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Effects of Nordic hamstring exercise combined with glider exercise on hip flexion flexibility and hamstring passive stiffness

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ABSTRACT

Eccentric training proved to be effective in hamstring injury prevention; however, little is known about effects of eccentric hamstring training at long muscle length on hamstring flexibility. Hence, the aim was to evaluate the effect of eccentric training at long muscle lengths on flexibility and passive properties of the hamstring muscles. 34 physically active young adults were randomized to either the control or intervention group (6 weeks of eccentric hamstring training at long muscle length; control group resumed with their usual activities). Maximal passive hip flexion range of motion (ROM), passive hamstring stiffness, shear modulus and tendon length of the biceps femoris long head (BFlh) were measured pre- and post-intervention. A significant time × group effect was observed for maximal passive hip ROM. Post-hoc testing revealed a significant increase in the intervention group (+11.2%; $p < 0.001$; $d = 1.55$). Additionally, a significant time effect was shown for shear modulus in a relaxed position ($p < 0.001$). No significant interaction was shown for other parameters. Results indicate that eccentric hamstring training at long muscle length elicits large gains in hamstring flexibility, which are most likely not related to changes in passive hamstring stiffness or BFlh distal tendon length.

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KEYWORDS
Biceps femoris; range of motion; elastography; stiffness; tendon length

Introduction

Hamstring strain injury (HSI) is one of the most common sports injuries in a number of high-speed running sports: track and field (Edouard et al., 2016), soccer (Ekstrand et al., 2011), American football (Beaulieu-Jones et al., 2017), Australian rules football (Saw et al., 2018) and rugby (Brooks et al., 2006). In addition, HSIs have a high recurrence rate of 12–48% (Liu et al., 2012). Thus, gaining knowledge about risk factors and preventive measures for HSIs is of particular scientific and practical importance.

Various modifiable HSI risk factors have been documented in the literature, one of them being hamstring length or flexibility (Freckleton and Pizzari, 2013). Hamstring flexibility has been defined as a maximal passive or active range of motion (ROM) around the hip (straight leg raise test) or knee joint (knee extension test) (Miyamoto et al., 2018). Earlier animal studies have shown that lengthening of an activated muscle causes muscle injury (Lieber and Friden, 1993). Thus, more extensible (i.e., flexible) muscle could endure larger strains before injury occurs (Garrett, 1990). However, evidence from prospective studies that evaluated hamstring flexibility as a risk factor for HSI is equivocal (Freckleton and Pizzari, 2013).

According to Blazevich (2019), maximal muscle elongation cannot be explicitly tested in vivo in humans because the maximum volitional stretch limit of a joint is determined by the subjective threshold at which a person terminates the stretch and not tissue stiffness. Additionally, fascial tissue remote from area of interest may restrict joint ROM (Wilke et al., 2020). Therefore, tissue (muscle, tendon) stiffness, which is its resistance to stretch within the ROM, could be a more important risk factor for muscle strain injury than maximum joint ROM as a measure of muscle flexibility (Kay et al., 2016). Indeed, Watsford et al. (2010) have reported that elevated muscle-tendon stiffness of hamstring muscles in the preseason discriminate between players who are likely to sustain an acute hamstring injury in Australian football. These results suggest that training interventions that could reduce passive hamstring stiffness and increase its length could also be effective in decreasing hamstring injury rate. However, since the authors (Watsford et al., 2010) assessed hamstring stiffness using a free oscilation technique that does not measure stiffness of individual hamstring muscles, more studies are needed to characterise associations between hamstring flexibility, passive stiffness of hamstring muscles and hamstring injury risk.

Eccentric training proved to be effective in reducing the risk of HSIs (for review, see Van Dyk et al. 2019). It has also been shown that the eccentric training increases hamstring flexibility; however, this effect was seen only if at least some eccentric hamstring exercises were performed at long muscle length (e.g., single-leg Roman dead lift, Askling's diver, eccentric hip extension) (Delvaux et al., 2020; Nelson and Bandy, 2004). The term long muscle length has been previously used to describe the leg curl exercise performed with the hip in a flexed position (= 90°) (Guex et al., 2016). In studies that used eccentric exercises only at short muscle length (exercises with an extended

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Abbreviations: BFlh - Biceps Femoris long headHSI - Hamstring strain injuryNHE - Nordic hamstring exerciseROM - range of motionSWE - Shear wave elastography hip = 0°, e.g., Nordic hamstring exercise, prone lying leg curl), no or minor changes in hamstring flexibility were observed (passive hip flexion or knee extension ROM) (Potier et al., 2009; Ribeiro-Alvares et al., 2018), despite obtaining large increases (22–34%) in biceps femoris long head (BFlh) fascicle length (fascicle length is decoupled from muscle length due to its pennation geometry). Notably, none of those studies evaluated the effects of eccentric training on hamstring muscle or tendon stiffness. To our knowledge, only Seymore et al. (2017) studied the effects of eccentric hamstring training at short muscle length (i.e. NHE) on hamstring passive stiffness measured by passive resistive torque and shear wave elastography (SWE) and reported no change after 6 weeks of training. When eccentric training was applied on other muscle groups, the observed increase in joint ROM (Kay et al., 2016; Kay et al., 2018; Mahieu et al., 2008) was accompanied by either a significant decrease (Mahieu et al., 2008) or no change in passive stiffness of the muscle-tendon complex (Kay et al., 2016, Kay et al., 2018). Thus, we lack knowledge on how passive mechanical characteristics of the hamstring muscle-tendon complex adapt to eccentric training, particularly at long muscle length.

To address this issue, we have designed the study in which we evaluated the effects of 6-week progressive eccentric hamstring training at long muscle length on hamstring flexibility (i.e. maximal hip ROM), passive resistive torque and muscle stiffness. We have employed ultrasound SWE for quantifying muscle shear modulus as a measure of passive muscle stiffness (Eby et al., 2013). Since BFlh is the most frequently injured hamstring muscle (Askling et al., 2007), SWE and tendon length were measured for the BFlh. Based on the findings of previous studies, we postulated that isoinertial eccentric training at long muscle length would increase maximal ROM and reduce passive resistive torque. Additionally, we hypothesized that BFlh passive stiffness would decrease, and BFlh tendon length would remain unchanged after the intervention programme. In a related study (Marušič et al., 2020), conducted with the same protocol and participants, changes in architectural and functional characteristics of the hamstrings were observed. Eccentric training at long muscle length improved knee flexion strength and voluntary activation level, and it increased BFlh fascicle length and reduced its pennation angle.

Materials and methods

Participants

Forty healthy volunteers were recruited to participate in this study and randomized to either the control or intervention group. Thirty-four of them successfully completed the study. Their body mass, muscle mass and body fat percentage, evaluated using a bio-impedance scale (Tanita MC-980 MA, Tanita, Tokyo, Japan), is presented in Table 1. Minimal sample size of 16 participants in each of the two groups was determined a priori for 80% statistical power, an alpha error of 0.05 and an effect size of 0.75. This effect size was selected based on previous eccentric hamstring training studies that reported moderate to large increases (effect sizes > 0.8) in hamstring flexibility in untrained and moderately trained individuals (Delvaux et al., 2020; Nelson and Bandy, 2004). All participants were either currently participating in sports or had experience in sports training and were physically active at least 3 times per week (>3 h/week). The inclusion criteria was the ability to perform the traditional NHE through at least 50% of the full ROM (= 90° of knee flexion). The exclusion criteria were the presence of neural, musculoskeletal and connective tissue injuries in the back or lower extremity during the past 12 months. Participants were assigned either to the control group (n = 18) or the intervention group (n = 22). Both groups underwent the measurement procedure before and after the intervention. The intervention group performed a 6-week intervention training programme of isoinertial eccentric strengthening in a lengthened position. Participants in the control group were instructed to continue with their regular physical activities and lifestyle. The study was approved by the National Medical Ethics Committee (0120–690/2017/8) and conducted according to the Declaration of Helsinki.

Study procedure

Before the start of the measuring protocol, the participants were informed about the purpose, course and possible risks of participating in the study. Afterwards, they signed the informed consent form. Before the warm-up, the participants underwent the assessment of body composition, passive resistive torque, panoramic ultrasound imaging and muscle shear modulus evaluation with ultrasound SWE. The warm-up included 3 minutes of stepping on a 40 cm high stepper, 10 squats, single leg deadlifts, lunges, front bending and hip thrusts. Afterwards, we evaluated maximal passive ROM of the hamstring muscles. After 6 weeks of the exercise intervention, we repeated the measurement protocol. In the post-intervention measurements, the participants were scheduled in the same part of the day as in the pre-intervention measurements, to avoid possible bias resulting from the circadian rhythm. All measurements were performed only on the preferred (i.e., kicking) leg.

Intervention training programme

The intervention training programme included two eccentric exercises at long muscle lengths: the modified NHE (eccentric knee flexion; Figure 1a) and the glider (eccentric hip extension; Figure 1b). The modified NHE was performed bilaterally in a custom-designed device with adjustable slope of the lower

Table 1. Descriptive statistics of the study participants (mean ± SD).

Group	Male/ female	Age [years]	Height [m]	Mass [kg]	BMI [kg/ m^2]	Body fat [%]	Muscle mass [%]
Intervention	12/6	24.2 ± 2.1	1.77 ± 0.09	73.0 ± 14.3	23.0 ± 3.2	15.1 ± 5.3	80.7 ± 5.1
Control	12/4	23.0 ± 2.8	1.79 ± 0.07	75.1 ± 15.1	23.2 ± 3.5	16.3 ± 6.7	79.6 ± 6.4

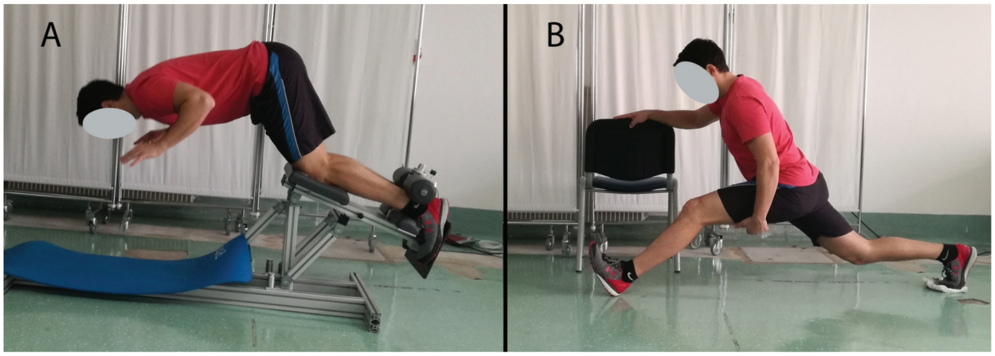

Figure 1. The modified Nordic hamstring exercise (A) and the glider (B).

leg support (S2P Ltd., Science to practice, Ljubljana, Slovenia). Šarabon and colleagues (2019) used the device in a previous study and demonstrated that modifying the NHE by adding hip flexion leads to achieving peak hip and knee torques and maximal hamstring muscle activity at longer muscle lengths. Participants were instructed to maintain the hip in 75° of flexion throughout each repetition of the NHE. A supervisor was in charge of correcting the hip angle using a goniometer when necessary. The glider exercise was previously described by Askling et al. (2014) and is part of the Askling L-protocol. Both legs were trained in the glider exercise. The frequency of training sessions was maintained throughout the study period (2 x week). The volume progression for both exercises is presented in Table 2. Weight was added to increase the difficulty of the exercise, based on the participants' value of their rate of perceived exertion during their last repetition (if it was below 8, the weight was increased). Extra weight ranged from 5 to 10 kg for the NHE (weight plate held with both hands in front of chest) and 8–20 kg for the glider (kettlebell held in the unsupported hand). Weight was added in the 3rd week, when the participant was able to perform at least 5 controlled repetitions until the last 20° of knee extension for the NHE. At the beginning and end of each training session participants reported their perceived muscle soreness from the previous session and the rate of perceived exertion for the current session, respectively.

Maximal passive range of motion

Maximal passive ROM assessment was performed with the participant lying supine on a therapeutic table with the passive straight leg raise test. The first examiner stabilized the opposite thigh and performed hip flexion while maintaining the knee fully extended. The second examiner measured the ROM using a goniometer (Baseline; Fabrication Enterprises Inc., New York, USA). The goniometer axis was aligned with the trochanter major, the stationary arm was parallel to the table and the moving arm aligned with the thigh oriented versus the lateral femoral condyle. The participant was instructed to completely relax while the examiner performed the desired movement. Two repetitions were performed, from which the higher ROM was recorded.

Table 2. Hamstring eccentric training programme in a lengthened position.

Week	1.	2.	3.	4.	5.	6.
NHE (sets x reps)	2 x 5	2 x 6	3 x 6	3 x 8	3 x 8	3 x 8
The glider (sets x reps)	2 x 5	2 x 6	3 x 6	3 x 8	3 x 8	3 x 8

NHE Nordic hamstring exercise

Passive resistive torque

Passive resistive torque was assessed in a supine position using an isokinetic dynamometer (Humac Norm, Computer Sports Medicine Inc., Massachusetts, ZDA). The performed movement was unilateral hip flexion with the knee fully extended. The pelvis and the non-measured thigh were fixated with straps. The measured leg was fixated with a strap superior to the ankle joint on a long bar. The hip was positioned in a neutral position in all three planes. The hip axis of motion was individually aligned with the dynamometer`s axis. Before the start of the measurement, we assessed the available amplitude of hip flexion. It was defined at the first point at which the participants felt tightness or discomfort in the posterior thigh. Afterwards, we weighted the relaxed leg, went through the range of motion again and set the safety locks. After patient setting was completed, five repetitions of full ROM were performed to account for possible viscoelastic changes within soft tissues and to verify whether the setting was appropriate. The velocity of the movement was set at 15°/s. After the familiarization trial, one measurement consisting of five repetitions was performed, from which ROM [degrees] and torque [Nm] data were obtained and a passive torque-angle relationship was calculated. Passive resistive torque was defined as the slope of the passive torque-angle curve in the last 50% of full ROM. An average value of passive resistive torque was calculated from five repetitions and taken into further analysis.

Tendon length

Length of the distal tendon of BFlh was measured with ultrasound imaging using Resona 7 diagnostic ultrasound (Mindray, Mindray Medical International Limited, Shenzen, China) and a middle-sized probe (3.8 cm field of view; model L11-3 U, Mindray, Shenzhen, China). An extended field of view was used to measure the tendon length of the BFlh. The measurement was performed both in a relaxed and stretched position. For the relaxed position, the participant lied prone with his hip in a neutral position and the knee fully extended. For the stretched position, the participant lied supine with the knee fully extended and hip positioned at 70% flexion of the ROM used for passive stiffness evaluation. The course of the BFlh was carefully drawn on the skin, with the help of palpation and isometric contraction. The ultrasound scan was then taken by continuously and steadily moving the probe along the marked line in a 3–5 s time window, starting at the insertion of BFlh. The medio-lateral angle of the probe was altered throughout the measurement so that it remained perpendicular to the skin and pointed according to the fascicle orientation of the BFlh (Pimenta et al., 2018). Constant minimal pressure was applied on the skin and a water-soluble, hypoallergenic ultrasound gel (AquaUltra Basic, Ultragel, Budapest, Hungary) was used to improve acoustic contact between the probe and skin. Images were analysed in a publicly available digitizing software program ImageJ (National Institutes of Health, Bethesda, Maryland, USA) by setting the appropriate scale and using the "straight/segmented line tool". Distal musculotendinous junction and insertion of the BFlh were identified on the recorded ultrasound image as shown in Freitas et al. (2018). The distal tendon length was marked and measured from the distal musculotendinous junction to the head of the fibula (Figure 2).

Strain quantification

SWE for strain quantification was performed following previously described measurement protocol for BFlh by Šarabon, Kozinc et al. (2019). Resona 7 diagnostic ultrasound (Mindray, Mindray Medical International Limited, Shenzen, China) was used and set to musculoskeletal SWE mode (assuming tissue density of 1000 kg/m^3). Shear modulus (kPa) data were acquired using the above-described middle-sized linear probe, in a relaxed and stretched position that were the same as for ultrasound imaging. The measurement point was set at the middle (50%) of the BFlh muscle-tendon complex, which was defined as the half distance from the ischial tuberosity to the head of the fibula. First, a transverse view was used to correctly identify the exact location of the BFlh. The probe was positioned in line with the BFlh so that several fascicles, superficial and deep aponeuroses were visible without interruption across the image. The probe's aligned position was outlined with a permanent marker. Before data acquisition, the limb was held in the testing position for 3 minutes to account for possible viscoelastic changes within the muscle (Morales-Artacho et al., 2017). During measurement, constant minimal pressure was applied on the skin, while the probe was held perpendicular to the skin. Water-soluble, hypoallergenic ultrasound gel (AquaUltra Basic, Ultragel, Budapest, Hungary) was used to improve acoustic contact between the probe and skin. Two repetitions were performed in each condition. For each single repetition, the mean value of eight quick consecutive scans (duration: 8 sec) was calculated. The size of the region of interest was set to 2 × 2 cm and the depth of region of interest was chosen based on the participants' anthropometric characteristics in a way that the whole region was targeting muscle tissue.

Statistical analysis

Statistical analysis was done in the SPSS 25 computer program (IBM, New York, USA). Descriptive statistics (mean ± standard deviation) and 95% confidence intervals were calculated. The normality of data was assessed using the Shapiro–Wilk test. To estimate inter-session reliability, coefficient of variation (CV), standard error of measurement (SEM) and intra-class correlation coefficient $ICC_{2,k}$ were calculated for the participants in the control group. Sphericity was tested using Mauchly's test and in case of assumption violation, a Greenhouse-Geisser correction was used. Repeated measures analysis of variance was used to analyse the differences among corresponding variables, testing for interaction between time (pre vs. post intervention) and group (control vs. intervention). Where interaction effect was statistically significant, paired sample two-tailed t-test was used to test the pre-post difference in each group. Effect sizes were calculated for the pairwise comparisons using the Cohen's *d* method and interpreted as trivial (≤ 0.20), small (0.20–0.59), moderate (0.60–1.19), large (1.20–1.99), or very large (≥2.00) (Hopkins, 2010). The level of statistical significance was set at $\alpha < 0.05$.

Results

At the end of the intervention programme, 18 participants from the intervention group and 16 from the control group were included in the analysis. Six participants did not complete the study, the reasons being: absence from post-intervention measurement (2), general illness (1), injury unrelated to intervention (2), technical problems during the post-intervention measurement (1). Participation in the training programme was registered each session; subjects in the intervention group performed 11 ± 1 sessions and had 92.6% participation rate

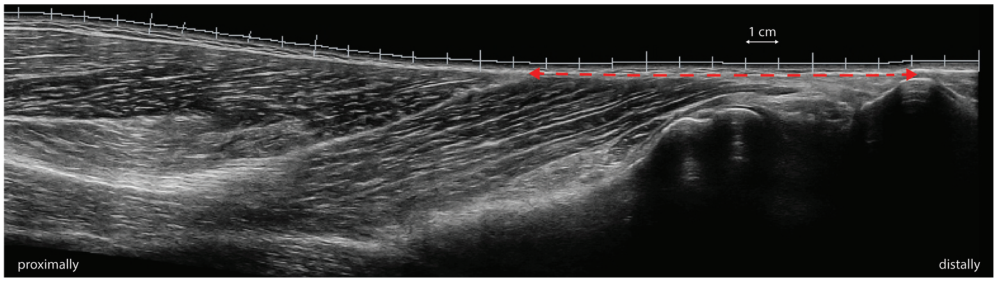

Figure 2. **Example of extended field of view ultrasound image of biceps femoris long head. Tendon length is marked with a dashed red line**. The arrows indicate the distal musculotendinous junction and the insertion of the biceps femoris long head on the head of the fibula.

Table 3. Reliability of measured parameters.

Task	CV	SEM	$ICC_{2,k}$	Bias F	Bias p
Maximal passive ROM [°]	2.88	0.33	0.99	0.58	0.46
Tendon length – relaxed [cm]	7.48	0.56	0.80	0.48	0.83
Tendon length – stretched [cm]	11.26	0.78	0.75	3.93	0.05
SWE elastic modulus – relaxed [kPa]	19.87	3.02	0.53	2.57	0.14
SWE elastic modulus – stretched [kPa]	22.03	6.46	0.74	1.83	0.20
Passive resistive torque [Nm/°]	16.47	0.10	0.74	0.49	0.50

CV – coefficient of variation (%); SEM – standard error of measurement; ICC – intra-class correlation coefficient; F – F ratio in analysis of variance; p – level of statistical significance

(out of 12 sessions, 8 subjects missed no sessions, 6 subjects missed 1 session, 2 subjects missed 2 sessions and 2 subjects missed 3 sessions). The average value of reported muscle soreness was 3.11 ± 0.53. The average rate of perceived exertion was 5.83 ± 1.31.

Inter-session reliability (pre – and post-sessions of the participants in the control group) of the measured parameters is shown in Table 3. With exception of SWE elastic modulus in a relaxed position, the analysis showed good to excellent test-retest reliability ($ICC_{2,k}$ = 0.74–0.99) without systematic bias for selected parameters. Maximal passive ROM and tendon length displayed acceptable within-individual variability (CV = ~3-11%), while SWE elastic modulus and passive resistive torque displayed relatively higher within-individual variability (CV = ~16-22%).

Table 4 shows descriptive statistics of dependent variables. Individual data of passive ROM are shown in the supplementary file. A significant interaction (time × group) effect was found for maximal passive ROM ($F = 34.32$, $p < 0.001$). Post-hoc testing revealed a significant increase in the intervention group for maximal passive ROM (11.2%; $p < 0.001$; $d = 1.55$), while no significant changes were observed in the control group ($p = 0.46$; $d = 0.19$).

No significant interaction effects were found for passive resistive torque ($F = 0.65$, $p = 0.43$), SWE elastic modulus in a relaxed ($F = 0.21$, $p = 0.65$) or stretched position ($F = 0.1$, $p = 0.76$), and BFlh tendon length in a relaxed ($F = 0.06$, $p = 0.81$) or stretched position ($F = 2.78$, $p = 0.11$).

Discussion

To our knowledge, this is the first study that has evaluated the effects of eccentric training at long muscle length on flexibility along with passive mechanical characteristics of the hamstring muscle-tendon complex. Our main finding is the large increase in maximal passive hip ROM following eccentric hamstring strengthening at long muscle lengths, without changes in passive hamstring stiffness or BFlh distal tendon length.

The observed gains in hamstring flexibility are generally comparable to previous research that also included eccentric hamstring exercises at long muscle length (Delvaux et al., 2020; Nelson and Bandy, 2004; Guex et al., 2016). Delvaux et al. (2020) have reported moderate gains (ES = 0.81) in hamstring flexibility in amateur athletes following 6-week eccentric hamstring training performed at short and long muscle lengths. Somewhat lower gains (ES ~ 0.4) in hamstring flexibility were observed in sprinters following 6-week eccentric hamstring training performed at short and long muscle lengths (Guex et al., 2016). In contrast, very large gains (ES ~ 2.0) in hamstring flexibility following 6-week eccentric hip flexion training have been reported by Nelson and Bandy (2004) in untrained high-school males. Notably, no or only minor gains in hamstring flexibility were observed after eccentric hamstrings training at short muscle length (Potier et al., 2009; Ribeiro-Alvares et al., 2018). Although studies that directly compare the effectiveness of eccentric hamstrings training at short vs. long muscle lengths in enhancing flexibility are lacking, the above-discussed results strongly suggest that eccentric hamstring exercises at long muscle lengths are needed for eliciting meaningful gains in hamstring flexibility.

Despite being effective in increasing hip joint ROM, the applied eccentric hamstring training at long muscle length had no significant effect on passive stiffness of hamstring muscle-tendon complex (i.e., passive resistive torque), shear modulus of the BFlh and BFlh distal tendon length. In the accompanying study, the applied training protocol also increased BFlh fascicle length and decreased BFlh pennation angle, while muscle thickness remained unchanged (Marušič et al., 2020). If we assume that the shear modulus of other hamstring muscles did not change substantially, then the above-mentioned adaptive changes strongly suggest the observed increase in maximal hip ROM is likely the result of increased stretch tolerance (Blazevich, 2019) and altered muscle architecture. However, as we did not measure the stiffness properties of the hamstring tendons and remote muscle-tendon units in series with the hamstrings (e.g., triceps surae), tissue-specific changes within the hamstring or neighbouring muscle-tendon complex cannot be excluded (see Discussion below).

In line with our results, Seymore et al. (2017) have reported no change in passive resistive torque and the elastic modulus of BFlh following eccentric hamstring training at short muscle length. Furthermore, Kay and co-workers (Kay et al., 2016; Kay et al., 2018) applied maximal isokinetic eccentric training to

Table 4. Descriptive statistics of measured parameters.

	CONTROL GROUP				INTERVENTION GROUP				
	PRE	95% CI	POST	95% CI	PRE	95% CI	POST	95% CI	SIG (p)
Maximal passive ROM [°]	81.0 ± 14.4	72.2– 89.6	80.4 ± 15.4	70.8– 89.5	80.1 ± 11.0	75.0– 86.4	89.1 ± 10.4	84.3– 95	0.001
Tendon length – relaxed [cm]	11.9 ± 1.5	11.1– 12.9	11.9 ± 1.5	11– 12.7	11.9 ± 1.3	11.3– 12.6	11.9 ± 1.3	11.2– 12.6	0.81
Tendon length – stretched [cm]	9.4 ± 1.6	8.3– 9.7	10.2 ± 2.1	8.8– 10.6	10.0 ± 1.7	9.2– 10.9	10.1 ± 1.3	9.4– 10.8	0.11
SWE elastic modulus – relaxed [kPa]	17.6 ± 3.4	15.8– 19.8	20.7 ± 2.6	19.4– 22.5	16.7 ± 2.8	15.5– 18.3	20.4 ± 3.8	18.8– 22.6	0.65
SWE elastic modulus – stretched [kPa]	42.8 ± 16.4	33.4– 53.6	38.5 ± 11.5	33.5– 46.5	52.6 ± 26.6	38.9– 67.1	49.8 ± 28.2	35.6– 65.3	0.76
Passive resistive torque [Nm/°]	0.84 ± 0.19	0.7– 0.9	0.8 ± 0.25	0.7– 0.9	0.91 ± 0.29	0.8– 1.1	0.93 ± 0.26	0.8– 1.1	0.43

PRE: pre-intervention; POST: post-intervention; CI – confidence interval; SIG: significant time (pre vs. post) x group (control vs. intervention) interaction; ROM: range of motion; SWE: shear wave elastography.

ankle plantar flexors and knee extensors, and also reported significant increases in joint ROM with no change in passive muscle-tendon complex stiffness. Notably, the authors also found significant increases in Achilles and vastus lateralis tendon stiffness, (Kay et al., 2016; Kay et al., 2018) and a decrease in passive stiffness of the gastrocnemius muscle (measured using ultrasonography) (Kay et al., 2016). In contrast, studies that used considerably lower loads during eccentric training of plantar flexors observed no change in Achilles tendon stiffness (Mahieu et al., 2008; Fouré et al., 2013), while the stiffness of the whole muscle-tendon complex either decreased (Mahieu et al., 2008) or remained unchanged (Fouré et al., 2013). Our findings and data from previous research suggest that (i) eccentric training can induce significant changes in passive mechanical properties that are tissue-specific, and (ii) these eccentric-training induced changes in passive mechanical characteristics of muscles and tendons are likely to be intensity-dependent. In other words, the adaptive potential of eccentric training seems to be dependent on both the length at which muscle-tendon complex is being eccentrically loaded, and the magnitude of load placed on the main agonist during eccentric contraction.

Muscle shear modulus proved to be a valid indicator of passive muscle stiffness (Koo et al., 2013). However, during joint ROM and passive resistive torque measurement, elongation does not only involve musculotendinous tissues crossing the studied joint, but also other tissues around that joint (e.g., nerves, skin), as well as remote myofascial tissues. Regarding the latter, due to mechanical force transmission between serially connected skeletal muscles, local alterations of the mechanical tissue properties may modify flexibility in remote joints (Wilke et al., 2020). This is partly supported by recent findings of Miyamoto et al. (2018) who reported only low to moderate negative correlations ($r = -0.31$ to -0.48) between hamstring flexibility tests and shear modulus of hamstring muscles. To advance our knowledge in this field, future eccentric training studies should, therefore, evaluate passive mechanical properties of both the local and serially connected remote musculotendinous tissues.

We have also examined the effects of eccentric hamstring training at long muscle length on BFlh distal tendon length. The observed distal tendon length in a relaxed position from pre-intervention measurement of all subjects (mean ± SD; 11.9 ± 1.4 cm) is comparable to results of a similar study using ultrasonography on healthy subjects or tape measurement on cadavers: 9.8 ± 1.2 cm on healthy subjects and 11.9 ± 2.0 cm on cadavers (Tosovic et al., 2016). In general, due to its higher potential to store and recoil elastic strain energy, longer tendon could be related to increased movement economy during stretch-shortening cycle activities like running (Hunter et al., 2015). Conversely, short tendon is likely to be limited in reaching an adequate stretch and elastic energy storing, which could lead to increased demands and strain of the muscle. The applied eccentric hamstring training had no significant effect on BFlh tendon length in either relaxed or stretched conditions. These results are consistent with previous research on the Achilles tendon (Fouré et al., 2013 ; Geremia et al., 2018), and add further support to the general viewpoint that changes in tendon mechanical properties, rather than tendon dimensions, are the main type of tendon adaptation to eccentric training (Bohm et al., 2015).

Progressive eccentric hamstring training at long muscle length significantly improves hamstring flexibility without affecting passive muscle stiffness or tendon length of biceps femoris long head. Our results, in combination with findings of the related study (Marušič et al., 2020), could have relevant practical implications. Namely, eccentric hamstring training at long muscle length elicits multiple favourable functional and structural adaptations that have been associated with increased risk of hamstring strain injury or reinjury (Freckleton and Pizzari, 2013; Opar et al., 2012). These include (i) increased hamstring flexibility (present study), (ii) increased BFlh fascicle length (Marušič et al., 2020), (iii) increased eccentric strength and voluntary activation of the hamstrings (Marušič et al., 2020), and (iv) shifts in the angle of hamstrings peak torque towards longer muscle length (Nelson and Bandy, 2004). From that perspective, the applied eccentric hamstring training at long muscle length could be an effective strategy for reducing the risk of HSI occurrence as well as for rehabilitation of HSIs. Future research should focus on evaluating possible effects of eccentric training at long muscle length for hamstring injury prevention.

Several limitations of the present study should be considered. The first is a relatively small number of training sessions conducted over a 6-week period, although a higher training frequency is questionable as eccentric training could lead to the development of delayed onset of muscle soreness and, thus, prevent engagement in the upcoming training sessions. Second, we did not quantify passive stiffness (i.e., shear modulus) of all hamstring muscles. Although we have included both hip-dominant and knee-dominant eccentric hamstring exercises that distinctly activate hamstring muscles (Hegyi et al., 2019), it is possible that the applied training resulted in muscle-specific adaptations of passive mechanical properties. Third, the amount of hip flexion during the modified NHE was set to 75° for all participants. To increase the stimulus for adaptation, hip flexion angle should have been adjusted individually based on the participant's flexibility level.

Conclusion

In conclusion, eccentric training at long muscle length proved to be effective in increasing maximal passive hip flexion ROM, without affecting passive muscle stiffness and distal tendon length of BFlh. Considering findings of the related study (Marušič et al., 2020), the observed increase in maximal hip flexion ROM is likely the result of increased stretch tolerance and altered muscle architecture, although other factors such as tendon stiffness or surrounding tissue changes cannot be excluded. Further research is needed to compare the effects of eccentric hamstring training at long and short muscle lengths.

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References

Askling, C. M., Tengvar, M., Saartok, T., & Thorstensson, A. (2007). Acute first-time hamstring strains during high-speed running: A longitudinal study including clinical and magnetic resonance imaging findings. *The American Journal of Sports Medicine*, *35*(2), 197–206. https://doi.org/10.1177/0363546506294679

Askling, C. M., Tengvar, M., Tarassova, O., & Thorstensson, A. (2014). Acute hamstring injuries in Swedish elite sprinters and jumpers: A prospective randomised controlled clinical trial comparing two rehabilitation protocols. *British Journal of Sports Medicine*, *48*(7), 532–539. https://doi.org/10.1136/bjsports-2013-093214

Beaulieu-Jones, B. R., Rossy, W. H., Sanchez, G., Whalen, J. M., Lavery, K. P., McHale, K. J., Vopat, B. G., Van Allen, J. J., Akamefula, R. A., & Provencher, M. T. (2017). Epidemiology of injuries identified at the NFL scouting combine and their impact on performance in the national football league: Evaluation of 2203 athletes from 2009 to 2015. *Orthop J Sport Med*, *5*(7), 7. https://doi.org/10.1177/2325967117708744

Blazevich, A. J. (2019). Adaptations in the passive mechanical properties of skeletal muscle to altered patterns of use. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *126*(5), 1483–1491. https://doi.org/10.1152/japplphysiol.00700.2018

Bohm, S., Mersmann, F., & Arampatzis, A. (2015). Human tendon adaptation in response to mechanical loading: A systematic review and meta-analysis of exercise intervention studies on healthy adults. *Sport Med - Open*, *1*(1), 1. https://doi.org/10.1186/s40798-015-0009-9

Brooks, J. H. M., Fuller, C. W., Kemp, S. P. T., & Reddin, D. B. (2006). Incidence, risk, and prevention of hamstring muscle injuries in professional rugby union. *The American Journal of Sports Medicine*, *34*(8), 1297–1306. https://doi.org/10.1177/0363546505286022

Delvaux, F., Schwartz, C., Decréquy, T.,Devalckeneer, T., Paulus, J., Bornheim, S., Kaux, J. F., & Croisier, J. L. (2020). Influence of a field hamstring eccentric training on muscle strength and flexibility. *International Journal of Sports Medicine*, *41*(4), 233–241. https://doi.org/10.1055/a-1073-7809

Eby, S. F., Song, P., Chen, S., Chen, Q., Greenleaf, J. F., & An, K. N. (2013). Validation of shear wave elastography in skeletal muscle. *Journal of Biomechanics*, *46*(14), 2381–2387. https://doi.org/10.1016/j.jbiomech.2013.07.033

Edouard, P., Branco, P., & Alonso, J. M. (2016). Muscle injury is the principal injury type and hamstring muscle injury is the first injury diagnosis during top-level international athletics championships between 2007 and 2015. *British Journal of Sports Medicine*, *50*(10), 619–630. https://doi.org/10.1136/bjsports-2015-095559

Ekstrand, J., Hägglund, M., & Waldén, M. (2011). Injury incidence and injury patterns in professional football: The UEFA injury study. *British Journal of Sports Medicine*, *45*(7), 553–558. https://doi.org/10.1136/bjsm.2009.060582

Fouré, A., Nordez, A., & Cornu, C. (2013). Effects of eccentric training on mechanical properties of the plantar flexor muscle-tendon complex. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *114*(5), 523–537. https://doi.org/10.1152/japplphysiol.01313.2011

Freckleton, G., & Pizzari, T. (2013). Risk factors for hamstring muscle strain injury in sport: A systematic review and meta-analysis. *British Journal of Sports Medicine*, *47*(6), 351–358. https://doi.org/10.1136/bjsports-2011-090664

Freitas, S. R., Marmeleira, J., Valamatos, M. J., Blazevich, A., & Mil-Homens, P. (2018). Ultrasonographic measurement of the biceps femoris long-head muscle architecture. *Journal of Ultrasound in Medicine : Official Journal of the American Institute of Ultrasound in Medicine*, *37*(4), 977–986. https://doi.org/10.1002/jum.14436

Garrett, W. E. (1990). Muscle strain injuries: Clinical and basic aspects. *Medicine and Science in Sports and Exercise*, *22*(4), 436–443. https://doi.org/10.1249/00005768-199008000-00003

Geremia, J. M., Baroni, B. M., Bobbert, M. F., Bini, R. R., Lanferdini, F. J., & Vaz, M. A. (2018). Effects of high loading by eccentric triceps surae training on Achilles tendon properties in humans. *European Journal of Applied Physiology*, *118*(8), 1725–1736. https://doi.org/10.1007/s00421-018-3904-1

Guex, K. J., Lugrin, V., Borloz, S., & Millet, G. P. (2016, February). Influence on strength and flexibility of a swing phase-specific hamstring eccentric program in sprinters' general preparation. *Journal Of Strength And Conditioning Research / National Strength & Conditioning Association*, *30*(2), 525–532. https://doi.org/10.1519/JSC.0000000000001103

Hegyi, A., Lahti, J., Giacomo, J. P., Gerus, P., Cronin, N. J., & Morin, J. B. (2019). Impact of hip flexion angle on unilateral and bilateral nordic hamstring exercise torque and high- density electromyography activity. *The Journal of Orthopaedic and Sports Physical Therapy*, *49*(8), 584–592. https://doi.org/10.2519/jospt.2019.8801

Hopkins, W. G. (2012). Linear models and effect magnitudes for research, clinical and practical applications. *Sportscience*, 14, 49–57. https://sportsci.org/2010/wghlinmod.htm. Accessed September 21, 2020.

Hunter, G. R., Mccarthy, J. P., Carter, S. J., et al. (2015). Muscle fiber type, achilles tendon length, potentiation, and running economy. *Journal Of Strength And Conditioning Research / National Strength & Conditioning Association*, *29*(5), 1302–1309. https://doi.org/10.1519/JSC.0000000000000760

Kay, A. D., Richmond, D., Talbot, C., Mina, M., Baross, A. W., & Blazevich, A. J. (2016). Stretching of active muscle elicits chronic changes in multiple strain risk factors. *Medicine and Science in Sports and Exercise*, *48*(7), 1388–1396. https://doi.org/10.1249/MSS.0000000000000887

Kay, A. D., Rubley, B., Talbot, C., Mina, M., Baross, A. W., & Blazevich, A. J. (2018). Stretch imposed on active muscle elicits positive adaptations in strain risk factors and exercise-induced muscle damage. *Scand J Med Sci Sport*, *28*(11), 2299–2309. https://doi.org/10.1111/sms.13251

Koo, T. K., Guo, J. Y., Cohen, J. H., & Parker, K. J. (2013). Relationship between shear elastic modulus and passive muscle force: An ex-vivo study. *Journal of Biomechanics*, *46*(12), 2053–2059. https://doi.org/10.1016/j.jbiomech.2013.05.016

Lieber, R. L., & Friden, J. (1993). Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *74*(2), 520–526. https://doi.org/10.1152/jappl.1993.74.2.520

Liu, H., Garrett, W. E., Moorman, C. T., & Yu, B. (2012). Injury rate, mechanism, and risk factors of hamstring strain injuries in sports: A review of the literature. *J Sport Heal Sci*, *1*(2), 92–101. https://doi.org/10.1016/j.jshs.2012.07.003

Mahieu, N. N., Mcnair, P., Cools, A., D'Haen, C., Vandermeulen, K., & Witvrouw, E. (2008). Effect of eccentric training on the plantar flexor muscle-tendon tissue properties. *Medicine and Science in Sports and Exercise*, *40*(1), 117–123. https://doi.org/10.1249/mss.0b013e3181599254

Marušič, J., Vatovec, R., Marković, G., & Šarabon, N. (2020). Effects of eccentric training at long-muscle length on architectural and functional characteristics of the hamstrings. *Scand J Med Sci Sport*, *30* (11), 2130–2142. sms.13770 https://doi.org/10.1111/sms.13770

Miyamoto, N., Hirata, K., Kimura, N., & Miyamoto-Mikami, E. (2018). Contributions of hamstring stiffness to straight-leg-raise and sit-and-reach test scores. *International Journal of Sports Medicine*, *39*(2), 110–114. https://doi.org/10.1055/s-0043-117411

Morales-Artacho, A. J., Lacourpaille, L., & Guilhem, G. (2017). Effects of warm-up on hamstring muscles stiffness: Cycling vs foam rolling. *Scand J Med Sci Sport*, *27*(12), 1959–1969. https://doi.org/10.1111/sms.12832

Nelson, R. T., & Bandy, W. D. (2004). Eccentric training and static stretching improve hamstring flexibility of high school males. *Journal of Athletic Training*, *39*(3), 254–258. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC522148/. Accessed September 21, 2020

Opar, D. A., Williams, M. D., & Shield, A. J. (2012). Hamstring strain injuries: Factors that lead to injury and re-injury. *Sport Med*, *42*(3), 209–226. https://doi.org/10.2165/11594800-000000000-00000

Pimenta, R., Blazevich, A. J., & Freitas, S. R. (2018). Biceps femoris long-head architecture assessed using different sonographic techniques. *Medicine and Science in Sports and Exercise*, *50*(12), 2584–2594. https://doi.org/10.1249/MSS.0000000000001731

Potier, T. G., Alexander, C. M., & Seynnes, O. R. (2009). Effects of eccentric strength training on biceps femoris muscle architecture and knee joint range of movement. *European Journal of Applied Physiology*, *105*(6), 939–944. https://doi.org/10.1007/s00421-008-0980-7

Ribeiro-Alvares, J. B., Marques, V. B., Vaz, M. A., & Baroni, B. M. (2018). Four weeks of nordic hamstring exercise reduce muscle injury risk factors in young adults. *Journal Of Strength And Conditioning Research / National Strength & Conditioning Association*, *32*(5), 1254–1262. https://doi.org/10.1519/JSC.0000000000001975

Šarabon, N., Kozinc, Ž., & Podrekar, N. (2019). Using shear-wave elastography in skeletal muscle: A repeatability and reproducibility study on biceps femoris muscle. *PLoS One*, *14*(8), 8. https://doi.org/10.1371/journal.pone.0222008

Šarabon, N., Marušič, J., Marković, G., & Kozinc, Ž. (2019). Kinematic and electromyographic analysis of variations in Nordic hamstring exercise. *PLoS One*, *14*(10), e0223437. https://doi.org/10.1371/journal.pone.0223437

Saw, R., Finch, C. F., Samra, D., Baquie, P., Cardoso, T., Hope, D., & Orchard, J. W. (2018). Injuries in Australian rules football: An overview of injury rates, patterns, and mechanisms across all levels of play. *Sports Health*, *10*(3), 208–216. https://doi.org/10.1177/1941738117726070

Seymore, K. D., Domire, Z. J., DeVita, P., Rider, P. M., & Kulas, A. S. (2017). The effect of Nordic hamstring strength training on muscle architecture, stiffness, and strength. *European Journal of Applied Physiology*, *117*(5), 943–953. https://doi.org/10.1007/s00421-017-3583-3

Tosovic, D., Muirhead, J. C., Brown, J. M. M., & Woodley, S. J. (2016). Anatomy of the long head of biceps femoris: An ultrasound study. *Clin Anat*, *29*(6), 738–745. https://doi.org/10.1002/ca.22718

Van Dyk, N., Behan, F. P., & Whiteley, R. (2019). Including the Nordic hamstring exercise in injury prevention programmes halves the rate of hamstring injuries: A systematic review and meta-analysis of 8459 athletes. *Br J Sports Med*, *53*(21), 1362–1370. https://doi.org/10.1136/bjsports-2018-100045

Watsford, M. L., Murphy, A. J., McLachlan, K. A.,Bryant, A. L., Cameron, M. L., Crossley, K. M., & Makdissi, M. (2010). A prospective study of the relationship between lower body stiffness and hamstring injury in professional Australian rules footballers. *The American Journal of Sports Medicine*, *38*(10), 2058–2064. https://doi.org/10.1177/0363546510370197

Wilke, J., Debelle, H., Tenberg, S., Dilley, A., & Maganaris, C. (2020). Ankle motion is associated with soft tissue displacement in the dorsal thigh: An in vivo investigation suggesting myofascial force transmission across the knee joint. *Frontiers in Physiology*, *11*, 180. https://doi.org/10.3389/fphys.2020.00180