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SUPERIMPOSING HIP EXTENSION ON KNEE FLEXION EVOKES HIGHER ACTIVATION IN BICEPS FEMORIS THAN KNEE FLEXION ALONE

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ABSTRACT

Hamstring muscle function during knee flexion has been linked to hamstring injury and performance. However, it is unclear whether knee flexion alone (KF) requires similar hamstring electromyography (EMG) activity pattern to simultaneous hip extension and knee flexion (HE-KF), a combination that occurs in the late swing phase of sprinting. This study examined whether HE-KF maximal voluntary isometric contraction (MVIC) evokes higher (EMG) activity in biceps femoris long head (BFlh) and semitendinosus (ST) than KF alone. Effects of shank rotation angles were also tested. Twenty-one males performed the abovementioned MVICs while EMG activity was measured along ST and BFlh. Conditions were compared using a one-way mixed functional ANOVA model under a fully Bayesian framework. Higher EMG activity was found in HE-KF in all shank rotation positions than in KF in the middle region of BFlh (highest in the 9th channel, by 0.022 mV [95%CrI 0.014 to 0.030] in neutral shank position). For ST, this was only observed in the neutral shank position and in the most proximal channel (by 0.013 mV [95%CrI 0.001 to 0.025]). We observed muscle- and region-specific responses to HE-KF. Future studies should examine whether hamstring activation in this task is related to injury risk and sprint performance.

1. INTRODUCTION

The hamstring muscle group is composed of three biarticular muscles, the biceps femoris long head (BFlh), the semitendinosus (ST), and the semimembranosus, as well as one mono-articular muscle, the biceps femoris short head. Besides their function as knee flexors, the bi-articular hamstrings are also strong hip extensors and substantially contribute to horizontal ground reaction force in acceleration running, which is essential for sprint performance (Morin et al., 2015). Hip extension and knee flexion are simultaneously required from the hamstrings in the late swing phase of high-speed running (Chumanov et al., 2011), where these muscles are highly activated (Hegyi et al., 2019b; Higashihara et al., 2010; Yu et al., 2008) and are also vulnerable to strain injury. As running speed increases, electromyography (EMG) activity also increases. This results in similar peak muscle lengths in the highly vulnerable BFlh in the late swing phase (Chumanov et al., 2007) across running speeds, which may be protective against strain injury (Garrett et al., 1987). Therefore, examining hamstring EMG activity is important both from an athletic performance and an injury risk perspective.

EMG activity of hamstring muscles in the late swing of high-speed running can exceed that recorded during a maximal isometric knee flexion (KF) contraction (Hegyi et al., 2019b; Kyröläinen et al., 2005). Higher EMG activity in high-speed running could be due to the fact that the hamstrings are simultaneously generating moments about the hip and knee in the late swing phase (Chumanov et al., 2011), which is not accounted for in traditional maximal KF contractions. It has been suggested that hamstring fascicles act isometrically in late swing of sprinting (Van Hooren and Bosch, 2017). However, there is a lack of experimental evidence about fascicle mechanics in sprinting, so the effect of hip extension should be tested in isolated conditions.

At present, it is unclear whether adding hip extension onto knee flexion would similarly affect the activation of different hamstring muscles. For example, based on some studies using muscle functional magnetic resonance imaging (mfMRI) to examine metabolic activation, Bourne et al. (2018) suggested that BFlh-to-ST activation ratio is higher in hip extension than in knee flexion-based exercises. Accordingly, larger BFlh hypertrophy has been observed after hip extension training on a roman chair than after knee-oriented Nordic hamstring training (Bourne et al., 2017a). These exercises also show preferential activation of the BFlh and ST muscles, respectively, when measured with traditional EMG (Bourne et al., 2017b) or high-density EMG (Hegyi et al., 2019a, 2018). Another study showed that hip extension increases the activation of BFlh relative to ST in the Nordic hamstring exercise, at least at a near-fully extended knee angle (Hegyi et al., 2019c). These findings may imply that superimposing hip extension on knee flexion would increase BFlh activation preferentially. However, other studies have reported no clear differences between hip- and knee-oriented exercises in the intermuscular distribution of EMG activity (Bourne et al., 2017b; Hegyi et al., 2019a; McAllister et al., 2014; Tsaklis et al., 2015). Additionally, of all hamstring muscles, the largest difference in muscle size between sprinters and non-sprinters is in ST (Handsfield et al., 2017; Miller et al., 2020), and it is currently unclear whether this is a result of adaptation to sprinting. If so, superimposing hip extension on knee flexion might be expected to target ST muscle rather than BFlh. Thus, superimposing hip extension on knee flexion may increase the activation of either or both of the BFlh and ST.

Shank rotation during knee flexion is another factor that may alter the interplay between hamstring muscles. EMG signals recorded during isometric knee flexion show that the medial (semimembranosus and ST) and lateral hamstrings (BFlh and biceps femoris short head) can be preferentially activated by adjusting shank rotation (J´onasson et al., 2016). Of the medial hamstrings, ST seems to be more sensitive to shank rotation than semimembranosus (Mohamed et al., 2003). Furthermore, during exercises requiring submaximal hamstring excitation, external and internal rotation of the leg increases the relative activity of the lateral and medial hamstrings, respectively (Beuchat and Maffiuletti, 2019; Lynn and Costigan, 2009).

Based on the above observations, it seems plausible that some MVIC variations, including hip extension and/or shank rotation, evoke higher maximal voluntary isometric EMG activity in the BFlh and ST muscles than knee flexion alone. In this study, we hypothesised that hip extension superimposed on knee flexion MVIC would result in higher hamstring EMG activity than during knee flexion only MVIC. We assumed that internal shank rotation would further increase ST activity, while external rotation would further increase BFlh activity. According to recent studies, proximo-distal distribution of EMG activity in these muscles is heterogeneous in several exercises (Hegyi et al., 2019a, 2018; Schoenfeld et al., 2015), as well as in running (Hegyi et al., 2019b). This suggests that the examined MVIC variations may alter muscle activation in certain muscle regions only, which was also tested in this study.

2. METHODS

2.1. PARTICIPANTS

Twenty-one young male university students (age 26.3 ± 4.2 yrs, height 1.86 ± 0.04 m; body mass 85.9 ± 10.8 kg; mean ± SD) who were engaged in strength training and recreational running on a weekly basis participated in this study. Exclusion criteria were known history of hamstring strain, previous anterior cruciate ligament injury, and any current musculoskeletal or metabolic disorder. All participants provided written informed consent for this study, which was approved by the ethics committee of the University of Physical Education (TE-KEB/ No02/2018). Testing procedures were performed in accordance with the Declaration of Helsinki.

2.2. STUDY DESIGN

Participants first completed a familiarisation session, which included a standardised warm-up protocol consisting of five minutes cycling and eight submaximal isometric hip extension and knee flexion contractions (from ~ 30 to ~ 90% of MVIC). This was followed by the practice of maximal voluntary isometric contractions (MVICs). Participants subsequently completed two randomly ordered measurement sessions (A and B) separated by 3 to 7 days to minimise fatigue. Each measurement session began with preparation and the warm-up protocol followed by the corresponding MVICs. During MVICs, participants lay prone on the testing bench with trunk and hips secured in neutral position (Fig. 1). The right (kicking) leg was tested with the knee flexed to 30◦ as defined with a goniometer. The left knee was flexed to ~ 5◦ to minimise discomfort. In session A, participants performed knee flexion MVICs (KF), as well as internal and external shank rotation, each superimposed on KF (KFI and KFE, respectively). In session B, KF was followed by hip extension superimposed on knee flexion MVICs (HE-KF), and HE-KF with internal (HE-KFI) and external shank rotation (HE-KFE). In both sessions, KF was always performed first, followed by the remaining MVICs in a random order. KF was repeated at the end of each session to monitor fatigue.

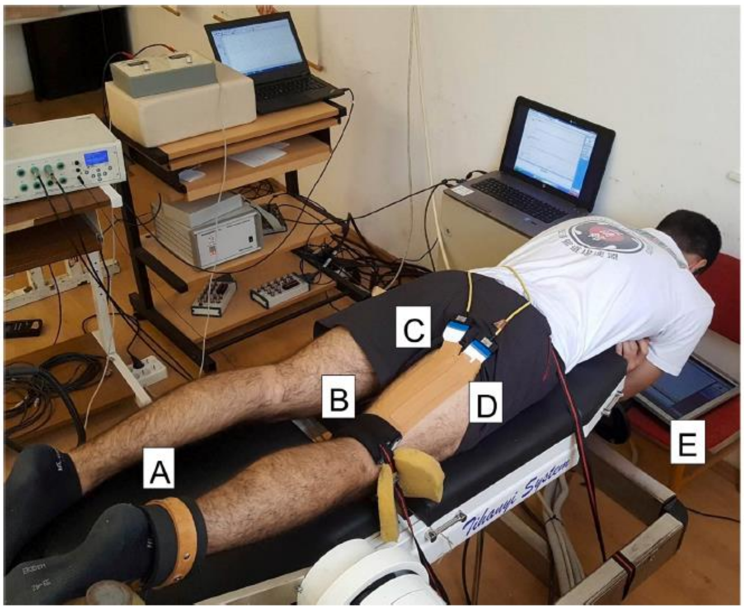

FIGURE 1 PARTICIPANTS LAY PRONE ON THE TESTING BENCH WITH THE RIGHT KNEE FLEXED TO 30°. LINEAR EMG ARRAYS WERE PLACED OVER THE RIGHT BICEPS FEMORIS LONG HEAD (D) AND SEMITENDINOSUS (C) AFTER DEFINING MUSCLE BORDERS WITH ULTRASONOGRAPHY. KNEE FLEXION AND HIP EXTENSION FORCES WERE MEASURED WITH FORCE TRANSDUCERS ATTACHED PROXIMAL TO THE MALLEOLI (A) AND PROXIMAL THE POPLITEAL FOSSA (B), RESPECTIVELY. VISUAL FORCE–TIME CURVE FEEDBACK WAS PROVIDED ON A SCREEN PLACED IN FRONT OF THE PARTICIPANT (E).

KF was performed with a neutral shank position to mimic previous studies that used KF for normalization (e.g. Brown et al., 2014; Contreras et al., 2016; Tsaklis et al., 2015). HE-KF was performed by reaching maximum effort in hip extension and knee flexion simultaneously. Although HE-KF was used for EMG normalisation in recent studies (Hegyi et al., 2019a, 2018), its effect on EMG activity has not been compared to other MVICs including knee flexion. To practice this contraction, participants squeezed a fitness ball placed over the back of the thigh by performing knee flexion while lifting their thigh from the bench with hip extension. During KFI and KFE, participants were asked to position their shank to maximal rotation while avoiding femoral rotation. They then performed knee flexion while maintaining the rotational position of the shank.

HE-KFI and HE-KFE were performed by rotating the shank first, followed by HE-KF as described above. To ensure that each participant maintained similar shank rotation positions during and between contractions, an investigator visually monitored shank rotation angle. Although this did not provide quantitative information, this approach was sufficient to distinguish between rotational positions. It was also important to apply test conditions that could realistically be performed as part of a typical MVIC protocol.

During each session, subjects performed two repetitions of each MVIC condition, followed by a third if peak torque differed by > 10% between the first two contractions. For each contraction, maximal effort was reached within two seconds then maintained for two seconds. Two-minute rest intervals were applied between trials. During all contractions, visual force–time feedback was provided on a screen in front of the participant (Fig. 1) to increase performance (Kellis and Baltzopoulos, 1996), as well as to ensure correct task performance (i.e. no substantial fluctuations around peak force at any of the measured joints). Verbal encouragement was given during all maximal efforts.

2.3. DATA COLLECTION

Ultrasonography (Hitachi-Aloka EUB 405 plus, Japan) was used to identify and mark the borders of the right BFlh and ST muscles, and these locations were used to position the high-density surface electromyography (HD-EMG) arrays as far from the muscle borders as possible to minimise cross talk. After the skin was shaved, abraded and cleaned with alcohol, a 16-channel linear EMG array (10 mm inter-electrode distance, OT Bioelectronica, Torino, Italy) was placed over each muscle (Fig. 1). Standard electrode positioning was used so that in BFlh, channel 8–9 from the distal end was aligned with the midpoint of the distance between the ischial tuberosity and popliteal fossa, while in ST the EMG array was placed one cm below the tendinous inscription of the muscle (Woodley and Mercer, 2005). Arrays were fastened over the skin using double-sided tape. EMG arrays were connected to a 12-bit A/D converter and amplifier (EMG-USB, OT Bioelectronica), and digital signals were recorded in BioLab software (v3.1, OT Bioelectronica). To maintain skin-electrode contact, 20 μl of conductive gel was injected into the electrode cavities. A reference electrode strap was placed over the left wrist. Signal quality was confirmed visually during submaximal knee flexion 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 all isometric trials, force was measured with two strain gauges at a sampling frequency of 1000 Hz. Digitised force signals were recorded in the BioLab software in synchrony with the EMG signals. A leather collar was fixed ~ 5 cm above the lateral malleolus and attached to the strain gauge, which was fixed to the testing table. The strain gauge was positioned perpendicular to the shank during contractions. Another collar was fixed proximal to the popliteal fossa and attached to a strain gauge positioned perpendicular to the thigh. 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 leather collar at the thigh. For knee flexion the lever arm was measured as the distance between the lateral epicondyle of the femur and the middle of the leather collar at the shank.

2.4. DATA PROCESSING

After inspecting the collected signals, channels over the identified innervation zones were excluded from the analysis. Raw signals were processed in Matlab (Mathworks Inc., Natick, MA, USA). EMG signals were band-pass filtered between 10 and 500 Hz using a zero-phase fourth-order Butterworth filter. To quantify the surface EMG amplitude during maximal isometric contractions, the root mean square (RMS) amplitude was calculated for each channel, over a 1 s epoch corresponding to the highest torque attained in each MVIC task, to avoid inclusion of rapid changes at the transient phases. Force signals were off-line low-pass filtered with a cut-off frequency of 15 Hz using a zero-phase, fourth-order Butterworth filter. Task-specific torque was calculated as the average from the same plateau as used for the EMG analysis.

3. STATISTICAL INFERENCE

3.1. ELECTROMYOGRAPHY ACTIVITY

HD-EMG data from ST and BFlh were treated as one-dimensional spatial functional data, and each reflected an outcome variable. The dependent variable was the MVIC conditions (9 levels). Let $yij(x)$ denote the jth functional observation in the ith MVIC condition, where a one-way mixed functional ANOVA model with a random subject-intercept of the following form was fitted:

$$yij(x) = \mu(x)+\alpha i(x)+\mu j(x)+eij(x) \tag{1}$$

where $\mu(x)$ is the grand mean function,$\alpha i(x)$ is the ith level main effect function, $\mu j(x)$ is the random intercept for the jth participant, and $eij(x)$ is the residuals. We fitted model (1) under a fully Bayesian framework. To estimate one-dimensional effect functions, we used a second-order random walk (RW2) model (Yue et al., 2019). The resulting Bayesian mixed model can be efficiently estimated using integrated nested Laplace approximations (INLA) (Liew et al., 2019; Wang et al., 2018). INLA provides accurate approximated posterior distributions of all parameters (e.g. β coefficients) given the data (Rue et al., 2009), and these distributions are needed for fully Bayesian inference (i.e. posterior mean with credible intervals [CrI]).

From model (1) we computed the pairwise mean with 95%CrI differences in EMG for each muscle between the five MVIC conditions (KFI, KFE, HE-KF, HE-KFI, HE-KFE) and the reference condition (KF). Note that here the effect of shank rotations were compared to near neutral position. Therefore, to examine differences between internally and externally rotated shank positions, pairwise mean with 95%CrI differences was also computed for each muscle from model (1) by contrasting KFE vs KFI and HE-KFE vs HE-KFI. To probe for possible intra-session fatigue-effects on EMG activity in each testing session, the pairwise mean with 95%CrI differences in EMG for each muscle was computed by contrasting KF at the end of session A vs KF at the start of session A, and KF at the end of session B vs KF at the start of session B. Significant changes were defined within a Bayesian framework as a non-zero crossing of the 95% CrI.

3.2. KNEE FLEXOR TORQUE

Similar to the EMG data, a one-way mixed model with a random subject intercept was used to test the influence of different MVIC conditions (9 levels) on knee flexor torque. For this analysis, we were principally interested in understanding changes in knee flexor torque within each session, as evidence of possible muscular fatigue. The pairwise mean with 95%CrI differences in knee flexor torque was computed by contrasting KF at the end of session A vs KF at the start of session A, and KF at the end of session B vs KF at the start of session B. Significant changes were defined as a non-zero crossing of the 95% CrI.

4. RESULTS

4.1. FATIGUE

There were no significant differences in EMG activity in ST or BFlh during KF at the start and end of session A or B (Fig. 2). However, there was a reduction in knee flexor torque at the end compared to the start of session A by - 7.8 Nm (95%CrI - 15.3 to - 0.3), whereas the change in session B of - 5.0 Nm (95%CrI - 12.6 to 2.7) was not statistically significant.

4.2. EMG ACTIVITY

4.2.1. KNEE FLEXION VS OTHER MVICS

Fig. 3 and Fig. 4 represent the EMG activity of ST and BFlh, respectively. Fig. 5 shows the pairwise differences between KF and each of the other MVIC conditions. For simplicity, here we report the region of greatest difference if the condition resulted in greater EMG activity compared to KF. For BFlh muscle, HE-KF (by 0.022 mV [95%CrI 0.014 to 0.030] in the 9th channel), HE-KFE (by 0.021 mV [95%CrI 0.013 to 0.029] in the 10th channel), and HE-KFI

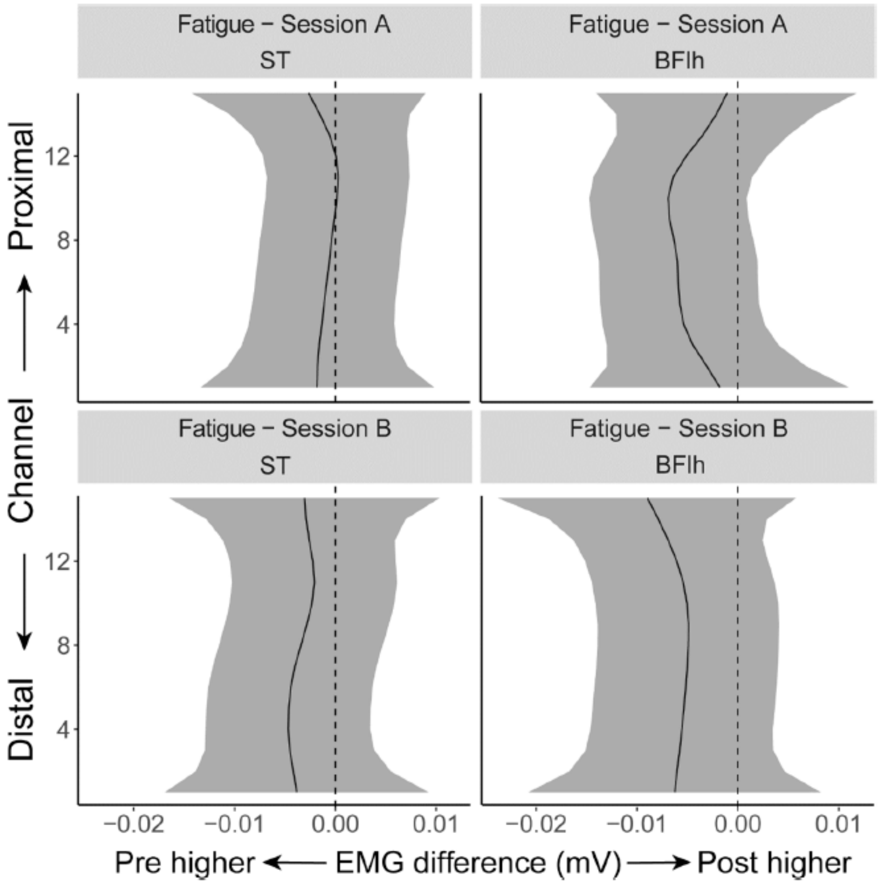

FIGURE 2 THE PAIRWISE MEAN WITH 95%CRI DIFFERENCES IN ELECTROMYOGRAPHY (EMG) ACTIVITY FOR EACH MUSCLE CONTRASTING KNEE FLEXION (KF) AT THE END VS THE START OF SESSION A, AND KF AT THE END VS THE START OF SESSION B, SHOWING NO SIGNIFICANT DIFFERENCES IN SEMITENDINOSUS (ST) OR BICEPS FEMORIS LONG HEAD (BFLH).

(by.010 mV [95%CrI 0.003 to 0.018] in the 9th channel) all resulted in significantly greater EMG activity than KF (session B). For ST muscle, only HE-KF resulted in greater EMG activity than KF (session B), in the 15th channel by 0.013 mV (95% CrI 0.001 to 0.025). Other differences were either not detected, or were in favour of KF.

4.2.2. INTERNAL VS EXTERNAL ROTATION

Significantly higher EMG activity occurred in KFE than in KFI in the proximal region of BFlh, by 0.020 mV (95%CrI 0.005 to 0.034) in the 15th channel, whilst significantly lower EMG activity was observed in the middle region of ST, by - 0.009 mV (95%CrI - 0.017 to - 0.001) in the 6th channel (session A). Compared to HE-KFI (session B), significantly higher EMG activity occurred in HE-KFE in the proximal region of BFlh, by 0.012 mV (95%CrI 0.003to 0.021) in the 12th channel, whilst significantly lower EMG activity occurred in the middle region of ST, by - 0.009 mV (95%CrI - 0.017 to - 0.001) in the 7th channel (Fig. 6).

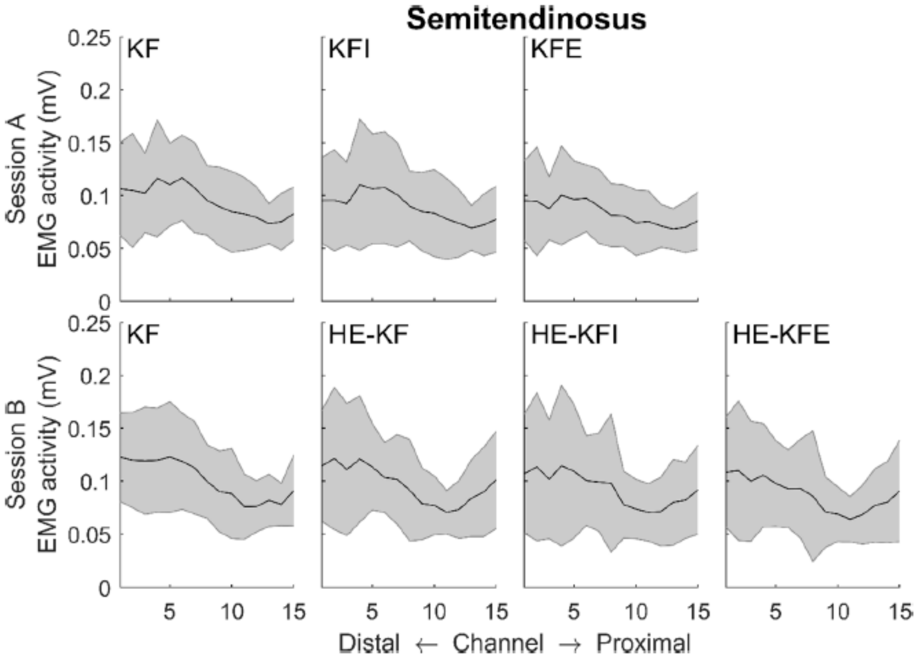

Figure 3 Mean and SD electromyography (EMG) activity of semitendinosus in each maximal voluntary isometric contraction. Fifteen EMG channels were recorded along the muscle. KF, knee flexion; KFI, knee flexion with internal shank rotation; KFE, knee flexion with external shank rotation; HE-KF, hip extension superimposed on knee flexion; HE-KFI, hip extension superimposed on knee flexion with internal shank rotation; HE-KFE, hip extension superimposed on knee flexion with external shank rotation.

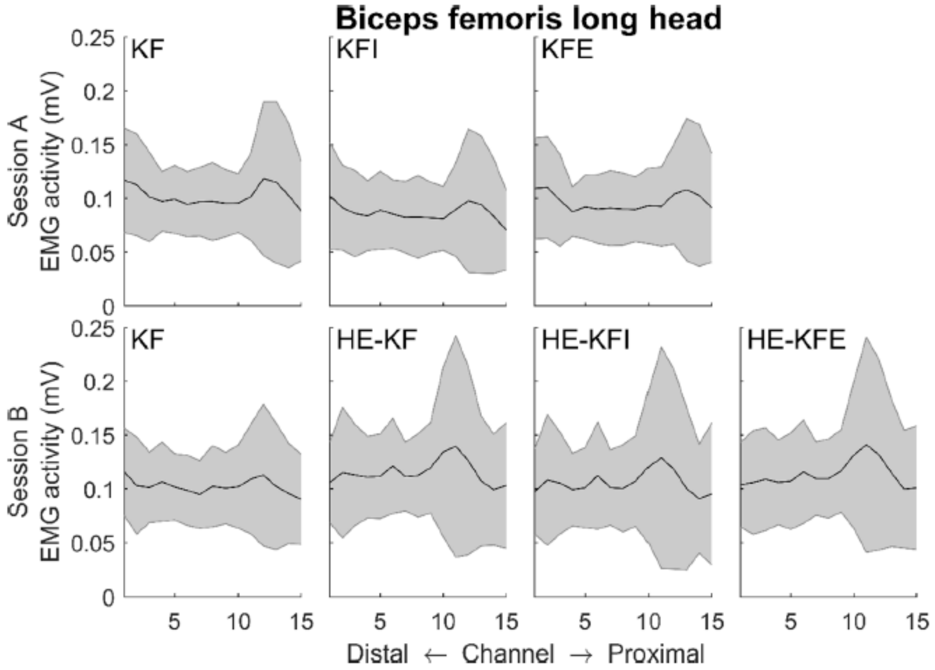

Figure 4 Mean and SD electromyography (EMG) activity of biceps femoris long head in each maximal voluntary isometric contraction. Fifteen EMG channels were recorded along the muscle. KF, knee flexion; KFI, knee flexion with internal shank rotation; KFE, knee flexion with external shank rotation; HE-KF, hip extension superimposed on knee flexion; HE-KFI, hip extension superimposed on knee flexion with internal shank rotation; HE-KFE, hip extension superimposed on knee flexion with external shank rotation.

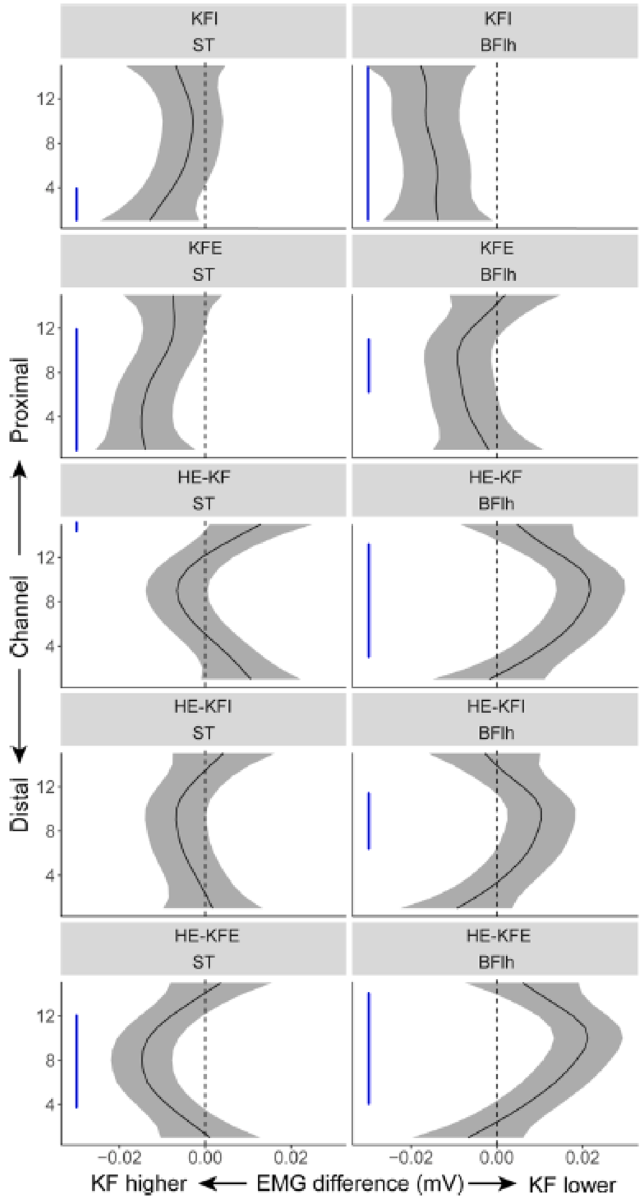

Figure 5 The pairwise mean with 95%CrI differences in electromyography (EMG) activity for semitendinosus (ST) and biceps femoris long head (BFlh) between the five maximal voluntary isometric (MVIC) conditions (KFI, KFE, HE-KF, HE-KFI, HE-KFE) and the reference condition (KF MVIC). Blue vertical lines indicate channels where 95%CrI does not include zero (i.e. statistical significance). KF, knee flexion; KFI, knee flexion with internal shank rotation; KFE, knee flexion with external shank rotation; HE-KF, hip extension superimposed on knee flexion; HE-KFI, hip extension superimposed on knee flexion with internal shank rotation; HE-KFE, hip extension superimposed on knee flexion with external shank rotation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

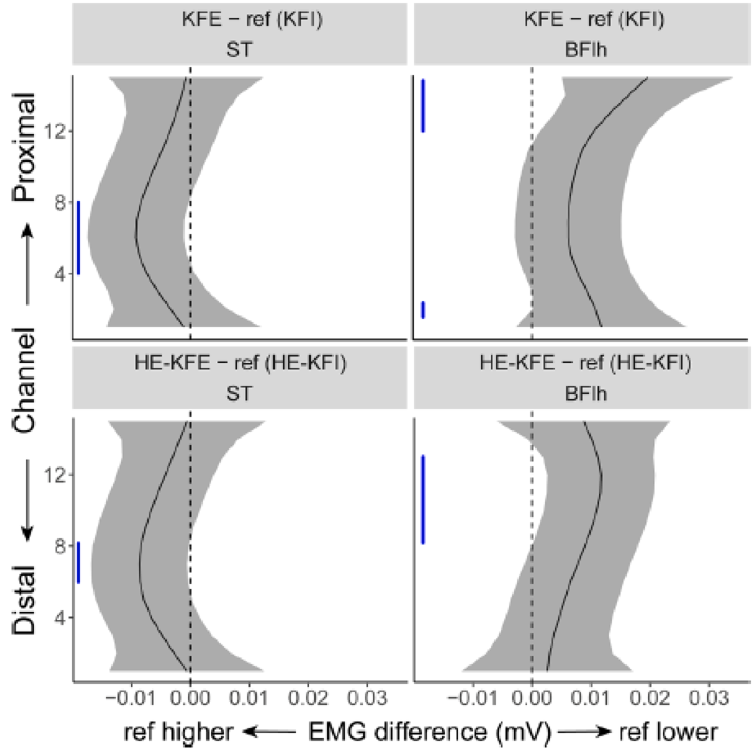

FIGURE 6 THE PAIRWISE MEAN WITH 95%CRI DIFFERENCES IN ELECTROMYOGRAPHY (EMG) ACTIVITY OF SEMITENDINOSUS (ST) AND BICEPS FEMORIS LONG HEAD (BFLH) CONTRASTING KNEE FLEXION WITH EXTERNAL SHANK ROTATION (KFE) VS KNEE FLEXION WITH INTERNAL SHANK ROTATION (KFI), AND SIMULTANEOUS HIP EXTENSION AND KNEE FLEXION WITH EXTERNAL SHANK ROTATION (HE-KFE) VS SIMULTANEOUS HIP EXTENSION AND KNEE FLEXION WITH INTERNAL SHANK ROTATION (HE-KFI). BLUE VERTICAL LINES INDICATE CHANNELS WHERE 95%CRI DOES NOT INCLUDE ZERO (I.E. STATISTICAL SIGNIFICANCE). (FOR INTERPRETATION OF THE REFERENCES TO COLOUR IN THIS FIGURE LEGEND, THE READER IS REFERRED TO THE WEB VERSION OF THIS ARTICLE.)

4.3. TORQUE

Table 1 shows the mean and SD of hip extension and knee flexion torque in each MVIC condition. Hip extension torque did not differ between neutral and rotated shank positions ($p > 0.05$). Knee flexion torque in KF was not different between sessions A and B, and was higher than in any other condition ($p < 0.05$). Modelled differences are shown in Table 2.

TABLE 1 RAW TORQUE DESCRIPTIVE STATISTICS.

Joint	**Condition**	**Mean**	**SD**
Hip	HE-KF	86.0	26.3
	HE-KFI	80.8	32.4
	HE-KFE	80.2	30.7
Knee	KF (session A)	150.3	27.4
	KF (session B)	144.5	22.6
	KFI	125.6	21.6
	KFE	135.2	22.3
	HE-KF	79.5	20.3
	HE-KFI	68.5	18.1
	HE-KFE	78.9	18.6

TABLE 2 MODELLED TORQUE DIFFERENCES BETWEEN CONDITIONS.

Joint	**Contrasting conditions**	**Mean**	**Lower 95% CrI**	**Upper 95% CrI**
Hip	HE-KFI – HE-KF	-4.9	-17.5	7.8
	HE-KFE – HE-KF	-5.4	-18.0	7.3
	HE-KFE – HE-KFI	-0.5	-13.4	12.4
Knee	KF (session B) – KF (session A)	-3.7	-11.2	3.8
	KFI – KF*	-22.5	-30.0	-15.0
	KFE – KF*	-13.0	-20.5	-5.5
	HE-KF – KF*	-64.5	-72.1	-56.9
	HE-KFI – KF*	-75.4	-83.1	-67.8
	HE-KFE – KF*	-65.1	-72.7	-57.5
	KFE – KFI*	9.5	1.9	17.2
	HE-KFE – HE-KFI*	10.4	2.7	18.0

5. DISCUSSION

This study confirmed our assumption that hip extension superimposed on knee flexion (HE-KF) evokes higher BFlh EMG activity than knee flexion alone (KF) during a maximal voluntary isometric contraction. This difference was most pronounced in the mid-region of BFlh. However, a superior effect of hip extension on knee flexion was not evident in ST, except in the most proximal channel. Differences between internal and external rotation were observed mainly in the proximal (KF) or middle (HE-KF) region of BFlh (external > internal shank rotation), and in the middle region of ST (internal > external shank rotation). However, EMG activity in rotated shank positions remained similar or lower when compared to the neutral position during KF or during HE-KF MVICs.

Compared to knee flexion alone, the largest increase in EMG activity when superimposing hip extension on knee flexion was in the mid-region of the BFlh. The mid-region of the BFlh shows the largest cross-sectional area in this muscle (Kositsky et al., 2020) with relatively high pennation angle (Woodley and Mercer, 2005), highlighting the high force producing capacity of this region. Along the fusiform ST muscle (Woodley and Mercer, 2005), the differences between HE-KF and KF were more uniform along the muscle, and showed statistical difference only in the most proximal channel. Based on the fact that ST is relatively large in sprinters (e.g. Handsfield et al., 2017; Miller et al., 2020), one would expect relatively higher activation of this muscle in HE-KF, which is more similar to hamstring muscle function in the late swing phase of sprinting than KF alone. This was not confirmed in the present study, which may be explained by the fact that our participants were non-sprinters. It may be that sprinters would produce higher ST activation in HE-KF, which should be clarified in future studies.

A few studies have suggested a link between the intermuscular distribution of muscle activation during knee flexion and hamstring injuries. For example, a prospective mfMRI study on football players showed that higher activation of ST relative to other hamstrings during dynamic leg curl is associated with a decreased hamstring injury risk (Schuermans et al., 2016). Another study showed that BFlh atrophy after a hamstring injury leads to a decreased contribution of this muscle to knee flexion torque (estimated from bipolar EMG activity, muscle volume, and moment arm of each hamstring) after a hamstring injury (Avrillon et al., 2020) during submaximal knee flexion contraction. These studies suggest a link between hamstring coordination and hamstring injury risk. However, they examined hamstring muscle function in knee flexion only, and the present study suggests that this does not reflect the intermuscular distribution of hamstring activation in a combined hip extension and knee flexion contraction, which is similar to hamstring muscle function in the late swing phase of sprinting. In future studies it may be of value to examine the association between intermuscular distribution of EMG activity in HE-KF and injury risk. Additionally, when assessing the intermuscular distribution of EMG activity (and corresponding intermuscular coordination), the interpretation of intermuscular differences may be hampered by the normalisation method, as discussed below.

Normalising EMG activity using the EMG amplitudes recorded during MVIC as the reference contraction is typical in order to define what percentage of the maximal EMG activity the task under investigation represents (Allison et al., 1993; Yang and Winter, 1984). KF is usually used as a reference for all hamstring muscles, implying that they are all maximally or at least similarly activated during KF. The current study shows that KF is not suitable to maximally activate BFlh in isometric conditions because HE-KF resulted in higher EMG activity in this muscle. Importantly, the largest difference between HE-KF and KF was found in the middle region of BFlh, which is also the region where traditional bipolar EMG electrodes are usually placed. Whether normalising BFlh EMG activity to HE-KF would allow more precise comparisons to ST activity than using KF alone remains to be further clarified. Additionally, inter- and intra-individual variability of HE-KF should be tested before implementing this as a novel EMG normalisation method for hamstrings. Normalising to KF in future studies would allow the results to be compared to those of most previous studies, but based on our results, KF may not be the optimal choice when aiming to compare the EMG activity of different hamstring muscles. Due to such potential pitfalls of normalisation, comparisons of EMG activity between different muscles should always be interpreted cautiously

It should be noted that normalising to MVIC *per se* has been criticised. For example, EMG activity during rapid or lengthening contractions can exceed that recorded during an MVIC (Perry, 1992). Indeed, recent hamstring studies also confirm that EMG activity exceeds 100% MVIC in high-speed running (Hegyi et al., 2019b; Kyr¨ol¨ainen et al., 2005), as well as in the Nordic hamstring exercise (Hegyi et al., 2018) when normalised to KF, and it is very likely that normalising to HE-KF would not solve this problem. As an alternative, some authors normalised hamstring EMG activity to that of sprint running (e.g. van den Tillaar et al., 2017). However, inter-individual differences in sprinting may allow for individual intermuscular coordination strategies, so we cannot be sure that all hamstrings are activated to the same extent. MVICs enable more uniform performance technique between individuals. Additionally, it is likely safer to normalise to MVIC considering that most hamstring strain injuries happen in sprinting (Verrall et al., 2003). Further discussion of different normalisation methods can be found elsewhere (e.g. Burden, 2010).

The anatomical paths of BFlh and ST reflect their rotational functions. The conjoined proximal tendon of BFlh and ST originates from the ischial tuberosity (Sato et al., 2012; Stępie´n et al., 2019). Distal to the common tendon, the ST runs distally and medially, inserting into the pes anserinus near the medial condyle of the tibia (Tubbs et al., 2006). BFlh runs distally and laterally, attaching on the styloid process of the fibula (Terry and LaPrade, 1996; Tubbs et al., 2006). Accordingly, ST and BFlh have the potential to rotate the shank medially and laterally, respectively. This seems to be reflected in the intermuscular differences in EMG activity of these muscles in rotated positions during many submaximal hamstring exercises (Beuchat and Maffiuletti, 2019; Lynn and Costigan, 2009). Similar results have also been reported during KF (Fiebert et al., 1997; J´onasson et al., 2016; Mohamed et al., 2003). A previous study found that medial rotation resulted in higher ST EMG activity compared to the neutral position, whereas no difference in BFlh activity between the neutral position and lateral rotation was reported (Mohamed et al., 2003) Mohamed et al. (2003) also found that BFlh activity was lower in the internally rotated position than in the neutral position, resulting in a lower BFlh EMG activity relative to ST EMG activity. This inhibitory effect was also seen in the current study. The increased ST EMG activity relative to BFlh activity in KFI and decreased ST EMG activity relative to BFlh activity in KFE were mainly attributable to decreased EMG activity in BFlh and ST in each position, respectively. Further, we did not observe higher EMG activity in any muscle in the rotated positions when compared to the neutral rotation position. It should be noted that Mohamed et al. (2003) used a different knee angle (70◦ flexion) compared to the current study, and sampled from a small number of motor units using fine-wire EMG. In contrast, the current study used HD-EMG, which suggests that some rotational effects are specific to certain muscle regions. Using traditional bipolar EMG, a recent study (Beyer et al., 2019) showed that internal shank rotation can increase ST activity at a relatively flexed knee position (90◦ flexion), which was not observed at 30◦ of knee flexion, while the opposite was observed in BFlh. Regional responses to shank rotations at different knee angles should be tested in future studies.

As a limitation of this study, surface EMG is prone to cross talk. To minimise this effect, we used HD-EMG arrays with a small pick-up area and 10 mm inter-electrode distance, and guided the placement of the arrays with ultrasonography. We also only included male participants with a relatively thin subcutaneous layer, which ensures the electrodes are close to the muscle and further minimises the risk of signal contamination by the activation of neighbouring muscles. An additional limitation of surface EMG is the shift of the muscle under the electrodes during contraction, although this effect was likely minimal in this study considering that EMG activity was recorded at the same hip and knee angles and during maximal contractions in all conditions. As another potential source of bias, the small decrease in knee flexion torque in the absence of changes in EMG activity during session A may have been an effect of fatigue (Kallenberg et al., 2007). However, the fact that EMG activity did not change from pre to post suggests that this is unlikely to affect our conclusions. Indeed, after excluding participants who showed the largest decrease in torque, we found similar results (see Supplementary file 1). It is also possible that the reduction in knee flexion torque is a result of the lower contribution of other knee flexors than BFlh and ST, which were not examined in this study. As an additional limitation, the degree of shank rotation was not quantified, potentially increasing variability within- and between individuals. However, maximum individual rotation is likely repeatable. We also used the same approach as previous studies, facilitating the comparison of results (Beuchat and Maffiuletti, 2019; J´onasson et al., 2016; Mohamed et al., 2003). Our results may be limited to the applied joint angles. Regarding hip flexion angle, an extended hip position (as used in the present study) seems to result in the highest BFlh EMG activity (Lunnen et al., 1981). Others found no effect of hip angle on overall hamstring EMG activity (Worrell et al., 2001). Regarding knee angle, a slightly flexed knee position (as used in this study) seems to result in higher overall hamstring EMG activity compared to fully extended (Mohamed et al., 2002) or more flexed knee angles (Worrell et al., 2001). This was similar in Onishi et al. (2002) for BFlh but not for ST and semimembranosus, where maximum EMG activity was observed at 90-105◦ of knee flexion. In this study we aimed to measure in a knee flexion position that was relatively close to that observed in the late swing of sprinting. However, in future studies it may be of value to test at different joint configurations, including flexed knee positions, when the aim is to identify a configuration that evokes the highest EMG activity for each hamstring muscle.

To conclude, non-uniform responses to hip extension superimposed on knee flexion were found within- and between hamstring muscles. Regional differences highlight the need for spatially robust methods to examine hamstring muscle function comprehensively. The fact that combined hip extension and knee flexion resulted in higher EMG activity in BFlh but not in ST compared to knee flexion alone suggests that intermuscular differences should be interpreted cautiously when EMG activity is normalised to knee flexion alone. Combined hip extension and knee flexion, which is similar to hamstring muscle function in sprinting, resulted in different EMG activity patterns compared to KF alone. Therefore, future studies should examine possible links between hamstring function in HE-KF and hamstring injury risk and sprint performance.

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

Appendix A. Supplementary material Supplementary data to this article can be found online.

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