The problem of Achilles tendon overuse-related injuries is unresolved for athletes, especially for habitual runners who are exposed to high injury rates. Considering such injuries initiate unilaterally and are suggested to occur due to overloading, inter-limb differences occurring during running might be a possible link to its etiology. Current knowledge on inter-limb differences during running and on the bilateral muscle-tendon characteristics of habitual runners might provide directions to preventive strategies designed by coaches and clinicians.

This thesis presents four articles in which bilateral evaluations were conducted in habitual runners. In Study I, athletes were evaluated during running after cycling while kinetic, kinematic and neuromuscular variables previously associated to Achilles tendon injury were analyzed bilaterally. In Study II, a similar approach was used while habitual runners running at two submaximal running speeds were investigated. In Study III, variables associated to limb stiffness and center of mass kinematics were analyzed bilaterally at the same speeds adopted in Study II. In Study IV, the neuromechanical and tendon properties of habitual runners were evaluated bilaterally during isometric contractions.

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BILATERAL KINETIC, KINEMATIC, NEUROMECHANICAL AND MUSCLE-TENDON PROPERTIES OF HABITUAL RUNNERS
Bilateral kinetic, kinematic, neuromechanical, and muscle-tendon properties of habitual runners

Tiago Canal Jacques
“Il bene si fa, ma non si dice. E certe medaglie si appendono all’anima, non alla giacca.”

Gino Bartali
ABSTRACT

Achilles tendon overuse-related injuries are a frequent problem to habitual runners. Such injuries occur more often unilaterally and its etiology is associated to overloading of the tendon tissue. Inter-limb differences during running are a possible cause for overload due to eventual differences in the mechanical loading provided to each limb. Furthermore, inter-limb differences in Achilles tendon properties were found in athletes due to sport-induced differences in the mechanical loading and in non-athletes due to limb preference. Currently, inter-limb differences in the Achilles properties of habitual runners is unknown. The present thesis investigated the existence of inter-limb differences in biomechanical, neuromechanical and Achilles tendon properties in habitual runners. In Study I, thirteen triathletes performed a cycle-run simulation while vertical ground reaction force (GRFv), lower limb kinematics and triceps surae and tibialis anterior activation were evaluated bilaterally during the start, mid and end stages of the 5 km running segment. In Study II, GRFv, lower limb kinematics, triceps surae and tibialis anterior activation and Achilles tendon strain were evaluated bilaterally in habitual runners at two running speeds (2.7 m.s\(^{-1}\) and 4.2 m.s\(^{-1}\)). In Study III, spatiotemporal variables, vertical (kVert) and limb (kLimb) stiffness and center of mass (COM) kinematics were evaluated bilaterally in habitual runners at the same running speeds adopted in Study II. In Study IV, maximal plantarflexion isometric force, triceps surae activation and activation ratios, and Achilles tendon morphological, mechanical and material properties were evaluated bilaterally in habitual runners. In Study I the Soleus activation was lower in the preferred limb from 53.4% to 75.89% of the stance phase (p<0.01, ES range = 0.59 to 0.80) at the end stage of running. In Study II, hip extension velocity was greater in the non-preferred limb from 71% to 93% of the stance phase (p<0.01) during running at 4.2 m.s\(^{-1}\) while no other inter-limb differences were observed. In Study III, no inter-limb differences were observed in spatiotemporal, kVert and kLimb at investigated running speeds. However, COM horizontal velocity was greater from 67% to 87.40% of stance the phase (p<0.05, ES >0.60) when the non-preferred limb was in contact with the ground. In Study IV, no inter-limb differences were observed in triceps surae activation or Achilles tendon properties. The activation ratios of MG and SOL, however, were observed to correlate in the preferred limb only.

In summary, neuromuscular and kinematic inter-limb differences were observed when healthy, non-injured habitual runners performed in running conditions similar to their
ecological conditions. Moreover, the Achilles tendon seem to adapt similarly among limbs of habitual runners, while triceps surae activation strategies might differ between limbs. Findings of inter-limb differences occurring during running may result in over-load during running and therefore might be implicated in the etiology of Achilles tendon overuse-related injuries in habitual runners. Findings of similar tendon properties among limbs suggest both limbs have similar chances of incurring in the injury process. Coaches and clinicians might improve current preventive strategies for Achilles tendon overuse-related injuries by monitoring tendon properties and running biomechanical and neuromuscular variables bilaterally across the season.
This thesis is based on the following original manuscripts:


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1 INTRODUCTION

1.1 Background

High rates of Achilles tendon (AT) overuse-related injuries are observed among the running population [1-5], although its etiology are poorly understood. Biomechanical and neuromuscular variables were associated to AT injury occurrence [6-15], but uncertainty exist on whether these are cause or consequence of injury. Studies have focused on inter-limb differences during running since they have been associated to increased injury risk [16, 17], although variables related to internal loading of the AT were not investigated bilaterally. Considering excessive loading is suggested to result in tendon tissue damage [18-21] and AT overuse injuries occur more often unilaterally [22], inter-limb differences occurring during running requires further investigation. Moreover, inter-limb differences were found in athletes and non-athletes due to sport-mediated inter-limb differences in the mechanical loading [23-25] or due to the preferential use of a given limb during daily life activities [26-28]. Currently there is no information regarding if inter-limb differences exists in bilateral muscle and tendon properties of habitual runners. The present thesis adopted experimental designs aimed to fill the mentioned gaps. Directions to coaches and clinicians on possible strategies to prevent or treat AT overuse-related injuries in habitual runners would arise from such investigation.

1.2 Bilateral lower limb biomechanics during running

Inter-limb differences have been investigated during running [17, 29-33] since they could result in greater mechanical loading being experienced by one limb during training and competition. A larger loading of a given limb would make it more susceptible to overloading, which has been associated to musculoskeletal overuse injuries [20, 34]. A summary of studies focused on the effects of running speed on biomechanical inter-limb differences is presented in Table 1. Kinetic and kinematic variables were investigated at
speeds below 5 m.s\(^{-1}\) (Table 1). The majority of these studies investigated a few sub-maximal speeds and focused on summarized metrics such as average or peak values extracted from the investigated biomechanical continuums (Table 1). Previous studies on inter-limb differences (Table 1) investigated kinetics and kinematics separately, limiting insights on the relationship between those variables. Furthermore, the majority of studies investigating inter-limb differences in running adopted a side-to-side limb classification, while functional classifications (e.g. dominant vs. non-dominant, preferred vs. non-preferred) were much less adopted (Table 1). Although functional classification methods adopted in the literature differ between studies, the side-to-side limb comparison may limit comparisons considering functional roles lower limbs are thought to play during locomotion [35-40].

Finally, the investigation of inter-limb differences during running have previously been limited to runners, therefore not including other habitual runners also exposed to high running training volumes such as triathletes. In this regard, triathletes represent a special case in the running population since during racing they run immediately after cycling [41-44]. The literature provides evidence for effects from prior cycling on subsequent running biomechanics [41-44], and evidence for inter-limb biomechanical differences occurring during cycling [45, 46]. However, investigations are lacking on possible biomechanical inter-limb differences during running preceded by cycling.

Table 1. Summary of published studies on the effects of running speed on inter-limb biomechanical differences during running.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Experience / Sex</th>
<th>Dependent variable</th>
<th>Surface/Speed</th>
<th>Analysis</th>
<th>Limb classification / Method</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamill et al. 1984</td>
<td>5</td>
<td>Non-runners / 8 Males, 2 Females</td>
<td>GRF</td>
<td>Track / 4.87 m.s(^{-1})</td>
<td>Summarized metrics</td>
<td>P-NP / kicking limb</td>
<td>No difference</td>
</tr>
<tr>
<td>Williams et al. 1987</td>
<td>14</td>
<td>Elite runners / Female</td>
<td>GRF</td>
<td>Track / 5.36 m.s(^{-1})</td>
<td>Summarized metrics</td>
<td>Right-left</td>
<td>No limb-specific</td>
</tr>
<tr>
<td>Karamanidis et al. 2003</td>
<td>12</td>
<td>Long-distance runners / Female</td>
<td>Joint kinematics</td>
<td>Treadmill / 2.5, 3 and 3.5 m.s(^{-1})</td>
<td>Summarized metrics</td>
<td>Right-left</td>
<td>No limb-specific</td>
</tr>
<tr>
<td>Zifchock et al. 2006</td>
<td>24-25</td>
<td>Not-specified / Female</td>
<td>GRF</td>
<td>Track / 3.7 m.s(^{-1})</td>
<td>Summarized metrics</td>
<td>Right-left</td>
<td>No difference</td>
</tr>
<tr>
<td>Pappas et al. 2015</td>
<td>22</td>
<td>Non-runners /Male</td>
<td>GRF</td>
<td>Treadmill / 2.22 m.s(^{-1})</td>
<td>Summarized metrics</td>
<td>D-N/ forward jumping test</td>
<td>Greater in the D</td>
</tr>
<tr>
<td>Hughes-Oliver et al. 2019</td>
<td>20</td>
<td>Runners/Male</td>
<td>Joint kinematics</td>
<td>Track / 3.35 m.s(^{-1})</td>
<td>Summarized/time-varying metrics</td>
<td>D-N/ not specified</td>
<td>No difference</td>
</tr>
</tbody>
</table>

GRF = ground reaction force; P = preferred limb; NP = non-preferred limb; D = dominant limb; ND = non-dominant limb.
1.3 Running biomechanics and tendon overuse injuries

AT overuse-related injuries are a frequent problem for habitual runners such as triathletes [3, 5] and runners [1, 2]. High training loads and improper recovery time between training sessions may result in inadequate tendon cellular matrix response, leading to a weakened tendon structure that may limit tendon remodeling [18-21]. Cross-sectional and prospective studies observed associations between biomechanical and neuromuscular variables in AT overuse injury. For example, GRF [6], anterior-posterior displacement of the center of pressure [10], lower limb kinematics [8, 9, 12], and limb stiffness [13, 14] were all associated to the occurrence of AT overuse injury. Regarding neuromuscular variables, EMG timing [11], EMG amplitude [7, 47], and the relative contribution of each muscle to total triceps surae normalized activation [47, 48] were also associated to the occurrence of AT overuse injury. Furthermore, excessive tendon strain (e.g. tendon deformation) was found to be detrimental to tendon health [49-51] and to be greater in individuals sustaining AT overuse injury [52]. A summary of studies investigating the association between biomechanical and neuromuscular variables and AT overuse injury can be found in Table 2.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Experience / Sex</th>
<th>Surface / Speed</th>
<th>Dependent variable</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donoghue et al. 2008</td>
<td>12-22</td>
<td>Non-runners / mixed</td>
<td>Treadmill / 2.8 m.s⁻¹</td>
<td>Ankle kinematics</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Donoghue et al. 2008, Azevedo et al. 2009</td>
<td>21-24</td>
<td>Runners, non-runners / mixed</td>
<td>Runway-Treadmill / 2.8 - 2.97 m.s⁻¹</td>
<td>Knee kinematics</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Van Ginckel et al. 2009</td>
<td>129</td>
<td>Novice runners / 19 males, 110 females</td>
<td>Runway</td>
<td>GRF, COP</td>
<td>Prospective</td>
</tr>
<tr>
<td>Baur et al. 2011</td>
<td>30</td>
<td>Runners / mixed</td>
<td>Treadmill / 3.3 m.s⁻¹</td>
<td>EMG</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Munteanu et al. 2011</td>
<td>-</td>
<td>Mixed</td>
<td>Mixed</td>
<td>GRF, COP, knee and ankle kinematics</td>
<td>Systematic review</td>
</tr>
<tr>
<td>Wyndow et al. 2013</td>
<td>-</td>
<td>Non-runners / males</td>
<td>Track / 4 m.s⁻¹</td>
<td>EMG</td>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Davis et al. 2015</td>
<td>249</td>
<td>Runners / females</td>
<td>Runway / 3.7 m.s⁻¹</td>
<td>GRF</td>
<td>Prospective</td>
</tr>
<tr>
<td>Lorimer &amp; Hume 2014, 2016</td>
<td>-</td>
<td>Runners, triathletes / mixed</td>
<td>-</td>
<td>Stiffness</td>
<td>Systematic review</td>
</tr>
</tbody>
</table>

GRF = ground reaction force; COP = center of pressure; EMG = electromyography.
1.4 External and internal loading

External loading during running refers to the action of forces external to the body due to acceleration of its center of mass, while internal loading refers to the action of forces internal to body segments (e.g. muscle and tendon forces) produced to control segment motion due to center of mass accelerations. Studies investigating inter-limb differences during running focused on biomechanical variables related to external loading (Table 1). However, it has been shown that external loading is sometimes poorly associated to internal loading [53-56]. For example, the load in the tibia are not well correlated to GRF metrics such impact peak and loading rate [53]. Regarding the AT, its peak internal force is much greater than measured peak GRF [55, 57], while timing of peak AT forces and peak GRF do not coincide [55, 57]. However, studies investigating inter-limb biomechanical differences during running (Table 1) and the association of running biomechanics to AT overuse injury (Table 2) have been limited to the evaluation of variables related to external loading. There is a lack of investigation regarding the occurrence of inter-limb differences when considering variables related to tendon internal loading during running.

1.5 Achilles tendon loading, properties and structure

1.5.1 Structure

The AT is the strongest tendon in the human body, experiencing tensional forces of up to 9000 N during overground running at 6 m.s\(^{-1}\) [57]. The AT is comprised of tendon material transferring force from the triceps surae muscles [58]. The triceps surae is a synergistic muscle group composed of the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL) muscles. The MG originates approximately in the medial femoral supracondylar tubercle while the LG originates approximately at the lateral femoral supracondylar tubercle, both at the femoral distal epiphysis of the femur [59]. The SOL has two origins arising from the tibia and fibula, at the inferior border of the soleal line and at the posterior aspect of the head and upper fourth of the dysplasia respectively [59]. The gastrocnemii (MG and LG) are bilateral muscles since they actuate both the knee and the ankle joints [59]. Estimated triceps surae volumes obtained from magnetic resonance imaging (MRI) are approximately 257, 150 and 438 cm\(^3\) for the MG, LG and SOL respectively [60]. The gastrocnemii and soleus aponeuroses combine distally to form the AT [59]. Handsfield, Slane [58], suggests the AT is comprised of sub-tendons structured by tropocollagen, microfibril, fibril, fibre, and fascicles. The free portion of the AT is defined by the portion of tendon between the calcaneus insertion and the soleus muscle-tendon junction [59]. Although this portion represents the tendon per se, the AT is commonly investigated in the literature considering the free tendon and MG aponeurosis up to the MG muscle-tendon junction (MTJ). The MG-MTJ is more frequently adopted due to its more superficial position relative to the SOL-MTJ.

1.5.2 Morphological, mechanical and material properties

Tendons adapt to mechanical load by changing their morphological, mechanical and material properties [61-63]. Morphological properties relate to the tendon’s anatomical structure. Tendon length and tendon cross-sectional area (CSA) are examples of tendon morphological properties. Due to its low cost and reliability, ultrasonography (US) have been used to assess those properties [23, 24, 27]. The slack length (e.g. the length prior to that at which the tendon starts to develop tension) is another commonly assessed morphological property. Tendon slack length is usually adopted in the calculation of tendon strain, although tendon resting length might also be used for strain estimations [26].

Tendon CSA is assessed by estimating the area of a given coronal cross-section of the tendon. The tendon’s mid portion (e.g. 3-4 cm proximal to the calcaneal insertion) is a commonly assessed site for CSA measurements [64, 65]. The mean CSA values can vary from \(\approx 35 \text{ mm}^2\) up to \(\approx 70 \text{ mm}^2\) [23, 24, 26, 64]. Although the AT moment arm (MA) is determined by tendon and bone morphology, it is included in this thesis as a morphological property. The AT MA is calculated as the perpendicular distance from the tendon force line of action to the rotation axis of the ankle joint [66] and can be assessed by combining US and motion capture [67, 68] or by MRI [24].

Commonly assessed AT mechanical properties are strain and stiffness. Tendon strain is defined as the relative displacement of the tendon, and is assessed by normalizing the variation in its length by its initial length (e.g. slack or rest length) when no load is present. Strain is proposed as an important mechanical property driving tendon adaptation (e.g. changes in tendon properties) [61-63]. Excessive strain was found to be detrimental to tendon tissue integrity [49-51] and linked to the occurrence of tendon overuse injuries [52]. Direct estimations of the AT length and strain can be obtained by combining ultrasonography with motion capture [69], although it can also be indirectly estimated using musculoskeletal models [70, 71].

Stiffness is defined as the amount of tendon lengthening per unit of tendon force and is calculated as the slope of a given portion of the AT force-elongation relationship [23,
Tendon force can be estimated by dividing the ankle extension torque by the estimated or directly measured AT MA [23, 24, 26]. The tendon modulus of elasticity (or elastic modulus) is a material property commonly assessed in studies investigating AT adaptation to mechanical loading [23, 24, 26]. The modulus of elasticity represents the relation between tendon stress per unit of tendon strain and is calculated as the slope of a given portion of the tendon stress-strain relationship [23, 24, 26].

1.5.3 Adaptation to load

Inter-limb differences in tendon properties are well documented in athletes involved in sport modalities in which a leading limb is predominant [23-25]. For example, AT stiffness [23, 25] and elastic modulus [23] are increased in the leading limb (e.g., propulsive limb) relative to the contralateral limb in jumpers. Similarly, patellar tendon stiffness is greater in the leading limb of fencers and badminton players in relation to the non-leading limb [24]. Interestingly, differentiation in tendon properties among limbs seem to also occur due to mechanical loading induced by limb preference during long-term, daily use. A greater isometric ankle extensor torque was found in the dominant limb of healthy non-athlete individuals [28], while AT CSA [27], stiffness and elastic modulus [26] are also greater in the dominant limb of healthy, non-athlete individuals. These findings provide evidence indicating differences in tendon properties due to sport practice and due to limb preference. Currently, the literature lacks information of possible inter-limb differences in neuromuscular and tendon properties of habitual runners.

1.6 Analysis of continuous data

The statistical analysis procedures employed in prior studies investigating inter-limb biomechanical differences during running have been conducted using summarized metrics (e.g., peaks, means; zero-dimensional data) extracted from the biomechanical continuum of interest (Table 1). The adoption of such a method oversimplifies the analysis of complex data such one-dimensional biomechanical and neuromuscular trajectories, since their time-varying nature cannot be adequately represented using a single value. Summarized metrics have also frequently been adopted for the investigation of biomechanical inter-limb differences in healthy individuals. The adoption of summarized information to that purpose is not a priori supported, since there is limited evidence from the literature showing inter-limb biomechanical differences to occur in healthy runners when such summarized metrics are considered (Table 1). Statistical parametric mapping (SPM) is one possibility to overcome these limitations. The SPM method “is an n-di-
2 AIMS

The general aim of this thesis is to investigate possible biomechanical inter-limb differences in habitual runners during running and due to running. Specific aims were:

1. To investigate inter-limb differences in kinetic, kinematic and triceps surae activation in triathletes during running after a cycle-run transition. This aim was addressed in Study I.
2. To investigate inter-limb differences in kinetic, kinematic, triceps surae activation and AT strain in habitual runners during submaximal running. This aim was addressed in Study II.
3. To investigate inter-limb differences in lower limb stiffness in habitual runners during submaximal running. This aim was addressed in Study III.
4. To investigate inter-limb differences in neuromuscular characteristics of triceps surae and AT properties of habitual runners. This aim was addressed in Study IV.

3 METHODS

3.1 Subjects and study designs

The studies presented in this thesis were approved by the Regional Ethics Review Board in Stockholm, Sweden and followed the principles outlined in the Declaration of Helsinki. Participants were informed about studies procedures and provided signed consent of their participation. Participants were informed that at any time point they were allowed to terminate their participation in the study, and that no reason would be required for such purpose. Subject characteristics and study designs are presented in Table 3.
### 3.2 Testing protocols

Bilateral evaluation during a cycle-run simulation

Triathletes visited the laboratory twice. In their first visit, triathletes performed an incremental cycling test for determination of maximal cycling power output on a cycle ergometer (Monark LC7, Monark Exercise AB, Sweden). Triathletes performed a warm-up at 100 W at a self-selected pedaling cadence. The incremental test consisted of a cycling trial starting at 150 W with 20 W workload increments each minute at a constant pedaling cadence (90 ± 2 rpm) until volitional exhaustion. Maximal power output was determined as the last stage triathletes were capable to maintain for more than 30 seconds. During their second visit to the laboratory triathletes performed a simulated cycle-run transition consisting of 20 minutes cycling at 70% of maximal power output immediately followed by a 5 kilometers time-trial treadmill run on a motorized treadmill (RL2500E, Rodby Innovation AB, Sweden) (Figure 1). Pedaling cadence and workload were visually inspected and controlled during cycling trials using visual feedback provided by the cycle-ergometer’s head unit. Triathletes were instructed to run at their race-pace and were allowed to increase or decrease treadmill speed but were not informed of their actual running speed.

Figure 1. Laboratory setup adopted during the cycle-run simulations (Study I).
3.2.1 Bilateral evaluation during treadmill running
Runners and triathletes visited the laboratory once for the bilateral evaluation of biomechanical variables during running. Prior to testing they warmed-up for 10 minutes at a self-selected speed below 2.7 m.s\(^{-1}\). Subsequently participants performed three running trials at each speed of 2.7 m.s\(^{-1}\) and 4.2 m.s\(^{-1}\) interspersed by 30 seconds rest on a motorized treadmill (RL2500E, Rodby Innovation AB, Sweden). The first 10 seconds of each running trial were used to allow participants to reach a steady-state running pattern, and the last 20 seconds of the trial were used for data registration.

3.2.2 Bilateral evaluation during isometric contractions
Runners and triathletes visited the laboratory once for the bilateral evaluation of isometric torque, EMG and AT properties. The isometric protocol consisted of 4 maximal plantarflexion contractions performed against a foot plate attached to a dynamometer (IsoMed 2000, D&R Ferstl GmbH, Germany). Each maximal contraction was interspersed by 1 minute rest period. During contraction participants were instructed to gradually increase force production from 0 to 5 seconds, therefore producing a ‘ramp’ torque. Three trials for warm-up and familiarization were conducted prior to testing by runners and triathletes performing submaximal contractions while receiving visual feedback of their ramp torque production. After maximal isometric torque evaluations, the AT MA and CSA were evaluated at rest using the same limb configuration adopted during the isometric trials.

3.2.3 Limb preference
The self-reported Waterloo Footedness Questionnaire [78] was applied in order to determine limb preference (Appendix I). Limb preference was defined whenever more than 60% of questionnaire answers were associated to a given limb.

3.3 Equipment and procedures

3.3.1 Ground reaction force
The GRF was registered using an instrumented insole system (Pedar® Mobile System, Novel GmBh, Munich, Germany) placed inside each participant’s running shoes (Figure 2) operating with a sampling rate of 100 Hz. The insoles were calibrated according to manufacturer instructions, while the system’s accuracy, validity and repeatability have been addressed elsewhere [79].

3.3.2 Motion capture
A twelve camera motion capture system (Oqus 4-series, Qualisys AB, Gothenburg, Sweden) operating with a sampling rate of 300 Hz registered the three-dimensional coordinates of 35 reflective markers placed on bone landmarks and segments during running [7]. A six camera motion capture system (Oqus 4-series, Qualisys AB, Gothenburg, Sweden) operating with a sampling rate of 300 Hz was used for registration of three-dimensional coordinates of reflective markers attached to the US probe, lateral and medial malleoli, and the calcaneus during maximal isometric contractions and AT-MA protocols.

3.3.3 Maximal isometric torque assessment
The ankle extensor torque produced during a maximal isometric contraction was registered using an isokinetic dynamometer (IsoMed 2000, D&R Ferstl GmbH, Germany) operating with a sampling rate of 3000 Hz (Figure 3). The knee joint was positioned at 45 degrees of flexion while the ankle joint was in neutral position (0 degrees, foot perpendicular to the tibia). The participant’s foot was securely strapped to the dynamometer’s foot plate to avoid foot movement, in particular the heel rising from the foot plate.
3.3.4 Moment arm assessment
The AT-MA was assessed using a hybrid method combining ultrasonography and motion capture [68, 80]. A 60 mm field-of-view linear array B-mode US probe operating with a sampling rate of 75 Hz (Echo Blaster 128, LV 7.5/60/128Z-2, Telemed, Lithuania) was positioned longitudinally to the AT line of action to register a sagittal cross-section of the AT as close as possible to the rotation axis of the ankle joint (Figure 4). The three-dimensional coordinates of reflective markers identifying the extrapolated US probe surface and on the lateral and medial malleolus were registered simultaneously during US imaging.

3.3.5 Cross-sectional area assessment
Cross-sectional imaging of the AT was registered using a 35 mm field-of-view linear array B-mode US probe operating with a sampling frequency of 50 Hz (EnVisor M2540, L7535, Philips Electronics N.V., the Netherlands). Two coronal cross-sectional images were registered from each limb at the free AT mid-portion defined as the point 40 mm from the AT calcaneal insertion.

3.3.6 Muscle-tendon junction displacement
The MG-MTJ displacement was registered using a 60 mm field-of-view linear array B-mode US probe operating with a sampling rate of 75 Hz (Echo Blaster 128, LV 7.5/60/128Z-2, Teledem, Lithuania). During the isometric contraction trials the US probe was positioned as illustrated in Figure 3. During running trials the US probe position was positioned as illustrated in Figure 5.

3.3.7 Electromyography
Surface EMG was used to register the myoelectric activity of MG, LG, SOL and tibialis anterior (TA) bilaterally using a telemetered system (Noraxon Telemyo 2400T G2, Noraxon, USA) operating with a sampling rate of 3000 Hz. Pairs of surface Ag/AgCl bipolar electrodes (Neuroline 720, Ambu Inc., Denmark) were placed parallel to the muscle fibers on each muscle bilaterally. The skin was carefully shaved and cleaned with alcohol wipe prior to electrode placement in order to reduce skin impedance. Electrodes were carefully positioned over the MG, LG, SOL and TA muscles observing an inter-electrode distance of 20 millimeters, while electrode location was replicated among
limbs. All surface EMG procedures followed guidelines established by the *Surface EMG for Non-Invasive Assessment of Muscles* (SENIAM) concerted action [81].

### 3.4 Data analysis

#### 3.4.1 Ground reaction force

GRF data were exported using the instrumented insole acquisition software (Pedar® Mobile System, Novel GmBh, Munich, Germany) into Matlab® (The MathWorks Inc., Natick, Massachusetts, USA). Raw GRFv signals were filtered at 10 Hz using a 2nd order low pass Butterworth filter and subsequently up-sampled to 300 Hz by a Fast Fourier Transform (FFT) interpolation method. Touch-down and toe-off were determined using a 50 N threshold [82]. Ten consecutive steps were averaged for each limb and considered as representative of each participant’s GRF pattern during stance.

#### 3.4.2 Kinematics

Marker trajectories registered during running and during a static trial were tracked and labeled using the Qualisys Track Manager software (Qualisys AB, Gothenburg, Sweden) and exported as .c3d files. The data were subsequently converted to the standard .trc file format adopted in OpenSim [83] using an open source toolbox (BTK, https://code.google.com/archive/p/b-tk/) in Matlab®. A generic musculoskeletal model [84] consisting of head, torso, pelvis, right and left femur, patella, tibia and fibula, calcaneus and toes was scaled to each participant’s anatomy using three-dimensional coordinates of reflective markers attached to participants’ body registered during a static trial. Segment mass properties were scaled proportionally to the total participant body mass. Segment lengths were scaled in order to represent participants’ anthropometry based on the relation between bone landmarks, and muscle-tendon units were subsequently scaled relative to segment lengths considering the model’s predefined origin and insertions for triceps surae muscle-tendon units. During scaling, the virtual markers located on the model were relocated to match the location of experimental markers during the static trial by solving an inverse kinematic problem that minimizes the weighted square error between experimental and virtual marker coordinates. The scaling process resulted in a subject-specific musculoskeletal model reflecting each participant’s anthropometrics (Figure 6). All subject-specific musculoskeletal models were built using the same model [84]. Detailed information such model’s degrees of freedom, rigid body coordinate system, joint types, and other are presented in detail by Rajagopal, Dembia

[84]. Joint generalized coordinates during running were estimated using the scaled subject-specific musculoskeletal models and registered marker trajectories were calculated using inverse kinematics in OpenSim. Generalized coordinates generated by the model were subsequently filtered using a 3rd order infinite impulse response (IIR) Butterworth filter with a cutoff frequency of 10 Hz using the Analysis Tool in OpenSim. Joint kinematics and muscle-tendon lengths extracted from ten steps were averaged for each limb and regarded as representative of runners and triathletes’ patterns during stance.

![Figure 6](image_url)  
*Figure 6. A musculoskeletal model scaled to participants’ anthropometrics was used for inverse kinematics procedures in OpenSim.*

#### 3.4.3 Limb and vertical stiffness

Modelled maximal force ($F_{max}$), vertical stiffness ($k_{vert}$) and limb stiffness ($k_{limb}$) were calculated using methods presented in more detail elsewhere [85-87]:

**Maximal force**

$$F_{max} = mg \frac{\pi}{2} \left( \frac{t_f}{t_c} + 1 \right)$$

where $F_{max} = $ maximal force, $m = $ body mass (kilograms), $g = $ gravitational acceleration ($\text{m} \cdot \text{s}^{-2}$), $t_f = $ flight time (seconds), $t_c = $ contact time (seconds).

**Vertical stiffness**

$$k_{vert} = \frac{F_{max}}{\Delta COM}$$

where $k_{vert} = $ vertical stiffness, $\Delta COM = $ vertical displacement of the center of mass (meters).
Limb stiffness

\[ k_{\text{Limb}} = \frac{F_{\text{max}}}{\Delta L_{\text{Limb}}} \]

\[ \Delta L_{\text{Limb}} = L - \sqrt{L^2 - \left(\frac{v_{t}^2}{2}\right) + \Delta \text{COM}} \]

where \( \Delta L_{\text{Limb}} \) = limb displacement (meters), \( L \) = limb length (meters), \( v \) = running speed (m s\(^{-1}\)), \( t_c \) = contact time (s), \( \Delta \text{COM} \) = vertical displacement of the center of mass (meters); Contact time (\( t_c \)) and flight time (\( t_f \)) were calculated by differentiating reference frames identified from touch-down and toe-off, while COM displacement during stance was obtained using the musculoskeletal model and inverse kinematics results from OpenSim. \( t_c \), \( t_f \), and \( \Delta \text{COM} \) averaged from ten consecutive steps from each submaximal speed were used as representative of each limb.

### 3.4.4 Tendon length and strain during running

The MG-MTJ displacement registered by US during running was exported to an open source video analysis software (Tracker 5.0.7, Open Source Physics, https://www.compadre.org/osp/index.cfm). The origin of a local coordinate system was defined as the superior left corner of the US field of view and the \( x \) coordinates defined the tendon’s proximal-distal displacement. The two dimensional coordinates of the MG-MTJ relative to the coordinate system were determined by tracking the MG-MTJ displacement during running frame-by-frame (Figure 14). AT lengthening (mm) was estimated by summing the instantaneous vector length determined from the calcaneus marker to the US probe to the tracked MG-MTJ displacement. AT strain was calculated during running as AT lengthening divided by the AT length at toe-off.

### 3.4.5 Moment-arm

The linear distance from the AT mid-portion line (Figure 5) to the true US probe surface was measured using an open source video analysis software (Tracker 5.0.7, Open Source Physics, https://www.compadre.org/osp/index.cfm) after proper ultrasound image calibration using known distances from the ultrasound field of view. Since the distance from true US probe surface to the extrapolated US probe surface was known, AT-MA was simply determined by subtracting those distances from the distance of US markers to the mid-point of the ankle joint as previously described [68, 80].

### 3.4.6 Cross-sectional area

Registered US images of the AT in the coronal plane were imported to ImageJ image analysis software (ImageJ 1.5i, National Institute of Health, USA). The polygon tool was used in ImageJ to determine the AT CSA (Figure 8) as previously described [64, 65] after proper image calibration and accounting for pixel aspect ratio. The CSA was considered as the average of three measurements taken from the AT from each limb.

---

*Figure 7. A video analysis software (Tracker 5.0.7, Open Source Physics, https://www.compadre.org/osp/index.cfm) was used to track the medial gastrocnemius muscle-tendon junction. Small red dots represent history of the MG-MTJ tracked position each time frame.*
3.4.7 Tendon force and stress
Ankle extensor torque registered during maximal isometric contractions were filtered at 10 Hz using a 4th order low pass Butterworth filter. The ramp portion of torque production was determined from 1% of peak torque to 100% of peak torque. The AT force in each limb was approximated by dividing ankle extensor torque by its respective estimated AT MA. The AT stress (N.mm⁻²) was calculated for each limb dividing the AT force by its CSA.

3.4.8 Tendon rest length, length and strain
The MG-MTJ displacement registered during the isometric contractions was analyzed using the same procedures as for the running trials. The AT resting length (mm) was calculated as the sum of the instantaneous vector length determined from the calcaneus marker to the US probe marker and the MG-MTJ local coordinates with the foot attached to dynamometer footplate at rest. The AT lengthening was calculated as the variation in the AT length during the isometric contractions by using the same method as for calculating the AT rest length. The AT strain (%) was calculated as the AT lengthening (mm) divided by rest length (mm).

3.4.9 Tendon stiffness and modulus of elasticity
The AT stiffness (N.mm⁻¹) was estimated as the linear regression from 30% to 90% of the AT force-elongation relationship. The AT modulus of elasticity (GPa) was estimated as the linear regression from 30% to 90% of the AT stress-strain relationship curve.

3.4.10 Electromyography
Raw EMG signals registered during the cycle-run and submaximal running trials (Figure 15) were band-pass filtered at 20-500 Hz using a 5th order Butterworth filter. Subsequently the root man square (RMS) envelopes were calculated from the filtered signal using a 40 ms moving-window. Raw EMG signals registered during isometric contractions were band-pass filtered at 30-850 Hz using a 4th order Butterworth filter and RMS envelopes were calculated using a 300 ms moving-window. EMG RMS in Study I was normalized using the peak EMG RMS found between the Start, Mid and End stages of the 5 km run. The EMG RMS in Study II was normalized using the peak RMS value found in each respective speed. In Study IV the EMG RMS was normalized using the peak RMS from the trial in which maximal torque occurred. The relative contribution each triceps surae muscle to total triceps surae activation and of the MG to total gastrocnemii (MG + LG) activation was determined. These MG, LG, and SOL ratios were calculated by dividing each muscle normalized activation by the sum of all triceps surae normalized activations as in Crouzier, Lacourpaille [88]. The ratio of MG normalized activation by the gastrocnemii normalized activation [88] was defined as ‘GAS’ ratio.

3.5 Statistical analysis
Discrete data analysis was adopted in Studies II and IV. For all the other inter-limb comparisons, the SPM analysis was adopted for testing the analysis of time-series data. All data were tested for normal distribution. In the case of non-normal data distribution, Wilcoxon signed-rank tests or statistical non-parametric tests (SnPM) were adopted. Paired t-tests were used in Studies I, II, III and IV to identify possible difference between the preferred and non-preferred limb. Pearson’s correlation coefficients were used in Studies III and IV for the assessment of the level of relationship between variables. Effect sizes were calculated for the zero-dimensional and one-dimensional data as differences between the means weighted by the mean of standard deviations [89].
In Study I, results from the GRFv and COPAP during the stance phase show no cluster crossing the SPM threshold at any time-point (Figure 9). Similarly, in Study II no differences were found in GRF during either slow (2.7 m s\(^{-1}\)) or fast (4.2 m s\(^{-1}\)) running. No inter-limb differences were observed in the analysis of limb and vertical stiffness, or spatiotemporal variables during slow and fast running (Table 5, Figure 10).
Figure 10. Mean, standard deviation and 95% confidence intervals for modelled maximal force (\(F_{\text{max}}\)), vertical stiffness (\(k_{\text{Vert}}\)), limb stiffness (\(k_{\text{Limb}}\)), and limb displacement (\(\Delta \text{Limb}\)) at slow (2.7 m.s\(^{-1}\)) and fast (4.2 m.s\(^{-1}\)). Black dots representing participant’s values for the preferred (P) and non-preferred (NP) limbs.

Table 5. Statistical results from inter-limb comparisons (preferred vs non-preferred) conducted in Study III.

<table>
<thead>
<tr>
<th></th>
<th>2.7 m.s(^{-1})</th>
<th>4.2 m.s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td>ES</td>
</tr>
<tr>
<td>(F_{\text{max}})</td>
<td>0.52</td>
<td>-0.19</td>
</tr>
<tr>
<td>(k_{\text{Vert}}^{\text{breaking}})</td>
<td>0.17</td>
<td>-0.41</td>
</tr>
<tr>
<td>(k_{\text{Vert}}^{\text{propulsion}})</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td>(k_{\text{Limb}}^{\text{breaking}})</td>
<td>0.87</td>
<td>0.04</td>
</tr>
<tr>
<td>(k_{\text{Limb}}^{\text{propulsion}})</td>
<td>0.54</td>
<td>0.17</td>
</tr>
<tr>
<td>(\Delta \text{Limb}^{\text{breaking}})</td>
<td>0.89</td>
<td>0.03</td>
</tr>
<tr>
<td>(\Delta \text{Limb}^{\text{propulsion}})</td>
<td>0.66</td>
<td>-0.12</td>
</tr>
<tr>
<td>Limb length</td>
<td>0.71</td>
<td>-0.16</td>
</tr>
<tr>
<td>Contact time</td>
<td>0.86</td>
<td>-0.04</td>
</tr>
<tr>
<td>Flight time</td>
<td>0.23</td>
<td>-0.36</td>
</tr>
<tr>
<td>Stride time</td>
<td>0.75</td>
<td>0.09</td>
</tr>
<tr>
<td>Braking time</td>
<td>0.53</td>
<td>0.18</td>
</tr>
<tr>
<td>Propulsion time</td>
<td>0.53</td>
<td>-0.18</td>
</tr>
</tbody>
</table>

p = p value, ES = effect size; CI = confidence interval.

In Study II hip joint extension velocity was greater in the non-preferred limb at both tested speeds from 71\% to 93\% (p<0.01) of the stance phase (Figure 11). Furthermore, in Study III a greater COM\(_{\text{horiz}}\) velocity in the preferred limb was observed during 0-12\% of the stance phase (p=0.05, ES=0.60), and greater COM\(_{\text{horiz}}\) velocity in the non-preferred limb during 67-87.40\% of stance (p<0.05, ES>0.60) during slow running (Figure 12). During fast running, the COM\(_{\text{horiz}}\) velocity was found to be greater in the preferred limb during 0-1.6\% of stance (p=0.05, ES=0.60). Furthermore, no inter-limb differences were observed in Study I and II regarding the MG, LG and SOL tendon strains estimated from the musculoskeletal model and in Study II considering strain from in vivo estimations (Figure 13).
Figure 11. Joint velocities during slow and fast running across the stance phase (touch down to ipsilateral toe off); Upper panels: the preferred limb is represented by blue lines while the non-preferred limb is represented by orange lines. Lower panels: statistical parametric mapping analysis (SPM, blue lines) and effect size (ES, orange lines) results are presented respectively to upper panels. Slow running (2.7 m.s$^{-1}$) = dashed lines; fast running = solid lines represent (4.2 m.s$^{-1}$).

Figure 12. Upper panels: center of mass vertical (COM$_{vert}$) and horizontal (COM$_{horiz}$) positions and velocities at slow (2.7 m.s$^{-1}$) and fast (4.2 m.s$^{-1}$) running speeds. Slow running: black dashed lines; fast running: black solid lines; vertical black dashed and solid lines indicate the transition from breaking to propulsion at slow and fast running speeds respectively. Lower panels: statistical parametric mapping (SPM, blue lines) and effects size (ES, orange lines) results are presented respectively to upper panels. Vertical solid red lines indicate data clusters which crossed the t threshold indicating p < 0.05.
In Study I the SOL activation was lower in the preferred limb from 53.4% to 75.89% (p < 0.01, ES range = 0.59 to 0.80) of the stance phase at the final stage of running after cycling Figure 14. The LG activation was also lower in the preferred limb from 67.75% to 82.66% of the stance phase at the same stage (p < 0.01), although the effects was considered of small magnitude (ES range = 0.41 to 0.51) Figure 14. The MG activation was observed to be lower in the non-preferred limb relative to the preferred at the mid stage from 75.24% to 78.7% of the stance phase (p < 0.01) although the difference was considered small (ES range = 0.39 to 0.58) Figure 14. No inter-limb differences were observed in the EMG analysis across a stride during submaximal running in Study II (Figure 15).
The results from Study IV indicate no inter-limb differences in AT morphological, mechanical or material properties (Table 6). No inter-limb differences were observed in triceps surae ratios and TA activation patterns during maximal isometric contractions (Figure 16). Furthermore, the results from an intra-limb analysis showed that MG and LG ratios correlated significantly to SOL ratio (MG-SOL: r=-0.80, p<0.01) and to GAS ratio (MG-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=0.86, p<0.01), and LG ratio to SOL ratio (LG-SOL: r=-0.65, p=0.01). All results from the Pearson’s correlation analysis are presented in the Appendix.

### Table 6. Achilles tendon morphological, mechanical and material properties presented as mean (standard deviation) and the corresponding Pearson’s correlation coefficient (r) for the association between limbs.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P limb</th>
<th>NP limb</th>
<th>p</th>
<th>ES</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA (mm²)</td>
<td>15</td>
<td>68.76 (12.14)</td>
<td>68.52 (10.58)</td>
<td>0.93</td>
<td>0.02</td>
<td>0.54*</td>
</tr>
<tr>
<td>Elongation (mm)</td>
<td>15</td>
<td>14.24 (4.41)</td>
<td>12.67 (4.81)</td>
<td>0.13</td>
<td>0.41</td>
<td>0.65*</td>
</tr>
<tr>
<td>Emodulus (GPa)</td>
<td>15</td>
<td>0.34 (0.14)</td>
<td>0.43 (0.22)</td>
<td>0.09</td>
<td>0.46</td>
<td>0.58*</td>
</tr>
<tr>
<td>Force (N)</td>
<td>15</td>
<td>2088 (891)</td>
<td>1940 (849)</td>
<td>0.14</td>
<td>0.39</td>
<td>0.90*</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>15</td>
<td>44.34 (5.34)</td>
<td>46.28 (6.75)</td>
<td>0.15</td>
<td>0.39</td>
<td>0.68*</td>
</tr>
<tr>
<td>Rest length (mm)</td>
<td>15</td>
<td>186.68 (21.77)</td>
<td>185.87 (21.83)</td>
<td>0.87</td>
<td>0.04</td>
<td>0.62*</td>
</tr>
<tr>
<td>Stiffness(N.mm⁻¹)</td>
<td>15</td>
<td>150.32 (50.62)</td>
<td>167.36 (67.28)</td>
<td>0.08</td>
<td>0.62</td>
<td>0.85*</td>
</tr>
<tr>
<td>Strain (%)</td>
<td>15</td>
<td>7.7 (2.7)</td>
<td>6.8 (2.5)</td>
<td>0.17</td>
<td>0.37</td>
<td>0.56*</td>
</tr>
<tr>
<td>Stress (N.mm⁻²)</td>
<td>15</td>
<td>30.82 (12.52)</td>
<td>27.79 (9.6)</td>
<td>0.10</td>
<td>0.44</td>
<td>0.84*</td>
</tr>
<tr>
<td>Torque (N.m⁻¹)</td>
<td>15</td>
<td>95.81 (40.01)</td>
<td>93.44 (43.65)</td>
<td>0.58</td>
<td>0.14</td>
<td>0.92*</td>
</tr>
</tbody>
</table>

CSA = cross sectional area; MA = moment arm; P = preferred; NF = non-preferred; ES = effects size. *statistically significant correlation (p<0.05).

Figure 16. Upper panels: triceps surae and tibialis anterior EMG normalized by peak RMS across 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-test analysis across the ramp portion of the maximal isometric contractions.
5 METHODOLOGICAL CONSIDERATIONS

5.1 Cycle-run simulation, treadmill run and fatigue

It was not possible to control for exclusive effects of fatigue due to the absence of a control run (e.g. no preceding cycling) in Study I. Furthermore, a fixed pedaling cadence and workload were adopted for standardization purposes, although it may not mimic ecological conditions of pedal power production in triathlon [90]. Running speeds investigated in Study II were chosen to complement prior studies focused on inter-limb differences when investigating external load related variables, which were up to 3.7 m.s\(^{-1}\). Fatigue was not present in Study II since trials were of short duration and interspersed by enough recovery time.

5.2 Kinetics

Inter-limb differences in high frequency components of the GRF were not registered since the used instrumented insole system is limited to a low operating sampling rate. Stiffness parameters during running were estimated using spatiotemporal parameters, and the validity and reliability of the applied method were demonstrated previously [85-87]. High intra and inter-day reliability have been reported during treadmill running at 4.4 m.s\(^{-1}\) [85]. Regarding to validity, prior studies showed acceptable bias when comparing the method to gold standard reference values for both overground and treadmill running [87]. Finally, high intra-class correlation coefficients (values of 0.61 and 0.99 for leg length and force estimations respectively) and -5%, -1% and 6% difference between the reference and the indirect method have been reported for limb stiffness, force and limb length [86].

5.3 Kinematics

The knee joint is constrained to the sagittal plane in the musculoskeletal model adopted in this thesis [84]. Habitual runners were using shoes and therefore the foot marker set did not allow adequate quantification of subtalar and metatarsophalangeal joint kinematics. Therefore, the subtalar and metatarsophalangeal joints were constrained to zero degrees following procedures elsewhere [84]. In Study I slack length from the model was used for tendon strain estimations, whereas in Study II tendon length at toe-off was adopted for tendon strain estimations. This was to account for possible differences in knee and ankle kinematics at toe-off, since MG, LG and SOL muscle-tendon unit paths are dependent on those joint configurations. Segment and muscle-tendon unit lengths are defined during the scaling process [84], and are defined symmetrically in OpenSim [83]. Thus, slack length, tendon length and therefore tendon strain derived from the musculoskeletal models fail to accurately identify subject-specific inter-limb differences. Future studies may investigate the effect of adding degrees of freedom to the knee joint, adopting unconstrained subtalar and metatarsophalangeal joints, and improving scaling accuracy when evaluating muscle-tendon strains bilaterally using musculoskeletal models.

5.4 Electromyography

Surface electromyography was previously adopted in studies investigating neuromuscular adaptations to long duration cycling protocols (e.g. >3 hours) in triathletes [91, 92], and also in cycle-run transition studies [93, 94]. Electrodes might be sensitive to the time they are in use mainly due to sweating, which affects the electro-mechanical stability skin-sensor interface with possible implications to the EMG signal registration [95]. In Study I triathletes cycled with EMG electrodes already attached to the skin to allow a faster transition from cycling to run as in prior studies [93, 94]. Although not mentioned in prior studies [91-94], it is not possible to exclude any effects of sweating on the electro-mechanical stability of the sensors and thus in the registered EMG signal across the cycle-run simulation. In addition, different EMG normalization procedures were adopted in Studies I and II. In Study I the independent variables were both running stages and limb preference, which required normalization by the peak EMG RMS achieved in the entire running trial. In Study II, the independent variable was limb preference only, which in turn allowed EMG normalization using the peak EMG RMS achieved during each tested running speed.
5.5 In vivo estimations

Tendon length and strain estimations derived from motion capture combined to MG-MTJ displacement registered using ultrasonography requires some caution. The ‘external’ Achilles tendon length differs depending upon whether the anatomical curvature is accounted for [96]. Furthermore, a great range of ultrasound sampling rates (30 to 146 Hz) have been used for MG-MTJ displacement registration during running [97-101]. Those sampling rates are considered to be well below the ideal for the accurate estimation of muscle-tendon dynamics [102, 103]. Future studies would benefit from the adoption of greater sampling rates than used so far [97-101] and from including tendon curvature [96] when aiming for accurate tendon length and strain estimations.

5.6 Limb preference assessment

Some of the previous studies on bilateral analysis have adopted questionnaires for determination of limb preference/dominance (see Table 1). In this thesis limb preference was assessed by applying the Waterloo footedness questionnaire [78] following similar methods in the literature where questionnaires were applied [26, 46]. However, the relation between limb preference/dominance classification and its relevance to each limb’s functional biomechanical role during locomotion is not well known. Studies on bilateral biomechanical analysis during bilateral tasks such as running might benefit from standardization of methods aiming to improve definition of functional roles of each limb during locomotion.

6 DISCUSSION

Investigations on inter-limb biomechanical differences during running have been conducted based upon the rationale that they would result in differences in the mechanical loading experienced by each limb. Previous studies have focused on discrete data extracted from the biomechanical continuum, and on evaluating a limited range of sub-maximal speeds. Moreover, those studies have not examined running populations apart from runners, and have focused on external loading variables, therefore not investigating variables associated to internal loading. Moreover, information is lacking regarding the bilateral muscle-tendon characteristics of habitual runners. The present thesis adopted experimental designs aimed to fill these knowledge gaps, therefore providing a broader analysis of inter-limb biomechanical differences during running and of bilateral muscle-tendon status in habitual runners. The present thesis focused on the Achilles tendon since overuse injuries in this tendon are a frequent problem to habitual runners [1-5].

6.1 Inter-limb differences during running after cycling

The SOL activation was reduced in the preferred limb at the final stage of running after cycling from mid-stance to the beginning of toe-off. Besides triceps surae EMG, no inter-limb differences were found in kinetics or kinematics, including tendon strains estimated from the musculoskeletal model. Since there are more actuators than necessary to actuate the ankle joint, direct effects from reductions in EMG RMS to ankle joint kinematics may not be expected. Furthermore, it is not possible to directly translate myoelectric information obtained from EMG to muscle force production [104]. However, although the studies in this thesis were not designed to explain the nature of inter-limb differences, a reduction in SOL activation could be speculated to be related to its role during cycling and running. During cycling the SOL has a major role in transferring power to the cranks [105] while during running SOL has a major role during support and propulsion [106-108]. An early observation of decreases in SOL activation during a run preceded by cycling in triathletes [94] may be linked to the important roles of SOL in cycling and running. In addition, a large neural drive to SOL in the preferred limb was previously suggested to occur during walking [37]. A greater neural drive to the SOL in...
the preferred limb might also have occurred in the triathletes during the cycle-run simulation. This might have reduced the central nervous system capacity to maintain the same level of neural drive to both limbs throughout the entire protocol. Although speculative, this theory might be supported by recent findings that neural drive is not equally shared by synergistic muscle groups such as the triceps surae [109]. A greater neural drive to SOL might also occur to counteract the less optimal MG fascicle length occurring during knee flexion, which was associated to reductions in MG firing rates [110]. Currently, information concerning LG fascicles and firing rates is lacking. With that in mind, the modulation of neural drive to a given limb and to a given muscle within a synergistic muscle group should be further investigated considering the triceps surae during running in triathletes.

In summary, Study I filled current gaps in the literature by investigating inter-limb differences in GRF, lower limb kinematics and triceps surae EMG in triathletes during a cycle-run simulation. No inter-limb differences in external load-related variables were found during running preceded by cycling. The observed reduction in SOL EMG RMS in the preferred limb might occur due to a prolonged greater neural drive to that limb and to the important roles of SOL in both cycling and running.

6.2 Inter-limb differences during submaximal running

In Study II greater hip extension velocity was found in the non-preferred limb after the mid-stance towards toe-off at both slow and fast running. Furthermore, a greater COMhoriz velocity was found during the braking portion of stance performed with the preferred limb at slow and fast running. The COMhoriz velocity was also greater in the non-preferred limb during the propulsion phase of stance at slow running speed in Study III, with a similar but non-significant difference also observed during fast running. Moreover, when a side-to-side rather than a limb preference comparison was conducted in Study III, inter-limb differences in kLimbm and braking and propulsion times were found. However, no inter-limb differences in GRF, triceps surae EMG or AT length and strain were found. Furthermore, the MG, LG, SOL and TA tendon strain analyses from musculoskeletal models showed no inter-limb differences, in agreement with joint displacement results.

Study III suggested that the preferred limb may be limited in controlling COMhoriz velocity during braking in relation to the non-preferred limb, since COMlimbm velocity was found to be lower in the beginning of preferred limb stance. In contrast, the non-preferred limb appears more prone to generating greater propulsion, since the COMlimbm velocity was greater when that limb was in contact with the ground. The greater hip velocity found in the non-preferred limb in Study II may be one possible mechanism helping to generate the greater COMhoriz velocity during a similar portion of the stance phase in Study III. It is noteworthy that no inter-limb differences in knee or ankle joint kinematics accompanied the differences in hip joint kinematics. If kinematic symmetry during running is an aim of the central nervous system (CNS), then results from Study II suggest it is more difficult to achieve similar joint velocities in the proximal than in the distal joints. On the other hand, if neural drive [37] or other CNS strategies determine functional roles for each limb during running, then inter-limb differences in hip and therefore COMhoriz velocity may be innate in human locomotion modes. A final speculation considers the intrinsic musculoskeletal properties of each limb. Adding a mass between 0.5 to 2 kg to able-bodied individuals was found to alter hip and knee joint kinetics during walking [111, 112]. Masses ranging from 150 to 350 g added to the foot were also found to affect stride frequency, anterior-posterior impulse, limb stiffness, and mechanical work during running [113]. If inter-limb differences in segments mass are innate to habitual runners as identified in sprinters [114] and active individuals [115], they may affect joint and COM kinematics, with possible implications to propulsion.

In summary, Studies II and III filled current gaps in the literature by investigating inter-limb differences in external and internal load-related variables during submaximal running. Inter-limb differences were observed in hip and COM kinematics and may be responsible for side-to-side differences in propulsion during running. The literature suggests joint torques and powers are modulated across the hip, knee and ankle joints when running speed varies [116-119]. However, the bilateral distribution of joint moments across the stance due to inter-limb differences in propulsion and its possible implication to AT loading are not known and requires investigation.

6.3 Bilateral neuromuscular and tendon properties

No inter-limb differences in triceps surae EMG or AT properties were found in Study IV. However, correlations among EMG ratios differed between limbs, suggesting muscle coordination may not be identical between the preferred and non-preferred limbs. Bilateral deficits in neuromechanical properties were suggested to occur due to homologous muscle groups being treated as single one by the CNS (e.g. common drive theory) or due to an innate, chronic asymmetric neural drive to the preferred and non-preferred limbs [120]. Therefore, assuming running as a task involving the coordination of both limbs simultaneously, bilateral rather than unilateral isometric contractions may have been able to mimic possible bilateral deficits occurring in the habitual runners investigated in Study IV. Although inter-limb differences were not observed in EMG ratios, a significant intra-limb correlation between MG and SOL was observed only in the preferred limb. If muscle synergism is an aim of the CNS during a given task, then inter-
limb differences in the correlation among triceps surae muscles ratios observed in Study IV may indicate an improved ability of the preferred-limb to perform such a task. Since information of bilateral EMG ratios are still scarce, the results from Study IV should be further confirmed.

AT properties investigated in Study IV were chosen based upon prior studies showing that limb preference may induce inter-limb differences in some AT properties in non-athletes [26-28]. Speculations can be made relative to findings of no inter-limb differences in AT properties of habitual runners. Inter-limb biomechanical differences during running was not a criteria for participation in Study IV, which possibly included individuals in a wide spectrum of inter-limb differences in AT loading during training and competitions. Thus, any inter-limb differences in AT properties resulting from limb preference as observed in non-athletes [26-28] might be mitigated rather than exacerbated in habitual runners due to training and competition routines. Whichever are the reasons for findings from Study IV, the quantification and the magnitude of the mechanical loading during running leading to alterations in AT properties are difficult problems to address. The magnitude problem is illustrated by the fact that in trained runners with 5 years of experience and 80 km/week training volume only AT CSA was found to be greater than in non-runners, while mechanical and neuromechanical properties were not different between groups [121]. Similarly, a nine month running training program performed by non-runners resulted in no alteration in AT mechanical properties [122]. The quantification and magnitude problems could be addressed in prospective studies aiming to monitor AT properties in habitual runners in combination with internal and external loading quantification aiming to understand the relationship between mechanical loading and muscle-tendon adaptation across the season.

In summary, Study IV investigated possible inter-limb differences in triceps surae neuromuscular properties concomitantly with AT properties in habitual runners. The results suggest that habitual running training does not lead to differential adaptation in muscle activation and tendon properties, at least considering the experimental design adopted in Study IV. However, triceps surae coordination strategies inferred from EMG ratios seem to differ between limbs during unilateral fixed-end isometric contractions. Inter and intra-limb differences in triceps surae EMG ratios necessitate further investigation during isometric and dynamic bilateral tasks.

6.4 Implications to overuse-related tendon injury

Inter-limb differences in SOL EMG RMS found in Study I may be related to the high rates of AT overuse-related injuries in triathletes [3, 5]. Unequal displacements observed within the AT during different conditions [123-126] are proposed to be the result of differential sliding occurring between triceps surae sub-tendons [58, 127]. Assuming intra-tendon sliding is similar among limbs, it is possible that a differential SOL activation occurring between limbs may also result in differential tendon displacements between limbs. If those unequal displacements are resulting in differential strain within the AT in each limb, than inter-limb differences in tendon strain may arise. Strain drives important tendon adaptive responses that, depending on the magnitude, can be positive or negative to tendon’s health [18, 19, 49, 50, 62, 128]. Although a possible influence of muscle activation on AT fiber gliding could be linked to the high rates of tendon injury in triathletes, the role of differential displacements within AT are still not completely understood. Studies have shown that aging [123], repair [124] and overuse-related tendon injury [129] seem to result in more uniform displacement occurring within the AT, suggesting that some degree of differential displacement is actually an innate characteristic of the healthy tendon. Although further investigation is necessary to unveil healthy or optimal levels of differential displacement within the AT and the effects of triceps surae activations on it, triathletes may benefit from a strength training program for ankle extensors aiming to prevent possible decreases in neural drive. This in turn could prevent differences in triceps surae activation among limbs to occur during running after cycling.

The results from Study II and III indirectly suggest the preferred limb has a functional role as a propulsive limb. A limb dedicated to propulsion was previously suggested to occur during walking [37-40] and running [35, 36], and therefore might be an innate characteristic of humans’ locomotion. Considering triceps surae muscle forces are important contributors to propulsion in running [106-108], and that peak AT forces occur after mid-stance [55], inter-limb differences during propulsion would theoretically result in greater AT loading in the limb producing greater propulsion. Simple strategies may be employed to monitor inter-limb differences during propulsion such feedback of the anterior-posterior GRF or COMsaxial velocity, which could be achieved by adapting current available wearable technologies such inertial measuring units of instrumented insoles. However, it is important to bear in mind the low correlation between external and internal loading [53-55]. The lack of effective strategies to reduce AT injury rates in the running population may therefore be linked to the fact that predominantly variables related to external loading were addressed in biomechanical studies investigating the association between running biomechanics and tendon overuse-related injury [6, 8-10]. Although coaches and clinicians are encouraged to register external loading data during training and competitions to monitor the magnitude of inter-limb differences in propulsion, future research is necessary to address the effects of propulsion on AT loading by using internal loading related variables.

The results from Study IV showed that muscle and tendon properties are not different between the preferred and non-preferred limb of habitual runners. It is well documented
that AT overuse-related tendon injury results in altered tendon properties [52, 64, 130, 131]. Modalities in which high magnitudes of mechanical loading occur to one limb are well known to induce unilateral changes in tendon properties [23, 24, 132]. Those modalities pose the question of which magnitudes of AT loading are responsible for optimal and non-optimal tendon adaptive responses. The overuse injury process usually occurs first unilaterally and only in some cases progresses to the contralateral limb [133].

What causes one limb to develop symptoms at first seems to be the missing puzzle in the AT overuse-related injury problem. With that in mind, in order to establish effective strategies to prevent overloading it is crucial to identify which magnitudes of inter-limb differences are necessary to trigger differential adaptations in muscle-tendon properties in habitual runners. Therefore, from findings of Study IV it seems plausible to recommend coaches and clinicians to monitor AT properties bilaterally in order to identify abrupt changes in tendon properties that could result in pathological conditions. Finally, few studies have focused on the individual contribution of activation and forces from triceps surae muscles to AT loading and change in its properties [47, 127]. With that in mind, it seems relevant to future studies to clarify i) which magnitudes of mechanical loading are important for tendon adaptation in habitual runners and ii) how inter and intra-limb differences in muscle coordination strategies (e.g. EMG ratios) occurring within triceps surae muscles would affect AT loading and possibly differential displacements and strains during running.

6.5 Strengths and limitations

Strengths of this thesis are

- The inclusion of non-usually investigated habitual runners such as triathletes;
- The inclusion of submaximal running speeds not previously investigated;
- Standardization of limb preference assessment;
- The implementation of a statistical analysis considering the entire biomechanical continuum of interest;
- The bilateral investigation of kinetic, kinematic and EMG variables simultaneously during running;
- The first-time bilateral registration of MG-MTJ displacement during running;
- The inclusion of both male and female habitual runners.

Limitations of this thesis are:

- Lack of a control run in Study I;
- A limited number of internal-loading related variables;
- A limited ultrasound sampling rate for MG-MTJ registration during running;
- To not address independently males and females;

A few other limitations were already considered in the Methodological Considerations section.

6.6 Ethical considerations

This thesis aimed to be inclusive by having men and women as participants which is an important aspect for equality in research practice. All participants were informed they could stop participation in the study at any time, and received feedback on their evaluations. All procedures and their purposes were made as clear as possible to participants. Experimental protocols were designed to not have participants run longer than necessary and any sort of psychological pressure on participants was avoided. Considering findings from this thesis, it would be unethical from coaches and clinicians to not monitor bilateral biomechanics and tendon characteristics of habitual runners throughout the seasons if equipment is available for doing so. Preventive strategies to avoid injury might be the most ethical path to follow.

6.7 Conclusions

Inter-limb differences in biomechanical and neuromuscular variables previously associated to AT overuse-related injuries occur in running conditions common to habitual runners.

Inter-limb differences occurred in portions of the stance phase where AT loading is known to be greater.

Triceps surae neuromechanical and Achilles tendon properties are similar among limbs of habitual runners.

From a perspective of muscle-tendon capacity for managing running loads, tendons in both limbs have equal chances of developing injury.

Coaches and clinicians should monitor neuromuscular and biomechanical inter-limb differences during running concomitantly with neuromechanical and tendon properties adaptation across the season in order to prevent potentially harmful conditions for the AT.
7 FUTURE DIRECTIONS

Investigations have been performed aiming to identify biomechanical causes of tendon overuse injuries in runners [7-10, 13, 14, 52]. Regardless of valuable effort from those studies, not only tendon but running overuse-related injury rates did not decline in the past years [1, 2]. It seems therefore that experimental designs adopted in previous studies were not able to adequately address the ‘missing puzzle’ of the running injury problem [54, 134].

A strong evidence that inter-limb differences may be an important part of the that puzzle and should be addressed in future studies resides in the fact that the majority of tendon injuries occur unilaterally [22]. Furthermore, future studies on inter-limb differences might further focus on the entire biomechanical continuum of interest approach for statistical analysis considering limitations of the summarized metrics procedure. Future studies should also explore the effects of timing of gait events in the analysis of biomechanical continuums, since they were found to affect the results of methods designed for time-varying analysis such as the SPM [135]. In conjunction with this, a more thorough examination of internal-loading should be conducted, since there is a dearth of literature on bilateral joint and muscle kinetics during running at different submaximal speeds and fatigue levels. In this regard, recent advances in techniques for personalized musculoskeletal models [136-138] and wearables [139, 140] have the potential to enhance current musculoskeletal loading estimations and to allow data to be registered during more ecological conditions, which will certainly help to get closer to real-world running loads. Another important direction will be the implementation of ultrafast ultrasound which has the potential to adequately capture muscle-tendon dynamics [103, 141, 142], in combination with algorithms capable of real-time tracking of muscle-tendon behavior [143]. In addition to that, accurately quantifying the amount of mechanical stimulus capable to generate alterations in tendon morphological, mechanical and material properties in habitual runners by adopting prospective experimental designs [25, 144] is paramount to improve prevention and treatment strategies designed by coaches and clinicians regarding overuse-related injuries.

Lastly, overuse-related tendon injuries may not occur due to overloading solely. In vitro studies [19] suggest tendon micro trauma followed by sub-optimal mechanical loading could be an alternative explanation to the cascade of degenerative processes associated with tendon damage and injury [19]. This proposed mechanism might be related to compensations in the mechanical loading occurring between limbs after initiation of the unilateral injury process, which could result in sub-loading of the injured tendon since the other limb is still adequately managing loads. Currently no study has adopted the sub-optimal loading theory in experimental designs involving frequently injured populations such as habitual runners.
Överbelastningsskador på hälsenan är ett vanligt förekommande problem hos löpare. Dessa skador uppträder inte sällan ensidigt och etiologin är förknippad med överbelastning av senvävnaden. Bilaterala skillnader mellan ben under löpning är en möjlig orsak till överbelastning på grund av eventuella skillnader i den mekaniska belastningen som varje ben utsätts för. Sidoskillnader har beskrivits i hälsenans egenskaper hos idrottsare på grund av sportrelaterade skillnader i mekanisk belastning, och hos icke-idrottsare på grund av benpreferens. Sidoskillnader i hälsenans mekaniska egenskaper mellan ben hos löpare har dock inte beskrivits tidigare. Denna avhandling undersökte föremålet av skillnader mellan benen i biomekaniska-, neuromekaniska- och hälsensegnskaper hos löpare. I studie I utförde 13 triatlater en simulerad cykel till löpning transition och den vertikala reaktionskraft (ground reaction force, GRFv), nedre extremiteternas kinematik, och muskelaktivering i triceps surae och tibialis anterior musklerna utvärderades bilateralt under start-, mitten- och slutfasen av 5 km löpning på löphand. I studie II utvärderades GRFv, nedre extremiteternas kinematik, muskelaktivering i triceps surae och tibialis anterior samt hälsenans töjning bilateralt hos löpare under två löphastigheter (2.7 m.s⁻¹ och 4.2 m.s⁻¹). I studie III utvärderades spatiotemporalas variabler, den vertikala (kVert) och extremitetens (kLimb) styvhet samt masscentrumets (centre of mass, COM) kinematik bilateralt hos löpare under samma løphastigheter som i studie II. I studie IV utvärderades den maximala isometriska plantarflexionsstyrkan, triceps surae-aktivering och aktivitetsnivån av löpare. I studie I var soleus-aktiveringen lägre i det icke-föredragna benet mellan 53.4% och 75.8% av stödfasen (p <0.01, ES-intervall = 0.59 till 0.80) i slutfasen av 5 km löpning. I studie II var höftextensionshastigheten snabbare i det icke-föredragna benet mellan 71% och 93% av stödfasen (p <0.01) under löpning vid 4.2 m.s⁻¹. Inga andra skillnader mellan benen observerades. I studie III observerades inga skillnader mellan extremiteterna i spatiotemporalas variabler, kVert eller kLimb vid de undersökta løphastigheterna. COM-horizontaltastigheten var snabbare från 67% till

87.40% av stödfasen (p <0.05, ES >0.60) för det icke-föredragna benet. I studie IV observerades inga skillnader mellan extremiteterna i triceps surae-aktivering eller hälsenans egenskaper. Aktiveringsförhållandena för gastrocnemius medialis och soleus korrelerade endast i det föredragna benet.

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skillnader mellan extremiteterna när friska, icke-skadade löpare sprang under förhållanden som liknade deras ekologiska träningssituation. Dessutom verkade hälsenan anpassa sig lika bilateralt hos löpare, medan aktiveringssstrategier för triceps surae kan skilja sig mellan benen. Upptäckterna av skillnader mellan extremiteterna under löpning kan vara relevanta för att undvika överbelastning och skillnaderna kan vara viktiga för etiologin av överbelastningsskador i hälsenan hos löpare. Att senegenskaperna inte skilte sig bilateralt tyder på att båda ben är lika utsatta för skaderisker. Tränare och kliniker kan förbättra nuvarande förebyggande strategier för överbelastningsskador på hälsenan genom att övervaka senegenskaper och biomekaniska och neuromuskulära variabler bilateralt under löpning över säsongen.
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10 REFERENCES


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**APPENDIX**

Pearson’s correlation analysis between activation ratios and peak activations in the preferred (upper plots) and non-preferred (lower plots) limb. MG = medial gastrocnemius; LG = lateral gastrocnemius; SOL = soleus; GAS = gastrocnemii.