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This is the published version of a paper published in *Journal of Biomechanics*.

Citation for the original published paper (version of record):

Jacques, T C., Bini, R., Arndt, A. (2021)

Bilateral In Vivo Neuromechanical Properties Of Thetriceps Surae And Achilles Tendon
In Runners And Tri-Athletes

Journal of Biomechanics, 123: 110493

<https://doi.org/10.1016/j.jbiomech.2021.110493>

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Bilateral *in vivo* neuromechanical properties of the triceps surae and Achilles tendon in runners and triathletes

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ARTICLE INFO

Article history:

Accepted 23 April 2021

Keywords:

Inter-limb
Running
Overuse
Injury

ABSTRACT

Inter-limb differences in Achilles tendon mechanical, material and morphological properties have previously been described in non-athletes and attributed to the preferential use of a given limb. Achilles tendon overuse tendon injury generally initiate unilaterally and alters triceps surae activation and Achilles tendon properties. The investigation of inter-limb differences in muscle activation and tendon properties may provide directions for injury prevention in habitual runners. In this study triceps surae and Achilles tendon properties were investigated bilaterally in habitual runners during unilateral maximal isometric contractions. Morphological, mechanical and material Achilles tendon properties were assessed using isokinetic dynamometry, motion capture and ultrasonography while triceps surae activation strategies were assessed using electromyography. Lower limb preference was assessed for inter-limb comparisons using the Waterloo questionnaire. Zero and one-dimensional statistical analysis and Cohen's *d* were employed to investigate possible inter-limb differences. Inter-limb associations in Achilles tendon properties and intra-limb associations between triceps surae activations were assessed using Pearson's correlation coefficients. No differences were observed between the preferred and non-preferred limb in terms of triceps surae muscle activation amplitude and Achilles tendon properties. However, intra-limb association among triceps surae activation ratios were not identical between limbs. Runners and triathletes present similar Achilles tendons properties between limbs, and thus initial observations of unilateral changes in the Achilles tendon properties might be used as a strategy to prevent the onset of overuse tendon injury. The non-similar associations within activation ratios between limbs should be further explored since triceps surae activation strategies may alter loading of the Achilles tendon.

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1. Introduction

It is well documented that inter-limb differences in tendon properties arise when one limb is exposed to greater mechanical loading (Couppe et al., 2008; Bayliss et al., 2016; Karamanidis et al. 2020). For example, the patellar tendon in the leading limb of fencers and badminton players differs in morphological and mechanical tendon properties compared to the non-leading limb (Couppe et al. 2008). Similarly, in jumping athletes the mechanical and material properties of the Achilles tendon (AT) in the take-off limb differ to the AT in the swing limb (Bayliss et al. 2016, Karamanidis & Epro 2020).

Less intuitively, individuals not involved in sport practice were also found to demonstrate inter-limb differences in plantar flexor strength and AT properties. For example, inter-limb differences in maximal isometric ankle extensor torque (Valderrabano et al. 2007), AT cross-sectional area (CSA) (Pang et al. 2006), AT resting length and modulus of elasticity (e.g. E-modulus) (Bohm et al. 2015), and stiffness (Chiu et al. 2016) were all observed in healthy non-athletes. Those inter-limb differences were associated to the preferred use of a given limb during daily life activities, suggesting that differential adaptation of muscle-tendon components can occur not only when mechanical loading is exacerbated, as in athlete training (Couppe et al. 2008, Bayliss et al. 2016, Karamanidis & Epro 2020). Moreover, those studies (Pang & Ying 2006, Valderrabano et al. 2007, Bohm et al. 2015, Chiu et al. 2016) introduce the question of whether or not individuals trained in theoretically symmetrical activities such as runners and triathletes would

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develop such inter-limb differences due to the above mentioned daily preferred limb usage.

AT overuse injuries are a frequent problem for the running population including runners and triathletes (Vleck et al., 1998; Gosling et al., 2008; Janssen et al., 2018; Dallinga et al., 2019). Clinical symptoms generally develop at first unilaterally (Paavola et al., 2000; Janssen et al., 2018), although some unilateral cases develop into bilateral cases (Paavola et al. 2000). Overuse injuries are well known to alter tendon morphological, mechanical and material properties (Arya et al. 2010; Chang et al., 2015; Obst et al. 2018). Achilles tendon overuse injury was also found to result in alterations in triceps surae activation patterns, affecting the triceps surae activation amplitude (Masood et al., 2014a; 2014b; Crouzier et al., 2020) and the relative activation contribution of a given muscle to its synergistic group total activation (Chang & Kulig, 2015; Crouzier et al., 2020). Those findings corroborate with suggestions that triceps surae activation may be implicated in AT overuse injuries (Arndt et al. 1998; Bojsen-Møller et al. 2015; Hug et al. 2017). Among the main variables significantly affecting ankle extensor torque production and thus triceps surae activation is the AT moment arm (MA) (Baxter and Piazza, 2014, 2018; Holzer et al., 2020), which is defined as the distance from muscle force line of action to the joint center of rotation. The MA has direct implications to the net ankle extensor torque production since for a given muscle force a greater MA results in greater torque about a joint. Thus, inter-limb differences in MA may result in inter-limb differences in triceps surae activation and AT force. Only one prior study was found investigating MA bilaterally, which found no inter-limb differences in non-athletes (Bohm et al. 2015). However, a bilateral MA investigation in habitual runners is still lacking.

Preventive strategies for AT overuse injuries would benefit from understanding whether inter-limb differences in muscle activation and tendon morphological, mechanical and material properties exist in habitual runners such as in runners and triathletes. To the best of our knowledge these variables have not previously been investigated bilaterally in this population. The aim of this study was to explore possible inter-limb differences in triceps surae activation and AT properties in runners and triathletes, which are running populations showing high incidences of AT overuse injuries (Vleck et al., 1998; Gosling et al., 2008; Janssen et al., 2018; Dallinga et al., 2019).

2. Methods

2.1. Participants

Fifteen habitual runners [10 runners and 5 triathletes; 11 males (body mass: 73.9 ± 2.5 kg, height: 183.3 ± 2.5 , age: 33.2 ± 4.0 years); 4 females (body mass: 64.2 ± 3.5 kg, height: 168.2 ± 5.9 cm, age: 33.2 ± 4.0 years); mean weekly running volume: 50.6 ± 21.5 km; minimum and maximum weekly running volume: 20 and 85 km respectively] gave informed written consent to participate in the study. Runners and triathletes declared no lower limb injury in the past six months prior to their testing session. This study was approved by the regional ethics committee and followed the Declaration of Helsinki guidelines.

3. Experimental procedures

Participants performed maximal isometric contractions while ankle extensor torque, electromyography (EMG), and the MG muscle-tendon junction (MG-MTJ) displacement were registered. Participants were evaluated while lying prone on an isokinetic dynamometer (Isomed 2000, D&R Ferstl GmbH, Germany) with a 45° knee flexion to approximately replicate the knee angle occur-

ring at mid-stance during running at speeds varying from 2.6 to 3.7 m s^{-1} (Orendurff et al. 2018). The ankle joint was securely strapped to dynamometer's footplate in neutral position (foot sole perpendicular to the tibia, 90°), while the shank (resting on a 45° wedge) and thigh were strapped to dynamometer's bench. Prior to the testing protocol, participants warmed-up performing ten submaximal contractions against the footplate while receiving visual feedback of torque production.

The testing protocol consisted of four ramp isometric contractions aiming to achieve maximal torque production within 5 s (Arya & Kulig, 2010; Bohm et al., 2015) while torque was registered by the isokinetic dynamometer at 3000 Hz. Bipolar electrodes were placed over triceps surae [medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL)] and the tibialis anterior (TA) muscles following SENIAM recommendations (Hermens et al. 2000). EMG signals were registered at 3000 Hz using electromyography (Noraxon TeleMyo 2400R G2, Noraxon Inc., USA). The MG-MTJ displacement was registered at 75 Hz by an ultrasound transducer (96-element linear probe, 60 mm field of view, B-mode, 7 MHz, Echoblaster 128, Telemed, Lithuania). The three-dimensional coordinates of passive reflective markers placed on the posterior portion of the calcaneus tuberosity and on the ultrasound transducer identifying the distal border of the transducer field of view were registered at 300 Hz by a six-camera motion capture system (Oqus 4-series, Qualisys AB, Sweden). For the evaluation of the Achilles tendon moment arm (AT MA), ultrasound imaging and the motion capture system were combined as described elsewhere (Manal et al. 2013), with participants in the same position as adopted during the ramp isometric protocol. After the maximal isometric contractions and MA registrations, the AT cross-sectional area (AT CSA) was registered by imaging the AT cross-section at four centimeters (Arya & Kulig, 2010; Stenroth et al., 2012) above the reference position used for the calcaneus marker. Torque production, EMG, motion capture and ultrasonography imaging during ramp contractions were temporally synchronized via a 5 V analog pulse.

3.1. Data analysis

EMG signals were band-pass filtered at 30–850 Hz using a 4th order Butterworth filter and subsequently enveloped by a root mean square (RMS) procedure calculated over consecutive 300 ms windows (Crouzier et al. 2020). The highest EMG RMS found between trials was used for normalization and the averaged normalized EMG across trials was considered as representative of the activation for each muscle. The MG, LG, SOL activation ratios were calculated by dividing their normalized activations to the sum of all triceps surae muscle activations (Crouzier et al. 2018). The ratio of MG normalized activation to the sum of the gastrocnemii (e.g. MG + LG) activations was also calculated (Crouzier et al. 2018) and was defined as 'GAS ratio'. Ankle extensor torque data were filtered at 10 Hz using a 4th order low pass Butterworth filter and the ramp portion of the isometric contraction was determined from 1% to 100% of peak torque. Considering TA activation was observed to result in negligible cocontraction during ramp maximal isometric contractions (Raiteri et al. 2015), no agonist torque correction was performed.

The MG-MTJ behavior (Fig. 1-A) registered during the isometric contractions was tracked using a video analysis software (Tracker 5.0.7, Open Source Physics, <https://www.compadre.org/osp/index.cfm>). The tracked local MG-MTJ coordinates were up-sampled by a Fast Fourier Transformation interpolation method to match the motion capture sampling rate and filtered at 10 Hz using a 2nd order low pass Butterworth filter. AT resting length was calculated as the distance between the calcaneus marker and MG-MTJ at rest. AT elongation was assessed during the isometric contractions as

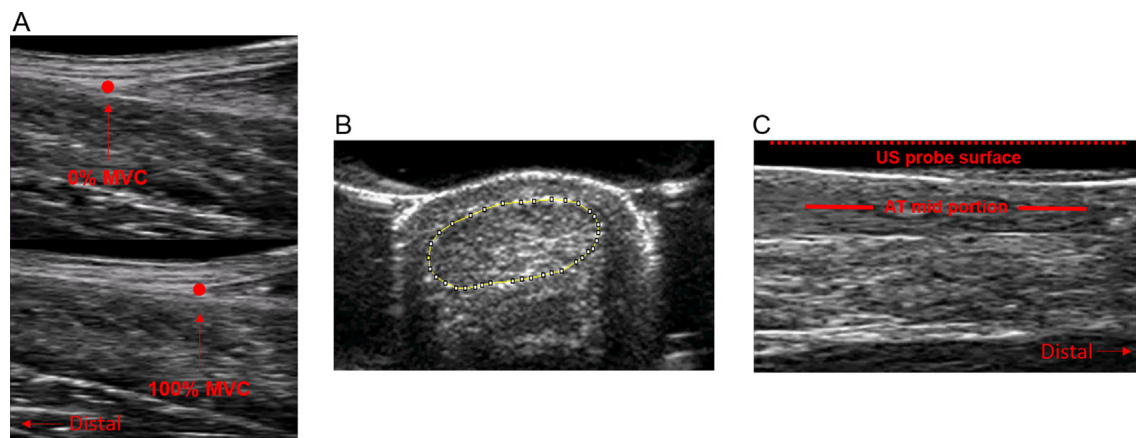


Fig. 1. Registration of the medial gastrocnemius muscle–tendon junction (MG–MTJ, red dot) displacement during a maximal isometric contraction (1–A); registration of the Achilles tendon cross-sectional area (yellow dotted line) (1–B); registration of the Achilles tendon (AT) mid portion (red solid line) and probe surface (red dotted line) (1–C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the MG–MTJ displacement. AT strain was calculated as the ratio of AT elongation to the AT resting length. After image calibration and compensation for pixel aspect ratio (Fig. 1–B), the AT CSA was manually determined using a free imaging software package (ImageJ, version 1.50i, National Institutes of Health, USA) as reported elsewhere (Arya & Kulig, 2010; Stenroth et al., 2012). In order to calculate the AT MA, the linear distance from the mid portion of the AT to the US probe skin-contact surface (Fig. 1–C) was assessed using video analysis software (Tracker 5.0.7, Open Source Physics, <https://www.compadre.org/osp/index.cfm>). Since the linear distance from the mid-point between US probe markers to the US probe skin-contact surface was known from US imaging (Fig. 1C), the AT MA was calculated by subtracting US probe to skin and skin to AT mid portion linear distances from the linear distance of the US probe mid-point marker to the malleolus mid-point as previously described (Manal et al. 2013). The AT force (N) was calculated by dividing ankle extension torque by the estimated AT MA. AT stress (N mm^{-2}) was calculated as the ratio between AT force and the estimated AT CSA. AT stiffness (N mm^{-1}) and the modulus of elasticity (E-modulus) were calculated by applying a linear regression from 30% to 90% of the AT force–elongation and stress–strain relationships respectively. A similar range was adopted by Stenroth et al. (2012) and justified by their participants being limited in performing maximal torque, which may also have occurred in the present study due to the adopted knee flexion angle. In order to identify possible effects of ankle joint rotation during ramp maximal contractions (Arampatzis et al. 2005) the instantaneous length from the calcaneus marker to the US probe marker ($\text{Heel to Probe}_{\text{vector}}$) was also included in the statistical analysis.

3.2. Statistical analysis

The Waterloo questionnaire (Elias et al. 1998) was adopted for determination of limb preference when more than 60% of the answers indicated preference for the use of a given limb. Possible effects of limb preference were investigated using paired t-tests for both zero-dimensional and one-dimensional data. Zero-dimensional variables (peak activations, activation ratios, peak forces, AT properties) were analyzed by paired t-tests in JASP (version 0.11, jasp-stats.org). One-dimensional data (EMG_{RMS} during ramp portion of isometric contractions) were analyzed using statistical parametric mapping (SPM) paired t-tests (SPM1D statistical package for Matlab®, version 0.40, www.spm1d.org). Normality of data distribution was tested and Wilcoxon signed-rank (zero-

dimensional) t-tests or non-parametric t-tests (one-dimensional) were employed when appropriate. The standardized effect sizes (ES) were calculated and interpreted as follows: trivial: $\text{ES} < 0.2$; small: $0.2 \leq \text{ES} < 0.6$, moderate: $0.6 \leq \text{ES} < 1.2$, large: $\text{ES} \geq 1.2$ (Hopkins et al. 2009). Statistically significant differences between limbs were assumed when $p < 0.05$ and $\text{ES} > 0.60$. The correlation between triceps surae ratios and peaks within limbs was assessed by Pearson's correlation coefficient (r). A strong positive correlation was assumed as $r \geq 0.8$, a moderate positive correlation as $0.5 \leq r < 0.8$, and a weak positive correlation as $0.3 \leq r < 0.5$, while significant correlations were assumed when $p < 0.05$. Final analysis for the EMG activations, peak activations and activation ratios were conducted with $n = 14$ as data for one participant was excluded due to poor quality of the raw EMG data.

4. Results

Normalized triceps surae and TA EMG RMS during the ramp portion of the maximal isometric contractions are presented in Fig. 2. No clusters were found crossing the significance threshold at any time point during the ramp portion of the MVC. Maximum, minimum and mean ES were 0.83, 0.68, and 0.46 for the MG; 0.79, 0.61, and 0.43 for the LG; 0.83, 0.65, 0.54 for the SOL; and 0.58, 0.47 and 0.29 for the TA respectively. The group mean, maximum and minimum EMG peak values are presented in Supplementary material I (Table 1), while no differences were observed between limbs [Supplementary material I (Table 2)]. Inter-individual mean, maximum and minimum peak activations for all muscles are presented in the Supplementary material II.

Mean values of activation ratios are presented in Supplementary material I (Table 1). The mean activation ratios were found to be balanced within and between limbs (Fig. 3A), while no differences were found between limbs considering the mean activation ratios [Supplementary material I (Table 2)]. The low variability of activation ratios is illustrated by the concentrated spatial distribution of activation ratios within each limb (Fig. 3B).

The Pearson's correlation analysis showed that MG and LG ratios correlated significantly to SOL ratio (MG–SOL: $r = -0.53$, $p = 0.04$; LG–SOL: $r = -0.80$, $p < 0.01$) and to GAS ratio (MG–GAS: $r = 0.56$, $p = 0.03$; LG–GAS: $r = -0.86$, $p < 0.01$) in the preferred limb. In the non-preferred limb, MG and LG ratios correlated significantly to GAS ratio (MG–GAS: $r = 0.79$, $p < 0.01$; LG–GAS: $r = -0.86$, $p < 0.01$), and LG ratio to SOL ratio (LG–SOL: $r = -0.65$, $p = 0.01$). MG and LG peaks were found to correlate significantly in the preferred ($r = 0.75$, $p < 0.01$) and non-preferred limbs

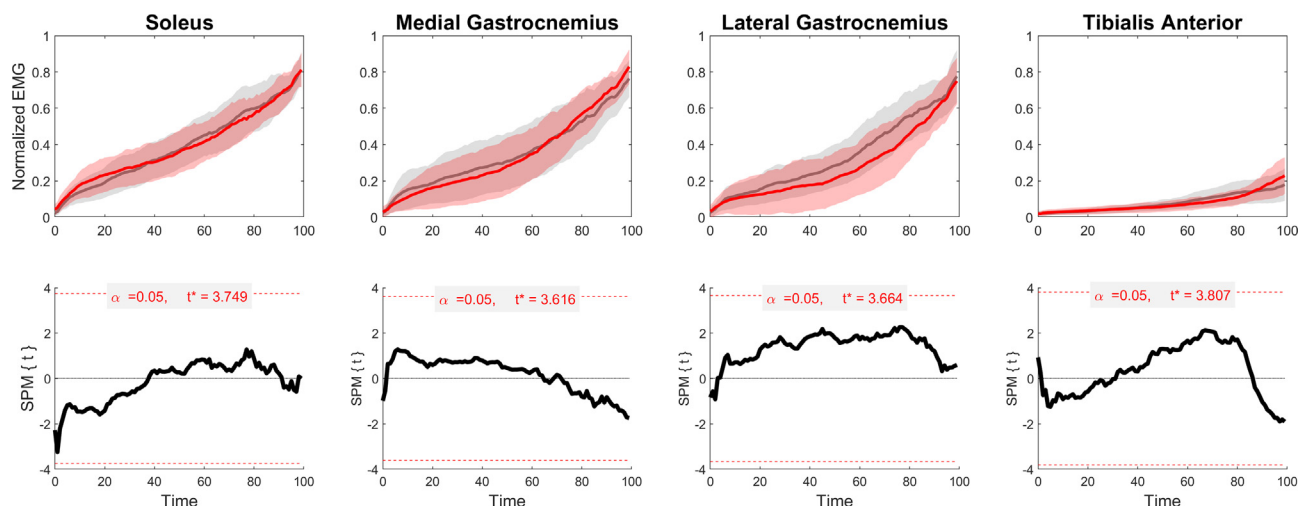


Fig. 2. Upper panels: EMG normalized by peak RMS during the ramp portion of maximal isometric contractions. Solid gray lines: preferred limb; solid red lines: non-preferred limb; Shaded areas: ± 1 standard deviation. Lower panels: statistical parametric mapping (SPM) paired t-tests analysis across the ramp portion of the maximal isometric contractions.

Table 1

Achilles tendon morphological, mechanical and material properties presented as mean \pm standard deviation.

	N	P limb	NP limb	p	ES	r
CSA (mm ²)	15	68.7 \pm 12.1	68.5 \pm 10.5	0.93	0.02	0.54*
Elongation (mm)	15	14.2 \pm 4.4	12.6 \pm 4.8	0.13	0.41	0.65*
E-modulus (GPa)	15	0.34 \pm 0.1	0.43 \pm 0.2	0.09	0.46	0.58*
Force (N)	15	2088 \pm 891	1940 \pm 849	0.14	0.39	0.90*
MA (mm)	15	44.3 \pm 5.3	46.2 \pm 6.7	0.15	0.39	0.68*
Resting length (mm)	15	186.7 \pm 21.7	185.8 \pm 21.8	0.87	0.04	0.62*
Stiffness (N mm ⁻¹)	15	150.3 \pm 50.6	167.3 \pm 67.2	0.08	0.62	0.85*
Strain (%)	15	7.7 \pm 2.7	6.8 \pm 2.5	0.17	0.37	0.56*
Stress (N mm ⁻²)	15	30.8 \pm 12.5	27.7 \pm 9.6	0.10	0.44	0.84*
Torque (N m ⁻¹)	15	95 \pm 40	93 \pm 43	0.58	0.14	0.92*

N = sample size; P = preferred; NP = non-preferred; ES = effect size; r = Pearson's correlation coefficient; CSA = cross-sectional area; MA = moment arm; *statistically significant correlation ($p < 0.05$).

($r = 0.64$, $p = 0.01$). Regarding the correlation analysis among activation peaks and activation ratios, peak LG correlated significantly to LG ratio ($r = 0.89$, $p < 0.01$) and to GAS ratio ($r = -0.77$, $p < 0.01$) in the preferred and in the non-preferred limbs (LGpeak-LG ratio: $r = 0.80$, $p < 0.01$; LGpeak-GAS ratio: $r = -0.64$, $p = 0.01$). All results regarding the Pearson's correlation analysis can be found in the [Supplementary material I \(Fig. 1\)](#).

The experimentally measured torque, force and AT mechanical, material and morphological properties were not different between limbs ([Table 1](#)), although significant correlations were observed between limbs ([Table 1](#)).

The HeeltoProbe_{vector} maximal length variations during contractions were 7.9 (4.5) and 5.6 (4.9) mm in the preferred and non-preferred limbs respectively, which was not statistically different ($p = 0.05$, ES = 0.54) and correlated significantly between limbs ($r = 0.62$). Inter-individual values for AT properties are presented in [Fig. 4](#).

5. Discussion

The present study investigated inter-limb differences in triceps surae activation and AT properties during maximal isometric contractions in runners and triathletes. To the best of our knowledge this is the first study investigating such characteristics in habitual runners. The present study was motivated by i) findings indicating inter-limb differences in ankle extension torque capacity and AT properties in healthy non-athletes ([Pang & Ying, 2006](#); [Valderrabano et al., 2007](#); [Bohm et al., 2015](#); [Chiu et al., 2016](#)),

by ii) studies indicating that triceps surae activation is altered in individuals sustaining AT overuse injury ([Chang & Kulig, 2015](#); [Crouzier et al., 2020](#)), and iii) by the fact that AT overuse injuries occur mainly unilaterally ([Paavola et al., 2000](#); [Janssen et al., 2018](#)). No significant differences were found between limbs regarding triceps surae activations, although correlations within triceps surae activation ratios presented distinct correlations between limbs. Furthermore, the AT can be considered similar between limbs of runners and triathletes since no significant inter-limb differences were observed in morphological, mechanical or material tendon properties.

This study found similar time-varying EMG patterns and EMG peaks between limbs. These results agreed with previous studies showing no difference in EMG amplitude between limbs during maximal ([Valderrabano et al. 2007](#); [Simon et al. 2008](#)) and sub-maximal ([Masood et al., 2014a; 2014b](#)) isometric contractions in healthy individuals. Inter-limb differences in triceps surae EMG have previously been described during locomotion ([Öunpuu and Winter, 1989](#); [Pierotti et al., 1991](#); [Olree and Vaughan, 1995](#); [Jacques et al., 2020](#)). Inter-limb differences observed during a bilateral task were suggested to occur due to common drive generalization of homologous muscle groups or due to an innate larger neural drive to one limb ([Simon & Ferris 2008](#)). Considering locomotion as a bilateral task may help explain why inter-limb differences are found in EMG during locomotion and not during unilateral isometric contractions, similarly to when comparisons were made between bilateral and unilateral isometric contractions ([Simon & Ferris 2008](#)). If that is the case, possible inter-limb differences

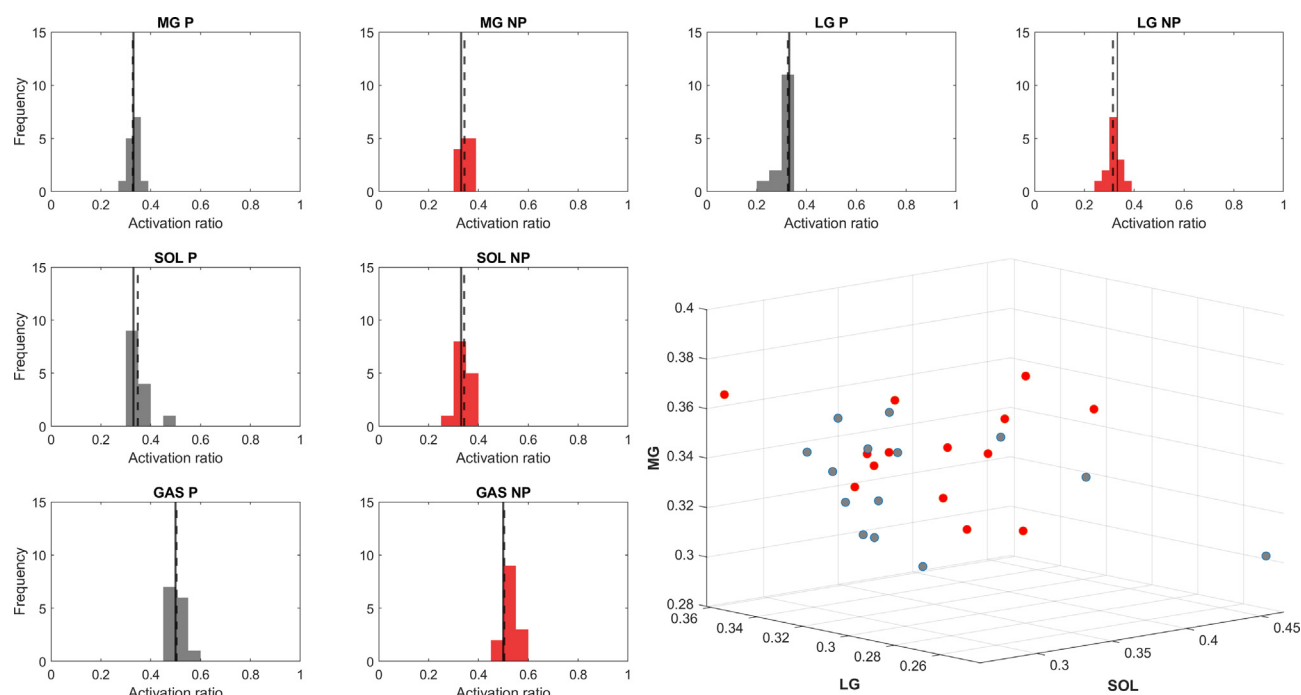


Fig. 3. Group distribution of activation ratios within limbs: gray and red bars illustrate the number of runners and triathletes distributed among activation ratios; vertical solid black lines illustrate group mean and dashed black lines a balanced activation ratio (note the proximity between solid and dashed lines; balanced activation ratios for MG, LG and SOL = 0.33 and for GAS = 0.5). A balanced activation ratio means an equal contribution from each muscle to the total muscle group activation. Right lower corner: gray and red dots illustrate triceps surae activation ratios from each limb for each runner/triathlete. The concentrated spatial distribution around balanced activation ratios (e.g. 0.33) for MG, LG and SOL suggests a low variability occurring in activation ratios between limbs and participants when performing maximal isometric contractions. MG = medial gastrocnemius, LG = lateral gastrocnemius, SOL = soleus; GAS = gastrocnemii; P = preferred limb; NP = non-preferred limb; gray dots = preferred limb, red dots = non-preferred limb.

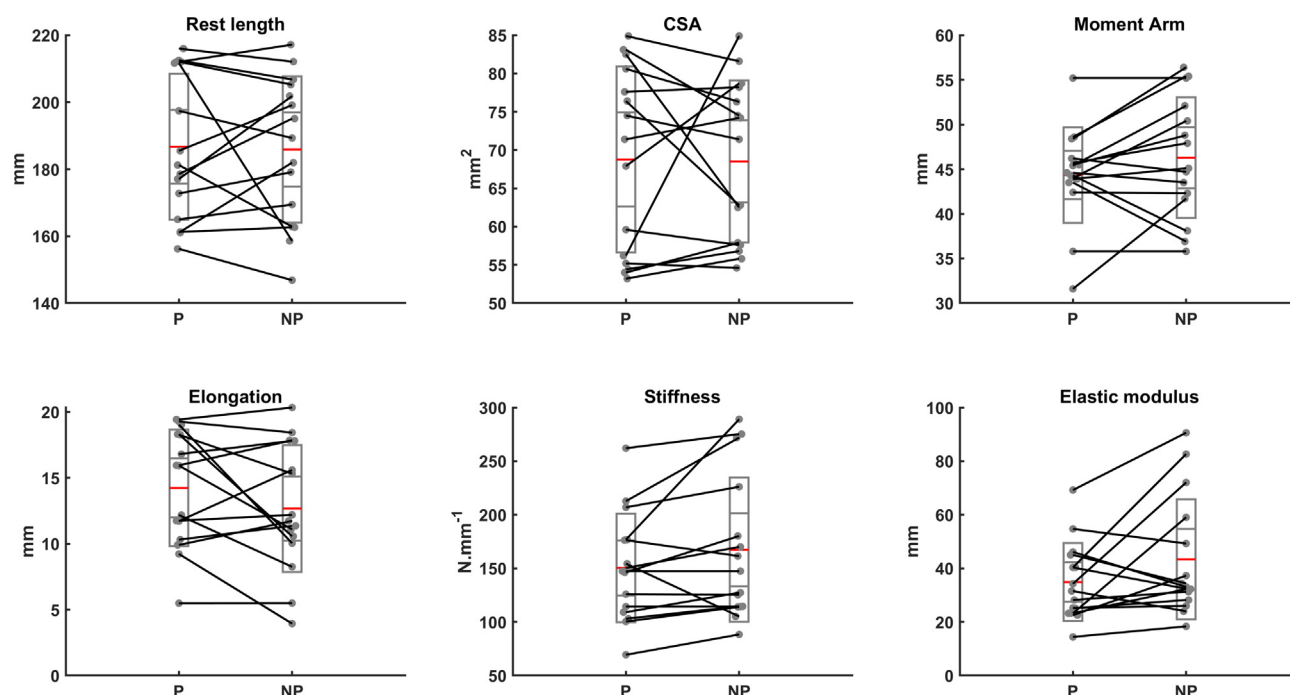


Fig. 4. Inter-subject variability of AT properties in the preferred (P) and non-preferred (NP) limbs. Group mean is represented by the solid red horizontal lines within each box; solid gray horizontal lines within boxes representing the standard error of measurement (95% of confidence interval); outer boxes limits representing ± 1 standard deviation.

occurring in the triceps surae EMG amplitudes would be optimally investigated in the running population during bilateral tasks, while including the analysis of one-dimensional nature of the EMG signals as in the present study.

In this study the relative contribution from each muscle to total triceps surae and gastrocnemii activations was investigated using the activation ratio analysis (Hug & Tucker, 2017; Crouzier et al., 2020). Muscle activation ratios are expected to be balanced during

maximal contractions (Hug & Tucker, 2017; Crouzier et al., 2020), which was confirmed and no-differences observed between limbs. However, the intra-limb correlation analysis between activation ratios showed that MG and SOL correlated significantly in the preferred but not in the non-preferred limb. These findings suggest that intra-limb activation strategies may not be similar between limbs even when activation ratios show no differences among limbs. Possible explanations to these observations may relate to different strategies being adopted in each limb to deal with functional differences of muscle–tendon units (e.g. mono vs. biarticular) or related to limb-specific neuromechanical strategies developed to deal with the contribution of other than triceps surae muscles to the net ankle extensor torque production. However, as the present study seems to be the first to investigate activation ratios among triceps surae muscles bilaterally and their respective intra-limb associations, further comparisons are limited. Due to possible implications of activation strategies to tendon stresses and overuse injury development (Arndt et al., 1998; Bojsen-Møller & Magnusson, 2015; Hug & Tucker, 2017), the intra-limb association between triceps surae activation ratios warrants further examination. Future studies might explore bilateral activation ratios during maximal and submaximal contractions, concomitantly with the assessment of indexes of force as recently implemented (Crouzier et al., 2018, 2020) to account for the interplay between muscle activation and muscle morphological characteristics occurring within limbs.

The observed values of AT morphological, mechanical and material properties were in general in line with prior studies using ultrasonography (Pang & Ying, 2006; Arya & Kulig, 2010; Stenroth et al., 2012; Manal et al., 2013; Bayliss et al., 2016; Cenni et al., 2018) and MRI methods (Bohm et al. 2015). However, it is important to have in mind that experimental designs of studies on AT properties vary considerably, and therefore caution is necessary when comparing findings among studies. A few common differences between studies are joint configuration during the isometric contractions, variability in ankle extensor torque production, contraction mode (e.g. sustained, ramp, explosive), the portion of the tendon where CSA is quantified and how (e.g. US vs MRI), the adopted range of the force–elongation and stress–strain relationships for mechanical and material properties quantification, direct (e.g. hybrid method, MRI) or indirect (e.g. reference values from the literature or scaled musculoskeletal models) determination of AT MA, and others.

This study did not observe inter-limb differences in ankle extensor torque production or AT properties, although prior studies found inter-limb differences in some of these variables in non-athletes (Pang & Ying, 2006; Valderrabano et al., 2007; Bohm et al., 2015; Chiu et al., 2016). The theoretical background in these prior studies considered the preferential use of one limb during daily life activities would elicit inter-limb differences in AT properties. Considering findings of the present study, it could be that habitual runners attenuate the effects of limb preference during daily life due to their regular bilateral training routine, thus limiting differential adaptation of strength and tendon properties. Alternatively, studies have reported different functional roles of limbs during locomotion, reporting one limb as having a propulsive role while the contralateral limb a supporting role (Hirokawa, 1989; Sadeghi et al., 1997; Dalleau et al., 1998; Potdevin et al., 2008). Such functional roles may elicit some degree of differential mechanical load to tendons, and then differential adaptation among limbs would be expected. Assuming such functional roles were occurring during training of the habitual runners investigated here, we should speculate that differences in the mechanical load to each limb may not be of enough magnitude to generate differential adaptation among limbs, although they might still be effective to counteract eventual limb preference effects from daily life usage.

A further perspective relates to the notion that external loading poorly matches internal loading (Scott and Winter, 1990; Nigg et al., 2017; Impellizzeri et al., 2019; Matijevich et al., 2019). This notion reduces the expectation of some degree of AT adaptation due to functional roles of each limb when considering external load related variables. Future studies should consider prospectively investigate triceps surae characteristics in runners and triathletes in a similar design as recently conducted in jumping athletes (Karamanidis & Epro, 2020). Monitoring external and internal loading variables is also necessary to determine which sort of tendon biomechanical properties, if any, better correlate to which sort of mechanical loading variable. As a practical application from this study, coaches and clinicians might perform a bilateral long-term control of AT properties aiming for detection of the expected alterations in tendon properties that generally occur first unilaterally in AT overuse injuries (Paavola et al., 2000; Janssen et al., 2018).

This study has some limitations. The adopted knee angle was implemented to mimic the stance phase of running, although it could limit the MG-MTJ displacement due to a less favorable MG muscle–tendon unit length. However, as observed by Karamanidis & Epro (2020) the MG force–length relationship was not significantly influenced by knee flexion angle in their study, which corroborates with this study as the elongation and strain found here were similar to those reported previously (Bohm et al., 2015; Bayliss et al., 2016). We did not conduct corrections for possible ankle joint rotation as suggested earlier (Arampatzis et al. 2005) although we believe our measurements were not significantly affected, as the absolute differences in heel movement were approximately 2 mm throughout contractions. Moreover, foot strapping on the dynamometer's footplate was conducted by the same evaluator and levels of ankle extensor torque were similar between limbs, diminishing chances of ankle joint rotations, and thus AT elongation, to differ between limbs. The Waterloo questionnaire (Elias et al. 1998) provides a simplified method for definition of limb preference, and thus a future standardization of functional tasks (Pappas et al. 2015) for preference determination would benefit studies in the field of bilateral lower limb biomechanics. A final limitation may relate to the adoption of a non-homogenous sample including men and women, triathletes and runners and therefore differences in weekly training volume among participants. This non-homogeneity may have contributed to increase variability in part of the data, although there is no evidence in the literature that gender and training volume have any effect on the bilateral muscle–tendon properties investigated here.

In conclusion, no differences in triceps surae activation and AT properties were observed between the preferred and non-preferred limb of habitual runners during unilateral maximal isometric contractions. The results presented here suggest that the AT of runners adapts equally between limbs, although correlations within triceps surae activation ratios were not similar between limbs. Further investigation is warranted to enhance current knowledge on how the AT of healthy athletes is loaded when considering triceps surae activation strategies and muscle structure bilaterally.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thanks all runners and triathletes who volunteered to this study and Olga Tarassova and Bas Van Hooren for assisting during data registration. This work was supported by the Swedish Research Council for Sport Science (CIF)

and the Brazilian National Council for Scientific and Technological Development (CNPq).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbiomech.2021.110493>.

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