

Biceps Femoris Architecture and Strength in Athletes with a Previous Anterior Cruciate Ligament Reconstruction

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1 **Title:**

2 Biceps femoris architecture and strength in athletes with a prior ACL reconstruction

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19 Biceps femoris architecture and ACL injury

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22 Catholic University.

23 **ABSTRACT**

24 **Purpose:** To determine if limbs with a history of anterior cruciate ligament (ACL) injury
25 reconstructed from the semitendinosus (ST) display different biceps femoris long head (BF_{lh})
26 architecture and eccentric strength, assessed during the Nordic hamstring exercise, compared
27 to the contralateral uninjured limb. **Methods:** The architectural characteristics of the BF_{lh}
28 were assessed at rest and at 25% of a maximal voluntary isometric contraction (MVIC) in the
29 control (n=52) and previous ACL injury group (n=15) using two-dimensional
30 ultrasonography. Eccentric knee-flexor strength was assessed during the Nordic hamstring
31 exercise. **Results:** Fascicle length was shorter ($p=0.001$; d range: 0.90 to 1.31) and pennation
32 angle (p range: 0.001 to 0.006; d range: 0.87 to 0.93) was greater in the BF_{lh} of the ACL
33 injured limb when compared to the contralateral uninjured limb at rest and during 25% of
34 MVIC. Eccentric strength was significantly lower in the ACL injured limb than the
35 contralateral uninjured limb (-13.7%; -42.9N; 95% CI = -78.7 to -7.2; $p=0.021$; $d=0.51$).
36 Fascicle length, MVIC and eccentric strength were not different between the left and right
37 limb in the control group. **Conclusions:** Limbs with a history of ACL injury reconstructed
38 from the ST have shorter fascicles and greater pennation angles in the BF_{lh} compared to the
39 contralateral uninjured side. Eccentric strength during the Nordic hamstring exercise of the
40 ACL injured limb is significantly lower than the contralateral side. These findings have
41 implications for ACL rehabilitation and hamstring injury prevention practices which should
42 consider altered architectural characteristics.

43 **Key Terms:** Hamstring injury; eccentric strength; anterior cruciate ligament injury; fascicle
44 length

45

46 **INTRODUCTION**

47 **Paragraph 1**

48 Anterior cruciate ligament (ACL) injuries are debilitating and result in a significant amount
49 of time out from training and competition (5, 29, 30). In addition, a history of severe knee
50 injury (including ACL injury) increases the risk of a future hamstring strain injury (HSI)(38).
51 However, there has been little scientific investigation into why an athlete is at an increased
52 risk of a HSI following an ACL injury (38). Reconstruction of the ACL following an injury is
53 highly invasive and typically involves one of two types of autogenous grafts, harvested from
54 either the semitendinosus/gracilis (ST) or patella tendon (8). These procedures, independent
55 of graft type, have been reported to result in long term deficits in eccentric and concentric
56 knee extensor(16, 17, 36) and flexor(19, 35, 36) strength up to 25 years following the
57 reconstruction. Despite the known link between prior ACL injury and future HSI risk,
58 research into compromised function of the knee flexors following ACL reconstruction, has
59 mostly focused on strength (19, 36) and rate of force development(16). Investigations into
60 structural differences of the hamstrings following ACL reconstruction have shown
61 differences in hamstring muscle volume, with the gracilis and ST of the surgically repaired
62 limb being significantly smaller, with the biceps femoris long head (BF_{lh}) being larger, when
63 compared to the contralateral uninjured limb (33). However, the presence of other deficits in
64 hamstring structure and/or function following ACL reconstruction remains largely unknown.

65 **Paragraph 2**

66 Of all the hamstring muscles, the BF_{lh} is the most commonly injured (18, 24). Therefore a
67 greater understanding of the factors which might alter the risk of HSI in this muscle is
68 needed. Recently it has been shown that limbs with a previous BF_{lh} strain injury display
69 architectural differences when compared to the contralateral uninjured BF_{lh} (37). Most
70 notably the previously injured BF_{lh} displays shorter fascicles compared to the contralateral

71 uninjured muscle (37). It is well accepted that limbs with a previous hamstring strain injury
72 display low levels of eccentric strength, which may be the result of (13, 27, 34) or cause (24)
73 of injury. Since a previous ACL injury is considered a risk factor for a future HSI in athletes
74 (18, 38) and considering the evidence which has shown reductions in eccentric strength in
75 limbs with a previous ACL injury (19, 35, 36) , it is of interest to determine if alterations in
76 hamstring architecture exist, given that eccentric contractions are thought to be a powerful
77 stimulus for in-series sarcomereogenesis (3) and hypertrophy (31). As the BF_{lh} is the most
78 commonly injured of the knee flexor muscles, it is also of interest to know if limbs with a
79 previous ACL injury can lead, indirectly, to alterations in BF_{lh} architecture.

80 **Paragraph 3**

81 The purposes of this study were to: 1) determine if a limb with a previous ACL injury
82 displays reduced eccentric knee flexor strength during the Nordic hamstring exercise when
83 compared to the contralateral uninjured limb and a healthy control group and; 2) determine if
84 the architectural characteristics of the BF_{lh} of the previous ACL injured limb is different to
85 the contralateral limb without a prior history of ACL injury and a healthy control group. It
86 was hypothesized that the previous ACL injured limb will exhibit reduced eccentric strength
87 and will present with shorter BF_{lh} fascicles when compared to the contralateral uninjured
88 limb.

89 **METHODS**

90 **Participants**

91 **Paragraph 4**

92 Sixty seven males (n=67) were recruited to participate in this case-control study. Fifty two
93 (n=52) elite athletes (age 22.6 ± 4.6 years; height 1.77 ± 0.05 m; body mass 74.4 ± 5.9 kg) with
94 no history of lower limb injury and in the past 12 months and no history at all of ACL injury

95 were recruited as a control group. Fifteen elite (n=15) athletes with a unilateral ACL injury
96 history (age 24.5 ± 4.2 years; height 1.86 ± 0.06 m; body mass 84.2 ± 8.1 kg) were recruited to
97 participate and form the ACL injured group. All athletes in both groups were currently
98 competing at national or international level in soccer or Australian Football. Inclusion criteria
99 for the ACL injured group were; (i) aged between 18 and 35 years, (ii) date of surgery
100 between 2004 and 2013, (iii) ACL reconstruction autograft from the ipsilateral ST, (iv) no
101 history of HSI in the past 12 months and (v) returned to pre injury levels of competition and
102 training. All ACL injured athletes reported standard rehabilitation progression as directed by
103 the physiotherapist of their respective clubs (21) and reported the use of some eccentric
104 hamstring conditioning at the time of assessment (10). The ACL injured athletes (9 soccer
105 players and 6 Australian Rules Football players) were recruited to assess the differences in
106 the BF_{lh} architectural characteristics, maximum voluntary isometric contraction (MVIC) knee
107 flexor strength and average peak force during the Nordic hamstring exercise of their ACL
108 injured limb and the contralateral uninjured limb. All participants provided written informed
109 consent prior to testing which was undertaken at the Australian Catholic University, Fitzroy,
110 Victoria, Australia. Ethical approval for the study was granted by the Australian Catholic
111 University Human Research Ethics Committee.

112 **Experimental design**

113 **Paragraph 5**

114 The test-retest reliability of real-time two-dimensional ultrasound derived measures of muscle
115 thickness, pennation angle and estimates of BF_{lh} fascicle length at rest and during different
116 isometric contraction intensities has previously been investigated (37). Nordic hamstring
117 exercise strength was assessed using a custom made device (25). All participants (ACL
118 injured group and control group) had their BF_{lh} architectural characteristics, eccentric and
119 MVIC knee flexor strength assessed during a single session. All ACL injured athletes were

120 assessed during early pre-season in their chosen sport (Soccer: June to July 2014, Australian
121 Rules Football: November to December 2014).

122 **BF_{th} architecture assessment**

123 **Paragraph 6**

124 Muscle thickness, pennation angle and estimates of BF_{th} fascicle length were determined
125 from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two
126 dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm)
127 (GE Healthcare Vivid-i, Wauwatosa, U.S.A). The scanning site was determined as the
128 halfway point between the ischial tuberosity and the knee joint fold, along the line of the
129 BF_{th}. Once the scanning site was determined, the distance of the site from various anatomical
130 landmarks were recorded to ensure reproducibility of the scanning site for future testing
131 sessions. These landmarks included the ischial tuberosity, fibula head and the posterior knee
132 joint fold at the mid-point between BF and ST tendon. All architectural assessments were
133 performed with participants in a prone position and the hip in a neutral position following at
134 least five minutes of inactivity. Assessments at rest were always performed first followed by
135 the isometric contraction protocol. Assessment of BF_{th} architecture at rest was performed
136 with the knee at 0° (fully extended). Assessment of BF_{th} architecture during isometric
137 contractions was always performed with the knee at 0° of knee flexion and preceded by a
138 MVIC in a custom made device (25). Participants were positioned prone on top of a padded
139 board with both the hip and knee fully extended. The ankles were secured superior to the
140 lateral malleolus by individual ankle braces which were secured atop custom made uniaxial
141 load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data
142 acquisition capabilities (Mantracourt, Devon, UK). Participants were then instructed to
143 contract maximally over a five second period, and the instantaneous peak force was used to
144 determine the MVIC. The active architectural assessment was performed in the same device

145 at 25% of MVIC with the participants shown the real-time visual feedback of the force
146 produced to ensure that target contraction intensities were met.

147 **Paragraph 7**

148 To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel
149 was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the
150 posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the
151 probe as this may influence the accuracy of the measures (15). Finally, the orientation of the
152 probe was manipulated slightly by the sonographer if the superficial and intermediate
153 aponeuroses were not parallel. Reliability of the sonographer when assessing the BF_{th}
154 architectural characteristics has been reported previously(37).

155 **Paragraph 8**

156 Once the images were collected, analysis was undertaken off-line (MicroDicom, Version
157 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazeovich and
158 colleagues (1). Following the digitising process, muscle thickness was defined as the distance
159 between the superficial and intermediate aponeuroses of BF_{th}. A fascicle of interest was
160 outlined and marked on the image (Fig. 1). The angle between this fascicle and the
161 intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis
162 angle for both aponeuroses was determined as the angle between the line marked as the
163 aponeurosis and an intersecting horizontal line across the captured image (1, 14). Fascicle
164 length was estimated from the length of the outlined fascicle between aponeuroses. As the
165 entire fascicle was not visible in the field of view of the probe its length was estimated via the
166 following validated equation from Blazeovich and colleagues (1, 14):

167 $FL = \sin(AA + 90^\circ) \times MT / \sin(180^\circ - (AA + 180^\circ - PA))$.

168 Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation
169 angle.

170 **Paragraph 9**

171 Fascicle length was reported in absolute terms (cm) and also relative to muscle thickness
172 (fascicle length/muscle thickness). The same assessor (RGT) collected and analysed all scans
173 and was blinded to participant identifiers during the analysis.

174 **Eccentric hamstring strength**

175 **Paragraph 10**

176 The assessment of eccentric hamstring strength using the Nordic hamstring exercise field
177 testing device has been reported previously (25). Participants were positioned in a kneeling
178 position over a padded board, with the ankles secured superior to the lateral malleolus by
179 individual ankle braces which were secured atop custom made uniaxial load cells (Delphi
180 Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities
181 (Mantracourt, Devon, UK). The ankle braces and load cells were secured to a pivot which
182 allowed the force to always be measured through the long axis of the load cells. Following a
183 warm up set, participants were asked to perform one set of three continuous maximal bilateral
184 repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean
185 forward at the slowest possible speed while maximally resisting this movement with both
186 lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands
187 held across the chest. Following each attempt a visual analogue scale was given to assess the
188 level of pain that was experienced. None of the participants reported any pain during testing.
189 Verbal encouragement was given throughout the range of motion to ensure maximal effort.
190 The peak force for each of the three repetitions was averaged for all statistical comparisons.

191 **Data analysis**

192 **Paragraph 11**

193 Whilst positioned in the custom made device, shank length (m) was determined as the
194 distance from the lateral tibial condyle to the mid-point of the brace which was placed around
195 the ankle. This measure of shank length was used to convert the force measurements
196 (collected in N) to torque (Nm). Knee flexor eccentric and MVIC strength force data were
197 transferred to a personal computer at 100Hz through a wireless USB base station
198 (Mantracourt, Devon, UK). The peak force value during the MVIC and the three Nordic
199 hamstring exercise repetitions for each of the limbs (left and right) was analysed using
200 custom made software. Eccentric knee flexor strength, reported in absolute terms (N and Nm)
201 and relative to body mass (N/kg and Nm/kg), was determined as the average of the peak
202 forces from the 3 repetitions for each limb, resulting in a left and right limb measure (25).
203 Knee flexor MVIC strength, reported in absolute terms (N and Nm) and relative to body mass
204 (N/kg and Nm/kg), was determined as the peak force produced during a 5 second maximal
205 effort for each limb.

206 **Statistical analyses**

207 **Paragraph 12**

208 All statistical analyses were performed using SPSS version 19.0.0.1 (IBM Corporation,
209 Chicago, IL). Where appropriate, data were screened for normal distribution using the
210 Shapiro-Wilk test and homoscedasticity of the data using Levene's test. Reliability of the
211 assessor (RGT) and processes used for the determination of the BFlh architectural
212 characteristics has previously been reported(37).

213 **Paragraph 13**

214 At both contraction intensities, a split-plot design ANOVA, with the within-subject variable
215 being limb (left/right or uninjured/ACL injured, depending on group) and the between-
216 subject variable being group (control or ACL injured group) was used to compare BF_{th}
217 architecture, MVIC and Nordic hamstring exercise strength variables. For the control group,
218 all architectural and strength measurements from the left and right limbs were averaged, as
219 the limbs did not differ ($p > 0.05$; Table 1.), in order to allow a single control group measure.
220 Where significant limb x group interactions were detected, post hoc t-tests with Bonferroni
221 adjustments to the alpha level were used to identify which comparisons differed.

222 **Paragraph 14**

223 Further between group analyses were undertaken to determine the extent of the between limb
224 asymmetry in BF_{th} architecture, MVIC and Nordic hamstring exercise strength, in the control
225 and ACL injured groups. The control group between limb asymmetry was determined as the
226 right limb minus the left and then converted to an absolute value (34, 37), whereas in the
227 ACL injured group asymmetry was determined as the uninjured limb minus the ACL injured
228 limb. Independent t-tests were used to assess differences in the extent of the between limb
229 asymmetry in the control compared to the ACL injured group. Bonferroni corrections were
230 employed to account for inflated type I error due to the multiple comparisons made for each
231 dependent variable. Significance was set at a $p < 0.05$ and where possible Cohen's d (4) was
232 reported for the effect size of the comparisons, with the levels of effect being deemed small
233 ($d = 0.20$), medium ($d = 0.50$) or large ($d = 0.80$) as recommended by Cohen (1988).

234 **RESULTS**

235 **Power calculations**

236 **Paragraph 15**

237 Power analysis was undertaken *a-priori* using G-Power(7). The analysis was based on the
238 anticipated differences between the ACL injured limb and the contralateral uninjured limb in
239 the ACL injured group. Estimates of effect size were based on previous research investigating
240 differences between limbs in athletes with a unilateral HSI history(37). This previous study
241 reported differences in BF_{th} fascicle length, between the previously injured limb and the
242 contralateral uninjured limb, to have an effect size of 1.34 when assessed at rest. Therefore an
243 effect size of 0.8 was deemed reasonable as a starting point. Power was set at 80% with an
244 alpha of 0.05 returning a calculated sample size of 15. As a cross-reference to confirm this
245 sample size calculation, previous studies that have used similar designs have used samples
246 from 13 to 15(27, 28, 34, 37).

247 **Participants**

248 **Paragraph 16**

249 The participants in the ACL injured group were 10.1±8.1kg heavier and 6.1±0.06cm taller
250 compared to the control group (p<0.05). All athletes from the ACL injured group had
251 suffered at least 1 ACL injury in the past 9 years (median time since surgery = 3.5years
252 [range = 1 year to 9 years]).

253 **BF_{th} architectural comparisons**

254 **Paragraph 17**

255 A significant limb-by-group interaction effect was found for fascicle length and fascicle
256 length relative to muscle thickness at both contraction intensities (p=0.004). Post hoc analysis
257 showed that fascicle length and fascicle length relative to muscle thickness were significantly

258 shorter in the BF_{lh} of the ACL injured limb compared to the contralateral uninjured limb in
259 the ACL injured group at both contraction intensities ($p < 0.05$, d range = 0.87 to 1.31; Table
260 1; Fig 2.). A significant limb-by-group interaction effect was detected at both contraction
261 intensities ($p = 0.003$) for pennation angle. Post hoc analysis showed that pennation angle was
262 greater in the injured limb compared to the contralateral uninjured limb in the ACL injured
263 group at both contraction intensities ($p < 0.05$, d range = 0.87 to 0.93; Table 1; Fig 2.).
264 Comparisons of muscle thickness displayed no significant main effects ($p > 0.05$, d range: 0.27
265 to 0.42; Table 1; Fig 2.), however when comparing the ACL injured limb to the contralateral
266 uninjured limb, at rest, there was a small effect size ($d = 0.42$; Table 1; Fig 2.) where the
267 uninjured limb was thicker than the injured. No significant differences in any BF_{lh}
268 architectural characteristics were found when comparing either limb in the ACL injured
269 group to the average of both limbs in the control group ($p > 0.05$, d range = 0.11 to 0.21).

270 **Paragraph 18**

271 Comparing the extent of between-limb asymmetry in all the BF_{lh} architectural characteristics
272 in the control group to the ACL injured group, the asymmetry in fascicle length, fascicle
273 length relative to muscle thickness and pennation angle was greater in the ACL injured group
274 ($p < 0.05$, d range = 0.86 to 1.13; Supp Table; Fig 3.).

275 **Knee flexor strength measures**

276 **Paragraph 19**

277 A significant limb-by-group interaction effect was found for average peak force during the
278 Nordic hamstring exercise ($p = 0.001$). Post hoc analysis showed that the ACL injured limb
279 ($269.9N \pm 81.4$) was 13.7% weaker than the contralateral uninjured limb ($312.9N \pm 85.1$) in the
280 ACL injured group (between limb difference: $43.0N$; 95% CI = 7.2 to 78.7; $p = 0.022$; $d = 0.51$;
281 Table 2). Independent of whether it was relative to body weight or an absolute measure of

282 force or torque, the ACL injured limb was weaker than the average of both limbs in the
283 control group ($p < 0.05$; d range = 0.58 to 0.74). There were no significant relative or absolute
284 differences in force or torque between the uninjured limb in the ACL injured group and the
285 average of both limbs in the control group (mean difference: 7.1N; 95% CI = -39.4 to 53.5;
286 $p = 0.763$; $d = 0.08$).

287 **Paragraph 20**

288 Between-limb asymmetry during the Nordic hamstring exercise was greater in the ACL
289 injured group (between group difference 36.0N; 95% CI = 12.2 to 59.7; $p = 0.003$; $d = 0.71$;
290 Supp Table.).

291 **Paragraph 21**

292 Comparisons of knee flexor MVIC strength of the ACL injured limb to the contralateral
293 uninjured limb and the average of both limbs in the control group displayed no significant
294 differences ($p > 0.05$, d range = 0.34 to 0.45).

295 **Paragraph 22**

296 Finally, no significant differences were found when comparing the extent of between limb
297 asymmetry in knee flexor MVIC between the ACL injured group and control group (between
298 group difference: -3.8N; 95% CI = -34.7 to 27.1; $p = 0.807$, $d = -0.07$; Supp Table.).

299 **DISCUSSION**

300 **Paragraph 23**

301 The major findings were that elite athletes with a unilateral ACL injury, which was
302 reconstructed with a graft from the ipsilateral ST, had shorter fascicles and greater pennation
303 angles in the BF_{th} of the previously ACL injured limb than the contralateral uninjured limb
304 both at rest and during a 25% MVIC. Furthermore, between limb asymmetry of fascicle

305 length and pennation angle was greater in the previous ACL injured group than the control
306 group. Moreover, eccentric strength during the Nordic hamstring exercise was significantly
307 lower in the previous ACL injured limb when compared to the contralateral uninjured limb,
308 whereas comparisons of isometric knee flexor strength displayed a small difference between
309 limbs as determined by effect size ($d=0.31$). Additionally the previous ACL injured group
310 had a greater between limb asymmetry in eccentric knee flexor strength compared to the
311 control group. To the authors' knowledge this is the first study that has investigated the BF_{lh}
312 architectural differences in a limb with a previous ACL injury, reconstructed from the
313 ipsilateral ST, in comparison to uninjured limbs (both from the contralateral limb and the
314 control group). In addition, no prior work has examined the between limb differences in
315 eccentric strength during the Nordic hamstring exercise in individuals with a history of
316 unilateral ACL injury.

317 **Paragraph 24**

318 Observations of shorter muscle fascicles and greater pennation angles have been reported in
319 previously strain injured BF_{lh} compared to the contralateral uninjured limb (37). However, no
320 prior study had investigated the effect that a previous ACL injury has on hamstring muscle
321 architecture. Athletes in the current study with a prior ACL injury, reconstructed from the ST,
322 have somewhat comparable BF_{lh} fascicle lengths in their injured limb, at rest (10.13cm±1.39;
323 Table 1) and 25% of MVIC (9.08cm±1.38; Table 1) compared to previously strain injured
324 BF_{lh} (rest: 10.40cm±1.12; 25% of MVIC: 9.50cm±1.10) (37). Additionally, the extent of
325 between limb asymmetry in BF_{lh} fascicle length in the athletes from the current study, when
326 assessed at rest (13.7%; 1.61cm±0.31) and 25% of MVIC (12.9%; 1.35cm±0.25) is
327 comparable to individuals with a unilateral history of BF_{lh} strain injury (rest: 12.9%;
328 1.54cm±0.12; 25% of MVIC: 10.9%; 1.17cm±0.10) (37). The similarities in BF_{lh} fascicle
329 length and between limb asymmetry in individuals with two different injuries are of great

330 interest as a history of both ACL injury and HSI increases the risk of future HSI (18, 38).
331 However the maladaptations which influence the increase in HSI risk in individuals with a
332 previous ACL injury are unknown. It has been hypothesized that possessing shorter muscle
333 fascicles, with fewer in-series sarcomeres, may result in an increased susceptibility to
334 eccentrically-induced muscle damage (2, 22). Therefore the shorter BF_{th} fascicle length in the
335 limb with a history of ACL injury may increase its susceptibility to muscle damage during
336 powerful eccentric contractions that occur during periods of high speed running. This
337 increased susceptibility to muscle damage may then contribute to the increased HSI risk in
338 individuals with a history of ACL injury.

339 **Paragraph 25**

340 Although speculative from the current data, changes in muscle activation throughout the
341 entire knee range of motion may contribute to variations in muscle architecture in individuals
342 with a history of ACL injury. Certainly individuals with a previous hamstring strain injury
343 display less BF_{th} activation at long muscle lengths, which hypothetically might be mediated
344 by the pain associated with the initial injury (11, 27, 34). Investigations into experimentally
345 induced pain have shown alterations in muscle activation, mechanical behaviour and motor
346 unit discharge rates in an apparent effort to reduce stress (force per unit area) and protect the
347 painful structures from further discomfort(11, 12, 20). Therefore the pain associated with an
348 ACL injury, as well as the surgical reconstruction, may alter knee flexor muscle activation so
349 as to protect the knee from further discomfort. If these alterations in muscle activation are
350 accentuated at long knee flexor muscle lengths, this may then result in architectural
351 maladaptations of the knee flexors. However it is possible that reductions in fascicle length
352 can occur despite compensatory increases in BF_{th} muscle volume in the ACL injured limb
353 (33), as changes in muscle architecture can occur independent of muscle size (23). What is
354 still to be determined is why and/or how ACL reconstruction using the ipsilateral ST might

355 influence BF_{lh} architecture. It is possible that reductions in activation and eccentric strength
356 may have contributed to the architectural alterations within the BF_{lh}, however other factors
357 may influence these changes. Without architectural data of the other knee flexor muscles (see
358 limitations section), it is impossible to know if these architectural deficits are evident in all
359 the hamstring muscles in the previous ACL injured limb. It is unlikely, however, that there is
360 a unique stimulus to the BF_{lh} compared to the medial hamstrings. Future research should
361 investigate if the architectural differences, found in the BF_{lh}, exist in the neighbouring knee
362 flexors.

363 **Paragraph 26**

364 In this study, individuals with a unilateral ACL injury reconstructed from the ipsilateral ST
365 displayed a significantly lower amount of eccentric strength during the Nordic hamstring
366 exercise in the previously ACL injured limb when compared to the contralateral uninjured
367 limb (15.9%; $d = 0.51$), despite smaller differences in MVIC strength (5.1%; $d = 0.31$).
368 Similar between limb differences in eccentric knee flexor strength (16.9%) are evident in
369 individuals with a unilateral ACL injury when assessed via isokinetic dynamometry more
370 than 20 years following the injury (36). With respect to the link between prior ACL injury
371 and HSI, elite Australian footballers who subsequently went on to sustain a HSI were ~14%
372 weaker compared to those that remained injury free when assessed prospectively(24). This is
373 a similar magnitude of weakness seen in the previously ACL reconstructed limb compared to
374 the contralateral uninjured limb in the current study. Given that approximately 60% of HSIs
375 occur during high speed running, these low levels of eccentric strength may suggest a
376 reduced ability to decelerate the lower limb during the terminal swing phase of high speed
377 running(24, 26). This coupled with the previously hypothesized increased susceptibility for
378 muscle damage due to shorter muscle fascicles (2, 9), may increase the risk of a future strain
379 injury of the BF_{lh} in individuals with a previous ACL injury during high speed running or

380 other repetitive eccentric contractions. Additionally, the lower levels of eccentric strength,
381 without any differences in MVIC, in the previously ACL injured limb may be due to a
382 maladaptive tension limiting mechanism (9). As the stresses and strains on the
383 musculoskeletal structures are greater during eccentric contractions compared to isometric
384 efforts (6), it is possible that the lower levels of force during the Nordic hamstring exercise
385 may act to reduce tissue loading in the ACL injured limb.

386 **Paragraph 27**

387 We acknowledge that there are limitations associated with the study. Firstly, the investigation
388 of the muscle architectural characteristics only occurred in the BF_{lh} and therefore it is
389 unknown as to what extent the other knee flexors may also be altered. Indeed previous
390 research suggests that compensatory adaptations may occur where inter-muscular
391 coordination is altered to accommodate the injured muscle (32). We have attempted imaging
392 of the ST and initial data did not display acceptable reproducibility. Previous studies have
393 also reported lower reliability when assessing ST when compared to BF_{lh} with intra-class
394 correlations 0.77 and 0.91 respectively (14). Additionally, as the BF_{lh} is the most commonly
395 injured hamstring muscle (18, 24), we believe that the findings reported in BF_{lh} architectural
396 differences between limbs with and without ACL reconstruction are of importance. Future
397 work should examine if these architectural differences are present in the other knee flexors,
398 particularly the harvested ST. Secondly the retrospective nature of the study limits the
399 determination of whether the differences in muscle architecture and eccentric strength existed
400 prior to the ACL injury and reconstruction or were the result of the incident. Prospective
401 investigations are required to determine any existence of a causal relationship and should be
402 the focus of future research. Finally, the current study only included athletes with an ACL
403 injury which was reconstructed with a graft from the ipsilateral ST. Future research should
404 aim to investigate the architectural variations in athletes with a non-ST graft.

405 **Paragraph 28**

406 In conclusion, the current study provided evidence that BF_{lh} fascicle length, pennation angle
407 and eccentric knee flexor strength during the Nordic hamstring exercise, in individuals with a
408 unilateral ACL injury which was reconstructed from the ipsilateral ST, is significantly
409 different to limbs without a history of ACL injury. Despite the retrospective nature of these
410 findings, they provide significant insight into the architectural and eccentric strength
411 asymmetries of the BF_{lh} which exist in those who have a history of ACL injury. These
412 differences should be considered when attempting to limit the risk of future HSI in those with
413 a history of ACL injury. Much work is still required to determine if hamstring muscle
414 architecture and eccentric knee flexor strength play a role in the aetiology of an ACL injury.

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416 **Paragraph 29**

417 Dr. Anthony Shield and Dr. David Opar are listed as co-inventors on an international patent
418 application filed for the experimental device (PCT/AU2012/001041.2012). Results of this
419 study do not constitute endorsement of the American College of Sports Medicine

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423

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531 Figure 1: A two dimensional ultrasound image of the biceps femoris long head. This image of
532 the biceps femoris long head was taken along the longitudinal axis of the posterior thigh.
533 From these images it is possible to determine the superficial and intermediate aponeuroses,
534 muscle thickness, angle of the fascicle in relation to the aponeurosis. Estimates of fascicle
535 length can then be made via trigonometry using muscle thickness and pennation angle.

536

537 Figure 2: Architectural characteristics of the BF_{lh} in ACL injured limb and the contralateral
538 uninjured limb in the previously ACL injured group at both contraction intensities. A)
539 fascicle length B) pennation angle C) muscle thickness D) fascicle length relative to muscle
540 thickness. Error bars illustrate the standard deviation. * $p < 0.05$ injured vs uninjured.

541

542 Figure 3: Comparisons of between leg asymmetry for the architectural characteristics of the
543 BF_{lh} in the previously ACL injured group (uninjured minus injured) to the absolute between
544 leg differences of the control group at both contraction intensities. A) fascicle length B)
545 pennation angle C) muscle thickness D) fascicle length relative to muscle thickness. Error
546 bars illustrate the standard deviation. * $p < 0.05$ injured vs control.

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