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Inter-limb differences in in-vivo tendon behavior, kinematics, kinetics and muscle activation during running

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ABSTRACT

Overloading of tendon tissue may result in overuse tendon injuries in runners. One possible cause of overloading could be the occurrence of biomechanical inter-limb differences during running. However, scarce information exists concerning the simultaneous analysis of inter-limb differences in external and internal loading-related variables in habitual runners. In this study ground reaction force, joint kinematics, triceps surae and tibialis anterior activations, and medial gastrocnemius muscle-tendon junction displacement were assessed bilaterally during treadmill running at 2.7 m.s⁻¹ and 4.2 m.s⁻¹. Statistical parametric t-tests and effect sizes were calculated to identify eventual inter-limb differences across the stance phase and stride cycle. Hip flexion angle was 9° greater (p = 0.03, ES = 0.30) in the non-preferred limb during the flight phase at 4.2 m.s⁻¹. Hip extension velocity was 45 deg.s⁻¹ greater (p = 0.04, ES = 0.41) during ground contact and 25 deg.s⁻¹ greater (p = 0.02, ES = 0.41) immediately after toe-off in the non-preferred limb at 4.2 m.s⁻¹. Hip extension velocity was also 40 deg. s⁻¹ greater (p = 0.01, ES = 0.46) in the non-preferred limb prior to touch-down at 4.2 m.s⁻¹. Brief inter-limb differences in joint kinematics were not accompanied by inter-limb differences in variables associated to internal loading, suggesting they are unlikely to be underlying factors leading to tendon overloading in healthy non-injured runners.

1. Introduction

Overuse running injuries are suggested to occur due to non-gradual increases in the mechanical load (Hreljac 2004) and due to reduced recovery time between training sessions (Hreljac 2004). Biomechanical inter-limb differences during running might lead to both greater mechanical load to one limb and a relative lower recovery time between sessions for that limb. This combination of factors might limit the capacity of the musculoskeletal system to fully recover between sessions, predisposing the musculoskeletal system to injuries (Hreljac 2004, Magnusson et al. 2010).

Despite the fact that speed is one important variable to manipulate running training load, scarce information exists in the literature regarding biomechanical inter-limb differences at submaximal running speeds when considering non-fatigued conditions. Hamill et al. (1984) found no differences in ground reaction force (GRF) variables between limbs when examining a single running speed of 4.48 m.s⁻¹. Moreover, Zifchock et al. (2006) found no differences in GRF variables when comparing the right and left limbs in healthy runners at the single speed of 3.7 m.s⁻¹ (Zifchock et al. 2006). Regarding lower limb kinematics, Karamanidis et al. (2003) found sagittal joint angular displacements and velocities to differ between the right and left limbs of female runners at different submaximal speeds (2.5, 3.0 and 3.5 m.s⁻¹) and stride cycle phases, although joint kinematic values were not reported for each limb and different stride frequencies were investigated. In addition, apart from those studies’ specificities and their relevant contribution to the field, three gaps can be identified in this field of knowledge: i) only one submaximal running speed (Hamill et al. 1984, Zifchock et al. 2006) or a limited range of submaximal speeds have been simultaneously investigated (Karamanidis et al. 2003), ii) the flight phase of the stride cycle was not yet explored regarding inter-limb differences and might be the result of inter-limb differences during the stance phase; and iii) with the exception of one study (Hamill et al. 1984), limb preference was not considered in all other studies. Controlling for limb preference during...
cyclic tasks such as running may be important since differential adaptation of the musculotendinous components was observed between limbs (Bohm et al. 2015, Chiu et al. 2016, Kulas et al. 2018). Limb preference can be defined as the preferential use of a given limb to perform one (Bohm et al. 2015) or a set of refined motor tasks (Elias et al. 1998).

The previously mentioned studies and many other studies on running biomechanics have used zero-dimensional data extracted or summarized from the original one-dimensional data for hypothesis testing, therefore excluding or reducing the majority of the information contained in the biomechanical continuum of interest (e.g. stride cycle, stance phase). In this regard, Hughes-Oliver et al. (2019) recently investigated inter-limb differences in joint kinematics across the entire stance phase of running while accounting for limb dominance. They found no inter-limb differences in sagittal and frontal plane lower limb joint angular displacements in either young and old runners, although the authors investigated only one submaximal running speed (3.35 m.s\(^{-1}\)).

However, the investigation of inter-limb differences across the stride cycle and different submaximal running speeds are still lacking. Although external loading variables such as the GRF and joint kinematics variables can be more easily assessed and monitored by coaches and clinicians, there are difficulties in relating them to the internal loading experienced by the musculoskeletal system (Scott and Winter, 1990, Nigg et al. 2017, Impellizzeri et al. 2019, Mattiejevich et al. 2019). Achilles tendon (AT) overuse injuries are one of the most frequent overuse injuries occurring in habitual runners such as runners and triathletes (Van Gent et al. 2007, Lorimer and Hume, 2014, Dallingga et al. 2019). Thus, biomechanical variables associated with AT internal loading requires further attention when considering possible inter-limb differences during running. Eventual differences in the internal loading experienced by each tendon may represent an increased risk of overloading in the collagenous tendon material (Wren et al. 2001, Scott et al. 2005, Ros et al. 2019). The strain experienced by the tendon relates to its internal loading and has been observed to be critical in tendon adaptation (Arampatzis et al. 2009, Bohm et al. 2014), to induce tendon structural damage (Wren et al. 2001, Scott et al. 2005, Ros et al. 2019) and to be implicated in tendon injury incidence (Obst et al. 2018). Tendon strain is often estimated by musculoskeletal models using the force-strain relationship (Arnold et al. 2013, Rajagopal et al. 2016) or by direct registration of the muscle–tendon junction (MTJ) displacement using ultrasonography (Leitner et al. 2019, Kharazi et al. 2021). Furthermore, muscle activation is an outcome variable assessed using electromyography (EMG) which assists in understanding muscle function during running (Arnold et al. 2013, Werkhausen et al. 2019). Considering the AT is connected to the triceps surae muscles, possible inter-limb differences in muscle function might translate to inter-limb differences in MTJ displacement. However, to our knowledge inter-limb differences in GRF, joint kinematics, MTJ displacement, and triceps surae activation have not been simultaneously investigated during submaximal running.

Based upon the above mentioned gaps in the literature, the aim of this exploratory study was to investigate possible inter-limb differences in variables associated to external and internal loading while accounting for limb preference at two submaximal running speeds (2.7 m.s\(^{-1}\) and 4.2 m.s\(^{-1}\)) in a group of healthy runners.

2. Methods

2.1. Participants

Eleven experienced runners [five males (mean ± SD): 33.2 ± 2.5 years; 183.3 ± 2.3 cm; 73.9 ± 2.5 kg, and six females (mean ± SD) 34.8 ± 6.3 years; 168.1 ± 5.4 cm; 63.7 ± 3.5 kg] participated in this study after giving informed consent. Runners self-reported a race pace of 3.9 ± 0.7 min.km\(^{-1}\) for a 5 km race distance. After conversion to age graded scores, (https://www.runnersworldonline.com.au/age-grading-calculator/) this indicated that the runners’ classification ranged from local to regional class. This study was approved by the Swedish Ethical Review Authority and follows the Declaration of Helsinki ethical guidelines. Runners reported no acute or chronic lower limb injury during the six months prior to testing.

2.2. Experimental procedures

Runners performed trials at slow (2.7 m.s\(^{-1}\)) and fast (4.2 m.s\(^{-1}\)) running speeds on a motorized treadmill (RL2500E, Rodby Innovation AB, Sweden). Prior to start, a ten minutes warm-up was performed by the runners at a self-selected speed up to 2.7 m.s\(^{-1}\). Trials consisted of running on a treadmill in which the first 10 s were used to allow runners to reach a steady state running pattern at the target speeds (2.7 m.s\(^{-1}\) and 4.2 m.s\(^{-1}\)). After reaching a steady state, the GRF, torso and lower limb motion, triceps surae [medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL)], and tibialis anterior (TA) EMG and the MG muscle–tendon junction (MG-MTJ) displacement were registered for 20 s. Runners performed three trials at each speed interspersed by 30 s to 1 min resting intervals. After finishing the set of trials for both speeds the ultrasound probe was moved to the opposite limb for the execution of another set of three trials per speed. The order of the starting limb for the ultrasonography registration was randomized and all subjects started testing by the slowest speed. The GRF normal component relative to the foot was sampled at 100 Hz by a pressure measuring insole system (Pedar®, Novel GmbH, Germany) placed inside each runner’s shoes (Hurkmans et al. 2006). Muscle activation was sampled at 3000 Hz by a surface EMG system (TeleMyo 2400R G2, Noraxon Inc., USA). Prior to placement of EMG electrodes, the skin was carefully shaved and cleaned with alcohol swabs to reduce skin impedance. Bipolar surface electrodes (Neuroline 720, Ambu Inc., Denmark) were placed parallel to muscle fibers with a 20 mm inter-electrode distance. All EMG procedures followed SENIAM recommendations (Stegeman and Hermens, 2007). Torso and lower limb motion were determined by tracking the three-dimensional coordinates of thirty-five passive reflective markers with a twelve-camera motion analysis system sampling at 300 Hz (Oqus 4-series, Qualisys AB, Sweden). Torso markers were placed on the spinal process of the C7 vertebral and on the right and left acromion. Pelvis markers were placed on the right and left anterior and posterior superior iliac spines. Lower limb markers were placed bilaterally on the medial and lateral epicondyles of the femur, medial and lateral malleoli, on the 1st and 5th metatarsophalangeal joints and on the calcaneus. Four rigid clusters of four non-collinear passive reflective markers were strapped bilaterally to the thigh and shank. The marker set was adapted from (Rajagopal et al. 2016) who reported many sagittal kinetic and kinematic variables at 4.0 m.s\(^{-1}\) using the same musculoskeletal model as in this study. The MG-MTJ displacement was sampled at 75 Hz by an ultrasound transducer (96-element linear probe, 60 mm field of view, B-mode, 7 MHz, Echoblaster 128, Telemed, Vilnius, Lithuania) firmly strapped to the shank (Fig. 1) to avoid transducer movement. Data synchronization between GRF, EMG and motion capture was performed by an analog pulse sent from the QTM® software acquisition system (QTM® software, Qualisys AB, Sweden). Temporal synchronization of the ultrasound imaging registration was performed by an analog pulse sent from the ultrasound system (Echoblaster 128, Telemed, Vilnius, Lithuania) to the QTM® software acquisition system (QTM® software, Qualisys AB, Sweden).

2.3. Data analysis

GRF data were filtered at 10 Hz using a second order low pass Butterworth filter and subsequently up sampled to 300 Hz by a Fast Fourier Transform (FFT) interpolation method to match the joint kinematics sampling frequency. EMG data were filtered at 20–500 Hz using a fifth
order band-pass Butterworth filter and the root mean squared (RMS) envelopes were subsequently computed using a 40 ms window. Marker coordinates were exported to c3d format files from the QTM software acquisition system and converted to the OpenSim native format using the open-source freely available BTK biomechanical toolkit for Matlab® (v2019a, MathWorks Inc., USA). A musculoskeletal model (Rajagopal et al. 2016) composed of feet, shanks, thighs, pelvis and torso was scaled to each participant’s anthropometrics using the Scale Tool in OpenSim 3.3 (Delp et al. 2007). After scaling, the Inverse Kinematics tool in OpenSim was used to estimate lower limb kinematics during running based upon each participant’s individualized musculoskeletal model. Joint and muscle-tendon kinematics estimated using the subject-specific musculoskeletal models were filtered at 10 Hz by the inbuilt third order low pass IIR Butterworth digital filter using the Analysis tool in OpenSim. MG and SOL muscle–tendon unit (MTU) strains were calculated by dividing the instantaneous length by the length at touch-down. The ultrasound recording of the MG-MTJ displacement registered during running trials was exported as AVI uncompressed files using ultrasound recording of the MG-MTJ displacement registered during running trials was exported as AVI uncompressed files using ultrasound’s system software (EchoWave II, Telemed, Vilnius, Lithuania) and its 2-D local coordinates were tracked manually frame-by-frame using a video tracking analysis software (Tracker 5.0.7, Open Physics, https://www.compadre.org/osp). Due to limitations in fully tracking the MG-MTJ in one or both limbs for some runners, the final analysis of the MG-MTJ manually tracked data included eight runners.

Five stance phases [GRF and center of pressure (COP) displacement] or stride cycles (joint kinematics, triceps surae and tibialis anterior EMG, MG-MTJ displacement) selected from each running trial were combined and averaged to be considered as representative of a given limb pattern. The stance phase was determined as the time from touch-down to toe-off while stride cycle was defined from touch-down to subsequent ipsilateral touch-down. Touch-down was determined as the frame in which the center of mass reached minimum vertical velocity, while toe-off was determined as the frame where the maximum knee extension occurred (King et al. 2019). These methods for touch-down and toe-off determination were validated for rear and non-rear foot strikes during treadmill running at a variety of speeds (King et al. 2019). We opted for this method since the native sampling rate of the motion capture system was three times greater than the native sampling rate used for GRF registration. Due to technical problems to synchronize the EMG signal with the other signals touch-down for all EMG muscles was defined as the time-point where 20% of peak SOL RMS occurred (Arnold et al. 2013, Werkhausen et al. 2019). This decision was based on prior studies on EMG during running showing this level of SOL activation occurring at touch-down for a range of running speeds and loads (Arnold et al. 2013, Werkhausen et al. 2019).

The vertical GRF was normalized to runners’ bodyweight while EMG was normalized to the peak RMS values determined at each running trial since only effects from limb preference were investigated. Data for the TA muscle are presented for five runners due to excessive background noise in one or both limbs of the remaining runners. The stance and stride cycles were time-normalized to 100 data points by a fast Fourier interpolation (FFT) method. The Waterloo questionnaire for footedness assessment (Elias et al. 1998) was employed to assign either the right or left limb as the preferred (P) or non-preferred (NP) limb. A preferred limb was defined when the questionnaire indicated more than 60% preference for a given limb. Apart from data analysis in OpenSim, all other data processing was conducted in Matlab®.

2.4. Statistical analysis

Statistical parametric mapping (SPM) two-tailed paired t-tests were employed to test for inter-limb differences occurring across the whole stance and stride cycles. The SPM analysis was conducted using the spm1d package for Matlab (https://www.spm1d.org) (Pataky et al. 2016). The SPM allows statistical inference over the original one-dimensional field. SPM employs Random Field Theory which corrects for multiple comparisons necessary when calculating the probability (p value) by which a cluster of data crosses a given “t” threshold by chance (Pataky 2016). The standardized difference of the means (Cohen’s D effect size, ES) was also calculated when $p < 0.05$. The magnitude of the ES was considered as follows: $<0.2$, trivial; $0.2$ to $0.6$, small; $0.6$ to $1.2$, moderate; greater than $1.2$, large (Hopkins et al. 2009). The ES was reported as the average of the ES value of the cluster crossing the t-threshold identified by the SPM t-test.

3. Results

No inter-limb differences were identified in GRF and anterior-posterior COP displacement (Fig. 2) across the stance phase. Hip flexion angle was $9°$ greater ($p = 0.03$, $ES = 0.30$) in the non-preferred limb from $77.71\%$ to $81.77\%$ of the stride cycle at $4.2 \text{ m.s}^{-1}$ (Fig. 3). Hip extension velocity was $45 \text{ deg.s}^{-1}$ greater ($p = 0.04$, $ES = 0.41$) in the non-preferred limb from $23.41\%$ to $24.49\%$ of the stride cycle at $4.2 \text{ m. s}^{-1}$. Hip extension velocity was also greater by $25 \text{ deg.s}^{-1}$ ($p = 0.02$, $ES = 0.41$) in the non-preferred limb from $35.18\%$ to $37.29\%$ of the stride cycle at $4.2 \text{ m. s}^{-1}$ (Fig. 4). Finally, hip extension velocity was $40 \text{ deg.s}^{-1}$ greater ($p = 0.01$, $ES = 0.46$) in the non-preferred limb from $84.32\%$ to $87.52\%$ of the stride cycle also at $4.2 \text{ m. s}^{-1}$ (Fig. 4). The one-dimensional ES relative to the surpassed SPM t-thresholds for both hip angular displacement and velocity are presented in Fig. 5.

The MG-MTJ displacements, and the MG and SOL MTU strains and velocities did not present inter-limb differences across the stride cycle (Fig. 6). Similarly, the MG, SOL and TA activations did not show inter-limb differences at any time-point of the stride cycle (Fig. 7).

4. Discussion

The present study was motivated by the rationale that overloading of the musculoskeletal system through inter-limb differences could occur during running. A second motivation resides in the observation that inter-limb differences have not been simultaneously investigated - even indirectly - in terms of both external and internal loading at submaximal speeds and non-fatigued conditions. In this study the full stance and stride cycle were considered for inter-limb comparisons. Brief inter-limb differences were observed in hip flexion angle and hip extension velocities during fast running across the stride cycle. However, no inter-limb differences were observed in GRF and COP trajectories, triceps surae activation, MTU strains and velocities, and in the in vivo MG-MTJ displacement.
The absence of inter-limb differences in GRF observed in the current study is in line with prior findings (Hamill et al. 1984, Zifchock et al. 2006), but provides new information that, for the entire stance phase of running, the preferred and non-preferred limbs are exposed to similar GRF (Hamill et al. 1984, Williams et al., 1987, Zifchock et al. 2006). Regarding joint angular displacements, results from this study are in line with those of Hughes-Oliver et al. (2019) who found no inter-limb differences in lower limb sagittal joint displacements across the stance phase during treadmill running at 3.35 m.s$^{-1}$. Findings from Hughes-Oliver et al. (2019) were extended here to the whole stride cycle at a faster submaximal speed (4.2 m.s$^{-1}$). The present results also extend observations from Hughes-Oliver et al. (2019) by showing that no differences occur between limbs of healthy runners in the sagittal knee and ankle joint velocities across the stride cycle at slow and fast submaximal running speeds.

Findings of inter-limb differences in hip angular displacement after toe-off complement findings from Hughes-Oliver et al. (2019) who analyzed hip angle across the stance phase solely, and that of Karamanidis et al. (2003) who reported inter-limb differences in hip kinematics during the flight phase although using zero-dimensional data. The results of this study showing inter-limb differences in hip joint velocity during both stance and flight phases of the stride cycle are apparently novel but in line with suggestions that joint velocities tend to be less symmetric than joint displacements during submaximal running (Karamanidis et al. 2003). Under the assumption that both limbs produce similar hip joint torques, the observed inter-limb differences in hip joint velocity would result in inter-limb differences in hip joint power across the stride cycle.

The observation of inter-limb differences in hip kinematics may be the result of different functional mechanical roles played by each limb during locomotion. Such proposed functional mechanical roles of each limb were similar between studies (“strut and propelling” (Cavanagh 1990), “supportive and propulsive” (Sadeghi et al. 1997), “stick and propulsive” (Dalleau et al. 1998), “braking and propulsive” (Potdevin et al. 2008)). Unfortunately, here we cannot state a functional mechanical role to each limb based solely on hip kinematics results.

**Fig. 2.** Ground reaction force (GRF) and center of pressure (COP) anterior-posterior displacement across the stance phase at slow (2.7 m.s$^{-1}$) and fast (4.2 m.s$^{-1}$) running. Black solid lines: preferred limb; red solid lines: non-preferred limb; Dashed lines: inter-individual observations; SPM: statistical parametric mapping.
However, based on the applied footedness questionnaire (Elias et al. 1998) the preferred limb can be considered the chosen limb to execute refined motor tasks while the non-preferred limb should provide support during the execution of such tasks. If the non-preferred limb as a supporting limb would also produce greater hip joint power is yet to be determined during submaximal treadmill running in fresh-state non-injured runners.

Regarding its possible contribution to injury development, a potential greater positive hip power being produced by the non-preferred limb during stance would result in greater levels of mechanical energy being produced and released by hip extensors in that limb, potentially increasing internal loading of hip extensors. In addition, the greater hip extension velocity during the end of the flight phase may result in greater acceleration of the non-preferred limb masses, thus increasing

Fig. 3. Hip, knee and ankle joint angular displacements across the stride cycle at slow (2.7 m.s$^{-1}$) and fast (4.2 m.s$^{-1}$) running. Black solid lines: preferred limb; red solid lines: non-preferred limb; Dashed lines: inter-individual observations; vertical black and red lines representing respectively the preferred and non-preferred limb’s toe-off; SPM: statistical parametric mapping. *p < 0.05 indicates a statistical difference between limbs.

Fig. 4. Hip, knee and ankle joint angular velocities across the stride cycle at slow (2.7 m.s$^{-1}$) and fast (4.2 m.s$^{-1}$) running. Black solid lines: preferred limb; red solid lines: non-preferred limb; Dashed lines: inter-individual observations; vertical black and red lines representing respectively the preferred and non-preferred limb’s toe-off; SPM: statistical parametric mapping. *p < 0.05 indicates a statistical difference between limbs.
impact loading at touch down (Schmitz et al. 2014) which showed to be harmful to passive musculoskeletal structures (Hreljac 2004). However, although differences were observed during brief portions of the stride cycle (e.g. ~ 3%) and were of low magnitude, assuming symmetry (or asymmetry) should be made with caution due to the high variability in the bilateral biomechanics of runners (Hanley & Tucker 2018). Future studies prospectively investigating the relation between injury incidence, inter-subject indices of bilateral differences (Hanley & Tucker 2018), as well as the possible role of limb preference on this relation are still warranted.

To the best of our knowledge this is the first study investigating inter-limb differences in AT internal loading-related variables during running. In this study the in vivo MG-MTJ displacement was considered as relating to AT strain. The in vivo MG-MTJ displacements observed here are comparable in magnitude and shape to those recently reported by Kharazi et al. (2021) for a single limb during submaximal treadmill running. No prior studies were found reporting an inter-limb comparison of in vivo MG-MTJ displacements or ‘true’ AT strain during submaximal running. Considering the combined results of MG-MTJ displacements with knee and ankle joint kinematics, MTU strains, and triceps surae activation similar AT tendon strain might be occurring between limbs at the submaximal speeds and runners examined here.

The present study has limitations. Our approach on adopting a specific threshold for EMG at touchdown may have neglected neuromuscular strategies in the triceps surae dependent on foot strike patterns (Ervilha et al. 2017). Another limitation of a bilateral EMG analysis relies on the assumption that muscle conformation and motor unit locations are similar between limbs, which might not be the case. Relative to our sample size, although a larger one would have identified inter-limb differences not detected here, the adoption of SPM analysis guaranteed a tight control under Type I and II errors (Pataky 2016). Moreover, caution is needed when interpreting the results since MTJ displacements should not be directly translated to AT internal loading. We decided upon reporting solely the MG-MTJ displacement as a strain-
related variable after recent studies reporting experimental procedures that are crucial to an accurate estimation of AT strain but couldn’t be implemented here. For example, US sampling rates for musculoskeletal tissues are higher than zero-dimensional metrics, and that external loading and joint kinematics variables might have low predictive power to explain inter-limb differences in hip angular displacement and velocity were observed across the stride phase of running but no other inter-limb differences were observed in non-fatigued healthy runners at submaximal treadmill running. The observed inter-limb differences in hip kinematics are unlikely to be an underlying factor for tendon overloading in healthy habitual runners, while other musculoskeletal tissues cannot be disregarded.

CRediT authorship contribution statement

Tiago Jacques: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. Rodrigo Bini: Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. Anton Arndt: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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