# Hamstrings Hypertrophy Is Specific to the Training Exercise: Nordic Hamstring versus Lengthened State Eccentric Training

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#### ABSTRACT

MAEO, S., T. G. BALSHAW, D. Z. NIN, E. J. MC DERMOTT, T. OSBORNE, N. B. COOPER, G. J. MASSEY, P. W. KONG, M. T. G. PAIN, and J. P. FOLLAND. Hamstrings Hypertrophy Is Specific to the Training Exercise: Nordic Hamstring versus Lengthened State Eccentric Training. Med. Sci. Sports Exerc., Vol. 56, No. 10, pp. 1893-1905, 2024. Introduction: The hamstring muscles play a crucial role in sprint running but are also highly susceptible to strain injuries, particularly within the biceps femoris long head (BFlh). This study compared the adaptations in muscle size and strength of the knee flexors, as well as BFIh muscle and aponeurosis size, after two eccentrically focused knee flexion training regimes: Nordic hamstring training (NHT) vs lengthened state eccentric training (LSET, isoinertial weight stack resistance in an accentuated hip-flexed position) vs habitual activity (no training controls: CON). Methods: Forty-two healthy young males completed 34 sessions of NHT or LSET over 12 wk or served as CON (n = 14/group). Magnetic resonance imaging-measured muscle volume of seven individual knee flexors and BFlh aponeurosis area, and maximum knee flexion torque during eccentric, concentric, and isometric contractions were assessed pre- and post-training. Results: LSET induced greater increases in hamstrings (+18% vs +11%) and BFlh (+19% vs +5%) muscle volumes and BFlh aponeurosis area (+9% vs +3%) than NHT (all  $P \le 0.001$ ), with no changes after CON. There were distinctly different patterns of hypertrophy between the two training regimes, largely due to the functional role of the muscles; LSET was more effective for increasing the size of knee flexors that also extend the hip (2.2-fold vs NHT), whereas NHT increased the size of knee flexors that do not extend the hip (1.9-fold vs LSET; both  $P \le 0.001$ ). Changes in maximum eccentric torque differed only between LSET and CON (+17% vs +4%; P = 0.009), with NHT (+11%) inbetween. Conclusions: These results suggest that LSET is superior to NHT in inducing overall hamstrings and BFlh hypertrophy, potentially contributing to better sprint performance improvements and protection against hamstring strain injuries than NHT. Key Words: MUSCLE VOLUME, APONEUROSIS SIZE, ECCENTRIC STRENGTH

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The hamstrings are the primary knee flexors and play a major role in horizontal force production during sprinting (1). Indeed, sprint performance is associated with hamstrings muscle size (2,3) and can be improved by resistance training of the knee flexors (4). However, hamstring strain injuries (HSI) are highly prevalent in many sports such as American football (5), rugby (6), and track and field (7), and account for 12–22% of all injuries in football/soccer (8,9). HSI typically occur during high-speed running, specifically during the late swing phase of sprinting (i.e., when the hip is flexed and the knee is extended) (10,11). The late swing phase involves peak force production by the hamstrings (12), while contracting eccentrically at a relatively long length (peak length for the gait cycle) (13). Thus, establishing an effective training modality for increasing hamstrings muscle size as well as knee

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flexor strength, particularly eccentric strength, will benefit many athletes and coaches for both performance improvement and injury prevention purposes (1,14,15).

Nordic hamstring training (NHT), an eccentric training modality for the knee flexors, has been widely demonstrated to reduce the risk of new and recurrent HSI (16-18). This may be at least partly explained by an increase in hamstring muscle size and eccentric knee flexor strength induced by NHT (14). However, the hip joint remains relatively extended (i.e., ~ anatomical position) throughout NHT. Furthermore, weaker participants may lack the strength to control the lowering movement beyond the initial phase of the contraction during NHT (thereafter presumably falling with relatively low neuromuscular activation at more extended angles). Therefore, the length of the biarticular hamstring muscles during the active phase of NHT appears shorter than during the late swing phase of running (19). In addition, growing evidence suggests that training at long muscle length promotes muscle hypertrophy (15,20,21). Importantly, hamstrings muscle hypertrophy was found to be >50% greater after knee flexion (leg curl) training performed at long lengths (hip flexed) compared with short lengths (hip extended) (15). Furthermore, rehabilitation emphasizing eccentric knee flexion training at long lengths (accentuated hip-flexed position), named lengthened state eccentric training (LSET) (22), resulted in a significantly lower HSI recurrence rate compared with noncompliant athletes (23). Considering these findings, LSET may produce greater increases in hamstrings muscle size and strength than NHT, with implications for injury prevention. However, no study has compared the functional and morphological adaptations of LSET versus NHT.

The biceps femoris long head (BFlh) has the highest susceptibility to HSI (5,16,18). Thus, morphological adaptations of the BFlh to training interventions are of particular interest, including size of the muscle, and its aponeurosis, which is integral to force transmission. Furthermore, a small BFlh proximal aponeurosis has been suggested as a risk factor for HSI by concentrating mechanical strain on the surrounding muscle tissue (24–27). In the vastus lateralis muscle, aponeurosis size appears responsive to resistance training (28–30). To the authors' knowledge, however, no study has investigated whether BFlh aponeurosis size changes after resistance training.

The main purpose of this study was to compare changes in muscle size and strength of the knee flexors, as well as BFlh muscle and aponeurosis size, after 12 wk of LSET versus NHT or habitual activity (control, CON). We hypothesized that LSET would induce greater increases in hamstrings muscle size, BFlh aponeurosis size, as well as eccentric knee flexor strength than NHT.

## METHODS

### Participants

Forty-eight healthy young males with no history of lower extremity injury or systematic exercise training of any kind in the last 18 months provided written informed consent and completed preintervention measurements within this study, which was approved by the Loughborough University Ethics Review Sub-Committee (R17-P054) and Nanyang Technological University Institutional Review Board (IRB-2017-07-030). Participants were first assigned to either CON or training in a 1:2 ratio depending on schedule availability (i.e., whether they could visit the laboratory two to three times a week for 12 wk), and then training participants were randomly assigned to LSET or NHT after the preintervention measurements. A total of six participants withdrew from the study because of personal reasons unrelated to study participation; 42 participants completed the study.

#### **Overview**

Participants visited the laboratory for a familiarization session involving voluntary maximum isometric, concentric, and eccentric contractions. Height, body mass, and physical activity levels with the International Physical Activity Questionnaire (IPAQ, short format [31]) were also measured in this session. Thereafter, two duplicate neuromuscular measurement sessions were conducted both pre (sessions 4-5 d apart before the first training session) and post (2-3 d after the last training session and 4-5 d apart) 12 wk of training or control intervention. This approach of duplicate measurement sessions at each time point is thought to reduce measurement error and may be particularly useful in the context of training adaptations (e.g., Heritage Family Study [32]) and between-group comparisons across two time points (29,33). All measurements were of the dominant leg, and the neuromuscular measurement sessions involved recordings of knee flexion torque and surface electromyography (EMG) of hamstring muscles during voluntary maximum isometric, concentric, and eccentric contractions. Axial T1-weighted magnetic resonance imaging (MRI) scans of the thigh were also conducted pre- (5 d before the first training session) and post-training (2-3 d after the final training session), always preceding the first neuromuscular measurement sessions. Participants in the training groups completed 12 wk (34 sessions) of systematic, progressive (load and volume of repetitions increased) knee flexor training of both legs. All participants were instructed to maintain their habitual physical activity and diet throughout the study, other than the supervised training interventions. Participants were instructed to eat and drink normally and avoid strenuous exercise and alcohol intake for 36 h, and caffeine consumption for 6 h, before all measurement sessions. Measurement sessions were conducted at a consistent time of day for each participant between 9:00 and 20:00 for both MRI and neuromuscular sessions.

#### **Resistance Training Interventions**

The training program consisted of 34 supervised sessions over 12 wk (three times per week apart from weeks 1 and 12 (two times per week); Supplemental Table 1, Number of eccentric sets × repetitions completed in each session of both training programs, Supplemental Digital Content, http://links. lww.com/MSS/D32) with each session separated by  $\geq$ 36 h. The two training regimes were inherently different as NHT is a bilateral, primarily body weight, exercise involving purely eccentric contractions, whereas for LSET, we employed a conventional isoinertial weight stack machine (modified for greater hip flexion) and concentric contractions/lifts to then be able to lower/return the load eccentrically (see Supplemental Fig. 1, Illustrations and training load of the lengthened state eccentric training and Nordic hamstring training, Supplemental Digital Content, http://links.lww.com/MSS/D32). To achieve a high level of eccentric loading with LSET, the concentric load was lifted with two legs and lowered eccentrically with one leg. Nonetheless, the number of eccentric sets and repetitions were standardized across both training regimes. The number of eccentric sets with each leg increased from 2 to 4, and the number of eccentric repetitions per set from 6 to 10, throughout the training program. All training sessions began with a standardized cycling warm-up (5 min, 70 rpm, 150 W; Ergomedic 874 E, Monark Exercise AB, Sweden). This was followed by 2× of ~15 s of static stretches of the hamstrings of each leg in a standing position (with the involved knee extended, contralateral knee flexed, and hip flexed to lean the upper body forward toward the extended leg). Consistent verbal encouragement was provided for both groups throughout the training.

Lengthened state eccentric training. Participants were positioned on a modified seated leg curl machine (Seated Leg Curl SL40, LifeFitness, USA), specifically with a modified back rest so that the hip was maintained in a flexed position ( $120^\circ$ ,  $0^\circ$  = anatomical position) to ensure the hamstrings were trained in a lengthened state (Supplemental. Fig. 2A, Supplemental Digital Content, http://links.lww.com/MSS/ D32). The knee joint center was aligned with the axis of rotation of the machine's lever arm, with each participant's seating position and lever arm length noted and replicated throughout the study. Adjustable straps were tightly secured across the hips, chest, and knee to prevent extraneous movement and to maintain the hip angle. To facilitate high eccentric knee flexor loading, participants first flexed the knee by pulling the lever arm down and back (i.e., concentric knee flexor contraction) using both legs until the knee joint angle was  $\sim 90^{\circ}$ , then using only one leg performed a slow and controlled knee extension ~4 s (i.e., eccentric knee flexor contraction) returning the lever arm to its original position. Participants alternated the leg that performed eccentric sets until the required number of sets was completed on both legs with a rest period of 5 min between legs and 2 min between sets. The starting load/weight of each participant was based on pretest strength measurements then iteratively adjusted/increased (typically by 2 kg) when participants could perform all the prescribed repetitions of the final (if two or three sets) or penultimate (if four sets) set, and all training loads were recorded in training logs/sheets. To reduce the chance of hamstring injury from contracting the muscle at unaccustomed long lengths in the early weeks of the training, knee joint range of motion, and thus the lengthened state of the hamstrings muscle, was progressively increased during the first 5 wk of training. This was done by manipulating the length of the cable between the training machine lever arm and the weight stack and ensuring that participants fully lowered the weight between each repetition. Specifically, knee joint angle at the start/end of each repetition (i.e., most extended position) was increased weekly by  $5-7^{\circ}$  in weeks 1-5 from  $37^{\circ}$  to  $32^{\circ}$ ,  $26^{\circ}$ ,  $19^{\circ}$ , and finally to  $14^{\circ}$  ( $0^{\circ}$  = full extension) in week 5 onward, based on the goniometer measurements.

Nordic hamstring training. Participants knelt on a padded 30-cm high box, with the lower leg horizontal and both ankles protruding over the rear end of the box, while the thighs and torso were initially vertical (Supplemental Fig. 2B, Supplemental Digital Content, http://links.lww.com/MSS/D32). Each ankle was restrained by an inextensible strap, placed 4 cm above the medial malleolus, and in series with an S-beam strain gauges (Force Logic, Swallowfield, UK). From this initial position, participants slowly leaned/lowered themselves forward from the knees by eccentrically contracting their hamstrings. They were instructed to take ~4 s to perform this controlled lowering eccentric contraction, keeping the hips and torso straight and their arms close to their chest for as long as possible, before being unable to further control the descent and falling onto a crash matt placed on the ground in front of them. The analogue force from both strain gauges was sampled at a frequency of 2000 Hz using an A/D converter (CED Power 1401 mk II, CED, UK) and a personal computer (Spike 2, CED, UK), and displayed on a screen placed on the ground in front of the participant to provide real-time visual feedback of force during the NHT. When participants could control their descent to within 15° of horizontal, progression involved additional load, added by use of a weighted vest starting at 1 kg and progressing up to 21 kg in one participant.

#### **Pre- and Postintervention Measurements**

Dynamometry and EMG. While seated on an isokinetic dynamometer (Contrex MJ, CMV AG, Dubendorf, Switzerland) with a hip flexion angle of  $60^{\circ}$  ( $0^{\circ}$  = full extension), strapping was secured across the participant's waist, shoulders, and distal thigh just above the patella of the involved lower extremity to minimize extraneous bodily movement during contractions. A high-density foam shin pad was secured behind the shank of the dominant leg  $\sim$ 2 cm above the medial malleolus. The shank was then strapped to the dynamometer crank arm at ~15% of shank length (i.e., the distance between the lateral malleolus and knee joint space) above the medial malleolus. The knee joint space was aligned with the dynamometer axis of rotation during a submaximal knee flexor contraction, whereas the knee joint was positioned at a midrange angle. Analogue torque, crank angle, and crank angular velocity signal outputs from the dynamometer were recorded using an A/D converter (Micro 1401, CED, UK) and associated computer software (Spike 2, CED, UK) during isometric, concentric, and eccentric knee flexion contractions. Torque, crank position, and crank velocity data were smoothed at 15 Hz for analysis purposes.

Surface EMG from the lateral and medial hamstrings was recorded using a wireless EMG system (Trigno, Delsys, USA) during all maximum knee flexion contractions. Skin preparation (shaving, abrading, and swabbing with 70% ethanol) preceded sensor fixation on the skin with the use of adhesive interfaces. Single differential Trigno Standard EMG sensors, constituting a bipolar configuration, were situated over the lateral and medial hamstrings at 50% of thigh length above the popliteal fossa. EMG signals were amplified at source (x300, 20 to 450 Hz bandwidth) before further amplification (x909, overall effective gain) and sampled at 2000 Hz. Correction for the inherent 48-ms delay of the analogue signal from the Trigno EMG system was performed before analysis to time align EMG data with torque, angle, and angular velocity signals, with all variables synchronously recorded using the same A/D converter and computer software.

Maximum isometric knee flexion contractions. Participants performed two isometric maximum voluntary contractions (MVC) of the dominant limb for 3-5 s at each crank angle of  $10^\circ$ ,  $95^\circ$ ,  $38^\circ$ , and  $66^\circ$  (in the order listed,  $0^\circ$  = full extension), following a series of incremental isometric knee flexion contractions ( $\sim$ 3–5 s per contraction; 3 × 50%, 3 × 75%, and  $1 \times 90\%$  of perceived maximum effort) at the initial crank angle (10°). During MVC, participants were instructed to "pull as hard as possible" until they were provided with the signal to cease the contraction, with intense verbal encouragement provided during all maximum isometric efforts. Real-time biofeedback, displayed on a computer screen in front of the participant, was provided to indicate the highest isometric torque achieved at each angle and motivate participants to improve their performance relative to the previous maximum effort. Isometric torque data were gravity corrected by subtracting baseline (passive) torque. Within an individual test session, isometric maximum voluntary torque was defined at each crank position as the highest torque achieved during the two maximum efforts. Hamstrings EMG amplitude was the average of the root mean square of both hamstrings sensors measured during a 500-ms epoch at isometric knee flexion maximum voluntary torque (250 ms either side) at each crank position.

Isometric maximum knee flexion torque at each crank angle was taken as a mean of the two test sessions at pre or post if there was less than a 10% difference; otherwise, a weighted mean was derived (weighting, in favor of the higher score, increased as the percentage difference between the two scores increased). Actual knee joint angles during the maximal contractions were derived from video camera recordings as detailed in the Supplemental Digital Content (Supplemental Digital Content, http://links.lww.com/MSS/D32). Knee joint angles at each crank position (10°, 38°, 66°, and 95°) were collapsed across all four test sessions and corresponded to knee joint angles of  $35 \pm 5^{\circ}$ ,  $55 \pm 6^{\circ}$ ,  $77 \pm 8^{\circ}$  and  $98 \pm 8^{\circ}$ , respectively. Quadratic functions were fitted to the relationship between the measured torque-angle relationship for each participant at each time point (pre and post) and used to derive knee flexion torque at 10° intervals between 35° and 95° knee joint angles for each participant. Maximum isometric torque was taken as the highest value produced at any of the 10° intervals in this range. Hamstring EMG amplitude was taken as the mean of the two test sessions at each time point.

Maximum concentric and eccentric knee flexion contractions. Isovelocity concentric and eccentric strength measurements involved passive torque assessment, followed by warm-up and MVC. Passive torque was assessed during four passive knee flexion-extension repetitions (middle two used for analysis) through 0–95° crank arm range of motion at an isovelocity of  $50^{\circ} \cdot s^{-1}$ , while the participant was instructed to remain completely relaxed. Thereafter, participants performed two warm-up sets (at ~50% and 80% of maximum effort) of two concentric–eccentric repetition cycles at  $50^{\circ} \cdot s^{-1}$ throughout their full range of movement (0-95°) with 30 s between sets. Participants were then instructed to "pull as hard as you can throughout the range of movement" during both the concentric and eccentric phases, and completed two maximum effort sets of two concentric-eccentric repetition cycles at  $50^{\circ} \cdot s^{-1}$  with 45 s rest between sets. Real-time torque biofeedback was provided throughout the contractions with the highest concentric and eccentric torque achieved so far indicated and intense verbal encouragement provided during all maximum efforts.

Maximum concentric and eccentric knee flexion torque was defined as the instantaneous highest torque registered within the isovelocity period (i.e., within 10% of the target velocity of  $50^{\circ} \cdot s^{-1}$ ), corrected for angle-specific passive limb torque. Root mean square EMG amplitude of both hamstrings sensors was measured during 200-ms epochs (100 ms either side) at both concentric and eccentric flexion maximum torque, and then averaged across both sensors. Concentric and eccentric maximum torque values were taken as a mean of the two test sessions at pre or post if there was less than a 10% difference between measurements from each session; otherwise, a weighted mean was derived. Concentric and eccentric hamstring EMG amplitude was taken as the mean of the two test sessions at each time point.

Magnetic resonance imaging. A 3-T MRI scanner (GE Healthcare Discovery MR750w 3.0-T MRI scanner) was used to scan the dominant leg in the supine position with the hip and knee in the extended/anatomical position. Using body array and spine coils, T1-weighted axial images were acquired from the anterior superior iliac spine to below the insertion of the popliteus (POP) on the tibia, in three overlapping blocks. Fish oil capsules were placed on the lateral aspect of the participant's thigh to allow blocks to be aligned during analysis. The following imaging parameters were used: imaging matrix =  $512 \times 512$ , field of view =  $260 \text{ mm} \times 260 \text{ mm}$ , spatial resolution =  $0.508 \text{ mm} \times 0.508 \text{ mm}$ , slice thickness = 5 mm, interslice gap = 0 mm, repetition time = 600 ms, echo time = 7.648 ms. MRI data were anonymized before analysis (i.e., investigators were made blinded to the conditions/groups). Preand postimages were analyzed side by side to allow for consistent analysis (in terms of inclusion/exclusion of noncontractile tissues such as aponeurosis, blood vessels and nerves) within each participant, with a convolution filter (sharpen  $5 \times 5$ ) applied to sharpen the images.

*Muscle volume.* Anatomical cross-sectional areas of the biceps femoris short head (BFsh), BFlh, semitendinosus (ST),

semimembranosus (SM), sartorius (SAR), and gracilis (GRA) muscles were outlined every third slice, and that of the POP was outlined every slice, from the most distal to proximal images using image analysis software (Horos software, version 1.1.7). The volume of each muscle was calculated using cubic spline interpolation of the anatomical cross-sectional areas along the limb (100 points, Origin 2021, OriginLab Corporation). The volumes of the hamstrings (HAMS) and overall knee flexors (KF) were calculated by summing the volumes of the four hamstrings and seven knee flexors muscles, respectively. We also calculated the volume of the knee flexors that extend the hip (KF and HE; sum of BFlh, ST, SM) and the knee flexors that do not extend the hip (KF not HE; sum of BFsh, GRA, SAR, POP).

**BFIh aponeurosis morphology.** The contact interface distance between the BFIh muscle and the proximal aponeurosis was outlined in each image in which the aponeurosis was identifiable (24). The contact interface distance in each slice included both the internal and external aponeurosis, and the highest contact interface distance across slices was considered maximum width. BFIh aponeurosis area was calculated as the product of the contact interface distance multiplied by the slice thickness (24). In addition, aponeurosis length was calculated by multiplying the number of images in which the aponeurosis was identifiable by slice thickness.

#### **Data and Statistical Analysis**

All statistical analyses were performed using SPSS software (v22, IBM Corporation, USA). Data normality was assessed using the Shapiro-Wilk test for each variable on pretest values pooled across all groups. Three variables (SAR muscle volume, isometric EMG, and concentric EMG) were found to be nonnormally distributed, and these data were log10 transformed for further analysis. One-way analysis of variance (ANOVA) was conducted on all pretest variables to assess whether baseline differences existed between groups. To examine training load progression within each training group, oneway ANOVA was conducted on the eccentric phase load at week 1, 4, 8, and 12 followed by least significant differences (LSD) tests corrected for multiple comparisons. Withingroup pre- to postintervention changes for absolute data were evaluated using paired *t*-tests. Comparisons of between-group adaptations to the intervention were assessed with repeated measures analysis of covariance (ANCOVA; group (LSET vs NHT vs CON)  $\times$  time (pre vs post)), with corresponding pretraining values used as covariates. When group × time interaction effects displayed P < 0.05, post hoc tests were conducted. Specifically, absolute change values were calculated for the variables that had significant interaction effects, and were compared among groups by one-way ANCOVA followed by LSD tests corrected for multiple comparisons. Data are presented as mean ± SD in the text/tables and mean  $\pm$  SE within the figures. Of the 42 participants who completed all the measurements (n = 14/group), the following MRI variables were excluded from the analysis because of poor image quality: all MRI variable data from three CON participants; SAR/GRA/POP volume data from one NHT participant; POP volume data from one LSET participant; aponeurosis data from one NHT participant (detailed in each table and figure).

# RESULTS

**Group characteristics at baseline.** Height (LSET  $1.78 \pm 0.06$ ; NHT  $1.76 \pm 0.08$ ; CON  $1.78 \pm 0.07$  m), body mass (LSET  $77 \pm 11$ ; NHT  $76 \pm 13$ ; CON  $73 \pm 6$  kg), age (LSET  $25 \pm 4$ ; NHT  $27 \pm 3$ ; CON  $24 \pm 3$  yr) and habitual physical activity (IPAQ: LSET  $1580 \pm 479$ ; NHT  $1198 \pm 392$ ; CON  $1342 \pm 458$  MET·min·wk<sup>-1</sup>) did not differ between groups at baseline (ANOVA,  $0.073 \le P \le 0.752$ ). Similarly, there were no baseline between-group differences in knee flexor muscle volume (individual muscles and muscle groups;  $0.065 \le P \le 0.976$ ), BFIh aponeurosis morphology ( $0.375 \le P \le 0.834$ ), maximum knee flexion torque (across contraction types;  $0.256 \le P \le 0.912$ ) (Tables 1 and 2).

**Training quantification for LSET and NHT.** The eccentric phase load in the LSET group had increased by week 4 (+26% vs week 1; P < 0.001) and increased further by week 12 (+41% vs week 1; P = 0.017 vs week 4) (Supplemental Fig. 2A', Supplemental Digital Content, http://links. lww.com/MSS/D32). Maximum eccentric force during the Nordic hamstring exercise in the NHT group increased by week 4 (+25% vs week 1; P = 0.047), with a subtle nonsignificant further increase by week 12 (+37% vs week 1) (Supplemental Fig. 2B', Supplemental Digital Content, http://links. lww.com/MSS/D32).

Muscle size. Following LSET, with the exception of the POP (P = 0.066), within-group increases occurred (paired t-test, all P < 0.001) in the volume of all four individual constituent hamstrings muscles (BFsh +6%; BFlh +19%; ST +27%; SM +14%), SAR (+8%), GRA (+24%), overall hamstrings (+18%), KF and HE (+20%), KF not HE (+11%), and overall knee flexors (+17%; Table 1). After NHT, with the exception of the SM (P = 0.423) and POP (P = 0.130), there were pre- to postincreases in all individual constituent muscles of the hamstrings (BFsh +22%; P < 0.001, BFlh +5%; *P* < 0.021, ST +20%; *P* < 0.001), SAR (+18%; *P* < 0.001), GRA (+30%; P < 0.001), overall hamstrings (+11%; P < 0.001), KF and HE (+9%; P < 0.001), KF not HE (+22%; P < 0.001), and overall knee flexors (+14%;P < 0.001). After CON, there were no within-group changes in the volume of any muscle or muscle group (paired *t*-test,  $0.173 \le P \le 0.955$ ).

All the muscle volume measurements (ANCOVA (all) P < 0.001), except for POP (P = 0.437; Table 1), showed significant group × time effects. LSET resulted in greater absolute muscle volume increases in the BFlh and SM compared with NHT (LSD (all) P < 0.001) and CON ((all) P < 0.001), but the changes in these muscles did not differ between NHT

TABLE 1. Muscle volume of constituent knee flexor muscles, anatomical and functional muscle groups, and BFIh aponeurosis morphology pre and post LSET (n = 14), NHT (n = 14), and control (CON, n = 11) interventions.

	LSET		NHT		CON		ANCOVA Interaction
	Pre	Post	Pre	Post	Pre	Post	Р
Volume of individual muscle	s (cm <sup>3</sup> )						
BFsh	108 ± 22	115 ± 20***	103 ± 31	126 ± 33***	106 ± 32	105 ± 33	< 0.001
BFIh	189 ± 32	224 ± 34***	200 ± 50	211 ± 51*	184 ± 34	185 ± 37	< 0.001
ST	202 ± 44	257 ± 54***	227 ± 61	273 ± 70***	206 ± 40	201 ± 46	< 0.001
SM	237 ± 38	270 ± 35***	248 ± 72	252 ± 77	236 ± 36	239 ± 35	< 0.001
SAR	153 ± 44	166 ± 44***	140 ± 28	166 ± 35***	146 ± 39	144 ± 39	< 0.001
GRA	94 ± 34	117 ± 41***	98 ± 32	127 ± 37***	103 ± 38	102 ± 39	< 0.001
POP	21.7 ± 3.8	22.1 ± 4.0	18.0 ± 3.4	18.4 ± 3.4	21.9 ± 5.3	22.0 ± 5.4	0.437
Volume of muscle groups (c	:m <sup>3</sup> )						
Hamstrings	736 ± 95	867 ± 99***	778 ± 191	861 ± 207***	732 ± 113	731 ± 122	< 0.001
KF and HE	628 ± 82	752 ± 88***	675 ± 166	735 ± 178***	626 ± 87	625 ± 97	< 0.001
KF not HE	377 ± 91	420 ± 100***	355 ± 81	432 ± 90***	377 ± 97	374 ± 101	< 0.001
Overall KF	1008 ± 168	1176 ± 179***	994 ± 161	1129 ± 182***	1003 ± 179	999 ± 192	< 0.001
Aponeurosis							
Area (cm <sup>2</sup> )	35.3 ± 7.8	38.5 ± 8.2***	37.6 ± 9.4	38.8 ± 9.5*	33.0 ± 5.9	33.5 ± 6.3*	< 0.001
Maximum width (cm)	3.42 ± 0.76	3.68 ± 0.72***	3.72 ± 0.95	3.74 ± 0.95	3.29 ± 0.77	3.34 ± 0.80	0.016
Length (cm)	19.2 ± 3.6	19.1 ± 3.6	18.4 ± 4.2	18.4 ± 4.2	18.5 ± 2.7	18.5 ± 2.7	0.300

Data are means  $\pm$  SD. Within-group effects of time were determined from paired *t*-tests and are denoted by \*P < 0.05 or \*\*\*P < 0.001. ANCOVA interaction effects of time (pre vs post) × group (LSET vs NHT vs CON) are reported. Post hoc comparisons of between-group changes are shown in Figures 1, 2, and 4. Hamstrings, the sum of BFsh, BFlh, ST, and SM. KF and HE, the sum of BFsh, ST, and SM. KF not HE, the sum of BFsh, SAR, GRA, and POP. Overall KF, the sum of all seven individual knee flexors. Participant numbers are as stated previously other than the following: POP, KF not HE, and overall KF in LSET (n = 13); SAR, GRA, POP, KF not HE, overall KF, and all aponeurosis variables in NHT (n = 13).

and CON (LSD  $0.053 \le P \le 0.949$ ; Fig. 1A). In contrast, NHT produced greater increases in absolute volume of the BFsh and SAR (Fig. 1B) than LSET (LSD 0.001  $\leq P \leq 0.010$ ) or CON ([both] P < 0.001), and these muscles also had greater increases after LSET than CON ( $0.010 \le P \le 0.027$ ). LSET and NHT produced similar muscle volume increases in ST (P = 0.072) and GRA (P = 0.113), and both training groups increased by more than CON (LSET, LSD (all) P < 0.001; NHT LSD (all) P < 0.001). Overall hamstring volume change was different between all three groups (LSET > NHT > CON; both P < 0.001; Fig. 2). KF and HE as well as KF not HE volume changes also showed differences between all three groups but with opposite patterns LSET > NHT > CON for KF and HE (both P < 0.001), but NHT > LSET > CON for KF not HE (both  $P \le 0.001$ ). Overall knee flexor volume increases were greater for both LSET and NHT ((all) P < 0.001) compared with CON but did not differ between the two training groups (P = 0.095). Percentage change values (based on pre to post mean changes [34]) for each muscle and muscle groups are summarized in Figure 3.

**BFIh aponeurosis.** BFIh aponeurosis area showed withingroup increases from pre to post after LSET (+9%; paired *t*-test, P < 0.001), NHT (+3%; P = 0.026), and CON (+2%; P = 0.030; Table 1). The absolute increases in BFlh aponeurosis area were greater for LSET than NHT (LSD P = 0.001) and CON (P < 0.001; Fig. 4A) but did not differ between NHT and CON (P = 0.292). Within-group increases in BFlh aponeurosis maximum width only occurred after LSET (+8%; paired *t*-test, P < 0.001), not NHT (P = 0.788) or CON (P = 0.446; Table 1). Absolute increases in BFlh aponeurosis maximum width were greater for LSET than NHT (LSD P = 0.031) or CON (P = 0.038; Fig. 4B) but did not differ between NHT and CON (P = 0.876). BFlh aponeurosis length did not increase within any group (paired *t*-test,  $0.336 \le P \le 0.337$ ) and showed no group × time effect (ANCOVA P = 0.300; Table 1).

**Maximum eccentric, isometric and concentric knee flexion strength.** Maximum eccentric torque increased from pre to post within the LSET (+17%; paired *t*-test, P = 0.002) and NHT groups (+11%; P = 0.048), but not for CON (+4%; P = 0.397; Table 2). The absolute increase in maximum eccentric torque following LSET was greater than CON (LSD, P = 0.009; Fig. 5A) but did not differ between LSET and NHT (P = 0.237) or NHT and CON (P = 0.104). Within-group increases in maximum isometric torque occurred after LSET (+27%; paired *t*-test, P < 0.001), NHT (+25%; P < 0.001), and CON (+14%; P < 0.001). The absolute increases in maximum isometric

TABLE 2. Maximum knee flexion torque during eccentric, isometric, and concentric contractions pre and post LSET (n = 14), NHT (n = 14), and control (CON, n = 14) interventions.

	LSET		NHT		CON		ANCOVA Interaction
	Pre	Post	Pre	Post	Pre	Post	Р
Knee flexion torque (N·m	1)						
Eccentric (50°/s)	145 ± 23	169 ± 24**	143 ± 45	159 ± 39*	128 ± 33	133 ± 27	0.013
Isometric (0°/s)	124 ± 26	158 ± 23***	132 ± 48	166 ± 39***	111 ± 32	127 ± 27***	< 0.001
Concentric (50°/s)	120 ± 20	142 ± 19**	122 ± 39	139 ± 30*	108 ± 29	118 ± 27*	0.063
Hamstring EMG (mV)							
Eccentric (50°/s)	0.099 ± 0.048	0.135 ± 0.063***	0.086 ± 0.043	0.106 ± 0.068	0.087 ± 0.040	0.092 ± 0.050	0.125
Isometric (0°/s)	0.122 ± 0.073	0.177 ± 0.077***	0.099 ± 0.059	0.155 ± 0.088**	0.086 ± 0.032	0.104 ± 0.038*	0.046
Concentric (50°/s)	0.118 ± 0.056	0.148 ± 0.058**	0.111 ± 0.054	0.137 ± 0.076*	0.111 ± 0.050	0.119 ± 0.053	0.278

Data are means  $\pm$  SD. Within-group effects of time were determined from paired *t*-tests and are denoted by \**P* < 0.05, \*\**P* < 0.01, or \*\*\**P* < 0.001. ANCOVA interactions for time (pre vs post) × group (LSET vs NHT vs CON) are reported. Post hoc comparisons of between-group changes are shown in Figure 5.



FIGURE 1—Absolute changes (pre to post) in the volume of seven constituent knee flexor muscles following LSET (n = 14), NHT (n = 14), and control (CON, n = 11) interventions. Symbols indicate between-group differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON, †different from LSET, §different from NHT. Data are means ± SE. Participant numbers are as stated previously other than the following: POP in LSET (n = 13); SAR, GRA, and POP in NHT (n = 13).

torque for LSET (LSD P = 0.002) and NHT (P = 0.001) were both greater than CON but did not differ between LSET and NHT (P = 0.697). Maximum concentric torque showed within-group increases following LSET (+18%; paired *t*-test, P = 0.001), NHT (+13%; P = 0.042), and CON interventions

200

150

100

50

0

∆ Muscle Volume (cm<sup>3</sup>)

(+9%; P = 0.027), but no group × time effect was observed (ANCOVA P = 0.063).

#### **Isometric knee flexion torque–angle relationships.** After LSET (+18% to +27%; paired *t*-test, (all) $P \le 0.001$ ;



FIGURE 2—Absolute changes (pre to post) in the volume of anatomical and functional muscle groups following LSET (n = 14), NHT (n = 14), and control (CON, n = 11) interventions. Symbols indicate between-group differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON, §different from NHT, †different from LSET. Data are means ± SE. Overall KF, the sum of all seven individual knee flexors. HAMS, the sum of the four hamstring muscles. KF and HE, the sum of BFlh, ST, and SM. KF not HE, the sum of BFsh, SAR, GRA, and POP. Participant numbers are as stated previously other than the following: overall KF and KF not HE in LSET and NHT (n = 13).



FIGURE 3—Summary of the percentage changes in muscle volume of the individual knee flexor muscles, and anatomical and functional muscle groups based on pre to post mean changes for each muscle or muscle group after LSET, NHT and control (CON) interventions. Symbols indicate betweengroup differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON, §different from NHT, †different from LSET. KF, the sum of all seven individual knee flexors. HAMS, the sum of BFsh, BFlh, ST, and SM. KF and HE, the sum of BFlh, ST, and SM. KF not HE, the sum of BFsh, SAR, GRA, and POP.

Fig. 6B), there were within-group increases in isometric torque at all knee joint angles between 35° and 95°. After CON, there were pre- to postincreases in isometric torque between 35° and 55° (+11% to +18%; paired *t*-test, 0.001  $\leq P \leq$  0.012), but not between 65° and 95° (0.061  $\leq P \leq$  0.636; Fig. 6C). Significant group × time effects were observed for isometric torque at all knee joint angles between 35° and 95° (0.001  $\leq P \leq$  0.020). Absolute increases in isometric torque were greater for NHT compared with CON for all knee joint angles (i.e., 35° to 95°; LSD 0.001  $\leq P \leq$  0.017; Fig. 6D) and were also greater for NHT compared with LSET for 55° and 75° (LSD 0.035  $\leq P \leq$  0.040), but not at other angles (0.062  $\leq P \leq$  0.766). Greater increases in absolute isometric

torque for LSET compared with CON occurred from  $35^{\circ}$  to  $75^{\circ}$  (LSD  $0.008 \le P \le 0.047$ ), but not at  $85^{\circ}$  and  $95^{\circ}$  ( $0.082 \le P \le 0.088$ ).

**Surface EMG.** After LSET, there were within-group increases in eccentric (+37%; paired *t*-test, P < 0.001), isometric (+45%; P < 0.001), and concentric (+25%; P = 0.002) hamstring EMG (Table 2). After NHT, there were pre- to postincreases in isometric (+56%; P = 0.004) and concentric (+23%; paired *t*-test, P = 0.027), but not eccentric (P = 0.081), hamstring EMG. After CON, there were within-group increases in isometric (+21%; P = 0.044), but not eccentric or concentric (paired *t*-test, 0.475  $\leq P \leq 0.651$ ), hamstring EMG. No group × time effects were detected for eccentric or concentric hamstring EMG



FIGURE 4—Absolute changes (pre to post) in BFlh aponeurosis area (A) and maximum width (B) following lengthened state training (LSET, n = 14), NHT (n = 13), and control (CON, n = 11) interventions. Symbols indicate differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON, §different from NHT. Data are means ± SE.



FIGURE 5—Absolute changes (pre to post) in maximum knee flexion torque and hamstring EMG during eccentric, concentric, and isometric contractions following lengthened state training (LSET, n = 14), NHT (n = 14), and control (CON, n = 14) interventions. Symbols indicate differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON. Data are means ± SE.

(ANCOVA  $0.125 \le P \le 0.278$ ). A significant group × time effect was observed for isometric hamstring EMG (ANCOVA P = 0.046), but post hoc comparisons of absolute change data did not reveal any between-group differences (LSD  $0.065 \le P < 1.00$ ; Fig. 5B).

# DISCUSSION

The main findings of this study were that LSET induced greater increases in the volume of the hamstrings and BFlh muscle as well as BFlh aponeurosis size than NHT. In addition, there was a distinctly different pattern of hypertrophy between the training regimes, with larger increases in the BFlh and SM after LSET (more than threefold vs NHT), but greater increases in BFsh and SAR after NHT (more than twofold vs LSET). These hypertrophic differences between exercises appeared to be largely due to the functional role of the muscles; LSET was more effective for increasing KF and HE size (more than twofold vs NHT) and NHT was more effective for increasing KF not HE size (approximately twofold vs LSET). The different pattern and magnitude of responses after LSET supported the first part of our hypothesis and suggests that LSET is superior to NHT in inducing greater hypertrophy of the hamstrings as well as the size of the BFlh muscle and aponeurosis, potentially contributing to better sprint performance and protection against HSI, which frequently occur within this muscle. However, contrary to the second part of our hypothesis, there were no differences in knee flexor eccentric strength gains between the two training regimes, perhaps because of similar increases in overall KF muscle volume.

**Hypertrophic adaptations.** After 12 wk of the intervention, both LSET and NHT significantly increased the volume of all the knee flexor muscles, except for SM after NHT and the smallest muscle (POP) in both groups, whereas the control group remained very consistent across all seven muscles (Table 1). However, there was no significant difference in overall knee flexor volume changes between LSET and NHT (Fig. 2).



FIGURE 6—Knee flexion maximum isometric torque–angle relationships pre and post (A) LSET (n = 14), (B) NHT (n = 14), and (C) control (CON, n = 14) interventions. D, Absolute changes (pre to post) in maximum knee flexion torque at knee joint angles from 35° to 95° (0° = full extension). A–C, Symbols denote significant within-group increases in torque from pre to post at the angle marked determined by paired *t*-tests as follows: \*P < 0.05, \*\*P < 0.01. Data are means ± SD. D, Symbols indicate differences in the magnitude of pre to post changes where post hoc tests displayed LSD P < 0.05: \*different from CON, †different from LSET. Data are means ± SE.

The fact that overall knee flexor hypertrophy was similar for LSET and NHT may suggest that the hypertrophic stimulus was comparable between the two types of training despite their many differences, including different muscle lengths and postures, bilateral versus unilateral eccentrics, concentric contractions with LSET (even if at a low load), and body weight vs weight stack resistance. Despite the similar overall knee flexor hypertrophy, there were many differences between the training regimes for smaller muscle groups and individual muscles as discussed below in detail.

LSET not only resulted in greater hypertrophy of the hamstrings compared with NHT (1.7-fold) but also produced a different pattern of hypertrophy between muscles, larger increases in BFlh (3.5-fold) and SM (9.7-fold), similar increases in the ST (1.3-fold), but smaller increases in BFsh (3.8-fold greater after NHT; Fig. 3). Thus, this study found pronounced evidence for training-specific adaptations in the amount and pattern of hypertrophy with different knee flexion exercises. In accordance with Maeo et al. (15,20), this suggests that exercise selection can markedly affect the morphological changes with resistance training even when exercises involve the same joint action. The greater hamstrings hypertrophy after LSET than that after NHT and the pattern of the individual hamstrings muscle changes are similar to the findings of Maeo et al. (15) who also found greater hamstrings muscle hypertrophy after 12 wk of knee flexion training at long lengths (hip flexed, seated) versus short lengths (hip extended, prone), with the most pronounced differences for the BFlh (2.2-fold, +14.4% vs +6.5%) and SM (2.3-fold, +8.2% vs +3.6%) compared with a more modest difference in the ST (1.2-fold, +23.6% vs 19.3%). Kellis and Blazevich (19) suggest that the contribution of the BFlh and SM to knee flexion torque production is much higher than the other two constituents when the hamstrings are in a lengthened position (i.e., in a hip-flexed and knee-extended position; see Figs. 4 and 5 of Ref. [19]). Therefore, the current study together with these previous studies (15,19) indicates that LSET is the better choice than NHT when aiming to elicit hamstrings hypertrophy and especially of the constituent BFlh and SM muscles.

Interestingly, NHT resulted in no/small hypertrophy of the BFlh and SM (similar to CON and <LSET) but clear hypertrophy of the ST and BFsh (>CON, and similar or >LSET, respectively). The lack of BFlh hypertrophy after NHT may be surprising based on acute EMG studies that indicate a high level of BFlh activation during this exercise (35,36). However, EMG studies may be misleading because of difficulties in accurately locating electrodes over individual muscles and cross-talk (37). Using functional MRI, Bourne et al. (38) found BFlh and SM activation during NHT to be significantly lower than the BFsh and especially ST, which broadly mimics the pattern of hypertrophy seen after NHT in the current study. Moreover, Bourne et al. (39) observed similar hypertrophic effects of NHT to the current study after 10 wk (20 sessions), with no changes in BFlh and SM compared with CON, but BFsh and ST showing marked hypertrophy. The current study with nearly double the training sessions (34 sessions in 12 wk) reinforces the finding that NHT produces no/negligible hypertrophy of the SM and BFlh but substantial hypertrophy of the BFsh and ST (20–23%).

In fact, NHT produced greater hypertrophy of the BFsh and SAR compared with LSET, with no between-group difference in GRA (Fig. 3) and POP showing no hypertrophic response to either type of training, perhaps because of either reduced accuracy in assessing the volume of this small muscle, or its primary role as a knee joint stabilizer rather than a knee flexor (40,41). Collectively, the nonhip extending knee flexors (KF not HE; BFsh, SAR, GRA, POP) were more responsive to NHT (approximately twofold LSET), whereas the hip extending knee flexors (KF and HE; BFlh, SM, ST) were more responsive to LSET (more than twofold NHT). As discussed previously, during LSET, the long length of the KF and HE muscles (i.e., biarticular hamstrings), but not the KF muscles that are not HE muscles, is the likely explanation for their differing hypertrophic response to this type of training. Considering NHT, although no convincing data are available, the lack of high external resistance to hip extension during this exercise (i.e., hip extension torque is restrained by gravity acting on the trunk and antagonist co-activation) may limit the contribution/ activation of the hip extending knee flexors and place greater reliance on the nonhip extending knee flexors. This point is partly supported by the finding that peak forces during NHT coincided with low BFlh and SM muscle activities (40), suggesting other muscles may be more heavily involved in this exercise, and this agrees with our finding of no hypertrophy of BFlh and SM after NHT. The SAR, being a biarticular hip flexor, would also have likely been at longer lengths during NHT (hip extended) than LSET (hip flexed), which may also explain its greater hypertrophic response to NHT. Maeo et al. (15) also found greater SAR hypertrophy when trained by knee flexion exercise at long (hip extended, prone) vs short (hip flexed, seated) lengths, collectively indicating that muscle lengths during exercise influence training-induced muscle hypertrophy.

Aponeurosis adaptations. BFlh aponeurosis size, assessed as contact interface area and maximum width, had larger increases after LSET (+8-9%) compared with NHT (+1-3%) and CON (+1-2%), with no significant difference found between NHT and CON (Fig. 4). Although previous studies have found vastus lateralis aponeurosis size to increase with training (28,29), this is the first study to document traininginduced increases in BFlh aponeurosis size after LSET but not NHT. As mentioned earlier, a small BFlh aponeurosis size has been suggested as a risk factor for HSI by concentrating mechanical strain on the surrounding muscle tissue (24-27). Given that NHT, which induced no/negligible increase in BFlh aponeurosis size in this study, has been shown to be effective in reducing the risk of new and recurrent HSI (16-18), it is possible that LSET may be more effective than NHT in preventing strain injuries. However, it is also possible that a small BFlh aponeurosis size is unrelated to future injury occurrence, as currently there is no prospective study confirming this relationship. Another possibility is that the benefits of NHT reducing injury risk in the BFlh are not due to adaptations of the BFlh (muscle and aponeurosis size was unchanged) but perhaps because increases in size and strength of the other muscles (BFsh particularly, but also SAR, ST) reduce the demands placed on the BFlh. Finally, it is notable that BFlh fascicle length has been shown to be associated, prospectively, with HSI (longer BFlh fascicles, lower HSI risks) (42), and NHT is reported to increase BFlh fascicle length (14). Although the capability of LSET to increase BFlh fascicle length is unknown, it is likely possible because increased muscle volume, which occurred in BFlh after LSET, can result from both longitudinal and radial growth of muscles (43). Thus, further research is needed to investigate whether BFlh aponeurosis size as well as fascicle length and their change after LSET and/or other training interventions are related to future HSI.

**Functional adaptations.** Maximum eccentric knee flexion torque increased in LSET (+17%) and NHT (+11%) but not in CON (+4%) (Table 2). Although only LSET increased eccentric strength compared with CON, there was no significant difference between LSET and NHT. Eccentric knee flexion strength is considered a key factor in HSI prevention (14,42,44), and the current results suggest that LSET and NHT may have similar efficacy for improving eccentric strength. However, as with the BFIh aponeurosis size, longitudinal investigation of training-induced increases in eccentric knee flexion strength on HSI needs to be examined in future studies.

Maximum isometric and concentric torque increased in all groups including CON (Table 2), suggesting some learning effects despite the familiarization session and two duplicate measurement sessions at each time point in the current study. This learning effect may be because the knee flexor muscle group gets relatively low habitual use in daily life, particularly for performing maximum contractions at long lengths where the largest isometric strength improvements occurred. Our previous study (33) using the same approach (one familiarization and two duplicate measurement sessions at each time point), but measurements of the knee extensors in the middle of the range of motion, did not find such learning effects in a control group. Nevertheless, the greater gains in maximum isometric strength of both LSET and NHT compared with CON (Fig. 5A) may be at least partly attributable to similar increases in overall knee flexor volume for LSET and NHT (Fig. 2), although this did not translate into between-group differences in concentric strength. Changes in hamstring EMG during the maximum contractions appeared to have a similar pattern to those of maximum knee flexion torque (Fig. 5B), but none of these changes were significantly different between groups. This may be partly because EMG measurements were from only two hamstring muscles, whereas knee flexion torque is produced by up to nine individual muscles. Indeed, training-induced changes in EMG often align with those of torque when EMG is taken from most of the muscles producing the intended torque (33,45-48). Thus, future studies should consider more careful familiarization (multiple sessions) and a greater range of EMG measurement sites when assessing the knee flexor muscles in training studies.

Isometric knee flexion torque increased at a wide range of joint angles after both LSET (+18-22%) and NHT (+25-29%), whereas CON also increased torque at extended knee joint angles (+11–18%), again suggesting some learning effects (Fig. 6A–C). The isometric strength changes across the range of knee joint angles were overall greater for both NHT (all angles) and LSET (the five most extended angles out of the seven) than CON, and also greater for NHT than LSET at intermediate angles 55-75° (Fig. 5D). The reason for the differences between NHT and LSET is unclear but may be partly attributable to the fact that NHT involves contracting at relatively short muscle lengths than LSET. It should be recognized that the measurements in this study did not extend beyond the angle of peak knee flexion torque. This was because of the difficulties in measuring knee flexion torque at long muscle lengths due to the discrepancy in crank angle and actual knee joint angle; during MVC at extended angles, the discrepancy was >25°, likely resulting from the compliance and misalignment of the segments to the crank. Manipulating hip joint angle (e.g., accentuated hip-flexed position, similar to LSET) during knee flexion torque measurements could help overcome this issue. This should be taken into account in future studies to better understand the effects of training interventions including LSET and/or NHT on strength improvements across wide joint angles/muscle lengths.

Limitations. This study compared the effects of two eccentrically focused knee flexor training regimes that are inherently different exercises: e.g., loading mechanism (weight stack, LSET vs mainly body weight, NHT), joint positions (hip flexed, LSET vs hip extended, NHT), bilateral (NHT) vs unilateral (LSET) eccentrics, and concentric component (LSET only). Therefore, this study did not isolate a single experimental variable; rather, it compared two quite distinct training regimes. Given the differential training effects we have observed, further studies should strive to isolate the specific variables accounting for these differences. Moreover, LSET was designed to provide a practical (i.e., widely accessible) resistance training regime for high eccentric loading of the knee flexors at long lengths with a minor modification (adjusted backrest hip angle) to a widely used type of knee flexion weight stack machine rather than sophisticated inaccessible equipment (e.g., a motorized isokinetic dynamometer) previously used for LSET (20,21). However, to achieve high eccentric loading without motorized apparatus or manual assistance, LSET involved

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concentrically lifting the load with two legs to eccentrically lower/return the load with one leg. Thus, LSET involved a significant volume of concentric work, albeit at a relatively low load. Nonetheless, despite these numerous differences between the two training regimes, they produced similar overall knee flexor hypertrophy but very different patterns of hypertrophy within the individual muscles. Furthermore, adding more work/training volume to NHT seems unlikely to significantly affect SM and BFlh hypertrophy or BFlh aponeurosis size as discussed previously (Figs. 1 and 4). Finally, the distinct patterns of hypertrophy within the knee flexors after LSET vs NHT (e.g., hamstrings vs SAR) seem likely to be specific to the nature of the exercise performed rather than the loading magnitude or volume per se.

#### CONCLUSIONS

In summary, the main findings of this study were that LSET induced greater increases in hamstring muscle size including larger increases in BFlh muscle volume (Fig. 3) and BFlh aponeurosis size (Fig. 4). Moreover, the training regimes induced distinctly different patterns of hypertrophy that appeared to be largely due to the functional role of the muscles; LSET was more effective for increasing KF and HE muscle size (2.2-fold vs NHT) and NHT for increasing KF not HE size (1.9-fold vs LSET). These results suggest that LSET is superior to NHT for inducing hypertrophy of the hamstrings and BFlh muscle, potentially contributing to better sprint performance improvements and providing a stronger protective effect against HSI, which often occur in the BFlh muscle.

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T.G.B., D.Z.N., P.W.K., M.T.G.P., and J.P.F. conceived and designed the study. All authors contributed to data collection or analysis, as well as interpretation of the results. S.M. drafted the manuscript, and all authors read and approved the final version of the manuscript, including the order of presentation of the authors. This study was approved by the Loughborough University Ethics Review Sub-Committee (Ethics approval number R17-P054) and Nanyang Technological University Institutional Review Board (Reference number IRB-2017-07-030). Written informed consent was obtained from each participant. All data are available in the main text. Additional data related to this study will be made available from the corresponding author upon reasonable request.

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