JYU DISSERTATIONS 178

Annamária Péter

Neural and Mechanical Function of Flexor Hallucis Longus at Different Walking Speeds and in Different Footwear

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND HEALTH SCIENCES

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ABSTRACT

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Ankle plantar flexor muscles make a major contribution to body propulsion in walking. Besides the triceps surae, deep ankle plantar flexors such as flexor hallucis longus (FHL) may also contribute to this. However, FHL function has not been extensively examined *in vivo*. Therefore, the aim of this thesis was to examine the effects of walking speed on FHL electromyography (EMG) activity, fascicle behaviour, and forces measured under the hallux in shod walking. Agreement between surface and intramuscular EMG was also tested in shod walking at different speeds for FHL, soleus, gastrocnemii, and tibialis anterior. Furthermore, intramuscular EMG activity of FHL and triceps surae was examined in different footwear at self-selected walking speed. As expected, FHL was highly active in the push-off phase of walking, similar to other plantar flexors. Increased walking speed was associated with higher FHL EMG activity and higher forces under the hallux, indicating an increase in the relative importance of FHL at faster walking speeds. FHL muscle fascicles operated at a near-constant length throughout the stance phase of slow walking, and shortened at faster speeds. This is similar to the fascicle mechanics of medial gastrocnemius in walking, with which FHL also shares similar architectural properties. When surface and intramuscular EMG methods were compared, there was often (~60% of all cases) poor agreement between methods for FHL, likely due to the challenge of minimising cross-talk in this muscle. Walking in shoes at preferred speed required higher plantar flexor muscle activity for body propulsion than walking in flip-flops or barefoot in most individuals, however individual variability was substantial. In shod walking, peak muscle activity occurred at the same relative time in the contact phase between participants. This may be due to the fact that shoes limit individual-specific natural foot and ankle function, imposing a restrictive motion pattern. This thesis provides *in vivo* evidence for the important role of FHL in walking. Using intramuscular EMG and ultrasonography, future studies should examine FHL function in individuals with Achilles tendinopathy or flatfoot, which are associated with altered FHL morphology, and perhaps also altered muscle function.

Keywords: walking, footwear, plantar flexors, flexor hallucis longus, electromyography, force, fascicle behaviour

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TIIVISTELMÄ (FINNISH ABSTRACT)

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Isovarpaan pitkän koukistajalihaksen hermostollinen ja mekaaninen toiminta eri kävelynopeuksilla ja erilaisilla jalkineilla
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Kävelyssä nilkan ojennukseen osallistuvat lihakset ovat merkittävässä roolissa erityisesti kontaktivaiheen kehoa eteenpäin työntävässä vaiheessa. Kolmipäisen pohjelihaksen lisäksi syvemmät pohjelihakset, kuten isovarpaan pitkä koukistajalihas (flexor hallucis longus, FHL), osallistuvat nilkan ojentamiseen. Tutkimuksen tarkoituksena oli selvittää kävelynopeuden vaikutuksia FHL:n lihasaktiivisuuteen, lihas-solukimppujen toimintaan ja isovarpaaseen kohdistuviin reaktiovoimiin kävelyn aikana. Lisäksi tutkittiin lihasaktiivisuuksia eri jalkineilla itsevalitulla kävelynopeudella. Lihaksen pinnalta mitatun lihasaktiivisuuden (elektromyografian, EMG) lisäksi EMG:tä mitattiin lihasten sisältä lankaelektrodeilla, ja EMG-signaalien amplitudien ja ajoitusten yhteneväisyyksiä näillä menetelmillä vertailtiin FHL-lihaksesta, leveästä kantalihaksesta, kaksoiskantalihaksesta sekä etummaisesta säärilihaksesta. Oletuksen mukaisesti isovarpaan pitkä koukistajalihas oli erittäin aktiivinen kävelyn työntövaiheessa samoin kuin muut nilkkaa ojentavat lihakset. Suurempi kävelynopeus oli yhteydessä korkeampaan FHL:n EMG-aktiivisuuteen ja suurempiin voimiin isovarpaan alla. Tämä viittaa isovarpaan pitkän koukistajalihaksen suhteellisen merkityksen lisääntymiseen suuremmilla kävelynopeuksilla. Isovarpaan pitkän koukistajalihaksen lihassolukimput toimivat lähes vakiopituudella koko kontaktivaiheen ajan hitaassa kävelyssä, mutta suuremmilla kävelynopeuksilla FHL työskenteli konsentrisesti. Tämä toimintatapa on samankaltainen kuin sisemmän kaksoiskantalihaksen lihas-solukimpun mekaniikka kävelyssä. Onkin huomattava, että kaksoiskantalihaksella ja FHL:lla on samankaltaisia rakenteellisia ominaisuuksia. Kun verrattiin pinta- ja lanka-EMG -menetelmiä, ~60% tapauksissa pinta-EMG ei vastannut lanka-EMG:n tuloksia. Useimmilla tutkittavilla jalkineilla kävely vaati suurempaan aktiivisuutta nilkkaa ojentavilta lihaksilta kävelyn työntövaiheessa kuin sandaaleilla tai paljasjaloin kävely. Yksilöllinen vaihtelu oli kuitenkin huomattavaa. Jalkineilla kävellessä lihasten huippuaktiivisuus ajoittui kontaktivaiheessa samaan kohtaan kaikilla osallistujilla. Tämä voi johtua siitä, että kengät rajoittavat yksilökohtaisia luonnollisia jalkaterän ja nilkan toimintoja, ja täten rajoittavat liikemallia. Tämä tutkielma tarjoaa *in vivo* todisteita isovarpaan pitkän koukistajalihaksen tärkeästä roolista kävelyssä. Tulevissa tutkimuksissa tämän tutkimuksen menetelmiä voisi soveltaa tutkittaessa henkilöitä, joilla on akillesjänteen tendinopatia tai matala jalkaholvi, jotka liittyvät muuttuneeseen morfologiaan ja luultavasti myös muuttuneeseen lihaksen toimintaan.

Avainsanat: Kävely, jalkineet, plantaarifleksio, isovarpaan pitkä koukistajalihas, elektromyografia, voima, lihassolukimppujen käyttäytyminen

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on four original papers, which are referred to by the following roman numerals:

I Péter A, Hegyi A, Stenroth L, Finni T & Cronin NJ. 2015. EMG and force production of the flexor hallucis longus muscle in isometric plantarflexion and the push-off phase of walking. Journal of Biomechanics 48(12), 3413–3419.

II Péter A, Hegyi A, Finni T & Cronin NJ. 2017. In vivo fascicle behavior of the flexor hallucis longus muscle at different walking speeds. Scandinavian Journal of Medicine & Science in Sports 27(12), 1716–1723.

III Péter A, Andersson E, Hegyi A, Finni T, Tarassova O, Cronin NJ, Grundström H & Arndt A. 2019. Comparing Surface and Fine-Wire Electromyography Activity of Lower Leg Muscles at Different Walking Speeds. Frontiers in Physiology 10:1283.

IV Péter A, Arndt A, Hegyi A, Finni T, Andersson E, Alkjær T, Tarassova O, Rönquist G & Cronin NJ. Effect of footwear on intramuscular EMG activity of plantar flexor muscles in walking. Submitted for publication.

The author of this thesis was mainly responsible for study design, data collection and analysis, data interpretation, and all steps in the publication process, reflecting her major role in all stages of the research process.

CONTENTS

1 INTRODUCTION

In human locomotion, ankle plantar flexor muscles are the primary contributors to forward propulsion of the body (e.g. Farris and Sawicki, 2012; Winter, 1983). Besides the triceps surae muscles, the deep ankle plantar flexors also seem to significantly contribute to this work in walking (Jacob, 2001). Although several studies have examined the role of plantar flexor muscles in walking, they have mainly focused on the triceps surae and were mostly performed at preferred speed. However, walking at different speeds or in different types of footwear, as occurs in everyday life, likely requires different intermuscular coordination between the ankle plantar flexor muscles.

Flexor Hallucis Longus (FHL) is a deep, multi-articular ankle plantar flexor muscle that attaches on the distal phalanx of the hallux. The hallux provides the last link between the human body and the ground in walking, so it is possible that FHL also plays an important role in the push-off phase of locomotion. FHL has recently been studied (Bojsen-Moller et al., 2010; Masood et al., 2014b; 2014a) due to its significant physiological cross-sectional area, which is related to force production capacity (Friederich and Brand, 1990; Kura et al., 1997). FHL has been suggested to perform numerous functions. For example, it is supposed to contribute to load transmission from the foot to the ground during locomotion (Ferris et al., 1995), and the significance of this contribution to propulsion is reflected by the large forces acting along its tendon in walking (Jacob, 2001). FHL has also been suggested to support the medial longitudinal arch of the foot (Sarafian, 1993; Waldeyer and Mayet, 1993). The physiological movement of the medial longitudinal foot arch has been found to be significantly smaller in shod walking compared to barefoot walking (Morio et al., 2009). Furthermore, due to its anatomy, FHL is believed to contribute to foot inversion (Hintermann et al., 1994), which is smaller in shoes than barefoot (Morio et al., 2009). Thus, it seems that footwear restricts foot motion in walking, which could have important implications for FHL and triceps surae function.

Lower leg health is very important since this body part is critical for a range of activities of daily living. Examining human walking in healthy, asymptomatic individuals may help to identify and understand how different ankle plantar flexor muscles function in a coordinated manner, and how their function changes with changes in walking speed or footwear. Increasing our knowledge in this field may help to identify sources of impairments in symptomatic individuals, improve the rehabilitation process after injury or surgery, and contribute to effective injury prevention strategies.

FHL seems to play an important role in propulsion during walking, and yet very little is known about its neuromechanical function. Furthermore, it has been suggested that the relative contribution of FHL to propulsion may significantly change with changes in walking speed or footwear. Most studies that previously examined FHL function involved cadavers or indirect methods, so there is a need for *in vivo* studies performed during real-life movements. Thus, the aim of this thesis was to examine the neural and mechanical function of FHL *in vivo* during human walking at different walking speeds and in different types of footwear.

2 LITERATURE REVIEW

2.1 Walking

Walking is one of the most common activities of our everyday lives. It is a complex integrated activity that requires all the contributing components to work simultaneously together. It is important to understand the individual contributions of the different muscles that are involved in walking because further understanding mechanisms of the simplest way of transport in humans can be applied to different domains such as maintaining health through daily physical activity, injury risk reduction, rehabilitation, and aging.

2.1.1 The gait cycle

Walking is a cyclical movement that can be separated into individual gait cycles. Typically, a gait cycle is defined to start when the heel contacts the ground (referred to as heel contact) and it ends when the same heel contacts the ground again (Perry, 1992). A gait cycle consists of two primary phases: stance phase and swing phase. During the whole stance phase the foot is on the ground and is thus weight bearing (~60-65% of the whole normal adult gait cycle) (Phillips, 2006). The stance phase starts with heel contact and it ends when the last toe leaves the ground (toe-off). During the whole swing phase, the leg is in the air and moving forward (~35-40% of the gait cycle) (Phillips, 2006). This phase starts at toe-off and it ends when the same foot contacts the ground again. Both the stance and swing phases can be divided into several sub-phases. The stance phase can be divided into early stance, mid stance, late stance and pre-swing (Neptune et al., 2001). Within the stance, it can also be useful to define the push-off phase, which starts at the end of mid stance when the heel leaves the ground, i.e. when the anterior-posterior ground reaction force crosses 0 N and becomes an anterior, propulsive force. The swing phase can be divided into initial swing, mid swing and terminal swing (Stöckel et al., 2015).

2.1.2 The role of ankle plantar flexors

In the push-off phase of walking, the highest power of all leg joints is produced around the ankle joint (Hof et al., 1983; Winter, 1983) (Figure 1). Accordingly, ankle plantar flexors make a major contribution to moving the centre of mass forward in walking. Ankle plantar flexor muscles consist of superficial and deep muscles. The superficial muscles are the so-called triceps surae group, which includes the soleus, medial and lateral gastrocnemii. The deep ankle plantar flexor muscles are the flexor digitorum longus, flexor hallucis longus (FHL), tibialis posterior, peroneus longus and peroneus brevis. Of these muscles, triceps surae muscles have been examined most extensively (e.g. Kepple et al., 1997; Neptune et al., 2001), but recent studies suggest that the deep ankle plantar flexors also play a significant role in walking (e.g. Jacob, 2001). Accordingly, ankle plantar flexors are mainly active in the stance phase of walking, and their peak activity occurs in the push-off phase (Maharaj et al., 2016; Zelik et al., 2015). In spite of several studies in this field, experimental evidence on the role of deep ankle plantar flexors is scarce, even in healthy individuals walking at a self-selected speed.

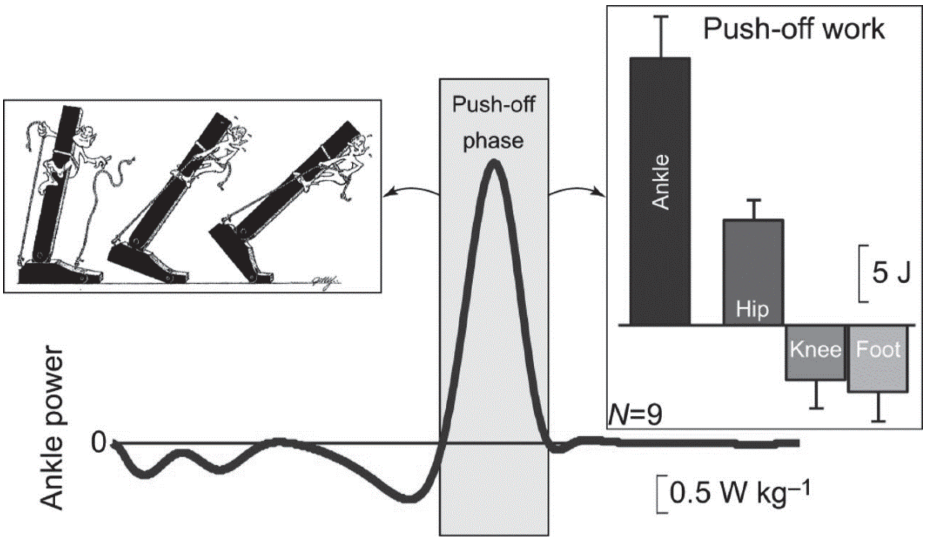

FIGURE 1 Ankle power and work during walking. Ankle power of one leg is plotted across a full gait cycle. The left inset illustrates ankle push-off behaviour. The right inset shows the push-off work of the different leg joints (mean ± SD; walking speed: 1.4 m/s, n=9). Figure reproduced with permission from The Company of Biologists Ltd (Zelik and Adamczyk, 2016).

In everyday life, we walk at different speeds, in different types of footwear, on different terrain, etc. All of these may markedly change the relative contribution of the ankle plantar flexor muscles to walking. For example, gait kinematics and muscle force requirements change with the speed of walking (e.g. Lelas et al., 2003), as does the electromyography (EMG) activity of soleus and medial gastrocnemius muscles in late stance (Den Otter et al., 2004; Lai et al., 2015; Neptune et al., 2008). Thus, it can be assumed that the different modalities of walking require different intermuscular coordination between the plantar flexor muscles. Although most people walk at a range of speeds, previous studies examining the EMG activity of different muscles have mainly only done so at preferred walking speed (e.g. Sacco et al., 2010). Thus, studies examining speed-related changes in EMG activity of superficial and deep ankle plantar flexor muscles are lacking.

Among the deep ankle plantar flexor muscles, FHL has recently attracted attention in the form of several studies (e.g. Bojsen-Moller et al., 2010; Masood et al., 2014b). One reason for this is that increased muscle thickness has been observed in flatfooted individuals, and FHL thickness also seems to be related to Achilles tendon injuries, although the mechanisms are currently unclear.

2.2 Flexor hallucis longus

2.2.1 Morphology and architecture

Flexor hallucis longus (FHL) is a multi-articular, bi-pennate, deep ankle plantar flexor muscle that is positioned on the fibular side of the leg. It originates from the distal 2/3 of the fibula and the membrane interossea, proceeds behind the medial malleolus over the ankle and the 1st metatarsophalangeal joints, and inserts on the distal phalanx of the great toe on the plantar surface of the foot (Gray, 1980) (Figure 2).

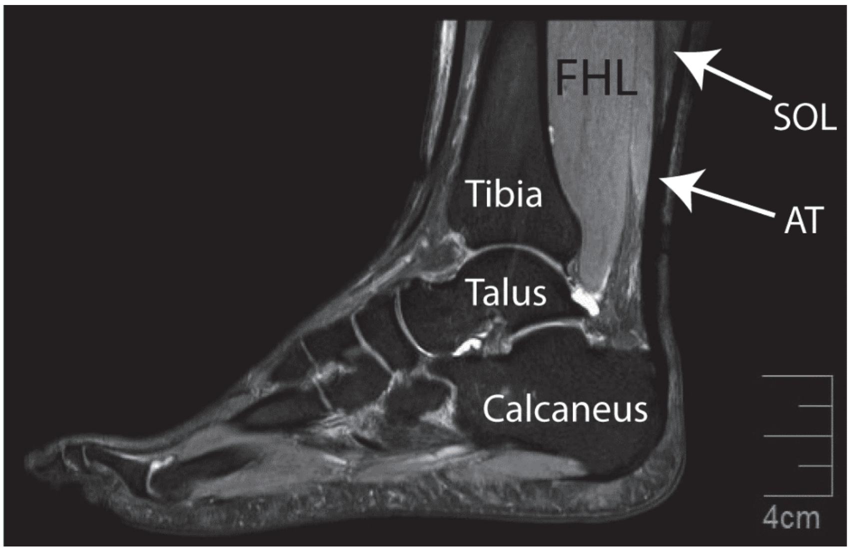

FIGURE 2 Sagittal plane magnetic resonance image of the distal shank showing the distal portion of the flexor hallucis longus (FHL) relative to other structures. Note that the FHL muscle-tendon junction is more distal than the soleus (SOL) muscle-tendon junction. Achilles tendon, AT.

Considerable variation in FHL morphology has been found between individuals (n=80, 40 males; mean age: 70.0 years, between the age of 48 and 90 years) (Figure 3) (Pichler et al., 2005). In most cases (70 cases, 88% of all cases), the lateral muscle belly ended more distally than the medial one. In five cases (6%), the medial muscle belly ended more distally than the lateral one, while in three cases (4%) muscle bellies of the two parts ended in the same place. In two further cases (3%), there was a lateral muscle belly but no medial belly. No statistical correlation was found between tibia length and FHL muscle length, or between the lengths of flexor digitorum longus and FHL. Furthermore, there was a significant range in muscle length: 82 mm for the lateral and 120 mm for the medial muscle belly.

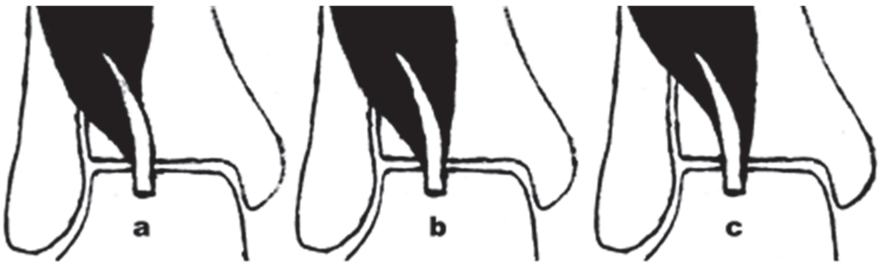

FIGURE 3 Three typical variations of FHL muscle ending. A The lateral muscle belly ends more distally. B Both bellies end in the same place. C Medial belly ends more distally. Figure reproduced with permission from Springer Nature (Pichler et al., 2005).

According to a cadaver study (Ward et al., 2009) where lower leg muscle architectural properties were examined, the following parameters were found for FHL (n=19): muscle mass: 38.9±17.1 grams; muscle length: 26.9±3.6 cm; fibre length: 5.3±1.3 cm; sarcomere length: 2.4±0.2 µm; pennation angle: 16.9±4.6°; and physiological cross-sectional area: 6.9±2.7 cm^2. The mean fibre length of the ankle plantar flexors (peroneus longus, peroneus brevis, gastrocnemius medialis, gastrocnemius lateralis, soleus, FHL, flexor digitorum longus and tibialis posterior) was 4.8±1.1 cm, while their total physiological cross-sectional area was 124.3±30.4 cm^2. This study was conducted on relatively old specimens (83±9 years), thus, the observed values may be even greater in younger individuals.

In the study of Fukunaga et al. (1992), magnetic resonance imaging was used to estimate muscle volumes of the lower leg muscles *in vivo* (n=12; age: 32.6±8.2 years ranging from 20 to 49 years). Participants were lying in prone position with the ankle at ~120° and the knee at 180°. FHL muscle length was 24.9±3.6 cm, fibre length was 3.8±0.5 cm, muscle volume was 74.0±10.6 cm^3, physiological cross sectional area was 19.3±2.8 cm^2, maximum anatomical cross-sectional area was 4.9±1.1 cm^2, and mean anatomical cross-sectional area was 3.0±0.4 cm^2. The mean fibre length of the ankle plantar flexors (gastrocnemius medialis, gastrocnemius lateralis, soleus, FHL, flexor digitorum longus and tibialis posterior) was 3.2±1.1 cm, their total physiological cross-sectional area was 391.4±83.2 cm^2 and the total maximum anatomical cross-sectional area was 69.5±10.4 cm^2.

Total physiological cross-sectional area of the triceps surae muscles was ~326±44 cm² and the mean muscle length ranged from 19 (flexor digitorum longus) to 32 cm (soleus) (Figure 4).

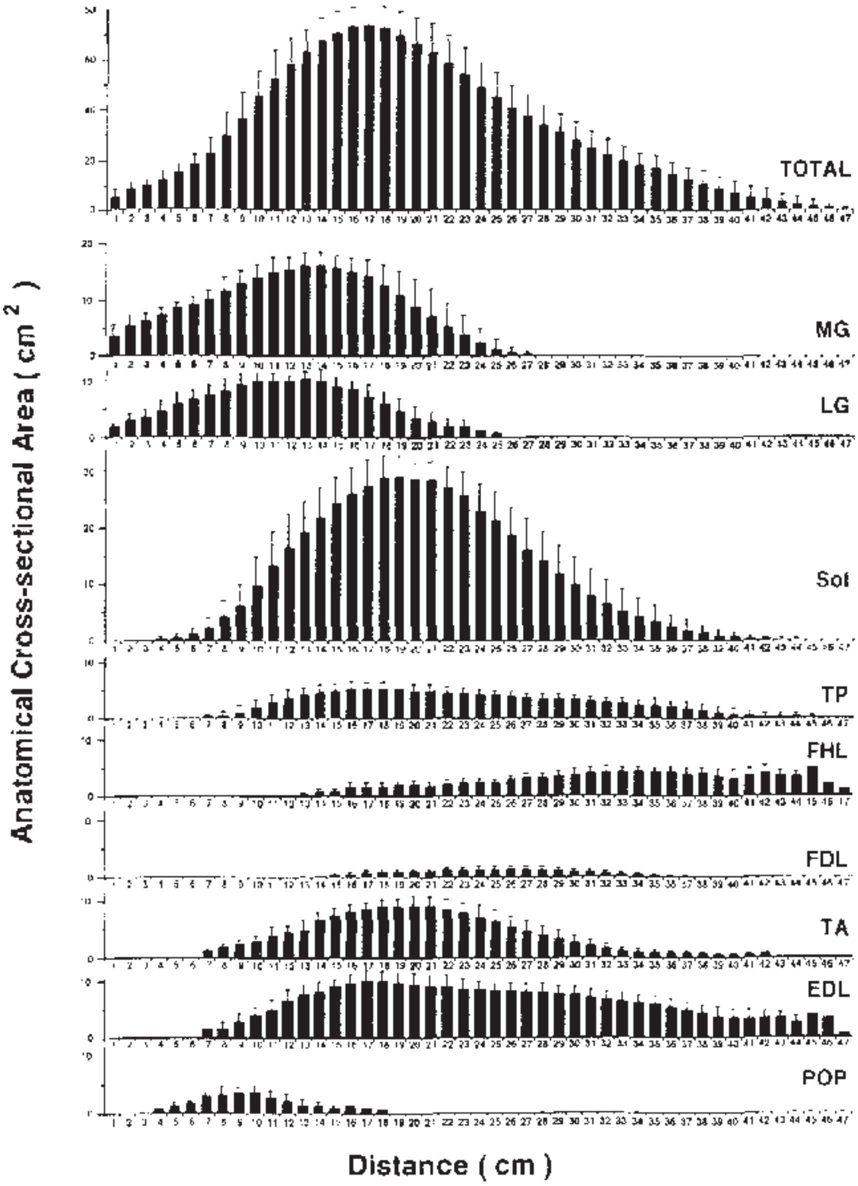

FIGURE 4 Anatomical cross-sectional area of lower leg muscles along the length of the leg. Slice 1 represents the proximal edge of the patella. The top figure shows the total cross-sectional area of all examined muscles, while other figures show the cross-sectional area of each examined muscle separately (MG=medial gastrocnemius; LG=lateral gastrocnemius; Sol=soleus; TP=tibialis posterior; FHL=flexor hallucis longus; FDL=flexor digitorum longus; TA=tibialis anterior; EDL=extensor digitorum longus, extensor hallucis longus, peroneus tertius, peroneus longus and brevis together; POP=popliteus). Each bar shows mean ± SD of all participants (n=12). Figure reproduced with permission from John Wiley and Sons (Fukunaga et al., 1992).

In another study (Mickle et al., 2013), the cross-sectional area of FHL was measured *in vivo* with ultrasonography (n=10, between the age of 23 and 50 years). The measurement was taken in supine position, hip externally rotated and knee slightly flexed from two locations: at 40% and 50% of the distance between the inferior margin of the medial malleolus and medial condyle of the tibia. The cross-sectional area of FHL was 4.3±0.7 cm^2 and 4.2±0.9 cm^2 at 40% and 50%, respectively.

The different studies gave similar values for muscle length and anatomical cross-sectional area of FHL. However, differences can be seen in other values that may be due to differences in methodology. For example, FHL fibre length was ~1.5 cm shorter in the study of Fukunaga et al. (1992) than in the cadaver study of Ward et al. (2009). In the study of Fukunaga et al. (1992) the ankle was in a somewhat plantar flexed position (~120°), which may have resulted in shorter FHL fibre length. A similar difference between these studies can also be seen in the mean fibre length of the ankle plantar flexor muscles (~1.6 cm), which further supports the notion that the differences between studies are due to different ankle joint positions. Furthermore, there were notable differences between studies in physiological cross-sectional area for both the FHL and the total ankle plantar flexor muscles. However, when examining the physiological cross-sectional area of FHL relative to the total physiological cross-sectional area of the ankle plantar flexor muscles, the results are similar (0.05 for Fukunaga et al., 1992; 0.06 for Ward et al., 2009). Thus, differences in the magnitude of these values between studies may have been caused by the different calculation methods that were used. In spite of these differences, it is clear that FHL's physiological cross-sectional area, which is related to force production capacity, is significant, approximately 70% of the physiological cross-sectional area of the lateral gastrocnemius muscle (Friederich and Brand, 1990; Fukunaga et al., 1992; Ward et al., 2009). FHL also has the largest cross-sectional area among all the long and short toe flexor muscles (Friederich and Brand, 1990; Kura et al., 1997; Wickiewicz et al., 1983). These findings suggest that FHL may be a functionally important muscle relative to several other muscles of the lower leg and foot.

2.2.2 Moment arms

In the study of Klein et al. (1996), moment arms of the ankle plantar flexor muscles were examined at the talocrural and subtalar joints *in vitro* (10 specimens from 8 donors, age over 60 years) (Table 1). When the talocrural motion was examined, the subtalar joint was fixed in neutral position, and when the subtalar motion was examined, the talocrural joint was held in a plantar flexed position. Considering the mean moment arm at the taloclural joint, the most important plantar flexor muscles were the triceps surae and the FHL, while at the subtalar joint these muscles had the smallest mean moment arms compared to the other examined ankle plantar flexor muscles.

During weight-bearing activities (e.g. walking), the longitudinal arch of the foot tends to flatten due to the weight of the body, resulting in eversion at the subtalar joint. Considering that at the subtalar joint tibialis posterior had a much higher mean moment arm than FHL, tibialis posterior muscle is better suited to counteracting the eversion of the foot. Thus, tibialis posterior seems to play a major role in maintaining the structure of the arch of the foot (Klein et al., 1996).

However, it is important to note that the position of the talocrural joint (being either in plantar- or dorsiflexion) did not influence the moment arm at the subtalar joint consistently across individuals but induced very large variations between individuals (mean moment arms ranging from -12.4 to 25.0, from -11.6 to 0.0 (i.e. no displacement registered) and from -23.8 to -1.9 mm for triceps surae, FHL and tibialis posterior respectively) (Klein et al., 1996).

TABLE 1 Moment arms of plantar flexor muscles. Adopted from (Klein et al., 1996).

	Moment arm (mm)					
	At the talocrural joint			At the subtalar joint		
	Mean	std ind	std rot	Mean	std ind	std rot
Triceps surae	52.8	5.1	0.7	-5.3	7.4	10.6
Peroneus longus	12.8	2.9	0.5	21.8	4.3	3.4
Peroneus brevis	9.9	2.4	0.5	20.5	3.9	2.8
Tibialis posterior	8.0	3.6	1.7	-19.2	3.6	1.3
FHL	26.6	4.4	1.6	-7.8	3.0	3.9
	positive number: plantar flexion			positive number: eversion negative number: inversion		

std ind = inter-individual standard deviation, *std rot* = standard deviation due to joint rotation. N = 10.

2.2.3 Innervation

According to a cadaver study (Yu et al., 2016) (n=8, age ranged from 25 to 60 years), FHL is innervated by branches of the tibial nerve. At the posteriolateral margin of FHL the tibial nerve separates into 2-3 branches, which enter the muscle. Two different intramuscular nerve distribution patterns have been observed. In most cases (n=11 out of 15 cases; Figure 5), the nerve separates into 3-4 branches. These branches enter the FHL on the posterior surface at the upper one-third of the muscle. The proximal branch innervates the upper part, the middle 1 or 2 branches innervate the middle part while the distal branch innervates the distal part of FHL. In fewer cases (n= 4 out of 15, Figure 5), the nerve enters the muscle near to its origin and then divides into two branches. Both of these branches run parallel distally through the muscle and innervate the anterior and the posterior parts.

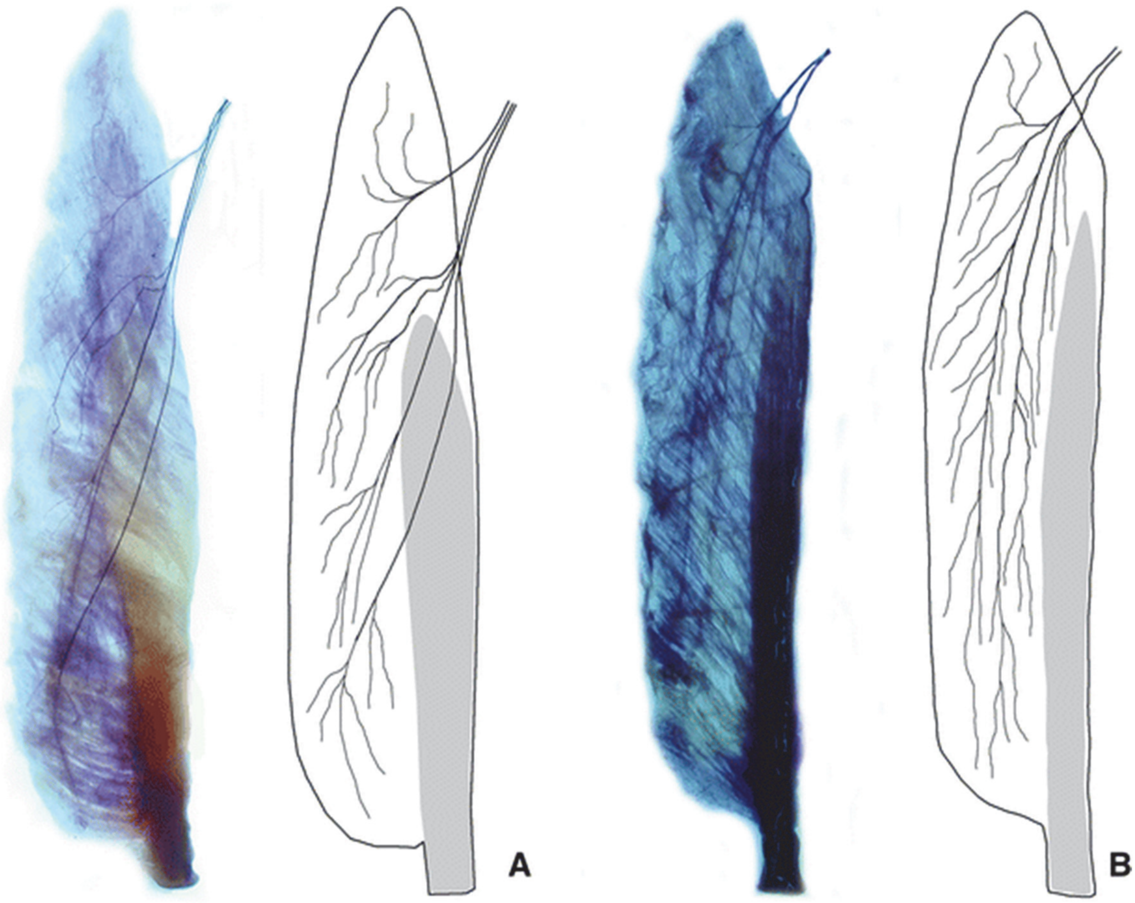

FIGURE 5 Panels A and B represent two distinct innervation patterns of flexor hallucis longus muscle. Figure reproduced with permission from Springer Nature (Yu et al., 2016).

2.2.4 Possible functions of FHL

A number of studies have shown the multifunctionality of FHL, but most of them have relied on indirect methods, and thus had to make certain assumptions about FHL function.

Maximum force is related to skeletal muscle size (Fukunaga et al., 2001b; Maughan et al., 1984). However, FHL is multiarticular, and contributes to torque at different joints. In the study of Kurihara et al. (2014) (n=26; age: 20.4±1.6 years), relationships were examined between maximum isometric toe flexion strength and cross-sectional area of plantar intrinsic (n=10) and extrinsic muscles (FHL, flexor digitorum longus). Cross-sectional area was measured using magnetic resonance imaging while toe flexor strength was measured using a dynamometer. There were positive correlations between toe flexor strength and the cross-sectional area of all examined muscles except for flexor digitorum longus (Figure 6). Thus, it seems that FHL (as well as intrinsic muscles) makes a meaningful contribution to toe flexion.

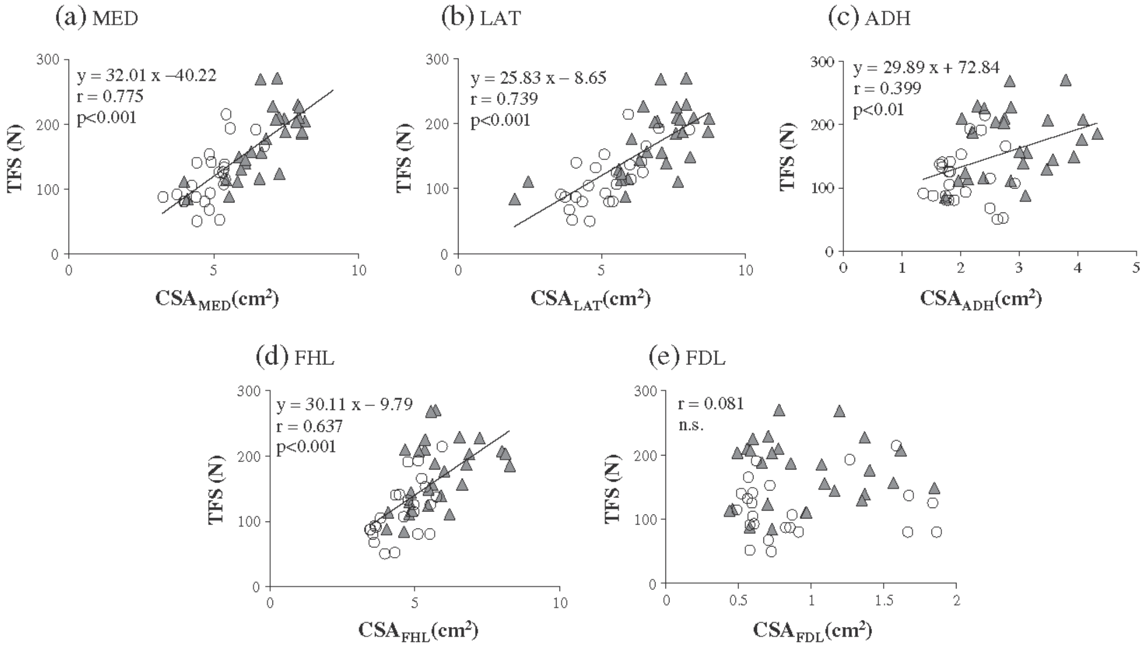

FIGURE 6 The relationship between toe flexor muscle strength and cross-sectional area (n=52, triangle=men, open circle=women). (a) medial parts of plantar intrinsic muscles, (b) lateral parts of plantar intrinsic muscles, (c) adductor hallucis muscle, (d) flexor hallucis longus muscle, (e) flexor digitorum longus muscle. Figure reproduced with permission from BioMed Central Ltd. (Kurihara et al., 2014).

Aside from its potential contribution to toe flexion, FHL may also contribute to plantar flexion at the ankle (Klein et al., 1996). In isometric conditions, the contribution of the triceps surae to the total plantar flexion moment ranges somewhere between ~65% (Gregor et al., 1991), ~70% (Masood et al., 2014a) and ~85% (Van Zandwijk et al., 1998). Although it is not clear how the different plantar flexor muscles contribute to plantar flexion, these study results imply that other muscles such as deep ankle plantar flexor muscles (including FHL) also contribute to ankle plantar flexion, and may thus play an important functional role during human gait.

The potential importance of FHL (and other toe flexors) in the push-off phase of locomotion is also supported by Goldmann et al. (2013), who showed that 7 weeks of isometric toe flexor muscle strength training improved horizontal jump performance significantly in previously untrained men. In walking, FHL is active in the stance phase (Perry, 1992). Therefore, it is likely that FHL plays an important role in this phase. The first metatarsophalangeal joint and the hallux together represent the last link between the ground and the human body in walking. It has been estimated (Jacob, 2001) that in the push-off phase of walking the forces are large under the first ray (first metatarsal head and hallux together) of the foot (~53% body weight), and they are also large along the FHL tendon (~52% body weight). Furthermore, the hallux has been found to transfer as much as 24% of body weight to the ground (Jacob, 2001). Thus, the first ray, which is crossed by FHL, seems to have prime importance in propulsion (Jacob, 2001). Such large forces may originate from two mechanisms: (1) force production of the hallux flexors (FHL and flexor hallucis brevis), and (2) force transmission from the proximal joints. In both ways, FHL might have the main role since it has the largest physiological cross-sectional area among all long and short toe flexor muscles (Friederich and Brand, 1990) and crosses multiple joints including the ankle and first metatarsophalangeal joints (Gray, 1980). Multi-articular muscles are hypothesized to function isometrically or near-isometrically during the contact phase of gait (Zajac et al., 2002). These mechanisms contribute to optimal energy transfer between proximal and distal joints, making muscle work economical. Based on modelling and ultrasonography studies, these mechanisms have been observed for the rectus femoris (hip-knee transfer) (Bobbert and van Ingen Schenau, 1988) and gastrocnemius muscles (knee-ankle transfer) (Neptune et al., 2001; van Ingen Schenau et al., 1992). Understanding the mechanical behaviour of FHL in walking would help to clarify its contribution to propulsion. The multiarticular nature of FHL suggests that this muscle may also contribute to foot supination (Ferris et al., 1995), medial longitudinal arch support (Thordarson et al., 1995), and rearfoot inversion (Hintermann et al., 1994).

2.3 Challenges of assessing FHL function

2.3.1 Mechanical behaviour

To date, only a few studies have examined FHL mechanics, so it is often necessary to make assumptions about how this muscle functions. Muscle structural characteristics are usually good predictors of how the muscle works (Lieber and Ward, 2011). FHL is a multi-articular muscle. The ratio of FHL muscle fibre length to muscle-tendon unit length is small, at around 0.20 (Ward et al., 2009). This small ratio implies that the muscle belly shortens minimally during contractions, implying isometric or near-isometric function. Based on the results of cadaver studies, FHL tendon excursion in the stance phase of simulated walking has been found to be 7.2±1.8 mm and 6.6±3.1 mm on average (Hofmann et al., 2013; Kirane et al., 2008, respectively). This tendon excursion is very small relative to the muscle fibre length, which is 52.7±12.9 mm (Ward et al., 2009). In the push-off phase of walking and running, as the heel leaves the ground there is plantar flexion at the ankle joint and dorsal flexion at the metatarsophalangeal joint (Bobbert and van Ingen Schenau, 1988; Leardini et al., 1999; Stefanyshyn and Nigg, 1998). Thus, during locomotion, the ankle and the metatarsophalangeal joints work in opposite directions in the stance phase (Leardini et al., 1999). Both of these joints are crossed by FHL, which means that FHL can facilitate energy transfer from the proximal leg muscles to the distal foot segments (Kirane et al., 2008). It is assumed that in multi-articular muscles, muscle shortening at one joint can be offset by lengthening at the other (Kaya et al., 2005). All of these findings support the idea of isometric or near-isometric function of FHL in walking. Nonetheless, although relatively small FHL tendon excursion has been found in the stance phase of simulated walking (Hofmann et al., 2013; Kirane et al., 2008), the magnitude of this excursion showed high variation between individuals (Hofmann et al., 2013). In the study of Hofmann et al. (2013), 8 specimens were examined, and FHL tendon excursion (based on myotendinous junction displacement) ranged between 4.3 and 10.2 mm. This suggests that quasi-isometric behaviour does not seem to be evident in all individuals. Importantly, FHL mechanical behaviour has not been examined *in vivo*, and it is unlikely that cadaver gait simulations can accurately describe *in vivo* muscle function.

Ultrasonography is a widely used non-invasive method to study *in vivo* muscle geometrical properties such as fascicle length, pennation angle, muscle thickness and cross-sectional area (Cronin et al., 2013a; Farris and Sawicki, 2012; Kawakami et al., 1998; Mickle et al., 2013). Measurements can be done in static and dynamic conditions with brightness-mode (B-mode) ultrasonography. The acquired ultrasonography videos can be analysed manually (time consuming, somewhat subjective) or with semi-automated tracking algorithms (Cronin et al., 2011; Farris and Lichtwark, 2016). To reveal muscle mechanics *in vivo*, muscle fascicle length changes are often examined during locomotion using this method. Fascicle length and pennation angle measurements in medial gastrocnemius show high reproducibility during walking throughout the gait cycle within the same day and on different days (Aggeloussis et al., 2010). As few as six steps may provide a reliable estimate of muscle fascicle function in walking (Aggeloussis et al., 2010). Most studies that examined the fascicle behaviour of plantar flexor muscles during walking at different speeds focused on the soleus and medial gastrocnemius muscles (Cronin et al., 2013a; Farris and Sawicki, 2012; Lai et al., 2015). These studies have found that the fascicle behaviour of soleus and medial gastrocnemius muscles is modified with changing walking speed. For example, fascicle shortening velocity of medial gastrocnemius has been found to increase with increasing walking speed (Farris and Sawicki, 2012). Ultrasonography may allow *in vivo* examination of FHL muscle mechanics at different walking speeds.

2.3.2 Muscle activity

Electromyography (EMG) is a frequently used method to examine the electrical activity of muscles during various physical activities (e.g. walking) in both research and clinical environments. EMG signals are usually recorded to define the magnitude and/or timing of muscle activity (onset/offset, peak activity timing) (Mann et al., 1986; Mann and Inman, 1964; Sutherland, 1966). The magnitude (i.e. amplitude) of EMG activity represents the number of active motor units and their firing frequency. EMG measurements can be done using either surface or intramuscular electrodes (Farina and Negro, 2012).

Surface EMG is used much more often than intramuscular EMG since surface EMG is non-invasive, cheaper, easier and more convenient to use. During static and dynamic movements, the surface EMG method is usually used to study superficial and large muscles that are easily accessible. Surface EMG electrode location is usually guided by recommendations such as SENIAM (Hermens et al., 2000), although these may need to be slightly adjusted depending on individual muscle morphology. In spite of the popularity of surface EMG, it has some limitations. With surface EMG, the EMG activity of deep muscles cannot be recorded. Furthermore, since signals are acquired from a large area (Roy et al., 1986), the recorded signals may not exclusively come from the desired muscle, but instead may also come from adjacent or deep muscles (Dimitrova et al., 2002; Lowery et al., 2003; Perry et al., 1981). This phenomenon is called cross-talk. Due to cross-talk, the recorded EMG signals may be misinterpreted (De Luca, 1997), and this can in turn lead to inaccurate conclusions. Thus, cross-talk needs to be minimised as much as possible, e.g. by locating the surface electrodes properly (Roy et al., 1986), and by using an appropriate electrode size and inter-electrode distance (Merletti et al., 2001). A further limitation of the surface EMG method is that the electrodes are positioned on the skin, but during contractions the muscle moves under the skin and the electrodes and this may have a large effect on the recorded surface EMG signal (De Luca, 1997; Farina et al., 2001; Rainoldi et al., 2000).

In spite of the fact that FHL is a deep ankle plantar flexor muscle, it has a small part behind the medial malleolus where it becomes superficial (Figure 2). In the study of Bojsen-Moller et al. (2010), FHL EMG activity was measured with surface EMG for the first time during maximal and submaximal isometric contractions from the small superficial part of the muscle. In Figure 7, which was presented in the study of Bojsen-Moller et al. (2010), we can see a submaximal ankle isometric plantar flexion contraction that was performed with added periods of toe flexion. During this contraction, FHL, soleus, and medial gastrocnemius surface EMG activity and the force under the hallux were measured. When soleus and medial gastrocnemius muscles were active but there was no force detected under the hallux, there was no activity detected from FHL. Conversely, when hallux force was detected, FHL EMG activity was also detected. Further studies (Masood et al., 2014a; 2014b) also measured FHL EMG activity with surface EMG during isometric plantar flexion contractions. In all of the above-mentioned studies the location of the FHL surface electrodes was defined by palpation.

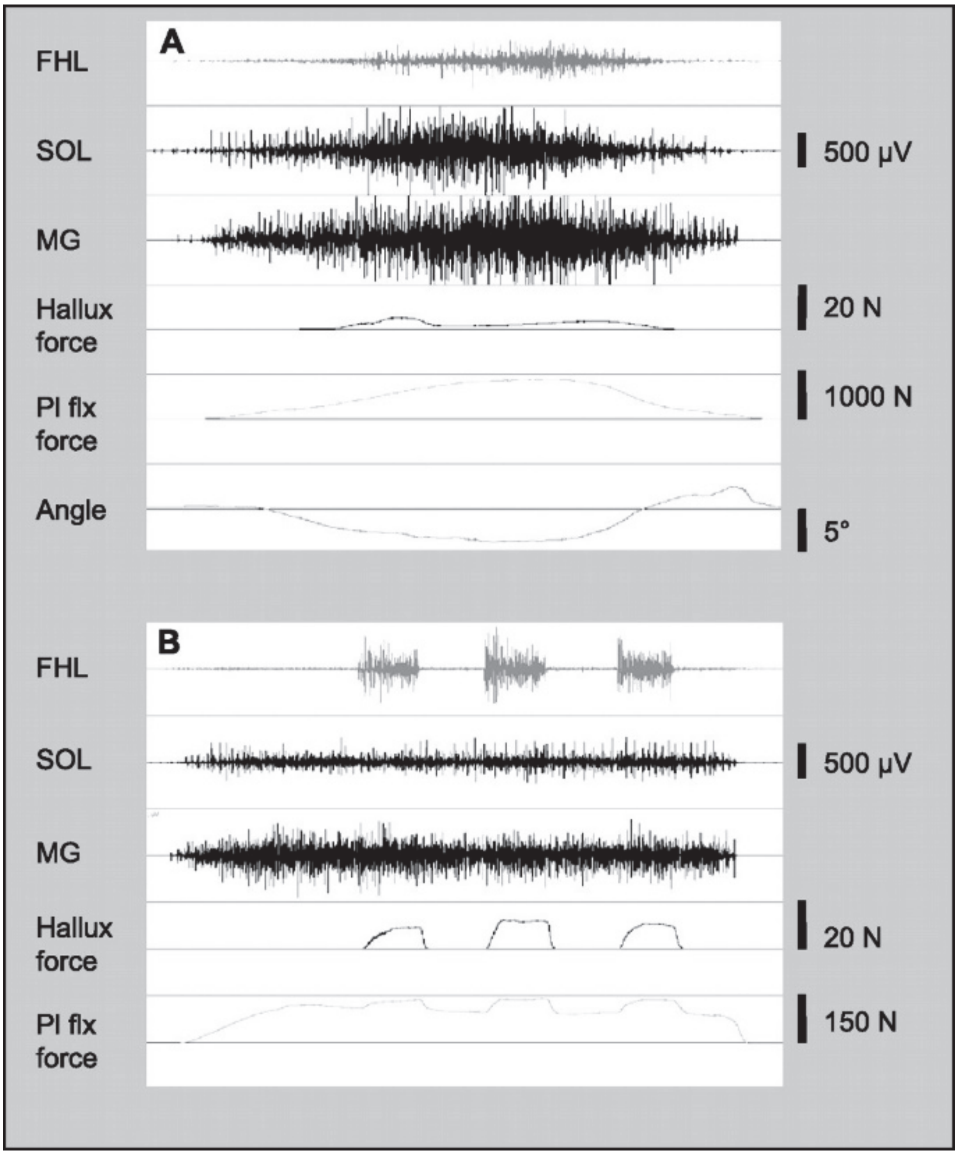

FIGURE 7 (A) Raw data from a maximal voluntary isometric plantarflexion contraction. (B) Selective hallux flexion force and corresponding FHL electromyography activity can be measured during a submaximal plantarflexion contraction. SOL=soleus, MG=medial gastrocnemius, Pl flx=plantarflexion. Figure reproduced with permission from 2010 The American Physiological Society (Bojsen-Moller et al., 2010).

Based on the above-mentioned studies (Bojsen-Moller et al., 2010; Masood et al., 2014a; 2014b), it seems that surface EMG can be used to study FHL muscle activity during isometric ankle plantar flexion contractions and, based on our own pilot testing, also during locomotion. However, this has not been extensively examined. There are also a number of challenges that could affect signal quality and validity when using surface EMG for this muscle, and this issue has not been addressed in the literature. For example, FHL is surrounded by several other muscles and has a relatively small area that can be recorded with surface EMG, so there is a potential risk of detecting cross-talk from other muscles. Clearly, there is a need to examine the validity of the surface EMG method for this muscle, for example, by comparing the signals with those obtained from intramuscular electrodes implanted within the FHL muscle.

As an alternative to surface EMG, intramuscular EMG is an invasive method that is typically used to examine the EMG activity of small (Andersson et al., 1997; Sutherland, 2001) or deep muscles (Andersson et al., 1997; Onishi et al., 2000; Õunpuu et al., 1997). Intramuscular electrode insertion is a time-consuming process that requires special skills (Õunpuu et al., 1997) and experienced or specialized personnel (e.g. a radiologist). To maximise the safety of the electrode insertion procedure, it is also advantageous to use real-time, high-resolution, Doppler ultrasonography. Intramuscular EMG electrodes have a small pick-up area that enables them to selectively detect motor unit activity in static and dynamic contractions. This method helps to decrease the effects of cross-talk and therefore reduces the risk of misinterpretation of the recorded signals (De Luca, 1997; De Luca and Merletti, 1988; Onishi et al., 2000; Perry et al., 1981). Furthermore, the inserted intramuscular wires follow the muscle movement during the contractions (Chapman et al., 2010; Hodges and Gandevia, 2000). Although intramuscular EMG may be painful for some individuals, it seems that the presence of intramuscular EMG electrodes does not affect human gait patterns (Winchester et al., 1996).

Good agreement between surface and intramuscular EMG activity is considered to be a sign of minimised cross-talk in the surface EMG signal. However, the agreement between these methods has not been comprehensively examined in FHL or other lower leg muscles in walking, especially at different speeds; this was one of the aims of this thesis, as detailed in the next section.

3 PURPOSE OF THE STUDY

The aim of this thesis was to provide a comprehensive overview of FHL function at different walking speeds and in different footwear.

Previous studies examining FHL function have shown that FHL is a multi-functional muscle (Ferris et al., 1995; Goldmann et al., 2013; Hintermann et al., 1994; Hofmann et al., 2013; Jacob, 2001; Kirane et al., 2008; Klein et al., 1996; Thordarson et al., 1995). However, all of these studies used cadavers or indirect methods to estimate FHL function. Thus, studies using direct *in-vivo* methods are needed in order to understand FHL function during real-life movements.

In everyday life, we walk at different speeds and in different footwear types. These variables may considerably change the relative contribution of ankle plantar flexor muscles to locomotion. Although most people walk at a range of different speeds, previous studies mainly focused on preferred speed walking (Sacco et al., 2010). Thus, studies examining speed-related changes in EMG activity of triceps surae and deep ankle plantar flexor muscles, such as FHL, are lacking.

Therefore, the aim of this thesis was to investigate FHL muscle function *in vivo* during isometric plantarflexion contractions and during walking at different speeds and in different footwear in healthy individuals. We further investigated the validity of surface EMG activity measurement of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles at different walking speeds.

The specific aims of the current thesis were:

1) To examine FHL surface EMG activity and the resulting force under the hallux in sustained isometric plantarflexion contractions at different torque levels and in the push-off phase of walking at different speeds to determine differences in the use of FHL between an isolated and a functional task, as well as the effect of increasing intensity of plantarflexion and locomotion (I).
2) To investigate FHL fascicle length changes with ultrasonography at different walking speeds to study FHL mechanical behaviour during locomotion *in vivo* (II).

3) To examine simultaneously recorded surface and intramuscular EMG activity of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles at different walking speeds to determine whether EMG signals obtained with the two methods show differences in amplitude at any time point throughout the stance phase of walking (III).
4) To study intramuscular EMG activity of FHL and triceps surae muscles in shod, barefoot and flip-flop walking to examine the effect of footwear on plantar flexor muscle function (IV).

4 MATERIALS AND METHODS

4.1 Study design

A cross-sectional study design was used in all studies. Studies I and II consisted of a single session, while studies III and IV consisted of two sessions: familiarization and testing. In the familiarization session (1-3 days before the testing session) the locations of surface EMG electrodes were determined and marked with permanent pen. In all studies, healthy adults were recruited as participants, and the right leg was always tested.

In all studies, participants walked overground at a range of speeds in their own sports shoes, and in study IV, different footwear conditions were also tested. In study I, four walking speeds were examined: preferred speed, 30% slower and 30% faster than preferred speed, and maximum walking speed. They also performed maximal and submaximal isometric plantar flexion contractions at different torque levels in a dynamometer. During all tasks, surface electromyography activity of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles and the force under the hallux were recorded. In study II, participants walked at three speeds (preferred, 30% faster and 30% slower than preferred speed) while fascicle behavior and surface EMG activity of FHL were recorded. In study III, the same four speeds were tested as in study I while surface and intramuscular EMG activity of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles were simultaneously recorded. In study IV, participants walked overground in their own sports shoes, barefoot and in standardized flip-flops at preferred speed, while intramuscular EMG activity of FHL, soleus, medial and lateral gastrocnemii muscles was recorded. Barefoot and flip-flop conditions were also performed at the same speed as the preferred shod walking condition (hereafter referred to as matched).

4.2 Participants

In total, 31 healthy participants (27 males and 4 females) volunteered to participate. In all studies, healthy male and female participants aged 20-48 years were recruited. The same participants took part in both study III and study IV. All participants were habitually shod walkers without any history of neuromuscular disorders, and without current or recent (< 6 months) leg or foot injuries. Participants with self-reported flatfoot were excluded in all studies (Angin et al., 2014). Participants were recruited among students and staff of the University of Jyväskylä and The Swedish School of Sport and Health Sciences via public advertisement, personal contact or e-mail.

In study I, eleven males were studied (age: 24.7±3.7 years; height: 180.6±6.6 cm; body mass: 79.2±9.1 kg). In study II, twenty males were recruited, however ultrasound data from only ten individuals (age: 24.7±3.5 years; height: 177.7±3.9 cm; body mass: 71.9±4.9 kg) were included in the paper due to the quality of the recorded ultrasound videos. In studies III and IV, ten individuals (six males and four females; age: 29.6±7.4 years, height: 174.0 ± 12.5 cm, body mass: 70.6 ± 12.7 kg, body mass index: 23.1 ± 1.7 m^{-2} kg) were examined. Convenience sampling was applied in all studies.

4.3 Ethics

Participants were informed about study procedures and gave their written consent prior to participation. Studies were approved by the ethics committee of the University of Jyväskylä (Studies I and II) or the Stockholm regional ethics committee (Studies III and IV; Approval No.: 2017/261-31/4). Participant anonymity and data privacy were maintained at all times. Each study was performed in agreement with the Declaration of Helsinki.

4.4 Data collection and analysis

4.4.1 Walking conditions

In studies I and II, the measurement area was 10 m long, while in studies III and IV it was 7 m long. In each study, custom-made photocells were placed at the beginning and end of the assigned measurement area to determine walking speeds. All overground walking trials started and ended 2-3 meters before and after the photocells, respectively, so that participants could maintain a steady speed within the measurement area. Participants were asked to accelerate and decelerate before and after the photocells, respectively.

In each study, participants first performed 5 walking trials at preferred speed in their own sports shoes. The slowest and fastest walking trials were excluded and the remaining three trials were averaged to define preferred walking speed (preferred shod walking speed in study IV). Walking tasks were then performed at different speeds (studies I, II and III) or in different footwear (study IV). In addition to preferred speed walking, walking tasks were performed in sports shoes at the following speeds: 30% slower and 30% faster than preferred speed walking (studies I, II and III), as well as maximum speed walking (studies I and III). In study IV, in addition to preferred shod walking, walking tasks were performed barefoot (in socks) and in standardized flip-flops at preferred speed and at the same speed as preferred shod walking (matched condition). The walking condition performed at preferred speed in sports shoes was always performed first in each study, while subsequent walking conditions were performed in a random order. In each condition, three successful trials within ± 5% of the target speed were performed.

4.4.2 EMG activity

4.4.2.1 Surface EMG

Surface EMG activity of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles was measured in studies I and III, while surface EMG activity of FHL was measured in study II. In all studies, inter-electrode distance for FHL was decreased to 16 mm to minimize cross-talk (Bojsen-Moller et al., 2010; Masood et al., 2014b), and was always 22 mm for other muscles.

After shaving, abrading and cleaning the skin with alcohol, silver-silver chloride surface electrodes (Ambu BlueSensor N, Ambu A/S, Ballerup, Denmark) were put on each muscle in bipolar configuration. Behind the medial malleolus, the FHL muscle belly becomes superficial; FHL surface electrodes were placed in this location. The placement of these electrodes was defined with ultrasonography as follows. On the medial side of the leg, the soleus insertion and FHL muscle-tendon junction were located and marked with permanent pen. Electrodes were placed between these markings, posterior to the medial malleolus. Medial and lateral gastrocnemii and tibialis anterior surface electrodes were located following SENIAM recommendations (Hermens et al., 2000), although positioning was sometimes slightly modified according to individual-specific muscle morphologies. Based on SENIAM recommendations (Hermens et al., 2000), soleus electrodes should be placed two-thirds of the way between the medial femur condyle and the medial malleolus on the medial side of the shank. However, this choice of positioning has been shown to be prone to cross-talk during walking (Bogey et al., 2000). Thus, in the studies in this thesis, soleus electrodes were placed laterally at the same proximal-distal location. In study III, the distance between the soleus insertion and distal FHL muscle tendon junction was also measured to examine if the size of the space where FHL surface electrodes could be placed had any effects on FHL surface EMG activity compared to intramuscular EMG.

EMG activity was recorded with a telemetric EMG system (Noraxon Inc., Scottsdale, AZ, United States) at a sampling frequency of 1500 (studies I and II) or 3000 Hz (study III). Analogue signals were sent wirelessly to an A/D converter (Cambridge Electronic Design, Cambridge, United Kingdom) that was connected to a personal computer. Signals were recorded and visualized in Spike2 software. In studies III and IV, a single surface reference electrode (silver–silver chloride Ambu BlueSensor N, Ambu A/S, Ballerup, Denmark) was also placed on the medial aspect of the tibia bone. In studies III and IV, surface and intramuscular EMG activity of FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles was simultaneously recorded. However, in study IV, surface EMG data were not included in the analysis for any muscle, nor were intramuscular data from tibialis anterior.

In all studies, EMG analyses were performed in Matlab (MathWorks Inc, Natick, MA, USA) and focused on the stance (studies II, III, IV) and push-off (studies I, II) phases of the step cycles. Raw EMG signals were band-pass filtered between 20 and 450 Hz (studies I and II) or between 20 and 500 Hz (studies III and IV) using a fourth-order zero lag Butterworth filter. Signals were then rectified, and the following analyses were performed.

In study I, root mean square (RMS) EMG activity was calculated for each muscle in the push-off phase. RMS values were calculated for each step and averaged within each task and for each individual. EMG activity was normalized to the maximal voluntary isometric contraction where the highest torque was detected as follows. After filtering, RMS activity was calculated from a stable 1-s plateau around the peak maximal voluntary isometric contraction torque. FHL EMG activity was also determined relative to the activity levels of soleus, medial and lateral gastrocnemii muscles, and to the average activity of the triceps surae. In this study, RMS was also calculated for submaximal isometric contractions from a 1-s stable plateau, and used to characterize the surface EMG activity of each muscle at the different torque levels.

In study II, RMS values were calculated for both the stance and push-off phases for all steps and were averaged within each participant and task. To decrease inter-individual variability, EMG signals were normalized to the peak activity of preferred speed walking using the peak dynamic method (Burden, 2010; Cronin et al., 2015). In this study, time to peak activity relative to stance phase duration was also calculated.

In studies III and IV, EMG signals were analysed in the stance phase. Signals were smoothed with a 10 Hz zero-lag low-pass filter (4th order Butterworth). Using linear interpolation, signals were then time-normalized (1-101 frames) for all steps, and EMG curves were averaged within each condition for each participant and muscle. The averaged signals were then normalised to the peak activity of preferred shod walking using the peak dynamic method (Burden, 2010; Cronin et al., 2015) to decrease inter-individual variability. Statistical Parametric Mapping analysis was performed on these time- and peak-normalized EMG signals. In study IV, time between heel contact and peak activity was also calculated and is hereafter referred to as time to peak activity. Inter-individual coefficients of variation (%) for time to peak activity were calculated for each individual, muscle and footwear condition.

4.4.2.2 Intramuscular EMG

Data collection for studies III and IV was conducted at the same time. In both cases intramuscular EMG activity was measured from FHL, soleus, medial and lateral gastrocnemii and tibialis anterior muscles. However, in study IV, tibialis anterior data were not included in the analysis. Inter-electrode distance was ≈5 mm for all muscles.

Intramuscular wire electrode insertion was conducted by an experienced radiologist under real-time, high-resolution, Doppler and B-mode ultrasonography (Logiq E9, GE, United States) guidance. After cleaning the skin with alcohol, Teflon-coated seven-stranded silver hook-wire electrodes (diameter 0.25 mm, stripped length of 2.0 mm forming the recording surface; previously sterilized in an autoclave) were inserted, two in each muscle with hypodermic needles (diameter 0.8 mm; carefully withdrawn after insertion) in bipolar configuration. Since surface EMG electrodes were also placed on each muscle (see above), the recording ending of every intramuscular electrode pair for a given muscle was inserted underneath the surface electrodes, except for FHL (Figure 8). For FHL, electrodes were placed 5-10 cm proximal to the surface electrodes on the lateral side of the lower leg depending on muscle thickness and vascularisation. In studies III and IV, intramuscular EMG signals were collected and analysed in the same way as surface EMG signals in study III.

In study IV, EMG onset and offset were defined for each muscle and step in the flip-flop walking condition. These values were first determined with Teager-Kaiser Energy Operator (Solnik et al., 2010) by amplifying the signal-to-noise ratio of the filtered EMG data, then using approximated generalized likelihood ratio (Staude and Wolf, 1999). The resulting data were used to estimate the timings of heel contact and toe-off (described in detail below), since it was not possible to use Pedar insoles for this purpose in the flip-flop condition.

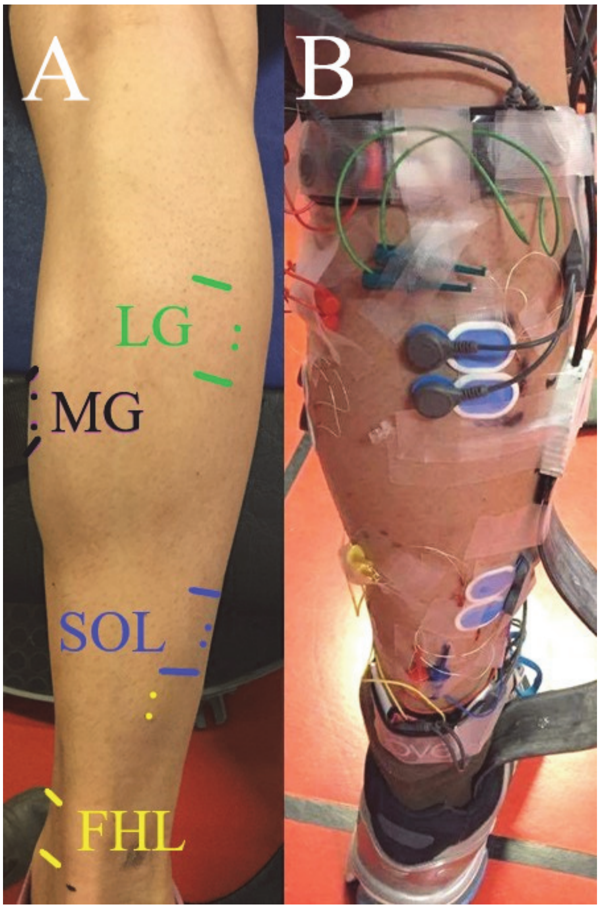

FIGURE 8 Surface and intramuscular electromyography electrode locations for plantar flexor muscles. (A) Electrodes were positioned in FHL, soleus (SOL), medial (MG) and lateral gastrocnemius (LG) muscles of the right leg. Surface electromyography electrodes were located between the horizontal lines, while intramuscular electrodes were inserted where the dots are shown. (B) Surface and intramuscular electrodes are attached. Data were also collected from tibialis anterior muscle, but it is not visible in this figure. Reused from study III.

4.4.3 Force

In studies I and II, overground walking trials were performed over two custom-made force platforms (10.0 m long, 0.5 m wide; University of Jyväskylä, Finland) positioned parallel to each other. Force platforms were used to record 3-dimensional ground reaction forces for each step, and to define the phases of the different step cycles. Data were sampled at 1000 Hz.

In studies III and IV, two 0.6 m x 0.4 m force platforms (Kistler type 9281 EA, Kistler AG, Winterthur, Switzerland) were placed in series in the middle of the measurement area. Force platforms were used to collect 3-dimensional ground reaction force data from one stride per trial (three strides per condition). Trials were repeated if the participant's right leg did not hit either of the force platforms. Data were sampled at 3000 Hz via Qualysis Track Manager software (Qualisys, AB, Sweden).

In flip-flop walking (study IV), it was not possible to use Pedar insoles to define the timings of heel contact and toe-off for steps that were performed within the measurement area but did not hit either of the force platforms. Thus, these timings were estimated based on EMG onset/offset and ground reaction forces of the steps (n=3) that hit the force plates. For each of the steps (n=3) that were performed on the force plates, EMG onset was determined relative to heel contact while EMG offset was determined relative to toe-off. This was performed for all muscles. The muscle that showed the lowest variability in EMG onset or offset for these three steps was chosen to be a reference to estimate the timings of heel contact and toe-off for the steps that were performed within the measurement area but did not hit either of the force platforms. The mean EMG onset/offset of the three steps was then used for this estimation.

4.4.4 Torque

In study I, isometric ankle plantarflexion tasks were performed in a custom-made ankle dynamometer (University of Jyväskylä, Finland) while torque was recorded at 1000 Hz. During these contractions, hip, knee, ankle and first metatarsophalangeal joints were fixed at 120°, 0°, 90° and 0°, respectively. The foot and thigh were strapped firmly to the dynamometer. After some familiarization contractions, participants were asked to maintain different isometric plantarflexion torque levels (20, 40, 60, 80, 100 and 120 Nm, performed in a random order) for two seconds with visual feedback. After the submaximal contractions, participants performed several sets of maximal contractions (3 times each, 1.5-2.0 min rest between contractions) which were used to normalise EMG data: maximal ankle dorsiflexion, maximal ankle plantarflexion, and hallux flexion superimposed on maximal ankle plantarflexion.

4.4.5 Plantar pressure

During walking (studies I, III and IV) and submaximal isometric tasks (study I), plantar pressure was recorded with a Pedar-X 99-sensor in-shoe dynamic pressure measuring system (Novel Inc., Munich, Germany) sampling at 100 Hz. An insole was placed in the right shoe (studies I, III and IV) or in the right sock during barefoot walking (study IV). Data were collected via Bluetooth with Pedar's own software. To synchronize data acquisition, a trigger signal was sent from Pedar software to Spike software (studies I, III and IV) and to Qualysis Track Manager software (studies III and IV) at the start of each recording. Before each task, an assistant pressed down the hallux to help map the pressure area under the hallux (study I), and to ensure that the insole did not move in the shoe between trials and tasks (studies I, III and IV). In studies III and IV, plantar pressure data were further used to define the timings of heel contact and toe-off for all steps performed within the measurement area.

When the hallux was pressed down by an assistant to define the hallux area, sensors that were at the edge of this area were removed from the analysis due to possible interference from other toes or foot regions. During walking, forces were calculated in the push-off phase by dividing the pressure by the pressed hallux area. Peak force was determined under the hallux and the foot for all steps and averaged for every trial and for each individual. Impulse was calculated as the integral of the force-time curve for sensors under the hallux and under the whole foot. Force and impulse values collected during walking were normalized to body mass. During isometric plantarflexion contractions, the average force was calculated over the same time interval as the RMS EMG calculation. Hallux contributions to peak or average force and impulse were calculated by dividing hallux values by the values for the whole plantar surface (referred to as relative hallux force/impulse).

In studies III and IV, plantar pressure data were used to define the timings of heel contact and toe-off for all steps over the measurement area. All analyses were performed in Matlab as follows. First, vertical force data (sum of forces from all insole sensors) were extrapolated to 3000 Hz in order to coordinate with the ground reaction force data measured with the force plates. Then, a 10.0 N vertical ground reaction force threshold was used to define heel contact and toe-off for the steps that hit either of the force platforms (Osis et al., 2016). This was used to help define the thresholds for vertical forces measured with Pedar insoles and to minimise errors (e.g. mismatch between foot and insole size). Thresholds were defined for each participant and task. EMG activity was analysed between heel contact and toe-off for each step.

In study III, the stance phase was divided into four sub-phases: early stance (0–16.5%), mid stance (16.5–50%), late stance (50–83%) and pre-swing (83–100%) based on previous literature (Neptune et al., 2001).

4.4.6 Ultrasonography

To estimate FHL fascicle length changes in walking, a personal computer-based portable ultrasound system (EchoBlaster 128; Telemed, Vilnius, Lithuania) was used with a linear probe (96-element, B-mode; 7 MHz, transducer field width: 60.0 mm). Sampling frequency was 80 Hz. The probe was placed over the posterior site of the right lower leg, firmly fixed over the mid-belly and aligned with FHL fascicles. To minimize probe rotation, a flat-shaped probe was used. The ultrasound probe was connected to a portable ultrasound unit (5.0 kg), which was carried by an assistant who walked next to the force plate, around one meter behind the participants during the walking tasks. Data collection was synchronized with force and EMG signals using a digital output signal that was sent from the ultrasound system to Spike2 software.

To determine FHL fascicle length changes during the walking tasks, a previously validated semi-automated fascicle tracking algorithm was used (Cronin et al., 2011; Farris and Lichtwark, 2016; Gillett et al., 2013). A region of interest was defined between the deep and middle aponeuroses, since this part of FHL is thicker than the more superficial part (Mickle et al., 2013). Due to longer fascicles in this region, analysis error may also be relatively small. To estimate fascicle length, the initial and end points of a straight line were positioned on the first ultrasound frame of each video from the deep to the middle aponeurosis, parallel to the lines of collagenous tissue. The remaining frames were then tracked automatically. Fascicle length data were smoothed (20-Hz fourth-order Butterworth low-pass filter) in Matlab. In total, 244 steps were analysed (94, 70 and 80 steps for slow, preferred and fast walking speeds, respectively). Fascicle analysis focused on the stance phase since FHL is mainly active in this phase and not in swing (Perry, 1992; Zelik et al., 2015). The push-off phase was also analysed separately, since ankle plantar flexor muscles have a significant role in this phase in propelling the body forward (Neptune et al., 2001). The fascicle length at heel strike was defined as initial fascicle length. In both phases (i.e. stance and push-off), minimum and maximum FHL fascicle length were calculated separately as well as the range of fascicle length change, i.e. the difference between minimum and maximum fascicle length. Fascicle length was also calculated at toe-off and at the time of peak FHL EMG activity. Mean fascicle velocity was calculated by averaging the first derivation of fascicle length change within each phase.

4.4.7 Statistical analyses

4.4.7.1 SPSS analyses

All statistical analyses in studies I and II and some parts of study IV were performed using SPSS software (IBM, New York, NY, USA). For each test, significance level was set at $P<0.05$. First, Shapiro-Wilk's W test was used to determine the normality of data distribution. Other procedures are outlined for each study below.

In study I, homogeneity of variance was defined with Levene's test. To correlate FHL muscle activity with the force measured under the hallux in isometric and walking trials, Spearman's rank was used. When normal data distribution and homogeneity of variances were confirmed, one-way ANOVA and Tukey HSD post-hoc tests were used to define the differences between EMG, force or impulse data that were measured at different walking speeds and at different submaximal plantar flexion torque levels. One participant did not perform trials at maximal walking speed, so differences between the maximal walking speed task and other walking tasks were determined with Gabriel's procedure. In all other cases, non-parametric Kruskal-Wallis ANOVA and Games-Howell post hoc tests were used to detect differences.

In study II, to test the differences between variables across walking speeds, one-way repeated-measures ANOVA was used. If the assumption of sphericity (determined by Mauchly's test) was violated, Greenhouse-Geisser adjustment was used. In cases where significant main effects were detected, Bonferroni post hoc tests were used to determine the location of the differences.

In study IV, two different statistical analyses were performed. First, for each of the barefoot and flip-flop conditions, statistical differences between preferred and matched conditions were tested in terms of stance phase duration, push-off phase duration, and walking speed with paired samples t-tests. As there were no differences ($P>0.05$) between preferred and matched conditions in any of the examined variables, later analysis focused on only those trials that were performed at preferred speed (since preferred speed seems to be highly repeatable (Boonstra et al., 1993; Kadaba et al., 1989; Stolze et al., 1998; Wirth et al., 2011)). Secondly, time to peak activity was tested. To test for statistical differences between the different footwear conditions for each muscle, one-way repeated measures ANOVA was used.

4.4.7.2 Statistical Parametric Mapping

All statistical analyses in study III and some parts of study IV were performed using Statistical Parametric Mapping (SPM; Friston, 2007). SPM analyses were performed in Matlab using the open-source spm1d code (v.M0.11). Data were tested at each point of the time-normalised stance phase.

In study III, to compare surface and intramuscular EMG amplitudes, SPM two-tailed paired t-tests were used. This was performed for all muscles at all walking speeds. In study IV, to compare intramuscular EMG amplitudes of each muscle between the different footwear conditions, SPM one-way repeated measures ANOVA was used. Statistical testing was performed as follows. First, scalar output statistic variable (SPM{t} and SPM{F} for studies III and IV, respectively) was calculated for each time point. This is a scalar trajectory variable that shows the magnitude of the differences. This variable was calculated to form a Statistical Parametric Map. A critical threshold was then calculated (set to 5%) to test the null hypothesis. If any values of the SPM trajectory variable (SPM{t} or SPM{F}) exceeded the critical threshold, EMG time-series were considered to be significantly different. In these cases, cluster-specific P-values were also calculated. In study III, the magnitude of the differences was calculated and expressed as mean difference ± 95% confidence intervals (CI).

5 RESULTS

This chapter provides a summary of the results of the studies in this thesis. For further details, please see the original papers (I-IV). Table 2 presents the number of analysed steps and their characteristics for each study.

5.1 FHL surface EMG activity and hallux force in isometric plantar flexion and at different walking speeds

5.1.1 Force

In isometric ankle plantarflexion tasks, the average force under the hallux generally increased ($P<0.001$) with increasing torque levels, however, there was no difference in relative hallux force.

In walking, the absolute peak force measured under the hallux increased with increasing walking speed ($P=0.005$), and this increase was higher under the hallux than in the other foot regions ($P=0.018$). The impulse under the hallux did not change with speed, but the relative hallux impulse increased with increasing walking speed ($P=0.005$). Hallux force increased linearly with increasing surface FHL activity during both the isometric ($P<0.001$) and walking tasks ($P=0.003$).

5.1.2 EMG activity

FHL EMG activity increased with increasing plantarflexion level and walking speed ($P=0.024$ and $P<0.001$, respectively). During the sustained isometric plantarflexion contractions, FHL/soleus surface EMG activity ratio was higher at 20.0 Nm ($P<0.001$) compared to other torque levels. Other surface EMG activity ratios (i.e. FHL/medial gastrocnemius, FHL/lateral gastrocnemius and FHL/triceps surae) did not change with the increasing torque levels. None of the activity ratios changed with increasing walking speed.

TABLE 2 Number of analysed steps and their characteristics for each study.

		Shod				Barefoot		Flip-flops	
	Study	Slow	Preferred	Fast	Maximum	Pref.	Matched	Pref.	Matched
Included steps (n)	I	16 ± 2	16 ± 6	12 ± 7	15 ± 6	-	-	-	-
	II	9 ± 4	7 ± 5	8 ± 2	-	-	-	-	-
	III	14 ± 3	12 ± 3	11 ± 2	9 ± 1	-	-	-	-
	IV	-	12 ± 3	-	-	13 ± 4	12 ± 2	12 ± 1	12 ± 0
Speed (m/s)	I	1.05 ± 0.08	1.36 ± 0.10	1.93 ± 0.16	2.51 ± 0.31	-	-	-	-
	II	1.06 ± 0.08	1.37 ± 0.10	1.97 ± 0.19	-	-	-	-	-
	III	1.01 ± 0.13	1.43 ± 0.19	1.84 ± 0.23	2.20 ± 0.38	-	-	-	-
	IV	-	1.43 ± 0.19	-	-	1.38 ± 0.22	1.43 ± 0.20	1.41 ± 0.21	1.43 ± 0.20
Stance phase duration (s)	I	-	-	-	-	-	-	-	-
	II	0.73 ± 0.04	0.63 ± 0.03	0.52 ± 0.04	-	-	-	-	-
	III	0.81 ± 0.06	0.67 ± 0.06	0.58 ± 0.04	0.50 ± 0.04	-	-	-	-
	IV	-	0.67 ± 0.06	-	-	0.63 ± 0.08	0.61 ± 0.06	0.68 ± 0.07	0.67 ± 0.05
Push-off phase duration (s)	I	0.35 ± 0.04	0.31 ± 0.04	0.25 ± 0.03	0.22 ± 0.03	-	-	-	-
	II	0.30 ± 0.03	0.26 ± 0.02	0.22 ± 0.03	-	-	-	-	-
	III	0.42 ± 0.05	0.36 ± 0.04	0.30 ± 0.04	0.23 ± 0.05	-	-	-	-
	IV	-	0.36 ± 0.04	-	-	0.36 ± 0.05	0.34 ± 0.04	0.37 ± 0.07	0.37 ± 0.05

Included steps are expressed as median ± interquartile range. Other values are mean ± standard deviation.

5.2 Fascicle behaviour of FHL at different walking speeds

FHL fascicle length measured at heel strike (56.7±5.7, 56.0±6.1 and 56.5±7.7 mm in slow, preferred and fast walking, respectively), at toe off (56.2±5.7, 55.2±6.9 and 55.6±8.1 mm) and at the time of peak FHL surface EMG activity (57.1±6.1, 56.1±6.0 and 56.2±7.8 mm) did not change with speed. Time to peak activity of FHL (73±3%, 73±3% and 70±3%) also did not change with speed. Figure 9 shows a typical example of raw fascicle length changes at the different walking speeds throughout the step cycle.

5.2.1 Stance phase

Range of fascicle length change (3.5±1.3, 3.9±1.4 and 4.5±1.8 mm in slow, preferred and fast walking, respectively), minimum length (54.9±5.1, 54.0±5.9 and 53.5±6.9 mm), maximum length (58.4±6.1, 58.0±6.4 and 58.0±7.7 mm) and mean fascicle velocity (-0.2±3.2, -1.2±4.9 and -4.4±6.6 mm/s) did not change with speed. However, FHL RMS EMG activity increased with increasing walking speed (mean values: 31±6%, 44±8% and 77±22%) ($P<0.02$ in all cases).

5.2.2 Push-off phase

Range of fascicle length change (1.9±1.2, 2.3±0.9 and 2.9±1.4 mm in slow, preferred and fast walking, respectively), minimum length (55.7±5.5, 54.6±5.6 and 53.8±7.0 mm), and maximum length (57.7±6.2, 57.0±6.1 and 56.8±7.7 mm) did not change with speed. However, mean fascicle velocity increased from slow to fast walking (0.3±5.7 and -9.4±11.9 mm/s in slow and fast walking, respectively; $P=0.21$) and FHL RMS EMG activity increased with increasing walking speed (mean values: 41±1%, 60±16% and 105±39%) ($P<0.02$ in all cases).

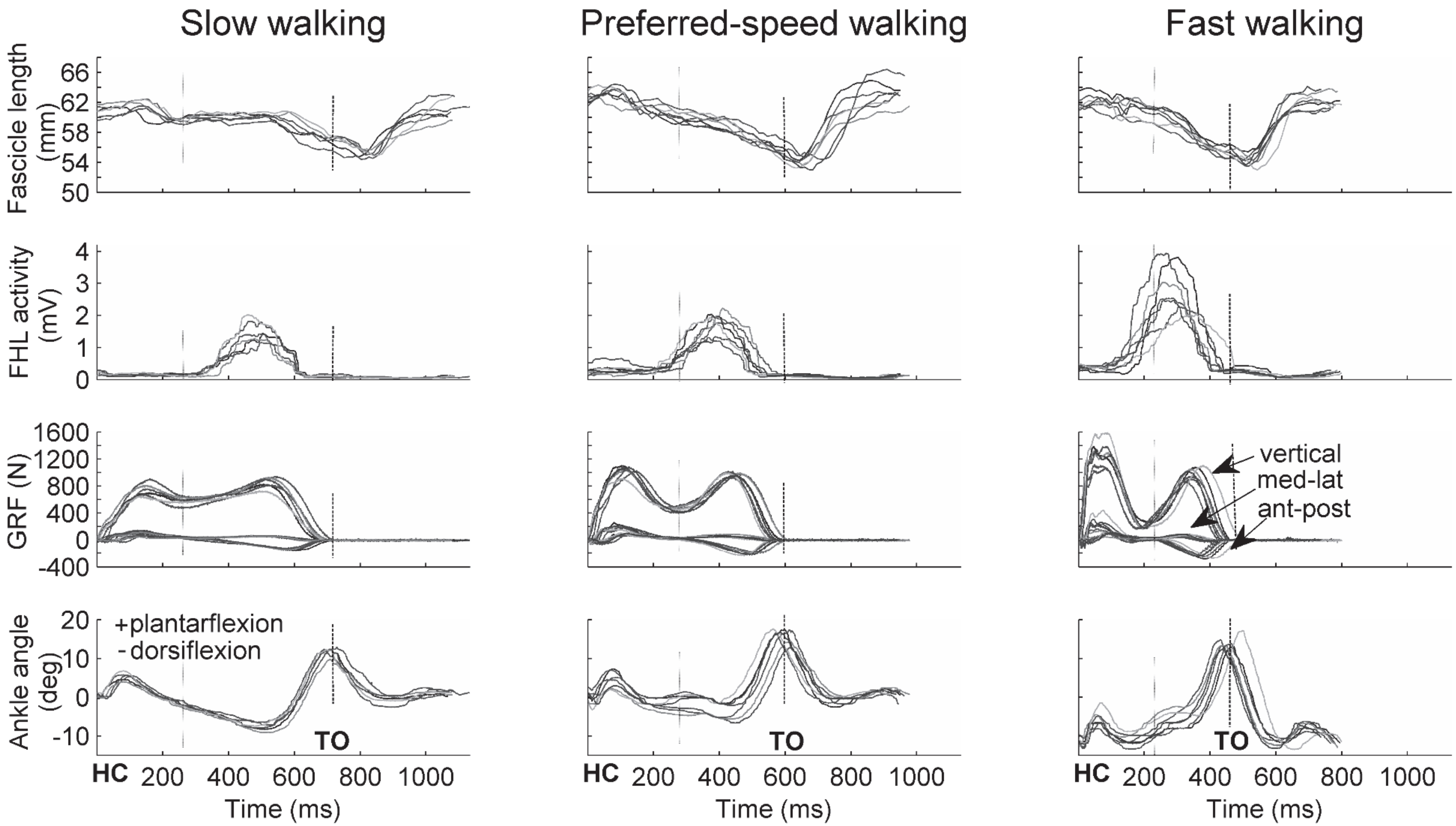

FIGURE 9 Typical example of FHL fascicle and surface EMG behaviour of one participant at different walking speeds. Each curve within each subfigure represents one whole step from heel contact (HC) to the subsequent heel contact. Dotted vertical lines represent toe-off (TO). Dashed vertical lines represent the start of push-off. EMG signals were smoothed (100-ms root-mean-square moving window) for better visualization. Ankle angle was measured with an electro-goniometer. GRF, ground reaction force; med-lat, medial-lateral GRF; ant-post, anterior-posterior GRF. Reused with permission from study II.

5.3 Surface *versus* intramuscular EMG activity of lower leg muscles at different walking speeds

Mean EMG activity group results for each muscle and walking speed are presented in figures 10-13.

When comparing surface and intramuscular EMG activities, differences in FHL in late stance at all walking speeds except fast were observed (slow: 66–74% of stance phase, $P<0.001$, preferred: 65–71%, $P=0.003$, maximum speed walking: 60–67%, $P=0.005$). Subject-specific differences were also seen at all walking speeds in FHL, showing that surface and intramuscular FHL activity patterns are similar for some individuals but very different for others (Figure 14).

In soleus, differences were found in three phases: in early stance at all speeds (slow: 0–7.3%, $P=0.004$, preferred: 0–5.3%, $P=0.008$, fast: 0–1.4%, $P=0.045$, maximum: 0–5.2%, $P=0.02$), in late stance at preferred, fast and maximum speed (preferred: 72.7–73.6%, $P=0.047$, fast: 68.5–84.2%, $P<0.001$, maximum: 63.8–86.6%, $P<0.001$), and in pre-swing at slow, fast and maximum speed (slow: 95.3–98.6%, $P=0.03$, fast: 68.5–84.2% and 95.3–100%, $P<0.001$ and $P=0.016$, respectively, maximum: 63.8–86.6% and 95.2–100%, $P<0.001$ and $P=0.023$, respectively).

In tibialis anterior, differences in three phases were found: in mid-stance at all speeds (slow: 43.7–44.3 and 47.5–67.2%, $P=0.049$ and $P<0.001$, respectively, preferred: 45.9–85.3%, $P<0.001$, fast: 39.4–70.2%, $P<0.001$, maximum: 25.7–30.4 and 35.6–67.2%, $P=0.015$ and $P<0.001$, respectively), in late stance at all speeds (slow: 47.5–67.2 and 78.6–83.3%, $P<0.001$ and $P=0.023$, respectively, preferred: 45.9–85.3%, $P<0.001$, fast: 39.4–70.2%, $P< 0.001$, maximum: 35.6–67.2%, $P<0.001$) and in pre-swing at slow and preferred speed walking (slow: 78.6–83.3%, $P=0.023$, preferred: 45.9–85.3%, $P<0.001$).

In medial gastrocnemius, there were no differences at any speed, or in lateral gastrocnemius at slow and preferred speed. However, differences in lateral gastrocnemius at faster speeds in the late stance and pre-swing phases were observed (fast: 71.8–86.3%, $P<0.001$, maximum speed walking: 82–85%, $P=0.036$).

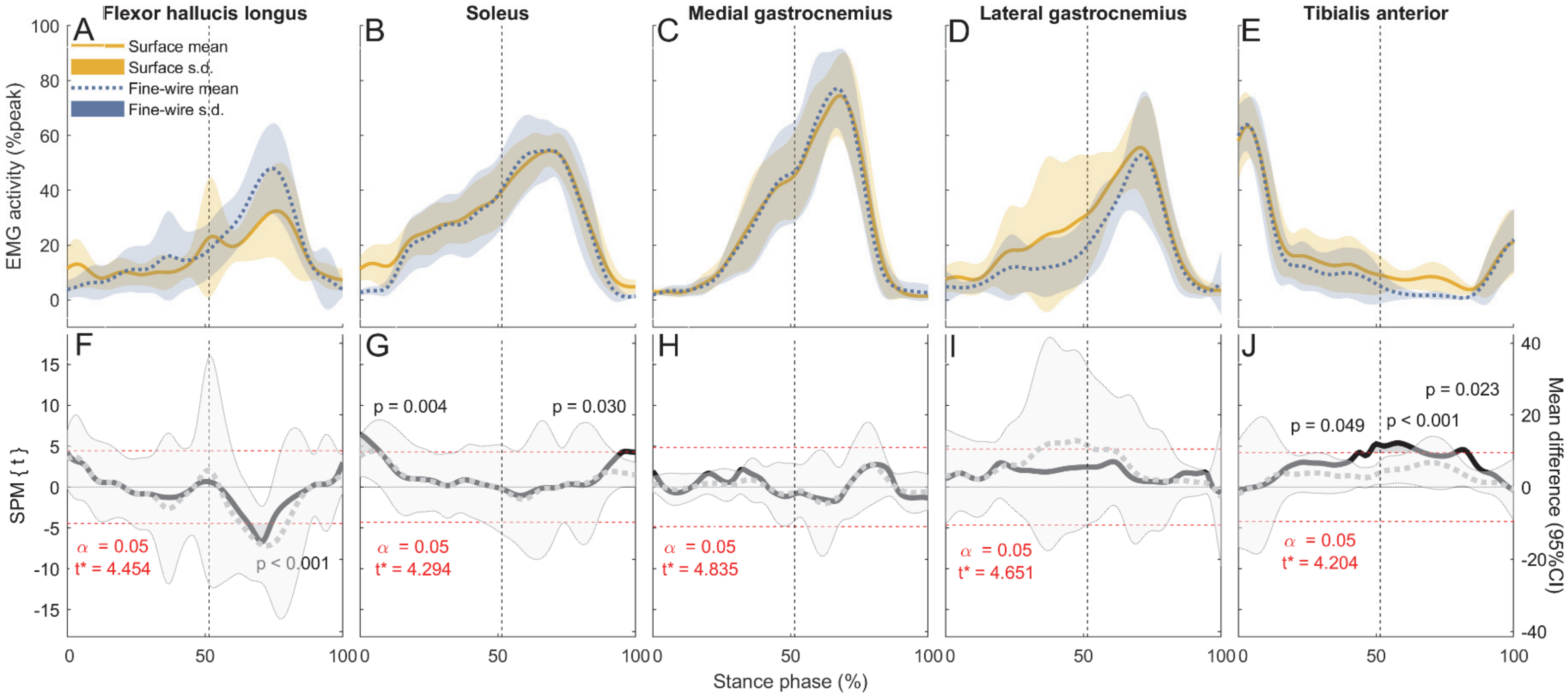

FIGURE 10 Electromyography (EMG) activity of all muscles (mean ± SD) (A-E) and corresponding comparisons of surface and intramuscular EMG signals (F-J; SPM{t}, statistics for two-tailed t-tests represented by solid black lines) throughout the stance phase of **slow walking**. EMG signals were normalized to the peak activity of preferred speed walking (%peak). Mean difference is also presented with 95% confidence intervals (grey dotted lines and shaded areas). Vertical dotted lines show the start of push-off, while dashed red lines show the critical thresholds (t*). The calculated P-values are also presented for each supra-threshold cluster. Reused from study III.

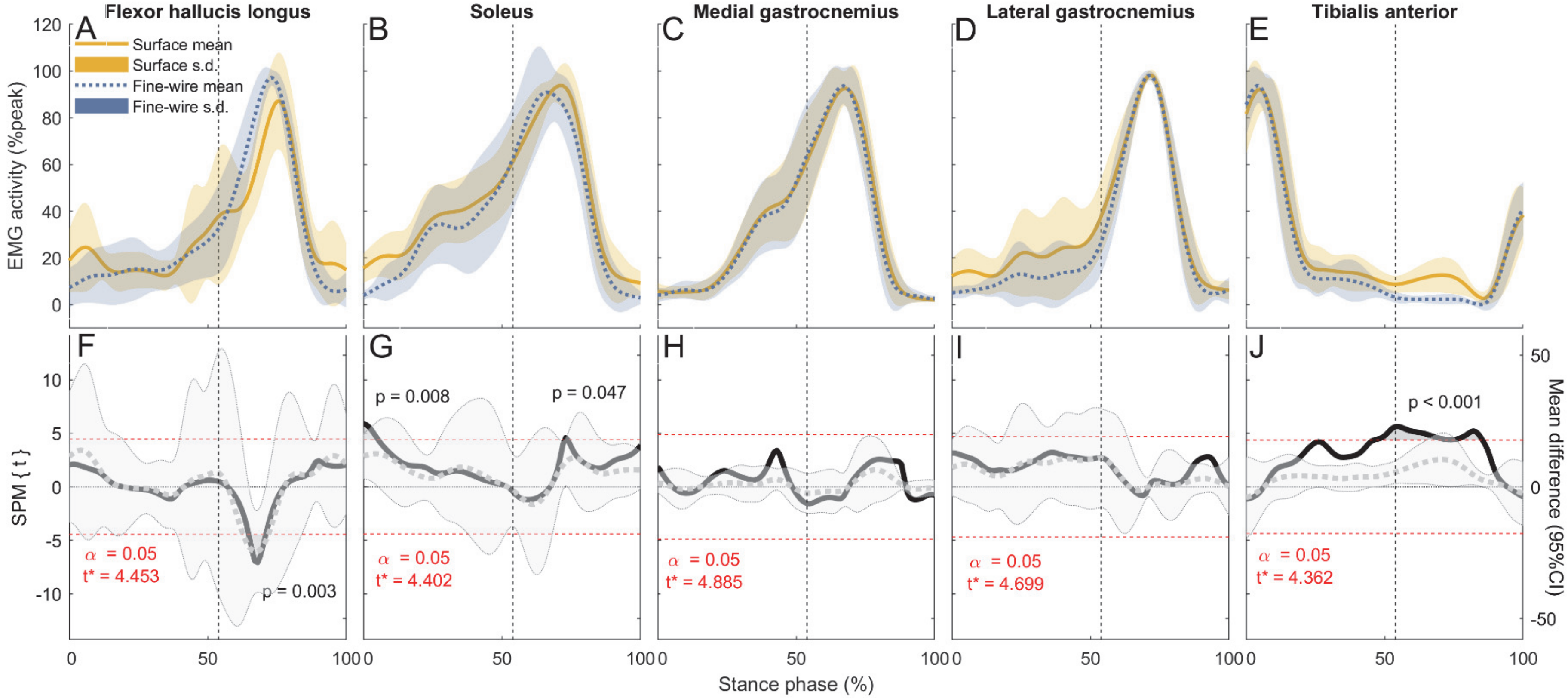

FIGURE 11 Electromyography (EMG) activity of all muscles (mean ± SD) (A-E) and corresponding comparisons of surface and intramuscular EMG signals (F-J; SPM{t}, statistics for two-tailed t-tests represented by solid black lines) throughout the stance phase of **preferred speed walking**. EMG signals were normalized to the peak activity of preferred speed walking (%peak). Mean difference is also presented with 95% confidence intervals (grey dotted lines and shaded areas). Vertical dotted lines show the start of push-off, while dashed red lines show the critical thresholds (t*). The calculated P-values are also presented for each supra-threshold cluster. Reused from study III.

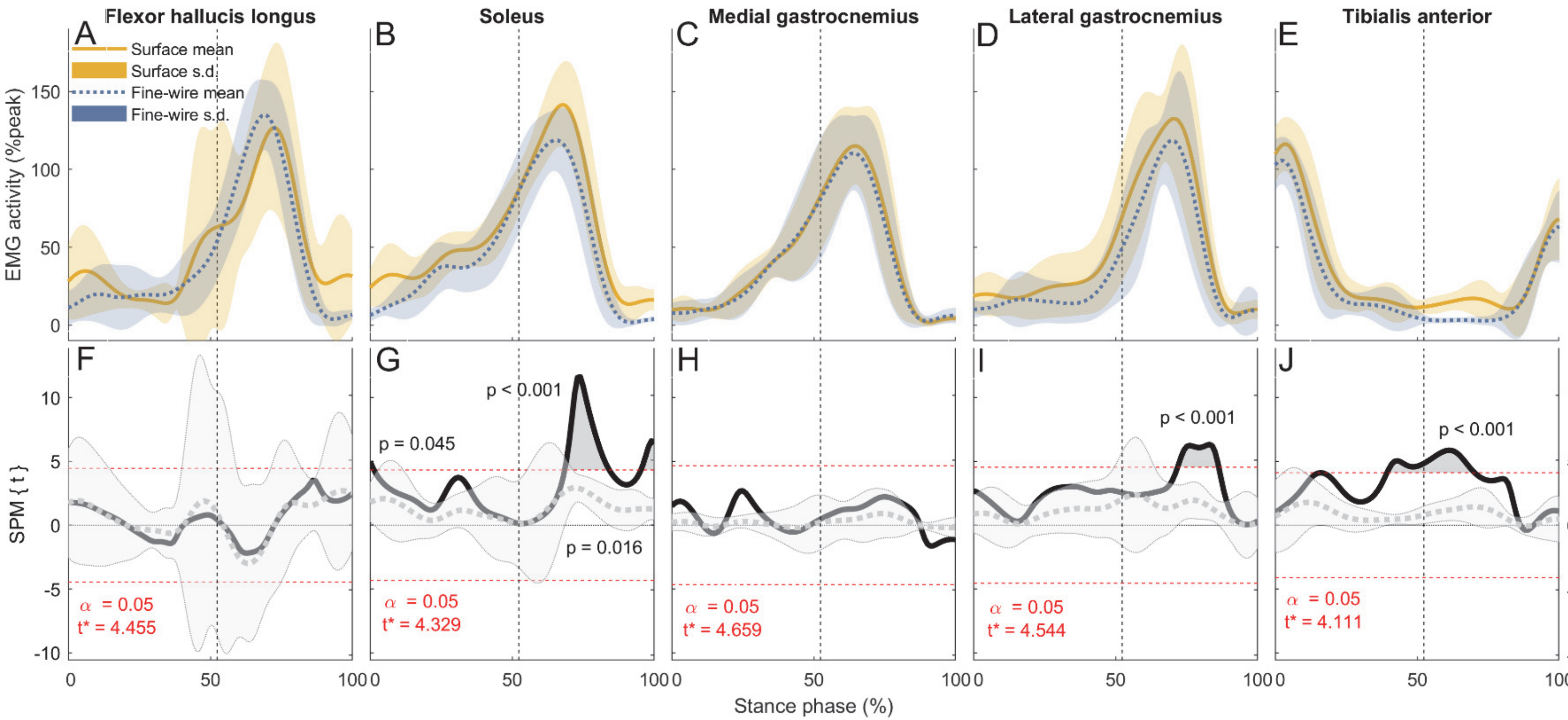

FIGURE 12 Electromyography (EMG) activity of all muscles (mean ± SD) (A-E) and corresponding comparisons of surface and intramuscular EMG signals (F-J; SPM{t}, statistics for two-tailed t-tests represented by solid black lines) throughout the stance phase of **fast walking**. EMG signals were normalized to the peak activity of preferred speed walking (%peak). Mean difference is also presented with 95% confidence intervals (grey dotted lines and shaded areas). Vertical dotted lines show the start of push-off, while dashed red lines show the critical thresholds (t*). The calculated P-values are also presented for each supra-threshold cluster. Reused from study III.

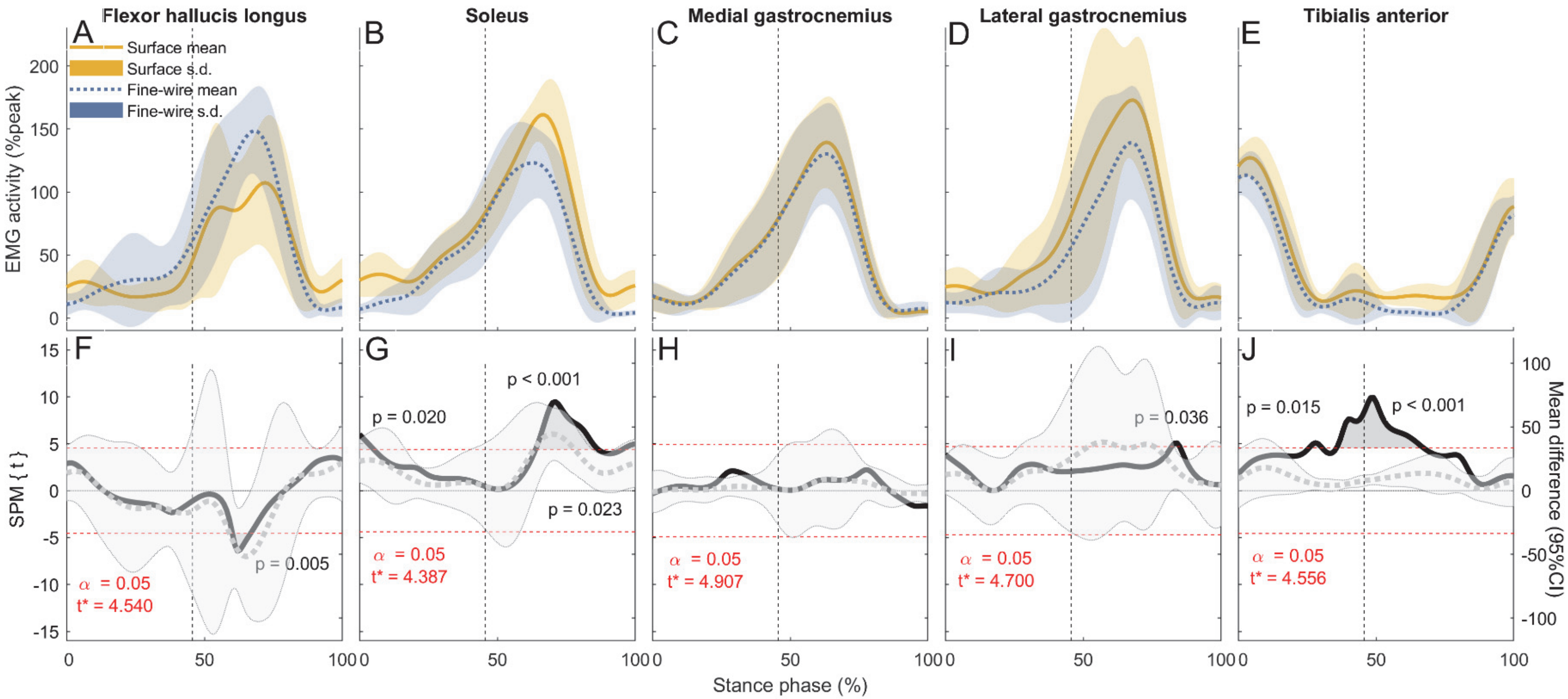

FIGURE 13 Electromyography (EMG) activity of all muscles (mean ± SD) (A-E) and corresponding comparisons of surface and intramuscular EMG signals (F-J; SPM{t}, statistics for two-tailed t-tests represented by solid black lines) throughout the stance phase of **maximum speed walking**. EMG signals were normalized to the peak activity of preferred speed walking (%peak). Mean difference is also presented with 95% confidence intervals (grey dotted lines and shaded areas). Vertical dotted lines show the start of push-off, while dashed red lines show the critical thresholds (t*). The calculated P-values are also presented for each supra-threshold cluster. Reused from study III.

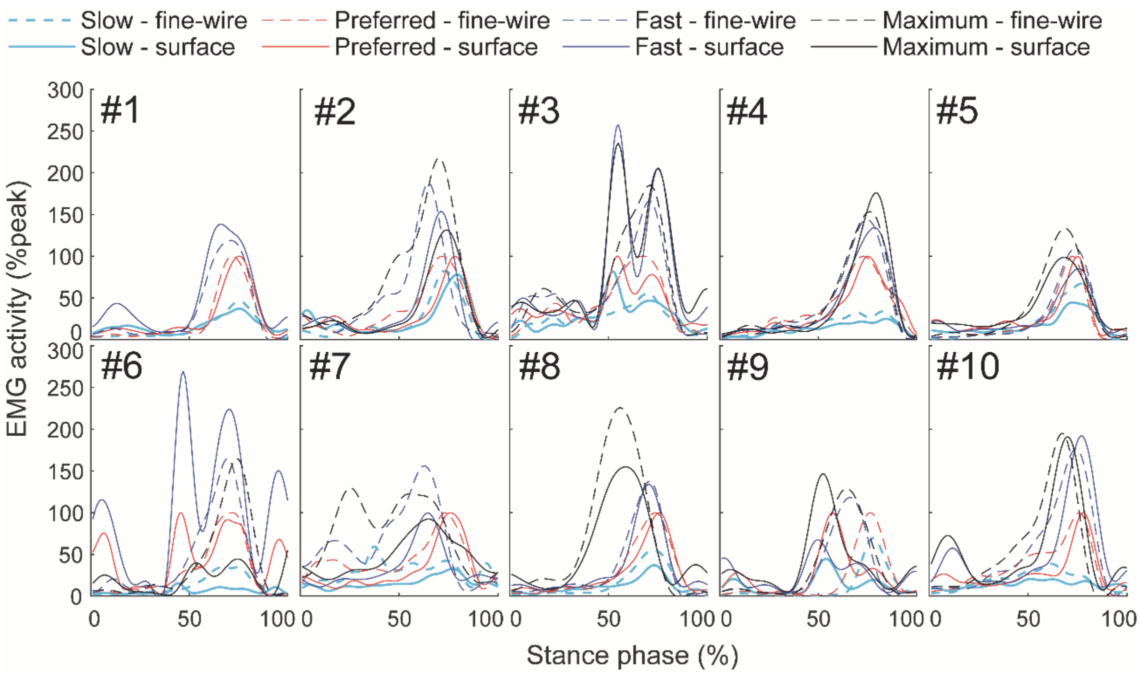

FIGURE 14 Flexor hallucis longus surface (solid) and fine-wire (dashed) electromyography activity in the stance phase of slow, preferred, fast and maximum speed walking for each individual (#1-10, respectively). EMG signals were normalized to the peak activity of preferred speed walking (%peak). All steps at a given speed were averaged. Adopted from study III.

5.4 Effects of footwear on plantar flexor intramuscular EMG activity in walking

Figure 15 demonstrates a typical example of intramuscular EMG activity from all steps and walking conditions for one participant.

There were no differences in walking speed, stance or push-off phase durations between footwear conditions (Table 2). Peak EMG activity was highest during shod walking in 66% of all conditions (25 out of 38 conditions) while it was lowest during barefoot walking in 58% of all conditions (22 out of 38 conditions). For the individuals for whom this was the case, differences in peak intramuscular EMG activity between shod and barefoot conditions were 65%, 32%, 16%, and 42% in FHL, soleus, medial and lateral gastrocnemii, respectively. At the group level, intramuscular EMG activity was higher in shod walking than in barefoot walking by 26%, 19%, 7% and 29% in FHL, soleus, medial and lateral gastrocnemii, respectively. Nonetheless, at the group level we found no statistical difference between footwear conditions in EMG activity in any of the examined muscles. This may have been caused by the high inter-individual variability in EMG amplitudes in the different footwear conditions (e.g. Figure 16).

For all muscles, inter-individual coefficients of variation (%) of the time to peak activity showed the lowest value of all conditions in shod walking (Table 3).

TABLE 3 Inter-individual coefficients of variation (%) of time to peak activity defined with intramuscular electromyography.

	Walking condition		
Muscles	**Shod**	**Barefoot**	**Flip-flops**
Flexor hallucis longus	3.5	6.9	6.5
Soleus	7.0	10.5	11.7
Medial gastrocnemius	8.0	12.8	11.9
Lateral gastrocnemius	3.4	8.7	9.8

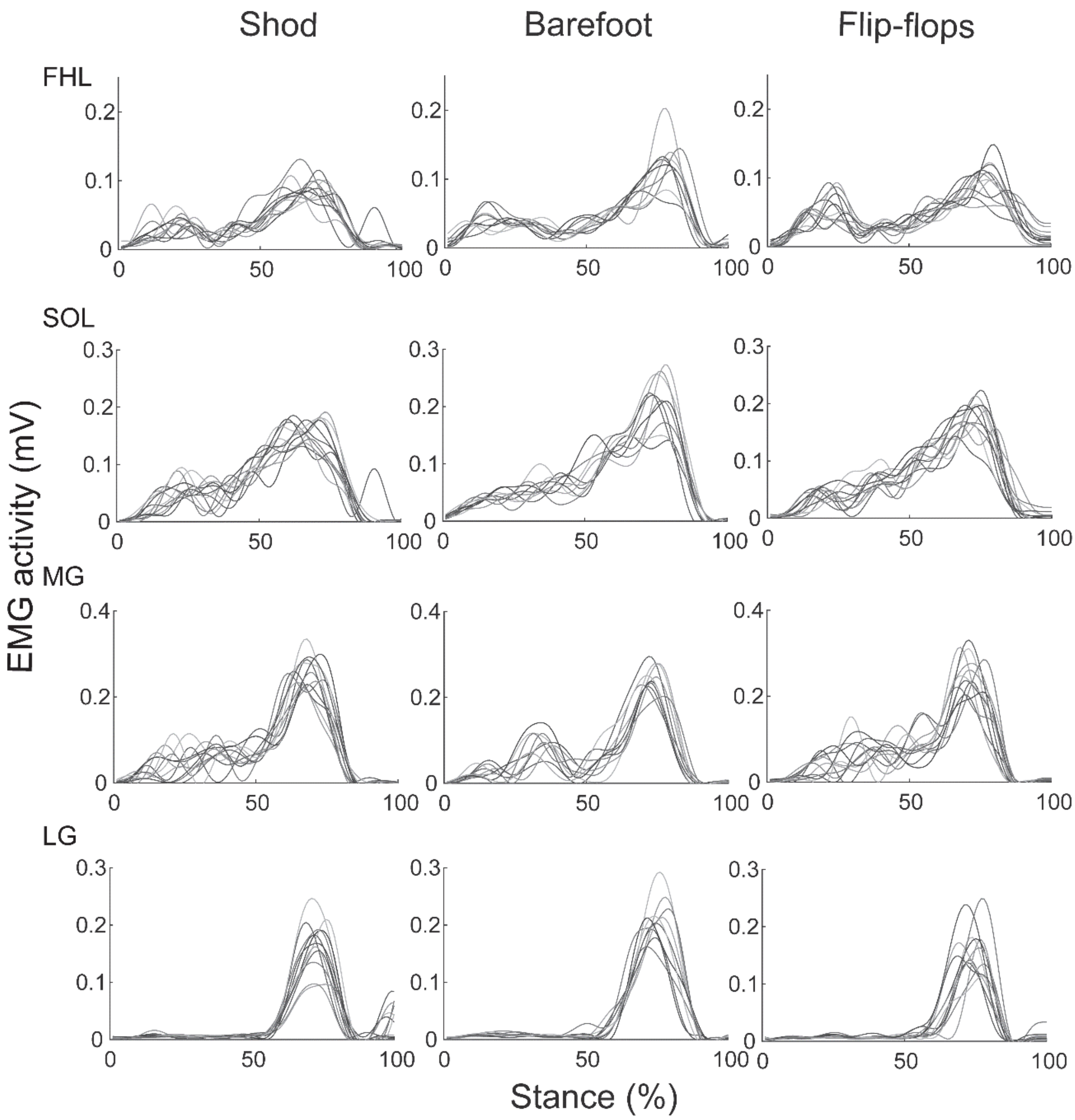

FIGURE 15 Smoothed intramuscular electromyography (EMG) activity of one individual that shows all steps for all muscles (FHL=flexor hallucis longus, SOL=soleus, MG=medial gastrocnemius, LG=lateral gastrocnemius) and preferred speed walking conditions. Steps are shown from heel contact (0%) to toe-off (100%). EMG amplitudes are not normalised.

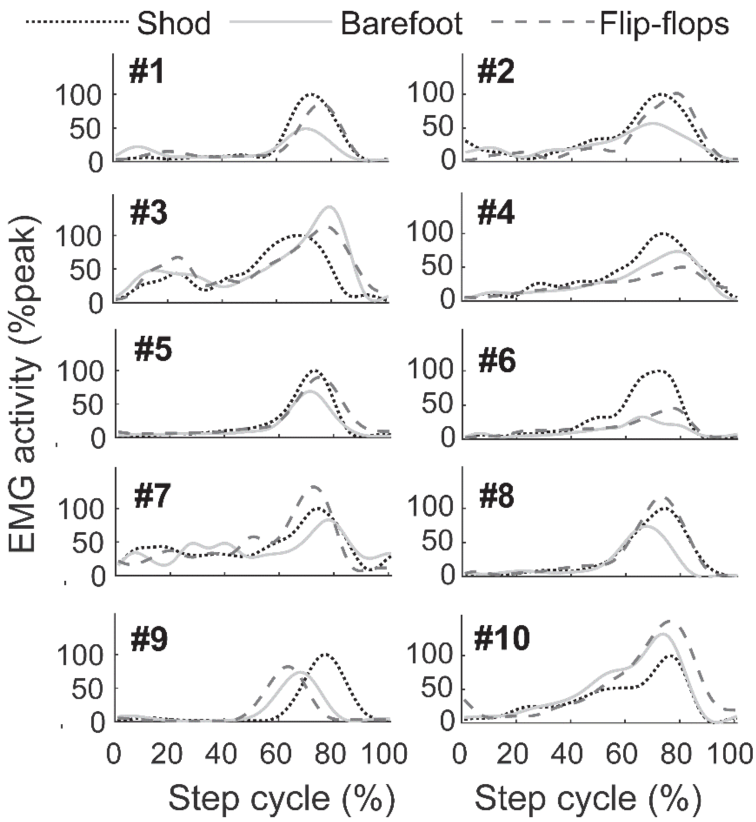

FIGURE 16 Peak normalized (to the peak activity of preferred speed shod walking, %peak) intramuscular electromyography activity for each participant (#1-10) from flexor hallucis longus in all footwear conditions in the stance phase. Steps start from heel contact and terminate at toe-off. Each line demonstrates the average of all analysed steps for each footwear condition.

6 DISCUSSION

The main findings of this thesis were as follows:

1) FHL surface EMG activity generally increased in parallel with force under the hallux with increasing isometric plantarflexion torque levels and increasing walking speeds. Furthermore, as walking speed increased, the force under the hallux increased at a higher rate than the force under other foot regions. Thus, the impulse under the hallux did not change with increasing walking speed, but the relative contribution of hallux flexion to the total foot flexion impulse did increase, highlighting the increasing importance of FHL with increasing walking speed. However, in isometric plantarflexion tasks, the relative force under the hallux did not increase with increasing torque levels (I), suggesting that FHL functions in a task-specific manner.

2) Throughout the stance phase of walking, the range of FHL fascicle length change was relatively small. Mean fascicle velocity in the push-off phase increased with increasing walking speed. Although the FHL fascicle operating range was relatively constant across all speeds, surface EMG activity of FHL increased in both the stance and push-off phases with increasing walking speed (II).

3) When comparing surface and intramuscular EMG activity during walking at different speeds, there were differences in FHL in the late stance phase at all speeds except fast walking. In soleus and tibialis anterior muscles, surface EMG activity was detected in phases where the intramuscular method registered no activity. In soleus and lateral gastrocnemius muscles, we also found differences between surface and intramuscular EMG values around the time of peak activity at faster speeds. However, there were no differences in EMG amplitudes between the two methods in medial gastrocnemius at all walking speeds, and in lateral gastrocnemius at slow and preferred walking speeds (III).

4) When examining the acute effects of barefoot walking and walking in flip-flops compared to shod walking, at the group level we found no differences in intramuscular EMG activity of FHL, soleus, medial and lateral gastrocnemii muscles between footwear types in the stance phase. Although there were highly individual directions of change in EMG magnitude, most individuals showed the highest peak EMG activity in every muscle during shod walking and the lowest peak EMG activity during barefoot walking. Additionally, in all muscles, the lowest inter-individual variability in time to peak intramuscular EMG activity during shod walking was observed (IV).

6.1 Surface EMG activity and force production of FHL in walking and isometric plantar flexion

In this study, increased walking speed and increased isometric plantarflexion torque were found to be associated with higher FHL surface EMG activity and higher hallux forces. In response to an increase in walking speed, there is a decrease in the duration of the stance phase, as well as in the duration of each subphase (Liu et al., 2014) such as push-off, as observed in the present study. However, with increasing walking speed, the force under the hallux increased relatively more than the force under other foot regions. Therefore, due to the increasing hallux force level, the impulse under the hallux was maintained across walking speeds. Relative to the total foot flexion impulse, hallux flexion impulse increased with increasing walking speed, which highlights the increasing importance of FHL at faster walking speeds. On the contrary, with increasing isometric plantarflexion torque levels, no change was observed in relative hallux force, suggesting that FHL's functional role does not change across torque levels, but is task-dependent.

Isometric ankle plantarflexion is an isolated contraction type, in which proximal leg joints do not generate forces, and thus compared to walking, force transmission from proximal joints is minimal. In the present study, with increasing walking speed, FHL surface EMG activity increased in parallel with lateral gastrocnemius EMG activity. Gastrocnemius has been found to transmit forces in the direction of the forefoot (Chen et al., 2012; Fukunaga et al., 2001a). In push-off, the elastic energy reuse of the medial longitudinal foot arch supports the process of positive work generation (Ker et al., 1987) due to the windlass mechanism (Hicks, 1954), a key contributor to which is the plantar aponeurosis (Erdemir et al., 2004). At higher walking speeds, the increased tension in the plantar aponeurosis is the result of more dorsiflexed toes in push-off (Caravaggi et al., 2010). The foot has also been found to become stiffer at higher walking speeds, resulting in a decreased foot length (Stolwijk et al., 2014), which may be due to increased tissue tension along the plantar foot surface. Besides the passive foot elements, an increase in foot stiffness can be achieved by increasing foot flexor muscle activity.

In a study (Kelly et al., 2014) in which increasing external load was applied to the medial longitudinal arch, increased EMG activity was found in intrinsic foot flexor muscles (i.e. abductor hallucis, flexor digitorum brevis and quadratus plantae). These findings suggest that the increase in EMG activity of these foot muscles serves to decrease stress on the passive foot elements by increasing the stiffness of the foot. In the current study, surface EMG activity of FHL increased with increasing walking speed. As FHL supports the medial longitudinal arch (Thordarson et al., 1995), it may also contribute to foot stiffness during walking.

In walking, the foot starts to shorten as the heel lifts off of the ground, and peak shortening occurs after the heel strike of the contralateral foot, after which the foot rapidly lengthens just before toe-off (Stolwijk et al., 2014). Peak FHL EMG activity has been found to occur in the terminal phase of stance, and activity then rapidly decreases before toe-off (Perry, 1992). Therefore, it seems that changes in foot length and EMG activity of FHL are linked. In the present study, FHL surface EMG activity and hallux force were correlated. Assuming that FHL contributes to foot stiffness and body propulsion in walking, hallux force may be expected to increase at a greater rate than the force in other parts of the foot as walking speed increases. The results of the present study confirm this hypothesis. The cross-sectional area of FHL is among the largest of all intrinsic and extrinsic foot flexor muscles (Friederich and Brand, 1990; Kura et al., 1997). This indicates that FHL has a higher potential to produce toe flexion force than other muscles. However, the measured force under the hallux may not solely originate from FHL. Since other muscles and the plantar aponeurosis also attach on the hallux (e.g. flexor hallucis brevis) (Gray, 1980), these structures could also affect hallux force output. With increasing walking speed, there is increased tension in the plantar aponeurosis (Caravaggi et al., 2010), which is in line with the observed increase in hallux force. The contribution of other foot structures to force production measured under the hallux is somewhat reflected in the findings of this study, since surface EMG activity of FHL and the measured hallux force did not correlate perfectly. However, the numerous difficulties associated with surface EMG measurements (discussed below) likely also contributed to this issue.

6.2 Fascicle behaviour of FHL at different walking speeds

This study examined fascicle behaviour of FHL *in vivo* with ultrasonography during overground walking. The range of FHL fascicle length changes was relatively small in the stance phase, but fascicle velocity increased with increasing walking speed. The results of this study further showed that FHL fascicles worked at a near-constant length in the stance phase of slow walking, while there was a trend toward shortening at faster speeds. Surface EMG activity of FHL increased with increasing walking speed in both the stance and push-off phases. Thus, the findings of this study suggest that FHL and medial gastrocnemius muscles exhibit similar neuromechanical behaviour during walking.

Anatomically, FHL originates from the distal 2/3 of the fibula and membrane interossea and inserts on the hallux, while medial gastrocnemius muscle arises from the medial condyle of the femur and inserts on the calcaneus (Gray, 1980). Although the origins and insertions of these muscles are different, they are both plantar flexor muscles that cross more than one joint. They also have similar muscle belly lengths (26.9±3.6 cm for FHL, 26.9±4.7 cm for medial gastrocnemius), similar fibre lengths (5.3±1.3 cm for FHL, 5.1±1 cm for medial gastrocnemius), and thus similarly small fibre to muscle belly length ratios (0.20 for FHL, 0.19 for medial gastrocnemius) (Ward et al., 2009). The small fibre to muscle belly length ratio predicts near-isometric function of the muscle fascicles, which is commonly reported for medial gastrocnemius in most of the stance phase of walking (Cronin and Finni, 2013; Fukunaga et al., 2001a), and was also observed for FHL in the present study, especially during slow walking. In push-off, medial gastrocnemius muscle fascicles typically shorten (Farris and Sawicki, 2012; Krishnaswamy et al., 2011), while in the present study, FHL fascicles worked at a near-constant length in the whole stance phase of slow walking, with a trend toward shortening at preferred and fast speed walking.

Gait kinematics and muscle force requirements have been found to change with increasing walking speed (Dubbeldam et al., 2010; Lelas et al., 2003). For example, based on modelling studies, walking at faster speeds is predicted to require increased ankle plantar flexor work (Neptune et al., 2008), and this is also supported by *in vivo* mechanical data. Higher ankle joint torques, increased soleus surface EMG activity and reduced soleus fascicle length have been found with increasing walking speed (0.7, 1.4, 2.0 m/s) (Lai et al., 2015). While medial gastrocnemius fascicle length changes have been found to be relatively similar as walking speed increases (0.75, 1.25, 1.75, and 2.0 m/s), fascicle shortening velocity does seem to increase (Farris and Sawicki, 2012). In the present study, similar FHL fascicle behaviour was observed with increasing walking speed, since there was no change in FHL fascicle length at heel strike or in the range of fascicle lengths, but mean fascicle velocity in the push-off phase increased with speed. This was the first study to examine FHL fascicle behaviour, so it is not possible to compare the findings of the present study to previous studies. Although mean FHL fascicle shortening velocity increased with increasing walking speed, implying similar speed-dependent changes in FHL fascicle behaviour as in medial gastrocnemius and soleus (Farris and Sawicki, 2012; Lai et al., 2015), these results cannot be quantitatively compared.

According to cadaver studies (Hofmann et al., 2013; Kirane et al., 2008), the tendon excursion of FHL is quite small (7.2 and 6.6 mm on average, respectively; high inter-individual variation) relative to FHL muscle fibre length (52.7 mm) (Ward et al., 2009) in simulated walking. In our work, the muscle-tendon junction of FHL, imaged *in vivo* with ultrasonography, showed minimal movement during simulated push-off (supplementary video of Péter et al., 2015). The range of FHL fascicle length changes was also relatively small in both the stance and push-off phases in walking, and the operating length of FHL fascicles was constant across speeds. Although these results imply near-isometric FHL fascicle behaviour, there is no data available concerning the behaviour of the FHL distal tendon. In the push-off phase of human locomotion, as the heel leaves the ground there is plantarflexion at the ankle joint and dorsiflexion at the first metatarsophalangeal joint (Leardini et al., 1999). The opposing movements of these joints imply near-isometric function at the level of the FHL muscle-tendon unit. However, joint displacement parameters may not reflect muscle contraction mode, particularly in muscles that have long tendons (Cronin et al., 2013b) such as the FHL. Additionally, foot length changes during walking (Stolwijk et al., 2014) and the lever arm of FHL at the joints that it crosses also affect the length change of the muscle-tendon unit in response to angular displacements of the joint.

6.3 Lower leg EMG activity – surface or intramuscular?

Shank muscle surface and intramuscular EMG activity was compared at different walking speeds. In FHL, differences between methods were detected in late stance at all walking speeds except fast. In soleus and tibialis anterior, in phases where intramuscular EMG indicated inactivity, activity was detected with surface EMG. In soleus and lateral gastrocnemius, differences were detected around the time of peak muscle activity at relatively fast walking speeds. These findings can all likely be at least partly explained by the influence of cross-talk on surface EMG. Conversely, no differences were found between methods in medial gastrocnemius activity at any speed or in lateral gastrocnemius at slow and preferred walking speeds.

According to previous walking studies, FHL is active in the stance phase (Perry, 1992), and this was confirmed in the current study. FHL was mainly active in the push-off phase of walking, suggesting a possible role of this muscle in body propulsion. Although it is primarily a deep ankle plantar flexor muscle, behind the medial malleolus, part of FHL becomes superficial. Previously (Bojsen-Moller et al., 2010), FHL surface EMG activity was recorded from this superficial area during submaximal contractions with a decreased inter-electrode distance (16 mm). To improve the accuracy of surface electrode location, ultrasonography guidance was also used, as was the case in studies I and II of this thesis. Although surface electrodes were placed carefully and inter-electrode distance was decreased, subject-specific differences were found in the present study between surface and intramuscular EMG methods in FHL at most walking speeds. However, it is important to note that surface and intramuscular EMG activity of four participants (Figure 14: A, D, E and H) followed similar patterns, as opposed to the other six participants. There may be several possible reasons for this. Firstly, the FHL muscle-tendon complex seems to exhibit individual-specific mechanical behaviour, which may affect surface EMG signal recordings because of tissue movement under the skin and electrodes. A previous cadaver gait simulation study (Hofmann et al., 2013) found large differences in FHL tendon excursion between specimens during the stance phase of walking, with values ranging from

4.3 to 10.2 mm (mean: 7.2 mm, n = 8). Additionally, we found high inter-individual differences in FHL fascicle length changes at different walking speeds (Study II). The size of the region where FHL surface electrodes could be placed (3.2 cm on average, ranging from 2.5 to 4.7 cm) may also affect surface EMG recordings. However, EMG data recorded from individuals with a relatively small suitable region showed good agreement between surface and intramuscular EMG methods compared to other individuals with a larger suitable region for surface electrodes. Other factors could also influence surface EMG recordings such as different subcutaneous tissue thicknesses under the surface electrodes and changes in skin impedance. Some factors that may potentially increase cross-talk are difficult to examine or control, thus based on the findings of the current study, intramuscular EMG is preferable over surface EMG to study the activity of FHL.

In walking, soleus is typically active from the mid-stance phase to the beginning of pre-swing (Cuccurullo, 2004; Perry, 1992). Previous studies detected no activity in swing from soleus with intramuscular EMG but some activity was detected with surface EMG during walking at preferred speed, potentially due to cross-talk from tibialis anterior (Bogey et al., 2000; 2003). Although soleus activity patterns recorded with surface and intramuscular EMG were similar in the stance phase of preferred speed walking [figure 1 in Bogey et al., 2000 and figure 2 in Bogey et al., 2003], higher activity was detected with surface EMG than with intramuscular EMG in early stance and at the end of pre-swing. In the current study, similar results were found, since surface EMG detected activity in early stance at all walking speeds and in pre-swing at all walking speeds except preferred, while no activity was detected with intramuscular EMG. This suggests that surface EMG was prone to cross-talk in these phases. Differences between EMG methods were also detected around the time of soleus peak activity at all speeds except slow. These results, based on lateral placement of the surface electrodes, are in agreement with previous findings where surface electrodes were placed medially (Bogey et al., 2000; 2003). Based on these results, it seems that soleus surface EMG activity is affected by cross-talk during walking, and thus soleus surface EMG data should be interpreted cautiously, especially when surface EMG is used to define muscle onset/offset. Furthermore, these findings highlight the importance of careful surface electrode positioning. After finding the correct location of the surface electrodes following SENIAM recommendations, this location should be modified depending on the thickness of the muscle belly.

In line with previous studies (Cuccurullo, 2004; Perry, 1992), we found the gastrocnemius muscles to be active in the stance phase. In medial gastrocnemius, there were no differences in EMG amplitudes between surface and intramuscular EMG at any speed. Thus, surface EMG with this electrode location and inter-electrode distance seems to be suitable for assessing activity in this muscle at all examined walking speeds. The good agreement between the two EMG methods can be explained by the large cross-sectional area of medial gastrocnemius, which allows the surface electrodes to be located sufficiently far from other muscles, thereby minimising cross-talk. Although lateral gastrocnemius has a smaller muscle volume than medial gastrocnemius (Ward et al., 2009), it seems that surface EMG is also valid in this muscle at slow and preferred walking speeds, although small differences between methods were detected in three individuals. However, at faster walking speeds, surface EMG electrodes placed over lateral gastrocnemius seem to pick up signals from other muscles. This may be caused by the neighbouring muscles' increased activity and a change in intermuscular coordination strategies with increasing walking speed (Cronin et al., 2013a).

Previous intramuscular EMG studies on walking have found that tibialis anterior exhibited peaks in activity at two distinct times in the stance phase, near heel strike and toe off, while no activity was detected in the mid-stance phase (Gray and Basmajian, 1968). The intramuscular results of the current study are in agreement with these previous findings. However, with surface EMG, activity was detected from tibialis anterior in the mid and late stance phases at all speeds. Ankle plantar flexor muscles are highly active in these phases, which may result in cross-talk being recorded in the tibialis anterior surface EMG data. Therefore, the process of defining muscle onset/offset based on surface EMG recordings may be affected by cross-talk in these phases. Furthermore, differences between surface and intramuscular EMG signals were detected in the pre-swing phase at slow and preferred walking speeds, which may have been caused by the abovementioned speed effects.

6.4 Individual EMG responses to different footwear

In study IV, the acute effects of walking barefoot and in standardized flip-flops on the intramuscular EMG activity of ankle plantar flexor muscles (i.e. FHL, soleus, medial and lateral gastrocnemii) were examined in individuals who were habitually shod. Across the whole stance phase, the different footwear types did not affect the activity of any of the examined muscles at the group level. However, large inter-individual differences were detected in the direction of changes in EMG magnitude.

Consistent footwear effects were expected across individuals in the different footwear conditions, as all examined individuals were habitually shod and did not have any foot abnormalities. On the contrary, large individual differences were present, which may somewhat explain the absence of differences at the group level. In most cases, the highest peak EMG activity was detected during shod walking and the lowest activity was found during barefoot walking in all of the examined muscles. This is in line with the idea that footwear may increase the compliance of the leg, requiring higher muscle activity from the ankle plantar flexor muscles in the propulsion phase, as recently found in intrinsic foot muscles (Kelly et al., 2016). On the contrary, the lower muscle activity observed during barefoot walking may have been caused by a lower ankle stiffness in barefoot walking compared to shod walking. FHL, contrary to the triceps surae, spans the ankle and the medial longitudinal arch of the foot, and inserts on the distal phalanx of the hallux. FHL is believed to support the medial longitudinal arch of the foot (Sarafian, 1993), along with intrinsic foot muscles and passive foot elements. We speculate that in the absence of shoes, passive foot structures may make a larger contribution to the function of the medial longitudinal arch, thus decreasing the need for high FHL activity. FHL has also been suggested to contribute to foot inversion (Hintermann et al., 1994), and foot inversion is more pronounced during barefoot walking compared to shod walking (Morio et al., 2009). Although foot kinematics were not recorded, the results of this study suggest that FHL is not the primary contributor to the acute increase in foot inversion in barefoot walking, since lower FHL EMG activity was detected during barefoot walking compared to shod. However, it is important to note that the acute responses to barefoot walking examined in this study may differ from habitual barefoot walking, since our participants regularly wore shoes. In the long-term, walking in minimalistic footwear has been found to increase the strength of ankle plantar flexors and toe flexors, as well as the cross-sectional area of FHL and flexor digitorum longus (Brüggemann et al., 2005). Thus, it may be that habitual barefoot (or minimalistic footwear) walkers actually exhibit higher EMG activity during walking.

In all muscles, inter-individual variability in time to peak activity was the lowest in shod walking. This finding is consistent with the idea that footwear restricts the individual-specific and natural motion of the foot by imposing a rather consistent motion pattern for all individuals, especially during push-off (Morio et al., 2009). Individuals who are habitually shod are used to the properties (e.g. medial longitudinal arch support) of their own footwear. Additionally, the height of the arch of habitually shod people shows high inter-individual variability compared to habitually barefoot people (D'Août et al., 2009). Due to the fact that habitually shod people are used to different types of footwear, and that they each have a correspondingly different foot structure, each of these individuals may be expected to respond differently to the absence of their familiar footwear. This may explain why studies of habitually shod people that examine the acute or short-term effects of different types of footwear result in inconsistent, unexpected or no effects on lower leg muscle and foot function. This explanation may also apply in the present study, where no differences were detected in the EMG amplitudes of the ankle plantar flexor muscles between the different footwear conditions at the group level, while relatively high variability in the time to peak activity was found between individuals in barefoot and flip-flop walking. Interestingly, previous intervention studies reported consistent effects across participants after walking/running in minimal footwear or barefoot for several weeks. These effects include increased foot muscle size and strength, increased leg and foot stiffness, and better use of the spring-like function of the medial longitudinal foot arch (Brüggemann et al., 2005; Chen et al., 2016; De Wit et al., 2000; Johnson et al., 2015; Miller et al., 2014; Perl et al., 2012; Ridge et al., 2018).

6.5 Limitations

In study I, force was measured under the hallux and presumed to be representative of FHL force production. Although FHL has the largest physiological cross-sectional area of all extrinsic and intrinsic foot flexor muscles (Friederich and Brand, 1990; Kura et al., 1997), indicating a large capacity to contribute to toe flexion force, the measured force may not exclusively originate from FHL. In fact, the measured force under the hallux may also come from other foot structures that attach on the hallux such as muscles (e.g. flexor hallucis brevis) or the plantar aponeurosis (Gray, 1980). Another limitation of this study was the use of surface EMG, especially for FHL. To minimize cross-talk, ultrasonography was used to precisely locate FHL surface electrodes as far from the soleus insertion as possible. Furthermore, inter-electrode distance was decreased to 16 mm from the usually used ~20 mm. Nonetheless, as discussed above, this method does not appear to yield valid data for some individuals and tasks.

Although measuring muscle fascicle behaviour (e.g. fascicle length changes) with ultrasonography has been found to be highly reproducible (Aggeloussis et al., 2010) during walking, ultrasonography has some limitations. For example, it is a two-dimensional method that is frequently used to study the movement of complex, three-dimensional structures. Therefore, ultrasonography may lead to errors such as over- or underestimation of fascicle length changes in cases where the probe is not aligned with the plane of the fascicles (Bénard et al., 2009). The magnitude of this error may also change throughout the step cycle. To minimize this error, image quality was confirmed with hallux flexion and calf raise movements. Another limitation is that the probe might move relative to the examined muscle area. To minimise probe movement, a flat-shaped probe was firmly fixed to the shank. Although this may compress muscle structures, there are currently no alternative methods available to examine fascicle length changes in-vivo during locomotion.

Due to the invasive and expensive nature of intramuscular EMG studies, the sample size was quite low in studies III and IV. This may potentially result in type II errors. Furthermore, in study III, intramuscular EMG electrodes were placed close to the surface EMG electrodes for all muscles except FHL, due to rich vascularization near FHL surface electrodes. Thus, regional differences in FHL activation might have resulted in differences between surface and intramuscular EMG signals. Additionally, compared to surface EMG, intramuscular EMG electrodes record activity from a relatively small number of motor units, which might have also resulted in differences between surface and intramuscular EMG signals. Nonetheless, intramuscular electrodes were placed as close to the surface electrodes as possible to record EMG activity from the same muscle region. Inter-electrode distance may also cause differences between the two EMG methods. Typically an inter-electrode distance of ~20 mm is used in surface EMG studies (as well as in this study, except for FHL), but a smaller inter-electrode distance (e.g. 10 mm) could further decrease cross-talk (De Luca et al., 2012).

In study IV, where only intramuscular EMG results were presented, the small pick-up area of the intramuscular electrodes may not represent whole muscle activity. However, it was important to use intramuscular EMG in this study to minimise cross-talk, especially in light of the finding that the surface EMG method seems to only be useful in some but not all individuals for FHL. In this study, the properties of the running shoes (e.g. stiffness of the sole, cushioning) were not taken into account, and may have been different between individuals. However, participants were asked to wear their own shoes to which they were accustomed, in order to eliminate the effects of acute adaptations to new shoes. Furthermore, due to pressure insole fixation, participants had to walk in socks during barefoot walking, and this may have affected foot sensation.

In all studies, healthy, non-injured individuals without clear foot deformities were recruited, and all had a relatively thin subcutaneous fat layer over the shank muscles. Thus, the application of these result may be restricted to this population.

6.6 Future directions

Although FHL muscle has been suggested to have many functions, this thesis primarily considered FHL as an ankle plantar flexor muscle and examined its behaviour in different conditions in this context. Future studies should focus on its other roles and how these may change at different walking speeds, in different types of footwear, and in individuals with different foot types. For example, future studies could investigate the mechanical influence of FHL at the 1st metatarsophalangeal joint, since FHL is a primary flexor of this joint.

FHL is a multi-articular muscle that crosses the ankle and the 1st metatarsophalangeal joints. In locomotion, these joints move in opposite directions, implying a complex pattern of FHL muscle-tendon unit behaviour. Future studies should examine how the FHL muscle-tendon unit behaves in locomotion and how it changes with changes in gait parameters such as walking speed and footwear. These studies could also use larger sample sizes to determine whether there is any relationship between FHL muscle-tendon unit behaviour and foot type (e.g. flatfoot). Future studies could also explore the potential mechanisms behind the large inter-individual variability in ankle plantar flexor EMG activity during walking in different types of footwear.

7 PRIMARY FINDINGS AND CONCLUSIONS

FHL surface EMG activity increased in parallel with the force measured under the hallux in isometric ankle plantar flexion contractions and walking. With increasing walking speed, the contribution of the hallux to total ground reaction force also increased. This result indicates an increase in the relative importance of FHL with increasing walking speed. Future studies examining FHL muscle activity should use intramuscular EMG, since surface EMG only seems to provide valid data for some but not all individuals. Moreover, it would be of value to understand why the surface method is more valid for some individuals than others.

During walking at different speeds, FHL fascicles worked at a near-constant length in the stance phase of slow walking, while they showed a trend toward shortening when walking speed increased. With increasing walking speed, mean FHL fascicle velocity and surface EMG activity increased. Therefore, it seems that the FHL and medial gastrocnemius muscles exhibit similar muscle fascicle behaviour with increasing walking speeds. Future studies should focus on FHL fascicle behaviour of people who have flat-foot (Angin et al., 2014) or Achilles tendon problems (Finni et al., 2006; Masood et al., 2014b), since FHL morphology has been found to be altered in these individuals.

The validity of surface EMG for measuring shank muscle activity was muscle- and walking speed-specific. Based on the results of this thesis, surface EMG is generally appropriate for measuring medial and lateral gastrocnemii EMG activity across several walking speeds. In FHL, minimizing surface EMG cross-talk is challenging, so the use of surface EMG is not recommended in this muscle. The measured surface EMG activity of soleus and tibialis anterior muscles should be interpreted cautiously in certain phases of the step cycle, especially when using these data to define muscle onset/offset. In the future, studies should further explore possible sources of cross-talk and how they could be minimized (e.g. better locating the electrodes, decreasing inter-electrode distance) to improve the selectivity of surface EMG in shank muscles during walking.

The results of the current thesis suggest that for most individuals, walking at preferred speed in shoes requires higher peak intramuscular EMG activity from the ankle plantar flexor muscles for body propulsion than walking barefoot or in flip-flops. The similar time to peak muscle activity observed between individuals may be due to the restrictive nature of the shoes, which impairs individual and natural foot and ankle function by superimposing a specific motion pattern over the individual one. In the future, footwear studies should investigate the long-term effects of walking barefoot or in flip-flops on the function of ankle plantar flexor muscles, and how their relative roles change when the type of footwear changes. Furthermore, these intervention studies should examine habitually barefoot individuals, since they have very similar foot structures, unlike habitually shod individuals, whose foot structures show very high inter-individual variability, even if their foot is considered to be “normal”.

Because of the challenge of examining FHL muscle function *in vivo*, only a few studies have been conducted in this field. In these studies, considerably high inter-individual differences have been revealed in activation strategies of deep and superficial ankle plantar flexor muscles. This is true of ankle plantar flexion contractions (Finni et al., 2006; Masood et al., 2014b), force transmission mechanisms between the triceps surae and FHL (Bojsen-Moller et al., 2010), and maximal voluntary force production of the toe flexor muscles (Goldmann and Brüggemann, 2012). Altered anatomical or electrical characteristics of FHL have been found in people with flatfoot (Angin et al., 2014), Achilles tendinopathy (Masood et al., 2014b) or Achilles tendon rupture (Finni et al., 2006). Thus, high inter-individual differences in FHL function may also be associated with these above-mentioned foot disorders due to different load sharing capacities. To better understand the mechanisms of these foot disorders *in vivo*, functionally relevant studies using more direct methods are clearly needed to reveal the roles of FHL during locomotion and different tasks in healthy individuals. Further studies could then examine how these functions are altered in symptomatic individuals or as a result of aging. Task-dependent inter-muscular coordination of foot muscles (including FHL) has been found (Zelik et al., 2015), highlighting the importance of studies examining muscle behaviour during functional movements. Understanding the function of FHL has implications for rehabilitation, biomechanical modelling, and the design of footwear, prostheses and orthotics.

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ORIGINAL PAPERS

I

EMG AND FORCE PRODUCTION OF THE FLEXOR HALLUCIS LONGUS MUSCLE IN ISOMETRIC PLANTARFLEXION AND THE PUSH-OFF PHASE OF WALKING

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EMG and force production of the flexor hallucis longus muscle in isometric plantarflexion and the push-off phase of walking

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ABSTRACT

Large forces are generated under the big toe in the push-off phase of walking. The largest flexor muscle of the big toe is the flexor hallucis longus (FHL), which likely contributes substantially to these forces. This study examined FHL function at different levels of isometric plantarflexion torque and in the push-off phase at different speeds of walking. FHL and calf muscle activity were measured with surface EMG and plantar pressure was recorded with pressure insoles. FHL activity was compared to the activity of the calf muscles. Force and impulse values were calculated under the big toe, and were compared to the entire pressed area of the insole to determine the relative contribution of big toe flexion forces to the ground reaction force. FHL activity increased with increasing plantarflexion torque level ($F=2.8$, $P=0.024$) and with increasing walking speed ($F=11.608$, $P<0.001$). No differences were observed in the relative contribution of the force under the big toe to the entire sole between different plantarflexion torque levels ($F=0.836$, $P=0.529$). On the contrary, in the push-off phase of walking, peak force under the big toe increased at a higher rate than force under the other areas of the plantar surface ($F=3.801$, $P=0.018$), implying a greater relative contribution to total force at faster speeds. Moreover, substantial differences were found between isometric plantarflexion and walking concerning FHL activity relative to that of the calf muscles, highlighting the task-dependant behaviour of FHL.

1. Introduction

The first metatarso-phalangeal joint (MPJ) has a significant range of motion in the sagittal plane in walking (Caravaggi et al., 2010). This joint is crossed by the flexor hallucis longus muscle (FHL). The FHL originates from the distal 2/3 of the fibula and the membrana interossea, and inserts onto the distal phalanx of the big toe while also spanning the ankle (Gray, 1980). Several functions have been attributed to FHL, including flexion of the big toe, supination of the foot (Ferris et al., 1995), maintenance of the medial longitudinal arch of the foot (Thordarson et al., 1995), production of inversion torque on the rearfoot (Hintermann et al., 1994) and plantarflexion torque at the ankle (Klein et al., 1996). FHL may also play a role in accelerating the centre of mass in the push-off phase of locomotion (Goldmann et al., 2013), presumably via energy conservation (Hofmann et al., 2013; Kirane et al., 2008).

It has been estimated that during walking the forces within the foot are largest in the first ray, with 29% body weight under the first MPJ and 24% under the big toe (Jacob, 2001). FHL may make a major contribution to these large forces, since it has the largest physiological cross-sectional area of the big toe flexor muscles (Friederich and Brand, 1990). Jacob (2001) estimated that the force along the FHL tendon is around 52% of body weight at the second peak of the vertical ground reaction force in the stance phase of walking. FHL muscle activity is also maximal at the terminal stance phase of gait (Perry, 1992).

Numerous studies have shown the multifunctionality of FHL, but all of these studies used cadaver or indirect methods to estimate muscle function. Direct in vivo methods have not been used except for the study of Perry (1992), which did not examine the relationship between FHL activity and movement outcomes. The aim of this study was to examine FHL activity and the resulting force under the big toe during sustained isometric plantarflexions at different contraction levels, and in the push-off phase of walking at different speeds. This allowed us to determine differences in the use of this muscle between an isolated and a functional task, as well as changes with increasing intensity of locomotion and plantarflexion.

2. Methods

2.1. Participants

Eleven male subjects (age: 24.7 ± 3.7 years; height: 180.6 ± 6.6 cm; body mass: 79.2 ± 9.1 kg) with no history of neuromuscular disorder or

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injury volunteered for this study. Subjects with self-reported flat foot were excluded (Angin et al., 2014). The right (dominant) leg of each subject was measured. The experimental procedures were approved by the ethics committee of the University of Jyväskylä and all subjects gave written informed consent. Testing was conducted according to the Declaration of Helsinki.

2.2. Experimental protocol

After shaving and abrading the skin lightly then cleansing with alcohol, bipolar electromyography (EMG) electrodes were placed over tibialis anterior (TA), gastrocnemius lateralis (GL), gastrocnemius medialis (GM), soleus (SOL) and flexor hallucis longus (FHL) muscles. After checking that electrode impedance was below 5 kΩ the signal quality was confirmed with a few plantarflexion and toe flexion tasks. Subjects then performed overground walking trials over two 10 m force platforms positioned parallel to each other. Thereafter, submaximal isometric plantarflexions were executed in a custom-made ankle dynamometer. Finally, for normalisation of EMG signals, maximal voluntary isometric contractions (MVICs) were performed. During all walking and plantarflexion trials, foot pressures were recorded using pressure insoles (see Section 2.3 below).

2.2.1. Walking trials

After warm-up and familiarisation trials, subjects were asked to walk at a self-selected speed over the force plates five times. The slowest and fastest trials were discarded and the remaining values were averaged to signify the preferred speed of walking (PW). Subjects then walked at maximal walking speed (MW; subjects were asked to walk as fast as possible) as well as at slower (SW) and faster (FW) speeds than PW: target durations over the force platforms were PW+30% and PW−30%, respectively. PW trials were always performed first, but subsequent trials were performed in a random order. Three successful trials (within ±5% of target time) were recorded for the MW, SW and FW conditions.

2.2.2. Submaximal and maximal plantarflexions

After some familiarisation trials, subjects were asked to reach and then maintain different isometric plantarflexion torque levels for 2 s. Torque–time curves served as visual feedback for the subjects. Torque levels were set at 20, 40, 60, 80, 100 and 120 Nm and were performed in a random order. Absolute rather than relative values were chosen because low torques within this range were expected during walking. Submaximal trials served as an initial warm up for the maximal contractions. In addition, 3 submaximal contractions lasting 2–3 s at around 70% MVIC were performed before maximal trials. For TA, triceps surae and FHL muscles, dorsiflexion, plantarflexion and big toe flexion superimposed on plantarflexion were performed, respectively. Strong verbal encouragement was given to ensure maximal effort. MVICs were performed 3 times with 1.5 min rest between contractions. In cases where the greatest torque was recorded in the third trial, additional contraction(s) were performed until maximal torque no longer increased.

2.3. Instrumentation

2.3.1. EMG activity

EMG activity was recorded using a telemetric system (Noraxon Inc. Scottsdale, AZ, USA). Silver–silver chloride Ambu *BlueSensor* N bipolar surface electrodes (Ambu A/S, Ballerup, Denmark) were used to measure EMG activity according to the SENIAM recommendations for TA, GL and GM (Hermens et al., 1999). For FHL, proper placement of electrodes was determined using ultrasonography: the electrodes were placed between the insertion of SOL and the calcaneus on the medial side, posterior to the medial malleolus where FHL was identified

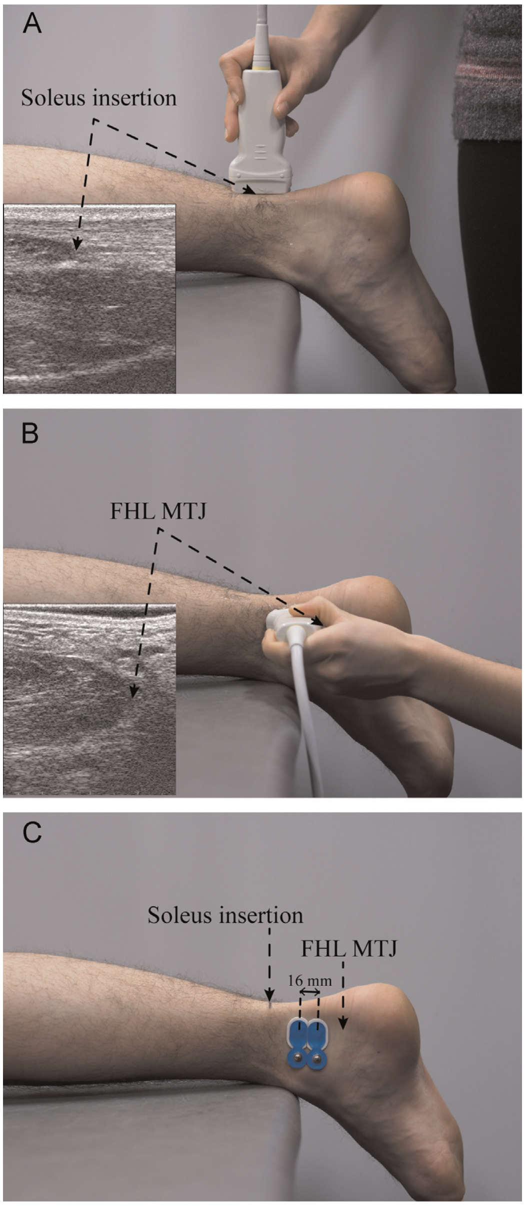

Fig. 1. FHL electrode placement. Insertion of the SOL (A) and the muscle–tendon junction (MTJ) of the FHL (B) were defined by ultrasonography, and EMG electrodes were placed between these anatomical landmarks with an interelectrode distance of 16 mm (C). Before attaching the electrodes, plantarflexion tasks were performed while displacement of the FHL MTJ was followed (not shown).

(Fig. 1). The inter-electrode distance for FHL was reduced to 16 mm to minimise cross-talk from other muscles (Bojsen-Møller et al., 2010; Masood et al., 2014). For SOL the electrodes were placed on the lateral side of the muscle to avoid cross-talk between SOL and FHL, but otherwise the SENIAM recommendations were followed. Inter-electrode distance for TA and triceps surae muscles was 22 mm. EMG signals were sampled at a frequency of 1500 Hz and transmitted wirelessly to an A/D converter (Cambridge Electronic Design, Cambridge, UK) connected to a personal computer. Digital signals were recorded and visualised online along with the ground reaction force (GRF) signals in Spike2 software (Cambridge Electronic Design, Cambridge, UK).

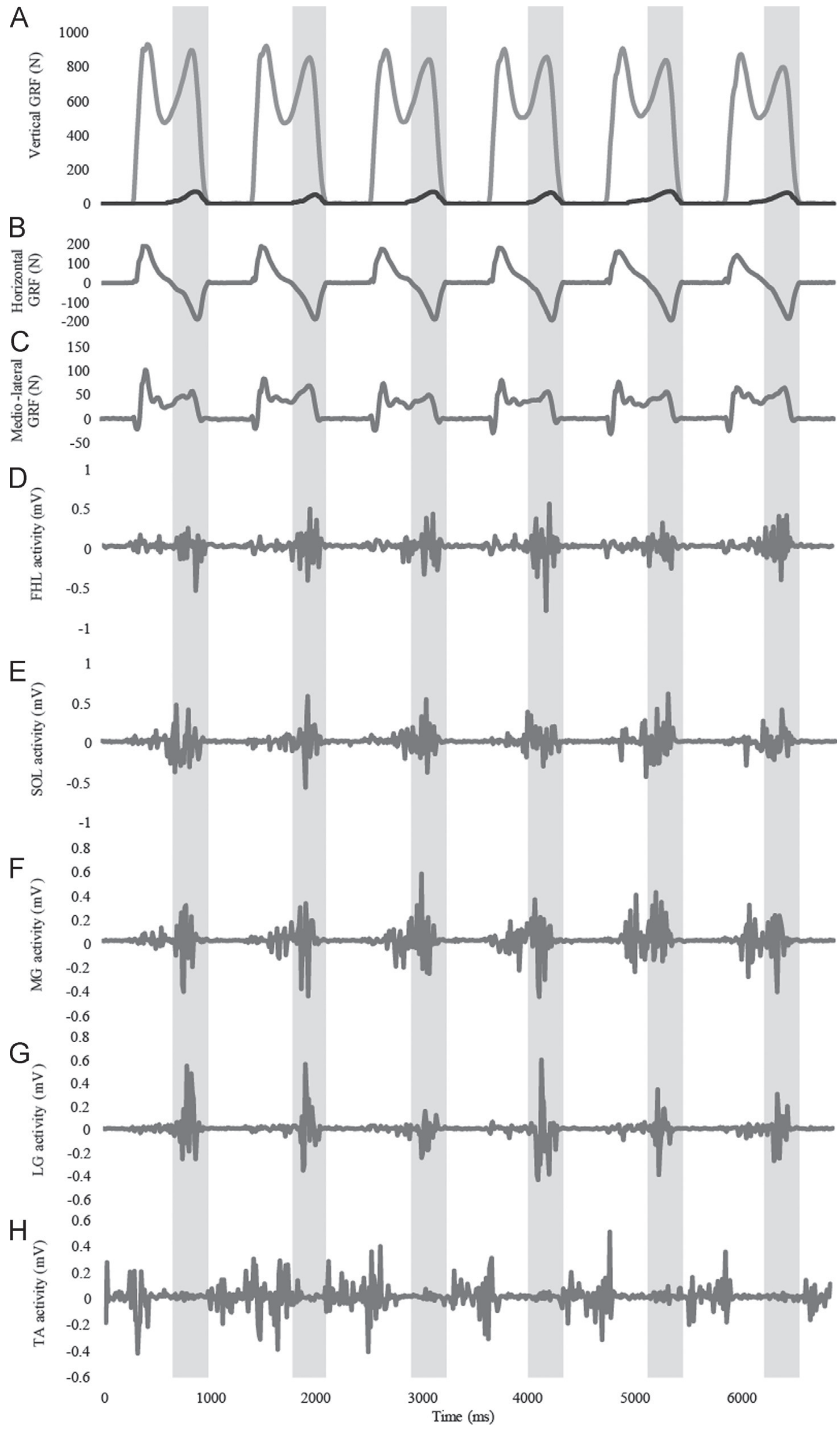

Fig. 2. Raw data from one walking trial and one subject. Panels A–C show ground reaction forces (GRF) measured from the force platform. In addition, the black line in A represents the force calculated for the area under the big toe. Panels D–H show EMG activity from the FHL, SOL, MG, LG and TA muscles. Shaded areas represent the push-off phase of steps that were analysed.

2.3.2. Force and torque

Walking trials were performed on two custom-made 10 m × 0.5 m force plates positioned parallel to each other (University of Jyväskylä, Finland; sampling frequency = 1000 Hz), allowing 3 dimensional GRFs to be recorded for each step and leg separately (Fig. 2). To avoid recording the acceleration and deceleration phases, participants were asked to start approximately 3 m prior to the force plates and continue for 2–3 m after them, allowing several steps to be recorded at a steady speed. Speeds were determined using photocells at the start and end of the force plates. During isometric tasks torque was measured using a custom-made ankle dynamometer (University of Jyväskylä, Finland) equipped with a strain gauge (Raute Precision, Model TB5, max. load = 2000 kg, error < ±0.02%, sensitivity < ±0.1%). The hip, knee, ankle and 1st MPJ were fixed at 120°, 0°, 90° and 0°, respectively. The right foot was firmly strapped to the pedal of the dynamometer, and the thigh was strapped to the seat to avoid postural changes between trials (Fig. 3). The seat was positioned as close as possible to the pedal to minimise heel lift during plantarflexion.

2.3.3. Plantar pressure

Plantar pressure was recorded using the Pedar-X in-shoe dynamic pressure measuring system (Novel, Munich, Germany) during walking and submaximal isometric trials. An insole including 99 pressure sensors was placed into the subject's right shoe. Data were collected at 100 Hz via bluetooth using Pedar's own software. At the start of the recordings a trigger signal was sent to the Spike software to synchronise the data. Before every task the big toe was pressed down by an assistant while the foot was unloaded, to help map the pressure area covered by the big toe.

2.4. Analysis

Trials including steps that deviated by more than ±5% of the average duration of all gait cycles within a trial were excluded from the analysis for each subject.

2.4.1. EMG activity

TA activity was recorded to monitor antagonist activity during walking. Activity of the other muscles was analysed in the following way. EMG signals were band-pass filtered between 20 and 450 Hz by a butterworth filter in MATLAB (MathWorks Inc., Natick, MA, US). Root mean square (RMS) EMG activity of each muscle was calculated in the push-off phase of walking for each step based on the horizontal GRF, and values within each trial were averaged. For submaximal isometric contractions, RMS was calculated from a 1-s stable plateau to characterize the EMG activity at different torque levels. The MVIC where the peak torque was highest was used to normalise the EMG. After filtering, RMS was calculated from a stable 1-s plateau around the peak MVIC torque and considered to be maximal voluntary EMG activity. FHL activity was also defined relative to the individual triceps surae muscles (FHL/LG, FHL/MG and FHL/SOL) and to the average activity of the triceps surae (FHL/TS) to establish FHL activity relative to the primary plantarflexors.

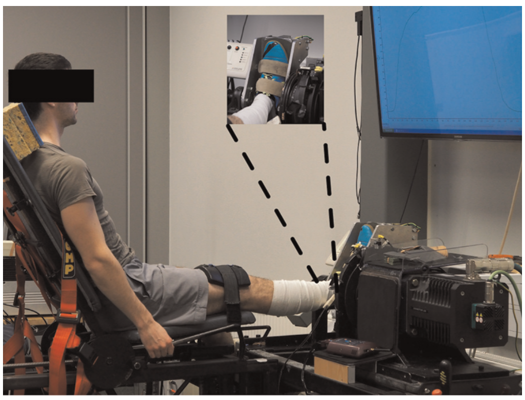

Fig. 3. In isometric tasks subjects were seated in a dynamometer. Hip, knee, ankle and 1st MPJ were positioned at 120°, 0°, 90°, and 0°, respectively. The foot was firmly strapped to the pedal of the dynamometer, which measured force using a strain gauge. The seat was adjusted to be as close as possible to the pedal to minimize the elevation of the heel during contractions.

2.4.2. Plantar pressure analyses

Before each trial, an assistant manually pressed down on the big toe. In the subsequent analysis, this was used to define the area of the big toe. The sensors at the edge of this area were excluded from the analysis to avoid interference from other toes or foot regions. Pressing the toe down before each trial also allowed us to ensure that the insole did not move inside the shoe between tasks.

Forces were calculated within the push-off phase of walking by dividing the pressure by the area of the pressed sensors. Peak forces were defined under the big toe and the whole foot for each step and averaged for every trial. Impulse was calculated as the integral of the force–time curve for the sensors under the big toe and under the foot. All force and impulse values in walking were normalised to body mass (BM). Average force during isometric plantarflexion was calculated over the same time interval as the calculation of RMS EMG. The contributions of the big toe to peak or average force and impulse were calculated by dividing the big toe values by the respective values for the whole plantar surface (hereafter referred to as *relative big toe force* and *relative big toe impulse*).

2.4.3. Statistical analysis

All statistical analyses were performed using IBM SPSS software (IBM New York, US). Normality of distribution was tested by Shapiro Wilk's W test, while homogeneity of variance was determined with Levene's test. Spearman's rank was used to perform correlations between FHL activity and force under the big toe in isometric and walking trials. Differences between EMG, force or impulse values at submaximal plantarflexion levels and different speeds of walking were defined by one-way ANOVA and Tukey HSD post-hoc test if normal distribution and homogeneity of variances were confirmed. One subject did not perform MW trials so differences between MW and other walking tasks were defined using Gabriel's procedure. In all other cases non-parametric Kruskal–Wallis ANOVA and Games–Howell post-hoc tests were used to signify differences. Significance level was set at $P<0.05$. Values in the text are expressed as mean ± standard deviation.

2. Results

Maximal isometric plantarflexion torque was 362.39 ± 78.35 Nm. In isometric tasks with varying torque level, average force under the big toe generally increased with torque ($F=5.41$, $P<0.001$), but there were no significant changes in relative big toe force ($F=0.836$, $P=0.529$) (Fig. 4). For walking trials, absolute peak force under the big toe increased significantly with increasing speed ($F=4.923$, $P=0.005$), and the increase was greater than in the other regions of the foot ($F=3.801$, $P=0.018$). Impulse under the big toe did not increase with speed ($F=0.078$, $P=0.972$), however relative big toe impulse did ($F=5.067$, $P=0.005$). Force under the big toe increased linearly as a function of increasing FHL activity, both in isometric tasks ($r=0.653$, $P<0.001$) and during walking ($r=0.439$, $P=0.003$).

Walking velocity, number of analysed steps for each walking trial and duration of the push-off phase are described in Table 1. Table 2 presents FHL EMG activity normalised to maximal voluntary activity, and compared to the activity of the superficial plantarflexors in different tasks. FHL activity increased with increasing plantarflexion level and walking speed ($F=2.8$, $P=0.024$ and $F=11.608$, $P<0.001$, respectively). In sustained isometric plantarflexions the FHL/SOL activity ratio was significantly higher at 20 Nm compared to all other torque levels ($F=6.298$, $P<0.001$). FHL/MG, FHL/LG and FHL/TS did not change with increasing

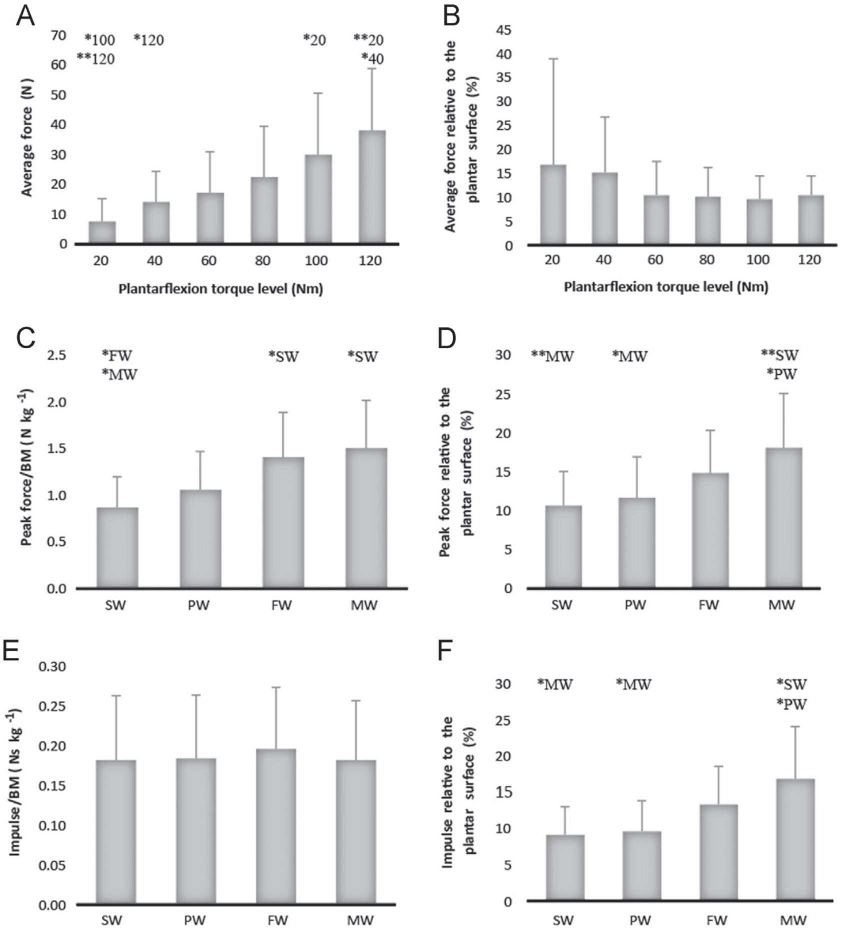

Fig. 4. Force and impulse in different tasks. A, C and E were calculated for the area under the big toe. Relative values (B, D, and F) indicate force and impulse under the big toe relative to the same values for the entire plantar surface. For walking, peak force and impulse were normalised to body mass (BM) and calculated in the push-off phase (C, E). Statistical differences: $^{*}P<0.05$, $^{**}P<0.01$.

Table 1
Descriptive statistics of walking trials included in the analysis. SW = slow walking, PW = preferred speed walking, FW = fast walking, MW = maximal speed walking. For SW and FW target times were PW + 30% and PW − 30%, respectively (± 5%).

	Included steps (n)			Velocity (m/s)			Duration of the push-off phase (ms)		
SW	16	±	2	1.05	±	0.08	352	±	42
PW	16	±	6	1.36	±	0.10	313	±	35
FW	12	±	7	1.93	±	0.16	253	±	29
MW	15	±	6	2.51	±	0.31	219	±	28

Included steps are expressed as median ± interquartile range. Other values are mean ± standard deviation.

torque. For walking trials, none of the activity ratios altered significantly with increasing speed.

3. Discussion

Our results show that increased isometric plantarflexion torque and increased walking speed were associated with higher FHL activity and concomitant higher forces under the big toe. It is well known that an increase in walking speed results in a decrease in stance phase duration, as well as the duration of all sub-phases (Liu et al., 2014) such as push-off (Table 1). On the contrary, force under the big toe increased at a higher rate than force in other regions of the plantar surface as walking speed increased. Thus, impulse was maintained across walking speeds by an increasing force level under the big toe. With increasing walking speed, big toe flexion impulse relative to total foot flexion impulse increased, highlighting the increasing importance of FHL at higher speeds. Conversely, in isometric tasks, as plantarflexion torque increased, significant changes in relative force under the big toe were not observed, indicating that the role of FHL did not change across torque levels.

Previously, based on surface EMG, Masood et al. (2014) found that the contribution of FHL to plantarflexion was about 31 ± 13% during intermittent isometric exercise at 30% of MVIC. In the current study, FHL activity was 16 ± 10% of the cumulative EMG activity of all four plantarflexors at a torque level of 100 Nm (28.72 ± 5.94% MVIC). Peak force under the big toe at preferred walking speed (11 ± 4% BM) was also lower than 24 ± 8% BM estimated by Jacob (2001). However, it should be emphasised that the method we used to map the big toe presumably underestimated the size of the target area, since we attempted to eliminate interference from other parts of the plantar surface.

During isometric plantarflexion FHL functions as a deep plantarflexor (Klein et al., 1996). In this isolated contraction mode, forces are not generated at proximal joints, and thus force

Table 2
FHL EMG activity alone and compared to EMG in the other calf muscles. For plantarflexion trials values refer to a 1-s plateau; for walking trials values were calculated as the mean in the push-off phase. Ratios were calculated after normalising to MVC.

	Task	FHL activity (%MVC)	FHL/SOL ratio	FHL/MG ratio	FHL/LG ratio	FHL/TS ratio
Submaximal sustained plantarflexion	20 Nm	3.78 ± 3.93^{*120}	15.64 ± 13.96$^{**40–100\ †120}$	0.72 ± 0.34	1.82 ± 2.19	0.58 ± 0.46
	40 Nm	5.44 ± 7.15	5.95 ± 2.99^{**20}	0.63 ± 0.16	1.44 ± 2.29	0.47 ± 0.46
	60 Nm	6.67 ± 6.91	4.04 ± 2.50^{**20}	0.73 ± 0.37	1.25 ± 2.03	0.45 ± 0.36
	80 Nm	9.52 ± 9.41	4.05 ± 2.84^{**20}	0.75 ± 0.21	1.23 ± 2.23	0.52 ± 0.42
	100 Nm	13.35 ± 14.49	3.93 ± 2.36^{**20}	0.75 ± 0.18	1.01 ± 1.28	0.59 ± 0.50
	120 Nm	16.71 ± 13.03^{*20}	3.46 ± 3.02^{†20}	0.79 ± 0.15	0.87 ± 0.76	0.66 ± 0.56
Walking	SW	51.49 ± 31.74^{**F†M}	1.54 ± 0.84	1.36 ± 0.85	3.27 ± 2.12	1.68 ± 1.04
	PW	70.38 ± 39.95^{*F†M}	1.50 ± 0.89	1.83 ± 1.37	2.73 ± 1.92	1.70 ± 1.10
	FW	134.34 ± 61.04^{*P**S}	1.72 ± 0.65	2.11 ± 1.05	2.72 ± 1.31	1.91 ± 0.74
	MW	200.34 ± 103.65^{†SP}	2.07 ± 0.84	2.41 ± 1.57	2.61 ± 1.19	2.16 ± 1.00

Differences between specific conditions are indicated.
Values are expressed as mean ± SD. Significant differences are shown as * if $P < 0.05$, ** if $P < 0.01$, † if $P < 0.001$. Differences between specific conditions are indicated.

transmission from proximal joints is minimal compared to the situation during locomotion. In this study, EMG activity increased in parallel in the gastrocnemius and FHL muscles with increasing speed. Gastrocnemius has been reported to transmit forces towards the forefoot (Chen et al., 2012; Fukunaga et al., 2001). In the second half of the stance phase elastic energy reuse in the longitudinal arch buttresses the generation of positive work (Ker et al., 1987). The main contributor to this so-called windlass mechanism (Hicks, 1954) is the plantar aponeurosis (Erdemir et al., 2004), with an increased tension level at higher walking speeds due to the more dorsiflexed toes in the push-off phase (Caravaggi et al., 2010). In vivo motion analysis has shown that at higher speeds, the foot becomes stiffer and so foot length decreases (Stolwijk et al., 2014). The decrease in foot length can be achieved by increased tension of the tissues on the plantar surface. In addition to the passive elements, further increases in foot stiffness are achieved by increasing the activity of the foot flexor muscles. Kelly et al. (2014) found increased EMG activity of the abductor hallucis, flexor digitorum brevis and quadratus plantae with increasing external load applied to the longitudinal arch, suggesting that intrinsic foot muscles serve to decrease stress on the passive elements and increase foot stiffness. In our study, we found that activity of the FHL also increased with increasing walking velocity. Since this muscle contributes to maintenance of the medial longitudinal arch (Thordarson et al., 1995), it may also contribute to foot stiffness.

Stolwijk et al. (2014) found that the foot begins to shorten upon heel elevation, achieves its peak rate of shortening after contralateral heel strike, and then rapidly lengthens just before toe off. Perry (1992) also found that FHL activity is highest in the terminal stance phase but rapidly decreases just before toe off. Thus, changes in foot length and FHL activity seem to be linked. We found that FHL activity and force under the big toe correlated significantly. If FHL contributes substantially to foot stiffness and propulsion of the body, the force under the big toe might be expected to increase at a higher rate than the force under the other parts of the foot as speed increases. Indeed, this is what we observed for peak force and impulse under the big toe. FHL has one of the largest cross-sectional areas of the deep extrinsic and intrinsic foot flexors (Friederich and Brand, 1990; Kura et al., 1997), which indicates a high force production capacity compared to the other muscles that contribute to toe flexion. However, it should be noted that forces under the big toe are not only due to force production of FHL. Other agonist and synergist muscles (such as flexor hallucis brevis, adductor and abductor hallucis) as well as antagonist muscles (such as extensor hallucis longus and extensor digitorum brevis) attach on the big toe (Gray, 1980), and may directly affect the vertical force output of the big toe. Moreover, the plantar aponeurosis inserts on the distal phalanx of the big toe. This structure experiences increased tension at higher walking velocities (Caravaggi et al., 2010), which is in line with the increased force under the big toe observed here. The contribution of structures other than FHL is reflected by the finding that FHL muscle activity and force under the big toe did not correlate perfectly in this study.

In the isometric conditions, the FHL/SOL ratio at 20 Nm was significantly higher than at higher isometric torque levels. However, absolute FHL activity increased gradually with increasing torque level, suggesting that soleus may not contribute substantially to plantarflexion torque at low torque levels. It should be noted that the FHL/SOL ratio was about two times greater in isometric tasks than in walking, whereas FHL/MG and FHL/LG were twofold higher in walking than in sustained isometric plantarflexions. Otherwise, FHL activity relative to the individual triceps surae muscles did not change generally across torque levels nor with increasing walking speed. These results highlight the task-dependent function of FHL, which is likely related to differences in joint mechanics between tasks.

It should be noted that the validity of FHL surface EMG measurement has not been examined. Due to the relatively small size of FHL, cross-talk from the soleus muscle may be present. To minimise this possibility, we defined the placement of the electrodes precisely and decreased the inter-electrode distance to 16 mm (Fig. 1). We followed the protocol of Bojsen-Møller et al. (2010) who visually demonstrated that cross-talk can be minimised using this protocol (see Fig. 3 in their paper). Another issue that may affect FHL EMG data is muscle belly displacement relative to the EMG electrodes, which has not been investigated previously. As shown in the Supplementary video that accompanies this paper, movement of the muscle–tendon junction is minimal both in maximal isometric toe flexion in a neutral position and in simulated push-off tasks. Thus, relative displacement between muscle and electrodes would have had a minimal effect on our EMG signals. Previous cadaver studies (Hofmann et al., 2013; Kirane et al., 2008) also indicate that the FHL muscle belly acts isometrically in simulated walking. Although we were as careful as possible while determining electrode placement, the assumption that cross-talk from neighbouring muscles has a minimal effect on FHL surface EMG during walking should be tested in the future. In any case, due to individual anatomical differences (Pichler et al., 2005), we believe that ultrasound evaluation of the correct placement is necessary.

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.jbiomech.2015.05.033

4. Conclusion

Our results show for the first time how force under the big toe and FHL EMG activity vary over different isometric plantarflexion levels and different speeds of locomotion. We found that FHL activity generally increased in parallel with the force under the big toe during isometric plantarflexion and walking. The contribution of big toe flexion to the ground reaction force also increased with increasing walking speed, indicating an increase in the relative importance of this muscle at faster speeds.

Conflict of interest statement

None of the authors have any conflict of interest to declare.

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jbiomech.2015.05.033

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II

IN VIVO FASCICLE BEHAVIOR OF THE FLEXOR HALLUCIS LONGUS MUSCLE AT DIFFERENT WALKING SPEEDS

by

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Title: In vivo fascicle behaviour of the flexor hallucis longus muscle at different walking speeds

Running head: FHL mechanics in walking

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ABSTRACT

Ankle plantar flexor muscles support and propel the body in the stance phase of locomotion. Besides the triceps surae, flexor hallucis longus muscle (FHL) may also contribute to this role, but very few in-vivo studies have examined FHL function during walking. Here we investigated FHL fascicle behaviour at different walking speeds. Ten healthy males walked overground at three different speeds while FHL fascicle length changes were recorded with ultrasound and muscle activity was recorded with surface electromyography (EMG). Fascicle length at heel strike, at toe off and at peak EMG activity did not change with speed. Range of FHL fascicle length change (3.5-4.5 and 1.9-2.9 mm on average in stance and push-off phase, respectively), as well as minimum (53.5-54.9 and 53.8-55.7 mm) and maximum (58-58.4 and 56.8-57.7 mm) fascicle length did not change with speed in the stance or push-off phase. Mean fascicle velocity did not change in the stance phase, but increased significantly in the push-off phase between slow and fast walking speeds ($P=0.021$). EMG activity increased significantly in both phases from slow to preferred and preferred to fast speed ($P<0.02$ in all cases). FHL muscle fascicles worked near-isometrically during the whole stance phase (at least during slow walking) and operated at approximately the same length at different walking speeds. FHL and medial gastrocnemius (MG) have similar fiber length to muscle belly length ratios and, according to our results, also exhibit similar fascicle behaviour at different walking speeds.

KEYWORDS: flexor hallucis longus mechanics, human locomotion, ultrasonography

INTRODUCTION

During the stance phase of human walking, ankle plantar flexor muscles support the body, and help to propel it during late stance (Winter, 1983; Kepple et al., 1997; Neptune et al., 2001).

Triceps surae muscle function in walking has been widely examined, but the deep plantar flexor muscles such as flexor hallucis longus (FHL) may also significantly contribute to this effort. Despite the relatively small anatomical cross-sectional area of FHL, its physiological cross-sectional area (PCSA), which is related to force production capacity (Blazevich et al., 2009), is significant, at around 70% of that of the lateral gastrocnemius (Fukunaga et al., 1992; Ward et al., 2009). FHL and flexor digitorum longus (FDL) together also constitute around 10% of the PCSA of all lower leg muscles (Wickiewicz et al., 1983), with around two times higher PCSA in FHL compared to FDL (Fukunaga et al., 1992). FHL contributes to load transmission from the shank to the ground during locomotion (Ferris et al., 1995), and its significant contribution to propulsion is reflected by the large forces acting along the FHL tendon during walking (52% body mass; (Jacob, 2001)).

One factor known to affect muscle function (and kinematics) in locomotion is the speed of movement (e.g. Lelas et al., 2003). Modelling studies have predicted that increased walking speed requires increased work from the plantar flexor muscles (Neptune et al., 2008). This is supported by in vivo measurements showing that increasing walking speed is related to higher ankle joint torque, increased muscle activity and altered fascicle behaviour of the soleus (SOL) (Lai et al., 2015) and medial gastrocnemius (MG) (Farris & Sawicki, 2012; Cronin et al., 2013a) muscles. Cronin et al. (2013a) found that shortening velocity in the push-off phase increases in MG but not in SOL muscle with increasing walking speed, changing the relative contribution of these muscles to propulsion. Similar to the calf muscles, FHL is also active in the stance phase of walking (Fujita, 1985; Perry, 1992), and exhibits increasing muscle activity and force under the big toe in the push-off phase with increasing walking speed (Péter et al., 2015). Thus, it might be expected that FHL also shows speed-dependent mechanical muscle function, that is, fascicle length changes, which could have important implications for force production and economy.

According to the results of cadaver studies (Kirane et al., 2008; Hofmann et al., 2013), FHL functions near-isometrically during simulated walking, as is the case for MG and SOL muscles (Cronin & Finni, 2013; Cronin & Lichtwark, 2013). However, to date, no in vivo studies of FHL mechanical behaviour during locomotion have been conducted to the best of our knowledge. Thus, in this study we used ultrasound to investigate FHL fascicle length changes in vivo during overground walking at different speeds. It was hypothesized that the range of FHL fascicle length change would be relatively small in the stance phase of walking, and that increased walking speed would result in increased fascicle velocity.

MATERIALS AND METHODS

Participants

Twenty healthy male individuals with no history of neuromuscular disorder or injury gave their written consent to participate in this study. Ultrasound data were obtained from ten participants (age 24.7±3.5 years; height 177.7±3.9 cm; body mass 71.9±4.9 kg) and included in this paper. The ethics committee of the University of Jyväskylä approved the study protocol. All procedures were in line with the Declaration of Helsinki.

Study protocol

After some familiarization trials, participants walked at three different speeds over two 10 m custom-made force platforms (University of Jyväskylä, Finland; sampling frequency 1000 Hz) positioned parallel to each other. Force plate data were sampled to define step cycle phases and photocells were placed at the beginning and end of the force platforms to determine walking speeds. Trials began 3 meters before and ended 2 meters after the force plates to ensure steady speed during the recording period. Firstly, participants performed 5 trials at their preferred speed. The slowest and fastest trials were discarded and the remaining three trials were averaged to yield preferred walking speed. Participants then performed faster and slower walking trials where the target times were 30% faster and 30% slower than at preferred speed, respectively. For each speed, at least 3 trials were obtained that were within ± 5% of the target time. Faster and slower walking trials were completed in a random order. During walking trials, FHL fascicle lengths were measured with ultrasound and muscle activity was recorded with surface electromyography (EMG). Ankle angle was measured with a custom-made electro-goniometer (University of Jyväskylä, Finland) for visualization purposes (Figure 2 and 3). For all participants, the right leg was tested.

Methodology and analysis

Ultrasound

A personal computer-based portable ultrasound system (EchoBlaster 128; Telemed, Vilnius, Lithuania) was used with a 96-element linear probe (B-mode; 7 MHz, 60 mm transducer field width, 80 Hz sampling frequency), which was placed over the posterior site of the right leg to estimate FHL fascicle length change during walking. The ultrasound probe was firmly fixed over the mid-belly of the FHL muscle (Figure 1A), placed so that the probe was aligned with the FHL muscle fascicles to minimize perspective and parallax measurement errors. A flat-shaped probe was used to minimize probe rotation. Before starting the measurements, image quality was tested during big toe flexion and calf raises during standing. The probe was connected to a portable ultrasound unit (5 kg), which was carried by an assistant during walking. The assistant walked next to the force plate and approximately one meter behind the participant to avoid influencing their performance. Participants reported no discomfort or disturbance due to the ultrasound probe or the cable during the measurements. To synchronize data collection with other sources, a digital output signal was sent from the ultrasound system to Spike2 software (Cambridge Electronic Design, Cambridge, UK), where force and EMG signals were also recorded.

A previously validated automated fascicle tracking algorithm using Lucas-Kanade optical flow and affine optic flow extension was used to determine FHL muscle fascicle length change during walking tasks (Cronin et al., 2011; Gillett et al., 2013; Farris & Lichtwark, 2016). The region of interest (Fig 1B) was between the deep and middle aponeuroses of the muscle since it was thicker than the more superficial part, as reported previously (Mickle et al., 2013). Thus, analysis error may also be relatively small in this region due to the longer fascicles. The initial and end points of a straight line from the deep to the middle aponeurosis were assigned parallel to the lines of collagenous tissue visible on the appointed ultrasound frame, and used to represent fascicle length. A typical example of the raw fascicle length changes at different walking speeds is shown in Figure 2.

Fascicle length data were smoothed with a 20-Hz fourth-order Butterworth low-pass filter in MATLAB (MathWorks Inc, Natick, MA, US). A total of 244 steps were analysed: 94, 70 and 80 for slow, preferred and fast walking trials, respectively. The stance phase of each cycle was analysed since FHL is active in this phase of the step cycle, but not in swing (Perry, 1992; Zelik et al., 2015). The push-off phase was also separately analysed, since plantar flexor muscles play a significant role in body propulsion in this phase (Neptune et al., 2001).

Initial fascicle length was defined as the fascicle length at heel strike. Minimum and maximum fascicle length were calculated in the stance and push-off phases separately, and range of fascicle length change was calculated as the difference between the minimum and maximum lengths. Fascicle length at toe-off and at the time of peak EMG activity were also calculated. Mean fascicle velocity was calculated by averaging the first derivation of fascicle length change data within stance and push-off separately. Values within a participant and walking speed were averaged and included in the statistical analysis.

EMG

After shaving and abrading the skin and wiping with alcohol, bipolar surface EMG silver-silver chloride Ambu BlueSensor N electrodes (Ambu A/S, Ballerup, Denmark) with an inter-electrode distance of 16 mm (Masood et al., 2014) were located over the FHL muscle belly on the medial side of the leg between the soleus insertion and the FHL muscle-tendon junction, where only the FHL muscle belly lies (Fukunaga et al., 1992). Proper placement was determined using ultrasonography (detailed description in (Péter et al., 2015)). Signal quality was confirmed during isolated ankle and big toe plantar flexion. A wireless EMG system (Noraxon Inc. Scottsdale, AZ, USA) was used to sample data at a frequency of 1500Hz. Data were sent to an A/D converter (Cambridge Electronic Design, Cambridge, UK) and recorded in Spike2 software.

Raw EMG data were band-pass filtered between 20 and 450 Hz using a fourth-order zero lag Butterworth filter in MATLAB. Root mean square (RMS) values were calculated for the stance and push-off phases for each step, and averaged within a participant and task. The peak dynamic method was used to normalize EMG (Burden, 2010) to the peak activity of preferred-speed walking (Cronin et al., 2015) in order to decrease inter-individual variability. Time of peak activity relative to stance phase duration was calculated based on the smoothing procedure used with the peak dynamic method.

Statistical analysis

Statistical analysis was executed using SPSS software (IBM, New York, NY, USA). Shapiro-Wilk's W test was applied to determine the normality of data distribution. One-way repeated-measures ANOVA was used to test differences between variables across speeds. Where the assumption of sphericity – determined by Mauchly's test – was violated, Greenhouse-Geisser adjustment was applied. Where significant main effects were found, Bonferroni post hoc test was performed to define the location of differences (e.g. between slow and fast, $_{SF}$; slow and preferred, $_{SP}$). For all tests, $p<.05$ was set as a minimum level of statistical significance.

RESULTS

The number of analysed steps and their characteristics are shown in Table 1. Figure 3 shows the group mean FHL fascicle behaviour and EMG activity at the three different speeds of walking.

Fascicle length at heel strike, at toe off and at peak EMG activity did not change with speed. The timing of peak activity relative to stance phase duration did not change (72.7±3.3%, 72.7±3.1% and 70.4±3% at slow, preferred and fast walking speeds, respectively).

Stance phase

The range of fascicle length change, as well as minimum and maximum lengths, and mean fascicle velocity did not change significantly with increasing speed (Table 2).

Flexor hallucis longus RMS EMG activity increased significantly with increasing walking speed, with mean values of 31±6%, 44±8% and 77±22% at slow, preferred and fast walking speeds, respectively ($F=43.622$, $P_{SP}=.003$, $P_{SF}=.001$, $P_{PF}=.001$).

Push-off phase

Range of fascicle length change, minimum and maximum length did not change with increasing speed. Mean fascicle velocity increased, with a significant difference between slow and fast walking speeds ($F=9.038$, $P_{SF}=.021$) (Table 2).

Flexor hallucis longus RMS activity increased significantly with increasing walking speed in the push-off phase, with mean values of 41±1%, 60±16% and 105±39% at slow, preferred and fast walking speeds, respectively ($F=28.752$, $P_{SP}=.011$, $P_{SF}=.001$, $P_{PF}=.002$).

DISCUSSION

In this study we investigated FHL fascicle behaviour in vivo at different speeds of overground walking. Concurring with our hypotheses, we found that the range of FHL fascicle length change was relatively small in the stance phase of walking, and that FHL fascicle velocity increased with increasing walking speed. Moreover, we observed a relatively constant FHL operating range across all speeds, whereas FHL EMG activity increased significantly with speed in both the stance and push-off phases. These findings suggest that FHL exhibits similar neuromechanical behaviour to the MG muscle during walking.

Flexor hallucis longus muscle arises from the distal 2/3 of the fibula and the membrana interossea and inserts on the distal phalanx of the big toe, while MG originates from the medial condyle of the femur and inserts on the posterior surface of the calcaneus (Gray, 1980). Although MG originates above the knee and FHL below the knee and they insert on different places, both plantar flexor muscles cross more than one joint. The muscle belly length of FHL has been reported to be 26.9±3.6 cm, compared with 26.9±4.7 cm in MG (Ward et al., 2009). FHL and MG also have similar fibre lengths, 5.3±1.3 cm and 5.1±1 cm respectively (Ward et al., 2009). Thus, the fibre length to muscle belly length ratio is small in both muscles (0.20 and 0.19, respectively; (Ward et al., 2009)). This small ratio predicts near-isometric fascicle function, which is commonly reported in MG in the early stance phase of locomotion (e.g. Cronin & Finni, 2013; Fukunaga et al., 2001), and which we also observed in FHL in this study. In the push-off phase, MG fascicles typically shorten (Krishnaswamy et al., 2011; Farris & Sawicki, 2012), whereas in this study, FHL fascicles maintained a near-constant length during the entire stance phase of walking at the slow speed, while exhibiting a trend towards shortening at preferred and fast walking speeds.

It has been found that the kinematics of gait and muscle force requirements change with increasing speed (Lelas et al., 2003; Dubbeldam et al., 2010). For example, modelling studies predict that increasing walking speed requires increased work from the plantar flexor muscles (Neptune et al., 2008), and some in vivo mechanical data support this suggestion. With increasing walking speed (0.7, 1.4, 2.0 m/s), Lai et al. (2015) found higher ankle joint torques, increased SOL muscle activity and reduced SOL fascicle length. In MG, Farris & Sawicki (2012) examined fascicle length changes at four different walking speeds (0.75, 1.25, 1.75 and 2.0 m/s), and found relatively similar length changes at all speeds, but shortening velocity increased with increasing speed. Similarly, in our study of FHL fascicle behaviour, we found no changes in fascicle length at heel strike or in the range of FHL lengths with increasing walking speed, but an increase in mean fascicle velocity in the push-off phase with increasing walking speed. As FHL fascicle behaviour has not been examined so far, mean fascicle velocity was calculated to provide an overall picture about its mechanical behaviour. However, this limits the capability to compare our results to previous findings where shortening velocity was calculated separately. Although a significant increase in mean fascicle velocity in the direction of shortening implies a similar response to increased walking speed in the FHL in this study as MG and SOL (Farris & Sawicki, 2012; Lai et al., 2015), the results are not quantitatively comparable. We also found that FHL EMG activity increased significantly with increasing speed, which is in agreement with the results of previous studies of plantar flexor muscles (Neptune et al., 2008; Lai et al., 2015; Péter et al., 2015; Maharaj et al. 2016). These speed-dependent findings collectively highlight further similarities between the function of FHL and triceps surae muscles.

Previous cadaver studies (Kirane et al., 2008; Hofmann et al., 2013) examined FHL tendon excursion and found it to be small (7.2 and 6.6 mm on average, respectively) relative to muscle fibre length (52.7 mm; (Ward et al., 2009)) during simulated walking. We have previously shown in vivo that movement of the FHL muscle-tendon junction is minimal during simulated push-off tasks (see Péter et al., 2015 supplementary video). In the present study we found a relatively small range of FHL fascicle length change both in stance and push-off, and a constant operating length across walking speeds. Although these findings collectively imply near-isometric function of the muscle fascicles/belly, we are lack of information regarding the behaviour of the long distal tendon of FHL. During the push-off phase of locomotion as the heel leaves the ground, the ankle joint plantarflexes and the first metatarsophalangeal joint dorsiflexes (e.g. Leardini et al., 1999). These opposing joint movements predict near-isometric function of the FHL muscle-tendon unit. However, it is well known that joint displacement parameters do not reflect the contraction mode of the muscle itself, especially for muscles with long tendons (e.g. Cronin et al., 2013b), as is the case for FHL. Furthermore, length change of the foot during walking (Stolwijk et al., 2014) and FHL lever arm at the crossed joints also affect muscle-tendon length change in response to joint angular displacements. Future studies are needed to examine fascicle and muscle-tendon unit length changes, taking all relevant variables into account, to determine whether muscle-tendon decoupling is similar to the triceps surae, or FHL tendon rather serves as a stiff force transducer in walking.

In conclusion, the results of this in vivo study show that FHL muscle fascicles work at a near-constant length during the whole stance phase at slow walking speeds, with a trend towards shortening with increasing walking speeds. Furthermore, mean fascicle velocity increased significantly with increasing speed, as did EMG activity. Thus, these results suggest that FHL and MG muscles exhibit similar neuromechanical behaviour with increasing walking speed.

PERSPECTIVES

In this study, FHL fascicle behavior during walking was determined with ultrasonography in healthy people. We found a relatively small range of FHL fascicle length changes in the whole stance phase of walking as well as increased fascicle velocity and EMG activity with increasing walking speed. The possibility of measuring FHL fascicle behaviour with this method may make it feasible to examine in vivo the mechanical consequences of altered FHL morphology, for example in flat-footed people (Angin et al., 2014), a disorder that increases the risk of overuse injuries in the lower leg. It may also be possible to study changes in the mechanics of FHL muscle in people with Achilles tendon rupture (Finni et al., 2006, Masood et al. 2014), contributing to understand load sharing mechanisms between plantar flexor muscles.

GRANTS

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest.

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TABLES, FIGURE LEGENDS AND FIGURES

Figure 1. Ultrasound probe placement. Panel A represents ultrasound probe position. The probe was fixed with tape and an elastic bandage to minimize its movement relative to the skin. Proper placement was determined as the orientation in which the probe was parallel to the flexor hallucis longus (FHL) fascicles, based on scanning before fixing the probe. Panel B shows an ultrasound image of FHL fascicles during standing. The region of interest and one FHL fascicle are marked with a dashed line and solid line, respectively. FHL-S, FHL superficial region; FHL-D, FHL deep region

Figure 2. Typical example of flexor hallucis longus (FHL) fascicle and electromyography (EMG) behaviour from one participant. Each curve within a subfigure represents one step. Curves begin with heel contact (HC). Dotted lines represent the start of the push-off phase and dashed lines the time of toe off (TO). For better visualization, EMG data were smoothed with a 100-ms root-mean-square moving window, and then consequent right shifts of the curves were corrected. Ankle angle represents the sagittal plane rotation of the ankle joint based on electro-goniometer data, where 0° represents the angle in standing, positive indicates plantarflexion, and negative indicates dorsiflexion. GRF, ground reaction force

Figure 3. Flexor hallucis longus (FHL) fascicle and electromyography (EMG) behaviour of all participants. Group mean and standard deviation data (N=10). Fascicle length and ankle angle are normalized to the length and angle at heel contact, respectively. FHL EMG activity is expressed as a percentage of peak activity in preferred-speed walking (%PW_{peak}). Note that for FHL activity, error bars do not only represent individual differences because of the normalization method that was used. For better visualization, EMG data were smoothed with a 100-ms root-mean-square moving window, and then consequent right shifts of the curves were corrected. Ground reaction forces (GRF) are normalized to body mass. Ankle angle represents the sagittal plane rotation of the ankle joint based on electro-goniometer data. Curves begin with heel contact (HC). Dotted lines represent the start of the push-off phase and dashed lines the time of toe off (TO).

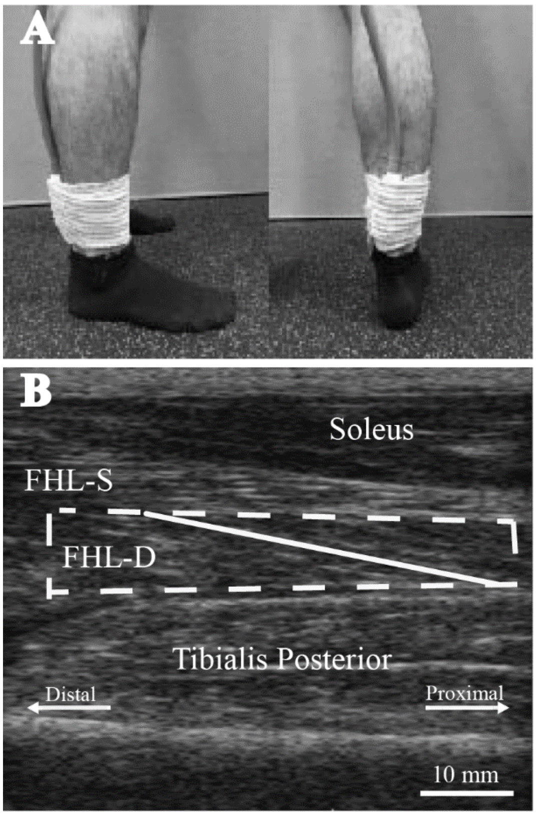

Figure 1

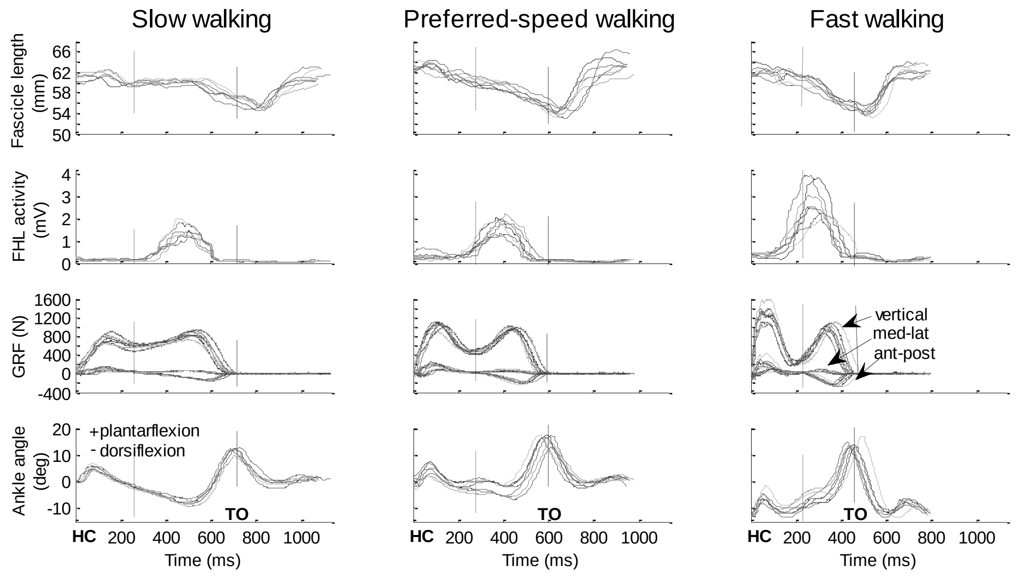

Figure 2

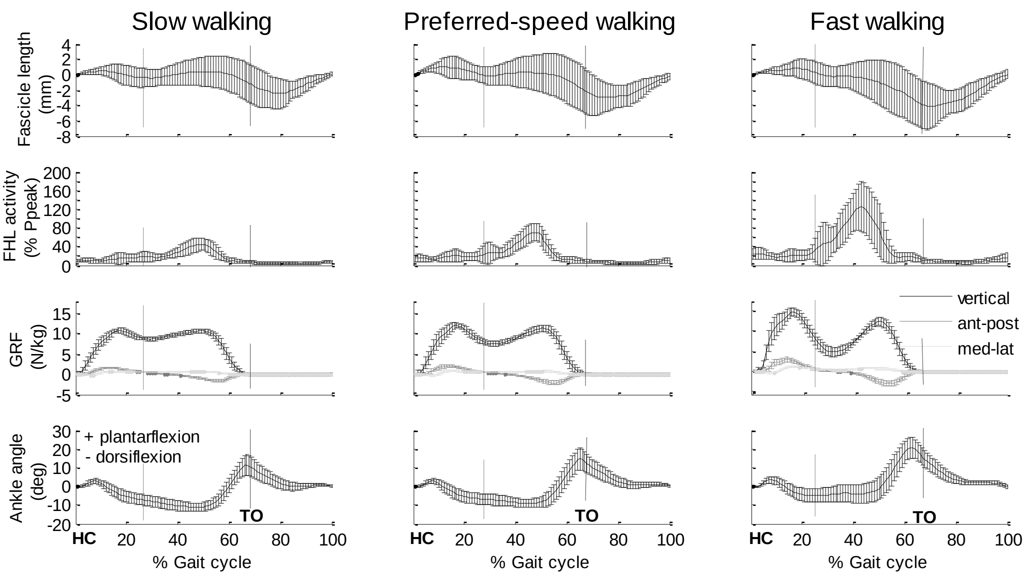

Figure 3

Table 1. Characteristics of the analysed steps

	Slow	Preferred	Fast
Steps (n)	9 ± 4	7 ± 5	8 ± 2
Speed (m/s)	1.06 ± 0.08	1.37 ± 0.1	1.97 ± 0.19
Step cycle duration (s)	1.22 ± 0.07*P,F	1.07 ± 0.05*S,F	0.91 ± 0.05*S,P
Stance phase duration (s)	0.73 ± 0.04*P,F	0.63 ± 0.03*S,F	0.52 ± 0.04*S,P
Swing phase duration (s)	0.49 ± 0.04*P,F	0.44 ± 0.02*S,F	0.39 ± 0.02*S,P
Push-off phase duration (s)	0.30 ± 0.03*P,F	0.26 ± 0.02*S,F	0.22 ± 0.03*S,P
Stance phase (% of step cycle)	60 ± 2*F	59 ± 1*F	57 ± 1*S,P
Swing phase (% of step cycle)	40 ± 2*F	41 ± 1*F	43 ± 1*S,P
Push-off phase (% of step cycle)	25 ± 3	25 ± 2	24 ± 2

Number of included steps is expressed as median ± interquartile range. Other values are mean ± SD.
* different from preferred (P), fast (F) or slow (S) walking speed ($p<.05$).

Table 2. Characteristics of flexor hallucis longus fascicle behaviour in walking

		Slow	Preferred	Fast
	Fascicle length at heel strike (mm)	56.7 ± 5.7	56 ± 6.1	56.5 ± 7.7
	Fascicle length at toe off (mm)	56.2 ± 5.7	55.2 ± 6.9	55.6 ± 8.1
	Fascicle length at peak EMG activity (mm)	57.1 ± 6.1	56.1 ± 6	56.2 ± 7.8
Stance	Minimum fascicle length (mm)	54.9 ± 5.1	54 ± 5.9	53.5 ± 6.9
	Maximum fascicle length (mm)	58.4 ± 6.1	58 ± 6.4	58 ± 7.7
	Range of fascicle length change (mm)	3.5 ± 1.3	3.9 ± 1.4	4.5 ± 1.8
	Mean fascicle length change velocity (mm/s)	-0.2 ± 3.2	-1.2 ± 4.9	-4.4 ± 6.6
Push-off	Minimum fascicle length (mm)	55.7 ± 5.5	54.6 ± 5.6	53.8 ± 7
	Maximum fascicle length (mm)	57.7 ± 6.2	57 ± 6.1	56.8 ± 7.7
	Range of fascicle length change (mm)	1.9 ± 1.2	2.3 ± 0.9	2.9 ± 1.4
	Mean fascicle length change velocity (mm/s)	0.3 ± 5.7 *F	-2.8 ± 8.1	-9.4 ± 11.9 *S

F and S denote fast and slow walking speeds, respectively.
Values are mean ± SD.
* $p<.05$.

III

COMPARING SURFACE AND FINE-WIRE ELECTROMYOGRAPHY ACTIVITY OF LOWER LEG MUSCLES AT DIFFERENT WALKING SPEEDS

by

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Comparing Surface and Fine-Wire Electromyography Activity of Lower Leg Muscles at Different Walking Speeds

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Ankle plantar flexor muscles are active in the stance phase of walking to propel the body forward. Increasing walking speed requires increased plantar flexor excitation, frequently assessed using surface electromyography (EMG). Despite its popularity, validity of surface EMG applied on shank muscles is mostly unclear. Thus, we examined the agreement between surface and intramuscular EMG at a range of walking speeds. Ten participants walked overground at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) while surface and fine-wire EMG activities of flexor hallucis longus (FHL), soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG), and tibialis anterior (TA) muscles were recorded. Surface and intramuscular peak-normalised EMG amplitudes were compared for each muscle and speed across the stance phase using Statistical Parametric Mapping. In FHL, we found differences around peak activity at all speeds except fast. There was no difference in MG at any speed or in LG at slow and preferred speeds. For SOL and LG, differences were seen in the push-off phase at fast and maximum walking speeds. In SOL and TA, surface EMG registered activity during phases in which intramuscular EMG indicated inactivity. Our results suggest that surface EMG is generally a suitable method to measure MG and LG EMG activity across several walking speeds. Minimising cross-talk in FHL remains challenging. Furthermore, SOL and TA muscle onset/offset defined by surface EMG should be interpreted cautiously. These findings should be considered when recording and interpreting surface EMG of shank muscles in walking.

Keywords: bipedal locomotion, ankle plantar flexor muscles, surface electromyography, EMG, intramuscular electromyography

Abbreviations: 95% CI, 95% confidence intervals; EMG, electromyography; FHL, flexor hallucis longus; HC, heel contact; LG, lateral gastrocnemius; MG, medial gastrocnemius; SOL, soleus; SPM, Statistical Parametric Mapping; TA, tibialis anterior; TO, toe-off.

INTRODUCTION

Electromyography is often used in research and clinical environments to examine muscle excitations in normal and pathological conditions. There are two predominant forms of EMG measurement; surface and intramuscular EMG (Farina and Negro, 2012). Non-invasive surface EMG is widely used for superficial, large, and easily accessible muscles. With surface EMG, excitation level is acquired from a large area including several motor unit populations (Roy et al., 1986). Despite its popularity and relatively simple use, surface EMG has some inherent limitations. For example, selective recordings from deep muscles are not possible. Furthermore, due to the relatively large pick-up area of the electrodes, unwanted signals can be recorded from adjacent or deep muscles (Perry et al., 1981; Dimitrova et al., 2002; Lowery et al., 2003), an error source termed cross-talk. Cross-talk needs to be rigorously considered since it may lead to the misinterpretation of the recorded EMG signal (De Luca, 1997). General suggestions for minimising cross-talk include proper surface electrode location (Roy et al., 1986), and proper electrode size and inter-electrode distance (Merletti et al., 2001). Proper surface electrode location is also important since electrodes located close to the innervation zone or the tendon region may cause large signal amplitude variation (Sadoyama et al., 1985; Roy et al., 1986; Rainoldi et al., 2000, 2004; Farina et al., 2001; Merletti et al., 2001). Furthermore, during muscle contractions the muscle moves under the skin and the electrodes, which may also have considerable effects on the recorded surface EMG signal (De Luca, 1997; Rainoldi et al., 2000; Farina et al., 2001).

Intramuscular EMG is an invasive method and is therefore seldom used. It is primarily used to study deep muscles (Andersson et al., 1997; Õunpuu et al., 1997; Onishi et al., 2000), and muscles that have a small cross-sectional area (Andersson et al., 1997; Sutherland, 2001). Furthermore, special skills are required to insert the electrodes, and this process takes longer compared to the placement of surface electrodes (Õunpuu et al., 1997). A major advantage of intramuscular EMG compared to surface EMG is that it is suitable to selectively detect EMG signals of a muscle during static and dynamic conditions, while minimising cross-talk (Perry et al., 1981; De Luca and Merletti, 1988; De Luca, 1997; Onishi et al., 2000). By inserting fine wires intramuscularly, the EMG recording wires will follow the movement of the muscle underneath the skin during dynamic contractions (e.g., in walking) (Hodges and Gandevia, 2000; Chapman et al., 2010). Since it does not seem to affect human gait patterns (Winchester et al., 1996), this method can be used to detect EMG activity in human walking.

Human walking is a common movement requiring substantial work from lower leg muscles, especially in the stance phase. Therefore, recording EMG activity from different muscles of the lower leg with high accuracy is of primary interest in gait studies. Since both surface and intramuscular EMG methods aim to examine muscle excitation timing and amplitude, previous studies have used intramuscular EMG to validate the idea that surface EMG signals contain no or negligible cross-talk (i.e., measure from the target muscle explicitly) (Kadaba et al., 1985; Bogey et al., 2000, 2003). These studies focused on the SOL and TA muscles only, explicitly measured during gait at self-selected speed. However, the speed of locomotion seems to affect muscle function and kinematics (Lelas et al., 2003; Cronin et al., 2013). It is assumed that increased walking speed requires increased ankle plantar flexor work (Neptune et al., 2008). The ankle plantar flexors' relative contribution to propulsion also changes with speed besides a general increase in surface EMG activity in these muscles (Cronin et al., 2013). To the best of our knowledge, there is no study yet in which the validity of the surface EMG method was examined for lower leg muscles apart from SOL and TA across a range of walking speeds.

Thus, in this study we simultaneously recorded surface and intramuscular EMG activity from flexor hallucis longus (FHL), SOL, MG, LG, and TA muscles at different walking speeds to examine whether EMG signals recorded with the two methods show differences in EMG amplitude at any point across the stance phase of walking. We hypothesised that there would be no difference between surface and intramuscular EMG activity of FHL, SOL, MG, LG, and TA muscles at relatively slow walking speeds, but disagreement between these methods would appear at faster speeds.

MATERIALS AND METHODS

Participants

After convenience-based sampling, 10 healthy, physically active individuals (six males, four females; age 29.6 ± 7.4 years, height 174.0 ± 12.5 cm, body mass 70.6 ± 12.7 kg, body mass index: 23.14 ± 1.7 m^{-2} kg) without history of neuromuscular disorders or previous/current leg injuries gave written consent to participate in this study. The study was approved by the Stockholm regional ethics committee (Approval No.: 2017/261-31/4) and was performed in agreement with the Declaration of Helsinki.

Study Protocol

Participants first attended a familiarisation session where they were acquainted with the study protocol and surface EMG electrode locations were marked with permanent pen (**Figure 1A**). The main testing session was 1–3 days later, where after standardised warm-up in a dynamometer (submaximal and maximal isometric plantar flexion and dorsiflexion contractions) and 5 min preferred-speed walking, data collection started. First, participants walked at their self-selected steady speed along the measurement area five times in typical cushioned running shoes while surface and intramuscular EMG activities from the right shank and ground reaction forces were recorded. Out of the five trials, the slowest and fastest trials were excluded and the speeds of the remaining three trials were averaged to define preferred walking speed of each individual. Then they walked at three randomly ordered speeds, which were 30% slower and 30% faster than preferred walking speed (within ±5% of target speed), and maximum walking speed where participants were asked to walk as fast as they could. Three successful trials were recorded at each speed. Overground walking trials started and terminated 2–3 m before and after the measurement area, respectively, to aim for a steady speed over the 7 m measurement area. To define walking speeds, custom-made photocells were installed at the beginning and end of the measurement area. Participants reported no discomfort and their walking pattern seemed normal during the recordings.

Instrumentation

Surface and Intramuscular EMG Activity

After shaving, lightly abrading, and cleaning the skin with alcohol, silver–silver chloride Ambu BlueSensor N (Ambu A/S, Ballerup, Denmark) surface electrodes were put on the examined muscles in bipolar configuration (**Figure 1B**). FHL electrodes were placed behind the medial malleolus where the muscle belly was superficial as defined by ultrasonography (Echo Blaster 128; Telemed, Vilnius, Lithuania) [detailed description in Péter et al. (2015)]. For MG, LG, and TA muscles, the SENIAM recommendations were followed with slight adjustments if individual-specific muscle morphologies required. According to SENIAM (Hermens et al., 2000), SOL surface electrodes are recommended to be placed on the medial side of the shank at two-third of the line between medial femur condyle to medial malleolus. However, it has been shown previously that surface electrodes placed as recommended were prone to register activity from muscles other than SOL during self-selected speed walking (Bogey et al., 2000). Therefore, in this study we placed SOL surface electrodes laterally, at the same proximal–distal location. For FHL, inter-electrode distance was decreased to 16 mm to minimise cross-talk (Bojsen-Moller et al., 2010; Masood et al., 2014), while inter-electrode distance for other muscles was 22 mm. Furthermore, we also measured the distance between SOL insertion and distal FHL muscle tendon junction to examine if the magnitude of the space where surface electrodes can be placed has any effects on the surface EMG activity compared to intramuscular EMG.

Intramuscular electrodes were inserted by an experienced radiologist (co-author HG) under real-time, high-resolution B-mode, and Doppler ultrasonography (Logiq E9, GE, United States) guidance. After cleaning the skin with alcohol, Teflon-coated seven-stranded silver hook-wire electrodes were inserted in bipolar configuration with an inter-tip distance of ≈5 mm (wire diameter 0.25 mm, stripped length of 2 mm forming the recording surface, previously sterilised in an autoclave), two in each muscle with hypodermic needles (diameter 0.8 mm, after wire insertion the needles were carefully withdrawn). The recording ending of the intramuscular EMG electrodes for SOL, MG, LG, and TA muscles were inserted underneath the surface electrodes. FHL intramuscular electrodes were inserted 5–10 cm proximal to the surface electrodes, on the lateral side of the shank, depending on the thickness of the muscle belly and vascularisation (**Figure 1A**).

Surface and intramuscular EMG activity of all muscles was simultaneously recorded using a telemetric system (MyoSystem 1400A, Noraxon Inc., Scottsdale, AZ, United States) with a sampling frequency of 3000 Hz. Signals were transmitted wirelessly to an A/D converter (Cambridge Electronic Design, Cambridge, United Kingdom) that was connected to a personal computer. Digital signals were collected and visualised online in Spike2 software (Cambridge Electronic Design, Cambridge, United Kingdom). A single surface reference electrode (silver–silver chloride Ambu BlueSensor N, Ambu A/S, Ballerup, Denmark) was attached to the skin over the medial aspect of the tibia bone.

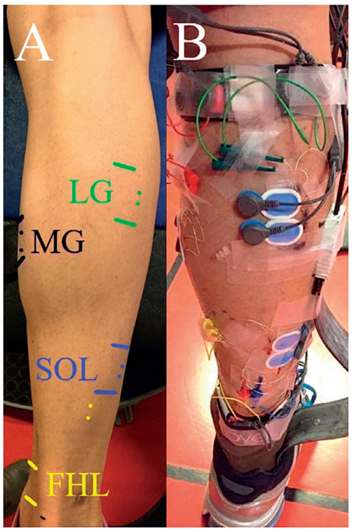

FIGURE 1 | (A) The locations of the surface and intramuscular electrodes on the investigated leg's flexor hallucis longus (FHL), soleus (SOL), and medial and lateral gastrocnemii (MG and LG). Surface electrodes were placed between the horizontal lines; intramuscular electrodes were inserted approximately where the dots are shown. **(B)** The investigated leg with the surface and intramuscular electrodes attached. Tibialis anterior (TA) was also measured (not shown).

Gait Events

A plantar pressure insole was placed in the right shoe to define the timing of HC and TO for all steps (Pedar-X 99-sensor in-shoe dynamic pressure measuring system, Novel Inc., Munich, Germany, 100 Hz sampling frequency). Data were sent to a personal computer via Bluetooth to record in the Pedar software. To ensure that insoles did not move in the shoes between trials, the big toe was pressed down by an assistant before each trial and we ensured that the same pressure sensors were activated this way (Péter et al., 2015).

Halfway along the walking distance of the measurement area, two 0.6 m × 0.4 m 3-D force platforms (Kistler type 9281EA, Kistler AG, Winterthur, Switzerland) were installed in series. Three-dimensional ground reaction force data were collected from the right leg, one stride per trial to define the start of the push-off phase. Walking trials were repeated if the participant's right leg did not hit at least one of the force platforms. Data were recorded with Qualisys Track Manager software (Qualisys AB, Sweden), with a sampling frequency of 3000 Hz.

To synchronise data acquisition a trigger signal was sent to the Spike software from the Pedar system at the start of each recording. This trigger signal was sent simultaneously to the Qualisys Track Manager software, which started recordings in this software.

Analysis

Gait Events

First, HC and TO timings were defined for each step over the measurement area in Matlab (MathWorks Inc., Natick, MA, United States) as follows. Vertical force as the sum of forces measured with all sensors of the insole was first extrapolated to 3000 Hz to coordinate with ground reaction force data. Then, HC and TO were defined for the steps on the force plates based on a 10 N vertical ground reaction force threshold (Osis et al., 2016). The timings based on ground reaction force helped to define thresholds for vertical forces measured with the insole to minimise errors due to potential mismatch between the size of the foot and the insole. Based on this subject- and task-specific threshold, HC and TO were defined for all steps from steady-speed trials within the measurement area (i.e., steps where force signals were recorded by pressure insoles only, as well as those recorded by the force plates). EMG activity was analysed between HC and TO for all steps as detailed below.

For the steps that occurred on force plates, push-off initiation was defined as the time when the anterior–posterior ground reaction force crossed 0 N and became an anterior, propulsive force, for visualisation (**Figures 2–6**). Sub-phases of the stance were defined as early stance (0–16.5%), mid stance (16.5–50%), late stance (50–83%), and pre-swing (83–100%) based on previous literature (Neptune et al., 2001), to assist data interpretation.

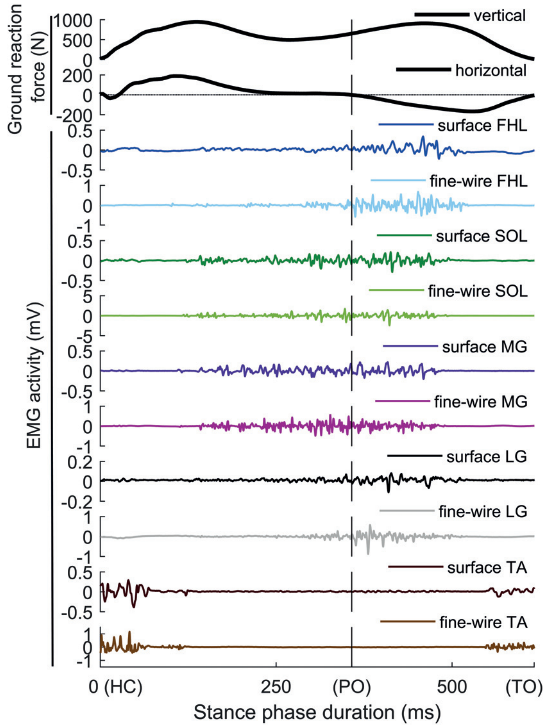

FIGURE 2 | Raw data from one step of one participant recorded during preferred speed walking. The top two panels show ground reaction forces (vertical and horizontal) measured with the force platform. The remaining panels show surface and intramuscular EMG activity of flexor hallucis longus (FHL), soleus (SOL), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tibialis anterior (TA). HC, heel contact; PO, push-off start; TO, toe off.

EMG Activity

Medial gastrocnemius surface EMG from one and LG surface EMG from another participant were not recorded due to technical issues. Additionally, one of the participants did not perform maximum walking speed trials. All other recordings were included in the analysis.

Electromyography signals of all muscles were analysed in the stance phase of the step cycles as defined above. EMG analyses were conducted in Matlab. Surface and intramuscular EMG signals were band-pass filtered between 20 and 500 Hz using a zero-lag 4th order Butterworth filter. The filtered and rectified signals were smoothed with a 10 Hz zero-lag low-pass filter (4th order Butterworth). Signals for each stance phase were time-normalised (1–101 frames) using linear interpolation. Subsequently, EMG signals were averaged within every walking condition for each participant and muscle. To decrease inter-individual variability, EMG signals were normalised to the peak activity of preferred speed walking (Cronin et al., 2015). These time- and peak-normalised signals were included in further analysis as detailed below.

Statistical Analysis

We used SPM (Friston, 2007) to statistically examine the difference between surface and intramuscular EMG amplitudes at each point of the time-normalised stance phase. SPM analysis was performed in Matlab using the open-source spm1d code (v.M0.1[1]). SPM two-tailed paired t-tests were used to compare surface and intramuscular EMG signal curves of all muscles during slow, preferred, fast, and maximum walking speeds as follows. Firstly, the scalar output statistic SPM{t} was calculated forming a Statistical Parametric Map. SPM{t} is a scalar trajectory variable, that shows the magnitude of differences between surface and intramuscular EMG signals. The magnitudes of the differences were also expressed as mean difference $\pm$ 95% CI. In order to test the null hypothesis, we calculated the critical threshold at which only α% (set to 5%) of smooth random curves would be expected to traverse. This critical threshold calculation is based on estimates of trajectory smoothness via temporal gradients (Friston, 2007) and, based on that smoothness, Random Field Theory expectations regarding the field-wide maximum (Adler and Taylor, 2007). EMG time-series were considered significantly different if any values of SPM{t}exceeded the critical threshold. In the final step, cluster specific p-values were calculated.

[1]www.spm1d.org

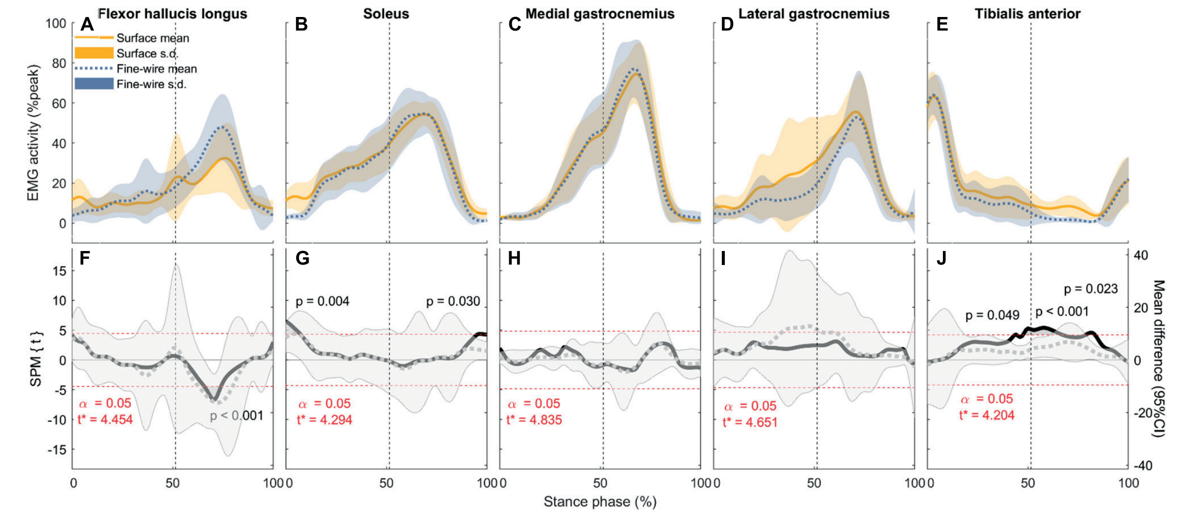

FIGURE 3 | Electromyography (EMG) activity (mean and standard deviation) of each muscle (**A–E**: normalised to peak activity in preferred speed walking, %peak) and the corresponding comparisons between surface and intramuscular EMG recordings **(F–J)** in the stance phase of slow walking (1.01 ± 0.13 m s^{-1}). Vertical dotted lines represent the beginning of push-off. Panels **(F–J)**: SPM{*t*} statistics for two-tailed *t*-tests (solid black lines, left *y*-axis), and mean difference with 95% confidence intervals (grey dotted lines with shaded areas). Dashed red lines are critical thresholds (*t**) calculated for α significance level defining supra-threshold clusters for SPM{*t*} trajectories. *P*-values were calculated for each supra-threshold cluster.

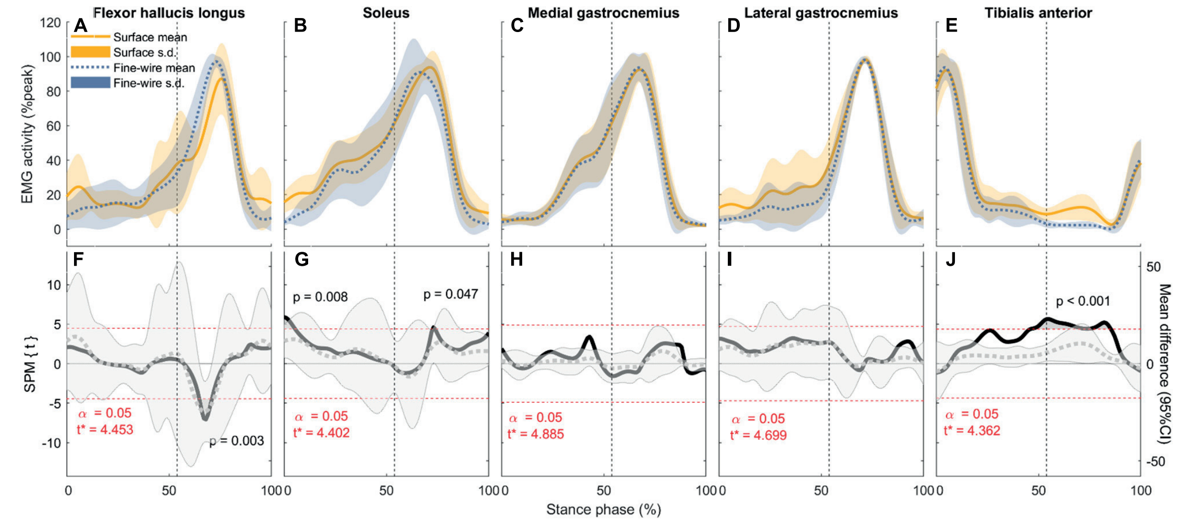

FIGURE 4 | Electromyography (EMG) activity (mean and standard deviation) of each muscle (**A–E**, normalised to peak activity in preferred speed walking, %peak) and the corresponding comparisons between surface and intramuscular EMG recordings **(F–J)** in the stance phase of preferred speed walking (1.43 ± 0.19 m s^{-1}). Vertical dotted lines represent the beginning of push-off. Panels **(F–J)**: SPM{*t*} statistics for two-tailed *t*-tests (solid black lines, left *y*-axis), and mean difference with 95% confidence intervals (grey dotted lines with shaded areas). Dashed red lines are critical thresholds (*t**) calculated for α significance level defining supra-threshold clusters for SPM{*t*} trajectories. *P*-values were calculated for each supra-threshold cluster.

RESULTS

Walking speeds were 1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1} (mean ± standard deviation), and number of steps included in the analysis were 14 ± 3, 12 ± 3, 11 ± 2, and 9 ± 1 (median ± interquartile range) at slow, preferred, fast, and maximum walking speeds, respectively. Stance phase durations were 0.81 ± 0.06, 0.67 ± 0.06, 0.58 ± 0.04, and 0.5 ± 0.04 s (mean ± standard deviation) at slow, preferred, fast, and maximum walking speeds, respectively. The space between

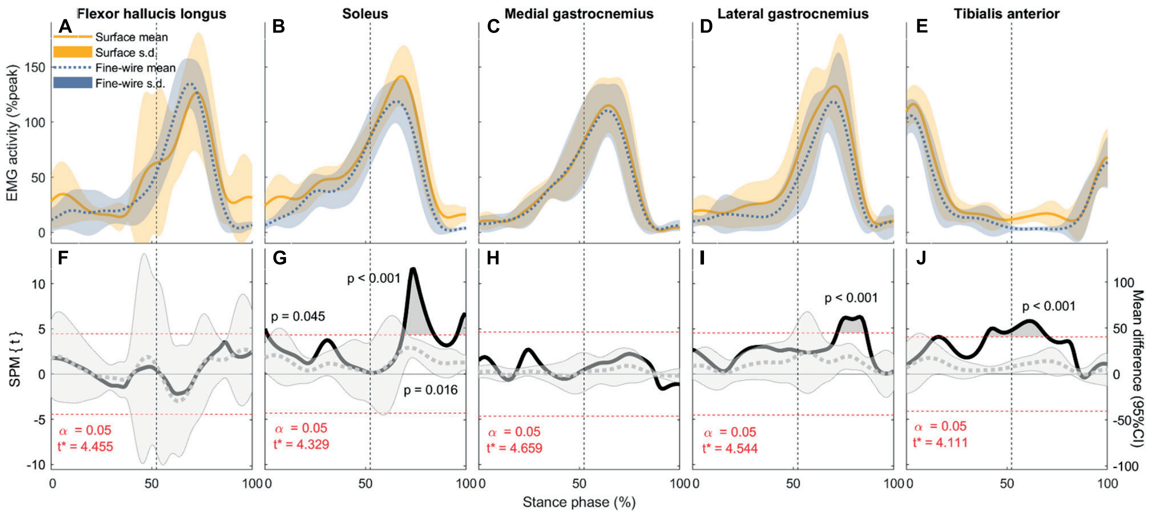

FIGURE 5 | Electromyography (EMG) activity (mean and standard deviation) of each muscle (**A–E**: normalised to peak activity in preferred speed walking, %peak) and the corresponding comparisons between surface and intramuscular EMG recordings **(F–J)** in the stance phase of fast walking (1.84 ± 0.23 m s^{-1}). Vertical dotted lines represent the beginning of push-off. Panels **(F–J)** SPM{*t*} statistics for two-tailed *t*-tests (solid black lines, left *y*-axis), and mean difference with 95% confidence intervals (grey dotted lines with shaded areas). Dashed red lines are critical thresholds (*t**) calculated for α significance level defining supra-threshold clusters for SPM{*t*} trajectories. *P*-values were calculated for each supra-threshold cluster.

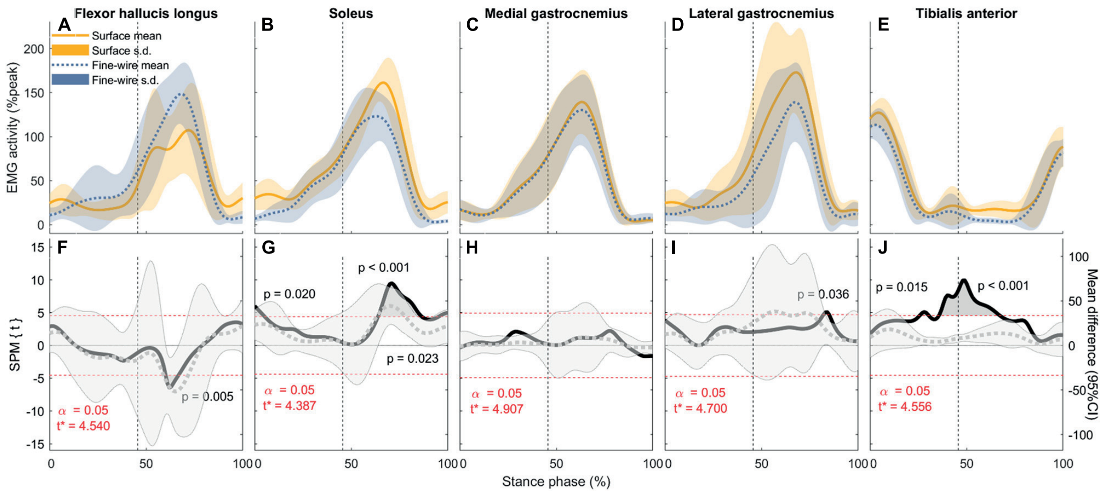

FIGURE 6 | Electromyography (EMG) activity (mean and standard deviation) of each muscle (**A–E**: normalised to peak activity in preferred speed walking, %peak) and the corresponding comparisons between surface and intramuscular EMG recordings **(F–J)** in the stance phase of maximum speed walking (2.20 ± 0.38 m s^{-1}). Vertical dotted lines represent the beginning of push-off. Panels **(F–J)** SPM{*t*} statistics for two-tailed *t*-tests (solid black lines, left *y*-axis), and mean difference with 95% confidence intervals (grey dotted lines with shaded areas). Dashed red lines are critical thresholds (*t**) calculated for α significance level defining supra-threshold clusters for SPM{*t*} trajectories. *P*-values were calculated for each supra-threshold cluster.

SOL insertion and distal FHL muscle–tendon junction where surface electrodes could be placed was 3.22 cm on average, ranging from 2.5 to 4.7 cm.

Figure 2 shows typical EMG signals from the five muscles recorded both with surface and intramuscular electrodes. Mean EMG activity results for all muscles and walking speeds are presented for the whole group of participants in **Figures 3–6** and for each individual in **Figures 7–11**.

In FHL we found differences in late stance at slow, preferred, and maximum speed walking (slow: 65.7–74% of stance phase, $p < 0.001$, preferred: 64.8–70.9%, $p = 0.003$, maximum speed walking: 60.1–66.7%, $p = 0.005$). There was no difference in FHL in fast walking. However, we found subject-specific differences between surface and intramuscular EMG patterns in FHL at all walking speeds (**Figure 7**).

In SOL, there were differences in early stance at all walking speeds (slow: 0–7.3%, $p = 0.004$, preferred: 0–5.3%, $p = 0.008$, fast: 0–1.4%, $p = 0.045$, maximum: 0–5.2%, $p = 0.02$), and in late stance at all speeds except slow (preferred: 72.7–73.6%, $p = 0.047$, fast: 68.5–84.2%, $p < 0.001$, maximum: 63.8–86.6%, $p < 0.001$). There were also a differences in pre-swing at all speeds except preferred speed (slow: 95.3–98.6%, $p = 0.03$, fast: 68.5–84.2% and 95.3–100%, $p < 0.001$ and $p = 0.016$, respectively, maximum: 63.8–86.6% and 95.2–100%, $p < 0.001$ and $p = 0.023$, respectively).

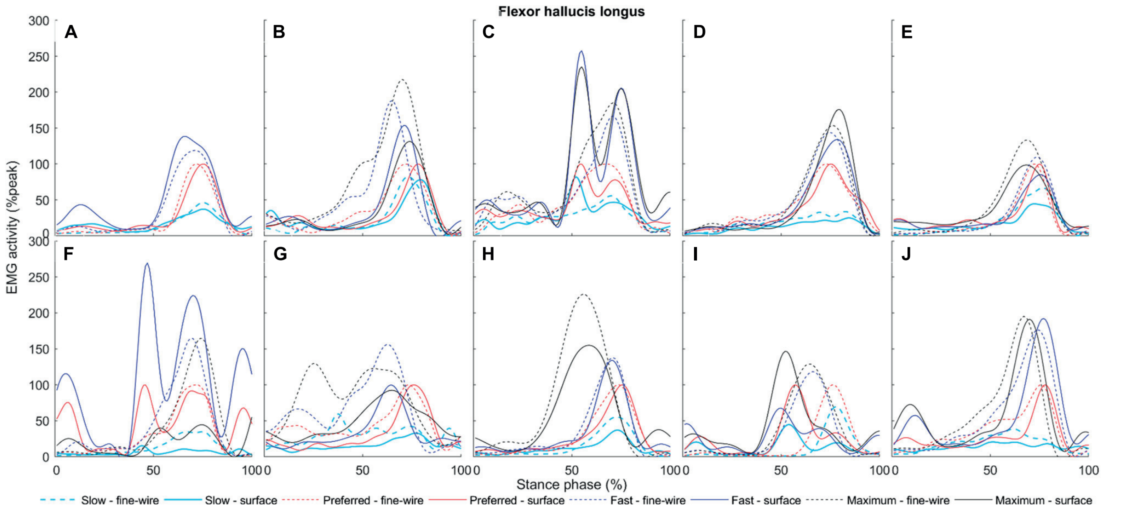

FIGURE 7 | Flexor hallucis longus surface (solid) and intramuscular (dashed) electromyography (EMG) activity at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) in the stance phase for each individual (**A–J**, respectively). The amplitudes of smoothed and time-normalised EMG curves were normalised to peak activity in preferred speed walking (%peak). Each curve represents the average of all steps (number of steps included in the analysis were 14 ± 3, 12 ± 3, 11 ± 2, and 9 ± 1, median ± interquartile range, at slow, preferred, fast, and maximum walking speeds, respectively) at a given walking speed.

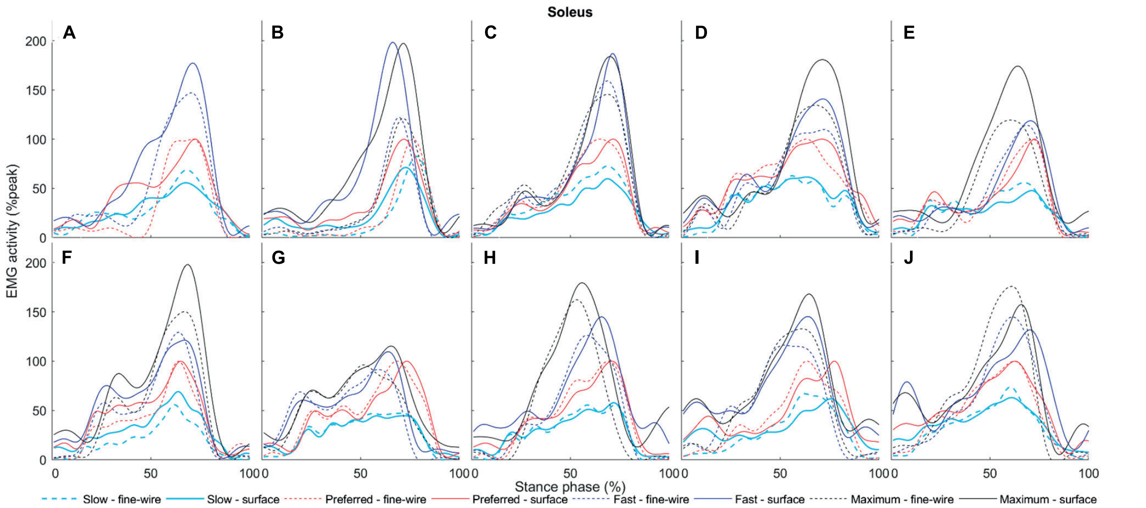

FIGURE 8 | Soleus surface (solid) and intramuscular (dashed) electromyography (EMG) activity at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) in the stance phase for each individual (**A–J**, respectively). The amplitudes of smoothed and time-normalised EMG curves were normalised to peak activity in preferred speed walking (%peak). Each curve represents the average of all steps at a given walking speed.

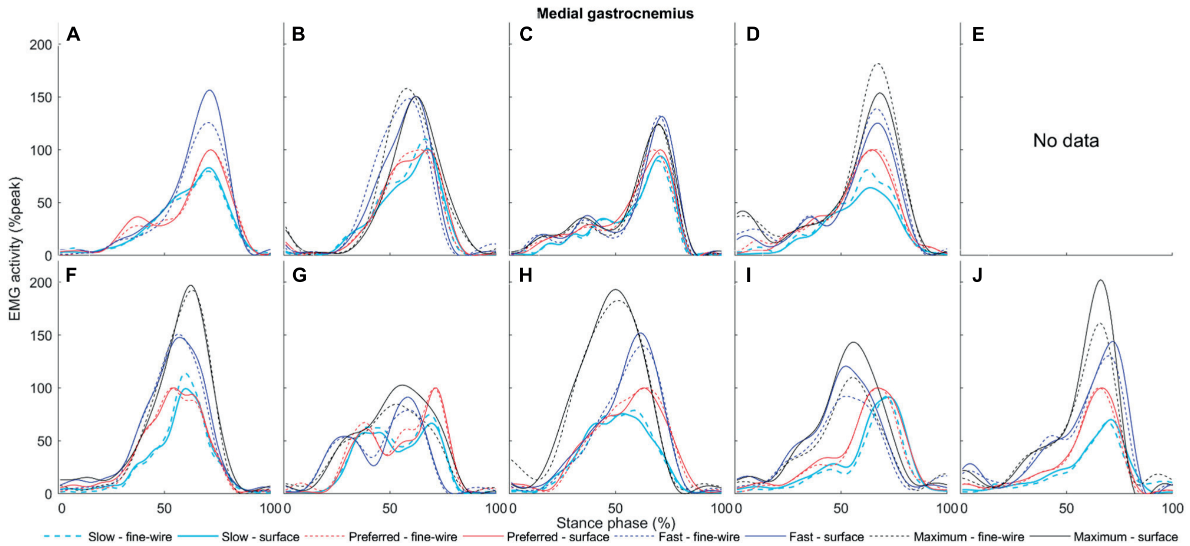

FIGURE 9 | Medial gastrocnemius surface (solid) and intramuscular (dashed) electromyography (EMG) activity at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) in the stance phase for each individual (**A–J**, respectively). The amplitudes of smoothed and time-normalised EMG curves were normalised to peak activity in preferred speed walking (%peak). Each curve represents the average of all steps (number of steps included in the analysis were 14 ± 3, 12 ± 3, 11 ± 2, and 9 ± 1, median ± interquartile range, at slow, preferred, fast, and maximum walking speeds, respectively) at a given walking speed.

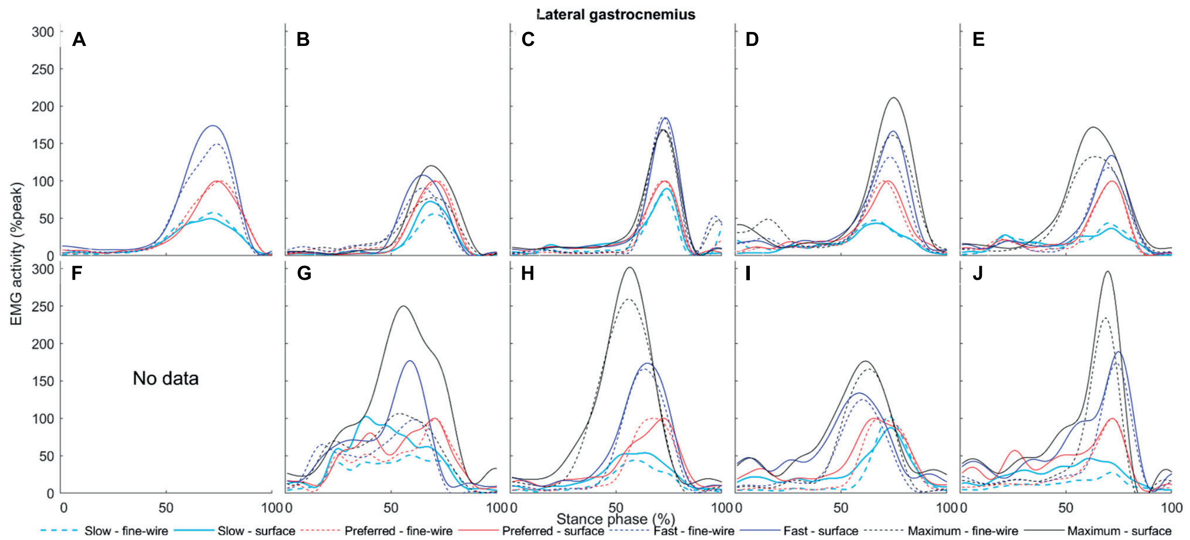

FIGURE 10 | Lateral gastrocnemius (solid) and intramuscular (dashed) electromyography (EMG) activity at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) in the stance phase for each individual (**A–J**, respectively). The amplitudes of smoothed and time-normalised EMG curves were normalised to peak activity in preferred speed walking (%peak). Each curve represents the average of all steps (number of steps included in the analysis were 14 ± 3, 12 ± 3, 11 ± 2, and 9 ± 1, median ± interquartile range, at slow, preferred, fast, and maximum walking speeds, respectively) at a given walking speed.

In TA, there were differences in mid stance at all walking speeds (slow: 43.7–44.3 and 47.5–67.2%, p = 0.049 and p < 0.001, respectively, preferred: 45.9–85.3%, p < 0.001, fast: 39.4–70.2%, p < 0.001, maximum: 25.7–30.4 and 35.6–67.2%,p = 0.015 and p < 0.001, respectively). Similarly, there were differences in late stance at all walking speeds

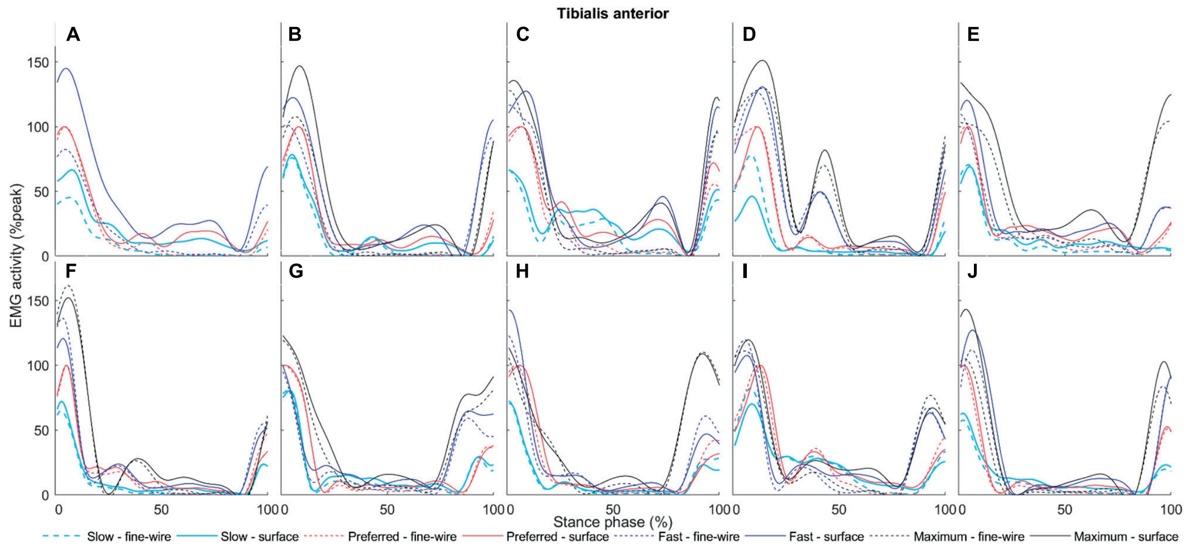

FIGURE 11 | Tibialis anterior surface (solid) and intramuscular (dashed) electromyography (EMG) activity at slow, preferred, fast, and maximum walking speeds (1.01 ± 0.13, 1.43 ± 0.19, 1.84 ± 0.23, and 2.20 ± 0.38 m s^{-1}, respectively) in the stance phase for each individual (**A–J**, respectively). The amplitudes of smoothed and time-normalised EMG curves were normalised to peak activity in preferred speed walking (%peak). Each curve represents the average of all steps (number of steps included in the analysis were 14 ± 3, 12 ± 3, 11 ± 2, and 9 ± 1, median ± interquartile range, at slow, preferred, fast, and maximum walking speeds, respectively) at a given walking speed.

(slow: 47.5–67.2 and 78.6–83.3%, $p < 0.001$ and $p = 0.023$, respectively, preferred: 45.9–85.3%, $p < 0.001$, fast: 39.4–70.2%, $p < 0.001$, maximum: 35.6–67.2%, $p < 0.001$). There were also a differences in pre-swing at slow and preferred speed (slow: 78.6–83.3%, $p = 0.023$, preferred: 45.9–85.3%, $p < 0.001$).

In MG there was no difference between surface and intramuscular EMG at any walking speed, or at slow or preferred speed walking in LG. However, at faster speeds we found differences in LG in late stance and pre-swing (fast: 71.8–86.3%, $p < 0.001$, maximum speed walking: 82–85%, $p = 0.036$).

DISCUSSION

This study compared surface and intramuscular EMG activity of key plantar flexor muscles in walking at different speeds by comparing the amplitudes of surface and intramuscular EMG recordings from healthy participants. In FHL, we found differences in the late stance phase at all speeds except fast, therefore questioning the validity of surface EMG for this muscle. In SOL and TA, surface EMG registered activity during phases in which intramuscular EMG indicated inactivity. For SOL and LG, differences between the two EMG methods were also seen around peak activity at relatively fast walking speeds. These results suggest that surface EMG signals were influenced by cross-talk in these cases. No differences in EMG amplitudes were found between the two methods for MG at any speed and LG at slow and preferred speeds, supporting the validity of surface EMG for these muscles within the range of walking speeds studied.

Early studies indicate that FHL is active in the stance phase of walking (Perry, 1992). This study confirmed that FHL is mainly active in push-off, therefore indicating a potential role of this muscle in propulsion. Bojsen-Moller et al. (2010) demonstrated that surface EMG recordings from FHL are possible with minimal cross-talk in submaximal contractions when electrodes are placed behind the medial malleolus with 16 mm interelectrode distance. To further improve electrode location accuracy, we used ultrasonography guidance (Péter et al., 2015, 2017). Despite careful electrode location and the decreased inter-electrode distance, the current study revealed significant subject-specific differences between surface and intramuscular EMG patterns in FHL at a range of walking speeds. Indeed, **Figure 7** shows that surface and intramuscular EMG activity of participants A, D, E, and H follow similar patterns in contrast to the six other participants. The reason why the amplitudes of surface and intramuscular EMG recordings are in agreement for some but not other participants could be manifold. One explanation is that the mechanical behaviour of FHL muscle–tendon complex seems to be individual-specific, and this presumably affects surface EMG recordings due to tissue motion underneath the skin. For example, in cadaver gait simulations, Hofmann et al. (2013) reported large differences between FHL tendon excursion in the stance phase ranging between 4.31 and 10.16 mm (mean = 7.18 mm, n = 8). Regarding muscle mechanics *in vivo*, we previously detected high inter-individual differences in FHL fascicle length change at similar walking speeds to those applied in the current study

(Péter et al., 2017). It should also be mentioned that the defined space where surface electrodes were placed (distance between SOL insertion and distal FHL muscle tendon junction) was as short as 3.22 cm (ranging from 2.5 to 4.7 cm), increasing the potential for cross-talk, although data from some participants with relatively shorter distance presented good agreement between methods compared to others with more space for electrodes. Other factors such as changes in skin impedance and inter-individual differences in subcutaneous tissue thickness behind the medial malleolus can further influence surface EMG recordings. Some of the potential factors that increase cross-talk are challenging to examine and control, therefore based on our current knowledge we suggest using intramuscular instead of surface EMG to define the EMG activity for FHL muscle.

Soleus is mostly active from mid stance to the beginning of the pre-swing phase during walking, and is inactive in the swing phase (Perry, 1992; Cuccurullo, 2004). Previous studies found no intramuscular EMG activity in the swing phase but some activity was seen from surface EMG in preferred-speed walking, suggesting potential cross-talk from TA (Bogey et al., 2000, 2003). Although surface and intramuscular EMG activity followed similar patterns in the active phase, both studies [see Figure 1 in Bogey et al. (2000) and Figure 2 in Bogey et al. (2003)] showed higher SOL surface activity in early stance and at the end of the pre-swing phase compared to intramuscular EMG at preferred speed walking. Concurrently, we detected activity with surface EMG in early stance at all speeds and in the pre-swing phase at all speeds except preferred speed while there was no activity with intramuscular EMG, suggesting that surface EMG was subject to cross-talk. Furthermore, we found differences between surface and intramuscular EMG signal amplitudes in SOL around peak activity at preferred, fast, and maximum speed walking. Our results show that at all walking speeds when surface EMG electrodes are placed laterally, they are prone to detect activity in those phases in which intramuscular EMG indicates that the muscle is actually inactive. This is similar to previous findings (Bogey et al., 2000, 2003) where medial surface electrode placement was used. Thus, SOL surface EMG activity may be affected by cross-talk and based on these results, SOL onset/offset during walking defined by surface EMG signals should be interpreted cautiously. Based on our results SOL surface electrodes should be placed over the skin with great caution. After checking SOL surface electrode location based on SENIAM recommendations, the location should be reconsidered based on muscle belly thickness to decrease cross-talk and moved to a location where SOL muscle belly is larger but also sufficiently far from MG and LG.

Similar to previous reports (Perry, 1992; Cuccurullo, 2004), the gastrocnemii muscles were activated in the stance phase. In MG we found no difference between surface and intramuscular EMG amplitudes at any walking speeds, suggesting that surface EMG is a suitable method with the used surface electrode location and inter-electrode distance. Compared to other plantar flexors, full agreement between methods across a range of walking speeds can be explained by the large cross-sectional area of MG, which enables electrodes to be placed a sufficient distance from other muscles, thereby minimising cross-talk. Although LG has substantially lower volume (Ward et al., 2009), surface EMG seems valid at slow and preferred speed walking, but small differences were seen in three participants (participant G, I, and J; **Figure 10**). However, LG surface electrodes seem to pick up some activity from surrounding muscles at faster speeds. This may be due to increased activity of neighbouring muscles and alteration in intermuscular coordination strategies as walking speed increases (Cronin et al., 2013). Our results suggest that surface EMG cross-talk is minimised for LG, at least at slow and preferred speed walking.

Previous studies showed two distinct peaks in intramuscular TA EMG activity in the stance phase of walking, near heel-strike, and TO, respectively, whereas TA was not active in the mid stance (or mid-swing) phases in healthy individuals (Gray and Basmajian, 1968). These findings are all in agreement with our intramuscular results. However, we detected surface but not intramuscular EMG activity in the mid and late stance phases at all walking speeds. In these phases, plantar flexors are highly active, providing a potential source of cross-talk. This suggests that defining onset/offset may be affected by cross-talk in these inactive periods. Additionally, at slow and preferred speed walking we also found a difference in the pre-swing phase, which may be due to the speed-effects mentioned above.

Limitations

Sample size in intramuscular EMG studies is relatively low in general, mainly due to the invasive and costly nature of the study. Similarly, in the current study, the relatively low sample size might have led to increased type II error rate and increased uncertainty in the magnitude of the differences. This can be seen in the 95% CIs shown in the figures comparing EMG activity acquired with the two methods. Additionally, we placed intramuscular EMG electrodes in close proximity to the surface electrodes in all muscles except FHL, where intramuscular electrodes were inserted 5–10 cm proximal to the surface electrodes, and on the lateral side of the shank due to rich vascularisation close to the surface electrodes. Therefore, potential regional differences in activation might have affected the detected differences between surface and intramuscular EMG recordings. It should also be mentioned that intramuscular EMG records from a relatively smaller number of motor units compared to surface EMG, which might have been an additional source of differences detected between EMG amplitudes acquired with the two methods. However, we placed the intramuscular EMG wires as close as possible to the surface electrodes to make sure we record from the same muscle region. Inter-electrode spacing can also affect differences between the two methods. Although an inter-electrode spacing of ∼20 mm is typical in surface EMG studies (and was used in the current study for all muscles except FHL), smaller (i.e., 10 mm) inter-electrode spacing may decrease the potential for cross-talk (De Luca et al., 2012). The application of our results may be restricted to healthy and non-injured individuals with a relatively thin subcutaneous fat layer over the examined muscles.

CONCLUSION

The validity of surface EMG to measure shank muscle activity is muscle- and walking speed-specific. Our results suggest that surface EMG is generally a suitable method of measuring muscle activity in MG and LG across several walking speeds. SOL and TA activity measured with surface EMG should be interpreted with caution in relevant sub-phases of walking. For FHL, surface EMG is not recommended. Future studies should explore potential sources of cross-talk and whether they can be further minimised (e.g., by decreasing inter-electrode distance), thereby improving the ability to selectively record from each shank muscle.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The study was approved by the Stockholm Regional Ethic Committee (Approval No: 2017/261-31/4) and was performed in agreement with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AP, EA, TF, AH, NC, and AA conceived and designed the study, interpreted the study results, and edited and revised the manuscript. AP, EA, AH, OT, HG, and AA performed the experiments. AP and AH analysed the data and prepared the figures. AP drafted the manuscript. All authors approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

IV

EFFECT OF FOOTWEAR ON INTRAMUSCULAR EMG ACTIVITY OF PLANTAR FLEXOR MUSCLES IN WALKING

by

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