



Antagonist muscle architecture and aponeurosis/tendon strain of biceps femoris long head during maximal isometric efforts

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Abstract

Introduction Examination of the mechanical behavior of the hamstrings when acting as antagonists provides information about loading of this muscle group and its role for joint stability during forceful quadriceps contractions. The aim of this study was to quantify biceps femoris long head fascicle length (FL), angle of pennation (PA) and distal tendon/aponeurosis strain during maximum voluntary contraction efforts of the knee extensors using real-time ultrasound.

Methods Fourteen participants performed passive joint movements and maximum voluntary knee extension and flexion efforts of the knee flexors at 0°, 45° and 90° of knee flexion. An ultrasound probe was used to visualize FL, PA and tendon/aponeurosis strain from the distal part of the muscle.

Results Two-way analysis of variance designs indicated that: (a) antagonist BF_{lh} tendon/aponeurosis strain increased significantly up to $2.77 \pm 1.25\%$ relative to rest ($p < 0.05$). The FL increased non-significantly ($2.86 \pm 6.81\%$) while the PA was unaltered during isometric MVC efforts of the knee extensors ($p > 0.05$) (b) FL, PA and tendon/aponeurosis strain of the BF_{lh} when acting as antagonist were not significantly affected by knee joint angular position ($p > 0.05$).

Conclusions Antagonist hamstring function takes the form of a lengthened tendon/aponeurosis, no fascicle shortening and submaximal neural activation. Future research could examine whether exercise interventions that aim to alter tendon/aponeurosis mechanical properties of the hamstrings when acting as antagonists are beneficial for injury prevention and rehabilitation.

Keywords Hamstrings · Architecture · Ultrasound · Muscle mechanics · Agonist and antagonist · EMG activity · Isometric contraction

Abbreviations

BF _{lh}	Biceps femoris long head
EMG	Electromyography
FL	Fascicle length
FL _e	Effective fascicle length
MVC	Maximum voluntary contraction
LMTU	Muscle–tendon unit length
PA	Pennation angle
RMS	Root mean square
US	Ultrasound

Introduction

Hamstring muscle function is important for the performance of demanding skills, such as high-intensity sprinting or changes in direction (Thelen et al. 2005; Opar and Serrall 2014). Alterations in hamstring activation during late swing phase of running may be critical for hamstring injury (Timmins et al. 2014). Early and sufficient activation of the hamstrings prior to ground contact is considered important for controlling knee joint stability (Gazendam and Hof 2007; Kellis and Liassou 2009).

The function of the hamstrings as antagonists during maximal efforts of the knee extensors has been extensively examined using electromyography (EMG) (Baratta et al. 1988; Kellis and Baltzopoulos 1997). These studies have shown that antagonist activation during isometric and isokinetic tests can reach 15% of maximum voluntary contraction (MVC) value (Baratta et al. 1988; Kellis and Baltzopoulos 1997; Kellis et al. 2014). Using EMG-based models, the

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predicted antagonist torque of the hamstrings could account for approximately 5–13% of the torque recorded by the isokinetic dynamometer (Kellis and Baltzopoulos 1997; Kellis 2003; Kellis and Katis 2008). However, the EMG signal provides an indication of neural activation of the muscle and cannot describe the role of the non-contractile components.

Like any striated muscle, the force exerted by the hamstrings is a result of both contractile and passive (in-series and parallel elastic) components of the muscle. During an isometric knee flexion contraction, the biceps femoris long head (BF_{lh}) muscle fascicles shorten by as much as 20% (Bennett et al. 2014) while distal tendon/aponeurosis lengthens up to 7% (Kellis 2016). These data, however, may not apply to conditions where the muscle acts as antagonist, because in the latter condition, the neural activation is submaximal and the muscle–tendon unit may lengthen due to maximum force exertion by the agonist muscle (quadriceps) (Simoneau et al. 2012). Consequently, it is possible that when acting as antagonist, muscle fascicles and tendon/aponeurosis show a different mechanical behavior from the one predicted based on corresponding agonist contraction data.

The mechanics of antagonist muscle co-contraction have received less attention in the literature (Simoneau et al. 2012; Raiteri et al. 2015). Particularly, Simoneau et al. (2012) found that when acting as antagonists, the fascicle length (FL) of the gastrocnemius remained relatively unchanged while the tibialis anterior FL increased approximately by 5%. Nevertheless, at the same level of activation, the FL was greater and pennation angle (PA) was smaller, during antagonist than during agonist contractions. This was attributed to a change in joint position which increased FL and cancelled fascicle shortening when the muscle co-contracts (Simoneau et al. 2012). This was confirmed by Raiteri et al. (2015) who reported insignificant changes in FL of ankle muscles when acting as antagonists. To our knowledge, the changes in muscle architecture and tendon strain when the hamstrings function as antagonists are unknown.

The contribution of the tendon and muscle to the mechanical properties of the muscle–tendon unit varies depending on the changes in initial length (Herbert and Crosbie 1997; Herbert et al. 2002; Hoang et al. 2007; Kwah et al. 2012). Antagonist hamstring muscle activation and predicted moment of force vary with joint angle, being greater near knee extension than more flexed knee joint angles (Kellis and Baltzopoulos 1997; Kellis 2003; Kellis and Katis 2008). In addition, changes in neuromuscular properties of the muscle during isometric contractions may depend on the initial muscle length (Muraoka et al. 2004) and this effect differs between the hamstrings and the quadriceps (Hannah et al. 2014). Consequently, during agonist contractions of the quadriceps, a different response of BF_{lh} may be expected than when acting as agonist muscle itself.

Examination of the mechanical behavior of the muscle fascicles and the tendinous tissue of the hamstrings may provide interesting information regarding the mechanisms of force production of this muscle when acting as antagonist. This could be useful for the understanding of the mechanical loading of the hamstrings during maximal contractions of the quadriceps and the role of this load for knee joint stability. Consequently, the purpose of this study was to quantify BF_{lh} muscle and tendon/aponeurosis architecture during agonist and antagonist contractions. Based on the reviewed literature, the following hypotheses were tested: (a) that FL of the BF_{lh} would increase while PA would be unaltered during antagonist contractions, (b) antagonist tendon/aponeurosis strain would increase from rest to MVC, and (c) that FL, PA and tendon/aponeurosis strain of the antagonist muscle contractions would differ between joint angular positions tests.

Methods

Participants

A total of 14 males (age 20.1 ± 0.3 years; mass 81.5 ± 2.3 kg; height 1.77 ± 0.02 m) volunteered to participate in this study after signing written informed consent. The participants were healthy, and they had no injury of the lower limbs including history of hamstring strain or any other muscle or ligamentous injury of the knee. The participants were physically active, but they did not engage in a specific sport or exercise program during the measurement period. The procedures are confirmed with the Declaration of Helsinki and were approved by the University ethics review committee.

Procedure

The tests were performed on a Cybex (Humac Norm, CSMI, MA, USA) dynamometer with the subject in the prone position. The axis of rotation of the dynamometer was carefully aligned with the approximate center of rotation of the knee, on the posterior aspect of the lateral femoral condyle. Axis alignment was checked both at rest and maximum contractions to reduce errors occurring due to joint movement. A twin-axis goniometer (Model TSD 130B, Biopac Systems, Inc., Goleta, CA, USA) was used to record knee angular position (0° = full knee extension). The goniometry signal was fed through BNC connectors (Model CLB107, Biopac Systems) to a 12-bit analog-to-digital converter sampling at a rate of 1000 Hz per channel using the Acknowledge (version 3.9.1, Biopac Systems) software. Ultrasonic images from the BF_{lh} were recorded using an ultrasonic apparatus (SSD-3500, ALOKA, Japan) with an electronic linear array probe of 10 MHz wave frequency and a length of 6 cm.

Dynamometer torque and knee joint angular position were simultaneously recorded at 1000 Hz. The video-capturing module of the system software allowed simultaneous recording of the ultrasound video images at a rate of 30 Hz.

The EMG signal from the BFlh was collected using a pair of bipolar bar surface electrodes (inter-electrode distance 1 cm, TSD 150B, Biopac System Inc., Goleta, CA, USA) which were positioned on the BFlh muscle belly, proximally to the US probe. The skin was shaved and cleaned with alcohol wipes. The ground electrode was placed over a bony landmark on the lateral epicondyle. The EMG signal was amplified (gain $\times 1000$) with an input impedance of 10 M Ω and a common rejection ratio of 130dB. The signal was filtered using a band pass filter (low 15 Hz and high 450 Hz, full-wave rectified) and the root mean square (RMS) was displayed on-line during each trial.

To quantify the effects of changes in joint position on recorded measurements, the participants first performed five passive extension–flexion trials at a very slow angular velocity (2° s^{-1}). The range of motion was from 100° of knee flexion to about 5° hyper-extension ($0^\circ =$ full knee extension). Each trial started from 90° to 45° of knee flexion, a 2-s pause, and then passive movement from 45° to 0° of knee flexion. Participants were asked to remain completely relaxed throughout all trials.

The main protocol included a total of 18 maximum isometric contractions (MVCs) from 0° , 45° and 90° knee flexion angles. Specifically, in each angular position the participant performed five submaximal (warm-up) and three maximal isometric contractions (MVCs) of the knee extensors followed by three MVCs of the knee flexors. The duration of the contraction was 5 s. The order of performance was randomized across angular positions and muscles.

The limb gravitational torque was recorded from a static position of 30° of knee flexion to allow correction of the recorded torque for gravitational effects. Knee flexion and extension MVC torque obtained in each angular position were further analyzed.

The EMG produced during the isometric MVC was measured during a 2-s epoch at the torque plateau. EMG activity obtained during knee extension MVC (antagonist contraction) was expressed as a percentage of maximum EMG during the flexion MVC at the corresponding angular position (agonist contraction).

Ultrasound measurements

Ultrasound measurements (US) were performed by the same operator with more than 10 years of experience in musculoskeletal US of the hamstrings. Particularly, starting from the distal origin, the probe was positioned approximately at 40–45% of muscle–tendon unit length (defined as the distance between distal and proximal ends of muscle) and

the position was marked on the skin. This location allowed visualization of the most distal fascicles and intermediate tendon of the BFlh. An echo absorptive marker was placed in the US field of view such that any random movement of the probe is recorded. Ultrasound video footages were automatically stored in digital format by the software.

In each video ultrasound footage, using a video-based software (Max Traq Lite version 2.09, Innovision Systems, Inc., Columbiaville, Mich. U.S.A) the ends of two fascicles were manually digitized in the distal region of the BFlh, starting at the fascicle's superficial origin and ending at the fascicle's insertion onto the intermediate (deeper) aponeurosis (Fig. 1). When the FL exceeded the field of view, a

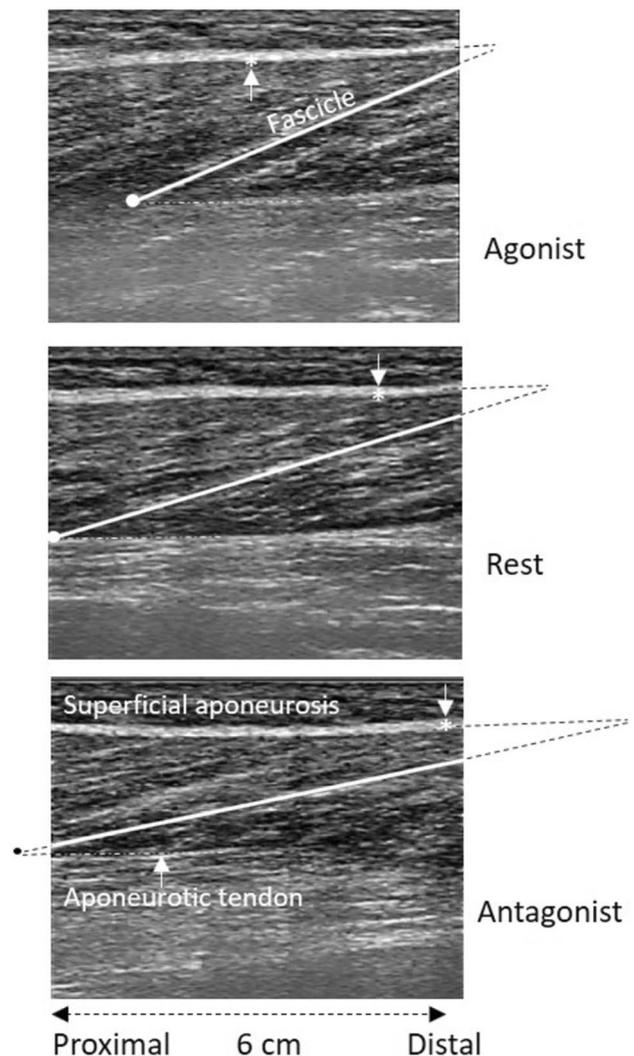


Fig. 1 Example US images of the BFlh at rest and when the muscle was activated as agonist (knee flexor contraction) and antagonist (knee extension contraction). The fascicle length was defined as the linear distance between the superficial and central aponeurosis. Further, in each image a vertical arrow points to the intersection of a fascicle and the superficial aponeurosis (indicated as an asterisk)

straight line was drawn along the visible trajectory of the fascicle until it intersected with the corresponding straight line of the intermediate aponeurosis. Subsequently, the FL was directly measured as the distance between the visible end and the intersection between the extended FL and intermediate tendinous lines (Ando et al. 2014). The angle between the line marking the outlined fascicle and the deep aponeurosis was then measured giving the PA. An average of the lengths of three muscle fascicles was further analyzed.

In addition, the absolute FL was multiplied by the cosine of the pennation angle. This provided a measure of the effective length of the fascicles (FLe), which is the length of the fascicles projected onto the long axis of the muscle (Diong et al. 2012).

Tendon/aponeurosis elongation

Tendon/aponeurosis elongation was quantified by re-digitizing the same US video footages in two points: the most distal intersection of a fascicle with the superficial aponeurosis of the muscle (Fig. 1) and a reference skin hyperechoic marker to account for any displacement due to probe movement.

Resting length was measured at 40° of knee flexion (where the passive moment is almost zero (Silder et al. 2007)). The curved path from the distal origin to each of the markers along the skin surface corresponding to the digitized points (Kellis et al. 2009) was quantified using a flexible tape. The mean (\pm SD) distance of each marker from their origin was 209.55 ± 22.8 mm, corresponding to $58.3 \pm 5.2\%$ of whole muscle–tendon length. Since rest length values were taken at 40° degrees, displacement of the tendon/aponeurosis point from 40° to 0° (when muscle–tendon unit (MTU) lengthened) was considered as negative whilst displacement from 40° to 90° (when MTU shortened) was considered as positive. Validity and reliability of the US measurements have been reported elsewhere (Kellis et al. 2009, 2017; Kellis 2018). Tendon/aponeurosis strain was estimated by dividing the recorded displacement of the specific tendon/aponeurosis segment by its rest length.

During isometric contractions, the knee may show some motion which can introduce an overestimation of the changes in each architecture parameter (Fig. 2). From the goniometer data, it was found that the average change in angular position during knee extension MVCs (antagonist BFlh contraction) was $4.4^\circ \pm 1.6^\circ$, $9.1^\circ \pm 1.2^\circ$ and $6.3^\circ \pm 1.4^\circ$, for 0°, 45° and 90° knee flexion angles, respectively. The corresponding values for knee flexion MVCs (agonist BFlh contraction) were $4.3^\circ \pm 0.4^\circ$, $4.9^\circ \pm 0.7^\circ$ and $3.1^\circ \pm 0.4^\circ$, for 0, 45 and 90° knee flexion angles, respectively. The change due to passive motion ranged from 0.52 ± 0.13 mm to 1.82 ± 0.37 mm (FL), $0.60^\circ \pm 0.22^\circ$ to $1.61^\circ \pm 0.35^\circ$ (PA) and from 2.40 ± 0.41 mm to 5.21 ± 1.54 mm (tendon/aponeurosis displacement).

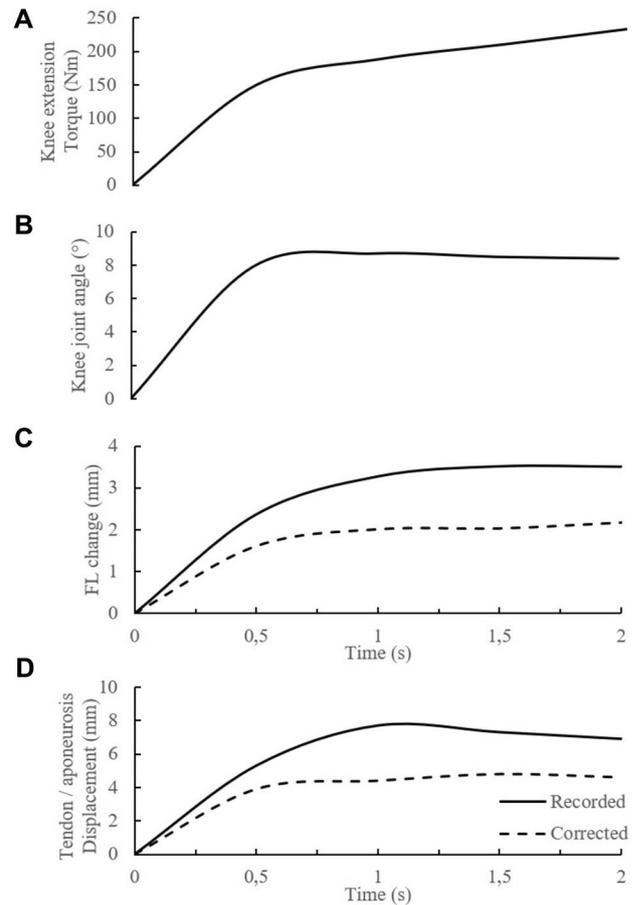


Fig. 2 Torque (a) and knee joint angle (b) changes for one subject during the first 2 s of the maximum isometric contraction of the knee extensors at an angular position of 45°. Changes in architecture were recorded during passive joint motion and used to correct the recorded values during contraction. In c, the changes in recorded (solid lines) and corrected (dashed lines) fascicle lengths of the biceps femoris long head are presented. In d, the corresponding changes in the recorded and corrected for passive motion tendon/aponeurosis strain are illustrated

Subsequently, passive motion values were subtracted from the changes of each variable during contraction (Fig. 2).

In each angular position test, torque, absolute FL, PA, FL strain, FLe, tendon/aponeurosis elongation and strain were determined during knee extension and flexion efforts (Fig. 3).

Statistical analysis

MVC torque differences between knee joint angles and direction (extension–flexion) were examined using a two-way analysis of variance (ANOVA). Antagonist EMG was compared between the three joint positions using a one-way ANOVA. Two-way analysis of variance (ANOVA) designs were used to examine the effects of knee joint

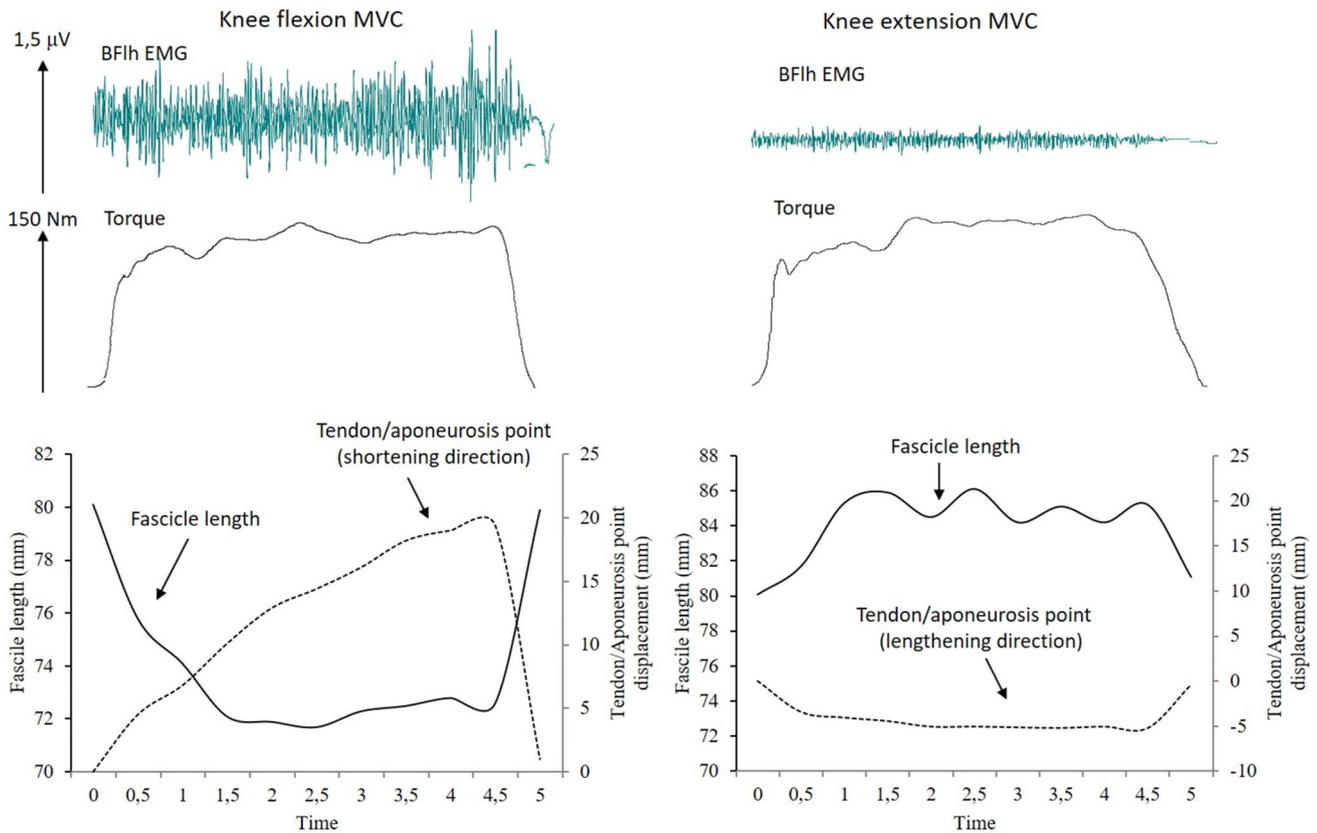


Fig. 3 An example of change in EMG, torque, measured fascicle length and tendon/aponeurosis displacement during maximum voluntary contraction of the knee extensors (left panel of graphs) and flexors (right panel of graphs) performed at full extension (0° knee flexion angle). EMG and torque were extracted from the recording software while fascicle length and tendon/aponeurosis displacement

were estimated after digitizing the synchronized ultrasound video. In knee flexion contractions, tendon/aponeurosis lengthens as the fascicle shortens, and this is referred to as “shortening” direction. In contrast, during knee extension contraction, the tendon/aponeurosis point moves in the opposite direction and this is referred to as “lengthening” direction

angle (0°, 45° and 90°) and level of effort (rest, MVC) on FL and PA, for knee extension and flexion tests. Two-way ANOVA designs were also applied to examine the effects of knee joint angle and direction (extension–flexion) on fascicle strain, FLe, tendon/aponeurosis displacement and strain. Significant interactions were followed up with simple effect tests and, if significant, post hoc Tukey tests were applied to examine significant differences between pairs of means. Statistical significance was set at $p < 0.05$.

Results

Torque and activation

The MVC torque values and antagonist EMG values are presented in Table 1. There was a significant interaction (angle by direction) effect on recorded torque ($F_{2,26} = 16.10$, $p < 0.05$). Post hoc Tukey tests showed that knee flexion MVC torque was significantly greater at 0° and 45° compared with 90° while knee extension MVC torque was

Table 1 Mean (\pm SD) Torque during knee flexion and extension maximum isometric contractions and antagonist normalized EMG (*significantly greater compared with 90° at $p < 0.05$)

Angle (°)	Torque (Nm)		Antagonist normalized EMG (% MVC) during knee extension
	Knee flexion	Knee extension	
0	122.13 \pm 25.62*	127.01 \pm 26.99	7.41 \pm 3.11*
45	124.32 \pm 22.99*	193.97 \pm 39.35*	6.32 \pm 2.99
90	93.68 \pm 17.16	127.12 \pm 33.35	5.34 \pm 1.83

significantly greater at 45° compared with the rest positions. The normalized antagonist EMG ranged from 5.36 ± 2.18 to $7.57 \pm 2.87\%$ and it was significantly different between joint angles ($F_{2,26} = 8.83, p < 0.05$). Post hoc Tukey test indicated that antagonist EMG at 0° was significantly greater than EMG recorded at 90° ($p < 0.05$).

Architecture measurements

The FL and PA values for each testing condition are presented in Table 2. There was not a significant interaction (angle by level of effort) effect on FL and PA in all testing conditions ($p > 0.05$).

For agonist BFlh contractions, there was a significant main effect for level of effort ($F_{1,13} = 157.59, p < 0.05$) and angle ($F_{2,26} = 39.01, p < 0.05$) on FL. Post hoc Tukey tests showed that FL significantly decreased from rest to MVC and it was greater at 0° compared with 45° and 90° ($p < 0.05$). Similarly, the main effect for level of effort

($F_{1,13} = 115.05, p < 0.05$) and angle ($F_{2,26} = 8.13, p < 0.05$) on PA was also significant. Post hoc Tukey tests showed that the PA significantly increased from rest to MVC and it was smaller at 0° compared with 45° and 90° ($p < 0.05$).

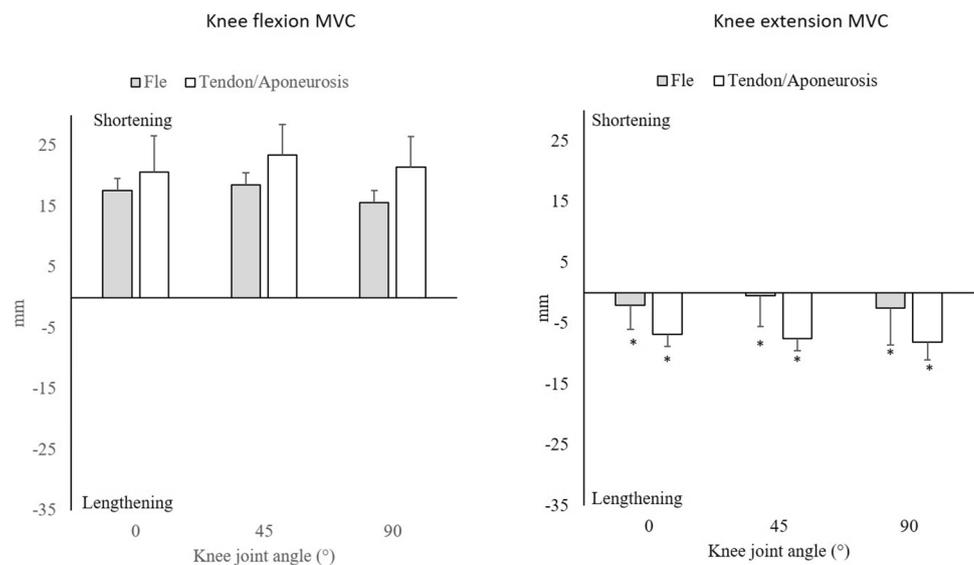
When the BFlh functioned as antagonist, the main effect for angle ($F_{2,26} = 61.98, p < 0.05$) was significant while level of effort did not have a significant effect ($F_{1,13} = 2.72, p > 0.05$). Post hoc Tukey tests showed that FL was greater at 0° compared with 45° and 90° ($p < 0.05$). In contrast, non-significant effects of independent variables on PA during knee extension were found ($p > 0.05$).

Figure 4 presents the FLe and tendon/aponeurosis segment length in each condition. The FLe was significantly higher during knee flexion MVC compared with knee extension MVC ($F_{1,13} = 161.75, p < 0.05$) but no other significant effects were found ($p > 0.05$). Tendon/aponeurosis displacement was significantly greater during extension compared with flexion ($F_{1,13} = 83.3, p < 0.05$) but it did not differ between the three joint angular positions ($p > 0.05$).

Table 2 Mean (\pm SD) fascicle length and pennation angle during each testing condition (*value collapsed across levels of effort significantly different compared with 45° and 90° at $p < 0.05$, ^ value collapsed across angles significantly different compared with rest value, $p < 0.05$)

Joint angle	Flexion		Extension	
	Rest	MVC	Rest	MVC
Fascicle length (mm)				
0°	86.75 \pm 5.62* [^]	71.51 \pm 7.15*	84.92 \pm 5.60*	86.64 \pm 7.01*
45°	81.63 \pm 5.85 [^]	65.77 \pm 8.05	80.45 \pm 6.71	81.62 \pm 7.33
90°	77.44 \pm 6.07 [^]	60.23 \pm 5.19	76.23 \pm 6.66	78.37 \pm 7.83
Pennation angle (°)				
0°	16.19 \pm 3.95* [^]	23.43 \pm 3.62*	17.05 \pm 3.83	18.14 \pm 4.71
45°	18.82 \pm 3.27 [^]	26.72 \pm 4.14	18.35 \pm 3.79	18.78 \pm 4.22
90°	20.50 \pm 4.05 [^]	25.83 \pm 4.62	19.92 \pm 4.60	18.87 \pm 4.50

Fig. 4 Mean (\pm SD) change in effective fascicle length and tendon/aponeurosis strain (mm) of the BFlh during MVC of knee extensors (right graph) and flexors (left graph). Positive values indicate shortening of the fascicles and lengthening of the tendon/aponeurosis in the shortening direction of the muscle. Negative values indicate lengthening of the fascicles and lengthening of the tendon/aponeurosis in the direction opposite to the contraction. Error bars indicated standard deviation (*significantly different compared with knee flexion value at $p < 0.05$)



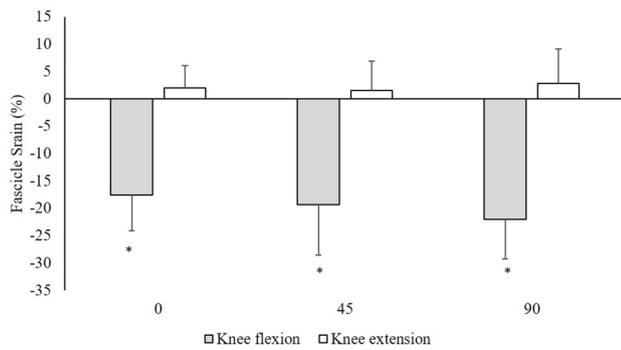


Fig. 5 Mean (\pm SD) fascicle strain values of the biceps femoris long head (BFLh) during MVC of knee extensors and flexors at 0°, 45° and 90° angular positions (*significant difference with extension value at $p < 0.05$)

Table 3 Mean (\pm SD) strain (%) of the distal tendon/aponeurosis segment during each testing condition (positive value indicates displacement in the shortening direction, *significantly different compared with 90°, $p < 0.05$, #significantly different compared with 90°, $p < 0.05$, ^value collapsed across angles significantly different compared with extension, $p < 0.05$)

Angle (°)	Passive	Knee flexion MVC	Knee extension MVC
0	-4.44 \pm 1.39*#	8.80 \pm 3.73^	-2.32 \pm 0.88
45	0.41 \pm 0.10*	8.32 \pm 2.14^	-2.55 \pm 1.01
90	7.30 \pm 1.73	7.39 \pm 1.75^	-2.77 \pm 1.25

Fascicle and tendon/aponeurosis strain

The FL strain per condition is reported in Fig. 5. During knee flexion, the FL shortened up to a maximum of $21.96 \pm 7.24\%$ (at 90° of knee flexion). During knee extension, the FL increased up to $2.86 \pm 6.96\%$ (at 90° of knee flexion). The ANOVA showed a non-significant interaction (angle x direction) effect on FL strain values during MVC efforts ($p > 0.05$). The main effect for angle was also non-significant ($p > 0.05$). There was, however, a significant difference in FL strain between extension and flexion ($F_{1,13} = 192.97, p > 0.05$).

The recorded tendon/aponeurosis strain values are reported in Table 3. The ANOVA showed a non-significant interaction (angle x direction) effect on tendon/aponeurosis strain values during MVC efforts ($p > 0.05$). Strain was significantly greater during extension compared with flexion ($F_{1,13} = 101.52, p < 0.05$) but it did not differ between the three joint angular positions ($p > 0.05$). Strain recorded as the knee passively moved from one position to another (passively) was significantly different between all pairs of joint angles ($F_{2,26} = 102.08, p < 0.05$).

Discussion

The main findings of this study are that antagonist co-contraction of the BFLh was achieved via an increase in tendon/aponeurosis strain while the FL and PA remain unaltered. Further, changes in BFLh architecture during antagonist contraction were not significantly affected by knee joint angular position. To our knowledge, no previous studies have examined BFLh muscle–tendon mechanics when acting as antagonist.

Performance of maximum isometric contractions is accompanied by passive rotation of the dynamometer lever arm and the joint as the level of effort increases, and this can lead to an overestimation of the changes in architecture (Karamanidis et al. 2005). For antagonist co-contractions, this effect may be relatively higher, as the antagonist muscle works at submaximal levels of effort and changes in architecture due to contraction may be masked by changes in architecture due to passive joint rotation. Consequently, this methodological issue is important to consider prior to interpretation of antagonist mechanical behavior. In the present study, the change in joint position when performing MVCs was monitored and all architectural data obtained due to passive joint motion were removed from the final measurements. If correction is not performed, the antagonist FL and tendon/aponeurosis strain would be overestimated. The effect varies between joint positions, being greater for knee extension MVCs performed from 45° knee joint angle (the highest quadriceps forces cause highest joint rotation) and can result in an overestimation of antagonist BFLh tendon/aponeurosis strain of almost 50% (Fig. 2). It should be noted, however, that despite the special care in stabilizing the participant in chair position, complete prevention or control of changes in angular position and soft tissue deformation is difficult, and it varies significantly between participants (Karamanidis et al. 2005).

The first hypothesis was that the FL increases and PA is unaltered when BFLh acts as antagonist. Our results partly confirmed this hypothesis, as both antagonist FL and PA did not change from rest to knee extension MVC (Table 1). Some studies (Simoneau et al. 2012; Raiteri et al. 2015) reported similar changes of the antagonist gastrocnemius FL and PA from rest to MVC efforts. However, Simoneau et al. (2012) found that antagonist tibialis anterior FL increased and PA remained unaltered from rest to MVC. This might indicate that responses of antagonist architecture to agonist maximum contractions may vary between muscles. For the BFLh, it appears that fascicles operated at an isometric mode when the quadriceps worked maximally. Further, the insignificant change in antagonist PA indicates that the force transfer from the muscle fascicles to the tendon did not change from rest to MVC.

The increase in tendon/aponeurosis strain of the BFlh when acting as antagonist confirms our second hypothesis (Table 3). The direction of superficial tendon/aponeurosis elongation differed between agonist and antagonist contractions. When the muscle contracted as agonist, its distal superficial tendon/aponeurosis point shifted to the fascicle shortening direction (Table 3). When the muscle co-contracted as antagonist, the tendon/aponeurosis shifted to the lengthening direction (opposite to fascicle shortening). This indicates that the mechanical behavior of the BFlh whole muscle–tendon unit differs between agonist and antagonist activation.

Interpretation of changes in tendon/aponeurosis strain should be seen relative to the change in muscle FL, PA as well as the level of muscle activity when the BFlh acts as antagonist. When the BFlh acted as agonist (knee flexion MVCs), the effective FL was reduced by approximately 17 mm and the distal tendon/aponeuroses segment lengthened (in the same direction) by almost 20 mm (Fig. 4). In contrast, antagonist co-contraction was accomplished via a very small increase in FL of the muscle (~2 mm) and, a relatively greater increase in superficial tendon/aponeurosis displacement (~6 to 8 mm) in the lengthening direction (Fig. 4). This suggests that when the hamstrings co-contrast, their muscle–tendon unit lengthens while the fascicles contract almost isometrically. It should be mentioned, however, that during agonist contraction, the deep aponeurotic tendon shifts distally to accommodate fascicle shortening and muscle thickening. In contrast, when activated as antagonist, the deep aponeurotic tendon showed variable behavior. This might have resulted in high individual variability of antagonist FL strain (Fig. 5).

Simoneau et al. (2012) proposed that the changes in antagonist muscle FL during isometric MVCs may be transient, reaching a “steady state” when tension is maintained for a certain period of time. They observed that the antagonist lengthens at the beginning of the contraction and it remains at the same length for the rest of the MVC. They attributed this behavior to the changes in joint angular position during contraction. This appears to be supported by the present findings. Particularly, although time-related changes during MVC were not quantified, it was observed that lengthening occurs mainly during the initial phase of the contraction and it still exists even after correction of joint motion was performed (Fig. 3). Consequently, compared with the rest condition, the distal tendon/aponeurosis lengthens due to knee extension contraction and perhaps some changes of internal bones’ position or soft tissue deformation, which is unidentifiable by the digital goniometer attached to the knee joint.

Some factors may have contributed to the aforementioned observations. First, it was suggested that at submaximal activation levels, the muscle exerts higher force when it

contracts as an antagonist rather than as an agonist, meaning that neuromuscular efficiency would be higher during antagonist than agonist contractions in vivo (Simoneau et al. 2012). This was attributed to a “residual force enhancement” which is activated when the muscle is stretched prior to isometric contraction (Herzog and Leonard 2002; Simoneau et al. 2012); the additional force is provided by both passive and active muscle components of the muscle (Herzog and Leonard 2002). Second, Hannah et al. (2014) reported that hamstring electromechanical delay was almost double than that of the quadriceps which was attributed to a longer series elastic component and less type II fiber percentage of the hamstrings compared with the quadriceps. Hence, hamstring muscle fibers need to contract for a longer time to take the slack of the tendons (Hannah et al. 2014). It is not clear whether this longer mechanical response of the hamstrings takes place when they are activated as antagonists. If this is the case, however, then for a short initial period of the knee extension contraction, the antagonist muscle would lengthen almost passively. Third, passive elongation of the BFlh tendon (including the aponeuroses) contributes about half of the total compliance of the relaxed MTU, mainly because the tendinous tissue is much longer than the muscle fascicles (Kellis 2018). This provides an explanation for the observed changes in tendon/aponeurosis and fascicle architecture when the muscle co-contracts.

Variations in joint position between agonist and antagonist co-contractions may also result in different moment arms of the muscle between the two conditions (Simoneau et al. 2012). The moment arm of the BFlh increases from 90° to 25° and decreases thereafter (Kellis and Baltzopoulos 1999). This means that, when the muscle acted as antagonist, its moment arm was probably reduced during MVCs performed from 0° knee flexion angle while the opposite occurred at 45° and 90° tests. This indicates that antagonist torque of the muscle would be less than corresponding agonist torque at 0° but it would be greater at 45° and 90° tests. Given that the amount of joint rotation during MVC is less than 10°, the effect of change in moment arm is likely to be small but it is not minimal. The net effect of these changes would be that antagonist torque predicted using EMG-torque models would be slightly overestimated at 0° and underestimated at 45° and 90°.

In the present study, the EMG activity of the BFlh was approximately 5–9% MVC indicating that the hamstrings were activated submaximally as antagonists (Table 1). It has been reported that for the same level of activation, FL is greater when the muscle acts as antagonist compared with agonist FL, indicating that the muscle fibers act in an eccentric mode (Simoneau et al. 2012). Others, however, have suggested that the minimal changes in antagonist FL point to a low capacity to produce active force, suggesting that the recorded antagonist EMG activity is simply cross-talk

(Raiteri et al. 2015). Based on our ultrasound findings, complete relaxation of antagonist musculature to agonist maximum contractions seems unlikely. It appears that tendon/aponeurosis mechanical behavior differs between agonist and antagonist contractions, such that during agonist contractions the MTU shortens while during antagonist contractions, the MTU lengthens. Whether these changes indicate a greater efficiency when the muscle acts as antagonist, as previously suggested (Simoneau et al. 2012), is unclear.

The third hypothesis examined in this study was that changes in mechanical responses of the antagonist muscle–tendon unit during MVC would differ between joint positions. Our results, however, did not confirm this suggestion (Table 2). The non-significant effect of angular position on recorded parameters was also observed during agonist MVC knee flexion contractions which is in line with previous findings (Kellis 2016). This indicates that for this specific range of joint angle positions, initial muscle length had no particular effect on any of the architectural parameters tested. This is not in line with previous studies who have shown that vastus lateralis (Fukunaga et al. 1997) and tibialis anterior (Fukutani et al. 2017) fascicle shortening during contraction was greater when the contraction was performed from shorter initial length compared with longer lengths. This was attributed to a greater slackness of the muscle–tendon unit at shorter lengths than at longer lengths (Fukunaga et al. 1997; Fukutani et al. 2017). The absence of such difference in our results might be related to the range of tested joint angles. Particularly, it has been shown that when the hip is in prone position and the knee is flexed from 90° to 0°, the BFlh length is in the range of shortest lengths, at the ascending part of the force–length relationship (Chleboun et al. 2001; Kellis 2018). This might indicate that changes in length from 90 to 0° might have not been significant enough to cause a different amount of FL and tendon displacement between joint angular positions as in all cases the tendon might have been slack. In addition, the BFlh has a greater overall tendon length compared with vasti muscles and thus a greater slack is likely (Kellis et al. 2009; Hannah et al. 2014).

The present findings have some implications for quantification of antagonist muscle function, injury and rehabilitation. First, examination of hamstring antagonist muscle function using EMG recordings and estimation of antagonist torque using EMG–torque models provide only a rather inaccurate picture of its function. Future models incorporating both EMG and changes in architecture may yield more accurate predictions of hamstring antagonist torque. Second, the observation that hamstring antagonist muscle function is accomplished by tendon/aponeurosis lengthening at low-EMG activation levels may also provide additional insights into the function of this muscle during high-speed movements, such as running. For example, it has been reported

that during the terminal swing phase, the BFlh EMG activity is rather low [less than 25% MVC (Higashihara et al. 2015)] and the hamstrings lengthen either actively or passively. In these conditions, the hamstrings operate as antagonists as the knee extends from mid to late swing phase. If the large dominant quadriceps muscles provide the main power for knee extension and hip flexion, then one may suggest that for some period of time the hamstrings operate as antagonists by lengthening either passively or by contracting. There are some suggestions that the hamstrings work quasi-isometrically during this type of movement (Van Hooren and Bosch 2017). Consequently, our results may suggest that hamstring muscle lengthening may be initiated by tendon/aponeurosis lengthening and a quasi-isometric contraction of the fascicles. Future studies could investigate whether individuals who have stiffer or compliant tendon/aponeurosis of the BFlh are at greater risk to sustain injury. Further, future research could examine whether exercise interventions that aim to alter the mechanical properties of the BFlh tendon/aponeurosis are beneficial for injury prevention and rehabilitation.

Future research could examine whether exercise programs that aim to alter tendon/aponeurosis mechanical properties are beneficial for injury prevention or rehabilitation of the hamstrings or the anterior cruciate ligament.

There are several limitations of this study. First, US analysis refers to two-dimensional displacements while the tendinous muscle morphology is three-dimensional. Consequently, it was assumed that the aponeuroses and FLs are linear. Second, the estimated strain is specific to the chosen fascicles and tendon/aponeurosis segment. It is highly possible that FL and tendon/aponeurosis displacement differ along the BFlh. Third, quantification of tendon/aponeurosis strain was based on the evaluation of indirect skin-based measurement of rest length and only the superficial (distal) aponeurosis displacement. Implementation of new techniques, such as extended field of view US (Palmer et al. 2015) or three-dimensional US (Haberfehlner et al. 2016) may assist in better imaging of these structures but currently their application is restricted to static conditions. Nevertheless, the combination of such data with non-linear modeling of the FL and tendon/aponeurosis segments may provide further insights into muscle–tendon mechanical behavior *in vivo*. Although the reliability and validity of the protocol have been previously reported (Kellis et al. 2009, 2017; Kellis 2018), it should be mentioned that the quality of US images obtained from the hamstrings using conventional US systems such as the one used in this study is not as high as those reported for ankle muscles. This may be due to the presence of subcutaneous and intramuscular fat which influences US imaging of the hamstrings. Fourth, in the present study, BFlh architecture was evaluated from a specific combination of hip and knee joint angles (knee

flexes from 0° to 90° and hip is flexed 90°). These joint positions correspond to the short and intermediate lengths of the muscle (Chleboun et al. 2001). It is highly possible that changes in tendon and muscle architecture are different when tests are performed from greater muscle lengths. Finally, although special attention was given to ensure appropriate body stabilization, the effect of joint motion and soft tissue deformation on the recorded torque and architecture values was reduced but it was not neglected.

Conclusion

Antagonist co-contraction of the BFLh during isometric voluntary efforts of the knee extensors is accompanied by an increase in tendon/aponeurosis strain and insignificant changes in FL and PA. Prediction of hamstring torque from EMG data may provide inaccurate estimates of antagonist hamstrings torque. The role of tendon/aponeurosis is important for hamstring muscle loading and mechanical behavior during movements that are driven by forceful quadriceps contraction. Future research could examine whether exercise programs that aim to alter tendon/aponeurosis mechanical properties are beneficial for injury prevention or rehabilitation of injuries that are affected by hamstring function, such as hamstring strains or anterior cruciate ligament injuries.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

References

- Ando R, Taniguchi K, Saito A et al (2014) Validity of fascicle length estimation in the vastus lateralis and vastus intermedius using ultrasonography. *J Electromyogr Kinesiol* 24:214–220. <https://doi.org/10.1016/j.jelekin.2014.01.003>
- Baratta R, Solomonow M, Zhou BH et al (1988) Muscular coactivation. The role of the antagonist musculature in maintaining knee stability. *Am J Sports Med* 16:113–122
- Bennett HJ, Rider PM, Domire ZJ et al (2014) Heterogeneous fascicle behavior within the biceps femoris long head at different muscle activation levels. *J Biomech* 47:3050–3055. <https://doi.org/10.1016/j.jbiomech.2014.06.032>
- Chleboun GS, France AR, Crill MT et al (2001) In vivo measurement of fascicle length and pennation angle of the human biceps femoris muscle. *Cells Tissues Organs* 169:401–409
- Diong J, Herbert RD, Kwah LK et al (2012) Mechanisms of increased passive compliance of hamstring muscle-tendon units after spinal cord injury. *Clin Biomech* 27:893–898. <https://doi.org/10.1016/j.clinbiomech.2012.07.003>
- Fukunaga T, Kawakami Y, Kuno S et al (1997) Muscle architecture and function in humans. *J Biomech* 30:457–463
- Fukutani A, Misaki J, Isaka T (2017) Relationship between joint torque and muscle fascicle shortening at various joint angles and intensities in the plantar flexors. *Sci Rep*. <https://doi.org/10.1038/s41598-017-00485-1>
- Gazendam MG, Hof AL (2007) Averaged EMG profiles in jogging and running at different speeds. *Gait Posture* 25:604–614
- Haberfehlner H, Maas H, Harlaar J et al (2016) Freehand three-dimensional ultrasound to assess semitendinosus muscle morphology. *J Anat* 229:591–599. <https://doi.org/10.1111/joa.12501>
- Hannah R, Minshull C, Smith SL, Folland JP (2014) Longer electromechanical delay impairs hamstrings explosive force versus quadriceps. *Med Sci Sport Exerc* 46:963–972. <https://doi.org/10.1249/MSS.000000000000188>
- Herbert RD, Crosbie J (1997) Rest length and compliance of non-immobilised and immobilised rabbit soleus muscle and tendon. *Eur J Appl Physiol* 76:472–479. <https://doi.org/10.1007/s004210050277>
- Herbert RD, Moseley AM, Butler JE, Gandevia SC (2002) Change in length of relaxed muscle fascicles and tendons with knee and ankle movement in humans. *J Physiol* 539:637–645
- Herzog W, Leonard TR (2002) Force enhancement following stretching of skeletal muscle: a new mechanism. *J Exp Biol* 202:1275–1283. [https://doi.org/10.1016/S0021-9290\(97\)00079-1](https://doi.org/10.1016/S0021-9290(97)00079-1)
- Higashihara A, Nagano Y, Ono T, Fukubayashi T (2015) Differences in activation properties of the hamstring muscles during overground sprinting. *Gait Posture* 42:360–364. <https://doi.org/10.1016/j.gaitpost.2015.07.002>
- Hoang PD, Herbert RD, Todd G et al (2007) Passive mechanical properties of human gastrocnemius muscle tendon units, muscle fascicles and tendons in vivo. *J Exp Biol* 210:4159–4168. <https://doi.org/10.1242/jeb.002204>
- Karamanidis K, Stafiliadis S, DeMonte G et al (2005) Inevitable joint angular rotation affects muscle architecture during isometric contraction. *J Electromyogr Kinesiol* 15:608–616
- Kellis E (2003) Antagonist moment of force during maximal knee extension in pubertal boys: effects of quadriceps fatigue. *Eur J Appl Physiol* 89:271–280
- Kellis E (2016) Biceps femoris and semitendinosus tendon/aponeurosis strain during passive and active (isometric) conditions. *J Electromyogr Kinesiol* 26:111–119. <https://doi.org/10.1016/j.jelekin.2015.11.007>
- Kellis E (2018) Biceps femoris fascicle length during passive stretching. *J Electromyogr Kinesiol* 38:119–125. <https://doi.org/10.1016/j.jelekin.2017.11.015>
- Kellis E, Baltzopoulos V (1997) The effects of antagonist moment on the resultant knee joint moment during isokinetic testing of the knee extensors. *Eur J Appl Physiol Occup Physiol* 76:253–259
- Kellis E, Baltzopoulos V (1999) In vivo determination of the patella tendon and hamstrings moment arms in adult males using videofluoroscopy during submaximal knee extension and flexion. *Clin Biomech* 14:118–124
- Kellis E, Katis A (2008) Hamstring antagonist moment estimation using clinically applicable models: Muscle dependency and synergy effects. *J Electromyogr Kinesiol* 18:144–153
- Kellis E, Liassou C (2009) The effect of selective muscle fatigue on sagittal lower limb kinematics and muscle activity during level running. *J Orthop Sports Phys Ther* 39:210–220. <https://doi.org/10.2519/jospt.2009.2859>
- Kellis E, Galanis N, Natsis K, Kapetanios G (2009) Validity of architectural properties of the hamstring muscles: correlation of ultrasound findings with cadaveric dissection. *J Biomech* 42:2549–2554. <https://doi.org/10.1016/j.jbiomech.2009.07.011>

- Kellis E, Mademli L, Patikas D, Kofotolis N (2014) Neuromuscular interactions around the knee in children, adults and elderly. *World J Orthop* 5:469–485. <https://doi.org/10.5312/wjo.v5.i4.469>
- Kellis E, Ellinoudis A, Intziegianni K (2017) Reliability of sonographic assessment of biceps femoris distal tendon strain during passive stretching. *Ultrasound Med Biol* 43:1769–1779. <https://doi.org/10.1016/j.ultrasmedbio.2017.04.018>
- Kwah LK, Herbert RD, Harvey LA et al (2012) Passive mechanical properties of gastrocnemius muscles of people with ankle contracture after stroke. *Arch Phys Med Rehabil* 93:1185–1190. <https://doi.org/10.1016/j.apmr.2012.02.009>
- Muraoka T, Muramatsu T, Fukunaga T, Kanehisa H (2004) Influence of tendon slack on electromechanical delay in the human medial gastrocnemius in vivo. *J Appl Physiol* 96:540–544. <https://doi.org/10.1152/jappphysiol.01015.2002>
- Opar DA, Serpell BG (2014) Is there a potential relationship between prior hamstring strain injury and increased risk for future anterior cruciate ligament injury? *Arch Phys Med Rehabil* 95:401–405. <https://doi.org/10.1016/j.apmr.2013.07.028>
- Palmer TB, Akehi K, Thiele RM et al (2015) Reliability of panoramic ultrasound imaging in simultaneously examining muscle size and quality of the hamstring muscles in young, healthy males and females. *Ultrasound Med Biol* 41:675–684. <https://doi.org/10.1016/j.ultrasmedbio.2014.10.011>
- Raiteri BJ, Cresswell AG, Lichtwark GA (2015) Ultrasound reveals negligible cocontraction during isometric plantar flexion and dorsiflexion despite the presence of antagonist electromyographic activity. *J Appl Physiol* 118:1193–1199. <https://doi.org/10.1152/jappphysiol.00825.2014>
- Silder A, Whittington B, Heiderscheid B, Thelen DG (2007) Identification of passive elastic joint moment-angle relationships in the lower extremity. *J Biomech* 40:2628–2635. <https://doi.org/10.1016/j.jbiomech.2006.12.017>
- Simoneau EM, Longo S, Seynnes OR, Narici MV (2012) Human muscle fascicle behavior in agonist and antagonist isometric contractions. *Muscle Nerve* 45:92–99. <https://doi.org/10.1002/mus.22257>
- Thelen DG, Chumanov ES, Hoerth DM et al (2005) Hamstring muscle kinematics during treadmill sprinting. *Med Sci Sport Exerc* 37:108–114
- Timmins RG, Opar DA, Williams MD et al (2014) Reduced biceps femoris myoelectrical activity influences eccentric knee flexor weakness after repeat sprint running. *Scand J Med Sci Sports*. <https://doi.org/10.1111/sms.12171>
- Van Hooren B, Bosch F (2017) Is there really an eccentric action of the hamstrings during the swing phase of high-speed running? Part I: a critical review of the literature. *J Sports Sci* 35:2313–2321. <https://doi.org/10.1080/02640414.2016.1266018>