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ORIGINAL ARTICLE:

QUADRATUS FEMORIS: AN EMG INVESTIGATION DURING WALKING AND RUNNING

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ABSTRACT

Dysfunction of hip stabilizing muscles such as quadratus femoris (QF) is identified as a potential source of lower extremity injury during functional tasks like running. Despite these assumptions, there are currently no electromyography (EMG) data that establishes the burst activity profile of QF during any functional task like walking or running. The objectives of this study were to characterize and compare the EMG activity profile of QF while walking and running (primary aim) and describe the direction specific action of QF (secondary aim). A bipolar fine-wire intramuscular electrode was inserted via ultrasound guidance into the QF of 10 healthy participants (4 females). Ensemble curves were generated from four walking and running trials, and normalized to maximum voluntary isometric contractions (MVICs). Paired *t*-tests compared the temporal and amplitude EMG variables. The relative activity of QF in the MVICs was calculated. The QF displayed moderate to high amplitude activity in the stance phase of walking and very high activity during stance in running. During swing, there was minimal QF activity recorded during walking and high amplitudes were present while running (effect size = 4.23, $P<0.001$). For the MVICs, external rotation and clam produced the greatest QF activity, with the hip in the anatomical position. This study provides an understanding of the activity demands placed on QF while walking and running. The high activity in late swing during running may signify a synergistic role with other posterior thigh muscles to control deceleration of the limb in preparation for stance.

INTRODUCTION

There is increasing awareness of the importance of hip muscle function for local and distal joint health (Reiman et al., 2009). Deficits in hip muscle strength, activity or hip joint mechanics have been identified in lower limb pathology (Bolgla et al., 2011; Sims et al., 2002; Smith et al., 2014). It is important then, to understand the function of hip muscles in order to facilitate the clinical assessment and rehabilitation of these conditions.

A key hip muscle group identified in a recent narrative review by Retchford and colleagues are the deep hip external rotators (Retchford et al., 2013). With reference to biomechanical modelling (Torry et al., 2006) and radiological literature (Miokovic et al., 2011), the authors identified that these muscles have favorable morphological features to contribute to stability of the hip joint, much like the rotator cuff of the shoulder. It is for this reason that targeted interventions for improving deep hip external rotator strength are becoming a fundamental component of contemporary hip rehabilitation protocols (Bennell et al., 2014). Quadratus femoris (QF) is considered a particularly important constituent of the deep external rotators (Miokovic et al., 2011), yet due to the technical difficulty and perceived discomfort associated with accessing this deep muscle with fine wire electrodes, and the proximity to the sciatic nerve, it is only recently that a published account of QF EMG has been documented (Hodges et al., 2014). The investigation by Hodges et al. on ten healthy participants confirmed that QF was highly active in maximum isometric hip external rotation and extension. While this was an important contribution to the QF literature, there are still no published data that evaluate the activity of QF during commonly performed functional tasks like walking or running.

Clinicians and researchers often use walking and running to examine the influence of muscles on stability (Pandy and Andriacchi, 2010), movement (Gazendam and Hof, 2007; Pandy and Andriacchi, 2010) and pathology (Smith et al., 2014). Valuable insights into the role of some hip muscles such as gluteus minimus (Semciw et al., 2014) and the adductors (Green and Morris, 1970) have been provided through EMG analysis of gait. Knowledge of QF activity during walking and running will help to establish the functional significance of this muscle to movement and stability; provide normative data for further evaluation in healthy and pathological populations; and allow comparisons to predicted muscle activity generated through less invasive techniques such as 3D musculoskeletal modelling (Lenhart et al., 2014).

The objective of this study was to illustrate, quantify and compare the activation properties of QF in walking and running. Given the lack of QF research, a secondary aim was to describe the relative contribution of QF in a range of maximum voluntary isometric contractions (MVICs). This will add to our current understanding of the direction specific function of this muscle.

METHODS

Participants.

A convenience sample of ten healthy participants (4 female) volunteered for this study. To ensure participants represented an active sample, inclusion into the study required participants to satisfying a Tegner activity score of greater than 3 (Tegner and Lysholm, 1985); and be active in competitive or recreational activities that induced sweating for at least one hour, three times per week over the last year (Queen et al., 2006). Approval was obtained from the University Human Ethics Committee (UHEC 13-005) and informed written consent was obtained from all eligible volunteers and the rights of subjects were protected.

Instrumentation and electrode placement.

Bi-polar, 75 μm fine wire electrodes (A-M Systems, Washington, USA) were prepared as described previously by Basmajian and Stecko (1962) from 25 cm stainless steel Teflon® coated wires. To mark the location of electrode insertion, participants were asked to lay on their side with their stance leg (Bullock-Saxton et al., 2001) placed uppermost, and their hips and knees in 45° flexion. The mid-point of a line between the ischial tuberosity and the greater trochanter (center) was marked to identify QF under real time ultrasound (HDI 3000; Advanced Technology Laboratories, Washington, USA) (Perotto et al., 2005). An electrode was inserted with the aid of a 9 cm spinal needle (Terumo, Tokyo, Japan) under ultrasound guidance (Fig. 1). Footswitches (Model: 402, Interlink Electronics, California, USA) were secured to the heel and great toe for the purpose of identifying the temporal phases of the gait cycle (Semciw et al., 2014), and a Trigno wireless 16-Channel EMG system (Delsys® Inc., Boston, USA) was used to collect raw signals from the footswitches and EMG electrode.

[Insert Figure 1 here]

Data Acquisition

Participants completed a series of six walking and running trials along a ten meter pathway, followed by MVIC data collection. Walking trials were paced at comfortable self-selected speed

(Semciw et al., 2014), and running trials paced for a 5 km run. Trials were repeated if a participants' speed exceeded ± 5% of their average speed (established during warm-up). The final four of the six walking and running trials were recorded for analysis, and the order of walking and running was randomly assigned. Participants were also asked to rate their level of discomfort (associated with the fine-wire electrodes) while walking and running by completing a 10 cm visual analogue scale (0 = no discomfort, 10 = maximum possible discomfort) at the end of their walking and running trials (Semciw et al., 2013a). The mean level of discomfort was classified according to the following criteria: 0.0 to 0.4 cm, no discomfort; 0.5 to 4.4 cm, mild discomfort; 4.5 to 7.4 cm, moderate discomfort; 7.5 to 10.0 cm, severe discomfort (Jensen et al., 2003).

MVIC data were then recorded for amplitude normalization and to further differentiate the functional properties of the QF across seven actions (secondary aim) (Table 1). Three MVICs were maintained for a total of 3 seconds for each position, with a 3 minute rest in-between. Consistent verbal encouragement was provided and the order of MVICs was randomly assigned.

[Insert Table 1 here]

Data Processing and Statistical Analysis

The processing of QF EMG signals (gait and MVIC data) in this study was performed as described on other muscles previously (Semciw et al., 2014). Briefly, raw signals collected by the EMG system (CMRR >80 dB @60Hz; gain of 1000; band pass filtered 20-900 Hz) were sampled at 2000Hz. Signals were then high-pass filtered (Butterworth 4th order, 50 Hz cut-off) to remove low frequency movement artefact, rectified and low-pass filtered (Butterworth 4th order, 6 Hz cut-off) to generate a linear envelope. Gait data were amplitude normalized to % MVIC and time normalized to 100 points (Fig 2).

[Insert Figure 2 here]

EMG profile. To provide an illustration of the QF EMG profile while walking and running, an ensemble average was generated from the two middle strides of the 4 walking and 4 running trials. The ensemble averages for all participants were summed and averaged to generate a grand ensemble curve ± 95% confidence interval (EMG profile) for walking and running (Semciw et al., 2014).

Temporal and amplitude variables. Temporal and amplitude EMG variables were collected from the linear envelope of each participant's walking and running trials during the stance phase, swing phase and the overall gait cycle. The variables acquired were peak amplitude (% MVIC), average amplitude (% MVIC) and time to peak (TTP) (stance phase, % stance; swing phase, % swing; overall gait cycle, % gait cycle).

MVIC data. The mean amplitude from the middle 1 second of the MVIC trials was recorded for each action. The highest value across all actions was used for amplitude normalization of gait variables. To provide an indication of the relative contribution of QF to each MVIC action (secondary aim), the highest MVIC was used to normalize activity across all actions, and graphically illustrated with box-plots from least QF activity to most activity. The relative activity of QF during each MVIC was then classified according to previously defined criteria into low (0%-20% MVIC), moderate (21%-40% MVIC), high (41%-60% MVIC) and very high activity (>60% MVIC) (Reiman et al., 2012).

Statistical Analysis. Means (SD) of the temporal and amplitude EMG variables across the three phases (stance, swing and overall) of the gait cycle, as well as walking speed and levels of discomfort were compared between walking and running with paired samples *t*-tests. Logarithm transformed data were used where original data were not normally distributed (assessed by Kolmogorov-Smirnov test). A standardized mean difference (SMD = mean difference/pooled SD) was calculated to indicate the magnitude of difference between walking and running EMG variables, where 0.2, 0.5 and 0.8 was considered small, medium and large respectively (Cohen, 1988). SPSS statistical software package (version 19, IBM SPSS Inc., Chicago, IL, USA) was used for all statistical comparisons and the significance level was set at $\alpha=0.05$.

RESULTS

Data from 9 participants were analyzed and reported, as one participants' data was affected by artefact. The mean (SD) age, height, body mass and weekly activity profiles are presented in Table 2, and ambulation characteristics are presented in Table 3. While running, participants ambulated at significantly faster speeds; had quicker stride times, and 'toe-off' occurred significantly earlier in the gait cycle. Some participants reported transient lightheadedness when standing up following electrode insertions; although all participants were able to complete the testing session. The discomfort levels were mild on average and not significantly different between ambulation speeds.

[Insert Table 2 and 3 here]

EMG profile.

The grand ensemble curve while walking illustrated two clear bursts; both were within the stance phase of gait (Figure 3A), with the first burst (≈0% to 20% gait cycle) being the largest. Within individual participant trials, the peak activity in stance was larger than the peak in swing in eight of the nine participants.

While running, the grand ensemble curve also illustrated two large bursts across the gait cycle (Figure 3B). One burst occurred in stance (≈15% to 20% gait cycle), and the other in late swing (≈80% to 100% gait cycle). Within individual participant trials, the peak in swing was greater than the peak in stance in five of the nine participants.

[Insert Figure 3 here]

Temporal and amplitude comparisons. The temporal and amplitude EMG data presented in Table 4 indicate that the activity of QF is generally greater in magnitude while running compared with walking, with the greatest differences present in the swing phase. Within stance, QF activity while walking is moderate to high in amplitude, while activity when running is very high. The difference in peak amplitude did not reach significance, however the average amplitude was significantly greater when running. In the swing phase, minimal activity was recorded during walking; however, high to very high amplitudes were present while running. There were significant differences in peak and average amplitude, with the magnitude of difference in peak activity being extremely large (ES=4.23). When considering the total stride, peak and average amplitude was significantly greater in running. Finally, the temporal data show that the TTP occurs later for running than walking within all phases analyzed, although this did not reach significance when considering the total stride.

[Insert Table 4 here]

Direction specific action during MVICs. The relative intensity of QF activity during the MVIC actions is illustrated in Fig 4. The QF was active at a very high intensity during external rotation and clam; a high intensity during extension and abduction; a moderate intensity during abduction in internal rotation; and was minimally active during internal rotation and flexion.

[Insert Figure 4 here]

DISCUSSION

This study presents a number of novel findings. It provides an illustrative guide for fine wire EMG electrode insertion, and is the first investigation to quantify QF muscle activity and discomfort levels during a functional task with fine wire electrodes *in-situ*. The results suggest that QF is highly active in stance and swing while running. The greatest difference in activity between walking and running was in the swing phase, where peak amplitude was markedly higher while running. Finally, across the seven MVICs tested in this study, activity of QF was highest during hip external rotation and clam; and lowest during hip internal rotation and flexion.

Level of discomfort

Discomfort levels while walking and running with fine-wire electrodes in QF were mild on average, and comparable to levels reported in other hip related fine-wire investigations (Semciw et al., 2013a). This novel information can be used to support participant recruitment and ethical approval for further EMG work on this understudied muscle.

Direction specific action

The results of the current study during the MVIC maneuvers are in general agreement with those of the only other EMG investigation into QF, reported by Hodges et al. (2014). Each study elicited high to very high activity during external rotation and extension MVICs of the hip. As a posterior hip joint muscle, the results support the biomechanical evidence (moment arms) of a potential contribution to external rotation and extension in the anatomical position (Dostal et al., 1986; Vaarbakken et al., 2015). On the other hand, the high activity elicited during hip joint abduction in this study is in contrast to the mild activity recorded by the ten participants of Hodges et al. (mean ≈ 17% MVIC). The difference in results between studies could be attributed to the testing position (Hodges et al. participants were tested in prone) or the reporting metric (means reported by Hodges et al.). Nevertheless, with a moment arm that does not favor hip abduction (Neumann, 2010), the results of each study imply that QF may be more involved in hip abduction than previously thought; perhaps to provide a co-contraction, or femoral head depressor action to counteract the high activity generated by prime movers of hip abduction, such as gluteus medius (Semciw et al., 2013b). It is also important to consider that slight deviations from the anatomical position could change the orientation of the moment arm, resulting in opposing actions, as has been observed in some deep hip muscles (Delp et al., 1999). Further work assessing the activity of QF during MVICs in different positions along a sagittal, coronal and transverse plane will help to further our understanding of the direction specific action of this muscle.

Walking and Running

Stance. According to biomechanical literature, posterior hip and thigh extensors (e.g. gluteus maximus) contribute primarily to the absorption of vertical ground reaction forces during walking and running, with minimal influence on forward propulsion (Hamner et al., 2010; Pandy and Andriacchi, 2010). As a posterior hip muscle with high EMG activity during hip extension (Fig 4), it is possible that QF acts synergistically with other lower limb extensors (e.g. gluteus maximus) to absorb the vertical ground reaction forces during stance in walking and running (Gazendam and Hof, 2007). The single burst of activity while running, and the two bursts of activity while walking correspond with the dominant peaks in vertical ground reaction forces reported in the literature (Ounpuu, 1994). Yet the QF is small in physiological cross-sectional area (Torry et al., 2006), thus not morphologically suited to generating the large torques required to absorb these forces; a role better suited to larger muscles like the quadriceps and gluteus maximus (Hamner et al., 2010). It is likely that the QF serves a local stabilizing role at the hip joint in stance, by drawing the head of femur into the acetabulum with its horizontally directed muscle fibers (Neumann, 2010). Additionally, with a large external rotation moment arm between 0° and 30° hip flexion (Vaarbakken et al., 2015), it is also possible that the burst of QF activity observed during stance in walking and running is related to its functional role as a hip external rotator, where it may contribute to the eccentric control of hip internal rotation occurring during initial stance of gait (Ounpuu, 1994).

During stance, peak and average amplitude was higher while running compared with walking, although peak amplitude did not reach statistical significance. The lack of significance in peak amplitude is likely due to the high degree of variability between participants (identified by large SDs) and the small sample size. Nevertheless, the relatively higher amplitude in running is not surprising given that it involves a greater magnitude of most descriptors of gait, including velocity, joint range and power (Ounpuu, 1994).

Swing. The swing phase had the greatest differences in amplitude between ambulation speeds. Very high amplitude was recorded in late swing while running (80% to 100% gait cycle), whereas QF was relatively quiet in this phase of walking. The high EMG activity of QF in the late swing phase of running is consistent with the high peak force (relative to the stance phase) estimated through 3D modelling by Lenhart et al. (2014). Forward propulsion in running requires the additional activity of not only the lower limb plantar flexors in stance (Hamner et al., 2010), but also the powerful contraction of the hip flexors in early to mid-swing to generate momentum (Gazendam and Hof, 2007; Montgomery et al., 1994). Hip extensors are recruited eccentrically to decelerate the lower limb towards the end of swing, in preparation for stance (Gazendam and Hof, 2007). This role has been attributed to the biarticular hamstring muscles, with several studies reporting high amplitude activity in late swing (Gazendam and Hof, 2007; Montgomery et al., 1994). Based on the moment arm and fiber length of QF through range, a recent cadaveric study concluded that the greatest capacity for QF to generate force was as an extensor of the flexed hip (Vaarbakken et al., 2015). It is possible that QF works eccentrically in synergy with the biarticular hamstrings in the late stage of swing to stabilize the head of femur in the acetabulum, while the hamstrings generate the large torques required to control the motion of the lower limb in preparation for stance. It is unlikely that QF has a role in controlling transverse plane motion in terminal swing, as the external rotation moment arm is markedly reduced at this corresponding hip flexion angle (Novacheck, 1998; Vaarbakken et al., 2015).

Clinical implications.

The results of the current study suggest that the QF is highly active in the stance phase of running. Dysfunction of the QF in runners may partially explain the deficits in hip external rotation strength (Cichanowski et al., 2007; Souza and Powers, 2009), and excessive hip internal rotation (Loudon and Reiman, 2012; Souza and Powers, 2009) commonly observed in athletes with running related injuries such as patellofemoral pain syndrome. Further work is required to understand the function of this muscle in people with running related injuries, and to identify and evaluate potential targeted QF rehabilitation exercise.

There also seems to be an intricate functional relationship between QF and the biarticular hamstring muscles. A direct association with hamstring and QF injury has previously been reported in a range of athletes including runners (Askling et al., 2007; Askling et al., 2008; Willick et al., 2002). The EMG activity of QF recorded in the current study appears to be synergistic with the biarticular hamstring muscle activity reported within the literature (Gazendam and Hof, 2007; Montgomery et al., 1994). Specific comparisons between QF and hamstring activity therefore warrants further research, with potential for important clinical outcomes. For example, it may be warranted for clinicians to consider screening QF dysfunction as a differential diagnosis of hamstring related injuries; and QF rehabilitation as a potential intervention to accelerate recovery and minimize the risk of these injuries.

Limitations.

The mean running speed of participants in this study was reflective of fast running speeds described in the literature (Gazendam and Hof, 2007; Montgomery et al., 1994; Novacheck, 1998), however speeds varied between participants. Speed can influence the pattern of muscle activity (Montgomery et al., 1994) and this may explain the variability in EMG activity (identified by large SD's in amplitude variables) between participants in this study.

The participants of this exploratory study consisted of a homogeneous, convenience sample of healthy, active University aged students. Further work is warranted to establish the activity patterns of QF within other populations (e.g. elite athletes or pathological groups).

Small sample size is a further potential limitation of this study. The sample was based on recent fine-wire investigations of deep hip muscles (Giphart et al., 2012; Hodges et al., 2014) and sufficiently powered to detect differences in activity between walking and running in the current study. Larger samples may be considered for detecting differences between healthy and pathological populations; and the mild discomfort levels reported in this study may be used to facilitate participant recruitment in those circumstances.

CONCLUSION

This is the first study to investigate QF muscle activity while walking and running. The data suggest that QF is highly active during stance across both ambulation speeds. The very high activity of QF in late swing while running was in stark contrast to walking, and may reflect a synergistic role with other hip extensors in controlling lower limb motion in preparation for stance. The average level of discomfort while ambulating with fine wire electrodes in QF was mild. This may encourage further research on this muscle to enhance our understanding of its role in pathology, elite performance and potential targeted rehabilitation programs.

CONFLICT OF INTEREST

None declared

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Figures

FIGURE 1. Ultrasound image of electrode insertion path into the quadratus femoris muscle. Line indicates electrode insertion path. GMax – gluteus maximus; GT - greater trochanter; IT - ischial tuberosity; QF – quadratus femoris muscle; Sc nn – sciatic nerve

FIGURE 2. Illustration of a rectified EMG signal (background) and the corresponding processed linear envelop for one participant across one stride while running. Horizontal arrow indicates peak amplitude. Vertical arrow indicates time to peak. Dotted vertical line represents toe-off and divides the stance and swing phase.

FIGURE 3: Grand ensemble curves ± 95% confidence intervals during (A) walking and (B) running. Dashed vertical line indicates toe-off. Note, peak bursts in this figure represent mean peak activity within and across participants, therefore do not reflect absolute peak values of each burst in Table 4.

FIGURE 4: Box plots illustrating relative amplitude (median, interquartile range and range) of muscle activity for the MVIC testing actions. Peak activity was recorded from 6 participants during external rotation, 2 participants during extension, and 1 participant during abduction in internal rotation.

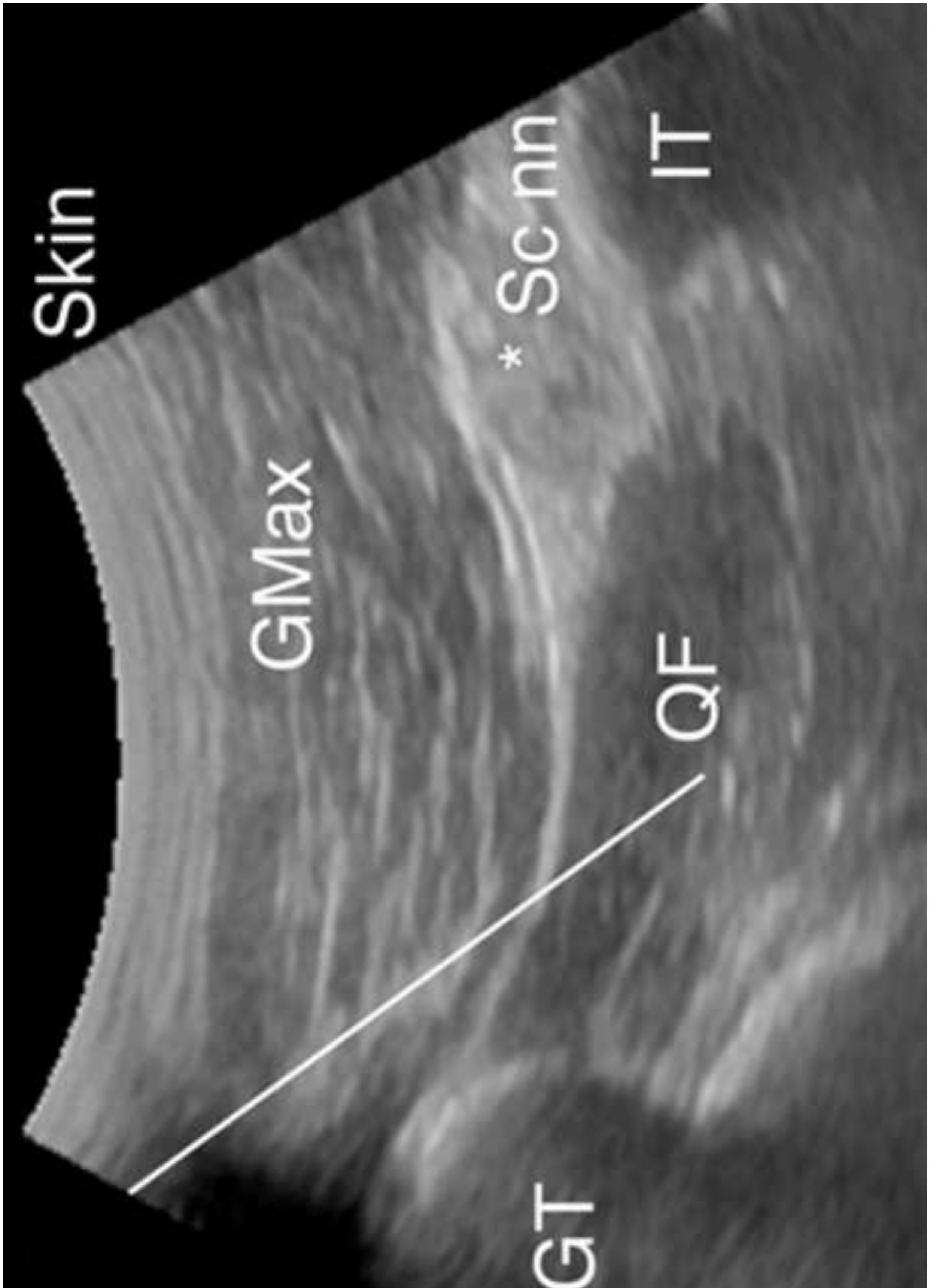


Figure 1

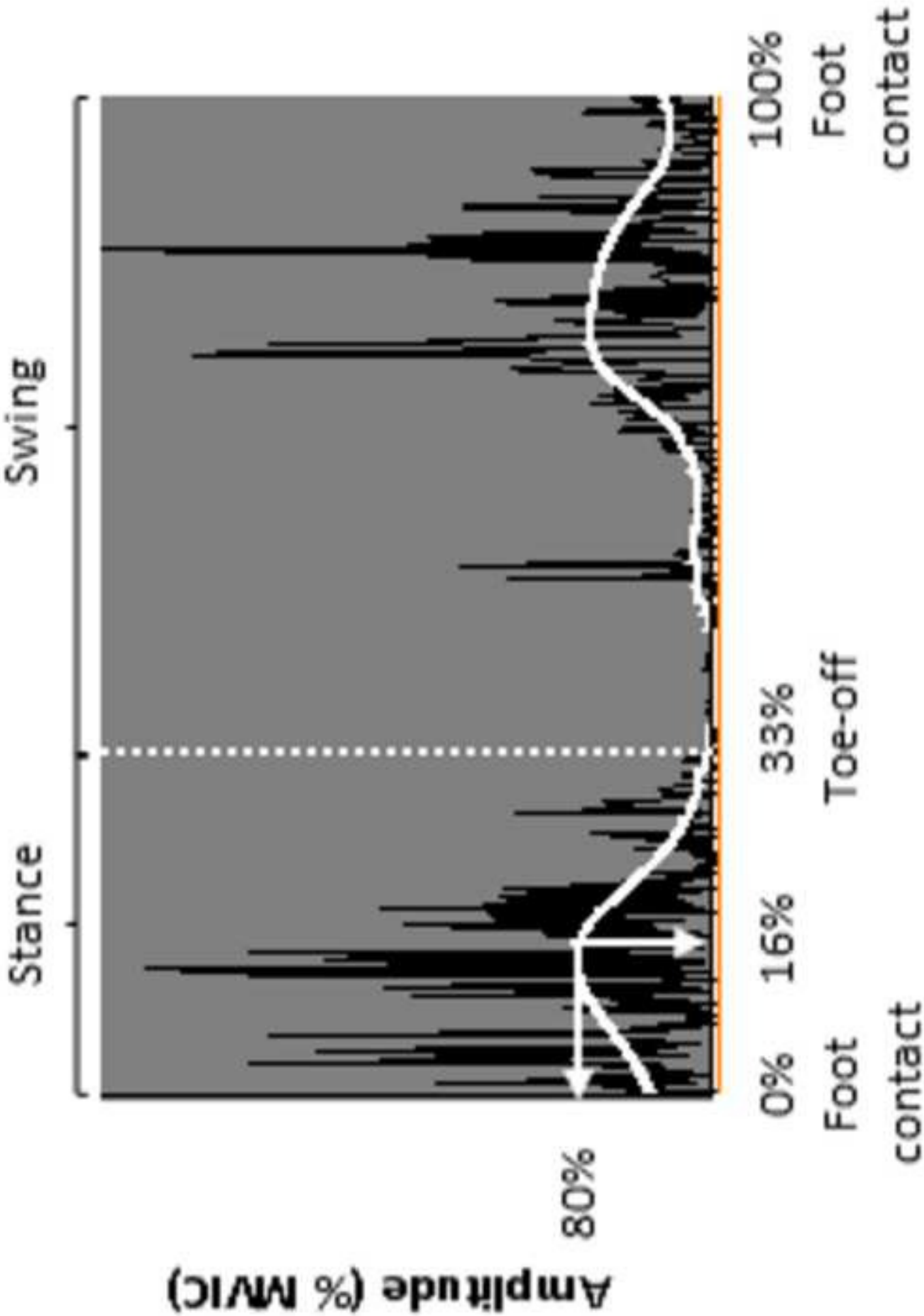
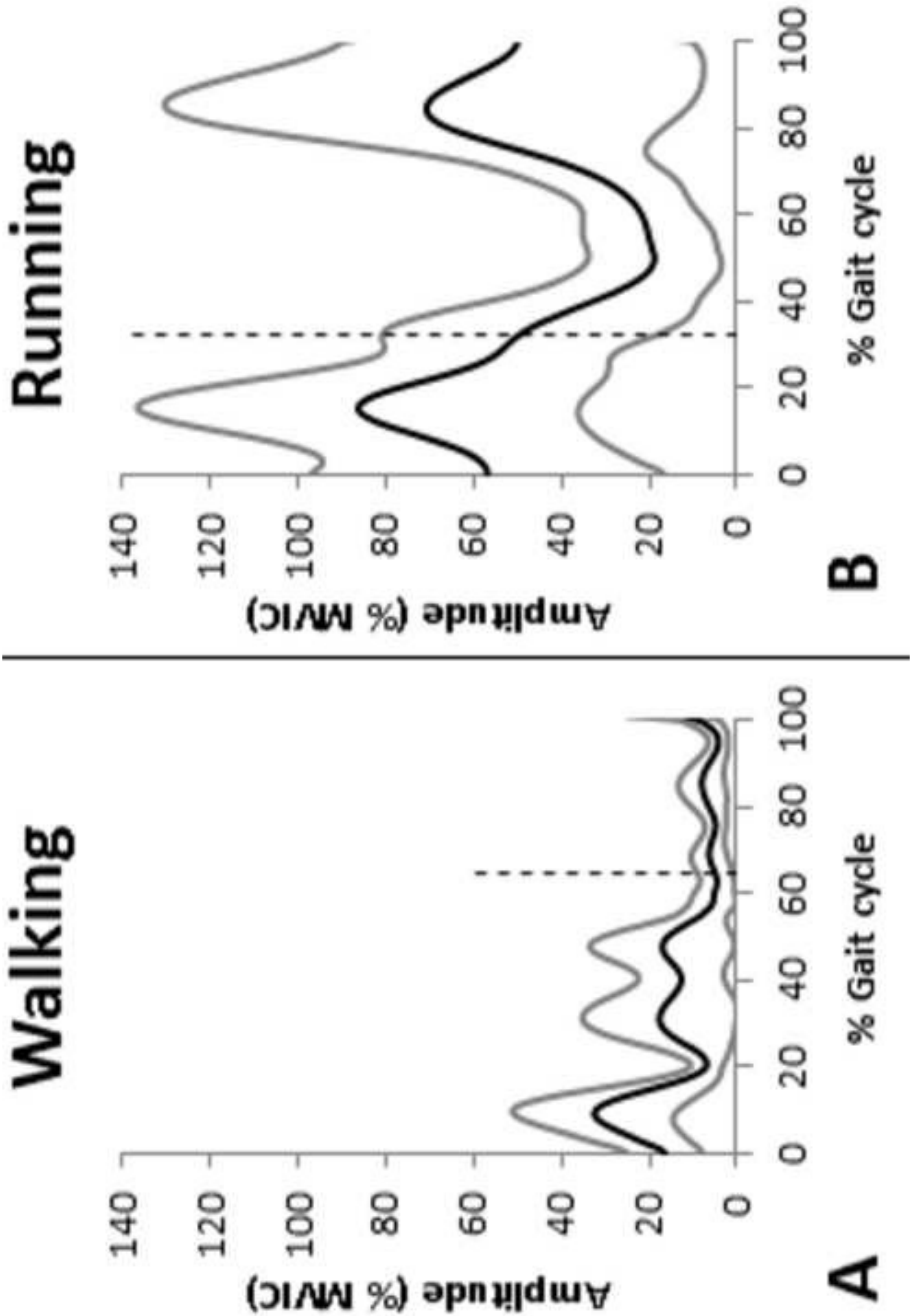

Stance
Swing
Amplitude (% MVIC)
80%
0%
Foot contact
16%
33%
Toe-off
100%
Foot contact
% Gait cycle

Figure 2

Figure 3


Walking
Running
Amplitude (% MVIC)
% Gait cycle
A
B
0
20
40
60
80
100
120
140

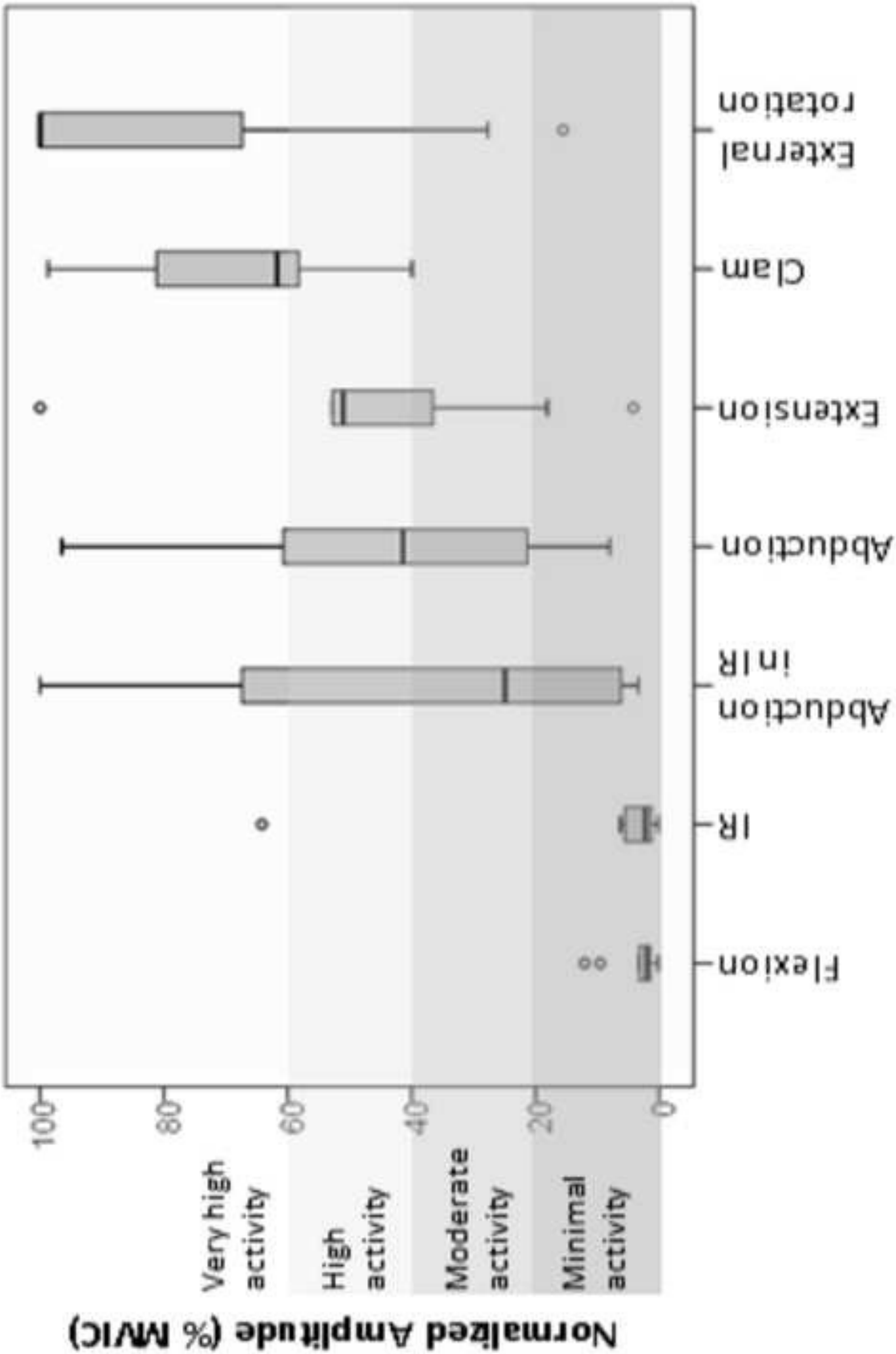

Normalized Amplitude (% MVIC)
Very high activity
High activity
Moderate activity
Minimal activity
100
80
60
40
20
0
Flexion
IR
Abduction in IR
Abduction
Extension
Clam
External rotation

Figure 4

Table 1: Maximum voluntary isometric contractions of the hip

Action	Position	Resistance
External rotation	Side lie- pillow between knees Hip- anatomical position Knee- 90° flexion	Applied by investigator at medial border of foot
Internal rotation	Side lie- pillow between knees Hip- anatomical position Knee- 90° flexion	Applied by investigator at lateral border of foot
Flexion	Side lie- pillow between knees Hip- anatomical position Knee- 90° flexion	Applied by investigator on the anterior aspect of the distal femur
Extension	Prone Hip- anatomical position Knee- 90° flexion	Belt secured around the plinth and the participants knee
Abduction	Side lie- pillow between knees Hip- anatomical position Knee- anatomical position	Belt secured around the plinth and the participants knee
Abduction in hip internal rotation	Side lie- pillow between knees Hip- internal rotation Knee- anatomical position	Belt secured around the plinth and the participants knee
Clam	Side lie- pillow between knees Hips- 45° flexion Knees- 90° flexion	Belt secured around the plinth and the participants knee
Note: The same investigator was responsible for setting-up and instructing the participant on the relevant action, observing for compensatory movement strategies and applying manual resistance where appropriate.		

Table 2: Participant characteristics, exercise profile and stance dominant limb (testing leg)

Gender	Number	Age (SD) years	Height (SD) cm	Body mass (SD) kg	Running training (SD) hrs/wk	Running related sports (SD) hrs/wk	Total exercise time (SD) hrs/wk	Stance dominant limb- left leg
Male	5	23.8 (1.6)	184.9 (8.5)	89.7 (19.7)	0.8 (1.4)	3.0 (2.8)	4.8 (1.5)	2
Female	4	23.5 (1.7)	167.0 (3.5)	65.8 (8.0)	2.0 (0.3)	3.1 (1.2)	7.5 (1.1)	3
Total	9	23.7 (1.6)	177.8 (10.6)	79.1 (19.4)	1.3 (1.2)	3.0 (1.2)	6.0 (1.9)	5

Table 3: Mean (SD) ambulation characteristics and level of discomfort

Ambulation type	Speed (ms^{-1})	Stride time (s)	Toe-off (% gait cycle)	Discomfort (cm)
Walking	1.60 (0.19)	0.96 (0.02)	64.7 (2.5)	2.5 (1.9)
Running	4.62 (1.26)	0.70 (0.05)	32.2 (3.6)	3.1 (2.5)
P-value	<0.001	<0.001	<0.001	0.575

Table 4: Temporal and amplitude EMG comparisons between running and walking

Phase	Outcome	Trial mean (SD)		Effect size	Paired *t*-test	
		Walk	Run		t_8-value	*P*-value
Stance	Peak (% MVIC)	49.1 (33.7)	105.3 (82.2)	-0.47	2.192	0.060
	Average (% MVIC)	20.9 (15.7)	71.0 (59.7)	-0.56	2.642	0.029*
	[1]TTP (% stance)	25.2 (23.1)	44.1 (17.7)	-0.58	2.769	0.024*
Swing	[1]Peak (% MVIC)	12.2 (9.1)	108.6 (104.3)	-4.23	12.629	<0.001*
	Average (% MVIC)	7.9 (5.7)	52.1 (51.6)	-0.58	2.771	0.024*
	TTP (% flight)	39.7 (16.7)	61.0 (19.0)	-0.49	2.255	0.054
Total stride	Peak (% MVIC)	49.3 (33.5)	128.5 (101.8)	-0.53	2.479	0.038*
	Average (% MVIC)	17.6 (12.7)	61.7 (57.0)	-0.52	2.425	0.041*
	[1]TTP (% gait cycle)	19.7 (20.1)	35.8 (22.9)	-0.50	2.301	0.050

Note. There were infrequent occasions within individual participant trials where peak amplitude occurred during different phases (stance versus flight). The mean values of these variables in the ‘total stride’ phase are therefore different than the mean values in the stance or flight phase. Abbreviations: Ave, average; TTP, time to peak.

[1]Analysis performed on logarithm transformed data, however untransformed data are presented for ease of interpretation.

*Significant findings.