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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.

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}

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.

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-A²⁷ 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.²⁰ 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 statement³² (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,

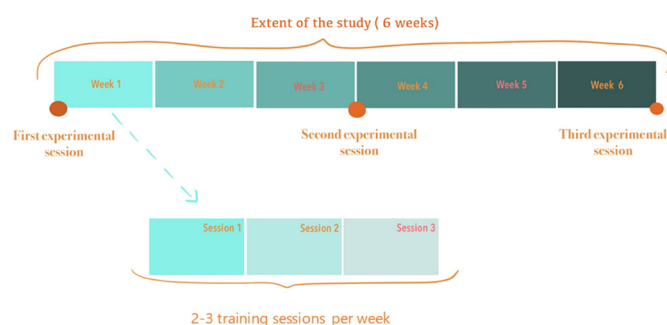


Figure 1 Overview of the study design.

$\alpha=0.01$, 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.

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 rest¹⁵ 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 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

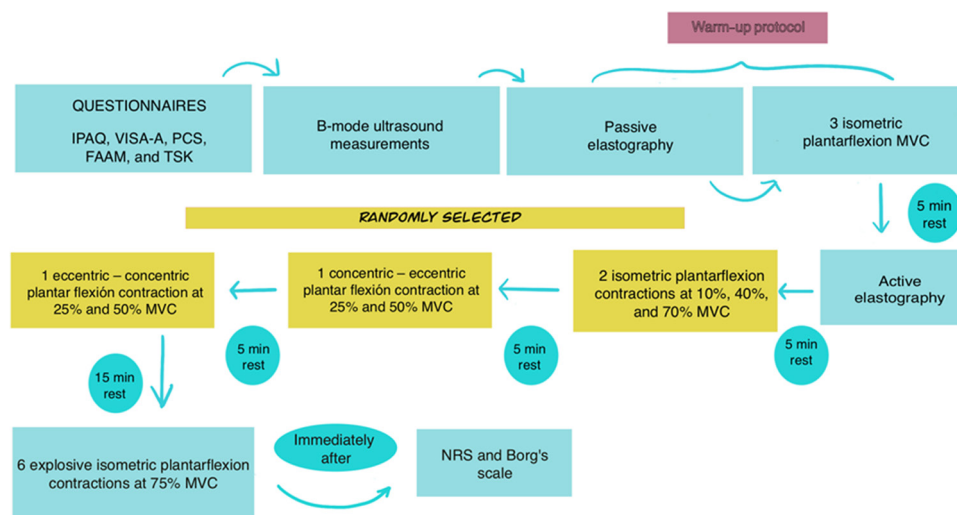


Figure 2 Experimental session design. FAAM, Foot and Ankle Ability Measure; IPAQ-SF, International Physical Activity Questionnaire short form; MVC, maximal voluntary contraction; NRS, Numerical Rating Scale; PCS, Pain Catastrophising Scale; TSK, Tampa Scale for Kinesiophobia; VISA-A, Victorian Institute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

at 10%, 40% and 70% MVC (10% MVC/s ramp-up, 10s hold, 10% MVC/s ramp-down and 30s 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 (1s ramp-up, 3s hold, 1s ramp-down and 10s 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 scale⁴⁰ 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 10s, 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 10s, 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} osteoarthritis⁴⁷ 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 Kulig¹⁴ 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

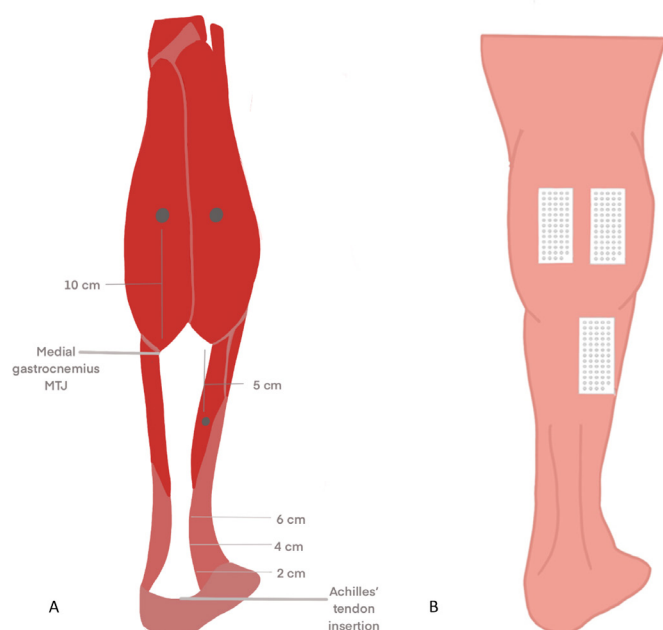


Figure 3 (A) Anatomical landmarks used for ultrasonography and (B) position of the electrodes in the MG, LG and SO muscles. LG, lateral gastrocnemius; MG, medial gastrocnemius, SO, soleus.

(<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 diameter⁴⁹ 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, Salerno, 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-OTBioelettronica, 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 $\alpha=0.05\%$ 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 tasks⁶⁰ 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 region⁶⁶ 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 (10s) 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 condition⁷⁰ 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.

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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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