

**Research Bank**

Journal article

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

**Timmins, Ryan G., Bourne, Matthew N., Shield, Anthony J., Williams, Morgan, Lorenzen, Christian and Opar, David A.**

This is a pre-copyedited, author-produced version of an article accepted for publication in *Medicine and Science in Sports and Exercise*.

The published version of record Timmins, R. G., Bourne, M. N., Shield, A. J., Williams, M., Lorenzen, C. and Opar, D. A. (2016). Biceps femoris architecture and strength in athletes with a prior ACL reconstruction. *Medicine and Science in Sports and Exercise*, 48(3), pp. 337-345 is available online at:

<https://doi.org/10.1249/MSS.0000000000000783>

This work © 2016 is licensed under [Creative Commons Attribution-NonCommercial 4.0 International](https://creativecommons.org/licenses/by-nc/4.0/).

1 **Title:**

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

3 **Authors:**

4 Ryan G Timmins<sup>1</sup>, Matthew N Bourne<sup>2</sup>, Anthony J Shield<sup>2</sup>, Morgan D Williams<sup>3</sup>, Christian  
5 Lorenzen<sup>1</sup>, David A Opar<sup>1</sup>

6 <sup>1</sup>School of Exercise Science, Australian Catholic University, Melbourne, Australia

7 <sup>2</sup>School of Exercise and Nutrition Sciences and Institute of Health and Biomedical  
8 Innovation, Queensland University of Technology, Brisbane, Australia

9 <sup>3</sup>School of Health, Sport and Professional Practice, University of South Wales, Pontypridd,  
10 Wales, UK

11 **Corresponding author:**

12 **Ryan G. Timmins**

13 School of Exercise Science, Australian Catholic University, 115 Victoria Parade, Fitzroy,  
14 3065, Melbourne, Victoria, Australia

15 [ryan.timmins@myacu.edu.au](mailto:ryan.timmins@myacu.edu.au)

16 Telephone: +61 3 9953 3742

17 Fax: +61 3 9953 3095

18 **Running title:**

19 Biceps femoris architecture and ACL injury

20 **Disclosure of funding:**

21 This study was partially funded by a Faculty of Health Research Grant from the Australian  
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<sub>lh</sub>)  
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<sub>lh</sub>  
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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub>) 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<sub>lh</sub> 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<sub>lh</sub> strain injury display  
69 architectural differences when compared to the contralateral uninjured BF<sub>lh</sub> (37). Most  
70 notably the previously injured BF<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> 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  $22.6 \pm 4.6$  years; height  $1.77 \pm 0.05$ m; body mass  $74.4 \pm 5.9$ 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  $24.5 \pm 4.2$  years; height  $1.86 \pm 0.06$ m; body mass  $84.2 \pm 8.1$ 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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> architecture assessment**

### 123 **Paragraph 6**

124 Muscle thickness, pennation angle and estimates of BF<sub>lh</sub> 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<sub>lh</sub>. 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<sub>lh</sub> architecture at rest was performed  
136 with the knee at 0° (fully extended). Assessment of BF<sub>lh</sub> 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<sub>th</sub>  
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<sub>th</sub>. 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<sub>th</sub>  
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<sub>th</sub> 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<sub>th</sub> 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<sub>th</sub> 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<sub>th</sub> 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<sub>lh</sub>  
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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> (rest: 10.40cm±1.12; 25% of MVIC: 9.50cm±1.10) (37). Additionally, the extent of  
325 between limb asymmetry in BF<sub>lh</sub> 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<sub>lh</sub> strain injury (rest: 12.9%;  
328 1.54cm±0.12; 25% of MVIC: 10.9%; 1.17cm±0.10) (37). The similarities in BF<sub>lh</sub> 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<sub>th</sub> 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<sub>th</sub> 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<sub>lh</sub> 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<sub>lh</sub> architecture. It is possible that reductions in activation and eccentric strength  
356 may have contributed to the architectural alterations within the BF<sub>lh</sub>, 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<sub>lh</sub> compared to the medial hamstrings. Future research should  
361 investigate if the architectural differences, found in the BF<sub>lh</sub>, 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<sub>lh</sub> 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<sub>lh</sub> 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<sub>lh</sub> with intra-class  
394 correlations 0.77 and 0.91 respectively (14). Additionally, as the BF<sub>lh</sub> is the most commonly  
395 injured hamstring muscle (18, 24), we believe that the findings reported in BF<sub>lh</sub> 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<sub>th</sub> 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<sub>th</sub> 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.

415 **ACKNOWLEDGMENTS**

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

420 **Disclosure of funding:**

421 This study was partially funded by a Faculty of Health Research Grant from the Australian  
422 Catholic University.

423

424 **REFERENCES**

- 425 1. Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human  
426 quadriceps femoris architecture assessed in vivo. *J Anat.* 2006 Sep;209(3):289-310.
- 427 2. Brockett CL, Morgan DL, Proske U. Predicting hamstring strain injury in elite  
428 athletes. *Med Sci Sports Exerc.* 2004 Mar;36(3):379-87.
- 429 3. Butterfield TA, Herzog W. The magnitude of muscle strain does not influence serial  
430 sarcomere number adaptations following eccentric exercise. *Pflugers Archiv.* 2006  
431 Feb;451(5):688-700.
- 432 4. Cohen D. *Statistical power analysis for the behavioral sciences.* Hillsdale (NJ):  
433 Erlbaum; 1988. 77-83.
- 434 5. Dallalana RJ, Brooks JH, Kemp SP, Williams AM. The epidemiology of knee injuries  
435 in English professional rugby union. *Am J Sports Med.* 2007 May;35(5):818-30.
- 436 6. Enoka RM. Eccentric contractions require unique activation strategies by the nervous  
437 system. *J Appl Physiol (1985).* 1996 Dec;81(6):2339-46.
- 438 7. Faul F, Erdfelder E, Lang AG, Buchner A. G\*Power 3: a flexible statistical power  
439 analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.*  
440 2007 May;39(2):175-91.
- 441 8. Frank CB, Jackson DW. The science of reconstruction of the anterior cruciate  
442 ligament. *J Bone Joint Surg Am.* 1997 Oct;79(10):1556-76.
- 443 9. Fyfe JJ, Opar DA, Williams MD, Shield AJ. The role of neuromuscular inhibition in  
444 hamstring strain injury recurrence. *J Electromyogr Kinesiol.* 2013 Jun;23(3):523-30.
- 445 10. Heiderscheit BC, Sherry MA, Silder A, Chumanov ES, Thelen DG. Hamstring strain  
446 injuries: recommendations for diagnosis, rehabilitation, and injury prevention. *J Orthop*  
447 *Sports Phys Ther.* 2010 Feb;40(2):67-81.

- 448 11. Hodges PW, Tucker K. Moving differently in pain: a new theory to explain the  
449 adaptation to pain. *Pain*. 2011 Mar;152(3 Suppl):S90-8.
- 450 12. Hug F, Hodges PW, van den Hoorn W, Tucker K. Between-muscle differences in the  
451 adaptation to experimental pain. *J Appl Physiol (1985)*. 2014 Nov 15;117(10):1132-40.
- 452 13. Jonhagen S, Nemeth G, Eriksson E. Hamstring injuries in sprinters. The role of  
453 concentric and eccentric hamstring muscle strength and flexibility. *Am J Sports Med*. 1994  
454 Mar-Apr;22(2):262-6.
- 455 14. Kellis E, Galanis N, Natsis K, Kapetanios G. Validity of architectural properties of the  
456 hamstring muscles: correlation of ultrasound findings with cadaveric dissection. *J Biomech*.  
457 2009 Nov 13;42(15):2549-54.
- 458 15. Klimstra M, Dowling J, Durkin JL, MacDonald M. The effect of ultrasound probe  
459 orientation on muscle architecture measurement. *J Electromyogr Kinesiol*. 2007  
460 Aug;17(4):504-14.
- 461 16. Knezevic OM, Mirkov DM, Kadija M, Nedeljkovic A, Jaric S. Asymmetries in  
462 explosive strength following anterior cruciate ligament reconstruction. *The Knee*. 2014  
463 Jul;21(6):1039-45.
- 464 17. Konishi Y, Kinugasa R, Oda T, Tsukazaki S, Fukubayashi T. Relationship between  
465 muscle volume and muscle torque of the hamstrings after anterior cruciate ligament lesion.  
466 *Knee Surg Sports Traumatol Arthrosc*. 2012 Nov;20(11):2270-4.
- 467 18. Koulouris G, Connell DA, Brukner P, Schneider-Kolsky M. Magnetic resonance  
468 imaging parameters for assessing risk of recurrent hamstring injuries in elite athletes. *Am J*  
469 *Sports Med*. 2007 Sep;35(9):1500-6.
- 470 19. Kramer J, Nusca D, Fowler P, Webster-Bogaert S. Knee flexor and extensor strength  
471 during concentric and eccentric muscle actions after anterior cruciate ligament reconstruction

472 using the semitendinosus tendon and ligament augmentation device. *Am J Sports Med.* 1993  
473 Mar-Apr;21(2):285-91.

474 20. Lund JP, Donga R, Widmer CG, Stohler CS. The pain-adaptation model: a discussion  
475 of the relationship between chronic musculoskeletal pain and motor activity. *Can J Physiol*  
476 *Pharmacol.* 1991 May;69(5):683-94.

477 21. Makihara Y, Nishino A, Fukubayashi T, Kanamori A. Decrease of knee flexion  
478 torque in patients with ACL reconstruction: combined analysis of the architecture and  
479 function of the knee flexor muscles. *Knee Surg Sports Traumatol Arthrosc.* 2006  
480 Apr;14(4):310-7.

481 22. Morgan DL. New insights into the behavior of muscle during active lengthening.  
482 *Biophys J.* 1990;57(2):209-21.

483 23. Noorkoiv M, Nosaka K, Blazevich AJ. Neuromuscular adaptations associated with  
484 knee joint angle-specific force change. *Med Sci Sports Exerc.* 2014 Aug;46(8):1525-37.

485 24. Opar D, Williams M, Timmins R, Hickey J, Duhig S, Shield A. Eccentric hamstring  
486 strength and hamstring injury risk in Australian Footballers. *Med Sci Sports Exerc.*  
487 2015;47(4):857-65.

488 25. Opar DA, Piatkowski T, Williams MD, Shield AJ. A novel device using the nordic  
489 hamstring exercise to assess eccentric knee flexor strength: a reliability and retrospective  
490 injury study. *J Orthop Sports Phys Ther.* 2013 Sep;43(9):636-40.

491 26. Opar DA, Williams MD, Shield AJ. Hamstring strain injuries: factors that lead to  
492 injury and re-injury. *Sports Med.* 2012 Mar 1;42(3):209-26.

493 27. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Knee flexor strength and  
494 bicep femoris electromyographical activity is lower in previously strained hamstrings. *J*  
495 *Electromyogr Kinesiol.* 2013 Jun;23(3):696-703.

- 496 28. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Rate of torque and  
497 electromyographic development during anticipated eccentric contraction is lower in  
498 previously strained hamstrings. *Am J Sports Med.* 2013 Jan;41(1):116-25.
- 499 29. Orchard JW, Seward H, Orchard JJ. Results of 2 decades of injury surveillance and  
500 public release of data in the Australian Football League. *Am J Sports Med.* 2013  
501 Apr;41(4):734-41.
- 502 30. Prodromos CC, Han Y, Rogowski J, Joyce B, Shi K. A meta-analysis of the incidence  
503 of anterior cruciate ligament tears as a function of gender, sport, and a knee injury-reduction  
504 regimen. *Arthroscopy.* 2007 Dec;23(12):1320-5 e6.
- 505 31. Roig M, O'Brien K, Kirk G, Murray R, McKinnon P, Shadgan B, et al. The effects of  
506 eccentric versus concentric resistance training on muscle strength and mass in healthy adults:  
507 a systematic review with meta-analysis. *Br J Sports Med.* 2009 Aug;43(8):556-68.
- 508 32. Silder A, Heiderscheit BC, Thelen DG, Enright T, Tuite MJ. MR observations of  
509 long-term musculotendon remodeling following a hamstring strain injury. *Skeletal Radiol.*  
510 2008 Dec;37(12):1101-9.
- 511 33. Snow BJ, Wilcox JJ, Burks RT, Greis PE. Evaluation of muscle size and fatty  
512 infiltration with MRI nine to eleven years following hamstring harvest for ACL  
513 reconstruction. *J Bone Joint Surg Am.* 2012 Jul 18;94(14):1274-82.
- 514 34. Sole G, Milosavljevic S, Nicholson HD, Sullivan SJ. Selective strength loss and  
515 decreased muscle activity in hamstring injury. *J Orthop Sports Phys Ther.* 2011  
516 May;41(5):354-63.
- 517 35. St Clair Gibson A, Lambert MI, Durandt JJ, Scales N, Noakes TD. Quadriceps and  
518 hamstrings peak torque ratio changes in persons with chronic anterior cruciate ligament  
519 deficiency. *J Orthop Sports Phys Ther.* 2000 Jul;30(7):418-27.

- 520 36. Tengman E, Brax Olofsson L, Stensdotter AK, Nilsson KG, Hager CK. Anterior  
521 cruciate ligament injury after more than 20 years. II. Concentric and eccentric knee muscle  
522 strength. *Scand J Med Sci Sports*. 2014 Dec;24(6):e501-9.
- 523 37. Timmins R, Shield A, Williams M, Lorenzen C, Opar D. Biceps femoris long head  
524 architecture: a reliability and retrospective injury study. *Med Sci Sports Exerc*.  
525 2015;47(5):905-13.
- 526 38. Verrall GM, Slavotinek JP, Barnes PG, Fon GT, Spriggins AJ. Clinical risk factors for  
527 hamstring muscle strain injury: a prospective study with correlation of injury by magnetic  
528 resonance imaging. *Br J Sports Med*. 2001 Dec;35(6):435-9.
- 529
- 530



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<sub>lh</sub> 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<sub>lh</sub> 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.

547

548