras et al., 2022, Alghamdi et al., 2022, Arya and Kulig, 2010, Aubry et al., 2015, Azevedo et al., 2009, Baur et al., 2011, Callow et al., 2022, Child et al., 2010, Chimenti et al., 2017, Chimenti et al., 2014, Crowley et al., 2022, Debenham et al., 2016, Fernandes et al., 2023, Finnamore et al., 2019, Grigg et al., 2012, Wang et al., 2011, Intziegianni et al., 2016, Lalumiere et al., 2020, Nadeau et al., 2016, Nuri et al., 2018, Pingel et al., 2013, Reid et al., 2012, Romero-Morales et al., 2019, Scholes et al., 2018, Shim et al., 2019, Syha et al., 2007, Szaro and Ghali Gataa, 2021, Schie et al., 2010, Wang et al., 2012, Wyndow et al., 2013, Zhang et al., 2017, Chang and Kulig, 2015, Fernandes et al., 2022, Crawford et al., 2023) published between 2004 and 2023. Thirty-two studies investigated individuals with NIAT, five studies investigated individuals with IAT and two studies investigated individuals with NIAT and IAT in different groups. Twenty-nine studies had a control group and ten used the asymptomatic leg as a control group. In total, 842 participants with NIAT, 130 with IAT and 741 healthy participants were

included. The average age for the individuals with NIAT was 43.9 (range from 24.2 to 56.3 years). The average age in the studies assessing individuals with IAT was 50.6 (range from 35 to 61 years) and the age of the healthy individuals was 41.0 (range from 23.2 to 58.2 years). The average VISA-A score for the NIAT participants was 64.1 (range from 37.2 to 87) and for the IAT participants the VISA-A score was 54.3 (range from 47.6 to 59.9).

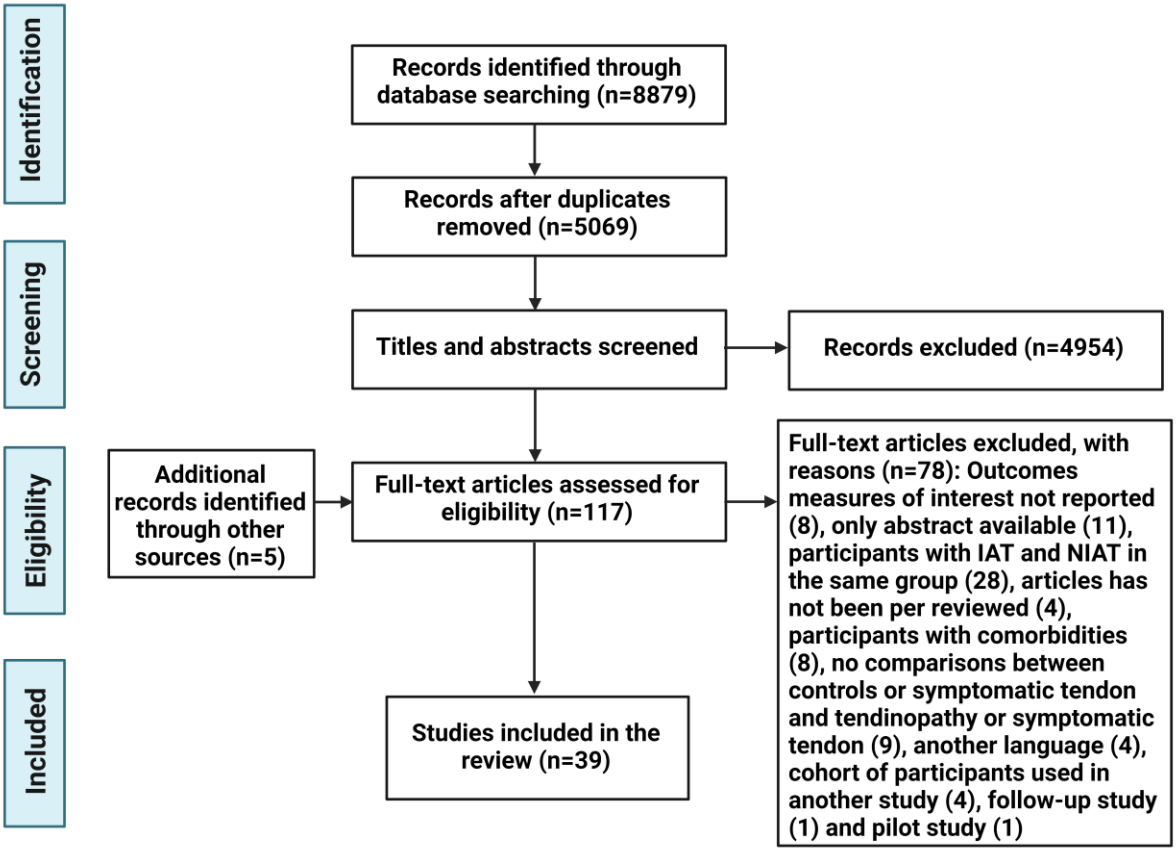

Figure 7. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 flow diagram for Step 1.
Other sources were identified through searching gray literature and hand searching.

2.4.1.3 Narrative synthesis of results

The studies included in Step 1 had a significant degree of heterogeneity with respect to the region of the tendon assessed, the control group (control or asymptomatic leg as a control), and type of task performed. Therefore, meta-analysis was inappropriate

and narrative synthesis was reported. To facilitate the interpretation of the results, the outcome measures of interest were grouped in morpho-mechanical and neuromuscular parameters, respectively.

2.4.1.4 Morpho-mechanical parameters

Thickness. Five studies investigated the maximal thickness of the Achilles tendon in individuals with NIAT (Callow et al., 2022, Crawford et al., 2023, Intziegianni et al., 2016, Lalumiere et al., 2020, Scholes et al., 2018), and one in individuals with IAT (Callow et al., 2022). In individuals with NIAT, the maximal tendon thickness was greater compared to controls (Callow et al., 2022, Intziegianni et al., 2016, Lalumiere et al., 2020). Similarly, the maximal tendon thickness was greater in the symptomatic leg compared to the asymptomatic (Crawford et al., 2023, Lalumiere et al., 2020, Scholes et al., 2018). In individuals with IAT, the maximal tendon thickness was greater compared to controls (Callow et al., 2022). Two and three studies assessed tendon thickness at the distal tendon insertion in individuals with NIAT (Callow et al., 2022, Child et al., 2010), and IAT (Aggouras et al., 2022, Callow et al., 2022, Chimenti et al., 2017), respectively, with no difference in tendon thickness at insertion between the symptomatic leg and controls in individuals with NIAT (Callow et al., 2022, Child et al., 2010). In individuals with IAT, tendon thickness at insertion was greater in the symptomatic leg compared to controls (Aggouras et al., 2022, Callow et al., 2022, Chimenti et al., 2017). Two studies measured the average thickness of the tendon in individuals with NIAT (Lalumiere et al., 2020, Nuri et al., 2018), and one in individuals with IAT (Zhang et al., 2017). In individuals with NIAT, average thickness was greater in the symptomatic leg compared to the asymptomatic and controls (Lalumiere et al., 2020, Nuri et al., 2018). In individuals with IAT, the average tendon thickness was greater in the symptomatic leg compared to controls (Zhang et al., 2017). Seven studies assessed the tendon thickness at different regions in individuals with NIAT (Alghamdi et al., 2022, Callow et al., 2022, Child et al., 2010, Grigg et al., 2012, Malmgaard-Clausen et al., 2021, Nadeau et al., 2016, Romero-Morales et al., 2019), and three in individuals with IAT (Alghamdi et al., 2022, Callow et al., 2022, Chimenti et al., 2014). In individuals with NIAT, tendon thickness at the myotendinous junction (MTJ) of

the SO muscle, at the moment arm and 2 cm proximal to the posterosuperior calcaneal edge were greater in the symptomatic leg compared to the controls (Child et al., 2010) and the asymptomatic leg (Callow et al., 2022, Malmgaard-Clausen et al., 2021). Additionally, tendon thickness at 2 cm, 4 cm and 6 cm from the tendon insertion was greater in the asymptomatic leg compared to the asymptomatic and controls (Grigg et al., 2012, Nadeau et al., 2016, Romero-Morales et al., 2019). Moreover, tendon thickness at 1 cm, 2 cm, and 3 cm from the posterosuperior calcaneal edge was greater in the symptomatic leg compared to controls (Syha et al., 2007). One study reported no difference in tendon thickness at the posterosuperior calcaneal edge between the symptomatic leg, the asymptomatic and controls (Alghamdi et al., 2022). In individuals with IAT, tendon thickness at the posterosuperior calcaneal edge, at the MTJ of the SO muscle and 2 cm proximal to the tendon insertion were greater in the symptomatic leg compared to the control (Chimenti et al., 2014) and the asymptomatic leg (Alghamdi et al., 2022, Callow et al., 2022).

Antero-posterior diameter. Eight studies assessed the maximal antero-posterior diameter of the Achilles tendon in individuals with NIAT (Finnamore et al., 2019, Lalumiere et al., 2020, Resteghini and Yeoh, 2012, Scholes et al., 2018, Schie et al., 2010, Szaro and Ghali Gataa, 2021, Tsehaie et al., 2017, Petersen et al., 2007), and none in individuals with IAT. In individuals with NIAT, the maximal antero-posterior diameter was greater compared to the control group (Finnamore et al., 2019, Lalumiere et al., 2020, Schie et al., 2010, Szaro and Ghali Gataa, 2021). Likewise, the maximal antero-posterior diameter was greater in the symptomatic leg compared to the asymptomatic one (Lalumiere et al., 2020, Resteghini and Yeoh, 2012, Scholes et al., 2018, Tsehaie et al., 2017, Petersen et al., 2007). The mean antero-posterior diameter and the antero-posterior diameter at 6 cm from the insertion were also measured in individuals with NIAT (Lalumiere et al., 2020, Nadeau et al., 2016), showing increased mean antero-posterior diameter (most painful region of the tendon) in the symptomatic leg compared to controls and the asymptomatic leg (Lalumiere et al., 2020) and increased antero-posterior diameter at 6 cm from the insertion in the symptomatic leg compared with the asymptomatic (Nadeau et al., 2016).

Cross-sectional area. Two studies investigated the maximal CSA of the Achilles tendon in individuals with NIAT (Callow et al., 2022, Tsehaie et al., 2017), and one in individuals with IAT (Callow et al., 2022). In individuals with NIAT, maximal CSA in the symptomatic leg was greater compared to the control group (Callow et al., 2022). Similarly, maximal CSA was greater in the symptomatic leg compared to the asymptomatic (Tsehaie et al., 2017). In individuals with IAT, maximal CSA in the symptomatic leg was greater compared to the control group (Callow et al., 2022). Three studies assessed the average CSA area in individuals with NIAT (Arya and Kulig, 2010, Nuri et al., 2018, Shim et al., 2019), and one in individuals with IAT (Zhang et al., 2017). In individuals with NIAT, the averaged CSA was greater in the symptomatic leg compared to controls (Arya and Kulig, 2010, Nuri et al., 2018, Shim et al., 2019) and the asymptomatic leg (Nuri et al., 2018). In individuals with IAT, no differences were found in averaged CSA between the symptomatic leg and controls (Zhang et al., 2017). Additionally, the CSA at 4 cm and 6 cm from the tendon insertion was determined in individuals with NIAT (Intziegianni et al., 2016, Nadeau et al., 2016, Romero-Morales et al., 2019), CSA at 4 cm and 6 cm from insertion was greater in the symptomatic leg compared to controls (Intziegianni et al., 2016, Nadeau et al., 2016, Romero-Morales et al., 2019). Furthermore, CSA at insertion was assessed in individuals with NIAT and in individuals with IAT (Callow et al., 2022). In the NIAT group, no differences were observed in the CSA at insertion between the symptomatic leg and controls; however, differences in CSA at insertion were observed between the symptomatic leg of the IAT group and controls (Callow et al., 2022). Finally, CSA at the MTJ of the SO was evaluated in individuals with NIAT, and in individuals with IAT (Callow et al., 2022). In individuals with NIAT and IAT, the CSA at the MTJ was greater in the symptomatic leg compared to controls (Callow et al., 2022).

Length. Four studies assessed the Achilles free tendon length in individuals with NIAT (Alghamdi et al., 2022, Nuri et al., 2018, Shim et al., 2019, Szaro and Ghali Gataa, 2021), and one in individuals with IAT (Alghamdi et al., 2022). In individuals with NIAT, no differences were observed in free tendon length (from the tendon insertion to the MTJ of the SO muscle) between the symptomatic leg and the asymptomatic (Alghamdi et al.,

2022), and controls (Shim et al., 2019); however, one study reported increased free tendon length in the symptomatic leg compared con controls (Szaro and Ghali Gataa, 2021). Similarly, no differences were observed in tendon free length (from the calcaneal notch to the MTJ of the SO muscle) between the symptomatic leg, the asymptomatic (Couppé et al., 2020) and controls (Nuri et al., 2018). In individuals with IAT, no differences were observed in the free tendon length between the symptomatic leg and the asymptomatic (Alghamdi et al., 2022). Two studies evaluated the tendon length (from the tendon insertion to the MTJ of the MG muscle) in individuals with NIAT (Arya and Kulig, 2010, Intziegianni et al., 2016), and one in individuals with IAT (Chimenti et al., 2014). In individuals with NIAT, no differences were observed in tendon length between the symptomatic leg and controls (Arya and Kulig, 2010, Intziegianni et al., 2016). In individuals with IAT, no differences were reported in tendon length between the symptomatic leg, the asymptomatic and controls (Chimenti et al., 2014). Additionally, two studies investigated the insertional length of Achilles tendon in individuals with NIAT (Alghamdi et al., 2022, Szaro and Ghali Gataa, 2021), and one in individuals with IAT (Alghamdi et al., 2022). In individuals with NIAT, one study showed no difference in the insertion tendon length between the symptomatic and the asymptomatic (Alghamdi et al., 2022); however, the other study found increased insertion tendon length in the symptomatic leg compared to the asymptomatic (Szaro and Ghali Gataa, 2021). In individuals with IAT, no difference was observed in the insertional tendon length between the symptomatic leg and the asymptomatic (Alghamdi et al., 2022).

Width. One study investigated the maximal width of the Achilles tendon in individuals with NIAT, and none in individuals with IAT. In individuals with NIAT, maximal width was greater in the symptomatic leg compared to controls (Szaro and Ghali Gataa, 2021). Additionally, one study assessed the averaged width of the tendon in individuals with NIAT (Nuri et al., 2018), and none in individuals with IAT. In individuals with NIAT, no difference was observed in the average width between the symptomatic leg, the asymptomatic leg nor controls (Nuri et al., 2018). Moreover, one study evaluated the tendon width at 6 cm from the insertion in individuals with NIAT (Nadeau et al., 2016), and none in individuals with IAT. In individuals with NIAT, the tendon width at 6 cm from

the insertion was greater in the symptomatic leg compared with the asymptomatic (Nadeau et al., 2016). Finally, one study assessed tendon width at insertion in individuals with NIAT (Szaro and Ghali Gataa, 2021), and none in individuals with IAT. In individuals with NIAT, tendon width at insertion was greater in the symptomatic leg compared to the control group (Szaro and Ghali Gataa, 2021).

Volume. Two studies evaluated the volume of the Achilles tendon in individuals with NIAT (Shalabi et al., 2004, Tsehaie et al., 2017), and none in individuals with IAT. In individuals with NIAT, tendon volume (2-12 cm proximal to the tendon insertion) was greater in the symptomatic leg compared to the asymptomatic (Shalabi et al., 2004). In contrast, one study reported no difference in tendon volume (2-7 cm proximal to the tendon insertion) between the symptomatic and the asymptomatic (Tsehaie et al., 2017). Two studies assessed free tendon volume in individuals with NIAT (Nuri et al., 2018, Shim et al., 2019), and none in individuals with IAT. In individuals with NIAT, free tendon volume (from the insertion to the MTJ of the SO muscle) was greater in the symptomatic leg compared to the asymptomatic and controls, during rest and isometric plantarflexion contractions at 50% MVIC (Nuri et al., 2018). However, one study reported no difference in the free tendon volume between the asymptomatic leg and controls (Shim et al., 2019).

Insertional angle. One study assessed the insertional angle of the Achilles tendon in individuals with NIAT, and individuals with IAT (Alghamdi et al., 2022). In individuals with NIAT and IAT, no differences were found in the insertional angle between the symptomatic leg and the asymptomatic (Alghamdi et al., 2022).

Stiffness and Young's modulus. Seven studies investigated the stiffness of the Achilles tendon in individuals with NIAT (Arya and Kulig, 2010, Aubry et al., 2015, Crawford et al., 2023, Intziegianni et al., 2016, Shim et al., 2019, Wang et al., 2012, Chang and Kulig, 2015), and two in individuals with IAT (Chimenti et al., 2014, Zhang et al., 2017), In individuals with NIAT, tendon stiffness was lower in the symptomatic leg compared to the asymptomatic (Wang et al., 2012, Chang and Kulig, 2015) and controls (Arya and Kulig, 2010, Chang and Kulig, 2015). Likewise, Young's modulus was lower in

the symptomatic leg compared to controls (Shim et al., 2019, Arya and Kulig, 2010). Regarding the shear-wave velocity (indirect measure of stiffness), at rest position, axial shear-wave velocity was lower in the symptomatic leg compared to controls, but no difference was observed in the sagittal shear-wave velocity; however, in 0° of plantarflexion axial and sagittal shear-wave velocities were lower in the symptomatic leg compared to controls (Aubry et al., 2015). Correspondingly, sagittal shear-wave velocity was lower in the symptomatic leg compared to the asymptomatic (Crawford et al., 2023). However, one study reported no difference in compliance (measure opposite of stiffness) between the symptomatic leg and controls (Intziegianni et al., 2016). In individuals with IAT, tendon stiffness was lower in the symptomatic leg compared to the asymptomatic and controls (Chimenti et al., 2014). However, one study reported greater mean hardness in the symptomatic leg compared to the control group (Zhang et al., 2017).

Strain and elongation. Five studies investigated the Achilles tendon strain in individuals with NIAT (Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Intziegianni et al., 2016, Nuri et al., 2018), and two studies in individuals with IAT (Chimenti et al., 2017, Chimenti et al., 2014). In individuals with NIAT, during plantarflexion contractions at 50% MVIC, antero-posterior strain, longitudinal strain and CSA strain were higher in the symptomatic leg compared to the asymptomatic and controls, with no differences in the diameter strain between the symptomatic, the asymptomatic and controls (Nuri et al., 2018). Likewise, during ramp plantarflexion MVIC, tendon strain was higher in the symptomatic leg compared to controls (Arya and Kulig, 2010, Child et al., 2010). However, after applying eccentric exercises, antero-posterior tendon strain was lower in the symptomatic leg compared to the asymptomatic and controls (Grigg et al., 2012). Additionally, during single leg vertical jump, no difference was observed in tendon strain between the symptomatic leg and the control group (Intziegianni et al., 2016). In individuals with IAT, during dorsiflexion tasks (standing and partial squatting in an inclined platform), transverse compressive strain and axial tensile strain were lower in the symptomatic leg compared to controls (Chimenti et al., 2017). In contrast, during passive movement from 10° of plantarflexion to 10° of dorsiflexion, tendon strain was higher in the symptomatic leg compared to the asymptomatic and

controls (Chimenti et al., 2014). Two studies investigated the tendon elongation in individuals with NIAT (Arya and Kulig, 2010, Intziegianni et al., 2016), and none in individuals with IAT. In individuals with NIAT, during ramp plantarflexion MVIC, tendon elongation was higher in the symptomatic leg compared with the control group (Arya and Kulig, 2010). Nevertheless, during single leg vertical jump, no difference was observed in tendon elongation between the symptomatic leg and controls (Intziegianni et al., 2016).

Tendon force and stress. One study assessed Achilles tendon force in individuals with NIAT(Arya and Kulig, 2010), and one study in individuals with IAT (Chimenti et al., 2014). In individuals with NIAT, no difference was observed in tendon force between the symptomatic leg and control group (Arya and Kulig, 2010). In individuals with IAT, no differences were observed between the symptomatic leg, the asymptomatic and controls (Chimenti et al., 2014). Two studies evaluated tendon stress in individuals with NIAT (Arya and Kulig, 2010, Shim et al., 2019), and none in individuals with IAT. In individuals with NIAT, tendon stress was lower in the symptomatic leg compared to controls (Arya and Kulig, 2010, Shim et al., 2019). However, no difference in peak stress location was observed between the symptomatic leg and controls (Shim et al., 2019).

Other parameters. One study assessed mechanical hysteresis, elastic energy stored, and elastic energy released in individuals with NIAT (Wang et al., 2012), and none in individuals with IAT. In individuals with NIAT, the symptomatic leg has greater mechanical hysteresis and lower elastic energy store and release than the asymptomatic leg (Wang et al., 2012).

2.4.1.5 Neuromuscular parameters

Amplitude. Four studies investigated the EMG amplitude of the triceps surae muscles in individuals with NIAT (Azevedo et al., 2009, Baur et al., 2011, Pingel et al., 2013, Reid et al., 2012), and none in individuals with IAT. In individuals with NIAT, during running trials at self-selected speed, no differences were observed in the EMG amplitude

of the LG before (20 ms) and after (20 ms) heel strike compared to controls (Azevedo et al., 2009); however, during running trials at a speed of 3.33 m/s, the EMG amplitude of the MG during the weight acceptance (from initial contact and the following 20% of the stride period) and push-off (from 20% of the stride period to toe off) were lower compared to the control group, with no differences in the EMG amplitude of the MG during the preactivation (from onset of EMG activity to touchdown) (Baur et al., 2011). Additionally, during 1-hour treadmill run, the EMG amplitude of the MG was lower in the NIAT group compared to controls, and the comparison between the first 10 minutes with the last 10 minutes of the running task, controls showed an increase in the EMG amplitude of the MG; however, this effect was not observed in individuals with NIAT (Pingel et al., 2013). The EMG amplitude of the LG was higher in the NIAT group compared to controls during the 1-hour treadmill run (Pingel et al., 2013). Furthermore, during the Alfredson protocol, EMG amplitude of the MG and SO was higher in the NIAT group compared to controls, irrespective of the knee position (straight or bent) (Reid et al., 2012).

Frequency. One study assessed the EMG spectral frequency parameters of the triceps surae muscles in individuals with NIAT (Pingel et al., 2013), and none in individuals with IAT. In individuals with NIAT, during 1-hour treadmill run, the EMG frequency of the MG was higher compared to the control group, with no differences in the EMG frequency of the LG (Pingel et al., 2013).

Timing of activation. Three studies evaluated the EMG timing of activation of the triceps surae muscles in individuals with NIAT (Debenham et al., 2016, Wyndow et al., 2013, Chang and Kulig, 2015), and none in individuals with IAT. In individuals with NIAT, during submaximal single-limb hopping, the EMG activity onset of the SO was longer compared to the control group, with no differences in the EMG peak and EMG offset of the SO (EMG onset, EMG peak and EMG offset calculated relative to the ground contact) (Debenham et al., 2016). Moreover, during running trials at 4 m/s, the offset timing of the SO relative to the LG was shorter in the NIAT group compared to controls, with no differences for onset timing between groups (Wyndow et al., 2013). Furthermore, during single-leg hopping, the preactivation (time between the onset of ground reaction force

and EMG amplitude of the MG) was longer in the symptomatic leg compared to the asymptomatic and between side differences were significant between NIAT and controls (Chang and Kulig, 2015).

Electromechanical delay. Two studies assessed the electromechanical delay (EMD) of the triceps surae muscles in individuals with NIAT (Wang et al., 2012, Chang and Kulig, 2015), and one in individuals with IAT (Crowley et al., 2022). In individuals with NIAT, during plantarflexion MVIC, the EMD was longer in the MG and SO muscles of the symptomatic leg compared to the asymptomatic (Wang et al., 2012). Similarly, the averaged EMD of the triceps surae muscles was longer in the symptomatic leg of NIAT group compared to the asymptomatic leg and controls (Chang and Kulig, 2015). In individuals with IAT, during plantarflexion MVIC, no differences were observed in the electromechanical delay of the MG compared to controls (Crowley et al., 2022).

Evoked reflexes. Two studies assessed the evoked reflexes of the triceps surae muscles in individuals with NIAT (Wang et al., 2011, Chang and Kulig, 2015), and none in individuals with IAT. In individuals with NIAT, the V/M_{sup} (V-wave with a superimposed supramaximal intensity stimulation/ superimposed supramaximal M-wave) of the SO muscle was higher in the symptomatic leg compared to the asymptomatic, the rate of EMG rise (between TA and SO) was higher in the symptomatic leg compared to the asymptomatic within the time spans of 0 to 30 ms and 0 to 50 ms, and the mean average voltage (between TA and SO) was higher in the symptomatic leg compared to the asymptomatic leg within the time spans of 0 to 30 ms (Wang et al., 2011). No differences were observed in M_{Hsup}/M_{sup}, M_{Hmax}/M_{max}, H_{max}/M_{max} at rest, and H_{sup}/M_{sup} (H_{max}=H-reflex, M_{Hmax}=M-wave to the H-reflex intensity, H_{sup}=superimposed H-reflex, and the $M_{Hsup=}$ M-wave at the superimposed H-reflex) (Wang et al., 2011). Additionally, the H/M (H-reflex/M-wave) ratio for the SO muscle was higher in the symptomatic leg compared to the asymptomatic leg in individuals with NIAT and side differences were significant between NIAT and controls (Chang and Kulig, 2015). Similarly, V/M (V-wave/M-wave) ratio for the SO muscle was higher in the symptomatic leg compared to the asymptomatic

and between side differences were significant between NIAT and controls (Chang and Kulig, 2015).

Co-contraction ratio. One study evaluated the co-contraction ratio of the ankle dorsiflexor muscles in terms of the ankle plantar flexors muscles in individuals with NIAT (Chang and Kulig, 2015), but none in individuals with IAT. In individuals with NIAT, during a single leg hopping task, the co-contraction ratio between the TA and the ankle plantar flexors (MG, SO, and plantaris longus) was lower on the symptomatic leg compared to the asymptomatic and between side differences were significant between NIAT and controls (Chang and Kulig, 2015).

Motor unit parameters. One study investigated the motor unit discharge rate and coefficient of variation of the discharge rate of the triceps surae muscles in individuals with NIAT (Fernandes et al., 2023), but none in individuals with IAT. During submaximal isometric plantarflexion contractions at 10% and 20% MVIC, discharge rate of the LG increased from 10% to 20% in the control group; however, this change was not observed in individuals with NIAT (Fernandes et al., 2023). Additionally, no differences in the discharge rate of the MG and SO and the coefficient of variation of the interspike interval were observed for the MG, LG, and SO muscles during the submaximal isometric contractions (Fernandes et al., 2023).

Other parameters. One study investigated the intracortical inhibition and the mean active motor threshold of the triceps surae muscles in individuals with NIAT (Fernandes et al., 2022), but none in individuals with IAT. During submaximal isometric plantarflexion contractions at 10% MVIC, the short-interval intracortical inhibition of the triceps surae muscle was higher in individuals with NIAT compared to controls (Fernandes et al., 2022). However, no difference was observed in the mean active motor threshold between NIAT and controls (Fernandes et al., 2022).

2.4.1.6 Methodological quality assessment

2 RCTs were analised with Rob 2, 5 NRSIs with ROBINS-I and 32 observational studies with the NOS. The results of the risk of bias assessment for the observational studies can be seen in **table 2.** The results of the risk of bias for NRISs and RCTs studies can be seen in **figure 8.**

2.4.1.7 Level of evidence (GRADE)

The certainty of the evidence for each outcome assessed by RCTs, NRSIs and observational studies was evaluated separately by two reviewers (Schünemann et al., 2020) and is presented in Appendix 5 (Table 13).

Table 1. Study Characteristics Step 1.

First Author, Year, Type of Study and Type of Tendinopathy	Experimental group. Sample size; sex (f/m); age (yrs); height (cm); weight (kg); level of physical activity (h/week, MET-min week^{-1}) or mileage (km/week)	Control group. Sample size; sex (f/m); age (yrs); height (cm); weight (kg); level of physical activity (h/week, MET-min week^{-1}) or mileage (km/week)	Intensity (VISA-A and VAS or NRS); duration of symptoms (mo); diagnosis	Task	Outcome measure(s)	Results
Aggouras et al. 2022 (Aggouras et al., 2022) Observational IAT	N=8; 3 f/5 m; 57.71 ± 9.89 yrs; BMI: 30.68 ± 7.69	N=8; 5 f/3 m; 39.13 ± 13.45 yrs; BMI: 24.30 ± 3.64	VISA-A 52.97 ± 23.37; Clinical examination	Passive heel lowering task	-Thickness at insertion	↑ Thickness at insertion IAT
Alghamdi et al. 2022 (Alghamdi et al., 2022) Observational NIAT and IAT	IAT. N=17; 9 f/8 m; 48 ± 14 yrs; 174.8 ± 11.6 cm; 74.5 ± 14.7 kg NIAT. N=17; 5 f/12 m; 50 ± 16 yrs; 175.5 ± 8.5 cm; 78.1 ± 16.1 kg	Asymptomatic leg as a control	IAT. VISA-A symptomatic 59.9 ± 18.7, asymptomatic 96.5 ± 3; 18.5 ± 30.8 mo; Clinical examination NIAT. VISA-A symptomatic 59.1 ± 19.7, asymptomatic 94.8 ± 3.9; 17.1 ± 36.2 mo; Clinical examination	Rest	-Thickness at posterosuperior calcaneal edge -Insertional length -Insertion angle -Free length	↑ Thickness at posterosuperior calcaneal edge in the symptomatic leg IAT, not in the NIAT. = Insertional length, insertion angle and free length between groups
Arya and Kulig 2010 (Arya and Kulig, 2010) Observational NIAT	N=12; 47.33 ± 8.3 yrs, 173 ± 9 cm; 86.01 ± 9.62 kg	N=12; 44.83 ± 7.2 yrs, 176 ± 9 cm; 77.97 ± 9.70 kg	NR. Clinical examination and ultrasound imaging	Rest and slow ramped plantarflexion isometric contractions to maximal force	-Average CSA -Length -Stiffness -Young's modulus -Strain -Elongation - Force -Stress	↑ Average CSA, strain, and elongation NIAT = Length and force ↓ Stiffness, Young's modulus, and stress NIAT
Aubry et al. 2015 (Aubry et al., 2015) Observational NIAT	N=25; 11 f/14 m; 56 (46–63) yrs	N=80; 43 f/37 m; 50 (31–57) yrs	NR. Clinical examination and ultrasound imaging	Measurements in two ankle positions: Passive maximal plantarflexion	-Sagittal mean velocity -Axial mean velocity	Position 1 = Sagittal mean velocity ↓ Axial mean velocity NIAT Position 2

				(position 1) and 0° plantarflexion (position 2)		↓ Sagittal and axial mean velocity NIAT
Azevedo et al. 2009 (Azevedo et al., 2009) Observational NIAT	N=21. 5 f/ 16 m; 41.8 ± 9.7 yrs; 177.8 ± 7.4 cm; 77.6 ± 12.6 kg	N=21; 5 f/16 m; 38.9 ± 10.1 yrs; 174.3 ± 8.0; 70.2 ± 10.9 kg	NR. Clinical examination	10 running trials at self-selected speed on a 10-meter pathway	-LG EMG amplitude before heel strike -LG EMG amplitude after heel strike	= LG EMG amplitude before heel strike and after heel strike
Baur et al. 2011 (Baur et al., 2011) Observational NIAT	N=30; 10 f/20 m; 41 ± 7 yrs; 175 ± 6 cm, 72 ± 10 kg; 45 ± 21 km/week	N=30; 10 f/20 m; 37 ± 10 yrs; 174 ± 10 cm; 67 ± 13 kg; 42 ± 14 km/week	NR. Clinical examination and ultrasound imaging	10 running trials at a speed of 3.33 m/s using neutral running shoes	-EMG MG amplitude preactivation -EMG MG amplitude weight acceptance -EMG amplitude push-off	= EMG MG amplitude preactivation ↓ EMG MG amplitude weight acceptance and push-off NIAT
Callow et al. 2022 (Callow et al., 2022) Observational NIAT and IAT	IAT. N=25; 17 f/8 m; 61 ± 18 yrs; 163 ± 11.4 cm; 81.7 ± 17.2 kg NIAT. N=41; 23 f/ 18 m; 55 ± 17 yrs; 170 ± 23.0 cm; 83.9 ± 16.4 kg	Control IAT. N=25; 17 f/8 m; 55 ± 15, 168 ± 8.65 cm; 78.0 ± 14.5 Control NIAT. N=41; 23 f/18 m; 52 ± 12; 171 ± 17.8 cm; 77.1 ± 15.8 kg	NR. Retrospective analysis of medical records and MRI	Rest	-Maximal thickness -Thickness insertion -Thickness MTJ SO -Maximal CSA -CSA insertion -CSA MTJ SO	↑ Maximal thickness, thickness insertion, thickness MTJ SO, maximal CSA, CSA insertion, CSA MTJ SO IAT. ↑ Maximal thickness and thickness MTJ SO, maximal CSA and CSA MTJ SO NIAT
Child et al. 2010 (Child et al., 2010) Observational NIAT	N=14; 40 ± 8; 177 ± 6; 80 ± 9 kg; 48 ± 18 km/week	N=15, 35 ± 9; 178 ± 5 cm; 79 ± 11 kg; 42 ± 13 km/week	VISA-A 70 ± 8; 27 ± 47 mo; Clinical examination and ultrasound imaging	Rest and during maximal isometric voluntary contractions	-Thickness moment arm -Thickness insertion -Strain	↑ Thickness moment arm and strain NIAT = Thickness insertion
Chimenti et al. 2017 (Chimenti et al., 2017) Observational IAT	N=10; 6 f/4 m; 50.3 ± 8.5 yrs; BMI 31.9 ± 6.7	N=10; 5 f/5 m 43.7 ± 15.9 yrs; BMI 25.0 ± 4.3	VISA-A 57 ± 24; Clinical examination and ultrasound imaging	Standing and partial squatting on an inclination platform	-Thickness insertion -Transverse compressive strain -Axial tensile strain	↑ Thickness insertion IAT ↓ Transverse compressive strain and axial tensile strain (standing and partial squat) IAT

Chimenti et al. 2014 (Chimenti et al., 2014) Observational IAT	N=20; 11 f/9 m; 58.6 ± 7.8 yrs; 170 ± 10 cm; 87.5 ± 17.5 kg. Asymptomatic leg also assessed.	N=20; 11 f/9 m; 58.2 ± 8.5 yrs; 170 ± 10 cm; 80.3 ± 16.0 kg	IAT. VISA-A 47.6 ± 26.8; 10 (4-24) mo; Clinical examination Control. VISA-A 100.0 ± 0.0	Rest and passive movement from 10° plantarflexion to 10° dorsiflexion	-Thickness 2 cm -Length -Stiffness -Strain -Elongation -Force	↑ Thickness 2 cm, strain, and elongation symptomatic leg vs asymptomatic vs control = Length and force ↓ Stiffness symptomatic leg vs asymptomatic vs control
Crawford et al. 2023 (Crawford et al., 2023) Observational NIAT	N=20; 7 f/13 m; 56.3 ± 6.1 yrs; 176.1 ± 10.2 cm; 86.3 ± 20.1 kg	Asymptomatic leg as a control	VISA-A 45. 4 ± 20.7; VAS: 7.0 ± 1.4; 14.5 ± 12.9 mo; Clinical examination	Rest	-Maximal thickness -Shear-wave velocity	↑ Maximal thickness symptomatic leg ↓ Shear-wave velocity symptomatic leg
Crowley et al. 2022 (Crowley et al., 2022) Observational IAT	N=34; 43.7 ± 10 yrs; 89.6 ± 16.3 kg; 68.2 ± 16.1 h/week	N=34; 42.8 ± 8.9 yrs; 87.2 ± 9.7 kg; 76.1 ± 21.3 h/week	IAT. VISA-A 54.1 ± 16.6; Clinical examination Control. VISA-A 97.7 ± 0.5	Plantarflexion MVIC	-EMD MG	= EMD MG
Debenham et al. 2016 (Debenham et al., 2016) Observational NIAT	N= 15; 6 f/9 m; 41.2 ± 12.7 yrs; 174.1 ± 9.6 cm; 82.0 ± 12.2 kg	N=11; 8 f/3 m; 23.2 ± 6.7 yrs; 170.1 ± 8.2 cm; 70.7 ± 13.3 kg	MP. VISA-A 64.7 ± 12.7; 12.06 ± 8.24 mo; Clinical examination Control. VISA-A 100	Sub-maximal single limb hopping	-EMG onset SO -EMG peak SO -EMG offset SO	↑ EMG onset SO NIAT = EMG peak SO and EMG offset SO
Fernandes et al. 2023 (Fernandes et al., 2023) Observational NIAT	N=12; 5 f/7 m; 44.3 ± 6.7 yrs; 173 ± 5.7 cm, 76.2 ± 9.3 kg; 38.7 ± 9.1 km/week	N=13; 6 f/7 m; 34.0 ± 4.2 yrs; 171 ± 5.6 cm; 64.8 ± 7.0 kg; 30.4 ± 8.4 km/week	MP. VISA-A 70.1 ± 5.7; Clinical examination Control. VISA-A 100 ± 0	Submaximal plantarflexion isometric contractions at 10% and 20% of the peak torque	-Motor unit discharge rate MG, LG, and SO -Coefficient of variation of motor unit discharge rate MG, LG, and SO	= DR MG, LG, and SO (DR LG interaction intensity x group) = Coefficient of variation of motor unit discharge rate MG, LG, and SO
Finnamore et al. 2019 (Finnamore et al., 2019) Observational NIAT	N= 10; 5 f/5 m; 48 ± 8.9; BMI 24 ± 3.4; 43 (15–80) km per week	N=15; 8 f/7 m; 50 ± 15 yrs; BMI 25 ± 2.8; 34 (8–125) km per week	MP. VISA-A 69 ± 8.1; Clinical examination and ultrasound imaging Control. VISA-A 99 ± 0.5	Rest	-Maximal AP diameter	↑ Maximal AP diameter NIAT

Grigg et al. 2011 (Grigg et al., 2012) NRIS NIAT	N= 11; 49.0 ± 4.5 yrs; 180.6 ± 2,2 cm; 92.6 ± 5,6 kg Asymptomatic leg also assessed	N=9; 48.2 ± 3.8 yrs; 181.6 ± 2.0 cm; 97.3 ± 6.9 kg	VISA-A 62 ± 4; 10 (2-30) mo; Clinical examination	One session of the Alfredson protocol	-Thickness 4 cm -AP Strain	↑ Thickness 4 cm symptomatic leg vs asymptomatic vs control ↓ Strain (after exercise) symptomatic leg vs asymptomatic vs control
Intziegianni et al. 2016 (Intziegianni et al., 2016) Observational NIAT	N=10; 3 f/7 m; 40 ± 7 yrs; 175 ± 9 cm; 77 ± 9 kg	N=10; 3 f/7 m; 37 ± 8 yrs; 178 ± 7 cm; 79 ± 10 kg	VISA-A 87 ± 7; Clinical examination and ultrasound imaging	Rest and single leg vertical jump	-Maximal thickness -CSA 4 cm -Length -Compliance -Strain -Elongation	↑ Maximal thickness and CSA NIAT = Length, compliance, strain, and elongation
Lalumiere et al. 2020 (Lalumiere et al., 2020) Observational NIAT	N=40; 13 f/27 m; 42.7 ± 8.7 yrs; 174.6 ± 8.2 cm; 78.4 ± 15.6 kg Asymptomatic leg also assessed	N=34; 15 f/ 19 m; 39.8 ± 10.8 yrs; 172.4 ± 8.0 cm; 73.3 ±11.9 kg	MP. VISA-A 60.7 ± 18.6; 27.0 ± 30.1 mo; Clinical examination Control VISA-A 100 ± 0	Rest	-Maximal thickness -Mean thickness -Maximal AP diameter -Mean AP diameter	↑ Maximal thickness and mean thickness symptomatic leg vs asymptomatic vs control ↑ Maximal AP diameter and mean AP diameter symptomatic leg vs asymptomatic vs control
Malmgaard-Clausen et al. 2021 (Malmgaard-Clausen et al., 2021) RCT NIAT	N= 35; 9 f/26 m; 40.7 ± 1.7 yrs; BMI 25.1 ± 0.4; 8.5 ± 0.9 hours per week	Asymptomatic leg as a control	VISA-A 70.5 ± 2.4; NRS 4.0 ± 0.3; 1.74 ± 0.12 mo; Clinical examination and ultrasound imaging	Rest	-Thickness 2 cm above the posterosuperior calcaneal region	↑ Thickness 2 cm above the posterosuperior calcaneal region in the symptomatic leg
Nadeau et al. 2016 (Nadeau et al., 2016) Observational NIAT	N= 20; 6 f/14 m; 41.8 ± 9.2 yrs; 175 ± 8 cm; 77.8 ± 16 kg Asymptomatic leg also assessed	N=23; 7 f/16 m; 39.9 ± 11.2 yrs; 174 ± 8 cm; 74.4 ± 10.9 kg	MP. VISA-A 60.9 ± 18.25; Clinical examination Control. VISA-A 100 ± 0	Rest	-Thickness 6 cm -AP diameter 6 cm -CSA 6 cm -Width 6 cm	↑ Thickness 6 cm symptomatic leg ↑ AP diameter 6 cm, CSA 6 cm, and width 6 cm symptomatic leg
Nuri et al. 2018 (Nuri et al., 2018) Observational NIAT	N=10; 42.2 ± 11.59 yrs; 176.6 ± 7.5 cm; 79.8 ± 7.8 kg; 5556 ±	N=10; 41.93 ± 12.26 yrs; 176 ± 9.85 cm; 82.2 ± 7.02 kg; 5969 ±	MP. VISA-A 54.2 ± 16.52; 42 ± 24 mo; Clinical examination and ultrasound	Before and after 10 submaximal plantarflexion isometric	-Average thickness -Average CSA -Free length -Average width	↑ Average thickness, average CSA, longitudinal strain, and CSA

	3067 (MET-min week^{-1}) Asymptomatic leg also assessed	3789 (MET-min week^{-1})	imaging Control. VISA-A 100 ± 0	contractions at 50% MVIC.	-Longitudinal strain -Diameter AP strain -CSA strain -Width strain -Volume	strain symptomatic leg vs asymptomatic vs control = Free length, average width, and width strain ↑ Volume at rest and under load in the symptomatic leg vs asymptomatic vs control
Pingel et al. 2013 (Pingel et al., 2013) NRIS NIAT	N= 9; 4 f/5 m; 53 ± 9 yrs; 180 ± 0 cm; 81 ± 17 kg	N= 9; 4 f/5 m; 54 ± 8 yrs; 175 ± 0 cm; 80 ± 15	VISA-A 47 ± 16	Before and after 1-hour treadmill run	-EMG amplitude MG and LG -EMG frequency MG and LG	↓ EMG amplitude MG NIAT ↑ EMG amplitude LG NIAT ↑ EMG amplitude MG at the end in controls, not NIAT ↑ EMG frequency MG NIAT = EMG frequency LG
Reid et al. 2012 (Reid et al., 2012) NRIS NIAT	N=18; 26 ± 7 yrs; 173 ± 7 cm; 77.6 ± 20 kg	N=18; 30 ± 4; 174 ± 7 cm; 80 ± 14 kg	VISA-A 61 ± 16; 7.6 ± 6.6 mo; Clinical examination	One session of the Alfredson protocol	-EMG amplitude MG and SO	↑ EMG amplitude MG and SO NIAT
Resteghini et al. 2012 (Resteghini and Yeoh, 2012) NRSI NIAT	N=32; 12 f/20 m; 40.3 (25–63) yrs;	Asymptomatic leg as a control	VISA-A 37.2 (10-60) VAS 6.6 (4-9); 17 (6–60) mo; Clinical examination and ultrasound imaging	Rest	-Maximal AP diameter	↑ Maximal AP diameter symptomatic side
Romero-Morales et al. 2019 (Romero-Morales et al., 2019) Observational NIAT	N= 71; 45.11 ± 12.75 yrs; 176 ± 11 cm; 76.00 ± 12.00 kg	N= 72; 37.61 ± 11.91 yrs; 176 ± 12 cm; 75.00 ± 18.50 kg	VISA-A 56.00 ± 14.00; VAS 2.00 ± 3.00; Clinical examination	Rest	-Thickness 4 cm -Thickness 6 cm -CSA 4 cm -CSA 6 cm	↑ Thickness 4 cm and 6 cm NIAT ↑ CSA 4 cm and 6 cm NIAT

Scholes et al. 2018 (Scholes et al., 2018) Observational NIAT	N=21;45.2 ± 13.3 yrs; 176 ± 10 cm; 77.9 ± 14.9 kg	Asymptomatic leg as a control	VISA-A 69 ± 16; Clinical examination and ultrasound imaging	Rest	-Maximal thickness -Maximal AP diameter	↑ Maximal thickness and maximal AP diameter symptomatic
Shim et al. 2019 (Shim et al., 2019) Observational NIAT	N=8; 2 f/6 m; 37 ± 11 yrs; 177 ± 9 cm; 78 ± 13 kg	N=8; 3 f/5 m; 29 ± 4 yrs; 180 ± 6 cm; 75 ± 14 kg	NR. Clinical examination	Rest and submaximal plantarflexion isometric contractions at 50% of the MVIC for MP group and 70% MVIC for the controls	-Average CSA -Length -Free volume -Young's modulus -Strain -Peak stress magnitude -Peak stress location	↑ Average CSA NIAT = Length, free volume, and peak stress location ↓ Young's modulus and peak stress magnitude NIAT Strain NR
Syha et al. 2007 (Syha et al., 2007) Observational NIAT	N=24; 45.8 ± 11.4 yrs	N=35; 38.7 ± 10.9 yrs	NR. Clinical examination and ultrasound imaging	Rest	-Maximal thickness at 1, 2 and 3 cm above the posterosuperior region of the calcaneus. -Mean thickness at 1, 2, and 3 cm above the posterosuperior region of the calcaneus	↑ Maximal thickness and mean thickness in each segment NIAT
Szaro & Ghali Gataa 2021 (Szaro and Ghali Gataa, 2021) Observational NIAT	N=74; 28 f/46 m; 52.9 ± 10.4 yrs	N=81; 33 f/48 m; 35.2 ± 13.6 yrs	NR. Clinical examination and MRI	Rest	-Maximal AP diameter -Free length -Insertion length -Maximal width -Insertion Width	↑ Maximal AP diameter, free length, insertion length, maximal width, and insertion width NIAT
Schie et al. 2010 (Schie et al., 2010) Observational NIAT	N=26; 14 f/12 m; 44.9 ± 6.2; BMI 24.8 ± 2.2	N=26; 10 f/16 m; 43.6 ± 12.6 yrs; BMI 26.1 ± 4.3	VISA-A 67.3 ± 18.2; 48.5 ± 69.4 mo; Clinical examination	Rest	-Maximal AP diameter	↑ Maximal AP diameter NIAT
Wang et al. 2011 (Wang et al., 2011) Observational NIAT	N=14; 4 f/10 m; 24.2 ± 1.7 yrs; 177.3 ± 8.4 cm; 69.2 ± 9.0 kg	Asymptomatic leg as a control	VISA-A 73.1 ± 4.5; NRS 4.3 ± 1.9; 5.4 ± 1.6 mo; Clinical examination and ultrasound imaging	Submaximal and supramaximal electrically evoked isometric plantarflexion contractions during rest	-H_{max}/M_{max} -H_{sup}/M_{sup} -V/M_{sup} - M_{Hmax}/M_{max} -M_{Hsup}/M_{sup} (Spinal reflexes were measured for the SO) -RER ratio (rate	= H_{max}/M_{max} H_{sup}/M_{sup} M_{Hmax}/M_{max} M_{Hsup}/M_{sup} ↑ V/M_{sup}, RER ratio (0 to 30 ms, and 0 to 50 ms), and MAV ratio (0 to

				and maximal voluntary isometric contractions	of EMG rise between TA and SO) -MAV ratio (mean average voltage between TA and SO)	30 ms) symptomatic leg
Wang et al. 2012 (Wang et al., 2012) Observational NIAT	N= 17; 27.3 ± 2.0 yrs; 183.2 ± 7.1 cm; 75.9 ± 10.8 kg	Asymptomatic leg as a control	VISA-A 70.7 ± 7.8; 5.9 ± 1.3 mo; Clinical examination and ultrasound imaging	Maximal isometric plantarflexion and dorsiflexion contractions gradually for 5 seconds	-Stiffness -Mechanical hysteresis -Elastic energy stored -Elastic energy released -EMD MG and SO	↓ Stiffness, stored and released elastic energy symptomatic leg ↑ Mechanical hysteresis symptomatic leg ↑ EMG MG and SO symptomatic leg
Wyndow et al. 2013 (Wyndow et al., 2013) Observational NIAT	N=15; 42 ± 7 yrs; 177 ± 5 cm; 80 ± 11 kg; 43 ± 15 km per week	N=19; 36 ± 8 yrs; 179 ± 5 cm; 77 ± 10 kg; 40 ± 16 km per week	VISA-A 70 ± 10; Clinical examination and ultrasound imaging	Running at 4 m/s along a 25 m walkway	-EMG onset timing MG, LG, and SO -EMG offset timing MG, LG, and SO	= EMG onset timing MG, LG, and SO ↓ Relative offset timing LG and SO NIAT
Zhang et al. 2017 (Zhang et al., 2017) Observational IAT	N=16; 4 f/12 m; 32 (15-53) yrs	N=29; 9 f/20 m; 33 (14-80) yrs	NR. Clinical examination	Rest	-Average thickness -Average CSA -Mean hardness	↑ Average thickness IAT = Average CSA ↑ Mean hardness middle portion IAT, not proximal portion
Chang and Kulig 2015 (Chang and Kulig, 2015) Observational NIAT	N=9; 46.8 ± 6.3 yrs; 170 ± 8 cm; 74.1 ± 13.4 kg; 4720 ± 3437.1 (MET-min) Asymptomatic leg also assessed	N=10; 48.7 ± 4.4 yrs; 170 ± 5; 84.9 ± 19.7 kg; 3983.3 ± 3080.0 (MET-min)	VISA-A MP 79.4 ± 22; Clinical examination and ultrasound imaging VISA-A Control 99.5 ± 1.2;	Plantarflexion MVIC	-Average stiffness -Average EMD -Average preactivation -H/M ratio -V/M ratio -Co-contraction ratio	↓ Stiffness and co-contraction ratio asymptomatic leg vs symptomatic vs control ↑ EMD, preactivation, H/M ratio and V/M ratio asymptomatic leg vs symptomatic vs control
Shalabi et al. 2004 (Shalabi et al., 2004) NRSI NIAT	N=25; 9 f/16 m; 51 yrs.	Asymptomatic leg as a control	NRS pre 5 (2-6); NRS post 3 (1-6); 18 (6-120) mo; Clinical examination	Rest	-Volume	↑ Volume symptomatic leg

Tsehaie et al. 2017 (Tsehaie et al., 2017) NRSI NIAT	N=25; 15 f/ 10 m; 46 ± 9.5 yrs; BMI: 25.9 ± 4.7	Asymptomatic leg as a control	9 (5-21) mo; Clinical examination	Rest	-Maximal diameter -Maximal CSA -Volume	↑ Maximal diameter and CSA symptomatic leg = Volume
Petersen et al. 2007 (Petersen et al., 2007) RCT NIAT	All groups. N=100; 40 f/60 m; 42.5 ± 11.07 yrs; 177.2 ± 8.89 cm; 79.7 ± 14.25 kg	Asymptomatic leg as a control	VAS all groups 1.23 ± 0.23, 7.4 ± 2.3 mo; Clinical examination and ultrasound imaging	Rest	-Maximal AP diameter	↑ Maximal AP diameter symptomatic leg
Fernandes et al. 2021 (Fernandes et al., 2022) Observational NIAT	N=11; 5 f/6 m; 44.1 (35.7 to 52.5) yrs; 172.0 (165.9 to 178.1) cm; 76.2 (65.8 to 86.6) kg; 37.4 (26.6 to 48.2) km/w	N=13; 6 f/7 m; 34 (30.0 to 38.2); 170.6 (165 to 176.2) cm; 64.8 (58 to 71.8) kg; 30.4 (22 to 38.8) km/w	NIAT. VISA-A 70.7 (63.7 to 77.7); Control. VISA-A 100; Clinical examination	Submaximal isometric plantarflexion contractions at 10% MVIC	-Short-interval intracortical inhibition -Mean active motor threshold	↑ Short-interval intracortical inhibition NIAT (all muscles) = Mean active motor threshold

NIAT, non-insertional Achilles tendinopathy; IAT, insertional Achilles tendinopathy; RCT, randomised controlled trial; NRSI, non-randomised interventional study; CSA, cross-sectional area; AP, antero-posterior; EMG, electromyography; MVIC, maximal voluntary isometric contraction; MG, medial gastrocnemius; LG, lateral gastrocnemius; SO, soleus; EMD, electromechanical delay; H, H-reflex; M, M-wave; V, V-wave; sup, superimposed; NR, not reported.

Table 2. Risk of bias assessment observational studies

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Total	Quality assessment
Aggouras et al. 2022	1	0	0	1	1	1	1	5	Fair
Alghamdi et al. 2022	1	1	1	1	0	1	1	6	Poor
Arya et al. 2010	0	1	1	1	2	1	1	7	Good
Aubry et al. 2015	1	0	1	1	1	1	1	6	Good
Azevedo et al. 2009	0	0	1	1	1	1	1	5	Fair
Baur et al. 2011	0	0	1	1	2	2	1	7	Fair
Callow et al. 2022	1	0	1	1	2	2	1	8	Good
Child et al. 2010	0	0	1	1	2	2	1	7	Fair
Chimenti et al. 2017	1	0	1	1	1	1	1	6	Good
Chimenti et al 2014	1	1	1	1	2	1	1	8	Good
Crawford et al. 2023	1	0	1	1	0	1	1	5	Poor
Crowley et al. 2022	0	1	1	1	2	1	1	7	Good
Debenham et al. 2014	1	0	1	1	1	1	1	6	Good
Fernandes et al. 2023	1	1	1	1	1	1	1	7	Good
Finnamore et al. 2019	1	1	1	1	2	2	1	9	Good
Intziegianni et al. 2016	1	1	1	1	2	1	1	8	Good

Lalumiere et al. 2020	1	0	1	1	2	1	1	7	Good
Nadeau et al. 2016	1	0	1	1	1	1	1	6	Good
Nuri et al. 2017	1	0	1	1	2	1	1	7	Good
Reid et al. 2011	1	0	1	1	1	1	1	6	Good
Romero-Morales et al. 2019	1	1	1	1	2	1	1	8	Good
Scholes et al. 2017	0	0	1	1	0	2	1	5	Poor
Shim et al. 2019	1	0	1	1	2	1	1	7	Good
Syha et al. 2007	1	0	1	1	2	2	1	8	Good
Szaro et al. 2021	1	0	1	1	1	1	1	6	Good
van Schie et al. 2010	1	0	1	1	2	2	1	8	Good
Wang et al. 2011	1	0	1	1	0	1	1	5	Poor
Wang et al. 2012	1	0	1	1	0	1	1	5	Poor
Window et al. 2013	0	0	1	1	1	1	1	5	Fair
Zhang et al. 2017	1	0	1	1	1	2	1	7	Good
Chan et al. 2015	1	0	1	1	2	1	1	7	Good
Fernandes et al. 2021	0	1	1	1	1	1	1	6	Good

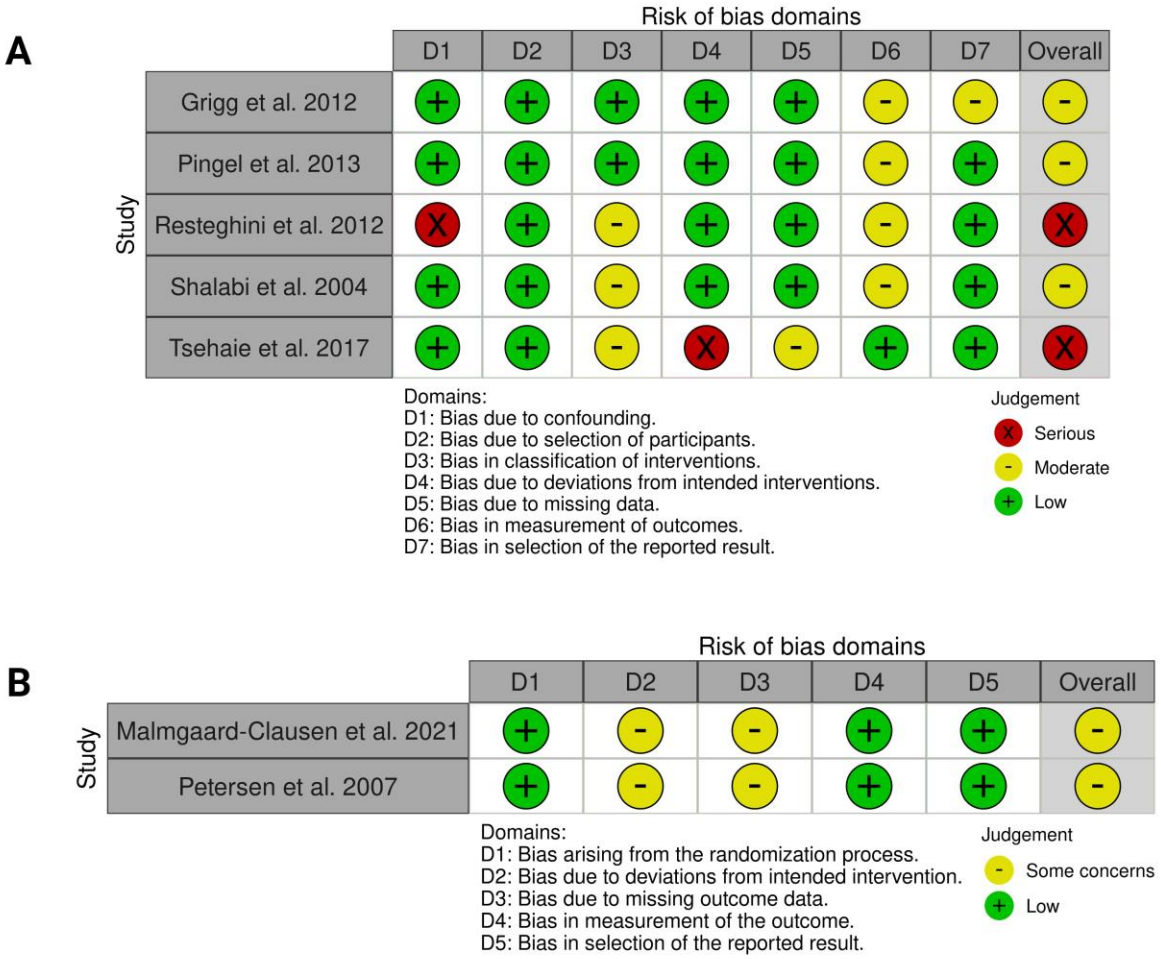

Figure 8. Risk of bias assessment of NRIS studies (A) and RCT studies (B) for Step 1.

2.4.2 STEP 2

2.4.2.1 Study selection

The database search retrieved a total of 4478 records. After the removal of duplicates, 2541 titles and abstracts were screened by two reviewers. A total of 39 studies were full text screened. Finally, 13 studies were included in the final narrative synthesis (**Figure 9**). Study characteristics for the 13 studies can be found in **Table 3.**

2.4.2.2 Characteristics of included studies

Of the 13 included studies 9 were RCT(Demir Benli et al., 2022, Beyer et al., 2015, Boesen et al., 2017, Johannsen et al., 2022, Malmgaard-Clausen et al., 2021, Solomons et al., 2020, Stefansson et al., 2019), and 4 were NRSI (Ohberg et al., 2004, Shalabi et al., 2004, Tsehaie et al., 2017, von Wehren et al., 2019). These studies were published between 2004 and 2022. All the included studies assessed individuals with NIAT. In total, 383 participants with NIAT that received an exercise intervention were included. 5 studies did not have a control group, 3 used the asymptomatic side as the control group, 4 used the exercise intervention as a control group, and 1 had a control group that did not receive the intervention. The average age was 45.8 (range from 40.7 to 52 years). The average VISA-A score pre intervention was 55.2 (range from 46.6 to 70.5) and the average VISA-A post intervention score was 75.8 (range from 68 to 83.2).

2.4.2.3 Narrative synthesis of results

The included studies had a significant degree of heterogeneity with respect to the exercise intervention, region of the tendon assessed and timepoints of the measurements. Therefore, meta-analysis was inappropriate, and a narrative synthesis was conducted. Outcome measures were reported before and immediately after completing the intervention. Since no studies with neuromuscular parameters or individuals with IAT were included, only outcome measures of morpho-mechanical parameters in individuals with NIAT were reported.

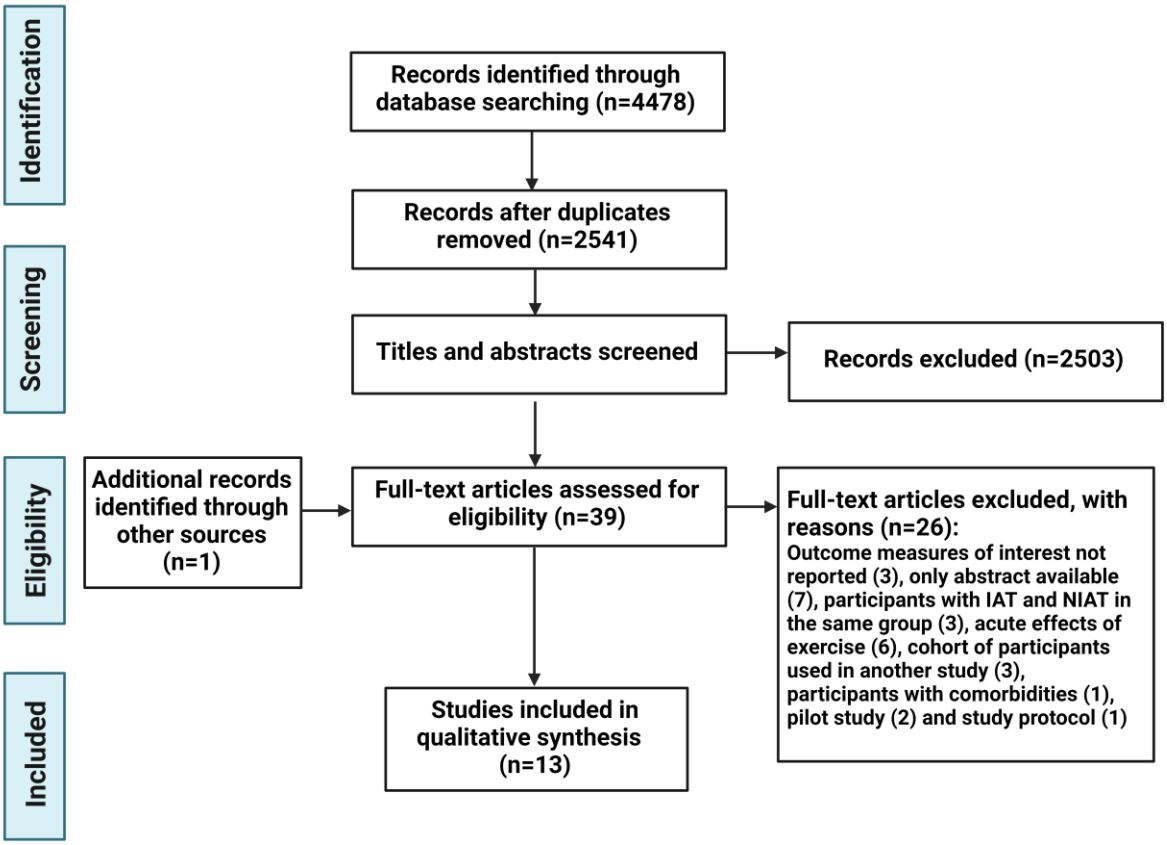

Figure 9. Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) 2020 flow diagram for Step 2.
No other articles were identified through searching gray literature and hand searching.

2.4.2.4 Morpho-mechanical parameters

Thickness. Eight studies assessed the effect of exercise on the maximal thickness of the Achilles tendon in individuals with NIAT (Beyer et al., 2015, Boesen et al., 2017, Johannsen et al., 2022, Malmgaard-Clausen et al., 2021, Ohberg et al., 2004, Solomons et al., 2020, Stefansson et al., 2019, Tsehaie et al., 2017). Maximal tendon thickness decreased after 3 months of eccentric exercises (Johannsen et al., 2022, Ohberg et al., 2004), and heavy-slow resistance training, with no differences between both interventions (Beyer et al., 2015). However, other studies reported no change in maximal tendon thickness after 3 months of eccentric exercises (Boesen et al., 2017, Stefansson et al., 2019, Tsehaie et al., 2017), 3 months of resistance training (Malmgaard-Clausen et al., 2021), or 3 months of progressive isometric, concentric and eccentric training (Solomons

et al., 2020). Three studies investigated the effect of exercise on tendon thickness in individuals with NIAT (Demir Benli et al., 2022, Malmgaard-Clausen et al., 2021, von Wehren et al., 2019). Tendon thickness at 3 cm above the posterosuperior edge of the calcaneus decreased after 3 months of eccentric exercises (von Wehren et al., 2019). However, one study reported no change in tendon thickness 2 cm above the posterosuperior edge of the calcaneus after 3 months of resistance training (Malmgaard-Clausen et al., 2021). Additionally, one study reported increased tendon thickness at 5 cm from the insertion after 3 months of eccentric exercises (Demir Benli et al., 2022).

Antero-posterior diameter. Two studies evaluated the effect of exercise on the maximal antero-posterior diameter of the Achilles tendon in individuals with NIAT (Rompe et al., 2007, Petersen et al., 2007). No difference was found in maximal antero-posterior tendon diameter after 3 months of eccentric training (Rompe et al., 2007, Petersen et al., 2007).

CSA. One study investigated the effect of exercise on the maximal CSA of the Achilles tendon in individuals with NIAT (Tsehaie et al., 2017). Maximal tendon CSA decreased after 3 months of eccentric exercises (Tsehaie et al., 2017). Two studies assessed tendon CSA at different regions in individuals with NIAT (Malmgaard-Clausen et al., 2021, von Wehren et al., 2019). No difference was observed in tendon CSA at 2 cm from the posterosuperior edge of the calcaneus after 3 months of resistance training (Malmgaard-Clausen et al., 2021). However, other study reported decreased tendon CSA at 3 cm from the posterosuperior edge of the calcaneus after 3 months of eccentric exercises (von Wehren et al., 2019).

Width. One study evaluated the effect of exercise on the maximal width of the Achilles tendon in individuals with NIAT (Stefansson et al., 2019). No difference on maximal tendon width was observed after 3 months of eccentric exercises (Stefansson et al., 2019).

Volume. Two studies investigated the effect of exercise on the volume of the Achilles tendon in individuals with NIAT (Shalabi et al., 2004, Tsehaie et al., 2017). Tendon volume assessed 2-12 cm proximal to the tendon insertion decreased after 3 months of eccentric exercises (Shalabi et al., 2004). Likewise, tendon volume assessed 2-7 cm proximally to the tendon insertion decreased after 3 months of eccentric exercises (Tsehaie et al., 2017).

Strain. One study investigated the effect of exercise on the relative strain of the Achilles tendon in individuals with NIAT (Demir Benli et al., 2022). Relative strain (strain ratio=Achilles tendon strain/Kager's fat strain) increased after 3 months of eccentric exercises (Demir Benli et al., 2022).

Table 3. Study characteristics Step 2.

First Author, Year, Type of Study and Type of Tendinopathy	Experimental group. Sample size; sex (f/m); age (yrs); height (cm); weight (kg); level of physical activity (h/week, MET-min week^{-1}) or mileage (km/week)	Control group. Sample size; sex (f/m); age (yrs); height (cm); weight (kg); level of physical activity (h/week, MET-min week^{-1}) or mileage (km/week)	Intensity (VISA-A and VAS or NRS), duration of symptoms (mo), diagnosis	Intervention	Outcome measures (s)	Results
Benli et al. 2022.(Demir Benli et al., 2022) RCT NIAT	N=40 (after dropouts N=32), 18 f/14 m, 34.8 ± 13.3 yrs, 170.8 ± 10.4 cm, 75.1 ± 13.5 kg; 9 (7-14) h/w	No control group	VISA-A pre 52.6 ± 20.5; VISA-A post 80.0 ± 24.3; VAS pre 5.9 ± 2.4; VAS post 2.6 ± 2.5.	Eccentric plantarflexion contractions (3 sets x 15 repetitions, twice per day for 3 months)	-Thickness 5 cm -Strain Ratio= Achilles tendon strain/Kager's fat strain	↑ Thickness and strain ratio
Beyer et al. 2015.(Beyer et al., 2015) RCT NIAT	ECC. N=25 (after dropouts N=20); 7 f/18 m; 48 ± 2 yrs; 179 ± 2 cm; Weight: 81 ± 2 kg; 5 ± 1 h/w HSR. N=22 (after dropouts N=16). 8f/14 m; 48 ± 2 yrs; Height: 178 ± 2 cm; Weight: 81 ± 2 kg; 5 ± 1 h/w	No control group	ECC. VISA-A Pre 58 ± 3.9; post 72 ± 3.7; VAS–heel rises pre 19 ± 5.0; post 12 ± 3.6; 19 ± 5 mo HSR. VISA-A pre 54 ± 3.2; post 76 ± 3.7; VAS–heel rises pre 29 ± 5.5; VAS-heel rises post 7 ± 2.4; 17 ± 3 mo; Clinical examination and ultrasound imaging	ECC. Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months). HSR. Resistance training in a fitness center	-Maximal thickness	↓ Maximal thickness, no differences between groups
Boesen et al. 2017.(Boesen et al., 2017) RCT NIAT	Exercise group. N=20 (after dropouts N=19); 40.9 ± 6.6 yrs; 183.5 ± 20.4 cm; 89.7 ± 22.1 kg;	The exercise group was the control group	VISA-A pre 59.2 ± 10.1; VISA-A post 69.8 ± 13.1; VAS pre 45 ± 23; VAS post 29.5 ± 6.1; 7.7 ± 9.35 mo; Clinical examination and ultrasound imaging	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months)	-Maximal thickness	= Maximal thickness
Johannsen et al. 2022.(Johannsen et al., 2022)	Exercise group (N=52 (after dropouts N=48); 20 f/32 m; 46 (44-48) yrs; 178 (176-180)	The exercise group was the control group	VISA-A pre 50 (46-54); VISA-A post 68 (63-72); VAS pre 48 (41-55); VAS post 16	Heavy slow resistance exercise program performed 3 times per week using standard	-Maximal thickness	↓ Maximal thickness

RCT. NIAT	cm; 81.4 (78.2-84.6) kg.		(10-22); 20 (14-26) mo; Clinical examination and ultrasound imaging	resistance equipment for 3 months		
Malmgaard-Clausen et al. 2021.(Malmgaard-Clausen et al., 2021) RCT. NIAT	Exercise group. N=35 (after dropouts N=30); 9 f/26 m; 40.7 ± 1.7 yrs; BMI: 25.1 ± 0.4; 8.5 ± 0.9 h/w	The exercise group was the control group	VISA-A pre 70.5 ± 2.4; VISA-A post 83.2 ± 2.4; NRS pre 4.0 ± 0.3; NRS post 0.4 ± 0.2; 1.87 ± 0.13 mo; Clinical examination	The rehabilitation program consisted of 12 weeks of resistance training 3 times per week with four exercises in total	-Maximal thickness -Thickness 2 cm above the posterosuperior calcaneal edge -CSA	= Maximal thickness, thickness 2 cm above the posterosuperior calcaneal edge and CSA
Ohberg et al. 2004.(Ohberg et al., 2004) NRSI NIAT	N=25 (No dropouts); 6 f/19 m; 50.4 ± 9.6 yrs; 173.6 ± 7.1 cm; 80.5 ± 12.3 kg.	Asymptomatic leg as a control	17.1 (6–120) mo; Clinical examination and ultrasound imaging	Eccentric plantarflexion contractions for 3 months	-Maximal thickness	↓ Maximal thickness NIAT = Maximal thickness asymptomatic leg
Petersen et al. 2007.(Petersen et al., 2007) RCT NIAT	ECC. N=37 (After dropouts N=32); 14 f/23 m; 42.1 ± 11 yrs; 176.4 ± 9.3 cm; 79.4 ± 17.1 kg	No control group	VAS improved significantly after 12 weeks of training in the ECC group. 7.1 ± 2.6 mo; Clinical examination and ultrasound imaging	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months).	-Maximal AP diameter	= Maximal AP diameter
Shalabi et al. 2004.(Shalabi et al., 2004) NRSI NIAT	N=25; 9 f/16 m; 51 yrs.	Asymptomatic leg as a control (Intervention performed only in the symptomatic leg)	NRS pre 5 (2-6); NRS post 3 (1-6); 18 (6-120) mo; Clinical examination	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months)	-Volume	↓ Volume symptomatic leg
Solomons et al. 2020.(Solomons et al., 2020) RCT. NIAT	Exercise group. N=8 (after dropouts N=7); 5 f/3 m; 47 ± 7.2 yrs.	The exercise group was the control group	VISA-A pre 56.1 ± 24.1; VISA-A post 82.1 ± 45.1; 7.6 ± 7.5 mo; Clinical examination	3 months of progressive isometric, concentric and eccentric training and kinetic chain strengthening exercises	-Maximal thickness	= Maximal thickness
Stefansson et al. 2019.(Stefansson et al., 2019)	N=19 (after dropouts N=15); 46.0 ± 12.9 yrs; 177.0 ± 7.7 cm; 93.1 ± 18.6 kg	No control group	VISA-A pre 54.14 ± 2.75; VISA-A post 70.69 ± 4.6; 28.8 ± 39.7 mo; Clinical	Eccentric plantarflexion contractions with extended and flexed knee (3 sets	-Maximal thickness -Maximal width	= Maximal thickness and maximal width

RCT. NIAT			examination and ultrasound imaging	x 15 repetitions each, twice per day, every day, for 3 months)		
Tsehaie et al. 2017.(Tsehaie et al., 2017) NRSI NIAT	N=25 (after dropouts N=20); 15 f/ 10 m; 46 ± 9.5 yrs; BMI: 25.9 ± 4.7	Asymptomatic side as a control	After the intervention VISA-A increased significantly by 12.3 points; 9 (5-21) mo; Clinical examination	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months)	-Maximal thickness -Maximal CSA -Volume	↓ Volume and CSA in the symptomatic leg = Maximal thickness after 6 months
von Wehren et al. 2019.(von Wehren et al., 2019) NRSI NIAT	Exercise group. N=25; 10 f/15 m; 52 ± 13 yrs.	The exercise group was the control group	VISA-A pre 46.6 ± 15.8; VISA-A post 80.7 ± 18.5; Clinical examination and MRI	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months)	-Thickness 3 cm above the posterosuperior calcaneal edge -CSA	↓ Thickness 3 cm above the posterosuperior calcaneal edge and CSA after 6 months
Rompe et al. 2007.(Rompe et al., 2007) RCT NIAT	ECC. N=25 (After dropouts N=23); 16 f/9 m; 48.1 ± 9.9 yrs.	N=25, 16 f/9 m; 46.4 ± 11.4. The control group did not receive the intervention.	ECC. VISA-A pre 50.6 ± 11.5; VISA-A post 75.6 ± 18.7. Control. VISA-A pre 48.2 ± 9.0; VISA-A post 55.0 ± 12.9; Clinical examination and ultrasound imaging	Eccentric plantarflexion contractions with extended and flexed knee (3 sets x 15 repetitions each, twice per day, every day, for 3 months).	-Maximal AP diameter	= Maximal AP diameter after 4 months

NIAT, non-insertional Achilles tendinopathy; RCT, randomised controlled trial; NRSI, non-randomised interventional study; CSA, cross-sectional area; NR, not reported. Time for the post assessment was reported if participants were not assessed immediately after completing the intervention.

2.4.2.5 Methodological quality assessment

9 RCTs studies were assessed with the Rob 2 and 4 NRSIs with the ROBINS-I. The results of the risk of bias assessment can be seen in **Figure 10**.

2.4.2.6 Level of evidence (GRADE)

The certainty of the evidence for each outcome assessed by RCT and NRSIs was evaluated separately by two reviewers (Schünemann et al., 2020) and is presented in Appendix 6 (Table 14).

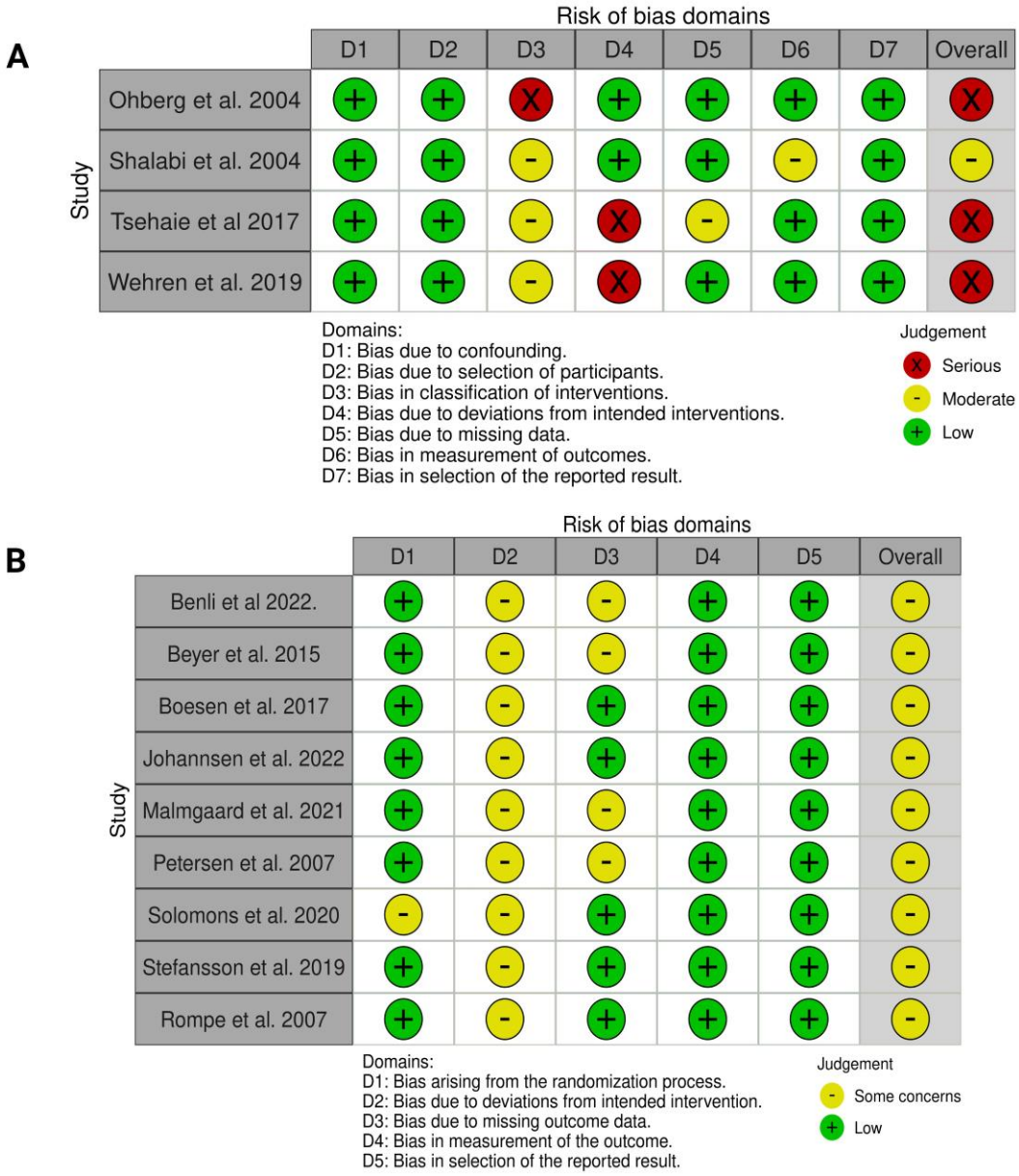

Figure 10. Risk of bias assessment of NRIS Studies (A) and RCT studies (B) for Step 2.

2.5 DISCUSSION

The present systematic review synthesises current evidence investigating neuromechanical changes induced by NIAT and IAT and the neuromechanical effects of exercise-induced Achilles tendon loading on these properties. As described previously, this systematic review was developed in a two-steps process to properly address each objective. Findings suggest that individuals with NIAT and IAT exhibit neuromechanical changes, as indicated by differences in triceps surae muscle's neuromuscular properties and Achilles tendon's morpho-mechanical parameters. However, studies investigating the effect of exercise-induced mechanical tendon loading showed inconsistent effects of exercise in modifying NIAT-induced alterations in Achilles tendon morpho-mechanical properties. Surprisingly, none of the studies focused on investigating the effect of exercise-induced mechanical loading on the neuromuscular properties of the triceps surae muscle in individuals with NIAT or IAT. These findings show that there is a lack of evidence about the neuromechanical processes involved in potential improvements in pain and motor performance following longitudinal exercise interventions in individuals with these conditions.

Concerning the certainty of evidence, the overall quality of evidence for the studies included in Step 1 was very low. This classification was primarily attributed to the inclusion of observational studies characterised by small sample size. However, depending on the specific outcome measure being assessed, the quality of evidence varied, ranging from very low to moderate. Similarly, the overall quality of evidence for the studies included in Step 2 was low. This assessment was due to the inclusion of studies with severe risks of bias, differences in outcome measures or small sample sizes. Nevertheless, depending on the outcome measure of interest, the quality of evidence ranged from very low to moderate.

2.5.1 Morpho-mechanical properties

Morphological properties of the included studies showed increased maximal tendon thickness and average tendon thickness in individuals with NIAT and IAT (Callow et al., 2022, Zhang et al., 2017, Nuri et al., 2018, Crawford et al., 2023, Intziegianni et al., 2016, Lalumiere et al., 2020, Scholes et al., 2018). Additionally, several of the included studies showed increase tendon thickness at different regions in individuals with NIAT and IAT (Chimenti et al., 2014, Alghamdi et al., 2022, Callow et al., 2022, Child et al., 2010, Grigg et al., 2012, Malmgaard-Clausen et al., 2021, Nadeau et al., 2016, Romero-Morales et al., 2019). Only one study has reported no difference in tendon thickness at the posterosuperior calcaneal edge in individuals with NIAT (Alghamdi et al., 2022). These results agree with evidence showing that increased tendon thickness is one of the most characteristics ultrasonographic and magnetic resonance imaging findings in individuals with AT (Paavola et al., 2002). Despite that thickness increased in most of the studies, it is important to highlight the variability in the methodology applied to determine this parameter, with studies assessing the tendon thickness at the MTJ of the SO (Callow et al., 2022), at the moment arm (Child et al., 2010), 1, 2, or 3 cm proximal to the posterosuperior edge of the calcaneus (Malmgaard-Clausen et al., 2021, Alghamdi et al., 2022, Callow et al., 2022, Chimenti et al., 2014, Syha et al., 2007), and at 2, 4, and 6 cm from the tendon insertion (Grigg et al., 2012, Nadeau et al., 2016, Romero-Morales et al., 2019). Interestingly, tendon thickness at insertion was greater in individuals with IAT, but not in individuals with NIAT (Aggouras et al., 2022, Child et al., 2010, Callow et al., 2022, Chimenti et al., 2017), supporting the idea NIAT and IAT are two different conditions with morphological differences (Maffulli et al., 2020). Regarding antero-posterior diameter, included studies indicate that maximal diameter increased in individuals with NIAT (Finnamore et al., 2019, Lalumiere et al., 2020, Resteghini and Yeoh, 2012, Scholes et al., 2018, Schie et al., 2010, Szaro and Ghali Gataa, 2021, Tsehaie et al., 2017, Petersen et al., 2007). Similarly, mean antero-posterior diameter at 6 cm from the insertion increased in individuals with NIAT (Lalumiere et al., 2020, Nadeau et al., 2016). Together, these results suggest that increased antero-posterior diameter is a common finding in individuals with NIAT. Maximal CSA and average CSA results showed increased maximal

CSA in individuals with NIAT, yet average CSA only increased in individuals with NIAT (Callow et al., 2022, Zhang et al., 2017, Shim et al., 2019, Nuri et al., 2018, Arya and Kulig, 2010, Tsehaie et al., 2017). Additionally, CSA was determined at different tendon regions, showing increased CSA at 4 and 6 cm from the tendon insertion in individuals with NIAT (Intziegianni et al., 2016, Nadeau et al., 2016, Romero-Morales et al., 2019), greater CSA at the MTJ of the SO in individuals with NIAT and IAT (Callow et al., 2022) and increased CSA at insertion in individuals with IAT (Callow et al., 2022), again reinforcing the idea that both conditions induce different morphological adaptations.

Overall, included studies indicate that free tendon length (Alghamdi et al., 2022, Nuri et al., 2018, Shim et al., 2019, Szaro and Ghali Gataa, 2021) and tendon length assessed from the insertion to the MTJ of the MG (Arya and Kulig, 2010, Intziegianni et al., 2016, Chimenti et al., 2014) were similar between individuals with NIAT and IAT compared to controls or the asymptomatic leg. Only one study reported increased free tendon length in individuals with NIAT (Szaro and Ghali Gataa, 2021). Insertional length showed conflictive results since one study found no differences (Alghamdi et al., 2022); however, another study reported increased insertional length in individuals with NIAT (Szaro and Ghali Gataa, 2021), yet no differences were reported for individuals with IAT (Alghamdi et al., 2022). It is important to point out that the only study that reported increased free tendon length and insertional length in individuals with NIAT describe the use of different MRI devices and imaging protocols in its methodology, which may have influenced their results. Collectively, these findings suggest that free tendon length and tendon length do not change in individuals with NIAT or IAT. Concerning maximal width and average width, included studies reported increased maximal width in individuals with NIAT (Szaro and Ghali Gataa, 2021); however, no difference was shown for average width in individuals with NIAT (Nuri et al., 2018). Furthermore, the width at 6 cm from the insertion (Nadeau et al., 2016) and at insertion were greater in individuals with NIAT (Szaro and Ghali Gataa, 2021). These results imply that tendon width changes in individuals with NIAT are region-specific. About tendon volume and free tendon volume, included studies showed inconsistent results, with studies indicating increased tendon volume (Shalabi et al., 2004) and free tendon volume (Nuri et al., 2018) and others

showing no change in tendon volume (Tsehaie et al., 2017) or free tendon volume (Shim et al., 2019) in individuals with NIAT. These differences may be explained by the different methodologies used to assess tendon volume. Insertional angle was assessed in only one study, showing no difference between symptomatic and asymptomatic legs in individuals with NIAT and IAT (Alghamdi et al., 2022). Since only one study assessed this parameter, no conclusion can be made.

Regarding the mechanical properties, most of the included studies reported decreased stiffness (Wang et al., 2012, Arya and Kulig, 2010, Chang and Kulig, 2015), Young's modulus (Shim et al., 2019, Arya and Kulig, 2010), and shear-wave velocity (Aubry et al., 2015, Crawford et al., 2023) in individuals with NIAT. Only one study reported no difference in compliance (measure opposite of stiffness) in individuals with NIAT (Intziegianni et al., 2016). In individuals with IAT, one study reported decreased stiffness (Chimenti et al., 2014) and another greater hardness (Zhang et al., 2017). These differences may be explained by the methodology applied since Zhang et al. 2017 used axial-strain sonoelastography to determine the hardness; however, this technique involves manual axial compression of the tissue using the ultrasound transducer to generate tissue deformation (Zhang et al., 2017); therefore, the external load applied may have influenced the results. Taken together, the results obtained from most studies suggest decreased stiffness in individuals with NIAT; however, no conclusion can be made for individuals with IAT. This conclusion is supported by a recent systematic review demonstrating lower tendon stiffness in individuals with AT; however, this systematic review included individuals with NIAT and IAT (Obst et al., 2018). In general, strain and elongation were greater in individuals with NIAT (Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Intziegianni et al., 2016, Nuri et al., 2018); however, these changes seem to be task dependent. For instance, a study reported that strain was lower during eccentric contractions (Grigg et al., 2012), while other study assessing single vertical jumps, did not find differences in strain and elongation in individuals with NIAT (Intziegianni et al., 2016). Similarly, inconsistent results in strain were reported in individuals with IAT, with one study showing decreased strain during dorsiflexion tasks (Chimenti et al., 2017) and another showing increased strain during passive movements

(Chimenti et al., 2014), again, indicating that changes in strain are task-dependent in individuals with IAT. Finally, included studies showed no difference in tendon force in individuals with NIAT and IAT compared to the asymptomatic leg and controls (Arya and Kulig, 2010, Chimenti et al., 2014). Since only one study assessed this parameter in each condition, no conclusion can be made. Tendon stress was lower in individuals with NIAT (Arya and Kulig, 2010, Shim et al., 2019), suggesting decreased tendon stress in individuals with NIAT.

2.5.2 Neuromuscular properties

EMG amplitude assessed in the included studies showed different results dependent on the task in individuals with NIAT. In running tasks, two studies reported lower EMG MG amplitude (weight acceptance, push-off, and overall EMG amplitude) (Baur et al., 2011, Pingel et al., 2013) and one showed increased EMG LG amplitude in individuals with NIAT (Pingel et al., 2013). However, during eccentric contractions, EMG MG and EMG SO were greater in individuals with NIAT (Reid et al., 2012). Therefore, it seems that changes in EMG amplitude of the triceps surae muscles in individuals with NIAT are task dependent. Only one study investigated the spectral frequency of the triceps surae muscles, showing increased EMG MG frequency in individuals with NIAT (Pingel et al., 2013). Regarding the timing of activation, included studies reported longer EMG onset for SO and preactivation of MG during single limb hopping (Debenham et al., 2016), during running, the offset of the SO relative to the LG was shorter in individuals with NIAT (Wyndow et al., 2013), and during single leg-hopping, the preactivation was longer in individuals with NIAT (Chang and Kulig, 2015). Together, these findings suggest longer onset and shorter activation offset of the SO muscle in individuals with NIAT during dynamic tasks. Similarly, the electromechanical delay of the MG and SO and the average triceps surae electromechanical delay were longer in individuals with NIAT (Wang et al., 2012, Chang and Kulig, 2015). Nevertheless, one study reported no difference in electromechanical delay of the MG during MVIC in individuals with IAT. Collectively, these results suggest an increase in the electromechanical delay of the triceps surae muscles in individuals with NIAT.

Motor-evoked reflexes reported in the included studies showed increased V/M_{sup} in the SO muscle (V-wave with a superimposed supramaximal intensity stimulation/ superimposed supramaximal M-wave), rate of EMG rise (between TA and SO), mean average voltage (between TA and SO), H/M (H-reflex/M-wave) ratio for the SO, and V/M (V-wave/M-wave) in individuals with NIAT (Wang et al., 2011, Chang and Kulig, 2015). Together, these results suggest increased α-motoneuron excitability and up-regulated descending neural drive to the triceps surae muscles in individuals with NIAT (Chang and Kulig, 2015). Additionally, the co-contraction ratio between the tibialis anterior muscle and the ankle plantarflexors was lower in individuals with NIAT during single-leg hopping (Chang and Kulig, 2015). Finally, discharge rate and coefficient of variation of the interspike interval were similar between individuals with NIAT and controls during submaximal isometric contractions at 10 and 20% MVC (Fernandes et al., 2023). Since only one study assessed co-contraction ratio and motor unit firing rate parameters, no conclusion regarding these parameters can be made.

2.5.3 Exercise-induced tendon loading adaptations on morpho-mechanical properties

The management with the highest level of evidence for AT is exercise rehabilitation (Martin et al., 2018). Exercise rehabilitation programs aim to provide a mechanical load to the tendon to induce remodelling, decrease pain, and improve calf muscle endurance and strength (Silbernagel et al., 2020). Included studies investigated the effect of different exercise intervention protocols on the Achilles tendon morpho-mechanical properties. Most of the exercise interventions applied were an adaptation of the Alfredson protocol (Demir Benli et al., 2022, Beyer et al., 2015, Boesen et al., 2017, Petersen et al., 2007, Shalabi et al., 2004, Stefansson et al., 2019, Tsehaie et al., 2017, von Wehren et al., 2019, Rompe et al., 2007); however, heavy-slow resistance exercises (Beyer et al., 2015, Johannsen et al., 2022), resistance exercises (Malmgaard-Clausen et al., 2021), and a combination of progressive isometric, concentric, eccentric, and kinetic chain strengthening exercises (Solomons et al., 2020) were also applied. Regarding maximal tendon thickness, included studies showed inconclusive results since some reported

decreased maximal tendon thickness (Johannsen et al., 2022, Beyer et al., 2015, Ohberg et al., 2004) and others reported no changes (Boesen et al., 2017, Solomons et al., 2020, Malmgaard-Clausen et al., 2021, Stefansson et al., 2019, Tsehaie et al., 2017) in individuals with NIAT. Tendon thickness assessed at different regions in the included studies showed a decrease (von Wehren et al., 2019), no change (Malmgaard-Clausen et al., 2021) or even increase in thickness after the exercise intervention (Demir Benli et al., 2022). A variety of factors may explain these results. First, the characteristics of the exercise interventions could influence morphological adjustments since different exercise protocols could influence the load placed in the Achilles tendon. Second, the duration of the exercise intervention could influence tendon remodelling since longer exercise interventions ($\geq$ 12 weeks) seem to be more effective to generate tendon morphological adaptations in asymptomatic individuals (Bohm et al., 2015). None of the included studies applied the exercise intervention for more than 3 months. Third, the supervision during the training sessions could have also influenced outcomes since most of the studies used home-based exercise protocols, relying on the engagement of the participants to perform the exercises, which could generate uncertainties regarding the control over the load, speed, and range of movement during the exercises. Fourth, the included studies did not control for the pathology stage, which may have influenced the tendon adaptations (Malliaras and Cook, 2011). Finally, technical limitations of the imaging equipment or the level of experience of the operators might have influenced the sensitivity to identify tendon adaptations after an exercise intervention (Färnqvist et al., 2020).

It is assumed that exercise interventions improve symptoms by inducing tendon morpho-mechanical changes in individuals with NIAT; however, there are still doubts about how specific tendon loading exercise interventions modify these properties. Regarding maximal antero-posterior diameter, included studies reported no change in maximal antero-posterior diameter after 3 months of eccentric exercise in individuals with NIAT (Rompe et al., 2007, Petersen et al., 2007). However, maximal CSA decreases after 3 months of eccentric exercise in individuals with NIAT (Tsehaie et al., 2017). Since only one study assessed maximal CSA, no conclusion can be drawn. Nevertheless, changes in CSA can differ at different tendon regions. For instance, one study reported no

difference in CSA at 2 cm from the posterosuperior calcaneal edge after 3 months of resistance training (Malmgaard-Clausen et al., 2021), and another showed decreased CSA at 3 cm from the posterosuperior calcaneal edge after 3 months of eccentric training (von Wehren et al., 2019). Only one study assessed maximal width, showing no changes after 3 months of eccentric exercises (Stefansson et al., 2019). Since we observed inconsistent results in CSA at different regions and only one study assessed maximal width, no conclusions can be drawn for these parameters. Concerning tendon volume, the included studies showed decreased tendon volume after 3 months of eccentric training (Shalabi et al., 2004, Tsehaie et al., 2017), suggesting that this type of exercise may induce changes in tendon volume after 3 months in individuals with NIAT. Finally, only one study assessed tendon strain, showing that relative strain increased after 3 months of eccentric training in individuals with NIAT (Demir Benli et al., 2022). Since only one study investigated this parameter, no conclusions can be made. Overall, there is a lack of evidence about the effect of different tendon loading exercise interventions in the morpho-mechanical properties of the Achilles tendon in individuals with NIAT.

Unfortunately, none of the included studies assessed the effect of exercise-induced mechanical tendon loading on the morpho-mechanical properties of the Achilles tendon in individuals with IAT. This could be possibly related to the lower incidence of this condition compared to NIAT in the population (Paavola et al., 2002). Likewise, no included studies assessed the effect of exercise-induced mechanical tendon loading on the neuromuscular properties of the triceps surae, highlighting a fundamental gap in the current literature about the effect of exercise intervention protocols in these conditions.

2.5.4 Methodological considerations

The current systematic review followed a rigorous methodology (Contreras-Hernandez et al., 2022b). Two reviewers performed screening, quality assessment, and data extraction independently for Steps 1 and 2. The risk of bias was determined using Rob 2, ROBINS-I, or an adapted version of the NOS according to the type of study assessed for each step. Due to the significant heterogeneity of the included studies in Steps 1 and 2, meta-analysis was not appropriate, and narrative synthesis was reported.

There are some limitations that should be considered. Some of the studies included in Step 1 assessed recreationally active individuals (i.e., runners), which would limit the generalisability of the results to a different population. Since repetitive loading may alter tendon morpho-mechanical properties, including recreationally active individuals may have influenced the observed tendon properties and the triceps surae neuromuscular parameters. Additionally, the included studies in Step 2 did not assess individuals with IAT nor neuromuscular parameters for individuals with NIAT or IAT; thus, data synthesis for Step 2 only included morpho-mechanical parameters. Furthermore, the overall certainty of evidence for Steps 1 and 2 were classified as very low and low, respectively. For Step 1 this classification was mainly due to the inclusion of observational studies, and for Step 2, due to the inclusion of studies with severe risks of bias, differences in outcome measures or small sample size.

2.6 CONCLUSION

Individuals with NIAT and IAT exhibit neuromechanical changes, as indicated by differences in the triceps surae muscle's neuromuscular properties and the Achilles tendon's morpho-mechanical parameters when compared to asymptomatic individuals or the asymptomatic leg. Additionally, exercise-induced mechanical tendon loading showed inconsistent results in modifying the morpho-mechanical properties of the Achilles tendon in individuals with NIAT. We did not find any study assessing these properties in individuals with IAT, suggesting that future investigations should address IAT specifically. Furthermore, there is a lack of studies investigating the effect of exercise-induced mechanical tendon loading on the neuromuscular properties of the triceps surae muscle in individuals with NIAT and IAT. Therefore, further research in this area is needed to elucidate the contributions of the neuromuscular system to the improvements in pain and function following exercise interventions for the management of NIAT and IAT.

CHAPTER 3

Achilles tendon morpho-mechanical parameters are related to triceps surae motor unit firing properties.

This chapter reports in full a manuscript which is under review in the Journal of Neurophysiology. Changes have been made in the text of this chapter for the purposes of the thesis to reflect the content of previous chapters and to increase cohesion with studies in later chapters.

Manuscript under review:

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Achilles tendon morpho-mechanical parameters are related to triceps surae motor unit firing properties (Journal of Neurophysiology).

Author Contributions:

IC-H, EM-V, and DF conceived and designed research; IC-H and MA performed experiments; IC-H and EM-V analysed data; IC-H and EM-V interpreted results of experiments; IC-H prepared figures; IC-H drafted manuscript; IC-H, EM-V, DF, FN and MA edited and revised manuscript.

Conference Presentations:

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Morphological and mechanical properties of the Achilles tendon and their relationship with triceps surae motor unit firing properties during isometric contractions. International Society of Electrophysiology and Kinesiology (ISEK). Quebec, Canada, June 22-25, 2022.

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Achilles tendon morpho-mechanical parameters are related to changes in triceps surae motor unit firing properties. UK Sensorimotor Conference, Newcastle, United Kingdom, June 26-28, 2023.

3.1 ABSTRACT

Recent studies combining high-density surface electromyography and ultrasound imaging have yielded valuable insights into the relationship between motor unit activity and muscle contractile properties, yet there is limited evidence of the relationship between motor unit firing properties and the morpho-mechanical properties of tendons. This study aimed to determine the relationship between triceps surae motor unit firing properties and the morpho-mechanical properties of the Achilles tendon. Motor unit firing properties (mean discharge rate, COVisi, and neuromechanical delay (NMD)) of the MG, LG, and SO muscles were assessed using HD-sEMG during isometric plantarflexion contractions at 10% and 40% of the maximal voluntary contraction (MVC). Morpho-mechanical properties of the Achilles tendon were determined using B-mode ultrasonography and shear-wave elastography. Multiple linear regression analysis showed that at 10% MVC, the discharge rate of the triceps surae muscles was able to explain 41.7% of the variance in AT stiffness. Additionally, at 10% MVC, the COVisi SO was able to predict 30.4% of the variance in Achilles tendon length. At 40% MVC, COVisi MG and COVisi SO were able to explain 48.7% of the variance in Achilles tendon length. NMD was not associated with any morpho-mechanical parameter. This study provides novel evidence that there is a contraction-intensity dependent relationship between motor unit firing parameters of the triceps surae muscle and the morpho-mechanical properties of the Achilles tendon.

3.2 INTRODUCTION

Human movement emerges from the interplay between descending output from the CNS, sensory input from the body and environment, muscle dynamics and whole-body dynamics (Nishikawa et al., 2007). Thus, the CNS plans, initiates and sends motor commands to the muscle fibres via motoneurons (Contreras-Hernandez et al., 2022b) that translate these neural commands into forces (Oliveira et al., 2022), which are then transmitted via connective tissue to the skeletal system to generate movement (Finni, 2006). In human locomotion, it is believed that elastic energy is stored within the elastic tissues of the muscle-tendon units that support the body and drive it upwards during the stance phase (Lichtwark and Wilson, 2006). However, this adaptability requires that the amount of elastic energy stored should be modulated by muscular contraction (Lichtwark and Wilson, 2006). Based on this, several studies have used the triceps surae muscle to investigate how elastic energy can be stored and released efficiently (Ishikawa et al., 2005, Ishikawa et al., 2007). In humans, plantarflexion is primarily accomplished by the triceps surae, which consists of the MG, LG and SO muscles (Héroux et al., 2014). Despite being agonist muscles that share the same common distal tendon, these muscles have anatomical, neurophysiological, and functional differences suggesting diverse functional roles (Héroux et al., 2014). These functional roles are associated with distinctive motor unit firing rate properties between muscles during different tasks (Héroux et al., 2014).

The muscle-tendon unit can be considered as a functional component of human movement capable of working as a motor, damper, and spring to exert, dissipate or store, and release energy (Alexander, 1991, Roberts and Azizi, 2011). These complex functions are possible by serial and parallel coupling of active force-generating tissues and passive force-transmitting tissues and by using the ability to shift energy between active and passive components (Bojsen-Møller and Magnusson, 2019). Passive elastic components include tendons and aponeurosis which transmit force in series with the active force generated by the muscle’s fibres (Biewener and Roberts, 2000). Within this framework, the Achilles tendon has been investigated extensively (Bojsen-Møller and Magnusson,

2019). The Achilles tendon is the largest, thickest, and strongest tendon of the human body (Freedman et al., 2014, Joseph et al., 2012, Ying et al., 2003), and it plays a fundamental role in the biomechanics of the lower limb (Pierre-Jerome et al., 2010). The Achilles tendon transmits forces generated by the strongest ankle plantar flexors (Dawe and Davis, 2011) and this muscle-tendon complex crosses and acts on the knee, ankle, and subtalar joint (Joseph et al., 2012).

Studies investigating the features of the Achilles tendon include morphological properties (i.e., length, thickness, CSA), mechanical characteristics (i.e., Young's modulus, tensile rupture stress) or both (Winnicki et al., 2020). In vivo methodologies to determine the mechanical properties of the Achilles tendon are becoming more frequently used due to their ability to assess the mechanical behavior of the Achilles tendon during various activities (Arampatzis et al., 2005, Kubo et al., 2004, Maganaris and Paul, 2002, Muramatsu et al., 2001). During the past few years, shear-wave elastography (SWE) has been increasingly used to study the mechanical properties of tendons (Taljanovic et al., 2017). SWE has the advantage of being able to measure the speed of shear stress wave propagation, allowing the calculation of the Young's modulus (i.e. tendon stiffness) (Gennisson et al., 2007). Overall, current literature suggests that shear waves propagate faster in healthy tendons compared with tendinopathic tendons (which show greater compliance), and faster in tendons that are under tension (Taljanovic et al., 2017).

Recent studies combining ultrasound imaging and electromyography techniques have assessed the mechanisms responsible for converting neural activity into muscle contractions (Barber et al., 2013, Brown and McGill, 2010, Day et al., 2013). These techniques have provided a more comprehensive description of the events underlying the generation of muscle force (Carbonaro et al., 2022), like the relationship between muscle activation and fascicle length during different postural conditions (Day et al., 2013); the spatiotemporal associations between electrical and mechanical properties of active motor units (Carbonaro et al., 2022), or the relationship between modulations in tibialis anterior muscle, motor unit discharge rate, fascicle length and dorsiflexion torque (Martinez-Valdes et al., 2022); yet there is limited evidence of the relationship between motor unit

firing properties and the morpho-mechanical properties of tendons. Studies investigating the effect of static-stretch interventions on the triceps surae have shed light on this relationship (Ye et al., 2016, Trajano et al., 2013, Mazzo et al., 2021). For example, Mazzo et al.(Mazzo et al., 2021) have shown that after a static-stretch intervention on the triceps surae, there is an increase in motor unit discharge rate and a decrease in motor unit recruitment threshold at low forces (10% of the maximum). Furthermore, Trajano et al.(Trajano et al., 2013) found similar increases in SO muscle discharge rate at low forces following calf-muscle stretching. It is possible that stretching-induced changes in Achilles tendon stiffness were related to changes in the motor unit discharge rate of the triceps surae muscles, however, this was not assessed in those studies.

We aimed to determine the relationship between triceps surae motor unit firing properties and the morpho-mechanical features of the Achilles tendon. For this purpose, we assessed which triceps surae motor unit discharge properties (e.g., mean discharge rate, discharge rate variability (estimated by the COVisi) and NMD) would explain most of the variance in tendon length, thickness, CSA, and the estimated stiffness via multiple regression analysis. Since force generation is the result of the relationship between the neural drive received by muscles (i.e., motor unit firing rate and recruitment) and muscle-tendon unit behavior, we hypothesised that there is a relationship between discharge rate and the mechanical properties of the Achilles tendon; thus, we expect that individuals with greater Achilles tendon stiffness will show lower discharge rate.

3.3 METHODS

3.3.1 Participants

Twenty-five healthy (17 males, 8 females, 28.60 ± 3.92 years, 74.00 ± 11.57 kg, 171.10 ± 9.22 cm) participants were recruited from the University of Birmingham staff/student population and the local community via leaflets, e-mail, and social media.

Men or women aged 18 to 55 years old were recruited; this age-range was selected to avoid ageing-related changes of the tendon, since previous studies have found lower stiffness and Young's modulus of the Achilles tendon in older than younger populations (Lindemann et al., 2020). Inclusion criteria include confirmation of a healthy Achilles tendon determined by an experienced physiotherapist through physical examination and ultrasound imaging. Ultrasound imaging included assessing normal tendon thickness (no focal or diffuse thickening) and echoic pattern (no focal hypoechoic and hyperechoic areas within the tendon) (Bleakney and White, 2005).

Exclusion criteria included the following: (1) systemic or inflammatory conditions including rheumatic, neuromuscular disorders, and malignancy, (2) current or previous history of chronic respiratory, neurological, or cardiovascular diseases, (3) history of Achilles tendinopathy or lower limb surgery, and (4) pain/injury in the lower limbs within the previous 6 months.

3.3.2 Study design

This cross-sectional study was conducted from October 2021 to December 2022 at a laboratory within the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), University of Birmingham, UK. The Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, UK, approved the study (ERN_20-0604A). The study was conducted according to the Declaration of Helsinki and all participants provided written informed consent prior to participation. The guideline for

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) was used to facilitate reporting (Vandenbroucke et al., 2007).

Participants visited the laboratory once for the experimental session (2.5 hours) and were asked to avoid any strenuous physical activity 24 hours before testing. The assessed leg was randomised across participants. A subgroup of participants (3 males, 3 females, 27.17 ± 4.49 years, 68.42 ± 7.17 kg, 167.33 ± 7.22 cm) visited the laboratory twice (one week apart) to confirm the intra-tester reliability of B-mode ultrasonography and SWE measurements. During this period, participants were instructed to maintain their level of physical activity and avoid any strenuous physical activity 24 hours before testing.

3.3.3 Experimental setup and tasks

Experimental sessions included physical examination, ultrasonography of the Achilles tendon, HD-sEMG of the triceps surae muscles and torque recordings. A representation of the experimental setup is shown in **Figure 11.**

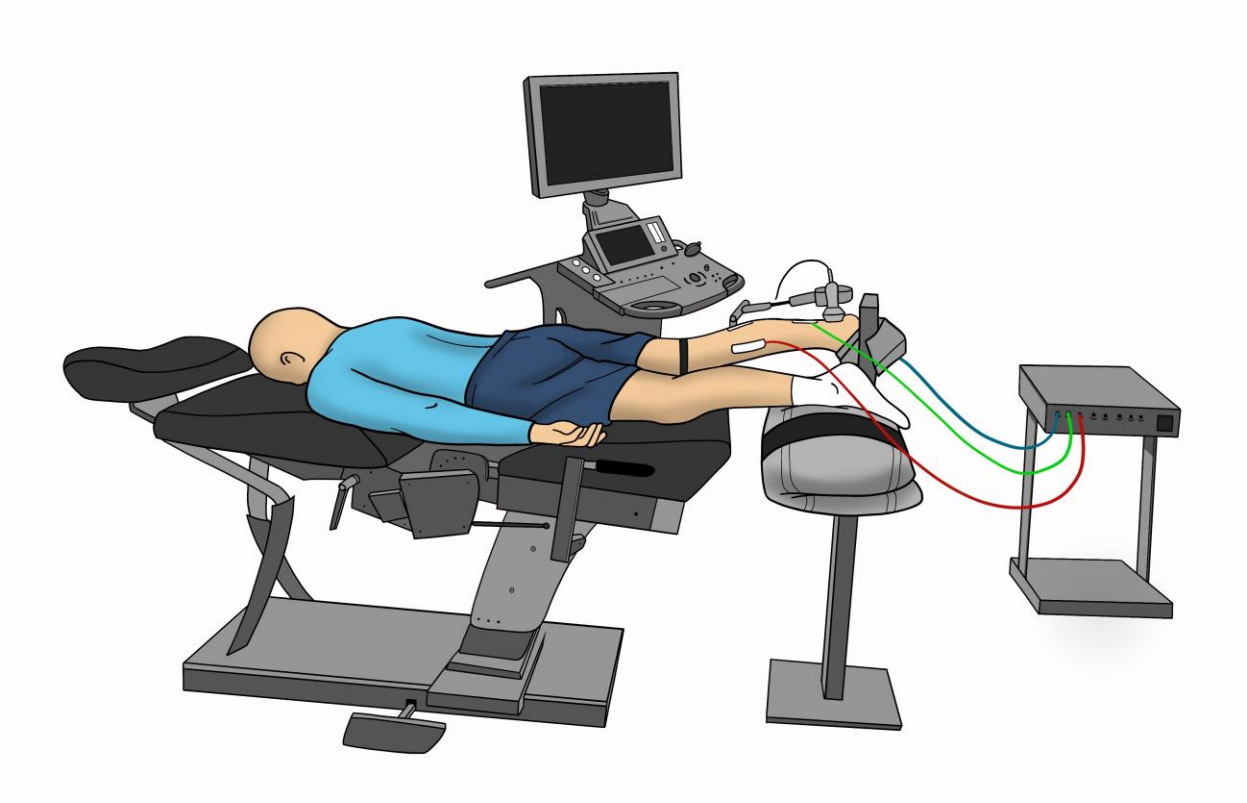

Figure 11. Representation of the experimental setup

Anthropometric data (age, gender, weight, height and foot dominance) was obtained. Foot preference in specific daily activities (foot dominance) was determined using a behavioral foot-preference inventory (Chapman et al., 1987). Participants lay prone on the chair of a Biodex System 3 dynamometer (Biodex Medical System), with their knees extended and their tested foot tightly strapped on the footplate. The pelvis was stabilised with another strap to minimise compensatory movements and the ankle was positioned in 0° of plantarflexion with the dynamometer axis aligned with the inferior tip of the lateral malleolus (Contreras-Hernandez et al., 2022a). Ultrasonography (LOGIQ S8 GE Healthcare, Milwaukee, USA) was used to confirm the normal structure of the Achilles tendon. Then, the Achilles tendon length, thickness, and CSA were determined during rest (see procedure below). Then, the skin was cleaned and prepared, and the electrodes were placed on the MG, LG, and SO muscles (see details below). Following the placement of the electrodes, we performed passive elastography assessments. HD-

sEMG was used to confirm that the muscles were not active during these measurements as this could influence the estimation of stiffness.

Next, participants performed a warm-up protocol consisting of 3 isometric plantarflexion contractions at their perceived 30% maximal voluntary force for 5 seconds with 30 seconds rest between the contractions. Then, the MVC was determined during three isometric plantarflexion contractions (5 seconds each and 2 minutes of rest between contractions) (Martinez-Valdes et al., 2018) at 0° of plantarflexion. The highest MVC value was used as the reference maximal torque. Following the ultrasound measurements, we measured the activity of the MG, LG, and SO muscles during two isometric plantarflexion contractions at 10%, and 40% MVC (10% MVC/s ramp-up, 10 s hold, 10% MVC/s ramp-down and 30 s rest) with HD-sEMG. The order of the contractions at different target torque levels was randomised using a randomisation app (Randomizer) and visual feedback of the target output was provided via a computer monitor positioned 1 m from the participant. A study schematic describing the experimental session is shown in **Figure 12.**

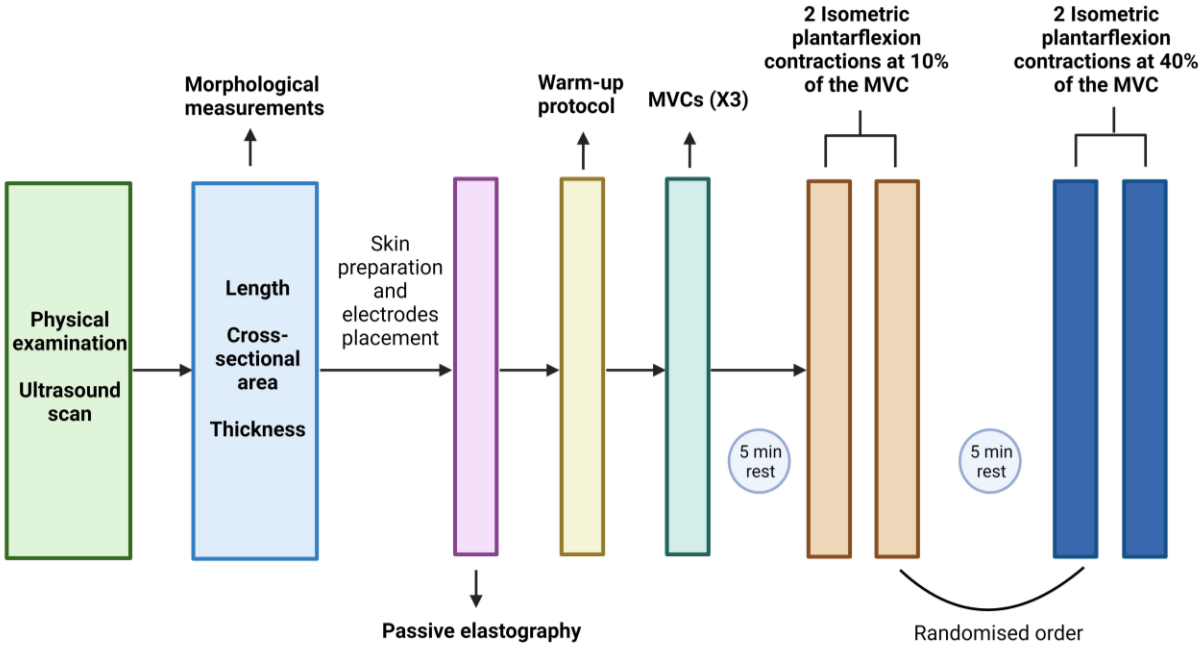

Figure 12. Schematic of the experimental procedure.
The order of the contractions performed at each target torque (10 and 40% MVC) was randomised. MVC, maximum voluntary contraction.

3.3.4 Ultrasonography

All ultrasound images were obtained using an ultrasound imaging device equipped with SWE (LOGIQ S8 GE Healthcare, Milwaukee, USA). All morphological tendon variables (tendon thickness, length and CSA) were recorded in B-mode with a 16-linear array probe (50 mm, 4-15 MHz). SWE was recorded in elastography mode with a 9-linear array probe (44 mm, 2-8 MHz).

An adaptation of the protocol developed by Arya and Kulig (Arya and Kulig, 2010) was used to measure the morphological properties of the Achilles tendon. Briefly, the ultrasound probe was placed longitudinally over the posterior aspect of the heel, and the calcaneal notch was identified. Then, a fine wire (3.2 x 40 mm) was used under the probe to create an artifact in the ultrasound image. The wire was then aligned with the distal part of the tendon and the corresponding point was marked on the skin with a marker. Then, the ultrasound probe was moved proximally to locate the MTJ of the MG, and again, a fine wire was used to create an artifact in the ultrasound image. The wire was aligned with the MTJ of the MG and the corresponding point was marked on the skin. The distance between these two points represented the resting length of the Achilles tendon. Subsequently, marks were made at 2, 4 and 6 cm above the Achilles tendon's insertion, these marks were used as reference to place the middle part of the ultrasound probe in the sagittal plane to determine the thickness of the Achilles tendon at 2, 4 and 6 cm of its insertion, and 3 ultrasound images were taken for each mark. Similarly, we used these marks to locate the probe in the transversal plane and obtain the CSA at 2, 4 and 6 cm of the Achilles tendon's insertion, and again three ultrasound images were taken for each mark.

For the HD-sEMG electrode grids placement, a tape and marker were used to draw a line following the direction of the Achilles tendon, indicating the mid-line of the posterior leg. For the MG HD-sEMG electrode grid placement, a mark was made 10 cm above the distal MTJ and 4 cm medial to the mid-line. Similarly, for the LG HD-sEMG electrode grid placement, the leg was marked 10 cm above the distal MTJ and 4 cm lateral to the mid-

line. Likewise, for the SO HD-sEMG electrode grid placement, the leg was marked 5 cm below the distal MTJ and 4 cm lateral to the mid-line. The central electrodes of the HD-sEMG grids (electrode in row 7 and column 3) were placed on top of all these marks. A representation of the anatomical landmarks used for ultrasonography and electrode placement is shown in **Figure 13.**

For the SWE measurements, the ultrasound probe was placed in the sagittal plane, with the middle part of the probe located at 4 cm above the Achilles tendon's insertion. Additionally, a probe holder was used to avoid applying pressure over the tendon and introducing movements that may interfere with the measurements. A test SWE measurement was done to check for possible voids in the estimation and if voids were detected, the ultrasound probe was removed, ultrasound gel was added, and the ultrasound probe was placed again. Passive elastography images were acquired during 12 s (twice). Due to the equipment features, a SWE image was obtained every 2.4 s, thus, in order to obtain at least 4 SWE images, the elastography measurements lasted 12 s. Elastography images were checked following each measurement to determine possible voids that may have affected our results.

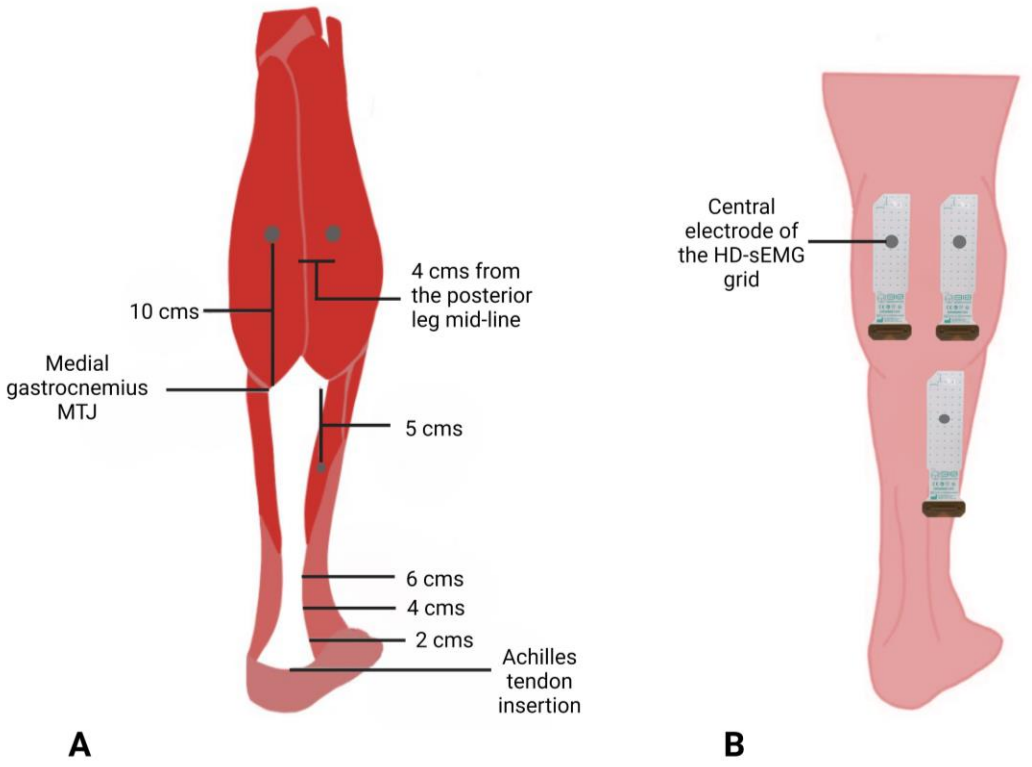

Figure 13. Anatomical landmarks and position of electrodes.
(A) Representation of the anatomical landmarks used for ultrasonography and (B) position of the HD-sEMG grids in the MG, LG, and SO muscles. MG, medial gastrocnemius; LG, lateral gastrocnemius; SO, soleus.

3.3.5 Intra-rater/inter-session reliability of the SWE measurements

Due to the very low to moderate reliability of the SWE results reported in a recent systematic review (Mifsud et al., 2023b), we performed an intra-rater/inter-session reliability analysis of the stiffness of the Achilles tendon to check the consistency of these measures. Briefly, a group of six participants came to the laboratory for a second experimental session one week apart. Following an identical protocol, the same researcher (ICH) conducted SWE measurements on both occasions.

3.3.6 HD-sEMG and torque recordings

HD-sEMG signals were recorded from the MG, LG and SO muscles using three two-dimensional (2D) adhesive grids (OT Bioelettronica, Italy) of 13 x 5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm) placed in the position described above. The HD-sEMG grid was prepared by attaching a double-side adhesive foam to the grid surface (SPES Medica, Genova, Italy) and by filling the grid cavities with conductive paste which provided adequate electrode-skin contact (AC-CREAM, SPES Medica, Genova, Italy). Additionally, participants' skin was shaved (if necessary), gently abraded (Nuprep, Skin Prep Gel, Weaver and Company, Aurora, Colorado) and cleaned with water.

All signals were converted from analog-to-digital by a 16-bit analogue-digital converter (Quattrocento- OT Bioelecttronica, Torino, Italy). Signals were amplified by a factor of 150, sampled at 2048 Hz, and filtered with a band-pass filter (bandwidth: 10-500 Hz, first order, -3 dB) (Arvanitidis et al., 2019). HD-sEMG signals were acquired in monopolar mode with ground electrodes (WhiteSensor WS, Ambu A/S, Ballerup, Denmark) positioned in the head of the fibula and with a wet strap in the thigh of the evaluated leg. All the grids and ground electrodes were connected to the same bioelectrical amplifier (Quattrocento-OT-Bioelecttronica, Torino, Italy). The torque exerted by the participants was assessed with a Biodex System 3 dynamometer (Biodex Medical System), which was synchronised with the HD-sEMG signals through the auxiliary input of the EMG amplifier (Arvanitidis et al., 2019).

3.3.7 Image analysis

3.3.7.1 Ultrasound images analysis.

After acquiring the ultrasound images, a reference of 1 cm was drawn using the ultrasound tools. Then, the software ImageJ (http://imagej.nih.gov/ij) was used to determine the Achilles tendon thickness at 2, 4, and 6 cm from its insertion. Briefly, the

reference was measured with the ImageJ tools, converted into pixels, and set as scale. Then, the length of the image was determined, and the middle point marked on the image. Next, the distance between the superficial and deep part of the paratenon was measured. After, the thickness at 2, 4, and 6 cm was averaged to obtain the Achilles tendon thickness. Conversely, ultrasound tools were used to determine the CSA of the Achilles tendon at 2, 4, and 6 cm of its insertion. A discontinuous line was drawn following the internal part of the paratenon as a reference and the CSA was measured. Then, the CSA at 2, 4, and 6 cm was averaged to obtain the Achilles tendon CSA.

For the SWE measurements, we obtained approximately 4 SWE colour maps (height x width, 2.5 cm x 1 cm) which were selected using elastography ultrasound tools to allow a better visualisation of the Achilles tendon. A region of interest (ROI) of 3 mm diameter (Siu et al., 2016) was selected and located in the middle of the tendon at 4 cm from its insertion to determine the stiffness (kPa). Lastly, mean stiffness was calculated over the ROIs of the 4 consecutive images recorded (Coombes et al., 2018).

3.3.8 HD-sEMG signal analysis

3.3.8.1 Torque signal analysis

The highest peak torque exerted during the MVCs (SI: Newton-meters) was used as a measure of maximal plantarflexion strength for each participant (Arvanitidis et al., 2022). The torque signal was low pass filtered at 15 Hz and then used to quantify the torque steadiness (coefficient of variation of torque, SD torque/mean torque * 100) from the steady phase of the contractions (Martinez-Valdes et al., 2020). A custom-made MATLAB script was used to plot the torque exerted by each participant, visually identify the steady phase (approximately of 10 s) of the contraction, and select the starting and ending point of the time window needed for the analysis (Arvanitidis et al., 2022).

3.3.8.2 Motor unit analysis

The HD-sEMG signals recorded during the isometric plantarflexion contractions (10% and 40% MVC) were decomposed into motor unit spike trains with an algorithm based on blind source separation, which provides automatic identification of multiple single motor units (Martinez-Valdes et al., 2022). Each identified motor unit was assessed for decomposition accuracy with a validated metric (Silhouette, SIL) that represents the accuracy of the decomposed spike train (Martinez-Valdes et al., 2022), which was set to $\geq$ 0.90 (Negro et al., 2016a). SIL is a normalised measure of the relative height of the peaks of the decomposed spike trains with respect to the baseline noise (Martinez-Valdes et al., 2022).

The signals were decomposed throughout the whole duration of the submaximal contractions, and the discharge times of the identified motor units were converted into binary spike trains (Martinez-Valdes et al., 2018). The mean discharge rate and COVisi were calculated during the steady phase of the torque signal (10 s duration). Motor unit recruitment threshold was defined as the plantarflexor torque (%MVC) at the time when the motor units began firing action potentials (Martinez-Valdes et al., 2020). Missing pulses producing non-physiological firing rates i.e., inter-spike intervals > 250 ms, were manually and iteratively excluded and the pulse train was re-calculated. Additionally, in cases where the algorithm incorrectly assigned two or three pulses for only a single firing, the operator removed this firing and the final pulse trains were re-estimated (Martinez-Valdes et al., 2022). All single motor unit data was recorded, analysed, and reported according to the consensus for experimental design in electromyography: single motor unit matrix (Martinez-Valdes et al., 2023).

3.3.8.3 Motor unit recruitment threshold matching

MG, LG, and SO motor units were matched by their recruitment threshold with a tolerance of ± 1% MVC. The matched motor units were then grouped into two groups according to their recruitment thresholds (0-10% MVC and 10-40% MVC) (Martinez-

Valdes et al., 2018), in order to avoid between-muscle differences in recruitment threshold affecting mean discharge rate results, due to potential identification of different populations (low vs high-threshold) of motor units across muscles.

3.3.8.4 Neuromechanical delay

Neuromechanical interactions between motor unit rate coding and force generation were determined using cross-correlation to assess similarities and delays between fluctuations in motor unit firing activity and torque. Delays between the motor unit firing activity and torque were used as a measure of the NMD. Motor unit discharge times obtained were summed to generate a CST that represents the cumulative activity of multiple motor units (Martinez-Valdes et al., 2022). The signals obtained from CST and torque were low pass filtered (4^{th} order zero-phase Butterworth, 2 Hz) and then high pass filtered (4^{th} order zero-phase Butterworth, 0.75 Hz) as presented previously (Martinez-Valdes et al., 2021). Then, filtered signals were cross-correlated to determine the similarities in their fluctuations (cross-correlation coefficient) and to obtain the NMD (calculated from the lags found from the cross-correlation function) between CST and torque (Martinez-Valdes et al., 2022). Finally, cross-correlation coefficient between signals was computed in 5-s segments with 50% overlap (Martinez-Valdes et al., 2022). The average cross-correlation coefficient and NMD obtained from these segments was reported.

3.3.9 Statistical analysis

Descriptive statistics were used to report the data which are presented as mean ± SD, unless otherwise stated. The Shapiro-Wilk test was used to assess data normality. Sphericity was assessed by Mauchly test, and if violated, the Greenhouse-Geisser correction was applied to the degrees of freedom. The level of significance for all statistical procedures was set at $P<0.05$ and 95% confidence intervals (CI) were reported. First, the intra-rater/inter-session reliability for the morpho-mechanical properties of the Achilles tendon was assessed. Intraclass Correlation Coefficient (ICC), a measure of relative reliability, was calculated using a two-way mixed effects model with absolute agreement. The following criteria were used to determine reliability: <0.5 poor, 0.5 – 0.75 moderate, 0.75–0.9 good, and >0.9 excellent (Koo and Li, 2016). Additionally, the standard error of the measurement (SEM) was included as a measure of absolute reliability. The SEM represents differences in measurements units, considering both the inter-variation within individuals and the variability of the measurement (Atkinson and Nevill, 1998), and was obtained from the residual error of a within-subject analysis of variance (ANOVA).

Discharge rate and COVisi variables were compared between muscles at each torque level with a linear mixed model analysis with factors muscle (MG, LG, and SO) and torque (10% and 40%) as fixed effects, and participants as random effect. Cross-correlation coefficients and NMD were compared between muscles and all muscles combined (ALL) at each torque level with a linear mixed model with factors muscle (MG, LG, SO, and ALL) and torque (10% and 40% MVC) as fixed effects, and participants as random effect. All motor unit data obtained from each participant was averaged and then compared across individuals. When the linear mixed model was significant, pairwise comparisons were performed with Tuckey post hoc analysis.

A multiple linear regression (stepwise) analysis was performed on the motor unit parameters and maximal voluntary torque to identify the variables that predicted changes in morphological and mechanical variables of the Achilles tendon. Therefore,

morphological and mechanical Achilles tendon properties (length, thickness, CSA, and stiffness) were used as dependent variables and motor unit parameters (Discharge rate, COVisi and NMD) and maximal voluntary torque were regarded as independent variables. Additionally, a multiple linear regression (stepwise) analysis was performed on the motor unit parameters to identify the variables that predicted changes in torque steadiness. Consequently, torque steadiness was used as a dependent variable and motor unit parameters (Discharge rate, COVisi and NMD) were regarded as independent variables.

IBM SPSS Statistics software, V. 29.0 (Armonk, New York, USA) and GraphPad Prism software V.8.0.2 (San Diego, California, USA) were used for statistical analysis of the data.

3.4 RESULTS

3.4.1 Intra-rater/Inter-session reliability

Intra-rater/Inter-session reliability analysis revealed excellent reliability for length and thickness (ICC: 0.99 and 0.99), good reliability for stiffness (ICC: 0.90) and moderate reliability for CSA (ICC: 0.64) **(Table 4).**

Table 4. Intra-rater/Inter-session reliability results of the ultrasonography morpho-mechanical measures.

	ICC	SEM
Length (cm)	0.99 (0.95-0.99)	0.25
Thickness (cm)	0.99 (0.97-0.99)	0.002
CSA (cm^2)	0.64 (-0.34-0.94)	0.011
Stiffness (kPa)	0.90 (0.50-0.99)	2.67

CSA, cross-sectional area; ICC, intraclass correlation coefficient; SEM, standard error of measurements.

3.4.2 Morphological and mechanical properties of the Achilles tendon

Length, thickness, CSA, and stiffness are presented in **Table 5**. Mean ± SD and minimum-maximum values are reported.

Table 5. Morphological and mechanical properties of the Achilles tendon

	Mean ± SD	minimum-maximum
Length (cm)	19.89 ± 2.57	15.40 – 25.50
Thickness (cm)	0.39 ± 0.04	0.35 – 0.50
CSA (cm^2)	0.41 ± 0.07	0.31 – 0.53
Stiffness (kPa)	75.95 ± 9.98	53.35 – 91.66

CSA, cross-sectional area; SD, standard deviation.

3.4.3 Motor unit decomposition

A total of 1892 motor units were identified in the triceps surae muscle during the submaximal contractions (across all participants). At 10% MVC, the average number of motor units identified were 13.6 ± 17.90, 5.56 ± 9.6, and 12.12 ± 8.95 for the MG, LG, and

SO muscles, respectively. At 40% MVC, the average number of motor units identified were 22.44 ± 23.49, 9.64 ± 11.21, and 12.32 ± 7.52 for the MG, LG, and SO muscles, respectively. Regarding the recruitment-threshold-matched motor units, a total of 397 motor units were matched between the MG, LG, and SO muscles, with an average of 6.64 ± 7.69 and 9.24 ± 8.94 for each participant during the 10% and 40% MVC tasks, respectively.

3.4.4 Discharge rate and discharge rate variability

Average discharge rate calculated from recruitment-threshold-matched motor units from MG, LG, and SO muscles at 10 and 40% MVC are presented in **Figure 14A**. Overall, average discharge rate increased as the target torque increased, but discharge rate was similar between muscles (torque effect: $P<0.0001$, mean difference=-2.11, CI=-2.79 to -1.45, muscle effect: $P=0.175$). Average COVisi calculated from recruitment-threshold-matched motor units from the MG, LG, and SO at 10 and 40% MVC are presented in **Figure 14B.** In general, average COVisi increased as the target torque increased, with a difference between muscles; however, no torque-muscle interaction was found (torque effect: $P<0.0001$, mean difference=-4.00, 95% CI=-5.73 to -2.27; muscle effect: $P=0.0069$, interaction force-muscle: $P=0.90$). Given that there were no between-muscle differences in discharge rate, we averaged discharge rate results from all muscles (Discharge rate ALL) and inserted this variable into the linear regression. Meanwhile, COVisi results from each muscle were inserted into the multiple regression independently since there were significant differences between muscles.

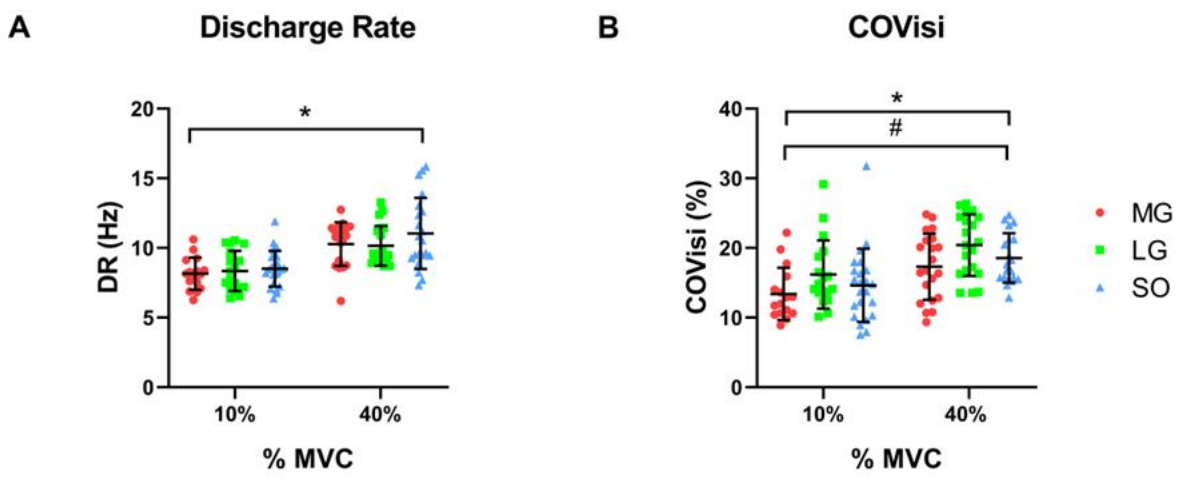

Figure 14. Motor unit discharge rate and COVisi.
A) Motor unit discharge rate calculated from recruitment-threshold-matched motor units from medial gastrocnemius (MG: red dot), lateral gastrocnemius (LG: green square) and soleus (SO: blue triangle) muscles at 10 and 40% maximum voluntary contraction (MVC). B) Motor unit coefficient of variation for the interspike interval (COVisi) calculated from recruitment-threshold-matched motor units from MG, LG, and SO muscles at 10% and 40% MVC. A linear mixed model was used for statistical comparisons. Motor unit discharge rate and motor unit COVisi values (means ± SD) were averaged for each subject and presented at each submaximal target torque (10 and 40% MVC). * Main effect of torque, P<0.0001. # Main effect of muscle, P=0.0069.

3.4.5 Cross-correlations and neuromechanical delay

Cross-correlation coefficient results calculated from motor units from MG, LG, and SO at 10% and 40% MVC are presented in **Figure 15A.** Overall, cross-correlation coefficients did not change as the target torque increased; however, the cross-correlation coefficient between CST and torque was greater when the CST from all muscles was combined (torque effect: P=0.28, mean difference=-0.034, CI=-0.098 to 0.029, muscle effect: P= 0.0022). Furthermore, NMD results calculated from the CSTs from MG, LG, and SO at 10% and 40% are presented in **Figure 15B.** NMD did not change as the target torque increased and did not differ between muscles (torque effect: P=0.06, mean difference 53.69, CI=-279 to 110.2, muscle effect: P=0.73). Therefore, we used the NMD obtained from all muscles (ALL) and we inserted this variable in the multiple regression.

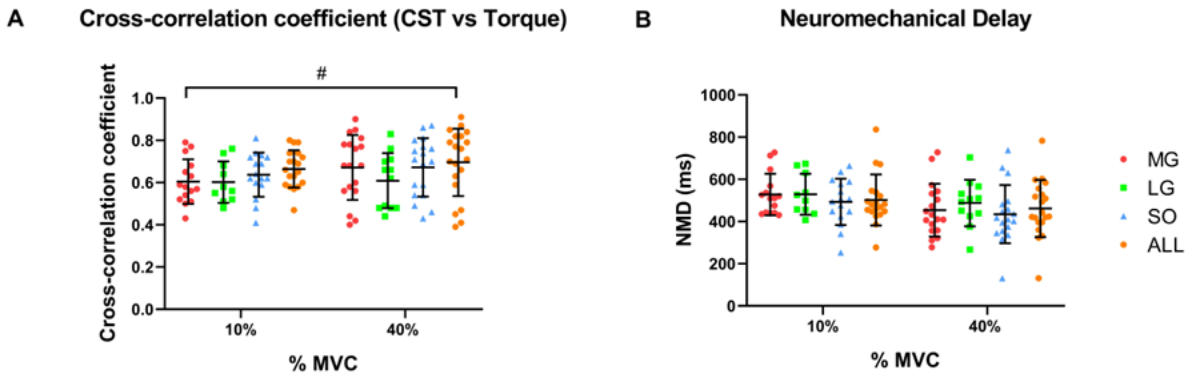

Figure 15. Cross-correlation coefficient between cumulative spike train and torque, and neuromechanical delay.
A) Cross-correlation coefficients between cumulative spike train (CST) vs torque from medial gastrocnemius (MG: red dot), lateral gastrocnemius (LG: green square), soleus (SO: blue triangle) and all muscles combined (ALL: orange dot) at 10% and 40% maximum voluntary contraction (MVC) B) Neuromechanical delay (NMD) from MG, LG, SO and ALL at 10% and 40% MVC. A linear mixed model was used for statistical comparisons. Cross-correlation coefficient and NMD values (means ± SD) were averaged for each subject and presented at each submaximal target torque (10 and 40% MVC). # Main effect of muscle, P=0.0022.

3.4.6 Multiple Linear Regression

Motor unit variables (Discharge rate ALL, COVisi MG, COVisi LG and COVisi SO) were entered into the multiple linear regression analysis to assess which of these variables was associated with the morpho-mechanical properties of the Achilles tendon (length, thickness, CSA, and stiffness). **Table 6** reports the results of the multiple regressions for these variables.

When the morphological properties were analysed as dependent variables, at 10% MVC, only COVisi SO was entered into the model, explaining 30.4% of the variance in the length. However, at 40% MVC, both COVisi MG and COVisi SO were entered into the model, explaining 48.7% of the variance in length. Additionally, at 40% MVC, COVisi SO was entered into the model, explaining 29% of the variance in the thickness. Maximal voluntary torque was not entered into the model for any of the morphological parameters.

When tendon stiffness was analysed as dependent variable, at 10% MVC, only Discharge rate ALL was entered into the model, explaining 41.7% of the variance in Achilles tendon stiffness. Discharge rate ALL was negatively associated with stiffness, meaning that the discharge rate of the triceps surae was higher in tendons with lower stiffness. MG motor unit discharge rate and SWE results for two representative participants with different levels of Achilles tendon stiffness can be seen in **Figure 16.** Maximal voluntary torque was not entered into the model for the mechanical parameter.

Furthermore, when the NMD was analysed as the dependent variable at both target torque levels, none of the morpho-mechanical tendon variables were entered into the model. Moreover, when we analysed torque steadiness as the dependent variable, none of the motor unit properties or morpho-mechanical parameters were entered into the model.

Table 6. Mean ± SD and correlation coefficients between dependent variables and independent variables.

Table 6. Mean ± SD and correlation coefficients between dependent variables (morphological-mechanical properties) and independent variables: Discharge rate ALL, COVisi MG, COVisi LG and COVisi SO

Dependent variable	Mean ± SD	Torque Level, %MVC	Discharge rate ALL (Hz)	COVisi MG (%)	COVisi LG (%)	COVisi SO (%)
Length (cm)	20.31 ± 2.80	10	8.23 ± 1.14, r=-0.27	12.92 ± 3.88, r=-0.31	14.09 ± 2.60, r=-0.005	13.43 ± 3.57, **r=-0.61***
	20.12 ± 2.73	40	10.56 ± 1.52, r=-0.20	17.17 ± 4.84, **r=-0.57***	20.41 ± 4.42, r=-0.28	18.28 ± 3.40, **r=-0.59***
Thickness (cm)	0.41 ± 0.04	10	8.23 ± 1.14, r=0.15	12.92 ± 3.88, r=-0.22	14.09 ± 2.60, r=0.24	13.43 ± 3.57, r=-0.14
	0.40 ± 0.04	40	10.56 ± 1.52, r=-0.21	17.17 ± 4.84, r=-0.15	20.41 ± 4.42, r=-0.05	18.28 ± 3.40, **r=-0.57***
CSA (cm^2)	0.41 ± 0.07	10	8.23 ± 1.14, r=-0.28	12.92 ± 3.88, r=-0.44	14.09 ± 2.60, r=-0.50	13.43 ± 3.57, r=0.11
	0.40 ± 0.06	40	10.56 ± 1.52, r= 0.21	17.17 ± 4.84, r=-0.39	20.41 ± 4.42, r=-0.27	18.28 ± 3.40, r=-0.08
Stiffness (kPa)	78.50 ± 8.51	10	8.23 ± 1.14, **r=-0.69***	12.92 ± 3.88, r=-0.65	14.09 ± 2.60, r=0.05	13.43 ± 3.57, r=0.12
	76.03 ± 9.81	40	10.56 ± 1.52, r=-0.16	17.17 ± 4.84, r=-0.11	20.41 ± 4.42, r=-0.10	18.28 ± 3.40, r=0.17

CSA, cross-sectional area; %MVC, percentage of the maximal voluntary contraction; DR, discharge rate; ALL, all muscles; COV_{isi} coefficient of variation of the interspike interval; MG, medial gastrocnemius; LG, lateral gastrocnemius; SO, soleus. *Significant correlation (P<0.05)

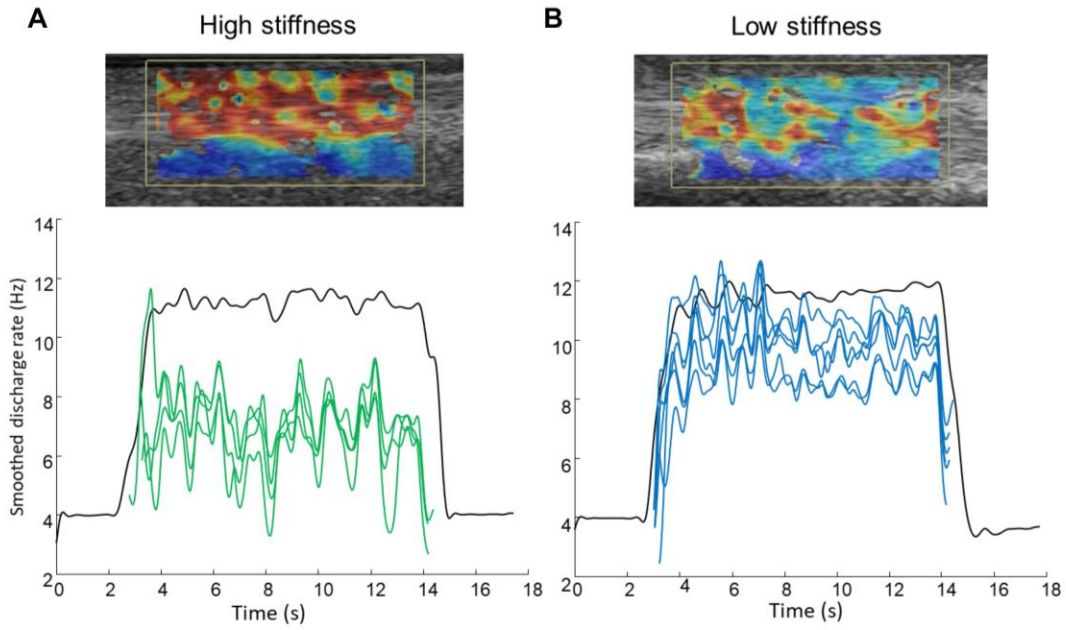

Figure 16. Motor unit discharge rate and Achilles tendon stiffness for two representative participants. A) Individual with high Achilles tendon stiffness (top, shear-wave elastography map) and low discharge rate (bottom). B) Individual with low Achilles tendon stiffness (top, shear-wave elastography map) and high discharge rate (bottom).

3.5 DISCUSSION

This study revealed that changes in tendon stiffness could be predicted by changes in triceps surae motor unit discharge rate at low forces. In addition, motor unit firing rate variability (estimated by COVisi) of individual triceps surae muscles was able to predict changes in tendon morphology in a load-dependent manner. Previous studies showed some evidence of the relationship between active/passive tendon mechanics and muscle activity; however, to our knowledge, this is the first study to observe a relationship between motor unit firing and tendon morpho-mechanical properties. By employing HD-sEMG, conventional B-mode ultrasonography, and SWE we were able to identify neuromechanical relationships relevant for the generation of force.

3.5.1 Neural drive to MG, LG, and SO muscles

It is difficult to compare the neural drive (motor unit discharge rate and recruitment) received by agonist muscles due to the limitations in HD-sEMG decomposition techniques which are only able to identify a subset of the populations of active motor units (Martinez-Valdes et al., 2018). However, an indirect assessment of the neural drive received by synergistic muscles can be estimated by comparing firing parameters from motor units matched by recruitment threshold (Martinez-Valdes et al., 2018). This approach minimises the effect of recruitment-threshold dependent variations in discharge rate between muscles, which can be due to the identification of different populations of motor units by the decomposition algorithm. By employing this approach, we observed no differences in motor unit discharge rate between muscles at 10% and 40% MVC (Fig. 4 A). Nevertheless, when we estimated the motor unit firing rate variability through COVisi, we observed differences between muscles. Specifically, the COVisi was higher in the LG compared with the MG at 40% MVC (Fig. 4B). This is an interesting finding, since a recent study has shown minimal common drive between MG and LG muscles during isometric plantarflexion contractions estimated by coherence analysis between CST of each muscle at similar target torques (Hug et al., 2021). The relative independent control of

these muscles may allow for flexible control of the ankle joint to comply with their functions (e.g., maintaining balance, joint stabilisation, distribution of tendon strain) during different tasks (Hug et al., 2021). This theory is partially supported by studies showing smaller volume and longer fascicles in the LG muscle compared to the MG (Crouzier et al., 2018), and different actions in the frontal plane (Lee and Piazza, 2008, Vieira et al., 2010, Cohen et al., 2020).

Another method to estimate the effective neural drive to muscles is to sum the spike trains of the involved motor units, and then smooth the resultant signal to produce a continuous estimate of the command signal (CST) (Mazzo et al., 2022). Our findings indicate that CST was moderately correlated with fluctuations in the isometric plantarflexion torque at 10% and 40% MVC (R= 0.67 and R=0.69, respectively). The strength of our cross-correlation coefficients results was higher compared with the one reported by Mazzo et al. 2022, in the triceps surae muscles at 10% (R=0.582) and 35% (R=0.612). However, the strength of the cross-correlation coefficients between the CST estimates and torque fluctuations for the triceps surae muscle was not as strong as the one observed in tasks where a single muscle is involved (Mazzo et al., 2022). For example, Thompson et al. 2018, found higher cross-correlations between the neural drive and torque in isolated SO muscles during evoked contractions (R=0.84). Even so, this difference may be explained by the different species assessed (human vs cat) or type of contraction (voluntary vs electrically evoked). Other possible explanations are the involvement of other muscles (e.g., intrinsic foot muscles or accessory lower leg muscles) during the plantarflexion tasks (Mazzo et al., 2022) or the variable level of activation among the triceps surae muscles without compromising the net force (Finni et al., 2017, Maas and Sandercock, 2010). Additionally, our results showed no differences in the cross-correlation coefficients between the MG, LG, and SO at 10% and 40% MVC, the only difference observed was between MG and ALL at 10% MVC (Fig. 5 A). Moreover, no differences were observed as the target torque increased. These results indicate the cross-correlation coefficient between the CST and torque of the MG, LG, and SO can be used separately to estimate the moment-to-moment fluctuations in force during isometric plantarflexion contractions at low and moderate target torques. For this reason, we used

the average delay quantified from the cross-correlation function from all muscles to calculate the NMD described below.

3.5.2 Neuromechanical delay

The conversion of neural signals to force output has a latency due to the dynamic sensitivity of the motor neurons and to the time needed to stretch the series elastic components of the muscle-tendon unit following the electrical activation of the muscle fibers (Del Vecchio et al., 2018). Previous studies have used the EMD to determine the time lapse between the onset of muscle electrical activation and onset of force/torque production (Cavanagh and Komi, 1979, Esposito et al., 2009, Esposito et al., 2011a, Esposito et al., 2011b, Hug et al., 2011a, Hug et al., 2011b). However, this method does not provide information on the delay between neural drive to muscle and force (Del Vecchio et al., 2018). Due to this reason, in our study we used the NMD, which is defined as the time difference between the neural drive and the generated force/torque during a voluntary contraction. The NMD can be estimated from the time lag of the peak of the cross-correlation between the CST and torque (Del Vecchio et al., 2018). Our results showed that the mean estimated NMD was 502.05 ± 120.48 ms and 461.80 ± 135.25 ms at 10% and 40%, respectively. Overall, our results showed higher NMD values compared with previous studies. Del Vecchio et al, 2018 and Martinez-Valdes et al., 2021 reported NMD values of ~300 ms in the tibialis anterior muscle during isometric dorsiflexion contractions modulated at low frequencies and low target torques (Martinez-Valdes et al., 2022). These differences may be explained by the different muscles assessed (tibialis anterior vs triceps surae), the different contraction evaluated (modulated vs not modulated), or the dynamometer used. Additionally, our results showed no difference in the NMD between muscles at 10% and 40% MVC, neither as the target torque increase (Fig. 5 B), which is in agreement with the findings from Martinez-Valdes et al., 2022 (Martinez-Valdes et al., 2022).

3.5.3 Relationship between motor unit firing rate and morpho-mechanical properties

During walking and running, the muscular contraction modulates the amount of energy stored in the elastic tissues (Lichtwark and Wilson, 2006). Therefore, muscles must have the ability to produce or absorb mechanical work, and this behavior is highly dependent on the interactions between the active (myofibrils) and passive (mainly tendon and aponeurosis) elements of the series elastic components (Lichtwark and Wilson, 2006, Nordez et al., 2009). During isometric contractions, there is a shortening of the muscle fascicle and lengthening of the tendon at a fixed ankle joint; therefore, a shortening of the active element produced a lengthening of the passive/elastic element of the series elastic components (Ito et al., 1998). Additionally, it has been observed that the slackness and compliance of the passive/elastic element allow the fascicle length and pennation angle changes to occur during isometric contractions (Ito et al., 1998). Furthermore, it has been shown that after a static-stretch intervention of the triceps surae muscles, there is an increase in the discharge rate and decrease in motor unit recruitment thresholds at low muscle forces, suggesting that the adjustment in motor unit activity is likely related to the change in the compliance of the muscle-tendon unit following stretching (Mazzo et al., 2021). Consequently, there is a relationship between discharge rate, tension development and force-generating capacity (Mazzo et al., 2021).

Our results showed that differences in Achilles tendon's stiffness could be predicted by changes in the discharge rate ALL at 10% MVC. This is consistent with a previous study, which reported changes in the neural drive of the triceps surae muscles during isometric plantarflexion contractions at 10% MVC but not at 35% MVC after stretching (which increases tendon compliance) (Mazzo et al., 2021). This load-dependent relationship between motor unit firing rate and stiffness may be partially explained by the tendon's mechanical behavior. Tendon stiffness increases at higher muscle contraction levels, however, the rate of change in tendon's stiffness varies according to the amount of tension placed on the tendon (Maganaris and Paul, 1999). Tendons are lengthened more easily at low forces and then, a plateau in tendon length

is reached at higher force levels (Ito et al., 1998). It can be speculated that individuals with greater tendon compliance at low forces might have required greater motor unit firing output to control the contraction, while at higher forces, the higher tendon stiffness might have allowed a more efficient conversion of neural drive into muscle contraction and then force transmission to the tendon. This greater efficiency in the conversion of neural output into contraction and subsequent transmission of muscle force at higher force levels may have possibly increased the difficulty of detecting variations in tendon stiffness of the participants measured in the current study. Nevertheless, this load-dependent relationship may also be in part explained by the motoneuron modulation that occurs at the motoneuron dendrites and depends on the interactions between descending monoaminergic drive and spinal circuits (Heckmann et al., 2005). Since Golgi tendon organ mechanoreceptors can detect rapid changes in contractile force (Davies et al., 1995), and Ib afferents mostly inhibit homonymous motoneurons through di/tri-synaptic connections (Brown and Fyffe, 1981), it can be hypothesised that tendons with reduced stiffness might decrease the sensitivity of the Golgi tendon organ, lessening the inhibitory input to the α-motoneuron and therefore explaining the increase in the discharge in tendons with greater compliance.

Additionally, multiple regression analysis results showed that changes in length could be predicted by changes in COVisi SO at 10% MVC; however, changes in length could be predicted by changes in COVisi MG and COVisi SO at 40% MVC. These results might be explained by differences in the contribution of each of the triceps surae muscles to the net force at different target torques. In support of this notion, a recent study reported that during isometric plantarflexion contractions at 10% MVC, the activation ratio of the SO muscle was higher than the activation ratio of the MG, and both were higher than the activation ratio of the LG; However, at 50% MVC, the activation ratio of the SO decreased to similar values of the activation ratio of the MG, and both were higher than the activation ratio of the LG (Crouzier et al., 2018), suggesting that the COVisi of both muscles was able to predict changes in the Achilles tendon's length probably because at 40% MVC, they have a similar level of activation. However, we also observed that changes in the Achilles tendon thickness could be predicted by changes in the COVisi SO at 40% MVC,

indicating that these relationships between the variability of the discharge rate and the morphological properties of the tendon are not just influenced by the level of activation of each individual muscle, it might be possible that the discharge rate modulation of each muscle transmits differently to the tendon, even when the resultant modulation (torque steadiness) seems to be not affected by this, since we did not find associations between COVisi and torque steadiness.

3.5.4 Methodological considerations

There are some methodological aspects of this study which should be considered. First, the measurements of tendon stiffness were performed at 0° of plantarflexion, which places the Achilles tendon under light tension; therefore, this may have increased our stiffness values. Second, the ankle attachment of the isokinetic dynamometer that was used had two lever arms to measure plantarflexion; thus, the time to detect the torque may have been longer, influencing the results of the NMD. Finally, current ultrasound imaging devices with SWE have very limited sampling resolution (0.5 to 2 SWE images per second); future developments in SWE technology might enable an improved and concurrent assessment of the interplay between tendon stiffness and motor unit firing properties.

3.5 CONCLUSIONS

This study shows a contraction-intensity dependent relationship between the motor unit firing parameters of the triceps surae and the morpho-mechanical properties of the Achilles tendon. The most relevant finding is that individuals with increased tendon stiffness showed lower discharge rate at low target torque force. This novel approach provides valuable insights into the complex neuromechanical interactions during low-force voluntary isometric tasks. Our research contributes to a more comprehensive understanding of the underlying mechanisms involved in neural coding and muscle-tendon unit behavior and how this interplay impacts force generation during such tasks.

CHAPTER 4

Load-dependent changes in triceps surae motor unit firing properties in individuals with non-insertional Achilles tendinopathy.

This chapter presents the full contents of a manuscript that has been prepared for publication, along with changes made in the text specifically for the content of this thesis. It presents the baseline comparisons between asymptomatic and individuals with non-insertional Achilles tendinopathy.

Manuscript in preparation:

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Load-dependent changes in triceps surae motor unit firing properties in individuals with non-insertional Achilles tendinopathy.

Author Contributions:

IC-H, EM-V, and DF conceived and designed research; IC-H and MA performed experiments; IC-H and EM-V analysed data; IC-H and EM-V interpreted results of experiments; IC-H prepared figures; IC-H drafted manuscript; IC-H, EM-V, DF, FN and MA edited and revised manuscript.

4.1 ABSTRACT

It has been proposed that asymmetrical load transmission to the Achilles tendon may play an essential role in the pathophysiology of NIAT; however, there is limited evidence of how the triceps surae motor unit firing rate parameters may influence the asymmetrical load on the Achilles tendon in this condition. This study investigated triceps surae motor unit firing parameters in individuals with NIAT compared to controls at low, intermediate, and high force levels. Motor unit firing parameters (recruitment and derecruitment thresholds, discharge rate, COVisi, NMD and cross-correlation coefficient (between CST and torque)) of the MG, LG, and SO muscles were assessed using HD-sEMG during isometric plantarflexion contractions at 10%, 40%, and 70% of the MVC. Additionally, morpho-mechanical properties of the Achilles tendon were determined using B-mode ultrasonography and shear-wave elastography. Linear mixed-effect model analysis showed that the discharge rate of the LG increased ($P=0.002$), and the derecruitment threshold of the LG decreased ($P=0.039$) in the NIAT group compared to the control group at 70% MVC. Additionally, the cross-correlation coefficient of the LG decreased at 10% MVC ($P<0.0001$) and increased at 70% MVC ($P=0.013$) in the NIAT group compared to the control group. This study demonstrates that individuals with NIAT have load-dependent changes in LG motor unit firing rate parameters, affecting the contribution of the LG to the net plantarflexion force during plantarflexion isometric contractions.

4.2 INTRODUCTION

NIAT is a common debilitating tendon disorder in the lower extremity, characterised by an insidious onset of pain accompanied by swelling, stiffness and thickening in the midportion of the Achilles tendon (Bahari et al., 2023, Sara et al., 2023, Maffulli et al., 1998, van Dijk et al., 2011). NIAT can affect athletes and the general population, causing disability, functional impairment, and decreasing performance (Kvist, 1994, de Jonge et al., 2011). It is estimated that more than half (55-65%) of the Achilles tendon problems occur about 2-7 cm proximal to the superior border of the calcaneus (Järvinen et al., 2005). The aetiology of the NIAT remains debated (Maffulli et al., 2020); however, excessive loading of the tendon is considered the major causative factor (Leadbetter, 1992). Recent evidence suggest that the triceps surae muscle may influence the magnitude and distribution of the Achilles tendon load, stress and strain (Debenham et al., 2017, O'Neill et al., 2015); therefore, deficits in the performance of this muscle have been identified as key factors (O'Neill et al., 2019). In humans, plantarflexion is primarily accomplished by the triceps surae muscle, which comprises the MG, LG and SO muscles (Héroux et al., 2014). The resultant force generated by these muscles is then transmitted to the Achilles tendon, which links the triceps surae muscle to the calcaneus (Crouzier et al., 2020). The Achilles tendon is composed of three subtendons, arising from each of the triceps surae muscles (Crouzier et al., 2020); together, these subtendons exhibit a twisted structure, with the deep layer of the tendon formed by fibers from the LG and SO, and the superficial layer formed by fibers from the MG and SO, with a wide range of rotation of the subtendons between individuals (Szaro et al., 2009). Nonuniform displacements within the Achilles tendon have been observed using ultrasound imaging during isometric contractions (Clark and Franz, 2018), which are consistent with nonuniform distribution of load within the tendon (Arndt et al., 1998). Additionally, 3D models suggest that the nonuniform distribution of load within the Achilles tendon is largely due to the distribution of force among the MG, LG, and SO muscles (Handsfield et al., 2017). Consequently, it has been proposed that an imbalance of the force produced by these three muscles may cause an heterogenous load within the tendon, contributing to the development of NIAT (Bojsen-Møller and Magnusson, 2015, Hug and Tucker, 2017).

The morpho-mechanical properties of the Achilles tendon in individuals with NIAT have been studied extensively. The morphological changes include an increase in tendon thickness, CSA, and volume (Shalabi et al., 2004, Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Docking and Cook, 2016) and the mechanical changes comprise an increase in longitudinal and transverse strain, hysteresis, and lower stiffness and Young's modulus compared to healthy tendons under the same tensile load (Arya and Kulig, 2010, Child et al., 2010, Wang et al., 2012, Chang and Kulig, 2015). Alterations in the Achilles tendon morpho-mechanical properties are relevant because of their potential to modify the stress-strain patterns experienced by the tendon during muscle contraction (Hansen et al., 2017), and muscle's capacity to function within the force-length curve's optimal region (Arya and Kulig, 2010), possibly influencing the triceps surae motor unit parameters.

For the generation of movement, the CNS must effectively coordinate the many degrees of freedom of the musculoskeletal system, considering the nonlinear features of muscles and the dynamic interactions between the segments of the body, as well as the interactions between the body and the environment (d'Avella and Bizzi, 2005). This complex task might be simplified by organising a modular and hierarchical control (Bizzi et al., 2002, Loeb et al., 1999, Full and Koditschek, 1999). It has been hypothesised that the CNS uses a set of muscle synergies, the coherent activation in space or time of a group of muscles, as output modules. Thus, supraspinal and afferent signals combine with a determined number of muscle synergies to generate a variety of muscle activation patterns (d'Avella and Bizzi, 2005). Based on this, it has been theorised that alterations in how the CNS control the coordination of the triceps surae muscles could create uneven loading within the Achilles tendon and contribute to tendinopathy (Cook and Purdam, 2009). Although these muscles act as agonists that share the same common distal tendon, they exhibit anatomical, neurophysiological, and functional differences, indicating diverse functional roles (Héroux et al., 2014). These functional roles are linked to unique properties in motor unit firing rates between muscles when performing different tasks (Héroux et al., 2014).

The force exerted by a muscle during a voluntary contraction depends, partly, on the recruitment and discharge rate of the motor units (Enoka and Duchateau, 2017). Hence, alterations in motor performance may be the consequence of the inability of the CNS to increase the recruitment and/or the discharge rate of motor units (Enoka and Duchateau, 2017). Within this framework, the most reliable approach to investigate how the CNS modulates the recruitment and discharge firing rates of the triceps surae motor units during a voluntary contraction is through the individual analysis of the MG, LG, and SO muscles (Fernandes et al., 2023, Hug et al., 2021). To the best of our knowledge, only one study has investigated the motor unit firing rates properties of the triceps surae muscles in individuals with NIAT. Fernandes et al. 2021 reported no differences in the discharge rate of the LG between a group of runners with NIAT and a control group at low forces; however, they observed that in the NIAT group the discharge rate of the LG remained unchanged as the target torque increased from 10% to 20% MVC, in contrast to the control group, suggesting that the neural drive to the LG is affected in individuals with NIAT (Fernandes et al., 2023). Nevertheless, the authors only assessed the motor unit firing parameters during low force isometric plantarflexion contractions; consequently, it's fundamental to investigate the triceps surae motor unit firing parameters at increasing target torque levels.

We aimed to investigate triceps surae motor unit firing parameters in individuals with NIAT compared to controls at low, intermediate, and high force levels during isometric plantarflexion contractions. Our primary hypothesis is that individuals with NIAT exhibit altered LG motor unit firing parameters. Additionally, we hypothesised that these alterations depend on the force exerted during the task.

4.3 METHODS

4.3.1 Ethical approval

This study was approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, UK (ERN_20-0604A).

4.3.2 Participants

Twenty-five healthy (17 males, 8 females, 28.60 ± 3.92 years, 74.00 ± 11.57 kg, 171.10 ± 9.22 cm) and twenty-six participants with NIAT (14 males, 12 females, 29.04 ± 8.46 years, 75.09 ± 17.20 kg, 173 ± 9.16 cm) were recruited from the University of Birmingham staff/student population and the local community via leaflets, e-mail, and social media.

Inclusion criteria included men or women aged 18 to 55 years old. This range was chosen to prevent the influence of ageing-related alterations in tendon properties, since previous studies have found that the Achilles tendon has lower stiffness and Young's modulus in older individuals (Lindemann et al., 2020). For the NIAT group inclusion criteria were as follows: presence of NIAT determined by an experienced physiotherapist based on defined clinical findings (VISA-A (Robinson et al., 2001) and NRS (Alghadir et al., 2018) and pain for more than 3 months (Beyer et al., 2015)), physical examination and ultrasound imaging. Ultrasound imaging included assessing echoic pattern (focal hypoechoic and hyperechoic areas within the tendon) and tendon thickness (focal or diffuse thickening) (Bleakney and White, 2005). VISA-A scores less than 90 were considered as a reference to identify individuals with NIAT (Iversen et al., 2012). Due to the high variability in the NRS scores in individuals with NIAT (Beyer et al., 2015, Rompe et al., 2009), we considered individuals with an NRS score ≥ 2. Inclusion criteria for the healthy group included confirmation of a healthy tendon determined by the same physiotherapist through physical examination and ultrasound imaging.

The exclusion criteria for both groups were as follows: (1) current or history of chronic neurological, respiratory, or cardiovascular diseases, (2) systemic or inflammatory conditions including rheumatic, neuromuscular disorders, and malignancy, and (3) history of lower limb surgery. Specific exclusion criteria for the participants with NIAT were if they had undergone a corticosteroid injection in the Achilles tendon within the last 12 months or if they had been diagnosed with IAT. Specific exclusion criteria for the healthy group were history of NIAT, lower limb surgery or pain/injury in the lower limbs within the previous 6 months.

4.3.3 Study design

This cross-sectional study was conducted from October 2021 to April 2023 at a laboratory within the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), University of Birmingham, UK. The study was conducted according to the declaration of Helsinki and participants signed written informed consent prior to participation. The STROBE guideline was used to facilitate reporting (Vandenbroucke et al., 2007).

Participants made a single visit to the laboratory for the experimental session (2.5 hours). They were instructed to avoid any vigorous physical activity within 24 hours prior to testing. The assessed leg was randomised in the control group, and the leg with tendinopathy symptoms was evaluated in the NIAT group. In the case of individuals with bilateral symptoms, the most painful leg was assessed.

4.3.4 Experimental setup and tasks

Experimental sessions included completing a battery of questionnaires, physical examination, ultrasonography of the Achilles tendon, HD-sEMG of the triceps surae muscles and torque recordings during plantarflexion isometric contractions. A representation of the experimental setup is shown in **Figure 17.**

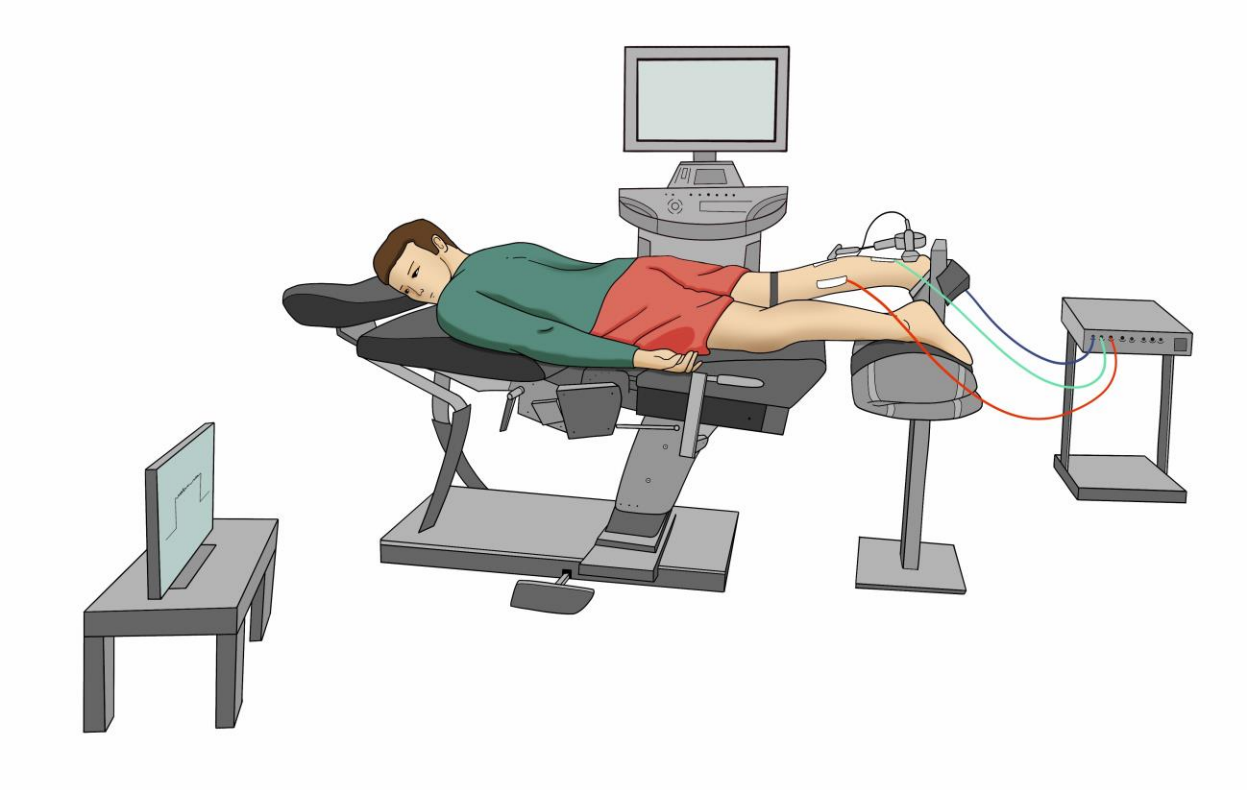

Figure 17. Representation of the experimental setup.

Anthropometric data (age, gender, weight, height, and foot dominance) was obtained across the participants. Foot preference in specific daily activities (foot dominance) was determined using a behavioral foot-preference inventory (Chapman et al., 1987). For the NIAT group, we obtained information regarding their symptoms (intensity of pain (NRS), duration of symptoms, and presence of bilateral symptoms). Then, all participants were asked to complete a battery of questionnaires, which included the VISA-A (Robinson et al., 2001), the International Physical Activity Questionnaire short form (IPAQ-SF) (Craig et al., 2003), Foot and Ankle Ability Measure (FAAM) (Martin et al., 2005), Pain Catastrophising Scale (PCS) (Sullivan et al., 1995) and Tampa Scale for Kinesiophobia (TSK) (Kori, 1990). Then, participants lay prone on the chair of a Biodex System 3 dynamometer (Biodex Medical System), with their knees extended and their assessed foot tightly strapped on the footplate. The ankle was positioned in 0° of plantarflexion with the axis of the dynamometer aligned with the inferior tip of the lateral

malleolus. The pelvis was stabilised with a strap to minimise compensatory movements. Ultrasonography was used to determine the length, thickness, and CSA of the Achilles tendon (see procedure below). Then, the skin was shaved, cleaned, and prepared, and the electrodes grids were placed on the MG, LG, and SO muscles (see details below). Following the placement of the electrode grids, we assessed Achilles tendon stiffness through SWE during rest conditions. HD-sEMG was used to confirm that the muscles were not active during these measurements as this could influence the estimation of stiffness.

Next, participants performed a warm-up protocol consisting of 3 submaximal isometric plantarflexion contractions at their perceived 30% of maximal voluntary force for 5 seconds with 30 seconds rest between contractions. The MVC was then determined during 3 isometric plantarflexion contractions of 5 seconds with 2 minutes rest between contractions (Martinez-Valdes et al., 2018) at 0° of plantarflexion. The highest MVC value was used as the reference maximal torque. Then, we measured the electromyographic activity of the MG, LG, and SO muscles during two isometric plantarflexion contractions at 10, 40 and 70% MVC (10% MVC/s ramp-up, 10 s hold, 10% MVC/s ramp-down and 30 s rest) with HD-sEMG. Visual feedback of the torque produced by the participant was provided through a computer monitor positioned 1 m in front of the participant. The order of the contractions at different target torque levels (10, 40, and 70% MVC) was randomised using a randomisation app (Randomizer). A schematic representation of the experimental session is shown in **Figure 18.**

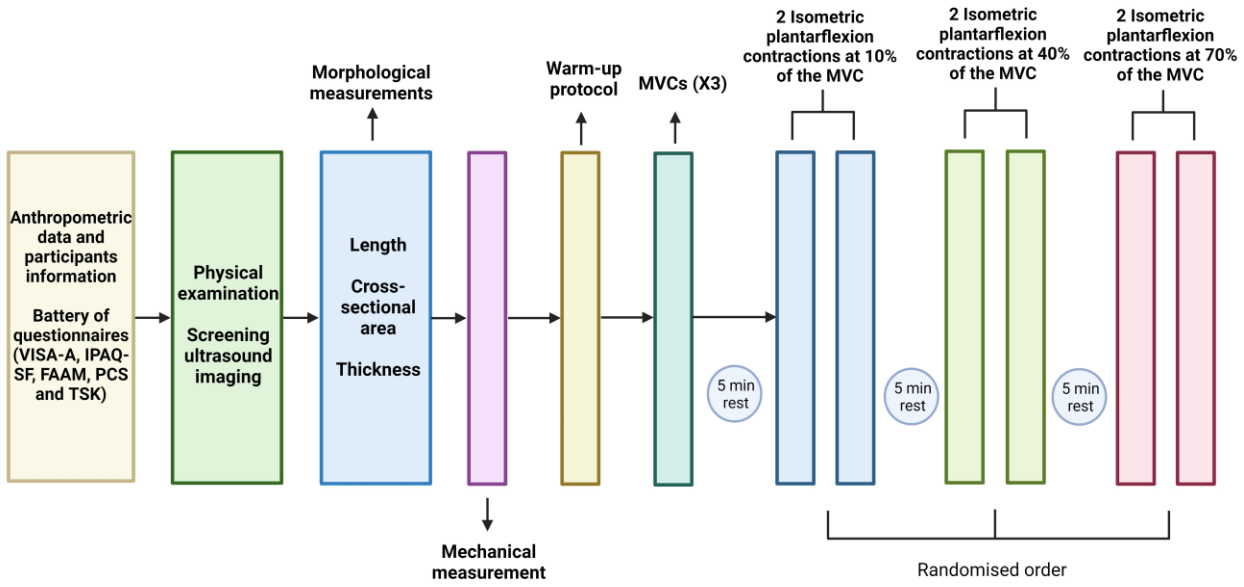

Figure 18. Schematic of the experimental procedure.
The order of the contractions performed at each target torque (10, 40, and 70% MVC) was randomised. VISA-A, Victorian Institute of Sports Assessment- Achilles Questionnaire; IPAQ-SF, International Physical Activity Questionnaire short form; FAAM, Foot and Ankle Ability Measure; PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia; MVC, maximum voluntary contraction.

4.3.5 Ultrasonography

All ultrasound images were obtained using an ultrasound imaging device equipped with SWE (LOGIQ S8 GE Healthcare, Milwaukee, USA). Tendon thickness, length, and CSA were recorded in B-mode with a 16-linear array probe (50 mm, 4-15 MHz). SWE was recorded in elastography mode with a 9-linear array probe (44 mm, 2-8 MHz).

The morphological properties of the Achilles tendon were determined using an adaptation of the protocol developed by Arya and Kulig (Arya and Kulig, 2010). The ultrasound probe was placed longitudinally over the posterior part of the heel with the ankle at 0° of plantarflexion, and the calcaneal notch was identified. Then, a fine wire (3.2 x 40 mm) was used under the probe to create an artifact in the ultrasound image. The artifact was aligned with the calcaneal notch and the corresponding point was marked on the skin with a marker (this mark represented the Achilles tendon's insertion). The ultrasound probe was moved proximally to identify the MTJ of the MG, and again, the

wire was aligned with the MTJ of the MG and the corresponding point was marked on the skin. The distance between these two marks (calcaneal notch and MTJ of the MG represented the length of the Achilles tendon). Subsequently, marks were made at 2, 4 and 6 cm above the Achilles tendon's insertion and these marks were used as reference to place the middle part of the probe in the longitudinal plane to obtain the thickness of the Achilles tendon at 2, 4 and 6 cm of its insertion. Similarly, these reference marks were used to place the probe in the transversal plane and determine the CSA at 2, 4 and 6 cm of the Achilles tendon's insertion. Three ultrasound images were obtained in the longitudinal and transverse planes for each mark.

For the HD-sEMG electrode grid placement, a tape and marker were used to draw a line following the direction of the Achilles tendon. This line and the distal MTJ of the MG were used as references for the positioning of the electrode grids. Briefly, for the MG electrode grid, a mark was made 10 cm above the distal MTJ of the MG and 4 cm medial to the line that followed the direction of the Achilles tendon. Similarly, for the LG electrode grid, the leg was marked 10 cm above the distal MTJ of the MG and 4 cm lateral to the line that followed the direction of the Achilles tendon. Likewise, for the SO electrode grid, the leg was marked 5 cm below the distal MTJ of the MG and 4 cm lateral to the line that followed the direction of the Achilles tendon. The central electrodes of the HD-sEMG grids (electrode in row 7 and column 3) were placed on top of all these marks.

For the mechanical parameters (i.e., estimated stiffness), the ankle was placed at 0° of plantarflexion and the ultrasound probe was placed longitudinally, with the middle part of the probe located at 4 cm above the Achilles tendon's insertion. A probe holder was used to avoid applying pressure over the tendon. A trial SWE measurement was done to check for possible voids in the stiffness estimation and if voids were detected, the probe was removed, ultrasound gel was added, and the probe was placed again. Elastography images were acquired for 12 seconds (twice). Due to the equipment properties, as a SWE image was obtained every 2.4 seconds; thus, to obtain 4 SWE images, the elastography measurements lasted 12 seconds.

4.3.6 HD-sEMG and torque recordings

Participants' skin was shaved, gently abraded (Nuprep, Skin Prep Gel, Weaver and Company, Aurora, Colorado), cleaned with water and dried with tissue paper. Three two-dimensional (2D) adhesive grids (OT Bioelettronica, Italy) of 13 x 5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm) were used to record the HD-sEMG signals from the MG, LG, and SO muscles (position of each grid was described above). The HD-sEMG grid was prepared by attaching a double-sided adhesive foam to the grid surface (SPES Medica, Genova, Italy) and by filling the grid cavities with conductive paste which provided adequate electrode-skin contact (AC-CREAM, SPES Medica, Genova, Italy).

HD-sEMG signals were converted by a 16-bit analogue-digital converter (Quattrocento- OT Bioelecttronica, Torino, Italy). Signals were amplified by a factor of 150, sampled at 2048 Hz, and filtered with a band-pass filter (bandwidth: 10-500 Hz, first order, -3 dB) (Arvanitidis et al., 2019). HD-sEMG signals were acquired in monopolar mode with ground electrodes (WhiteSensor WS, Ambu A/S, Ballerup, Denmark) located in the head of the fibula and with a wet strap in the thigh of the assessed leg. Electrode grids and ground electrodes were connected to an bioelectrical amplifier (Quattrocento-OT-Bioelecttronica, Torino, Italy) and the torque exerted by the participants was obtained with a Biodex System 3 dynamometer (Biodex Medical System), which was synchronised with the HD-sEMG signals through the auxiliary input of the EMG amplifier (Arvanitidis et al., 2019).

4.3.7 Image analysis

4.3.71 Ultrasound images analysis

After obtaining the ultrasound images, a reference line of 1 cm was drawn using the ultrasound device tools. Then, using the software ImageJ (http://imagej.nih.gov/ij), the reference line was converted into pixels, and set as a scale. Next, the width of the

ultrasound image was determined, and the middle point marked on the image. To obtain the Achilles tendon thickness, the distance between the superficial and deep part of the paratenon was measured at 2, 4, and 6 cm from the Achilles tendon's insertion. The measurements of three ultrasound images were averaged for each position. Conversely, ultrasound device tools were used to determine the CSA. Briefly, a discontinuous line was drawn following the internal part of the paratenon as reference and the CSA was measured at 2, 4, and 6 cm of its insertion. Similarly, the measurements of three ultrasound images were averaged for each position.

For the stiffness measurements, we obtained approximately 4 SWE colour maps (height x width, 2.5 cm x 1 cm) selected using the elastography tools. Then, using the ultrasound device tools, a line was drawn in the middle of the ultrasound image (this line indicates the 4 cm distance from the Achilles tendon). A ROI of 3 mm diameter (Siu et al., 2016) was aligned with the reference line and located in the middle of the tendon, and the stiffness (kPa) was determined. Lastly, mean stiffness was calculated over the ROIs of the 4 consecutive images (Coombes et al., 2018).

4.3.8 HD-sEMG signal analysis

4.3.8.1 Motor unit analysis

The HD-sEMG signals recorded during the two isometric plantarflexion contractions at each target torque (10, 40, and 70% MVC) were decomposed into motor unit spike trains with an algorithm based on blind source separation, which provides automatic identification of multiple single motor units (Martinez-Valdes et al., 2022). Single motor units were assessed for decomposition accuracy with a validated metric (Silhouette, SIL), which was set to $\geq$ 0.90 (Negro et al., 2016a). SIL is a normalised measure of the relative height of the peaks of the decomposed spike trains with respect to the baseline noise (Martinez-Valdes et al., 2022).

The HD-sEMG signals were decomposed throughout the duration of the submaximal contractions, and the discharge times of the identified motor units were converted into binary spike trains (Martinez-Valdes et al., 2018). Recruitment threshold was defined as the torque (%MVC) at the time when motor units began firing action potentials and derecruitment threshold was defined as the torque (%MVC) at the time when motor units ceased firing action potentials (Martinez-Valdes et al., 2020). Discharge rate and COVisi were calculated during the steady phase of the torque signal (10 s duration). Missing pulses producing non-physiological firing rates i.e., inter-spike intervals > 250 ms, were manually and iteratively deleted and the pulse train was re-estimated. Furthermore, if the algorithm assigned two or more pulses for a single firing, we removed the additional firing and the final pulse trains were re-calculated (Martinez-Valdes et al., 2022). Motor unit data was recorded, analysed, and reported according to the consensus for experimental design in electromyography: single motor unit matrix (Martinez-Valdes et al., 2023).

4.3.8.2 Neuromechanical delay and cross-correlation coefficient

Motor unit rate coding and force generation interactions were determined using cross-correlation to assess delays and similarities between fluctuations in cumulative motor unit firing of each individual muscle (MG, LG, and SO) and torque. Motor unit discharge times obtained were summed to generate a CST that represents the cumulative activity of multiple motor units (Martinez-Valdes et al., 2022). CST and torque parameters were low pass filtered (4^{th} order zero-phase Butterworth, 2 Hz) and then high pass filtered (4^{th} order zero-phase Butterworth, 0.75 Hz) as presented previously (Martinez-Valdes et al., 2021). Then, CST and torque were cross-correlated to determine the delay in their fluctuations (calculated from the lags obtained from the cross-correlation function) and the similarities in their fluctuations (cross-correlation coefficient) (Martinez-Valdes et al., 2022). Delays between the motor unit firing activity and torque were used as a measure of the NMD. Finally, cross-correlation coefficient between signals was computed in 5-s segments with 50% overlap (Martinez-Valdes et al., 2022). The average NMD and cross-correlation coefficient calculated from these segments was reported. Furthermore, the

NMD and cross-correlation coefficient of the triceps surae muscle (MG, LG, and SO combined) was also calculated.

4.3.8.3 Torque signal analysis

The highest peak torque (SI: Newton-meters) exerted during the MVCs was low pass filtered at 15 Hz and then used to quantify the torque steadiness (coefficient of variation of torque (Coefficient of variation of torque, SD torque/mean torque * 100) from the steady phase of the contractions (Martinez-Valdes et al., 2020). The torque exerted by each participant was plotted using a custom-made MATLAB script allowing us to identify and select the steady phase of the contraction needed for the analysis (Arvanitidis et al., 2022).

4.3.9 Statistical analysis

Descriptive statistics were used to report the data which are presented as mean ± SD, unless otherwise specified. The Shapiro-Wilk test was used to determine the normality of the data, and the Levene test was used to evaluate the assumption of homogeneity of variance. As these assumptions were met, parametric statistical tests were deemed appropriate; however, if these assumptions were not met, non-parametric statistical tests were used. The level of significance for all statistical procedures was set at $P<0.05$ and 95% confidence intervals (CI) were reported. Participant characteristics, questionnaire scores, morpho-mechanical properties of the Achilles tendon and maximal voluntary torque were compared between groups using Independent T-test for continuous variables. IBM SPSS Statistics software, V. 29.0 (Armonk, New York, USA) was used for these comparisons.

All statistical analyses for the HD-sEMG were done using R (version 4.2.1, R Development Core Team, 2023). A generalised linear mixed-effects model (GLMM) analysis was performed with the R package lme4 (version 1.1.31). The following model was used *"variable of interest ~ group * muscle * torque (1| subject)".* This model is read as a "variable of interest predicted by each of the factors". The variable of interest (on the left) is the dependent variable and the ones on the right (except the subject) are the independent (explanatory) variables, or "fixed effects", group (Control, NIAT), muscle (MG, LG, SO), and torque (10, 40 and 70% MVC). The 1|subject in the model is the "random effect", which characterises the variation that is due to individual differences. The normality of residuals was assessed using the Shapiro-Wilk test after running the model. Residual outliers were removed using Cook's distance method (considering as threshold, a distance of 4 times the SD) when the normality assumption was not met. Post-hoc pairwise comparisons were conducted using Sidak correction and least-squares contrasts, as implemented in the R package emmeans (version 1.8.8). The post-hoc tests were used based on the interactions identified with the GLMM. Post-hoc results were reported as p-value and 95% confidence intervals (CI).

Torque steadiness was compared between groups at each torque level with a linear mixed model analysis with factors group (Control, NIAT) and torque (10, 40, 70% MVC) as fixed effects, and participants as random effect using GraphPad Prism software V.8.0.2 (San Diego, California, USA).

Relationships between pain level and questionnaire scores and neuromuscular and morpho-mechanical parameters were analysed through correlation analysis using IBM SPSS Statistics software, V. 29.0. Pearson correlation coefficients and p-values for each significant correlation were reported.

4.4 RESULTS

4.4.1 Participants characteristics

All 51 participants (26 NIAT, 25 asymptomatic controls) completed the study. Participants anthropometrics and information regarding their symptoms are reported in **Table 7.** No statistical differences were observed between the groups.

Table 7. Participants' characteristics between Control and Non-insertional Achilles Tendinopathy groups.

	NIAT group (N=26)	Control group (N=25)	p-value	95% CI
Age (years)	29.04 ± 8.46	28.60 ± 3.92	0.815	[-4.17,3.30]
Sex (males/females)	14/12	17/8		
Height (cm)	173.00 ± 9.16	171.10 ± 9.22	0.463	[-7.08, 3.27]
Weight (kg)	75.09 ± 17.20	74.00 ± 11.57	0.793	[-9.37, 7.20]
Laterality (Right/Left)	24/2	19/6		
Assessed leg (Right/Left)	16/10	17/8		
Bilateral symptoms	13/26	N/A		
Symptoms duration (months)	42.10 ± 38.45	N/A		
NRS	3.12 ± 1.24	N/A		

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. NIAT, non-insertional Achilles tendinopathy; NRS, Numerical Rating Scale; N/A, non-applicable. * Indicates significant statistical difference, $p<0.05$.

4.4.2 Questionnaires

Questionnaires scores between groups are reported in **Table 8.** Individuals in the NIAT group presented lower scores in the VISA-A ($p<0.001$), IPAQ-SF ($p=0.019$), FAAM (Subscale Activities of Daily Living) ($p<0.001$), and FAAM (Subscale Sports) ($p<0.001$) compared to the Control group. Additionally, individuals in the NIAT group showed higher TSK scores compared to the Control group ($p=0.021$). No differences were observed in the PCS scores between groups.

Table 8. Questionnaires score between Control and Non-insertional Achilles Tendinopathy groups.

	NIAT group (N=26)	Control group (N=25)	p-value	95% CI
VISA-A	65.73 ± 16.16	100 ± 0	<0.001*	[23.99, 37.35]
IPAQ-SF	128.46 ± 58.36	171.60 ± 68.19	0.019*	[7.47, 78.81]
FAAM (Subscale Activities of Daily Living)	85.42 ± 11.15	99.44 ± 1.83	<0.001*	[9.48, 18.56]
FAAM (Subscale Sports)	68.15 ± 17.04	98.68 ± 3.68	<0.001*	[23.52, 37.53]
PCS	12.15 ± 10.65	8.72 ± 8.25	0.205	[-8.81, 1.94]
TSK	36.08 ± 7.64	30.80 ± 8.21	0.021*	[-9.74, -0.82]

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. NIAT, non-insertional Achilles tendinopathy; VISA-A, Victorian Institute of Sport Assessment – Achilles Questionnaire; IPAQ-SF, International Physical Activity Questionnaire short form; FAAM, Foot and Ankle Ability Measure, PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia. * Indicates significant statistical difference, $p<0.05$.

4.4.3 Morpho-mechanical properties of the Achilles tendon

Morpho-mechanical properties of the Achilles tendon between groups are reported in **Table 9.** Individuals in the NIAT group presented higher thickness at 6 cm (p=0.034), and CSA at 4 cm (p=0.039) and 6 cm (p<0.001) of the Achilles tendon compared to Control group. Additionally, individuals in the NIAT group showed lower stiffness of the Achilles tendon compared to the Control group (p=0.003). No differences were observed in length, thickness at 2 and 4 cm and CSA at 2 cm of the Achilles tendon between groups.

Table 9. Morpho-mechanical properties of the Achilles tendon between Control and Non-insertional Achilles Tendinopathy groups.

	NIAT group (N=26)	Control group (N=25)	p-value	95% CI
Length (cm)	21.23 ± 3.07	19.89 ± 2.57	0.096	[-2.94, 0.25]
Thickness 2 cm (cm)	0.35 ± 0.06	0.37 ± 0.04	0.400	[-0.02, 0.04]
Thickness 4 cm (cm)	0.45 ± 0.10	0.41 ± 0.05	0.074	[-0.08, 0.004]
Thickness 6 cm (cm)	0.47 ± 0.12	0.40 ± 0.05	0.008*	[-0.12, -0.02]
CSA 2 cm (cm^2)	0.46 ± 0.08	0.44 ± 0.09	0.315	[-0.07, -0.02]
CSA 4 cm (cm^2)	0.44 ± 0.09	0.40 ± 0.06	0.039*	[-0.09, -0.002]
CSA 6 cm (cm^2)	0.48 ± 0.08	0.38 ± 0.08	<0.001*	[-0.14, -0.05]
Stiffness (kPa)	68.16 ± 7.80	75.95 ± 9.98	0.003*	[2.76, 12.82]

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. NIAT, non-insertional Achilles tendinopathy; CSA, cross-sectional area * Indicates significant statistical difference, $p<0.05$.

4.4.4 Motor unit decomposition

A total of 5681 motor units were identified in the triceps surae muscle during the submaximal contractions in the NIAT and control groups. At 10% MVC, in the NIAT group the average number of motor units identified were 14.3 ± 9.2, 6.5 ± 5.1, and 15.6 ± 8.4 and in the control group 18.9 ± 18.6, 8 ± 10.9, and 12.6 ± 8.8 for the MG, LG, and SO muscles, respectively. At 40% MVC, in the NIAT group the average number of motor units identified were 25.3 ± 19.5, 11.4 ± 9.6, and 16.5 ± 8.0 and in the control group 25.5 ± 23.4, 11.1 ± 11.3, and 12.3 ± 7.5 for the MG, LG, and SO muscles, respectively. At 70% MVC, in the NIAT group the average number of motor units identified were 18.5 ± 15.7, 9.3 ± 11.1, and 12.1 ± 8.1 and in the control group 10.4 ± 12.6, 3.9 ± 2.5, and 11.9 ± 8.0 for the MG, LG, and SO muscles, respectively.

4.4.5 Recruitment and derecruitment threshold

Recruitment thresholds obtained from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented in **Figure 19A.** Overall, no differences in recruitment threshold were observed between groups across torque levels (Group effect:

F=0.56, P=0.46). Within-group muscle differences at each torque level can be seen in **Figure 19A.** Derecruitment thresholds calculated from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented **in Figure 19B.** In general, derecruitment threshold increased differently across torque levels between muscles and groups (interaction effect: F=7.24, P<0.0001), with between groups differences showing decreased derecruitment threshold in the LG in the NIAT group compared to control group at 70% MVC (P=0.039, 95% CI= 0.11 to 10.41). Within-group muscle differences at each torque level can be seen in **Figure 19B.**

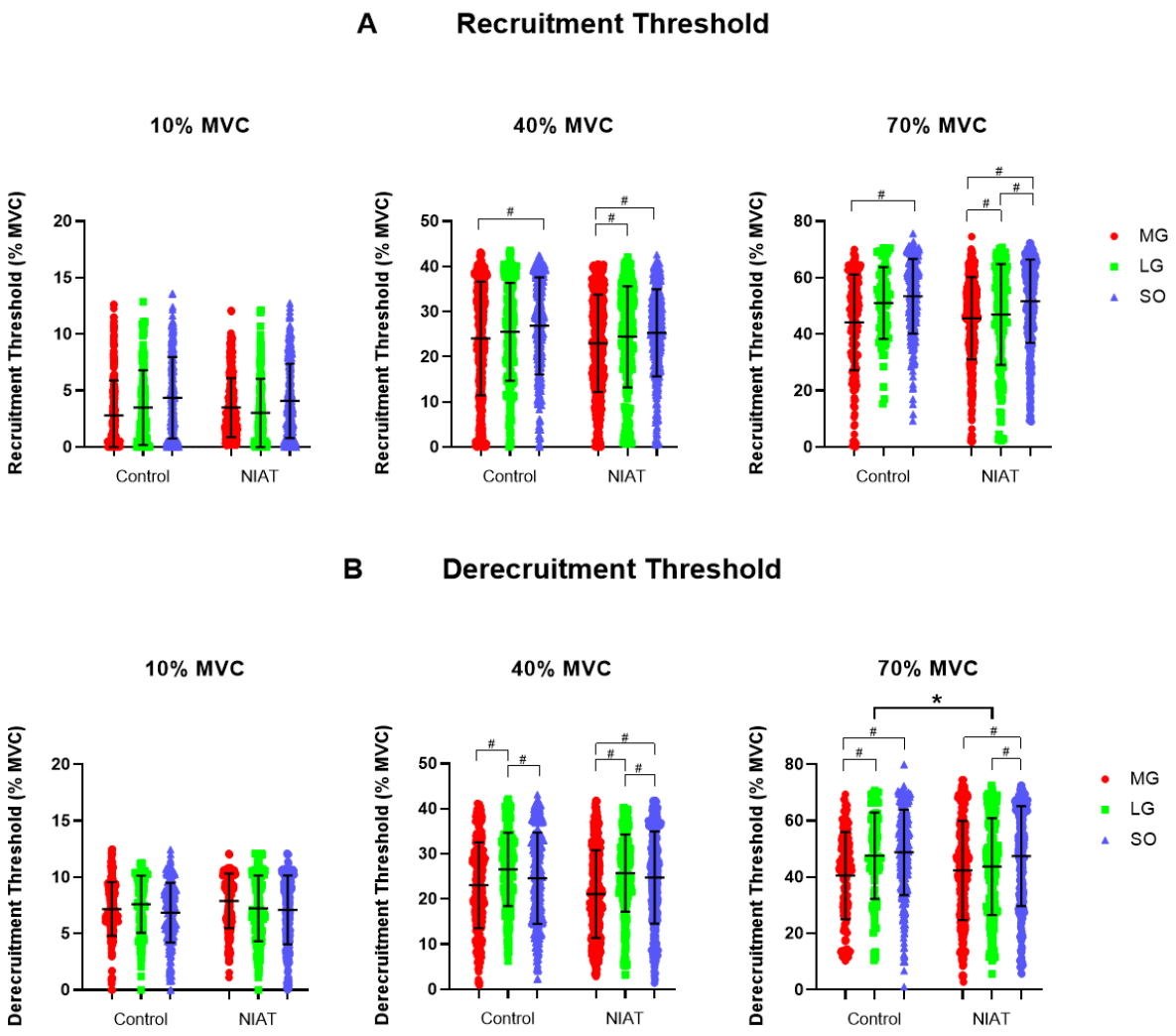

Figure 19. Recruitment and derecruitment thresholds between Control and Non-insertional Achilles Tendinopathy groups.
A) Recruitment threshold from medial gastrocnemius (MG: red bar), lateral gastrocnemius (LG: green bar) and soleus (SO: blue bar) muscles at 10, 40, and 70% maximum voluntary contraction (MVC) between control and non-insertional tendinopathy (NIAT) groups. B) Derecruitment threshold from MG, LG, and SO muscles at 10, 40, and 70% MVC between control and non-insertional tendinopathy (NIAT) groups. A linear mixed model was used for statistical comparisons. * Statistical differences between muscles and groups, $p<0.05$. # Statistical differences between muscles within groups, $p<0.05$.

4.4.6 Discharge Rate

Discharge rate obtained from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented in **Figure 20.** Overall, discharge rate increased differently across torque levels between muscles and groups (interaction effect: $F=7.52$, $P<0.0001$),

with between groups difference showing significantly increased discharge rate in the LG in the NIAT group compared to control group at 70% MVC (P=0.002, 95% CI= -3.27 to -0.34). Within-group muscle differences at each torque level can be seen in **Figure 20.**

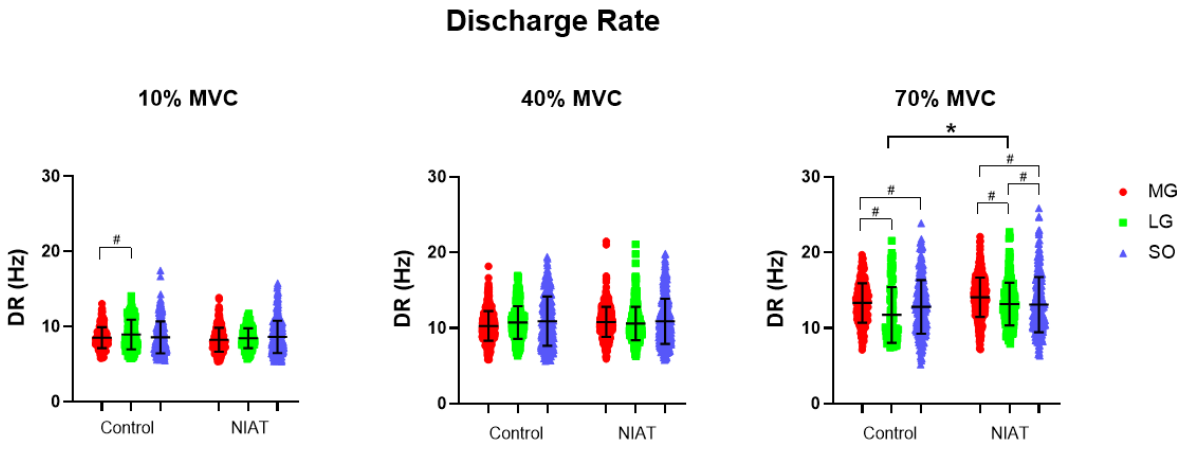

Figure 20. Discharge rate between Control and Non-insertional Achilles Tendinopathy groups. Motor unit discharge rate from medial gastrocnemius (MG: red dot), lateral gastrocnemius (LG: green square) and soleus (SO: blue triangle) muscles at 10, 40, and 70% maximum voluntary contraction (MVC) between control and non-insertional tendinopathy (NIAT) groups. A linear mixed model was used for statistical comparisons. * Statistical differences between muscles and groups, p<0.05. # Statistical differences between muscles within groups, p<0.05.

4.4.7 Discharge Rate Variability

COVisi calculated from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented in **Figure 21.** Overall, COVisi increased differently between groups and muscles (interaction effect: F=4.41, P=0.012); however, no differences between NIAT and control groups were observed. Within-group muscle differences at each torque level can be seen in **Figure 21.**

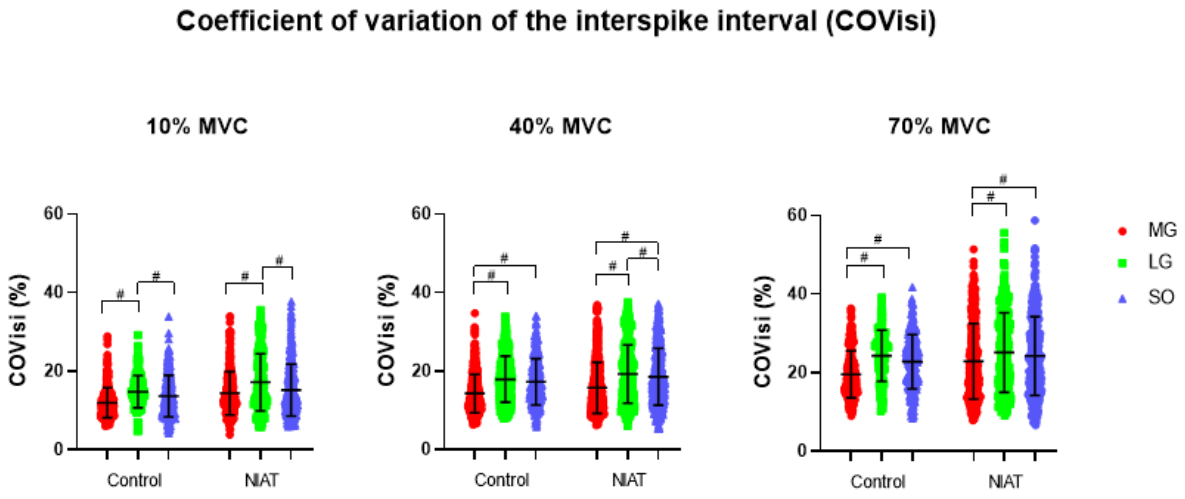

Figure 21. Coefficient of variation of the interspike interval between Control and Non-insertional Achilles Tendinopathy groups.
Motor unit coefficient of variation of the interspike interval (COVisi) from medial gastrocnemius (MG: red dot), lateral gastrocnemius (LG: green square) and soleus (SO: blue triangle) muscles at 10, 40, and 70% maximum voluntary contraction (MVC) between control and non-insertional tendinopathy (NIAT) groups. A linear mixed model was used for statistical comparisons. # Statistical differences between muscles within groups, $p<0.05$.

4.4.8 Neuromechanical delay and cross-correlation coefficient

NMD calculated from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented in **Figure 22A.** Overall, NMD decreased distinctly across torque levels between muscles and groups (interaction effect: $F=11.74$, $P<0.0001$); however, no differences between NIAT and control groups were observed. Within-group muscle differences at each torque level can be seen in **Figure 22A.** Cross-correlation coefficient calculated from motor units from MG, LG, and SO muscles at 10, 40, and 70% MVC are presented in **Figure 22B.** In general, cross-correlation coefficient vary across torque levels between muscles and groups (interaction effect: $F=84.27$, $P<0.0001$), with between groups differences showing significantly decreased cross-correlation coefficient in the LG in the NIAT group compared to control group at 10% MVC ($P<0.0001$, 95% CI= 0.07 to 0.27) and increased cross-correlation coefficient in the LG in the NIAT group compared to control group at 70% MVC ($P=0.013$, 95% CI= -0.21 to -0.01). Within-group muscle differences at each torque level can be seen in **Figure 22B.**

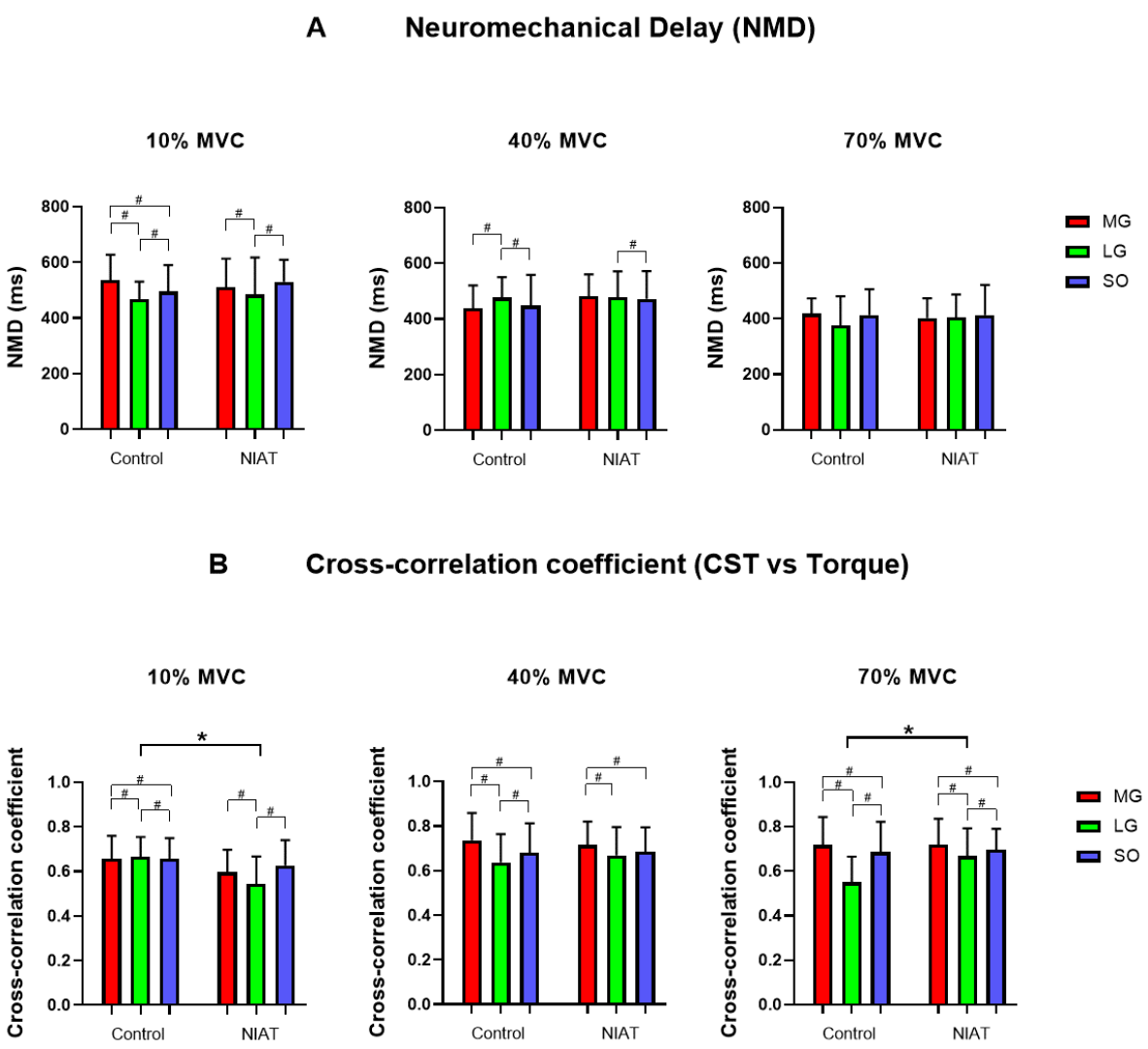

Figure 22. Neuromechanical delay and cross-correlation coefficient between Control and Non-insertional Achilles Tendinopathy groups.
A) Neuromechanical delay (MND) from medial gastrocnemius (MG: red bar), lateral gastrocnemius (LG: green bar) and soleus (SO: blue bar) muscles at 10, 40, and 70% maximum voluntary contraction (MVC) between control and non-insertional tendinopathy (NIAT) groups. B) Cross-correlation coefficients between cumulative spike train (CST) vs torque from MG, LG, and SO muscles at 10, 40, and 70% MVC between control and NIAT groups. A linear mixed model was used for statistical comparisons. * Statistical differences between muscles and groups, $p<0.05$. # Statistical differences between muscles within groups, $p<0.05$.

4.4.9 Maximal voluntary torque and torque steadiness

Maximal voluntary torque between control and NIAT groups is presented in **Figure 23A.** No difference between control and NIAT groups was observed (P=0.448, 95% CI = [-9.60, 21.38]). Torque steadiness between control and NIAT groups at 10, 40, and 70% MVC is presented in **Figure 23B.** Overall, torque steadiness varied across torque levels (Torque effect: F=29.94 P<0.0001, MD=0.28, 95% CI= -0.04 to 0.60); however, no differences between groups for the same force level were observed (Group effect: P=0.09).

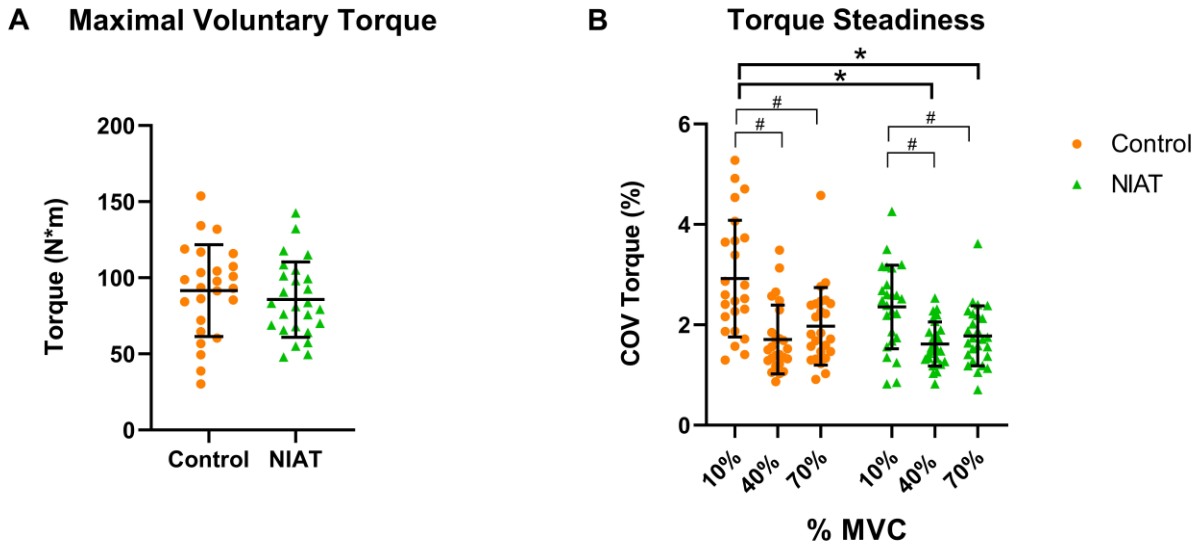

Figure 23. Maximal voluntary torque and torque steadiness between Control and Non-insertional Achilles tendinopathy groups.
A) Maximal voluntary torque between control and non-insertional Achilles Tendinopathy (NIAT) groups. B) Torque steadiness between control and NIAT groups at 10, 40, and 70 maximum voluntary contraction (MVC). * Statistical differences between groups, p<0.05. # Statistical differences between within groups, p<0.05.

4.4.10 Relationships between pain level and questionnaires and neuromuscular and morpho-mechanical parameters

A correlation analysis was conducted to assess the relationship between pain level and questionnaires and neuromuscular and morpho-mechanical parameters. The pain level was significantly negatively correlated with the LG derecruitment threshold at 70% MVC ($r= -0.52$, $P<0.05$). VISA-A was significantly negatively correlated with Achilles tendon thickness 6 cm ($r= -0.46$, $P<0.01$). Moreover, FAAM (Subscale Activities of Daily Living) was significantly negatively correlated with Achilles tendon thickness 6 cm ($r= -0.64$, $P<0.001$) and CSA 6 cm ($r= -0.42$, $P<0.05$). Finally, PCS was significantly positively correlated with Achilles tendon thickness 6 cm ($r=0.49$, $P<0.05$). No significant correlations were found for IPAQ-SF, FAAM (Subscale Sports) and TSK questionnaires.

4.5 DISCUSSION

This study shows that individuals with NIAT have load-dependent changes in triceps surae motor unit firing rate properties. We demonstrated that individuals with NIAT have increased motor unit discharge rate and decreased derecruitment threshold of the LG muscle compared to controls during isometric plantarflexion contractions at higher forces. Furthermore, our findings indicate that individuals with NIAT exhibit a diminished cross-correlation coefficient of the LG's CST-torque at low forces, alongside an augmented CST-torque cross-correlation coefficient at high forces. Taken together, these findings indicate that the contribution of the LG to the plantarflexion net torque is altered in individuals with NIAT, in a load-dependent manner.

4.5.1 Changes in firing properties to MG, LG, and SO muscles

There is evidence that some muscles are predominantly controlled by a common neural drive (Bremner et al., 1991, Gibbs et al., 1995, Laine et al., 2015, Kerkman et al., 2018), with the strength of this shared drive being greater among muscles closely related anatomically and functionally (Gibbs et al., 1995, Kerkman et al., 2018). However, recent evidence indicates reduced common neural drive between MG and LG, and SO and LG muscles during isometric plantarflexion contractions and heel-raise tasks in asymptomatic individuals (Hug et al., 2021). Since the Achilles tendon is composed of three subtendons with a twisted structure and different lengths, and nonuniform displacements within the Achilles tendon have been observed during isometric contractions (Crouzier et al., 2020), it can be speculated that motor unit firing rate properties of the triceps surae muscles could be adjusted differently in response to changes in the mechanical properties of the Achilles tendon. Within this framework, our findings revealed increased discharge rate and decreased derecruitment threshold in the LG in individuals with NIAT only at high forces. Consistent with our results, Fernandes et al. 2022 reported no significant differences in the discharge rate of the MG, LG, and SO muscles in individuals with NIAT at 10% and 20% MVC; however, they found that in the NIAT group the discharge rate of the LG remained unchanged as the target torque

increased from 10% to 20% MVC, in contrast to the control group (Fernandes et al., 2023). Since induced pain does not produce significant changes in the EMG during high submaximal voluntary contractions (Graven-Nielsen et al., 1997), it has been proposed that central adjustments to high-threshold motor units are necessary to reach high force levels in the presence of pain (Martinez-Valdes et al., 2020). Interestingly, Martinez-Valdes et al. 2020 using a model of experimental pain in the tibialis anterior muscle, reported increased discharge rate and decreased recruitment and derecruitment threshold in the high-threshold motor units at 70% MVC, with no changes in the low-threshold motor units (Martinez-Valdes et al., 2020). This reduction in the derecruitment threshold for high-threshold motor units at elevated forces enables this population of motor units to stay active for a longer duration throughout the contraction (Martinez-Valdes et al., 2020). Given that high-threshold motor units typically fatigue faster, this mechanism could underlie the observed increase in the discharge rate observed at such force levels (Martinez-Valdes et al., 2020). Therefore, our findings suggest an adaptation of the LG's high-threshold motor units to achieve high submaximal forces in individuals with NIAT. Nevertheless, a distinct examination focusing on both high and low-threshold motor units is essential to further validate this hypothesis. Regarding COVisi, we did not observe differences in the MG, LG, and SO muscles between groups at any force level. This is in accordance with the study of Fernandes et al., which did not observe changes in the COVisi of the triceps surae muscles in individuals with NIAT (Fernandes et al., 2023). Since changes in COVisi are related to changes in torque steadiness, and we did not observe differences in torque steadiness between groups at any force level, COVisi does not seem to be impaired in individuals with NIAT.

4.5.2 Cross correlation coefficients and neuromechanical delay

When an individual aims to sustain a constant force during a submaximal isometric contraction, the applied force is never constant instead varies around an average value (Jones et al., 2002, Laidlaw et al., 2000, Tracy and Enoka, 2002). Since the most important factor influencing torque steadiness for a muscle is the total motor unit activity (De Luca et al., 1982, Farina and Negro, 2015, Farina et al., 2014, Negro et al., 2016b,

Feeney et al., 2018), the CST can be used to estimate the effective neural drive to muscles (Mazzo et al., 2022). A recent study using the CST have shown that the neural drive to the triceps surae muscles was moderately correlated with fluctuations in isometric plantarflexion torque (Mazzo et al., 2022). Our results indicate that the cross-correlation coefficient between CST and torque decreased in the LG at low forces and increased at high forces in individuals with NIAT compared to controls. These findings suggest that the contribution of the LG to the net plantarflexion torque at low forces is reduced in individuals with NIAT, which is in agreement with a previous study that has indirectly shown that the contribution of the LG to the net plantarflexion torque is significantly reduced in individuals with NIAT at low forces; however, the same authors also reported decreased contribution of the LG to the net plantarflexion torque at intermediate forces (Crouzier et al., 2020), which was a difference that we did not find. This discrepancy may be explained by the methodology employed since the study of Crouzier et al. 2020 used the LG activation and relative physiological cross-sectional area of the LG to indirectly estimate the contribution of this muscle to the net plantarflexion torque. Since each subtendon has a different length and orientation, and the LG's subtendon is the longest, it can be theorised that the decrease in stiffness observed in individuals with NIAT may affect differently the force transmission throughout the LG muscle-tendon unit, explaining the force-dependent contribution of the LG to the net plantarflexion torque in individuals with NIAT.

The conversion of neural signals to force output has a latency caused by the dynamic responsiveness of motor neurons and the time required to stretch the series elastic components of the muscle-tendon unit after the activation of the muscle fibers (Del Vecchio et al., 2018). In this context, the NMD represents the time difference between the neural drive and the generated torque during a voluntary contraction and can be estimated from the time lag of the peak of the cross-correlation between the CST and torque (Del Vecchio et al., 2018). Our results showed no differences in NMD of the MG, LG, and SO between the NIAT group and control group at 10%, 40%, and 70% MVC; however, NMD decreased as the target torque increased in both groups. The decrease in the NMD as the target torque increase may be explained by the Henneman's size

principle (Henneman, 1957), since as the target torque increases larger motor units, which innervate fast-twitch muscle fibers, are recruited. Consequently, the recruitment of fast-twitch muscle fibers may contribute to the observed decrease in NMD. Theoretically, the stiffness of the Achilles tendon affects the time to stretch the elastic components of the muscle-tendon unit during a voluntary contraction. Given our observation of reduced tendon stiffness in individuals with NIAT, we were expecting that this difference may influence the NMD results; however, no differences between groups were observed for this parameter.

4.5.3 Relationships between pain, self-reported outcomes, and neuromuscular and morpho-mechanical parameters

Our results showed a negative correlation between pain level and LG derecruitment threshold at 70% MVC, suggesting that as the pain level increases, individuals with NIAT decrease the derecruitment threshold of the LG at high forces. As we discussed previously, the decrease in the derecruitment threshold likely involves high-threshold motor units, allowing them to be active for longer to accomplish the task. Consequently, constant activation of high-threshold motor units, which are not fatigue resistant, will eventually lead to increased muscle fatigue, which may produce a redistribution of the triceps surae muscle activity, affecting the distribution of load on the tendon. However, motor unit firing rate properties during fatiguing contractions in individuals with NIAT have not been explored. Additionally, we found a negative correlation between VISA-A and Achilles tendon thickness 6 cm, indicating that as the tendon thickness increases, the severity of the symptoms increases. Nevertheless, previous studies have shown inconsistent relationships between the extent or severity of tendon abnormalities and severity of symptoms (Martin et al., 2018). Similarly, we observed a negative correlation between FAAM (Subscale Activities of Daily Living) and Achilles tendon thickness 6 cm and CSA 6 cm, showing that as the tendon thickness and CSA increase, the physical function of individuals with NIAT decreases, providing additional evidence of the relationship between tendon morphology and physical function.

Furthermore, we found a positive correlation between PCS and Achilles tendon thickness 6 cm, revealing that pain catastrophising (rumination, magnification, and helplessness about pain) increases as tendon thickness increases. Since morphological changes to the Achilles tendon in individuals with NIAT are commonly linked to the duration of symptoms, our results support the idea that chronic NIAT is linked to changes in psychological attitudes about pain. Finally, we expected to find relationships between motor unit firing rate parameters and the mechanical properties of the Achilles tendon as we found in asymptomatic individuals (See Chapter 3); however, no correlations were found between these parameters. This may be explained by the methodology employed since changes in motor unit firing rate properties were observed mostly during high force contractions; however, tendon stiffness was assessed during rest conditions. Consequently, tendon stiffness assessment during high-force isometric contractions is necessary to elucidate this intricate relationship.

Understanding the interplay between symptoms, functionality, psychological attitudes towards pain, and neuromuscular and morpho-mechanical properties is essential for enhanced comprehension of the different factors involved in NIAT. Designing intervention strategies that consider the dynamic interplay between these components is imperative to achieve optimal outcomes in the management of this condition.

4.5.4 Limitations and future developments

There are some methodological aspects of this study which should be considered. First, the individuals in the NIAT group exhibited relatively low pain levels compared to previous studies. This observation may be attributed to most participants being physically active; thus, extrapolating our findings to more symptomatic populations should be done cautiously. Second, tendon stiffness measurements were performed only at 4 cm from the tendon insertion; however, tendon stiffness might differ across other tendon regions. The application of new technologies, such as 3D SWE (Götschi et al., 2023), presents a promising approach to uncovering the variations in tendon stiffness across different regions. Third, due to the limited resolution of the device employed to estimate tendon stiffness, we had to perform these assessments during resting conditions. The assessment of tendon stiffness during isometric plantarflexion contractions in a broader range of plantarflexion torques may provide more precise information about the relationship between tendon mechanical behaviour and triceps surae motor unit firing rate properties. Future studies employing devices allowing SWE measurements at higher temporospatial resolutions could concurrently assess the stiffness of the Achilles tendon and triceps surae motor unit firing rate properties during plantarflexion contractions to provide a more comprehensive understanding of the interplay of neural coding and muscle-tendon behaviour in this condition. Additionally, since recent studies have successfully identified motor units during shortening and lengthening contractions (Glaser and Holobar, 2019, Oliveira and Negro, 2021), future studies should investigate triceps surae motor unit firing rate properties during dynamic plantarflexion contractions in individuals with NIAT.

4.6 CONCLUSIONS

This study demonstrates that individuals with NIAT have load-dependent changes in triceps surae motor unit firing rate properties. Specifically, we observed an increase in the motor unit discharge rate and a decrease in the derecruitment threshold of the LG muscle in individuals with NIAT compared to controls during isometric plantarflexion contractions at high forces, providing relevant information regarding the underlying mechanisms involved in this condition. Furthermore, our findings suggest that the contribution of the LG to the net plantarflexion force is affected in individuals with NIAT, underscoring the significance of asymmetrical load transmission to the Achilles tendon in the pathophysiology of this condition.

CHAPTER 5

Eccentric and concentric torque feedback training induces similar improvements in pain, function, and adjustments in triceps surae motor unit firing properties in individuals with non-insertional Achilles tendinopathy. A randomised controlled trial.

This chapter presents the full contents of a manuscript that has been prepared for publication, along with changes made in the text specifically for the content of this thesis. It presents the results of the 6-week intervention based on either eccentric or concentric contractions in individuals with non-insertional Achilles tendinopathy. The development and execution of this chapter rigorously adheres to the published protocol of the randomised controlled trial (Contreras-Hernandez et al., 2022a).

Publication:

Ignacio Contreras-Hernandez, Deborah Falla, Eduardo Martinez-Valdes. Neuromuscular and structural tendon adaptations after 6 weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy: protocol for a randomised controlled trial. BMJ Open 2022;12:e058683

Manuscript in preparation:

Ignacio Contreras-Hernandez, Michail Arvanitidis, Deborah Falla, Francesco Negro, Eduardo Martinez-Valdes. Eccentric and concentric torque feedback training induces similar improvements in pain, function, and adjustments in triceps surae motor unit firing rate properties in individuals with non-insertional Achilles tendinopathy. A randomised controlled trial.

Authors contribution:

IC-H, EM-V, and DF conceived and designed research; IC-H and MA performed experiments; IC-H and EM-V analysed data; IC-H and EM-V interpreted results of experiments; IC-H prepared figures; IC-H drafted manuscript; IC-H, EM-V, DF, FN and MA edited and revised manuscript.

5.1 ABSTRACT

NIAT is a common disorder of the mid-portion of the Achilles tendon. Although ECC exercise has been widely used to treat this condition, the mechanisms by which ECC exercise may help to resolve tendon pain remain unclear. The purpose of this randomised controlled trial was to investigate the effects of a 6-week intervention based on either ECC or CON visuo-motor torque feedback training on triceps surae motor unit properties, pain, function, and Achilles tendon's morphological and material properties in individuals with NIAT. Twenty-six individuals with NIAT (14 males, 12 females, 29.04 ± 8.46 years, 75.09 ± 17.20 kg, 173 ± 9.16 cm) and 13 asymptomatic individuals (8 males, 5 females, 30.17 ± 3.41 years, 73.09 ± 11.65 kg, 170.01 ± 10.82 cm) participated in the study. Motor unit firing properties of the MG, LG, and soleus SO muscles were assessed using HD-sEMG during isometric plantarflexion contractions at 10, 40, and 70% of the MVC. Pain and function were determined using the VISA-A questionnaire and the NRS. The Achilles tendon's morphological and material properties were obtained using B-mode ultrasonography and SWE. Outcomes measures were assessed before the intervention (week 1), week 3, and week 6. Linear mixed-effect model analysis showed that derecruitment thresholds increased at 40% and 70% MVC with time ($P=0.02$ and $P=0.03$, respectively), with no differences between groups. The discharge rate of the triceps surae muscle increased at 40% MVC with time ($P=0.04$), with no differences between groups. Additionally, VISA-A improved differently between groups and time ($P=0.02$) and NRS decreased with time ($P<0.001$), with no differences between groups. Furthermore, Achilles tendon's thickness and CSA did not change with time; however, Young's modulus increased differently between groups and time ($P=0.02$). Together, these findings demonstrate that a 6-week torque feedback training intervention based on ECC or CON contractions induces similar load-dependent changes in triceps surae motor unit firing properties, pain and symptoms decrease, and tendon Young's modulus increases in individuals with NIAT.

5.2 INTRODUCTION

The Achilles tendon is the strongest and largest tendon in the human body, and it can be affected by both degenerative and traumatic processes (Aicale et al., 2018). NIAT is a clinical diagnosis characterised by an insidious onset of pain accompanied by swelling, perceived stiffness and thickening in the midportion of the Achilles tendon (Bahari et al., 2023, Sara et al., 2023, Maffulli et al., 1998, van Dijk et al., 2011), and it is the most prevalent occurring tendinopathy of the lower extremity (de Jonge et al., 2011). Albeit our improved understanding of this condition, it remains a devastating and slow-progressing injury (Silbernagel et al., 2020), with full recovery taking a year or longer, and its reinjury rate is high (Silbernagel et al., 2007b, Silbernagel et al., 2007a). The aetiology of the NIAT remains unclear and it is likely to be multifactorial (Vlist et al., 2019, Kader et al., 2002). However, from a mechanobiological perspective, repetitive mechanical tissue overuse seems to be a predominant cause (Kader et al., 2002, Abate et al., 2009). Thus, excessive loading of the tendon may result in structural damage that, with time, can lead to a degenerated and weakened tendon (Kjaer, 2004), affecting its material properties. Recent evidence has identified that deficits in the performance of the MG, LG, and SO muscles may be a key factor (O'Neill et al., 2019) since these muscles may influence the magnitude and distribution of the Achilles tendon load, strain, and stress (Debenham et al., 2017, O'Neill et al., 2015). Interestingly, despite the fact that the triceps surae muscles act as agonists that share the same common distal tendon, they exhibit morphological, neurophysiological, and functional differences, suggesting diverse functional roles (Héroux et al., 2014).

Morpho-mechanical and material changes induced by NIAT include an increase in tendon CSA, thickness, volume (Shalabi et al., 2004, Arya and Kulig, 2010, Child et al., 2010, Grigg et al., 2012, Docking and Cook, 2016), transverse strain, hysteresis, and a decrease in stiffness and Young’s modulus (Arya and Kulig, 2010, Child et al., 2010, Wang et al., 2012, Chang and Kulig, 2015). Nevertheless, the impact of different exercise interventions on the morpho-mechanical and material properties of the Achilles tendon in individuals with NIAT has shown some ambiguous results. One systematic review has

synthesised the evidence regarding the morphological adaptations of Achilles tendon to ECC exercise in individuals with NIAT, concluding that tendon thickness does not change in parallel with improved clinical outcomes (Färnqvist et al., 2020). However, a RCT comparing heavy-slow resistance exercises and ECC exercises in individuals with NIAT reported reduced tendon thickness in both groups (Beyer et al., 2015). Notably, following an exercise intervention, some tendons may experience an increase in collagen content and size while simultaneously reducing their water content, resulting in unchanged tendon thickness (Färnqvist et al., 2020). Therefore, the Achilles tendon adaptations to exercise might only be detected by assessing the material properties (Färnqvist et al., 2020).

The CNS regulates muscle forces by modifying the recruitment and discharge rate of the associated motor units (Enoka and Duchateau, 2017). Recent studies have shown that motor units firing properties can adapt to changes in muscle-tendon properties (Martinez-Valdes et al., 2022, Cudicio et al., 2022). Thus, changes in motor unit firing properties may be behind injury-induced adjustments in motor performance (Enoka and Duchateau, 2017). It has been proposed that neural control of agonist and antagonist muscles during dynamic movements requires precise inhibitory and excitatory synaptic inputs to spinal motor neurons, suggesting that appropriate training can potentially modify this neural control (Möck and Del Vecchio, 2023). Nevertheless, previous investigations examining the effects of exercise on motor unit firing properties have shown contradicting results (Martinez-Valdes et al., 2017a). For example, Martinez-Valdes et al. 2017 observed an increase in the motor unit discharge rate of the vastus medialis and lateralis at 50 and 70% MVC after high-intensity interval training, with no changes at lower forces (Martinez-Valdes et al., 2017a). In contrast, Vila Cha et al. 2010 reported an increase in the discharge rate of the vastus medialis obliquus and vastus lateralis at 10 and 30% MVC following strength training (Vila-Chã et al., 2010). Within this framework, limited studies have investigated the motor unit firing rate properties in individuals with chronic musculoskeletal conditions such as ACL injury (Nuccio et al., 2021) and Achilles tendinopathy (Fernandes et al., 2023). To the best of our knowledge, no studies have investigated the induced changes in motor unit firing properties of the triceps surae muscle after applying an exercise intervention protocol to individuals with NIAT.

Exercise interventions have the highest level of evidence for the management of NIAT (Martin et al., 2018). The primary goal of exercise interventions is to provide controlled mechanical loading to the tendon to promote remodelling, decrease pain, and enhance endurance and strength of the triceps surae muscle (Silbernagel et al., 2020). Although optimal loading parameters have not been determined, it has been suggested that tendons have a more favourable response to high loads with longer durations than undergoing low loads with shorter durations (Magnusson and Kjaer, 2019). Historically, exercise interventions based on Achilles tendon loading have focused on ECC contractions; however, exercise interventions including CON contractions or a combination of both types of contractions have also shown good results (Silbernagel et al., 2007a, Beyer et al., 2015). Recently, it has been proposed that isometric exercises may be helpful for the management of the NIAT at early stages (Gravare Silbernagel et al., 2019); however, there is no evidence supporting its superiority over any other exercise modality (Gatz et al., 2020). Consequently, the idea that the tendon's response to mechanical load depends on the type of muscle contraction applied has been questioned (Magnusson and Kjaer, 2019, Couppé et al., 2015). Furthermore, there is a lack of studies assessing the effects of pure ECC or CON contractions on NIAT since current rehabilitation protocols employed in clinical practice are unable to isolate these contractions (Franchi et al., 2014).

Studies assessing the effect of exercise in individuals with NIAT usually have insufficient control over the load, speed, pain tolerance, or range of movement in which the exercises were performed. Therefore, we have developed a novel exercise intervention approach based on slow-speed visuo-motor training that uses an isokinetic dynamometer to control tendon loads while providing visual feedback of the exerted torque to the participants. This innovative approach allows for increased time under tension, controlled loading throughout the range of movement, individualised contraction intensity based on each participant's capacity and pain tolerance and facilitate the interaction with the device to improve motor performance.

We aimed to investigate the changes in triceps surae motor unit firing properties, pain and function, and Achilles tendon's morphological and material properties after applying a 6-week intervention protocol based on either controlled ECC or CON contractions in individuals with NIAT. Furthermore, we aimed to examine whether exercise-induced changes in triceps surae motor unit firing properties differed from asymptomatic controls. We hypothesised that the intervention protocol based on ECC contractions will be more effective to induced changes in triceps surae motor unit firing properties, pain, function, and Achilles tendon's morphological and material properties in individuals with NIAT.

5.3 METHODS

5.3.1 Design

The study design was a two-arm, parallel-group, RCT conducted from October 2021 to April 2023 at a laboratory within the Centre of Precision Rehabilitation for Spinal Pain (CPR spine), University of Birmingham, UK. This study was approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, UK (ERN_20-0604A) and registered under ISRCTN registry (ISRCTN46462385). The study was conducted according to the declaration of Helsinki and reported following the CONSORT (Consolidated Standards of Reporting Trials) guidelines (Schulz et al., 2010). All participants signed written informed consent prior to participation. The participant flowchart can be seen in **Figure 24.**

Participants with NIAT visited the laboratory over six consecutive weeks for the experimental sessions (week 1, week 3, and week 6) and training sessions (2-3 sessions per week). Participants in the control group visited the laboratory once to allow comparisons with the NIAT groups. Participants with NIAT were randomly allocated into two groups: ECC or CON training.

The assessed leg was randomised in the control group, and the symptomatic leg was evaluated in the ECC and CON groups. If individuals with NIAT presented bilateral symptoms, the leg with more severe symptoms was assessed. All participants were instructed to avoid any vigorous physical activity within 24 hours before each experimental session. Each experimental session lasted 2.5 hours and each training session 40 minutes.

Based on power analysis calculations (G*Power software) (Faul et al., 2009), a total of 26 individuals with NIAT (ECC group= 13, and CON group= 13) and 13 controls were required for this study. This sample size considers a power= 0.80, alpha=0.01, 25% loss of participants and an effect size (d) of 1.7 calculated from the study of Yu et al. (Yu

et al., 2013), which investigated the effect of 8 weeks of either ECC or CON training on pain levels in individuals with NIAT.

5.3.2 Participants

Twenty-six participants with NIAT (14 males, 12 females, 29.04 ± 8.46 years, 75.09 ± 17.20 kg, 173 ± 9.16 cm) and 13 asymptomatic individuals (8 males, 5 females, 30.17 ± 3.41 years, 73.09 ± 11.65 kg, 170.01 ± 10.82 cm) were recruited from the University of Birmingham staff/student population and the local community via leaflets, e-mail, and social media.

Inclusion criteria included men or women aged 18 to 55 years old. This age range was specifically chosen to minimise the influence of age in tendon properties, since previous studies have observed lower stiffness and Young's modulus on the Achilles tendon in older individuals (Lindemann et al., 2020). Inclusion criteria for the NIAT were as follows: presence of NIAT determined by an experienced physiotherapist based on clinical findings, physical examination, and ultrasound imaging. Clinical findings encompassed the VISA-A (Robinson et al., 2001), NRS (Alghadir et al., 2018), and symptoms for more than 3 months (Beyer et al., 2015). Individuals with a VISA-A score less than 90 and NRS score ≥ 2 were considered for the ECC and CON groups (Iversen et al., 2012). Ultrasound imaging was used to assess the echoic pattern (focal hyperechoic and hypoechoic areas within the tendon) and tendon thickness (focal or diffuse thickening) (Bleakney and White, 2005). Inclusion criteria for the healthy group included confirmation of a healthy Achilles tendon determined by the same physiotherapist through physical examination and ultrasound imaging.

The exclusion criteria for the ECC, CON, and the control groups were as follows: (1) systemic or inflammatory conditions including malignancy, neuromuscular disorders, and rheumatic diseases, (2) current or history of chronic cardiovascular, neurological, or respiratory diseases, and (3) history of lower limb surgery. Additionally, specific exclusion criteria for the individuals with NIAT included if they had been diagnosed with IAT or if

they received a corticosteroid injection in the Achilles tendon in the last 12 months. Specific exclusion criteria for the control group were history of Achilles tendon pain, lower limb surgery or pain/injury in the lower limb within the previous 6 months.

5.3.3 Randomisation, allocation, and blinding

Individuals with NIAT were randomised by an independent researcher (Dr Eduardo Martinez-Valdes (EM-V)) in a 1:1 allocation ratio to either ECC or CON groups (parallel-groups) using a computer-generated simple scheme randomisation. EM-V secured the randomisation code using password-protected files; thus, allocation concealment was guaranteed. EM-V released the randomisation code once each participant with NIAT completed the first experimental session. To achieve double blinding, an independent researcher (JVH) encoded the participants ID using the blindr software (https://github.com/U8NWXD/blindr). IC-H and MA performed experimental sessions, and IC-H performed the training sessions. JVH released the participants ID code after data analysis was performed. Due to the nature of the interventions, participants' blinding was not possible.

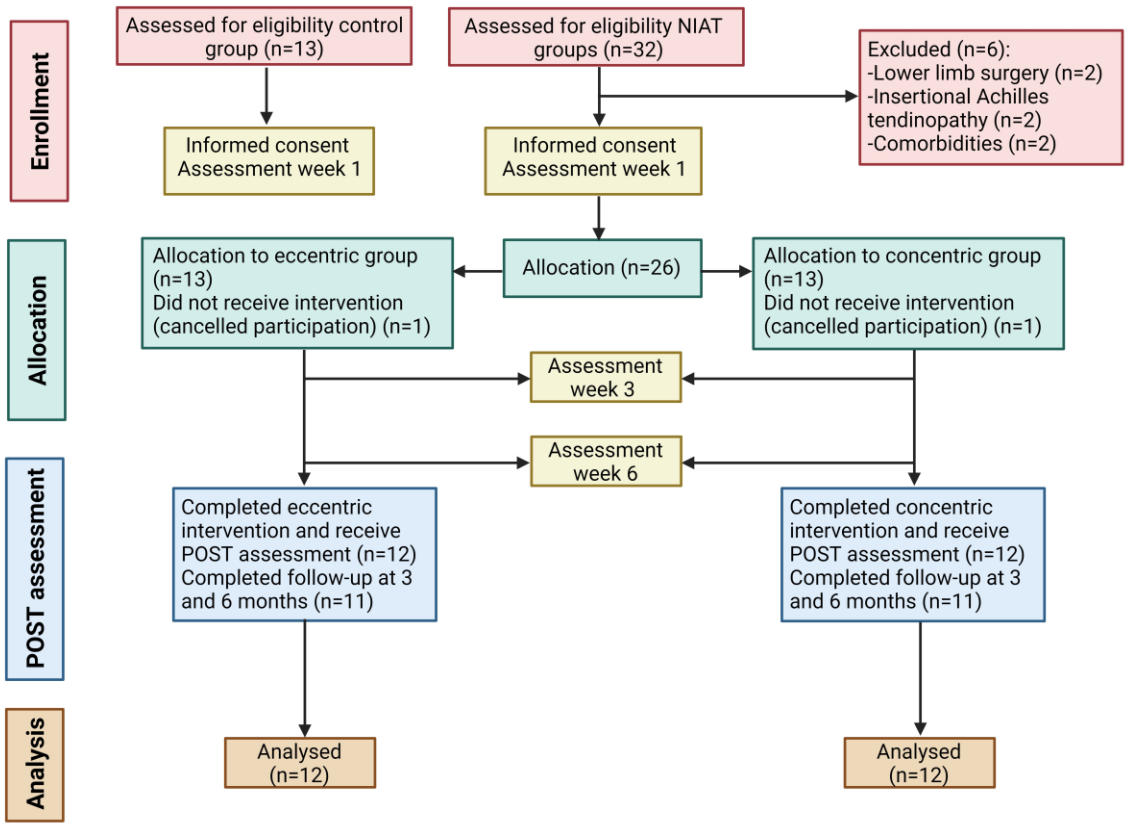

Figure 24. Participation flowchart according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines.
NIAT, non-insertional Achilles tendinopathy.

5.3.4 Experimental setup and tasks

Each experimental session included completing a battery of questionnaires, ultrasonography of the Achilles tendon, HD-sEMG of the triceps surae muscles during plantarflexion isometric contractions at 10%, 40%, and 70% MVC. A representation of the experimental setup is shown in **Figure 25.**

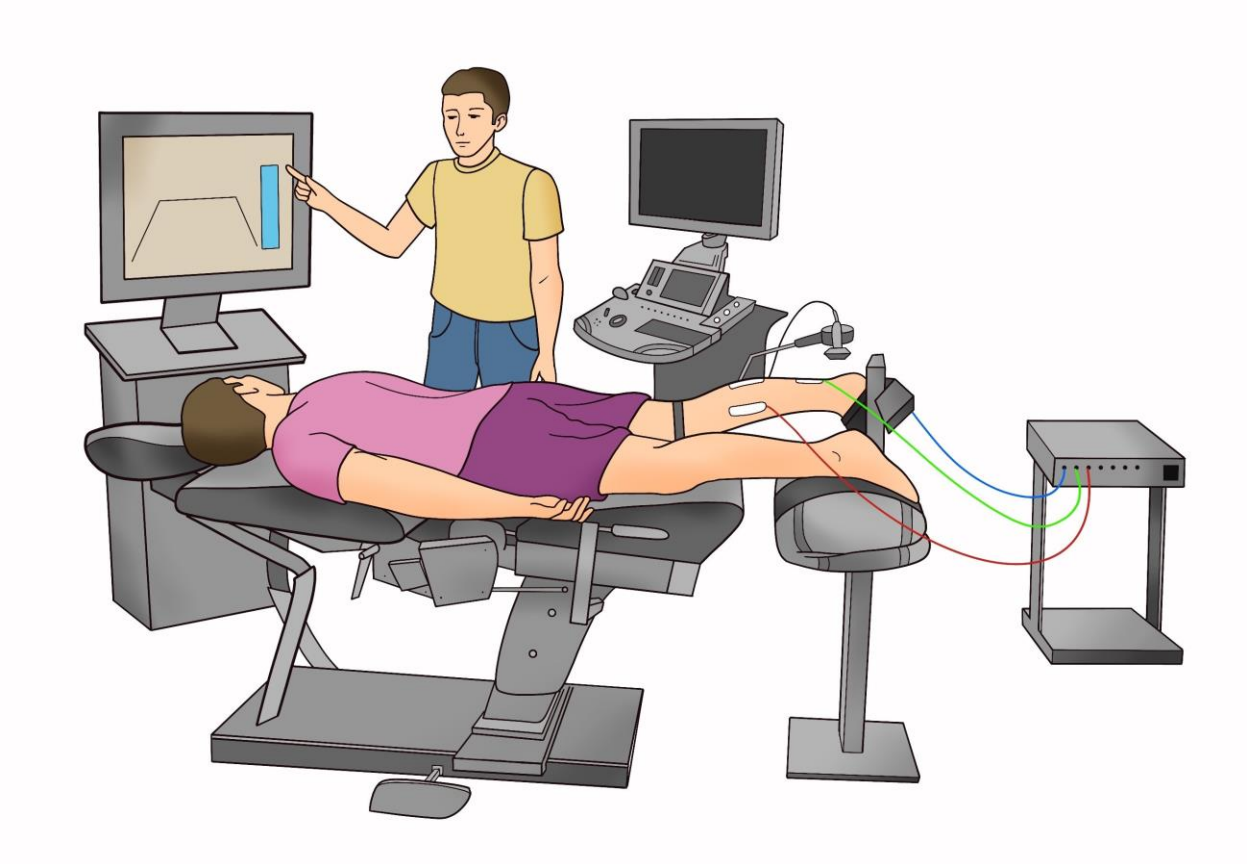

Figure 25. Representation of the experimental setup.

Anthropometric data (age, gender, weight, height, and foot dominance) was obtained in the baseline experimental session across participants. Foot preference (foot dominance) during daily life activities was determined using a behavioral-preference inventory (Chapman et al., 1987). For the individuals with NIAT, information regarding their symptoms (intensity of pain (NRS), duration of symptoms, and presence of bilateral symptoms) was obtained. Participants with NIAT reported their intensity of pain in each experimental session. After this assessment, all participants were asked to complete a battery of questionnaires, which included the VISA-A (Robinson et al., 2001), FAAM (Martin et al., 2005), TSK (Kori, 1990), PCS (Sullivan et al., 1995), and the IPAQ-SF (Craig et al., 2003). Then, participants lay prone on a Biodex System 3 dynamometer (Biodex Medical System), with their knees extended, the pelvis stabilised with a strap, and the assessed foot tightly strapped on the footplate. The ankle was orientated in 0° of plantarflexion and the axis of the dynamometer was aligned with the inferior tip of the

lateral malleolus. After, tendon morphological properties were assessed using ultrasonography. Following this procedure, the skin was shaved, cleaned, and prepared for the electrode grids placement. Electrode grids were placed on the MG, LG, and SO muscles in the same position across participants (see details below). Then, tendon material properties were estimated using SWE during rest conditions.

Next, participants underwent a warm-up protocol comprising 3 submaximal isometric plantarflexion contractions during 5 seconds at their perceived 30% of maximal voluntary force (30 seconds rest between contractions). After this warm-up, the MVC was determined during 3 maximal plantarflexion contractions during 5 seconds with 2 minutes of rest between contractions (Martinez-Valdes et al., 2018). The highest MVC value obtained was used as the reference maximal torque. Then, the electromyographic activity of the MG, LG, and SO muscles was assessed during two isometric plantarflexion contractions at 10, 40, and 70% MVC (10% MVC/s ramp-up, 10 s hold, 10% MVC/s ramp-down and 30 s rest) with HD-sEMG. A schematic representation of the experimental session is shown in **Figure 26.**

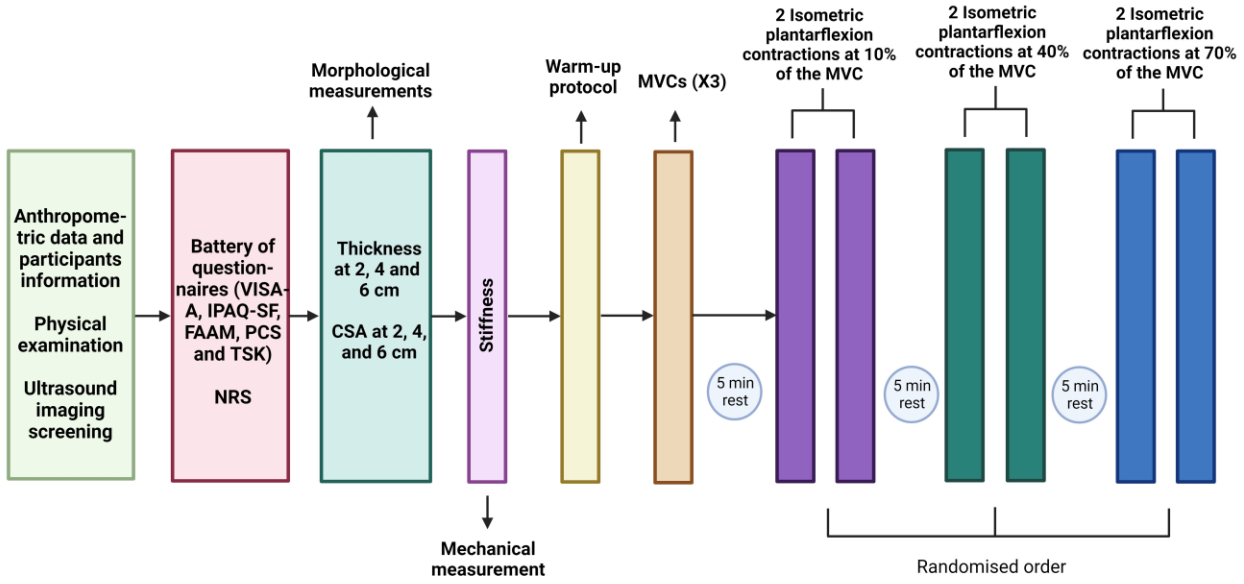

Figure 26. Schematic of the experimental procedure.
Physical examination and ultrasound imaging screening were performed only in the first experimental session. The order of the contractions performed at each target torque (10, 40, and 70% MVC) was randomised. VISA-A, Victorian Institute of Sports Assessment- Achilles Questionnaire; IPAQ-SF, International Physical Activity Questionnaire short form; FAAM, Foot and Ankle Ability Measure; PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia; CSA, cross-sectional area; MVC, maximum voluntary contraction.

5.3.5 Ultrasonography

All ultrasound images were acquired using an ultrasonography device equipped with SWE (LOGIQ S8 GE Healthcare, Milwaukee, USA). Morphological properties include tendon thickness and CSA. Material properties encompass Young's modulus. Tendon thickness and CSA were recorded in B-mode with a 16-linear array probe (50 mm, 4-15 MHz), and Young's modulus was recorded in SWE mode with a 9-linear array probe (44 mm, 2-8 MHz). An intra-rater/inter-session reliability assessment was performed in asymptomatic individuals to check for consistency of the ultrasound measures, showing excellent reliability for thickness (ICC: 0.99 and 0.99), good reliability for Young's modulus (ICC: 0.90) and moderate reliability for CSA (ICC: 0.64) (See more details in chapter 3)

The morphological properties were determined using an adaptation of the protocol developed by Arya and Kulig (Arya and Kulig, 2010). Briefly, the ultrasound probe was aligned longitudinally along the posterior part of the calcaneus, with the ankle positioned at 0° of plantarflexion, to identify the calcaneal notch. Subsequently, a fine wire (3.2 x 40 mm) was positioned beneath the probe to generate a visible artifact on the ultrasound image. This artifact was aligned with the calcaneal notch and the corresponding point was marked on the skin with a marker, representing the Achilles tendon's insertion. Then, the ultrasound probe was moved proximately to determine the MTJ of the MG. Similarly, a fine wire was placed beneath the ultrasound probe and aligned with the MTJ of the MG and the corresponding point was marked on the skin. The distance between the calcaneal notch and the MTJ of the MG represented the length of the Achilles tendon. Additional marks were drawn at 2, 4, and 6 cm above the calcaneal notch and these marks were used as reference to place the middle part of the ultrasound probe in the longitudinal plane to assess the Achilles tendon's thickness at 2, 4, and 6 cm of its insertion. Likewise, the same reference marks were used to place the ultrasound probe in the transversal plane and determine the Achilles tendon's CSA at 2, 4, and 6 cm of its insertion.

For the HD-sEMG electrode grid placement, a tape and a marker were used to draw a longitudinal line on the posterior part of the lower leg following the direction of the Achilles tendon. This line and the distal MTJ of the MG were used as references for the placement of the electrode grids. Specifically, for the MG electrode grid, a mark was made 10 cm above the distal MTJ of the MG and 4 cm medial to the posterior line. Similarly, for the LG electrode grid, a mark was made 10 cm above the distal MTJ of the MG and 4 cm lateral to the posterior line. Finally, for the SO electrode grid, a mark was made 5 cm below the distal MTJ of the MG and 4 cm lateral to the posterior line.

For the assessment of the Achilles tendon's Young's modulus, the ankle was positioned at 0° of plantarflexion and the ultrasound probe was placed longitudinal over the posterior aspect of the calcaneus. The middle part of the ultrasound probe was marked and aligned with the mark done at 4 cm above the Achilles tendon's insertion. To prevent applying pressure on the tendon, a probe holder was used. A test SWE

measurement was conducted to identify possible voids in the Young's modulus estimation and if voids were detected, the probe was temporarily removed, additional ultrasound gel was applied, and the probe was repositioned. A SWE image was acquired every 2.4 seconds; therefore, to obtain 4 SWE images, the SWE measurements lasted 12 seconds.

5.3.6 HD-sEMG and torque recordings

Participants' skin was prepared for the placement of the electrode grids. The skin was shaved, gently abraded (Nuprep, Skin Prep Gel, Weaver and Company, Aurora, Colorado), cleaned with water and dried with tissue paper. HD-sEMG signals from the MG, LG, and SO muscles were recorded using three two-dimensional (2D) adhesive grids (OT Bioelettronica, Italy) of 13 x 5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm). Each HD-sEMG was prepared by filling the grid cavities with conductive paste (AC-CREAM, SPES Medica, Genova, Italy) which provided adequate electrode-skin contact and by attaching a double-sided adhesive foam to the grid surface (SPES Medica, Genova, Italy).

HD-sEMG signals were digitised by a 16-bit analogue-digital converter (Quattrocento- OT Bioelecttronica, Torino, Italy), amplified by a factor of 150, sampled at 2048 Hz, and filtered with a band-pass filter (bandwidth: 10-500 Hz, first order, -3 dB) (Arvanitidis et al., 2019). HD-sEMG signals were collected in monopolar mode with ground electrodes (WhiteSensor WS, Ambu A/S, Ballerup, Denmark) positioned the head of the fibula and with a wet strap on the thigh of the assessed leg. HD-sEMG electrode grids and ground electrodes were connected to a bioelectrical amplifier (Quattrocento-OT-Bioelecttronica, Torino, Italy). The torque exerted by the participants was obtained with a Biodex System 3 dynamometer (Biodex Medical System), synchronised with the HD-sEMG signals using the auxiliary input of the bioelectrical amplifier.

5.3.4 Image analysis

Tendon thickness. Ultrasound images were analysed using the software ImageJ (http://imagej.nih.gov/ij) to assess tendon thickness. Briefly, a reference line of 1 cm was drawn using the ultrasound device tools. Using ImageJ software tools, the reference line was converted into pixels and set as a global scale. Next, the width of the ultrasound image was determined, and the middle point marked on the image. After, the distance between the superficial and deep aspects of the tendon were measured at 2, 4, and 6 cm from the insertion. The measurements of three ultrasound images were averaged for each position.

Tendon cross-sectional area. Ultrasound images were analysed using the ultrasound device tools to determine tendon CSA. A discontinuous line was drawn following the perimeter of the tendon as reference and the CSA was measured at 2, 4, and 6 cm of its insertion. The measurements of three ultrasound images were averaged for each position.

Tendon Young's modulus. Ultrasound images were analysed using ultrasound device tools to assess tendon Young's modulus. 4 SWE colour maps (height x width, 2.5 cm x 1 cm) were chosen and a line was drawn in the middle of the ultrasound image (this line indicates the 4 cm distance from the Achilles tendon's insertion). Then, a ROI of 3 mm diameter (Siu et al., 2016) was selected, aligned with the reference line, and positioned in the middle section of the tendon. Finally, mean tendon Young's modulus was calculated over the ROIs of 4 consecutive images (Coombes et al., 2018).

5.3.5 HD-sEMG signal analysis

5.3.5.1 Motor unit analysis

The HD-sEMG signals were decomposed into motor unit spike trains throughout the duration of the plantarflexion isometric plantarflexion contractions with an algorithm

based on blind source separation (Martinez-Valdes et al., 2022). The discharge times of the identified motor units were converted into binary spike trains (Martinez-Valdes et al., 2018). A validated metric (Silhouette, SIL) was used to determine the decomposition accuracy of identified motor units and was set to $\geq$ 0.90 (Negro et al., 2016a). SIL is a normalised measure of the relative height of the peaks of the decomposed spike trains with respect to the baseline noise (Martinez-Valdes et al., 2022). Motor unit properties were independently averaged for the MG, LG, and SO muscles at each target torque level (10, 40, and 70% MVC). This approach has been used in a previous study and has shown high reliability (Martinez-Valdes et al., 2016).

Discharge rate and COVisi were defined as the average number of action potentials discharged per second by a single motor unit and the time between two successive action potentials discharged by a motor unit, respectively (McManus et al., 2021). Discharge rate and COVisi were calculated from the steady phase of the contraction (10 seconds duration). Recruitment and derecruitment thresholds were defined as the torque (%MVC) when motor units began or ceased firing action potentials, respectively (Martinez-Valdes et al., 2020). Missing pulses producing non-physiological firing rates were manually removed and iteratively deleted, and the pulse train was re-calculated (Martinez-Valdes et al., 2022). Motor unit firing rate properties were recorded, analysed, and reported according to the consensus for experimental design in electromyography: single motor unit matrix (Martinez-Valdes et al., 2023).

5.3.5.2 Cross-correlation coefficient

The interactions between neural drive and force generation were examined using cross-correlation analysis. Motor unit discharge times obtained from the decomposition, were summed to generate a CST that depicts the cumulative activity of several motor units (Martinez-Valdes et al., 2022). Then, a low-pass filter (4^{th} order zero-phase Butterworth, 2 Hz) was applied to the CST and torque signals, followed by high-pass filtering (4^{th} order zero-phase Butterworth, 0.75 Hz) as reported previously (Martinez-Valdes et al., 2021). CST and torque values were cross-correlated to determine

similarities in their fluctuations (cross-correlation coefficient) (Martinez-Valdes et al., 2022). Cross-correlation coefficients were computed in 5-s segments with 50% overlap (Martinez-Valdes et al., 2022). The average cross-correlation coefficient calculated from these segments was reported.

5.3.5.2 Torque signal analysis

The highest peak torque (SI: Newton-meters) obtained during the isometric plantarflexion MVCs was low-pass filtered at 15 Hz and then used to determine the torque steadiness (coefficient of variation of torque (COV torque)). The coefficient of variation of torque was calculated according to the formula SD torque/mean torque * 100 from the steady phase of the contractions (Martinez-Valdes et al., 2020). The torque exerted by each participant was visualised using a custom-made MATLAB script to identify and select the steady phase of the contraction (Arvanitidis et al., 2022).

5.3.6 Training sessions

Participants in the ECC and CON groups came to our laboratory to perform the training sessions. The same experienced physiotherapist who performed the participants' screening guided the training sessions using a Biodex System 3 dynamometer. Participants in the ECC group were asked to perform a warm-up protocol consisting of 3 ECC plantarflexion (i.e., from plantarflexion to dorsiflexion) contractions at 25% MVC followed by the training protocol which consisted of 4 x 15 ECC plantarflexion contractions at 50% MVC with a range of motion from 0° to 30° of plantarflexion. The angular speed was kept at 3°/s (equating to a time under tension of 10 seconds) and three minutes of rest was provided between series. Participants in the CON group were asked to complete the same protocol, but with CON contractions (i.e., from dorsiflexion to plantarflexion). Visual feedback of the exerted torque was provided in all training sessions and participants were instructed to match the torque output as closely as possible to the target torque during the duration of the contraction. The MVC was adjusted for the ECC and

CON groups every two weeks of training and was based on the maximal isometric MVC torque produced. Both training interventions were designed to apply controlled mechanical loading to the tendon, using moderate loads at slow speeds during a relatively short period of time (6 weeks) and incorporating visuo-motor torque feedback. This approach aimed to achieve an increase in triceps surae strength, enhance motor performance, and ultimately reduce pain. The primary mechanisms for strength gains in the triceps surae muscle likely involved supraspinal and spinal adaptations. Additionally, improvements in motor performance were likely driven by motor learning processes, supported by further supraspinal and spinal adaptations.

5.3.7 Follow-up

Participants were contacted via email after 3 and 6 months following the completion of the ECC or CON exercise intervention, where they were asked to complete the NRS and VISA-A questionnaire to assess their pain and function.

5.3.8 Statistical analysis

Descriptive statistics were reported as means and SDs, unless otherwise specified. The Shapiro-Wilk test was used to determine the normality of the data, and the Levene test was used to assess the assumption of homogeneity of variance. As these assumptions were met, parametric statistical tests were deemed appropriate. Questionnaire scores and morphological and material properties of the Achilles tendon were compared between groups using a linear mixed model analysis with factors group (ECC, CON) and time (week 1, week 3, and week 6) as fixed effects, and participants as random effect. Similarly, motor unit firing rate properties of one of the isometric contractions were compared between groups at each torque level independently using a linear mixed model analysis with factors group (ECC, CON) and time (week 1, week 3, and week 6) as fixed effects, and participants as random effect. Additionally, motor unit firing rate properties were compared between the control group at baseline and the ECC and CON groups at week 6 using a linear mixed model analysis with factors group (control, ECC, and CON) and muscle (MG, LG, and SO) as fixed effects, and participants as random effect. Maximal voluntary torque was compared between control at baseline and ECC and CON groups after the intervention (week 6) using ANOVA. Torque steadiness was also compared using ANOVA between control group at baseline and the ECC and CON groups post-intervention (week 6) at each torque level independently. Post-hoc pairwise comparisons were conducted using Tuckey test correction. Post-hoc results were reported as p-value and 95% confidence intervals (CI). Linear mixed-model and ANOVA analysis were performed using GraphPad Prism software V.8.0.2 (San Diego, California, USA).

5.4 RESULTS

5.4.1 Participants characteristics

Participants anthropometrics and information regarding their symptoms in the ECC and CON groups are reported in **Table 10**. No statistical differences were observed between the groups at baseline (pre intervention).

Table 10. Participants characteristics between Eccentric and Concentric groups.

	ECC group (N=12)	CON group (N=12)	p-value	95% CI
Age (years)	28.83 ± 6.85	29.67 ± 10.20	0.816	[-8.19, 6.52]
Sex (males/females)	7/5	6/6		
Height (cm)	174.38 ± 8,28	171.31 ± 10.62	0.437	[-4.99, 11.14]
Weight (kg)	74.57 ± 19.40	74.57 ± 16.54	1.00	[-15.26, 15.26]
Laterality (Right/Left)	11/1	11/1		
Assessed leg (Right/Left)	6/6	8/4		
Bilateral symptoms	4/12	7/12		
Symptoms duration (months)	33. 42 ± 34.35	49.50 ± 40.47	0.305	[-47.86, 15.70]
NRS	2.92 ± 1.16	3.25 ± 1.29	0.513	[-1.37, 0.71]

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. ECC, eccentric; CON, concentric; NRS, Numerical Rating Scale; N/A, non-applicable. * Indicates significant statistical difference, $p<0.05$.

5.4.2 Questionnaires

Questionnaires scores between ECC and CON groups at baseline are reported in **Table 11**. No differences between ECC and CON groups were observed for the VISA-A, IPAQ-SF, FAAM (Subscale activities of daily living and subscale sports), PCS and TSK questionnaires.

Table 11. Questionnaire scores between Eccentric and Concentric groups.

	ECC group (N=12)	CON group (N=12)	p-value	95% CI
VISA-A	68.67 ± 11.96	63.00 ± 19.07	0.393	[-7.81, 19.14]
IPAQ-SF	128.33 ± 71.55	121.67 ± 46.73	0.789	[-44.49, 57.83]
FAAM (Subscale Activities of Daily Living)	89.58 ± 7.24	81.67 ± 12.18	0.066	[-0.56, 16.40]
FAAM (Subscale Sports)	70.42 ± 15.66	65.75 ± 18.36	0.510	[-9.78, 19.11]
PCS	11.75 ± 9.23	13.42 ± 12.81	0.718	[-11.12, 7.78]
TSK	37.08 ± 4.42	37.17 ± 9.04	0.977	[-6.11, 5.94]

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. ECC, eccentric; CON, concentric; VISA-A, Victorian Institute of Sport Assessment – Achilles Questionnaire; IPAQ-SF, International Physical Activity Questionnaire short form; FAAM, Foot and Ankle Ability Measure, PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia. * Indicates significant statistical difference, $p<0.05$.

VISA-A scores between ECC and CON groups at week 1, week 3, week 6, 3-month follow-up and 6-month follow-up are presented in **Figure 27A**. Overall, VISA-A scores increased similarly between groups (Time effect: $F=23.18$, $P<0.001$); however, no differences between groups were observed (Group effect: $P=0.07$). Within-group differences across assessment times can be seen in **Figure 27A**. NRS scores between ECC and CON groups at week 1, week 3, week 6, 3-month follow-up and 6-month follow-up are presented in **Figure 27B**. NRS decreased with time (Time effect: $F= 47.61$, $P<0.0001$) in the ECC and CON groups; however, no differences were observed between groups (Group effect: $P=0.73$). Within-group differences across assessment times can be seen in **Figure 27B**.

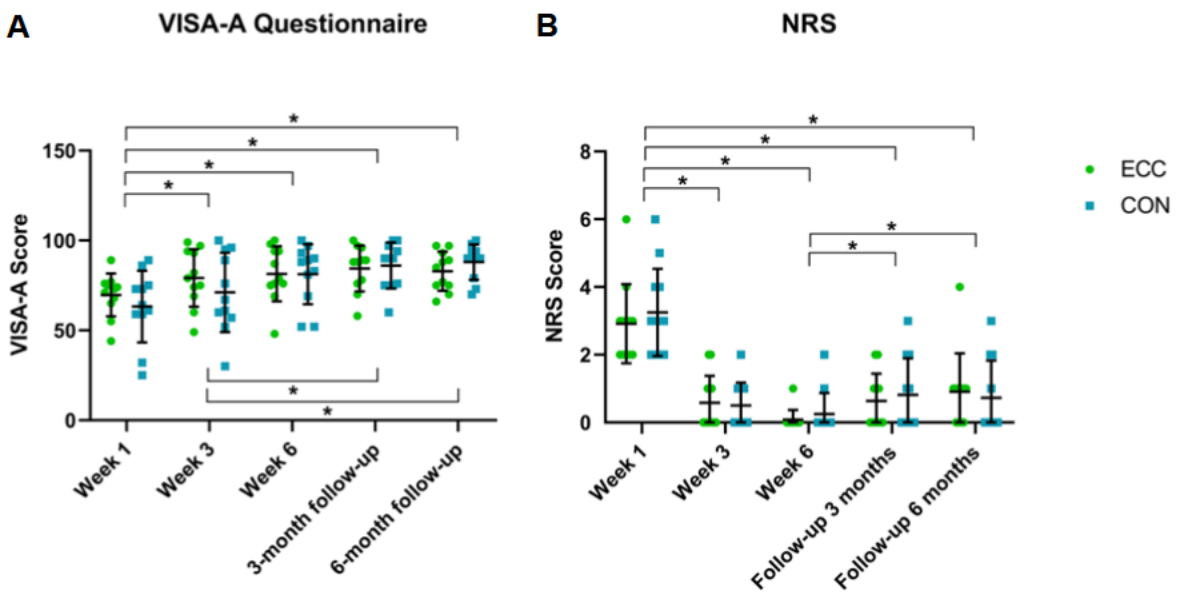

Figure 27. VISA-A and NRS scores between Eccentric and Concentric groups.
A) Victorian Institute of Sports Assessment – Achilles (VISA-A) questionnaire scores between eccentric (ECC) and concentric (CON) groups at week 1, week 3, week 6, follow-up 3 months and follow-up 6 months. B) Numerical Rating Scale (NRS) scores between eccentric (ECC) and concentric (CON) groups at week 1, week 3, week 6, follow-up 3 months and follow-up 6 months. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, $p<0.05$.

IPAQ-SF scores between ECC and CON groups at week 1, week 3, and week 6 can be seen in **Figure 28A**. Overall, IPAQ-SF scores did not change across time in the ECC and CON groups (Time effect: P=0.27), with no differences between groups (Group effect: P=0.75). FAAM (Activities Daily Living) scores between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 28B**. In general, FAAM (Activities Daily Living) scores increased differently between groups and time (Interaction effect: F=3.67, P=0.046). Within-group differences across assessment times can be seen in **Figure 28B**. FAAM (Sport) scores between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 28C**. Overall, FAAM (Sport) scores increased with time (Time effect: F= 7.24, P= 0.0038), with no differences between groups (Group effect: P= 0.80). Within-group differences across assessment times are shown in **Figure 28C**. PCS scores between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 28D**. In general, PCS scores decreased with time (Time effect: F= 6.38, P= 0.01),

with no differences between groups (Group effect: P= 0.87). Within-group differences across assessment times are presented in **Figure 28D**. TSK scores between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 28E**. Overall, TSK scores decreased with time (Time effect: F= 9.66, P= 0.002); however, no differences between groups were observed (Group effect: P= 0.70). Within-group differences across assessment times are shown in **Figure 28E**.

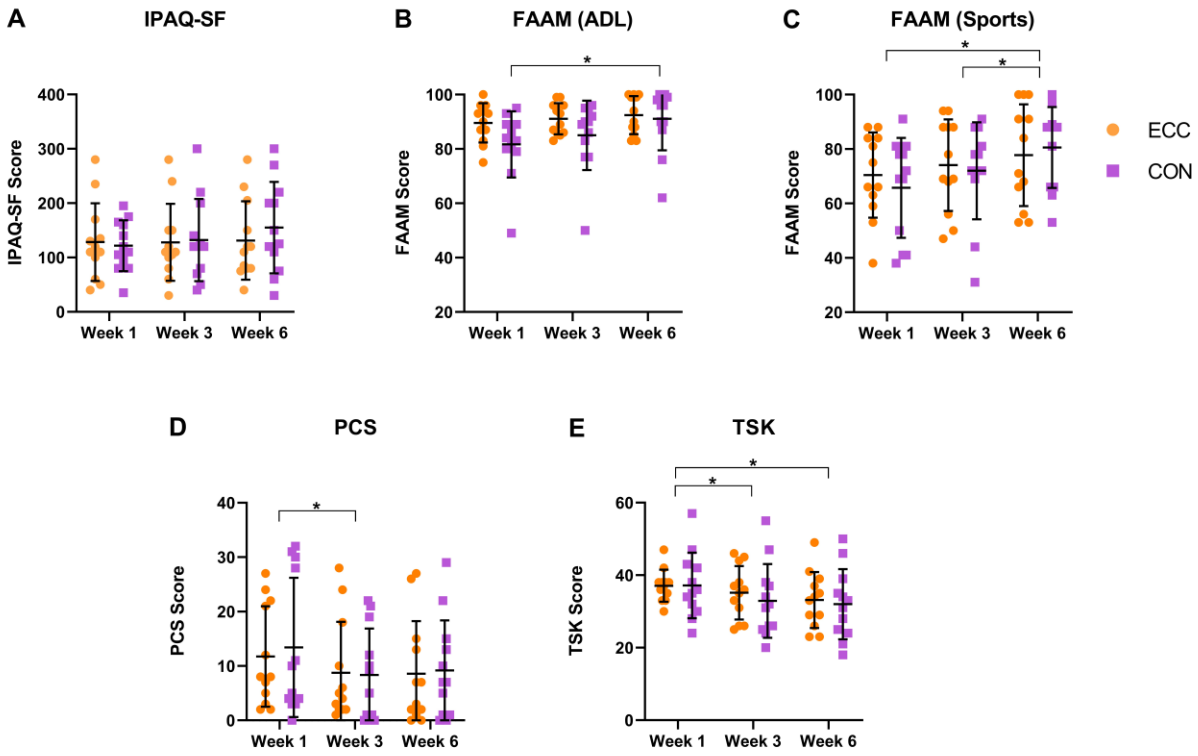

Figure 28. Questionnaires scores between Eccentric and Concentric groups.
A) International Physical Activity Questionnaire short form (IPAQ-SF) scores, B) Foot and Ankle Ability Measure (FAAM) subscale Activities of Daily Living (ADL) scores, C) FAAM subscale Sports scores, D) Pain Catastrophising Scale (PCS) scores and E) Tampa Scale for Kinesiophobia (TSK) scores between eccentric (ECC) and (CON) groups at week 1, week 3, and week 6. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, p<0.05.

5.4.3 Morphological and material properties of the Achilles tendon

Morphological and material properties of the Achilles tendon between ECC and CON groups at baseline are reported in **Table 12**.

Table 12. Morphological and material properties of the Achilles tendon between Eccentric and Concentric groups.

	ECC group (N=12)	CON group (N=12)	p-value	95% CI
Length (cm)	21.16 ± 4.01	21.19 ± 2.20	0.980	[-2.77, 2.71]
Thickness 2 cm (cm)	0.34 ± 0.05	0.37 ± 0.07	0.396	[-0.07, 0.03]
Thickness 4 cm (cm)	0.42 ± 0.09	0.49 ± 0.11	0.103	[-0.16, 0.02]
Thickness 6 cm (cm)	0.43 ± 0.05	0.51 ± 0.16	0.134	[-0.17, 0.02]
CSA 2 cm (cm^2)	0.43 ± 0.07	0.49 ± 0.08	0.078	[-0.12, 0.01]
CSA 4 cm (cm^2)	0.41 ± 0.07	0.47 ± 0.10	0.102	[-0.13, 0.01]
CSA 6 cm (cm^2)	0.46 ± 0.07	0.50 ± 0.10	0.266	[-0.11, 0.03]
Young's modulus (kPa)	63.58 ± 6.45	72.90 ± 6.3	0.002*	[-14.71, -3.92]

Values are presented as mean ± SD (Standard deviation). 95% Confidence intervals are reported. ECC, eccentric; CON, concentric; CSA, cross-sectional area * Indicates significant statistical difference, $p<0.05$.

Thickness at 2 cm between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 29A**. Overall, thickness at 2 cm did not change across time in the ECC and CON groups (Time effect: P=0.25), with no differences between groups (Group effect: P=0.38). Thickness at 4 cm between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 29B**. Similarly, thickness at 4 cm did not change across time in the ECC and CON groups (Time effect: P=0.75), with no differences between groups (Group effect: P=0.15). Thickness at 6 cm between ECC and CON groups at week 1, week 3, and week 6 can be seen in **Figure 29C**. Likewise, thickness at 6 cm did not change across time in the ECC and CON groups (Time effect: P=0.37), with no differences between groups (Group effect: P=0.15). CSA at 2 cm between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 29D**. Overall, CSA at 2 cm did not change across time in the ECC and CON groups (Time effect: P=0.07), with no differences between groups (Group effect: P=0.12). CSA at 4 cm between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 29E**.

Likewise, CSA at 4 cm did not change across time in the ECC and CON groups (Time effect: P=0.70), with no differences between groups (Group effect: P=0.15). CSA at 6 cm between ECC and CON groups at week 1, week 3, and week 6 can be seen in **Figure 29F**. Similarly, CSA at 6 cm did not change across time in the ECC and CON groups (Time effect: P=0.59), with no differences between groups (Group effect: P=0.35). Due to the differences in Young's modulus at baseline between ECC and CON groups, the percentage of Young's modulus change at weeks 3 and 6 was calculated (**Figure 29G)**. Overall, Young's modulus increased differently across groups and times (Interaction effect: F=7.01, P= 0.02) with higher Young's modulus in the ECC compared to the CON group at week 6 (P=0.01, 95% CI=2.66 to 19.09) and increased Young's modulus in the ECC group at week 6 compared to week 3 (P=0.01, 95% CI=-24.29 to -3.01).

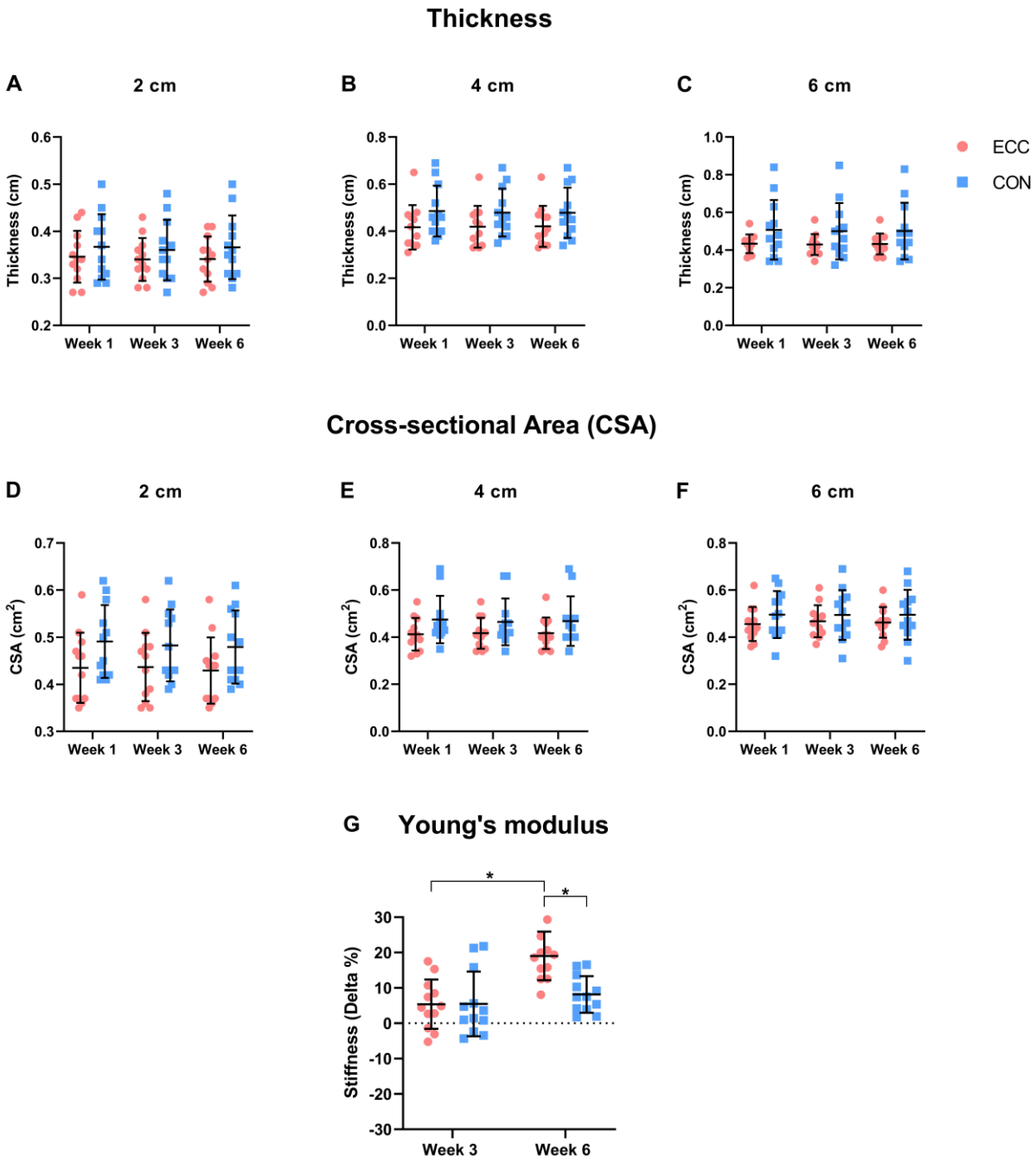

Figure 29. *Morphological and material properties of the Achilles tendon between Eccentric and Concentric groups.*
A) Achilles tendon thickness at 2 cm (A), 4 cm (B), and 6 cm (C) from its insertion between eccentric (ECC) and concentric (CON) groups at week 1, week 3, and week 6. Achilles tendon cross-sectional area (CSA) at 2 cm (D), 4 cm (E), and 6 cm (F) from its insertion between ECC and CON groups at week 1, week 3, and week 6. Percentual change in Achilles tendon Young's modulus (G) between ECC and CON groups at week 3 and week 6. A linear mixed model was used for statistical comparisons. * Statistical differences between and within groups, $p<0.05$.

5.4.4 Recruitment and derecruitment thresholds

Recruitment and derecruitment thresholds of the MG, LG, and SO muscles were averaged since no muscle x group interaction was found across time at each force level (P>0.09 in all cases). Recruitment threshold of the triceps surae muscle at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 30A**. Overall, recruitment threshold did not change across time at any force level (Time effect: P=0.06, P=0.07, P=0.36, for the 10, 40, and 70% MVC respectively), with no differences between groups (Group effect: P=0.45, P=0.45, P=0.45, for the 10, 40, and 70% MVC respectively). Derecruitment threshold of the triceps surae muscle at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 30B**. In general, derecruitment threshold did not change across time at 10% MVC (Time effect: P=0.16) and was not different between groups (Group effect: P=0.99). Nevertheless, derecruitment threshold increased across time at 40% MVC (Time effect: F=5.22, P=0.02), with higher derecruitment threshold at week 6 compared to week 1; however, no differences between groups were observed (Group effect: P=0.85). Finally, derecruitment threshold increased across time at 70% MVC (Time effect: F=4.12, P=0.03), with greater derecruitment threshold at week 3 compared to week 1; however, no differences between groups were found (Group effect: P=0.93).

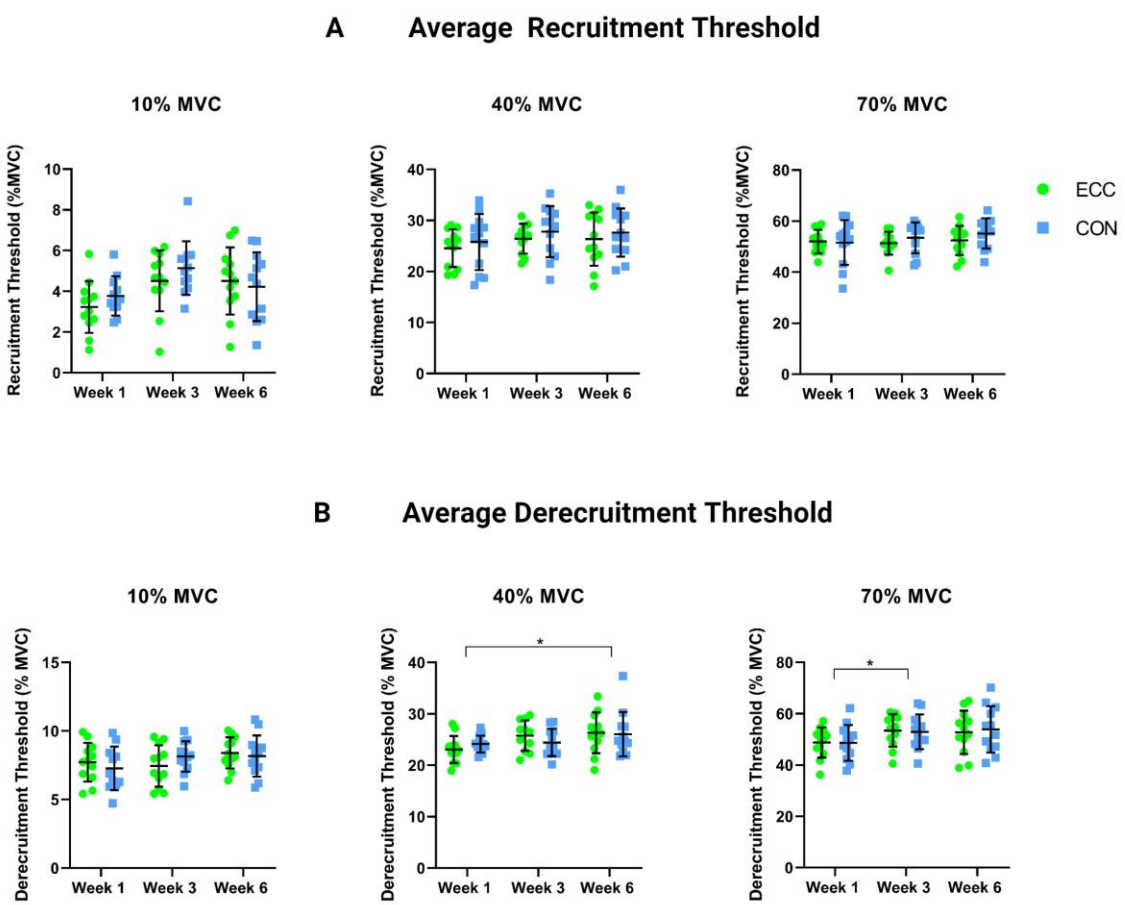

Figure 30. Recruitment and derecruitment thresholds between Eccentric and Concentric groups. A) Recruitment threshold from triceps surae muscles at 10, 40, 70% maximum voluntary contraction (MVC) between eccentric (ECC) and concentric (CON) groups at week 1, week 3, and week 6. B) Derecruitment threshold from triceps surae muscles at 10, 40, 70% MVC between ECC and CON groups at week 1, week 3, and week 6. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, $p<0.05$.

5.4.5 Discharge rate and COVisi

Discharge rate and COVisi of the MG, LG, and SO muscles were averaged since no muscle x group interaction was found across time at each force level. The discharge rate of the triceps surae muscle at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 31A**. Overall, discharge rate at 10% MVC did not change across time (Time effect: P=0.26), with no differences between

groups (Group effect: P=0.77). However, discharge rate at 40% MVC increased across time (Time effect: F=4.16, P=0.04), with significant differences between week 1 and week 3; yet no differences between groups were observed (P=0.90). Discharge rate at 70% MVC did not change across time (Time effect: P=0.23), with no differences between groups (Group effect: P=0.06). COVisi of the triceps surae muscle at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 31B**. In general, COVisi did not change across time at any force level (Time effect: P=0.14, P=0.11, P=0.45, for the 10, 40, and 70% MVC respectively), with no differences between groups (Group effect: P=0.1, P=0.42, P=0.98, for the 10, 40, and 70% MVC respectively).

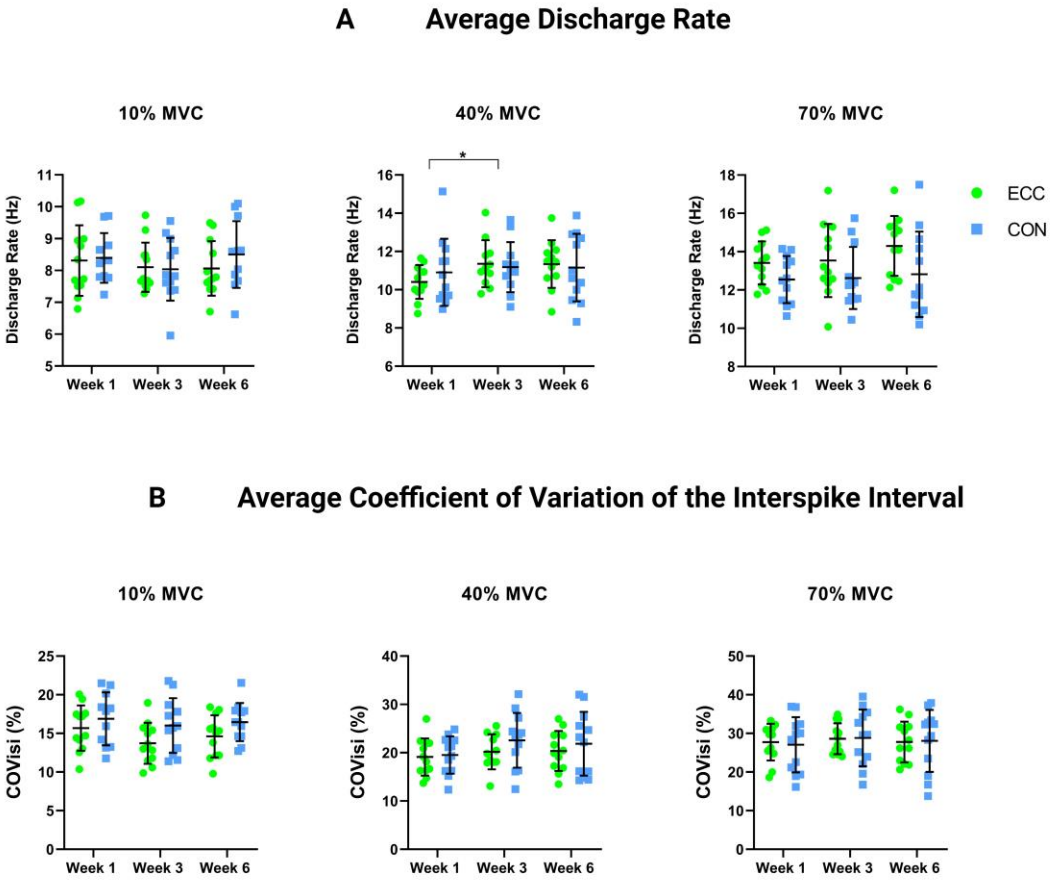

Figure 31. Discharge rate and coefficient of variation of the interspike interval between Eccentric and Concentric groups.
A) Discharge rate from triceps surae muscles at 10, 40, 70% maximal voluntary contraction (MVC) between eccentric (ECC) and concentric (CON) groups at week 1, week 3, and week 6. B) Coefficient of variation of the interspike interval (COVisi) from triceps surae muscles at 10, 40, 70% MVC between ECC and CON groups at week 1, week, 3, and week 6. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, $p<0.05$.

5.4.6 Cross-correlation coefficient

Cross-correlation coefficient at 10% MVC between ECC and CON groups at week 1, week 3, and week 6 for each individual muscle are presented in **Figure 32A**. Overall, cross-correlation coefficient at 10% MVC did not change across time for any muscle (Time effect: P=0.35, P=0.31, P=0.12, for the MG, LG, and SO muscles respectively), with no differences between groups (Group effect: P=0.80, P=0.65, P=0.96, for the MG, LG, and SO muscles respectively). Cross-correlation coefficient at 40% MVC between ECC and CON groups at week 1, week 3, and week 6 for each individual muscle are presented in **Figure 32B**. Cross-correlation coefficient of the MG at 40% MVC did not change across time (Time effect: P=0.07), with no differences between groups (Group effect: P=0.57). Nevertheless, cross-correlation coefficient of the LG at 40% MVC decreased across time (Time effect: F=9.59, P=0.001), with lower cross-correlation coefficient at week 3 compared to week 1 and week 6 compared to week 1; however, no differences between groups were observed (Group effect: P=0.35). Similarly, cross-correlation coefficient of the SO at 40% MVC decreased across time (Time effect: F=9.37, P=0.002); with lower cross-correlation coefficient at week 3 compared to week 1 and week 6 compared to week 1; however, no differences between groups were observed (Group effect: P=0.76). Cross-correlation coefficient at 70% MVC between ECC and CON groups at week 1, week 3, and week 6 for each individual muscle are presented in **Figure 32C**. Overall, cross-correlation of the MG at 70% MVC did not change across time (Time effect: P=0.07), with no differences between groups (Group effect: P=0.40). Yet, cross-correlation of the LG at 70% MVC decreased different between time and groups (Interaction effect: F=3.92, P=0.04), with decreased cross-correlation coefficient in the ECC group at week 6 compared to week 3; however, no differences between groups were observed. Finally, cross-correlation of the SO at 70% MVC decreased across time (Time effect: F=4.75, P=0.03), with decreased cross-correlation at week 6 compared to week 1, with no differences between groups (Group effect: P=0.23).

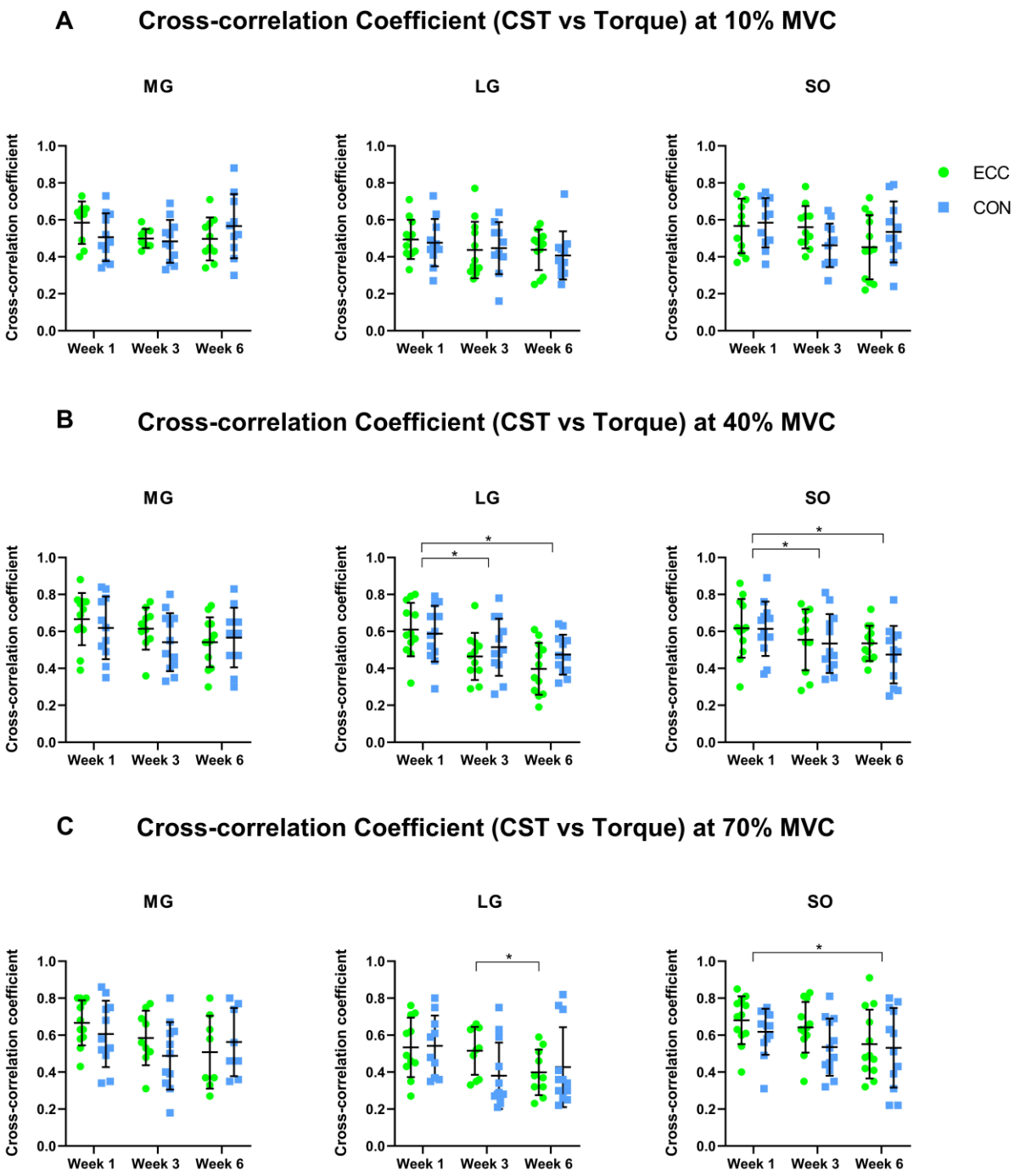

Figure 32. Cross-correlation coefficients between Eccentric and Concentric groups.
A) Cross-correlation coefficient (cumulative spike train (CST) and torque) at 10% MVC between eccentric (ECC) and concentric (CON) groups for the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SO) muscles at week 1, week 3, and week 6. B) Cross-correlation coefficient at 40% MVC between ECC and CON groups for the MG, LG, and SO muscles at week 1, week 3, and week 6. C) Cross-correlation coefficient at 70% MVC between ECC and CON groups for the MG, LG, and SO muscles at week 1, week 3, and week 6. A *linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, p<0.05.*

5.4.7 Maximal voluntary torque and torque steadiness between Eccentric and Concentric groups

Maximal voluntary torque between ECC and CON groups at week 1, week 3, and week 6 are presented in **Figure 33A**. Overall, maximal voluntary torque increased across time (Time effect: $F=17.53$, $P<0.0001$, with higher maximal voluntary torque at week 3 and week 6 compared to week 1, and higher maximal voluntary torque at week 6 compared to week 3; with no differences between groups (Group effect: $P=0.49$). Torque steadiness at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6 are shown in **Figure 33B**. In general, torque steadiness did not change across time at any force level (Time effect: $P=0.36$, $P=0.10$, $P=0.12$, for the 10, 40, and 70% MVC respectively), with no differences between groups (Group effect: $P=0.47$, $P=0.12$, $P=0.92$, for the 10, 40, and 70% MVC respectively).

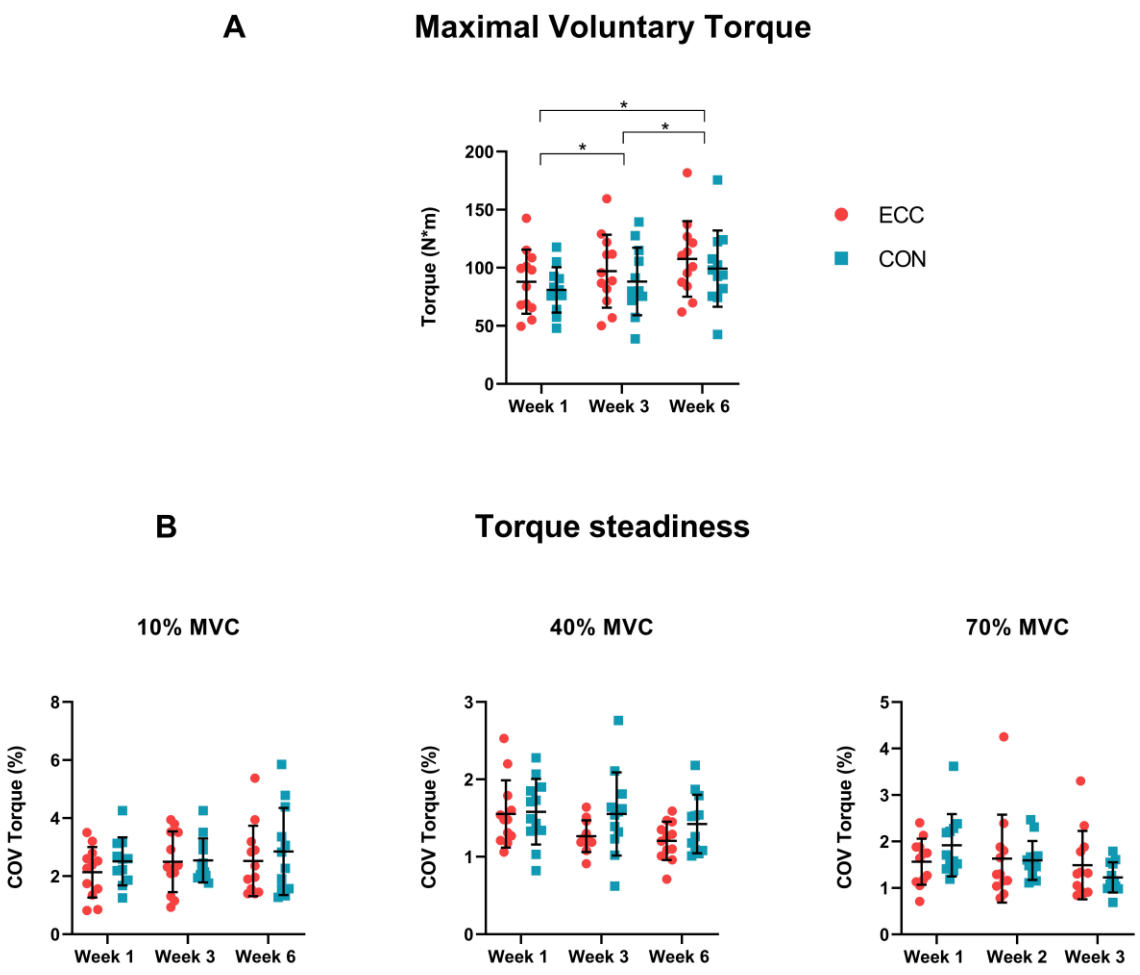

Figure 33. Maximal voluntary torque and torque steadiness between Eccentric and Concentric groups. A) Maximal voluntary torque between eccentric (ECC) and concentric (CON) groups at week 1, week 3, and week 6. B) Torque steadiness at 10, 40, and 70% MVC between ECC and CON groups at week 1, week 3, and week 6. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, $p<0.05$.

5.4.8 Recruitment and derecruitment threshold between Control, ECC, and CON groups

Recruitment and derecruitment threshold of the MG, LG, and SO muscles were compared between the control group at baseline and the ECC and CON groups at week 6. Recruitment threshold of the MG, LG, and SO muscles at 10, 40, and 70% MVC for the control, ECC, and CON groups are shown in **Figure 34A**. Overall, recruitment threshold was different between muscles across force levels (Muscle effect: $P<0.02$ in all

cases); however, no differences between groups were observed (Group effect: P>0.09 in all cases). Derecruitment threshold of the MG, LG, and SO muscles at 10, 40, and 70% MVC for the control, ECC, and CON groups are presented in **Figure 34B**. In general, derecruitment threshold at 10 and 40% MVC did not change between muscles (Muscle effect: P=0.09 and P=0.15, at 10 and 40% MVC respectively), with no differences between groups (Group effect: P=0.09 and P=0.62, at 10 and 40% MVC respectively). Nevertheless, derecruitment threshold at 70% MVC change between muscles (Muscle effect: F=4.25, P=0.03); however, no differences between groups were observed (Group effect: P=0.08).

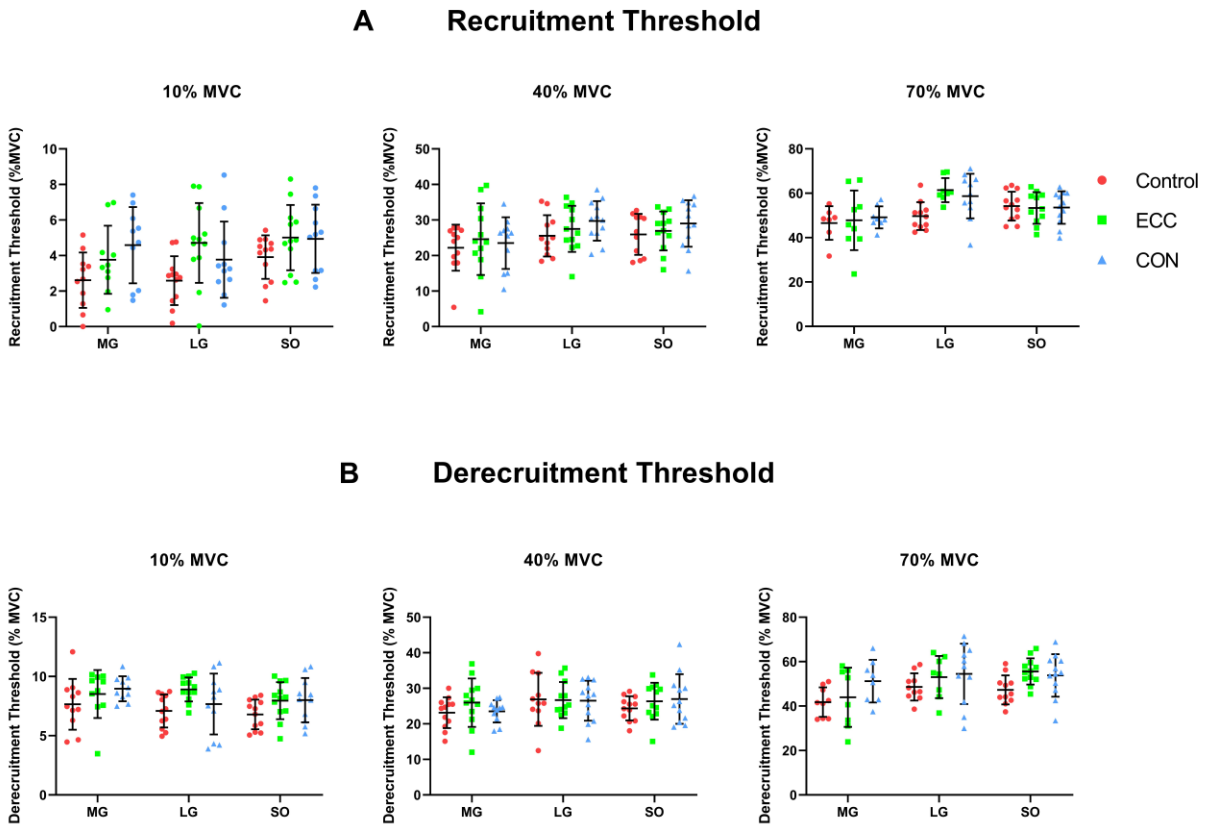

Figure 34. Recruitment and derecruitment thresholds between the Control, Eccentric, and Concentric groups.
A) Recruitment threshold from medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SO) muscles at 10, 40, and 70% maximal voluntary contraction (MVC) for the control group at baseline compared to the eccentric (ECC) and concentric (CON) groups after the intervention (week 6). B) Derecruitment threshold from MG, LG, and SO muscles at 10, 40, and 70% MVC for the control group at baseline compared to the ECC and CON groups after the intervention. A linear mixed model was used for statistical comparisons. * Statistical differences between assessment times, p<0.05.

5.4.9 Discharge rate, COVisi, and cross-correlation coefficient between Control, ECC, and CON groups

Discharge rate, COVisi, and cross-correlation coefficient of the MG, LG, and SO muscles were compared between the control group at baseline and the ECC and CON groups after the intervention (week 6). Discharge rate of the MG, LG, and SO muscles at 10, 40, and 70% MVC for the control, ECC, and CON groups are shown in **Figure 35A**. Overall, discharge rate at 10 and 40% MVC did not change between muscles (Muscle effect: P=0.12 and P=0.47, for the 10 and 40% MVC, respectively), with no differences between groups (Group effect: P=0.13 and P=0.36, for the 10 and 40% MVC, respectively). Yet, discharge rate at 70% MVC was different between muscles (Muscle effect: F=4.27, P=0.04); however, no differences between groups were observed (Group effect: P=0.12). COVisi of the MG, LG, and SO muscles at 10, 40, and 70% MVC for the control, ECC and CON groups are presented in **Figure 35B**. In general, COVisi at 10% MVC was different between muscles (Muscle effect: F=4.57, P=0.02), with no differences between groups (Group effect: P=0.06). However, COVisi at 40% MVC was different between muscles (Muscle effect: F=10.65, P=0.003) and groups (Group effect: F=3.92, P=0.04). COVisi at 70% MVC was different between groups (Group effect: F=4.07, P=0.04), but did not change between muscles (Muscle effect: P=0.05). Cross-correlation coefficients of the MG, LG, and SO muscles at 10, 40, and 70% MVC for the control, ECC and CON groups are presented in **Figure 35C.** Overall, cross-correlation coefficient at 10% MVC was different between muscles (Muscle effect: F=10.11, P=0.003), with no differences between groups (Group effect: P=0.08). Cross-correlation coefficient at 40% MVC was different between muscles (Muscle effect: F=11.17, P=0.001) and groups (Group effect: F=9.58, P=0.001). Finally, cross-correlation coefficient at 70% MVC was different between muscles (Muscle effect: F=6.17, P=0.008), with no differences between groups (Group effect: P=0.15).

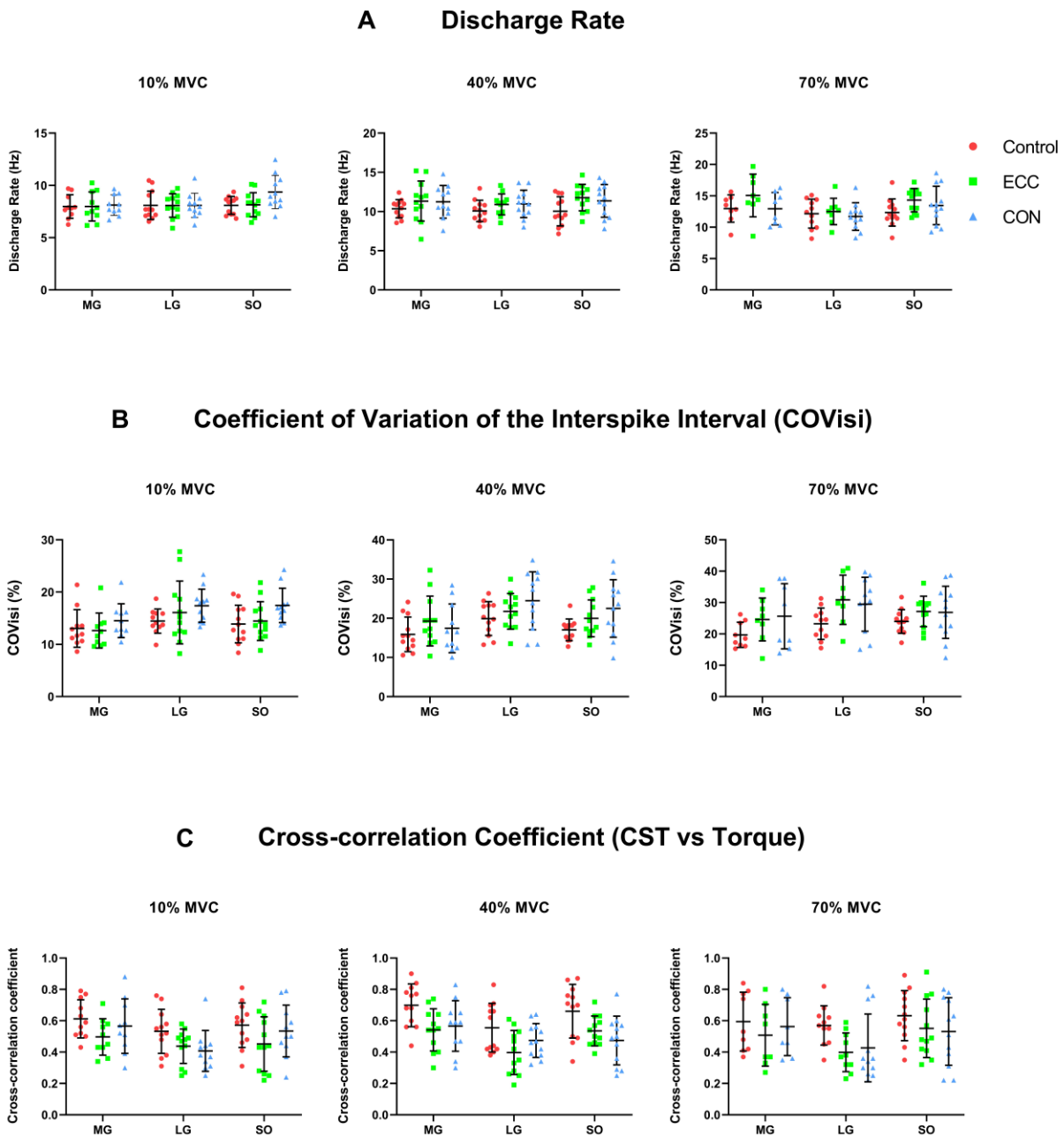

Figure 35. Discharge rate, coefficient of variation of the interspike interval and cross-correlation coefficients between the Control, Eccentric, and Concentric groups.
A) Discharge rate from medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SO) muscles at 10, 40, and 70% maximal voluntary contraction (MVC) for the control group at baseline compared to the eccentric (ECC) and concentric (CON) groups after the intervention (week 6). B) Coefficient of variation of the interspike interval (COVisi) from MG, LG, and SO muscles at 10, 40, and 70% MVC for the control group at baseline compared to the ECC and CON groups after the intervention. C) Cross-correlation coefficient (cumulative spike train (CST) and torque) from MG, LG, and SO muscles at 10, 40, and 70% MVC for the control group at baseline compared to the ECC and CON groups after the intervention. A linear mixed model was used for statistical comparisons. * Statistical differences between groups, $p<0.05$.

5.4.10 Maximal voluntary torque and torque steadiness between Control, ECC, and CON groups

Maximal voluntary torque and torque steadiness were compared between the control group at baseline and the ECC and CON groups after the intervention (week 6). Maximal voluntary torque for the control, ECC and CON groups are presented in **Figure 36A.** No differences in maximal voluntary torque between groups were observed (P=0.16). Torque steadiness at 10, 40, and 70% MVC for the control, ECC and CON groups are shown in **Figure 36B.** Overall, no differences in torque steadiness were observed between groups at any torque level (P=0.77, P=0.051, P=0.07, for the 10, 40, and 70% MVC respectively).

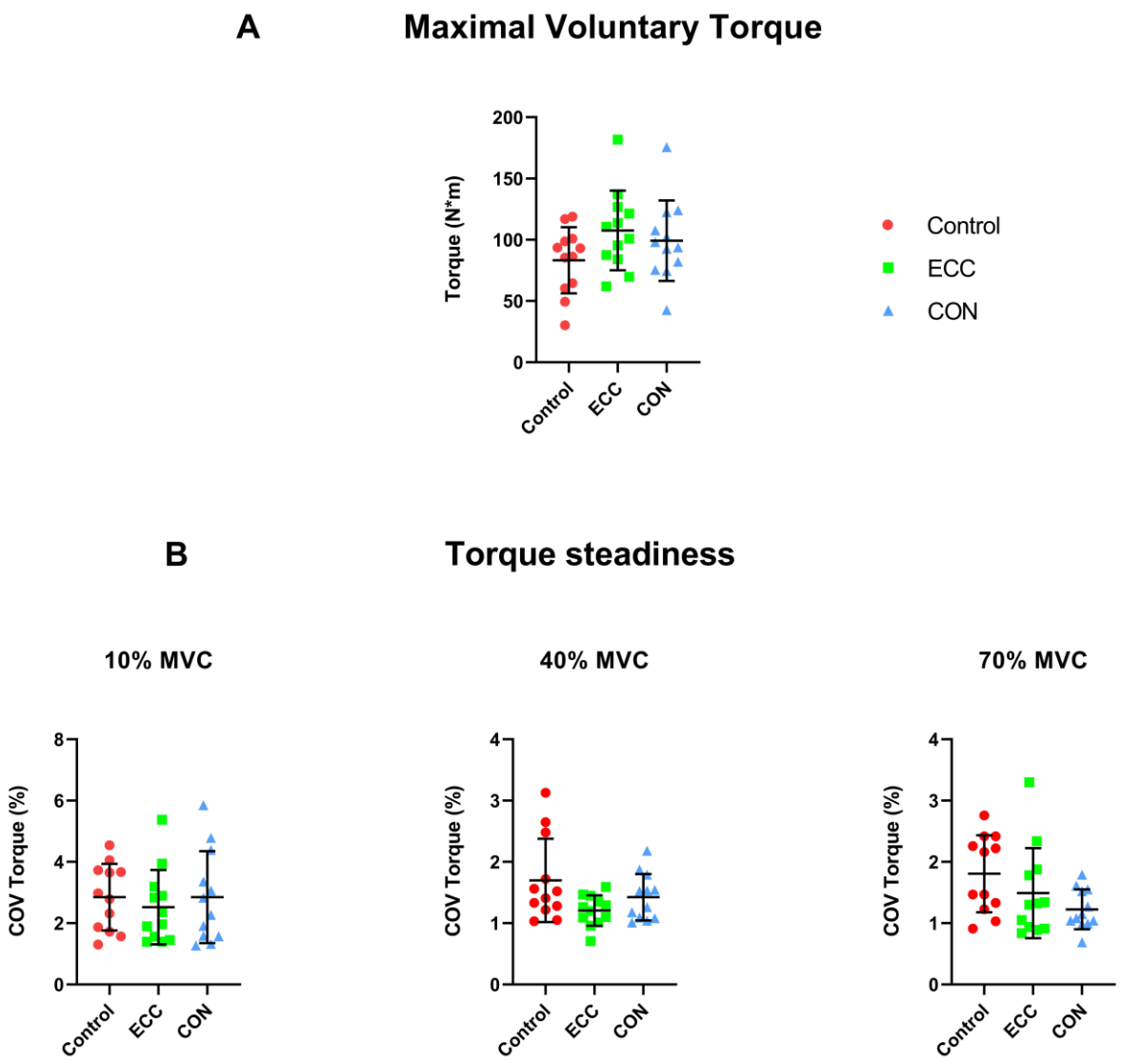

Figure 36. Maximal voluntary torque and torque steadiness between Control, Eccentric and Concentric groups.
A) Maximal voluntary torque for the control group at baseline compared to the eccentric (ECC) and concentric (CON) groups after the intervention (week 6). B) Torque steadiness at 10, 40, and 70% MVC for the control group at baseline compared to the ECC and CON groups after the intervention. A one-way ANOVA was used for statistical comparisons. * Statistical differences between groups, $p<0.05$.

5.5 DISCUSSION

This is the first study to show that a 6-week torque feedback training intervention based on either controlled ECC or CON contractions induced load-dependent changes in triceps surae motor unit firing rate properties. Moreover, our results demonstrate that this intervention was effective in decreasing pain levels and increasing function and tendon material properties independently of the protocol applied, supporting the idea that controlled progression of the load, range of movement, time under tension, speed of the contraction, and the provision of visuomotor torque feedback are more relevant for the rehabilitation of individuals with NIAT than the type of contraction used as part of an exercise intervention. Furthermore, our results indicate that individuals in the ECC and CON groups presented similar triceps surae motor unit firing rate properties to controls after the intervention, suggesting that exercise-induced changes in neuromuscular parameters could be behind the improvement in pain and function in individuals with NIAT.

5.5.1 Differences between training protocols

Although ECC exercise has been widely used for the management of NIAT, the mechanism by which ECC exercises may help to improve tendon symptoms is still unclear (Maffulli et al., 2020). However, exercise interventions combining ECC and CON contractions or isolating ECC or CON contractions, have been successfully used in individuals with this condition (Silbernagel et al., 2007a, Beyer et al., 2015). Within this framework, current recommendation for the management of NIAT include progressive mechanical loading, which can be either in the form of ECC exercise, or a heavy-load, slow speed (CON/ECC) exercise program (Martin et al., 2018). As a result, the concept that the tendon's response to mechanical load varies based on the specific type of contraction exerted has come under scrutiny (Magnusson and Kjaer, 2019, Couppé et al., 2015). For this reason, we focused our exercise intervention on isolating ECC or CON contractions with an isokinetic dynamometer, to be able to make an adequate comparison of the adaptations induced by each training type. Regarding motor unit firing properties,

most studies have reported no differences in recruitment thresholds between ECC and CON contractions when lifting and lowering an inertial load (Bawa and Jones, 1999, Garland et al., 1996, Laidlaw et al., 2000, Søgaard, 1995, Stotz and Bawa, 2001) or resisting a torque motor (Altenburg et al., 2009, Pasquet et al., 2006, Stotz and Bawa, 2001), suggesting that the recruitment order of motor units is usually similar between ECC and CON contractions (Duchateau and Enoka, 2008). Additionally, most studies have reported a decrease in discharge rate during lowering an inertial load compared with lifting the load (Del Valle and Thomas, 2005, Kallio et al., 2013, Laidlaw et al., 2000, Semmler et al., 2002, Stotz and Bawa, 2001, Tax et al., 1989), when resisting a torque motor with a similar fascicle length for both ECC and CON contractions (Pasquet et al., 2006) or when the two anisometric contractions were performed at a same relative torque level (Altenburg et al., 2009), indicating that rate coding is modulated differently between ECC and CON contractions (Duchateau and Baudry, 2014). Despite this different modulation between types of contraction, our results showed no differences in triceps surae motor unit firing properties after 6 weeks of training between the ECC and CON groups. We hypothesise that these findings may be related to the study's design since, during the training sessions, the participants only performed dynamic contractions; however, during the experimental sessions, the motor unit firing properties were assessed during isometric contractions. Thus, it seems plausible that a difference in the adaptations of motor unit firing properties with lengthening and shortening contractions may be observed only during assessments involving these types of contractions. Another possible explanation may be related to the features of the intervention itself, since in addition to controlling the type of contraction, load, range of movement, and speed of the contraction, we provided individualised visuomotor feedback during each training session; therefore, both training interventions may have induced similar supraspinal or spinal adaptations. Some studies involving motor skill training have reported increased representation of the muscles in the primary motor cortex and enhanced excitability of the corticospinal pathways (Jensen et al., 2005, Pascual-Leone et al., 1995). Nevertheless, strength training does not seem to produce the same adaptations (Carroll et al., 2002, Jensen et al., 2005). Alternatively, it has been suggested that training-induced changes in Ia afferent feedback can influence motor unit discharge rate (Duchateau et al., 2006). Furthermore, it has been observed in

animal models that physical activity can change the biophysical properties of motor neurons, which will likely produce changes in the ionic conductances of motor neurons and, therefore, influence their recruitment thresholds and discharge rate patterns (Duchateau et al., 2006).

5.5.2 Self-reported outcome measures

Our results showed that VISA-A scores increased in both groups with time. Interestingly, VISA-A scores increased after 3 weeks of training, and this increase was maintained after 6 weeks of training. Additionally, the increase in the VISA-A scores was maintained during the 3-month and 6-month follow-ups, indicating that the effect of both interventions on the symptoms was still observed after 6 months. Similar findings have been reported extensively in the literature, with a recent systematic review showing increased VISA-A scores after 2 weeks of exercise training in individuals with NIAT (Murphy et al., 2018). Notably, the authors also reported that peak VISA-A scores were obtained after 12 weeks of training regardless of the protocol used (Murphy et al., 2018); however, we observed that peak VISA-A scores were obtained after 3 weeks of training in both groups, suggesting that the torque feedback training intervention applied was more efficient to reduce symptoms in individuals with NIAT. NRS scores decreased in both groups with time; however, pain levels decreased after 3 weeks of training, and this decrease was maintained after 6 weeks of training. Consistent with VISA-A scores, the effect of both exercise interventions on the pain level was still visible during the 3-month and 6-month follow-up. Regarding the level of physical activity, IPAQ-SF scores showed no difference with time or between groups, results that we expected since we instructed the participants to maintain their level of physical activity during the intervention period. The physical function of the lower leg during activities of daily living (FAAM Subscale Activities Daily Living) increased differently between groups and time, probably due to the lower FAAM (Subscale Activities of Daily Living) score at baseline in the CON group. Nevertheless, the physical function of the lower leg during sports activities (FAAM Subscale Sports) increased with time, with no differences between groups. This result agrees partially with the VISA-A and NRS scores observed, indicating that the decrease

in symptoms and pain was reflected in the physical function of the lower leg during sports activities. Furthermore, PCS and TSK scores decreased with time, with no differences between groups. We theorised that the decrease in the symptoms may reduce the participants' anxiety about their condition, possibly explaining the change in their psychological attitudes about pain with the intervention.

5.5.3 Morphological and material properties of the Achilles tendon

Tendons play a crucial role in the mechanical transmission of muscle forces to bones, thereby permitting locomotion and increasing joint stability (Wang, 2006). Moreover, tendons are dynamic living tissues that adapt their metabolism, morphology, and material properties in response to mechanical forces (Wang, 2006). Our results revealed that Achilles tendon's thickness and CSA at 2, 4, and 6 cm did not change with time, with no differences between groups. Together, these results showed that 6-week ECC or CON intervention training did not induce changes in the morphological properties of the Achilles tendon in individuals with NIAT. Our findings agree with current evidence that tendon morphological adaptations with high magnitude loading training are more evident with longer intervention durations (> 12 weeks) (Bohm et al., 2015). Nevertheless, in individuals with NIAT, there are still ambiguous results about the effect of training on the Achilles tendon's morphological properties, with studies showing a decrease in thickness and CSA and others showing no change in these parameters (Färnqvist et al., 2020). It is noteworthy that the participants in both the ECC and CON groups exhibited relatively modest increases in tendon thickness and CSA at baseline compared to previous studies (Romero-Morales et al., 2019, Coombes et al., 2018, Sleeswijk Visser et al., 2024). This discrepancy could be attributed to differences in the methodology, as previous studies focused on measuring the thickest portion of the Achilles tendon. Even so, these results, along with the low to moderate pain levels reported, suggest that most individuals with NIAT were likely in the early stages of tendinopathy.

Over the past two decades, ultrasonography combined with torque assessments has remained as the gold standard method to assess the mechanical and material

properties of tendons in vivo (Maganaris and Paul, 2002, Maganaris and Paul, 2000, Magnusson, 2002, Arampatzis et al., 2005, Kubo et al., 2002). This is accomplished by analysing force-elongation and stress-strain curves generated from applying incremental tension during controlled isometric contractions or passive stretching of the muscle-tendon unit (Fouré, 2016). Nevertheless, this approach has methodological limitations such as the restricted spatial coverage of the ultrasound probes (e.g., 2D assessment of 3D strain, restricted planar field of view), the need for data normalisation to calculate force, stress, and strain values (Fouré, 2016), and the inability to determine regional differences in tendon stiffness (Mifsud et al., 2023a). Given these limitations, SWE has emerged as a valuable ultrasound technique for assessing the viscoelastic properties of tendons (Sukanen et al., 2024). SWE has shown reliable results assessing the Young's modulus of lower limb tendons (Schneebeli et al., 2021, Payne et al., 2018); however, its association with in vivo dynamometry and ultrasound-based tendon stiffness is limited to small tendon strains (up to 10% MVC), suggesting that SWE is an appropriate method for assessing tendons in resting conditions rather than under load (Mifsud et al., 2023a). Regarding the tendon material parameters, our results showed an increased Achilles tendon's Young's modulus with time, with differences between groups. These results showed that 6-week ECC or CON intervention training induced adaptation in the Achilles tendon's material properties in individuals with NIAT, supporting the idea that intervention-induced changes in tendon Young's modulus seem to be more attributed to adaptations of the material properties rather than morphological properties (Bohm et al., 2015), at least with short interventions. Additionally, the difference in tendon Young's modulus after the ECC and CON training interventions may be linked to greater force production during lengthening compared to shortening contractions (Franchi et al., 2017). According to Huxley's model, this greater force production during ECC contractions could be due to a further stretch of S2 portions of the myosin, occurring first at a slow velocity of lengthening (Franchi et al., 2017). Consequently, it could be speculated that the higher force production with the ECC training may have induced a higher increase in tendon Young' modulus than the CON training in individuals with NIAT.

5.5.4 Motor unit firing rate properties between ECC and CON groups

Research into how different types of training induce adaptations in motor unit firing parameters has been limited (Duchateau et al., 2006). Only a few studies have investigated motor unit firing behavior following strength training, and the results remain controversial (Vila-Chã et al., 2010). Our study is the first to investigate the adaptations in motor unit firing rate properties after a training protocol based on ECC or CON contractions in individuals with NIAT. Our results showed that the average recruitment threshold of the triceps surae muscles did not change with time at any force level, with no differences between the ECC and CON groups. These results align with a previous study that has shown no changes in the recruitment threshold of the vastus medialis and vastus lateralis muscles after 2 weeks of endurance or high-intensity interval training in a broad range of forces (10, 30, 50 and 70% MVC) (Martinez-Valdes et al., 2017a). Nevertheless, a recent study has observed a decreased recruitment threshold in the tibialis anterior muscle after 4 weeks of resistance training (Del Vecchio et al., 2019). These contrasting results may be explained by the training protocol applied, the duration of the intervention or the muscle assessed. Conversely, derecruitment thresholds increased with time at 40 and 70% MVC in both intervention groups. This result shows that after both exercise interventions, the force trajectory during the ramp-down part of the contraction was achieved by derecruiting motor units early in the contraction, suggesting an adaptation in low-threshold motor units after the exercise intervention to sustain the decrease in the force (Del Vecchio et al., 2019). Regarding discharge rate parameters, we observed an increase in the average discharge rate of the triceps surae muscles with time at 40% MVC, with no differences between groups. Specifically, our results indicate that the average discharge rate increased at 40% MVC after 3 weeks of ECC or CON training. Interestingly, Martinez-Valdes et al. also found an increase in the discharge rate of the vastus medialis and vastus lateralis muscles after 2 weeks of high-intensity interval training (Martinez-Valdes et al., 2017a). Similarly, Del Vecchio et al. observed an increase in the discharge rate of the tibialis anterior muscle after 4 weeks of strength training (Del Vecchio et al., 2019). Nevertheless, Del Vecchio et al. reported an increase in the discharge rate at different force levels (35, 50 and 70% MVC) (Del Vecchio et al., 2019),

which we did not observe. This difference may be explained by the exercise intervention applied since Del Vecchio et al. 2019 used maximal ballistic contractions and sustained isometric contractions at 75% of the maximal voluntary force. It can be speculated that the increase in the discharge rate at 40% MVC observed in our study may be related to the characteristics of the exercise intervention applied since the individuals were trained with a similar load (50% MVC); however, comparing loads between isometric and dynamic contractions could be misleading. Together, these results support the idea that strength training induces early motor unit discharge rate adaptations. Concerning COVisi parameters, our results showed no change in the averaged COVisi of the triceps surae muscles with time at any force level, with no differences between ECC and CON groups. These findings contrast the results observed by Vila-Chã et al. 2016 which reported decreased COVisi of the vastus medialis obliquus and vastus lateralis after 6 weeks of strength training. This finding can be explained by the lack of change in torque steadiness after both interventions since changes in COVisi are linked to changes in torque variability, which does not seem to be impaired in individuals with NIAT (Fernandes et al., 2023) (See Chapter 4).

Recently, it has been observed that the CST can be used to estimate the effective neural drive to synergistic muscles (Mazzo et al., 2022). Specifically, it has been determine that the neural drive to the triceps surae muscles is moderately correlated with fluctuations in the isometric plantarflexion torque (Mazzo et al., 2022). Our results showed that the cross-correlation coefficient between CST and torque in the MG did not change with time at any force level, with no differences between the ECC and CON groups. However, the cross-correlation coefficient decreased in the LG and SO at 40 and 70% MVC with time, with no differences between groups. These results suggest that ECC and CON interventions decrease the contribution of the LG and SO muscles to the net plantarflexion torque at moderate and high forces in individuals with NIAT. Although alterations in force distribution among the MG, LG, and SO muscles has been indicated as a potential contributor to AT (Bojsen-Møller and Magnusson, 2015, Hug and Tucker, 2017, O'Neill et al., 2019), a few studies have experimentally addressed this question (Crouzier et al., 2020). Crouzier et al. 2020 observed that the contribution of the LG to the

total plantarflexion torque is lower in individuals with NIAT compared to controls at 20 and 40% MVC (Crouzier et al., 2020). Nevertheless, we observed in our previous study (Chapter 4) an increase in the contribution of the LG to the net plantarflexion torque at 70% MVC in individuals with NIAT. These load-dependent changes may partially explain our results since both training interventions decrease the contribution of the LG to the net plantarflexion torque. However, we did not expect a decrease in the contribution of the SO to the net plantarflexion torque. It can be hypothesised that the decrease in the contribution of the LG and SO muscles to the net plantarflexion torque with the interventions may be linked to the induced changes in the Achilles tendon's material properties observed in our study, where increased Young's modulus of the tendon allows an improved transmission of muscle forces, requiring less neural input to exert the contractions. Nevertheless, a concurrent assessment of the Young's modulus of each subtendon and motor unit firing rate properties during isometric contractions at different forces is necessary to elucidate this problem.

5.5.5 Maximal voluntary torque and torque steadiness

Muscle strength is typically measured as the peak force achieved during an MVC. Increased maximal voluntary torque is attributable to adaptations in the muscle fibers' ability to produce force and the activation characteristics of the involved motor units (Duchateau et al., 2006). Our results indicate that ECC and CON groups increased their maximal voluntary torque after 3 weeks and 6 weeks of training, without differences between groups. This result agrees with a previous study that observed increased peak plantarflexion torque after an 8-week ECC or CON strengthening training in individuals with AT (Yu et al., 2013). Since changes in muscle architecture with ECC or CON exercise interventions have been observed with longer periods of training (> 8 weeks) (Schoenfeld et al., 2017), our results suggest that the increase in the maximal voluntary torque may be influenced by a neuromechanical component. The increased Young's modulus observed after both interventions may have enhanced the force transmission in the muscle-tendon unit and, therefore, increased the motor unit twitch forces despite not observing changes in the discharge rate at high forces. Nevertheless, changes in

supraspinal excitability, spinal pathways, or motoneurons membrane properties may also contributed to the increased maximal voluntary torque (Duchateau et al., 2006). Regarding torque steadiness, our findings show that the COV torque did not change in the ECC and CON groups at any torque level across time. This result agrees with previous studies showing that torque steadiness is not usually affected in individuals with NIAT (Chapter 4) (Fernandes et al., 2023). Since variations in motor output observed during steady contractions are significantly affected by the variability of the discharge rate (Laidlaw et al., 2000, Moritz et al., 2005, Taylor et al., 2003), and we did not find changes in COVisi at any torque level with both training interventions, our results suggest that the load-dependent adaptations in motor unit properties observed in the ECC and CON groups after training were not reflected in this motor performance task.

5.5.6 Motor unit firing rate properties between the Control, ECC, and CON groups.

Motor unit firing rate properties have been recently investigated in individuals with NIAT (Fernandes et al., 2023); however, no studies have investigated whether ECC or CON training interventions on individuals with NIAT would induce changes in motor unit function that could resemble those observed in asymptomatic individuals. Our findings revealed no differences in the recruitment and derecruitment thresholds between the control, ECC, and CON groups at each torque level. Interestingly, in our previous study (Chapter 4), we observed a decreased derecruitment threshold in the LG at high forces in individuals with NIAT; therefore, it can be speculated that both training interventions may have increased the derecruitment threshold of the LG muscle at high forces. Similarly, no differences were observed in the discharge rate, COVisi and cross-correlation coefficient between the control, ECC, and CON groups at each torque level when comparisons were performed for the same muscle. Regarding the discharge rate, in our previous study (Chapter 4), we reported an increase in the discharge rate of the LG at high forces in individuals with NIAT. Thus, it can be theorised that both interventions may have decreased the discharge rate of the LG at high forces. Concerning COVisi, in our previous study (Chapter 4), we did not observe differences in COVisi at any force level in individuals with NIAT compared to asymptomatic individuals. Hence, it seems

plausible that both training interventions did not change this parameter. Additionally, in our previous study (Chapter 4), we observed a decrease in the cross-correlation coefficient of the LG at 10% MVC and an increase in the cross-correlation coefficient of the LG at 70% MVC in individuals with NIAT; consequently, it seems that both training interventions were able to normalise the contribution of the LG to the net plantarflexion torque at different force levels. Collectively, these findings demonstrate that ECC and CON training interventions were effective in inducing changes in motor unit firing rate properties of the triceps surae muscle in individuals with NIAT, suggesting that both training modalities could successfully restore altered neuromuscular parameters to normal levels observed in asymptomatic individuals. Finally, it is important to mention that in our previous study (Chapter 4) the analysis of motor unit properties was performed considering individual motor unit firing rate values at each level of force, which is different to the analysis performed in the current study; therefore, comparisons between both studies should be done cautiously.

5.5.7 Limitations and future developments

There are some methodological aspects of this study which should be considered. First, participants in both the ECC and CON groups showed relatively modest baseline increases in tendon thickness and CSA, along with low to moderate pain levels. Therefore, caution is warranted when extrapolating these findings to populations with more pronounced morphological changes or severe symptoms. Second, it was not possible to blind the participants to the exercise intervention. Unfortunately, due to the features of the exercise intervention, participants' blinding was not possible since, during the training sessions, it was required to explain the type of contraction the participants needed to perform. Third, tendon Young's modulus measurements were done only during rest conditions. Concurrent assessment of tendon Young's modulus and triceps surae motor unit firing rate properties may provide a more comprehensive understanding of the relationship between neural coding and muscle-tendon unit. Fourth, triceps surae motor units were not individually tracked across sessions. Although the approach used in this study has demonstrated high reliability (Martinez-Valdes et al., 2016), individual tracking of motor unit firing properties allows us to follow the same motor unit across sessions, providing a more accurate interpretation of the effects of exercise interventions on motor unit firing rate properties. Finally, future studies should explore whether the changes induced by exercise interventions observed during isometric contractions are also present during dynamic plantarflexion contractions in individuals with NIAT. Such investigations would enable a more direct assessment of the effect of the exercise on the same tasks employed during the training.

5.6 CONCLUSIONS

This study demonstrates that a 6-week torque feedback training intervention based on either controlled ECC or CON exercises induces load-dependent changes in triceps surae motor unit firing properties, decreases pain, improves function, and increases tendon Young's modulus in individuals with NIAT. Furthermore, our results show that after the intervention individuals in the ECC and CON groups presented similar triceps surae motor unit firing rate properties compared to controls, suggesting a neuromuscular adaptation induced by exercise in individuals with NIAT.

CHAPTER 6

Thesis Summary and Future Work

This chapter aims to summarise the most relevant findings presented in this thesis, providing fundamental insights into the intricate interaction between neuromuscular and morpho-mechanical properties of the muscle-tendon unit, elucidating some of the underlying mechanisms of NIAT, and demonstrating the neuromuscular adaptations induced by controlled exercises interventions in individuals with this condition. Additionally, this chapter presents the limitations of each of the studies presented in this thesis and suggests possible future investigations.

6.1 Neuromechanical changes in individuals with AT and the effect of exercise-induced mechanical tendon loading

As outlined in *Chapter 2,* a systematic review of the existing literature was conducted in a two-step process. Step 1 synthesises the current literature regarding triceps surae-Achilles tendon neuromechanical changes in individuals with NIAT and IAT, and Step 2 synthesises the existing literature concerning the effect of exercise-induced mechanical tendon loading on neuromechanical changes in individuals with NIAT and IAT. Step 1 findings suggest that individuals with NIAT and IAT showed neuromechanical changes evidenced by the differences in the morpho-mechanical parameters of the Achilles tendon and triceps surae muscle's neuromuscular properties. However, Step 2

showed inconclusive results regarding the effect of exercise in changing NIAT-induced alterations in the morpho-mechanical properties of the Achilles tendon.

Regarding the morphological properties of the Achilles tendon, included studies showed increased thickness and anteroposterior diameter in individuals with AT. However, tendon thickness at the tendon insertion was greater only in individuals with IAT, and CSA increased only in individuals with NIAT. Together, these findings support the idea that NIAT and IAT are conditions with different morphological features (Maffulli et al., 2020). Additionally, included studies suggest that tendon length does not change in individuals with NIAT or IAT. Furthermore, the included studies showed an increased tendon width and no change in tendon volume in individuals with NIAT. Concerning the mechanical properties, most of the included studies showed decreased stiffness, Young's modulus, and shear-wave velocity in individuals with NIAT. Moreover, most included studies showed increased strain and elongation in individuals with NIAT. Overall, there is insufficient evidence about the mechanical properties of the Achilles tendon in individuals with IAT.

Concerning the neuromuscular properties, included studies suggest that changes in the triceps surae muscle in individuals with NIAT are task-dependent. Additionally, included studies suggest that individuals with NIAT present longer onset and shorter activation offset of the SO muscle. Similarly, electromechanical delay was longer in individuals with NIAT. Finally, regarding motor-evoked reflexes, included studies suggest increased α-motoneuron excitability and up-regulated descending neural drive to the triceps surae muscles in individuals with NIAT (Chang and Kulig, 2015).

The effect of exercise-induced tendon loading adaptations on morpho-mechanical properties of Achilles tendon in individuals with NIAT showed inconclusive results regarding tendon thickness, CSA, and width. However, included studies suggest that tendon volume decreases after 3 months of ECC training in individuals with NIAT. Unfortunately, none of the included studies assessed the effect of exercise-induced mechanical tendon loading in the morpho-mechanical properties of the Achilles tendon in

individuals with IAT, and none of the included studies investigated the effect of exercise-induced mechanical tendon loading in the neuromuscular properties of the triceps surae muscle in individuals with NIAT or IAT.

6.2 Relationships between Achilles tendon morpho-mechanical parameters and triceps surae motor unit firing properties

The aim of *Chapter 3* was to determine the relationship between triceps surae motor unit firing properties and the morpho-mechanical features of the Achilles tendon in asymptomatic individuals. Our results revealed that differences in Achilles tendon stiffness could be predicted by changes in the discharge rate of the triceps surae muscle at low forces (10% MVC). Since the mechanical properties of the Achilles tendon are linked to the capacity of the triceps surae muscle to produce force (Maganaris et al., 2008), it can be theorised that individuals with decreased stiffness at low forces might have required a higher motor unit discharge rate to control the contraction. Conversely, at higher force levels, increased tendon stiffness may facilitate a more efficient transformation of the neural input into muscle contraction and then the force transmission to the tendon. Additionally, we found that changes in tendon length could be predicted by changes in COVisi SO at 10% MVC; however, changes in tendon length could also be predicted by changes in COVisi MG and COVisi SO at 40% MVC. It can be speculated that these differences may be explained by the contribution of each of the triceps surae muscles to the net plantarflexion torque at different target torque levels. Nevertheless, we also found that changes in the Achilles tendon thickness could be predicted by changes in the COVisi SO at 40% MVC, suggesting that the relationship between COVisi and morphological properties of the Achilles tendon is not just governed by the level of activation of each of the triceps surae muscles.

6.3 Induced changes in triceps surae motor unit firing rate properties in individuals with NIAT

The aim of *Chapter 4* was to investigate triceps surae motor unit firing rate properties in individuals with NIAT compared to asymptomatic individuals at low, moderate, and high force levels during isometric plantarflexion contractions. Our findings indicate an increased discharge rate and decreased derecruitment threshold in the LG in individuals with NIAT at high forces. Given that induced pain does not lead to notable alterations in EMG activity during high submaximal voluntary contractions (Graven-Nielsen et al., 1997), it seems that central adjustments to high-threshold motor units are essential for achieving high-force outputs in the presence of pain (Martinez-Valdes et al., 2020). Supporting this idea, Martinez-Valdes et al. 2020 found increased DR and decreased recruitment and derecruitment threshold only in the high-threshold motor units at 70% MVC in an experimental pain model (Martinez-Valdes et al., 2020). Based on these observations, our findings suggest an adaptation of the LG's high-threshold motor units during isometric plantarflexion contractions at high force levels in individuals with NIAT. Additionally, our results indicate that the cross-correlation coefficient between CST and torque decreased in the LG at low forces and increased at high forces in individuals with NIAT. These findings indicate a decrease in the contribution of the LG at low forces to the net plantarflexion torque and an increase in the contribution of the LG at high forces to the net plantarflexion torque in individuals with NIAT. Given that the subtendons of the Achilles tendon vary in length and orientation, and the LG's subtendon is the longest, it is plausible to suggest that the reduction in stiffness observed in individuals with NIAT might influence force transmission through the LG muscle-tendon unit. This could account for the force-dependent contribution of the LG to the overall plantarflexion torque in individuals with NIAT.

6.4 Effect of eccentric and concentric exercise training in triceps surae motor unit firing rate properties, pain and function, and the morpho-mechanical parameters of the Achilles tendon in individuals with NIAT

The main aim of *Chapter 5* was to investigate the changes in triceps surae motor unit firing properties, pain and function, and the morpho-mechanical parameters of the Achilles tendon after applying a 6-week intervention based on controlled ECC or CON exercises in individuals with NIAT. Moreover, we aimed to examine whether exercise-induced changes in triceps surae motor unit firing properties differed from asymptomatic controls. Our results showed that derecruitment thresholds increased with time at 40 and 70% MVC in the ECC and CON groups, indicating that the ramp-down part of the contraction was achieved by derecruiting motor units early in the contraction. These findings may suggest an adaptation in low-threshold motor units after both exercise interventions to sustain the decrease in force (Del Vecchio et al., 2019). Additionally, our results showed an increase in the average discharge rate of the triceps surae muscles at 40% MVC after 3 weeks of ECC or CON exercises in individuals with NIAT. It can be theorised that the increase in the discharge rate at 40% MVC may be linked to the features of exercise intervention since ECC and CON groups trained with a similar load (50% MVC). Our findings provide supporting evidence for the idea that strength training induces early motor unit firing rate adaptations.

Regarding the effect of exercise on the cross-correlation coefficient between CST and torque, our results revealed that the cross-correlation coefficient CST-torque decreased in the LG and SO muscles at 40% and 70% MVC in the ECC and CON groups, indicating that ECC and CON exercise interventions decrease the contribution of the LG and SO muscles to the net plantarflexion torque at moderate and high forces in individuals with NIAT. It can be speculated that the observed reduction in the contribution of the LG and SO muscles to the overall plantarflexion torque following both exercise interventions may be related to the increase in the stiffness observed in the ECC and CON groups

since increased tendon stiffness might enhance the efficiency of muscle force transmission, thus reducing the neural effort required to perform the contractions.

Comparisons between exercise-induced changes in triceps surae muscle motor unit firing rate properties in individuals with NIAT and asymptomatic controls showed no differences in the recruitment and derecruitment thresholds between the control, ECC, and CON groups at each torque level. Since we observed a decrease in the derecruitment threshold in the LG at high forces in individuals with NIAT (*Chapter 4*), it can be hypothesised that ECC and CON training interventions may have induced an increase in the derecruitment threshold of the LG muscle at high forces. Likewise, no differences in the discharge rate were observed between the control, ECC and CON groups at each torque level. Since in our previous study (*Chapter 4*), we observed an increase in the discharge rate of the LG at high forces in individuals with NIAT; it seems plausible that both training interventions may have decreased the discharge rate of the LG at high forces. Furthermore, no differences were observed in the cross-correlation coefficient CST-torque between the control, ECC and CON groups at each torque level. Since we observed a decrease in the cross-correlation coefficient of the LG at low force levels and an increase in the cross-correlation coefficient of the LG at high forces in individuals with NIAT (*Chapter 4*), it is possible that ECC and CON training interventions were able to normalise the contribution of the LG to the net plantarflexion torque. Together, these results suggest that ECC and CON exercise interventions could successfully restore altered motor unit firing rate properties to normal levels observed in asymptomatic individuals.

About the effect of the exercise interventions in pain and function, our results reveal that VISA-A scores increased after 3 weeks of ECC or CON training, and this increase was maintained after 6 weeks of training. Additionally, our results show that the increase in VISA-A scores was still present after 6 months of completing the intervention in the ECC and CON groups. Similarly, our results indicate that NRS scores decreased after 3 weeks of ECC or CON training, and this decrease was maintained after 6 weeks of training. Furthermore, our findings demonstrate that the decrease in the NRS scores

was still present after 6 months of completing the intervention in the ECC and CON groups. Regarding the physical function of the lower limb, our results show that both training interventions increased the FAAM Subscale Sports scores. However, FAAM Subscale Activities of Daily Living score increased differently between ECC and CON groups. Together, these results suggest that both exercise interventions were effective in reducing pain and increasing function in individuals with NIAT.

Regarding the effect of exercise interventions on the morpho-mechanical properties of the Achilles tendon, our findings indicate no changes in thickness and CSA at 2, 4, and 6 cm from the tendon insertion in the ECC and CON groups. These findings align with current evidence suggesting that tendon morphological adaptations require longer periods of training (>12 weeks) (Bohm et al., 2015). Finally, concerning the mechanical properties, our results demonstrate an increase in tendon stiffness in both the ECC and CON groups after training; however, the increase was higher in the ECC group. This difference between groups may be explained by the greater force production during lengthening compared to shortening contractions, as lengthening contractions engage the passive elastic components of the muscle-tendon unit more (Franchi et al., 2014). These results support the idea that exercise induced changes in tendon stiffness are more related to adaptations in the material properties rather than the morphological parameters (Bohm et al., 2015).

6.5 Limitation of the studies

The findings of this thesis provide valuable insights into the neuromuscular properties of the triceps surae muscle, the morpho-mechanical parameters of the Achilles tendon and the effect of controlled exercises on these properties in individuals with NIAT. However, it is important to acknowledge several limitations that may influence the interpretation and generalisability of the outcomes. These limitations include methodological challenges, potential bias emerging from the study design, and specific characteristics of the participants.

As outlined in *Chapter Two,* the included studies in Steps 1 and 2 presented significant heterogeneity. Specifically, the included studies in Step 1 presented significant heterogeneity regarding the region of the tendon assessed, the control group (asymptomatic participants or the contralateral asymptomatic leg), and the type of task performed. The studies included in Step 2 showed significant heterogeneity regarding the type of exercise intervention applied, the region of the tendon assessed and the time points of the measurements. Thus, meta-analysis was inappropriate, and narrative synthesis was developed for both Steps. Additionally, the overall quality of evidence of the studies included in Step 1 was very low, mostly due to the inclusion of observational studies with small sample size. However, the overall quality of evidence of the studies included in Step 2 was low due to the inclusion of studies with severe risks of bias, differences in the outcome measures or a small sample size. Furthermore, since NIAT and IAT are more prevalent in individuals involved in recreational activities, some studies included in Step 1 assessed runners. Since mechanical loading modify the morpho-mechanical properties of the Achilles tendon and subsequently affect the neuromuscular parameters of the triceps surae muscle (Maganaris et al., 2008), caution should be taken when generalising these findings to sedentary individuals with NIAT or IAT.

Several challenges regarding the use of SWE to assess the mechanical properties of the Achilles tendon were observed. As described in *Chapter 3, Chapter 4, and Chapter 5,* measurements of Achilles tendon stiffness were performed at 0° of plantarflexion,

which may induce light tension on the tendon. Therefore, comparisons with other studies that have measured the Achilles tendon's stiffness in a resting position (Dirrichs et al., 2016) should be done cautiously. Additionally, tendon stiffness assessments were performed only at 4 cm from the tendon insertion. Due to the twisted structure of the Achilles tendon, the intersubject differences between the subtendons of the MG, LG, and SO, and the nonuniform distribution of load observed in the Achilles tendon (Handsfield et al., 2017), tendon stiffness might vary in different regions. Consequently, comparing stiffness values between distinct tendon regions may not be appropriate. Tendon stiffness evaluations were done only during rest conditions. Despite the efforts to determine tendon stiffness during low force isometric contractions (10% MVC), the movement of the tendon induced by the contraction affected the quality of the SWE color maps, producing voids on the image that ultimately led to unreliable results.

Certain limitations emerged concerning the motor unit firing rate properties analysis. As detailed in *Chapter 3*, *Chapter 4,* and *Chapter 5,* the ankle attachment of the isokinetic dynamometer used had two lever arms to assess plantarflexion forces. Thus, this ankle attachment may have influenced the NMD results, as the lever arms could increase the time required to detect the torque signal during isometric plantarflexion contractions. Additionally, as outlined in *Chapter 4* and *Chapter 5,* the motor unit firing rate properties of the MG, LG, and SO were analysed independently for each force level; however, no motor unit recruitment threshold matching was performed. Matching synergistic muscles motor units by recruitment threshold enables the direct comparison of the motor unit firing rate parameters across muscles (Martinez-Valdes et al., 2018). Nevertheless, this process is highly time-consuming, particularly when the number of motor units identified is high. Finally, as presented *in Chapter 3*, *Chapter 4*, and *Chapter 5*, the number of motor units identified in each of the triceps surae muscles varies significantly between individuals. This variations may be attributed to the challenges in decomposing EMG signals in females (Lulic-Kuryllo and Inglis, 2022), resulting in a low number of identified motor units in these participants.

Some issues about the characteristics of the participants were detected. As shown in *Chapter 4,* the control and NIAT groups were not matched by sex. Despite this, no differences between groups in age, height, and weight were observed; therefore, we believe that sex differences between groups had a minimal impact on our results. Additionally, as presented in *Chapter 5,* ECC and CON groups were not matched by sex due to the difficulties in the recruitment of participants with NIAT. However, no differences between groups were observed regarding age, height, weight, physical activity level, duration of symptoms and level of pain; thus, sex differences between groups may had minimal influence in our findings. Furthermore, as outlined in *Chapter 4* and *Chapter 5*, individuals in the NIAT group exhibited relatively low pain levels. This result may be due to most participants being physically active. Given that physical activity is related to the morphology of the Achilles tendon in individuals with NIAT (Corrigan et al., 2018), extrapolating our findings to more symptomatic populations should be done cautiously.

Few limitations regarding the design of the RCT were observed. As described in *Chapter 5,* ECC and CON groups were not blinded to the exercise intervention. During the training sessions, it was mandatory to explain the type of contraction the participants needed to perform; consequently, blinding was not possible. Additionally, the torque feedback training intervention based on ECC or CON contractions included 2-3 weekly training sessions. Unfortunately, some participants could complete only 2 training sessions per week, significantly reducing the total volume of work of the training intervention. These differences in the training frequency may have influenced our findings. Finally, the exercise intervention applied was shorter (6 weeks) compared with most of the interventional studies in individuals with NIAT (8 to 12 weeks) (Färnqvist et al., 2020). Hence, the duration of the exercise intervention might account for the unchanged morphological properties of the Achilles tendon observed in the ECC and CON groups.

6.6 Future Lines of Work

Exploring the relationship between the neuromuscular parameters and morpho-mechanical properties of the muscle tendon-unit, the underlying mechanism of tendinopathies, and the effect of controlled exercise interventions on the neuromuscular parameters are essential directions for future research. Upcoming investigations should consider assessing tendon stiffness at different regions and joint positions to have a broader representation of the tendon's mechanical behavior. Additionally, assessing tendon stiffness during isometric contractions may provide a more comprehensive understanding of the interplay between the neuromuscular and morpho-mechanical components in both asymptomatic individuals and those with tendinopathies. Moreover, further research and developments in SWE technology with higher sampling resolution might allow a concurrent assessment of the tendon's mechanical properties and motor unit firing rate parameters, providing relevant evidence regarding the underlying mechanisms involved in tendinopathies.

Subsequent research should consider designing studies that match the control and tendinopathy groups by age, sex, height, weight, and level of physical activity. This approach may reduce the influence of these factors on the outcome measures of interest. Additionally, future studies should investigate whether similar exercise interventions produce equivalent changes in motor unit firing rate parameters and morpho-mechanical properties in individuals with increased levels of pain. Since alterations in the morpho-mechanical properties of tendinopathic tendons have been linked to the severity of the symptoms (Maffulli et al., 2020), the effect of exercise interventions in individuals with higher levels of pain may differ from our findings.

Concerning the motor unit firing rate properties, recent studies have successfully identified motor units during dynamic contractions (Glaser and Holobar, 2019, Oliveira and Negro, 2021); thus, future studies should consider assessing the motor unit firing rate parameters and the effect of exercise interventions on these parameters during dynamic contractions in individuals with tendinopathies. Furthermore, since recent developments

in motor unit firing rate analysis allow the individual tracking of the same motor unit across sessions (Martinez-Valdes et al., 2017b), subsequent studies should consider investigating the effect of exercise interventions on motor unit firing rate properties with this methodology. Moreover, future research should consider using motor unit recruitment threshold matching analysis to identify if the effects of exercise interventions on motor unit firing rate properties are specific to low or high-threshold motor unit populations.

Finally, since the morphological changes in tendons due to exercise interventions may require longer periods (greater than 12 weeks) (Bohm et al., 2015), future RCTs should include prolonged exercise interventions for individuals with tendinopathies. Given the nature of the exercise intervention (torque feedback training with an isokinetic dynamometer), such studies can be challenging, as they require participants to attend the laboratory multiple times for both experimental and training sessions, heavily relying on their commitment to the study. However, these studies are crucial for understanding the effect of exercise interventions on tendinopathies, allowing us to develop more effective exercise interventions and improve patient outcomes.

6.6 Concluding Remarks

This thesis provides novel evidence supporting the relationship between the neuromuscular properties of the triceps surae muscle and the morpho-mechanical properties of the Achilles tendon in asymptomatic individuals. Our findings show a contraction-intensity dependent relationship between motor unit firing rate parameters of the triceps surae muscle and the morpho-mechanical properties of the Achilles tendon. Specifically, our results indicate that individuals with increased tendon stiffness showed a lower discharge rate during isometric plantarflexion contractions at low target forces. Additionally, this thesis supports the concept that people with NIAT exhibit altered neuromuscular parameters of the triceps surae muscle. Our outcomes reveal that individuals with NIAT show an increased discharge rate and a decreased derecruitment threshold of the LG muscle during isometric plantarflexion contractions at high forces. Furthermore, our results demonstrate that the contribution of the LG to the net plantarflexion torque is affected in individuals with NIAT, providing additional evidence supporting the theory that uneven load transmission between the triceps surae muscles may play a central role in the development of NIAT. In addition, this thesis presents evidence that a controlled exercise intervention induced changes in the neuromuscular properties of the triceps surae muscle in individuals with NIAT. Our findings demonstrate that a 6-week torque feedback training intervention based on ECC or CON contractions induced load-dependent changes in the triceps surae motor unit firing rate properties, decreased pain, improved function, and increased tendon stiffness in individuals with NIAT. Specifically, changes in the motor unit firing rate parameters included increased derecruitment thresholds at moderate and high forces and an increased discharge rate at moderate forces. Moreover, our results suggest that a training intervention involving controlled ECC, or CON contractions induced similar adaptations in triceps surae motor unit firing rate parameters, pain, and function, questioning the idea that ECC exercises are better for the management of individuals with NIAT. Finally, our outcomes show no differences in motor unit firing rate adaptations between individuals with NIAT after the training intervention and asymptomatic controls, possibly indicating a normalisation of these properties after the intervention in individuals with this condition. Collectively, this

research provides fundamental insights into the intricate interaction between neuromuscular and morpho-mechanical properties of the muscle-tendon unit, elucidates some of the underlying mechanisms of NIAT, and demonstrates the neuromuscular adaptations induced by training interventions based on controlled exercises in individuals with this condition.

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Appendix

Appendix 1. Contreras-Hernandez et al. (2022a)

Open access Protocol

BMJ Open

Neuromechanical changes in Achilles tendinopathy and the effects of exercise-induced mechanical tendon loading: a protocol for a systematic review

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ABSTRACT

Introduction Achilles tendinopathy (AT) is a debilitating overuse injury characterised by pain, impaired functional performance, morpho-mechanical changes to the Achilles tendon and triceps surae neuromuscular alterations. Loading-based exercise has become the principal non-surgical choice for the treatment of AT; however, mechanistic evidence by which loading-based treatment may help to resolve tendon pain remains unclear. This systematic review aims to summarise the evidence of the neuromechanical changes produced by AT and by exercise-induced mechanical loading.

Methods and analysis This systematic review protocol was informed and reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA-P) and the Cochrane Handbook for Systematic Reviews of Interventions. Pubmed, MEDLINE, EMBASE, CINAHL Plus, Web of Science and SPORTDiscus electronic databases will be searched from inception to February 2021. Additionally, grey literature and key journals will be reviewed. Risk of bias will be determined independently by two reviewers using the version 2 of the Cochrane risk-of-bias tool for randomised trials (RoB 2) and the risk of bias in non-randomised studies - of interventions (ROBINS-I) tool according to Cochrane recommendations. Quality of the cumulative evidence will be assessed with the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) guidelines. If homogeneity exists between groups of studies, a random-effects meta-analysis will be conducted. If not, results will be synthesised narratively.

Ethics and dissemination Our findings will be disseminated through publication in a peer-reviewed journal and presented at conferences. No ethical approval was required.

PROSPERO registration number CRD42021231933.

Strengths and limitations of this study

- This will be the first systematic review to synthesise evidence of neuromechanical changes produced by Achilles tendinopathy (AT).
- This will also be the first systematic review examining the effect of exercise-induced mechanical loading protocols on neuromechanical changes observed in individuals with AT.
- In accordance with the Grading of Recommendations, Assessment, Development, and Evaluation framework, the inclusion of observational studies might reduce the quality of evidence.

INTRODUCTION

Tendinopathy describes a spectrum of pathological changes to the tendon, leading to pain and reduced function. The essence of tendinopathy is a failed healing response, characterised by abnormalities in the microstructure, composition and cellularity of the tendon,[1] with degeneration and disorganised proliferation of tenocytes, disruption of collagen fibres and subsequent increase in non-collagenous matrix.[2]

Achilles tendinopathy (AT) is a debilitating overuse injury which causes considerable morbidity and functional impairment among the athletic and general population.[3 4] Achilles tendon injuries can be separated into non-insertional tendinopathy (55%–65% of the injuries), insertional tendinopathy (20%–25%) and proximal musculotendinous junction injuries (9%–25%), according to the location of pain.[5] Nevertheless, individuals may present symptoms at the insertion and mid-portion concurrently, and approximately 30% have bilateral pain.[6] Morphological comparisons of tendinopathic and healthy tendons have demonstrated a larger cross-sectional area (CSA) for the degenerated Achilles tendon.[7] It is believed that this increase is due to an accumulation of water and increased ground substance as a result of the pathology.[8] In addition, changes to the tendon's stiffness has been reported.[7] Typically, a thicker tendon is considered mechanically stronger due to its ability to dissipate high stresses (force/area) across the tendon and yield

lower strain energy.[7] However, degenerated Achilles tendon has lower stiffness and Young's modulus compared with healthy tendons.[7]

Human movement emerges from the interplay among descending output from the central nervous system (CNS), sensory input from the body and environment, muscle dynamics and the whole body dynamics.[9] Thus, neuromechanics is the study of the coupling between neural information processing and mechanical behaviour.[9] Fundamentally, the CNS plans, initiates and sends motor commands to muscle,[10] then the muscle executes the motor command producing force to pull the tendon, and finally, the tendon transmits and modulates muscle force controlling the movement.[11] Additionally, peripheral components of this hierarchical system (ie, muscle spindles and Golgi tendon organs) send feedback signals to assist the CNS in motor commands planning.[12]

Tendons require the ability to withstand, store and then deliver substantial force to perform day-to-day activities.[13] During sports-related activities, where the repetition and speed of the loading are drastically increased, the mechanical force placed on the tendon becomes substantially amplified.[14] Therefore, a decrease in tendon stiffness will cause the muscle fascicles to shorten more to account for the increased compliance of the tendon.[7] This may limit the muscle's ability to function within the force–length curve's optimal region, thereby affecting movement economy.[7]

In addition to the morpho-mechanical changes induced by AT, several neuromechanical adaptations have been reported in individuals with AT.[11] Neuromechanical adaptations include how the mechanical system may offload a task that the neural system needs to accomplish[9] and it is possible only through a tight connection between sensory and motor systems. One of these neuromechanical adaptations is the electromechanical delay (EMD), which is defined as the time lag between muscle activation and the mechanical force produced, which dictates the muscle-tendon unit's temporal efficiency.[11] Longer EMD has recently been reported for the affected side compared with the non-affected side in individuals with Achilles tendinosis, indicating a compromised triceps surae musculotendinous unit temporal efficiency (ability to transmit force from the muscle to the tendon as quickly as possible).[11]

Other neuromechanical adaptations are the evoked spinal reflexes assessed by Ia-afferent-mediated H-reflex and the net excitation of the neuron pool determined by the first volitional wave (V-wave).[15] Higher V-wave but not H-reflex values of the soleus muscle have been observed in the affected legs of athletes with chronic middle-portion AT.[16] This greater V-wave may result from an enhanced neural drive in the descending corticospinal pathways, elevated net excitability of both large and small motor neurons, and/or alterations in the presynaptic inhibition during voluntary activation of the soleus muscle.[17] However, a recent study using a similar approach has shown higher H-reflex and V-wave values in individuals with mid-portion AT compared with controls,[11] showing that there is some discrepancy across studies.

Once symptoms develop, ensuing movement dysfunction may contribute to the chronicity of symptoms.[18] Pain in the Achilles tendon causes widespread motor inhibition in the affected region, evidenced by the lower electrical activity of the agonist, synergist and antagonist muscles.[19] Individuals with tendinopathy also tend to use movement patterns that place excessive or abnormal load on their tendons; the faulty movement may represent either a root cause or a reason for chronicity or slow resolution.[18] This may be attributed to a protective mechanism that prevents further injury or even tendon rupture.[20]

Research on the treatment of AT is somewhat scarce despite the prevalence.[21] Over the past decade, loading-based treatment in the form of eccentric training (exercises where tendon lengthening during active contractions is emphasised) has become the main non-surgical choice of treatment for AT,[22] although there is no convincing evidence showing that this form of exercise is the most effective for AT. A recent systematic review concluded that there is little clinical or mechanistic evidence supporting the use of isolated eccentric exercises alone.[23] Well-conducted studies comparing different loading programmes are largely lacking.[23] The purpose of exercise is to provide mechanical loading to the tendon in order to promote remodelling, decrease pain and improve calf-muscle endurance and strength.[6 24] It seems that loading itself yields positive clinical, structural and biochemical effects with respect to tendinopathy.[6 25–28] However, the successful management of AT remains challenging, possibly due to a lack of knowledge about the effect of loading parameters that is, load progression, load magnitude, frequency (sets and repetitions) and restitution between treatment sessions.[21]

There are several systematic reviews about the effects of exercise in individuals with AT. However, most are focused on pain and function and usually only use self-reported outcome measures as a main outcome. Self-reported outcomes have high variability among the population, thus, the conclusion of these studies may be partially biased. Although two recent systematic reviews explored the effects of exercise on the morphological properties of the Achilles tendon in individuals with mid-portion AT,[29 30] to our knowledge, there are currently no systematic reviews examining the neuromechanical changes that occur in individuals with AT or the effects of exercise on these properties. Therefore, the aim of this systematic review is to synthesise the current literature regarding (1) triceps surae–Achilles tendon complex neuromechanics in individuals with AT and (2) the effect of exercise-induced mechanical tendon loading on neuromechanical changes induced by AT.

METHODS

This systematic review protocol has been developed following the Cochrane Handbook for Systematic

Reviews of Interventions and the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) 2015 checklist (online supplemental file 1).[31–33] This protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 26 January 2021 (registration number: CRD42021231933). The Cochrane Handbook mainly focuses on the synthesis of evidence from intervention studies which is related only to the second objective of this systematic review; unfortunately, the first objective is commonly addressed by observational studies. Therefore, we followed these guidelines in addition to the COSMOS-E guidance for the following reasons: First, there are many similarities in the general structure and procedures used in both types of reviews. Second, the information in the Cochrane Handbook is described in more detail and includes important information related to observational studies. Third, a similar approach has been made in other systematic review protocols.[34 35] Fourth, widely accepted standards of systematic reviews meta-analysis of observational studies are lacking.[36] We know that some methodological differences exist, but we will pay particular attention to certain steps of the conducted process (eg, choice of statistical methods, sources of heterogeneity, etc).[36]

Eligibility criteria

The PICOS framework (Population, Intervention, Comparison, Outcomes, and Study Design) has been used to define the eligibility criteria for the inclusion and exclusion of studies in this systematic review.[32 37] However, due to the characteristics of this research, we will use 'Indicator' in the same category of 'Intervention' as it has been used previously.[34 35]

Population

The population of interest is adults (aged 18–65 years) with mid-portion or insertional AT and pain-free adults as a control group. However, we will also include studies that have used the asymptomatic lower limb as a control. In order to avoid excluding relevant articles identified during the scoping search, participants with bilateral AT will also be included. There will be no restrictions in terms of gender or ethnicity. Studies that include individuals with AT who have been diagnosed with an underlying medical pathology or disorder (eg, systemic inflammatory conditions, cardiovascular diseases, neurological disorders) and/or history of Achilles tendon surgery will be excluded.

Intervention/indicator

In order to address the first aim of this systematic review, eligible studies will be those which include the use of any electrophysiological technique (eg, surface electromyography, intramuscular electromyography, high-density surface electromyography, transcranial magnetic stimulation, cervicomedullary magnetic stimulation or peripheral nerve stimulation) to determine neuromuscular, spinal, subcortical or supraspinal changes in people with AT. Additionally, eligible studies will be those which include the use of ultrasonographic or MRI to measure the morphological or mechanical properties of the Achilles tendon in people with AT. Similarly, eligible studies to address the second aim of this systematic review will be those which include the use of any electrophysiological technique to determine neuromuscular, spinal, subcortical or supraspinal changes produced by any exercise-induced mechanical tendon loading protocols (eg, eccentric, isometric or concentric contractions, plyometric exercises, stretching or rehabilitation protocols) in people with AT. Moreover, eligible studies will be those which include the use of ultrasonographic or MRI to measure changes in the morphological or mechanical properties of the Achilles tendon produced by any exercise-induced mechanical loading in individuals with AT.

Comparison

Studies must include a comparison of the neuromuscular properties of the triceps surae muscle or morpho-mechanical properties of the Achilles tendon between individuals with AT and controls or between symptomatic and asymptomatic lower limbs. Likewise, studies assessing the effects of exercise-induced mechanical tendon loading should include a comparison of the neuromuscular features of the triceps surae muscle or morpho-mechanical properties of the Achilles tendon in individuals with AT and controls or between symptomatic and asymptomatic lower limbs.

Outcomes

Primary outcomes will include neuromuscular properties of the triceps surae muscle and morpho-mechanical features of the Achilles tendon. We will include studies assessing the amplitude and timing of EMG activity of the gastrocnemius-soleus (millivolts and milliseconds, respectively), tibialis anterior and gastrocnemius-soleus coactivation (co-contraction ratio, %), gastrocnemius-soleus motor-evoked potentials (MEPs, microvolts) obtained from transcranial and cervicomedullary magnetic stimulation (TMS and CMEPs), H-reflex (peak-to-peak amplitude, microvolts), F-wave or V-wave obtained by peripheral nerve stimulation (tibial nerve) and motor unit data (motor unit discharge rate, Hz) obtained from intramuscular and/or high-density surface EMG recordings (millivolts). The present systematic review will also include morpho-mechanical properties such as length (cm), thickness (mm), CSA (mm^2), volume (cm^3), stiffness (N/mm or kPa), modulus (kPa), creep, elasticity (kPa), strain (%) and stress (kPa) of the Achilles tendon. Only studies that measure any of these neuromechanical properties quantitatively will be included. Secondary outcomes will include duration of symptoms (months), the severity of symptoms (Victorian Institute of Sports Assessment-Achilles questionnaire (VISA-A) or Visual Analogue Scale (VAS)), type of tendinopathy (mid-portion, insertional

or both), diagnostic confirmation (clinical assessment and/or ultrasound evaluation) and physical activity level (International Physical Activity Questionnaire (IPAQ), hours of physical activity per week, etc).

Study design

Based on scoping searches, randomised controlled trials and non-randomised controlled trials (ie, cohort, cross-sectional and cohort studies) will be considered to address both objectives of this systematic review adequately. Non-original literature (eg, systematic and narrative reviews) or other types of studies will be excluded and reported in the PRISMA flow diagram.

Information sources

The following electronic databases will be used from inception to February 2021: Pubmed, MEDLINE (Ovid Interface), EMBASE (Ovid Interface), CINAHL Plus (EBSCO Interface), Web of Science (WOS; Clarivate Analytics) and SPORTDiscus (EBSCO Interface). Specific research strategies have been designed considering medical subject heading (MESH) terms to improve search results. Moreover, hand searching of key journals will be conducted, including *Journal of Applied Physiology*, *Journal of Orthopaedic & Sports Physical Therapy*, *Journal of Electrophysiology and Kinesiology*, *Journal of Biomechanics*, *Clinical Biomechanics*, *British Journal of Sports Medicine*, *Medicine and Science in Sports and Exercise*, *Journal of Science and Medicine in Sports*, and *Isokinetic and Exercise Science*. The eligibility of the manuscripts found in hand searching will be defined using the PICOS framework. Additionally, relevant authors in the field will be contacted to identify unpublished articles in preparation. To minimise risk of bias publication, grey literature will be also included, and searches will be conducted using the British national bibliography for report literature (BNBRL), ProQuest Dissertations & These Global, OpenGrey database and EThOs. Reference lists of included studies and relevant systematic reviews will be checked for any further studies, accordingly with the MECIR standards.[38]

Search strategy

Two independent reviewers (IC-H and AS) will complete the search and identify potential studies to be included in this systematic review. There will be no restrictions in terms of date, design or language, to ensure inclusion of all relevant articles.

Due to the inability to obtain maximal retrieval of articles during the scoping search and to adequately address both objectives of this systematic review, this search will be conducted in a two-step process:

1. Initial search to identify studies with neuromuscular properties of the triceps surae or morpho-mechanical features of the Achilles tendon in individuals with AT.
2. Secondary search identifying studies assessing the effects of exercise-induced tendon mechanical loading on neuromuscular properties of the triceps surae or the morpho-mechanical characteristics of the Achilles tendon in individuals with AT.

A search strategy example for MEDLINE (Ovid Interface) database of each step is reported in online supplemental file 2 and includes MESH terms, keywords and search strings to ensure maximal retrieval.[39] The specific search terms will be modified to reflect differences in keywords and syntax between databases but search strategy consistency will be guaranteed.

Data management

Literature search results, including citation and abstract of potentially eligible studies, will be imported into EndNote V.X9 (Clarivate Analytics PCL) reference manage software by one reviewer (IC-H), allowing the identification and removal of any duplicates before the screening process. Abstracts and full texts of potentially eligible studies will be saved in an individual folder for each reviewer (IC-H, AS) and eligible studies will be retrieved and stored in EndNote V.X9. To effectively accomplish the screening process, forms that have been developed to reflect the inclusion and exclusion criteria will be used.

Study selection

Before the screening process, screening forms will be tested by two reviewers (IC-H, AS) in a small number of articles to ensure their effectiveness. The screening process will then start with the assessment of titles and abstracts of identified studies by two reviewers (IC-H, AS), and they will subcategorise them into definitely eligible, definitely ineligible or doubtful.[40] In the event of disagreement, reviewers will first attempt to resolve through discussion; however, if consensus cannot be reached, a third reviewer (EM-V) will mediate the process. Then, the reviewers will perform full-text screening of potentially eligible studies independently. Similarly, if no consensus is possible, a third reviewer will support the process. The agreement between the reviewers during both screening stages will be determined using the kappa statistic, and the PRISMA flow diagram will be used to summarise the study selection process.[32]

Data collection process

The data collection process will begin developing a standardised form based on the Cochrane data extraction template, objectives of the systematic review and inclusion criteria as a guide. A standardised form will be piloted a priori on a subgroup of studies. IC-H will extract data, and AS will check the accuracy of this process. Any discrepancies will be discussed between the two reviewers; however, the third reviewer (EM-V) will determine which data are relevant if no agreement is achieved. Authors of the primary studies will be contacted if any critical information that needs to be extracted is missing. If multiple publications of the same study exist, they will be collated, the primary authors contacted for further clarification and the duplicates removed. Likewise, if potentially eligible studies appear

Table 1 Characteristics of included studies

Date of data extraction	
Information about data	**Data extracted**
General study information	Title Authors Year of publication
Study methodology	Study design Sample size Individuals' characteristics (age, gender, weight, height, physical activity level, etc) Diagnostic confirmation (clinical evaluation, ultrasound/MRI assessment, use of questionnaires, etc) Achilles tendinopathy group characteristics (location, side, pain intensity, duration of symptoms, etc) Type of instrument used to measure the neuromuscular properties Type of instrument used to determine the morpho-mechanical properties (ultrasonography or MRI) Type of exercise-induced mechanical tendon loading protocol applied
Outcome	The neuromuscular properties include the amplitude and timing of EMG activity of the gastrocnemius-soleus, tibialis anterior and gastrocnemius-soleus coactivation, gastrocnemius-soleus MEPs, H-reflex, F-wave and motor unit data. The morpho-mechanical properties of the Achilles tendon include length, thickness, cross-sectional area, volume, stiffness, modulus, creep, elasticity, strain and stress. The comparison could be within groups (eg, affected vs non-affected side) or between groups (eg, Achilles tendinopathy group vs control group).
Funding, declaration of conflict of interest	Funding information Conflict of interest of authors

MEPs, motor-evoked potentials.

to use the same data during the data collection process, the primary authors will be contacted, and a specific report will be selected. The decision of the selected report will be justified.

Data items

Data items to be extracted include general study information, participants' characteristics, measurement methods and outcome measures. These items are presented in table 1. We will use the same extraction sheet for each step of the systematic review; the only difference will be the item regarding 'Type of exercise-induced mechanical tendon loading protocol applied'. If any eligible studies include more than two groups, data will be extracted only from the control group and the one that meets the eligibility criteria.

Risk of bias

The risk of bias will be determined independently by two reviewers (IC-H and AS) using the RoB 2 and ROBINS-I tools according with Cochrane recommendations.[31] The RoB 2 is a tool to determine the risk of bias in randomised trials and includes the following domains: bias arising from the randomisation process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, bias in selection of the reported result and overall bias.[41] The domains were selected to address all important mechanisms by which bias can be introduced into the results of a trial, based on a combination of empirical evidence and theoretical considerations.[41] Each domain is required, and no additional domains should be added. For each domain, the tool comprises a series of 'signalling questions', a judgement about risk of bias for the domain, free text boxes to justify responses to the signalling questions and risk-of-bias judgement, and an option to predict (and explain) the likely direction of bias.[31] Signalling questions aim to elicit information relevant to an assessment of risk of bias.[41] The questions seek to be reasonably factual in nature.[41] The response options are 'yes,' 'probably yes,' 'probably no,' 'no' and 'no information'.[41] Based on these responses, the options for a domain-level risk-of-bias judgement are 'Low', 'Some concerns' and 'High' risk of bias.[41]

The ROBINS-I will be used to determine the risk of bias in non-randomised studies of interventions and include the following domains: bias due to confounding, bias in selection of participants into the study, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes and bias in the selection of the reported result.[42] Each domain is mandatory, and no additional domain should be added. The tool comprises, for each domain, a series of 'signalling questions', a judgement about risk of bias for the domain, free text boxes to justify responses to the signalling questions and risk-of-bias judgements and an option to predict (and explain) the likely direction of bias.[31] The signalling questions aim to elicit information relevant to the risk-of-bias judgement for the domain, and work in the same way as for RoB 2.[31] The response options are 'yes,' 'probably yes,' 'probably no,' 'no' and 'no information'.[42] Based on these responses, the options for a domain-level risk-of-bias judgement are 'Low', 'Moderate', 'Serious' or 'Critical' risk of bias, with an additional option of 'no information'.[42]

Disagreements between the reviewers regarding the risk of bias in a study will be resolved by discussion, with the involvement of a third review author (EM-V) if necessary.

Data synthesis

A meta-analysis will be considered if outcomes and methodology of the selected studies are homogeneous. If possible, the two reviewers (IC-H, AS) will independently

group studies that are more homogeneous using the following characteristics:

- Parameter used to determine neuromuscular properties of the triceps surae.
- Parameter used to measure morpho-mechanical properties of the Achilles tendon.
- Type of exercised-induced mechanical tendon loading protocol applied.

Disagreement between the reviewers will be resolved by discussion, but if no agreement is possible, a third reviewer (EM-V) will mediate the process.

Whether clinical or methodological homogeneity across studies investigating the same outcome domain is sufficient, statistical heterogeneity will be performed. The amount of inconsistency among studies will be assessed using the I^2 statistic.[43] As in previous reviews, the grouping of studies will be eligible for meta-analysis if an I^2 value of <50% (low heterogeneity) is determined.[44] Then, a random-effects meta-analysis will be performed for each subgroup following the recommendations of Deeks *et al*.[45] Extracted data will be converted into a common rubric, which most likely be ORs with 95% CIs since we are dealing mostly with binary data.[46] If the data are not sufficiently homogeneous, the results of the considered outcomes will be described using the vote-counting procedure (direction of difference or no difference) and a narrative synthesis will be developed.[47] The narrative synthesis will be conducted following the recommendations of Popay and Snowden.[46]

Confidence in cumulative evidence

Data pooled quality (certainty) will be assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.[48 49] This process include five steps described by Goldet and Howick,[50] and the final quality of evidence will be presented as 'High', 'Moderate', 'Low' or 'Very Low'. The certainty of evidence for each outcome across studies can be decreased by risk of bias, inconsistency, indirectness, imprecision and publication bias.[51] Conversely, certainty of evidence can be increased by large effect size, dose–response gradient and plausible confounding biases that underestimate the effect size.[51] Finally, recommendations for the interpretation of the evidence quality will be given, following the criteria of Guyatt *et al*.[52]

Patient and public involvement

The topic of this systematic review protocol was not discussed at our established patient and public involvement meetings (PPI), due to COVID-19 pandemic. Patients will not be involved in the analysis and data collection of this project, but our results will be presented at PPI meetings at the University of Birmingham in the future.

Ethics and dissemination of results

Ethical approval is not required for this review, as it will only involve the collation of previously published data. Our findings will be disseminated through publication in a peer-reviewed journal and presented at national and/or international conferences.

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Contributors IC-H and EM-V are responsible for the conception of the research question and development of the protocol. IC-H wrote the first draft of the protocol with guidance from EM-V and DF. IC-H and AS will be the first and second reviewers, respectively. EM-V will be the third reviewer. All drafts were revised and reviewed by all the authors before the final approval of the last version of the manuscript.

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Appendix 2. Contreras-Hernandez et al. (2022b)

Open access Protocol

BMJ Open

Neuromuscular and structural tendon adaptations after 6 weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy: protocol for a randomised controlled trial

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ABSTRACT

Introduction There is limited evidence on the neural strategies employed by the central nervous system to control muscle force in the presence of non-insertional Achilles tendinopathy (NIAT). Additionally, the neuromuscular mechanisms by which exercise may help to resolve tendon pain remain unclear.

Objective This study aims to first establish changes in the gastrocnemius-soleus motor unit firing properties after applying a training protocol of 6 weeks based on either controlled eccentric or concentric contractions in individuals with NIAT. Second, we want to determine changes in the level of pain and function and mechanical and structural properties of the Achilles tendon after applying the same training protocol. Additionally, we want to compare these variables at baseline between individuals with NIAT and asymptomatic controls.

Methods and analysis A total of 26 individuals with chronic (>3 months) NIAT and 13 healthy controls will participate in the study. Individuals with NIAT will be randomised to perform eccentric or concentric training for 6 weeks. Motor unit firing properties of the medial gastrocnemius, lateral gastrocnemius and soleus muscles will be assessed using high-density surface electromyography, as well as Achilles tendon length, cross-sectional area, thickness and stiffness using B-mode ultrasonography and shear wave elastography. Moreover, participants will complete a battery of questionnaires to document their level of pain and function.

Ethics and dissemination Ethical approval (ERN-20-0604A) for the study was obtained from the Science, Technology, Engineering and Mathematics Ethical Review Committee of the University of Birmingham. The results of the study will be published in peer-review journals.

Trial registration number ISRCTN46462385.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The use of high-density surface electromyography to obtain gastrocnemius-soleus motor unit firing properties in response to a training protocol.
- ⇒ The use of B-mode ultrasound and Shear wave elastography to assess the morphological and mechanical properties of the Achilles tendon in response to a training protocol.
- ⇒ The training protocol will be based on pure eccentric or concentric contractions controlled using an isokinetic dynamometer.
- ⇒ Participants' age range is a limitation of our study, as it might affect the reproducibility of our findings in older populations.

INTRODUCTION

Achilles tendinopathy (AT) is a painful overuse injury of the Achilles tendon and it is common among athletes, especially those involved in running and jumping sports.[1–3] AT is clinically diagnosed when the patient presents with a combination of localised pain, swelling of the Achilles tendon and loss of function.[4] The essence of tendinopathy is a failed healing response, with degeneration and proliferation of tenocytes, disruption of collagen fibres and subsequent increase in non-collagenous matrix.[5] These structural changes in the tendon result in increased cross-sectional area, reduced tendon stiffness and altered viscoelastic properties in both symptomatic and asymptomatic tendons.[6]

The aetiology of AT remains debated and is likely caused by intrinsic and extrinsic factors.[7] One of the most accepted theories is that pain perception during early support loading may trigger inhibition of neuromuscular activity of the calf muscles detected as a reduction in electromyographic (EMG) amplitude.[8 9] Thus, the decrease in the ability to generate force in patients with AT could also reflect the decline in neuromuscular activity observed.[10 11] Moreover, it has been observed that this motor inhibition also

affects synergist and antagonist muscles.[12] Additionally, individuals with tendinopathy tend to use movement patterns that place an excessive or abnormal load on their tendons,[6] and it is believed that these motor adaptations may generate greater torsional stress in the tendon.[12 13] Finally, studies have shown that AT reduces tendon's stiffness,[14] which impairs the mechanisms responsible for transmitting force to the bone. Therefore, it is very likely that these alterations in tendon properties may produce changes in the neural drive received by the calf muscles.

Until now, most studies examining the neuromuscular impairments induced by AT have focused on investigating changes in interference EMG amplitude which is an indirect estimate of neural activity with many factors of influence.[15–17] Clearer information about the neural strategies employed by the central nervous system to control muscle force in the presence of AT can be obtained through motor unit recordings, since motor unit firing properties represent the direct neural output from the spinal cord to muscles.[18] Nevertheless, there are no studies that have measured motor unit firing properties in individuals with non-insertional Achilles tendinopathy (NIAT).

Although eccentric exercise has been widely used for the treatment of NIAT, the mechanisms by which eccentric exercises may help to resolve tendon pain remain unclear.[7] Regarding eccentric exercise alone, two prospective studies have reported a significant reduction in pain intensity and change on the Victorian Institute of Sports Assessment-Achilles questionnaire (VISA-A) in recreational athletes following a 12-week exercise programme.[19 20] In contrast, another study in non-athletic individuals found no significant improvement after a similar 12-week exercise programme.[21] Concerning eccentric exercises with an adjunctive treatment (eg, pulsed ultrasound, ice, sensory motor training), a 4-week intervention study resulted in decreased pain and higher plantarflexion peak torque in individuals with NIAT compared with controls.[22] However, studies that include eccentric exercises with an adjunctive treatment showed limited evidence of improvement over eccentric exercises alone.[23]

There are few studies where the effectiveness of eccentric versus concentric exercises has been compared. Mafi *et al* showed that patient satisfaction and return to previous activity were significantly superior after participating in a 12-week rehabilitation protocol based on eccentric exercise compared with concentric exercise. Although pain intensity decreased significantly in both groups, the amount of pain reduction was significantly greater for those that performed eccentric exercise.[24] Likewise, Yu *et al*[25] demonstrated that 8 weeks of eccentric exercise was more effective at reducing pain than concentric in individuals with chronic NIAT. Additionally, they found that eccentric exercise was more effective than concentric exercise at increasing muscle strength and endurance, and improving function.[25] In these investigations, participants performed the rehabilitation protocols with insufficient control over the load, speed, pain tolerance or the range of motion in which the exercises were performed. Moreover, it is essential to consider that when participants perform an eccentric plantar flexion exercise without adequate equipment, it is difficult to achieve pure eccentric contractions, which could have influenced the results obtained in these studies.

Based on the above, the aims of this study are to (1) establish changes in the gastrocnemius-soleus motor unit firing properties after applying a training protocol of 6 weeks based on either controlled eccentric or concentric contractions in individuals with NIAT; (2) determine changes in the level of pain and function and mechanical and structural properties of the Achilles tendon after applying the same training protocol; (3) compare these properties at baseline between individuals with NIAT and asymptomatic controls.

METHODS

Participants

Twenty-six individuals with NIAT and 13 asymptomatic controls will be recruited from the University of Birmingham staff/student population and the local community via leaflets, e-mail and social media.

Men or women aged 18–55 years old will be recruited. This age range was selected based on previous findings showing lower stiffness and Young's modulus of the Achilles tendon in older than younger population.[26] Inclusion criteria are NIAT determined by an experienced physiotherapist based on defined clinical findings (VISA-A[27] and NRS (Numerical Rating Scale)[28] scores), physical examination and ultrasound assessment, as well as having pain for at least 3 months.[29] VISA-A scores less than 90 will be considered as a reference to identify individuals with NIAT.[30] Regarding the NRS scores, previous studies have shown high variability in individuals with NIAT,[29 31] thus we will consider individuals with an NRS score≥2. Physical examination will include palpation of the Achilles tendon along its whole length in a proximal to distal direction, and gentle squeezing the tendon between the thumb and the index finger to identify tenderness over the tendon.[29] Ultrasound evaluation of the tendon's mid-portion will include identifying local thickening of the tendon and/or irregular tendon structure with hypoechoic areas and/or irregular fibre orientation.[31]

The exclusion criteria for both groups will include the following: (1) systemic or inflammatory conditions including rheumatic, neuromuscular disorders and malignancy, (2) current or history of chronic respiratory, neurological or cardiovascular diseases and (3) history of lower limb surgery. Specific exclusion criteria for the participants with NIAT are participation in any other treatment or rehabilitation programme for AT, corticosteroid injections in the previous 12 months and insertional AT. Additionally, if any participants present non-insertional and insertional AT concurrently in the same limb, they will be excluded. Specific exclusion criteria for the control group are pain/injury in the lower

limbs within the previous 6 months, history of AT or lower limb surgery.

Study design

This two-arm, parallel-group, randomised controlled trial will be conducted from October 2021 to December 2022 at a laboratory within the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), University of Birmingham, UK. The Science, Technology, Engineering and Mathematics Ethical Review Committee, University of Birmingham, UK, approved the study (ERN-20-0604A). All participants will provide written informed consent prior to participation. The study will be conducted according to the Declaration of Helsinki. This protocol has been designed following the SPIRIT 2013 statement[32] (see online supplemental file 1). The Trial Registration Data can be found as online supplemental file 2. Time schedule of enrolment, interventions, assessments and visits can be found as online supplemental file 3. The consent forms provided to healthy controls and patients can be also found as supplementary files (online supplemental files 4 and 5). Reporting will follow the Consolidated Standards of Reporting Trials (CONSORT) statement and the CONSORT flow diagram will be used to describe the flow of participants throughout the trial (see online supplemental file 6).

Participants with NIAT will visit the laboratory over six consecutive weeks for the experimental sessions (at weeks 1, 3 and 6) and training sessions (2–3 sessions per week) (figure 1). We will randomly allocate these participants into two groups: eccentric (ECC) or concentric (CON) training. Healthy participants will visit the laboratory once to allow baseline comparison with ECC and CON groups. Additionally, we will randomise the assessed leg in the healthy control group, and the most symptomatic leg in the ECC and CON groups will be evaluated. Finally, foot preference in specific daily activities (foot dominance) will be determined using a behavioural foot-preference inventory.[33] Each experimental session will last 2.5 hours, and each training session will last 40 min.

Sample size

According to power calculations (G*Power software),[34] a total of 26 individuals with NIAT (ECC group=13, and CON group=13) and 13 healthy controls will be required for this study. This sample size considers a power=0.80, alpha=0.01, 25% loss of participants and an effect size (d) of 1.7 calculated from the study of Yu *et al*,[25] where the authors compared reductions in pain after an 8-week concentric and eccentric training protocol in individuals with NIAT.

Figure 1 Overview of the study design.

Experimental sessions

These sessions will involve the completion of questionnaires, ultrasound imaging of the gastrocnemius-soleus muscles and the Achilles tendon, surface electromyography and torque recordings. All the procedures during the experimental sessions will be done by Ignacio Contreras-Hernandez (IC-H) and Joeri van Helden (JVH). IC-H is a PhD student at the University of Birmingham, Master in Physiology, Physiotherapist and member of the CPR Spine group. Joeri is a PhD student at the University of Birmingham, Master in Neuroscience, Psychologist and member of the CPR Spine group.

Anthropometric data (age, gender, weight, height, leg dominance and body mass index) will be obtained, and the participants will then be asked to complete a battery of questionnaires. This includes the International Physical Activity Questionnaire short form (IPAQ-SF),[35] VISA-A,[27] Foot and Ankle Ability Measure (FAAM),[36] Pain Catastrophising Scale (PCS)[37] and Tampa Scale for Kinesiophobia (TSK).[38] Additionally, participants will be asked to report their current level of pain using the NRS score. After that, participants will lie prone on the chair of a Biodex System 3 dynamometer (Biodex Medical Systems), and ultrasonography (LOGIQ S8 GE Healthcare, Milwaukee, USA) will be used to measure the length, thickness and cross-sectional area of the Achilles tendon, fascicle length, thickness and pennation angle of the medial gastrocnemius (MG), and thickness of the lateral gastrocnemius (LG) and soleus (SO) muscles during rest. Then, we will prepare the skin and place the electrodes on the MG, LG and SO muscles, and using high-density surface electromyography (HD-sEMG); we will ensure minimal electrical activity of these muscles during rest conditions for the measurements of the Achilles tendon stiffness (passive elastography).

Following the ultrasound assessment the maximal voluntary contraction (MVC) will be recorded during three isometric plantarflexion contractions of 5 s each.[15] Between each MVC, the volunteers will have 2 min of rest[15] and all MVCs will be performed at 0° of plantarflexion. The highest MVC value will be used as the reference maximal torque. We will use this MVC value as a reference for the isometric and dynamic plantarflexion contractions during the experimental and training sessions, to avoid multiple MVC measurements that may be produce pain and discomfort in individuals with NIAT. Afterwards, we will measure the stiffness of the tendon during two isometric plantarflexion contractions at 10% MVC (1 s ramp-up, 12 s hold, 1 s ramp-down and 30 s rest) (active elastography). Subsequently, using HD-sEMG, we will record motor unit activity of the MG, LG and SO muscles during two isometric plantarflexion contractions

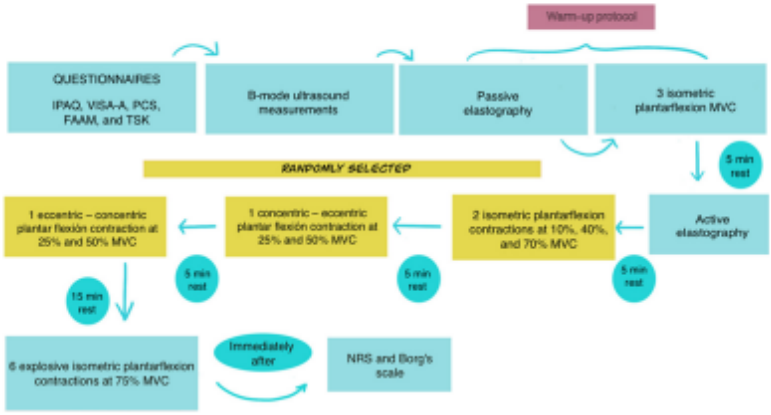

Figure 2 Experimental session design. FAAM, Foot and Ankle Ability Measure; IPAQ-SF, International Physical Activity Questionnaire short form; MVC, maximal voluntary contraction; NRS, Numerical Rating Scale; PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia; VISA-A, Victorian Institute of Sports Assessment-Achilles questionnaire

at 10%, 40% and 70% MVC (10% MVC/s ramp-up, 10 s hold, 10% MVC/s ramp-down and 30 s rest), one concentric–eccentric plantarflexion contraction at 25% and 50% MVC and one eccentric–concentric plantarflexion contraction at 25% and 50% MVC (the order of the different types of contractions will be randomly selected). Volunteers will have 5 min of rest at the end of each type of contraction (isometric, concentric–eccentric and eccentric–concentric). After 15 min of rest, HD-sEMG will be recorded from the MG, LG and SO muscles during six explosive (fast force development) isometric plantarflexion contractions at 75% MVC (1 s ramp-up, 3 s hold, 1 s ramp-down and 10 s rest).[39] Finally, both the rate of perceived exertion and the level of pain will be monitored regularly throughout the session, using the Borg ratings of perceived exertion scale[40] and the NRS (figure 2).

During all contractions, visual feedback of the target torque output will be provided via computer monitor positioned 1 m from the participant. Prior to the contractions, participants will be instructed to match the force output as closely as possible to the target force for the full duration of the contraction. For the dynamic contractions, the range of motion will be set at the total of 30° (neutral position 0°–30° of plantarflexion) and the angular speed will be set at 3°/s.

Training sessions

The training sessions will be done by Michalis Arvanitidis (MA). MA is a PhD student at the University of Birmingham, Master in Advanced Manipulative Physiotherapy, and Specialist Musculoskeletal Physiotherapist (Member of the Musculoskeletal Association of Chartered Physiotherapist) and member of the CPR Spine group.

All the training sessions will be done in prone position on the Biodex System 3 dynamometer.

The participants in the ECC group will be asked to perform a warm-up consisting of three eccentric plantarflexion contractions at 25% MVC; this will be followed by the eccentric training protocol. This protocol consists of 4×15 eccentric plantarflexion contractions at 50% MVC, range of motion of 30° (neutral position 0°–30° of plantarflexion), time under tension of 10 s, angular speed of 3°/s and 3 min of rest between each series. Visual feedback of the exerted torque will be provided. Participants in the CON group will perform a warm-up consisting of three concentric plantarflexion contractions at 25% MVC, and then, the concentric training protocol. This protocol consists of 4×15 concentric plantarflexion contractions at 50% MVC, range of motion of 30° (neutral position 0°–30° of plantarflexion), time under tension of 10 s, angular speed of 3°/s, and 3 min of rest between each series.

Preceding the contractions, participants will be instructed to match the torque output as closely as possible to the target torque for the full duration of the contraction.

Follow-up

Participants with NIAT will be asked to report their level of pain and function at 3 and 6 months after completing the training protocol.

Outcome measures

Primary outcomes measure

The primary outcomes for this study will be GM, GL and SO muscles motor unit firing properties assessed using HD-sEMG and decomposition techniques. These properties include motor unit discharge rate, recruitment and de-recruitment thresholds and discharge rate variability.

Secondary outcomes measure

Secondary outcomes will include level of pain and function assessed using the NRS and VISA-A questionnaire, Achilles tendon length, thickness, cross-sectional area and stiffness using B-mode ultrasonography and shear wave elastography (SWE). Additionally, secondary

outcomes will include GM, GL and SO muscles thickness, and GM muscle fascicle length and pennation angle evaluated using B-mode ultrasonography, as well as the level of physical activity, physical function, pain catastrophising and fear of movement assessed using the IPAQ-SF, FAAM, PCS and TSK questionnaires, respectively.

Questionnaires

In each experimental session, participants will be asked to complete the IPAQ-SF, VISA-A, FAAM, PCS and TSK questionnaires to measure physical activity level, symptoms in individuals with AT, physical function, pain catastrophising and fear of movement, respectively. The IPAQ has become the most widely used physical activity questionnaire,[41] and it has acceptable measurement properties for monitoring population levels of physical activity among 18–65 years old adults in diverse settings.[35] The VISA-A was developed with the aim of evaluating the symptoms of AT and their impact on physical activity. This questionnaire is valid, reliable, easy to use and ideal for comparing patients' progress in clinical settings.[27] The FAAM was developed to meet the need for a self-reported evaluative instrument that comprehensively assesses the physical function of individuals with musculoskeletal disorders of the leg, foot and ankle. The FAAM is a reliable, valid and responsive measure of self-reported physical function.[36] Additionally, we will use the PCS to understand the psychological processes that lead to heightened physical and emotional distress in response to aversive stimulation. This questionnaire is a reliable and valid measure of catastrophising.[37] Finally, we will apply the TSK to measure the fear of movement/(re)injury. This questionnaire has been validated in patients with chronic back pain,[42–44] acute back pain,[45 46] osteoarthritis[47] and fibromyalgia.[43 44]

Measurement set-up

For the measurements of the Achilles tendon, MG, LG and SO muscles, participants will lie prone on the dynamometer, with their knees extended and their tested foot tightly strapped on the footplate. The pelvis will be stabilised with another strap to minimise compensatory movements. The ankle will be positioned in 0° of plantarflexion and the axis of the dynamometer will be aligned with the inferior tip of the lateral malleolus. The setting and position of the set up (ie, chair and isokinetic device) will be saved, so the participants' position will be similar in each experimental session.

Ultrasound measurements

All ultrasound images will be obtained using an ultrasound imaging device equipped with SWE (LOGIQ S8 GE Healthcare, Milwaukee, USA). For the measurements of the length, thickness and cross-sectional area of the Achilles tendon, and the measurements of the fascicle length, thickness and pennation angle of the calf muscles, B-mode will be used with a 16-linear array probe (50 mm, 4–15 MHz). Subsequently, for the measurements of the Achilles tendon's stiffness during rest conditions and isometric plantarflexion contraction, the elastography mode will be used with a 9-linear array probe (44 mm, 2–8 MHz).

An adaptation of the protocol developed by Arya and Kulig[14] will be used to measure the structural properties of the Achilles tendon. Briefly, to obtain tendon length, the ultrasound transducer will be placed longitudinally over the posterior aspect of the heel, and the distal part of the Achilles tendon will be imaged and the corresponding point will be marked on the skin with a marker. Then, the ultrasound probe will be moved proximally to locate the musculotendinous junction of the MG, and this point will be marked on the skin. The distance between these two points will be measured with a tape and this distance will represent the resting length of the Achilles tendon. Marks at 2, 4 and 6 cm above the Achilles tendon insertion will then be made on the skin. Later, these marks will be used to place the probe in the transversal plane and determine the cross-sectional area of the Achilles tendon at 2 4 and 6 cm of its insertion. Additionally, we will use these marks to place the probe in the sagittal plane and determine the thickness of the Achilles tendon at 2, 4 and 6 cm of its insertion.

For muscle ultrasound images, the mid-line of the leg will be marked in the direction of the Achilles tendon. Additionally, a mark will be made on the leg 10 cm above the musculotendinous junction of the MG muscle and 4 cm medial to the mid-line. In this position, we will place the middle point of the HD-sEMG electrode grid and mark the contour of the grid on the skin. We will use these marks to place the probe in the sagittal plane and obtain the images of the MG muscle. Similarly, the leg will be marked 10 cm above the musculotendinous junction of the MG muscle and 4 cm lateral to the mid-line. Then, we will repeat the procedure mentioned above, but now for the LG muscle. Next, the leg will be marked 5 cm below the musculotendinous junction of the MG and 4 cm lateral to the mid-line. Again, we will repeat the procedure mentioned above, but now for the SO muscle (figure 3). The middle column of the HD-sEMG electrode grid will be used as a reference (see HD-sEMG and torque section below) to place the probe in the same position during all the experimental sessions and the images will be acquired with the probe oriented in the sagittal plane, and perpendicular to the skin, according to the recommendations of Bolsterlee *et al*.[48] To ensure that we are measuring the exact location of interest, we will use the procedure described above in each experimental session, and we also will mark the middle point of the ultrasound probe. Then, during the acquisition of the ultrasound images, we will align the mark in the ultrasound probe with the marks on the skin at 2, 4 and 6 cm from the Achilles tendon insertion and with the mark of the middle point of the HD-sEMG electrode grid of each muscle. This procedure will allow us to identify the location of interest during the analysis of the ultrasound images since we know that the middle point of the image represents the location of interest. The software Image J

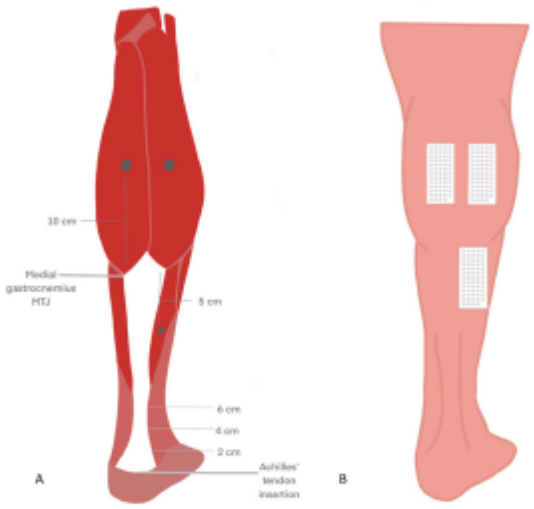

Figure 3 (A) Anatomical landmarks used for ultrasonography and (B) position of the electrodes in the MG, LG and SO muscles. LG, lateral gastrocnemius; MG, medial gastrocnemius, SO, soleus.

(https://imagej.nih.gov/ij/) will be used to analyse these images and determine the fascicle length, thickness and pennation angle.

For tendon stiffness measurements, the probe will be placed in the sagittal plane, with the middle part of the probe located at 4 cm above the Achilles tendon insertion. A probe holder will be used to avoid applying pressure over the tendon and movements that may interfere with the measurements. Then, we will perform a trial SWE to check for possible voids. If voids are detected at this stage, we will remove the probe holder and place the ultrasound probe again. The passive elastography images will be acquired for 12 s (twice), and the active elastography images will be acquired during two isometric plantarflexion contractions at 10% MVC (1 s ramp-up, 12 s hold, 1 s ramp down, 1 min rest). We will check the elastography images following each measurement to determine possible voids that may affect our results. If voids in the middle part of the tendon (at 4 cm of the insertion) are detected in this stage, we will repeat the procedure. A shear elastography colour map (height x width, 2.5 cm x 1 cm) will be chosen using elastography ultrasound tools to allow complete visualisation of the Achilles tendon width, and a region of interest (ROI) of 3 mm diameter[49] will be selected in the middle of each image to determine the shear wave velocity (m/s) and Young's modulus (kPa). Finally, we will average the mean shear wave velocity and Young's modulus over the ROIs of consecutive images.[50]

HD-sEMG and torque recording

Prior to electrode placement, the skin will be shaved (if necessary), gently abraded (Nuprep, Skin Prep Gel, Weaver and Company, Aurora, Colorado) to reduce skin impedance and cleaned with water. Three two-dimensional (2D) adhesive grids (SPES Medica, Salermo, Italy) of 13×5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm) will be used to record the HD-EMG signals. Conductive paste (AC-CREAM, SPES Medica, Genova, Italy) will be placed into the cavities of the grid, and the HD-sEMG electrodes will be placed in the exact position described for the ultrasound measurements of the triceps surae (one electrode grid for each muscle).

All signals will be converted from analog-to-digital by a 16-bit converter (Quattrocento- OTBiolettronica, Torino, Italy). The sampling frequency will be 2048 Hz and the amplifier gain will be set to 150. HD-sEMG signals will be digitally filtered with a bandwidth set up to 10 Hz for high pass cut frequency and to 500 Hz for low pass cut frequency.[51] HD-sEMG will be acquired in monopolar mode with reference electrodes (WhiteSensor WS, Ambu A/S, Ballerup, Denmark) positioned in the head of the fibula and with a strip in the thigh of the evaluated leg. All the electrode grids and reference electrodes will be connected to the same bioelectrical amplifier (Quattrocento-OT-Bioelettronica, Torino, Italy).

The torque exerted by the volunteers will be assessed with the isokinetic dynamometer, which will be synchronised with the HD-sEMG signals. Synchronisation will be obtained by recording torque signals generated by the isokinetic dynamometer through the auxiliary input of the EMG amplifier.[51]

Signal analysis

The torque signal will be low pass filtered at 15 Hz and then used to quantify torque steadiness (coefficient of variation of torque, SD torque/mean torque * 100) from the stable part of the contractions.[52]

The HD-sEMG signals will be decomposed into motor unit spike trains with an algorithm based on blind source separation, which provides automatic identification of motor unit activity,[52] and the accuracy of the decomposition will be tested with the silhouette measure, which will be set to ≥0.90.[53] The signals will be decomposed during the entire duration of the contractions, and the discharge times of the motor units will be transformed in binary spike trains.[54] The mean discharge rate and the discharge rate variability (CoV of the interspike interval (CoV_{isi})) will be determined during the stable plateau of torque signal. Additionally, motor unit recruitment and derecruitment thresholds will be defined as the ankle plantarflexion torques (%MVC) at the times when the motor units began and stopped discharging action potentials, respectively.[52] Discharge rates at recruitment and derecruitment will be determined using the first and last six discharges of the motor units.[52] Erroneous discharges will be visually inspected and edited using a custom algorithm.[52 55] Motor unit activity will be monitored longitudinally with a recent method proposed by Martinez-Valdes *et al*,[56] which allows tracking the same motor units across different experimental sessions.

Adverse event management

Participants will be informed that they may experience some pain during or after the experimental and training sessions. Monitoring of participants' pain will be done in each experimental and training sessions using the NRS. Appropriate rest time will be provided throughout the experimental and training sessions, and extra rest periods will be given to the participants at any time if required. If a participant experiences moderate pain (>6NRS) during the contractions, they will be given additional time to rest. If the pain intensity is maintained or worsens, we will terminate and reschedule the session. The session will be rescheduled in the upcoming 3 days. If pain intensity is maintained or worsened during these days and rescheduling the session is not possible, the participant will be removed from the study. This will be considered as an adverse effect, and it will be reported to the Science, Technology, Engineering and Mathematics Ethical Review Committee of the University of Birmingham.

Randomisation and blinding

Individuals with NIAT will be randomised by an independent researcher (Dr Eduardo Martinez-Valdes (EM-V)) in a 1:1 allocation ratio to either ECC or CON groups (parallel-groups) using computer-generated simple scheme randomisation (https://sigdaan.com/randomization/app/randomization-app). Allocation concealment will be ensured, as EM-V will secure the randomisation code using password-protected files. EM-V will give access to MA to the randomisation code once each participant has completed the first experimental session.

In order to achieve double-blinding, IC-H and JVH will perform the experimental sessions and MA will perform the training sessions. IC-H will use the blindr (https://github.com/U8NWXD/blindr) software to encode the results from different participants and will be blinded to the training protocol applied to the participants. MA will be blind to the participant's results, but not to the training protocol applied. EM-V will unmask the results after the data analysis is performed. Due to the nature of the interventions, participants' blinding is not possible.

Statistical analysis

IBM SPSS Statistics for Windows, V. 25.0 (Armonk, New York, USA) computer software will be used for statistical analysis of the data. Intention-to-treat and per protocol analysis will be performed. Descriptive statistics will be used to interpret the data which will be presented as mean±SD The Shapiro-Wilk Test will be used to assess data normality. The level of significance for all statistical procedures will be set at α=0.05% and 95% CI will be reported. Independent t-test will be used to determine the differences between individuals with NIAT and healthy controls at baseline. If the data are normally distributed, repeated measures analysis of variance (ANOVA) will be used. Factors of group (ECC and CON) and time (at weeks 1, 3 and 6) will be used to analyse each variable. Bonferroni post hoc analysis will be used if ANOVA is significant. The partial eta-squared (η_p^2) for ANOVA will be used to examine the effect size of changes after the training intervention. An η_p^2 less than 0.06 will be classified as 'small', 0.07–0.14 as 'moderate' and greater than 0.14 as 'large'.[57] If data are not normally distributed, appropriate non-parametric tests will be used.

DISCUSSION

To the best of our knowledge, this is the first study aiming to establish changes in motor unit firing properties of the gastrocnemius-soleus muscles after applying a training protocol based on either controlled eccentric or concentric contractions in individuals with NIAT. Additionally, this study will be the first to determine motor unit firing properties of the gastrocnemius-soleus muscles in individuals with NIAT compared with asymptomatic controls.

Regarding the variables related to the EMG activity and motor unit firing properties (ie, discharge rate, recruitment and discharge rate variability) of the gastrocnemius-soleus muscles; previous studies have estimated the activation of the triceps surae muscles in people with AT during walking,[58] running,[59] isometric plantarflexion tasks[60] and dynamic plantarflexion tasks.[61 62] Currently, there is no agreement in the literature in terms of plantarflexion torque measured during maximal contractions in individuals with NIAT; some authors did not find any difference between groups,[14 63] while others found statistically significant differences.[60 64] Interestingly, an investigation has observed a significant increase in LG activation during isometric plantarflexion tasks in people with AT following a 12-week training programme.[60] Despite all of these efforts, currently there are no studies that have evaluated motor unit firing properties of calf muscles in individuals with NIAT.

A strength of this study is that we will perform a detailed assessment of the mechanical and structural properties of the Achilles tendon and the calf muscles. One study showed that tendinopathy alters both the mechanical and material properties of the human Achilles tendon.[14] Morphological comparisons of tendinopathic and healthy tendons demonstrated a larger cross-sectional area for the degenerated Achilles tendon. Typically, a larger tendon is considered mechanically stronger due to its ability to dissipate stresses across the tendon and yield lower strain energy. Nonetheless, in the study of Arya and Kulig,[14] they demonstrated that despite having a larger cross-sectional area, the degenerated tendon had lower stiffness and Young's modulus compared with healthy tendons. Additionally, our study includes the use of SWE. This procedure has been used to measure tissue elasticity in tendons and might add to a better understanding of the effects of different types of exercises in tendons.[65] Furthermore, SWE is able to measure the Young modulus (slope of the stress–strain curve in the linear region[66] of Achilles tendon with high reliability).[67] Previous studies

suggest that SWE might be a useful tool for diagnosing and monitoring AT.[68] For instance, one study demonstrated that symptomatic Achilles tendons had lower Young modulus compared with healthy tendons and that stiffness increases in correlation with VISA-A scores after 6 months of treatment.[69]

Another strength of our study is using an isokinetic dynamometer to perform the training sessions. This device will allow us to control the intensity (50% MVC), range of motion (0°–30° of plantarflexion) and angular speed (3°/s) of the contractions, enabling us to have close control over the time under tension (10 s) of the Achilles tendon. Although the use of isokinetic dynamometers is common to measure peak torque in musculoskeletal research, its use during a training protocol is limited. To the best of our knowledge, there are no studies using isokinetic dynamometers to train individuals with NIAT. This represents an essential aspect of our RCT because previous studies investigating the effects of exercise in individuals with NIAT usually apply training protocols with insufficient control over the load, speed, pain tolerance or the range of motion, and this could have influenced their results.

Regarding the study's limitations, the relatively short training protocol (6 weeks) might influence the changes expected in the mechanical and structural properties of the Achilles tendon; therefore, longer training interventions might be required to assess changes in these parameters in the long term. Moreover, due to the nature of the training protocol applied (eccentric and concentric exercises), blinding of participants is not possible because we need to explain how to do the different types of exercises on the isokinetic dynamometer. We are aware this introduces bias into the RCT, but unfortunately, it is not possible to achieve participants' blinding. Another study limitation is the inclusion of participants with bilateral symptoms, which could potentially affect the results. Since morphological changes to the asymptomatic tendon are common in this condition[70] and 45% of thickened Achilles tendons progress to develop clinical symptoms within 12 months,[71] we decided to also include these patients in the study. Additionally, participants' age range is another limitation of our study, as it might affect the reproducibility of our findings in older populations; however, we have decided to recruit participants in this age range based on previous studies showing age-related differences in Achilles tendon's stiffness and Young's modulus, which could confound the results of the intervention.

The study of motor units is an area in continuous development, which in recent years has allowed a more profound understanding of the neural mechanisms involved in muscle contractions. However, much of the research in this area has focused on the normal neurophysiology of muscle rather than its relationship with alterations of the musculoskeletal system.

This research will therefore provide new insights regarding the neuromechanical effects of ECC and CON exercises in the management of individuals with NIAT. A more precise understanding of the mechanisms involved in this pathology is essential to improve the rehabilitation programmes commonly used in the management of this condition.

Patient and public involvement

The research question in this study forms part of a larger discussion about exercise and pain relief within our patient and public involvement meetings. Patients will not be involved in the analysis and data collection but will contribute to data interpretation and production of a lay summary of findings.

ETHICS AND DISSEMINATION

Ethical approval and trial registration

The research protocol has been approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee of the University of Birmingham (ERN-20-0604A).

Researchers will inform all participants of the characteristics of the research and will obtain written consent. Participants will be informed that they are free to withdraw from the study at any time without needing to provide a reason. In any unlikely adverse events, this will be immediately reported by the principal investigator to the ethics committee.

The results of this study will be submitted for publication in a peer review journal and presented at conferences.

Confidentiality

All information collected will be kept strictly confidential. Personal information will be retained but only available to the researchers using password-protected files. In addition, all data for presentations will be anonymised and aggregated, so the participants' identities will not be revealed in any way.

Twitter Deborah Falla @Deb_Falla and Eduardo Martinez-Valdes @mredumartinez

Contributors IC-H and EM-V are responsible for the conception, design and development of the protocol. EM-V is the lead supervisor of IC-H and DF is the cosupervisor. EM-V and DF have provided guidance on methodological decisions and critical revision. All authors have read and subsequently approved the final manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

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Appendix 3. Search strategy Step 1 used in MEDLINE (OVID interface)

Search string

("Corticospinal excita*" OR "cortical excita*" OR “spinal excita*” OR "spinal reflex" OR "H-reflex" OR "V-wave" OR "F-wave" OR "neural drive" OR "motor evoked potential*" OR "evoked muscle response*" OR "electromyogra*" OR "activation" OR "co-activation" OR "preactivation" OR "electromechanic*" OR "motor unit*" OR "motor neuron*" OR "length" OR "thickness" OR "cross sectional area" OR "modulus" OR "viscoelasticity" OR "elasticity" OR "strain" OR "stress" OR "stiffness" OR "volume" OR "creep" OR "mechanic*" OR "morphologic*") AND ("tendin*" OR "tenosynovitis" OR "paratenonitis" OR "tendovaginitis" OR "peritendinitis" OR "achillodynia") AND ("Achilles" OR "TendoAchilles" OR "Tendo-Achilles" OR "calcaneal tendon" OR "gastrocnemius" OR "triceps surae" OR "calf muscle*" OR "tibialis anterior")

Keywords

1- Corticospinal excita*.mp
2- Cortical excita*.mp
3- Spinal excita*.mp
4- Spinal reflex.mp
5- H-reflex.mp
6- V-wave.mp
7- F-wave.mp
8- Neural drive.mp
9- Motor evoked potential*.mp
10- Evoked muscle response*.mp
11- Electromyogra*.mp
12- Activation.mp
13- Co-activation.mp
14- Preactivation.mp
15- Electromechanic*.mp
16- Motor unit*.mp
17- Motor neuron*.mp
18- Length.mp
19- Thickness.mp
20- Cross sectional area.mp
21- Modulus.mp
22- Viscoelasticity.mp
23- Elasticity.mp
24- Strain.mp
25- Stress.mp

26-Stiffness.mp
27-Volume.mp
28-Creep.mp
29-Mechanic*.mp
30-Morphologic*.mp
31-1-30
32-Tendin*.mp
33-Tenosynovitis.mp
34-Paratenonitis.mp
35-Tendovaginitis.mp
36-Peritendinitis.mp
37-Achillodynia.mp
38-32-37
39-Achilles.mp
40-TendoAchilles.mp
41-Tendo-Achilles.mp
42-Calcaneal tendon.mp
43-Gastrocnemius.mp
44-Triceps surae.mp
45-Calf muscle*.mp
46-Tibialis anterior.mp
47-39-46
48-31 AND 38 AND 47

Appendix 4. Search strategy Step 2 used in MEDLINE (OVID interface)

Search string

("corticospinal excita*" OR "cortical excita*" OR “spinal excita*” OR "spinal reflex" OR "H-reflex" OR "V-wave" OR "F-wave" OR "neural drive" OR "motor evoked potential*" OR "evoked muscle response*" OR "electromyogra*" OR "activation" OR "co-activation" OR "preactivation" OR "electromechanic*" OR "motor unit*" OR "motor neuron*" OR "length" OR "thickness" OR "cross sectional area" OR "modulus" OR "viscoelasticity" OR "elasticity" OR "strain" OR "stress" OR "stiffness" OR "volume" OR "creep" OR "mechanic*" OR "morphologic*") AND ("mechanical load*" OR "Exercise*" OR "physical activity*" OR "eccentric" OR "concentric" OR "isometric" OR "training" OR "strengthening" OR "stretching" OR "vibration" OR "oscillation" OR "plyometric" OR "running" OR "walking") AND ("tendin*" OR "tenosynovitis" OR "paratenonitis" OR "tendovaginitis" OR "peritendinitis" OR "achillodynia") AND ("Achilles" OR "TendoAchilles" OR "Tendo-Achilles" OR "calcaneal tendon" OR "gastrocnemius" OR "triceps surae" OR "calf muscle*" OR "tibialis anterior")

Keywords

1- Corticospinal excita*.mp
2- Cortical excita*.mp
3- Spinal excita*.mp
4- Spinal reflex.mp
5- H-reflex.mp
6- V-wave.mp
7- F-wave.mp
8- Neural drive.mp
9- Motor evoked potential*.mp
10- Evoked muscle response*.mp
11- Electromyogra*.mp
12- Activation.mp
13- Co-activation.mp
14- Preactivation.mp
15- Electromechanic*.mp
16- Motor unit*.mp
17- Motor neuron*.mp
18- Length.mp
19- Thickness.mp
20- Cross sectional area.mp
21- Modulus.mp
22- Viscoelasticity.mp

23-Elasticity.mp
24-Strain.mp
25-Stress.mp
26-Stiffness.mp
27-Volume.mp
28-Creep.mp
29-Mechanic*.mp
30-Morphologic*.mp
31-1-30
32-Mechanical load*.mp
33-Exercise*.mp
34-Physical activity*.mp
35-Eccentric.mp
36-Concentric.mp
37-Isometric.mp
38-Training.mp
39-Strengthening.mp
40-Stretching.mp
41-Vibration.mp
42-Oscillation.mp
43-Plyometric.mp
44-Running.mp
45-Walking.mp
46-32-45
47-Tendin*.mp
48-Tenosynovitis.mp
49-Paratenonitis.mp
50-Tendovaginitis.mp
51-Peritendinitis.mp
52-Achillodynia.mp
53-47-52
54-Achilles.mp
55-TendoAchilles.mp
56-Tendo-Achilles.mp
57-Calcaneal tendon.mp
58-Gastrocnemius.mp
59-Triceps surae.mp
60-Calf muscle*.mp
61-Tibialis anterior.mp
62-54-61
63-31 AND 46 AND 53 AND 62

Appendix 5. Table 13. GRADE assessment for Step 1

Studies (Total participants)	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Certainty (Overall score)
Outcome: Thickness							
Malmgaard-Clausen et al 2021 (35)	RCT	Downgraded one level for bias due to deviations from intended intervention and bias due to missing outcome data	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**
Grigg et al. 2012 (20)	NRSI	Downgraded one level for bias in measurement outcomes and bias in selection of reported results	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**

Callow et al. 2022 Crawford et al. 2023 Intziegianni et al. 2016 Lalumiere et al. 2020 Scholes et al. 2018 Child et al. 2010 Aggouras et al. 2022 Chimenti et al. 2017 Nuri et al. 2018 Zhang et al. 2017 Alghamdi et al. 2022 Nadeau et al. 2016 Romero-Morales et al. 2019 Chimenti et al. 2014 (632)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Antero-posterior diameter							
Petersen et al. 2007 (100)	RCT	Downgraded one level for bias due to deviations from intended intervention and	No downgrading	No downgrading	No downgrading	Not detected	**Moderate**

		bias due to missing outcome data					
Resteghini et al. 2012 Tsehaie et al. 2017 (57)	NRIS	Downgraded one level for serious bias due to confounding and bias due to deviation of intended interventions	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**
Finnamore et al. 2019 Lalumiere et al. 2020 Scholes et al. 2018 van Schie et al. 2010 Szaro et al. 2021 Nadeau et al. 2016 (370)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Cross-sectional area							
Tsehaie et al. 2017 (25)	NRIS	Downgraded one level for serious bias due to deviation of intended interventions	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**

Callow et al. 2022 Arya et al. 2010 Nuri et al. 2018 Shim et al. 2019 Zhang et al. 2017 Intziegianni et al. 2016 Nadeau et al. 2016 Romero-Morales et al. 2019 (418)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Length							
Alghamdi et al. 2022 Nuri et al. 2018 Shim et al. 2019 Szaro et al. 2021 Arya et al. 2010 Intziegianni et al. 2016 Chimenti et al. 2014 (309)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Width							

Szaro et al. 2021 Nuri et al. 2018 Nadeau et al. 2016 (218)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Volume							
Shalabi et al. 2004 Tsehaie et al. 2017 (50)	NRIS	Downgraded one level for serious bias due to deviation of intended interventions	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Nuri et al. 2018 Shim et al. 2019 (36)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Insertional angle							
Alghamdi et al. 2022 (34)	Observational	Downgraded one level for comparability item	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Stiffness							

Arya et al. 2010 Aubry et al 2015 Crawford et al. 2023 Intziegianni et al. 2016 Shim et al. 2019 Wang et al. 2012 Chang et al. 2015 Chimenti et al. 2014 Zhang et al. 2017 (306)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Strain							
Grigg et al. 2012 (20)	NRIS	Downgraded one level for bias in measurement outcomes and bias in selection of reported results	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**
Arya et al. 2010 Child et al. 2010 Intziegianni et al. 2016 Nuri et al. 2018 Chimenti et al. 2017	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**

Chimenti et al. 2014 (153)							
Outcome: Force							
Arya et al. 2010 Chimenti et al. 2014 (64)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Stress							
Arya et al. 2010 Shim et al. 2019 (40)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Mechanical hysteresis							
Wang et al. 2012 (17)	Observational	Downgraded one level for comparability item	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Elastic energy stored and released							

Wang et al. 2012 (17)	Observational	Downgraded one level for comparability item	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: EMG amplitude							
Pingel et al. 2013 (18)	NRIS	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Moderate**
Azevedo et al. 2009 Baur et al. 2011 Reid et al. 2012 (138)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: EMG frequency							
Pingel et al. 2013 (18)	NRIS	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Moderate**
Outcome: Timing of activation							
Debenham et al. 2016 Wyndow et al. 2013 Chang et al. 2015 (79)	Observational	No downgrading	Downgraded one level for serious differences in outcome measures	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Electromechanical Delay							
Wang et al. 2012 Chang et al. 2015 (36)	Observational	Downgraded one level for comparability item	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Evoked reflexes							

Wang et al. 2012 Chang et al. 2015 (36)	Observational	Downgraded one level for comparability item	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Co-contraction ratio							
Chang et al. 2015 (19)	Observational	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Motor unit discharge rate							
Fernandes et al. 2023 (25)	Observational	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Motor unit coefficient of variation of the interspike interval							
Fernandes et al. 2023 (25)	Observational	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Intracortical inhibition							
Fernandes et al. 2021 (24)	Observational	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Very low**

RCT, randomised controlled trial; NRSI, non-randomised interventional study

Appendix 6. Table 14. GRADE assessment for Step 2

Studies (Total participants)	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Certainty (Overall score)
Outcome: Thickness							
Beyer et al. 2015 Boesen et al. 2017 Johannsen et al. 2022 Malmgaard-Clausen et al. 2021 Solomons et al. 2020 Stefansson et al. 2019 Benli et al. 2022 (221)	RCT	No downgrading	No downgrading	Downgraded one level for serious differences in outcome measures	Downgraded one level for small sample size	Not detected	**Low**
Ohberg et al. 2004 Tsehaie et al. 2017 von Wehren et al. 2019 (75)	NRIS	Downgraded one level for serious bias due in classification of interventions and deviation from intended interventions	No downgrading	Downgraded one level for serious differences in outcome measures	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Antero-posterior diameter							
Rompe et al. 2007 Petersen et al. 2007 (87)	RCT	No downgrading	Downgraded one level for serious differences in	No downgrading	Downgraded one level for small sample size	Not detected	**Low**

			measurements times				
Outcome: Cross-sectional area							
Malmgaard-Clausen et al. 2021 (35)	RCT	Downgraded one level for bias due to deviations from intended intervention and bias due to missing outcome data	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Low**
Tsehaie et al. 2017 von Wehren et al. 2019 (50)	NRIS	Downgraded one level for serious bias due to deviation from intended interventions	No downgrading	Downgraded one level for serious differences in outcome measures	Downgraded one level for small sample size	Not detected	**Very low**
Outcome: Width							
Stefansson et al. 2019 (19)	RCT	No downgrading	No downgrading	No downgrading	Downgraded one level for small sample size	Not detected	**Moderate**
Outcome: Volume							
Shalabi et al. 2004 Tsehaie et al. 2017 (50)	NRIS	Downgraded one level for serious bias due to deviation from intended interventions	No downgrading	Downgraded one level for serious differences in outcome measures	Downgraded one level for small sample size	Not detected	**Very low**

Outcome: Strain							
Benli et al. 2022 (40)	RCT	Downgraded one level for bias due to deviation of intended intervention and bias due to missing outcome data	No downgrading	No downgrading	Downgraded one level for small sample size	Not Detected	**Low**

RCT, randomised controlled trial; NRSI, non-randomised interventional study.

Appendix 7. Application for Ethical Review

UNIVERSITY OF BIRMINGHAM

APPLICATION FOR ETHICAL REVIEW

Who should use this form:

This form is to be completed by PIs or supervisors (for PGR student research) who have completed the University of Birmingham's Ethical Review of Research Self-Assessment Form (SAF) and have decided that further ethical review and approval is required before the commencement of a given Research Project.

Please be aware that all new research projects undertaken by postgraduate research (PGR) students first registered as from 1st September 2008 will be subject to the University's Ethical Review Process. PGR students first registered before 1st September 2008 should refer to their Department/School/College for further advice.

Researchers in the following categories are to use this form:

1. The project is to be conducted by:
    - Staff of the University of Birmingham; or
    - Postgraduate research (PGR) students enrolled at the University of Birmingham (to be completed by the student's supervisor);
2. The project is to be conducted at the University of Birmingham by visiting researchers.

Students undertaking undergraduate projects and taught postgraduate (PGT) students should refer to their Department/School for advice.

NOTES:

- An electronic version of the completed form should be submitted to the Research Ethics Officer, at the following email address: aer-ethics@contacts.bham.ac.uk. Please **do not** submit paper copies.
- If, in any section, you find that you have insufficient space, or you wish to supply additional material not specifically requested by the form, please it in a separate file, clearly marked and attached to the submission email.
- If you have any queries about the form, please address them to the Research Ethics Team.

☒ **Before submitting, please tick this box to confirm that you have consulted and understood the following information and guidance and that you have taken it into account when completing your application:**

- **The information and guidance provided on the University's ethics webpages (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-of-Research.aspx)**
- **The University's Code of Practice for Research (http://www.as.bham.ac.uk/legislation/docs/COP_Research.pdf)**

UNIVERSITY OF BIRMINGHAM APPLICATION FOR ETHICAL REVIEW	*OFFICE USE ONLY:* Application No: Date Received:

1. TITLE OF PROJECT

Neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy

2. THIS PROJECT IS:

University of Birmingham Staff Research project ☐
University of Birmingham Postgraduate Research (PGR) Student project ☒
Other ☐ (Please specify):

3. INVESTIGATORS

a) PLEASE GIVE DETAILS OF THE PRINCIPAL INVESTIGATORS OR SUPERVISORS (FOR PGR STUDENT PROJECTS)

Name: Title / first name / family name	Dr Eduardo Martinez-Valdes
Highest qualification & position	PhD
School/Department	Sports, Exercise, and Rehabilitation Sciences
Telephone:	

b) PLEASE GIVE DETAILS OF ANY CO-INVESTIGATORS OR CO-SUPERVISORS (FOR PGR STUDENT PROJECTS)

Name: Title / first name / family name	Professor Deborah Falla
Highest qualification & position held:	PhD
School/Department	Sports, Exercise, and Rehabilitation Sciences
Telephone:	
Email address:	

c) In the case of PGR student projects, please give details of the student

Name of student:	Ignacio Contreras-Hernandez	Student No:	[redacted]
Course of study:	PhD Sport, Exercise and Rehabilitation	Email address:	[redacted]
Principal supervisor:	Dr Eduardo Martinez-Valdes		

4. ESTIMATED START OF PROJECT Date: October

ESTIMATED END OF PROJECT Date: Dec 2022

5. FUNDING

List the funding sources (including internal sources) and give the status of each source.

Funding Body	Approved/Pending /To be submitted
BECAS CHILE	Ongoing

If you are requesting a quick turnaround on your application, please explain the reasons below (including funding-related deadlines). You should be aware that whilst effort will be made in cases of genuine urgency, it will not always be possible for the Ethics Committees to meet such requests.

6. SUMMARY OF PROJECT

Describe the purpose, background rationale for the proposed project, as well as the hypotheses/research questions to be examined and expected outcomes. This description should be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.

Background rationale

Achilles tendinopathy (AT) is characterized by pain, impaired performance, and swelling in and around the tendon (Longo et al., 2009). It can be categorized as insertional and non-insertional, two distinct disorders with different underlying pathophysiology and management options (Mafulli et al., 2019). AT is characterized by an increase in the cross-sectional area of the tendon with histological evidence of increased fundamental substance, hypercellularity, and disruption of collagen fibers (Archambault and cols, 1998) (Kader and cols, 2002). Previous studies have shown that these changes in the morphology and composition of the tendon modify its mechanical properties, specifically, decrease its stiffness and Young's modulus (Arya and Kulig, 2010).

The exact etiology is unknown and is considered multifactorial. One of the most accepted theories is that pain perception during early support loading may trigger inhibition of neuromuscular activity leading to a reduction in electromyographic amplitudes (Henriksen and cols, 2007) (Tucker and cols, 2009). Thus, the decrease in the ability to generate force in patients with AT could also be a reflection of the decline in neuromuscular activity observed (Haglund and cols, 1993) (Mahieu and cols, 2006). These motor impairments not only affect the maximal force exerted but also influenced the ability of an individual to produce steady force during submaximal voluntary contractions, this is known as a force steadiness (or torque steadiness) and is an essential aspect of force control (Tracy and Enoka, 2002). Until now, electromyographic studies in these patients have focused on quantifying muscle activation times during specific tasks; consequently, there are no studies that have measured the behavior of motor units (number, discharge rate, variability in the discharge rate, and the amplitude of the action potentials) in individuals with non-insertional AT.

The management of AT lacks evidence-based support, and tendinopathy sufferers are at risk of long-term morbidity with unpredictable clinical outcomes. Although the eccentric exercises have been widely used for the treatment of this pathology, evidence of histological changes following a program of eccentric exercise is lacking, and the mechanisms by which eccentric exercises may help to resolve the pain of tendinopathy remain unclear (Mafulli et al., 2019). Additionally, there are few studies where the effectiveness of eccentric exercises versus concentric exercises has been compared, but in these studies, patients performed the exercises at home, so there was no adequate control over the load used nor the range of motion in which the exercises were performed. Currently, there are no studies that have evaluated the behavior of motor units of calf muscles after applying a protocol based in force steadiness during eccentric or concentric contractions.

Objectives

1) To determine the behavior of motor units of the medial gastrocnemius, lateral gastrocnemius, and soleus muscles, and the mechanical and structural properties of these muscles and the Achilles tendon, in patients with non-insertional AT compared with healthy controls **(Experiment 1).**

2) To establish changes in the behavior of motor units of the medial gastrocnemius, lateral gastrocnemius and soleus muscles and the mechanical and structural properties of these muscles, and the Achilles tendon, after applying a protocol based in force steadiness during eccentric or concentric contractions in individuals with non-insertional AT **(Experiment 2).**

Expected outcomes

We hypothesize that the behavior of the motor units of the gastrocnemius-soleus muscles is altered in individuals with non-insertional AT. Additionally, the application of an exercise protocol based in force steadiness during eccentric or concentric contractions modifies the behavior of the motor units of the gastrocnemius-soleus muscles, but no the mechanical and structural properties of these muscles and the Achilles tendon in individuals with non-insertional AT.

7. CONDUCT OF PROJECT

Please give a description of the research methodology that will be used

Design

Experiment 1: Cross-sectional study
Experiment 2: Randomized prospective study

Participants with non-insertional AT will visit our laboratory during six consecutive weeks for the experimental sessions (at week 1, 3, and 6) and training sessions (2-3 sessions per week). These participants we will be randomly allocated in two different groups, eccentric (ECCT) or concentric (CONT) training. Healthy control participants will visit the CPR Spine laboratory in three occasions at week 1, 3 and 6 to allow comparison with ECCT and CONT regarding: Achilles tendon mechanical and structural properties, gastrocnemius-soleus motor unit behavior, and pain and function. Each experimental session will last approximately 2 hours and each training session 45 minutes.

Setting

The project will take place in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham and all measurements will be collected within the laboratories of the Centre of Precision Rehabilitation for Spinal Pain (CPR spine). This project will last from October 2021 until December 2022.

General procedures

Experimental session

Before the first experimental session, we will contact each participant and send them a document with all relevant information of the study. At the beginning of the first experimental session all participants will provide written consent prior to participation. Then, we will collect anthropometric data (i.e., age, weight, height, and BMI). After, we will apply a battery of questionnaires (see details in the Specific Procedures section).

Mechanical and structural properties

Using ultrasound techniques, we will measure the length, thickness, and cross-sectional area of the Achilles tendon during rest condition. Later on, we will determine the fascicle length, thickness, and pennation angle of the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SO) muscles during rest condition. Then, we will measure the stiffness of the Achilles tendon during rest condition (passive elastography) and during isometric plantarflexion contractions (active elastography).

Training session

For the training sessions, we will measure the isometric plantarflexion maximal voluntary contraction (MVC) of the ECCT and CONT groups every two weeks.
The participants in the ECCT group will be asked to perform the warm-up consisting of three eccentric plantarflexion contractions, and then, the eccentric training protocol. This protocol consists of 3 x 15 eccentric plantarflexion contractions at 50% of the MVC with visual feedback of the exerted force in each contraction. Similarly, participants in the CONT group will perform the warm-up consisting of three concentric plantarflexion contractions, and then, the concentric training protocol. This protocol consists of 3 x 15 concentric plantarflexion contractions at 50% of the MVC. Again, visual feedback of the exerted force in each contraction will be provided. **(Figure 2).**

Follow-up

Participants with non-insertional Achilles tendinopathy will be asked to report their pain level and function by email 3 and 6 months after completing the study. Pain level will be assessed with the Numeral Rating Scale (NRS) and function with the Victorian Institute of Sports Assessment-Achilles (VISA-A) questionnaire.

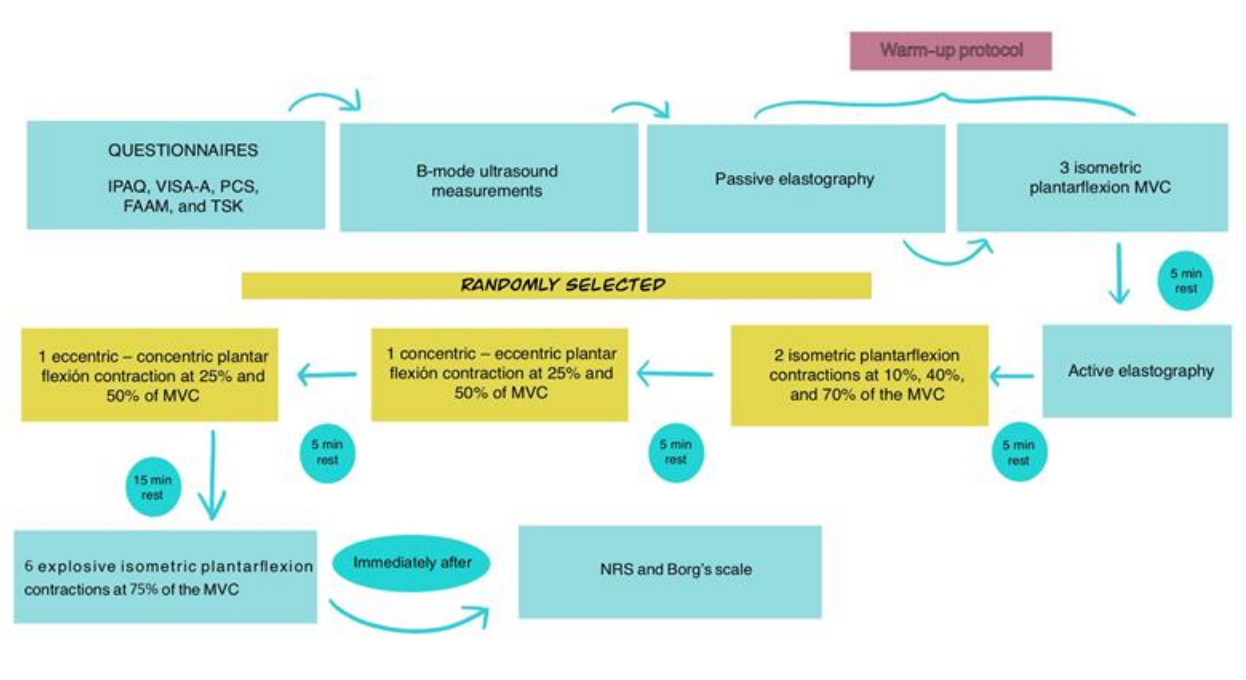

Figure 1. Experimental session design.

From the aforementioned measurements, the following outcome measures will be compared.

- Level of pain (NRS, 0-10)
- Rate of perceived exertion (Category Ratio Scale, 0-11)
- International Physical Activity Questionnaire (IPAQ short form), VISA-A, Foot and Ankle Ability Measure (FAAM), Pain Catastrophizing Scale (PCS), and Tampa Scale for Kinesiophobia (TSK) scores.
- Length, thickness, cross-sectional area, and stiffness of the Achilles tendon
- Fascicle length, thickness, and pennation angle of the calf muscles
- Number of motor units, discharge rate, variability of the discharge rate, and amplitude of the action potentials of the calf muscles.
- Force steadiness (quantified as standard deviation (SD) and coefficient of variation (CV) of force)

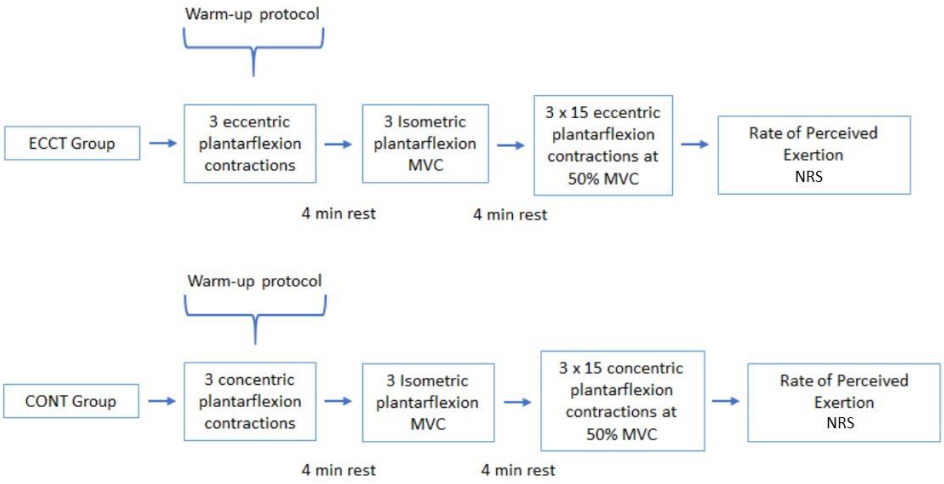

Figure 2. Training session design.

Statistical analysis

Descriptive statistics will be used to interpret the data and it will be presented as mean ± SD. The Shapiro-Wilk Test will be used to check data normality. If the criteria are met, repeated measures analysis of variance (ANOVA) will be used. A combination of different factors; group (ECCT, CONT, and CONTROL) and time (at week 1, 3, and 6) will be used to analyse each variable. Bonferroni corrected t-tests will be used if ANOVA is significant. If data is not normally distributed, relevant nonparametric tests will be used.

Specific Procedures

Questionnaires

At the beginning of each experimental session, all participants will have to fill various questionnaires. They will also have to report the current level of pain using the NRS.

The following questionnaires will be provided to the participants:

- International Physical Activity Questionnaire (IPAQ short form)
- Victorian Institute of Sports Assessment-Achilles (VISA-A)
- Pain Catastrophizing Scale (PCS)
- Foot and Ankle Ability Measure (FAAM)
- Tampa Scale for Kinesiophobia (TSK)

The IPAQ (short form) has reasonable measurement properties for monitoring population levels of physical activity among 18 to 65 years old adults in diverse settings (Craig et al., 2003). The VISA-A questionnaire is recommended to assess pain and stiffness, while FAAM questionnaire is recommended to evaluate the activity and participation in patients with a diagnosis of mid-portion AT (Martin et al., 2018).

At the end of each experimental session, we will use the Rating of Perceived Exertion Category-Ratio Scale (0-11) and the NRS.

Measurement set-up:

For the measurements of the Achilles tendon, MG, LG, and SO muscles, the participants will be placed in a prone position on the CYBEX Norm dynamometer (Computer Sports Medicine Inc., Massachusetts, USA), with their knees extended, and their testing foot tightly strapped on the foot plate. The pelvis will be stabilised with another strap, to avoid compensatory movements. The ankle will be placed at 0˚ of plantarflexion and the axis of the dynamometer will be aligned with the presumed axis of the ankle (the inferior tip of the lateral malleolus) (Lundberg et al., 1989; Breuning et al., 2008) **(Figure 3).**

Figure 3. Ankle torque (force) measurements setup.

Ultrasound measurements

All ultrasound images will be obtained using an ultrasound imaging device equipped with SWE (LOGIQ S8 GE Healthcare, Chicago, USA). For the B-mode measurements, we will use a 16-linear array probe, and for the shear wave elastography (SWE) a 9-linear array probe. The tendon ultrasound images will be obtained with the probe oriented in the sagittal and transversal planes, and perpendicularly to the skin. The muscle ultrasound images will be acquired with the probe oriented in the sagittal plane, and perpendicularly to the skin, according to the recommendations of Bolsterlee et al., 2016.

After the participants answer the questionnaires, we will measure some structural and mechanical properties of the Achilles tendon. First, with the probe in the sagittal plane, we will measure tendon length according to the recommendations of Barfod et al., 2015. Then, we will measure the thickness and stiffness (passive elastography) of the Achilles tendon in the sagittal plane, and the cross-sectional area in the transversal plane. Second, we will assess some structural properties of the MG, LG, and SO muscles. With the probe oriented in the sagittal plane, we will measure the fascicle length, thickness, and pennation angle during rest conditions. Then, we will ask the participants to perform three isometric plantarflexion MVCs. Finally, with the probe in the sagittal plane, we will measure the stiffness of the Achilles tendon during isometric plantarflexion contractions at 10% and 20% of the MVC (active elastography).

HDEMG and torque recording

Two-dimensional (2D) adhesive grids (SPES Medica, Salerno, Italy) of 13 x 5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm) will be used to record the HDEMG signals during the experimental sessions. We will use one electrode grid for each muscle (MG, LG, and SO). The position of the electrodes will follow specific guidelines (Barbero et al. 2012).

After the ultrasound measurements, we will measure the EMG activity of the MG, LG, and SO muscles. The participants will perform two isometric plantarflexion contractions at 10%, 40%, and 70% of the MVC. After five minutes of rest, they will perform one concentric-eccentric plantarflexion contraction at 25%, and 50% of the MVC. Subsequently, after five minutes, the volunteers will perform one eccentric-concentric plantarflexion contractions at 25%, and 50% of the MVC (the order of the isometric, concentric, and eccentric contractions will be randomly selected). Between each MVC the volunteers will have a minute of rest. The highest MVC value will be used as the reference maximal force. Finally, after 15 minutes of rest, the participants will be asked to perform 6 explosive isometric plantarflexion contractions.

Warm-up protocol
Participants will perform three isometric plantarflexion contractions at 25% of perceived maximal voluntary force (MVF) during 10 s, each separated by 15 s.

Isometric contractions
Participants will be asked to perform two sustained isometric plantarflexion contractions at 10%, 40%, and 70% of the isometric MVC.

Range of motion and angular speed
For the dynamic contractions, the range of motion will be set at the total of 40˚ (0˚ dorsiflexion and 40˚ plantarflexion) and the angular speed will be set at 5˚/s.

Dynamic contractions
Participants will perform 1 concentric-eccentric plantar flexion contraction at 25% and 50% of the MVC. Additionally, participants will perform 1 eccentric-concentric plantar flexion contraction at 25% and 50% of the MVC.

Explosive contractions
Participants will perform six sustained isometric explosive plantarflexion contractions at 75% of the isometric MVC.

During all contractions, visual feedback of the target force output will be provided via a computer monitor. Prior to the contractions, the participants will be instructed to match the force output as closely as possible to the target force for the full duration of the contraction. No verbal encouragement will be provided during testing. Moreover, if some of the volunteers experience moderate pain (>6 NRS) during the contractions, we will give them additional time to rest. If the pain persists, we will reduce the load by 20% during the contractions. Nevertheless, if after this adaptation, the pain intensity is maintained, we will end the measurements.

8. DOES THE PROJECT INVOLVE PARTICIPATION OF PEOPLE OTHER THAN THE RESEARCHERS AND SUPERVISORS?

Yes ☒ No ☐

Note: 'Participation' includes both active participation (such as when participants take part in an interview) and cases where participants take part in the study without their knowledge and consent at the time (for example, in crowd behavior research).

If you have answered NO please go to Section 18. If you have answered YES to this question please complete all the following sections.

9. PARTICIPANTS AS THE SUBJECTS OF THE RESEARCH

Describe the number of participants and important characteristics (such as age, gender, location, affiliation, level of fitness, intellectual ability etc.). Specify any inclusion/exclusion criteria to be used.

Sample characteristics

According to power calculations, a total of 26 individuals with non-insertional Achilles tendinopathy (ECCT group=13, and CONT group= 13) and 13 healthy controls will be required for this study. This sample size considers a power=0.80, alpha=0.01, 25% loss of participants and an effect size (d) of 1.7 calculated from the study of Yu et al. (Yu et al., 2013), where the authors compared reductions in pain after an 8-week concentric and eccentric training protocol in individuals with non-insertional Achilles tendinopathy.

Inclusion criteria (Achilles tendinopathy groups)

- Males and females aged between 18 and 55 years old
- Non-insertional AT will be confirmed by case history (eg, morning stiffness, pain after loading, altered functional status, self-reported calf muscle weakness or impaired endurance) and a careful physical examination (eg, pain with palpation of the mid portion of the Achilles tendon, focal or diffuse swelling, muscle weakness)
- Age and gender matched control participants without history of AT or lower limb disorders.
- Participants should not be receiving treatment during the course of the study.

Inclusion criteria (Healthy control group)

- Males and females aged between 18 and 55 years old
- Pain/injury in the lower limbs within the previous 6 months
- No history of Achilles tendinopathy

10. RECRUITMENT

Please state clearly how the participants will be identified, approached and recruited. Include any relationship between the investigator(s) and participant(s) (e.g. instructor-student).

Note: Attach a copy of any poster(s), advertisement(s) or letter(s) to be used for recruitment.

Participants who meet the criteria, will be recruited from the University of Birmingham staff / student population and Birmingham's community. Recruitment methods will consist of information leaflets posted throughout the University of Birmingham's departments, via e-mails, via social media (e.g. Facebook, Instagram and Twitter) and word of mouth.

11. CONSENT

a) Describe the process that the investigator(s) will be using to obtain valid consent. If consent is not to be obtained explain why**.** If the participants are minors or for other reasons are not competent to consent, describe the proposed alternate source of consent, including any permission / information letter to be provided to the person(s) providing the consent.

All information regarding the experiment will be provided in the 'Participant Information Sheet' (see attached) which will include a description of the study, inclusion and exclusion criteria, risk and benefits. The information about the study will also be provided verbally by an investigator. This information will be provided to the interested participants before they attend to the first experimental session. Written informed Consent (see attached) will be obtained after the study

Note: Attach a copy of the Participant Information Sheet (if applicable), the Consent Form (if applicable), the content of any telephone script (if applicable) and any other material that will be used in the consent process.

b) Will the participants be deceived in any way about the purpose of the study? **Yes** ☐ **No** ☒

If yes, please describe the nature and extent of the deception involved. Include how and when the deception will be revealed, and who will administer this feedback.

12. PARTICIPANT FEEDBACK

Explain what feedback/ information will be provided to the participants after participation in the research. (For example, a more complete description of the purpose of the research, or access to the results of the research).

If participants show interest in the study's findings, a summary report of our research findings will be sent to them via e-mail. Additionally, the researchers will be happy to answer any questions regarding the purpose of the study and/or measurement procedures via e-mail.

13. PARTICIPANT WITHDRAWAL

a) Describe how the participants will be informed of their right to withdraw from the project.

At any time up to two weeks post data collection, participants will have the right to withdraw from the project without any justification. This will be clearly reported on the Consent Form and Participant Information sheet and will be also expressed to the participants verbally.

b) Explain any consequences for the participant of withdrawing from the study and indicate what will be done with the participant's data if they withdraw.

In case of participants' withdrawal, all data acquired till that moment will be deleted and will not be used for analysis. Participants will be informed about this and their withdrawal will not result in any consequences.

14. COMPENSATION

Will participants receive compensation for participation?

i) Financial **Yes** ☒ **No** ☐

ii) Non-financial **Yes** ☒ **No** ☐

If **Yes** to **either** i) or ii) above, please provide details.

Participants with non-insertional AT will be compensated with £60, only for the assistance to the experimental sessions (3 sessions - £20 per session). If they are students, they will be offered paid compensation (£60) or 6 research hours. Each experimental session will last approximately 2 h.

Asymptomatic controls will not receive monetary compensation, but they will be offered 6 research hours for participating in the experimental sessions if they are students.

If participants choose to withdraw, how will you deal with compensation?

If participants withdraw prior to completion of the full study (i.e. 3 experimental sessions), they will be compensated as following:

- Withdrawal after session 1 (2 research hours) or (£20)
- Withdrawal after session 2 (4 research hours) or (£40)

15. CONFIDENTIALITY

a) Will all participants be anonymous? **Yes** ☐ **No** ☒

b) Will all data be treated as confidential? **Yes** ☒ **No** ☐

Note: Participants' identity/data will be confidential if an assigned ID code or number is used, but it will not be anonymous. Anonymous data cannot be traced back to an individual participant.

Describe the procedures to be used to ensure anonymity of participants and/or confidentiality of data both during the conduct of the research and in the release of its findings.

Participant's confidentiality will be kept all times, by assigning each one of them to a randomised ID number. This number will be used to collect and store all data accordingly. The necessary personal information that will be collected on the consent form during this study (e.g. participant's name, telephone number and e-mail) will be securely stored and locked in a cabinet in Dr Martinez-Valdes' Office. Only the researchers will have access to it.

All the collected data from measurements will be saved on a secure (password protected) strictly confidential University of Birmingham server. The data will only be shared with the participant, investigator and research team. Personal data will not be disclosed to a third party.

After data collection, all the data will be randomized and anonymised to the researchers with special software "Blindr" (https://github.com/U8NWXD/blindr) which allows to randomize filenames reversely in order to avoid bias when analysing the results.

Data will be kept for 10 years in line with University of Birmingham Regulations.

Please see attached the Participant Information Sheet.

If participant anonymity or confidentiality is not appropriate to this research project, explain, providing details of how all participants will be advised of the fact that data will not be anonymous or confidential.

16. STORAGE, ACCESS AND DISPOSAL OF DATA

Describe what research data will be stored, where, for what period of time, the measures that will be put in place to ensure security of the data, who will have access to the data, and the method and timing of disposal of the data.

All electronic data will be securely stored (in an electronic format) on the secure University of Birmingham server. Only the researchers will be able to access the data. Access to data will require a password and all data will be managed in accordance with the General Data Protection Regulation (GDPR).
After the completion of all measurements, all consent forms containing personal data will be securely stored in a locked filing cabinet in Dr Martinez-Valdes' office with authorised access only to the investigators.
The acquired data will be securely stored for 10 years according to University's Regulations.

17. *OTHER APPROVALS REQUIRED?* ***E.G. CRIMINAL RECORDS BUREAU (CRB) CHECKS OR NHS R&D APPROVALS.***

☐ YES ☒ NO ☐ NOT APPLICABLE

If yes, please specify.

18. SIGNIFICANCE/BENEFITS

Outline the potential significance and/or benefits of the research

The study of motor units is an area in continuous development, which in recent years has allowed a deeper understanding of the neural mechanisms involved in muscle contractions. However, much of the research in this area has focused on the normal neurophysiological study of muscle rather than its relationship with alterations of the musculoskeletal system, so this research will contribute to generate applied knowledge in a pathological condition.

This is the first study to determine the behavior of motor units of the medial gastrocnemius, lateral gastrocnemius, and soleus muscles, and the mechanical and structural properties of these muscles and the Achilles tendon in individuals with non-insertional AT. Additionally, this study is the first to address if a protocol of 6 weeks based in force steadiness during eccentric or concentric contractions could change the behavior of motor units of the calf muscles, and the mechanical and structural properties of these muscles and the Achilles tendon in individuals with this disorder.

19. RISKS

a) Outline any potential risks to **INDIVIDUALS**, including research staff, research participants, other individuals not involved in the research and the measures that will be taken to minimise any risks and the procedures to be adopted in the event of mishap

We believe that the risk from the procedures proposed within this project is very small. All participants will be advised that they can stop the experiment at any time.

Non-invasive mounting/attaching procedures of surface electrodes include slight discomfort from minor abrasion of the skin area. Prior electrode placement, the skin will need to be shaved to remove any hair. However, single use disposable razors will be used thus there is no expected risk from this procedure.

Concerning the risks during the MVCs, we will only measure maximal isometric voluntary contractions, which produces less stress and tension in the tendon compared with maximal dynamic voluntary contractions. Additionally, we will measure maximal isometric voluntary contractions at three time points throughout the study (at week 1, 3, and 6), and we will use this value as a reference in all the different types of contractions. Furthermore, we will use a limited range of motion from 0° to 40° in the dynamic contractions to avoid pain or discomfort at the end of the range of motion.

Participants will be informed that they may experience some mild discomfort while performing the contractions. Because of their simplicity, the motor tasks accomplished by the participants will not lead to anxiety or stress higher than that already expected from everyday life. To deal with the above, appropriate rest time will be provided throughout the experimental trials. Additionally, extra rest periods will be given to the participants at any time, if they need it. Moreover, if some of the volunteers experience moderate pain (>6 NRS) during the contractions, we will give them additional time to rest. If the pain persists, we will reduce the load by 20% during the contractions. Nevertheless, if after this adaptation, the pain intensity is maintained, we will end the session.

Covid-related risks protocol

The following pertains to entry and exit from the building/labs and whilst in attendance whereby there is a potential opportunity for close contact to transfer certain bodily fluids, predominantly in the form of respiratory droplets via sneezing, coughing and/or speaking.

1) On recruitment, the experimenter is to inform the prospective participant that they will be contacted 24 hours by experimenter prior to attendance to confirm that they are symptom free using the **NHS COVID-19 Track and Trace App,** or if this is not possible, **a COVID-19 Symptom Screening questionnaire** (i.e. https://www.sdcep.org.uk/wp-content/uploads/2020/05/Patient-COVID-19-screening-250520.pdf). Strictly no entry will be permitted for positive COVID-19 symptom screening. These individuals must be informed with strict instruction not to attend. The testing, if appropriate, will be rescheduled after a minimum of 14 days symptom free have lapsed. Similarly, participants who have been advised to isolate via a COVID-19 tracking system must not enter SportExR until their isolation period has expired.

2) The protocol will be first piloted using people from the within UoB community (i.e., staff, postdocs, PG/UG students) before external participants are recruited and brought in for testing.

3) Upon arrival to SportExR, all participants will be re-screened using the COVID-19 Symptom Screening questionnaire. Unsuccessful screened participants must be politely asked to leave. A Researcher will meet the participant in the atrium of SportExR, wearing a face mask/visor, and administer this check-in screening questionnaire.

4) Participants will be asked to wash their hands on arrival at the building at the relevant station (https://canvas.bham.ac.uk/courses/45482) and wear a mask upon entry until departure (one will be provided by researcher on arrival). Where appropriate (i.e. medical exemption), a medical visor (i.e. face shield) will be provided with instruction on how to apply whilst behind Perspex glass with the SportExR atrium. Participant is advised to wash hands before and after experiment.
5) Participant to be greeted by Researcher at the Entrance of SportExR and given a brief induction informing them of the precautions required. They will then be carefully guided to the lab and form the lab to exit, maintaining at least 2m distance at all times, to ensure safe and effective movement through SportExR complying with infection control and encourage avoidance of unnecessary touching of objects.

6) A maximum of 3 people (i.e. 1 participant and 2 experimenters) will be permitted at one time when the experiment is taking place (described below).

7) Experimenters will wear medical visors/face masks throughout.

8) Experimenters will also wear single use gloves during experiment and cleaning.

Further details can be found in the risk assessment form.

b) Outline any potential risks to **THE ENVIRONMENT and/or SOCIETY** and the measures that will be taken to minimise any risks and the procedures to be adopted in the event of mishap.

Not applicable

20. ARE THERE ANY OTHER ETHICAL ISSUES RAISED BY THE RESEARCH?

Yes ☐ **No** ☒

If yes, please specify

21. EXPERT REVIEWER/OPINION

You may be asked to nominate an expert reviewer for certain types of project, including those of an interventional nature or those involving significant risks. If you anticipate that this may apply to your work and you would like to nominate an expert reviewer at this stage, please provide details below.

Name
Contact details (including email address)
Brief explanation of reasons for nominating and/or nominee's suitability

22. CHECKLIST

Please mark if the study involves any of the following:

- Vulnerable groups, such as children and young people aged under 18 years, those with learning disability, or cognitive impairments ☐
- Research that induces or results in or causes anxiety, stress, pain or physical discomfort, or poses a risk of harm to participants (which is more than is expected from everyday life) ☒
- Risk to the personal safety of the researcher ☐
- Deception or research that is conducted without full and informed consent of the participants at time study is carried out ☐
- Administration of a chemical agent or vaccines or other substances (including vitamins or food substances) to human participants. ☐
- Production and/or use of genetically modified plants or microbes ☐
- Results that may have an adverse impact on the environment or food safety ☐
- Results that may be used to develop chemical or biological weapons ☐

Please check that the following documents are attached to your application.

	ATTACHED	NOT APPLICABLE
Recruitment advertisement	☒	
Participant information sheet	☒	
Consent form	☒	
Questionnaires	☒	
Interview Schedule		☒

23. DECLARATION BY APPLICANTS

I submit this application on the basis that the information it contains is confidential and will be used by the

University of Birmingham for the purposes of ethical review and monitoring of the research project described

herein, and to satisfy reporting requirements to regulatory bodies. The information will not be used for any

other purpose without my prior consent.

I declare that:

- The information in this form together with any accompanying information is complete and correct to the best of my knowledge and belief and I take full responsibility for it.
- I undertake to abide by University Code of Practice for Research (http://www.as.bham.ac.uk/legislation/docs/COP_Research.pdf) alongside any other relevant professional bodies' codes of conduct and/or ethical guidelines.
- I will report any changes affecting the ethical aspects of the project to the University of Birmingham Research Ethics Officer.
- I will report any adverse or unforeseen events which occur to the relevant Ethics Committee via the University of Birmingham Research Ethics Officer.

Name of principal investigator/project supervisor:	Eduardo Martinez-Valdes
Date:	13/06/2022

Please now save your completed form, print a copy for your records, and then email a copy to the Research Ethics Officer, at aer-ethics@contacts.bham.ac.uk. As noted above, please do not submit a paper copy.

Appendix 8. Consent form asymptomatic individuals

Consent Form

Study Title	Neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy		
Participant Name:		**Date:**	
Researcher Name:		**Ethics Number:**	

This information is being collected as part of a research project, which investigates the relationship between the neuromuscular control of the calf muscles and some characteristics of these muscles and the Achilles tendon in individuals with and without Achilles tendinopathy.

The research will be conducted at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. The information that you supply and that which may be collected as part of the research project will be entered into a filing system or database and will only be accessed by authorised personnel involved in the project. The information will be retained by the University of Birmingham and will only be used for the purpose of research, and statistical and audit purposes. By supplying this information, you are consenting to the University storing your information for the purposes stated above. The information will be processed by the University of Birmingham in accordance with the provisions of the Data Protection Act 2018. No identifiable personal data will be published.

This section to be completed by the participant:

Please initial the boxes at the end of each statement if you agree with it.

1. I confirm that I have read and understood the Participant Information Sheet for the above study. I have had the opportunity to ask questions and these have all been answered satisfactorily ☐
2. I. understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, up to two weeks after my last visit to the lab. ☐
3. I agree to the storage and use of my data for the purposes of this research study. ☐
4. I confirm that I have read and understand the paragraph relating to COVID-19 related risks in the participant information leaflet for this study and will abide by the measures put in place by the University of Birmingham. I have had the opportunity to ask questions if necessary and have had these answered satisfactorily. ☐
5. Based on the above, I agree to take part in this research study.

Signed:

Name in capitals:

Date:

This section to be completed by the researcher

I certify that this participant has read, properly completed and signed the screening and consent forms, witnessed by myself:

Signed: Date:

By supplying this information you are consenting to the University storing your information for the purposes of the stated research study. The information will be processed by the University of Birmingham in accordance with the provisions of the Data Protection Act 2018. No identifiable personal data will be published

Appendix 9. Consent form individuals with NIAT.

Consent Form

Study Title	Neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy		
Participant Name:		**Date:**	
Researcher Name:		**Ethics Number:**	

This information is being collected as part of a research project, which investigates the relationship between the neuromuscular control of the calf muscles and some characteristics of these muscles and the Achilles tendon in individuals with Achilles tendinopathy. Additionally, we want to determine which type of exercise protocol has better results in the treatment of this condition.

The research will be conducted at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. The information that you supply and that which may be collected as part of the research project will be entered into a filing system or database and will only be accessed by authorised personnel involved in the project. The information will be retained by the University of Birmingham and will only be used for the purpose of research, and statistical and audit purposes. By supplying this information, you are consenting to the University storing your information for the purposes stated above. The information will be processed by the University of Birmingham in accordance with the provisions of the Data Protection Act 2018. No identifiable personal data will be published.

This section to be completed by the participant:

Please initial the boxes at the end of each statement if you agree with it.

6. I confirm that I have read and understood the Participant Information Sheet for the above study. I have had the opportunity to ask questions and these have all been answered satisfactorily ☐
7. I. understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, up to two weeks after my last visit to the lab. ☐
8. I agree to the storage and use of my data for the purposes of this research study. ☐
9. I confirm that I have read and understand the paragraph relating to COVID-19 related risks in the participant information leaflet for this study and will abide by the measures put in place by the University of Birmingham. I have had the opportunity to ask questions if necessary and have had these answered satisfactorily. ☐
10. Based on the above, I agree to take part in this research study.

Signed:

Name in capitals:

Date:

This section to be completed by the researcher

I certify that this participant has read, properly completed and signed the screening and consent forms, witnessed by myself:

Signed:

Date:

By supplying this information, you are consenting to the University storing your information for the purposes of the stated research study. The information will be processed by the University of Birmingham in accordance with the provisions of the Data Protection Act 2018. No identifiable personal data will be published

Appendix 10. Participants info sheet (Asymptomatic)

Neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy.

Invitation

You are being invited to take part in a research study. Before you decide to participate, it is important you understand why the research is being done and what it will involve. Please take the time to read the following information sheet carefully and discuss it with the researcher or others if you wish.

What is the purpose of the study?

This study investigates the relationship between the neuromuscular control of the calf muscles and some characteristics of these muscles and the Achilles tendon in individuals with and without Achilles tendinopathy. The structural properties of the tendon will be assessed using ultrasound imaging, the electrical activity of your muscles will be evaluated using a non-invasive technique called high-density surface electromyography (HDEMG), and the calf muscle force will be assessed with a special device (dynamometer), designed to measure the movements of the ankle.

Why have I been chosen?

You have been chosen because we understand that you, as a healthy participant, might represent a normal individual of the population. The inclusion criteria to take part in the study is to be between 18 and 55 years old. The exclusion criteria include history of systemic or inflammatory conditions, chronic respiratory or neurological problems, cardiovascular diseases, pain/injury within the lower limbs in the previous six months, lower limb surgery, and pregnancy.

We will ask you to complete a brief screening assessment to ensure you are eligible to participate. This will be performed by an experienced researcher and will include questions about your general health.

Do I have to take part?

You are free to decide whether you participate or not. You will be given an information sheet to keep, and you will be asked to complete a brief screening questionnaire and answer some questions. If you meet the criteria described above, you will be invited to sign a consent form before taking part in the study. If you do agree to take part, you are free to withdraw at any time up to 2 weeks following the data collection without giving a reason by communicating with any

member of the research team by email. If you withdraw from the study, your personal data and all the data acquired until the point of withdrawal will be deleted and destroyed. Furthermore, the researchers will provide further information about the purpose of this study if you want, and you will be invited to leave your email address to receive the publication of the study results.

Healthy participants will visit our laboratory once for the experimental session. The experimental session will last approximately 2 hours, and if you are interested, you will receive 2 research hours for completing the session.

Due to the Covid-19 pandemic, we were forced to reduce the time of the experimental session. Thus, we will send you an email with a set of questionnaires 24 h before the experimental session. Please remember it is important to answer these questionnaires before you arrive at the lab.

Covid-19

For your safety we need to perform a covid-19 screening prior participation. Therefore, you will be contacted 24 hours prior to attendance to confirm that you are symptom free using the NHS COVID-19 Track and Trace App, or if this is not possible, a COVID-19 Symptom Screening questionnaire (i.e. https://www.sdcep.org.uk/wp-content/uploads/2020/05/Patient-COVID-19-screening-250520.pdf). Strictly no entry will be permitted for positive COVID-19 symptom screening. If you show positive COVID-19 symptoms, the testing can be rescheduled after a minimum of 14 days symptom-free have lapsed. Similarly, if you have been advised to isolate via a COVID-19 tracking system you must not enter the University building until your isolation period has expired.

Upon arrival to the School of Sport, Exercise and Rehabilitation Sciences, you will be re-screened using the COVID-19 Symptom Screening questionnaire in the atrium. If your screening is unsuccessful you will be politely asked to leave.

You will be asked to wash your hands on arrival at the building and wear a mask upon entry until departure (one will be provided by us on arrival). Where appropriate (i.e. medical exemption), a medical visor (i.e. face shield) will be provided with instruction on how to apply whilst behind Perspex glass. You are advised to wash hands before and after experiment.

We will provide a brief induction about the precautions required. We will carefully guide you to the laboratory and form the lab to exit, maintaining at least 2m distance at all times, to ensure safe and effective movement through the building complying with infection control and encourage avoidance of unnecessary touching of objects.

What will happen if I decide to take part?

After, covid-19 screening, we will move to the Centre of Precision Rehabilitation for Spinal Pain Laboratory (Room 218). Before you start with the experiment, you will be asked to read and sign the informed consent form.

The experimental session will include:

- Collection of the anthropometric data (i.e., age, height, weight, and body mass index).
- Measurements of the structural properties of the calf muscles and Achilles tendon during rest condition.
- Perform three maximal (full strength) isometric (you will push against resistance, but your ankle will not move) plantarflexion (downward movement of the foot away from the leg, like pressing the accelerator pedal in a car) contractions and measurements of the structural properties of the Achilles tendon during isometric contractions.
- Assessment of the electromyographic activity of the calf muscles during isometric plantarflexion contractions at different loads.
- Measurements of the electromyographic activity of the calf muscles during eccentric (i.e., you will be asked to try to do a plantarflexion movement, while the isokinetic device will move your foot to the opposite direction) plantarflexion contractions at different loads.
- Assessment of the electromyographic activity of the calf muscles during concentric (i.e., you will be asked to try to do a plantarflexion movement, while the isokinetic device will move your foot in the same direction) plantarflexion contractions at different loads.
- Evaluation of the electromyographic activity of your calf muscles during explosive (as hard and fast as you can) contractions.
- Complete questionnaires about rate of perceived exertion and the current level of pain.

We will ask you to bring shorts that leave your legs accessible and to avoid any strenuous exercise (48 h) before the experimental sessions.

What are the potential benefits of taking part?

This study will provide important information about some properties of the calf muscles and the Achilles tendon, and the relationship with the neuromuscular control of these muscles during different types of contractions. You will also get information regarding your calf muscles strength and control of force.

What are the potential risks of taking part?

The potential risks from the procedures proposed within this project are minimal. Non-invasive mounting/attaching procedures of surface electrodes include that the skin of three small areas of the leg needs to be shaved (to remove any hair) and then cleaned with abrasive paste. This could produce slight discomfort from minor abrasion of the skin area. Finally, you might feel some level of muscle soreness up to 24 to 48 hours after the experiment, this is a normal response to exercise (similar to the muscle soreness felt after going to the gym), and your muscles will recover fully after this period.

In the unlikely event that you suffer an injury, you should report this situation to any member of the research team, and then, we will contact an experienced physiotherapist for initial assessment. The physiotherapist will contact you by phone and monitor your symptoms over the next three days. Furthermore, if you do not show any improvement over this period, we will schedule a clinical evaluation with the members of the research team, and we will stop your participation in our study.

Furthermore, we have applied a series of measures in order to prevent covid-19 transmission. Therefore, both you and the research team will:

- Use protective personal equipment
- Wash hands before and after the experimental procedures
- Maintain a 2m distance for most of the experiment

In addition, we will make sure that the experimental area and equipment is adequately sanitized before and after the measurements.

You will be provided with instructions on how to enter the building and will be informed about all the preventive measures taking place prior to the experiments.

Will my participation be confidential?

All information collected on you will be kept strictly confidential. Personal information will be retained, but only available to the researchers using password protected files. Data will be kept for 10 years in accordance with the EU General Data Protection Regulation 2018 and the University of Birmingham Research Guidelines. All data for presentation will be anonymized and aggregated, so your identity will not be revealed in any way. You can withdraw your data until two weeks from data collection.

What will happen at the end of the research study?

The findings from this study will be presented in the form of presentations and scientific papers as appropriate. All data for presentation will be anonymized, which means your identity will not be revealed in any way.

Does the study follow ethics procedures?

This study underwent the ethical review processes of the University of Birmingham and received official approval from the University Ethics Committee.

Who is organizing and funding the research?

The study has been designed and organized by Ignacio Contreras-Hernandez and is overseen by Dr. Eduardo Martinez-Valdes, Lecturer in Spinal and Musculoskeletal Physiotherapy (

What if I have a problem or concern?

If you have doubts about any aspect of this study, please speak with Dr. Eduardo Martinez-Valdes or Ignacio Contreras-Hernandez. If you still have any concerns, you could also talk to the Head of School, Dr. Sarah Aldred (

For further information please contact Ignacio Contreras Hernandez

Ignacio Contreras-Hernandez Centre of Precision Rehabilitation for Spinal Pain School of Sports, Exercise and Rehabilitation Sciences University of Birmingham B15 2TT	

Thank you for your interest in participating in our study!

Appendix 11. Participants info sheet (Individuals with NIAT)

Neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy.

Invitation

You are being invited to take part in a research study. Before you decide to participate, it is important you understand why the research is being done and what it will involve. Please take the time to read the following information sheet carefully and discuss it with the researcher or others if you wish.

What is the purpose of the study?

This study investigates the relationship between the neuromuscular control of the calf muscles and some characteristics of these muscles and the Achilles tendon in individuals with Achilles tendinopathy. Additionally, we want to determine which type of exercise protocol has better results in this condition. The structural properties of the tendon will be assessed using ultrasound imaging, the electrical activity of your muscles will be evaluated using a non-invasive technique called high-density surface electromyography (HDEMG), and the calf muscle force will be assessed with a special device (dynamometer), designed to measure the movements of the ankle.

Why have I been chosen?

You have been chosen because we understand that you have experienced pain in the Achilles tendon at least for the last three months, or you have a diagnostic of non-insertional Achilles tendinopathy. The inclusion criteria to take part in the study is to be between 18 and 55 years old. The exclusion criteria include history of systemic or inflammatory conditions, chronic respiratory or neurological problems, cardiovascular diseases, lower limb surgery, and pregnancy.

We will ask you to complete a brief screening assessment to ensure you are eligible to participate. This will be performed by an experienced researcher and will include questions about your general health.

Do I have to take part?

You are free to decide whether you participate or not. You will be given an information sheet to keep, and you will be asked to complete a brief screening questionnaire and answer some questions. If you meet the criteria described above, you will be invited to sign a consent form before taking part in the study. If you do agree to take part, you are free to withdraw at any time up to 2 weeks following the data collection without giving a reason by communicating with any member of the research team by email. If you withdraw from the study, your personal data and all the data acquired until the point of withdrawal will be deleted and destroyed. Furthermore, the researchers will provide further information about the purpose of this study if you want, and you will be invited to leave your email address to receive the publication of the study results.

Participants with non-insertional Achilles tendinopathy will visit our laboratory over six consecutive weeks for the experimental sessions (at week 1, 3, and 6) and training sessions (2-3 sessions per week). Each experimental session will last approximately 2 hours, and you will be given £90 or 9 research hours for completing the three experimental sessions. However, if you decide to withdraw during the experimental sessions, we will proportionally give you the number of research hours or money that you dedicated to the experiments. Each training session will last approximately 30-40 minutes, but we will not compensate for your participation in these sessions.

Finally, you will be contacted by email 3 and 6 months after completing the study to report your level of pain and function.

Due to the Covid-19 pandemic, we were forced to reduce the time of the experimental sessions. Thus, we will send you an email with a set of questionnaires 24 h before the experimental sessions. Please remember it is important to answer these questionnaires before you arrive at the lab.

Covid-19

For your safety we need to perform a covid-19 screening prior participation. Therefore, you will be contacted 24 hours prior to attendance to confirm that you are symptom free using the NHS COVID-19 Track and Trace App, or if this is not possible, a COVID-19 Symptom Screening questionnaire (i.e. https://www.sdcep.org.uk/wp-content/uploads/2020/05/Patient-COVID-19-screening-250520.pdf). Strictly no entry will be permitted for positive COVID-19 symptom screening. If you show positive COVID-19 symptoms, the testing can be rescheduled after a minimum of 14 days symptom-free have lapsed. Similarly, if you have been advised to isolate via a COVID-19 tracking system you must not enter the University building until your isolation period has expired.

Upon arrival to the School of Sport, Exercise and Rehabilitation Sciences, you will be re-screened using the COVID-19 Symptom Screening questionnaire in the atrium. If your screening is unsuccessful you will be politely asked to leave.

You will be asked to wash your hands on arrival at the building and wear a mask upon entry until departure (one will be provided by us on arrival). Where appropriate (i.e. medical exemption), a medical visor (i.e. face shield) will be provided with instruction on how to apply whilst behind Perspex glass. You are advised to wash hands before and after experiment.

We will provide a brief induction about the precautions required. We will carefully guide you to the laboratory and form the lab to exit, maintaining at least 2m distance at all times, to ensure safe and effective movement through the building complying with infection control and encourage avoidance of unnecessary touching of objects.

What will happen if I decide to take part?

After, covid-19 screening, we will move to the Centre of Precision Rehabilitation for Spinal Pain Laboratory (Room 218). Before you start with the experiment, you will be asked to read and sign the informed consent form (Session 1 only).

The experimental sessions will include:

- Collection of the anthropometric data (i.e., age, height, weight, and body mass index).
- Measurements of the structural properties of the calf muscles and Achilles tendon during rest condition.
- Perform three maximal (full strength) isometric (you will push against resistance, but your ankle will not move) plantarflexion (downward movement of the foot away from the leg, like pressing the accelerator pedal in a car) contractions and measurements of structural properties of the Achilles tendon during isometric contractions.
- Assessment of the electromyographic activity of the calf muscles during isometric plantarflexion contractions at different loads.
- Measurements of the electromyographic activity of the calf muscles during eccentric (i.e., you will be asked to try to do a plantarflexion movement, while the isokinetic device will move your foot to the opposite direction) plantarflexion contractions at different loads.
- Assessment of the electromyographic activity of the calf muscles during concentric (i.e., you will be asked to try to do a plantarflexion movement, while the isokinetic device will move your foot in the same direction) plantarflexion contractions at different loads.
- Evaluation of the electromyographic activity of your calf muscles during explosive (as hard and fast as you can) contractions.
- Complete questionnaires about rate of perceived exertion and the current level of pain.

The training sessions will include:

- Perform a warm-up protocol consisting of three eccentric or concentric plantarflexion contractions.
- Perform three maximal isometric plantarflexion contractions (every two weeks)
- Complete the eccentric or concentric training protocol. The eccentric protocol consists of 4 x 15 eccentric plantarflexion contractions at 50% of the maximal force with visual feedback of the exerted force in each contraction. Similarly, the concentric protocol will include 4 x 15 repetitions concentric plantarflexion contractions at 50% of the maximal force with visual feedback of the exerted force in each contraction.
- Complete questionnaires about rate of perceived exertion and the current level of pain.

We will ask you to bring shorts that leave your legs accessible and to avoid any strenuous exercise (48 h) before the experimental sessions.

What are the potential benefits of taking part?

This study will provide important information about some properties of the calf muscles and the Achilles tendon, and the relationship with the neuromuscular control of these muscles during different types of contractions. Additionally, we will get a depth understanding of the effects of two different exercise protocols in this condition. Moreover, you will also get information regarding your calf muscles strength and control of force.

What are the potential risks of taking part?

The potential risks from the procedures proposed within this project are minimal. Non-invasive mounting/attaching procedures of surface electrodes include that the skin of three small areas of the leg needs to be shaved (to remove any hair) and then cleaned with abrasive paste. This could produce slight discomfort from minor abrasion of the skin area. You can feel up to moderate tendon pain during the first training sessions, which is normal during the treatment of tendinopathy. We will make sure that it does not reach levels equal or greater than 6 out of 10 during the sessions. Finally, you might feel some level of muscle or tendon soreness up to 24 to 48 hours after the experiment. This type of discomfort is usually observed after the first sessions in patients with non-insertional Achilles tendinopathy.

In the unlikely event that you suffer an injury, you should report this situation to any member of the research team, and then, we will contact an experienced physiotherapist for initial assessment. The physiotherapist will contact you by phone and monitor your symptoms over the next three days. Furthermore, if you do not show any improvement over this period, we will schedule a clinical evaluation with the members of the research team, and we will stop your participation in our study.

Furthermore, we have applied a series of measures in order to prevent covid-19 transmission. Therefore, both you and the research team will:

- Use protective personal equipment
- Wash hands before and after the experimental procedures
- Maintain a 2m distance for most of the experiment

In addition, we will make sure that the experimental area and equipment is adequately sanitized before and after the measurements.

You will be provided with instructions on how to enter the building and will be informed about all the preventive measures taking place prior to the experiments.

Will my participation be confidential?

All information collected on you will be kept strictly confidential. Personal information will be retained, but only available to the researchers using password protected files. Data will be kept for 10 years in accordance with the EU General Data Protection Regulation 2018 and the University of Birmingham Research Guidelines. All data for presentation will be anonymized and aggregated, so your identity will not be revealed in any way. You can withdraw your data until two weeks from data collection.

What will happen at the end of the research study?

The findings from this study will be presented in the form of presentations and scientific papers as appropriate. All data for presentation will be anonymized, which means your identity will not be revealed in any way.

Does the study follow ethics procedures?

This study underwent the ethical review processes of the University of Birmingham and received official approval from the University Ethics Committee.

Who is organizing and funding the research?

The study has been designed and organized by Ignacio Contreras-Hernandez and is overseen by Dr Eduardo Martinez-Valdes, Lecturer in Spinal and Musculoskeletal Physiotherapy

What if I have a problem or concern?

If you have a concern about any aspect of this study, please speak with Dr. Eduardo Martinez-Valdes or Ignacio Contreras-Hernandez. Should you still have any concerns, you could also speak to the Head of School, Dr. Sarah Aldred [redacted]

For further information please contact Ignacio Contreras Hernandez

Ignacio Contreras-Hernandez Centre of Precision Rehabilitation for Spinal Pain School of Sports, Exercise and Rehabilitation Sciences University of Birmingham B15 2TT	[redacted]

Thank you for your interest in participating in our study!

Appendix 12. Recruitment poster for asymptomatic individuals

UNIVERSITY OF BIRMINGHAM | CENTRE OF PRECISION REHABILITATION FOR SPINAL PAIN

Do you have a healthy Achilles tendon?

Purpose:

To determine the behavior of the motor units of the calf muscles, and the mechanical and structural properties of these muscles and the Achilles tendon in healthy individuals.

We are recruiting people who:

Have **no** medical history of...

- Neurological problems
- Cardiovascular diseases
- Systemic or inflammatory conditions
- Chronic respiratory problems
- Injury or pain within the lower limbs in the previous 6 months
- Lower limb surgery

AGE: 18+ -55

What do you need to do?

This study involves **1 experimental session** (2 hours)

We will use **ultrasound imaging** to measure the mechanical and structural properties of the leg muscles and Achilles tendon.

and **high-density surface electromyography** to measure muscle activity.

For completing this study, participants will receive 3 research hours if needed

Ignacio Contreras-Hernandez – [redacted]

Appendix 13. Recruitment poster individuals with NIAT

UNIVERSITY OF BIRMINGHAM | CENTRE OF PRECISION REHABILITATION FOR SPINAL PAIN

Are you suffering from Achilles tendinopathy?

Purpose:

To determine the neuromuscular and structural tendon adaptations after 6-weeks of either concentric or eccentric exercise in individuals with non-insertional Achilles tendinopathy.

We are recruiting people who:

Have **no** medical history of...

- Neurological problems
- Cardiovascular disease
- Systemic or inflammatory conditions
- Chronic respiratory problems
- Injury within the lower limbs in the previous 6 months
- Lower limb surgery

AGE: 18+ -55

Have had **pain** during the last 3 months in the **Achilles tendon**.

What do you need to do?

This study involves **3 experimental sessions** and **2-3 training sessions** per week for **6 weeks.**

We will use **ultrasound imaging** to measure the mechanical and structural properties of the leg muscles and the Achilles tendon and **high-density surface electromyography** to measure muscle activity.

For completing this study, participants will receive 90 £ or 9 research hours.

Ignacio Contreras-Hernandez –