Biceps femoris long head sarcomere and fascicle length adaptations after 3 weeks of eccentric exercise training

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Abstract

Background: Eccentric exercise increases muscle fascicle lengths; however, the mechanisms behind this adaptation are still unknown. This study aimed to determine whether biceps femoris long head (BFlh) fascicle length increases in response to 3 weeks of eccentric exercise training are the result of an in-series addition of sarcomeres within the muscle fibers.

Methods: Ten recreationally active participants (age = 27 ± 3 years; mass = 70 ± 14 kg; height = 174 ± 9 cm; mean ± SD) completed 3 weeks of Nordic hamstring exercise (NHE) training on a custom exercise device that was instrumented with load cells. We collected in vivo sarcomere and muscle fascicle images of the BFlh in 2 regions (central and distal) by using microendoscopy and 3 dimension ultrasonography. We then estimated sarcomere length, sarcomere number, and fascicle length before and after the training intervention.

Results: Eccentric knee flexion strength increased after the training (15%; p < 0.001; \( \eta^2_p = 0.75 \)). Further, we found a significant increase in fascicle length (21%; p < 0.001; \( \eta^2_p = 0.81 \)) and sarcomere length (17%; p < 0.001; \( \eta^2_p = 0.90 \)) in the distal but not in the central portion of the muscle. The estimated number of sarcomeres in series did not change in either region.

Conclusion: Fascicle length adaptations appear to be heterogeneous in the BFlh in response to 3 weeks of NHE training. An increase in sarcomere length, rather than the addition of sarcomeres in series, appears to underlie increases in fascicle length in the distal region of the BFlh. The mechanism driving regional increases in fascicle and sarcomere length remains unknown, but we speculate that it may be driven by regional changes in the passive tension of muscle or connective tissue adaptations.

Keywords: Hamstrings; Nordic hamstring exercise; Sarcomerogenesis

1. Introduction

Eccentric training is beneficial for enhancing muscle strength, power, and speed.\(^1,2\) It can improve mechanical muscle function more than other exercise modalities can (e.g., concentric and isometric).\(^3\) Longer fascicle lengths have been measured in training programs that sufficiently loading the eccentric phase of a movement,\(^1,5,7\) which is attributable to either longer fiber resting lengths or longitudinal fiber growth. Although this adaptation has been linked to muscle protection during subsequent exercise-induced muscle damage\(^6\) as well as to reduced strain-related injury risk,\(^7,9,10\) the mechanisms underlying these fascicle length changes are still unclear.

Nordic hamstring exercise (NHE) is such an eccentric exercise that elicits changes in fascicle length.\(^5,7\) However, the macroscopic and microscopic changes contributing to the increase in hamstring fascicle lengths remain poorly understood. The hypothesis put forward is that repetitive eccentric contractions cause an addition of sarcomeres in series (sarcomerogenesis) within muscle fibers,\(^11\) thus reducing the likelihood of over-stretching the muscle in subsequent eccentric loading. However, this hypothesis has not been tested due to the lack of simultaneous in vivo measurements of fascicle and sarcomere changes in human subjects. Additionally, methodological limitations, such as extrapolation when measuring fascicle length (e.g., using linear trigonometry to extrapolate fascicle length to...
regions where the fascicle is not visible) may hamper the interpretation of previous findings.\textsuperscript{2, 3–7} It is necessary to establish whether the increases in resting fascicle length observed after eccentric training result from sarcomerogenesis or from a change in resting sarcomere length in order to provide a scientific basis for training program design.

Muscle remodeling processes are related to the stress and strain applied to the muscle,\textsuperscript{13, 14} and muscle strain may be heterogeneous across different regions of the same muscle.\textsuperscript{15, 16} This heterogeneity suggests that morphological, structural, and molecular adaptations to eccentric training may be nonuniform within the muscle.\textsuperscript{17, 18} For example, in vivo measures of sarcomere length in the human tibialis anterior muscle have shown that sarcomeres are longer in the distal compared to the proximal region, despite fibers being of a similar length.\textsuperscript{19} Animal studies also suggest that different muscle architectures and fiber lengths may influence whether and how serial sarcomere adaptations occur.\textsuperscript{20} This highlights the need to use multiscale measurements to understand the nature of region-specific adaptations due to eccentric exercise.

The in vivo measurement of sarcomere and fascicle length has historically been unattainable, but promising new methods have emerged to examine both fascicle length and individual sarcomere numbers in live subjects. Freehand 3D ultrasound (3DUS) combines 2D ultrasound scanning and 3D motion analysis to provide direct in vivo measurements of fascicle length within a wide area of tissue structure.\textsuperscript{21} As such, it is possible to measure fascicles that are much longer than the field of view of conventional B-mode imaging.\textsuperscript{12} Minimally invasive optical microendoscopy visualizes individual sarcomeres and their dynamical length variations in vivo.\textsuperscript{22, 23} The technology can be used to sample sarcomere lengths from multiple fibers and, hence, provide an indication of mean sarcomere lengths in various regions of muscle.\textsuperscript{10, 22, 23} Combining freehand 3DUS with optical microendoscopy may provide evidence of whether eccentric exercise induces sarcomerogenesis in live human muscle.

The purpose of the present study was to measure in vivo changes in both full fascicle lengths and sarcomere lengths of the biceps femoris long head (BFlh) in response to eccentric training in order to determine whether changes in fascicle length are associated with changes in sarcomere number measured. We provide the first concurrent in vivo measurement of both fascicle length and median sarcomere length in multiple human BFlh muscle regions before and after an eccentric training intervention. We sought to investigate adaptations in the hamstring muscle because of the large increases in fascicle length that are reported to occur after relatively short intervention periods.\textsuperscript{2–7} Also, the hamstrings are important muscle groups to study, considering that BFlh strains are the most prevalent noncontact injury in sports involving running.\textsuperscript{24} We hypothesized that in BFlh, fascicle length would increase in response to NHE and that such adaptations would be accompanied by an addition of sarcomeres in series within fascicles.

2. Methods

2.1. Participants

Ten recreationally active volunteers (3 females: age = 25 ± 1 years, mass = 55 ± 3 kg, height = 164 ± 4 cm; 7 males: age = 29 ± 4 years, mass = 77 ± 11 kg, height = 181 ± 9 cm; mean ± SD) with no prior experience with NHE training and no active training for an athletic competition participated in the study. Participants were excluded if they had any lower-limb, trunk, or wrist injuries or any surgeries within the past 18 months, or if they had performed NHE within the past 6 months. Participants provided written informed consent to participate. The protocol was approved by the Stanford University Institutional Review Board, ratified by the University of Queensland ethics committee, and conducted according to the Declaration of Helsinki.

2.2. Study design

The NHE training lasted 3 weeks. Previous studies have shown that short periods of eccentric training are sufficient to elicit lengthening of passively measured BFlh fascicles in untrained individuals.\textsuperscript{3–7} On Day 1, baseline measurements of BFlh fascicle and sarcomere lengths were conducted. Then participants were familiarized with the NHE device and underwent a baseline assessment of eccentric knee flexor strength. All measurements were performed on the right leg, which was the dominant leg for all participants (i.e., the leg that participants used to kick a ball). On Day 2, fascicle length was measured again prior to the first NHE training session. Participants underwent a total of 9 training sessions in the 3 weeks that followed (Table 1). During the training period, participants were allowed to maintain their normal levels of physical activity (~3–5 h/week), but were asked to avoid any hamstring-specific strength training. Post-training BFlh fascicle and sarcomere lengths were measured 1 day after the last training session.

2.3. NHE training

Participants performed the NHE training using a custom-made device, similar to previous studies.\textsuperscript{2, 25} Participants kneeled on a padded board with their ankles secured by braces located superior to the lateral malleolus. The ankle braces were attached to uniaxial load cells (HT Sensor Technology, Shaanxi, China) secured to the board via a rod and swivel. From this position, participants leaned forward slowly with control (~30°/s) as they followed along with a metronome. To minimize concentric loading, once participants’ hands touched the floor, they would push themselves back to the starting position with their arms. Strong verbal encouragement was provided to the participants during all testing and training sessions to ensure maximal effort in each repetition.

2.4. Eccentric knee flexor strength measurement

The hamstrings’ eccentric strength was measured on the same custom device used for the training. Before testing, participants completed a warm-up set of 4 repetitions at 75% of their perceived

| Week | Session/week | Set | Repetition | Total number of repetitions | Rest between sets (min) |
|------|--------------|-----|------------|---------------------------|------------------------|
| 1    | 3            | 4   | 6          | 72                        | 2                      |
| 2    | 3            | 4   | 6          | 72                        | 2                      |
| 3    | 3            | 4   | 8          | 96                        | 2                      |
maximum effort. After a 3-min rest period, participants then completed 1 set of 4 maximal NHE repetitions. The peak knee flexion moment was computed from the load cell measurement at the ankle. The average of the 3 largest strength measurements of the 4 trials was analyzed. This measure produces consistent results between consecutive days (Pearson correlation coefficient \( r = 0.97 \), confidence interval: 0.65–0.99; \( r^2 = 0.95 \); mean absolute error (MAE) = 34 \( \pm \) 25 N).

2.5. Fascicle length

Fascicle lengths were measured using freehand 3DUS, as described elsewhere.\(^{21}\) The 3DUS scan involved B-mode ultrasound imaging (ArtUS EXT-1H beamformer and L15-7H40-A5 transducer (40 mm); Teledem, Vilnius, Lithuania) with synchronous optical tracking (Optitrack; NaturalPoint, Corvallis, OR, USA) of the position and orientation of the transducer. The scan was collected and analyzed using open-source software (Stradwin laboratory, Enspectra Health, Mountain View, CA, USA) of the position half-transverse orientation that allowed identification of the collagenous tissue running along the length of the muscle. Six free-hand 3DUS scans were performed in each session. The 2 scans with the best image quality (e.g., clear and visible fascicles and aponeuroses) were selected for 2D image extraction.

From the stack of 2D B-mode ultrasound images collected (slice thickness: \( \sim 0.36 \) mm; Fig. 1B), a 2D plane covering the entire length of the BFHh fascicles (Fig. 1C) was collected and positioned on the thickest part of the proximal cross-sectional area of the muscle. From this image, the central and distal regions for fascicle length estimation were determined (Fig. 1D). Fascicle orientation may change between regions of interest,\(^{12,26}\) so the location of the 2D plane was adjusted separately for each region by rotating the image along the 3 axes of the muscle volume recorded, optimizing for fascicle continuity. In each image and region, 3 entire fascicles were visually digitalized using the 3D coordinates of 6 points per fascicle. Then the coordinates were imported into Matlab (Version 2018b; The MathWorks, Natick, MA, US), and fascicle length was calculated as the length of a parametric spline approximation fitted to the 3D coordinates. This method allowed the consideration of fascicle curvature in the length estimations. Finally, 6 fascicle length estimations were averaged in each region.

One researcher (PAP) completed all fascicle-length measurements while blinded to participant and time. Between-day fascicle length variability due to measurement error was found to be low (Pearson correlation coefficient \( r = 0.74 \), 95% confidence interval: 0.22–0.94; \( r^2 = 0.55 \); MAE = 0.49 \( \pm \) 0.25 mm).

2.6. Sarcomere length and number

Sarcomere lengths were measured using second harmonic generation microendoscopy (Zebrascope, Zebra Medical Technology, Enspectra Health, Mountain View, CA, USA).\(^{22,23}\) The needle probe consisted of a pair of 2 cm needles with side-mounted lenses and 1 mm separation. One needle emits the laser light, while the other receives the second harmonic generation signals from the muscle fibers. The signal appears as repeating dark (myosin) and light bands. To reduce participant discomfort, the needle probe was inserted using a rapid, spring-loaded device. Because \textit{in vivo} sarcomere lengths are not uniform across intact muscles,\(^{19,27}\) 2 different insertion sites were defined: the central insertion site at 50% of the distance between the ischial tuberosity and the lateral side of the top of the patella and the distal insertion site at 70% of the same distance from the ischial tuberosity. Thus, the needle insertion sites were consistent with the central region of the fascicle length estimation sites (Fig. 1A and 1D). To ensure the fibers remained uncompromised, the probe was inserted such that the needles were approximately perpendicular to the fascicle plane (as determined by ultrasound).

Sarcomere lengths were analyzed as described by Lichtwark et al.\(^{19}\) A sequence of images (image depth: 5–15 mm;
sampling rate = 1 Hz) was collected in real time while slowly moving the microscope and needle through the muscle in order to sample from different fibers and different positions along the length of those fibers. During postprocessing, a Gaussian filter was used to subtract white noise, and a Fourier transform was used to calculate the strongest frequency spectrum between 1.5 µm and 5 µm. A region on the sharpest fiber image was selected for analysis. Across that region (~100 µm in length), the Fourier transform was repeated to estimate the average sarcomere length. One measurement per individual fiber was taken, totaling between 6 and 22 separate muscle fiber estimations (mean = 9.2 estimations) for each region per imaging session, to encompass the variability in sarcomere length measurements across different locations. The number of sarcomere-length estimations varied among subjects, so the median sarcomere length was used as a measure of central tendency for each participant and each session.

The number of sarcomeres in series for each fiber was estimated by dividing the fascicle length (used as a proxy for fiber length) by the median sarcomere length. One researcher (MAB) completed all sarcomere length calculations while blinded to participants’ names and times.

2.7. Statistical analysis

A paired t test was used to evaluate changes in eccentric knee flexor strength, fascicle length, sarcomere length, and the number of sarcomeres in series from baseline until the end of the training. All statistical tests were conducted in Prism (Version 8.4; GraphPad Software, San Diego, CA, USA) with an α level set at p < 0.05. All values are presented as mean ± SD. In the results, each participant is individualized with the same color across figures. Effect sizes are presented as partial eta squared (η²p). Effects at the 0.2, 0.5, and 0.8 were considered as small, medium, and large, respectively, as described by Cohen.26

3. Results

All participants completed all of the allocated training sessions. Eccentric knee flexion strength was greater at the end of the 3-week NHE training than at baseline (Baseline: 3.27 ± 0.85 N·m/kg; End: 3.76 ± 0.67 N·m/kg; p < 0.001; η²p = 0.75) (Fig. 2). Fascicle length increased in the distal region (Baseline: 7.0 ± 0.9 cm; End: 8.5 ± 0.8 cm; p < 0.001; η²p = 0.81) but not in the central region of the muscle (Baseline: 5.3 ± 0.7 cm; End: 5.1 ± 0.7 cm; p = 0.21; η²p = 0.07) (Fig. 3A). Similarly, median sarcomere length increased in the distal region (Baseline: 2.9 ± 0.28 µm; End: 3.4 ± 0.23 µm; p < 0.001; η²p = 0.90) but not in the central region of the BFlh muscle (Baseline: 3.1 ± 0.17 µm; End: 3.2 ± 0.27 µm; p = 0.28; η²p = 0.03) (Fig. 3B). The estimated number of sarcomeres in series did not change in the distal region (Baseline: 24,611 ± 4461 sarcomeres; End: 25,382 ± 2799 sarcomeres; p = 0.22; η²p = 0.07) nor in the central region (Baseline: 32,212 ± 2778 sarcomeres; End: 32,092 ± 4271 sarcomeres; p = 0.46; η²p < 0.01) (Fig. 3C). In Fig. 4, we present images displaying architectural adaptations for a representative subject. In Fig. 5, we present all participants’ sarcomere length measurements for all individual measures in each session and for each region of muscle. This provides an indication of how the distribution of sarcomere length measures changed between sessions.

4. Discussion

In this study, we measured in vivo fascicle and sarcomere lengths in the human BFlh before and after an eccentric training intervention. Three weeks of NHE led to increased resting fascicle and median sarcomere lengths by 21% and 17%, respectively, in the distal portion of the BFlh, which suggests that a large portion of the changes in fascicle length can be accounted for by increases in sarcomere length. However, we found no changes in the estimated number of sarcomeres in series. These findings provide new information about the early adaptations of the BFlh muscle in response to eccentric training.

4.1. Fascicle and sarcomere adaptations to eccentric training

BFlh fascicle length increased after a short period of eccentric training (9 training sessions distributed over 3 weeks), which is similar to previous observations in the midportion of the muscle.5–7 However, we found heterogeneous fascicle length adaptations across the BFlh; fascicle length increased in the distal but not in the central region of the muscle. The average change in fascicle length that we measured (~1.5 cm) is smaller than the change observed previously (~2 cm in the mid-BFlh).5–7 These differences may be related to the differences between the 3D full-field measures used in this study and previous measures made using limited field of view ultrasound imaging5–7 as well as to subtle differences in training load,5 scanning site, and/or instruction to the participants.

We found that sarcomere length increased only in the distal portion of the muscle. In vivo measurement of both fascicle and median sarcomere lengths from the same region of muscle allowed us to estimate the number of sarcomeres in series per fascicle. These estimates revealed no addition of sarcomeres in series in either region of the muscle; length changes of fascicles were proportional to changes in median sarcomere lengths.

Fig. 2. Eccentric knee flexion strength changes at group level. Values for each participant (different colors) are presented at baseline and at the end of the 3-week Nordic hamstring exercise training. Dotted lines are used to denote female participants. The average across all participants is presented in black. * p < 0.05, significant difference when comparing baseline and end time points.
length. Previous studies have suggested that sarcomerogenesis underlies the in vivo changes in hamstrings muscle fascicle lengths after eccentric training.5–7 However, these assertions were supported only indirectly, based on changes in fascicle lengths estimated with 2D ultrasonography or on changes in the angle/torque relationship.29 Thus, to our knowledge, this is the first study to show that eccentric training (such as 3 weeks of NHE) does not induce the addition of sarcomeres in series in humans, despite site-specific increases in fascicle length. Although this result may be unexpected, animal studies show that some muscles experience sarcomerogenesis in response to eccentric exercise,30,31 but others do not.20 Further, serial sarcomere number adaptations appear to differ between muscles of the same limb31 and within regions of the same muscle.32,33 Overall, reported sarcomere adaptation is not consistent in animal models after eccentric exercise, calling into question the predominant theory that sarcomerogenesis underpins the changes in BFllh fascicle lengths after eccentric training.

4.2. Interpretation of findings: Potential mechanisms of adaptation

We did not see an addition of serial sarcomeres in the muscle, but the increase in fascicle length seen here and in previous studies5–7 may represent adaptations to noncontractile
tissue. Although the current study did not investigate noncontractile tissue adaptation, the change in length measured (in resting muscle) may represent changes in passive tension governed by adaptations in connective tissue, titin function, or both. Extracellular matrix components related to muscle fiber compliance (Tenascin-C) and force transmission to sarcomeres (Collagen XII) adapt quickly in response to eccentric exercise \(^7,10\) and may account for our observations. Adaptations in titin stiffness or titin—actin interaction \(^35,36\) seen after eccentric exercise may also play a role in increasing sarcomere and fascicle length. Furthermore, changes in tendon stiffness \(^37\) cannot be disregarded as a potential source of adaptation. Overall, the mechanisms underpinning BF\(h\) architectural adaptations are still not well understood and warrant further investigation.

The observed heterogeneous adaptation to fascicle length at different muscle regions may be important for understanding how a training stimulus relates to muscle adaptation. Previous studies suggest that when muscles are placed under significant strain, muscle adaptations tend to predominate distally. \(^38\) Thus, a heterogeneous distribution of fiber strain \(^39\) and muscle activity \(^40\) toward the distal portion of the BF\(h\) may have influenced this region’s adaptations. Because the hip joint angle remains constant during the NHE, changes in fascicle excursion and tissue strain could be larger in the distal (shorter) portion of the BF\(h\) muscle. \(^41\) Such differences in strain could provide stimulus (e.g., mechanotransduction \(^1\)) for differential adaptations in fascicle lengths across different muscle regions. Further studies are required to better understand the stress and strain distribution across the muscle during eccentric exercises like the NHE and to explain how this relates to muscle adaptation.

4.3. Practical implications

Clinical interventions that employ NHE can expect increasing fascicle lengths in as short a time as 3 weeks; however, this may occur only in the distal region of BF\(h\). This finding presents a unique insight into the immediate (within 3 weeks) adaptations that occur in response to NHE, which may be necessary for tailoring injury-prevention training regimes and for identifying muscles at higher risk for injury. Previous studies suggest that, for the BF\(h\) muscle, longer fascicles may help protect athletes from a hamstring injury. \(^7,10\) However, this study questions the proposed mechanism for such protection. Although “long and strong” \(^7,10\) is considered an effective muscle adaptation to eccentric hamstring exercise, the goal of inducing these changes needs further consideration. The addition of sarcomeres in series may play a role in longer-term training, but we did not detect these changes early in the adaptive process, when most of the change in fascicle length occurs. \(^6\) There is likely to be little muscle-injury protection due to sarcomerogenesis in early training. Analysis of NHE interventions of longer durations using sarcomere and fascicle imaging could provide essential evidence to further evaluate this commonly used training method.

4.4. Strengths and limitations

Combining measurements in macro (fascicle) and micro (sarcomere) adaptations allowed us to overcome some of the methodological limitations of past studies. In particular, it allowed us to assess the full length of the BF\(h\) fascicles (i.e., to consider fascicle curvature) in various muscle regions and to visualize human \(in vivo\) sarcomere-length adaptations. Nevertheless, we recognize several limitations of our study. Although our intervention successfully increased BF\(h\) fascicle lengths, this was a relatively short-term study; hence, further studies are needed to explore whether sarcomerogenesis arises in response to more prolonged training periods (of various intensities). For instance, region-specific adaptations in mechanotransductor pathways related to muscle growth seem to peak at 4 weeks and 8 weeks after eccentric resistance training; \(^1\) thus, sarcomerogenesis may still occur at later stages in NHE training. Additionally, we did not include a control group. Another limitation is that our postintervention measurements were made 1 day after the end of the training, at which point a forced elongation in the sarcomeres may have remained; but further study is needed to test this speculation. Our study was not designed to detect differences in adaptations between genders, and it included a smaller proportion of female participants, so caution must be exercised when extrapolating the results of this study to other populations. For instance, females have shorter optimal lengths of hamstring muscle compared to males. \(^42\) Thus, the mechanical stress imposed by the training intervention may elicit differential adaptations between genders. \(^43\) Muscle activation within the hamstrings is greater in the semitendinosus during the NHE, \(^40\) so adaptations may have predominated in this muscle rather than in the BF\(h\). However, as mentioned before, the NHE has effectively increased fascicle lengths in the BF\(h\). \(^5,7\) Hence, we believe our exercise selection was appropriate for our research question. As an additional limitation, we estimated only passive sarcomere and fascicle lengths, \(^27\) and the estimates of sarcomere length represent only those from the sampled area. However, because we sampled at different depths of pennate BF\(h\) muscle (due to the microendoscopy sampling technique), it is likely that we sampled sarcomere lengths from different regions of each fiber imaged, giving a better estimate of the central tendency of sarcomere length.

5. Conclusion

NHE training (9 sessions in 3 weeks) increased resting fascicle and sarcomere lengths in the distal, but not the central, region of the BF\(h\) muscle. These results suggest a heterogeneous distribution of strain and adaptation within the muscle. The addition of sarcomeres in series did not accompany the adaptations observed in either region. The mechanisms behind these findings remain unclear, but adaptations in connective tissue, titin, and the extracellular matrix may play an important role.

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Authors’ contributions

PAP and MAB collected and analyzed the data and drafted the paper. All authors contributed to the study conception and design.
All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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