

1 **Title:** The functional significance of hamstrings composition: Is it really a ‘fast’ muscle
2 group?

3 **Running head:** Is hamstrings really a ‘fast’ muscle group?
4

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20 **ABSTRACT**

21 Hamstrings muscle fibre composition may be predominantly fast-twitch and could explain
22 the high incidence of hamstrings strain injuries. However, hamstrings muscle composition *in*
23 *vivo*, and its influence on knee flexor muscle function, remains unknown. We investigated
24 biceps femoris long head (BFH) myosin-heavy chain (MHC) composition from biopsy
25 samples, and the association of hamstrings composition and hamstrings muscle volume
26 (using MRI) with knee flexor maximal and explosive strength. Thirty-one young men
27 performed maximal (concentric, eccentric, isometric) and explosive (isometric) contractions.
28 BFH exhibited a balanced MHC distribution (mean±SD (min-max); 47.1±9.1% (32.6-71.0%)
29 MHC-I, 35.5±8.5% (21.5-60.0%) MHC-IIA, 17.4±9.1% (0.0-30.9%) MHC-IIX). Muscle volume
30 was correlated with knee flexor maximal strength at all velocities and contraction modes ($r=$
31 $0.62-0.76$, $P < 0.01$), but only associated with late phase explosive strength (time to 90 Nm;
32 $r = -0.53$, $P < 0.05$). In contrast, BFH muscle composition was not related to any maximal or
33 explosive strength measure. BFH MHC composition was not found to be 'fast', and
34 therefore composition does not appear to explain the high incidence of hamstrings strain
35 injury. Hamstrings muscle volume explained 38-58% of the inter-individual differences in
36 knee flexor maximum strength at a range of velocities and contraction modes, while BFH
37 muscle composition was not associated with maximal or explosive strength.

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

40 INTRODUCTION

41 The hamstrings muscle group is the primary knee flexor and a major hip extensor and as
42 such the strength of this muscle group is important for human locomotion and athletic
43 activities such as running and jumping. Hamstrings function, particularly rapid 'explosive'
44 force production (i.e. the development of contractile force as quickly as possible from a low
45 or resting level), is also considered important for dynamic knee joint control and stability,
46 and thus maintaining joint integrity (Aagaard et al., 2000; Zebis et al., 2011; Hannah et al.,
47 2014). Furthermore, hamstrings strain injuries are the most common injury in a variety of
48 sprint-based sports (e.g. football and track sprinting; Ekstrand et al., 2011; Alonso et al.,
49 2012) leading to large amounts of time loss in professional and recreational athletes. These
50 injuries most commonly affect the biceps femoris long head muscle (BFH; Woodley &
51 Mercer, 2004) and appear to occur during high velocity eccentric muscle actions (e.g. late
52 swing phase of sprinting; Chumanov et al., 2012). Therefore, understanding the function of
53 the hamstrings muscle, including its capacity for maximum strength at a wide range of
54 velocities and contraction modes (i.e. the full torque-velocity relationship) as well as
55 explosive strength, is of considerable interest. However, our understanding of hamstrings
56 morphology (size and composition) and how this relates to contractile function is relatively
57 limited.

58 For example, hamstrings myosin heavy chain (MHC) composition in young healthy
59 individuals remains unknown, as current BFH muscle composition data are derived solely
60 from cadavers (Johnson et al., 1973; Garrett et al., 1984; Dahmane et al., 2006). In a much
61 cited study of cadaver specimens, Garret et al. (1984) reported that hamstrings contained a
62 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 ($15\text{-}240^\circ \text{ s}^{-1}$) 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 BF_{lh} MHC isoform distribution and to
93 examine the association of hamstrings muscle size and BF_{lh} 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.

96 **METHODS**

97 **Participants**

98 Thirty-one healthy, recreationally active participants (age 21 ± 3 y (range: 18-29 y); height
99 1.79 ± 0.07 m; body mass 71.8 ± 7.3 kg; mean \pm SD) took part in this study. Participants had
100 a low to moderate level of physical activity and were not involved in systematic physical
101 training or had any previous experience of strength/power training (i.e. weight training,
102 plyometrics) of the lower body musculature. Their physical activity was assessed with the
103 International Physical Activity Questionnaire (iPAQ) short format (Craig et al., 2003) and
104 their average energy expenditure was 1739 ± 814 metabolic equivalent-minutes per week.
105 After completing the physical activity and health screen questionnaires, participants
106 provided written informed consent for their participation in this study, which was approved
107 by the Loughborough University Ethical Advisory Committee. All participants were healthy
108 with no history of musculoskeletal problems or injuries of the lower back, pelvis or legs.
109 Participants were instructed not to take part in any unaccustomed or strenuous physical
110 activity for at least 2 days prior to each laboratory visit and to refrain from alcohol and
111 caffeine for the last 24 h before each visit.

112 **Overview**

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

120 session. Seven days after the isokinetic dynamometer testing, hamstrings muscle size was
121 assessed by magnetic resonance imaging (MRI) (session 6). In the final session, muscle tissue
122 samples were obtained from the BF_{lh} muscle.

123 **Measurements and Data analysis**

124 Torque-velocity relationship

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

142 2000 Hz with a PC using Spike 2 software (CED, Cambridge, UK) and smoothed with a
143 moving average process over 0.065 s time epochs before any further analysis.

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

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

164 The high velocity torque ratio was defined as the concentric strength at 350° s⁻¹ divided by
165 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 4th 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 $\geq 80\%$ 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

213 used: imaging matrix: 512 x 512, field of view: 260 mm x 260 mm, spatial resolution: 0.508
214 mm x 0.508 mm, slice thickness: 5 mm, inter-slice gap: 0 mm.
215 MR images were analysed with Osirix software (version 4.0, Pixmeo, Geneva, Switzerland).
216 The BFLh, biceps femoris short head, semitendinosus and semimembranosus muscles were
217 manually outlined in every third image starting from the most proximal image in which the
218 muscle appeared. All manual segmentation measurements were completed by the same
219 investigator. Muscle volume was calculated using cubic spline interpolation (GraphPad
220 Prism 6, GraphPad Software, Inc.). To examine reliability of the analysis procedures, the
221 images from 6 randomly selected participants were re-analysed a week later and the
222 coefficient of variation (CV) was calculated. The CV for muscle volume was on average 0.6%.

223 Muscle sampling and myosin heavy chain composition

224 Muscle samples (~0.04 g) from the mid-section BFLh (~50% thigh length) of the dominant
225 leg were obtained under local anaesthesia (1% lidocaine) using the microbiopsy technique
226 (Pro-Mag Ultra, Angiotech, Medical Device Technologies, FL, USA) performed under direct
227 ultrasound guidance. Samples were immediately frozen in liquid nitrogen and stored at -
228 80°C for further analysis. MHC content was determined by sodium dodecyl sulphate (SDS)
229 polyacrylamide gel electrophoresis using a method derived from that previously described
230 (Fauteck & Kandarian, 1995). Electrophoresis (Mini-Protean 3, Bio-Rad) was performed on
231 6% (crosslinking 2.7%) polyacrylamide resolving gels with 4% (crosslinking 2.7%) stacking
232 gels at ~4°C. The gels were electrophoresed at a constant 100 V for 1 h, and thereafter at a
233 constant 6 mA for ~18 h. Gels were immediately silver stained (SilverQuest Silver Staining
234 Kit, Invitrogen) and protein bands quantified by densitometry (ChemiDoc XRS+ System, Bio-
235 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 \pm 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).

249 RESULTS

250 Descriptive data on BFlh MHC isoform distribution, hamstrings muscle size and knee flexor 251 strength

252 On average, the BFlh muscle exhibited a balanced, mixed MHC distribution with $47.1 \pm 9.1\%$
253 MHC-I, $35.5 \pm 8.5\%$ MHC-IIA and $17.4 \pm 9.1\%$ MHC-IIX, but with considerable variation
254 between individuals (Table 1 and Fig. 2). Total hamstrings muscle volume was on average
255 $794.1 \pm 122.2 \text{ cm}^3$ (CV= 15.4%), while the BFlh had smaller volume ($210.0 \pm 37.9 \text{ cm}^3$) than
256 the other biarticular muscles (ST; $228.6 \pm 45.4 \text{ cm}^3$, $P < 0.05$ and SM; $234.8 \pm 47.7 \text{ cm}^3$, $P <$
257 0.01 , Table 1).

258 The knee flexors exerted their highest torque during slow eccentric contractions ($131.1 \pm$
259 27.4 Nm), and there was considerable inter-individual variability at all contraction modes
260 and velocities (CV= 16.9-22.3%, Table 1). The high velocity torque ratio ($T_{\text{con350}}/T_{\text{isom}}$) was
261 0.51 ± 0.10 (CV= 18.6%). Knee flexor explosive strength characteristics, measured as time to
262 specific torques, were found to vary between individuals, particularly during the later stages
263 of the explosive contractions (Fig. 3).

264 Relationships of hamstrings muscle size and BFlh MHC isoform distribution with knee 265 flexion strength

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

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

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

277

278 **DISCUSSION**

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

288 The present study is the first to directly examine the BFlh muscle composition *in vivo* and
289 our results showed that, on average, the BFlh muscle had a balanced distribution of slow
290 and fast MHC isoforms ($47.1 \pm 9.1\%$ MHC-I and $52.9 \pm 9.1\%$ total MHC-II) in young healthy
291 men. In a much cited study, Garret et al. (1984) reported a BFlh muscle composition within a
292 small cohort of elderly cadavers to be similar to our data ($54.5 \pm 2.8\%$ type II fibres and 45.5
293 $\pm 2.8\%$ type I of total number of sampled fibres). Nevertheless, based on the small
294 differences they observed between the hamstrings and other muscles (quadriceps, 51.9%;
295 adductor magnus, 44.8% type II fibres) Garrett et al. (1984) argued that the 'high
296 proportion' of fast fibres in the hamstrings may contribute to their susceptibility to injury.
297 However, our *in vivo* hamstrings muscle composition data do not support this proposition
298 when compared to equivalent VL data. For example, Staron et al. (2000) reported the VL to
299 contain a greater proportion of MHC-II isoform (66.1% total MHC-II in 95 physically active
300 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

324 our findings; however similar studies on knee extensors reported mixed results for the
325 relationship of maximum/explosive strength with muscle composition. Some of the studies
326 on knee extensors examined these relationships with participants from diverse training and
327 athletic backgrounds e.g. untrained, endurance, and strength and power athletes (Viitasalo
328 & Komi, 1978; Viitasalo et al., 1981; Gür et al., 2003). Whilst this approach produces a wide
329 range of muscle composition values, numerous other neuromuscular characteristics also
330 likely vary between these groups (e.g. muscle size, architecture, neural drive) and these
331 could confound any relationship of maximum/explosive strength and muscle composition.
332 In the present study within a group of non-athletic young men, muscle composition did not
333 explain their differences in maximal or explosive strength despite the large inter-individual
334 variability in these measures (CV; maximal strength: 16.9-22.3%; explosive strength: 15.7-
335 44.1%). Whilst theoretically it may be surprising that muscle composition was not more
336 strongly predictive of function, this may reflect the extensive range of factors that influence
337 in-vivo function; most obviously muscle size (discussed below), but also including muscle
338 moment arm, neuromuscular activation of the agonists and antagonists, fibre length and
339 pennation, specific tension, parallel and series connective tissue (Folland & Williams, 2007).
340 Inter-individual differences in these factors may oppose or supersede any functional
341 differences due to muscle composition and thus conceal its contribution to maximal or
342 explosive strength *in vivo*. For example, agonist neural activation seems to be the primary
343 determinant of voluntary explosive contractions, particularly during the initial phase of
344 contraction (<75 ms), explaining up to 83% of the differences within healthy individuals (de
345 Ruyter et al., 2006, 2007; Klass et al., 2008; Folland et al., 2014). However, when neural drive
346 is controlled – via electrical stimulation – the differences in MHC content (Harridge et al.,

347 1996) or intrinsic contractile properties, that reflect muscle composition, become more
348 evident (Andersen & Aagaard, 2006; Folland et al., 2014).

349 Our results revealed that hamstrings volume explained a significant portion of the variance
350 in isometric (38%), concentric (50-55%) and eccentric (48-58%) knee flexor strength. These
351 values are within the range of previous reports of hamstrings isometric/concentric strength
352 (Kanehisa et al., 1994; Masuda et al., 2003; Akagi et al., 2012). The importance of muscle
353 size for eccentric hamstrings strength is a more novel finding. Considering that hamstrings
354 strain injury appears to predominantly occur during eccentric contractions (Chumanov et al.,
355 2012), this finding supports the notion that strength training with an emphasis on
356 hypertrophic adaptations may be a valid approach for preventing hamstrings injury. The
357 proportion of the variance in knee flexor strength explained by hamstrings size may be
358 partly due to the fact that other muscles (i.e. gastrocnemius, gracilis, sartorius and
359 popliteus) contribute to knee flexor torque in addition to the hamstrings, but also the
360 extensive range of neuromuscular factors found to influence function (discussed above).
361 Overall our finding that muscle size explains a substantial proportion of the variance in
362 maximum strength, but composition does not account for any variance, is similar to
363 observations made in two small studies ($n < 16$) in the knee extensors (Maughan & Nimmo,
364 1984; Johansson et al., 1987).

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

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

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

379 **Perspectives**

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

387 **REFERENCES**

- 388 Aagaard P, Andersen JL. Correlation between contractile strength and myosin heavy chain
389 isoform composition in human skeletal muscle. *Med Sci Sports Exerc* 1998; 30: 1217-1222.
- 390 Aagaard P, Simonsen EB, Andersen JL, Magnusson SP, Bojsen-Møller F, Dyhre-Poulsen P.
391 Antagonist muscle coactivation during isokinetic knee extension. *Scand J Med Sci Sports*
392 2000; 10: 58-67.
- 393 Akagi R, Tohdoh Y, Takahashi H. Muscle strength and size balances between reciprocal
394 muscle groups in the thigh and lower leg for young men. *Int J Sports Med* 2012; 33: 386-389.
- 395 Alonso JM, Edouard P, Fischetto G, Adams B, Depiesse F, Mountjoy M. Determination of
396 future prevention strategies in elite track and field: analysis of Daegu 2011 IAAF
397 Championships injuries and illnesses surveillance. *Br J Sports Med* 2012; 46: 505-514.
- 398 Bamman MM, Newcomer BR, Larson-Meyer DE, Weinsier RL, Hunter GR. Evaluation of the
399 strength-size relationship in vivo using various muscle size indices. *Med Sci Sports Exerc*
400 2000; 32: 1307-1313.
- 401 Bottinelli R, Pellegrino MA, Canepari M, Rossi R, Reggiani C. Specific contributions of various
402 muscle fibre types to human muscle performance: an in vitro study. *J Electromyogr Kinesiol*
403 1999; 9: 87-95.
- 404 Brockett CL, Morgan DL, Proske U. Predicting hamstring strain injury in elite athletes. *Med*
405 *Sci Sports Exerc* 2004; 36: 379-387.
- 406 Chumanov ES, Schache AG, Heiderscheit BC, Thelen DG. Hamstrings are most susceptible to
407 injury during the late swing phase of sprinting. *Br J Sports Med* 2012; 46: 90.

408 Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U,
409 Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability
410 and validity. *Med Sci Sports Exerc* 2003; 35: 1381-1395.

411 Dahmane R, Djordjevic S, Smerdu V. Adaptive potential of human biceps femoris muscle
412 demonstrated by histochemical, immunohistochemical and mechanomyographical
413 methods. *Med Biol Eng Comput* 2006; 44: 999-1006.

414 de Ruyter CJ, Van Leeuwen D, Heijblom A, Bobbert MF, de Haan A. Fast unilateral isometric
415 knee extension torque development and bilateral jump height. *Med Sci Sports Exerc* 2006:
416 38: 1843-1852.

417 de Ruyter CJ, Vermeulen G, Toussaint HM, de Haan A. Isometric knee-extensor torque
418 development and jump height in volleyball players. *Med Sci Sports Exerc* 2007; 39: 1336-
419 1346.

420 Denadai BS, de Oliveira FB, Camarda SR, Ribeiro L, Greco CC. Hamstrings-to-quadriceps
421 strength and size ratios of male professional soccer players with muscle imbalance. *Clin*
422 *Physiol Funct Imaging* 2014 Oct 27 doi: 10.1111/cpf.12209; Epub ahead of print.

423 Ekstrand J, Hägglund M, Waldén M. Epidemiology of muscle injuries in professional football
424 (soccer). *Am J Sports Med* 2011; 39: 1226-1232.

425 Erskine RM, Fletcher G, Folland JP. The contribution of muscle hypertrophy to strength
426 changes following resistance training. *Eur J Appl Physiol* 2014; 114: 1239-1249.

427 Evangelidis PE, Massey GJ, Pain MT, Folland JP. Biceps Femoris Aponeurosis Size: A Potential
428 Risk Factor for Strain Injury? *Med Sci Sports Exerc* 2015; 47: 1383-1389.

429 Fauteck SP, Kandarian SC. Sensitive detection of myosin heavy chain composition in skeletal
430 muscle under different loading conditions. *Am J Physiol* 1995; 268: C419-424.

431 Folland JP, Buckthorpe MW, Hannah R. Human capacity for explosive force production:
432 neural and contractile determinants. *Scand J Med Sci Sports* 2014; 24: 894-906.

433 Folland JP, Williams AG. The adaptations to strength training: morphological and
434 neurological contributions to increased strength. *Sports Med* 2007; 37: 145-168.

435 Fridén J, Lieber RL. Structural and mechanical basis of exercise-induced muscle injury. *Med*
436 *Sci Sports Exerc* 1992; 24: 521-530.

437 Fridén J, Sjöström M, Ekblom B. Myofibrillar damage following intense eccentric exercise in
438 man. *Int J Sports Med* 1983; 4: 170-176.

439 Garrett WE Jr, Califf JC, Bassett FH 3rd. Histochemical correlates of hamstring injuries. *Am J*
440 *Sports Med* 1984; 12: 98-103.

441 Gür H, Gransberg L, vanDyke D, Knutsson E, Larsson L. Relationship between in vivo muscle
442 force at different speeds of isokinetic movements and myosin isoform expression in men
443 and women. *Eur J Appl Physiol* 2003; 88: 487-496.

444 Hannah R, Minshull C, Smith SL, Folland JP. Longer electromechanical delay impairs
445 hamstrings explosive force versus quadriceps. *Med Sci Sports Exerc* 2014; 46: 963-972.

446 Inbar O, Kaiser P, Tesch P. Relationships between leg muscle fiber type distribution and leg
447 exercise performance. *Int J Sports Med* 1981; 2: 154-159.

448 Johansson C, Lorentzon R, Sjöström M, Fagerlund M, Fugl-Meyer AR. Sprinters and
449 marathon runners. Does isokinetic knee extensor performance reflect muscle size and
450 structure? *Acta Physiol Scand* 1987; 130: 663-669.

451 Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in
452 thirty-six human muscles. An autopsy study. *J Neurol Sci* 1973; 18: 111-129.

453 Kanehisa H, Ikegawa S, Fukunaga T. Comparison of muscle cross-sectional area and strength
454 between untrained women and men. *Eur J Appl Physiol* 1994; 68: 148-154.

455 Klass M, Baudry S, Duchateau J. Age-related decline in rate of torque development is
456 accompanied by lower maximal motor unit discharge frequency during fast contractions. *J*
457 *Appl Physiol* 2008; 104: 739-746.

458 Knapik JJ, Wright JE, Mawdsley RH, Braun J. Isometric, isotonic, and isokinetic torque
459 variations in four muscle groups through a range of joint motion. *Phys Ther* 1983; 63: 938-
460 947.

461 Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force
462 development: physiological and methodological considerations. *Eur J Appl Physiol* 2016: doi
463 10.1007/s00421-016-3346-6 [Epub ahead of print]

464 Masuda K, Kikuhara N, Takahashi H, Yamanaka K. The relationship between muscle cross-
465 sectional area and strength in various isokinetic movements among soccer players. *J Sports*
466 *Sci* 2003; 21: 851-858.

467 Maughan RJ, Nimmo MA. The influence of variations in muscle fibre composition on muscle
468 strength and cross-sectional area in untrained males. *J Physiol* 1984; 351: 299-311.

469 Maughan RJ, Watson JS, Weir J. Strength and cross-sectional area of human skeletal muscle.
470 J Physiol 1983; 338: 37-49.

471 Metzger JM, Moss RL. Calcium-sensitive cross-bridge transitions in mammalian fast and slow
472 skeletal muscle fibers. Science 1990; 247: 1088-1090.

473 Morgan DL, Allen DG. Early events in stretch-induced muscle damage. J Appl Physiol 1999:
474 87: 2007-2015.

475 Schantz P, Randall-Fox E, Hutchison W, Tydén A, Astrand PO. Muscle fibre type distribution,
476 muscle cross-sectional area and maximal voluntary strength in humans. Acta Physiol Scand
477 1983; 117: 219-226.

478 Schwartz FP, Bottaro M, Celes RS, Brown LE, Nascimento FA. The influence of velocity
479 overshoot movement artifact on isokinetic knee extension tests. J Sports Sci Med 2010; 9:
480 140-146.

481 Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, Toma K. Fiber
482 type composition of the vastus lateralis muscle of young men and women. J Histochem
483 Cytochem 2000; 48: 623-629.

484 Taylor AD, Humphries B, Smith P & Bronks R (1997). Electrophoretic separation of myosin
485 heavy chain isoforms in the human m. vastus lateralis: references to reproducibility and
486 relationships with force, electromechanical delay, fibre conduction velocity, endurance and
487 electromyography. Arch Physiol Biochem 105, 10-18.

488 Thelen DG, Chumanov ES, Hoerth DM, Best TM, Swanson SC, Li L, Young M, Heiderscheid BC.
489 Hamstring muscle kinematics during treadmill sprinting. *Med Sci Sports Exerc* 2005; 37: 108-
490 114.

491 Thorstensson A, Grimby G, Karlsson J. Force-velocity relations and fiber composition in
492 human knee extensor muscles. *J Appl Physiol* 1976; 40: 12-16.

493 Tillin NA, Pain MT, Folland JP. Identification of contraction onset during explosive
494 contractions. Response to Thompson et al. "Consistency of rapid muscle force
495 characteristics: influence of muscle contraction onset detection methodology" [*J*
496 *Electromyogr Kinesiol* 2012;22(6):893-900]. *J Electromyogr Kinesiol* 2013; 23: 991-994.

497 Viitasalo JT, Hakkinen K, Komi PV. Isometric and dynamic force production and muscle fibre
498 composition in man. *J Hum Mov Stud* 1981; 7: 199-209.

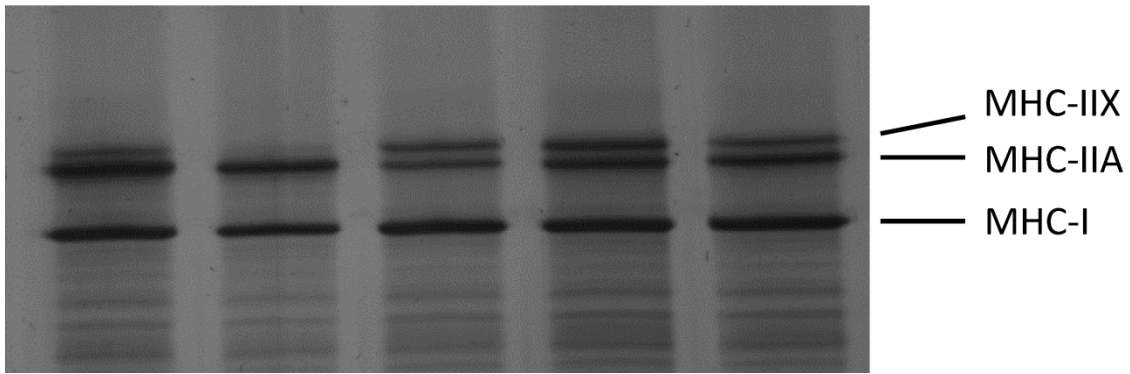
499 Viitasalo JT, Komi PV. Force-time characteristics and fiber composition in human leg
500 extensor muscles. *Eur J Appl Physiol* 1978; 40: 7-15.

501 Woodley SJ, Mercer SR. Hamstring strains - where do they occur? *N Z J Physiother* 2004; 32:
502 22-28.

503 Zebis MK, Andersen LL, Ellingsgaard H, Aagaard P. Rapid hamstring/quadriceps force
504 capacity in male vs. female elite soccer players. *J Strength Cond Res* 2011; 25: 1989-1993.

505

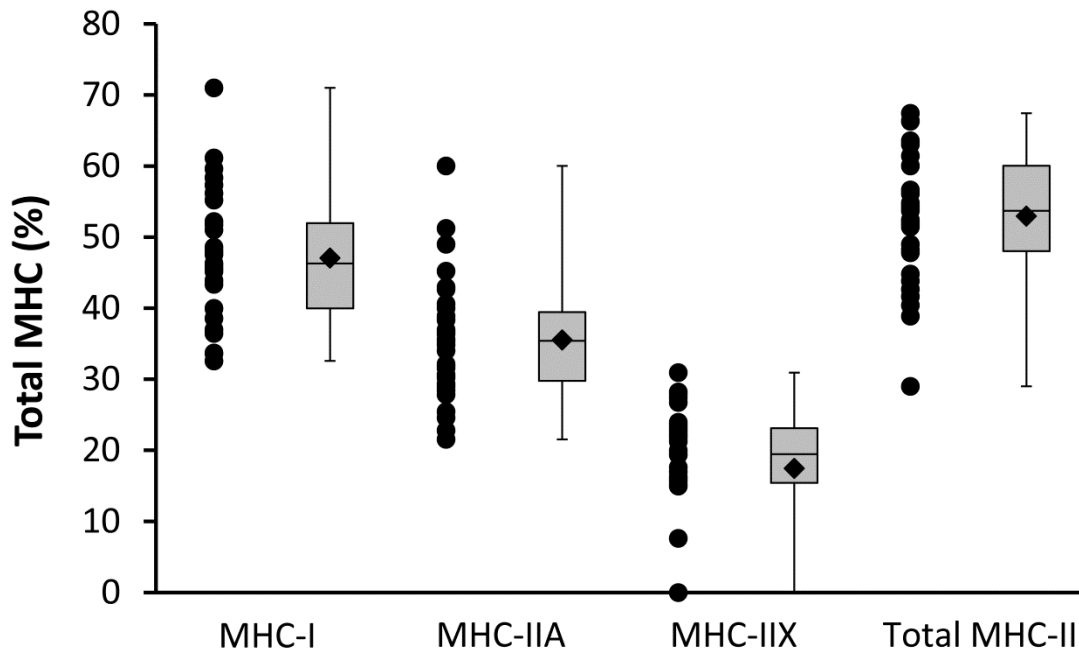
506 **Figure Captions**



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

511

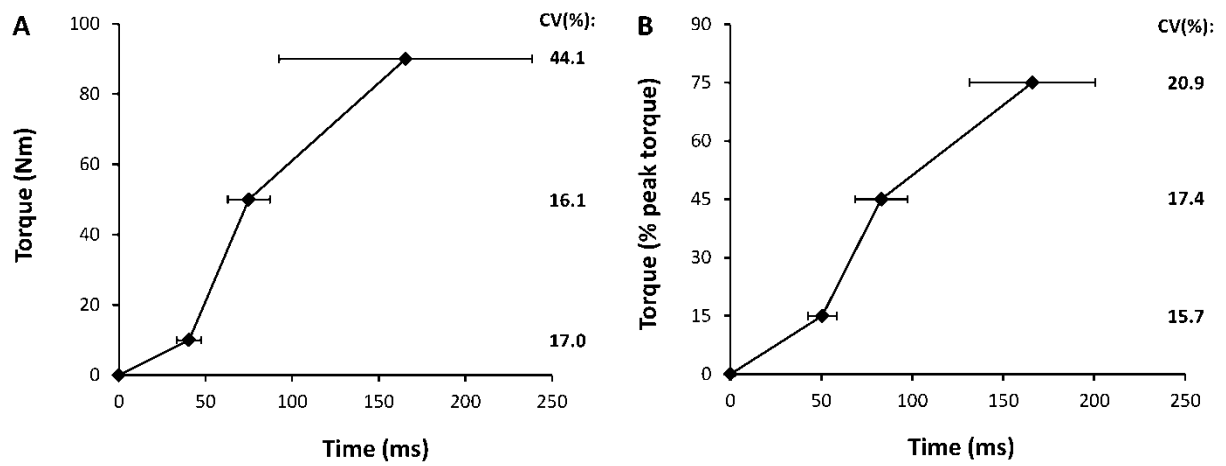
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513

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

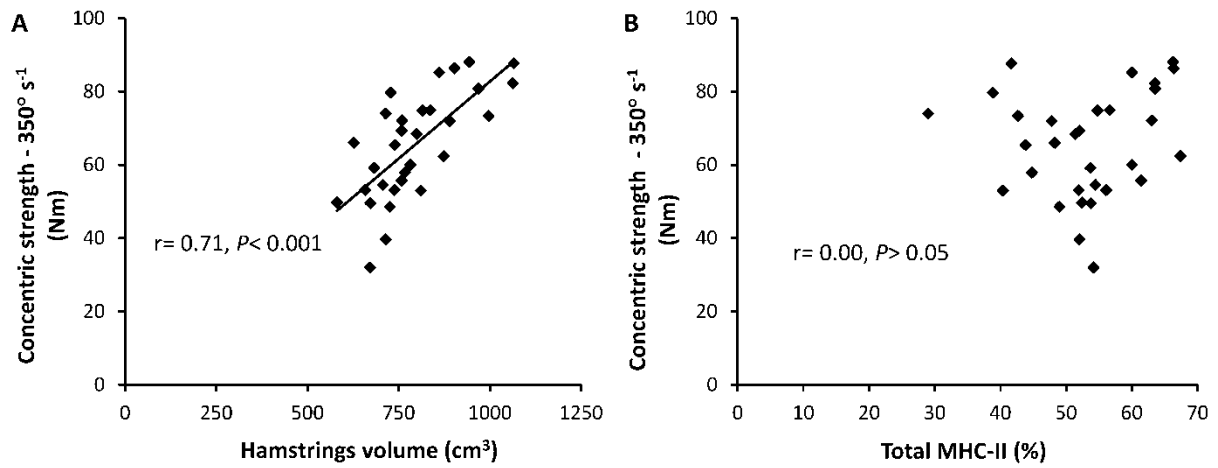
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518

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 \pm 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

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 (cm³)	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
	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

537 **Table 2.** Bivariate correlation coefficients of knee flexor maximal strength with hamstrings
 538 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

539 MHC, myosin heavy chain; BFlh, biceps femoris long head; Ecc, eccentric; Con, concentric;

540 [†] $P < 0.01$, [‡] $P < 0.001$

541

542 **Table 3.** Bivariate correlation coefficients between knee flexor explosive strength
 543 characteristics, measured as time to specific (absolute and relative) torques, with hamstrings
 544 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

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

546