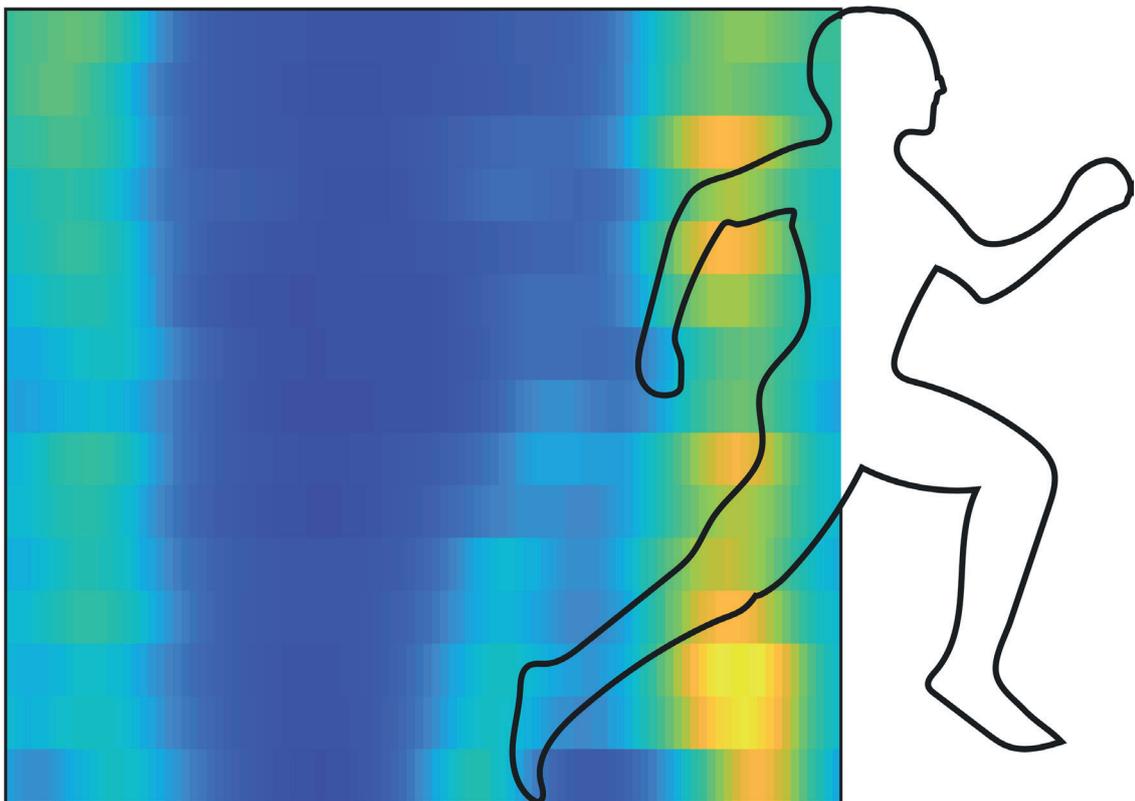


**András Hegyi**

# **Within- and between-muscle hamstring electromyography activity in various exercises and at different running speeds**

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JYU DISSERTATIONS 187

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András Hegyi

# Within- and between-muscle hamstring electromyography activity in various exercises and at different running speeds

Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella  
julkisesti tarkastettavaksi yliopiston Liikunta-rakennuksen salissa L304  
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## ABSTRACT

Hegyi, András

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In the early era of muscle research it was assumed that activation is uniform within muscles. However, recent research revealed regional differences in activation within several muscles, which has been linked to region-specific muscle adaptations to stimuli such as pain, fatigue, and training. Non-uniform within- and between-muscle distribution of hamstring injuries may be linked to non-uniform within- and between-muscle distribution of muscle activity. However, there is a lack of information about regional differences in hamstring muscle activity during hamstring exercises, and in high injury-risk movements such as high-speed running. Therefore, the aim of this thesis was to examine within- and between-muscle differences in biceps femoris and semitendinosus electromyography (EMG) activity using high-density surface EMG in typical hamstring exercises and at different running speeds. Our results showed that in most examined exercises, biceps femoris activity was lowest in the proximal region, which is the region where most hamstring injuries occur. The magnitude of regional differences was exercise-specific. In semitendinosus, some exercises presented uniform EMG activity proximo-distally, while others activated the middle and/or proximal region(s) the most. Generally, knee-oriented movements resulted in larger regional differences than hip-oriented movements in both muscles. Between-muscle differences were exercise-specific, whereby some knee-oriented movements activated semitendinosus preferentially, while hip extension performed on a roman chair activated biceps femoris more than semitendinosus. It was also found that performing Nordic hamstring exercise with hips flexed to 90° shows higher knee flexion torque and lower hamstring EMG activity as compared to performing Nordic hamstring exercise with neutral hips. In running, large inter-individual variability was observed in regional and muscle-specific EMG activity patterns, which were qualitatively consistent across a range of running speeds. These results suggest that a single pair of EMG electrodes placed over biceps femoris or semitendinosus does not accurately describe the activity of the whole muscle. Instead, spatially robust methods such as high-density EMG should be used to comprehensively describe hamstring activity. The sources and consequences of variations in regional activity patterns between exercises and between individuals should be further examined.

Keywords: hamstrings, high-density EMG, muscle function, Nordic hamstring exercise, running

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## TIIVISTELMÄ (FINNISH ABSTRACT)

Hegyí, András

Erot takareisilihasten sisäisessä ja lihasten välisessä aktiivisuudessa erilaisissa voimaharjoitteissa ja erilaisilla juoksunopeuksilla

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Viimeaikaiset tutkimukset ovat osoittaneet useissa lihaksissa olevan alueellisia eroja lihasaktiivisuudessa. Takareidessä vammojen ilmenemisen voidaan ajatella olevan yhteydessä lihasaktiivisuuden epätasaiseen jakautumiseen sekä lihaksen sisällä että lihasten välillä. Takareisiharjoitteiden ja korkean loukkaantumisriskin omaavien liikkeiden, kuten korkeatehoisen juoksun lihasaktiivisuuden alueellisista eroista on kuitenkin puutteellista tutkimusnäyttöä. Tässä väitöskirjassa tutkittiin sekä lihaksen sisäisiä että lihasten välisiä eroja kaksipäisen reisilihaksen (m. biceps femoris) ja puolijänteisen lihaksen (m. semitendinosus) aktiivisuuksissa monikanavaisella pinta-elektromyografialla (EMG) mitattuna tyypillisissä takareisiharjoitteissa ja eri juoksunopeuksilla. Tulokset osoittivat, että useimmissa harjoitteissa kaksipäisen reisilihaksen aktiivisuus oli pienintä lihaksen proksimaalisessa, eli vamma-alttiissa osassa. Lihasten alueelliset erot EMG-aktiivisuudessa olivat riippuvaisia harjoitteesta. Puolijänteisen lihaksen osalta EMG-aktiivisuus jakautui osassa harjoitteista tasaisesti koko lihaksen pituudelta, kun taas toiset liikkeet aktivoivat lihaksen keski- tai proksimaalista osaa eniten. Polvinivelen liikettä vaativissa harjoitteissa havaittiin suurempia alueellisia eroja kuin lonkanivelen liikettä vaativissa harjoitteissa. Lihasten välisten aktiivisuuserojen ollessa harjoitespesifisiä havaittiin joidenkin polvinivelen liikettä sisältävien harjoitteiden suosivan puolijänteisen lihaksen aktivointia, kun taas lonkan ojennusta vaativassa selkälihaspenkkiharjoitteessa kaksipäisen reisilihaksen EMG oli suurempaa kuin puolijänteisellä lihaksella. Kun Nordic hamstring -harjoitetta tehtiin lonkan ollessa 90° kulmassa, havaittiin suurempi polven koukistusvoima ja pienempi EMG-aktiivisuus kuin lonkkakulman ollessa suorana. Juostessa havaittiin suurta vaihtelevuutta yksilöiden välillä sekä alueellisissa että lihaskohtaisissa EMG-aktiivisuusmalleissa, jotka kuitenkin olivat yksilöittäin johdonmukaisesti samanmallisia kaikilla juoksunopeuksilla. Saadut tulokset viittaavat siihen, että kaksipäiseen reisilihakseen tai puolijänteiseen lihakseen asetetulla yksittäisellä EMG-elektrodiparilla ei saada tarkasti kuvattua koko lihaksen aktiivisuutta vaan kattavan lihasaktiivisuuden kuvaamiseksi tulisi käyttää monikanavaista EMG:tä. Lisätutkimuksia tarvitaan lihasten alueellisten aktiivisuus-mallien erojen syistä ja seurauksista eri harjoitteiden ja yksilöiden välillä.

Avainsanat: takareiden lihakset, lihasaktiivisuus, EMG, voimaharjoitus, Nordic hamstring, juoksu

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Jyväskylä 29.11.2019

András Hegyi

## LIST OF ORIGINAL PUBLICATIONS

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

- I. Hegyi, A., Péter, A., Finni, T. & Cronin, N.J. Region-dependent hamstrings activity in Nordic hamstring exercise and stiff-leg deadlift defined with high-density electromyography. *Scandinavian Journal of Medicine and Science in Sports*. 2018; 28(3): 992–1000.
- II. Hegyi, A., Csala, D., Péter, A., Finni, T. & Cronin, N.J. High-density electromyography in various hamstring exercises. *Scandinavian Journal of Medicine and Science in Sports*. 2019; 29(1): 34–43.
- III. Hegyi, A., Lahti, J., Giacomo, J.P., Gerus, P., Cronin, N.J. & Morin, J.B. Impact of hip flexion angle on unilateral and bilateral Nordic hamstring exercise torque and high-density electromyography activity. *Journal of Orthopaedic & Sports Physical Therapy*. 2019; 49(8): 584–592.
- IV. Hegyi, A., Gonçalves B.A.M., Finni, T. & Cronin, N.J. Individual Region- and Muscle-specific Hamstring Activity at Different Running Speeds. *Medicine & Science in Sports & Exercise*. 2019; 51(11): 2274–2285.

The author of this thesis, who is the first author of these publications, was mainly responsible for designing the studies, collecting and analyzing the data, interpreting the results, preparing the manuscripts, and managing the review process during publication procedures.

## ABBREVIATIONS

BB	Bent-knee bridge
BFlh	Biceps femoris long head
BFsh	Biceps femoris short head
CI	Confidence interval
CP	Cable pendulum
EMG	Electromyography
FL	Fascicle length
GM	Good morning exercise
HD-EMG	High-density surface electromyography
HE	45° hip extension
mfMRI	Muscle functional magnetic resonance imaging
MRI	Magnetic resonance imaging
MTJ	Muscle-tendon junction
MTU	Muscle-tendon unit
N	Sample size
NHE	Nordic hamstring exercise
NHE0	Nordic hamstring exercise performed with neutral hips
NHE90	Nordic hamstring exercise performed with hips flexed to 90°
PA	Pennation angle
PCSA	Physiological cross-sectional area
PLC	Prone leg curl
RDL	Unilateral Romanian deadlift
SB	Straight-knee bridge
SD	Standard deviation
SLC	Slide leg curl
SM	Semimembranosus
SPM	Statistical Parametric Mapping
ST	Semitendinosus
UHC	Upright hip extension conic-pulley

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ABSTRACT

TIIVISTELMÄ (FINNISH ABSTRACT)

ACKNOWLEDGEMENTS

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ABBREVIATIONS

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# 1 INTRODUCTION

Hamstring strain injuries have the highest incidence and a high re-injury rate in sports that involve repetitive sprints such as rugby union (Brooks et al., 2006), football (Ekstrand et al., 2016), track and field (Opar et al., 2014), Gaelic football (Roe et al., 2018), and Australian rules football (Saw et al., 2018). This type of injury places a significant financial burden on teams (Hickey et al., 2014). Hamstring injuries also lead to substantial player time loss from training and competition, which has been linked to lower final league ranking in professional football (Hägglund et al., 2013). Despite increased research efforts in this field, hamstring injury incidence has not decreased over the last few decades (Ekstrand et al., 2016). One reason might be that the mechanisms of hamstring strain injury are not fully understood. It seems that some exercise interventions can decrease hamstring injury rate substantially. For example, studies have shown that exercises requiring long hamstring lengths may be effective at reducing hamstring injury risk (Askling et al., 2013; Askling et al., 2014; Tyler et al., 2014). Additionally, exercise interventions that include the Nordic hamstring exercise can decrease injury incidence by around 50% (Van Dyk et al., 2019). Due to the potential role of training in hamstring injury prevention, understanding hamstring muscle function in different exercises and relating this information to muscle function in high injury-risk tasks (e.g. high-speed running) may help to reduce hamstring injury risk.

Electromyography (EMG) is frequently used to assess muscle function. The EMG signal represents the electrical activity generated in muscle fibers in response to the activation of innervating motor neurons (Farina et al., 2014). This method is typically used by placing one pair of electrodes over the examined muscle. It is generally assumed that the recorded signal represents the activity of the whole muscle. However, this assumption may not hold true. For example, in animal studies, it has been shown that the net force vectors produced by a muscle can be manipulated by region-specific muscle activation (e.g. Schieber, 1993). Human experiments have also found a link between region-specific muscle activity and certain muscle functions (e.g. Benn et al., 2018; Gallina et al., 2016). Addition-

ally, non-uniform adaptations in response to strength training have been reported in several muscles including triceps brachii (Kawakami et al., 1995; Wakahara et al., 2013), quadriceps (Ema et al., 2013; Narici et al., 1996b), and hamstrings (Housh et al., 1992). Region-specific adaptations may be linked to region-specific muscle activation (Wakahara et al., 2013). In hamstring muscles, non-uniform metabolic activation has been observed previously (Kubota et al., 2007; Mendez-Villanueva et al., 2016; Mendiguchia et al., 2013a; Mendiguchia et al., 2013b). In spite of this, proximo-distal differences are rarely considered when measuring the EMG activity of hamstring muscles. Therefore, this thesis will provide a comprehensive description of proximo-distal and intermuscular EMG activity of key hamstring muscles in different hamstring exercises and at different running speeds.

## **2 LITERATURE REVIEW**

### **2.1 Hamstring anatomy**

Three muscles of the hamstring muscle group, the biceps femoris long head (BF<sub>lh</sub>), semitendinosus (ST), and semimembranosus (SM; Figure 1), are bi-articular (spanning both the hip and knee joints). The fourth muscle of this group is the biceps femoris short head (BF<sub>sh</sub>), which is mono-articular (spanning the knee joint only). Although the hamstrings are considered to be synergistic hip extensor and knee flexor muscles, intermuscular and proximo-distal architectural differences suggest different functions of different hamstring heads and muscle regions. Therefore, a deeper understanding of hamstring anatomy may help to understand the versatile function and high injury susceptibility of this muscle group.

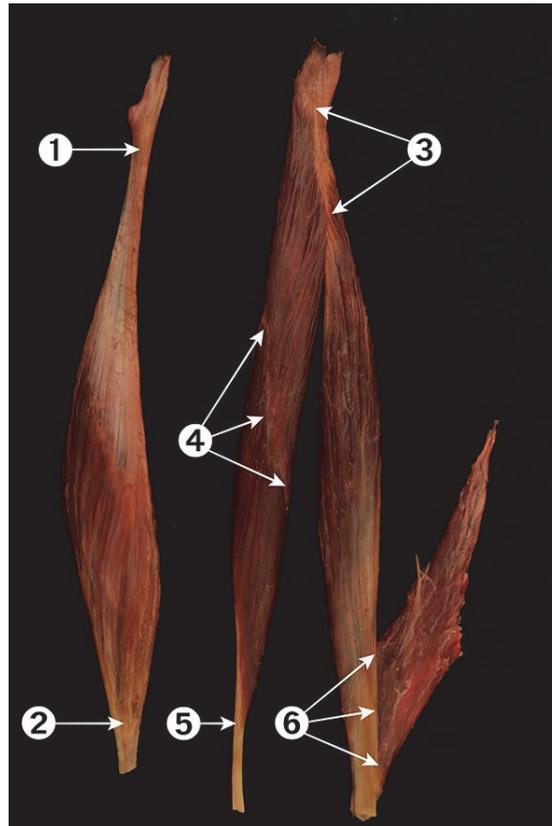


FIGURE 1 The hamstring complex. (1) Proximal tendon of the semimembranosus muscle; (2) distal tendon of the semimembranosus muscle; (3) conjoined tendon of the semitendinosus and the long head of the biceps femoris; (4) tendinous inscription (raphe) of the semitendinosus muscle; (5) distal tendon of the semitendinosus muscle; (6) common distal tendon of the long and short heads of the biceps femoris muscle. Source: Stępień et al., 2019. Reused with permission.

### 2.1.1 Attachment sites and course

The bi-articular components of the hamstrings originate from the ischial tuberosity. The SM has a crescent-shaped attachment zone on the anterolateral (Stępień et al., 2019), anterior (Feucht et al., 2015), or lateral (Miller et al., 2007) facet of the ischial tuberosity. Footprint (i.e. attachment area) dimensions have been reported to be 3.7 to 5.3 cm x 0.8 to 1.6 cm (length x height) (Feucht et al., 2015). The SM then runs caudally on the posteromedial side of the thigh towards the medial condyle of the tibia to insert in the pes anserinus. The BFlh and ST form a conjoined tendon proximally, which originates from an oval-shaped area on the posteromedial aspect of the ischial tuberosity according to most cadaveric dissections (Philippon et al., 2015; Sato et al., 2012; Stępień et al., 2019; van der Made et al., 2015), although some studies have reported a more lateral (Feucht et al., 2015; Neuschwander et al., 2015) or medial (Battermann et al., 2011; Miller et al., 2007) attachment zone of the BFlh/ST conjoined tendon. The conjoined tendon runs caudally ~10 cm from the origin and serves as an attachment zone for ST and BFlh fascicles (Battermann et al., 2011; Feucht et al., 2015; Miller et al., 2007; Philippon et al., 2015; van der Made et al., 2015). The morphology of this tendon

changes from roundish (several ST fascicles originate from here) to flat-shaped (both ST and BFlh fascicles originate from here) as it runs caudally (Battermann et al., 2011). The ST muscle then runs distally and medially towards the medial condyle of the tibia and its free distal tendon divides into several bands before inserting in the pes anserinus (Tubbs et al., 2006). Contrary to SM and ST, the BFlh runs laterally and distally from its conjoined tendon with the ST. The proximal aponeurosis of the BFlh provides an insertion area for the BFlh muscle fascicles. Nonetheless, the BFlh aponeuroses (both proximal and distal) are the smallest of all hamstring muscles (Woodley and Mercer, 2005). The morphology of the proximal aponeurosis seems to be crucial from an injury perspective. Several studies have suggested that a relatively narrow proximal BFlh aponeurosis is associated with larger tissue strain in fibers near the proximal muscle-tendon junction (MTJ) (Evangelidis et al., 2015; Fiorentino et al., 2012; Rehorn and Blemker, 2010), which seems to be the most frequently injured site of this muscle (De Smet and Best, 2000). Distally and laterally, the BFlh forms a common aponeurosis and distal tendon with the mono-articular BFsh, which originates from the mid-third of the posterolateral aspect of the femur. The common distal tendon of BFlh and BFsh attaches on the styloid process of the fibula (Terry and LaPrade, 1996; Tubbs et al., 2006).

### 2.1.2 Muscle-tendon lengths

Muscle-tendon unit (MTU) length is defined as the distance between the attachment and insertion of the muscle and its corresponding connective tissues. The attachments and course of these muscles define their MTU lengths. ST is usually found to be the longest of all hamstring MTUs, with a reported length of 43.8 to 47.0 cm (Kellis et al., 2012a; van der Made et al., 2015; Woodley and Mercer, 2005). ST has a relatively long distal tendon, taking up around 11.1 to 17.9% of its length (Kellis et al., 2012a; van der Made et al., 2015; Woodley and Mercer, 2005). Its free proximal tendon, on the contrary, is as short as 0.2 to 2.7% of its length (Kellis et al., 2012a; van der Made et al., 2015; Woodley and Mercer, 2005). SM has a relatively shorter MTU length of 38.7 to 43.8 cm, of which the proximal and distal free tendons make up 8.2 to 25.6% and 5.5 to 7.8% of its MTU length, respectively (Kellis et al., 2012a; van der Made et al., 2015; Woodley and Mercer, 2005). BFlh is around 38.9 to 43.8 cm long, and its proximal and distal tendons are 12.0 to 20.8% and 5.3 to 9.2% of its MTU length, respectively (Kellis et al., 2012a; van der Made et al., 2015; Woodley and Mercer, 2005). The proximal tendon of the ST and BFlh muscles forms a conjoined tendon, as described above. It has also been reported that SM has a wider proximal tendon than the conjoined tendon of BFlh/ST (3.1 to 6.2 cm and 2.6 to 4.1 cm, respectively), while the thickness of these tendons is similar (0.7 to 1.5 cm and 0.5 to 1.0 cm, respectively) (Feucht et al., 2015). The BFsh has a short muscle belly of around 28.5 to 29.1 cm with a short distal tendon (0.5 to 1.4% of MTU length), and its fascicles originate from the linea aspera, the upper two-thirds of the lateral supracondylar line, and from the lateral intermuscular septum, which separates BFsh from vastus lateralis muscle (Kellis et al., 2012a; Woodley and Mercer, 2005).

### 2.1.3 Architecture

The architecture of a skeletal muscle is defined as the spatial arrangement of muscle fibers (cells) within a muscle relative to the line of action, and is strongly associated with muscle function (Gans, 1982; Lieber and Fridén, 2000). In the human body, there are no two muscles with the same architectural arrangement, resulting in a unique force-generating capacity of each muscle (Lieber and Fridén, 2000). The muscle fibers of skeletal muscles are grouped in fascicles (bundles). Fascicle length is usually considered to be similar to that of the muscle fibers it includes. However, it should be noted that, typically in muscles with long fascicles, fibers do not span the entire length of the fascicle but terminate within the fascicle (so-called nonspanning fibers) and transmit force to neighboring muscle fibers (Ounjian et al., 1991). Nonetheless, fascicle length is usually easier to measure than fiber length due to its relatively larger cross-sectional area, and is therefore typically assessed as a proxy of muscle function. The most commonly reported architectural measures are fascicle length and pennation angle. Pennation angle is defined as the angle of the fascicles relative to the axis of muscle action. Relatively longer fascicles indicate high excursion capacity of a muscle (i.e. length change magnitude and velocity). Larger pennation angle (along with higher muscle volume or muscle mass) increases the force-generating capacity of a muscle by increasing its physiological cross-sectional area. Thus, fascicle length and physiological cross-sectional area are the functionally dominant architectural parameters.

#### 2.1.3.1 Intermuscular differences

Muscle architecture has long been studied with the use of 2-D B-mode ultrasonography in several human muscle groups (Fukashiro et al., 1995; Fukunaga et al., 1997; Narici et al., 1996a; Rutherford and Jones, 1992). However, due to the complex architecture and long fascicles of hamstring muscles, cadaver examinations are currently considered the most reliable method to define the architecture of this muscle group. Fascicle length and pennation angle of each hamstring muscle, as reported in cadaver studies, are shown in Table 1. Note that these results are typically defined as an average of several measurements along the muscle.

TABLE 1 Mean  $\pm$  standard deviation fascicle length and pennation angle of each hamstring muscle measured in cadaver studies.

Study	Biceps femoris long head		Biceps femoris short head		Semitendinosus		Semimembranosus		
	N	FL (cm)	PA (°)	FL (cm)	PA (°)	FL (cm)	PA (°)	FL (cm)	PA (°)
Weber, 1846	1	9.7	—	13.2	—	19.7	—	8.0	—
Wickiewicz et al., 1983	3	8.5 $\pm$ 0.9	0.0 $\pm$ 0.0	13.9 $\pm$ 0.6	23.3 $\pm$ 1.5	15.8 $\pm$ 0.3*	5.0 $\pm$ 0.0*	6.3 $\pm$ 0.8	15.0 $\pm$ 5.0
Friederich and Brand, 1990	2	7.3 $\pm$ 1.0	7.0 *	11.7 $\pm$ 0.8	15.0 *	15.3 $\pm$ 1.3	6.0 *	6.4 $\pm$ 1.5	16.0 *
Chleboun et al., 2001	5	8.0 $\pm$ 1.5	17.0 $\pm$ 4.2	—	—	—	—	—	—
Woodley and Mercer, 2005	6	7.0	—	12.4	—	9.0	—	5.0	—
Makihara et al., 2006	6	7.3 $\pm$ 1.3	28.0 $\pm$ 4.0	—	—	23.8 $\pm$ 1.8	0.0 $\pm$ 0.0	6.0 $\pm$ 0.8	31.0 $\pm$ 5.0
Klein Horsman et al., 2007	1	8.5	30.0	9.1	0.0	14.2	0.0	8.1	25.0
Ward et al., 2009	19	9.8 $\pm$ 2.6*	11.6 $\pm$ 5.5*	11.0 $\pm$ 2.1	12.3 $\pm$ 3.6	19.3 $\pm$ 4.1	12.9 $\pm$ 4.9	6.9 $\pm$ 1.8	15.1 $\pm$ 3.4
Kellis et al., 2009	6	7.6 $\pm$ 1.0	13.5 $\pm$ 2.3	—	—	18.7 $\pm$ 3.8	13.4 $\pm$ 3.3	—	—
Kellis et al., 2012a	8	7.0 $\pm$ 1.3	13.5 $\pm$ 2.9	10.4 $\pm$ 1.4	13.2 $\pm$ 2.6	14.9 $\pm$ 3.5	9.1 $\pm$ 3.5	5.4 $\pm$ 0.9	16.0 $\pm$ 2.4
Kumazaki et al., 2012	13	7.4 $\pm$ 1.1	—	13.1 $\pm$ 1.2	—	17.8 $\pm$ 1.2	—	6.3 $\pm$ 0.6	—
Ruggiero et al., 2016	1	7.8	—	15.2	—	8.4	—	5.3	—

N = sample size, FL = fascicle length, PA = pennation angle, \* sample size = N-1

In the human body, Ward et al. (2009) reported that ST, sartorius and gracilis muscles have the longest fascicles relative to their muscle lengths (0.65, 0.90, and 0.79, respectively), suggesting large excursion capacity of these muscles. In the bi-articular hamstrings, large excursion capacity has been considered crucial to protect the muscles from strain injury, which typically occurs in the late swing phase of running, where the ipsilateral hip is flexed and the knee is extended. Nonetheless, compared to ST, SM and BFlh have relatively lower capacity for excursion, as suggested by their relatively smaller fascicle length to muscle length ratios (SM = 0.17 to 0.37, BFlh = 0.19 to 0.28) (Friederich and Brand, 1990; Kellis et al., 2012a; Kumazaki et al., 2012; Ward et al., 2009; Wickiewicz et al., 1983). Even the monoarticular BFsh, which is not sensitive to hip flexion-extension, has a larger fascicle length to muscle length ratio (0.46 to 0.87) than those of the bi-articular SM and BFlh (Friederich and Brand, 1990; Kellis et al., 2012a; Kumazaki et al., 2012; Ward et al., 2009; Wickiewicz et al., 1983). These architectural features may be associated with the fact that among all of the hamstring muscles, the rate of sprinting-type strain injuries is highest in BFlh, and the rate of stretching-type strain injuries is highest in SM (Askling, 2011).

Compared to antigravity muscle groups such as the triceps surae or quadriceps femoris, the hamstrings have a relatively lower force production capacity (Ward et al., 2009). Apart from differences in fascicle lengths, pennation angle also differs between hamstring muscles (Table 1). ST has a physiological cross-sectional area of around 5 to 8 cm<sup>2</sup>, the lowest of all hamstring muscles (Kellis et al., 2012a; Ward et al., 2009; Woodley and Mercer, 2005), as well as a low pennation angle (Table 1) and moderate mass (69 to 100 g) (Kellis et al., 2012a; Ward et al., 2009; Wickiewicz et al., 1983). BFsh has a relatively large pennation angle but has the lowest mass (56 to 60 g) of all hamstring muscles, resulting in a relatively low PCSA (3 to 13 cm<sup>2</sup>), which suggests a modest force production capacity (Friederich and Brand, 1990; Kellis et al., 2012a; Ward et al., 2009; Woodley and Mercer, 2005). Compared to ST, the BFlh is relatively heavier (72 to 159 g) (Kellis et al., 2012a; Ward et al., 2009; Wickiewicz et al., 1983) and has a higher pennation angle according to most studies (Table 1). These characteristics result in a relatively larger PCSA in this muscle (9 to 27 cm<sup>2</sup>) (Friederich and Brand, 1990; Kellis et al., 2012a; Ward et al., 2009; Woodley and Mercer, 2005). The SM possesses the largest mass (94 to 134 g) (Kellis et al., 2012a; Ward et al., 2009; Wickiewicz et al., 1983) and pennation angle (Table 1) of all hamstring muscles, resulting in the largest PCSA (14 to 75 cm<sup>2</sup>) of these muscles (Kellis et al., 2012a; Ward et al., 2009; Wickiewicz et al., 1983; Woodley and Mercer, 2005).

The above-mentioned differences between muscles imply that the relative functional importance of each muscle may differ depending on the task being performed.

### 2.1.3.2 Intramuscular differences

Distinct muscle compartments (or regions) within a muscle seem to serve distinct functions (Schieber, 1993; Wakeling, 2009). In addition to intermuscular differences, intramuscular (i.e. regional) differences within hamstring muscles have

been reported, suggesting potential functional differences between muscle regions.

Among all hamstring heads, the regional architecture of BFlh (superficial compartment) has been examined most extensively. BFlh has a superficial (posterior) and a deep (anterior) compartment divided by an aponeurosis (i.e. internal tendon) (Kellis et al., 2012a; Woodley and Mercer, 2005). Although most studies agree that the architecture of this muscle is variable in the proximo-distal direction, some discrepancies are evident. For example, Tosovic et al. (2016) reported shorter fascicles and higher pennation angle distally than proximally, both *in vivo* and *in vitro*. In healthy participants, these values ranged proximo-distally from 7.1 to 8.3 cm and from 10.9 to 14.5°, respectively, when measured with ultrasonography. In the same study, using cadaver samples, fascicle lengths varied from 7.0 to 8.3 cm (ultrasonography) and from 7.4 to 8.9 cm (dissection), while pennation angle ranged from 9.3 to 12.3° (ultrasonography, ICC = 0.32) and from 8.0 to 8.8° (dissection). Similar proximo-distal differences can be identified qualitatively in the study of Ward et al. (2009). Similarly, other cadaver (Kellis et al., 2010) and *in vivo* ultrasound (Bennett et al., 2014) studies report longer fascicles proximally than distally. However, pennation angle shows some disagreement between studies, since it was higher in the proximal than in the distal site of the muscle (24.0 vs 17.8°) in the cadaver study of Kellis et al. (2010). These discrepancies may arise from methodological differences and inter-individual variability.

Semitendinosus also shows some proximo-distal variability. In ST, two compartments of the muscle have been identified, which are divided by a V-shaped tendinous inscription (also called the 'raphe') consisting of a lateral arm ~6.5 cm long and a medial arm ~2.4 cm long (Kellis et al., 2012b; Kellis and Balidou, 2014; Woodley and Mercer, 2005). This tendinous inscription serves as an attachment zone for most ST fascicles, although some fascicles span across this tendinous structure (Woodley and Mercer, 2005). Fascicle lengths have been reported to range between 12.3 to 15.5 cm and are longer in the distal than in the proximal region (Kellis et al., 2010; Woodley and Mercer, 2005). Pennation angle is smaller in the proximal than in the distal region and ranges between 8.8 and 14.7° (Kellis et al., 2010). PCSA seems to be slightly larger in the proximal (4.25 cm<sup>2</sup>) than in the distal (3.63 cm<sup>2</sup>) region (Woodley and Mercer, 2005).

Quantitative data on SM are relatively scarce. Qualitative examples from a cadaver (Ward et al., 2009, examples are available at [http://muscle.ucsd.edu/projects/architecture/maps\\_le.shtml](http://muscle.ucsd.edu/projects/architecture/maps_le.shtml)) show longer fascicles and larger pennation angle in the distal than in the proximal region of SM. Woodley and Mercer (2005) distinguished between three different muscle regions based on fascicle orientations. According to this study, the fascicles of the proximal region originate from the medial surface of the proximal tendon of the muscle and are positioned in the medial plane. They then attach onto the lateral surface of the distal tendon. Fascicle lengths in the proximal region range between 3.6 and 6.1 cm. Fascicles in the middle region originate from the lateral and medial surfaces of the proximal tendon and run posteriorly toward the lateral side of the distal tendon. Fascicle

lengths in the middle region range between 3.4 and 6.1 cm. The distal region was identified as a bulky bipennate muscle region originating from the lateral side of the proximal tendon and inserting in the broad distal tendon. Fascicle lengths in the distal region range between 3.4 and 6.1 cm. It has also been estimated that the PCSA is larger in the distal region (7.03 cm<sup>2</sup>) compared to the middle (4.43 cm<sup>2</sup>) and proximal (4.29 cm<sup>2</sup>) regions.

BFsh has been divided into proximal and distal regions based on nerve supply, fascicle orientations, and attachment zones (Woodley and Mercer, 2005). In some individuals, these two regions are also separated by thin connective tissue (Woodley and Mercer, 2005). In this muscle, shorter fascicles and larger pennation angle have been found in the distal than in the proximal region (Ward et al., 2009). Similarly, Woodley and Mercer (2005) reported shorter fascicles distally (10.2-13.1 cm) than proximally (12.1-14.6 cm), with slightly larger PCSA in the proximal (1.69 cm<sup>2</sup>) than in the distal (1.29 cm<sup>2</sup>) region.

The above regional differences suggest different functions of muscle regions, as discussed in section 2.2.

#### 2.1.4 Moment arms

The moment arm of force is the perpendicular distance from the line of action of force to the axis of rotation. A relatively longer internal moment arm provides a mechanical advantage for the muscle to produce torque. According to the anatomy of a joint and of the corresponding muscles spanning it, the moment arm can change with changes in joint angles. Therefore, some muscles gain a mechanical advantage over other muscles at certain joint angles.

The two main functions of the bi-articular hamstrings are hip extension and knee flexion, and moment arms have been examined during these movements in a few studies. At the knee joint, the cumulative moment arm of the hamstrings increases from knee extension (~1 cm) up to around 105° knee flexion (~3.5 cm) and then remains relatively constant as the knee is further flexed (Herzog and Read, 1993). ST has a longer moment arm in the sagittal plane compared to that of SM or BFlh, especially when the knee is relatively more flexed (Herzog and Read, 1993; Kellis et al., 2015). However, when the knee is almost fully extended, ST has a small negative (i.e. knee extensor) moment arm. This suggests that BFlh and SM have a mechanical advantage over ST to produce knee flexion torque at relatively more extended knee angles, while ST has a mechanical advantage over BFlh and SM to produce knee flexion torque at relatively more flexed knee angles. At the hip joint, the cumulative moment arm increases as a function of hip flexion from 4.0-6.0 cm to 6.0-7.5 cm (peaking between 35-50° hip flexion angle), then decreases to ~4.5 cm (90° knee flexion) (Németh and Ohlsén, 1985; Thelen et al., 2005b). This differs from other hip extensors such as the gluteus maximus (moment arm length decreases from 0 to 90° hip flexion angle) and adductor magnus (moment arm length increases from 0 to 90° hip flexion angle) (Németh and Ohlsén, 1985), and affects the relative mechanical advantage of hip extensor muscles through a range of hip flexion-extension angles. When hamstring muscles are considered separately, ST and BFlh show longer moment arms compared to

that of SM (Thelen et al., 2005b). This results in more muscle-tendon unit elongation in ST and BF<sub>lh</sub> than in SM when the hip is flexed.

### 2.1.5 Innervation

Hamstring muscles are innervated by different divisions of the sciatic nerve (Figure 2). The bi-articular components are innervated by the tibial division, while the BF<sub>sh</sub> is innervated by the fibular division. One or two motor branches innervate each of the SM and BF<sub>sh</sub> (Seidel et al., 1996; Stępień et al., 2019; Woodley and Mercer, 2005), two motor branches innervate the ST (Seidel et al., 1996; Stępień et al., 2019; Woodley and Mercer, 2005), and one-to-three motor branches have been reported for BF<sub>lh</sub> (Seidel et al., 1996; Stępień et al., 2019; Woodley and Mercer, 2005), each of which split into a series of further branches. Heterogeneous innervation may allow for heterogeneous muscle activation between and within hamstring muscles.

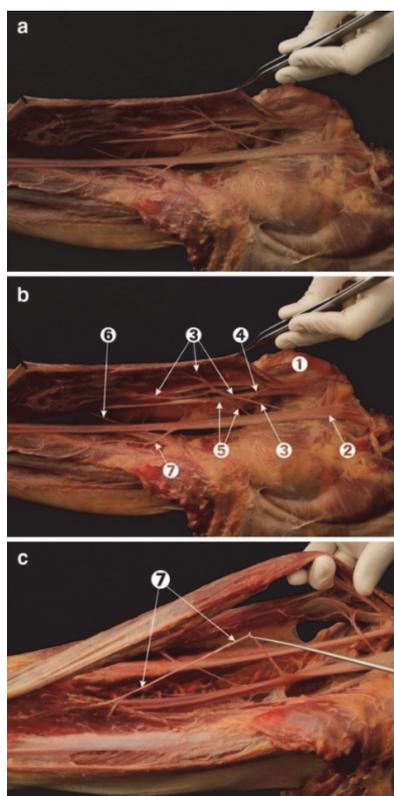


FIGURE 2 Lateral view of the thigh, innervation of the hamstring muscles. (1) Ischial tuberosity; (2) sciatic nerve; (3) motor branch to the long head of the biceps femoris muscle; (4) recurrent branch to the proximal attachment of conjoined tendon; (5) motor branch to the semitendinosus muscle; (6) motor branch to the semimembranosus muscle; (7) motor branch to the short head of the biceps femoris muscle. Source: Stępień et al. 2019. Reused with permission from the publisher.

## 2.2 Hamstring function

### 2.2.1 Muscle-tendon mechanics

Muscles and tendons are connected and are therefore required to interact. This interaction is affected by tissue properties, and has a substantial effect on the force production capacity and length change velocity of a muscle. Therefore, it may have important implications from a strain injury perspective. Current knowledge regarding hamstring muscle-tendon function, which is relevant for this thesis, is summarized in this section.

#### 2.2.1.1 Intermuscular differences

Mechanical behavior of the bi-articular hamstring muscle-tendon units (MTUs) is determined by the angular displacement of the hip and knee joints and respective moment arms. Some studies monitor these parameters to distinguish between the lengthening and shortening phases of different exercises (e.g. Bourne et al., 2017b). However, MTU behavior has also been examined in more complex movements such as running because of the high incidence of running-type hamstring injuries in many sports (Ekstrand et al., 2012; Ekstrand et al., 2016; Opar et al., 2014; Petersen et al., 2010; Roe et al., 2018; Saw et al., 2018). Studies show that hamstring MTUs undergo a stretch-shortening cycle during the running stride (Schache et al., 2012; Thelen et al., 2005b; Wan et al., 2017). During the stride, the hamstrings are lengthened in the swing phase and reach their peak MTU lengths in late swing, where most hamstring injuries seem to occur. Studies in this area have shown higher strain in the most frequently injured hamstring muscle (BFlh) compared to ST and SM in high-speed running. Modelling studies further suggest that high forces act along the hamstrings, and that peak forces occur in the late swing phase (Chumanov et al., 2007; Chumanov et al., 2011; Schache et al., 2013; Thelen et al., 2005a).

#### 2.2.1.2 Intramuscular differences

More than 80% of hamstring strain injuries occur in the BFlh (Connell et al., 2004; Koulouris et al., 2007; Woods et al., 2004), so most studies focus on this particular hamstring muscle when assessing intramuscular mechanisms. Although relatively high BFlh MTU strain could explain why this muscle is particularly susceptible to injury in the late swing phase, muscle-tendon decoupling should also be considered. There is currently a lack of experimental evidence, but modelling data shows that some of the MTU stretch occurs in the tendinous structures of the BFlh in the swing phase of running (Figure 3, Thelen et al., 2005a). This study also demonstrated that increased tendon compliance can further decrease the amount of stretch in the muscle fibers to some extent (provided that MTU lengthening and muscle activation are unchanged). It has been shown *in vitro* that the magnitude of fiber strain is a good predictor of strain injuries (Lieber and Friden,

1993). This implies that higher tendon compliance could potentially be associated with decreased strain injury risk at the level of muscle fibers. However, further experimental evidence is needed, and the range within which BFlh tendon compliance could realistically vary is currently unclear.

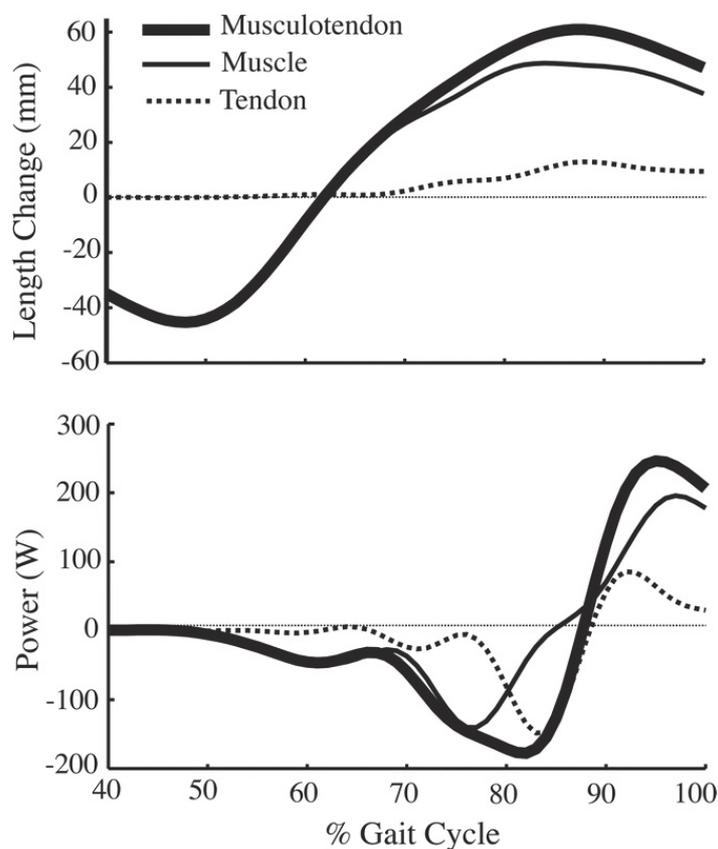


FIGURE 3 Ensemble curves (averaged over three successive strides) of the biceps femoris muscle, tendon, and musculotendon lengths, and power development during the swing phase of sprinting at a speed of  $9.3 \text{ m s}^{-1}$ . The stretch of the muscle component slows considerably when the biceps femoris is activated (starting at approximately 65% of the gait cycle), and the tendon begins to lengthen. The negative work done by the musculotendon actuator on the system is initially attributable to muscle stretch under load (50–80% of gait cycle). However, negative work done on the system between 80 and 90% of the gait cycle is due primarily to tendon stretch. The tendon recoils beyond 90% of the gait cycle, thereby returning energy to the system. Note that all length change measures are relative to lengths during quiet standing. Source: Thelen et al. 2005a. Re-used with permission.

Aside from muscle-tendon decoupling, some experimental and modelling studies suggest heterogeneous behavior of muscle fibers within the BFlh. For example, during isometric knee flexion contractions, BFlh fascicles shorten more in the proximal region (longer fascicles) than in the distal region (shorter fascicles), although individual variations are evident (Bennett et al., 2014). Additionally, in cyclic knee flexion-extensions, non-uniform tissue displacements were observed along the BFlh, as measured by MRI CINE phase-contrast imaging (Fiorentino et

al., 2012). This study showed increased regional differences in the active compared to passive muscle, with the largest strain observed near the proximal MTJ, which seems to be a typical site of strain injuries (De Smet and Best, 2000). Using the same method, another study reported higher localized strain at the proximal MTJ in previously injured compared to non-injured BFlh (Silder et al., 2010). Similarly, modelling data suggest that in the late swing phase of running, the strain of the fascicles is most pronounced close to the proximal MTJ, especially at high running speeds (Fiorentino et al., 2014; Fiorentino and Blemker, 2014). However, experimental data are scarce in running, and regional activation of the muscle may either amplify or counteract regional strain distribution in the BFlh (Garrett et al., 1987), potentially altering the risk of strain injury.

## **2.2.2 Muscle activation**

Adaptations gained through hamstring strengthening seem to be important to improve sprint performance (Guex et al., 2016; Mendiguchia et al., 2015; Morin et al., 2015) and to decrease strain injury risk (Bourne et al., 2018a; Timmins et al., 2016). The extent to which the muscles are activated is considered to be one of the main drivers of muscle adaptations to strengthening exercises. According to the heterogeneous anatomy of hamstring muscles, muscle activity seems to also be heterogeneous during different exercises, as briefly discussed in this section.

### **2.2.2.1 Intermuscular differences**

It has been well described that hamstring muscle activation varies between different hamstring exercises (Bourne et al., 2016; McAllister et al., 2014; Tsaklis et al., 2015; van den Tillaar et al., 2017; Zebis et al., 2013). For example, the Nordic hamstring exercise (NHE) requires high hamstring activation (Bourne et al., 2017b; Delahunt et al., 2016; Zebis et al., 2013), and has been shown to reduce hamstring injury risk in amateur and professional athletes, provided that athlete compliance was adequate. NHE is performed in a kneeling position, whereby the athlete performs a bilateral lengthening action of the hamstrings (at least at the MTU level) at a slow pace with neutral hips, or with some hip flexion during the movement. It should be mentioned that the relative load is typically higher in NHE than the load applied in other exercises because performing NHE with bodyweight only is usually close to (or exceeds) the eccentric one-repetition maximum of the athlete (i.e. the athlete is unable to resist up to full knee extension). This might explain why hamstring activity is so high in NHE. However, in addition to the magnitude of muscle activation, the activity of hamstring muscles relative to each other seems to also be meaningful. For example, in NHE, although all hamstring muscles are highly activated, preferential ST activation has been linked to higher increase in the size of this muscle compared to that of BFlh in response to training (Bourne et al., 2017a). Interestingly, some studies found no difference between the activity of these muscles when using surface electromyography (EMG) (Tsaklis et al., 2015; Zebis et al., 2013). As shown by Bourne et al. (2018), studies using muscle functional magnetic resonance imaging (mfMRI)

suggest that hip-dominant exercises show relatively higher BFlh than ST activation as compared to knee-dominant exercises (Figure 5). This pattern is less clear in studies using EMG (Bourne et al., 2017b; Tsaklis et al., 2015; van den Tillaar et al., 2017; Zebis et al., 2013). For example, hip-dominant kettlebell swing and Romanian deadlift were ST-dominant, and the knee-dominant supine leg curl was BFlh-dominant in the study of Zebis et al. (2013). Some potential reasons for discrepancies between studies are discussed in section 2.2.2.3. Some other studies show that, in accordance with the rotational function of each hamstring muscle, lateral rotation of the shank can increase BFlh activation, while medial rotation of the shank can increase ST activation (Lynn and Costigan, 2009), which may provide further opportunities for practitioners to target each of these muscles.

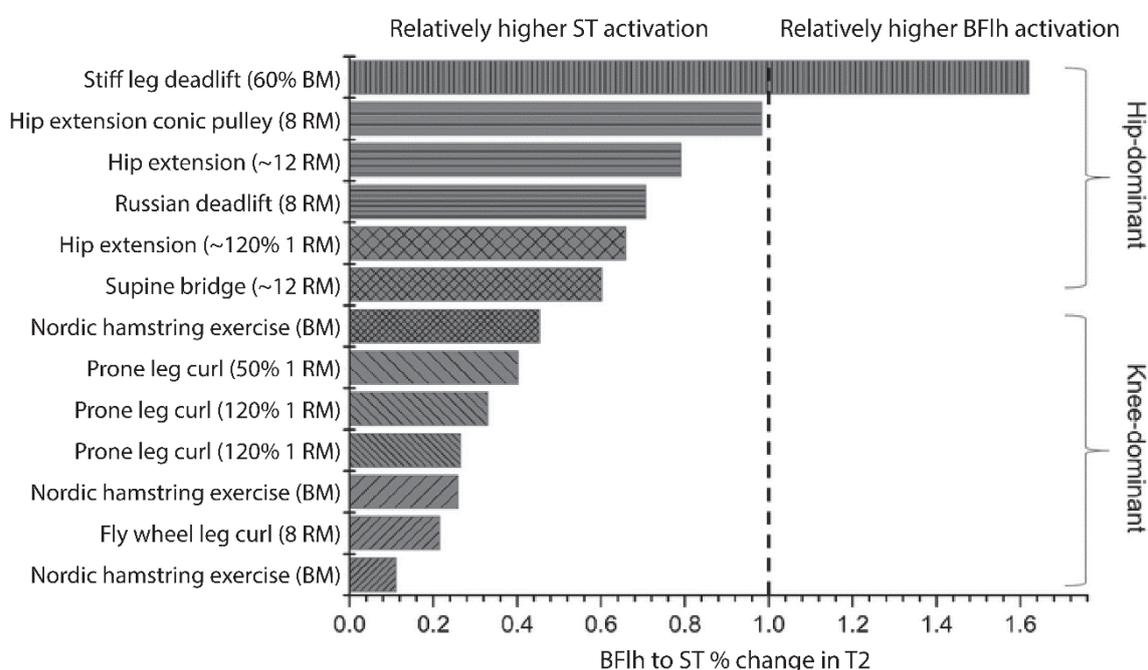


FIGURE 4 Ratio of BFlh to ST percentage change in T2 relaxation time in different exercises. Ratios > 1.0 indicate higher levels of BFlh than ST activity. Note the trend for relatively higher levels of BFlh activity during hip extension-oriented movements and more preferential ST activity during knee flexion-oriented movements. BFlh biceps femoris long head, BM body mass, RM repetition maximum, ST semitendinosus, T2 transverse relaxation time. Summarized from several studies by Bourne et al. 2018. Reused with permission.

Hamstring strain injuries typically occur in the late swing or early stance phase of high-speed running (Chumanov et al., 2012; Heiderscheit et al., 2005; Liu et al., 2017). Therefore, some studies have focused on hamstring activity in high-speed running in an attempt to further understand hamstring injury mechanisms (Higashihara et al., 2010; Higashihara et al., 2015; Schache et al., 2013; Yu et al., 2008). These EMG studies report high levels of hamstring activity in the late swing phase, suggesting that the hamstrings perform negative work in this phase to decelerate the shank. The *in vitro* study of Garrett et al. (1987) suggested that

increased activation of a muscle may be protective against strain injury because it increases the amount of energy the muscle absorbs while being stretched. It has also been suggested that increased ST activity can unload the BFlh to some extent, protecting the muscle from sustaining a strain injury (Schuermans et al., 2014). However, it is still unclear which of the hamstring muscles are activated the most in high-speed running. In one study (Higashihara et al., 2010), increasing running speed was associated with increased EMG activity of BFlh relative to ST in late swing. However, this study used a 30 mm interelectrode distance (increased potential for cross-talk, De Luca et al., 2012), and included only three strides from each of the eight participants, questioning the validity of these findings. It is clear that more sophisticated studies are needed to further understand hamstring activity in running.

### **2.2.2.2 Intramuscular differences**

A few studies using mfMRI showed that activation of hamstring muscles is heterogeneous proximo-distally in different hamstring exercises.

Kubota et al. (2007) were the first to report regional differences in hamstring activation using mfMRI. In this study, higher metabolic activity was found in the proximal and middle regions than in the distal region of ST during an eccentric knee flexion exercise. Some other studies also found regional differences in other hamstring muscles (Mendez-Villanueva et al., 2016; Mendiguchia et al., 2013b). It seems from these studies that different exercises activate different muscle regions within each hamstring muscle. To examine the neural representation of this phenomenon, Schoenfeld et al. (2015) recorded EMG from the proximal and distal regions of hamstring muscles. They found that the knee-dominant leg curl exercise activated the distal region more than the hip-dominant stiff-leg deadlift, although differences between exercises were not observed in the proximal region. It is currently unclear whether regional activity patterns are associated with regional muscle adaptations. In some muscles, non-uniform muscle activation has been linked to non-uniform adaptations (Hedayatpour et al., 2008a; Hedayatpour et al., 2008b; Wakahara et al., 2013). However, this question is especially interesting in light of the heterogeneous proximo-distal distribution of hamstring strain injuries (De Smet and Best, 2000).

In running, no study has examined regional activation of hamstring muscles. Although fiber strain in running seems to be larger in the proximal than in the distal region of the BFlh (Fiorentino et al., 2014), potential regional differences in hamstring activation likely affect strain distribution.

### **2.2.2.3 Methodological considerations**

Conventional EMG and mfMRI are the most common methods to estimate hamstring muscle activation. Each of these has some advantages but also some inherent limitations. This section provides a brief overview, and aims to shed light on

the potential advantage of using high-density surface EMG to provide further insights into hamstring muscle activation.

Conventional surface EMG is the most commonly used method to estimate hamstring activity. This method typically measures from a small area of the muscle using a single pair of electrodes attached over the muscle with a 10-30 mm inter-electrode spacing. EMG amplitudes provide information about the magnitude of muscle excitation, i.e. increased muscle activity is related to an increased number of active motor units and/or increased firing rate (for review, see Farina et al., 2014; Hug, 2011; Vigotsky et al., 2018). EMG amplitudes in the examined tasks are generally compared to those from a reference contraction, e.g. by normalizing to maximal voluntary isometric contraction, which is the most common approach. It should be mentioned here that comparing different muscles within and between individuals assumes that the reference contraction induces similar (submaximal or maximal) activation from all muscles and individuals. The main advantage of EMG is the high temporal resolution. For example, it is possible to selectively analyze the lengthening and shortening phases of an exercise or the activity in the late swing phase of a sprint stride. This method is also cheap, widely available, and relatively easy to use. One of the main disadvantages is the potential for cross-talk (i.e. contamination of the signals by the activation of neighboring muscles). Additionally, the recorded signal may not be representative of the whole muscle but restricted to a small area of the muscle. Although the pick-up area can be increased by selecting large electrodes and increasing interelectrode distance, these procedures also increase the amount of cross-talk. This is a crucial issue when selective signals are desired from each of the hamstring muscles. Because of the difficulties associated with this approach, some studies using EMG have reported that the signals were recorded from the medial (ST and SM) and lateral (BFlh and BFsh) hamstrings, without further distinguishing between hamstring muscles. On the other hand, when signals are selectively recorded from a single muscle, the pick-up area is relatively small compared to the size of the whole muscle, i.e. regional differences are not detected. Potential regional differences may lead to systematic or non-systematic errors when defining the activation of hamstring muscles using a single pair of EMG electrodes placed over each muscle. Although Schoenfeld et al. (2015) examined regional EMG activity in hamstring muscles by placing one pair of electrodes over each of the proximal and distal regions, further studies are needed to explore proximo-distal activity patterns along the whole length of hamstring muscles in real-time.

One method that can help to achieve this is the relatively more expensive mfMRI (Fleckenstein et al., 1988). Using this technique, it is assumed that the acute elevation of transverse relaxation time of tissue water is associated with the metabolic activation of the tissue (i.e. muscle) (Fisher et al., 1990). Several mfMRI scans can be done along the entire muscle of interest before and after exercise, and the changes in signal intensity are used to estimate changes in transverse relaxation time. Differences between pre and post measurements give an idea about the amount of muscle activation between the two scans. This method pro-

vides excellent spatial resolution and is associated with minimal cross-talk. However, perhaps the main disadvantage is that this method does not provide real-time data. Additionally, the shift in transverse relaxation time is sensitive to glycolysis, vascular dynamics, connective tissue content, muscle fiber type composition, and contraction mode (i.e. shortening *versus* lengthening). Moreover, use of this method usually requires several repetitions and/or high loading of the tissue (potential fatigue effect) to reliably estimate muscle metabolic activity (Fleckenstein et al., 1988; Jenner et al., 1994; Patten et al., 2003). Some of these factors may limit the ability to accurately compare the activation of different muscles within or between individuals.

To assess regional EMG activity, high-density surface EMG (HD-EMG) has been used in other muscles such as rectus femoris (Watanabe et al., 2014), biceps brachii (Staudenmann et al., 2014), vastus medialis (Gallina et al., 2016), and gastrocnemius lateralis (Cronin et al., 2015). The main improvement as compared to conventional EMG is that several small electrodes are placed over the muscle using one- (linear array) or two-dimensional (matrix) grids. The electrodes are smaller than used in typical EMG studies, minimizing cross-talk. This novel EMG configuration provides the possibility to record EMG signals with high spatial and temporal resolution, combining the main advantages of conventional EMG and mfMRI. Therefore, it can be assumed that the use of HD-EMG on hamstring muscles would likely provide additional insight into the neural representation of regional activity and intermuscular coordination of hamstring muscles.

### 3 PURPOSE OF THE STUDY

The purpose of this thesis was to comprehensively describe the EMG activity of hamstring muscles in various hamstring exercises and at different running speeds. To achieve this, HD-EMG was used to capture muscle activity with high spatial and temporal resolution.

Based on mfMRI studies, hip- and knee-dominant exercises preferentially activate BFlh and ST muscles, respectively (Bourne et al., 2018a). Additionally, Schoenfeld (2015), using conventional EMG electrodes placed over different muscle regions, reported higher proximal than distal hamstring activity in a hip-dominant exercise, but similar distal and proximal activity in a knee-dominant exercise. Similarly, in rectus femoris (a bi-articular muscle of the quadriceps femoris group), HD-EMG showed that the proximal region is preferentially activated in hip flexion, while the distal region is preferentially activated in knee extension (Watanabe et al., 2014). Following this research line, the present thesis used HD-EMG on hamstrings in hip- *versus* knee-dominant exercises to explore whether proximal activation is associated with hip extension and distal activation is associated with knee flexion. If regional differences exist in any of these exercises, it would suggest that future studies should take proximo-distal distribution of muscle activity into account when examining hamstring activity. This is pertinent because most previous studies have only examined activity from a small region of the muscle, which in this case would not be representative of the whole muscle.

NHE has recently gained attention because of the decreased injury rate observed in many studies implementing this exercise (for review, see Van Dyk et al., 2019). However, this exercise requires relatively short hamstring operating lengths, which is in contrast to the long muscle lengths at which the hamstrings must produce negative work in the late swing phase of running. Accordingly, previous exercise protocols that included exercises requiring long hamstring lengths have been found to be more successful in hamstring injury rehabilitation than those requiring relatively shorter muscle lengths (Askling et al., 2013; Askling et al., 2014; Tyler et al., 2014). Therefore, this thesis also includes a detailed biomechanical analysis of different NHE modes whereby the hamstrings operate at short *versus* long muscle lengths (i.e. by adjusting hip flexion angle),

performed either unilaterally or bilaterally. In this study a novel load-cell device allowing control over hip flexion angle and allowing knee range of motion to be matched between exercises was utilized. These factors are usually not considered when NHE is used for training or testing.

As mentioned before, understanding hamstring muscle function in running is important to further understand injury mechanisms. It was hypothesized that capturing the proximo-distal activity pattern of hamstring muscles as a function of increasing running speed would help us to further understand inter- and intramuscular coordination strategies in this high injury risk movement.

In addition to examining the most frequently injured hamstring muscle (BFlh), the least frequently injured hamstring muscle, ST, was also examined. This muscle forms a common proximal tendon with BFlh but has a different path and vastly different architecture, implying different function of these muscles. Additionally, ST has been suggested to play an important role in protecting BFlh from strain injury (Schuermans et al., 2014). Thus, further exploring the interplay between BFlh and ST would likely help to further our understanding of hamstring muscle function.

The specific aims of this thesis were as follows:

- 1) To define inter- and intramuscular differences in EMG activity in the knee-dominant Nordic hamstring exercise and the hip-dominant stiff-leg deadlift exercise at loads that are typically used in hamstrings training (I).
- 2) To assess inter- and intramuscular EMG activity patterns in the active lengthening and shortening phases of typical hamstring exercises performed at submaximal matched loads (II).
- 3) To examine the effects of different Nordic hamstring exercise modes on knee flexion torque and hamstring EMG activity using a novel load-cell device (III).
- 4) To explore the inter- and intramuscular EMG patterns of hamstring muscles at different running speeds to further understand hamstring muscle function in running (IV).

## 4 METHODS

### 4.1 Participants and ethics

In study I, healthy physically active participants with weightlifting experience were recruited to ensure they could perform the examined exercises with minimal practice. In studies II-IV, healthy amateur players were included from sports with high hamstring injury risk, such as football (II-IV), rugby union (II, III), and Gaelic football (II, IV). Additionally, due to the high-load eccentric exercises used in study III, engagement in strength training on a weekly basis was among the inclusion criteria. In all studies, exclusion criteria were any known previous hamstring and anterior cruciate ligament injuries, current or recent (< 3 years) lower back and leg injuries, as well as cardiovascular, metabolic, and neuromuscular disorders. Because subcutaneous tissue is generally thicker in females over the hamstrings and this increases the potential for cross-talk, only males were included, whose demographic data are presented in Table 2. Before enrolling participants into the studies, informed consent was collected from each of them based on their clear understanding of the facts, implications, and consequences of participating in the study. Testing procedures were approved by the University of Jyväskylä ethics committee (I, II, IV) and the review board of the Faculty of Sport Sciences, Université Côte d'Azur, Nice, France (III). All testing was performed according to the ethical principles of the seventh revision of the Declaration of Helsinki concerning research involving human participants.

TABLE 2 Morphometric data of the participants in each study.

Study	N	Age (years)	Body mass (kg)	Height (m)
I	12	24 ± 4	74 ± 8	1.79 ± 0.09
II	19	26 ± 3	80 ± 14	1.78 ± 0.09
III	13	23 ± 3	76 ± 6	1.77 ± 0.05
IV	13	25 ± 3	79 ± 9	1.80 ± 0.07

Values are mean ± standard deviation. N = number of participants

## 4.2 Study design

### 4.2.1 Familiarization sessions

Before the data collection day of each study, participants attended one or more (detailed below) familiarization sessions. During this period, participants refrained from additional strength training. Participants practiced maximal voluntary isometric contraction (MVIC) with real-time visual force-time curve feedback (Kellis and Baltzopoulos, 1996), which was used for EMG normalization in the main testing session of each study. In studies I-III, participants practiced and learned the proper technique of each exercise and the target loads were estimated, which were used in the main testing session of each study. The practiced exercises and some additional procedures were done as detailed below.

In study I, NHE and stiff-leg deadlift exercise were practiced in one session performed 10 to 14 days before data collection. In NHE, the exercise was performed with bodyweight only, which was supramaximal load for the participants (i.e. they were not able to control the movement until full knee extension). The pace of the movement ( $18^\circ$  per second) was controlled with a metronome. One-repetition concentric maximum was determined for stiff-leg deadlift exercise, and 80% of this load was used in the main testing session. These loads are typically used in training regimes.

In study II, four to five sessions (four to seven days apart) were used to practice nine hamstring exercises and to define 12 repetition maximum load for each. The selection of the exercises for this study were based on the criteria that the lengthening and shortening phases can be separated for analysis, and are typically used in previous studies or in typical training regimes targeting hamstrings. These exercises (Figure 5) were the good morning (GM), unilateral Romanian deadlift (RDL) cable pendulum (CP), bent-knee bridge (BB),  $45^\circ$  hip extension (HE); prone leg curl (PLC), slide leg curl (SLC), upright hip extension conic-pulley (UHC), straight-knee bridge (SB). Relatively low loads were applied to minimize fatigue during the measurement session.

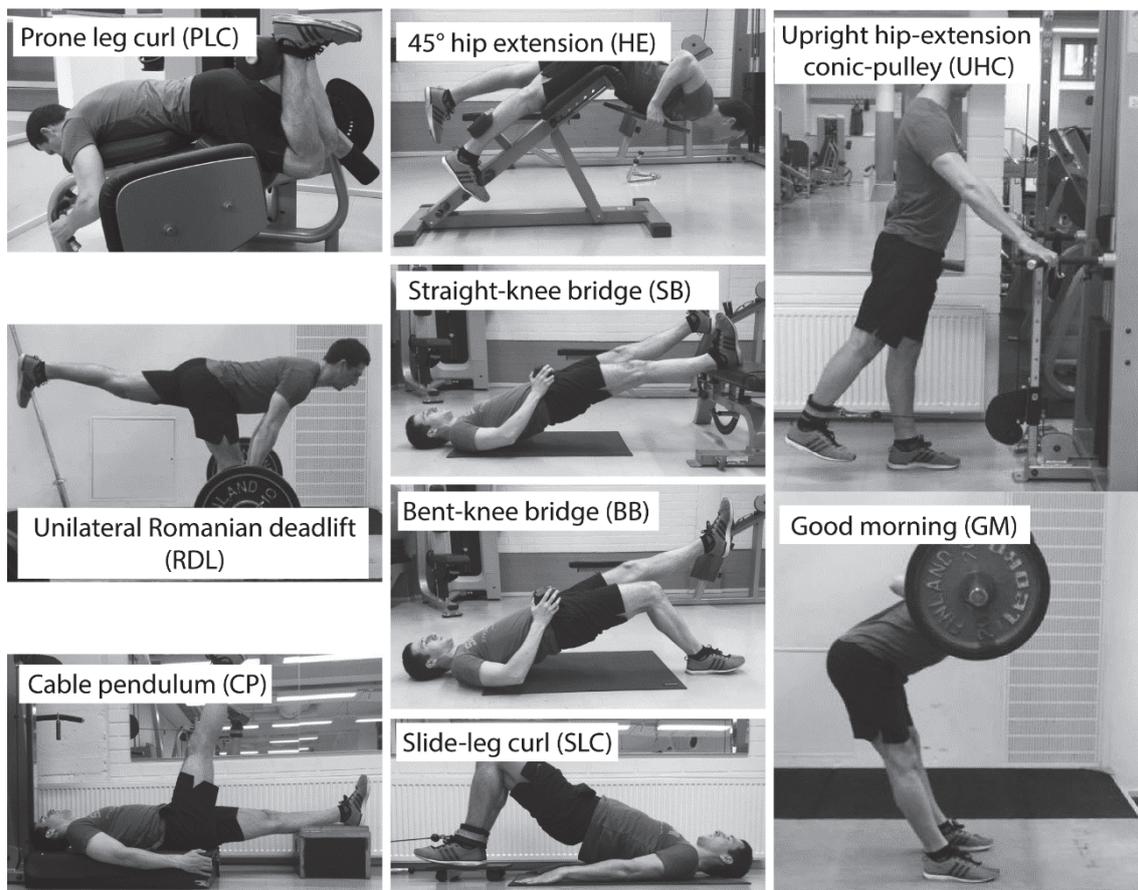


FIGURE 5 Nine typical hamstring exercises were tested in study II. Exercises were performed at 12-repetition maximum load after four or five familiarization sessions. Adopted from study II.

In study III, during a three-week period, participants attended six familiarization sessions before data collection. In this study, loads were set at eccentric one-repetition maximum for each of the four exercises. These exercises were: NHE performed (1) unilaterally or (2) bilaterally with neutral hips (unilateral and bilateral NHE0), and (3) unilaterally or (4) bilaterally with 90°-flexed hips (unilateral and bilateral NHE90). In each exercise, knee range of motion was approximately 90-15° performed at five-second pace, which was achieved by adjusting the load using resistance bands and weight plates mounted on the frame of the load-cell device (Figure 6). In addition to estimating these loads during the familiarization sessions, the aim was to elicit neural adaptations (Carolan and Cafarelli, 1992) and take advantage of the repeated bout effect during the measurement session (McHugh et al., 1999), which was five to eight days after the last familiarization session.

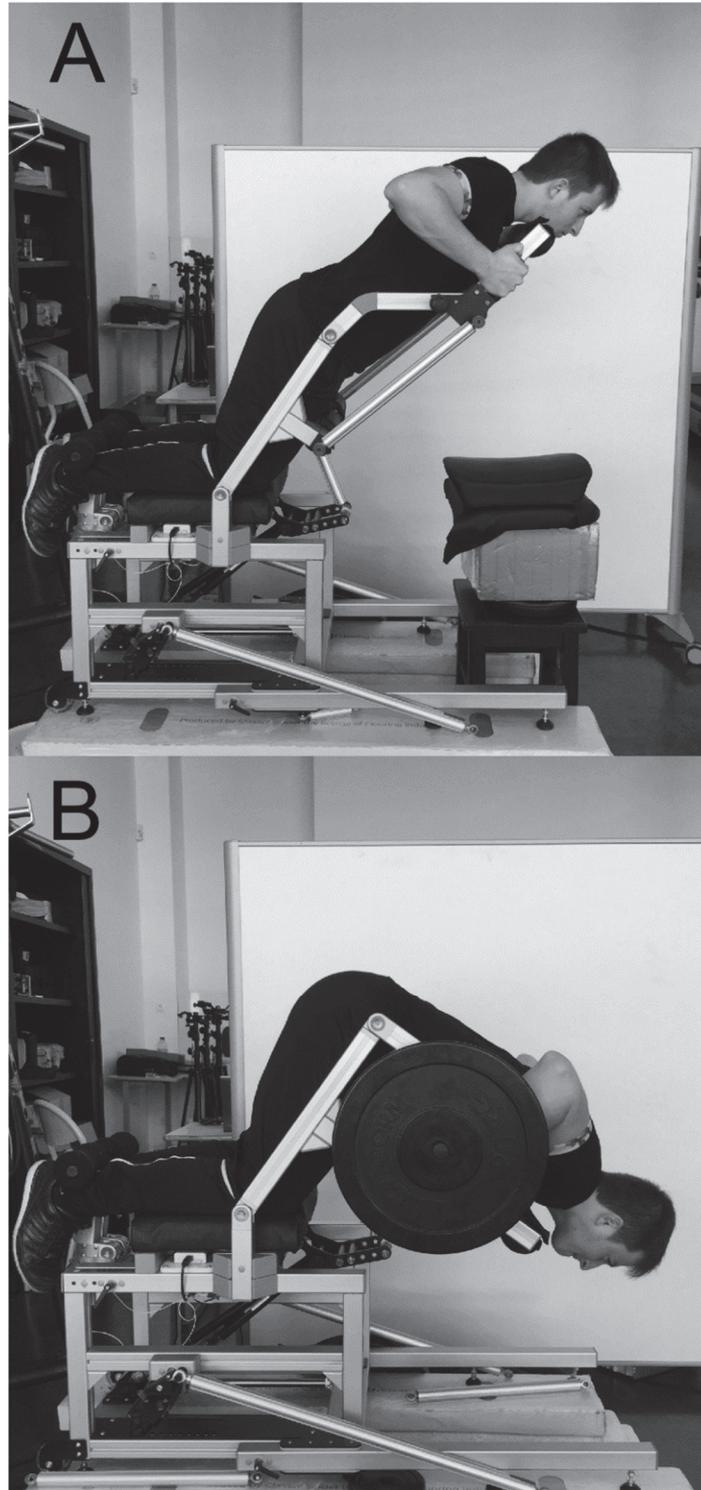


FIGURE 6 In study III, Nordic hamstring exercise (lengthening phase only) was performed either with neutral hips (A, bilateral) or with hips flexed to  $90^\circ$  (B, bilateral). These were performed in unilateral mode, too (not shown). Knee range of motion was set at  $90\text{--}15^\circ$  performed at a pace of five seconds. For each exercise, the load was set at one-repetition maximum using weight plates (B) or resistance bands (not seen, typically used in unilateral conditions) to increase or decrease the load, respectively. Adopted from study III.

In study IV, maximum running speed was tested on a motorized treadmill during a single session, and 45, 60, and 75% of this speed were the target speeds during the measurement session, which was four to seven days later. These speeds were chosen to ensure that several steps could be recorded at steady speed without technical failure or fatigue.

#### **4.2.2 Measurement sessions**

Each study included one measurement session. After preparation and warm-up, MVICs were performed in prone position with trunk and hips in neutral position and the knee of the tested leg flexed to 20° (I,II) or 30° (III, IV). Real-time visual force-time curve feedback was provided. In addition to the typically used knee flexion task, hip extension in study I was also tested, and hip extension combined with knee flexion in studies I and II. However, as these tasks did not lead to a clear increase in maximum EMG activity, these were not used in studies III and IV. The tested leg was the preferred kicking leg of each participant in studies I-III. In study IV, due to the restrictions of the laboratory, the left leg was tested for all participants. During the MVICs (I-IV), exercises (I-III), and running (IV), EMG activity was recorded from the BFlh and ST muscles using 16-electrode linear EMG arrays placed in the proximo-distal direction along each muscle. Additionally, during the exercises and in running, hip and knee joint kinematics were recorded using an electro-goniometer (I, II), potentiometer (III, knee only), or 3-D motion capture system (IV). Knee flexion force was measured during all NHE modes using a custom-made device (study I, University of Jyväskylä, Jyväskylä, Finland, designed based on previously used NHE testing device (Opar et al., 2013a)), and on a novel load-cell device (study III, Hamtech, Human Kinematic Inc., Carros, France). Five, six, and two repetitions of each exercise were performed in studies I, II, and III, respectively. In running (IV), a minimum of 15 strides were recorded at each speed. In each study, exercises and running tasks were performed in a randomized order, and with sufficient rest in-between. Participants reported that perceived fatigue was negligible during the measurement sessions.

#### **4.3 Data collection**

For studies I, II, and IV, data were collected at the Neuromuscular Research Center, University of Jyväskylä. Data collection for study III was done at the accredited research center Le Laboratoire Motricité Humaine Expertise, Sport, Santé in Nice, France.

### 4.3.1 High-density electromyography

Participants lay prone for HD-EMG preparation. After preparing the skin, linear arrays (high-density 1x16 electrodes, 10 mm interelectrode distance, OT Bioelettronica, Turin, Italy) were attached over the BFlh and ST muscles of the measured leg (Figure 7). The locations of the electrodes were defined with the use of high-resolution two-dimensional B-mode ultrasonography (Aloka  $\alpha$ 10, Tokyo, Japan (I, II), and Teleded EchoBlast 128, Vilnius, Lithuania (III, IV)) so that the electrodes were as far from the muscle borders and free tendons as possible. To standardize the proximo-distal positioning of the BFlh array, channel 8-9 was placed at 50% of the distance between the ischial tuberosity and the popliteal fossa. For ST, the array was positioned 1 cm below the proximal border of the tendinous inscription. The cavities of the electrodes were filled with conductive gel (Parker Laboratories, Fairfield, NJ). Then, after confirming good signal quality during submaximal contractions, the electrodes were firmly taped on the skin. A reference electrode strap was mounted on the contralateral wrist. An analog-digital converter (12-bit, EMG-USB, OT Bioelettronica) received the signals from the high-density arrays, and amplified ( $\times 1000$ ) digital signals were then transmitted to a personal computer and recorded in OT BioLab software (v 3.1, OT Bioelettronica). The sampling frequency was set at 2048 Hz. Fifteen differential channels were recorded from 15 cm along each muscle. Some of the electromyography, force, and kinematic recordings were sampled in different software, which were synchronized with a digital pulse.

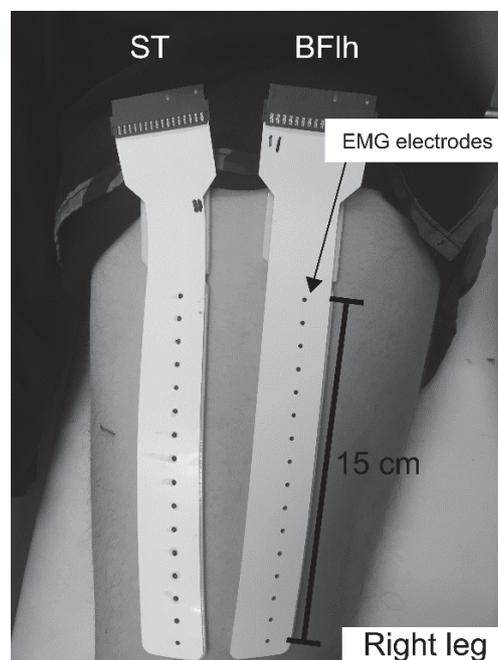


FIGURE 7 High-density electromyography (EMG) arrays were placed over the biceps femoris long head (BFlh) and semitendinosus (ST) to comprehensively describe the EMG activity of these muscles in various exercises and at different running speeds. This example shows the arrays attached over the right leg of a participant. After confirming signal quality, arrays were further secured with tape.

## 4.3.2 Force

### 4.3.2.1 Maximal voluntary isometric contractions

In MVICs, force signals were recorded at 1000 Hz in all studies, using the strain gauge of the dynamometer (I, II, UniDrive, University of Jyväskylä), the load cells of the Hamtech device (one per leg, ELAF, 1250 N, TE Connectivity, Shaffhausen, Switzerland), or with the strain gauge of the custom knee flexion force measuring device (IV, also used in study I to test NHE force, see 4.3.2.2). In hip extension, the strain gauge measured from above the knee fold. In the other tasks, the strain gauge measured from 2-5 cm above the lateral malleolus. Two to three maximal contractions were performed with 1-2 minutes rest in-between. Participants were asked to gradually increase force over 2-3 seconds and then maintain maximum contraction intensity for an additional 2 seconds.

### 4.3.2.2 Nordic hamstring exercise variations

Knee flexion force was measured from each leg separately during NHE using the strain gauges of the custom-made device (I) and the load cells of the Hamtech device (III). After converting analog signals to digital, signals were transmitted to a personal computer and recorded at 1000 Hz in Spike2 (I) or in a custom interface written in LabVIEW (III, v 8.5, National Instruments, Austin, TX). The distance between the lateral epicondyle of the femur and the center of the load cell (i.e. lever arm) was measured to calculate torque (III).

## 4.3.3 Kinematics

In studies I and IV, lower body kinematics were estimated by recording marker trajectories using a Vicon 3-D motion capture system (Vicon, Oxford, UK). Markers (14 mm diameter) were mounted on the pelvis, and either on both legs (I) or on the left leg only (IV). More specifically, markers were placed over the anterior and posterior superior iliac spine, lateral thigh, femur lateral epicondyle, lateral shank, lateral malleolus, calcaneus, and second metatarsal head. Marker displacements were sampled at 250 Hz in Nexus (v2.5, Vicon). Prior to collecting data in the exercises (I) and in running (IV), a standing trial was recorded to estimate the lengths of the segments, and the centers and coordinates of the joints. In study IV, foot strike (FS) and toe-off (TO) were defined based on force-sensitive resistors recording at 1000 Hz in Spike2 and positioned below the big toe, first and fifth metatarsal heads, and under the heel of the left foot.

In Study II, hip and knee joint sagittal plane kinematics were recorded using custom electro-goniometers sampling at 1000 Hz (University of Jyväskylä, Jyväskylä, Finland). Analog signals were converted to digital (EMG-USB, OT Bioelettronica) and then recorded in synchrony with the EMG signals in OT BioLab.

In Study III, knee joint angular displacement was recorded at a frequency of 1000 Hz with the potentiometer of the Hamtech device (P4500, Novotechnik,

Ostfildern, Germany). Digital signals were transmitted to a personal computer and recorded in a custom interface written in LabVIEW.

## 4.4 Data analysis

### 4.4.1 Joint angles and muscle-tendon lengths

Joint angles in Study II were used to define the lengthening and shortening phases of the exercises, which were used to assess contraction-mode effects on EMG activity. In Study III, knee joint angle was used to match the range of motion (90-15°) between exercises. Joint angles in running were also used to define sub-phases of the running stride as follows: 1) early stance (from FS to maximum knee angle in stance), 2) late stance (from maximum knee angle in stance to TO), 3) early swing (from TO to maximum knee angle in swing), 4) mid swing (from maximum knee angle in swing to maximum hip flexion angle), and 5) late swing (from maximum hip flexion angle to ipsilateral FS) (Higashihara et al., 2015). In Study I, to make stiff-leg deadlift exercise comparable to NHE, both exercises were analyzed in the active lengthening phase of each exercise only. In NHE, this was defined based on knee flexion force. However, in stiff-leg deadlift, the active lengthening phase was defined based on muscle-tendon lengths of the hamstrings. Similarly, muscle-tendon lengths were calculated in running. To estimate MTU lengths, 3-D marker displacements were low-pass filtered (8-10 Hz), joint angles were calculated in Nexus based on the plug-in gait model, then joint angles were exported and MTU lengths were calculated in Matlab based on modeling equations (Hawkins and Hull, 1990). Joint angles and muscle-tendon lengths were time-normalized (1-101 points) and then exercise repetitions (I) and stride cycles (IV) were averaged.

### 4.4.2 Torque

In the different NHE modes in Study III, torque (dominant leg) was calculated as the measured instantaneous force multiplied by the lever arm.

### 4.4.3 Region- and muscle-specific EMG activity

Electromyography signals were band-pass filtered with a zero-lag 4<sup>th</sup> order Butterworth filter in Matlab (MathWorks, Natick, MA, US). Bandwidth was set at 10-500 Hz (I-III), however, to remove movement artefacts in running, 20-500 Hz was used with an additional 50 Hz notch filter (IV). All filtered EMG signals were then rectified, and additional procedures were used as described below.

In MVICs, filtered and rectified signals over a one-second stable plateau were averaged. The highest value across repetitions and MVIC tasks for each channel was used to normalize the signals recorded from the corresponding channel during the exercises and running.

In Studies I and II, average normalized activity for each channel was calculated for the lengthening (I, II) and shortening (II) phases separately. Then, to define region-specific EMG activity levels, these values were averaged for channels 1-5 (distal region), 6-10 (middle region), and 11-15 (proximal region) for both BFlh and ST. To estimate the activity level of each muscle as a whole, all 15 channels were averaged proximo-distally along each muscle. Averaging several channels minimized the effects of muscle shift under the skin on the defined EMG amplitudes and increased statistical power.

While studies I and II focused on comparing muscle regions in different exercises including NHE, study III focused on the time-series comparison of muscle-specific EMG activity between different NHE modes and between muscles. To do so, each channel was low-pass filtered (5 Hz), normalized to MVIC, time-normalized (1-101 points), and then all channels along each muscle were averaged to define muscle-specific activity of BFlh and ST, respectively. These curves were included in the statistical analysis.

In running (IV), region- and muscle-specific EMG activity were calculated as follows. After smoothing the signals (10 Hz, low-pass) of each channel, EMG signals were normalized to MVIC, time-normalized (1-101 points), and then averaged across strides for each muscle and running speed. Region- and muscle-specific activity were defined similarly to the procedure of Studies I and II except that a few channels were excluded from the analysis due to the malfunction of some EMG arrays.

#### 4.4.4 Inter-individual differences

Due to large between-participant variability reported during gait in previous EMG studies (Ahn et al., 2011; Crouzier et al., 2019; Ivanenko et al., 2002; Winter and Yack, 1987), inter-individual coefficients of variation (CV%) for the EMG activity of each muscle and region in running, and also for MTU lengths (IV) were calculated.

#### 4.4.5 Statistical analysis

Univariate 0-D data (I, II) were analyzed in SPSS (IBM, Armonk, NY, US). Data normality was tested with Q-Q plots and Shapiro-Wilk tests. Repeated measures ANOVA was used to define interactions, which were followed up by pairwise comparisons with Bonferroni correction. Significance level was set at  $\alpha = 0.05$ . Cohen's *d* effect sizes are also reported with 90% confidence intervals (90% CI) in these studies. Cohen's *d* was interpreted as trivial  $< 0.2 \leq$  small  $< 0.5 \leq$  moderate  $< 0.8 \leq$  large (Cohen, 1988).

Univariate 1-D data (III, IV) were analyzed with Statistical Parametric Mapping (SPM, v0.4, open-source code available at [www.spm1d.org](http://www.spm1d.org)). The technical details of SPM analyses are detailed elsewhere (Adler and Taylor, 2007; Friston et al., 1995). In running (IV), two-way repeated measures ANOVAs were run across the running strides. This included the calculation of SPM{F} statistics for each time-point across the normalized time-series. The critical thresholds were

then defined (for  $\alpha = 0.05$ ) based on random field theory. Finally, P values were calculated for each of the supra-threshold clusters (i.e. regions where SPM trajectories crossed the critical threshold). In case of an interaction, SPM{t} test was run with Bonferroni correction to define the locations of the differences. Similarly, when comparing different NHE modes and the EMG activity of BFlh and ST across the time-normalized lengthening phase, SPM{t} values were calculated for each comparison to quantify the magnitude of the differences. Additionally, in study III, the sizes of the differences were also expressed as Cohen's  $d$ , with uncertainty presented by 95% confidence intervals (95% CI).

## 5 RESULTS

### 5.1 Nordic hamstring and stiff-leg deadlift exercises (I)

#### 5.1.1 Intramuscular differences

In NHE, significant regional differences were detected in both ST and BFlh ( $p = 0.003$  in both). In ST, activity in the proximal region was  $74 \pm 9$  %MVIC. Activity in the middle region ( $80 \pm 14$  %MVIC) was significantly higher than in the distal region ( $63 \pm 12$  %MVIC, Cohen's  $d = 1.41 \pm 0.79$ ,  $p = 0.002$ ). Other differences between regions were moderate to large ( $d$  range = 0.55-0.87), but did not reach statistical significance ( $p > 0.05$ ). In BFlh, distal activity ( $72 \pm 11$  %MVIC) was higher than middle ( $62 \pm 10$  %MVIC,  $d = 0.87 \pm 0.60$ ,  $p = 0.005$ ) and proximal activity ( $58 \pm 16$  %MVIC,  $d = 1.25 \pm 0.71$ ,  $p = 0.026$ ), respectively. Middle and proximal EMG activity did not differ significantly from each other ( $d = 0.38 \pm 0.53$ ,  $p > 0.05$ ).

In stiff-leg deadlift, activity in the proximal, middle, and distal regions of ST were  $37 \pm 7$ ,  $41 \pm 9$ , and  $37 \pm 9$  %MVIC, respectively. Small to moderate effects between ST regions ( $d$  range = 0.29-0.67) did not result in statistical differences ( $p > 0.05$ ), whereas significant region-dependent differences in EMG activity were detected in BFlh ( $p = 0.003$ ). Muscle activity was found to be significantly lower in the proximal ( $32 \pm 9$  %MVIC) compared to the middle ( $37 \pm 9$  %MVIC,  $d = 0.48 \pm 0.29$ ,  $p = 0.041$ ) and distal ( $39 \pm 10$  %MVIC,  $d = 0.63 \pm 0.35$ ,  $p = 0.024$ ) regions. No differences were detected between the distal and middle regions ( $d = 0.16 \pm 0.27$ ,  $p > 0.05$ ).

In both muscles, within-muscle differences were fairly consistent across participants, which is shown in the original article.

#### 5.1.2 Intermuscular differences

In NHE, when all 15 channels along each muscle were averaged, EMG activity was higher in ST ( $72 \pm 7$  %MVIC) than in BFlh ( $64 \pm 10$  %MVIC,  $d = 1.06 \pm 0.45$ ,  $p$

< 0.001). In SDL, EMG activity did not differ between ST ( $37 \pm 7$  %MVIC) and BFlh ( $36 \pm 9$  %MVIC,  $d = 0.19 \pm 0.34$ ,  $p > 0.05$ ).

## 5.2 Exercises at matched submaximal load (II)

### 5.2.1 Intramuscular differences

Regional differences were highly exercise- and muscle-specific ( $p < 0.001$ ). Descriptive data are presented on Figure 8. In ST, EMG activity was lower in the distal than in the middle or proximal regions during the slide leg curl, prone leg curl, and bent-knee bridge ( $d$  range = 0.53-1.20,  $p < 0.05$ ). In other exercises, regional differences were smaller ( $d$  range = 0.00-0.40) and not statistically significant ( $p > 0.05$ ). The largest regional differences within BFlh were observed in the shortening phase of the prone leg curl: distal ( $81 \pm 17$  %MVIC) larger than proximal ( $59 \pm 15$  %MVIC) by  $d = 1.28 \pm 0.30$ . Figure 9 shows that the share of whole-muscle activity shifts towards the middle and proximal regions within ST in some exercises. On the contrary, in BFlh, the middle and distal regions have more share of the whole-muscle activity than the proximal region (Figure 10).

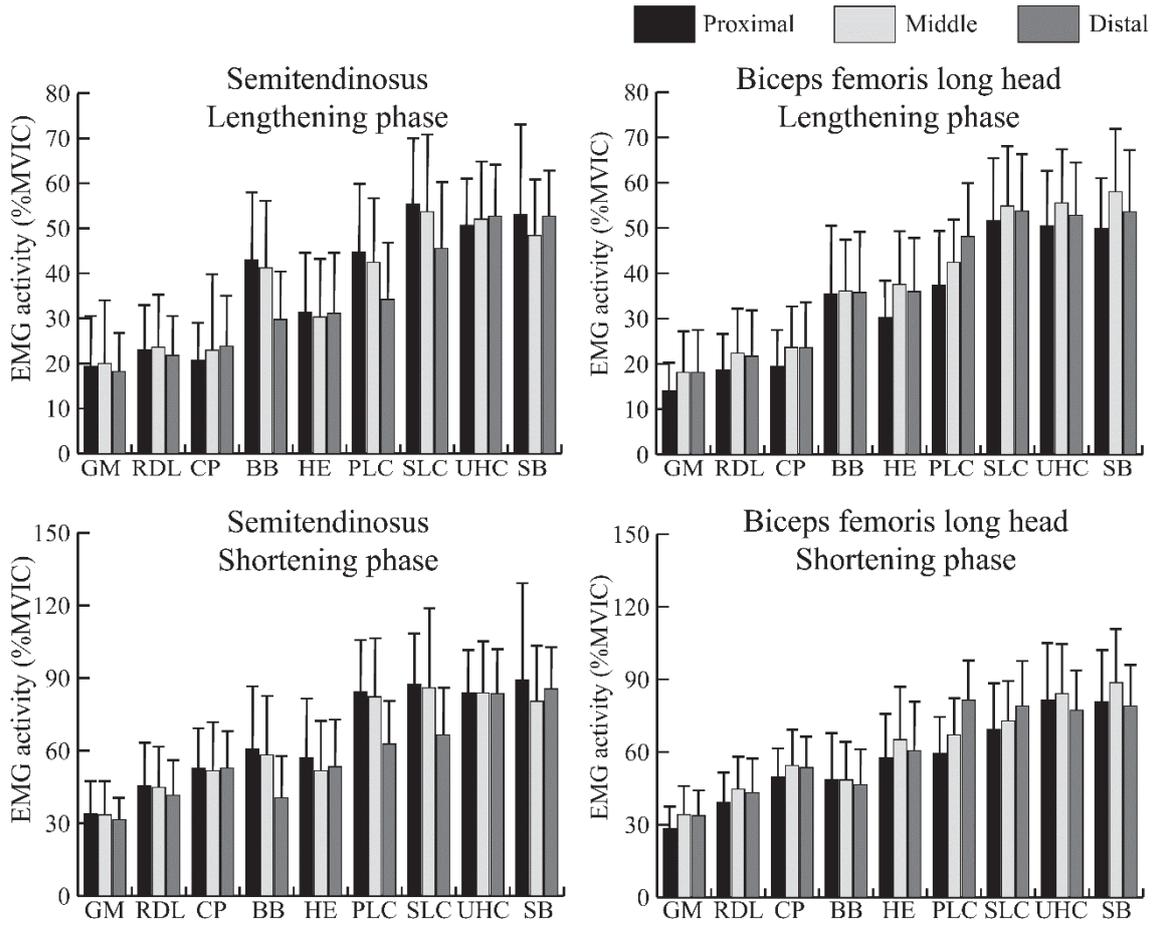


FIGURE 8 Mean and standard deviation of the normalized activity level (%MVIC, maximal voluntary isometric contraction, N=19) in the proximal, middle, and distal regions of each muscle during the eccentric and concentric phase of each exercise. GM, good morning; RDL, unilateral Romanian deadlift; CP, cable pendulum; BB, bent-knee bridge; HE, 45° hip extension; PLC, prone leg curl; SLC, slide leg curl; UHC, upright hip extension conic-pulley; SB, straight-knee bridge. Reused from study II.

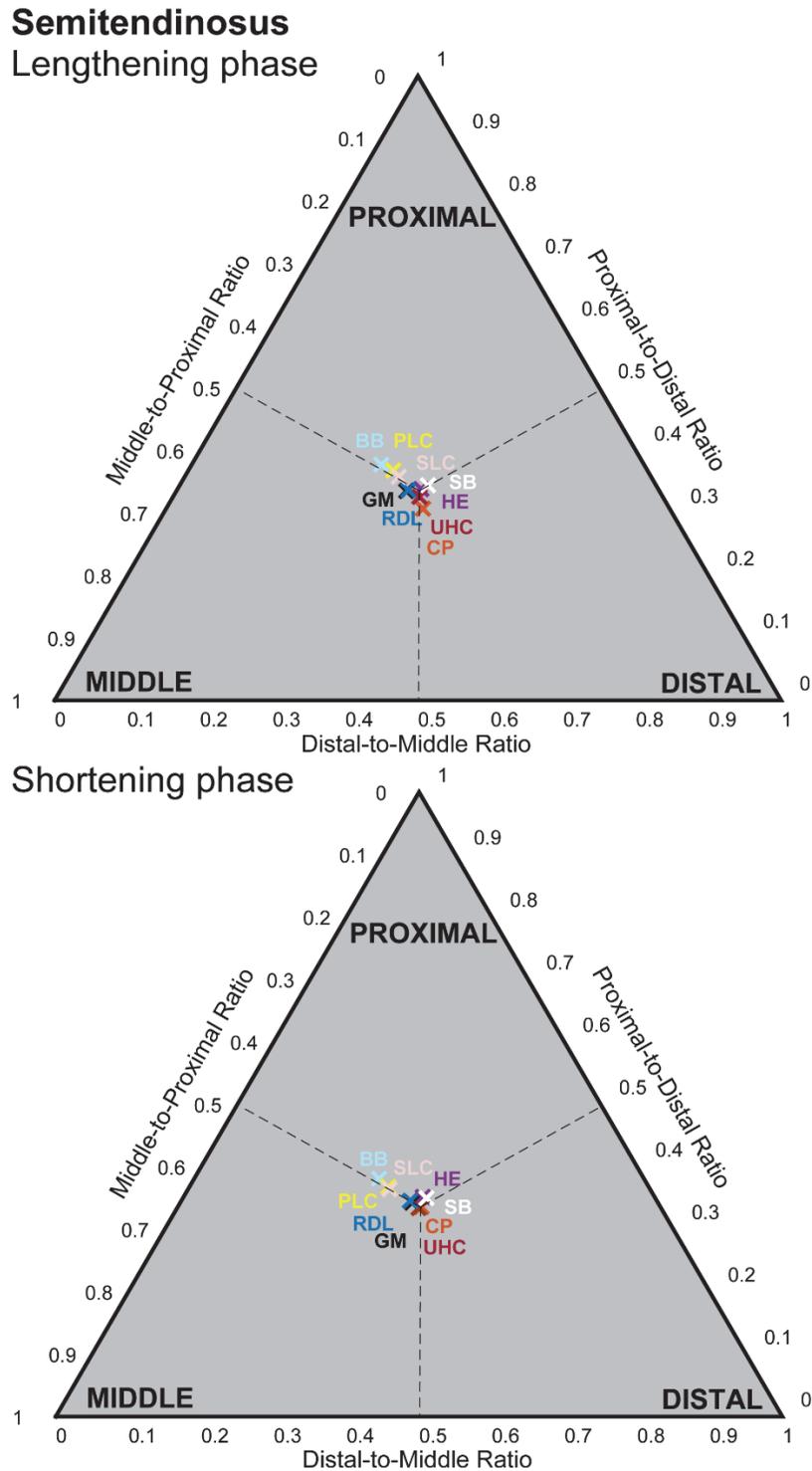


FIGURE 9 Ratios between the activity of different regions of the semitendinosus in the lengthening and shortening phases of nine hamstring exercises. The center of the triangle (where dashed lines meet) corresponds to homogeneous activity within the muscle. Regional are highly exercise-specific. For example, BB showed the largest difference between proximal and distal regions (Cohen's  $d = 1.20 \pm 0.44$  in the lengthening phase). GM, good morning; RDL, unilateral Romanian deadlift; CP, cable pendulum; BB, bent-knee bridge; HE, 45° hip extension; PLC, prone leg curl; SLC, slide leg curl; UHC, upright hip extension; SB, straight-knee bridge.

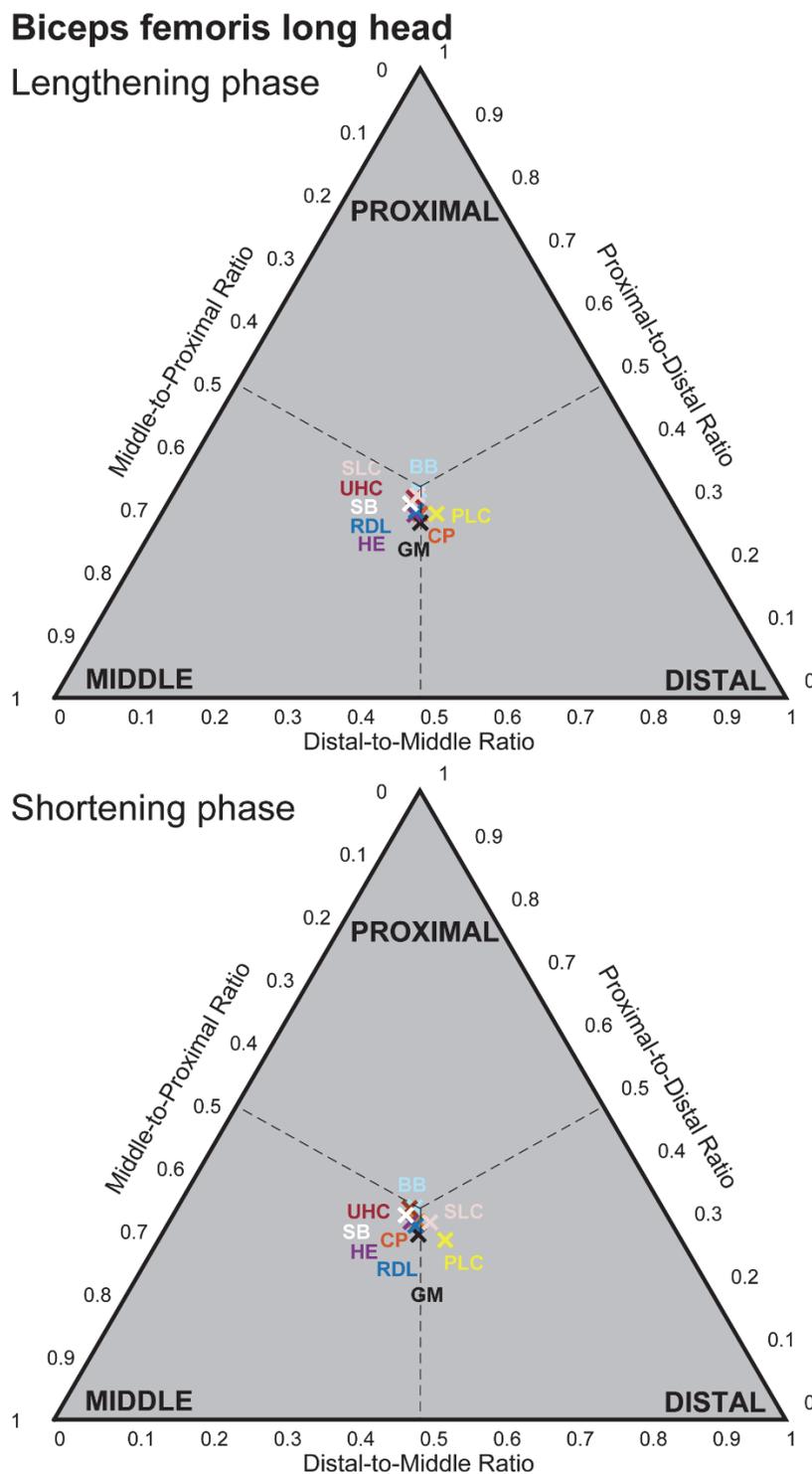


FIGURE 10 Ratios between the activity of different regions of the biceps femoris long head in the lengthening and shortening phases of nine hamstring exercises. The center of the triangle (where dashed lines meet) corresponds to homogeneous activity within the muscle. Exercises generally show higher activity in the middle/distal regions than in the proximal region. The largest difference between proximal and distal regions was observed in the shortening phase of PLC (Cohen's  $d = 1.28 \pm 0.30$ ). GM, good morning; RDL, unilateral Romanian deadlift; BB, bent-knee bridge; CP, cable pendulum; HE, 45° hip extension; PLC, prone leg curl; SLC, slide leg curl; UHC, upright hip extension conic-pulley; SB, straight-knee bridge.

## 5.2.2 Intermuscular differences

Both the exercise and the contraction mode affected the activity of BFlh and ST relative to each other. Nonetheless, differences in EMG activity between muscles were comparably smaller than within muscles. Significantly higher BFlh than ST activity was only observed in the shortening phase of the 45° hip extension ( $d = 0.33 \pm 0.24$ ,  $p = 0.026$ ). Significantly higher ST than BFlh activity was only observed in the shortening phase of the prone leg curl ( $d = 0.35 \pm 0.27$ ,  $p = 0.036$ ). Small differences in favor of greater ST activity were also observed in the shortening phase of the slide leg curl and bent-knee bridge, and in the lengthening phase of good morning ( $d$  range = 0.21-0.26), but these differences did not reach statistical significance ( $p > 0.05$ , Figure 11).

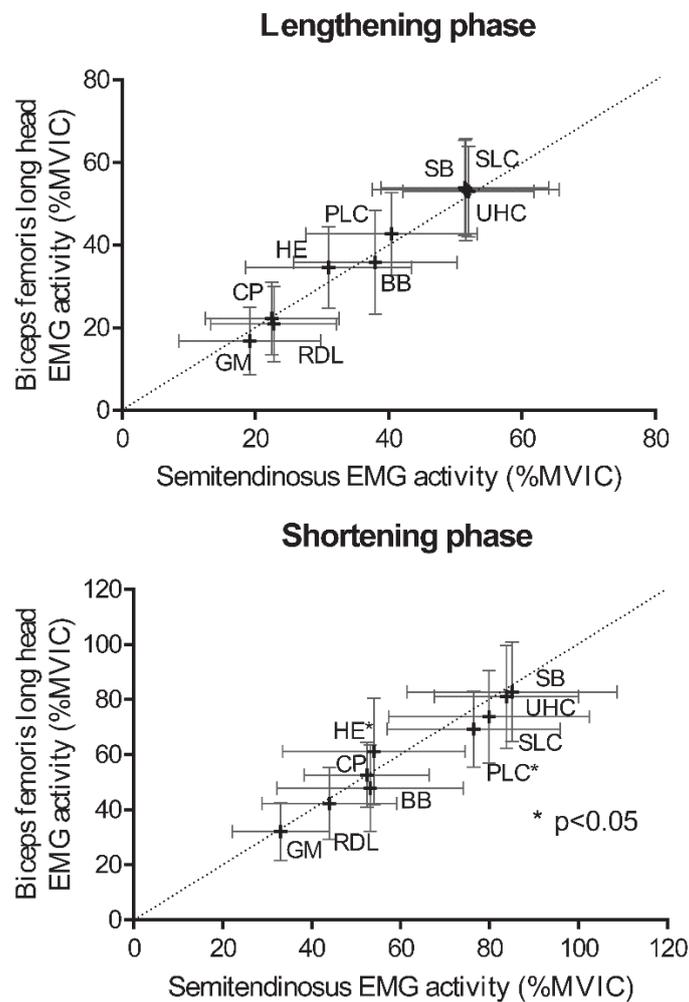


FIGURE 11 Whole-muscle electromyography (EMG) activity in the lengthening and shortening phase of each exercise. Group mean and standard deviation are presented (N=19). Dotted lines represent equal activity level between the two muscles when normalized to maximal voluntary isometric activity (MVIC). GM, good morning; RDL, unilateral Romanian deadlift; CP, cable pendulum; BB, bent-knee bridge; HE, 45° hip extension; PLC, prone leg curl; SLC, slide leg curl; UHC, upright hip extension conic-pulley; SB, straight-knee bridge. Reused from study II.

### 5.3 Nordic hamstring variations (III)

#### 5.3.1 Knee flexion torque

Significantly higher torque was reached in NHE90 than in NHE0 in both the bilateral (from start position until 87% movement phase,  $d = 2.74 \pm 0.85$ ,  $p < 0.001$ ) and unilateral (through the entire range of motion,  $d = 3.22 \pm 0.88$ ,  $p < 0.001$ ) modes (Figure 12). When comparing bilateral modes to unilateral modes, NHE0 showed higher torque in the bilateral mode in the midrange of the exercises ( $p = 0.005$ ), but NHE90 showed no difference in any subphase of the movements ( $p > 0.05$ ).

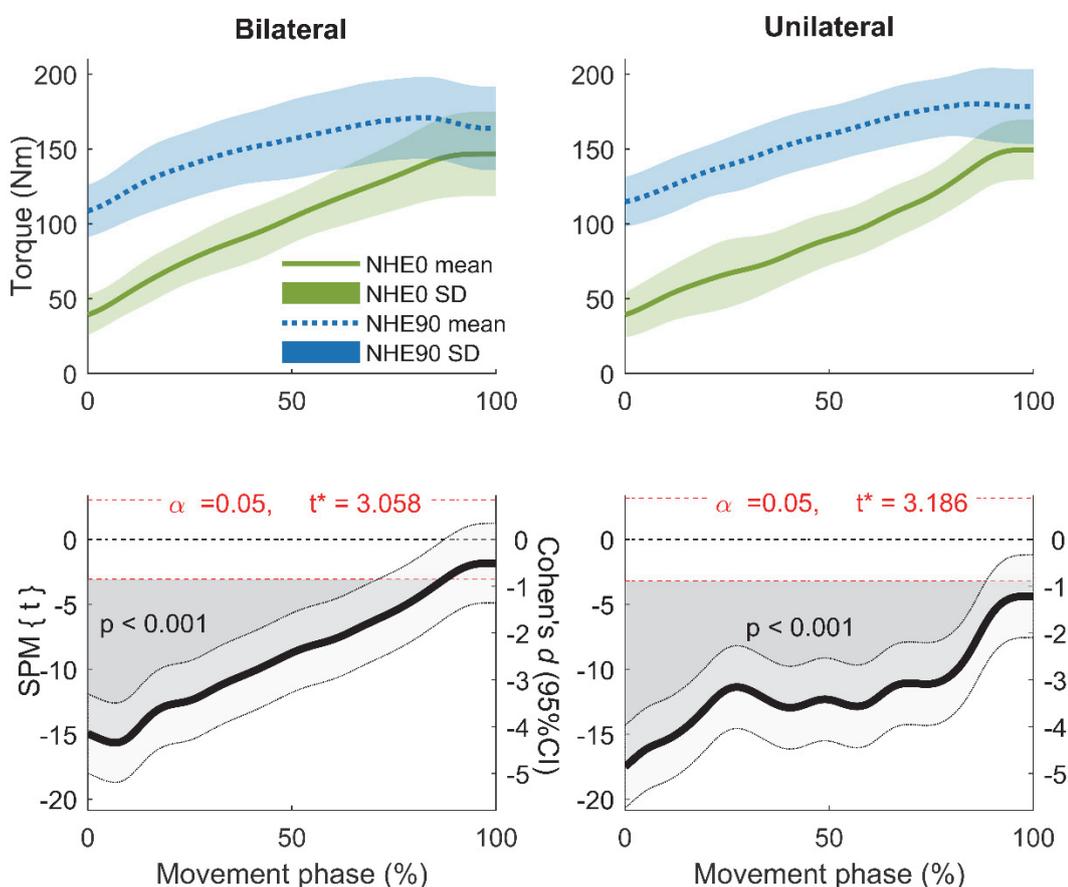


FIGURE 12 Knee flexion torque (mean  $\pm$  SD) in bilateral and unilateral Nordic hamstring exercises performed either with neutral hips (NHE0) or with 90°-flexed hips through a knee range of motion 90-15° (0-100% movement phase). Each was performed at eccentric one-repetition maximum load. Thick black lines (bottom plots) represent SPM{t} statistics and Cohen's d effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = 0.05$ ) are shown with dashed red lines. Probability values (P) are calculated for each supra-threshold cluster depicting statistically significant differences. Adopted from study III.

### 5.3.2 EMG activity

BFlh and ST showed higher activity in the NHE0 than in NHE90 in both the bilateral and unilateral modes, especially in the second half of the movement ( $d$  range = 1.10-2.18,  $p \leq 0.042$ ). Differences were not observed between unilateral and bilateral modes of NHE0 or NHE90 ( $p > 0.05$ ).

Intermuscular differences were highly dependent on the hip angle during NHE (Figure 13).

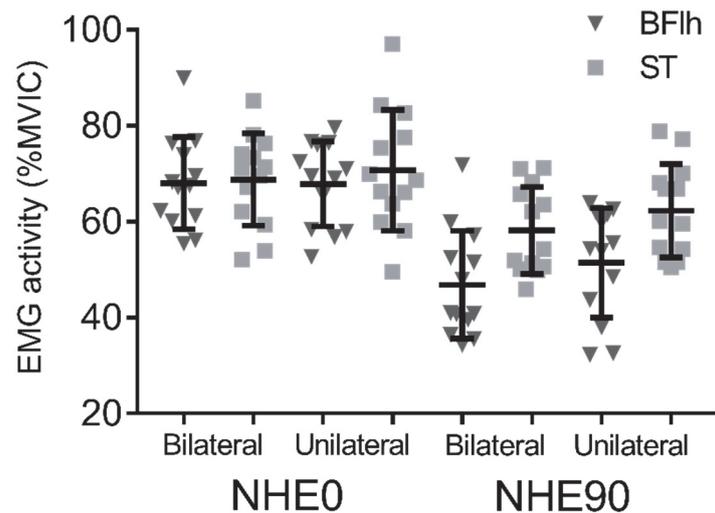


FIGURE 13 Electromyography (EMG) activity of the biceps femoris long head (BFlh) and semitendinosus (ST) in each exercise averaged across the entire knee range of motion and normalized to maximal voluntary contraction (%MVIC). Group results (mean, standard deviation) and the values of each participant are presented. NHE0, Nordic hamstring exercise performed in neutral hip position; NHE90, Nordic hamstring exercise performed with hips flexed to 90°.

Additionally, knee range of motion affected the activity of the BFlh and ST muscles relative to each other. For example, in bilateral NHE0, greater ST activity was seen in the early phase of the movement, as opposed to greater BFlh activity in the terminal phase of the movement ( $d = 1.07 \pm 1.07$ ,  $p = 0.043$ , and  $d = 1.11 \pm 1.07$ ,  $p = 0.030$ , respectively, Figure 14). In unilateral NHE0, no difference between muscles was observed ( $p > 0.05$ ). In NHE90, ST activity was generally higher than BFlh activity in both the bilateral and unilateral exercises ( $d = 1.09$ -1.25,  $p \leq 0.039$ ).

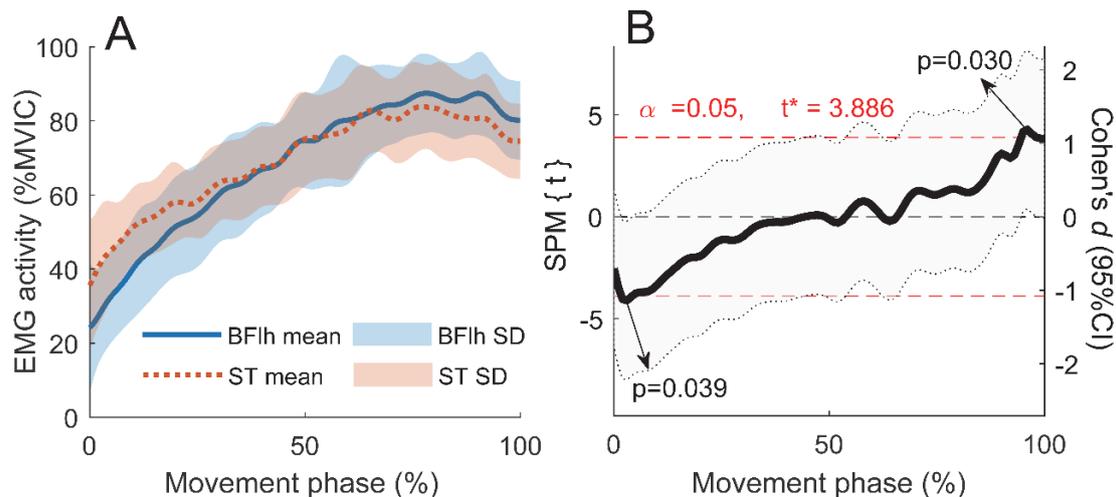


FIGURE 14 Knee range of motion affects which of the biceps femoris long head (BFlh) and semitendinosus (ST) is activated most during bilateral Nordic hamstring exercise performed with neutral hips (NHE0, knee range of motion 90-15°). Panel A shows mean and standard deviation (SD) of electromyography (EMG) activity normalized to maximal voluntary isometric contraction (MVIC). Panel B shows the Statistical Parametric Map (SPM{t}) and Cohen's *d* differences with 95% confidence intervals (CI). Phases where SPM{t} cross the critical threshold ( $t^*$ ) represent statistical differences between the activity of the two muscles. Adopted from study III.

## 5.4 Running (IV)

EMG activity and MTU behavior were examined at speeds of  $4.1 \pm 0.2 \text{ m s}^{-1}$  (slow),  $5.4 \pm 0.3 \text{ m s}^{-1}$  (moderate), and  $6.8 \pm 0.4 \text{ m s}^{-1}$  (fast), representing 45, 60, and 75% of maximum running speed, which was  $9.04 \pm 0.52 \text{ m s}^{-1}$ .

MTUs underwent typical stretch-shortening cycles through the running stride, with the longest length change observed in mid-swing. BFlh was longer than ST in all phases of the stride at all speeds ( $p < 0.001$ ). With increasing running speed, total length changes increased in both muscles, mainly because of decreased minimum MTU lengths (observed in early swing,  $p < 0.001$ ). Additionally, maximum lengths (observed in late swing) increased in both muscles when speed increased from slow to moderate (BFlh  $p = 0.013$ , ST  $p = 0.039$ ). MTU length changes were similar across participants in both muscles ( $\text{CV}\% \leq 3\%$ ).

### 5.4.1 Intramuscular differences (EMG)

In all regions, the highest EMG activity was observed in the late swing phase in both muscles at all speeds. Group mean results of each EMG channel recorded proximo-distally from BFlh at the fast running speed are shown in Figure 15.

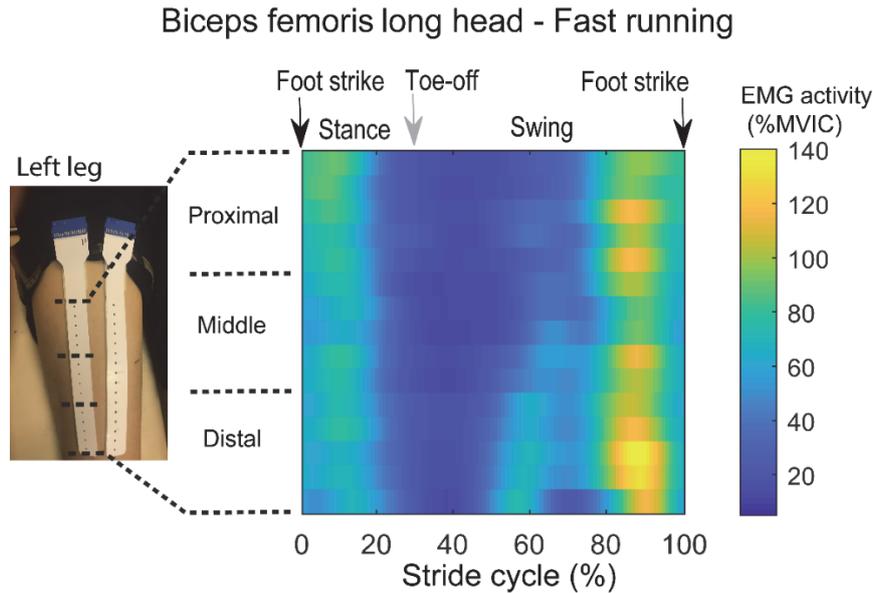


FIGURE 15 Group mean (N=13) electromyography (EMG) activity (normalized to maximal voluntary isometric contraction, MVIC) of biceps femoris long head at the fast running speed in each of the 15 EMG channels. Adopted from manuscript IV, in which proximo-distal patterns are illustrated for all speeds and muscles.

Although group mean results suggest higher distal than middle/proximal BFLh EMG activity in the late swing phase, the difference was not statistically significant at any of the running speeds ( $p > 0.05$ ). Similarly, no regional differences were observed within ST at any speed ( $p > 0.05$ ) at the group level. Nonetheless, this can partly be explained by large differences between participants in regional activity patterns. Regional patterns for each participant are shown in Figure 16 (fast running, BFLh) and in original article IV (all speeds, BFLh and ST).

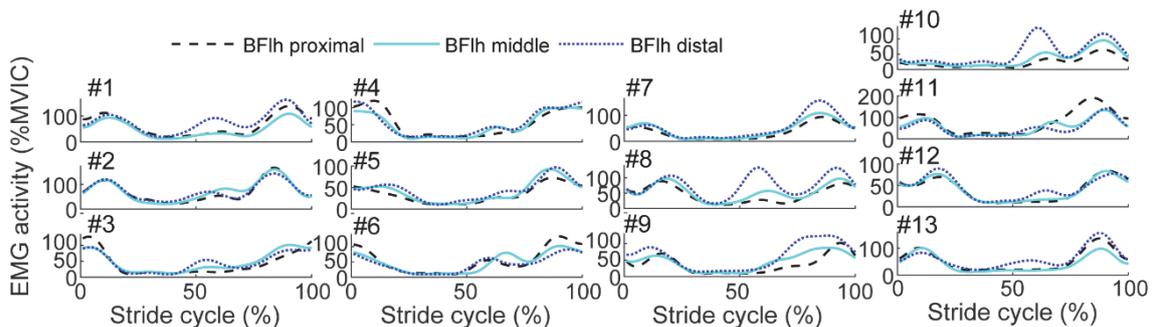


FIGURE 16 Regional activity patterns were highly specific to each participant (#1-13). Curves represent the average of all steps and channels recorded from the corresponding region at the fast running speed. Adopted from study IV.

#### 5.4.2 Intermuscular differences (EMG)

At the group level, no significant differences were observed between muscles at any of the running speeds when all channels along each muscle were averaged. However, as was the case for regional patterns, muscle-specific EMG activity was

highly specific to each participant (for typical examples see Figure 17). This resulted in large coefficients of variation, which ranged for example from 28% to 71% in the BFlh-to-ST activity ratio. Despite large differences between participants, qualitatively consistent intermuscular EMG patterns were observed across running speeds within most participants.

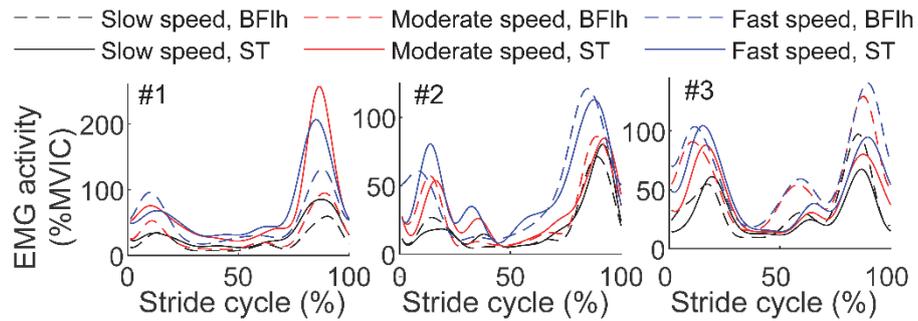


FIGURE 17 Typical example of different muscle-specific electromyography (EMG) patterns (normalized to maximal voluntary isometric contraction, MVIC) between participants (#1-3), suggesting participant-specific intermuscular coordination strategies. Adopted from study IV.

## 6 DISCUSSION

The current thesis used HD-EMG in different exercises and in running to reveal within-muscle differences in hamstring EMG activity, as well as defining muscle-specific EMG activity while taking proximo-distal differences into account. The main findings of this thesis were as follows:

- 1) Normalized EMG activity of the BFlh and ST muscles was non-uniform proximo-distally in several exercises. Proximo-distal differences substantially varied across exercises, whereby knee-dominant exercises presented generally larger differences between muscle regions (e.g. NHE, in both muscles) than most hip-dominant exercises. Additionally, proximo-distal EMG patterns were specific to the examined muscle: in BFlh, most exercises activated the middle or distal region the most, while in ST most exercises activated the middle or proximal region the most. Contraction mode (lengthening/shortening) only had a small effect on regional activity patterns. Intermuscular differences were rather small compared to intramuscular differences in exercises performed at submaximal loads: significantly higher activity in ST than BFlh was only observed in the shortening phase of prone leg curl, while higher BFlh than ST activity was only observed in the shortening phase of 45° hip extension. Conventional NHE resulted in higher ST than BFlh EMG activity (I, II).
- 2) NHE performance measures were highly affected by hip and knee angles. Performing NHE with 90°-flexed hips (NHE90) elicited higher knee flexion torque and lower hamstring EMG activity compared to NHE performed with neutral hips (NHE0). Additionally, when NHE0 was performed bilaterally, ST was preferentially activated in the early phase of the exercise, while BFlh was preferentially activated at the terminal phase of the exercise (i.e. near full knee extension) (III).

- 3) Compared to hamstring exercises, running resulted in relatively large inter-individual differences in region-specific and muscle-specific EMG activity patterns. However, these patterns were qualitatively consistent across a range of running speeds in most individuals (IV).

## 6.1 Region-specific hamstring activity

This thesis shows that regional hamstring EMG activity patterns are exercise-specific. This is in accordance with exercise-specific regional metabolic activity observed in studies using mfMRI. This section will discuss the current state of knowledge regarding regional activation of the BFlh and ST muscles.

### 6.1.1 Hamstring exercises

Kubota et al. (2007) were the first to report region-specific hamstring muscle use during the lengthening phase of the prone leg curl exercise. Five sets of 10 repetitions were performed at 120% of concentric 1-repetition-maximum load. In BFlh, acute changes in T2 relaxation time (a measure of metabolic activity) in response to the exercise showed higher changes in the distal and middle regions than in the proximal region. These changes persisted for seven days after the exercise. It should be mentioned that in a separate study, a similar exercise protocol did not induce significant changes in any regions of the BFlh (Mendiguchia et al., 2013b), however, this could be due to the low power in the latter study to detect statistically significant changes in BFlh. Using EMG, a method which is more sensitive to increases in muscle activity, Schoenfeld et al. (2015) found that electrodes placed over the distal region of the lateral hamstrings detected higher muscle activity than those placed over the proximal region in the same (prone leg curl) exercise. However, it should be noted that the interelectrode distance used in this study (20 mm) might not be suitable to record from the target muscles selectively (De Luca et al., 2012). Other mfMRI studies showed T2 changes only in the distal portion of the BFlh after four to five sets of eight repetitions of NHE (Mendez-Villanueva et al., 2016; Mendiguchia et al., 2013a). These results are similar to our findings wherein NHE (I) and prone leg curl (II) exercises activated distal BFlh the most. In the mechanically similar slide leg curl, EMG activity was higher than in prone leg curl, however regional differences were comparatively small (II). Similarly, T2 changes in response to Flywheel leg curl have been found to be smallest in the proximal region (Mendez-Villanueva et al., 2016). These results seem to be associated with higher strain in the proximal region than other muscle regions during cyclic knee flexion-extension contractions (Silder et al., 2010). Thus, previous findings collectively suggest that knee-dominant exercises activate the distal more than the proximal region of BFlh. However, our results further suggest that relatively low activity in the proximal region is also seen in several hip-dominant exercises; seven out of eight hip-dominant exercises showed significantly lower EMG activity in the proximal region than in the middle or

distal regions in the lengthening and/or shortening phase of the exercises (I, II). However, differences between regions in hip-dominant exercises were smaller than those observed in the knee-dominant prone leg curl and NHE. Additionally, hip-dominant bent-knee bridge showed no difference between muscle regions in BFlh. Some studies have suggested preferential proximal BFlh metabolic activation in the lunge (Mendiguchia et al., 2013b) and cable pendulum (Mendez-Villanueva et al., 2016). However, hamstring activation seems to be generally low in the cable pendulum (II) and lunge (Jönhagen et al., 2009), questioning whether meaningful adaptations can be achieved via these exercises.

In ST, acute changes in T2 relaxation time after prone leg curl and NHE were found to be most pronounced in the middle (Kubota et al., 2007; Mendiguchia et al., 2013a) or middle and proximal regions (Mendez-Villanueva et al., 2016; Mendiguchia et al., 2013b), and signal intensity further increased after 2-7 days, especially in the middle and proximal muscle regions (Kubota et al., 2007). Similarly, Flywheel leg curl has been shown to induce the greatest metabolic activation in the middle region (Mendez-Villanueva et al., 2016). This is consistent with our findings of higher ST activity in the middle and/or proximal regions compared to the distal region in knee-dominant NHE (I), prone leg curl, and slide leg curl (II). In contrast, most hip-dominant exercises showed relatively small or no regional differences within ST, except bent-knee bridge, which showed similar regional differences to those observed in knee-dominant exercises. Knee angle was 90° in bent-knee bridge compared to near full extension in all other hip-dominant exercises, but it is yet to be clarified whether this could be responsible for such marginal differences in regional activity patterns.

The evidence for exercise-specific regional activity patterns raises the question of whether regional activation of these muscles is associated with regional adaptations in response to training. In ST, most exercises seem to primarily activate the mid-to-proximal muscle region, and this is the region where the cross-sectional area is the largest within the muscle. In some fusiform muscles, e.g. triceps brachii, the region with the highest muscle activity was found to experience the largest increase in cross-sectional area in response to training (Wakahara et al., 2013). Moreover, in a different study, the largest increase in cross-sectional area was observed in the mid-region of ST after a knee-flexion based training intervention, although changes were also substantial in the distal region (Housh et al., 1992). It should be further clarified whether there is a direct link between regional ST activity and region-specific adaptations.

Contrary to ST, BFlh showed the highest activity in the distal region where muscle thickness is lower compared to the middle and proximal regions (Tosovic et al., 2016). In response to knee-flexion based training, the greatest hypertrophy within the muscle was observed in the middle and proximal regions, compared to small changes in the (presumably highly activated) distal region (Housh et al., 1992). This discrepancy may be explained by the architectural complexity within the BFlh. The fascicles of BFlh are longer proximally than distally (Kellis et al., 2010; Tosovic et al., 2016; Ward et al., 2009), and some studies have reported lower (Kellis et al., 2010) or higher (Tosovic et al., 2016) pennation angle in the

distal than the proximal muscle sites. Regions with higher pennation angle may be more suited for high force production, but intramuscular load distribution can also be modulated by region-specific muscle activation (Hedayatpour and Falla, 2012). The issue of how regional activation of BFlh affects architectural and morphological adaptations in different muscle regions is worthy of further study. This knowledge may be important from an injury perspective. In rehabilitation, selective activation of the injured muscle region may improve recovery, and relatively longer fascicles may be protective against hamstring strain injury (Timmins et al., 2016). Nonetheless, there is limited information available about region-dependent changes in BFlh fascicle length in response to training.

It should be noted that some adaptations to training may actually increase injury risk. For example, a relatively thick proximal BFlh seems to increase the amount of fiber strain near the proximal MTJ (where injuries typically occur) in running, thereby increasing hamstring injury risk (Fiorentino and Blemker, 2014). Conversely, relatively high aponeurosis thickness has the opposite effect (Evangelidis et al., 2015; Fiorentino et al., 2012; Fiorentino and Blemker, 2014; Rehorn and Blemker, 2010), and it is unclear whether BFlh aponeurosis morphology changes with training. From an injury point of view, it would be important to clarify the relative changes in muscle and aponeurosis thickness in response to training with different exercises.

### 6.1.2 Running

The early study of Garrett et al. (1987) suggested that increased activation of the muscle fibers may reduce strain injury risk because more energy is absorbed in the lengthening fibers. Following this idea, relatively high proximal BFlh activation may be advantageous in running, especially at high running speeds. However, this was not observed consistently for all individuals in this thesis. While some activated the proximal region of the muscle the most, others activated the middle or distal regions more strongly. It is currently unclear whether some proximo-distal activity patterns are related to higher injury risk. It may be that individual muscle morphologies and architectures require different proximo-distal muscle activation to achieve optimal load distribution within the muscles. An additional reason why variability between individuals was higher in running than in the exercises may be that running allowed individual differences in running technique, while exercise technique was relatively consistent between individuals. It should be noted that most individuals maintained their individual regional EMG patterns (i.e. similar qualitative patterns) across running speeds. It should be further clarified whether individual proximo-distal patterns in running require exercise selection based on the region-specific activity that each exercise evokes.

### 6.1.3 Impact on hamstring EMG research

Our results show that proximo-distal EMG amplitudes are different between ST and BFlh. This means that recording from a small region may lead to misinterpretation concerning which of the muscles is activated more, an issue that has been of interest in many studies (for review: Bourne et al., 2018). For example, in NHE, the highest EMG activity in ST was found in the upper mid-region of the muscle, which corresponds to the region where bipolar surface EMG electrodes are usually placed. However, in BFlh, the electrodes are usually placed over a region where EMG activity is rather low as compared to the distal region, which is activated the most. This might result in a systematic overestimation of ST activity relative to BFlh activity when using a traditional EMG approach. However, the fact that regional differences are highly variable between exercises implies that this error is non-systematic. Additionally, individual differences during running further increase the inaccuracy of interpreting muscle activity based on a single pair of EMG electrodes. Our results therefore suggest that spatially robust methods, such as HD-EMG, are necessary to comprehensively describe hamstring EMG activity.

## 6.2 Muscle-specific hamstring activity

To minimize region-bias, discussed in section 6.1.3, the studies included in this thesis defined muscle-specific EMG activity based on several (up to 15) channels located proximo-distally over each muscle. This section will discuss the magnitude of muscle activity and between-muscle differences during hamstring exercises, and individual EMG patterns in running.

### 6.2.1 Hamstring exercises

Similar to previous studies (Bourne et al., 2016; Tsaklis et al., 2015; Zebis et al., 2013), our results show that the magnitude of muscle activity can vary substantially between exercises. For example, when performed at 12-repetition-maximum load, good morning, unilateral Romanian deadlift, and cable pendulum exercises showed EMG activity below 30% relative to the activity measured during MVIC in the lengthening phase. Relatively small muscle activity has been reported previously (Bourne et al., 2017b; Zebis et al., 2013), which may be associated with relatively low forces produced by the hamstrings (Schellenberg et al., 2017). In our studies, the same relative load induced EMG activity corresponding to over 50% of that recorded during MVIC in the lengthening phase of other exercises such as slide-leg curl, upright hip-extension conic-pulley, and straight-knee bridge. In the concentric phase, EMG activity in these exercises ranged between 32% and 83% relative to MVIC. Exercises with high EMG activity may have more potential to induce adaptations to training as compared to exercises presenting relatively low EMG activity (II). Similar to previous studies (Bourne

et al., 2017b; Zebis et al., 2013), relatively high EMG activity was observed in the NHE, which was performed at relatively high loads compared to other exercises presented in this thesis (I, III). Furthermore, hip angle had a large effect on hamstring EMG activity (III). In NHE90, lower EMG activity was observed in both muscles than in NHE0 (except at the initial phase of the movements) when both were performed at 1-repetition-maximum load. In an isokinetic dynamometer, hip flexion angle had a relatively small effect on BFlh and ST EMG activity (Kellis et al., 2017). Additionally, it may be specific to dynamic (and perhaps lengthening) muscle actions because it seems that in isometric conditions, hip flexion angle does not affect hamstring EMG activity (Mohamed et al., 2002). Higher inhibition in the NHE90 (longer hamstring length) than in NHE0 (shorter hamstring length) could be an explanation for this discrepancy between lengthening exercises. Relatively low EMG activity in NHE90 was accompanied by higher knee flexion torque in NHE90 than in NHE0, suggesting relatively high passive force in NHE90. This is in agreement with isokinetic lengthening actions of the knee flexors (Kellis et al., 2017), and implies a more pronounced lengthening-type muscle action in NHE90 than in NHE0 and/or a superior contribution of other knee flexors to NHE90. Additionally, NHE90 requires hamstrings to operate at relatively long lengths compared to NHE0, with higher EMG activity compared to that observed in the exercises of the 'L-protocol' described by Askling et al. (Askling et al., 2013; Askling et al., 2014; Severini et al., 2018). Moreover, similar EMG and peak torque were reached in bilateral and unilateral NHE modes, which may be useful when a training goal is to decrease between-limb imbalances.

It has been shown that imbalance in activation between hamstring muscles may lead to increased fatigability and increased hamstring injury risk (Avrillon et al., 2018). Accordingly, as well as the magnitude of EMG activity, the activity of BFlh relative to ST has been the focus of many studies (Bourne et al., 2017b; Tsaklis et al., 2015; van den Tillaar et al., 2017; Zebis et al., 2013). Many of the exercises that were examined in this thesis required similar EMG activity from BFlh and ST. However, in accordance with mfMRI results (Bourne et al., 2017b), 45° hip extension exercise showed higher activity in BFlh than in ST, at least in the shortening phase of the movement (II). Correspondingly, this exercise seems to induce architectural (increased fascicle length) and morphological (increased muscle volume) adaptations in the BFlh (Bourne et al., 2017a). Higher ST than BFlh activity was observed in the shortening phase of prone leg curl (II), in traditional NHE (I), and in NHE90 (III). Similarly, studies using mfMRI showed higher ST than BFlh metabolic activity in prone leg curl (lengthening phase) (Kubota et al., 2007), and in NHE (Bourne et al., 2017b). This seems to translate into a superior increase in ST volume in response to NHE training (Bourne et al., 2017a). These findings cumulatively suggest that some hip-dominant exercises activate BFlh more strongly, while knee-dominant exercises activate ST more (Bourne et al., 2018a). However, some other exercises, whether hip- or knee-dominant, did not result in clear differences between the activity of ST or BFlh. A recent mfMRI study even found higher ST than BFlh activation in straight-knee bridge (Bourne et al., 2018b), an exercise that did not result in differences between

muscles in our study (II). Romanian deadlift exercise is subject to further discrepancy between a previous mfMRI study (Ono et al., 2011) and our results, whereby higher BFlh than ST activity was found with mfMRI, while similar activity was found in the two muscles with HD-EMG. These differences may be explained by differences in exercise technique (e.g. hip and knee rotations), individual differences, or the difference between measurement methods.

The results further suggest that range of motion should be considered when interpreting between-muscle differences in muscle activity. This study found that the relative activity of BFlh and ST muscles changes during the bilateral NHE, provided that the hips are in neutral position. In the early phase of the exercise, ST activity was higher than BFlh activity, whereas BFlh activity increased relative to ST activity towards knee extension. Significantly higher BFlh than ST activity was observed close to full knee extension. There may be a few reasons why most studies have found selective ST activation in NHE. Firstly, NHE is typically performed at supramaximal load, i.e. the participants cannot control the movement until near-full knee extension (Bourne et al., 2017b; Ditroilo et al., 2013; Marshall et al., 2015; Tsaklis et al., 2015; Zebis et al., 2013). This leads to a scenario where the hamstrings are inactive in the latter phase of the movement, where BFlh would be activated more than ST according to our results. Secondly, NHE is typically performed with some hip flexion, which seems to increase preferential ST activation throughout the entire range of motion. In our study, forceful hip extension was required in NHE0, and this hip extension effort presumably increased the relative activity of BFlh (Bourne et al., 2018a). Additionally, the longer knee flexion moment arm of BFlh compared to ST near full knee extension provides a mechanical advantage for BFlh to produce torque (Herzog and Read, 1993; Kellis et al., 2015), which may further explain higher BFlh activation near full knee extension in NHE0, although this did not seem to affect the terminal phase of NHE90.

### 6.2.2 Running

As hamstring injuries typically occur at high running speeds, speed-dependent changes in hamstring activity may provide insight into injury mechanisms. In our study, increasing running speed substantially increased hamstring activity, similar to previous studies (Kyröläinen et al., 2005; Schache et al., 2013). EMG activity was highest in late swing and early stance, also in accordance with previous studies (Higashihara et al., 2010; 2015; Kyröläinen et al., 2005; Schache et al., 2013; Yu et al., 2008). Although increased muscle activity in running may be beneficial at the level of muscle fibers (Garrett et al., 1987), a relatively stiff muscle requires the tendon to take up a larger proportion of the MTU lengthening (Cronin et al., 2008), which may predispose the tendon to injury.

Since more than 80% of hamstring injuries occur in the BFlh, comparing BFlh to ST activity could provide further information about intermuscular coordination. The fusiform architecture of ST suggests that this muscle is more suited to rapid length changes (which occur in high-speed running), as compared to the more pennate BFlh. Nonetheless, at the group level, no differences were found

between muscles at any of the running speeds when the activity from all regions were considered. This could likely be explained by large differences in activity patterns between individuals. Indeed, a recent study suggests that EMG activity patterns in several muscles seem to be unique to each individual (Hug et al., 2019). Individual differences in the activation of hamstring muscles in running may be related to the optimization of movement efficiency, which could be different between individuals because of unique characteristics of the neural and musculo-skeletal system. However, it is possible that individual coordination strategies increase injury risk in some individuals (Hug and Tucker, 2017). For example, a higher imbalance between the activation of hamstring muscles seems to be associated with decreased knee flexion performance in fatiguing isometric conditions (Avrillon et al., 2018), potentially affecting injury risk. However, others have suggested that in dynamic conditions, a relatively higher ST to BFlh activation ratio may be protective against BFlh injury (Schuermans et al., 2014). It should be clarified whether imbalances observed in isolated contractions and high-speed running are similar, and whether such imbalances affect fatigue and hamstring injury risk in sport-specific tasks.

### 6.3 Limitations

Surface EMG is prone to cross-talk. To limit this effect, this study used small electrodes and a short inter-electrode distance (De Luca et al., 2012), guided the placement of the linear arrays with B-mode ultrasonography, and recruited male participants who had only a thin subcutaneous layer over the target muscles. Although most of our results are aligned with mfMRI findings (neighboring muscles do not interfere with the results), the physiological mechanisms detected by the two methods are different from each other (detailed in section 2.2.2.3). Thus, mfMRI cannot be used as a validation tool. However, comparing HD-EMG data to intramuscular EMG recordings (minimized cross-talk) could further clarify the validity of HD-EMG. The movement of the muscle under the skin is also an inherent limitation of surface EMG. However, its effect was minimized by recording from a large area of the muscle relative to the amount of muscle movement. When comparing the EMG activity of muscle regions and different muscles, the method used to normalize EMG data may affect the interpretation of the results. The validity of the approach used in these studies is supported by the current evidence and some unpublished data. Nonetheless, it should be further examined whether knee flexion MVIC in the specific joint positions used can activate both BFlh and ST to a similar extent. Regarding kinematics, studies II and III used goniometers and a potentiometer, respectively, which are likely to be less accurate than 3-D motion analysis, which was used in studies I and IV. Additionally, MTU length calculations (I, IV) are affected by the model and cut-off frequency, although MTU behavior was not the main focus of these studies. In study IV, measurements took place on a treadmill (not overground) at submaximal (but not maximal) running speeds, which could both be limitations from an injury

perspective. However, this was due to methodological considerations, i.e. to record several steps at a constant speed, thereby obtaining more reliable EMG patterns. A further limitation is the relatively small sample size of the studies, which might have led to increased type II error rate, and the magnitudes of the differences might have been more descriptive of the population with larger sample sizes. Additionally, replication studies to eliminate potential Type I errors are encouraged. Finally, data obtained from recreational and amateur athletes in this thesis may not be applicable to other populations.

## 7 MAIN FINDINGS AND CONCLUSIONS

Hamstring proximo-distal EMG activity was non-uniform in several hamstring exercises and in running. This indicates that spatially robust methods, such as HD-EMG, provide a more comprehensive overview of hamstring EMG activity than traditional EMG, where a single pair of electrodes are placed over each muscle. Most exercises showed the lowest activity in the proximal region of the BFlh, and this may be associated with the high injury rate in this region (De Smet and Best, 2000). Intermuscular differences in hamstring activity were comparatively small. Nonetheless, intermuscular differences in hamstring activation seem to affect muscle-specific morphological and architectural adaptations (Bourne et al., 2017a). Further research should explore muscle- and region-specific adaptations in response to exercise interventions, as well as their impact on hamstring injuries. As such, it would be of value to examine the adaptations to NHE0 *versus* NHE90, since these exercises seem to require different intermuscular coordination and different contributions from active and passive structures. Our results suggest that NHE90 may be preferable when preferential ST activation and high passive torque at long hamstring muscle lengths are among the training goals. High passive torque in NHE90 suggests that this exercise may be more suitable than NHE0 for inducing fascicle lengthening, which seems to be valuable in hamstring injury risk reduction (Timmins et al., 2016). Intervention studies should test these biomechanics-based assumptions. It is also notable that during running, large inter-individual differences in both intermuscular and intramuscular EMG activity patterns were observed. Future studies should further examine the consequences of these differences for muscle performance and hamstring injury risk.

NHE is frequently used in sport settings as a tool for training hamstrings and monitoring athletes' strength. However, hip angle and knee range of motion are rarely controlled in these situations. Our results suggest that hip angle has a large effect on knee flexion torque and hamstring activation, so it may be of value to control hip position during NHE to ensure that knee flexor torque represents the same physiological mechanisms between measurement sessions and between athletes. Additionally, it may be useful to monitor the angle of peak torque in

NHE, since the early phase preferentially activates ST, while the latter phase preferentially activates BFlh, provided that hips are in neutral position. Depending on the strength level of the athlete and additional load applied, knee angle at peak torque can be different between athletes. Conversely, load can be adjusted so that angle of peak torque is similar between athletes, which may standardize the relative contributions of ST and BFlh to knee flexion torque between athletes.

Garrett et al. (1987) suggested that higher muscle activation can reduce the amount and speed of muscle fiber strain when the fiber is being elongated. It should be further examined whether there is a direct relationship between lower EMG activity and higher fiber strain *in vivo*. This could be especially important in high-speed running, where hamstring injuries occur. Nonetheless, *in vivo* examination of fiber or fascicle strain is very challenging. The MTU complex of BFlh undergoes a stretch-shortening cycle during the running stride (IV). However, muscle-tendon decoupling likely occurs due to tendon compliance. In some muscle-tendon units, e.g. the calf muscles, long and compliant tendons allow near-isometric fascicle behavior in locomotion (Cronin et al., 2013; Lichtwark and Wilson, 2006). It has recently been suggested that BFlh fascicles may also operate at near-constant lengths while the tendons take up the majority of the MTU lengthening in the swing phase of sprinting (Van Hooren and Bosch, 2017). However, there is a lack of experimental evidence to support this idea. Fascicle behavior is typically examined with ultrasonography in other MTUs. BFlh has long fascicles relative to the typical transducer field-of-view (4.7-6.0 cm), and it seems that substantial muscle gearing as well as out-of-plane fascicle and aponeurosis rotations occur in walking and running, especially at higher speeds (see supplementary video of study IV). An additional challenge may come from the heterogeneous strain distribution within the BFlh muscle, as suggested by modelling studies (Fiorentino et al., 2012; Fiorentino et al., 2014; Fiorentino and Blemker, 2014; Rehorn and Blemker, 2010). Ideally, the 3-D behavior of muscle fascicles (or fibers) should be examined along the entire length of the muscle in high-speed running. This information would likely further our understanding of hamstring injury mechanisms because the amount of fiber strain seems to be a good predictor of muscle strain injuries (Lieber and Friden, 1993). Because increased muscle activation may decrease injury risk (Garrett et al., 1987), *in vivo* fiber/fascicle behavior should then be related to the activation of the muscle in high-speed running. However, as also mentioned above, it is challenging to quantify BFlh fascicle length, even in static conditions (Franchi et al., 2020; Pimenta et al., 2018), and thus substantial methodological improvements are needed to enable BFlh fascicle mechanics to be examined in dynamic conditions.

EMG amplitude is indicative of the recruitment of motor units and their discharge rates in the measurement area, but is also influenced by the membrane properties of the muscle fibers (Farina et al., 2014). To further understand the source of the neural control of different hamstring muscles and their regions, it would be of value to decompose the EMG signals recorded from hamstring muscles. Recent developments in high-density surface EMG signal decomposition allow the tracking of a large number of motor units over time with high accuracy

in a spatially robust and non-invasive manner across the full recruitment range (Del Vecchio and Farina, 2019). Hamstring HD-EMG signal decomposition would further our understanding of neural inhibition, which has been linked to hamstring injuries (Fyfe et al., 2013; Opar et al., 2013b), and could provide further information about motor unit adaptations in response to hamstring exercises in healthy and injured muscles.

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## ORIGINAL PAPERS

### I

#### **REGION-DEPENDENT HAMSTRINGS ACTIVITY IN NORDIC HAMSTRING EXERCISE AND STIFF-LEG DEADLIFT DEFINED WITH HIGH-DENSITY ELECTROMYOGRAPHY**

by

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Running head: Regional hamstring activity in NHE and SDL

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**ABSTRACT**

Recent studies suggest region-specific metabolic activity in hamstring muscles during injury prevention exercises, but the neural representation of this phenomenon is unknown. The aim of this study was to examine whether regional differences are evident in the activity of biceps femoris long head (BF<sub>lh</sub>) and semitendinosus (ST) muscles during two common injury prevention exercises. Twelve male participants without a history of hamstring injury performed the Nordic hamstring exercise (NHE) and stiff-leg deadlift (SDL) while BF<sub>lh</sub> and ST activity were recorded with high-density electromyography (HD-EMG). Normalised activity was calculated from the distal, middle, and proximal regions in the eccentric phase of each exercise. In NHE, ST overall activity was substantially higher than in BF<sub>lh</sub> ( $d = 1.06 \pm 0.45$ ), compared to trivial differences between muscles in SDL ( $d = 0.19 \pm 0.34$ ). Regional differences were found in NHE for both muscles, with different proximo-distal patterns: the distal region showed the lowest activity level in ST (regional differences,  $d$  range = 0.55 – 1.41) but the highest activity level in BF<sub>lh</sub> (regional differences,  $d$  range = 0.38 – 1.25). In SDL, regional differences were smaller in both muscles ( $d$  range = 0.29 – 0.67 and 0.16 – 0.63 in ST and BF<sub>lh</sub>, respectively) than in NHE. The use of HD-EMG in hamstrings revealed heterogeneous hamstrings activity during typical injury prevention exercises. High-density EMG might be useful in future studies to provide a comprehensive overview of hamstring muscle activity in other exercises and high-injury risk tasks.

**Keywords:** electrical activity, bi-articular hamstrings, muscle function

## INTRODUCTION

Hamstring injuries are the most common in sports involving high-speed running<sup>1-3</sup>.

Optimising hamstring exercise selection for prevention of and rehabilitation from hamstring injury is of major interest since low eccentric strength is thought to be one of the most significant<sup>4-6</sup> but modifiable<sup>7-10</sup> risk factors for hamstring injury. In recent decades, injury incidence has not decreased<sup>11</sup>, highlighting the need for studies examining the possible mechanisms of injury.

Bi-articular components of the hamstrings (biceps femoris long head (BFlh), semitendinosus (ST) and semimembranosus) contribute to both hip extension and knee flexion<sup>12</sup>. Thus, complex within-muscle hamstring coordination may be required. Recent studies using muscle functional magnetic resonance imaging (mfMRI) suggest that hamstring exercises may cause heterogeneous elevation of transverse (T2) relaxation time within different hamstring muscles, implying inhomogeneous metabolic activity of different muscle regions<sup>13,14</sup>.

However, there is a lack of information about the neural representation of this phenomenon. Additionally, mfMRI cannot be used in real-time. Therefore, other methods of defining muscle activity are needed to comprehensively understand hamstring muscle function.

Electromyography (EMG) has been widely used to define real-time ST and BFlh activity in a variety of hamstring exercises<sup>15-18</sup>, but has relied on a single pair of EMG electrodes placed over each muscle. Assuming that the aforementioned mfMRI changes are reflected in the EMG activity, conventional EMG configurations may not be sufficient to describe overall muscle activity accurately.

High-density surface electromyography (HD-EMG) has been used recently to provide a more comprehensive overview of human muscle activity<sup>19</sup>. This method has revealed regional differences (e.g. proximal vs. distal) in the activity of different muscles during stimulation

and voluntary movements, e.g. walking<sup>20-23</sup>. The method has not yet been used in hamstrings, but would likely provide further insights to hamstring activity during strengthening exercises. Recent mfMRI studies suggest that hip-dominant exercises may activate proximal BFlh preferentially, while in knee-dominant exercises distal BFlh is relatively more activated<sup>14,24</sup>, which may be of interest from an injury prevention perspective. Thus in this study, to examine whether heterogeneous activity of hamstring regions exists, two exercises which are frequently used in injury prevention - the knee-dominant Nordic hamstring exercise (NHE) and the hip-dominant stiff-leg deadlift (SDL) - were investigated with HD-EMG. We aimed to examine the eccentric-only NHE and the eccentric phase of SDL to avoid contraction mode bias. We hypothesised that differences would be observed between normalised EMG activity of the proximal, middle and distal regions of BFlh and ST during NHE and SDL.

## **MATERIALS AND METHODS**

### **Participants**

Twelve recreationally active young males (age  $24.3 \pm 3.7$  years, body mass  $74.2 \pm 8.3$  kg, height  $179.3 \pm 8.8$  cm) with weightlifting experience and without any cardiovascular or musculoskeletal disorders volunteered for this study. Participants had no known history of hamstring injury, or any lower extremity/lower back injuries in the past 3 years. After informing the participants about the study details, they gave written consent before data collection. Testing procedures were approved by the ethics committee of the University of Jyväskylä and performed in accordance with the Declaration of Helsinki.

## Study design

In the familiarisation session (10-14 days before the testing), 1 repetition maximum (1RM) was defined for SDL<sup>25</sup>. Participants also practiced the NHE, and maximal voluntary isometric contractions (MVICs) with visual force-time curve feedback.

In the main testing session, after preparation and warm-up, participants lay prone in a custom-made dynamometer (UniDrive, University of Jyväskylä)<sup>26</sup> with hip joint and trunk fixed to the dynamometer bench in neutral position. For EMG signal normalisation, participants performed maximal hip extension and knee flexion MVICs after a specific warm-up including ten submaximal contractions with increasing intensity (from ~30 to ~90%). Activity along the BFlh and ST muscles was recorded during MVICs using HD-EMG arrays (Figure 1). During knee flexion contractions, the knee joint was fixed at ~20 degrees of flexion while the lever arm of the dynamometer was strapped 2 cm above the lateral malleolus. During hip extension, participant positioning was identical but the lever arm of the dynamometer was fixed 1 cm above the knee fold. Participants were asked to perform hip extension with a slightly flexed knee to match the position in the knee flexion tasks. Three maximal contractions were performed and maintained for 2 seconds for both knee flexion and hip extension (2 minutes rest in-between). Knee flexion or hip extension alone were assumed to be insufficient to evoke maximal muscle activity in all EMG channels. Thus, participants also performed hip extension MVICs superimposed on knee flexion MVICs (3 reps, 2 minutes rest in-between). In this task, the cuff of the dynamometer lever arm was fixed 2 cm above the lateral malleolus, and the thigh was strapped to the bench above the knee fold. Participants started by increasing knee flexion then adding hip extension, reaching maximal effort in both tasks in ~2 seconds and maintaining it for another 2 seconds. Thereafter, NHE and SDL exercises were performed in random order.

## Exercise description

### Nordic hamstring exercise

The knee-dominant NHE (5 repetitions, 2-min rest in-between) was performed on a custom-made device with force transducers attached above the ankles<sup>27</sup>. Participants started from a kneeling position, with arms crossed in front of the body. They then lowered the body forward as far as possible at a constant speed of  $18^\circ \text{ s}^{-1}$  controlled with a metronome. The hips and torso were in neutral position throughout the range of motion. NHE was performed as a bodyweight-only exercise, and participants were not able to resist until full knee extension. Force from each leg and EMG activity along the ST and BF<sub>lh</sub> were recorded during the exercise (Supplementary video 1).

### Stiff-leg deadlift

In the hip-dominant SDL, the starting position was upright. Throughout the range of motion, the knees were straight but not locked, and the back was in neutral position with closed scapulae. Participants lowered the bar close to the body towards the floor until the plates touched the floor or for as long as proper technique could be maintained. In the upward movement, the hips were extended to the starting position. The downward and upward movements were each performed in 2 seconds. Five repetitions were performed at 80% 1RM, with 2-min rest between repetitions. Joint kinematics and EMG activity along the muscles were recorded during the exercise (Supplementary video 2).

## Data collection

### EMG

HD-EMG preparation was performed while participants lay prone with neutral hip and knee joint angles. The right leg was measured for every participant. To determine proper HD-EMG array positioning, BFlh and ST muscle borders were determined with B-mode 2D ultrasonography (Aloka  $\alpha$ 10, Tokyo, Japan) and the skin over the borders was marked with a pen. After skin preparation, 15-channel semi-disposable EMG arrays (OT Bioelettronica, Torino, Italy) were attached along the midline between the borders for each muscle using adhesive foam, and connected to the amplifier of the EMG system. The cavities of the electrode arrays were filled with 20  $\mu$ l conducting gel for proper electrode-skin contact (Figure 1). Electrode arrays were further secured with adhesive tape to minimise movement artefact. For BFlh, channel eight/nine from the distal end of the array was aligned with the midpoint on the line between the ischial tuberosity and knee joint fold. For ST, the array was attached below the tendinous inscription<sup>28</sup> of the muscle defined with ultrasonography. A reference electrode was placed over the left wrist. Signal quality was checked during submaximal knee flexion contractions. EMG data were sampled at 2048 Hz, amplified (x1000) and digitised (EMG-USB 12-bit A/D converter, OT Bioelettronica). During each task, 15 differential signals were recorded from each muscle using BioLab software (v3.1, OT Bioelettronica).

### Kinematics

Before performing the exercises, reflective markers (14 mm diameter) were secured over the anterior and posterior superior iliac spine, lateral thigh, lateral epicondyle of the femur, lateral shank, lateral malleolus, calcaneus and second metatarsal head of each side, to determine hip

and knee joint angular displacements. 3D marker displacements were recorded using an 8-camera motion analysis system sampling at 250 Hz in Nexus software (Vicon Motion Systems Inc., Oxford, UK).

## Force

MVIC and NHE force data (strain gauge at the ankle, see supplementary video 1) were collected at 1000 Hz and digitised using an A/D converter (Cambridge Electronic Design, Cambridge, UK), and recorded in Spike2 software (Cambridge Electronic Design). Force and EMG data were synchronised by sending a pulse from Spike2 to the EMG software. Spike2 software was also used to send a digital pulse to the Nexus software to synchronise EMG and kinematic data.

## Data analysis

As NHE is an eccentric-only exercise, muscle activity levels were determined in the eccentric phase of each task to make exercises comparable. For NHE, force measured from the right leg was used to determine the active lengthening phase: from the start of force increment to the instant of peak force. For SDL, the eccentric phase was defined based on muscle-tendon length change: hip and knee joint angular displacements were calculated in Nexus software based on the Plug-in Gait Model after smoothing marker trajectories with an 8 Hz low-pass Butterworth filter. Joint angular data were then imported to Matlab (MathWorks Inc, Natick, MA, US), where ST and BFlh muscle-tendon lengths were calculated using modelling equations<sup>29</sup>.

EMG data were band-pass filtered using a 10-500 Hz fourth-order zero-phase Butterworth filter in Matlab. For MVICs, root-mean-square (RMS) EMG was calculated from a 1-second stable plateau for each EMG channel. In NHE and SDL, RMS activity in the eccentric phase

(defined as above) was calculated. RMS values across repetitions were averaged for each exercise and expressed as a percentage of the highest RMS activity of the corresponding channel during any of the MVIC tasks (%MVIC). Channels 1-5, 6-10, and 11-15 were then averaged to represent activity in the distal, middle and proximal regions, respectively. Overall activity was defined as the average normalised RMS activity of all 15 channels for each muscle. This approach minimised the effects of muscle shift under the skin on regional EMG activity.

### **Statistical analysis**

The magnitude of the differences (Cohen's  $d \pm 90\%$  confidence limits) between the overall activity levels of ST and BF<sub>lh</sub> muscles, and regional activity within each muscle in NHE and SDL were calculated using a custom spreadsheet<sup>30</sup>. Differences were classified as trivial ( $<0.2$ ), small ( $\geq 0.2$ ), moderate ( $\geq 0.5$ ), or large ( $\geq 0.8$ ). Nonetheless, differences with 90% confidence intervals overlapping both the positive ( $\geq 0.2$ ) and negative ( $\leq -0.2$ ) smallest worthwhile standardized effects were deemed to be unclear effects<sup>31</sup>.

### **RESULTS**

Peak NHE force was  $285 \pm 48$  N; load for SDL was  $86.9 \pm 25.8$  kg (mean  $\pm$  standard deviation). Figures 2 and 3 represent group average normalised activity for each EMG channel during NHE and SDL, respectively. Regional EMG activities for each individual are illustrated in Figure 4.

During NHE, activity within ST was highest in the middle region ( $80.48 \pm 13.78$  %MVIC), and differences between regions were substantial ( $d$  range = 0.55 to 1.41). BF<sub>lh</sub> activity was highest in the distal region ( $72.08 \pm 10.66$  %MVIC) and lowest in the proximal region ( $57.74 \pm 15.95$  %MVIC), with small to large differences between regions ( $d$  range = 0.38 to 1.25). In SDL, ST activity was highest in the middle region ( $40.70 \pm 9.44$  %MVIC), and differences between regions were small to moderate ( $d$  range = 0.29 to 0.67). In BF<sub>lh</sub>, the proximal region displayed the lowest activity level ( $32.23 \pm 8.55$  %MVIC) and the difference between the middle and distal regions was trivial ( $d = 0.16 \pm 0.27$ ). All within-muscle comparisons are shown in Table 1.

With respect to overall activity, ST presented substantially higher activity than BF<sub>lh</sub> in NHE ( $72.31 \pm 7.33$  %MVIC vs.  $63.97 \pm 10.46$  %MVIC,  $d = 1.06 \pm 0.45$ ), but in SDL the difference between muscles was negligible ( $37.46 \pm 6.74$  %MVIC in ST vs.  $36.07 \pm 8.54$  %MVIC in BF<sub>lh</sub>,  $d = 0.19 \pm 0.34$ ).

## DISCUSSION

This study has shown using HD-EMG that intramuscular distribution of normalised EMG activity is non-uniform along the BF<sub>lh</sub> and ST muscles; the middle-to-proximal region of ST and the distal region of BF<sub>lh</sub> showed the highest within-muscle activity, irrespective of exercise. Regarding inter-muscular differences, higher activity was observed in ST compared to BF<sub>lh</sub> in NHE, but no differences between muscles were observed in SDL.

### **Region-specific muscle activity**

In BF<sub>lh</sub>, the region effect was larger in NHE than in SDL, with large and moderate effects, respectively. In both tasks, the distal region was activated the most. This is consistent with preferentially distal BF<sub>lh</sub> muscle use based on mfMRI studies in NHE<sup>32</sup>, and in the mechanically similar eccentric knee extension task<sup>13</sup>. However, rather homogeneous muscle metabolic activity was previously found in a modified SDL<sup>14</sup>, contrary to our study wherein differences between regions were present, though smaller compared to NHE.

In ST, large differences in activity between regions were detected during NHE, whereby the middle and proximal regions were more active than the distal region. Similarly, in a previous study, middle and proximal regions of ST were more active than the distal region in eccentric knee extension<sup>13</sup>. Another previous study found that the middle region showed the highest activity in a modified SDL<sup>14</sup>, in accordance with the current study.

Abundant innervation of BF<sub>lh</sub> and ST likely contributes to the region-specific activity of these muscles. One-to-three primary nerves innervating different BF<sub>lh</sub> regions divide into two or more primary branches<sup>28,33</sup> that may contribute to region-dependent activity. ST muscle is divided into upper and lower parts by a tendinous inscription, which is an attachment zone for most of the fascicles proximally and distally<sup>28</sup>. Both parts are innervated by a separate primary motor nerve implying separate function of regions. In this study, activity was recorded from below the tendinous inscription due its relatively proximal location. ST showed heterogeneous distribution of muscle activity within this part. Small territories of motor units may be responsible for independent activity of hamstring muscle regions, which is yet to be examined.

In other muscles, e.g. in biceps brachii, region-specific activity has been linked to non-uniform hypertrophy after a training intervention<sup>34</sup>. Accordingly, ST is the thickest in the upper mid-region, which is the region where the highest activity was found in our study. On the contrary, BF<sub>lh</sub> showed the highest activity in the distal region where muscle thickness is lower compared to the middle and proximal regions<sup>35</sup>. However, BF<sub>lh</sub> architecture differs between regions, with shorter fascicles and larger pennation angle in the distal compared to the proximal region<sup>35</sup>. Thus, the distal region seems to be more suited to force production than the proximal region, which is more suited to larger excursions. On the contrary, Kellis et al.<sup>36</sup> found higher pennation angle in the proximal compared to the distal region in cadavers, which might be due to disagreement in BF<sub>lh</sub> pennation angles between cadaver and in vivo measurements<sup>35</sup>. Intervention studies are needed to examine associations between region-specific activity and architectural and morphological changes along the hamstrings.

### **Overall muscle activity**

Since hamstring injuries are most common in BF<sub>lh</sub>, muscle-specific exercise selection based on the activity of individual hamstring muscles is of significant interest. Architecturally, longer fascicle length and lower pennation angle of ST compared to BF<sub>lh</sub><sup>28,37</sup> suggests different functions of these muscles.

During NHE in the current study, ST showed higher relative activity compared to BF<sub>lh</sub>, which apparently associates with increased hypertrophy in ST relative to BF<sub>lh</sub> in response to NHE training<sup>38</sup>. On the contrary, in eccentric knee flexion<sup>39</sup> and NHE<sup>18</sup>, ST and BF<sub>lh</sub> were equally activated based on bipolar EMG. However, mfMRI studies during eccentric knee flexion<sup>13</sup> and NHE<sup>16</sup> found higher activity in ST compared to BF<sub>lh</sub>, similar to the current study. The latter mfMRI study analysed 3 slices at 40, 50 and 60% muscle length. During

NHE in the current study, between-muscle differences were not as high as those found with mfMRI, but it is likely that this mfMRI study ignored the region of highest activity within BF<sub>lh</sub>, which is distal to 40% muscle length according to the current study.

In SDL, overall activity did not differ between muscles in this study. In a previous study, the mechanically similar Romanian deadlift was classified as an ST-dominant exercise based on bipolar EMG. On the contrary, another study<sup>40</sup> also using conventional bipolar EMG showed higher activity in BF<sub>lh</sub> than in ST during SDL. However, the same study compared these results to mfMRI data, and found that differences in T2 changes between muscles were not observed when 5 slices along the muscles were analysed, which is in accordance with the current study using HD-EMG. Although methods were not directly compared, on the basis of the above results it seems that HD-EMG can provide a more comprehensive estimate of overall muscle activity than conventional bipolar EMG configurations.

As noted, previous studies are not in agreement concerning the relative activity of ST and BF<sub>lh</sub> in hamstring exercises. To estimate whole-muscle activity with mfMRI, 3-5 slices have been analysed along hamstring muscles<sup>15,38,40</sup>. However, this approach is not directly comparable to EMG studies wherein data are usually collected from a small muscle region. Large differences in spatial resolution between these methods may be one reason for discrepancies between studies, although mfMRI and EMG also assess different physiological mechanisms; metabolic and neural activity, respectively. As a limitation, EMG is prone to cross-talk. In this study, EMG activity measured from the ST and BF<sub>lh</sub> could have been contaminated by the activity of the semimembranosus and the short head of the biceps femoris, respectively. This possibility was presumably minimised by several factors: careful electrode array location using ultrasonography; applying 10 mm inter-electrode distance<sup>41</sup> and using electrodes with a relatively shallow pick-up area; examining male athletes with relatively thin subcutaneous tissue over the hamstrings. We also tried to improve EMG

normalisation by applying different MVIC tasks. However, studies targeting optimisation of EMG normalisation are needed to further improve comparability of muscles or muscle regions. Furthermore, deeper components of the hamstrings cannot be examined with surface EMG. HD-EMG may be a good complement to mfMRI to study ST and BFlh muscles, with real-time recording, financial considerations, and time-efficiency among the advantages of HD-EMG over mfMRI.

### **NHE and SDL for injury prevention**

The issue of which exercises can help to prevent hamstring injuries is under debate. Regarding the mechanism of injury, most running-type injuries seem to happen in the late swing phase of high-speed running<sup>42</sup>, whereas the hamstring muscle-tendon unit is actively lengthened and subjected to the highest forces and strain within the step cycle<sup>43,44</sup>. In this phase, hamstring muscles seem to be highly activated<sup>45</sup>. To mimic the injury mechanism, it is generally believed that eccentrically activating hamstring muscles at a longer muscle length would be optimal from an injury prevention perspective. Nonetheless, an increasing body of evidence suggests that the Nordic hamstring exercise, despite requiring a substantially shorter muscle operating length than in the late swing phase, has a preventive effect against hamstring injuries<sup>7,9,46</sup>. In NHE, ST was more active than BFlh, but both ST and BFlh were highly activated, supporting the idea of positive architectural changes (i.e. fascicle elongation) within BFlh in response to NHE intervention<sup>38</sup>. In SDL, hamstrings work at a longer muscle length than in NHE, and in this study relative activity of BFlh compared to ST was higher than in NHE (with negligible difference between muscles). However, absolute activity was substantially lower in both muscles (ST = 72% vs. 37%, BFlh = 64% vs. 36%, of MVIC on average in NHE vs. SDL). It should be noted that the load was not matched for the

two exercises. Instead loads that are generally used in training were applied, making these exercises comparable from a practical point of view. Due to the relatively low activity level in the eccentric phase of SDL, we speculate that SDL alone may not be as effective as NHE to prevent hamstring injuries.

Within BFlh, we observed the lowest activity in the proximal region in NHE. This may be associated with higher strain close to the proximal muscle-tendon junction in BFlh during cyclic knee-flexion extension contractions<sup>47</sup>. Silder et al.<sup>47</sup> also observed higher strain in previously injured BFlh, which may be associated with lower EMG activity in the BFlh of the injured limb<sup>48</sup>. The association between strain magnitude and EMG activity level should be further studied to reveal whether HD-EMG may be a useful tool for hamstring injury risk management.

Compared to the knee-dominant NHE, based on mfMRI studies<sup>14,24</sup> we expected relatively higher activity in the proximal BFlh compared to the distal region in the hip-dominant SDL. Instead, similar proximal-distal activity patterns were observed in NHE and SDL. Even though the most proximal region cannot be measured with surface EMG, this study suggests that hip-dominant exercises do not necessarily activate proximal BFlh preferentially. Future studies should further examine whether the relative activity of muscle regions can be modulated with different exercises.

### **Perspective**

Future studies should use HD-EMG to examine whether regional differences can be observed in other exercises and high-injury risk tasks. It should be emphasised that although proximal-distal differences seem to be significant, the clinical relevance of this phenomenon is yet to

be clarified. In previously strained BFlh, decreased EMG activity<sup>48,49</sup>, slower EMG onset<sup>50</sup> and decreased metabolic activity<sup>15</sup> were observed. It may be of value to examine whether inhibition is region-specific, and if exercise selection could affect the relative activity of muscle regions. This knowledge will help to identify the most suitable exercises for interventions and improve the restoration of neuromuscular function following a hamstring injury.

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## TABLES, FIGURE LEGENDS AND FIGURES

Table 1. Differences (Cohen's  $d \pm 90\%$  confidence limits) between the activity level of muscle regions for each muscle and exercise.

Region	Semitendinosus		Biceps femoris long head	
	middle	proximal	middle	proximal
<b>Nordic hamstring exercise</b>				
distal	1.41 $\pm 0.79^L$	0.87 $\pm 0.60^L$	-0.87 $\pm 0.38^L$	-1.25 $\pm 0.71^L$
middle		-0.55 $\pm 0.52^M$		-0.38 $\pm 0.53^S$
<b>Stiff-leg deadlift</b>				
distal	0.67 $\pm 0.63^M$	0.29 $\pm 0.44^S$	-0.16 $\pm 0.27^T$	-0.63 $\pm 0.35^M$
middle		-0.38 $\pm 0.39^S$		-0.48 $\pm 0.29^S$

<sup>T</sup> = trivial difference, <sup>S</sup> = small difference, <sup>M</sup> = moderate difference, <sup>L</sup> = large difference between regions. Positive and negative values refer to higher activity in the relatively more proximal and distal regions, respectively.

Figure 1. HD-EMG preparation.

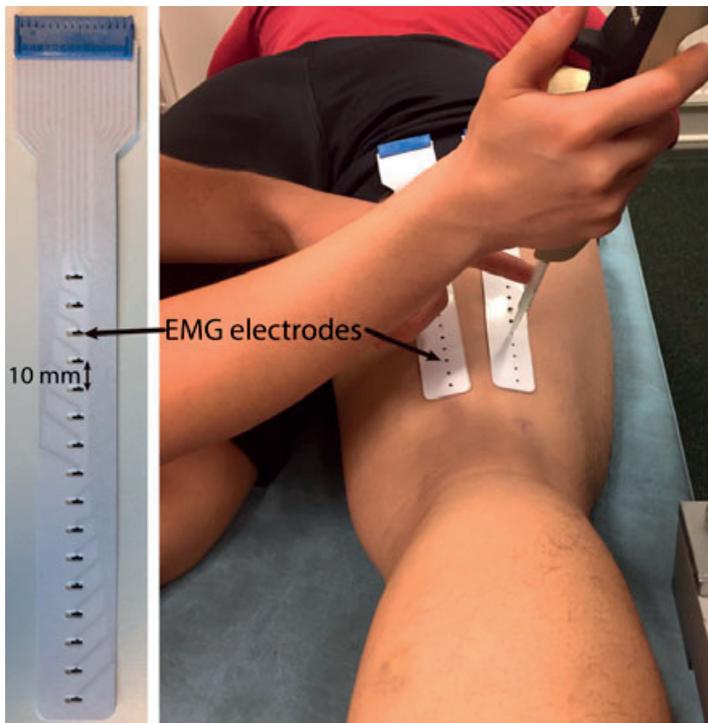


Figure 2. Distribution of normalised EMG signals in NHE.

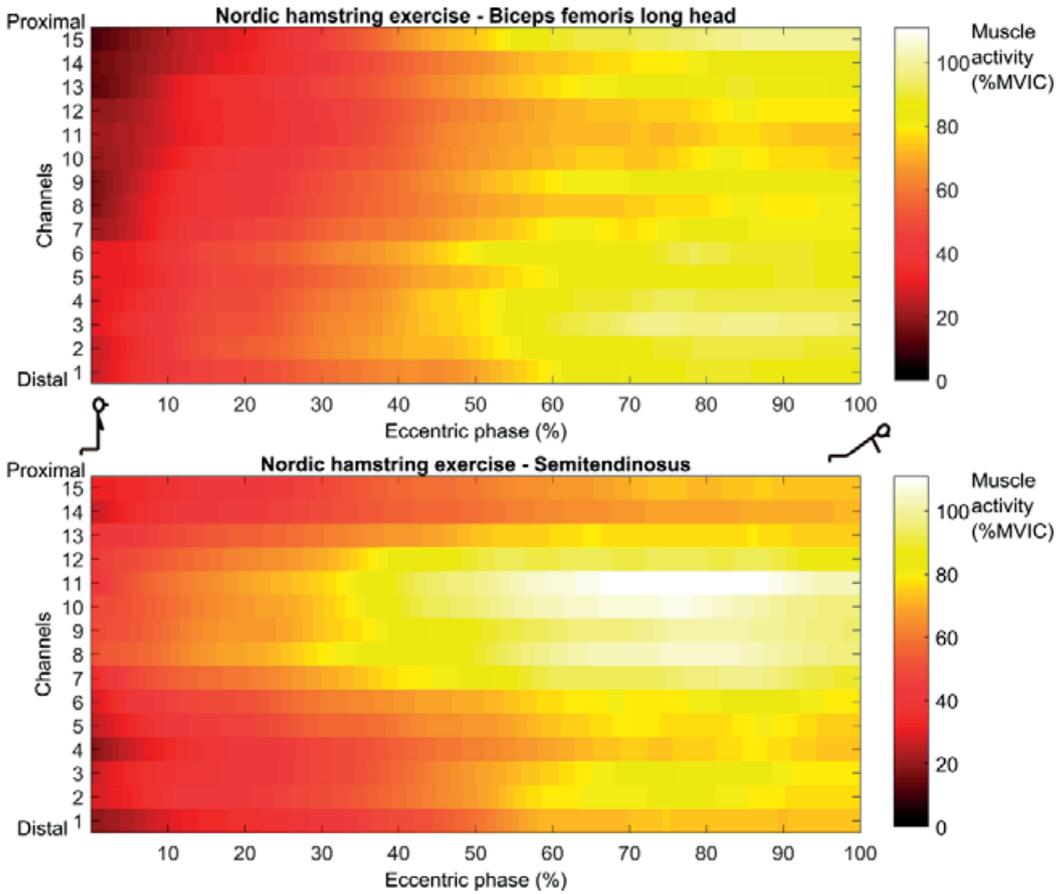


Figure 3. Distribution of normalised EMG signals in SDL.

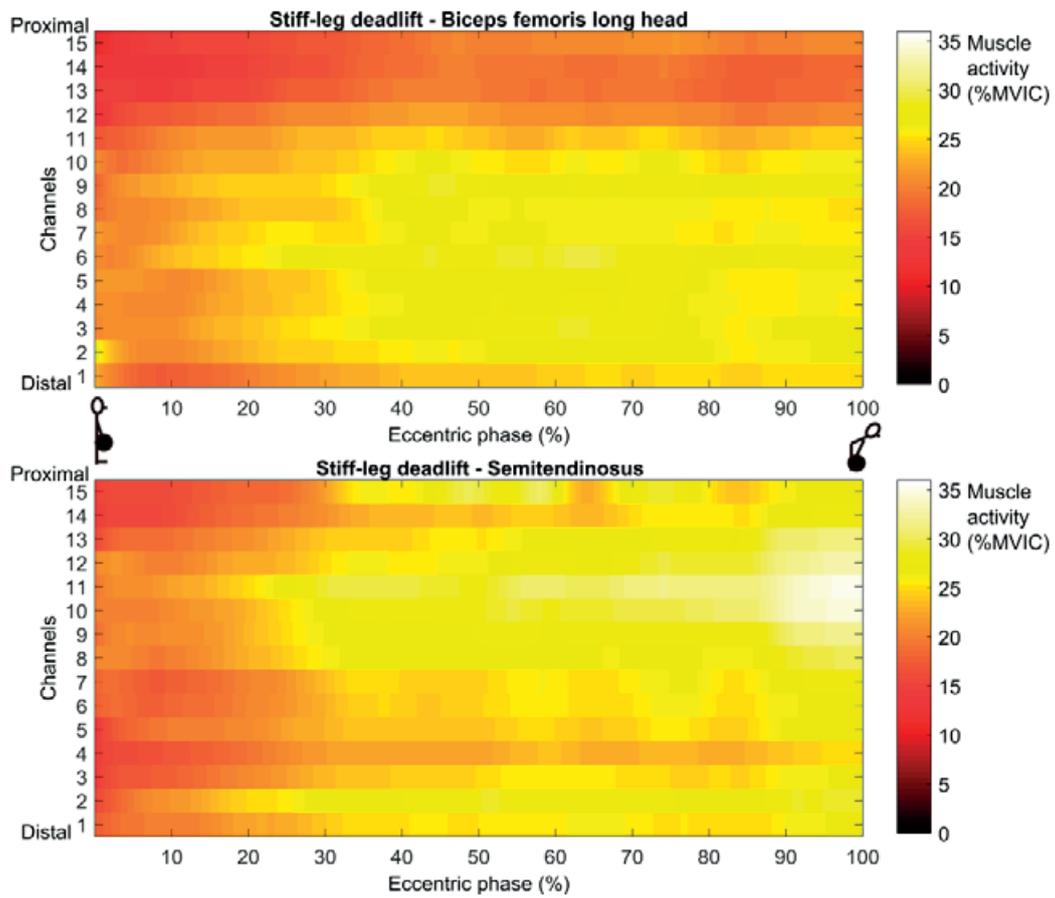
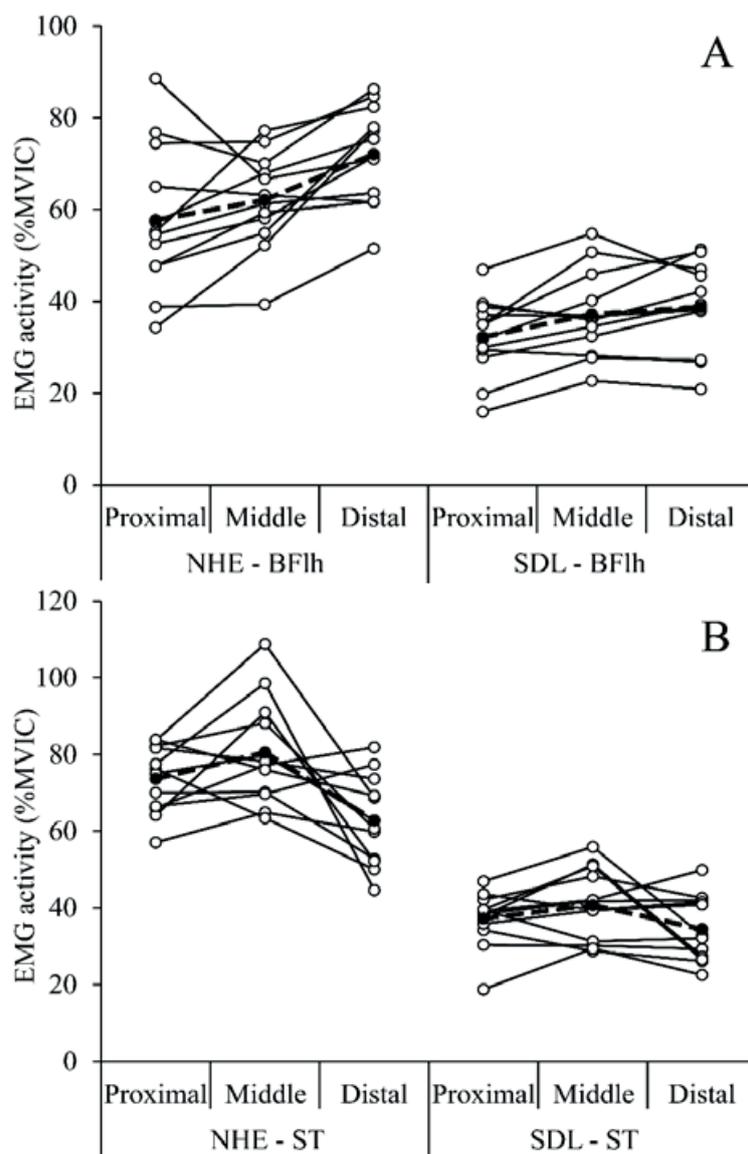


Figure 4. Regional activity for each participant.



## Figure legends

Figure 1. 15-channel high-density electromyography arrays were attached over the biceps femoris long head and semitendinosus to measure activity along the muscles. Electrode grids were filled with conductive gel for proper electrode-skin contact.

Figure 2. Normalised electromyographic (EMG) activity along the biceps femoris long head (upper panel) and semitendinosus (bottom panel) during Nordic hamstring exercise. The figure represents group average (N=12) for each channel of the EMG array along the corresponding muscle. EMG channels were time-normalised and low-pass filtered for visualisation. MVIC = maximal voluntary isometric contraction.

Figure 3. Normalised electromyographic (EMG) activity along the biceps femoris long head (upper panel) and semitendinosus (bottom panel) muscles during stiff-leg deadlift. The figure represents group average (N=12) for each channel of the EMG array along the corresponding muscle. EMG channels were time-normalised and low-pass filtered for visualisation. MVIC = maximal voluntary isometric contraction.

Figure 4. Regional electromyographic (EMG) activity as a percentage of the activity during maximal voluntary isometric contraction (%MVIC) in the biceps femoris long head (BF<sub>lh</sub>, panel A) and semitendinosus (ST, panel B) for each participant during Nordic hamstring exercise (NHE) and stiff-leg deadlift (SDL). Filled black markers connected with dashed lines represent group average.



## II

### **HIGH-DENSITY ELECTROMYOGRAPHY ACTIVITY IN VARIOUS HAMSTRING EXERCISES**

by

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High-density electromyography activity in various hamstring exercises

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**Running title:** HD-EMG activity in hamstring exercises

**ABSTRACT**

Proximal-distal differences in muscle activity are rarely considered when defining the activity level of hamstring muscles. The aim of this study was to determine the inter-muscular and proximal-distal electromyography (EMG) activity patterns of hamstring muscles during

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common hamstring exercises. Nineteen amateur athletes without a history of hamstring injury performed 9 exercises while EMG activity was recorded along the biceps femoris long head (BF<sub>lh</sub>) and semitendinosus (ST) muscles using 15-channel high-density electromyography (HD-EMG) electrodes. EMG activity levels normalized to those of a maximal voluntary isometric contraction (%MVIC) were determined for the eccentric and concentric phase of each exercise and compared between different muscles and regions (proximal, middle, distal) within each muscle. Straight-knee bridge, upright hip extension and leg curls exhibited the highest hamstrings activity in both the eccentric (40-54 %MVIC) and concentric phases (69-85 %MVIC). Hip extension was the only BF-dominant exercise (Cohen's  $d = 0.28$  (eccentric) and  $0.33$  (concentric)). Within ST, lower distal than middle/proximal activity was found in the bent-knee bridge and leg curl exercises ( $d$  range =  $0.53-1.20$ ), which was not evident in other exercises. BF<sub>lh</sub> also displayed large regional differences across exercises ( $d$  range =  $0.00-1.28$ ). This study demonstrates that inter-muscular and proximal-distal activity patterns are exercise-dependent, and in some exercises are affected by the contraction mode. Knowledge of activity levels and relative activity of hamstring muscles in different exercises may assist exercise selection in hamstring injury management.

**Keywords:** heterogeneous activity; rehabilitation; injury reduction

## INTRODUCTION

Hamstring strain is the most frequent injury in sports involving high-speed running<sup>1,2</sup>. For example in football, this type of injury results in a substantial player time loss<sup>1</sup>, decreased team performance<sup>3</sup>, and significant financial burdens on teams<sup>4</sup>. Re-injury rate can be as

high as 24% and is typical in the early stages of return to play<sup>5</sup>, suggesting suboptimal loading in the rehabilitation process.

Some interventions implementing eccentric exercises seem to mitigate hamstring injury occurrence<sup>6-10</sup>. In addition to low strength and short muscle length<sup>11,12</sup>, neural inhibition<sup>13</sup> and imbalances between the activity level of hamstring muscles<sup>14</sup> are also associated with hamstring injury. Proper exercise selection potentially allows the clinician to better succeed in (re-)injury prevention, but this is challenging for many reasons. For example, non-uniform adaptations to exercise interventions<sup>11,15,16</sup> may be associated with non-uniform hamstring activity patterns across exercises<sup>11,17-19</sup>. Moreover, study results are inconsistent concerning which hamstring muscles are activated in different exercises, as well as the extent of activation<sup>20</sup>, and it is questionable whether these differences are real or at least partly reflect the (in)accuracy with which different methods can define muscle activity.

Electromyography (EMG) is the most commonly used method to examine hamstring muscle activity<sup>20</sup>. In conventional EMG studies, electrodes are placed over the mid-belly of hamstring muscles, ignoring possible proximal-distal differences in muscle activity. Studies have shown non-uniform proximal-distal metabolic activity patterns within hamstring muscles<sup>18,19,21</sup>. Similarly, during two common hamstring exercises, we recently observed large differences in muscle activity within the semitendinosus (ST) and biceps femoris long head (BF<sub>lh</sub>) using high-density EMG (HD-EMG)<sup>22</sup>. Due to such regional differences, spatially robust methods may improve understanding of hamstrings activity patterns. This would potentially allow the clinician to selectively activate specific muscles or muscle regions.

In this study we aimed to define the excitation level of ST and BF<sub>lh</sub> muscles in the eccentric and concentric phases of 9 typical hamstring exercises. We also tested whether the relative activity of these muscles is similar in the eccentric and concentric phases, as well as if proximal-distal activity patterns are similar across exercises. According to the study aims, exercises were chosen that include clear eccentric and concentric phases (i.e. at the muscle-tendon unit level), and which are generally used in hamstring injury management.

## **MATERIALS AND METHODS**

### **Participants**

Nineteen young male amateur athletes (mean  $\pm$  standard deviation, age  $26.1 \pm 3.2$  years, body mass  $80.2 \pm 14.1$  kg, height  $178.3 \pm 9.3$  cm) from high injury-risk sports (9 soccer, 6 Gaelic football and 4 rugby players) and experienced at performing hamstring exercises participated in this study. Exclusion criteria were history of hamstring strain, previous anterior cruciate ligament or lower back injury, and cardiovascular or musculoskeletal disorders. Participants received detailed information about the study before they gave written informed consent.

Testing procedures were approved by the ethics committee of the University of Jyväskylä and performed according to the Declaration of Helsinki.

### **Study protocol**

The study was performed in the mid-season when the frequency of intense strength training was minimized. Participants refrained from additional strengthening exercises during the study to minimize training effects. Prior to data collection, 12-repetition maximum load

(12RM) was defined for 9 hamstring exercises across 4-5 sessions (4-7 days in-between). The examined exercises were: Good morning (GM), unilateral Romanian deadlift (RDL), cable pendulum (CP), bent-knee bridge (BB), 45° hip extension (45HE), prone leg curl (PLC), slide leg curl (SLC), upright hip-extension conic-pulley (UHC), and straight-knee bridge (SB) (Figure 1 and Video, Supplementary Content). In each session except the last one, 2-3 randomly selected exercises were practiced, then 12RM was tested<sup>23</sup>, while exercise technique was assessed and (if needed) corrected by an experienced practitioner to ensure standard technical performance. Unilateral exercises were performed with the dominant (kicking) leg (4 left, 15 right). In the last familiarisation session, maximal voluntary isometric contractions (MVICs) were practiced.

In the main testing session, after preparation and warm-up, participants performed knee flexion and hip extension MVICs for the purpose of EMG normalisation, followed by 6 repetitions of each exercise in a random order. The warm-up consisted of cycling, dynamic stretching (5 minutes each), and then 10 submaximal hip extension and knee flexion contractions performed in a custom-made dynamometer (UniDrive, University of Jyväskylä)<sup>24</sup>, with the intensity increasing from ~30 to ~90% MVIC. In the dynamometer where MVICs were performed, participants lay prone with the trunk and hip fixed to the dynamometer bench in neutral position. In the dominant (measured) leg, the knee joint was positioned in 20° of flexion while the other leg was extended. For knee flexion MVICs, the lever arm of the dynamometer was fixed ~5 cm above the lateral malleolus. For hip extension MVICs, the lever arm was strapped just above the knee joint fold, and participants were asked to maintain 20° of knee flexion, which was confirmed before each contraction using a goniometer. For both hip extension and knee flexion MVICs, two repetitions were performed, followed by a third if peak torque differed by > 5% between the first two contractions. For each contraction, maximum effort was maintained for 2 seconds and 2 minutes rest was applied between

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contractions. A simultaneous performance of knee flexion and hip extension was also performed, wherein the participants reached maximum effort in both tasks simultaneously, which was maintained for 2 seconds. For this task, the dynamometer lever arm was fixed ~5 cm above the lateral malleolus and the thigh was tightly fixed to the bench. Thereafter, 6 repetitions of the 9 selected exercises were performed in random order, at 12 RM load. For the exercises, hip and knee goniometers were aligned with the trochanter major and lateral epicondyle of the femur, respectively. Both the eccentric and concentric phases were performed in 2 seconds, controlled with a metronome. Four minutes rest was applied between exercises. Hip and knee joint angles were recorded as well as BFlh and ST EMG activity. Participants reported no substantial fatigue throughout the testing.

### Data collection

To determine correct HD-EMG array positioning, B-mode 2D ultrasonography (Aloka  $\alpha$ 10, Tokyo, Japan) was used to define and mark the borders of the BFlh and ST muscles as well as the location of their distal musculotendinous junctions. After skin preparation, a 15-channel EMG array (10 mm inter-electrode distance, OT Bioelettronica, Torino, Italy) was secured over each muscle (Figure 2) so that the electrodes were as far away from the muscle borders as possible, to minimize cross-talk. Electrode positioning was standardized so that in BFlh channel 8-9 from the distal end of the array was aligned with the midpoint along the ischial tuberosity – popliteal fossa distance, while in ST the EMG array was placed 1 cm below the tendinous inscription which was located relatively proximally. Arrays were fixed over the skin using adhesive foam and tape. EMG arrays were connected to an amplifier, and signals were digitized (EMG-USB 12-bit A/D converter, OT Bioelettronica) for recording in BioLab software (v3.1, OT Bioelettronica). To maintain skin-electrode contact, electrode

cavities were filled with 20  $\mu$ l conductive gel. A reference electrode was placed over the contralateral wrist. Signal quality was confirmed during submaximal contractions. EMG data were sampled at 2048 Hz and amplified by a factor of 1000. During the measurements, 15 differential channels were recorded from each muscle.

During MVICs, hip extension and knee flexion forces were measured with the dynamometer strain gauge at a sampling frequency of 1000 Hz, digitized (EMG-USB 12-bit A/D converter, OT Bioelectronica) and recorded in BioLab software in synchrony with the EMG signals.

Lever arms were measured to calculate torque. For hip extension the lever arm was measured as the distance between the trochanter major and the middle of the strain gauge. For knee flexion the lever arm was measured as the distance between the lateral epicondyle of the femur and the middle of the strain gauge. During muscle contractions, force-time curve feedback was provided.

Hip and knee joint angles were recorded using custom-made electro-goniometers (University of Jyväskylä, Finland). Angle data were digitized by the A/D converter of the EMG system and recorded in BioLab software simultaneously with the EMG data.

### **Data analysis**

A 10-500 Hz fourth-order zero-phase band-pass Butterworth filter was used to filter EMG data in Matlab (MathWorks Inc, Natick, MA, US). For MVICs, root-mean-square (RMS)

EMG activity was calculated from a 1-second stable force plateau for each EMG channel.

From the exercises, RMS activity was calculated in the entire eccentric and concentric phase (i.e.  $\sim$ 2 seconds for each) for each EMG channel based on hip and knee joint angular displacement. RMS values across the eccentric and concentric phases of the six repetitions

were averaged respectively and expressed as a percentage of the highest RMS activity of the corresponding EMG channel during any of the MVIC tasks (%MVIC).

Activity for each muscle was determined for the eccentric and concentric phases separately as the average RMS activity of all 15 channels along the corresponding muscle, which is hereafter referred to as overall activity. To determine the activity level of different muscle regions, average activity was calculated for channels 1-5 (distal region), 6-10 (middle region), and 11-15 (proximal region).

To provide estimates of hip extension and knee flexion strength, maximal torque during the isometric contractions was calculated as the maximum instantaneous force multiplied by the respective lever arm. The highest torque of all repetitions was used for the hip extension and knee flexion tasks.

### **Statistical analysis**

Normal distributions of studentized residuals were confirmed using Shapiro-Wilk test and Q-Q plots. For each exercise and contraction mode, the difference between BF1h and ST overall activity was tested with paired samples t-test in SPSS (IBM, Armonk, NY, USA).

Significance level was set at  $p < 0.05$ . Contraction mode\*region interaction for each exercise and region\*exercise interactions for each contraction mode were tested for each muscle with repeated-measures ANOVA. If Mauchly's test of sphericity was violated ( $p < 0.05$ ),

Greenhouse-Geisser adjustment was applied. Differences were located after Bonferroni correction. Cohen's  $d \pm 90\%$  confidence intervals (90% CI) were calculated to determine the magnitude of differences using a custom spreadsheet<sup>25</sup>. Differences were considered as

trivial ( $<0.2$ ), small ( $\geq 0.2$ ), moderate ( $\geq 0.5$ ), or large ( $\geq 0.8$ ). Differences where 90% CIs overlapped both 0.2 and -0.2 were considered unclear<sup>26</sup>.

## RESULTS

Maximal hip extension and knee flexion torque during the isometric contractions were  $236.5 \pm 84.1$  Nm and  $153.3 \pm 59.2$  Nm (mean  $\pm$  standard deviation), respectively.

### Overall activity

BFlh overall activity level ranged across exercises from an average of 17% to 54% in the eccentric, and 32% to 83% in the concentric phase, relative to MVIC (Figure 3). In ST, activity levels of 19% to 51% in the eccentric and 33% to 85% in the concentric phase were observed (Figure 3).

The only exercise with higher activity in BFlh compared to ST was 45HE: in both the concentric and eccentric phases, small differences between muscles were found ( $d = 0.28 \pm 0.28$  and  $0.33 \pm 0.24$ , respectively), which reached statistical significance in the concentric but not the eccentric phase ( $p = 0.026$  and  $0.100$ , respectively). ST activity was higher than BFlh activity in the eccentric phase of GM ( $d = 0.21 \pm 0.19$ ) and concentric phase of PLC, SLC and BB exercises ( $d = 0.35 \pm 0.27$ ,  $0.26 \pm 0.28$ , and  $0.24 \pm 0.25$ , respectively), from which only PLC reached statistical significance ( $p = 0.036$ ,  $0.118$ , and  $0.107$ , respectively).

Between-muscle differences are presented in Table 1.

## Regional activity patterns

Mean and standard deviation of regional activity levels are shown in Figure 4. Different exercises showed distinct regional patterns both in ST ( $p < 0.001$  in both eccentric and concentric) and in BF<sub>lh</sub> (eccentric:  $p = 0.001$ , concentric:  $p < 0.001$ ). The contraction mode affected the regional activity pattern of ST in BB, HE, PLC and SLC ( $p = 0.001$ ,  $p = 0.040$ ,  $p < 0.001$ , and  $p < 0.001$ , respectively), and the regional activity pattern of BF<sub>lh</sub> in UHC, PLC, SB and SLC ( $p = 0.012$ ,  $p < 0.001$ ,  $p = 0.016$  and  $p = 0.009$ , respectively).

Lower activity in the distal compared to the middle or proximal regions was found in BB, PLC and SLC ( $d$  range = 0.53 – 1.20,  $p < 0.05$ ), in both the eccentric and concentric phases. In all other exercises, no or only small differences between distal vs other regions were found ( $d$  range = 0.00 – 0.40,  $p > 0.05$ ). Similarly in BF<sub>lh</sub>, a large range in the magnitude of regional differences was observed across exercises (difference between regions,  $d$  range = 0.02 – 1.28), with PLC displaying the largest differences between muscle regions ( $d$  range = 0.41 – 1.28). Differences are detailed in Table 2.

## DISCUSSION

In the current study, muscle activity patterns were determined in 9 typical hamstring exercises using HD-EMG while taking proximal-distal differences into account. Small differences between the activity levels of BF<sub>lh</sub> and ST muscles were observed in the concentric phase of 45HE, SLC, PLC and BB, from which the only BF<sub>lh</sub>-dominant exercise-45HE- showed a difference in the eccentric phase. Proximal-distal distribution of EMG signals varied substantially across exercises, and showed different patterns between ST and BF<sub>lh</sub> muscles.

In addition to recent studies using muscle functional magnetic resonance imaging (mfMRI)<sup>18,19,21</sup> and our previous results using HD-EMG<sup>22</sup>, the exercise-dependent changes in proximal-distal activity patterns observed in this study reinforce the notion that spatially robust methods are needed to accurately describe the activity level of ST and BF<sub>lh</sub> muscles. This is further supported by the substantially different proximal-distal EMG activity patterns between muscles in most of the exercises. This was most pronounced in BB, wherein regional differences were moderate-to-large in ST but trivial in BF<sub>lh</sub>. This phenomenon likely leads to a non-systematic error when the activity levels of these muscles are compared based on a small region of the muscle.

Similar to previous studies<sup>17,27</sup>, we found high normalized activity levels in SB, SLC and PLC. Additionally, during UHC, which has not been the focus of many experiments, the activity level exceeded 80 %MVIC in the concentric phase. High activity levels in these exercises may facilitate training-induced adaptations in the hamstrings, although adaptations in response to these exercises are unclear. In accordance with previous literature<sup>28</sup>, particularly low overall hamstrings activity was observed in GM, which is apparently associated with low hamstring muscle forces in this exercise<sup>29</sup>. Exercises inducing limited hamstrings activity are likely suboptimal to facilitate meaningful muscle adaptations.

The relevance of the relative roles of individual hamstring muscles in hamstring injury are yet to be clarified. Training interventions should target the mitigation of injury risk factors. An imbalance between BF<sub>lh</sub> and ST muscle activity level seems to be associated with hamstring injuries<sup>14</sup>. Thus, balanced strengthening of these muscles should be a training goal. Although conventional EMG studies are not in agreement, previous mfMRI studies suggest that BF<sub>lh</sub> is relatively more active in hip-dominant exercises, while ST is relatively more active in knee-dominant exercises<sup>20</sup>. Based on the current study, it seems rather challenging to

preferentially activate BF<sub>lh</sub>. Previously, mfMRI showed relatively high activity in BF<sub>lh</sub> compared to ST in 45HE<sup>17</sup>, which is confirmed by our results. Other hip-dominant exercises did not induce higher activity in BF<sub>lh</sub> than in ST in this study.

Contraction-mode dependent between-muscle activity patterns were observed in some exercises in the current study. In the concentric phase, three exercises - SLC, PLC and UHC - showed higher activity in ST compared to BF<sub>lh</sub>. However, this difference was not evident in the eccentric phase of these exercises. This is inconsistent with previous results concerning eccentric PLC (120% concentric 1RM)<sup>18,30</sup> and the mechanically similar high-load eccentric-only Nordic hamstring exercise<sup>17,22,31</sup>, which seem to selectively activate ST. This discrepancy may be explained by the substantially lower load applied in the current study.

Similar to these exercises, no between-muscle differences were found in the eccentric phase of SB, BB or one-leg RDL. Based on the current study, these exercises should be used when balanced eccentric activation of ST and BF<sub>lh</sub> muscles is of interest. However, it is also likely important to include exercises with a relatively high overall hamstrings activity level to better facilitate muscle adaptations. The above observations suggest that ST-BF<sub>lh</sub> muscle selectivity cannot always be predicted based solely on the hip- or knee-dominant nature of the exercise, and may be affected by different neural control strategies in the eccentric and concentric phases.

In BF<sub>lh</sub>, eccentric stimuli may be of particular importance to elicit fascicle lengthening, which seems to reduce the risk for hamstring injury<sup>12</sup>. 45HE exhibited the largest activity in BF<sub>lh</sub> relative to ST, and has already been shown to effectively increase BF<sub>lh</sub> fascicle length<sup>11</sup>. Although activity level was higher in SB, UHC, SLC and PLC in our study, this does not necessarily imply that the eccentric phase of these exercises can more effectively elongate BF<sub>lh</sub> fascicles. Askling et al.<sup>7,8</sup> demonstrated that exercises performed at longer muscle

operating lengths are more effective for injury prevention than those requiring hamstrings to operate at a shorter length. Muscle length is clearly longer in 45HE compared to all four of the aforementioned high-activity exercises. Nonetheless, Nordic hamstring exercise also seems to reduce hamstring injuries<sup>6,9,10</sup>, even though the operating length is likely similar to that in SLC and PLC. Future studies should further clarify which of these exercises are the most beneficial to mitigate injury risk factors.

During rehabilitation, it may be of value to know regional activity patterns relative to the injury site to enable selective activation of the injured muscle region. In 80% of running-type hamstring injuries, the BFlh is affected primarily, and typically at the proximal site<sup>32</sup>. Within the BFlh, the proximal region seems to be the most challenging to activate since this region did not show higher activity compared to the distal or middle regions in any of the exercises in the current study. On the contrary, lunge<sup>19</sup> and CP<sup>21</sup> have been shown to activate the proximal BFlh in mfMRI studies. In the current study, CP showed the lowest activity in the proximal region. In any case, in both lunge<sup>33</sup> and CP, the overall hamstrings activity level is rather low, likely limiting meaningful adaptations in response to these exercises.

Manipulating the shin angle during a lunge may expose the hamstrings to substantially higher forces<sup>29</sup>, likely increasing hamstrings activity. However, it is unclear whether this manipulation alters the proximal-distal activity pattern. Future studies should examine whether targeting the injured muscle region during the rehabilitation process accelerates the restoration of muscle function after a hamstring injury.

It should be mentioned that some discrepancies exist when comparing some of our results with some previous mfMRI findings. Contrary to our finding that there are only trivial differences between ST and BFlh muscle activity levels in RDL, this exercise has been suggested to be a BFlh-dominant exercise based on mfMRI data<sup>34</sup>. However, in that study,

the exercise was performed bilaterally and included only 6 participants. In any case, in our study, hamstrings activity levels were 21% and 43% in the eccentric and concentric phases of RDL, the second lowest out of the examined exercises, likely minimizing the clinical relevance of this difference. On the contrary, hamstrings activity was particularly high in SB. In the current study, we did not detect clear differences between muscles in SB, contrary to Bourne et al. <sup>35</sup> who found higher metabolic activity in ST compared to BF<sub>lh</sub>, although the between-muscle difference seems to be smaller compared to most of the other exercises previously examined with mfMRI <sup>20</sup>. These discrepancies may arise from methodological issues: both mfMRI and EMG have limitations when comparing the relative contribution of different hamstring muscles. Metabolic activity estimated by mfMRI is sensitive to glycolysis <sup>36</sup>, vascular dynamics <sup>37</sup> and fiber type proportions <sup>38</sup>, which may differ between muscles and individuals. With respect to EMG, it is not clear whether reference contractions used for normalization activate all examined hamstring muscles to a similar extent. Accordingly, to examine the relative contribution of different hamstring muscles using these methods, it is likely most appropriate to compare within the same individuals and measurement session across exercises.

As a possible limitation of this study, surface EMG is prone to cross-talk. To minimize this effect, we used HD-EMG electrodes with a relatively shallow pick-up area and 10 mm inter-electrode distance <sup>39</sup>, ensured correct electrode location using ultrasonography, and measured male athletes with a relatively thin subcutaneous layer overlying the target muscles.

Furthermore, recording from 15 cm along each muscle likely minimized the effect of muscle movement relative to the skin, which is considered an inherent limitation of surface EMG.

Additionally, muscle regions were covered to a slightly different extent across individuals due to differences in muscle length relative to the length of the EMG arrays. As an additional

limitation, we measured amateur athletes without a history of hamstring injury, so our results may not be directly applicable to other populations, e.g. injured and/or professional athletes.

## **PERSPECTIVES**

HD-EMG revealed exercise-specific inter- and intramuscular hamstring activity patterns in 9 typical hamstring exercises. This study also revealed that the relative activity of different hamstring muscles may differ between the eccentric and concentric phases of an exercise. These findings highlight the potential impact of exercise selection procedure on hamstrings strengthening. The clinical implications of heterogeneous hamstrings EMG activity should be further examined, as well as the mechanisms and functional relevance of heterogeneous activity.

## **CONFLICT OF INTEREST**

The authors have no professional relationships with any company or manufacturer who would benefit from the current study results.

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#### FIGURE CAPTIONS

Figure 1: Examined hamstring exercises

Figure 2: High-density electromyography (HD-EMG)

Figure 3: Overall activity levels based on 15-channel arrays

Figure 4: Hamstrings regional EMG activity patterns

#### FIGURE LEGENDS

Figure 1. Nine typical rehabilitation exercises examined in this study. GM, Good morning; RDL, Unilateral Romanian deadlift; CP, Cable pendulum; BB, Bent-knee bridge; 45HE, 45° hip extension; PLC, Prone leg curl; SLC, Slide leg curl; UHC, Upright hip extension conic-pulley; SB, Straight-knee bridge.

Figure 2. High-density electromyography (HD-EMG) arrays (A) were attached and secured (B) over the semitendinosus (ST) and the long head of the biceps femoris (BF<sub>lh</sub>) to comprehensively describe muscle activity level during each exercise.

Figure 3. Electromyography (EMG) activity levels in the eccentric (A) and concentric (B) phase of each exercise. Mean and standard deviation are presented. Data represent the average of 15 EMG channels along each muscle. Dotted lines represent equal activity level between the two muscles when normalized to maximal voluntary isometric activity (MVIC). GM, Good morning; RDL, Unilateral Romanian deadlift; CP, Cable pendulum; BB, Bent-knee bridge; 45HE, 45° hip extension; PLC, Prone leg curl; SLC, Slide leg curl; UHC, Upright hip extension conic-pulley; SB, Straight-knee bridge.

Figure 4. Mean and standard deviation of the normalized activity level (%MVIC, maximal voluntary isometric contraction) in the proximal, middle and distal regions of each muscle during the eccentric and concentric phase of each exercise. GM, Good morning; RDL, Unilateral Romanian deadlift; CP, Cable pendulum; BB, Bent-knee bridge; 45HE, 45° hip extension; PLC, Prone leg curl; SLC, Slide leg curl; UHC, Upright hip extension conic-pulley; SB, Straight-knee bridge.

Table 1. Differences (Cohen's d  $\pm$  90% confidence limits) between BF<sub>lh</sub> and ST muscles in the eccentric and concentric phase of hamstring exercises.

	Eccentric		Concentric	
	Straight-knee bridge (SB)	0.19	$\pm 0.37^T$	-0.09
Upright hip extension conic-pulley (UHC)	0.11	$\pm 0.33^U$	-0.16	$\pm 0.29^T$
Slide leg curl (SLC)	0.12	$\pm 0.25^T$	-0.26	$\pm 0.28^S$
Prone leg curl (PLC)	0.17	$\pm 0.20^T$	-0.35	$\pm 0.27^S$
45° hip extension (45HE)	0.28	$\pm 0.28^S$	<b>0.33</b>	<b><math>\pm 0.24^S</math></b>
Bent-knee bridge (BB)	-0.17	$\pm 0.27^T$	-0.24	$\pm 0.25^S$
Cable pendulum (CP)	-0.02	$\pm 0.43^U$	0.01	$\pm 0.38^U$
Unilateral Romanian deadlift (RDL)	-0.19	$\pm 0.24^T$	-0.11	$\pm 0.22^T$
Good morning (GM)	-0.21	$\pm 0.19^S$	-0.09	$\pm 0.25^T$

Positive values: biceps femoris long head > semitendinosus (BF<sub>lh</sub> > ST)

Negative values: biceps femoris long head < semitendinosus (BF<sub>lh</sub> < ST)

T = trivial difference, S = small difference between muscles, U = unclear. **p < 0.05**

Table 2. Regional differences in the electromyography activity level of hamstring muscles in the eccentric and concentric phase of hamstring exercises.

Region	Eccentric						Concentric					
	Semitendinosus			Biceps femoris long head			Semitendinosus			Biceps femoris long head		
	middle	proximal	proximal	middle	proximal	proximal	middle	proximal	proximal	middle	proximal	proximal
distal middle	-0.40 ±0.42 <sup>S</sup>	0.04 ±0.67 <sup>U</sup>	0.31 ±0.24 <sup>S</sup>	0.31 ±0.24 <sup>S</sup>	-0.26 ±0.32 <sup>S</sup>	-0.58 ±0.27 <sup>M</sup>	-0.29 ±0.39 <sup>S</sup>	0.21 ±0.79 <sup>U</sup>	0.54 ±0.31 <sup>M</sup>	0.10 ±0.44 <sup>U</sup>	-0.45 ±0.33 <sup>S</sup>	
Upright hip extension conic-pulley (UHC)												
distal middle	-0.06 ±0.41 <sup>U</sup>	-0.17 ±0.34 <sup>U</sup>	0.22 ±0.19 <sup>S</sup>	0.22 ±0.19 <sup>S</sup>	-0.19 ±0.30 <sup>U</sup>	-0.42 ±0.26 <sup>S</sup>	0.01 ±0.40 <sup>U</sup>	0.02 ±0.38 <sup>U</sup>	0.40 ±0.23 <sup>S</sup>	0.25 ±0.41 <sup>S</sup>	-0.15 ±0.34 <sup>T</sup>	
Slide leg curl (SLC)												
distal middle	<b>0.53 ±0.33<sup>M</sup></b>	<b>0.63 ±0.32<sup>M</sup></b>	0.09 ±0.23 <sup>T</sup>	0.09 ±0.23 <sup>T</sup>	-0.16 ±0.28 <sup>T</sup>	-0.25 ±0.25 <sup>S</sup>	<b>0.95 ±0.43<sup>L</sup></b>	<b>1.02 ±0.32<sup>L</sup></b>	-0.31 ±0.26 <sup>S</sup>	<b>-0.49 ±0.28<sup>S</sup></b>	-0.18 ±0.20 <sup>T</sup>	
Prone leg curl (PLC)												
distal middle	<b>0.62 ±0.29<sup>M</sup></b>	<b>0.79 ±0.31<sup>M</sup></b>	<b>-0.46 ±0.28<sup>S</sup></b>	<b>-0.46 ±0.28<sup>S</sup></b>	<b>-0.87 ±0.30<sup>L</sup></b>	<b>-0.41 ±0.20<sup>S</sup></b>	<b>1.03 ±0.28<sup>L</sup></b>	<b>1.15 ±0.30<sup>L</sup></b>	<b>-0.84 ±0.32<sup>L</sup></b>	<b>-1.28 ±0.30<sup>L</sup></b>	<b>-0.45 ±0.24<sup>S</sup></b>	
45° hip extension (45HE)												
distal middle	-0.06 ±0.22 <sup>T</sup>	0.02 ±0.23 <sup>U</sup>	0.13 ±0.18 <sup>T</sup>	0.13 ±0.18 <sup>T</sup>	<b>-0.46 ±0.28<sup>S</sup></b>	<b>-0.59 ±0.23<sup>M</sup></b>	-0.08 ±0.24 <sup>T</sup>	0.17 ±0.26 <sup>T</sup>	0.21 ±0.17 <sup>S</sup>	-0.14 ±0.24 <sup>T</sup>	-0.35 ±0.23 <sup>S</sup>	
Bent-knee bridge (BB)												
distal middle	<b>1.03 ±0.34<sup>L</sup></b>	<b>1.20 ±0.44<sup>L</sup></b>	0.02 ±0.21 <sup>T</sup>	0.02 ±0.21 <sup>T</sup>	-0.02 ±0.22 <sup>U</sup>	-0.05 ±0.26 <sup>U</sup>	<b>0.98 ±0.30<sup>L</sup></b>	<b>1.13 ±0.37<sup>L</sup></b>	0.13 ±0.23 <sup>T</sup>	0.13 ±0.27 <sup>T</sup>	0.00 ±0.30 <sup>U</sup>	
Cable pendulum (CP)												
distal middle	-0.08 ±0.47 <sup>U</sup>	-0.27 ±0.36 <sup>S</sup>	0.00 ±0.14 <sup>T</sup>	0.00 ±0.14 <sup>T</sup>	<b>-0.39 ±0.18<sup>S</sup></b>	<b>-0.39 ±0.15<sup>S</sup></b>	-0.06 ±0.45 <sup>U</sup>	0.00 ±0.42 <sup>U</sup>	0.07 ±0.25 <sup>T</sup>	-0.28 ±0.35 <sup>S</sup>	-0.35 ±0.34 <sup>S</sup>	
Unilateral Romanian deadlift (RDL)												
distal middle	0.20 ±0.36 <sup>S</sup>	0.14 ±0.30 <sup>T</sup>	0.06 ±0.13 <sup>T</sup>	0.06 ±0.13 <sup>T</sup>	<b>-0.29 ±0.17<sup>S</sup></b>	<b>-0.35 ±0.13<sup>S</sup></b>	0.21 ±0.32 <sup>S</sup>	0.26 ±0.32 <sup>S</sup>	0.10 ±0.14 <sup>T</sup>	-0.26 ±0.19 <sup>S</sup>	<b>-0.36 ±0.18<sup>S</sup></b>	
Good morning (GM)												
distal middle	0.20 ±0.42 <sup>U</sup>	0.12 ±0.33 <sup>U</sup>	0.00 ±0.13 <sup>T</sup>	0.00 ±0.13 <sup>T</sup>	<b>-0.42 ±0.18<sup>S</sup></b>	<b>-0.42 ±0.14<sup>S</sup></b>	0.21 ±0.42 <sup>U</sup>	0.25 ±0.48 <sup>U</sup>	0.04 ±0.14 <sup>T</sup>	<b>-0.47 ±0.17<sup>S</sup></b>	<b>-0.51 ±0.20<sup>M</sup></b>	

Cohen's  $d$  ±90% confidence limits. T = trivial difference, S = small difference, M = moderate difference, L = large difference between regions, U = unclear.

Positive and negative differences correspond to higher activity level in the relatively more proximal and distal regions, respectively. **p<0.05**



GM



45HE



PLC



UHC



SB



RDL



BB

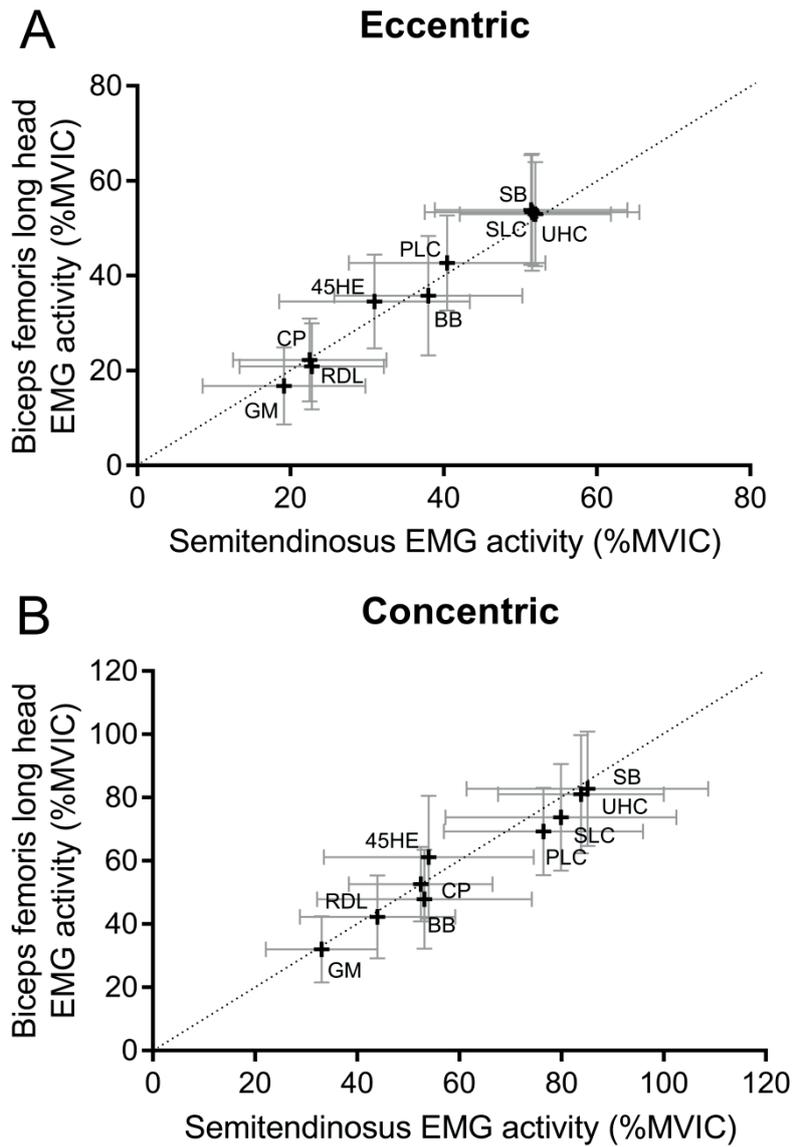


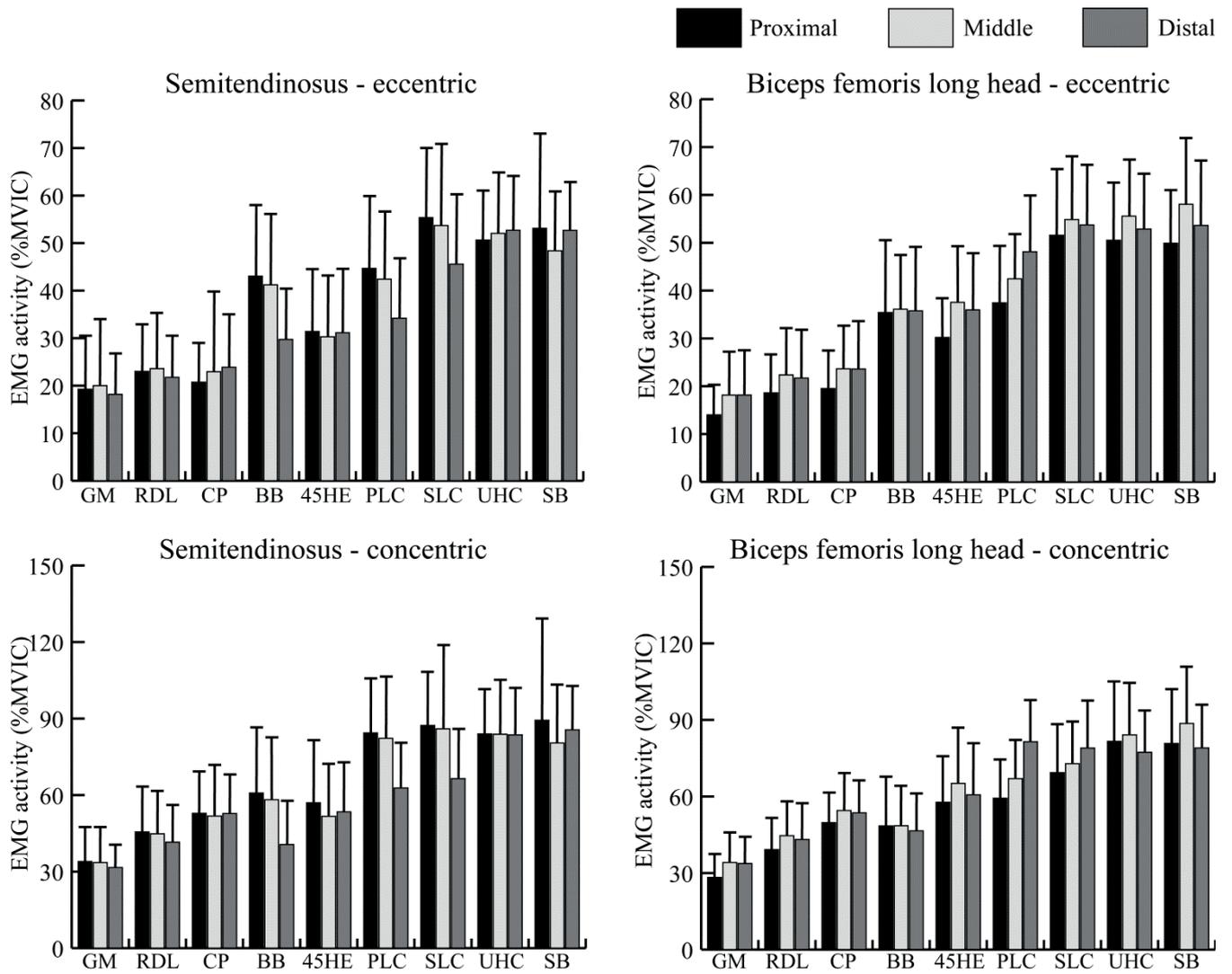
SLC



CP









### III

## **IMPACT OF HIP FLEXION ANGLE ON UNILATERAL AND BILATERAL NORDIC HAMSTRING EXERCISE TORQUE AND HIGH-DENSITY ELECTROMYOGRAPHY ACTIVITY**

by

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# [ RESEARCH REPORT ]

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## Impact of Hip Flexion Angle on Unilateral and Bilateral Nordic Hamstring Exercise Torque and High-Density Electromyography Activity

**H**amstring strain is one of the most common injuries in sports that involve repetitive sprints, such as football,<sup>15</sup> Gaelic football,<sup>35</sup> Australian rules football,<sup>36</sup> and rugby.<sup>8</sup> This type of injury results in significant financial consequences for clubs,<sup>24</sup> as well as substantial player time loss,<sup>22</sup> which are associated with a lower final league ranking, at least in top-level football.<sup>22</sup> Thus, hamstring injury risk reduction is of primary interest in many sports.



play an important role in sprinting.<sup>33</sup> Sprint-type hamstring injuries<sup>2</sup> seem to occur mainly during the late swing phase or early contact phase, in which hamstrings are highly activated

The biarticular hamstrings (biceps femoris long head [BFLH], semitendinosus [ST], and semimembranosus) span both the hip and knee joints, functioning as hip extensors and knee flexors, and

at longer lengths.<sup>37</sup> Most sprint-related hamstring injuries occur in the BFLH.<sup>11,26</sup> Although training regimes that aim to induce BFLH fascicle lengthening seem to be beneficial,<sup>40</sup> the injury mechanism is likely to be multifactorial,<sup>6</sup> and other synergist muscles, such as the ST, may play an important role in protecting the BFLH<sup>38</sup> from injury.

In addition to targeting specific muscles, Askling et al<sup>3,4</sup> suggested that for injury prevention, exercises performed at relatively long hamstring muscle lengths may be preferable to those involving short muscle lengths. However, the unilateral exercises in Askling's protocol<sup>39</sup> require relatively low hamstring muscle activity, potentially limiting protective adaptations such as fascicle lengthening and eccentric strength improvements.<sup>6,40</sup>

On the contrary, the Nordic hamstring exercise (NHE) results in higher hamstring muscle activity relative to other exercises.<sup>7,23,42</sup> The NHE has been associated with decreased hamstring injury

● **BACKGROUND:** In the bilateral Nordic hamstring exercise (NHE), hamstrings operate at relatively short lengths, which may limit the efficacy of the NHE in hamstring injury prevention.

● **OBJECTIVES:** To examine knee flexion torque and biceps femoris long head (BFLH) and semitendinosus (ST) high-density electromyography (EMG) activity during the unilateral and bilateral NHE, performed with either neutral (NHE0) or 90°-flexed (NHE90) hips.

● **METHODS:** In this laboratory study, exercises were performed on a novel device at the eccentric 1-repetition maximum load defined for 90° to 15° of knee range of motion. Torque and EMG signals normalized to maximal voluntary isometric activity were compared in different phases of the exercises with statistical parametric mapping.

● **RESULTS:** The EMG levels were lower in NHE90 than in NHE0, mainly in the second half of the movement. Knee flexor eccentric torque was higher in

NHE90 than in NHE0 from the beginning to 87% of the bilateral movement, and over the entire unilateral movement. In NHE0, ST activity compared to BFLH activity was higher during the initial movement phase and lower when the movement was close to knee extension. Torque and EMG activity were generally similar in the bilateral and unilateral modes.

● **CONCLUSION:** If performed with neutral hips, the NHE selectively activates the BFLH near full knee extension. Performing the NHE with hips flexed to 90° is preferable when higher passive torque and ST selectivity are targeted at a longer muscle length. Performing these exercises unilaterally could help train each limb separately, with similar torque and EMG output to those of the bilateral conditions. *J Orthop Sports Phys Ther* 2019;49(8):584-592. Epub 26 Mar 2019. doi:10.2519/jospt.2019.8801

● **KEY WORDS:** biceps femoris, rehabilitation, semitendinosus, strain injury

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incidence in many studies, provided that the athlete was sufficiently compliant.<sup>6</sup> The NHE is typically performed as an eccentric-only exercise, in which the athlete, in kneeling, performs a slow, bilateral, eccentric action of the knee flexors by resisting knee extension, with neutral or slightly flexed hips. During the NHE, the range of active hamstring lengthening is limited by the neutral hip position, and is further reduced when the athlete is unable to resist the external load at a certain point of knee extension (earlier in weaker individuals). Compared to Askling's protocol,<sup>3,4</sup> the relatively short hamstring operating length during the NHE is considered to be a limitation of this exercise. Although high force and electromyography (EMG) activity during the NHE<sup>23</sup> likely contribute to its preventive effect, it is unknown whether performing the NHE at longer hamstring lengths (ie, with flexed hips) or performing it unilaterally would alter knee flexion torque and hamstring EMG activity during the exercise. This information may help practitioners understand the potential impact of such exercise variations on muscle adaptations.

The primary aim of this study was to examine knee flexion torque and EMG activity of the BFLH and ST during a bilateral NHE performed at a neutral (0°) hip angle (NHE0) with a shorter muscle length, compared to a bilateral NHE performed at 90° of hip flexion (NHE90) with a longer muscle length. The secondary aims were to examine whether knee flexion torque and EMG activity levels differed when performing the exercises in the unilateral compared to the bilateral mode, and to explore intermuscular (BFLH versus ST) differences in EMG activity during the 4 exercises.

## METHODS

### Participants

**E**XCLUSION CRITERIA WERE HISTORY of hamstring or anterior cruciate ligament injury, recent (in the previous 6 months) leg injury, and current muscu-

loskeletal, cardiovascular, or metabolic disorders. Amateur football and rugby players aged 18 to 30 years who trained at least 3 times per week and engaged in strength training on a weekly basis were recruited. Athletes from local sports clubs who met the study criteria and were available to participate volunteered, resulting in a total of 13 participants (mean ± SD age, 23 ± 3 years; body mass, 76 ± 6 kg; height, 1.77 ± 0.05 m). All participants provided written informed consent prior to commencing familiarization with the study protocol. The testing procedures were approved by the Institutional Review Board of the Faculty of Sport Sciences, Université Côte d'Azur (number 2016-08), and performed in an accredited research center (Le Laboratoire Motricité Humaine Expertise, Sport, Santé), according to the Declaration of Helsinki.

### Study Design

Participants were familiarized with the study protocol in a 3-week process consisting of 6 sessions to facilitate initial neural adaptations<sup>9</sup> and to take advantage of the "repeated bout effect" on the testing day.<sup>29</sup> During these sessions, a standardized warm-up (**APPENDIX A**, available at [www.jospt.org](http://www.jospt.org)) was followed by practicing all 4 of the test exercises. For each exercise, eccentric action of the knee flexors was performed at a target pace of 5 seconds, controlled with a metronome (Pro Metronome; EUMLab-Xanin Technology GmbH, Berlin, Germany), throughout the 90° to 15° knee range of motion (full knee extension, 0°), as measured with the potentiometer of the load-cell device. Participants initiated the knee extension movement from approximately 110° prior to reaching 90°, from where the 5-second pacing began. Two of the 4 exercises were performed in a neutral hip position throughout the knee extension range (NHE0), and the other 2 were performed in 90° of hip flexion (NHE90), as maintained via the trunk support on the frame of the device (**FIGURE 1**).

Both the NHE0 and NHE90 were performed in a unilateral and a bilateral

mode (**FIGURE 1**). To match the relative load across exercises, resistance bands or weight plates were attached to the frame of the device to decrease or increase the external load, respectively (**FIGURE 1**). During each familiarization session, the maximal load at which the eccentric movement could be performed in the knee range of motion of 90° to 15° and at the desired pace was defined and used as a training load (1-repetition maximum). This provided a starting point on the testing day, when the eccentric 1-repetition maximum load for each exercise was redefined. In the last familiarization session, knee flexion maximal voluntary isometric contractions (MVICs) were also practiced to decrease interindividual variability when normalizing EMG signals.

The measurement session was 5 to 8 days after the last familiarization session. During this period, participants were asked to refrain from additional eccentric training. In the main measurement session, after preparation and standardized warm-up, MVICs were performed for EMG normalization purposes. During the MVICs, participants lay prone on the load-cell device, with their shin parallel to the ground and their knees flexed at 30°. Knee joints were aligned with the rotation axis of the frame of the device. Hips and trunk were secured in neutral position. The load cells of the device (1 for each leg) were placed above the proximal border of the lateral malleoli. Participants were instructed to perform forceful knee flexion with their self-reported kicking leg (1 left, 12 right; hereafter referred to as "dominant leg") and to maintain maximum contraction intensity for 2 to 3 seconds.

Three MVICs were performed, with a 1-minute rest. Then, 2 repetitions from each of the 4 exercises were performed in a randomized order, with a 1-minute rest between repetitions and a 4-minute rest between exercises. The target loads (eccentric 1-repetition maximum) were individually defined just before data collection for each exercise. Each

## RESEARCH REPORT

exercise was performed at the 5-second pace practiced before, which translated to approximately 15°/s. Knee flexion force, knee angular displacement, and EMG activity of the BFLH and ST were recorded during MVICs and NHEs. No substantial fatigue was reported throughout the measurement session.

### Data Collection

After shaving, abrading, and cleaning the skin, we placed 15-channel, high-density EMG arrays (OT Bioelettronica, Turin, Italy) at a 10-mm interelectrode distance along the midline between the borders of each of the BFLH and ST muscles,<sup>23</sup> guided by brightness-mode ultrasonography (Echo Blaster 128; TELEMED Ltd, Vilnius, Lithuania). Proximal-distal positioning of the elec-

trodes was standardized by aligning channel 8-9 (from distal) with the anatomical middle of the BFLH muscle, defined as the distance between the ischial tuberosity and popliteal fossa. To avoid placing the electrodes over the relatively longer distal tendon of the ST, the array for this muscle was placed relatively more proximally, at 1 cm below the proximal edge of the ST tendinous inscription (located with ultrasonography).

Conductive gel (signa gel; Parker Laboratories, Inc, Fairfield, NJ) was applied to the adhesive foam of the arrays, signal quality was checked during submaximal knee flexion, and the arrays were fixed to the skin using tape. The high-density EMG arrays were connected to a 12-bit analog-digital converter and amplifier (OT Bioelettronica), and digital signals

were recorded in OT BioLab Version 3.1 (OT Bioelettronica). A reference electrode strap was placed over the contralateral wrist. Electromyographic signals were amplified by a factor of 1000 and sampled at 2048 Hz. Fifteen EMG channels were recorded in the proximal-distal direction from each of the BFLH and ST simultaneously, covering 15 cm along each muscle.

Force was measured in the dominant leg during MVICs and the eccentric exercises using the load cells of the device (ELAF, 1250 N; TE Connectivity, Schaffhausen, Switzerland), and sagittal plane knee angle was measured with the built-in potentiometer of the device (P4500; Novotechnik, Ostfildern, Germany), both sampled at 1000 Hz. Digitized signals were recorded using a custom interface in LabVIEW (Version 8.5; National Instruments, Austin, TX). The NHE0 and NHE90 showed good to excellent between-session and within-session reliability, as tested previously (APPENDIX B, available at [www.jospt.org](http://www.jospt.org)). Torque was calculated using the length of the lever arm, measured as the distance between the femur's lateral epicondyle and the center of the load cells. The OT BioLab (OT Bioelettronica) and LabVIEW (National Instruments) recordings were synchronized with a digital pulse, sent via an external trigger to the load-cell device and EMG system simultaneously.

### Data Processing

Only data collected during the active-lengthening phase, standardized across exercises as the range of motion between the knee angles of 90° and 15°, were included in the analyses. Electromyographic signals were band-pass filtered (10-500 Hz) with a zero-lag, fourth-order Butterworth filter in MATLAB (The MathWorks, Inc, Natick, MA). To calculate reference activity levels used for normalization, root-mean-square activity was defined over a 1-second stable period for each EMG channel and MVIC, and for both muscles. Of these MVICs, the highest activity for each channel was used for



**FIGURE 1.** The Nordic hamstring exercise was performed with neutral hips (0°; panels A and C) and 90°-flexed hips (panels B and D). The Nordic hamstring exercise with neutral hips required forceful hip extension to minimize hip flexion. In the Nordic hamstring exercise with 90°-flexed hips, the trunk lay on the device, requiring no hip extension effort. Both exercises were performed bilaterally (A and B) and unilaterally (C and D). All exercises were performed at eccentric 1-repetition maximum load, defined for the knee range of motion of 90° to 15° at a pace of 15°/s. Load was adjusted using weight plates or resistance bands, or exercises were performed with body weight only, according to the strength level of the participant.

normalization. Normalized signals were smoothed with a 5-Hz, fourth-order, zero-lag, low-pass Butterworth filter. Smoothed curves of all 15 channels per muscle were averaged to estimate overall activity of the BFLH and ST muscles, taking proximal-distal differences<sup>23</sup> into account.

Torque (Newton meters) for the dominant leg (from which EMG signals were recorded) was calculated for each exercise and repetition as the instantaneous force (Newtons) multiplied by the lever arm (meters).

The EMG and torque curves were time normalized (0-100 points) for comparison and included in further analyses as detailed below.

### Statistical Analysis

Statistically significant differences between curves were detected using statistical parametric mapping (SPM<sup>20</sup>; Functional Imaging Laboratory, London, UK), by applying 2-tailed paired *t* tests in MATLAB (The MathWorks, Inc) after ensuring normal data distribution (Shapiro-Wilk test). The SPM analyses were conducted using an open-source code (Version 0.4; www.spm1d.org) in 4 steps. First, the SPM{*t*} test statistic was calculated for each point in the normalized time series. Then, the critical threshold, which only 5% (alpha level) of smooth random curves would be expected to exceed, was defined. Thereafter, the locations where the SPM{*t*} trajectory crossed the critical threshold in the normalized time series were determined to define so-called “supra-threshold clusters.” Finally, SPM was used to calculate cluster-specific *P* values. The technical details of SPM are described elsewhere.<sup>1,20</sup>

Using this method, differences in knee flexion torque and BFLH and ST EMG activity were examined between the 2 repetitions of each exercise. This way, we ensured that 2 repetitions per exercise could describe exercise-specific torque and EMG characteristics with high agreement across all points of the normalized time series (APPENDIX C FIGURES 1 through

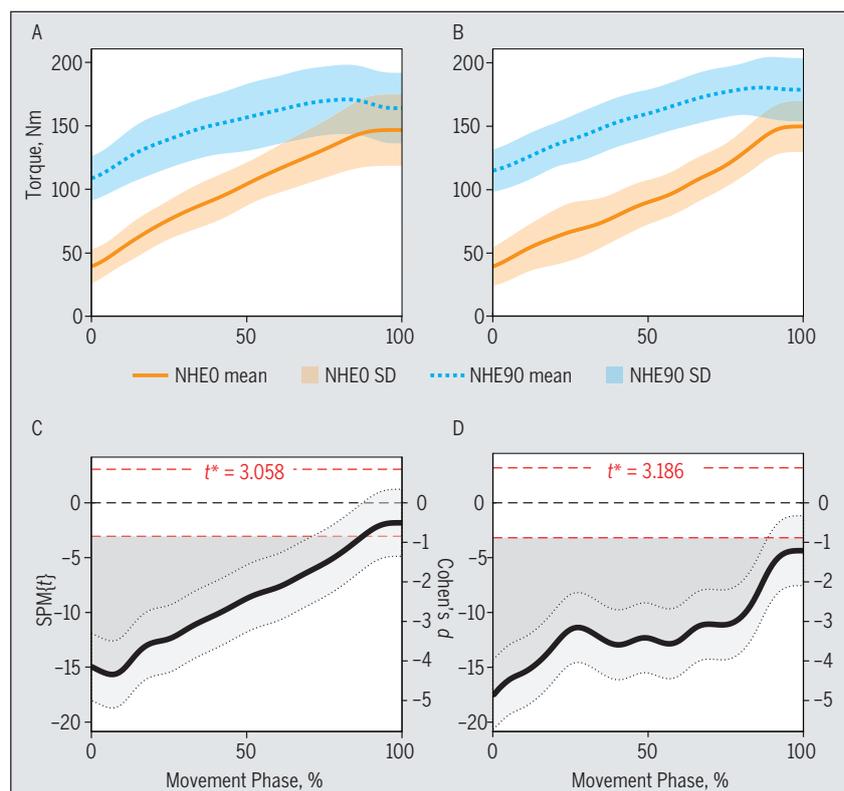
3, available at www.jospt.org). After averaging the 2 repetitions for each exercise, differences between exercises in torque and EMG variables were examined, as well as differences between EMG activity levels of the BFLH and ST for each exercise. Cohen’s  $d_z \pm 95\%$  confidence intervals (CIs) were calculated for each point of the normalized time series, presented in FIGURES 2 through 4 and in APPENDIX C (FIGURES 4 through 8). Cohen’s  $d_z$  was calculated as the difference between means divided by the SD of the difference scores. The average Cohen’s  $d_z \pm 95\%$  CI for each supra-threshold cluster are presented in the text. Cohen’s  $d_z$  differences were considered small ( $0.2 < 0.5$ ), moderate ( $0.5 < 0.8$ ), or large ( $\geq 0.8$ ).<sup>10</sup>

## RESULTS

### Bilateral NHE0 Versus Bilateral NHE90

**I**N BILATERAL MODE, TORQUE LEVELS IN the NHE90 were significantly higher than those in the NHE0 from 0% to 87% of the movement ( $d_z = 2.74 \pm 0.85$ ,  $P < .001$ ) (FIGURE 2).

Both the BFLH and ST exhibited higher EMG activity in the NHE0 than in the NHE90. The BFLH showed 1 supra-threshold cluster from 36% until the end of the movements ( $d_z = 2.18 \pm 1.07$ ,  $P < .001$ ) (FIGURE 3). The ST showed 2 supra-threshold clusters, between 45% and 84% and between 90% and 100% of the movements ( $d_z = 1.53 \pm 1.13$ ,  $P < .001$  and  $d_z = 1.37 \pm 1.13$ , respectively) (FIGURE 3).



**FIGURE 2.** Mean and SD values for knee flexion torque in the NHE0 and NHE90, performed (A) bilaterally and (B) unilaterally. Differences between the NHE0 and NHE90 are shown in panels C and D, respectively. Thick black lines represent SPM{*t*} statistics and Cohen’s *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences (all,  $P < .001$ ). Abbreviations: NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

## [ RESEARCH REPORT ]

### Unilateral NHE0 Versus Unilateral NHE90

In unilateral mode, significantly higher torque levels were found in the NHE90 than in the NHE0 during the entire movement ( $d_z = 3.22 \pm 0.88$ ,  $P < .001$ ) (FIGURE 2).

Both the BFLH and ST exhibited higher activity in the NHE0 than in the NHE90. The BFLH showed 1 supra-threshold cluster from 51% until the end of the movement ( $d_z = 1.65 \pm 1.06$ ,  $P < .001$ ) (APPENDIX C FIGURE 4). The ST showed 3 supra-threshold clusters, at 51% to 53%, 67% to 73%, and 84% to 85% of the movement ( $d_z = 1.13 \pm 1.13$ ,  $P = .034$ ;  $d_z = 1.19 \pm 1.13$ ,  $P = .003$ ; and  $d_z = 1.10 \pm 1.13$ ,  $P = .042$ , respectively) (APPENDIX C FIGURE 4).

### Differences Between Bilateral and Unilateral Exercises

The bilateral NHE0 showed higher torque than the unilateral NHE0 in the midrange of the movement (52%-78%,  $d_z = 0.95 \pm 0.91$ ,  $P = .005$ ) (APPENDIX C FIGURE 5). In the NHE90, no difference was found between bilateral and unilateral torques (APPENDIX C FIGURE 5).

No difference was found in EMG activity levels in any of the muscles when bilateral exercises were compared to unilateral exercises (APPENDIX C FIGURES 6 and 7).

### Intermuscular EMG Differences

As shown in FIGURE 4, in the bilateral NHE0, ST activity was higher than BFLH activity during the early phase of the movement (2%-4%,  $d_z = 1.07 \pm 1.07$ ,  $P = .039$ ) and lower during the final phase (94%-98%,  $d_z = 1.11 \pm 1.07$ ,  $P = .030$ ). Differences between muscles in the unilateral NHE0 did not reach statistical significance (APPENDIX C FIGURE 8).

In the bilateral NHE90 (FIGURE 4), ST activity, compared to BFLH activity, was statistically higher in 3 subphases in the second half of the movement (62%-69%,  $d_z = 1.12 \pm 1.05$ ,  $P = .016$ ; 72%-80%,  $d_z = 1.09 \pm 1.05$ ,  $P = .009$ ; and 83%-100%,  $d_z = 1.25 \pm 1.05$ ,  $P < .001$ , respectively). In the unilateral NHE90 (APPENDIX C FIGURE 8), higher ST activity, compared to BFLH activity, was observed at the initial and

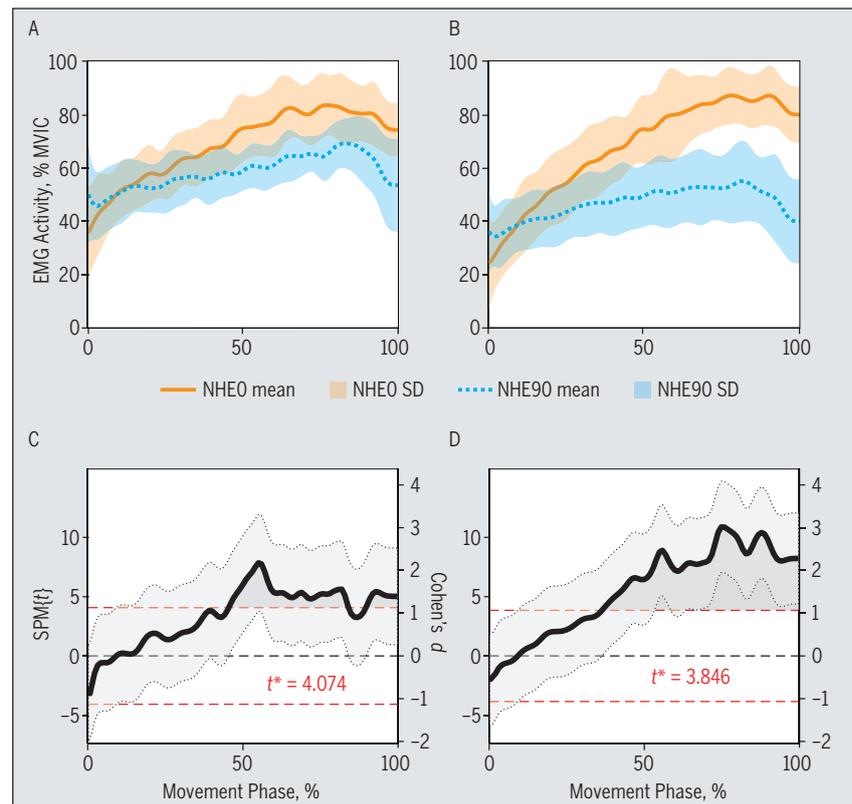
final phases of the movement (0%-3%,  $d_z = 1.14 \pm 1.04$ ,  $P = .039$ ; 66%-72%,  $d_z = 1.11 \pm 1.04$ ,  $P = .021$ ; 85%-89%,  $d_z = 1.10 \pm 1.04$ ,  $P = .034$ ; and 94%-100%,  $d_z = 1.15 \pm 1.04$ ,  $P = .024$ , respectively).

## DISCUSSION

THE PRESENT STUDY USED A NOVEL load-cell device and high-density EMG to assess knee flexion torque and hamstring EMG output for different NHE modes. Statistical parametric mapping showed remarkably higher torque and considerably lower hamstring EMG activity levels during the NHE90 compared with the NHE0 in most phases of

the movements. Muscle activity also differed between the NHE90 and NHE0 in the BFLH relative to the ST. There were similar peak torque and EMG activity levels in unilateral and bilateral modes of both the NHE0 and NHE90, provided that relative loads were matched.

During the bilateral NHE0, high EMG activity was observed in hamstring muscles, a finding similar to that of previous studies involving the NHE.<sup>7,23,42</sup> A recent study used high-density EMG and found higher ST than BFLH activity in the NHE,<sup>23</sup> similar to other studies that have used muscle functional magnetic resonance imaging.<sup>5,7,16,30,31</sup> However, we analyzed data across the entire move-



**FIGURE 3.** Mean and SD values for (A) semitendinosus and (B) biceps femoris long head EMG activity (normalized to percent knee flexion MVIC) in the bilateral exercises (NHE0 and NHE90). Differences between the bilateral NHE0 and NHE90 for each muscle are shown in panels C and D, respectively. Thick black lines represent SPM{t} statistics and Cohen's d effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (t\*) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (P) are calculated for each supra-threshold cluster depicting statistically significant differences (all,  $P < .001$ ). Abbreviations: EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

ment and found higher ST than BFLH activity only during the early phase of the movement. The relative activity of the BFLH compared to the ST then increased toward full knee extension. Ultimately, higher BFLH than ST activity reached statistical significance when the knee was relatively extended. Similar results have been reported in isokinetic and isometric knee flexion.<sup>25,34</sup>

In most studies of the NHE, participants performed the exercise at a supra-maximal eccentric load.<sup>7,14,23,28,41,42</sup> When doing so, the hamstrings are highly loaded, but participants are generally unable to maintain resistance over the entire range of motion until the knee

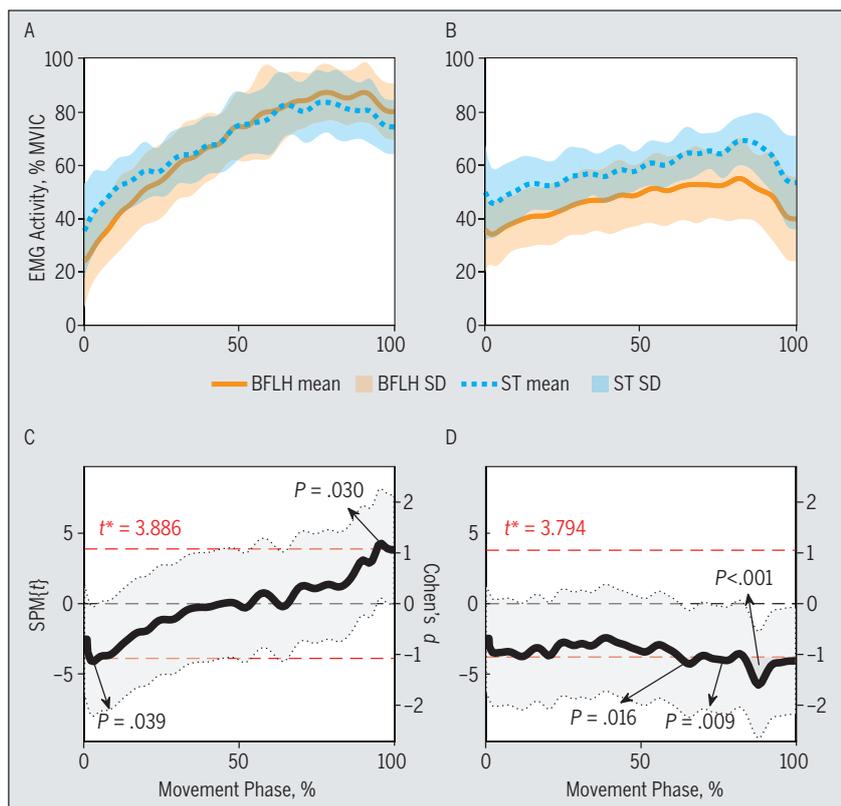
is nearly extended, at which point the BFLH would be selectively activated according to our results. Another difference between this study and previous studies is that the NHEO in this study was performed with hip extension effort throughout the movement, with which participants were highly familiarized. The relative activity of the BFLH compared to the ST is higher in hip extension compared to knee flexion exercises.<sup>6</sup> This suggests that hip extension effort in the NHE may improve BFLH activity and might also have contributed to the relatively higher BFLH activity in the current study than that observed in previous studies. From a practical

perspective, there seems to be a trade-off between adding extra load (limiting the range of motion) and selectively activating the BFLH in the NHEO, which should be considered when targeting different muscles in the training program.

In the bilateral NHE90, the hips were flexed to 90° and EMG activity levels of both muscles were substantially lower than those in the NHEO, with the hips in a neutral position. These differences were larger than those reported for eccentric isokinetic testing.<sup>25</sup> In contrast, this difference is not present in isometric knee flexion at different hip flexion angles.<sup>32</sup> This might be explained by eccentric inhibition in the NHE90, where muscle lengths are substantially longer than in the NHEO. In the NHE90, long muscle lengths are accompanied by higher hamstring activity relative to the exercises used in Askling's protocol.<sup>39</sup> Therefore, the NHE90 may be beneficial when aiming to decrease the inhibition associated with hamstring injuries.<sup>21</sup>

Although the protective mechanisms of exercises performed at long muscle lengths are unclear, the NHE90 may be useful when the training goal is to eccentrically activate the hamstrings at long muscle lengths. However, it may also be important that the activity of the BFLH relative to that of the ST differs between the NHEO and NHE90. Lower BFLH compared to ST activity might be explained by the fact that the NHE90 was performed without hip extension effort (the trunk was supported by the frame of the device). This suggests that performing the NHE with flexed hips might also be beneficial when attempting to selectively target the ST muscle during training.

Despite lower EMG activity levels in the NHE90 than in the NHEO, substantially higher knee flexion torque was observed in the NHE90 than in the NHEO. This agrees with results on isokinetic eccentric action of the knee flexors.<sup>25</sup> Although the difference between knee flexion torques decreased as the knee was extended in the current study, EMG



**FIGURE 4.** Mean and SD values for BFLH and ST EMG activity (normalized to percent knee flexion MVIC) in the bilateral (A) NHEO and (B) NHE90. Differences between muscles are shown in panels C and D, respectively. Thick black lines represent SPM(*t*) statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (*t*<sup>\*</sup>) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: BFLH, biceps femoris long head; EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHEO, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping; ST, semitendinosus.

activity was still lower in the NHE90 close to knee extension. One reason for relatively higher torque in the NHE90 could be that the passive stiffness of hamstring muscles increases with hip flexion.<sup>27</sup> However, the difference in passive stiffness between different hip flexion angles decreases as the knee moves from a flexed toward an extended position,<sup>27</sup> as opposed to what we observed in knee flexion torques. This suggests that passive stiffness has a negligible effect on the substantially higher torque in the NHE90 compared to the NHE0.

Additionally, higher passive stiffness would not explain why EMG activity was so much lower in the NHE90 than in the NHE0. A superior contribution of other knee flexor muscles cannot be totally excluded. However, it seems more likely that high torque coupled with low EMG activity is a result of more pronounced eccentric work at the fascicle level when performing the NHE90 compared to the NHE0, although this hypothesis should be supported experimentally. This, in theory, would further facilitate BFLH fascicle elongation, which seems to be associated with lower risk of hamstring injury.<sup>6,40</sup>

In addition to differences at the fascicle level, it is likely that higher knee flexion torque in the NHE90 compared to the NHE0 also exposes the tendinous structures of the hamstrings to relatively higher forces. Previous studies have shown that a lower ratio between the width of the BFLH proximal aponeurosis and the width of the BFLH muscle results in higher strain at the proximal muscle-tendon junction,<sup>17,18</sup> where most hamstring injuries occur.<sup>13</sup> It should be examined whether exercises with relatively high passive and low active forces—as observed in the NHE90—can increase this ratio, and whether that helps to protect against hamstring injuries.

When comparing bilateral to unilateral exercises, similar torque and EMG levels were reached in the NHE90. Although some differences were found be-

tween bilateral and unilateral torques in the NHE0, this was not evident around peak torque. Although the NHE is classically performed bilaterally, unilateral performance may improve the potential to selectively strengthen the weaker and/or injured side without impairing torque and EMG output compared to the bilateral modality. Whether unilateral or bilateral strengthening may be more effective at reducing side-to-side strength imbalances and the risk of hamstring injury warrants further study.<sup>19</sup>

### Clinical Implications Summary

The NHE90 may be preferable when selective ST activation and high passive force are targeted at relatively long hamstring operating lengths. Higher torque and lower EMG activity in the NHE90 than in the NHE0 implies (1) more pronounced eccentric work in the NHE90, which may facilitate protective adaptations (eg, fascicle elongation)<sup>39</sup>; and (2) potential for increasing the width ratio of the BFLH proximal aponeurosis and muscle belly, which may reduce injury risk.<sup>14,15</sup>

Performing the NHE with hip extension effort and control over the knee range of motion (applying loads that allow active hamstring lengthening close to knee extension) may be important when more balanced ST and BFLH activation is a training goal.

Performing NHE in unilateral mode may help to decrease interlimb differences in strength and muscle architecture.

### Limitations

As a study limitation, surface EMG recordings are prone to cross-talk. In order to limit this effect, we used high-density arrays with small electrodes and a short interelectrode distance,<sup>12</sup> positioned the electrodes with ultrasound guidance, and recruited male athletes who had a relatively thin subcutaneous layer over the hamstrings. Covering 15 cm along the muscles also minimized the effect of muscle movement relative to the skin. The EMG normalization method also af-

fects the observed activity level of these muscles, and it is still unclear whether knee flexion MVIC can activate both the BFLH and ST to a similar extent. Regarding kinematics, knee joint angle was not measured directly but rather with the potentiometer of the device, with some inherent inaccuracy.

A further limitation is the small sample size in this study, which might have led to increased false-negative error rate and biased Cohen's  $d_z$ . Although all observed statistically significant differences can be considered large ( $d_z$  greater than 1.0), and SPM exerts tight control over type I errors, we encourage replication studies to eliminate potential type I errors in this study. Finally, data obtained from amateur athletes in this study may not be applicable to other populations.

## CONCLUSION

**WE FOUND HIGHER TORQUE AND** lower EMG activity in the NHE90 compared to the NHE0, and performing these exercises unilaterally elicited similar torque and EMG levels to those in the bilateral exercises. Statistical parametric mapping revealed that controlling the knee range of motion can influence the relative activity of hamstring muscles in the NHE when hips are in a neutral position. These NHE variations may improve the diversity of exercise selection in hamstring-strengthening protocols. Adaptations in response to these exercises, as well as the impact of these exercises on injury prevention, should be further examined. ●

### KEY POINTS

**FINDINGS:** The Nordic hamstring exercise (NHE) selectively activated the semitendinosus (ST) in the early phase but the biceps femoris long head (BFLH) in the late phase, when hips were in neutral position (0°). On the contrary, performing the NHE with hips flexed to 90° resulted in higher torque and higher activity of the ST compared to the BFLH. The unilateral and bilateral

NHEs resulted in similar knee flexion torques and EMG activity when relative loads were matched.

**IMPLICATIONS:** The NHE with hips flexed to 90° may be preferable when selective ST activation and high passive force are targeted at relatively long hamstring lengths. Hip extension effort and utilizing the terminal phase of the NHE may be important when more balanced ST and BFLH activation is a training goal. **CAUTION:** Adaptations to these variations of the NHE should be further examined before implementing these findings in clinical practice.

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## APPENDIX A

### DESCRIPTION OF THE STANDARDIZED WARM-UP UTILIZED IN THIS STUDY

1. Five minutes of jogging on a treadmill (self-selected speed: 10-12 km/h)
2. Eight repetitions of “the extender” for each leg  
The participant lies supine, with the hip flexed at approximately 90° on 1 side and the other leg in neutral position. The participant stabilizes the thigh over the flexed hip with his or her hands and performs slow, cyclic knee flexion/extension in the full knee range of motion
3. Eight repetitions of “the diver” for each leg  
Standing on 1 leg (knee flexion, approximately 10°), the participant performs a simulated dive, that is, flexes the hip while lifting the other leg (similar to a 1-leg Romanian deadlift but with a 90° knee angle), and simultaneously extends the arms forward. The pelvis is kept horizontal and knee angles are constant during the exercise. After reaching maximal hip flexion, the participant returns to the starting position
4. Two repetitions of the traditional Nordic hamstring exercise, performed on the ground while the ankles are stabilized manually
5. Repeat steps 2 through 4
6. One maximal isometric contraction on the device, performed with neutral trunk and hips and the knee flexed to 30° (same position and instructions as when recording maximal voluntary isometric contractions for normalization purposes)

“The extender,” “the diver,” and the Nordic hamstring exercise are presented in the **ONLINE VIDEO**.

## APPENDIX B

### RELIABILITY OF THE LOAD-CELL DEVICE USED IN THIS STUDY

#### Participants

Prior to data collection in this study, the reliability of peak force measured by the device was tested on 20 male amateur athletes without prior hamstring injury (mean  $\pm$  SD age, 21.3  $\pm$  2.9 years; height, 1.78  $\pm$  0.05 m; body mass, 75.8  $\pm$  8.4 kg). All participants gave written consent to participate in this reliability study. Data collection was conducted in an accredited laboratory (Le Laboratoire Motricité Humaine Expertise, Sport, Santé, Nice, France), and study procedures were approved by the Faculty of Sport Sciences at the Université Côte d'Azur (number 2016-08) and done according to the Declaration of Helsinki.

#### Study Design

Participants performed the bilateral Nordic hamstring exercise with neutral hips (0°) and with hips flexed to 90°. A metronome was used to control the pace of each exercise at 15°/s, to minimize differences between repetitions, exercises, and participants. All exercises were performed with body weight only. Peak force was defined for the left and right legs. In each of the 2 sessions, 2 repetitions of each exercise were performed in a random order after warm-up. There were 7 to 10 days of rest between the 2 sessions. Within-session reliability results reflect the reliability of the 2 repetitions performed per session (TABLE 1). Between-session reliability is based on the average of the 2 repetitions per session (TABLE 2).

#### Calculated Reliability Measures

The intrasubject coefficient of variation (CV) was calculated as  $CV\% = \frac{SD_i}{\bar{z}_i} \times 100$ , where  $SD_i$  is the standard deviation of the 2 repetitions and  $\bar{z}_i$  is the mean of the 2 repetitions in each participant. The tables below show group averages, with 95% confidence intervals.

Typical error (TE) =  $\frac{SD(y_i - x_i)}{\sqrt{2}}$ , where SD is the standard deviation of the difference between  $y_i$  and  $x_i$  observations. Typical error is also expressed as a CV (TE %; percentage of the mean).

The intraclass correlation coefficient (model 3,1)  $r = 1 - \frac{TE^2}{SD^2}$ , where TE is the typical error and SD is the mean between-subject standard deviation.

Small-sample bias was tested, based on a factor calculated as  $1 + \frac{1-r^2}{2(n-3)}$ , where n is the sample size and r is the intraclass correlation coefficient.

Minimum detectable change at the 95% confidence level =  $TE \times 1.96 \times \sqrt{2}$ , where TE is the typical error.

**TABLE 1**  
Within-Session Reliability

	NHE0		NHE90	
	Left Leg	Right Leg	Left Leg	Right Leg
Session 1*				
Repetition 1	380.1 $\pm$ 63.2	375.7 $\pm$ 63.7	354.0 $\pm$ 46.7	345.6 $\pm$ 61.0
Repetition 2	367.7 $\pm$ 63.1	367.6 $\pm$ 68.5	358.1 $\pm$ 42.9	365.4 $\pm$ 56.5
Session 2*				
Repetition 1	388.4 $\pm$ 54.9	376.8 $\pm$ 53.6	364.5 $\pm$ 54.6	322.0 $\pm$ 52.6
Repetition 2	385.6 $\pm$ 62.5	374.2 $\pm$ 53.9	358.5 $\pm$ 46.3	330.6 $\pm$ 53.2
ICC†				
Session 1	0.93 (0.82, 0.97)	0.90 (0.77, 0.96)	0.94 (0.85, 0.97)	0.94 (0.87, 0.98)
Session 2	0.94 (0.85, 0.97)	0.87 (0.70, 0.95)	0.91 (0.78, 0.96)	0.95 (0.88, 0.98)
CV, %‡				
Session 1	4.40 (3.07, 5.74)	4.74 (3.28, 6.21)	3.17 (2.39, 3.96)	4.43 (2.11, 6.75)
Session 2	3.25 (2.10, 4.39)	4.28 (2.65, 5.92)	3.19 (1.97, 4.42)	3.36 (2.07, 4.64)

Table continues on page B3.

## APPENDIX B

	NHEO		NHE90	
	Left Leg	Right Leg	Left Leg	Right Leg
TE <sup>†</sup>				
Session 1	18.1 (13.8, 26.5)	21.7 (16.5, 31.7)	12.0 (9.1, 17.5)	14.6 (11.1, 21.3)
Session 2	15.5 (11.8, 22.6)	20.3 (15.4, 29.7)	16.2 (12.3, 23.7)	12.3 (9.2, 18.4)
TE, % of mean <sup>†</sup>				
Session 1	4.85 (3.69, 7.08)	5.84 (4.44, 8.54)	3.37 (2.56, 4.92)	4.11 (3.12, 6.00)
Session 2	4.00 (3.04, 5.85)	5.41 (4.11, 7.90)	4.49 (3.41, 6.56)	3.77 (2.83, 5.65)
MDC <sub>95</sub>				
Session 1	50.2	60.2	33.2	40.5
Session 2	42.9	56.3	45.0	34.1

Abbreviations: CV, intrasubject coefficient of variation; ICC, intraclass correlation coefficient; MDC<sub>95</sub>, minimum detectable change at the 95% confidence level; NHEO, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; TE, typical error.

\*Values are mean ± SD peak force, measured in each leg, in the NHEO and NHE90.

<sup>†</sup>Values in parentheses are 95% confidence interval.

**TABLE 2**  
**Between-Session Reliability**

	NHEO		NHE90	
	Left Leg	Right Leg	Left Leg	Right Leg
Session 1*	373.9 ± 61.9	371.6 ± 64.3	356.1 ± 44.1	351.3 ± 56.2
Session 2*	387.0 ± 57.8	375.5 ± 51.8	361.5 ± 49.3	326.3 ± 52.2
ICC <sup>†</sup>	0.91 (0.78, 0.96)	0.81 (0.59, 0.92)	0.89 (0.75, 0.96)	0.85 (0.65, 0.94)
CV, % <sup>†</sup>	4.38 (2.87, 5.90)	5.35 (3.31, 8.88)	3.86 (2.88, 4.84)	7.10 (5.46, 8.75)
TE <sup>†</sup>	19.4 (14.7, 28.23)	26.3 (20.0, 38.5)	16.2 (12.3, 23.6)	22.1 (16.7, 32.7)
TE, % of mean <sup>†</sup>	5.09 (3.87, 7.42)	7.05 (5.36, 10.30)	4.51 (3.43, 6.58)	6.53 (4.94, 9.66)
MDC <sub>95</sub>	53.6	73.0	44.8	61.3

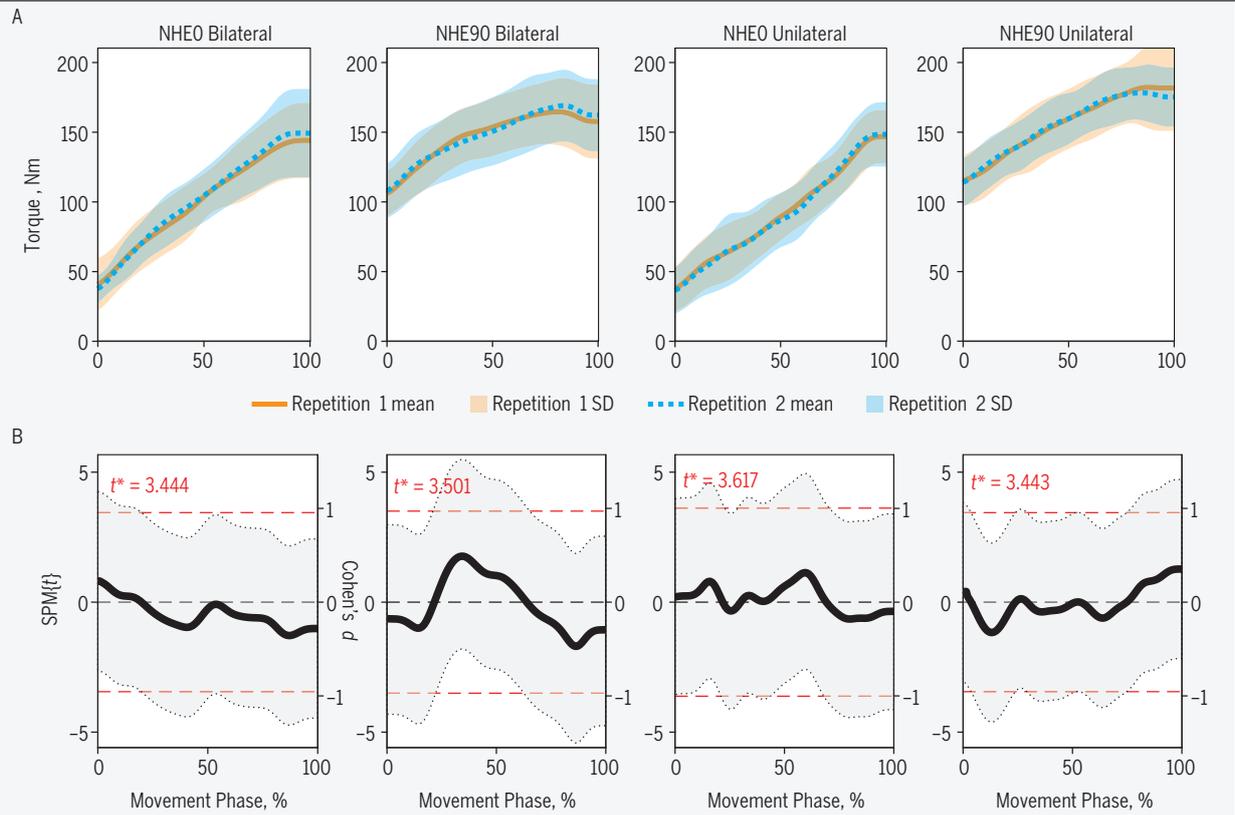
Abbreviations: CV, intrasubject coefficient of variation; ICC, intraclass correlation coefficient; MDC<sub>95</sub>, minimum detectable change at the 95% confidence level; NHEO, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; TE, typical error.

\*Values are mean ± SD peak force, measured in each leg, in the NHEO and NHE90. In session 2, right-leg force in the NHE90 from 1 participant was excluded due to data loss when exporting; therefore, in this comparison—as well as when the NHE90 right-leg force was compared between sessions—we had 19 instead of 20 observations.

<sup>†</sup>Values in parentheses are 95% confidence interval.

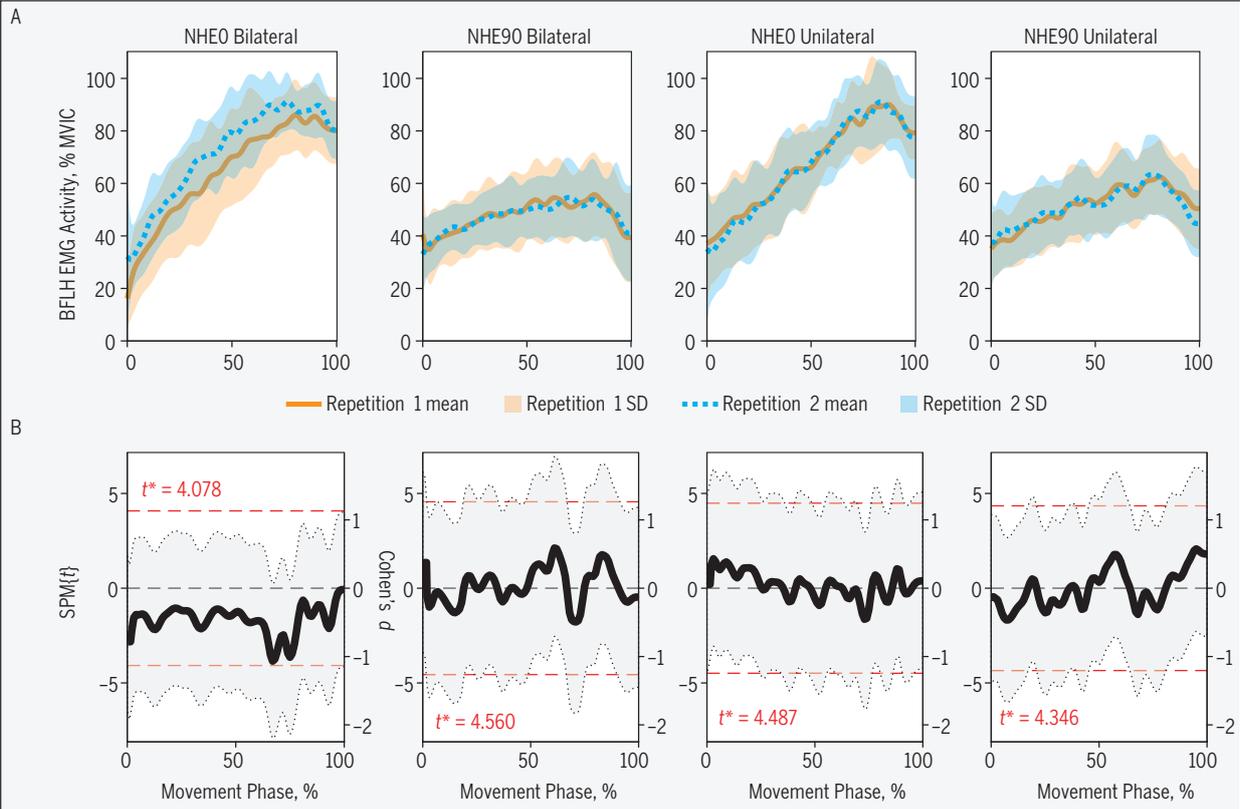
# [ RESEARCH REPORT ]

## APPENDIX C



**FIGURE 1.** Torque differences between the first and second repetitions. (A) Mean and SD values for knee flexion torque for each exercise. (B) Differences between repetitions for each exercise. Thick black lines represent SPM{t} statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (*t\**) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. No statistically significant differences were found between repetitions. Abbreviations: NHEO, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

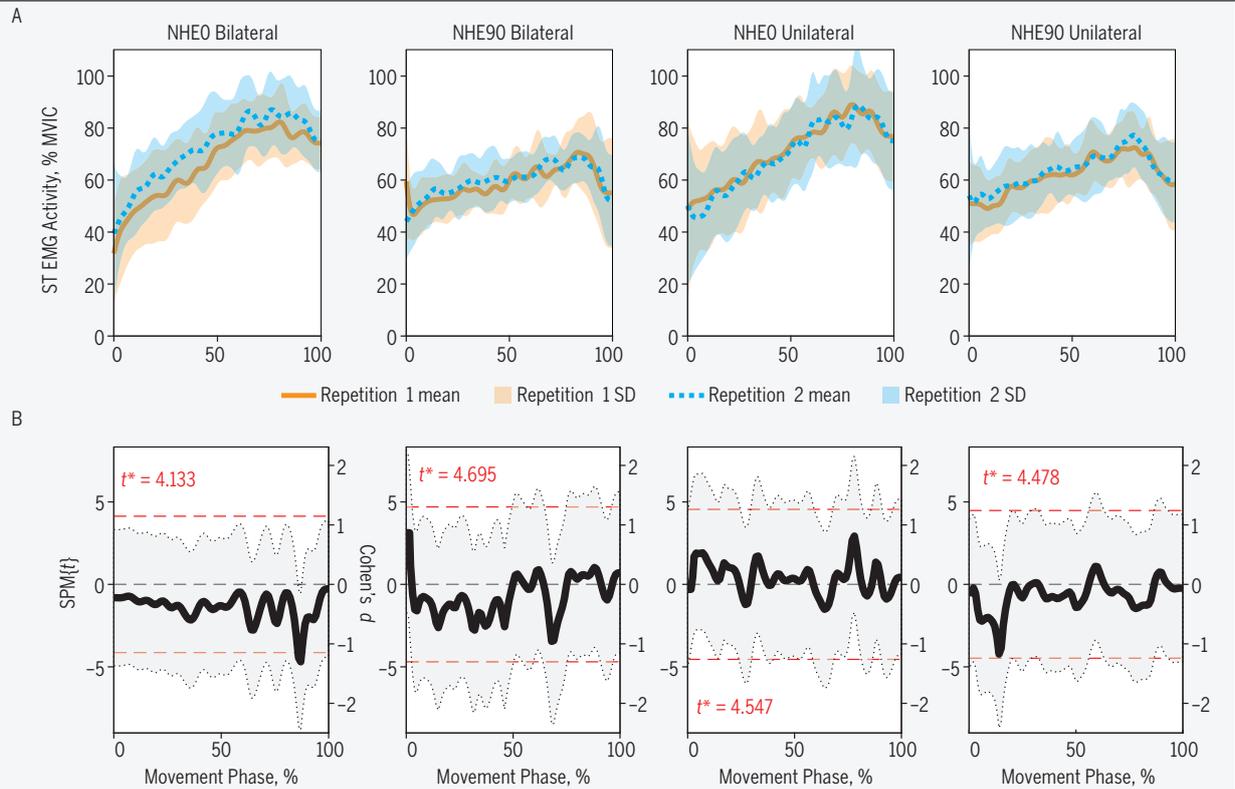
APPENDIX C



**FIGURE 2.** Differences for the BFLH between the first and second repetitions. (A) Mean and SD values for BFLH EMG activity for each exercise when normalized to percent MVIC. (B) Differences between repetitions for each exercise. Thick black lines represent SPM(*t*) statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (*t*<sup>\*</sup>) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. No statistically significant differences were found between repetitions. Abbreviations: BFLH, biceps femoris long head; EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHEO, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

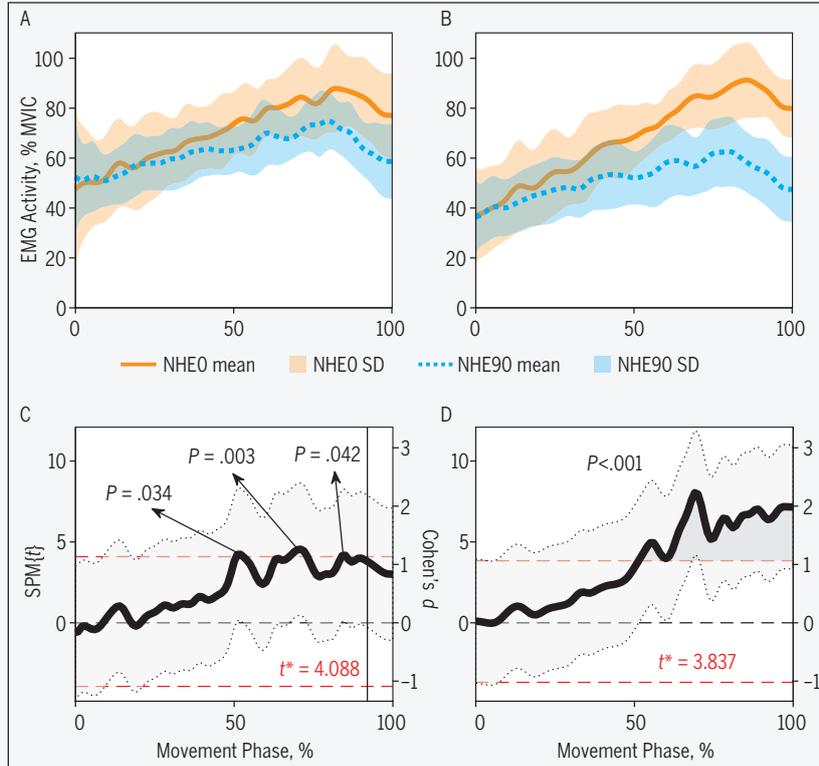
# [ RESEARCH REPORT ]

## APPENDIX C



**FIGURE 3.** Differences for the ST between the first and second repetitions. (A) Mean and SD values for ST EMG activity for each exercise when normalized to percent MVIC. (B) Differences between repetitions for each exercise. Thick black lines represent SPM{t} statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. The only statistically significant difference was found in the bilateral NHE0, between 85% and 86% of the movement phase ( $P = .038$ ). Abbreviations: EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping; ST, semitendinosus.

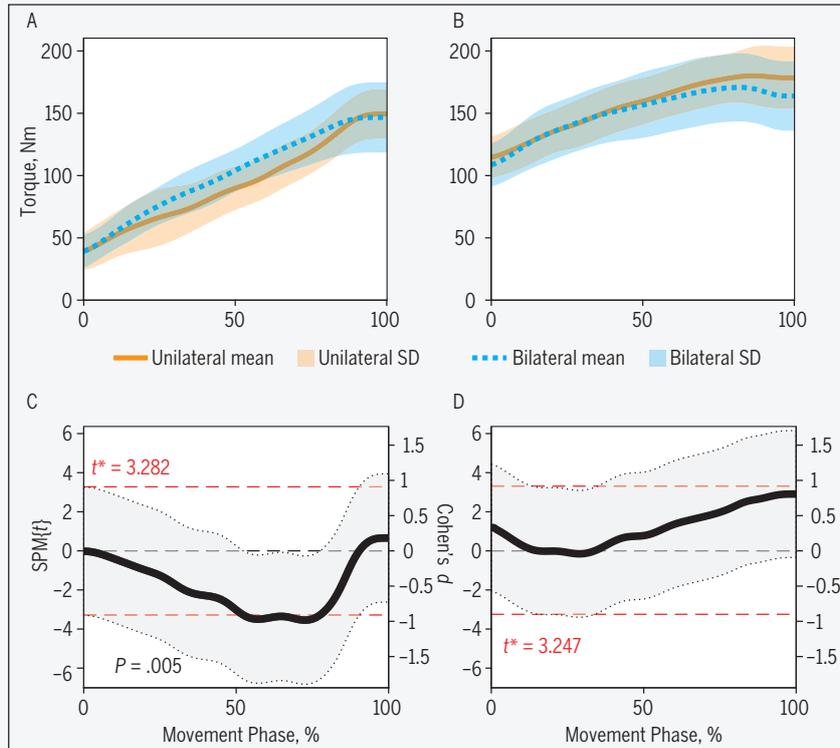
APPENDIX C



**FIGURE 4.** Differences between the unilateral NHE0 and NHE90 for each muscle. Mean and SD values for (A) semitendinosus and (B) biceps femoris long head EMG activity for each exercise when normalized to percent MVIC. Differences between (C) the unilateral NHE0 and (D) the unilateral NHE90 for each muscle. Thick black lines represent SPM{t} statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (*t*\*) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

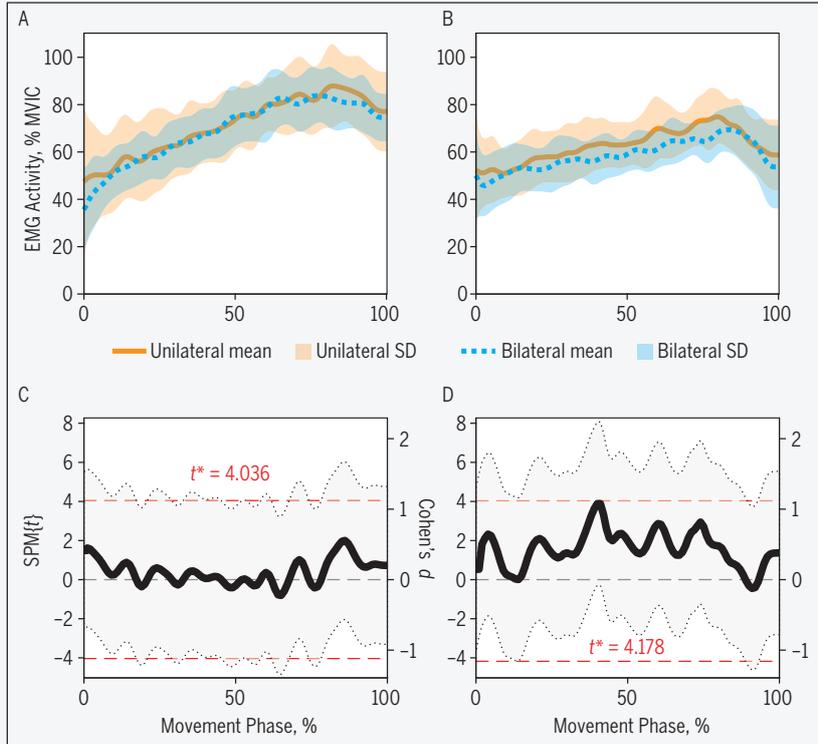
# [ RESEARCH REPORT ]

## APPENDIX C



**FIGURE 5.** Differences between unilateral and bilateral torque. Mean and SD values for knee flexion torque in (A) the bilateral and unilateral NHE0 and (B) the bilateral and unilateral NHE90. Differences between (C) unilateral and (D) bilateral exercises. Thick black lines represent SPM{t} statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

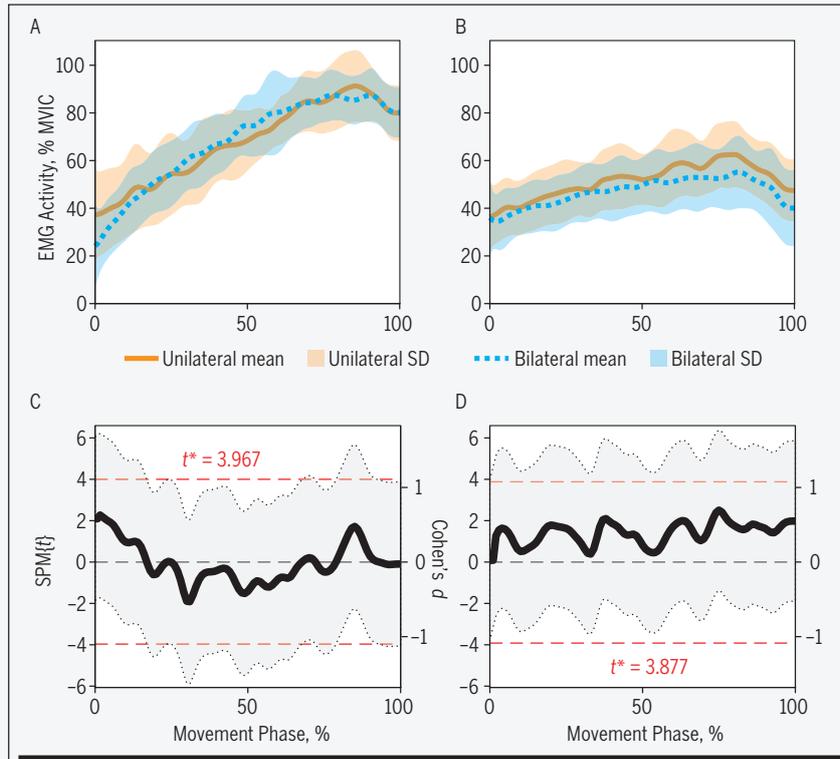
APPENDIX C



**FIGURE 6.** Differences in semitendinosus EMG activity between unilateral and bilateral modes of each exercise. Mean and SD values for semitendinosus EMG activity, when normalized to percent MVIC, in the (A) NHE0 and (B) NHE90. Differences between the unilateral and bilateral (C) NHE0 and (D) NHE90. Thick black lines represent SPM{t} statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds (*t*<sup>\*</sup>) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

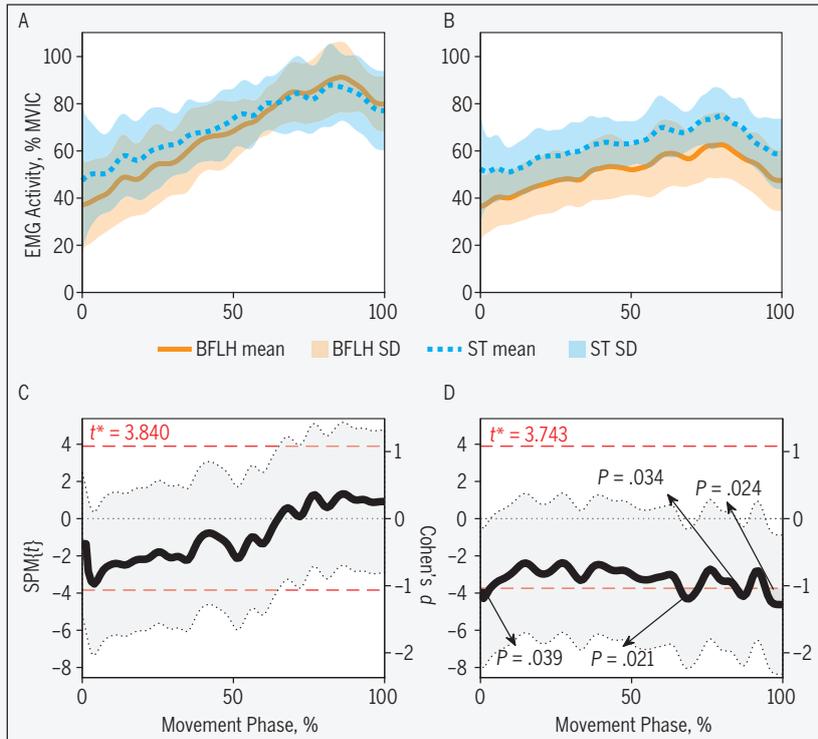
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## APPENDIX C



**FIGURE 7.** Differences in biceps femoris long head EMG activity between unilateral and bilateral modes of each exercise. Mean and SD values for biceps femoris long head EMG activity, when normalized to percent MVIC, in the (A) NHE0 and (B) NHE90. Differences between the unilateral and bilateral (C) NHE0 and (D) NHE90. Thick black lines represent SPM{t} statistics and Cohen's  $d$  effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values ( $P$ ) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping.

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**FIGURE 8.** Intermuscular differences in the unilateral exercises. Mean and SD values for BFLH and ST EMG activity when normalized to percent MVIC in the (A) NHE0 and (B) NHE90. Differences between muscles are shown in panels C and D, respectively. Thick black lines represent SPM(t) statistics and Cohen's *d* effect sizes, and the latter are accompanied by 95% confidence intervals. Critical thresholds ( $t^*$ ) calculated for significance level ( $\alpha = .05$ ) are shown with dashed red lines. Probability values (*P*) are calculated for each supra-threshold cluster depicting statistically significant differences. Abbreviations: BFLH, biceps femoris long head; EMG, electromyography; MVIC, maximal voluntary isometric contraction; NHE0, Nordic hamstring exercise with neutral hips; NHE90, Nordic hamstring exercise with 90°-flexed hips; SPM, statistical parametric mapping; ST, semitendinosus.



## IV

### **INDIVIDUAL REGION- AND MUSCLE-SPECIFIC HAMSTRING ACTIVITY AT DIFFERENT RUNNING SPEEDS**

by

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## Individual Region- and Muscle-specific Hamstring Activity at Different Running Speeds

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# **Individual Region- and Muscle-specific Hamstring Activity at Different Running Speeds**

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## Abstract

**Introduction:** Hamstring strain injuries typically occur in the proximal biceps femoris long head (BF<sub>lh</sub>) at high running speeds. Strain magnitude seems to be the primary determinant of strain injury, and may be regulated by muscle activation. In running, BF<sub>lh</sub> strain is largest in the proximal region, especially at high speeds. However, region-specific activity has not been examined. This study examined the proximal-distal and intermuscular activity of BF<sub>lh</sub> and semitendinosus (ST) as a function of increasing running speed. **Methods:** Thirteen participants ran at steady speeds of 4.1 (slow), 5.4 (moderate), and 6.8 m·s<sup>-1</sup> (fast) on a treadmill. Region- and muscle-specific electromyography (EMG) activity were recorded at each speed using high-density EMG, and were normalised to maximal voluntary isometric activity (MVIC). Muscle-tendon unit (MTU) lengths were calculated from kinematic recordings. Speed-effects, regional and intermuscular differences were tested with Statistical Parametric Mapping. **Results:** With increasing running speed, EMG activity increased in all regions of both muscles to a similar extent in the clinically relevant late swing phase. Increases in MTU lengths in late swing as a function of running speed were comparatively small. In fast running, EMG activity was highest in late swing in all regions, and reached 115 ±20% (proximal region, mean ±95% confidence limit), 106 ±11% (middle), and 124 ±16% (distal) relative to MVIC in BF<sub>lh</sub>. Regional and intermuscular EMG patterns were highly individual, but each individual maintained similar proximal-distal and intermuscular EMG activity patterns across running speeds. **Conclusion:** Running is associated with highly individual hamstring activity patterns, but these patterns are similar across speeds. It may thus be crucial to implement running at submaximal speeds early after hamstring injury for restoration of normal neuromuscular function.

**Keywords:** electromyography; biceps femoris; semitendinosus; muscle mechanics; injury mechanism; locomotion; proximal-distal differences

## 1 Introduction

Hamstring strain is a very common (1) and highly recurrent (1, 2) injury in running-based sports. This type of injury results in substantially decreased player availability, which negatively affects team performance (3), and leads to significant financial loss for teams (4). It seems that injury incidence has not decreased over the past few decades (1), but further understanding of injury mechanisms would likely help to reduce hamstring injury risk.

Over 80% of running-based hamstring injuries occur in the biceps femoris long head (BFlh), and only a few injuries are located in other bi-articular hamstring muscles (semitendinosus, ST, and semimembranosus, SM) (5). Anatomical and functional differences between hamstring muscles may partly explain the imbalanced intermuscular distribution of hamstring injuries. For example, the pennate architecture of BFlh suggests that it is suited to high force production rather than the rapid length changes that likely occur in high-speed running, contrary to the parallel-fibred ST (6), and these muscles also present different moment arm lengths. To reveal functional differences between muscles, muscle-tendon behaviour has been widely examined in high-speed running. Most of these studies show higher strain in BFlh than ST and SM (7–9), and high forces (10–13) acting along the lengthening BFlh muscle-tendon unit (MTU) in the late swing phase (10, 12). Although these results may explain why BFlh is particularly susceptible to injury, intermuscular coordination also seems to be important. Although bi-articular hamstrings seem to act as synergist muscles in running (14), different muscles and regions show distinct innervation patterns, which also show large inter-individual variability (6), potentially allowing for individual muscle- and region-specific activity patterns. As an example for the practical importance of intermuscular coordination between hamstring heads, ST, which shares a common

proximal tendon with BFlh (6), has been suggested to protect BFlh from injury (15). Both ST and BFlh electromyography (EMG) activity increase with increasing running speed, but differences in EMG activity between these muscles are unclear in the clinically relevant phases of the running stride, i.e. late swing and early stance (11, 16–18). Based on animal models, highly activated muscles are more protected against strain injury compared to less activated muscles (19). Thus, strain and muscle activity may be important determinants of hamstring injury risk.

Recent studies have demonstrated that both strain (20) and muscle activity (21) can vary substantially within hamstring muscles. Anatomical variations along the BFlh are large (22), leading to higher strain in the proximal compared to other muscle regions in running, especially at high running speeds (20). Correspondingly, most BFlh injuries occur near the proximal muscle-tendon junction (23). Proximal-distal variations in metabolic (24) and EMG activity (21, 25) in BFlh and ST are also considerable in several hamstring exercises. This suggests that proximal-distal variations should always be considered when examining hamstring function. Previous EMG studies only recorded from a small muscle region when examining the interplay between BFlh and ST in running, ignoring possible proximal-distal differences (11, 16–18). The existence of proximal-distal differences could affect the interpretation of hamstring activity based on a small region of the muscle (21).

Hamstring EMG studies generally report group results and, as mentioned above, show no clear differences between BFlh and ST activity in running. The inability to detect differences between muscles at the group level may arise from individual variations in the activity patterns of hamstring muscles (26). Large inter-individual differences in the relative activity of hamstring

muscles have been found recently during isometric contractions, and these differences seem to affect muscle performance (27). It is currently unknown whether individual variations in proximal-distal and intermuscular hamstring EMG patterns are evident in running.

In this study, the primary aim was to examine the impact of increasing running speed on proximal-distal and intermuscular EMG activity of the BFlh and ST muscles. An additional study aim was to examine BFlh and ST MTU length changes, allowing muscle-specific EMG activity to be interpreted in relation to muscle mechanics. We hypothesized that, with increasing speed, a general increase in hamstrings activity would occur with comparably smaller changes in MTU strains. We also hypothesized that, at the group level, BFlh activity would generally be lowest in the proximal region, as is the case in many hamstring exercises (21, 25). Moreover, we expected increasing running speed to increase regional differences within BFlh. Nonetheless, we expected individual intermuscular and proximal-distal activity patterns.

## **2 Methods**

### **2.1 Participants**

Participants from sports with high hamstring injury prevalence (football and Gaelic football) were recruited from local clubs. Exclusion criteria were previous hamstring and anterior cruciate ligament injuries, as well as recent (<6 months) lower body injuries and any current injury or illness. A total of 13 male participants (mean  $\pm$  standard deviation, age:  $25 \pm 3$  years, height:  $1.80 \pm 0.07$  m, body mass:  $79 \pm 9$  kg, preferred kicking leg: 3 left, 10 right) volunteered for this study. Participants were fully informed of the procedures and risks and gave written informed

consent before participating in the testing procedures. Study procedures were approved by the local ethics committee and were performed according to the Declaration of Helsinki.

Sample size was defined based on an *a priori* sample size calculation, with the goal of detecting expected regional differences in BFlh and ST. Since no such data are available from running, calculations were based on regional differences in the Nordic hamstring exercise (28), as this exercise requires similar hamstrings activity to that expected in running at the target running speeds (17). Cohen's *d* differences between proximal and distal regions in Nordic hamstring exercise ranged between 0.87 – 1.25 (BFlh) and 0.87 – 1.41 (ST). Therefore, we aimed to observe differences of  $d = 0.85$  with 80% power ( $\alpha = 0.05$ ).

## 2.2 Study design

The study consisted of two sessions with 4-7 days in-between. The first session started with a standardized warm-up consisting of whole-body mobilisation, five minutes jogging at self-selected speed, and running at increasingly faster speeds of  $3.3 \text{ m}\cdot\text{s}^{-1}$ ,  $4.4 \text{ m}\cdot\text{s}^{-1}$ ,  $5.6 \text{ m}\cdot\text{s}^{-1}$ , and  $6.7 \text{ m}\cdot\text{s}^{-1}$  (~15 strides per leg per speed) on a motorized treadmill (University of Jyväskylä, Finland; maximum treadmill speed:  $13.9 \text{ m}\cdot\text{s}^{-1}$ ). This was followed by a maximum running speed test on the same treadmill. Participants wore regular cushioned running shoes and a safety harness whenever running on the treadmill. The harness was fixed to an electric system that stopped the treadmill in the event of a fall. An additional emergency button was held by the participant while running. Each participant tested this safety system to ensure that they were not afraid of running at their maximum speed on the treadmill. To test maximum running speed, an investigator increased the speed of the treadmill gradually from  $1.4 \text{ m}\cdot\text{s}^{-1}$  until the participant stopped the treadmill or fell due to technical failure. Speed increments were steeper at slower speeds and

then slowed down to  $\sim 0.2 \text{ m}\cdot\text{s}^{-1}$  per second close to maximum speed. The highest speed reached without clear technical failure was estimated based on video recordings. This test was performed three times, with four-minutes rest periods in-between, and the highest speed of all three trials was defined as the maximum individual running speed. Thereafter, participants practiced maximum isometric knee flexion contractions (MVICs), which were used in the main testing session for normalisation.

In the main testing session, high-density electromyography (HD-EMG) preparation and the warm-up protocol were followed by MVICs in a custom-made knee flexion force measuring device (21). MVICs were performed lying prone with neutral trunk, neutral hips and the left knee flexed to  $30^\circ$ . The right leg was extended and hips were firmly fixed to the measurement device. We asked participants to gradually increase knee flexion contraction intensity in the left leg up to 100% MVIC over  $\sim 2$ -3 seconds, and then maintain MVIC for two seconds. Three repetitions were performed with two-minutes rest periods in-between. Reflective markers and force-sensitive resistors were then mounted on the participants to define lower body kinematics and event timings in running, respectively. Based on pilot testing, 75% of maximum running speed was as close to the maximum speed as possible while allowing  $\sim 15$  strides per leg without clear technical failure. This speed was thus chosen as the fastest testing speed, to maximise the chance of obtaining reliable EMG signals and minimise fatigue. In addition to 75% of maximum running speed, participants ran at 45% and 60% of maximum running speed to allow the effects of speed to be examined from the EMG data. Hereafter, these speeds are referred to as ‘fast’, ‘slow’, and ‘moderate’ speeds, respectively. Just before data collection in running, each target speed was practiced (slow, moderate, and fast speeds, in order) with four-minutes rest in-between. To practice, the investigator gradually increased the speed of the treadmill from

walking speed ( $\sim 1.4 \text{ m}\cdot\text{s}^{-1}$ ) up to the target speed within ten seconds, which was maintained for at least 15 strides per leg, after which the treadmill was slowed down to a complete stop. For actual data collection, the same three speeds were then repeated in random order with four-minute rest periods. Due to laboratory setup restrictions, kinematics and EMG were only recorded from the left leg for all individuals.

## **2.3 Data collection**

### **2.3.1 Proximal-distal electromyography activity**

Participants lay prone with neutral trunk, hip and knee angles for HD-EMG preparation. Sixteen-electrode HD-EMG arrays (10 mm inter-electrode distance, OT Bioelettronica, Torino, Italy) were attached over each of the BFlh and ST muscles (Figure 1). First, we defined the borders of each muscle, the position of the ST tendinous inscription (6), and the distal muscle-tendon junctions (in flexed knee position) using B-mode 2D ultrasonography (EchoBlast 128, Teledyne Inc., Vilnius, Lithuania). The skin was then shaved, abraded and cleaned with alcohol to decrease skin impedance. HD-EMG arrays were attached along each muscle so that they were as far away from the muscle borders as possible to minimise cross-talk from neighbouring muscles. To standardize proximal-distal positioning for BFlh, channel 8-9 from the distal end was aligned with 50% of the length between the ischial tuberosity and the popliteal fold. For ST, the array was placed 1 cm below the tendinous inscription, which is an anatomical landmark defining two distinct regions of the muscle. Due to the long distal tendon of ST, we also ensured that the most distal electrode was still proximal to the distal muscle-tendon junction. The arrays were attached using double-sided tape. Electrode cavities were filled with 20  $\mu\text{L}$  conductive gel (Signa gel, Parker Laboratories, NJ, USA), then further secured with adhesive tape to minimise movement

artefacts. A reference electrode strap was secured around the right wrist. Arrays were connected to an A/D converter (12-bit, OT Bioelettronica), amplified (x1000), and digital signals were recorded in BioLab software (v 3.1, OT Bioelettronica). Signal quality was checked visually during submaximal knee flexions. Fifteen differential EMG channels were recorded for each muscle at 2048 Hz sampling frequency.

### **2.3.2 MVICs for normalisation**

During MVICs, an ankle strap placed above the lateral malleolus of the left leg was connected to the strain gauge of the measurement device. Force signals were amplified (x1000) and digitised (A/D converter, Cambridge Electronic Designs, Cambridge, UK) to record at 1000 Hz using Spike2 software (Cambridge Electronic Desings). A digital synchronisation signal was sent from Spike2 to BioLab to synchronise force and HD-EMG recordings.

### **2.3.3 Kinematics**

To estimate hip and knee angular displacements and calculate muscle-tendon length changes in running, lower body kinematics were recorded with a 7-camera 3-D motion analysis system (VICON Inc., Oxford, UK). Reflective markers were mounted on the pelvis and left leg according to the plug-in gait model: anterior and posterior superior iliac spines on both sides, lateral surface of the thigh, flexion-extension axis of the knee, lateral surface of the shank, lateral malleolus, and over the calcaneus and second metatarsal head. Foot markers were placed on the left shoe. Marker displacements were recorded at 250 Hz in Nexus software (v2.5, VICON Inc.). To estimate segment lengths, joint centres, and joint coordinates, a standing trial was recorded prior to recording marker displacements in running.

### **2.3.4 Contact timing**

To define foot contact timings, four force-sensitive resistors were taped to the sole of the left foot under the big toe, the head of the first and fifth metatarsal joints, and the heel, respectively. Analogue force signals were digitised and recorded at 1000 Hz in Spike2. To synchronise all signals, a digital signal was sent from Spike2 to the BioLab and Nexus software.

## **2.4 Data analysis**

### **2.4.1 High-density electromyography**

A few EMG channels were excluded from the analysis due to array malfunctions. For BFlh a total of  $11 \pm 1$  (median  $\pm$  interquartile range) channels were analysed (proximal region:  $4 \pm 1$ , middle:  $3 \pm 2$ , distal:  $4 \pm 1$ ). For ST, a total of  $11 \pm 2$  channels were analysed (proximal:  $4 \pm 2$ , middle:  $3 \pm 1$ , distal:  $4 \pm 2$ ). EMG signals were offline band-pass (20-500 Hz) and notch (50 Hz) filtered with a 4<sup>th</sup> order zero-lag Butterworth filter, and then rectified in Matlab (MathWorks Inc., Natick, MA, USA). For MVICs, average EMG activity was defined for a 1-second stable plateau around peak torque. For each EMG channel and muscle, the highest MVIC activity was chosen for normalisation.

To smooth the signals for curve analyses, a 10-Hz low-pass zero-phase Butterworth filter was applied to each channel. Similar to the approach we used previously, the proximal, middle, and distal five channels of each array were averaged to estimate the EMG activity levels in different muscle regions (21, 25). Additionally, all functioning channels were averaged along each muscle to estimate the overall activity level of each muscle. Averaging several channels was performed to minimise the effects of muscle shift under the electrodes on EMG amplitudes. Foot strike (FS)

and toe-off (TO) timings were defined based on the sum of all four force-sensitive resistors, i.e. increasing (FS) or decreasing (TO) force for 20 consecutive frames. Then, EMG curves for each muscle and region were time-normalised for each stride (from FS until the next FS) and then averaged for each running speed and individual. These curves were included in the statistical analysis.

#### **2.4.2 Muscle-tendon lengths and stride sub-phases**

Marker trajectories were smoothed with a 10 Hz low-pass Butterworth filter, and hip and knee joint angles were then calculated in Nexus software applying the plug-in gait model. Sagittal plane hip and knee angles were exported and used to calculate BFlh and ST MTU lengths relative to thigh length (29) using Matlab. MTU lengths were calculated for each stride, time-normalised, then corresponding time-points across strides were averaged for each running speed and individual. Additionally, joint angles and contact timings were used to define stride sub-phases as (1) early stance (from FS to maximum knee angle in stance), (2) late stance (from maximum knee angle in stance to TO), (3) early swing (from TO to maximum knee angle in swing), (4) mid swing (from maximum knee angle in swing to maximum hip flexion angle), and (5) late swing (from maximum hip flexion angle to ipsilateral FS) (16). The number of strides defined and analysed were  $19 \pm 3$ ,  $18 \pm 2$ , and  $17 \pm 3$  (median  $\pm$  interquartile range) at slow, moderate, and fast running speeds, respectively.

#### **2.4.3 Inter-individual differences**

To quantify inter-individual differences in EMG activity and MTU lengths, inter-individual coefficients of variation (CV%) were calculated for each muscle's EMG activity (all channels

averaged), for each region of BF<sub>lh</sub> and ST, and for MTU lengths. Additionally, to see whether inter-individual variability in intermuscular coordination changed across the stride and between speeds, coefficients of variation were calculated for BF<sub>lh</sub> activity relative to ST activity (i.e. BF<sub>lh</sub>-to-ST ratio) for each speed. All CV%s were calculated for each sub-phase of the stride.

#### **2.4.4 Statistical analysis**

To compare curves with high temporal resolution, all statistical analyses were performed using Statistical Parametric Mapping (SPM (30), v0.4, [www.spm1d.org](http://www.spm1d.org)) in Matlab. Two-way repeated measures ANOVAs were run across the time-normalised running strides. SPM{F} test statistics were calculated to test speed x regional EMG interactions (for each muscle), speed x muscle-specific EMG interactions, and speed x MTU length interactions. Family-wise type I error rate was set at 0.05. In case of an interaction at any time-point across the stride, locations of the differences were tested using paired-sample t-tests with Bonferroni correction. In all SPM analyses, the test statistic (SPM{F} or SPM{t}) was calculated first, and the critical threshold (F\* or t\*) was then defined based on Random Field Theory so that only  $\alpha\%$  of random curves were expected to exceed the critical threshold in the long run. Whenever the test statistic trajectory crossed the critical threshold (forming so-called supra-threshold clusters) the difference was deemed statistically significant. Finally, p values were calculated for each supra-threshold cluster. SPM technical details are described elsewhere (30, 31).

### 3 Results

The maximum running speed was  $9.04 \pm 0.52 \text{ m}\cdot\text{s}^{-1}$  (mean  $\pm$  standard deviation). Thus, data were collected at speeds of  $4.07 \pm 0.23$ ,  $5.42 \pm 0.31$  and  $6.78 \pm 0.39 \text{ m}\cdot\text{s}^{-1}$  (slow, moderate, and fast speeds, respectively).

When all channels along each muscle were averaged, peak BFlh EMG amplitudes were observed in late swing and were  $63 \pm 9\%$  (mean  $\pm 95\%$  confidence limit, slow running speed),  $90 \pm 11\%$  (moderate),  $115 \pm 13\%$  (fast) relative to MVIC. Peak ST EMG activity values in late swing were  $69 \pm 9\%$  (slow),  $102 \pm 28\%$  (moderate),  $121 \pm 18\%$  (fast) relative to MVIC. Group means  $\pm$  standard deviations across the stride are shown for each speed in Figure 2. A speed  $\times$  muscle EMG interaction was only seen in a short period before toe-off (25-29% stride,  $p = 0.025$ ). Post-hoc analyses showed no difference between muscles at any speed across the stride [see Figure, Supplemental Digital Content 1, Upper panels show mean and standard deviation (s.d.) of biceps femoris long head (BFlh) and semitendinosus (ST) electromyography (EMG) activity (normalised to maximal voluntary isometric contraction, MVIC) at slow, moderate and fast running speeds. Lower panels represent corresponding SPM<sub>t</sub> test statistic trajectories (thick black lines), <http://links.lww.com/MSS/B641>].

Speed  $\times$  MTU length interactions were found in late stance (23-28% stride,  $p = 0.035$ ) and in swing (33-83% and 93-100% stride,  $p < 0.001$  and  $p = 0.027$ ). In both muscles, increasing speed increased the total length changes, mainly due to a decrease in minimum MTU lengths [ $p < 0.001$ ; see Figure, Supplemental Digital Content 2, Effects of running speed on muscle-tendon unit (MTU) lengths, <http://links.lww.com/MSS/B642>]. Maximum lengths only increased from slow to moderate speed but not from moderate to fast speed (see Figure, Supplemental Digital

Content 2, <http://links.lww.com/MSS/B642>). BFlh MTU was significantly longer than ST MTU across the entire stride at all speeds ( $p < 0.001$ ) (Figure 2). Individual MTU length changes were similar across participants [see Figure, Supplemental Digital Content 3, Similar patterns between individuals S1-S13 in biceps femoris long head (BFlh) and semitendinosus (ST) muscle-tendon unit (MTU) lengths, <http://links.lww.com/MSS/B643>], resulting in inter-individual CVs as small as 1-3% across speeds in both muscles [see Figure, Supplemental Digital Content 4, Inter-individual coefficients of variations in muscle-tendon unit lengths in the early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw); <http://links.lww.com/MSS/B644>].

### **3.1. Regional EMG activity**

Group mean EMG amplitudes defined proximal-distally along each muscle are shown in Figure 1.

In BFlh, the highest EMG activity was reached in late swing in all muscle regions. After dividing each muscle into three distinct regions, region-specific peak activity levels in BFlh in slow running were  $58 \pm 9\%$  (proximal region; mean  $\pm 95\%$  confidence limit),  $63 \pm 8\%$  (middle), and  $69 \pm 11\%$  (distal) relative to MVIC. At moderate speed, the corresponding values were  $93 \pm 16\%$ ,  $86 \pm 9\%$ , and  $94 \pm 14\%$ , respectively. At fast speed, EMG peak values were  $115 \pm 20\%$  (proximal),  $106 \pm 11\%$  (middle), and  $124 \pm 16\%$  (distal) relative to MVIC.

In BFlh, a speed x region interaction was found only in early swing (41-56% stride,  $p < 0.001$ ). With increasing speed, EMG activity increased in all BFlh regions across the stride ( $p < 0.001$ ) except in late stance (proximal: 20-29% middle: 16-30%, and distal: 17-29% stride,

respectively). Differences between speeds are shown for each region in Figure 3. Post hoc analyses showed no differences between muscle regions at any running speed at any time point across the stride [see Figure, Supplemental Digital Content 5, Regional differences in the electromyography (EMG) activity of biceps femoris long head (BF<sub>lh</sub>, normalised to maximal voluntary isometric contraction, MVIC); <http://links.lww.com/MSS/B645>.].

In ST, the highest EMG activity was reached in late swing in all muscle regions, similar to BF<sub>lh</sub>. Region-specific peak activity levels in ST in slow running were 70 ±17% (proximal region; mean ±95% confidence limit), 65 ±8% (middle), and 74 ±9% (distal) relative to MVIC. The corresponding values at moderate speed were 99 ±28%, 91 ±14%, and 113 ±46%, respectively. At fast speed, EMG peak values were 136 ±32% (proximal), 118 ±15% (middle), and 122 ±15% (distal) relative to MVIC.

In ST, similar to BF<sub>lh</sub>, a speed x region interaction was found only in early swing (52-56% stride,  $p = 0.028$ ). With increasing speed, EMG activity increased in all ST regions across the entire stride in the proximal and middle regions ( $p < 0.001$ ) and in most phases in the distal region (0-23%,  $p < 0.001$ ; 25-32%,  $p = 0.010$ ; 39-80%,  $p < 0.001$ ; 89-100%,  $p = 0.001$ ). Differences between speeds are shown for each region in Figure 4. No differences between muscle regions were detected at any of the running speeds [see Figure, Supplemental Digital Content 6, Regional differences in the electromyography (EMG) activity of semitendinosus (ST, normalised to maximal voluntary isometric contraction, MVIC); <http://links.lww.com/MSS/B646>.].

### 3.2 Individual EMG patterns

Individual region-specific activity patterns were evident in both BFlh (Figure 5) and ST (Figure 6; and see Figure, Supplemental Digital Content 7 [Large inter-individual coefficients of variations in regional activity levels in the early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw); <http://links.lww.com/MSS/B647>] for inter-individual coefficients of variations for each region and speed). Similar to regional patterns, the overall activity pattern of each muscle showed distinct individual patterns [see Figure, Supplemental Digital Content 8, Individual (S1-S13) muscle-specific electromyography (EMG) activity patterns of biceps femoris long head (BFlh) and semitendinosus (ST) (normalised to maximal voluntary activity, MVIC; <http://links.lww.com/MSS/B648>); and see Figure, Supplemental Digital Content 9, (Large inter-individual coefficients of variations in muscle activity levels at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds) for inter-individual CVs in muscle-specific EMG activity]. BFlh-to-ST EMG ratio ranged across speeds and sub-phases between 28% and 71%, depicting individual intermuscular coordination strategies (Figure, Supplemental Digital Content 9, <http://links.lww.com/MSS/B649>). In general, CVs were lowest in late swing at all speeds (1.6 times higher in other stride phases on average). Of all speeds, late swing CVs were smallest at the fast running speed (CV range across regions and muscles = 21-42%).

## 4 Discussion

In this study, we examined proximal-distal and intermuscular differences in BFlh and ST muscle activity across a range of running speeds. Contrary to our primary hypothesis, at the group level, proximal-distal and intermuscular statistical differences were not detected in BFlh or ST at any

of the running speeds. However, as expected, individual differences were evident across the entire stride at all speeds. According to our secondary hypothesis, with increasing speed, large increases in hamstring HD-EMG activity were accompanied by relatively small increases in maximum MTU lengths in the late swing phase, indicating higher hamstring stiffness at higher speeds.

Similar to previous EMG studies (11, 32), we found a substantial increase in hamstring EMG activity with increasing running speed (Figures 3-4), and the highest activity levels were observed in late swing and early stance at all speeds, as also observed previously (11, 16–18, 32). In these phases, BFlh and ST EMG activity reached a higher amplitude at 75% of maximum running speed (which is considered sprinting speed in this cohort,  $> 24 \text{ km}\cdot\text{h}^{-1}$ ) compared to that in typical hamstring exercises performed at submaximal or maximal loads (21, 25). EMG values in late swing and early stance also exceeded the muscle activity levels achieved during MVICs, as observed previously (32, 33). This reinforces the notion that it is challenging to activate hamstrings during regular exercises to the same extent as during sprinting. It should be noted that exercises are generally performed at a much slower pace relative to that of sprinting, and movement speed is known to affect EMG amplitudes (34). Our results further support the idea that in the early stance and late swing phases, high BFlh and ST EMG activities are reached at relatively long muscle lengths (11). This in turn supports the notion that exercises facilitating long hamstring operating lengths and high activation may help to prepare the hamstrings for the requirements of high-speed running (35).

The typical bi-phasic activity pattern (i.e. peaks at early stance and late swing) of BFlh and ST was seen for most individuals, and hamstring injuries seem to occur in these phases (36).

However, a third burst in the early-to-mid swing phase was seen for 6 individuals in BFlh (Figure 5) and for 3 individuals in ST (Figure 6). In this phase the hamstrings are believed to stabilize the hip and knee while these joints are being flexed and extended, respectively (17). At the same time, the rate of lengthening of the hamstring MTUs is the highest in this phase of running (Figure 2). The fusiform architecture of ST suggests that this muscle is built for such fast muscle actions (6). On the contrary, BFlh is not suited to rapid length changes (6). Rapid stretch in this phase lowers the potential for stretch relaxation, increasing tensile forces acting along the muscle (37). MTU strain was relatively constant across individuals, contrary to the large inter-individual variation of BFlh activity during this lengthening phase. According to Garrett et al. (19), increasing muscle activity increases the energy absorbed by the muscle during lengthening and decreases strain injury risk. If this applies to hamstrings in sprinting, increased BFlh activity could be a protective mechanism against muscle strain injury. However, increasing muscle activity (and thus stiffness) generally results in more of the MTU stretch being transferred to the aponeuroses and tendons (38), which are often involved in hamstring strain injuries. It is likely that there is an optimal range of BFlh activity in running, and that this optimum range is influenced by individual factors such as the mechanical properties and architecture of the hamstring muscle components. This could partly explain individual variations in BFlh activity in running. Although hamstring injuries seem to occur mainly in late swing, structural damage potentially accumulating in mid swing may predispose the BFlh to strain injury in late swing, where large tensile forces act at long hamstring lengths. Future studies should further examine the link between the magnitude of BFlh activity in these phases and strain injury risk.

BFlh EMG activity is likely affected by the activity of other synergist muscles like ST. BFlh-to-ST activity ratio showed large individual variability, suggesting individual strategies for the

relative activation of these muscles in running and sprinting. Statistical differences were not seen between BF<sub>lh</sub> and ST activity. However, the lack of detectable difference seems to result from large individual variations (Figure, Supplemental Digital Content 3, <http://links.lww.com/MSS/B643>). It seems that a similar magnitude of activity between hamstring muscles may be advantageous for muscle performance in isometric fatiguing contractions (27), and may be associated with decreased hamstring injury risk (15). It is currently unclear whether this directly implies that all individuals should activate these muscles similarly in running. Individual coordination strategies in running may require individual muscle-specific hamstring strengthening. For example, some may benefit from BF<sub>lh</sub>-dominant exercises such as 45° hip extension on a roman chair (25), while others may benefit more from ST-dominant exercises such as Nordic hamstring exercise performed with flexed hips (39) to achieve different adaptations. Morphological and architectural characteristics of the hamstring muscles show substantial individual variations (40), and it should be clarified whether these variations are linked to individual coordination strategies.

Aside from intermuscular variations, proximal-distal activity patterns were also highly specific to individuals, which may be the reason why regional differences were not statistically different when all individuals were pooled together for statistical analysis. Regional activity patterns may be associated with regional adaptations, although we are not aware of studies examining this phenomenon in hamstrings. Within the BF<sub>lh</sub>, it seems to be challenging to selectively activate the proximal region (the region where most injuries occur) via hamstring exercises (21, 25). Nonetheless, in the current study, one participant showed the highest activity in the proximal BF<sub>lh</sub> relative to other muscle regions in running (S11 in Figure 5) and some others showed similar proximal EMG activity to other regions (S2, S4, S6 in Figure 5). It may be that for some

individuals, potential regional adaptations gained through hamstring exercises may not be optimal to prepare the proximal region for the needs of high-speed running. Defining hamstring activity patterns for each player in a team could assist exercise selection, however the cost-benefit balance of this approach is currently unclear. Examining the link between individual patterns in running vs in hamstring exercises may help to improve exercise selection for hamstring injury risk reduction.

Since hamstring injuries typically occur at high running speeds, speed-dependent changes in intermuscular activity may be important to note. One study (17) showed that BFlh-to-ST activity ratio increased in the late swing phase with increasing running speed. On the contrary, we did not detect speed effects on intermuscular EMG activity patterns. Individual muscle-specific (and region-specific) activity patterns were similar across a range of running speeds. This supports the idea that early implementation of running, even at slow speeds, might be crucial to restore the neuromuscular function of the hamstring muscles after a hamstring injury.

Similar to previous studies (11, 12), we observed large increases in hamstring activity with increasing running speed without substantial changes in peak MTU lengths in late swing, implying higher MTU stiffness at higher speeds. Although the BFlh and ST MTUs undergo a stretch-shortening cycle throughout the running stride, muscle-tendon decoupling likely occurs to some extent. Based on simulations, increasing muscle activity from early to late swing slows the stretch of the BFlh muscle component, and the tendons take up part of the MTU lengthening (10). Individual variations in muscle activity in swing imply that muscle-tendon decoupling may also be individual-specific. Although the compliance of the series elastic elements affects the length change of the muscle component, even highly compliant tendons seem to require the

muscle component to bear most of the MTU lengthening within the BFlh (10). Although it has recently been suggested that the fascicles of the BFlh are quasi-isometric in late swing (41), this has not been supported with experimental data. We recently attempted to assess BFlh fascicle behaviour and relate that to muscle-tendon behaviour in running *in vivo*, using a combination of B-mode 2-D ultrasonography and kinematic analysis. However, we were unable to quantify fascicle length changes due to out-of-plane fascicle rotations (see Video, Supplemental Digital Content 10, example ultrasound recordings in locomotion, <http://links.lww.com/MSS/B650>). Fascicle length changes appear to be significant throughout the stride but accurately quantifying these changes remains challenging. According to magnetic resonance imaging-based modelling, a further challenge is that BFlh strain seems to be heterogeneous within the muscle in running, with the highest strain near the proximal muscle-tendon junction, where most injuries occur (20). Heterogeneous proximal-distal strain distribution may be associated with heterogeneous proximal-distal EMG activity patterns observed in the current study. Further technical improvements are needed to fully understand the association between BFlh muscle activity and muscle-tendon mechanics in running.

Although this study focused solely on BFlh and ST, it is likely that large inter-individual variations are also affected by individual contributions of other muscles. For example, modelling data suggest that several muscles in the lumbo-pelvic region affect BFlh behaviour in running (12). Further experimental data suggest that an increased activation of the gluteus maximus in running can decrease hamstring strain injury risk (42), which seems to be more pronounced in fatigued conditions (43). Our results suggest that these factors should be examined at an individual level in future studies to understand the interplay between muscles in running.

It is important to note some limitations of this study. An inherent limitation of surface EMG methods is the contamination of the signals by neighbouring muscle activity (i.e. cross-talk). To limit this effect, we attached the electrode arrays as far from the borders of the target muscle as possible (defined with ultrasonography), and applied short inter-electrode distances (10 mm) and electrodes with a small pick-up area. Since the subcutaneous layer over the hamstrings is generally thicker in females than in males (increasing the potential for cross-talk) we measured male athletes only. The EMG results might have been affected by the EMG normalisation method, although changes with increasing speed are not affected by this factor. It should also be noted that muscle-tendon length calculations are slightly affected by the model used and the cut-off frequency applied, although MTU behaviour was not the main focus of this study. Furthermore, we measured at submaximal (and not at maximal) running speeds, due to the methodological constraints previously mentioned. However, the fastest running speed used in this study is already considered sprinting in football codes. It remains unclear though whether higher sprinting speeds would result in different hamstring activity patterns from what we observed. Notably, running trials were performed on a motorized treadmill to ensure that the target speeds were reached and maintained across several strides. However, differences between hamstring activation and mechanics when running on a treadmill vs. overground are yet to be clarified. We also emphasize that we recruited amateur athletes, so the results may not be applicable to other populations.

To summarize the main findings of this study, hamstring intermuscular and proximal-distal activity patterns seem to be highly variable between individuals, but are qualitatively consistent within individuals across a range of running speeds. This means that slow-speed running results in a similar shaped activity pattern to high-speed running, and thus should be applied early after

a hamstring injury to facilitate neuromuscular recovery and prepare for high-speed running. Future studies should examine how fatigue affects hamstring activity patterns, and whether individual differences in hamstring coordination are linked to performance and hamstring injury susceptibility in running.

## **5 Conflict of interest**

The authors have no professional relationships with companies or manufacturers who will benefit from the results of the present study. The results of the present study do not constitute endorsement by ACSM. The results of this unfunded study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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## Figure Captions

Figure 1. Positioning of the high-density electromyography (EMG) arrays, and group mean (N=13) proximal-distal electromyography activity levels normalised to maximum voluntary isometric (MVIC) amplitudes. Fifteen differential channels were recorded along each muscle using high-density EMG arrays (10 mm inter-electrode distance) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).

Figure 2. Group mean and standard deviation (s.d.) of joint angular displacements, biceps femoris long head (BF<sub>lh</sub>) and semitendinosus (ST) muscle-tendon unit (MTU) lengths, and electromyography (EMG) activity (all EMG channels averaged per muscle; normalised to a maximum voluntary isometric contraction, %MVIC) at slow, moderate, and fast running speeds ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ,  $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ , and  $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ , respectively). Vertical dashed lines define the sub-phases of the running stride (0-100%) as: early stance (ESt, foot strike at 0% to maximum knee angle in stance), late stance (LSt, maximum knee angle in stance to toe-off), early swing (ESw, toe-off to maximum knee angle in swing), mid swing (MSw, maximum knee angle in swing to maximum hip angle in swing), and late swing (LWs, maximum hip angle in swing to foot strike at 100%).

Figure 3. Effects of running speed on the electromyography (EMG) activity of biceps femoris long head (BF<sub>lh</sub>) muscle regions (normalised to maximal voluntary isometric contraction, MVIC). Panels (A), (B), and (C) represent group mean and standard deviation (s.d.) across the

stride cycle at different speeds for each region. Panels (D) to(L) show the statistical parametric maps. (D), (E), and- (F) show differences between moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ) and slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ) running speeds, (G), (H), and(I) show differences between fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) and slow running speeds, (J), (K), and (L) show differences between fast and moderate running speeds, in the proximal, middle, and distal regions, respectively. Thick black lines are the SPM{t} test statistics representing the magnitude of the differences relative to the s.d. and sample size ( $N=13$ ). Critical thresholds ( $t^*$ ) were calculated for each comparison after Bonferroni correction (dashed red horizontal lines; family-wise  $\alpha = 0.05$ ). Probability (p) values are shown for each supra-threshold cluster depicting statistically significant differences. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).

Figure 4. Effects of running speed on the electromyography (EMG) activity of semitendinosus (ST) muscle regions (normalised to maximal voluntary isometric contraction, MVIC). Panels (A), (B), and (C) represent group mean and standard deviation (s.d.) across the stride cycle at different speeds for each region. Panels (D) to(L) show the statistical parametric maps. (D), (E), and- (F) show differences between moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ) and slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ) running speeds, (G), (H), and(I) show differences between fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) and slow running speeds, (J), (K), and (L) show differences between fast and moderate running speeds, in the proximal, middle, and distal regions, respectively. Thick black lines are the SPM{t} test statistics representing the magnitude of the differences relative to the s.d. and sample size ( $N=13$ ). Critical thresholds ( $t^*$ ) were calculated for each comparison after Bonferroni correction (dashed red horizontal lines; family-wise  $\alpha = 0.05$ ). Probability (p) values are shown for each supra-threshold

cluster depicting statistically significant differences. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).

Figure 5. Individual (rows S1-S13) region-specific biceps femoris long head (BF<sub>lh</sub>) electromyography (EMG) activity patterns normalised to maximal voluntary activity (MVIC) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds. Note that scaling of the y axis is optimised for each individual so that regional differences are easier to identify within each individual. Foot strike is at 0% and 100% of the stride.

Figure 6. Individual (rows S1-S13) region-specific semitendinosus (ST) electromyography (EMG) activity patterns when normalised to maximal voluntary activity (MVIC) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds. Note that scaling of the y axis is optimised for each individual so that regional differences are easier to identify within each individual. Foot strike is at 0% and 100% of the stride.

### **List of Supplemental Digital Content**

Figure, Supplemental Digital Content 1.pdf

Figure, Supplemental Digital Content 2.pdf

Figure, Supplemental Digital Content 3.pdf

Figure, Supplemental Digital Content 4.pdf

Figure, Supplemental Digital Content 5.pdf

Figure, Supplemental Digital Content 6.pdf

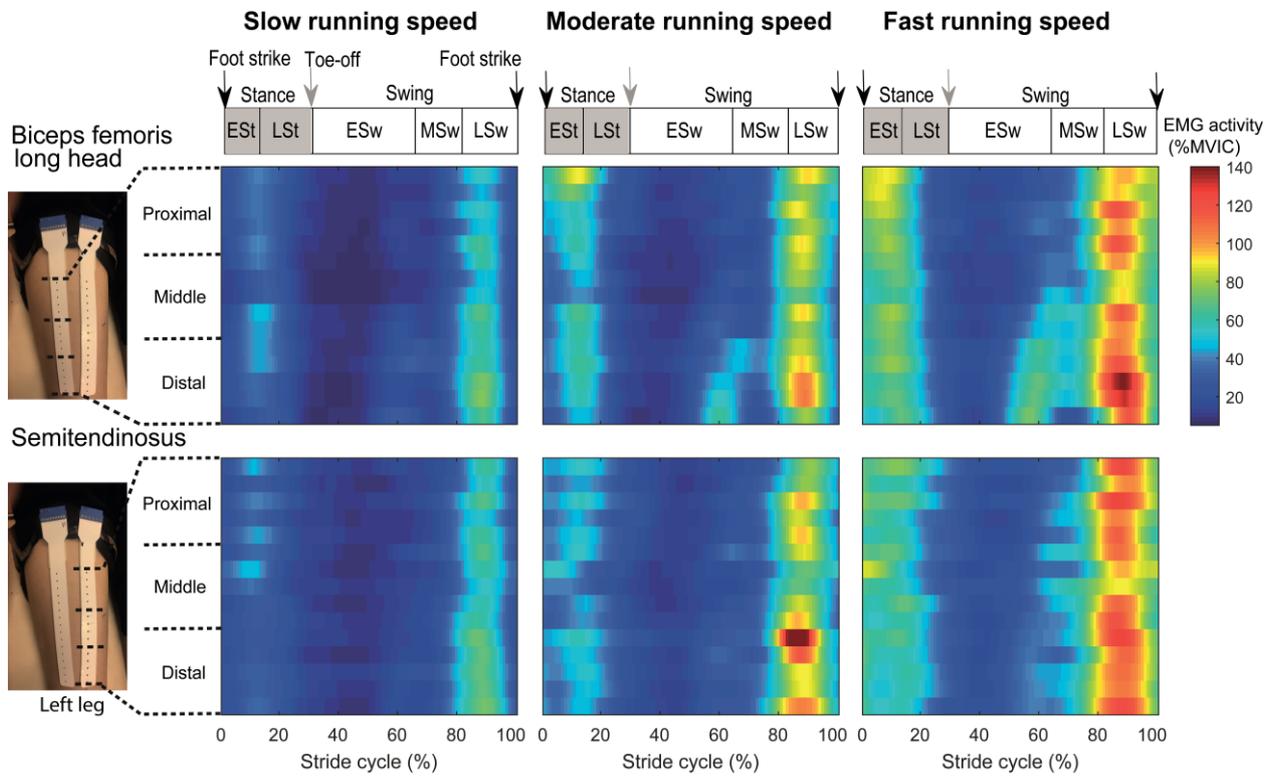
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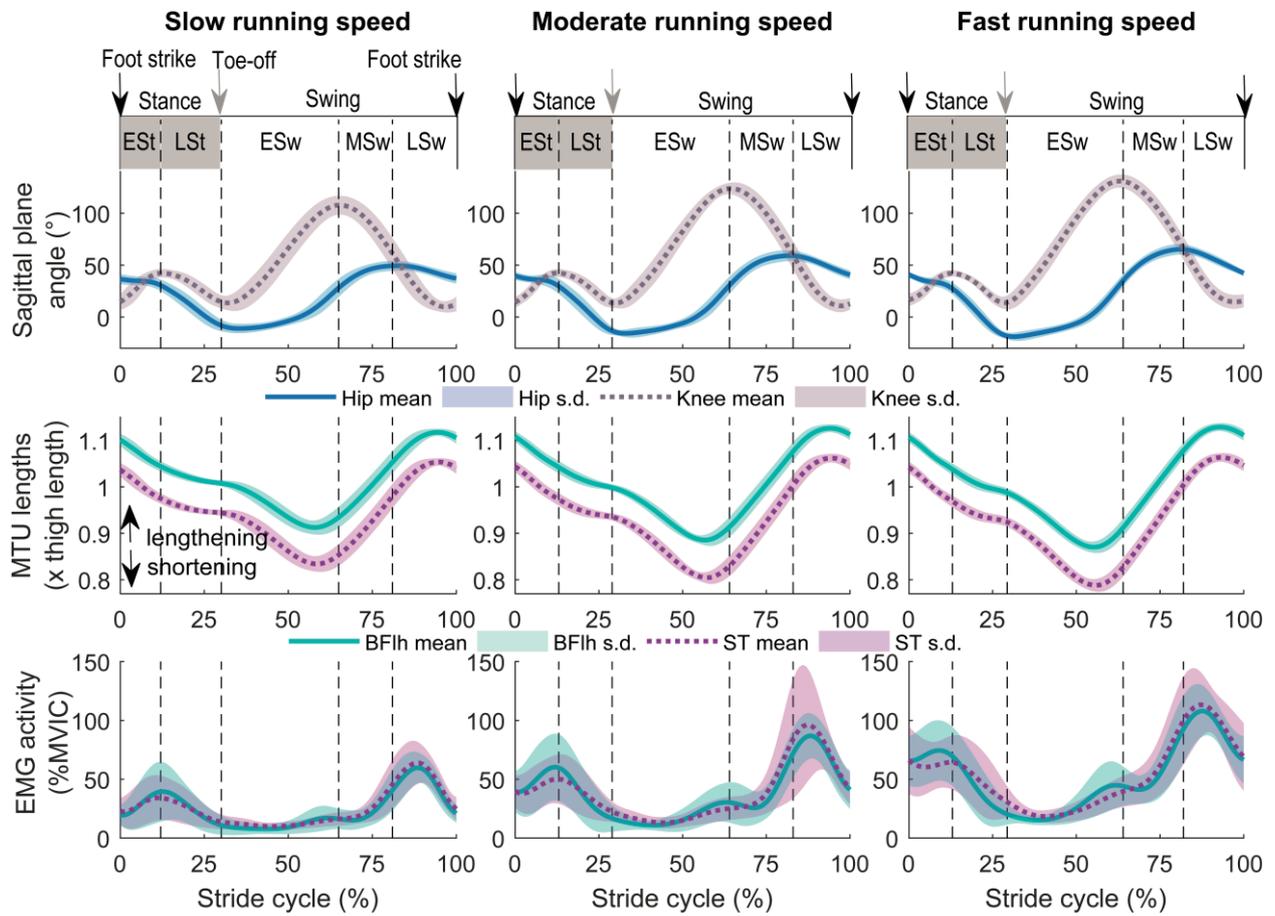
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Video, Supplemental Digital Content 10.mp4

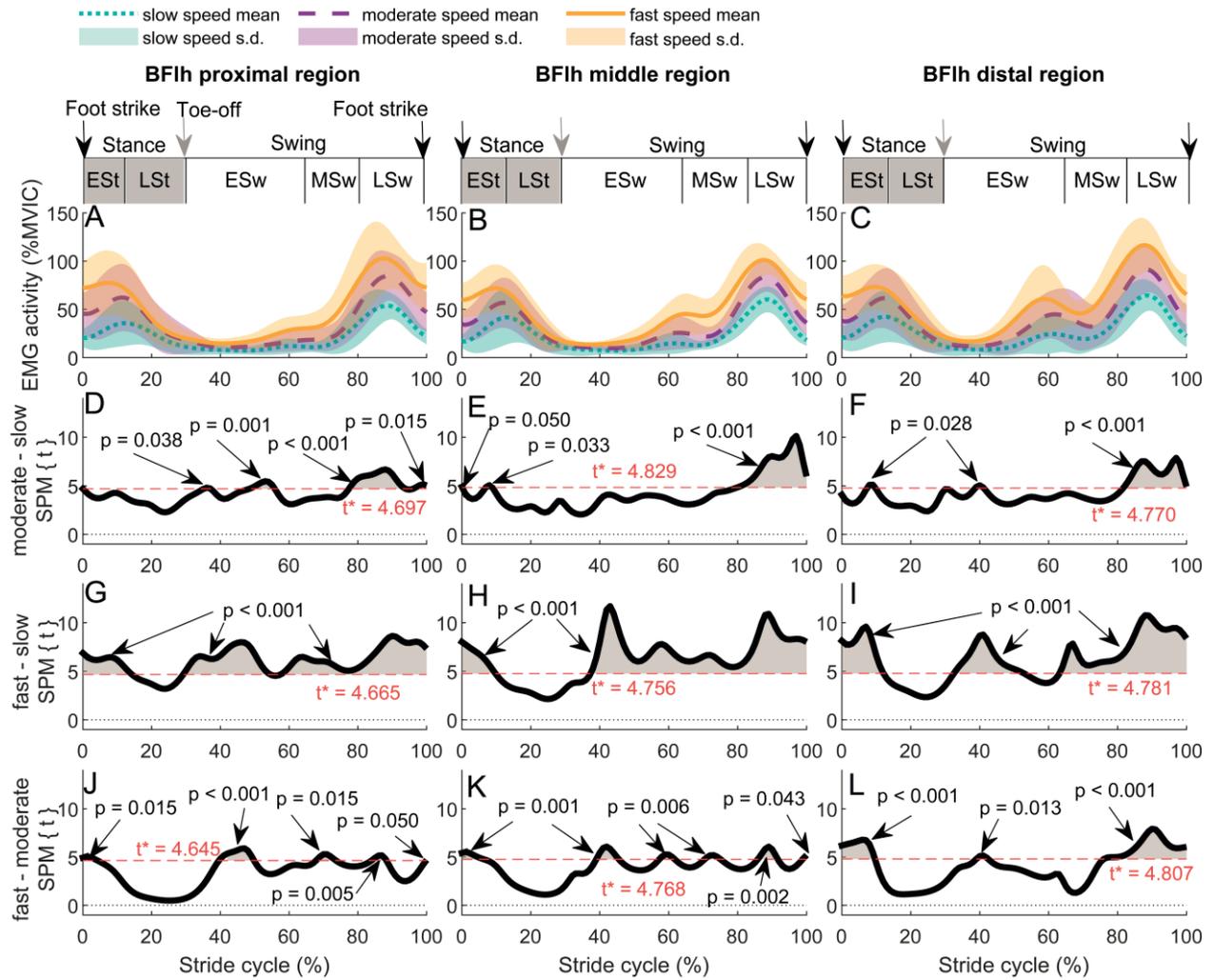
**Figure 1**



**Figure 2**



**Figure 3**



**Figure 4**

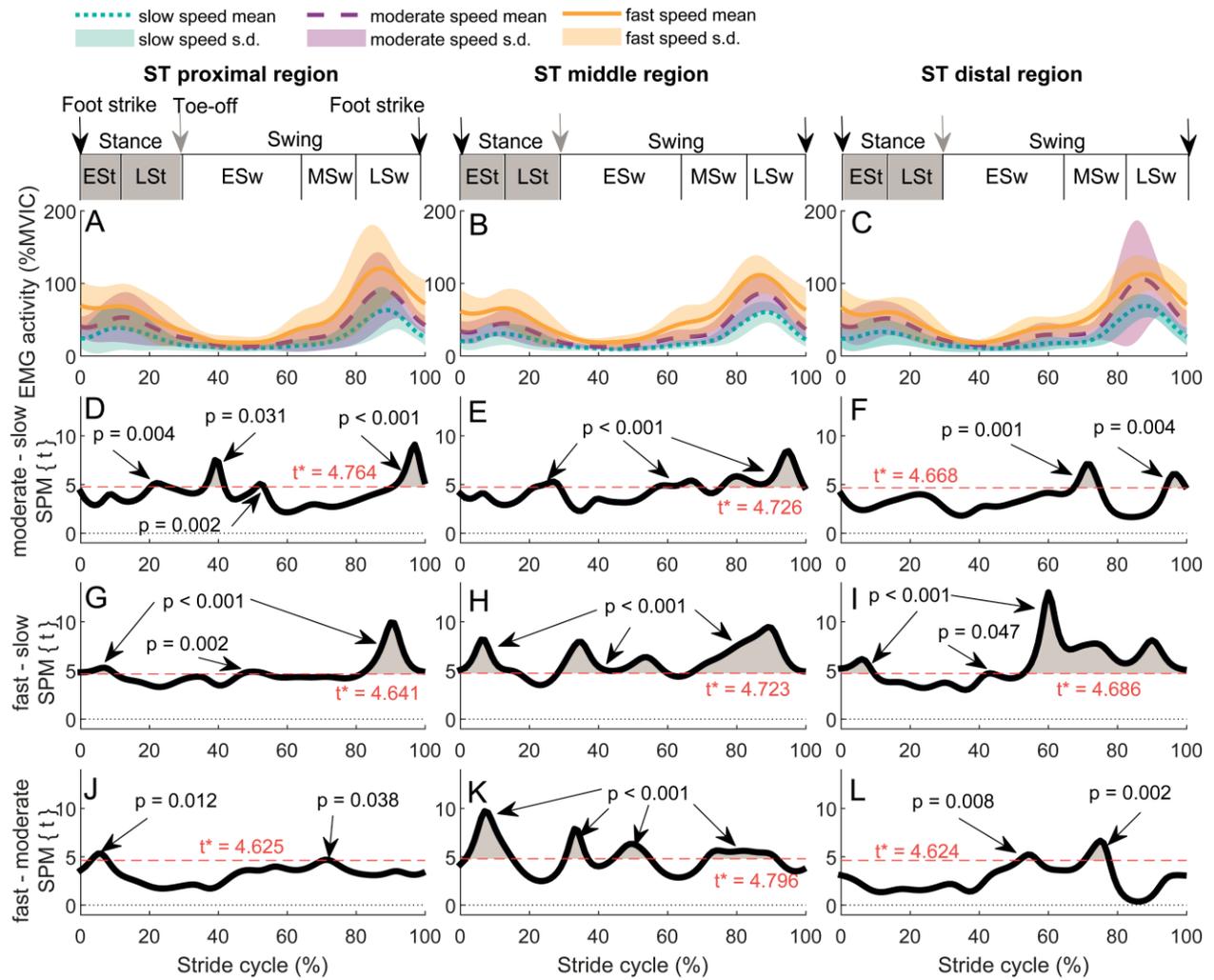


Figure 5

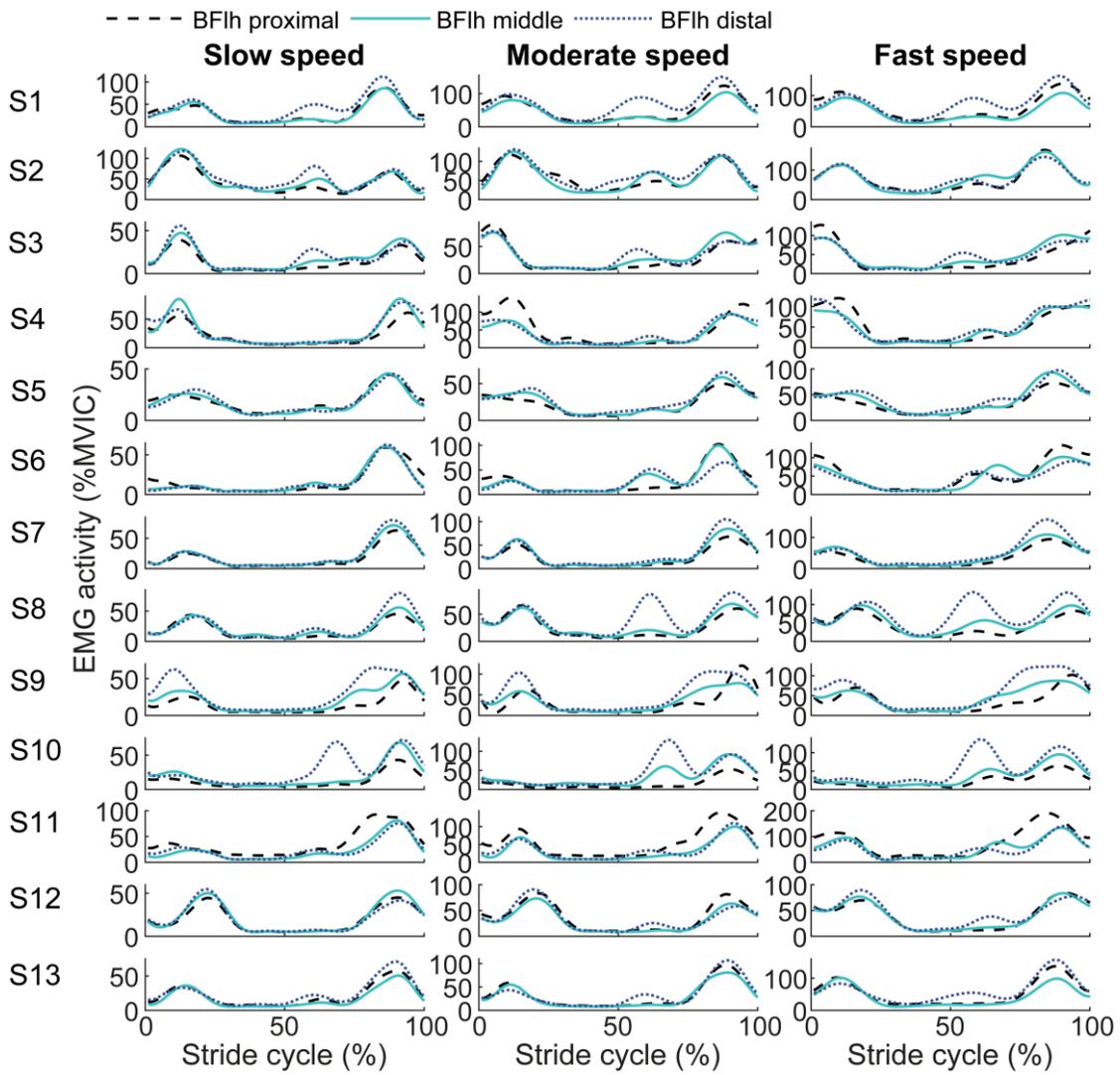
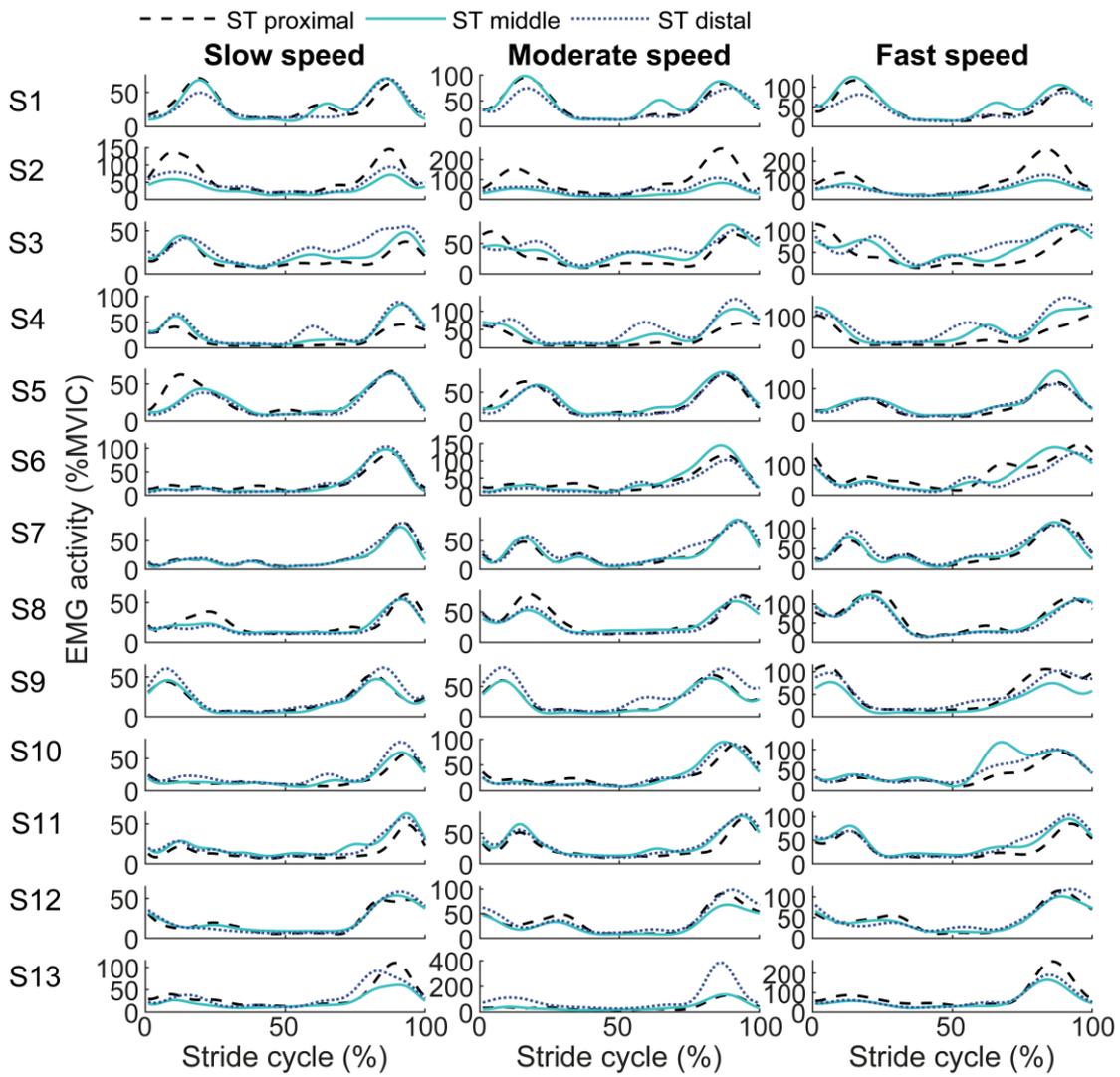
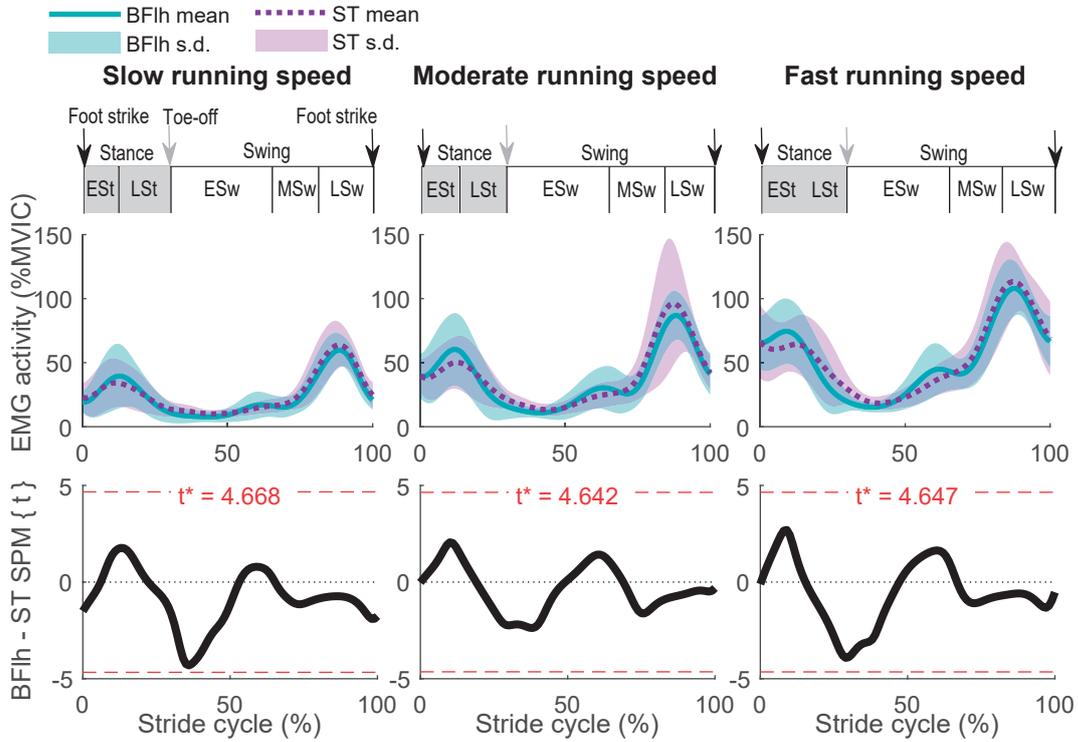


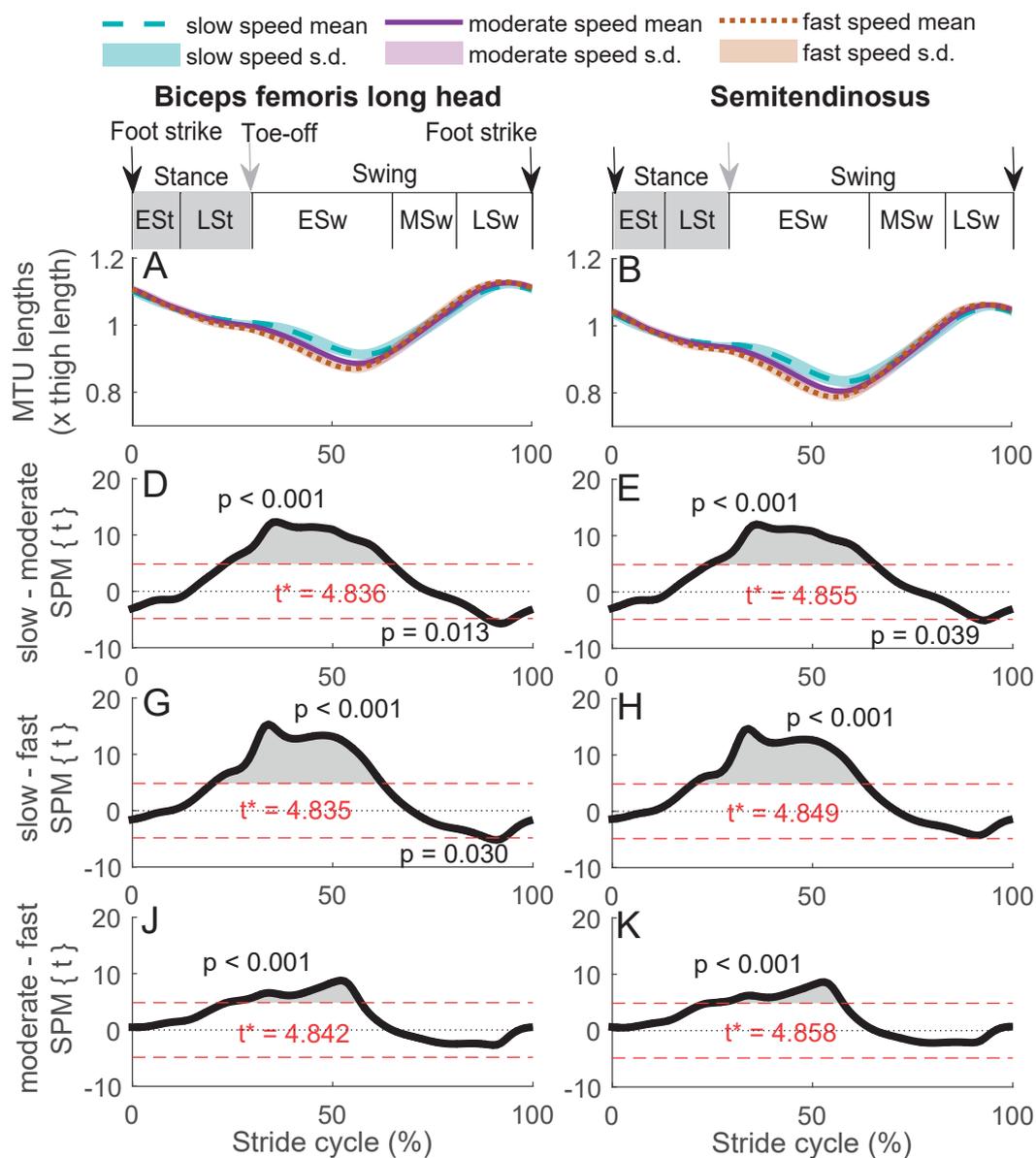
Figure 6



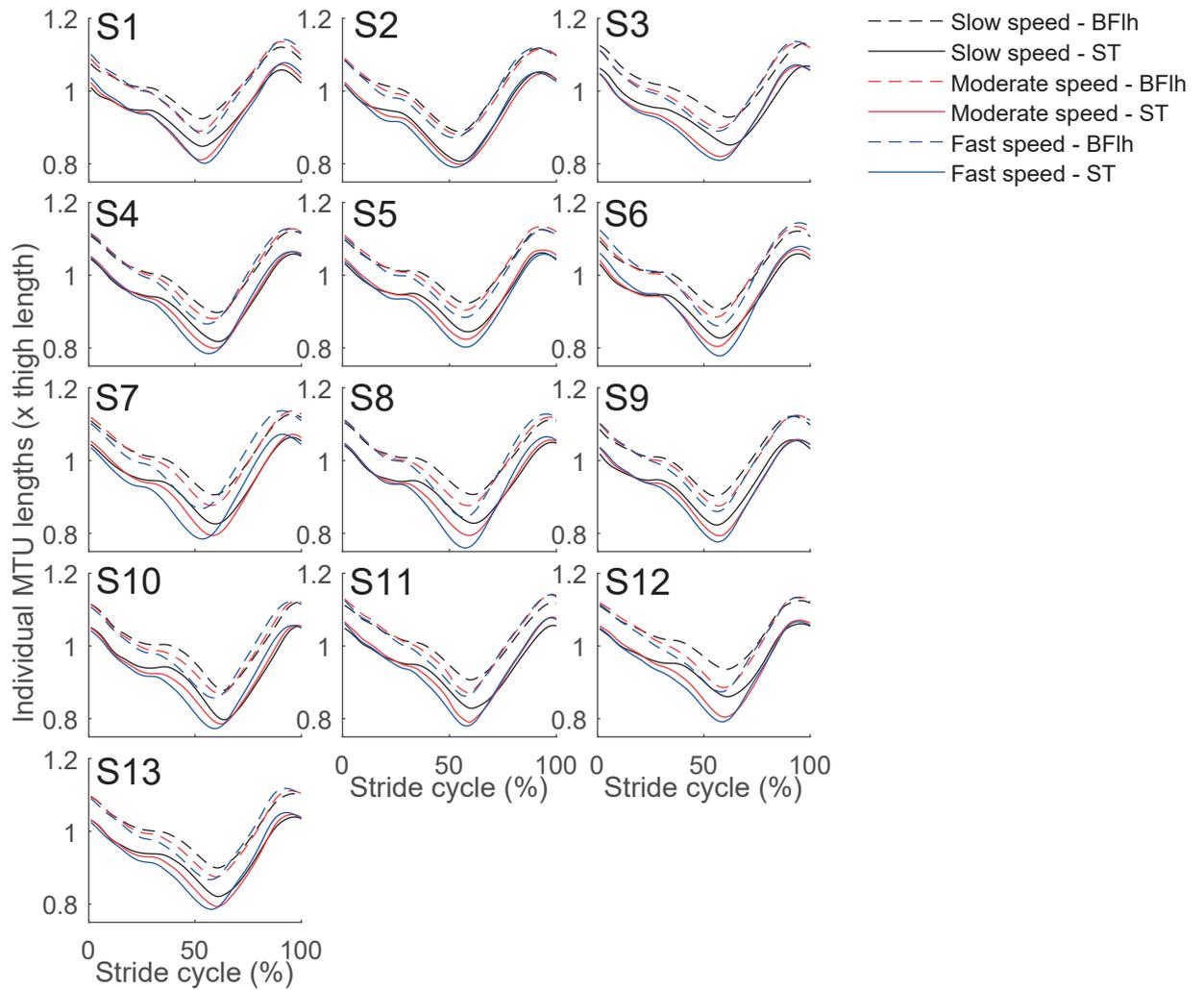
SUPPLEMENTARY FIGURE 1. Upper panels show mean and standard deviation (s.d.) of biceps femoris long head (BFIh) and semitendinosus (ST) electromyography (EMG) activity (normalised to maximal voluntary isometric contraction, MVIC) at slow, moderate and fast running speeds. Lower panels represent corresponding SPM{t} test statistic trajectories (thick black lines). Critical thresholds ( $t^*$ ) are calculated for each comparison after Bonferroni correction (dashed red horizontal lines). These thresholds are not crossed by the SPM{t}, indicating no statistically significant intermuscular differences at any time point of the stride at group level. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).



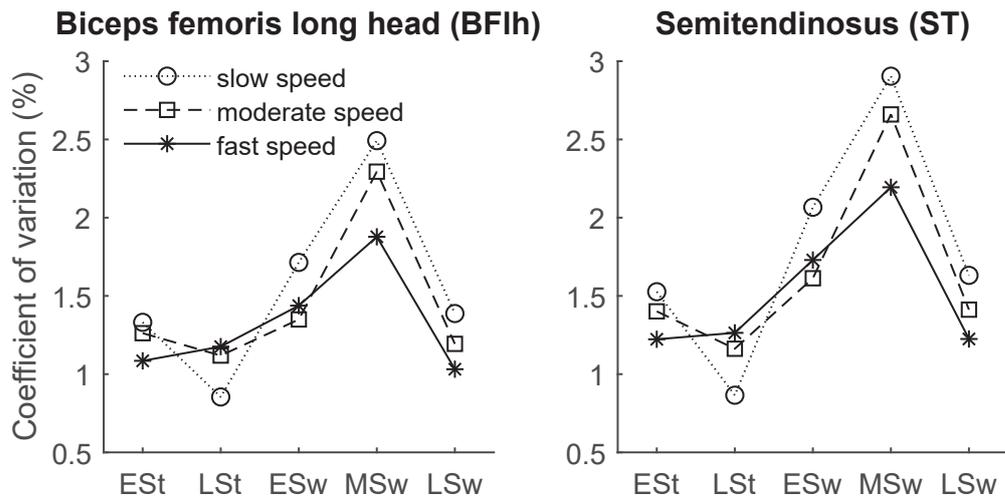
SUPPLEMENTARY FIGURE 2. Effects of running speed on muscle-tendon unit (MTU) lengths. Panels (A) and (B) represent mean and standard deviation (s.d.) across the stride cycle for all running speeds (slow =  $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ , moderate =  $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ , and fast =  $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) for each muscle respectively. Panels (C)-(H) show the statistical parametric maps depicting differences between slow-moderate (C), (D), slow-fast (E), (F), and moderate-fast (G), (H) running speeds in semitendinosus and biceps femoris long head, respectively. Thick black lines represent the SPM{t} test statistics showing the magnitude of the differences relative to s.d. and sample size (N=13). Critical thresholds ( $t^*$ ) are calculated for each comparison after Bonferroni correction (dashed red horizontal lines). Probability (p) values are shown for each supra-threshold cluster depicting statistically significant differences. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).



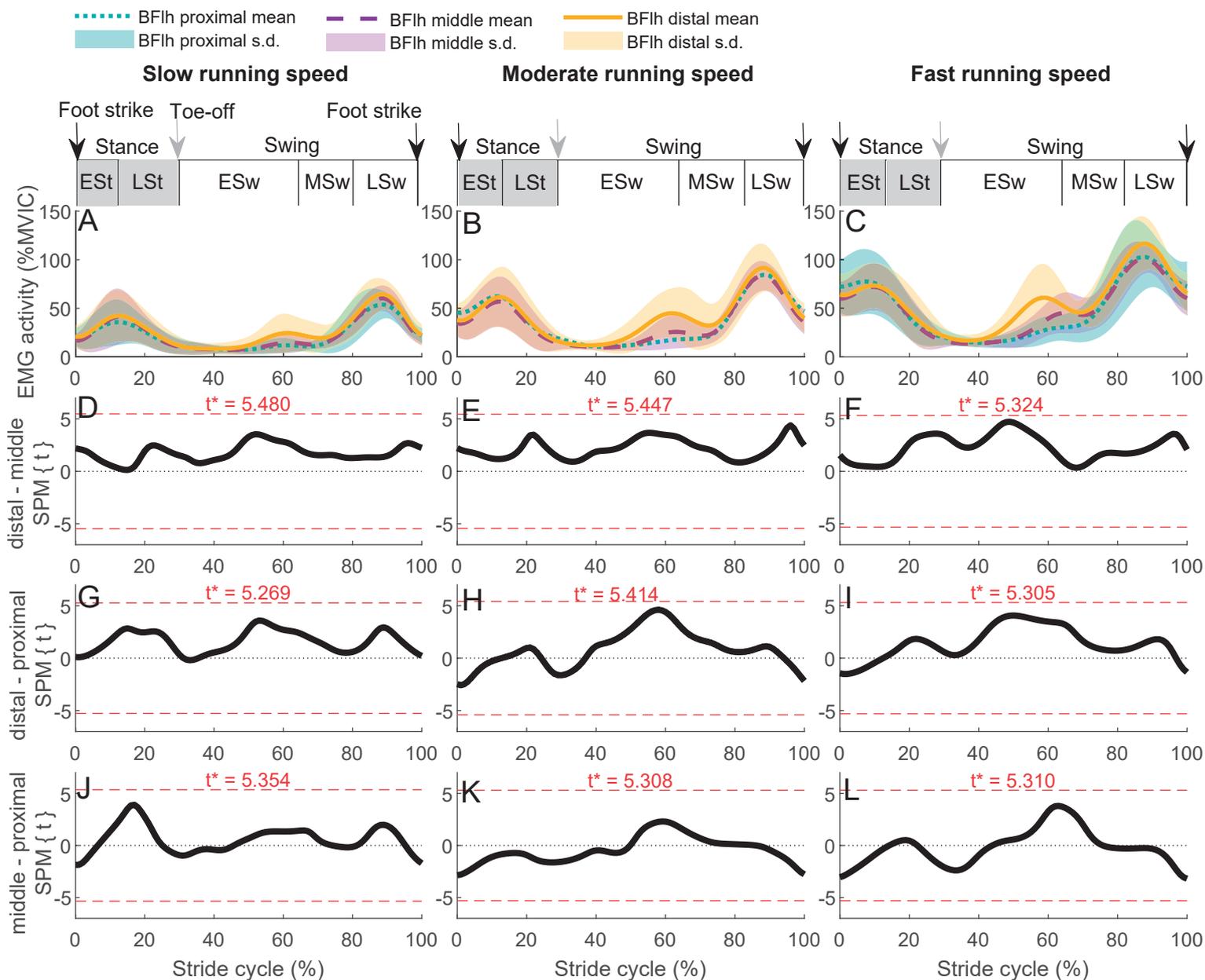
SUPPLEMENTARY FIGURE 3. Similar patterns between individuals S1-S13 in biceps femoris long head (BFH) and semitendinosus (ST) muscle-tendon unit (MTU) lengths at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds.



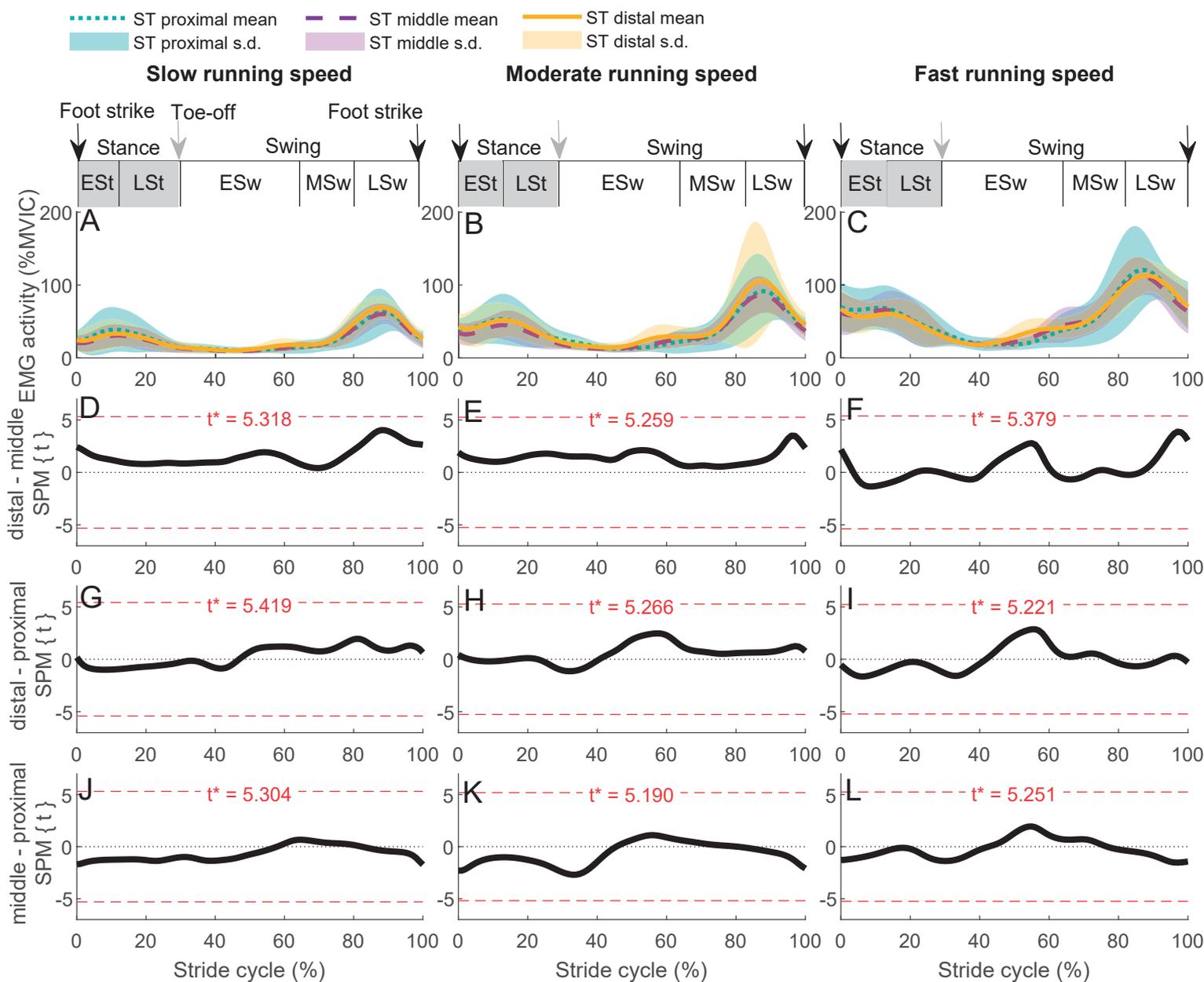
SUPPLEMENTARY FIGURE 4. Inter-individual coefficients of variations in muscle-tendon unit lengths in the early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds.



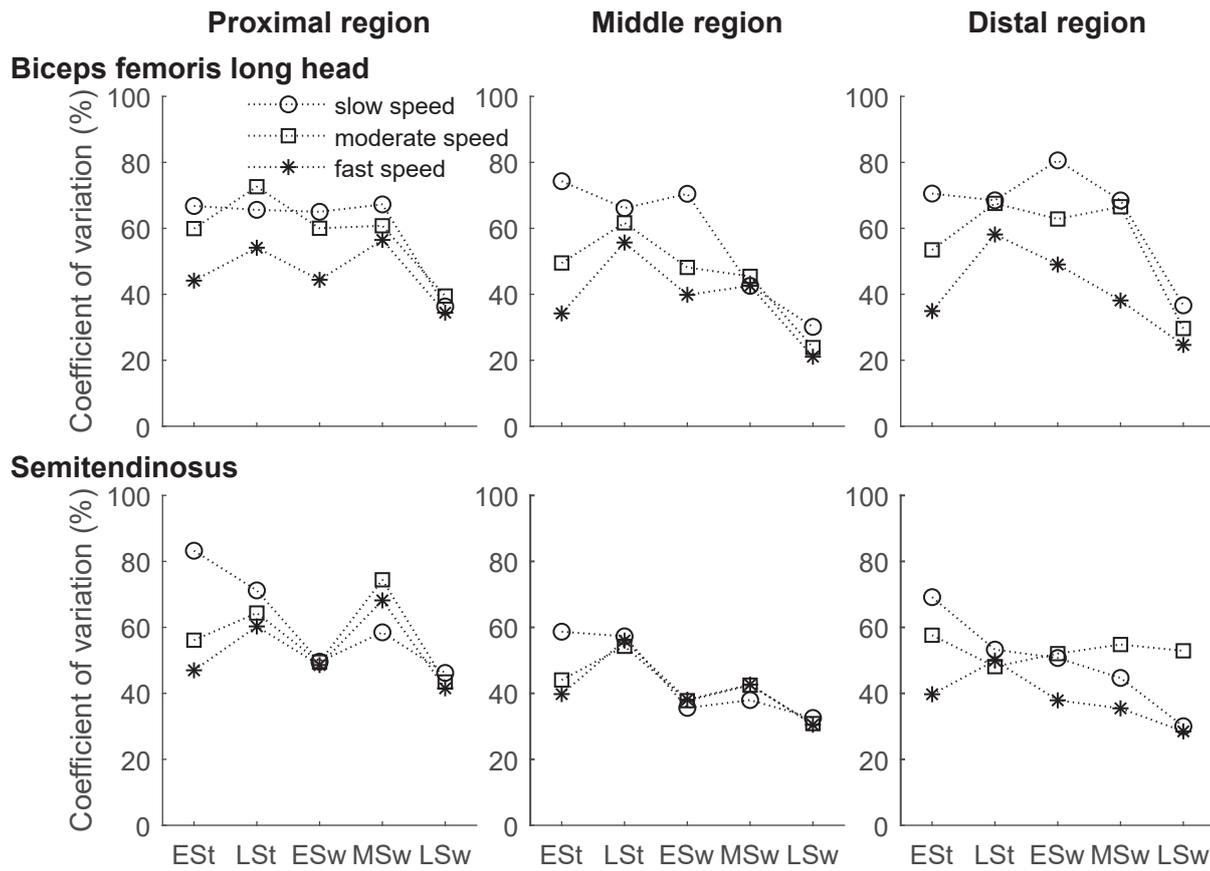
SUPPLEMENTARY FIGURE 5. Regional differences in the electromyography (EMG) activity of biceps femoris long head (BFIh, normalised to maximal voluntary isometric contraction, MVIC). Panels (A)-(C) represent group mean and standard deviation (s.d.) across the stride cycle in each region for each running speed. Panels (D)-(L) show the statistical parametric maps. (D), (E) and (F) show differences between distal and middle regions; (G), (H), and (I) show differences between distal and proximal regions; (J), (K), and (L) show differences between middle and proximal regions at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds, respectively. Thick black lines are the SPM $\{t\}$  test statistics representing the magnitude of the differences relative to the s.d. and sample size (N=13). Critical thresholds ( $t^*$ ) were calculated for each comparison after Bonferroni correction (dashed red horizontal lines; family-wise  $\alpha = 0.05$ ). SPM $\{t\}$  trajectory does not cross the  $t^*$  level in any of the comparisons, indicating no statistical differences between muscle regions. Running stride sub-phases were defined as early stance (ES<sub>t</sub>), late stance (LS<sub>t</sub>), early swing (ES<sub>w</sub>), mid swing (MS<sub>w</sub>), and late swing (LS<sub>w</sub>).



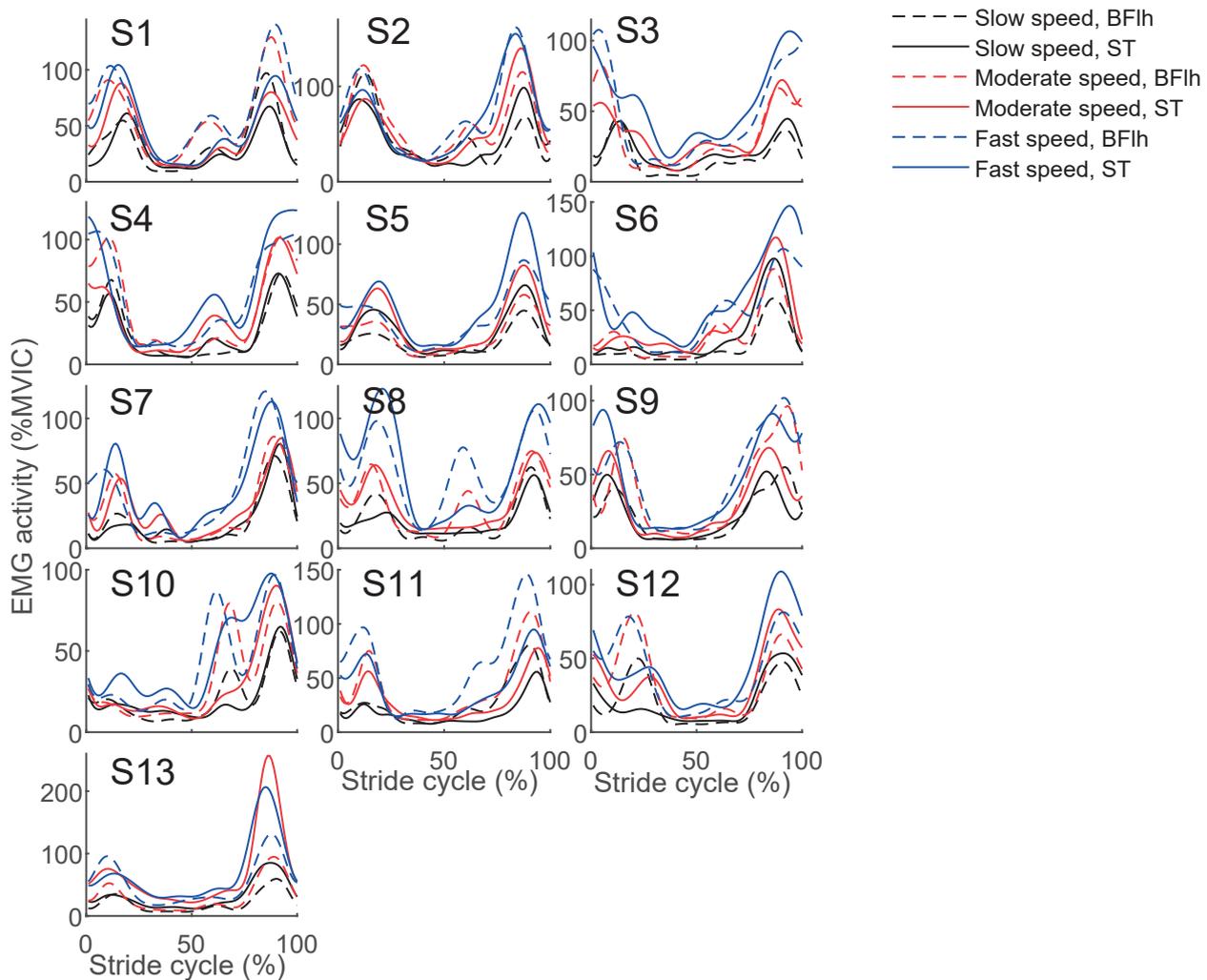
SUPPLEMENTARY FIGURE 6. Regional differences in the electromyography (EMG) activity of semitendinosus (ST, normalised to maximal voluntary isometric contraction, MVIC). Panels (A)-(C) represent group mean and standard deviation (s.d.) across the stride cycle in each region for each running speed. Panels (D)-(L) show the statistical parametric maps. (D), (E) and (F) show differences between distal and middle regions; (G), (H), and (I) show differences between distal and proximal regions; (J), (K), and (L) show differences between middle and proximal regions at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds, respectively. Thick black lines are the SPM $\{t\}$  test statistics representing the magnitude of the differences relative to the s.d. and sample size ( $N=13$ ). Critical thresholds ( $t^*$ ) were calculated for each comparison after Bonferroni correction (dashed red horizontal lines; family-wise  $\alpha = 0.05$ ). SPM $\{t\}$  trajectory does not cross the  $t^*$  level in any of the comparisons, indicating no statistical differences between muscle regions. Running stride sub-phases were defined as early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).



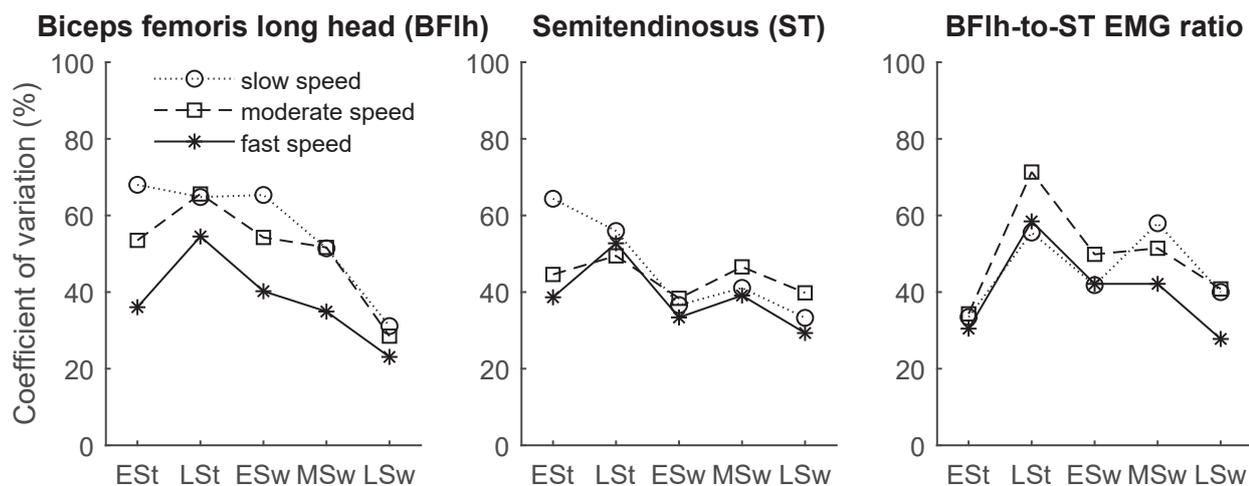
SUPPLEMENTARY FIGURE 7. Large inter-individual coefficients of variations in regional activity levels in the early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds.



SUPPLEMENTARY FIGURE 8. Individual (S1-S13) muscle-specific electromyography (EMG) activity patterns of biceps femoris long head (BFH) and semitendinosus (ST) (normalised to maximal voluntary activity, MVIC) at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds when all EMG channels are averaged along each muscle. Note that scaling of the y axis is optimised for each individual so that intermuscular differences are easier to identify. Foot strike is at 0% and 100% of the stride.



SUPPLEMENTARY FIGURE 9. Large inter-individual coefficients of variations in muscle activity levels at slow ( $4.1 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ), moderate ( $5.4 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ ), and fast ( $6.8 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ ) running speeds when all (up to 15) electromyography (EMG) channels are averaged along each muscle. Sub-phases of the running stride: early stance (ESt), late stance (LSt), early swing (ESw), mid swing (MSw), and late swing (LSw).



ACCEPTED