**Is soleus intrinsic motor neurone excitability altered in runners with mid-portion Achilles tendinopathy?**

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**Abstract** (295 words)

**Objective**: Soleus weakness is suggested to be a significant contributor Achilles tendinopathy (AT) in runners. Muscle force is influenced by the ability of motor units to discharge at high frequencies. Intrinsic motor neurone excitability contributes to motor units discharge rate and consequently, force production. Therefore, a reduction in intrinsic motor neurone excitability could be an important factor contributing to soleus weakness in AT. Persistent inward currents (PICs) are essential for normal muscle function and a reduction of the motor neurone intrinsic excitability (e.g. PICs amplitude). This study aimed to investigate the motor neurone intrinsic excitability properties of soleus, by comparing the estimates of PICs (ΔFs) and discharge rates of soleus between runners with and without mid-portion AT.

**Methods:** This was an observational study in which we compared the delta frequency (ΔF) of soleus of runners with (n=11) and without AT (n=12). ΔF estimates the contribution of persistent inward currents to motor neurones self-sustained firing during voluntary contractions and was calculated using the paired-motor unit technique from ramp-shaped isometric plantar flexor contractions of 20% maximal torque. The discharge rates of soleus motor units were assessed using high-density surface electromyography.

**Results:** We found no differences in soleus ΔF (mean difference = -0.05 pps; 95%CI -1.6 to 1.5; p=0.940), peak discharge rates (-0.16pps; 95%CI -1.7 to 1.3, p=0.826), or recruitment threshold (-1.54%; 95%CI -3.5 to 0.5; p=0.111) between groups.

**Conclusion:** This study demonstrates no differences in soleus intrinsic motor neurone excitability between runners with or without Achilles tendinopathy. Thus, intrinsic motor neurone excitability might not be a responsible mechanism or soleus might not be the main muscle contributing to the plantar flexor weakness reported in AT. Other neurophysiological mechanisms and the individual contribution of lateral and medial gastrocnemius in this plantar flexor weakness should be explored in future studies.

**Keywords**

Achilles tendon; running; motor unit; inhibitory circuits; persistent inward current; triceps surae

**Impact statement:**

The clinical significance of this study is that Soleus might not be the main muscle affected in runners with AT. Other muscles such as the lateral and/or medial gastrocnemius could be influencing deficits in plantar flexor performance. Rehabilitation strategies focusing on Soleus only might not be ideal to assist runners with AT.

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**1. Introduction**

Achilles tendinopathy (AT) is painful condition affecting the Achilles tendon. It often becomes chronic, and has a great affect in muscle tendon function1. AT is an overloading injury and although its aetiology is multifactorial, deficits in muscle performance are suggested to be key factors2,3, which seem to be maintained long after symptomatic recovery4. Some evidence suggests that soleus dysfunction is the main factor influencing the strength and endurance deficits observed in runners with AT2. Selective weakness or altered muscle coordination, may create unequal Achilles tendon5 loading and possibly contribute to tendon pain and pathology, and the development of chronic AT6. From a neurophysiological perspective, the force exerted by a muscle depends, partly, on the recruitment and discharge rates of motor units7. Thus, deficits in strength could be a result of a reduced ability to recruit motor units and/or to increase the rate at which motor neurones discharge7.

Motor neurones are responsible for integrating and amplifying excitatory synaptic input from descending pathways and afferent fibres into an appropriate motor output8. Persistent inward currents (PICs) are an intrinsic property of the motor neurone that amplify and prolong synaptic input9. PICs can amplify synaptic input 5-fold, facilitating increases in motor neurone discharge rate, and thus, force production8–10. This amplification from PICs is essential for normal muscle function, and a reduction of the motor neurone intrinsic excitability (e.g. PICs amplitude) can significantly reduce muscle force11,12. PICs are deactivated by localised inhibitory inputs (e.g. reciprocal13 and recurrent inhibition14) which reduce motor neurone excitability. Interestingly, runners with AT have greater levels of intracortical inhibition15. Also, the occurrence of pain in tendinopathies16 stimulates spinal inhibitory circuits, via descending inputs to the motor neurone pool, and could consequently inhibit PICs17,18. As PICs are essential for normal muscle function, inhibition-induced PICs reduction in runners with AT could significantly reduce muscle function in this population11,12.

PICs amplitude can be estimated in humans, by calculating the delta frequency (ΔF) with the paired-motor unit technique11,19. This is a valid and widely used method that measures the difference in discharge rate of a control unit (lower-threshold) at the times of recruitment and de-recruitment of a test unit (higher-threshold)11,19. ΔF is assumed to be an estimate of the contribution of PICs to motor neurone self-sustained firing19. Because soleus is suggested to be inhibited and driving the reduced strength observed in AT2, this study aimed to investigate the motor neurone intrinsic excitability properties of soleus, by comparing the estimates of PICs amplitudes (ΔFs) and motor unit discharge rates of soleus between runners with and without mid-portion AT. We hypothesised that the runners with AT would have lower ΔF amplitude and lower motor unit discharge rates than runners without AT.

**2. Methods**

Twenty-three endurance runners with (n=11, 6 males, 45.5±11.7 years old, **172.8**±9.5 **cm, 77.2**±15.0 **kg**, and without mid-portion AT (n=12, 7 males, **33.0** ± 6.4 **years old, 170.9** ± 8.7 **cm, 65.6** ± 11.9 **kg**) were recruited from running clubs around Southeast Queensland, Australia. **They had** a running routine of more than twice weekly for more than 4 months. T**he AT group had a running routine of 39.4** ± 16.6 **km/week and the control group, 29.1** ± 13.3 **km/week. These runners** also participated in a previous study from our laboratory15,20 where strength and endurance values were reported.

Diagnosis of mid-portion AT was confirmed by an experienced physiotherapist (GLF) during examination considering: a) localised mid-portion Achilles tendon pain, for more than three months, b) pain provoked by physical activities that load the Achilles tendon in a dose dependent way, and c) pain with palpation at the mid portion of tendon. Volunteers were excluded if presenting with insertional AT; if they have had previous rupture of or surgery on the Achilles tendon; clinical findings indicating a differential diagnosis for the Achilles tendon pain (such as tendon tear); regular participation in other sports involving high speed running (football, rugby, AFL etc), VISA-A score > 90 points for AT group, and < 100 for the control group; any other current musculoskeletal injuries of the lower limb; any neurological disorder; or mental health issues affecting consent. All participants reported being free of comorbidities such as cardiac, pulmonary, renal, endocrine or gastrointestinal disease and were not taking any medication for tendon pain or that would affect tendon structure21. Prior to testing, all participants read and signed a detailed informed consent document and completed the VISA-A questionnaire22. **The average VISA-A score for the AT group was 69.2 (95% CI 63.4 to 75) and 100 (0) for the control group.** This study was approved by the Queensland University of Technology Human Research and Ethics Committee in line with the Declaration of Helsinki. Data collection was conducted during the COVID-19 pandemic and all safety procedures followed local state government policies.

*2.1 Torque acquisition*

Plantar flexor isometric peak torque was measured using an isokinetic dynamometer (Biodex Medical Systems, Shirley, New York). For the bilateral AT presentations (n=3), the most symptomatic leg was used and for the control group, the dominant leg was used for testing. Leg dominance was selected by asking the participants which leg they would use to kick a ball. Participants were positioned in a seated position (75º of hip flexion) with their knee straight and ankle at 90º. Warm up consisted of 2 × 4 s isometric contractions of participant’s perceived 20, 40, 60 and 80% maximal voluntary isometric contraction intensity. After warm-up, participants performed at least 3 maximal voluntary isometric contractions, until <5% variation was observed between contractions, the highest value was used. Thereafter, participants were familiarised with triangular-shaped contractions of 20% of their peak torque, which has been extensively described for ΔF calculations using the paired motor unit technique11,23. Participants had 4 attempts to become familiarised with the task with ~ 30-s rest between contractions before recordings were made. Rate of torque rise and decline was standardised at ~ 2% peak torque/s (10-s up and 10-s down), followed by 1 min of rest between contractions. Participants received real-time visual feedback of the triangular pathway, displayed by monitor. When the torque trajectory was not closely followed the trial was excluded and repeated. Three successful contractions were recorded for each participant.

*2.2 Surface electromyography recordings*

During the plantar flexor triangular-shaped contractions, high-density surface electromyography (HD-EMG) (Sessantaquattro, OTBioelettronica, Torino, Italy) signals from soleus were recorded using OT Biolab+ software (version 1.3.0., OTBioelettronica, Torino, Italy. After skin preparation (shaving, light abrasion, and cleansing of area with alcohol), electrodes were positioned following the estimated muscle fibre orientation using a bi-adhesive layer with a conductive paste to ensure good skin-electrode contact and conductibility. Two 32-channels electrodes arrays (ELSCH032NM6, OTBioelettronica, Torino, Italy) were placed on soleus, one laterally and one medially to the Achilles tendon. Two electrodes were used on SOL to increase the number of identified motor units. Data from both electrodes were clustered into one file to increase motor unit yield, prior to analysis of SOL motor unit characteristics. The ground strap electrode (WS2, OTBioelettronica, Torino, Italy) was dampened and strapped in place at the height of the malleoli of the tested leg. The EMG signals were recorded in monopolar mode, amplified (256x), band-passed filtered (10-500Hz) and converted to digital signal at 2048Hz by a 16-bit wireless amplifier (Sessantaquattro, OTBioelettronica, Torino, Italy), before being stored for offline analysis.

*2.3 Motor unit identification*

Torque was recorded and analysed with OT Biolab+ software. HD-EMG signal was recorded and analysed offline, decomposed into motor unit spike trains, then converted into instantaneous discharge rates with specialised software using blind source separation decomposition technique, DEMUSE tool software (v.4.1; The University of Maribor, Slovenia)24. The single best contraction, with the lowest deviation from torque trajectory, was analysed. If two or three contractions presented similar torque trajectory, the one with more identified motor units was chosen for analysis. All motor units were visually inspected, erroneous discharge times were excluded, and missed discharges included24. Manual editing and visual inspection is required to reduce automatic decomposition discharge errors and improve data reliability25. Reliability for manual inspection is very high across operators for motor unit mean discharge rate and recruitment with intra-class correlation coefficient (ICC) of >0.9926. Only motor units with a pulse-to-noise ratio >30dB and sensitivity > 90%, were considered for data analysis27. The assessor who performed motor unit analysis process was not blinded by group.

*2.4 Motor neurone excitability (ΔF and peak discharge rate)*

After discharge events for each motor unit were converted into instantaneous discharge rates, a 5th-order polynomial was fitted to the discharge rates for each motor unit. The maximum value obtained from the polynomial curve was considered the peak discharge rate. Recruitment threshold was considered the relative torque (%) produced at the time each motor unit was recruited and it was used to characterise the populations of motor units identified by the decomposition algorithm for each group. PIC amplitude was estimated using the paired motor unit analysis19 (**Fig 1**). Motor units with a lower recruitment threshold (control units) were paired with others with higher-recruitment threshold (test units). ΔF was calculated as the change in discharge rates of the control motor unit in the time between recruitment to the de-recruitment of the test unit12,19. Pairs of motor units were produced using the following criteria: (1) rate-to-rate correlation between the smoothed discharge rate polynomials of the test and control units with an r ≥ 0.7; (2) test units were recruited 1.0 s after the control units; and (3) the control unit showed no discharge rate saturation after the moment of test unit recruitment (i.e. discharge rate from the control unit at the moment the test unit was recruited, minus the peak discharge rate at the control unit > 0.5 pps)14,19. ΔFs obtained for each control unit were averaged to obtain a single ΔF for each test motor unit. Peak discharge rate and recruitment threshold reported in this manuscript are only from the test motor units used in the ΔFs analysis.



**Fig 1** Data illustrating the paired motor unit method to measure the delta frequency (ΔF) from a single participant, during a triangular shaped contraction reaching 20% of peak isometric torque (**A**). Motor unit discharge rate for a test unit (**B**) and a control unit (**C**) are shown in different colours. The purple continuous line represents the 5th-order polynomial equation fit to the instantaneous discharge rate of the control unit. The shaded area represents an estimate of PICs amplitude (ΔF).

*2.5 Statistical analysis*

All statistical analyses were undertaken using R studio (version 1.3.1093). Models were fitted using the *lme4* and *lmerTest* package28. A linear mixed-effect model was used to compare ΔF of soleus between groups. The AT group was significantly older than the control group. As age is a factor that can affect the estimates of PICs8, a random intercept and slope (age) were included for each participant in the study to account for the influence of age and the correlation between repeated observations (cluster of motor unit) for each participant29. Recruitment threshold was not statistically different between groups, suggesting similar (lower-threshold) population of motor units were acquired from HD-EMG analysis, thus, it was not used in the statistical model. Separate linear mixed-effects models were used to analyse motor unit peak discharge rate and recruitment threshold between groups, these included a random intercept and slope for each participant. The estimated marginal mean difference and 95% confidence intervals (CI) for ΔF, peak discharge rate, and recruitment threshold between groups, were determined using the *emmeans* package30. An α level of 5% was used. Data is presented throughout the manuscript as mean (± 95% CI).

**3. Results**

Ninety-eight motor units (8.9 ± 5.4 per participant) were identified for the AT group and from those, 45 yielded paired analysis (ΔF), while 91 motor units (7.5 ± 7.0 per participant) were identified for the control group, where 60 were able to be used for ΔF analysis.

There was no group effect on ΔF (p=0.933, η2p<0.001, Fig 2A) and there was no influence of age (p=0.320, η2p=0.07) in the model. We investigated the influence of outliers in the results of ΔF, but our main results (ΔF analysis) remained unchanged (p=0.743, η2p=0.01). Also, there were no differences between groups in peak discharge rate (p=0.826, η2p=0.003) and on motor unit recruitment threshold between groups (p=0.111, η2p=0.16). Estimated marginal means and mean differences are presented in **Table 1.**



**Fig 2** ΔF (A), peak discharge rates (B), and recruitment thresholds (C) for each group. Each colour represents data points for one participant. Mean and 95% confidence intervals are offset to the left. pps = pulse per second.

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| **Table 1** |  |  |  |  |
| ΔF, peak discharge rates and recruitment threshold estimated marginal means per group. | | | | |
| Variable | AT | Control | AT – Control difference | |
| ΔF (pps) | 2.2 (1.2 to 3.3) | 2.3 (1.4 to 3.2) | -0.05 (-1.6 to 1.5) | |
| Peak discharge rate (pps) | 8.3 (7.2 to 9.5) | 8.5 (7.5 to 9.6) | -0.16 (-1.7 to 1.4) | |
| Recruitment threshold (% peak torque) | 7.6 (6.2 to 9.1) | 9.2 (7.8 to 10.5) | -1.54 (-3.5 to 0.5) | |
| Data is presented as mean and 95% confidence interval (lower and upper limits). | | |  |  |

**4. Discussion**

This study compared ΔFs and discharge rates, from lower-threshold motor units of soleus, between runners with and without mid-portion AT. Contrary to our initial hypothesis, ΔFs and peak discharge rates from soleus motor neurones are not altered in runners with AT during submaximal contractions (20% of each participant maximal torque). Therefore, our data indicate that intrinsic excitability of lower-threshold motor neurones from soleus of runners with AT is not different from those of runners without AT. ΔF values obtained in this study are similar to of other studies which also tested soleus, using triangular contractions with the same protocol in non-runners 10,11,31,32, indicating that chronic running activities do not increase or decrease them.

Similar intrinsic motor neurone excitability between runners with or without AT possibly indicates that i) other musculotendinous or neurophysiological mechanisms might be contributing to the reduced plantar flexor function2,15 observed in AT, ii) other muscles (e.g., gastrocnemii) might be affected, and iii) the intrinsic excitability might only be affected at higher intensity contractions. PICs are highly sensitive to inhibitory stimuli13 and increased intracortical inhibition has been reported15 in runners with AT. However, based on this study’s findings, this increased inhibition does not seem to be influencing soleus motor unit behaviour at low forces (0-20% MVC). Pain responses can also stimulate descending inhibitory inputs and affect motor neurone excitability18,33. Chronic pain has also been suggested to affect muscle activation in other chronic tendinopathies. Examples include, reduced corticospinal excitability and lower infraspinatus activation in individuals with chronic rotator cuff tendinopathy34, increased corticospinal inhibition of the rectus femoris in volleyball athletes with patellar tendinopathy35, and altered activation patterns in the hip abductors during walking (i.e. prolonged activation of gluteus minimus and gluteus medius), in individuals with gluteal tendinopathy. If soleus force production were affected in runners with AT, soleus motor neurone intrinsic excitability (i.e. PICs) would have been reduced, but differences in plantarflexor force levels were not observed in this sample of participants. Perhaps the deficits in plantar flexor force observed36 in runners with AT might be a result of changes in motor neurone excitability not in soleus, but in gastrocnemius lateralis and/or medialis.

The suggestion that soleus is the most involved muscle in the deficits in plantar flexor torque in AT was made based differences in torque measures between knee flexed and extended2. However, this analysis is limited to torque output, and it neglects some neurophysiological mechanisms that enable force production. Perhaps, the deficits in torque observed in this group are present in other muscles (i.e. gastrocnemius lateralis and/or medialis) rather than soleus. As individual muscles of the triceps surae receive independent neural drive37, we can hypothesise that the impact from increased inhibitory output from increased intracortical inhibition15 in runners with AT, may not be affecting all three muscles the same way. In fact, a study38 calculated the physiological cross-sectional area and normalised RMS surface electromyography (EMG) to calculate the index of force of each muscle of the triceps surae. This way the authors estimated the contribution of each individual muscle of triceps surae for force production in runners with AT. Interestingly, they found a significant reduction of gastrocnemius lateralis contribution in the AT groups, but not in soleus or in gastrocnemius medialis38. Additionally, a pilot study20 from our group also found a reduction in neural drive to gastrocnemius lateralis but not to gastrocnemius medialis or soleus in runners with AT compared to healthy runners during submaximal contractions.

Soleus motor unit peak discharge rate and recruitment threshold also showed no differences between groups. A reduction in neural drive to the motor neurone, would negatively impact soleus motor neurone11,12. This would have been seen as reduced discharge rate and force output; however, this was not observed in our study. Thus, it is safe to say that soleus motor neurone excitability during low intensity isometric contractions, is not affected in runners with AT. Recruitment threshold was not different between groups, outlining that similar populations (lower-threshold) of motor units were analysed and compared between groups. It is possible that different results would have been observed during higher contraction intensities, which would require recruitment of higher-threshold motor units and higher discharge rates. However, using higher intensity contractions reduces the number of motor units, yielded by the decomposition algorithm24,39,40. Future studies should try and use higher contraction intensities and try and replicate our studies using lower-threshold motor units.

All these findings imply that the ability of the soleus to produce force might not be affected in AT, as previously suggested in the literature2. Future studies should investigate motor neurone excitability of each individual muscle of the triceps surae to understand if these muscle deficits36 widely reported in runners with AT are in fact muscle specific.

*4.4 Strengths and Limitations*

The HD-EMG technology used in this study provides reliable estimates of individual motor unit discharge rates25. However, it requires data to be collected during isometric contractions to allow accurate motor unit recording and decomposition. Therefore, we cannot extrapolate our results into dynamic tasks such as calf raises or running. For optimal motor unit identification and ∆F calculation, submaximal intensity is required due to technological limitations. A peak torque of 20% MVC, used in this study, is a commonly used target torque in PIC studies8,11,19,23,32,41. This lower intensity contraction recruits lower-threshold motor units. Higher contraction intensities would require a recruitment of higher-threshold motor units, which could present different results, however these measures are not possible. Perhaps the data obtained in low level forces do not represent the motor neurone excitability when higher intensity contractions are required (e.g. higher intensity running). It is relevant to point out that there were no differences in isometric peak torque between groups. We would expect to see deficits in motor neurone excitability to be associated with deficits in force.

**5. Conclusion**

This study investigated soleus motor neurone intrinsic excitability by estimating PICs amplitude (i.e., ΔF) as a potential neurophysiological mechanism underpinning the motor function deficits reported in runners with chronic mid-portion AT. Our data suggest that runners with mid-portion AT do not present deficits in soleus motor neurone excitability or discharge rates compared to healthy controls. Future studies should investigate other musculotendinous and neurophysiological mechanisms and potential alterations of intrinsic motor neurone excitability and its influence on motor unit discharge rate of gastrocnemius lateralis and/or medialis to investigate the mechanisms underpinning muscle deficits reported in AT.

**Authors contribution**

GLF, LO and GST designed the study. GLF and LO conducted experiments. GLF analysed the data and drafted the first version of the manuscript. GLF, LO, AS and GST critically revised the manuscript. All authors read and approved this final version of the manuscript.

**Conflict of interests**

The authors declare no conflict of interest with the present research.

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**Data availability**

The datasets generated during and/or analysed during the current study are available in the GitHub repository, [PERSISTENT WEB LINK TO DATASETS]

**References**

1. Rio E, Moseley L, Purdam C, et al. The pain of tendinopathy: Physiological or pathophysiological? *Sport Med*. 2014;44(1):9-23. doi:10.1007/s40279-013-0096-z

2. O’Neill S, Barry S, Watson P. Plantarflexor strength and endurance deficits associated with mid-portion Achilles tendinopathy: The role of soleus. *Phys Ther Sport*. 2019;37:69-76. doi:10.1016/j.ptsp.2019.03.002

3. Mahieu NN, Witvrouw E, Stevens V, Van Tiggelen D, Roget P. Intrinsic risk factors for the development of Achilles tendon overuse injury: A prospective study. *Am J Sports Med*. 2006;34(2):226-235. doi:10.1177/0363546505279918

4. Silbernagel KG, Thomeé R, Eriksson BI, Karlsson J. Full symptomatic recovery does not ensure full recovery of muscle-tendon function in patients with Achilles tendinopathy. *Br J Sports Med*. 2007;41(4):276-280. doi:10.1136/bjsm.2006.033464

5. Hug F, Tucker K. Muscle Coordination and the Development of Musculoskeletal Disorders. *Exerc Sport Sci Rev*. 2017;45(4):201-208. doi:10.1249/JES.0000000000000122

6. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med*. 2009;43(6):409-416. doi:10.1136/bjsm.2008.051193

7. Enoka RM, Duchateau J. Rate coding and the control of muscle force. *Cold Spring Harb Perspect Med*. 2017;7(10):1-12. doi:10.1101/cshperspect.a029702

8. Orssatto LBR, Borg DN, Blazevich AJ, Sakugawa RL, Shield AJ, Trajano GS. Intrinsic motoneuron excitability is reduced in soleus and tibialis anterior of older adults. *GeroScience*. Published online 2021:2719-2735. doi:10.1007/s11357-021-00478-z

9. Binder MD, Powers RK, Heckman CJ. Nonlinear Input-Output Functions of Motoneurons. *Physiology (Bethesda)*. 2020;35(1):31-39. doi:10.1152/physiol.00026.2019

10. Orssatto LBRR, Mackay K, Shield AJ, Sakugawa RL, Blazevich AJ, Trajano GS. Estimates of persistent inward currents increase with the level of voluntary drive in low-threshold motor units of plantar flexor muscles. *J Neurophysiol*. 2021;125(5):1746-1754. doi:10.1152/jn.00697.2020

11. Trajano GS, Taylor JL, Orssatto LBR, McNulty CR, Blazevich AJ. Passive muscle stretching reduces estimates of persistent inward current strength in soleus motor units. *J Exp Biol*. 2020;(61 7):jeb.229922. doi:10.1242/jeb.229922

12. Heckman CJ, Gorassini MA, Bennett DJ. Persistent inward currents in motoneuron dendrites: Implications for motor output. *Muscle and Nerve*. 2005;31(2):135-156. doi:10.1002/mus.20261

13. Hyngstrom, A. S., Johnson, M. D., & Heckman CJ. Summation of Excitatory and Inhibitory Synaptic Inputs by Motoneurons With Highly Active Dendrites. *J Neurophysiol*. Published online 2008. doi:10.1152/jn.01253.2007

14. Vandenberk MS, Kalmar JM. An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *J Neurophysiol*. 2014;111(9):1877-1884. doi:10.1152/jn.00469.2013

15. Fernandes GL, Orssatto LBR, Shield AJ, Trajano GS. Runners with mid‐portion Achilles tendinopathy have greater triceps surae intracortical inhibition than healthy controls. *Scand J Med Sci Sports*. Published online 2021:1-18. doi:10.1111/sms.14111

16. Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: What is its merit in clinical practice and research? *Br J Sports Med*. 2016;50(19):1187-1191. doi:10.1136/bjsports-2015-095422

17. Sohn MK, Graven-Nielsen T, Arendt-Nielsen L, Svensson P. Inhibition of motor unit firing during experimental muscle pain in humans. *Muscle and Nerve*. 2000;23(8):1219-1226. doi:10.1002/1097-4598(200008)23:8<1219::AID-MUS10>3.0.CO;2-A

18. Farina D, Arendt-Nielsen L, Merletti R, Graven-Nielsen T. Effect of Experimental Muscle Pain on Motor Unit Firing Rate and Conduction Velocity. *J Neurophysiol*. 2004;91(3):1250-1259. doi:10.1152/jn.00620.2003

19. Gorassini M, Yang JF, Siu M, Bennett DJ. Intrinsic activation of human motoneurons: Possible contribution to motor unit excitation. *J Neurophysiol*. 2002;87(4):1850-1858. doi:10.1152/jn.00024.2001

20. Fernandes GL, Orssatto LBR, Sakugawa RL, Trajano GS. Lower motor unit discharge rates in gastrocnemius lateralis, but not in gastrocnemius medialis or soleus, in runners with Achilles tendinopathy: a pilot study. *Eur J Appl Physiol*. 2022;(accepted for publication):2022.05.05.22274750. doi:10.1007/s00421-022-05089-w

21. Knobloch K. Drug-Induced Tendon Disorders. In: Ackermann PW, Hart DA, eds. *Metabolic Influences on Risk for Tendon Disorders*. Springer International Publishing; 2016:229-238. doi:10.1007/978-3-319-33943-6\_22

22. Martin RL, Chimenti R, Cuddeford T, et al. The VISA-A questionnaire: An index of the severity of Achilles tendinopathy I. *J Orthop Sports Phys Ther*. 2018;48(5):9-11. https://pubmed.ncbi.nlm.nih.gov/11579069/

23. Hassan A, Thompson CK, Negro F, et al. Impact of parameter selection on estimates of motoneuron excitability using paired motor unit analysis. *J Neural Eng*. 2020;17(1):016063. doi:10.1088/1741-2552/ab5eda

24. Del Vecchio A, Holobar A, Falla D, Felici F, Enoka RM, Farina D. Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals. *J Electromyogr Kinesiol*. 2020;53:102426. doi:10.1016/j.jelekin.2020.102426

25. Martinez-Valdes E, Laine CM, Falla D, Mayer F, Farina D. High-density surface electromyography provides reliable estimates of motor unit behavior. *Clin Neurophysiol*. 2016;127(6):2534-2541. doi:10.1016/j.clinph.2015.10.065

26. Hug F, Avrillon S, Del Vecchio A, et al. Analysis of motor unit spike trains estimated from high-density surface electromyography is highly reliable across operators. *J Electromyogr Kinesiol*. 2021;58(February). doi:10.1016/j.jelekin.2021.102548

27. Del Vecchio A, Negro F, Holobar A, et al. You are as fast as your motor neurons: speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *J Physiol*. 2019;597(9):2445-2456. doi:10.1113/JP277396

28. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67(1). doi:10.18637/jss.v067.i01

29. Boccia G, Martinez-Valdes E, Negro F, Rainoldi A, Falla D. Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher in open compared with closed kinetic chain exercise. *J Appl Physiol*. 2019;127(4):950-958. doi:10.1152/japplphysiol.00310.2019

30. Lenth R V. Least-squares means: The R package lsmeans. *J Stat Softw*. 2016;69(1):1-33. doi:10.18637/jss.v069.i01

31. Kim EH, Wilson JM, Thompson CK, Heckman CJ. Differences in estimated persistent inward currents between ankle flexors and extensors in humans. *J Neurophysiol*. 2020;124(2):525-535. doi:10.1152/JN.00746.2019

32. Orssatto LBR, Fernandes GL, Blazevich AJ, Trajano GS. Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults. *J Physiol*. 2022;0:2022.08.08.503135. doi:10.1113/JP283708

33. Tucker K, Larsson AK, Oknelid S, Hodges P. Similar alteration of motor unit recruitment strategies during the anticipation and experience of pain. *Pain*. 2012;153(3):636-643. doi:10.1016/j.pain.2011.11.024

34. Ngomo S, Mercier C, Bouyer LJ, Savoie A, Roy JS. Alterations in central motor representation increase over time in individuals with rotator cuff tendinopathy. *Clin Neurophysiol*. 2015;126(2):365-371. doi:10.1016/j.clinph.2014.05.035

35. Rio E, Kidgell D, Purdam C, et al. Isometric exercise induces analgesia and reduces inhibition in patellar tendinopathy. *Br J Sports Med*. 2015;49(19):1277-1283. doi:10.1136/bjsports-2014-094386

36. McAuliffe S, Tabuena A, McCreesh K, et al. Altered strength profile in Achilles tendinopathy: A systematic review and meta-analysis. *J Athl Train*. 2019;54(8):889-900. doi:10.4085/1062-6050-43-18

37. Hug F, Del Vecchio A, Avrillon S, Farina D, Tucker KJ. Muscles from the same muscle group do not necessarily share common drive: evidence from the human triceps surae. *J Appl Physiol*. Published online 2020. doi:10.1152/japplphysiol.00635.2020

38. Crouzier M, Tucker K, Lacourpaille L, et al. *Force-Sharing within the Triceps Surae: An Achilles Heel in Achilles Tendinopathy*. Vol 52.; 2020. doi:10.1249/MSS.0000000000002229

39. McNeil CJ, Doherty TJ, Stashuk DW, Rice CL. The effect of contraction intensity on motor unit number estimates of the tibialis anterior. *Clin Neurophysiol*. 2005;116(6):1342-1347. doi:10.1016/j.clinph.2005.02.006

40. Hassan AS, Kim EH, Khurram OU, et al. Properties of Motor Units of Elbow and Ankle Muscles Decomposed Using High-Density Surface EMG. *Proc Annu Int Conf IEEE Eng Med Biol Soc EMBS*. Published online 2019:3874-3878. doi:10.1109/EMBC.2019.8857475

41. Powers RK, Nardelli P, Cope TC. Estimation of the contribution of intrinsic currents to motoneuron firing based on paired motoneuron discharge records in the decerebrate cat. *J Neurophysiol*. 2008;100(1):292-303. doi:10.1152/jn.90296.2008