

Research article

## Fascicle Behavior and Muscle Activity of The Biceps Femoris Long Head during Running at Increasing Speeds

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### Abstract

Hamstring strain injuries (HSIs) are prevalent in sports involving high-speed running and most of the HSIs are biceps femoris long head (BFLh) injuries. The primary cause for HSIs during high-speed running remains controversial due to the lack of in vivo measurement of the BFLh muscle behavior during running. Therefore, the purpose of this study was to quantify the muscle-tendon unit (MTU) and fascicle behavior of BFLh during running. Seven college male sprinters ( $22.14 \pm 1.8$  years;  $177.7 \pm 2.5$  cm;  $70.57 \pm 5.1$  kg; personal bests in 100m:  $11.1 \pm 0.2$  s) were tested on a motorized treadmill instrumented with two force plate for running at 4, 5, 6 m/s. The ground reaction force (GRF), 3D lower limb kinematics, EMG, and ultrasound images of biceps femoris long head (BFLh) in the middle region were recorded simultaneously. BFLh fascicles undergo little length change (about 1 cm) in the late swing phase during running at three submaximal speeds. BFLh fascicle lengthening accounted for about 30% of MTU length change during the late swing phase. BFLh was most active during the late swing and early stance phases, ranging from 83%MVC at a running speed of 4 m/s to 116%MVC at 6 m/s. Muscle fascicles in the middle region of BFLh undergo relatively little lengthening relative to the MTU in the late swing phase during running in comparison to results from simulation studies. These results suggest that there is a decoupling between the fascicle in the middle region and MTU length changes during the late swing phase of running.

**Key words:** Hamstring muscle, sprinting, injury mechanism, different speeds, muscle activation.

### Introduction

There are at least two types of Hamstring strain injuries (HSIs) in terms of injury situations and mechanism (Askling et al., 2012). The most common injury type occurs during high-speed running (Ekstrand et al., 2011; Brooks et al., 2006), which mainly affects the biceps femoris long head (BFLh) (Ekstrand et al., 2012) and the other type occurs as a result of an “over-stretch” movements (Askling et al., 2006). While the direct cause for the over-stretch type of HSIs is obvious, the primary cause for HSIs during high-speed running remains controversial in the literature (Ruan, 2018; Herzog, 2017).

Yu et al. (2017) argued that muscle over-strain during the late swing in high-speed running is the direct cause of HSIs. This argument is mainly based on in vitro animal muscle studies and computational modeling studies. Many in vitro animal muscle studies showed that strain injury was caused by reaching a critical strain rather than exceeding a critical force (William et al., 1987; Lieber and Friden, 1993). For example, (Lieber and Friden, 1993) demonstrated that muscle damage was identical when animal muscles were stretched by 25% of the muscle fascicle length at the same stretching rate but different muscle force production.

Furthermore, computational modeling studies (Fiorentino et al., 2014; Chumanov et al., 2007; Thelen et al., 2005a; Thelen et al., 2005b) demonstrated that both muscle-tendon unit (MTU) and muscle fascicles were stretched actively during the late swing of running at 70% - 100% of the maximal running speed, and the fascicle was stretched about 40 mm, accounting for about 80% of MTU length change (Thelen et al., 2005a). However, findings from in vitro animal or computational models may not be generalized to human HSIs during high-speed running. Excessive strain as the primary cause of HSIs was challenged by some authors. Van Hooren and Bosch, (2017a) argued that there might not be any eccentric action of the hamstrings during the swing phase of high-speed running. This could be attributed to the underestimated muscle slack, limitations of 2D models, overestimated tendon stiffness, and potential underestimation of the influence of muscle gearing ratio in previous studies. Even if the hamstrings are lengthening in the late swing phase of running, it is unlikely for excessive strain to occur (Ruan, 2018). The maximum length of BFLh during high-speed running in the injury trial was close to the muscle's optimal length (Heiderscheit et al., 2005; Schache et al., 2009). This is much less than the typical 12.5% or 25% strain from the optimal length, which was conducted in injury research using animal models (Lieber, 1993). Furthermore, connective tissue compliance may lead to decoupling between fascicle and MTU length, and hamstring muscle fascicles may conduct quasi-isometric contraction during the swing phase of running, which has been validated from animal in vivo studies (Gillis and Biewener, 2001; Gillis et al., 2005). Although there are no studies investigating the fascicle behavior of hamstring muscle in vivo during running, quasi-isometric muscle fascicle behavior during eccentric muscle-tendon action has been confirmed during hamstring exercises by

in vivo measurement (Pincheira et al., 2022; Van Hooren et al., 2022).

The purpose of this study was to assess BFlh fascicle behavior in vivo during running. We hypothesized that there is a decoupling between the BFlh fascicle and MTU length during running, and this decoupling pattern remains consistent across different running speeds. The findings of this study may provide new insight into HSI during running.

## Methods

### Participants

Seven college male sprinters (age:  $22.14 \pm 1.8$  years; height:  $177.7 \pm 2.5$  cm; body mass:  $70.57 \pm 5.1$  kg; personal bests in 100m:  $11.1 \pm 0.2$  s) who had received regular sprinting training (3 - 4 times per week) and strength training (once per week) for at least three years were recruited to participate in this study. Participants did not experience any lower limb injuries in the past 24 months and had never suffered a hamstring strain injury. The study protocol was approved by the ethical committee of the Shanghai University of Sport and consent forms were signed by all participants before testing. Participants wore spandex shorts and the same type of athletic shoes (Do-win RC2) during data collection.

### Experimental procedures

The participants performed trials at 4, 5, and 6 m/s running speeds in random order on a motorized treadmill (FIT 5, Bertec instrumented treadmill, U.S.A, two independent belts 1.75 x 0.5m each) instrumented with two force plates (1000 Hz). Following about 10-s stabilization at the target speed, ground reaction forces (GRF), 3D lower limb kinematics, EMG, and ultrasound images of the biceps femoris long head (BFlh) were recorded simultaneously for 15 seconds. Participants performed two trials at the same speed with 1-minute rest intervals between trials, followed by a 2-minute rest before trials at a different speed.

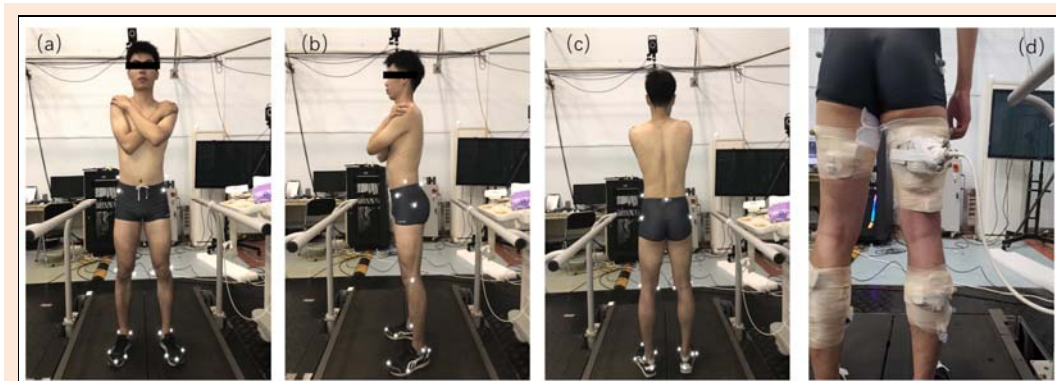
### Data collection

All running movements were captured using an eight-camera Vicon motion capture system (200 Hz, Oxford, UK). Retroreflective markers (14 mm) were used for both calibration and tracking. Calibration markers were attached bilaterally to the first metatarsal heads, medial and lateral epicondyles of the knee joint, and medial and lateral malleoli.

Tracking markers were attached to the iliac crest tubercle, anterior superior iliac spines, sacrum, fifth metatarsal heads, posterior surface of the calcaneus, and second metatarsal heads (Figure 1a-c). Additional rigid plates with 4 tracking markers were attached bilaterally to the thighs and shanks. Participants performed a static calibration trial with all markers presented. Joint angles for the hip, knee, and ankle measured during a static standing trial were defined as neutral. The calibration markers were then removed before data collection. It took about 5 seconds for the treadmill to reach the target speed. Participants had approximately 10 seconds to stabilize their gait and posture once the treadmill reached the target speed. Ground reaction force (GRF), 3D lower limb kinematics, electromyography (EMG), and ultrasound images of the biceps femoris long head (BFlh) were then recorded simultaneously for 15 seconds for each trial.

EMG signals of the BFlh from the right leg were collected using a surface EMG system (2000 Hz sampling frequency; common-mode rejection ratio >100dB; Noraxon, U.S.A). Before placing the EMG electrode, the skin was cleaned with alcohol. The electrode was placed according to the SENIAM (surface electromyography for non-invasive assessment of muscles) guidelines (Hermens et al., 2000) (Figure 1d). It was secured with strapping tape to minimize motion artifacts. Before running testing, the EMG signals during 5-second isometric maximal voluntary contraction (MVC) of the BFlh muscle were recorded with the subject in a prone position with the hip in neutral and the knee flexion of 45°.

Muscle fascicle action of right-sided BFlh was imaged with two-dimensional B-mode ultrasound using a probe (frequency: 7 MHz; width and depth: 40 and 65 mm; flat; 128-element; Philips HD7 XE, L12-3 probe) connected to a PC-based ultrasound system. The total weight of the ultrasound probe and the supporting device is about 130 g. The probe was secured in a custom-made foam holder and strapped using a Coban™ compression bandage (3M, St. Paul, MN, USA) and a transparent sponge athletic bandage. It was consistently placed at approximately 60% of the distance between the ischial tuberosity and the fibula head on the subjects (Figure 1d). A trial was then conducted to examine the quality of the ultrasound image before data collection. Images were recorded in Cine Loop at 22 frames/s. The quality of the ultrasound image was carefully monitored during the running test.



**Figure 1.** Experimental setup showing kinematic markers in (a), (b) and (c), the ultrasound probe and the EMG electrodes placement showing in (d).

### Data analysis

Marker trajectory data were processed using Vicon Nexus Version 2.6.1 (Oxford, UK). Then, Visual 3D (C-Motion, Germantown, MD, United States) was used to compute the 3D kinematic variables. GRF data was used to identify foot strike and toe-off events using a 20N threshold. A fourth-order Butterworth low-pass filter with a cutoff frequency of 10 Hz was used to smooth the 3D marker coordinates (Winter, 1990). A lower body model was used to calculate the angle of the hip and knee joint. Additionally, BFlh MTU length changes during running were estimated using OpenSim (Gait 2392, NCSRR, Stanford, CA, United States).

Full-wave rectified EMG signals were filtered using a moving root-mean-squared (RMS) filter with a window size of 50 ms and normalized to the maximum RMS value of the EMG signal in MVC testing. The root mean squared (RMS) envelopes were subsequently computed.

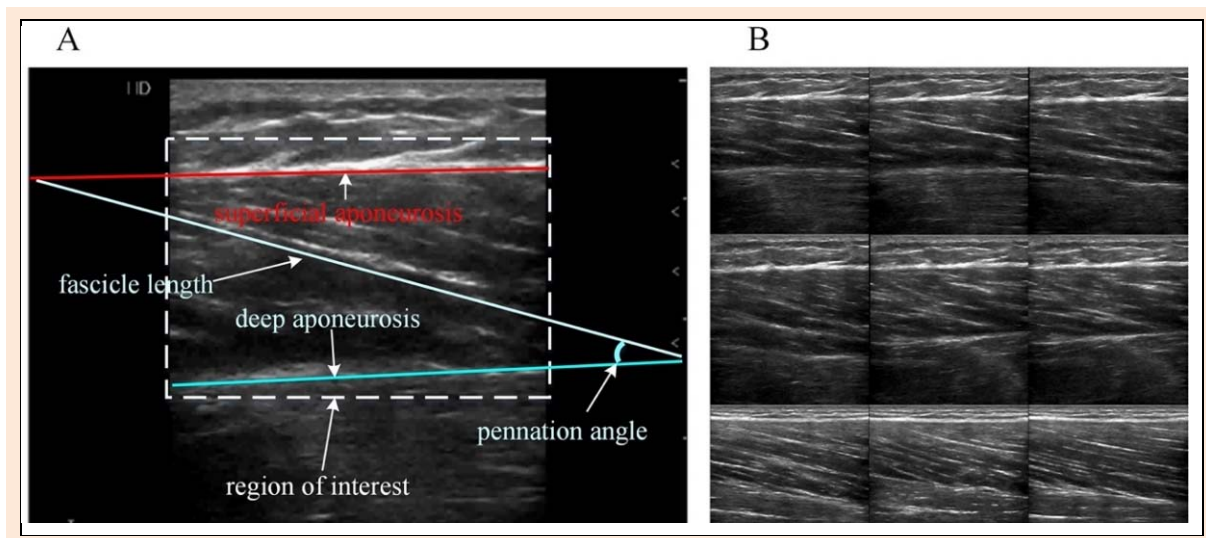
Changes in BFlh fascicle length were calculated by UltraTrack software (Farris and Lichtwark, 2016). Reliability for both automated and manual tracking approach have been confirmed (Gillett et al., 2012). In each ultrasound image, the fascicles were identified along their length from the superficial and deep aponeuroses and tracked continuously frame by frame using Affine Optic Flow Model (AOFM) (Farris and Lichtwark, 2016). Key-frame Correction Algorithm (KFCA) was applied to correct the accumulated error and drift of tracking data in the calculation process (Farris and Lichtwark, 2016). When the software could not automatically track the fascicle correctly,

manual corrections were made using the key-frame correction function. Data were then exported from UltraTrack and processed in Microsoft Excel.

For data reduction, joint kinematics, EMG, and length changes in MTU and fascicle of BFlh were time-normalized to the gait cycle, and the three continuous steps were analyzed and averaged for each trial. The gait cycle consists of the stance phase and the swing phase. The stance phase was defined as the interval from the right foot strike to the toe-off as determined by GRF data, whereas the swing phase was defined as the interval from the right toe-off to the right foot strike. Additionally, the late swing was defined as the interval from peak knee flexion in the swing phase to foot strike (Kenneally-Dabrowski et al., 2019a).

### Statistical analysis

Statistical analyses were performed by using SPSS (v25.0). A parametric test was performed after the normality of data for one-sample t-tests or the normality of residuals for within-subject comparisons was not rejected at an alpha level of 5% according to Shapiro-Wilk tests ( $P > 0.05$ ). A one-way repeated-measures ANOVA was used to test the possible differences among different running speeds. When significant main effects were found, an LSD Post-hoc multiple comparisons would be used to test the differences between the different running speed conditions for the EMG, kinematics, and fascicle length changes. The probability level accepted for statistical significance was  $P < 0.05$ . Values are presented as means.



**Figure 2.** Ultrasound image showing the region of interest, fascicle length, superficial and deep aponeuroses, and pennation angle (A). Ultrasound imaging screenshots during running (B).

**Table 1.** Maximum length changes in MTU and fascicle of BFlh.

Speeds	Swing phase			Late swing phase		
	MTU (cm)	Fascicle (cm)	Ratio (%)	MTU (cm)	Fascicle (cm)	Ratio (%)
4m/s	7.05 ± 1.55	1.31 ± 0.29	18.58 ± 5.1	4.41 ± 1.51	1.02 ± 0.35	23.03 ± 7.8
5m/s	7.59 ± 1.28	1.90 ± 0.32	25.03 ± 4.7	5.12 ± 2.08	1.28 ± 0.52	24.50 ± 8.9
6m/s	7.92 ± 1.33	1.60 ± 0.63	20.20 ± 8.4	3.27 ± 0.99	1.12 ± 0.34	31.98 ± 9.3

Values are mean ± SD. Ratio = Fascicle length/ MTU length. The differences between running speeds and ratios of fascicle length and MTU found no significant differences during both the swing and late swing phases. The maximum length changes represent the difference between the maximum length and the minimum length.

## Results

### The MTU behavior

Length changes in the MTU during running are shown in Figure 3A and Table 1. The MTU rapidly shortened throughout the whole standing phase regardless of running speed. Peak MTU lengths during running are  $36.85 \pm 2.95$ ,  $37.76 \pm 2.87$ , and  $37.65 \pm 3.15$  at 4, 5, and 6 m/s, respectively. The MTU lengthened during the swing phase, approximately from 40% to 50% of the gait cycle, and shortened towards the end of the late swing phase. With increased speed, the maximum length changes in MTU did not significantly increase during both the swing and late swing phases (Table 1). Furthermore, in the late swing phase (90% of the gait cycle), MTU undergoes an additional lengthening and shortening.

### The fascicle behavior

Length changes in the fascicle during running are shown in Figure 2B and Table 1. The fascicles experienced a shortening and then a lengthening during the swing phase. Peak BF<sub>lh</sub> fascicle lengths during running are  $6.13 \pm 0.85$ ,  $5.94 \pm 0.81$ , and  $5.73 \pm 0.82$  cm at 4, 5, and 6 m/s, respectively. There was no significant difference in the magnitude of lengthening during both the swing and the late swing phase between different running speeds ( $p > 0.05$ ). BF<sub>lh</sub> fascicle length changes accounted for less than 25.03% of MTU length changes during the swing phase, regardless of running speed.

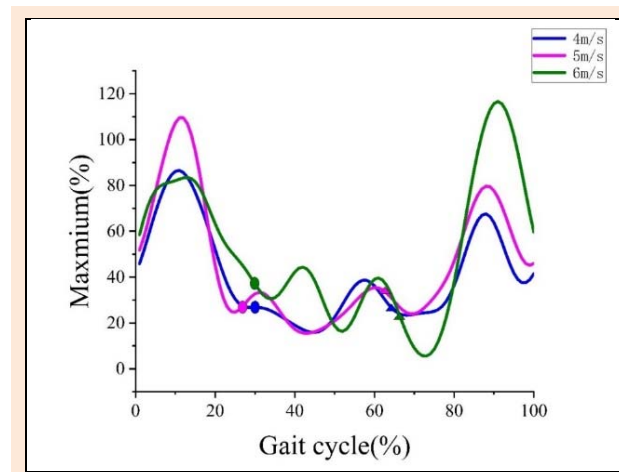
### The joint angle changes

No significant differences in peak value of knee flexion

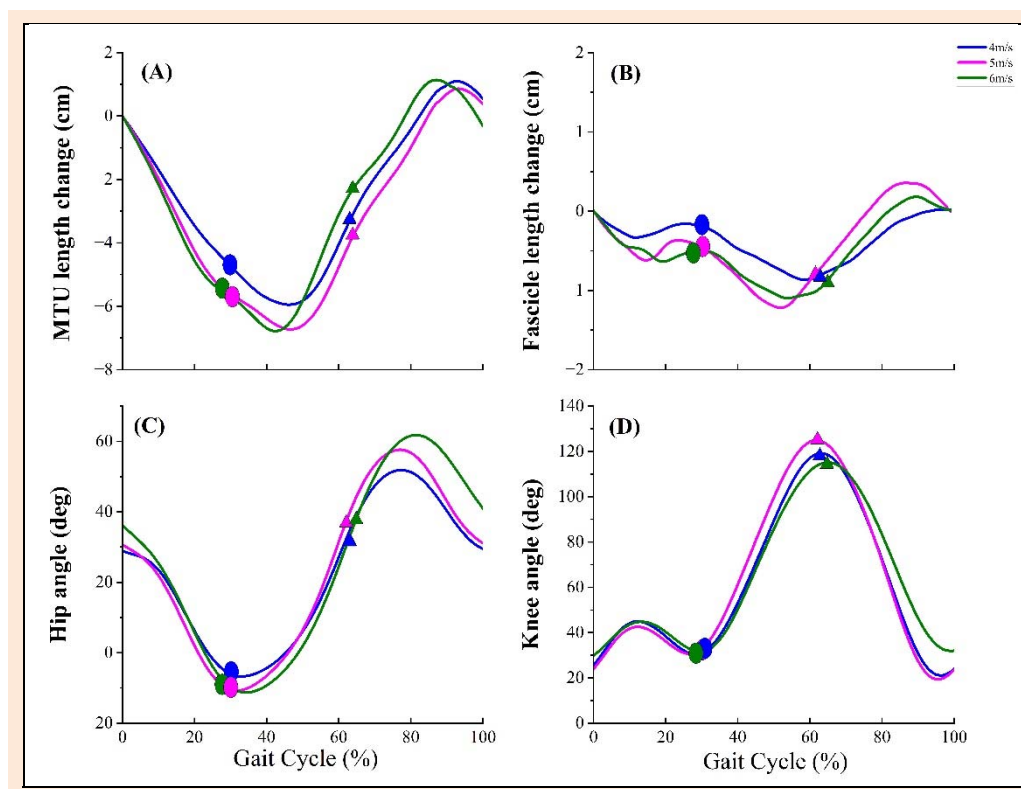
were found between different running speeds (Figure 3D). The peak knee flexion prior to foot strike has no significant difference from 4 to 6 m/s ( $p > 0.05$ ) (Figure 3D).

### The EMG changes

The study showed that BF<sub>lh</sub> was most active during the late swing and early stance phases, ranging from 83% MVC at a running speed of 4 m/s to 116% MVC at 6 m/s (Figure 4). There were significant differences in the EMG values between different running speeds during both the swing and the late swing phases ( $p < 0.05$ ).



**Figure 4.** BF<sub>lh</sub> muscle linear envelop EMG during a running gait cycle. Blue, pink, and green lines represent 4m/s, 5m/s, and 6m/s, respectively. Ellipses represent the time of toe-off during different running speeds; Triangles represent the starting time of the late swing phase.



**Figure 3.** Group mean curves for the variables assessed during running. Blue, pink, and green lines represent 4m/s, 5m/s, and 6m/s, respectively. Ellipses represent the starting time of the swing phase during different running speeds. Triangles represent the starting time of the late swing phase. The swing phase was defined as the interval from the right toe-off to the right foot strike. The late swing was defined as the interval from the peak knee flexion in the swing phase to the foot strike.

## Discussion

The objective of this study was to characterize BFlh fascicle behavior during running. Quantitative *in vivo* measurements are essential for understanding muscle mechanics and injury mechanisms of HSIs. As far as we know, this is the first study to measure BFlh fascicle behavior *in vivo* during running. The results of this study determined that BFlh fascicles undergo lengthening about 1 cm and account for about 30% of MTU length change during the late swing phase. Overall, these results suggested that there is a decoupling between the fascicle in the middle region of BFlh and MTU length changes during the late swing phase of running.

The highest speed (6m/s) tested in this study was about 70% of the maximum speed participants could run on the treadmill. There is a concern whether the findings from this study could be generalized to running at higher speeds. Thelen et al. (Chumanov et al., 2007, Thelen et al., 2005b) measured MTU length changes during running at 80% to 100% of maximum running speed (9.3 m/s). The results showed that peak muscle-tendon lengths did not vary significantly over the range of running speeds tested (Chumanov et al., 2007; Thelen et al., 2005b). Schache et al. (2013) also found that the maximum MTU stretch for BFlh during the swing phase of running did not increase significantly between 6.9 m/s and  $9.0 \pm 0.7$  m/s. MTU length was calculated using the 3-D coordinates of the muscle attachment points in corresponding segment reference frames (Chumanov et al., 2007, Thelen et al., 2005a; Schache et al., 2013; Yu et al., 2008). Their results showed that the peak value of BFlh MTU length occurred during the late swing phase (approximately 90% of the sprinting gait cycle), where both the hip and knee joint angles reached the peak value at about  $50^\circ$ . In our study, the peak value of BFlh MTU length occurred at 90% of the gait cycle, with both the hip and knee joint angles reaching about  $50^\circ$  at 6 m/s. Therefore, we believe that the peak MTU lengths during higher speeds running would be similar to those during 6 m/s running.

Computer simulation studies (Fiorentino et al., 2014; Chumanov et al., 2007; Thelen et al., 2005a) suggest that muscle fascicle length in BFlh is the main contributor to changes in MTU length during the later swing of running, accounting for about 70% - 80%. In contrast, our results showed that BFlh fascicle length change only accounted for about 20-32% of MTU length change, and the magnitude of lengthening of BFlh fascicle was only about 25% of that found in the simulation study (Thelen et al., 2005a). The relatively little lengthening of the fascicle relative to the MTU during the late swing phase may be related to some mechanisms proposed by Van Hooren and Bosch (Van Hooren and Bosch, 2017b; 2018). The connective tissue compliance may lead to limited fascicle lengthening (Pincheira et al., 2022) and tendon stiffness in the simulation studies has been overestimated (Bohm et al., 2018). Also, the presumed high gear ratio of the pennate muscle further reduced the fascicle lengthening during MTU eccentric action (Azizi and Roberts, 2014). Additionally, during higher speed running, with higher muscle activation, increased fascicle stiffness and less fascicle length-

ening could occur (Brooks et al., 1995). The results from the current study are consistent with the data from previous *in vivo* studies (Pincheira et al., 2022; Van Hooren et al., 2022). During exercises like Nordic hamstring exercise (NHE) and single-leg Roman chair exercise, BFlh muscle fascicles act almost isometrically and stretched much less than MTU for much of the exercise, and most fascicle lengthening during NHE occurs after the breakpoint, likely due to decreased muscle activation (Van Hooren et al., 2022). Combining these findings with our results, we are confident that a relatively little fascicle lengthening relative to the MTU and a decoupling between fascicle and MTU length could occur during running even if the running speed is higher than 6m/s. Furthermore, there seems to be a trend for a parabolic effect with the highest increase in fascicle length change at 5m/s but a decrease at 6m/s, possibly due to higher muscle activation. Such an effect could mean that the length change of the fascicle during max sprinting would be minimal. The minimal length change of the fascicle is associated with increasing the elastic strain energy contribution from tendons, which has been verified for Gastrocnemius Medialis (GM) during running at increasing speeds (Monte et al., 2020).

Knowledge about BFlh fascicle behavior and muscle activity during running from *in vivo* measurement can provide new insights into the mechanisms of HSIs. The results of this study showed that there are two peak values of muscle activation during the whole gait cycle across all running speeds, which are consistent with previous studies (Yu et al., 2008; Higashihara et al., 2010; Chumanov et al., 2011; Higashihara et al., 2015). Interestingly, the peak values of muscle activation even exceed 100% of maximal voluntary contraction, which is in line with a previous study (Kyrolainen et al., 2005). Additionally, the peak muscle activation of Bflh was synchronous with peak musculotendon length, indicating that the BFlh muscle is exposed to a high tensile force during the late swing phase (Higashihara et al., 2016). Serner et al. proposed that the coupling of rapid muscle activation and a rapid lengthening of MTU is a fundamental injury mechanism for muscle injuries regardless of open chain or close chain movements (Serner et al., 2019). These studies suggested that the larger MTU strains cause greater forces and thus muscle strain injury. Accordingly, muscle strain was often used as an indicator of injury risk (Perrin et al., 2020). However, Perrin et al. (2020) argued that the MTU strain is not a good indicator of injury risk (Perrin et al., 2020). As the cross-sectional area of muscle changes at different locations, they proposed that stress rather than strain is the principal cause of muscle damage (Perrin et al., 2020). Our results at least partially support their argument that overstrain of MTU is not the principal cause of muscle damage. Due to the decoupling between fascicle and MTU length changes, MTU strain is not representative of the amount of muscle fiber strain, at least for muscle fiber strain in the middle region of BFlh.

Our results may also contribute to optimizing the hamstring injury prevention program. The most important finding from this study is that the fascicles in the middle region of BFlh undergo limited stretching during the late swing phase, in which hamstrings are more likely to be in-

jured (Kenneally-Dabrowski et al., 2019b). Most authors agree eccentric strength training is an effective exercise to reduce HSIs injury risk (Van Hooren and Bosch, 2017a; Perrin et al., 2020). Still, more research is needed to determine the best eccentric strength training protocol to reduce HSIs risk.

### Limitations of current research

Several limitations to this study should be considered when interpreting the findings. Firstly, there are certain methodological limitations to the in vivo BFlh fascicle measurement used in this study. A 4 cm linear array probe was not able to collect the entire fascicle length during running. We recognized the limitations of extrapolating fascicle length using trigonometric equations. Franchi et al. (2020) compared BFlh fascicle length obtained from different ultrasound-based approaches and found that the extrapolation method resulted in a BFlh fascicle overestimation of 0.3 - 2 cm compared with fascicle length obtained from an extended field of view (EFOV) ultrasound scans. We recognized that the EFOV technique offers advantages compared with the extrapolation method, but it should not be considered as the “gold standard” (Franchi et al., 2020). Maintaining the transducer parallel to the fascicle plane over a large area of interest during scanning is challenging and, if not done correctly, can compromise the quality of the acquired images (Noorkoiv et al., 2010; Adkins and Murray, 2020). More importantly, they also found that fascicle lengths estimated with the same extrapolation method are highly reliable within the same session. Therefore, we are more confident about the fascicle length change obtained from this study. Also, due to regional heterogeneity in muscle architecture causing regional variation in fascicle strain (Azizi and Deslauriers, 2014), strain experienced by fascicle in the middle region may not be representative of the whole muscle. Certainly, fascicle length changes in other regions during running warrant further study. Additionally, since external compression could increase muscle belly length (Wakeling et al., 2013), the pressure applied by probe fixation would have increased the length changes of the fascicle during the swing phase. A previous study by (Bolsterlee et al., 2015) found that skin compression leads to a systematic underestimation of pennation angles but does not affect pennation angles and fascicle lengths. This indirectly suggests that image misalignment is the most significant source of error in measuring fascicle length and pennation angles.

Secondly, our tests were conducted on athletes in good physical condition, so the impact of fatigue on the activity of BFlh muscle fascicles during running is unclear. Significant changes in muscle architectural and physiological factors would occur with muscle fatigue (Li et al., 2016). Thirdly, we did not compare different models when we estimated MTU behavior. So, it is unclear whether the MTU behavior would differ if we use other models to estimate it. Finally, the relatively small sample size used in this study may affect some statistical results. However, the small inter-individual difference increases our confidence in our main findings.

### Conclusion

In summary, our results showed that muscle fascicles in the middle region of BFlh undergo relatively little lengthening relative to the MTU in the late swing phase during running in comparison to results from simulation studies. The findings suggest that there is a decoupling between the fascicle in the middle region and MTU length changes during the late swing phase of running.

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### Key points

- This is the first study to measure the biceps femoris long head muscle fascicle behavior during running in vivo.
- The study found that peak muscle activation of BFLh coincided with peak MTU length, indicating high tensile forces during the late swing phase.
- There seems to be a decoupling between fascicle and muscle-tendon unit length changes during the late swing phase.
- The study's findings are likely applicable to higher running speeds, as previous research shows similar muscle-tendon unit length changes across different speeds.
- The degree of fascial lengthening in the present study differed from the computer simulation and may be related to connective tissue compliance and overestimation of tendon stiffness in the simulation.

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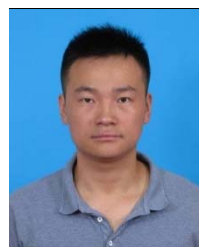
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