Title: The functional significance of hamstrings composition: Is it really a 'fast' muscle group?

Running head: Is hamstrings really a 'fast' muscle group?

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ABSTRACT

Hamstrings muscle fibre composition may be predominantly fast-twitch and could explain the high incidence of hamstrings strain injuries. However, hamstrings muscle composition *in vivo,* and its influence on knee flexor muscle function, remains unknown. We investigated biceps femoris long head (BFlh) myosin-heavy chain (MHC) composition from biopsy samples, and the association of hamstrings composition and hamstrings muscle volume (using MRI) with knee flexor maximal and explosive strength. Thirty-one young men performed maximal (concentric, eccentric, isometric) and explosive (isometric) contractions. BFlh exhibited a balanced MHC distribution (mean±SD (min-max); 47.1±9.1% (32.6-71.0%) MHC-I, 35.5±8.5% (21.5-60.0%) MHC-IIA, 17.4±9.1% (0.0-30.9%) MHC-IIX). Muscle volume was correlated with knee flexor maximal strength at all velocities and contraction modes (r= 0.62–0.76, $P< 0.01$), but only associated with late phase explosive strength (time to 90 Nm; r= -0.53, $P< 0.05$). In contrast, BFlh muscle composition was not related to any maximal or explosive strength measure. BFlh MHC composition was not found to be 'fast', and therefore composition does not appear to explain the high incidence of hamstrings strain injury. Hamstrings muscle volume explained 38-58% of the inter-individual differences in knee flexor maximum strength at a range of velocities and contraction modes, while BFlh muscle composition was not associated with maximal or explosive strength.

Keywords: Biceps femoris long head, myosin heavy chain, muscle volume, explosive strength, maximal strength, muscle biopsies, MRI

INTRODUCTION

The hamstrings muscle group is the primary knee flexor and a major hip extensor and as such the strength of this muscle group is important for human locomotion and athletic activities such as running and jumping. Hamstrings function, particularly rapid 'explosive' force production (i.e. the development of contractile force as quickly as possible from a low or resting level), is also considered important for dynamic knee joint control and stability, and thus maintaining joint integrity (Aagaard et al., 2000; Zebis et al., 2011; Hannah et al., 2014). Furthermore, hamstrings strain injuries are the most common injury in a variety of sprint-based sports (e.g. football and track sprinting; Ekstrand et al., 2011; Alonso et al., 2012) leading to large amounts of time loss in professional and recreational athletes. These injuries most commonly affect the biceps femoris long head muscle (BFlh; Woodley & Mercer, 2004) and appear to occur during high velocity eccentric muscle actions (e.g. late swing phase of sprinting; Chumanov et al., 2012). Therefore, understanding the function of the hamstrings muscle, including its capacity for maximum strength at a wide range of velocities and contraction modes (i.e. the full torque-velocity relationship) as well as explosive strength, is of considerable interest. However, our understanding of hamstrings morphology (size and composition) and how this relates to contractile function is relatively limited.

For example, hamstrings myosin heavy chain (MHC) composition in young healthy individuals remains unknown, as current BFlh muscle composition data are derived solely from cadavers (Johnson et al., 1973; Garrett et al., 1984; Dahmane et al., 2006). In a much cited study of cadaver specimens, Garret et al. (1984) reported that hamstrings contained a higher proportion of type II fibres than the quadriceps or adductor magnus and suggested

63 that this muscle composition may contribute to the high susceptibility of the hamstrings to
64 strain injuries. Type II fibres are known to be more susceptible to damage after eccentric
65 contractions (Fridén et al., 1983) and thus suggested to be more vulnerable to strain injury
66 (Brockett et al., 2004). Moreover, the methodological limitations of the study of Garret et al.
67 (1984) (a small sample of 10 elderly cadavers) highlight the need to determine hamstrings
68 muscle composition in healthy young, recreationally active adults in order to understand if
69 the composition of this muscle may contribute to its high incidence of strain injury.

70 As hamstrings muscle composition has only been determined within cadavers, its influence
71 on muscle function remains unknown. Within the quadriceps femoris, a significant
72 correlation between maximum isometric or isovelocity (15-240° s^{-1}) strength and
73 composition of the vastus lateralis (VL) has often (Thorstensson et al., 1976; Viitasalo &
74 Komi, 1978; Aagaard & Andersen, 1998; Gür et al., 2003) but not always been reported
75 (Inbar et al., 1981; Viitasalo et al., 1981; Schantz et al., 1983). Nevertheless, the balance of
76 evidence from quadriceps studies suggests that hamstrings muscle composition appears
77 likely to influence maximum strength of the knee flexors. Furthermore, *in vitro* studies
78 demonstrate that type II fibres (MHC-II) have a higher rate of force development (Metzger &
79 Moss, 1990) and greater maximum force at high velocities of shortening (Bottinelli et al.,
80 1999), yet the influence of MHC composition on *in vivo* hamstrings function remains
81 unknown.

82 Whilst the influence of hamstrings muscle composition on function *in vivo* remains to be
83 elucidated, muscle size has been consistently found to be a substantial determinant of
84 isometric strength in various muscles (e.g. elbow flexors, r= 0.81, Erskine et al., 2014;
85 plantar flexors, r= 0.65, Bamman et al., 2000; knee extensors, r= 0.59, Maughan et al.,

86 1983). Considering the hamstrings, the four studies we are aware of reported quite diverse
87 relationships between muscle size and isometric/concentric strength measures (r= -0.22 to
88 0.80; Kanehisa et al., 1994; Masuda et al., 2003; Akagi et al., 2012; Denadai et al., 2014).
89 However, none of these studies examined eccentric or explosive strength. It is also possible
90 that the combined influence of muscle composition and muscle size may further explain the
91 variability in hamstrings muscle function, although this has not been investigated.

92 Therefore, the aim of this study was to determine the BFlh MHC isoform distribution and to
93 examine the association of hamstrings muscle size and BFlh MHC composition with knee
94 flexor strength, including maximal strength measurements across the torque-velocity
95 relationship (concentric, isometric and eccentric) as well as explosive isometric strength.

METHODS

Participants

Thirty-one healthy, recreationally active participants (age 21 ± 3 y (range: 18-29 y); height 1.79 ± 0.07 m; body mass 71.8 ± 7.3 kg; mean ± SD) took part in this study. Participants had a low to moderate level of physical activity and were not involved in systematic physical training or had any previous experience of strength/power training (i.e. weight training, plyometrics) of the lower body musculature. Their physical activity was assessed with the International Physical Activity Questionnaire (iPAQ) short format (Craig et al., 2003) and their average energy expenditure was 1739 ± 814 metabolic equivalent-minutes per week. After completing the physical activity and health screen questionnaires, participants provided written informed consent for their participation in this study, which was approved by the Loughborough University Ethical Advisory Committee. All participants were healthy with no history of musculoskeletal problems or injuries of the lower back, pelvis or legs. Participants were instructed not to take part in any unaccustomed or strenuous physical activity for at least 2 days prior to each laboratory visit and to refrain from alcohol and caffeine for the last 24 h before each visit.

Overview

Participants visited the laboratory on seven separate occasions, each seven days apart at a consistent time of the day (11:00-16:00 h). All the measurements were conducted on the participants' dominant leg (defined as their kicking leg). The first session involved recording anthropometric data and familiarization with the procedures for testing knee flexor explosive isometric strength that was measured during the second and third sessions. The third and fourth sessions involved familiarization with the isokinetic dynamometer procedures, while the knee flexor torque-velocity relationship was examined in the fifth

session. Seven days after the isokinetic dynamometer testing, hamstrings muscle size was assessed by magnetic resonance imaging (MRI) (session 6). In the final session, muscle tissue samples were obtained from the BFlh muscle.

Measurements and Data analysis

Torque-velocity relationship

The participants were seated on an isokinetic dynamometer chair (Con-Trex MJ, CMV AG, Dübendorf, Switzerland) with a hip angle of 120° (180°= full extension). This hip angle is similar to that during late swing phase during sprinting (Thelen et al., 2005). Two 3-point belts secured the torso and additional straps tightly secured the pelvis and the distal thigh of their dominant leg. A brace was also placed in front of the non-involved leg. The alignment of the knee joint centre with the dynamometer rotational axis was performed during isometric knee flexion contractions of >50% of maximal isometric voluntary torque at a knee joint angle of ~115°. The dynamometer's shin brace was placed posterior to the shank ~2 cm above the medial malleolus before the shank was tightly secured to the dynamometer lever arm. The range of motion was established (total: 104°, most flexed-extended crank angle*:* 67-171°; 180° full extension) and anatomical zero was set at full extension of the knee joint. Passive torque measurements were recorded while the tested leg was passively moved through the full range of motion and thereafter active torque values were corrected for passive torque. Participants were instructed to grasp the handles next to the seat during maximal contractions. Standardized verbal encouragement was given by the same investigator and online visual feedback of the crank torque was provided on a computer screen. The torque, crank angle and crank velocity signals were sampled at

2000 Hz with a PC using Spike 2 software (CED, Cambridge, UK) and smoothed with a moving average process over 0.065 s time epochs before any further analysis.

For isometric strength measurement, participants first completed a standardized warm-up consisting of a progressive series of submaximal contractions before they performed two sets of three maximum contractions, one at each of three different crank angles (165°, 145° and 125° in a consistent order; 180°= full extension) near the angle where knee flexors exert their maximal torque (Knapik et al., 1983). Participants were instructed to flex their knee and "pull" as hard and as fast as possible for 3-5 s. One-minute rest was given between each contraction and 2 min between sets. The contraction with the highest torque irrespective of crank angle was selected for further analysis. Isometric strength was defined as the average torque over a 0.5 s period around the highest instantaneous torque.

For the concentric and eccentric strength measurement, participants first completed a standardized warm-up protocol with five submaximal concentric-eccentric contractions of progressively higher intensity. Then, they performed knee flexors maximal concentric-eccentric contractions at 50° s^{-1} (3 sets of 2 reciprocal contractions) and 350° s^{-1} (3 sets of 3 reciprocal contractions) over the full range of motion. There was ≥1 min rest between each set and ≥2 min rest between velocities. For the concentric-eccentric contractions, the acceleration and deceleration phases were excluded in order to disregard torque overshoot during these phases (Schwartz et al., 2010) and the constant isovelocity period was identified (within ±5% of the prescribed crank angular velocity). Finally, concentric and eccentric strength at each velocity was defined as the highest instantaneous torque recorded within the isovelocity range of the relevant contractions.

The high velocity torque ratio was defined as the concentric strength at 350° s^{-1} divided by the isometric strength (T_{con350}/T_{isom}).

166 Explosive isometric strength

167 Participants lay in a prone position on a custom-made isometric dynamometer at fixed hip
168 (140°, 180°= full extension) and knee (150°) joint angles selected to replicate the joint
169 positions during the late swing phase of sprinting (Thelen et al., 2005) when hamstrings
170 strains are thought to occur. To minimize any extraneous movements, participants were
171 fastened with two straps across the hips, a strap over the lower back and a strap over the
172 distal thigh just above the knee joint. A metal ankle cuff with a lining of high density
173 neoprene was placed ~4 cm above the medial malleolus and the distal leg was tightly
174 secured to the cuff with straps. Force was measured with a calibrated strain gauge (linear
175 response up to 500 N, Force Logic UK, UK) in series with the ankle cuff and perpendicular to
176 the tibia. The force signal was amplified (x370) and sampled at 2000 Hz with an external
177 analog-to-digital converter (Micro 1401-3, CED, Cambridge, UK). A PC recorded and
178 displayed the data using the Spike 2 software (CED, Cambridge, UK). The force signal was
179 filtered with a 4^{th} order Butterworth filter with a low pass cut-off frequency of 500 Hz. The
180 distance between the knee joint space and the centre of the ankle cuff was measured to
181 calculate knee flexion torque.

182 After a standardised warm-up, participants performed 3 maximal knee flexion contractions
183 to establish the target torque for the subsequent explosive contractions (see below). A
184 computer screen provided real time visual feedback by displaying the torque response.
185 Thereafter, participants completed 10 explosive contractions with 30 s rest between
186 contractions. They were instructed to contract 'as fast and as hard as possible' for ~1 s with
187 an emphasis on 'fast' without any countermovement or pre-tension. Real-time visual
188 feedback was provided on the computer screen displaying the torque response, with
189 specific performance feedback of the time from 1% to 80% of peak torque. For the

190 detection of any countermovement or pre-tension, the resting torque was displayed on a
191 sensitive scale. Standardized verbal encouragement was given throughout the maximal and
192 explosive contractions.

193 During offline analysis, the three valid explosive contractions (achieved torque ≥80% of peak
194 torque with no discernible counter-movement or pre-tension - change of baseline signal
195 <0.2 Nm for the 100 ms prior to the onset of contraction) with the fastest time from onset
196 to 50% of peak torque were selected for further analysis. Analysis of these contractions
197 consisted of measurement of the time from contraction onset to 10, 50, and 90 Nm and the
198 time from contraction onset to 15, 45 and 75% of peak torque. We used this approach as it
199 facilitates comparisons over the same range of torques for all participants, and may relate
200 more directly to physiological determinants specific to that range of torques (Maffiuletti et
201 al., 2016). Although this does not directly measure the explosive strength in Nm/s, it does
202 quantify the explosive strength characteristics. Force onsets were identified manually by
203 visual identification by a trained investigator using a systematic approach which is
204 considered to be more valid than automated methods (Tillin et al., 2013). The three
205 analysed explosive contractions were averaged within each measurement session, before
206 averaging across the two sessions when these measurements were made.

207 Magnetic resonance imaging (MRI)

208 A 1.5 T MRI scanner (Signa HDxt, GE) was used to scan the dominant leg in the supine
209 position with the hip and knee joints extended. T1-weighted axial plane images were
210 acquired from the anterior superior iliac spine to the knee joint space in two overlapping
211 blocks and oil filled capsules were placed on the lateral side of the participants' thigh to help
212 with the alignment of the blocks during analysis. The following imaging parameters were

used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm.

MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland). The BFlh, biceps femoris short head, semitendinosus and semimembranosus muscles were manually outlined in every third image starting from the most proximal image in which the muscle appeared. All manual segmentation measurements were completed by the same investigator. Muscle volume was calculated using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). To examine reliability of the analysis procedures, the images from 6 randomly selected participants were re-analysed a week later and the coefficient of variation (CV) was calculated. The CV for muscle volume was on average 0.6%.

Muscle sampling and myosin heavy chain composition

Muscle samples (~0.04 g) from the mid-section BFlh (~50% thigh length) of the dominant leg were obtained under local anaesthesia (1% lidocaine) using the microbiopsy technique (Pro-Mag Ultra, Angiotech, Medical Device Technologies, FL, USA) performed under direct ultrasound guidance. Samples were immediately frozen in liquid nitrogen and stored at -80°C for further analysis. MHC content was determined by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis using a method derived from that previously described (Fauteck & Kandarian, 1995). Electrophoresis (Mini-Protean 3, Bio-Rad) was performed on 6% (crosslinking 2.7%) polyacrylamide resolving gels with 4% (crosslinking 2.7%) stacking gels at ~4°C. The gels were electrophoresed at a constant 100 V for 1 h, and thereafter at a constant 6 mA for ~18 h. Gels were immediately silver stained (SilverQuest Silver Staining Kit, Invitrogen) and protein bands quantified by densitometry (ChemiDoc XRS+ System, Bio-Rad). Muscle samples were classified according to the relative expression of the three MHC

236 isoforms: type I, IIA, and IIX (Fig. 1). The MHC analysis was run in duplicate and the mean of
237 the 2 analyses was taken. When the first 2 analyses had a difference >10% a third analysis
238 was run. For each individual, the representative MHC distribution was defined as the mean
239 of all repeats in which the different MHC isoforms were within 10% between analyses. The
240 CV for repeat samples was 3.9% for MHC-I, 5.7% for MHC-IIA and 8.4% for MHC-IIX.

241 **Statistical analysis**

242 Data are presented as mean ± SD. One-way analysis of variance was used to examine for
243 differences in muscle volume between the constituents muscles of hamstrings and in knee
244 flexors torque at the different velocities. Bivariate relationships were examined using
245 Pearson product moment correlations between the dependent variables and the Holm-
246 Bonferroni correction was used to control for multiple tests. The level of significance was set
247 at $P < 0.05$. All statistical procedures were performed with IBM SPSS 22 (IBM Corporation,
248 Armonk, NY).

RESULTS

Descriptive data on BFlh MHC isoform distribution, hamstrings muscle size and knee flexor strength

On average, the BFlh muscle exhibited a balanced, mixed MHC distribution with 47.1 ± 9.1% MHC-I, 35.5 ± 8.5% MHC-IIA and 17.4 ± 9.1% MHC-IIX, but with considerable variation between individuals (Table 1 and Fig. 2). Total hamstrings muscle volume was on average 794.1 ± 122.2 cm^3 (CV= 15.4%), while the BFlh had smaller volume (210.0 ± 37.9 cm^3) than the other biarticular muscles (ST; 228.6 ± 45.4 cm^3, $P< 0.05$ and SM; 234.8 ± 47.7 cm^3, $P< 0.01$, Table 1).

The knee flexors exerted their highest torque during slow eccentric contractions (131.1 ± 27.4 Nm), and there was considerable inter-individual variability at all contraction modes and velocities (CV= 16.9-22.3%, Table 1). The high velocity torque ratio (T_{con350}/T_{isom}) was 0.51 ± 0.10 (CV= 18.6%). Knee flexor explosive strength characteristics, measured as time to specific torques, were found to vary between individuals, particularly during the later stages of the explosive contractions (Fig. 3).

Relationships of hamstrings muscle size and BFlh MHC isoform distribution with knee flexion strength

Hamstrings muscle volume had moderate to strong correlations with knee flexor torque at all velocities ($r= 0.62–0.76$, $P< 0.01$, Table 2). In contrast, no relationship was found between BFlh muscle composition and maximal strength at any velocity ($-0.22 < r < 0.20$, $P> 0.05$, Fig. 4) or T_{con350}/T_{isom} ($-0.16 < r < 0.24$, $P> 0.05$). When torque values at all velocities were

expressed relative to muscle volume there remained no association with BFlh muscle composition ($-0.29 < r < 0.35$, $P > 0.05$).

Hamstrings muscle volume was unrelated to explosive strength characteristics (Table 3), measured as time to achieve low absolute levels or relative measures of torque, however it was associated with explosive strength characteristics (time) to high absolute levels of torque (time to 90 Nm; $r = -0.53$, $P < 0.05$). BFlh MHC distribution was unrelated to any measure of explosive strength ($-0.20 < r < 0.24$, $P > 0.05$, Table 3).

DISCUSSION

This study examined the influence of hamstrings muscle size and BFlh muscle composition on knee flexors maximal and explosive strength. We found that within the examined cohort, the BFlh exhibited on average a balanced MHC isoform distribution that appears comparable to that of the vastus lateralis within the quadriceps (see below), and therefore does not support the suggestion that the high incidence of strain injury in this muscle is a result of BFlh composition. Further, we found that 38-58% of the variance in knee flexor maximum torque at isometric and at a range of concentric and eccentric velocities was attributable to differences in hamstrings muscle volume, while BFlh MHC distribution was not related to any measure of maximal or explosive strength.

The present study is the first to directly examine the BFlh muscle composition *in vivo* and our results showed that, on average, the BFlh muscle had a balanced distribution of slow and fast MHC isoforms (47.1 ± 9.1% MHC-I and 52.9 ± 9.1% total MHC-II) in young healthy men. In a much cited study, Garret et al. (1984) reported a BFlh muscle composition within a small cohort of elderly cadavers to be similar to our data (54.5 ± 2.8% type II fibres and 45.5 ± 2.8% type I of total number of sampled fibres). Nevertheless, based on the small differences they observed between the hamstrings and other muscles (quadriceps, 51.9%; adductor magnus, 44.8% type II fibres) Garrett et al. (1984) argued that the 'high proportion' of fast fibres in the hamstrings may contribute to their susceptibility to injury. However, our *in vivo* hamstrings muscle composition data do not support this proposition when compared to equivalent VL data. For example, Staron et al. (2000) reported the VL to contain a greater proportion of MHC-II isoform (66.1% total MHC-II in 95 physically active young men) compared to the BFlh in the current study. Nevertheless, other studies have

301 reported a more balanced VL MHC distribution (n= 28, 49 ± 18% MHC-I, 35 ± 16% MHC-IIA,
302 16 ± 10% MHC-IIX; Taylor et al., 1997). Based on our findings, the BFlh does not have a 'fast'
303 composition, and appears to have a MHC distribution similar or slower than the VL.
304 Consequently, the composition of the BFlh does not seem to explain the high incidence of
305 strain injuries within this muscle compared to other muscles. Therefore, other aspects of
306 hamstrings structure (e.g. aponeurosis size; Evangelidis et al., 2015) or function (eccentric
307 actions at long lengths; Thelen et al., 2005) are likely to explain the high incidence of strain
308 injuries in this muscle. On an individual basis however, it is possible that the proportion of
309 MHC-II isoforms could still be a risk factor for hamstrings strain injury. Type II fibres are
310 more susceptible to eccentric exercise-induced muscle damage (Fridén et al., 1983), possibly
311 due to structural differences between fibre types (e.g. thinner Z-disks in type II fibres; Fridén
312 & Lieber, 1992). The accumulation of microscopic eccentric exercise-induced muscle
313 damage and subsequent changes in function (reduction of force-generating capacity, shift of
314 optimum fibre length and impairment of the excitation-contraction coupling; Morgan &
315 Allen, 1999) could contribute to a macroscopic injury (Brockett et al., 2004). Within our
316 cohort, total MHC-II isoform content ranged from 29.0-67.4% and it is possible that
317 individuals with a high proportion of type II fibres could be at higher risk of injury. Future
318 retrospective and prospective studies are needed to elucidate the relationship between
319 muscle composition and the incidence of individual strain injuries.

320 Whilst MHC composition is a strong determinant of contractile function in single fibres
321 (Metzger & Moss, 1990; Bottinelli et al., 1999), in this study no correlation was found
322 between BFlh muscle composition and knee flexors maximal or explosive strength *in vivo*.
323 The lack of relevant previous data on the hamstrings prevents any direct comparison with

our findings; however similar studies on knee extensors reported mixed results for the relationship of maximum/explosive strength with muscle composition. Some of the studies on knee extensors examined these relationships with participants from diverse training and athletic backgrounds e.g. untrained, endurance, and strength and power athletes (Viitasalo & Komi, 1978; Viitasalo et al., 1981; Gür et al., 2003). Whilst this approach produces a wide range of muscle composition values, numerous other neuromuscular characteristics also likely vary between these groups (e.g. muscle size, architecture, neural drive) and these could confound any relationship of maximum/explosive strength and muscle composition. In the present study within a group of non-athletic young men, muscle composition did not explain their differences in maximal or explosive strength despite the large inter-individual variability in these measures (CV; maximal strength: 16.9-22.3%; explosive strength: 15.7-44.1%). Whilst theoretically it may be surprising that muscle composition was not more strongly predictive of function, this may reflect the extensive range of factors that influence in-vivo function; most obviously muscle size (discussed below), but also including muscle moment arm, neuromuscular activation of the agonists and antagonists, fibre length and pennation, specific tension, parallel and series connective tissue (Folland & Williams, 2007). Inter-individual differences in these factors may oppose or supersede any functional differences due to muscle composition and thus conceal its contribution to maximal or explosive strength *in vivo*. For example, agonist neural activation seems to be the primary determinant of voluntary explosive contractions, particularly during the initial phase of contraction (<75 ms), explaining up to 83% of the differences within healthy individuals (de Ruiter et al., 2006, 2007; Klass et al., 2008; Folland et al., 2014). However, when neural drive is controlled – via electrical stimulation – the differences in MHC content (Harridge et al.,

1996) or intrinsic contractile properties, that reflect muscle composition, become more evident (Andersen & Aagaard, 2006; Folland et al,. 2014).

Our results revealed that hamstrings volume explained a significant portion of the variance in isometric (38%), concentric (50-55%) and eccentric (48-58%) knee flexor strength. These values are within the range of previous reports of hamstrings isometric/concentric strength (Kanehisa et al., 1994; Masuda et al., 2003; Akagi et al., 2012). The importance of muscle size for eccentric hamstrings strength is a more novel finding. Considering that hamstrings strain injury appears to predominantly occur during eccentric contractions (Chumanov et al., 2012), this finding supports the notion that strength training with an emphasis on hypertrophic adaptations may be a valid approach for preventing hamstrings injury. The proportion of the variance in knee flexor strength explained by hamstrings size may be partly due to the fact that other muscles (i.e. gastrocnemius, gracilis, sartorius and popliteus) contribute to knee flexor torque in addition to the hamstrings, but also the extensive range of neuromuscular factors found to influence function (discussed above). Overall our finding that muscle size explains a substantial proportion of the variance in maximum strength, but composition does not account for any variance, is similar to observations made in two small studies (n< 16) in the knee extensors (Maughan & Nimmo, 1984; Johansson et al., 1987).

In contrast to maximal strength, explosive strength was not influenced by muscle size apart from at high levels of absolute torque (time from rest to 90 Nm; r= -0.53, *P*< 0.01). Whilst no similar data exist on hamstrings, elbow flexor explosive isometric strength has been related to muscle volume during a similar late phase of contraction (150 ms, r= 0.69, *P*< 0.001; Erskine et al., 2014). In contrast, explosive strength during the early phase of force/torque

production appears to be predominantly explained by agonist neural activation and the contractile (twitch) response to a single action potential, together explaining 77% of the variance in force after 50 ms of contraction (Folland et al., 2014).

In conclusion, the balanced MHC distribution found in BFlh muscle appears to be comparable or slower to that of the VL, and therefore seems unlikely to contribute to the high susceptibility of the BFlh to strain injury. Hamstrings muscle volume explained 38-58% of the inter-individual differences in knee flexors torque at a range of velocities and contraction modes, while BFlh muscle composition was not associated with maximal or explosive strength.

Perspectives

Our data show that, within recreationally active young men, hamstrings exhibit a balanced muscle composition that seems to be comparable to that of the VL. Based on these findings, the suggestion that hamstrings contain primarily fast-twitch fibres and that this muscle composition may explain the high rates of hamstrings strain injuries was not supported. From a functional perspective, muscle composition was not a determinant of knee flexor function. In contrast, muscle size explained a large proportion of knee flexor maximal strength and a moderate proportion of late phase explosive strength.

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Figure Captions

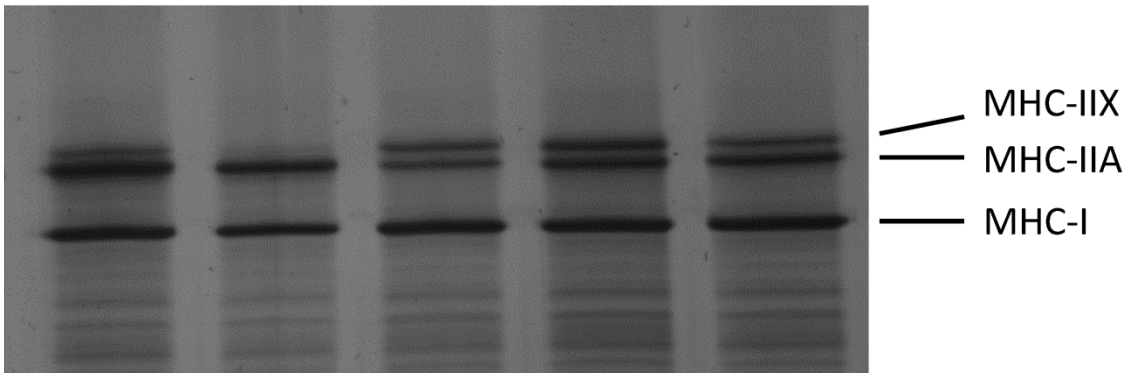


Figure 1. Example sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis separation of the different myosin heavy chain (MHC) isoforms in biceps femoris long head muscle sampled from 5 participants.

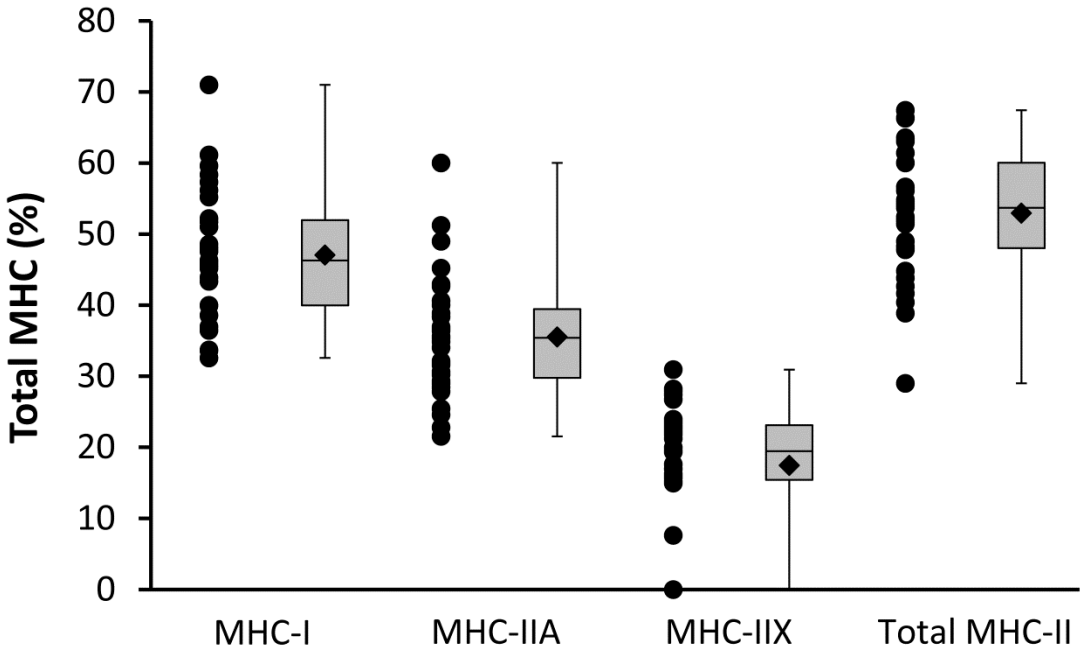
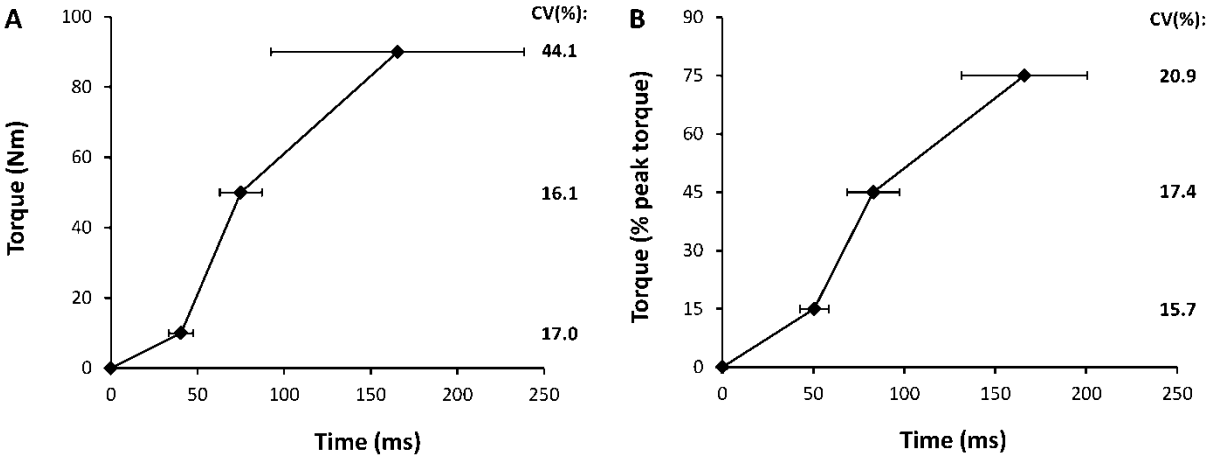


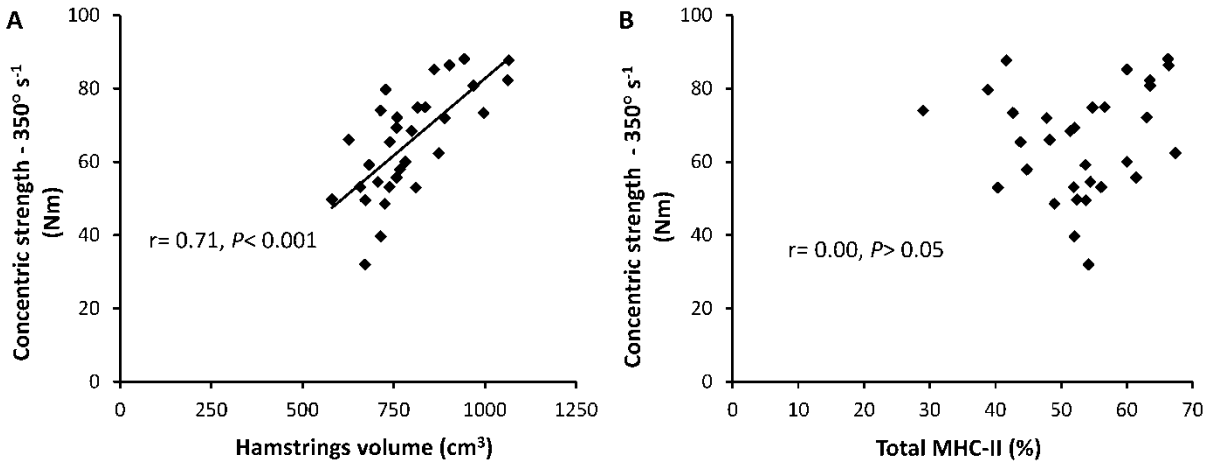
Figure 2. BFlh MHC isoform relative distribution (n= 31) presented in a combined box plot and scatterplot. Box plot's whiskers correspond to minimum and maximum values and the filled rhombi correspond to the group mean values.



518

519 **Figure 3.** Knee flexion explosive strength characteristics expressed as time from zero to
520 absolute (A) and relative (B) torque levels. Data expressed as mean ± SD (n= 31) with inter-
521 individual coefficient of variation (CV) presented at each torque level.

522



523

524 **Figure 4.** Relationships between concentric strength at 350° $s^{-1}$and (A) hamstrings volume

525 and (B) BFlh total MHC-II isoform content (n= 31). BFlh: biceps femoris long head, MHC:

526 myosin heavy chain.

527

528 **Tables**

529 **Table 1.** Descriptive data of biceps femoris long head muscle composition, hamstrings
530 muscle volume, and knee flexor strength.

		Mean ± SD	Range	CV (%)
Muscle composition (%)	**MHC-I**	47.1 ± 9.1	32.6 – 71.0	19.3
	MHC-IIA	35.5 ± 8.5	21.5 – 60.0	23.9
	MHC-IIX	17.4 ± 9.1	0.0 – 30.9	52.4
Muscle volume (cm^3)	**BFlh**	210.0 ± 37.9	157.4 - 289.4	18.1
	BFsh	120.6 ± 22.3†	76.6 - 170.4	18.5
	ST	228.6 ± 45.4*	120.5 - 342.5	19.9
	SM	234.8 ± 47.7†	125.3 - 330.2	20.3
	Total	794.1 ± 122.2	581.1 - 1065.6	15.4
Maximal strength (Nm)	**Ecc 350°** s^{-1}	115.7 ± 23.6†	58.0 - 164.5	20.4
	Ecc 50° s^{-1}	131.1 ± 27.4	66.9 - 174.2	20.9
	Isometric	128.3 ± 21.7	92.1 - 176.4	16.9
	Con 50° s^{-1}	108.3 ± 21.1†	65.9 - 154.0	19.5
	Con 350° s^{-1}	65.4 ± 14.6†	32.0 - 88.1	22.3
	$\mathbf{T_{con350}/T_{isom}}$	0.51 ± 0.10	0.29 - 0.71	18.6

531 Data presented as mean ± SD (n= 31). The muscle volumes of the constituent muscles were
532 compared to BFlh, and maximal strength measures were compared to isometric strength.
533 MHC, myosin heavy chain; BFlh, biceps femoris long head; BFsh, biceps femoris short head;

534 ST, semitendinosus; SM, semimembranosus; Ecc, eccentric; Con, concentric; * $P < 0.05$, † $P <$

535 0.01.

536

Table 2. Bivariate correlation coefficients of knee flexor maximal strength with hamstrings muscle volume and biceps femoris long head muscle composition

		Hamstrings volume	BFlh muscle composition		
			MHC-I	MHC-IIA	MHC-IIX
Maximal strength	**Isometric**	0.62†	0.01	0.20	-0.20
	Con 50° s^{-1}	0.74‡	0.08	0.15	-0.22
	Con 350° s^{-1}	0.71‡	-0.11	0.12	0.00
	Ecc 50° s^{-1}	0.76‡	0.04	0.05	-0.09
	Ecc 350° s^{-1}	0.69‡	0.01	0.09	-0.10
	T_{con350}/T_{isom}	0.27	-0.16	-0.09	0.24

MHC, myosin heavy chain; BFlh, biceps femoris long head; Ecc, eccentric; Con, concentric; † $P< 0.01$, ‡ $P< 0.001$

Table 3. Bivariate correlation coefficients between knee flexor explosive strength characteristics, measured as time to specific (absolute and relative) torques, with hamstrings muscle volume and biceps femoris long head composition.

	Explosive strength characteristics					
	Time to absolute torque			**Time to relative torque (%MVT)**		
	10 Nm	**50 Nm**	**90 Nm**	**15%**	**45%**	**75%**
Hamstrings volume (cm^3)	-0.10	-0.43	-0.53*	0.01	-0.16	-0.24
MHC-I (%)	-0.09	0.14	0.06	-0.01	0.14	0.24
MHC-IIA (%)	0.01	-0.06	-0.15	0.03	-0.05	-0.05
MHC-IIX (%)	0.08	-0.09	0.08	-0.02	-0.09	-0.20

MHC, myosin heavy chain; MVT, maximal voluntary torque; * $P< 0.05$