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Research article

Passive Muscle Stiffness of Biceps Femoris is Acutely Reduced after Eccentric Knee Flexion

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Abstract
Eccentric hamstring exercises reportedly prevent hamstring strain injury in the biceps femoris long head (BFlh). However, information on the favorable adaptive responses in the BFlh to eccentric hamstring exercises is limited. We aimed to examine the acute effect of maximal isokinetic eccentric knee flexion on passive BFlh stiffness as a potential risk factor for the hamstring strain injury using ultrasound shear wave elastography. Ten young participants randomly performed both tasks involving five consecutive repetitions of isokinetic concentric and eccentric knee flexion with maximal effort on different legs. Passive BFlh shear modulus was taken before and 30, 60, 90, and 120 s after each task. Passive BFlh shear modulus was significantly reduced at all time points after eccentric knee flexion, whereas there was no significant change in passive BFlh shear modulus after the concentric task. The present findings indicate that passive BFlh stiffness would reduce specifically after low-volume, slow-velocity eccentric knee flexion exercise. The findings may help provide practitioners with a basis to develop more effective exercise programs for preventing HSI.

Key words: Eccentric hamstring exercises, isokinetic knee flexion, ultrasound shear wave elastography, shear modulus.

Introduction

Hamstring strain injury (HSI) remains one of the most common injuries across a range of sprint-based sports, such as track-and-field (Opar et al., 2014), soccer (Ekstrand et al., 2011), rugby (Brooks et al., 2005), and baseball (Ahmad et al., 2014). Eccentric hamstring exercises (i.e., lengthening of the contracting hamstring) reportedly prevent HSI (van Dyk et al., 2019) through possible mechanisms of increasing hamstring strength (Mjolsnes et al., 2004) and consequently hamstring-to-quadriceps strength ratio (Severo-Silveira et al., 2021) and/or of increasing lower limb flexibility (O'Sullivan et al., 2012). On the other hand, although most of the sprint-type HSI occurs in the long head of the biceps femoris (BFlh) (Askling et al., 2007), available information on the favorable adaptive responses in the BFlh to eccentric hamstring exercises is limited to an increase in BFlh fascicle length (Gerard et al., 2020) which has been suggested as a possible mechanism for the prevention of HSI (Timmins et al., 2016). A more detailed elucidation of the favorable adaptive responses of eccentric hamstring exercises on the BFlh (rather than the hamstring) can lead to advanced strategies aimed at preventing HSI.

Stiffness of the hamstring muscle-tendon unit has been reported as a risk factor for HSI (Green et al., 2020; Watsford et al., 2010). Muscle-tendon unit stiffness is attributable, at least in part, to muscle stiffness (Gajdosik, 2001; Konrad and Tilp, 2020). Recently, high passive muscle stiffness measured with ultrasound shear wave elastography (SWE), which can quantify the stiffness of individual muscles, has been suggested as a factor related to muscle injury (Kumagai et al., 2019; Miyamoto-Mikami et al., 2021). Considering these observations, together with the fact that most HSI occurs in the BFlh as mentioned above (Askling et al., 2007), it is plausible to suppose that passive BFlh stiffness is a risk factor for HSI and that eccentric hamstring exercises prevent HSI by reducing passive BFlh stiffness. However, this is only speculation and not experimentally demonstrated. Therefore, it is important to pursue an understanding of whether and how eccentric hamstring exercise modifies passive BFlh stiffness.

As a first step toward the above goal, based on the notions that findings about the acute changes by exercises could provide insights into their chronic responses, the present study aimed to examine an acute effect of maximal isokinetic eccentric knee flexion on passive BFlh stiffness, with special emphasis on the comparison with concentric (i.e., shortening) contraction. Passive BFlh stiffness is reduced by hamstring stretching of passive knee extension (i.e., lengthening of the resting hamstring) in the hip flexed position (Miyamoto et al., 2017). Based on this observation, we hypothesized that passive BFlh stiffness would be acutely reduced by eccentric but not concentric knee flexion exercise.

Methods

Study design
Two tasks that consisted of isokinetic concentric (CON) and eccentric (ECC) knee flexion were performed to fulfill the goal of the present study. Except for the contraction type of hamstring exercise, the protocols and variables for analyses were the same between the two tasks (see below sections). In a randomized design, each participant performed a CON task on one leg and an ECC task on the other leg. The CON and ECC tasks were performed on the same day with a 10-min rest period in a randomized order. The

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room temperature was set to 24°C using an air conditioner to minimize the potential temperature-induced effects.

Participants

When calculating the necessary sample size, there was no direct data from previous studies to be referred to for a priori power analysis since no previous study has investigated the acute effect of eccentric contractions on muscle stiffness. Consequently, the necessary sample size was calculated based on the data of our preliminary study [n = 5, partial η^2 for TASK (2) × TIME (5) interaction = 0.689, partial η^2 for main effect of TIME in ECC task = 0.630], using a priori power analysis (G*Power, version 3.1.9.4 software, 26 Heinrich-Heine-Universitat Düsseldorf, Germany) with an assumed type 1 error of 0.05 and statistical power (1-β) of 0.8. The critical sample size was estimated to be at least 10 for each task. Ten healthy men (age: 24.1 ± 1.8 years, height: 173.2 ± 5.0 cm, weight: 64.9 ± 4.8 kg; mean ± SD) were recruited for the study. Participants with a history of thigh muscle or knee injuries within the last 6 months were excluded from the study. None of the participants had been involved in any resistance training exercise. They were asked to refrain from intense exercise within 48 h before the experiment. Before testing, the participants were informed of the study procedures and provided written informed consent. The study was approved by the local ethics committee (project identification code: 2021-96), and all experimental procedures were performed in accordance with the Declaration of Helsinki.

Experimental setup and procedure

Each participant was seated on an isokinetic dynamometer (Biodex System 4 Pro, Biodex Medical System, USA) with the hip flexed at 70° (0° = lying position). The participant was tightly secured to the dynamometer using non-elastic straps. The rotation axis of the motor was aligned with that of the knee joint. The lever arm of the dynamometer was attached 3-4 cm above the lateral malleolus. After a 5-min rest, passive BFlh shear modulus (a measure of stiffness, expressed in the unit of Pascal) was measured using ultrasound SWE (see below section) before isokinetic exercises (PRE) in the sitting position with the hip flexed at 70° and the knee of the tested leg fully extended. Subsequently, the participants performed a specific isokinetic warm-up exercise consisting of 6 sub-maximal (3 self-perceived 50% effort and 3 self-perceived 80% effort) and 2 maximal CON or ECC knee flexion on the tested leg at an angular velocity of 20°/s to familiarize themselves with the protocol (El-Ashker et al., 2019). This angular velocity was chosen based on the fact that the Nordic hamstring exercise, one of the major exercises of HSI prevention programs, was performed at approximately 20°/s (Alt et al., 2018). After a 3-min rest, each participant performed five consecutive repetitions of CON or ECC knee flexion with maximal efforts. In both tasks, the knee joint range of motion was between 0° and 90° (0° = full extension). Measurements of passive BFlh shear modulus were taken at 30, 60, 90, and 120 s after the end of the CON/ECC task (POST).

SWE measurement

An ultrasound SWE scanner (Aixplorer Ver. 12; Supersonic Imagine, France), coupled with a linear array probe (SL10-2), was used to assess passive BFlh shear modulus in SWE mode (MSK preset, persistence = off, smoothing = 5). According to a previous study (Miyamoto et al., 2018), the ultrasound probe was positioned at 50% of the thigh length (the distance between the greater trochanter and the lateral epicondyle of the femur). The probe orientation was adjusted to identify fascicles within the B-mode image, and the location was marked on the skin with a waterproof pen. At each of five measurement time points (i.e., PRE, POST30, POST60, POST90, POST120) in each task, SWE measurements were performed three times (i.e., three images were acquired). The images were acquired while ensuring that the color map was stable for a few seconds. Care was taken not to press and deform the muscle while scanning. A region of interest in the SWE color map was carefully selected as large as possible with the exclusion of subcutaneous tissues and aponeurosis for each image using the built-in software of the scanner, and the average value of the shear modulus over the region of interest was calculated (Figure 1). At each time point, the values of three measurements in each trial were averaged and used for further analyses.

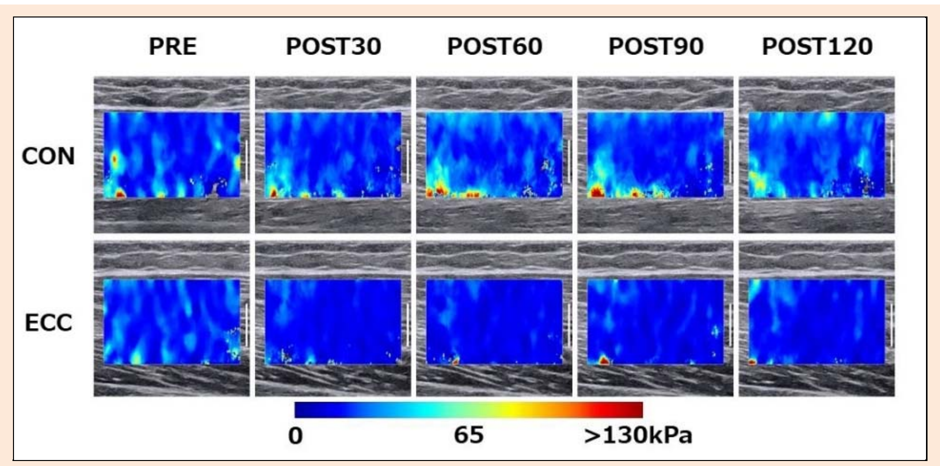

Figure 1. Typical examples of ultrasound shear wave elastography measurements of the biceps femoris long head (BFlh) in concentric (CON) and eccentric (ECC) tasks. Measurements were performed before (PRE) and 30, 60, 90, and 120 s after each task (POST30, POST60, POST90, POST120, respectively). The colored region represents the shear modulus map with the scale below the images. Scale bar = 1cm.

Before the above experiments commenced, to evaluate the inter-trial (intra-day, intra-rater) repeatability, passive BFlh shear modulus measurements were performed for both legs of 2 participants (i.e., total 4 legs) in the sitting position as mentioned above (i.e., the hip flexed at 70° and knee of the tested leg fully extended). Measurements were performed 4 times for each leg, with a 2-min rest between measurements. During the rest period, participants were allowed to flex their legs.

Statistical analysis

The intraclass correlation coefficient (ICC) and coefficient of variation (CV) were calculated to evaluate the repeatability. For the main experimental data, the normality of the data was confirmed using the Shapiro–Wilk test. A two-way analysis of variance (ANOVA) [TASK (CON, ECC) × TIME (PRE and POST30, POST60, POST90, and POST120)] with repeated measures was performed. When a significant interaction was identified, additional one-way ANOVA with post-hoc tests (Dunnett test and paired t-test) were performed. The significance level for all comparisons was set at $p = 0.05$. All statistical analyses were performed using statistical software (Prism, version 9.3.1; GraphPad Software). Data are expressed as mean ± SD.

Results

For the inter-trial repeatability, the CV was ≤ 2.7%, with an ICC of 0.94, indicating excellent inter-trial repeatability.

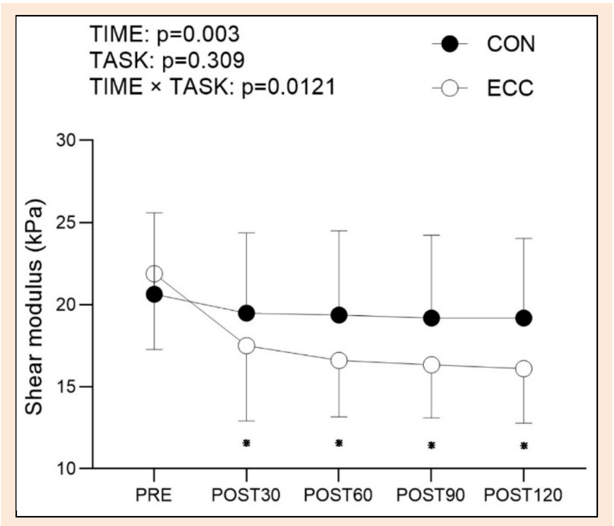

Figure 2. Passive biceps femoris long head (BFlh) shear modulus before (PRE) and 30, 60, 90, and 120 s after (POST30, POST60, POST90, POST120, respectively) concentric (CON) and eccentric (ECC) exercises. *Significant reduction compared with PRE in ECC task ($p < 0.05$).

Figure 2 shows the passive BFlh shear modulus in CON and ECC tasks. Two-way ANOVA revealed a significant TASK × TIME interaction ($p = 0.012$, partial $\eta^2 = 0.372$). Post-hoc tests showed that passive BFlh shear modulus in ECC task was significantly reduced at POST30 ($p = 0.023$, Cohen's $d = 0.95$), POST60 ($p = 0.004$, Cohen's $d = 1.30$), POST90 ($p = 0.002$, Cohen's $d = 1.39$) and POST120 ($p = 0.001$, Cohen's $d = 1.44$) compared with PRE while there was no significant main effect of TIME on passive BFlh shear modulus in CON task ($p = 0.473$, partial $\eta^2 = 0.062$).

Discussion

The finding of the present study was that the passive BFlh shear modulus was reduced after low-volume, slow-velocity ECC while there was no significant change in the CON task. The results support our hypothesis that eccentric knee flexion could acutely reduce passive BFlh stiffness. To the best of our knowledge, this is the first study to demonstrate contraction mode-dependent acute changes in passive muscle stiffness by using ultrasound SWE.

Passive muscle stiffness has been reported to reduce after an acute bout of passive lengthening (i.e., passive stretching exercise) (Hirata et al., 2017; Hirata et al., 2016; Miyamoto et al., 2017). Although several possible mechanisms have been proposed for the passive lengthening-induced reduction in passive muscle stiffness (Kay et al., 2015; Nakamura et al., 2011; Nakamura et al., 2015), the most likely and plausible explanation is intramuscular connective tissue lengthening (Gajdosik, 2001; Morse et al., 2008). Intramuscular connective tissues such as perimysium and endomysium have wavy fibrils, which are commonly known as crimp (Diamant et al., 1972). Previous studies have shown that the crimps within the connective tissues disappeared after lengthening the tissues (Franchi et al., 2007b). Furthermore, it is suggested that such crimps decrease in number, appear more flattened, and do not reappear after the tissue is physiologically elongated to an extent in vivo (Franchi et al., 2007a; Franchi et al., 2007b). It is reasonable to presume that these phenomena would occur during passive lengthening and during active lengthening (i.e., eccentric exercise).

Another possible mechanism for reduced passive BFlh stiffness is the increased muscle temperature (Sapin-de Brosses et al., 2010). In the present study, five repetitions of 4.5 s (calculated based on 90° range of motion and angular velocity of 20°/s) isokinetic knee flexion were performed with maximal efforts in both CON and ECC tasks. Thus, it is most unlikely that muscle temperature was elevated only in the ECC task, but not in the CON task, although the muscle temperature in BFlh has not been assessed.

The effect of static stretching duration on passive muscle stiffness has been examined using SWE in previous literature (Reiner et al., 2022; Sato et al., 2020). Reiner et al. (Reiner et al., 2022) showed that passive semitendinosus stiffness was significantly reduced after a 120-s static stretching. Also, Sato et al. (Sato et al., 2020) showed no significant change in passive medial gastrocnemius stiffness immediately after a 20-s static stretching. These findings together indicate that passive lengthening with a duration as short as 20 s could not reduce passive muscle stiffness. In contrast, the present study demonstrated that passive BFlh stiffness was significantly reduced after 5 repetitions of 4.5 s (i.e., 22.5 s in total) active lengthening. However, we should note that the procedure used in the present study included not only five consecutive repetitions of CON or ECC knee flexion, but also a warm-up exercise between the PRE and POST measurements, although a 3-min rest period was allocated after the warm-up exercise. Even when considering the effect of the specific warm-up exercise, the duration of eccentric contractions is less than 60 s in total. Although this concern needs to be kept in mind, it is likely that active lengthening (i.e., eccentric exercise) is preferred over passive lengthening (i.e., passive stretching exercise) to reduce passive muscle stiffness more efficiently in a shorter time.

Previous studies have reported that passive muscle stiffness increased after eccentric exercises aiming to induce muscle damage and/or delayed onset muscle soreness (Leung et al., 2017; Matsuo et al., 2015; Xu et al., 2019). This is in contrast with our finding that passive BFlh stiffness was reduced in ECC task. The contradiction between the present and previous studies is most likely due to the difference in the number of repetitions and set of eccentric exercises. In the previous studies, the volumes of eccentric exercises were relatively high (15 repetitions of heel drops × 10 sets (Leung et al., 2017), 10 maximal eccentric contractions × 6 sets (Matsuo et al., 2015), and 75 maximal eccentric contractions × 1 set (Xu et al., 2019)). In contrast, the eccentric exercise protocol in the present study was determined based on injury prevention warm-up programs including the Nordic hamstring exercise (e.g., FIFA 11+, Part 2-9, HAMSTRINGS for 'beginner': 1 set (3-5 repetitions) (Soligard et al., 2008)), and the volume (5 maximal isokinetic eccentric contractions × 1 set) was considerably lower than that of the previous studies. Although the threshold for eccentric exercise volume that stiffens the exercising muscle is unknown at this time, a well-defined threshold volume should be crucial to injury prevention programs. Further studies are required to clarify the relationship between eccentric exercise volume and subsequent change in muscle stiffness.

The effectiveness of eccentric hamstring exercise training (e.g., hamstring training intervention involving the Nordic hamstring exercise) for the prevention of HSI has been demonstrated (van Dyk et al., 2019). Although an increase in BFlh fascicle length induced by eccentric hamstring training has been suggested as a possible mechanism for the prevention of HSI, available information is limited on the favorable adaptive responses in the BFlh to eccentric hamstring training, and it remains to be elucidated how eccentric hamstring training influences the BFlh. The current findings that low-volume, slow-velocity eccentric knee flexion could acutely reduce passive BFlh stiffness, which is suggested as a potential risk factor for the HSI although this has not been epidemiologically demonstrated (Kumagai et al., 2019; Miyamoto-Mikami et al., 2021), will be evidence for the background of argument regarding the protective effect of eccentric hamstring training on the HSI. Longitudinal studies with eccentric hamstring training intervention are warranted to examine whether low-volume, slow-velocity eccentric hamstring training can simultaneously reduce passive BFlh stiffness and increase BFlh fascicle length and which exercises are more effective in concurrently reducing passive BFlh stiffness and lengthening BFlh fascicles.

The present study has some limitations. First, the passive BFlh shear modulus was measured only at 50% of thigh length. Kellis et al. (Kellis et al., 2010) reported that the biceps femoris architecture (e.g., fascicle length) is not uniform from proximal to distal positions. This previous finding suggests a possibility that mechanical stress imposed during passive lengthening (passive stretching exercise) is nonuniform within a muscle (Miyamoto et al., 2017), which may lead to inhomogeneity in muscle stiffness changes. Besides, Hegyi et al. (Hegyi et al., 2019) showed the proximal-distal differences in electromyographic activity of BFlh during common hamstrings exercises. Although it remains unknown whether such proximal-distal differences in electromyographic activity can lead to the regional difference in muscle stiffness changes at this time, the change in passive muscle stiffness after active lengthening (eccentric exercise) may be nonuniform along the BFlh. Further studies are required to reveal whether the current findings hold true for other regions. Second, we failed to observe the return to baseline, although passive BFlh stiffness was measured every 30 s until 2 min after the end of eccentric exercise. Prior to the study, because there are no studies on the time course of acute changes in passive muscle stiffness after low-volume eccentric contraction, we expected that the acute effect of eccentric contractions of duration as short as 22.5 s (5 repetitions of 4.5 s) would disappear within 2 min, based on the previous findings that the effect of static stretching of duration on passive muscle stiffness (Kay and Blazevich, 2009; Konrad et al., 2019; Konrad and Tilp, 2020). Additionally, in the present study, the acute effect of eccentric contractions was only examined at the angular velocity of 20°/s. Therefore, future studies with larger sample size are warranted to examine how long the eccentric exercise-induced acute reduction in passive muscle stiffness can last, with special emphasis on eccentric exercise volume (repetition, set, intensity, and/or duration) and angular velocity.

Conclusion

The present study demonstrated that passive BFlh stiffness was acutely reduced after five repetitions of maximal isokinetic eccentric but not concentric knee flexion, suggesting that passive BFlh stiffness would reduce specifically after low-volume, slow-velocity eccentric knee flexion exercise. The present findings may provide practitioners with a basis to develop more effective exercise programs for preventing HSI.

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Key points

- Passive muscle stiffness of the biceps femoris long head (BFlh) was directly assessed using ultrasound shear wave elastography.
- Passive BFlh stiffness was reduced after five consecutive repetitions of eccentric knee flexion, but not concentric contractions.
- The present findings may provide practitioners with a basis to develop more effective exercise programs for preventing HSI.

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