

1 **Title:**

2 Effect of prior injury on changes to biceps femoris architecture across an AFL season.

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18 **Running title:**

19 Hamstring architecture changes in-season

20 **Key words:** architecture, hamstring, muscle injury, AFL.

21

22 **ABSTRACT**

23 **Purpose:** To assess in-season alterations of biceps femoris long head (BF<sub>lh</sub>) fascicle length in  
24 elite Australian footballers with and without a history of HSI.

25 **Methods:** Thirty elite Australian football players were recruited. Twelve had a history of  
26 unilateral HSI. Eighteen had no HSI history. All had their BF<sub>lh</sub> architecture assessed at  
27 approximately monthly intervals, six times across a competitive season.

28 **Results:** The previously injured limb's BF<sub>lh</sub> fascicles increased from the start of the season and  
29 peaked at week 5. Fascicle length gradually decreased until the end of the season, where they  
30 were shortest. The contralateral uninjured limb's fascicles were the longest when assessed at  
31 week 5 and showed a reduction in-season where weeks 17 and 23 were shorter than the first.  
32 Control group fascicles were longest at week 5 and reduced in-season. The previously injured  
33 limb's BF<sub>lh</sub> fascicles were shorter than the control group at all weeks and the contralateral  
34 uninjured limb at week 5. Compared to the control group, the contralateral uninjured limb had  
35 shorter fascicles from weeks 9 to 23.

36 **Conclusion:** Athletes with a history of HSI end the season with shorter fascicles than they start.  
37 Limbs without a history of HSI display similar BF<sub>lh</sub> fascicle lengths at the end of the season as  
38 they begin with. All athletes increase fascicle length at the beginning of the season however the  
39 extent of these differed based on history of HSI. These findings show that a HSI history may  
40 influence structural adaptation of the BF<sub>lh</sub> in-season.

41

42 **INTRODUCTION**

43 For more than 20 years hamstring strain injuries (HSIs) have been the leading cause of lost  
44 playing and training time in elite Australian football (26). Furthermore, HSIs commonly re-occur  
45 and typically result in a reduced level of performance following a return to competitive match  
46 play.(35) These injuries represent a significant financial burden for the athlete and/or their  
47 organisation(14). Given that a history of HSI has been consistently shown to increase the risk of  
48 future a HSI (11, 25), investigations involving previously injured individuals have attempted to  
49 determine if retrospective deficits in structure and/or function of the hamstrings contribute to the  
50 elevated risk of re-injury (7, 21-23, 27, 30).

51  
52 Recently, variations in biceps femoris long head (BF<sub>lh</sub>) architectural characteristics and their  
53 role in the aetiology of HSI have been brought to the attention of researchers and practitioners  
54 (30-33). Elite soccer players with shorter BF<sub>lh</sub> fascicles were reported to have a 4.1 fold  
55 increased risk of future HSI and this was amplified in those athletes with a history of HSI (32).  
56 These data, coupled with the finding that a previously injured BF<sub>lh</sub> consistently displays shorter  
57 fascicles than the uninjured contralateral limb (30), suggests that architectural characteristics of  
58 those with a history of HSI likely contribute to the elevated rate of re-injury.

59  
60 Providing interventions for athletes that present with shorter fascicles following ultrasonic  
61 examination would appear to be relatively straight forward. This is due to the increasing  
62 evidence that resistance exercise, particularly eccentric training targeting the hamstrings, can  
63 increase BF<sub>lh</sub> fascicle length (6, 33, 34). However those with a prior HSI might exhibit a  
64 reduced scope for positive adaptation as a result of a diminished capacity to activate the

65 previously injured muscle, per the inhibition hypothesis (7, 10, 22). This reduced ability to  
66 activate the previously injured muscle may also limit the extent of strain within the contractile  
67 tissue, which in turn may dampen the stimulus needed to increase fascicle length and eccentric  
68 strength (4, 13, 18). One study has examined the impact of a prior HSI on the adaptation of the  
69 hamstrings, reporting that elite Australian footballers with a HSI in the prior 12 months increased  
70 eccentric knee flexor strength to a lesser extent across a pre-season training period than  
71 individuals without a HSI(24). A restricted capacity to improve eccentric knee flexor strength is  
72 at least one mechanism through which prior HSI could increase the risk of future injury (20, 32).

73  
74 Despite the aforementioned findings, it remains unclear as to whether a history of HSI impacts  
75 upon the adaptive capacity of other risk factors, such as BFlh fascicle length, particularly during  
76 the in-season period. Recovery time and competition travel schedules can also limit when  
77 physical training can be implemented during a season. It is well established that physical  
78 performance variables tend to decline across the in-season period in elite Australian footballers  
79 (8). However, it remains to be seen if a specific pathological history might influence these  
80 changes. An improved understanding of the in-season changes in BFlh fascicle length, in  
81 previously injured and uninjured limbs, may inform on whether those with a history of HSI  
82 respond differently to the demands of a competitive season. Such data may have implications for  
83 the provision of risk mitigating interventions that are tailored to individuals based on their injury  
84 history. Therefore, the purpose of this study was to observe the in-season time course of changes  
85 to BFlh architecture in elite Australian footballers, with and without a history of HSI.

86

87 **METHODS**

88 **Participants**

89 **Participants**

90 **Paragraph**

91 In total, 30 elite male Australian footballers participated in this study. All participants provided  
92 written informed consent prior to collection of any data. For all athletes, team medical staff  
93 completed a retrospective injury questionnaire that detailed their history of hamstring,  
94 quadriceps, groin and calf strain injuries and chronic groin pain in the past 12 months, as well as  
95 the history of anterior cruciate ligament (ACL) injury at any stage throughout their career. This  
96 information was sourced from club medical records via the team doctor or physiotherapist. Of  
97 the 30 participants, 18 had no history of HSI or any other significant lower limb injury (including  
98 ACL) and formed the control group. Twelve athletes had suffered a unilateral BFlh strain injury  
99 in the prior 12 months and formed the previously injured group. Ethical approval for the study  
100 was granted by the Australian Catholic University Human Research Ethics Committee (approval  
101 number 2016-145E).

102 **Study design**

103 **Paragraph**

104 This observational, retrospective cohort study was completed during the 2016 Australian  
105 Football League season which consists of 23 weeks of competitive matches (March 2016 to  
106 August 2016). All participants had their BFlh architecture assessed via two-dimensional  
107 ultrasound (Figure 1) approximately once every month on six separate occasions throughout the  
108 in-season period, at a consistent time of day. These assessments occurred at weeks 1, 5, 9, 13, 17  
109 and 23 (final week of competitive games) of the in-season period. . .

## 110 **BFlh architecture assessment**

### 111 **Paragraph**

112 The protocol for the collection of BFlh muscle architecture has been described previously (29-  
113 33). Muscle thickness, pennation angle and fascicle length of the BFlh was determined from  
114 ultrasound images taken along the longitudinal axis of the muscle belly utilising a two  
115 dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE  
116 Healthcare Vivid-*i*, Wauwatosa, U.S.A). The scanning site was determined as the halfway point  
117 between the ischial tuberosity and the knee joint fold, along the line of the BFlh. All architectural  
118 assessments were performed with participants in a prone position, with the hip in neutral and the  
119 knee fully extended, following at least 5 minutes of inactivity. To gather ultrasound images, the  
120 linear array ultrasound probe, with a layer of conductive gel, was placed on the skin over the  
121 scanning site and aligned longitudinally and perpendicular to the posterior thigh. Care was taken  
122 to ensure minimal pressure was placed on the skin by the probe. Finally, the orientation of the  
123 probe was manipulated slightly by the assessor (RGT) if the superficial and intermediate  
124 aponeuroses were not parallel. Reliability of the assessor (RGT) has been previously reported for  
125 the assessment of BFlh architectural characteristics (intraclass correlations range from 0.93 to  
126 0.98 and typical error as a % coefficient of variation range from 2.1 to 3.4)(30). The assessor  
127 (RGT) has experience in the assessment of muscle architecture utilising two-dimensional  
128 ultrasound, specifically when assessing the BFlh (6, 30-33).

### 129 **Paragraph**

130 Once the images were collected, analysis was undertaken off-line (MicroDicom, Version 0.7.8,  
131 Bulgaria). For each image (Figure 1), fascicle length estimation was performed as described by  
132 Blazeovich and colleagues(5). Muscle thickness was defined as the distance between the

133 superficial and intermediate aponeuroses of the BFlh. A fascicle of interest was outlined and  
134 marked on the image and the angle at which it inserted onto the intermediate aponeurosis was  
135 determined as the pennation angle. The superficial and intermediate aponeurosis angles were  
136 determined as the angle between the line marked as the aponeurosis and an intersecting  
137 horizontal reference line across the captured image(5, 16). As the entire fascicle was not visible  
138 in probe's field of view, it was estimated via the following equation from Blazeovich and  
139 colleagues(5, 16):

$$140 \quad FL = \sin(AA + 90^\circ) \times MT / \sin(180^\circ - (AA + 180^\circ - PA)).$$

141 Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation  
142 angle. Fascicle length was reported in absolute terms (cm) from a single image and fascicle. The  
143 same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers  
144 (name, limb and group) during the collection and analysis of the images.

## 145 **Statistical analyses**

### 146 **Paragraph**

147 All data (including age, height and weight) were analysed using a custom spreadsheet which  
148 assessed the magnitude of difference across the season within groups as well as the extent of any  
149 between group differences in muscle architecture, at each time point (15). As there were no  
150 differences between limbs in the control group at all weeks, the two-limb averages were used for  
151 all comparisons. In order to reduce bias associated with non-uniformity of error, all data were  
152 log-transformed and effect sizes (Cohen's *d*) with  $\pm$  90% confidence interval (CI) were  
153 calculated. Effect sizes of  $\geq 0.2$ ,  $\geq 0.5$  and  $\geq 0.8$  were defined as small, moderate and large,  
154 respectively, with effect sizes of  $< 0.2$  deemed as trivial. Finally, any effects where the 90% CI

155 simultaneously overlapped the positive ( $\geq 0.2$ ) and negative ( $\leq -0.2$ ) thresholds of a small effect,  
156 were defined as being unclear(2).

157

## 158 **RESULTS**

### 159 **Power calculations**

160 Power analysis was undertaken *a priori* using G-Power(9). The analysis was based on  
161 anticipated differences in BFlh fascicle length between the injured and contralateral uninjured  
162 limbs, using a split plot ANOVA model. Effect size estimates were based on previous  
163 research(30) which reported an effect size of 1.34 when comparing BFlh fascicle length between  
164 injured and uninjured limbs. Therefore an effect size of 1.2 was deemed as a reasonable and  
165 conservative starting point for determining sample size. A calculated sample size of 10 per group  
166 was determined utilising the below parameters:

- 167 • Power ( $1 - \beta$  err probability) = 0.80
- 168 •  $\alpha = 0.05$
- 169 • Effect size = 1.2

### 170 **Participant details**

171 There were no clear differences between the two groups with respect to age (unclear effect;  $d =$   
172  $0.11 \pm 0.60$ ), height (unclear effect;  $d = 0.06 \pm 0.59$ ) and body mass (unclear effect;  $d = 0.26 \pm$   
173  $0.59$ ) (previously injured group age =  $22.9 \pm 2.6$  yrs, height =  $1.87 \pm 0.06$  m, body mass =  $86.0 \pm$   
174  $6.3$  kg; control group age =  $23.5 \pm 3.9$  yrs, height =  $1.88 \pm 0.10$  m, body mass =  $88.7 \pm 10.4$  kg).  
175 Percentage of total time on ground throughout the entire competitive season did not differ  
176 between the previously injured ( $80.6 \pm 3.7\%$ ) and the control group ( $79.8 \pm 5.4\%$ ; unclear effect;



177  $d = 0.17 \pm 0.58$ ). There were also no within group differences, across the season, in the  
178 percentage of total time on ground for either the previously injured (trivial effects;  $d$  range: 0.15  
179 to 0.17) or control groups (trivial effects:  $d$  range: 0.13 to 0.17).

180 Throughout the study, three participants suffered a HSI. Two of these were from the control  
181 group with one being from the previously injured group. The injuries for the control group  
182 participants occurred between weeks 13 and 17. As a result, these two participants were excluded  
183 from analysis at weeks 17 and 23. The previously injured participant's incident occurred after  
184 week 23 and was not removed from any analysis due to the injury occurring after the final  
185 assessment was completed.

## 186 **BF<sub>th</sub> architectural characteristics**

### 187 **Fascicle length**

#### 188 **Temporal changes across the in-season period**

##### 189 *Previously injured limbs*

190 Fascicle length in the previously injured limbs increased from week 1 to week 5 (small effect;  $d$   
191 =  $0.20 \pm 0.32$ ) and fascicles were longer at all time points when compared to week 23 (small to  
192 moderate effects;  $d$  range: 0.22 to 0.75; Table 1 and 2, Figure 2). Furthermore, fascicles were  
193 longer at weeks 5 and 9 compared to weeks 13 and 17 (small effect;  $d$  range = 0.22 to 0.31;  
194 Table 1 and 2, Figure 2)

##### 195 *Contralateral uninjured limbs*

196 Fascicle length was longest at week 5 compared to all other weeks (small to large effects;  $d$   
197 range = 0.40 to 0.89; Table 1 and 2, Figure 2). Furthermore, fascicle lengths were longer at  
198 weeks 1 and 9 compared to weeks 17 and 23 (small to moderate effects;  $d$  range = 0.35 to 0.50;

199 Table 1 and 2, Figure 2). Week 9 also displayed longer fascicles compared to week 13 (small  
200 effect;  $d = 0.21 \pm 0.19$ ; Table 1 and 2, Figure 2), whilst at week 13 fascicles were longer  
201 compared to week 23 (small effect;  $d = 0.22 \pm 0.17$ ; Table 1 and 2, Figure 2).

## 202 *Control group*

203 Longer fascicles were observed in the control group at weeks 5, 9 and 13 when compared to  
204 weeks 1, 17 and 23 (small to large effects;  $d$  range: 0.34 to 1.01; Table 1 and 2, Figure 2).  
205 Furthermore, fascicles were longer at week 5 compared to week 13 (small effect;  $d = 0.33 \pm$   
206  $0.23$ ; Table 1 and 2, Figure 2) and longer at week 17 compared to week 23 (small effect;  $d = 0.42$   
207  $\pm 0.26$ ; Table 1 and 2, Figure 2).

## 208 **Between group comparisons**

### 209 *Previously injured limbs compared to contralateral uninjured limb*

210 The previously injured limb displayed shorter fascicle lengths compared to the contralateral  
211 uninjured limb only at week 5 (moderate effect;  $d = -0.76 \pm 0.68$ ; Table 3).

### 212 *Previously injured limbs compared to control group*

213 Fascicle length of the previously injured limb was shorter than the control group at all time  
214 points (moderate to large effects;  $d$  range: -1.15 to -0.77; Table 3).

### 215 *Contralateral uninjured limb compared to control group*

216 The contralateral uninjured limb displayed shorter fascicles compared to the control group  
217 average at weeks 9, 13, 17, 23 (moderate to large effect;  $d$  range = -0.87 to -0.54; Table 3)

218 **Pennation angle**

219 **Temporal changes across the in-season period**

220 *Previously injured limbs*

221 Pennation angle in the previously injured limb was smaller at all weeks compared to week 23  
222 (moderate to large effects;  $d = -1.13$  to  $-0.60$ , Table 1). Pennation angle was also lesser at week 5  
223 compared to week 17 (small effect;  $d = 0.26 \pm 0.44$ , Table 1).

224 *Contralateral uninjured limb*

225 Pennation angle was less at week 5 compared to all other weeks (moderate to large effect;  $d$   
226 range =  $-1.61$  to  $-0.71$ , Table 1). In contrast, pennation angle was larger at week 23 compared to  
227 all other time points (small to large effects;  $d$  range =  $1.61$  to  $0.35$ , Table 1). Pennation angle was  
228 also lesser at week 1 compared to week 13 (small effect;  $d = 0.36 \pm 0.50$ , Table 1).

229 *Control group*

230 Pennation angle was greatest at weeks 1 and 23 when compared to all other weeks (small to large  
231 effects;  $d$  range =  $0.21$  to  $0.94$ , Table 1). Further, pennation angle was greater at weeks 13 and 17  
232 when compared to weeks 5 and 9 (small effects;  $d$  range =  $0.23$  to  $0.33$ , Table 1).

233 **Between group comparisons**

234 **Paragraph**

235 *Previously injured limbs compared to contralateral uninjured limb*

236 Pennation angle in the previously injured limb was larger compared to the contralateral uninjured  
237 limbs at weeks 5 and 23 (moderate to large effects;  $d$  range =  $0.61$  to  $1.04$ ; Table 3).

238 *Previously injured limbs compared to control group*

239 When compared to the control group, previously injured limbs had greater pennation angles at  
240 weeks 5, 9, 13 and 23 (moderate to large effects;  $d$  range =  $0.50$  to  $1.01$ ; Table 3).

241 *Contralateral uninjured limb compared to control group*

242 The contralateral uninjured limb's pennation angle was greater than the control group average at  
243 week 9 ( $d = 0.61 \pm 0.60$ ) and 13 ( $d = 0.46 \pm 0.62$ ).

244

245 **Muscle thickness**

246 **Temporal changes across the in-season period**

247 *Previously injured limbs*

248 Muscle thickness was greater at week 23 compared to week 1 (small effect;  $d = 0.26 \pm 0.45$ ,  
249 Table 1).

250 *Contralateral uninjured limb*

251 No small, moderate or large effects were detected for muscle thickness across all time points.

252 *Control group*

253 Muscle thickness was greater at week 5 ( $d = 0.29 \pm 0.19$ , Table 1) and week 13 ( $d = 0.20 \pm 0.13$ ,  
254 Table 1) compared to week 17.

255

256 **Between group comparisons**

257 *Previously injured limbs compared to contralateral uninjured limb*

258 No small, moderate or large effects were detected for muscle thickness between the previously  
259 injured and uninjured contralateral limbs.

260 *Previously injured limbs compared to control group*

261 Compared to the control group the previously injured limbs had decreased muscle thickness at  
262 weeks 1, 5 and 13 (moderate effect;  $d$  range -0.56 to -0.48; Table 3)

263 *Contralateral uninjured limb compared to control group*

264 No small, moderate or large effects were detected.

## 265 **DISCUSSION**

266 The main findings of this study were 1) those with a history of unilateral HSI end the in-season  
267 period with shorter BFlh fascicles compared to the start of the in-season period in both their  
268 previously injured and contralateral uninjured limb; 2) uninjured limbs display similar BFlh  
269 fascicle lengths at the start of the in-season period compared to the end of the in-season period;  
270 3) increases in BFlh fascicle length were observed early in-season across all athletes, however  
271 the magnitude of this increase differed based on history of HSI.

272

273 BFlh fascicle length has been identified as a modifiable risk factor for HSI (32), however, it was  
274 previously unclear as to how or if this parameter changed across a season in elite Australian  
275 footballers. In the current study all groups increased BFlh fascicle length during the early part of  
276 the in-season period, which then progressively shortened until the end of the competitive season.  
277 Of note, the increase was largest in the control group (moderate effect,  $d = 0.67 \pm 0.33$ ), followed  
278 by the contralateral uninjured limbs (small effect,  $d = 0.47 \pm 0.27$ ) and finally the previously  
279 injured limbs (small effect,  $d = 0.20 \pm 0.32$ ). This divergence in early in-season responses across  
280 groups appears to be a factor that ultimately results in both limbs from the previously injured  
281 athlete possessing shorter fascicles at the conclusion of the season compared to the start of the  
282 season. From weeks 5 to 23, the control group displays the largest decline in fascicle length  
283 (large effect,  $d = -1.01 \pm 0.31$ ), followed by the contralateral uninjured limbs (large effect,  $d = -$   
284  $0.89 \pm 0.35$ ) and then the previously injured limbs (moderate effect,  $d = -0.75 \pm 0.37$ ). These  
285 findings differ to work which has examined in-season alterations in vastus lateralis fascicle  
286 length, in softball and track and field (3, 19). In these studies, an initial decline in the first half of

287 the competitive season was counteracted by an increase at the end of the season(3, 19). However  
288 as the vastus lateralis acts in an anti-gravity nature, it is likely that the differing roles of the knee  
289 extensors and flexors contribute to these divergent findings, as would the differing demands  
290 between the sports examined.

291

292 The current data suggest that the early in-season period (i.e. within the first one to two months of  
293 the commencement of the season) may be an important time to continue to implement  
294 interventions to increase BFlh fascicle length, particularly in Australian footballers with a history  
295 of HSI. Simplistically, there is the possibility that this could be achieved with high-intensity,  
296 eccentric loading strategies that can elicit favourable adaptations within 2 weeks (33). However,  
297 there are likely a number of practical considerations that may limit or preclude such a strategy in  
298 elite sporting environments compared to those observed from lab-based studies in recreational  
299 athletes. These may include coach/athlete apprehension towards eccentrically induced muscle  
300 damage often reported in response to unaccustomed training(1) (which can be accentuated by the  
301 extent of the muscle strain undertaken during lengthening contractions (17)). Also a greater  
302 emphasis placed on recovery between matches at the expense of loading exposures (12, 28), as  
303 well as the presence or accumulation of other lower limb injuries that might not result in on-field  
304 time loss but do require modifications to resistance exercise prescription. Prior evidence has  
305 suggested that the de-training effect for BFlh fascicles following eccentric training interventions  
306 can occur in as little as four weeks(33), which would justify the need for constant application of  
307 an eccentric strength training stimulus, yet implementation appears to be challenging in practice  
308 (1).

309

310 It should be acknowledged that the current paper is limited as no architecture data was captured  
311 during the pre-season period, which spans November to February. It is certainly possible that the  
312 previously injured athletes increased fascicle length substantially during this period and future  
313 work should seek to explore this possibility. Nevertheless, across the entire in-season period, the  
314 previously injured hamstrings possessed shorter fascicles than the control group at all weeks  
315 (moderate to large effects throughout). These findings are likely to at least partly explain the high  
316 rates of HSI recurrence seen in Australian footballers (26). Therefore consideration should be  
317 given to what previously injured Australian footballers are capable of doing during their off-  
318 season program as a means of minimising any deficits at the commencement of the season. As  
319 exposure to high speed running can be minimised in the off-season, this may allow for the  
320 application of high-intensity strength training interventions targeted at increasing or at least  
321 minimising reductions in BFlh fascicle length, leading into the next pre- and in-season periods.

322

323 The current study indirectly infers the possibility that previously injured athletes/limbs are less  
324 capable of adapting positively to the rigours of in-season demands compared to those without a  
325 history of injury. Similar observational research has found that previously injured Australian  
326 footballers display less improvement in eccentric knee flexor strength across the pre-season  
327 compared to their uninjured counterparts(24). Such limited adaptation in previously injured  
328 athletes could be partly attributed to prolonged neuromuscular inhibition(10), which has been  
329 noted in previously injured athletes even after returning to pre-injured levels of competition(7,  
330 22, 23, 30). For example, a previously injured BFlh has been shown to be significantly less  
331 active than uninjured contralateral muscles during performance of the Nordic hamstring curl(7),  
332 which is an exercise commonly used in HSI rehabilitation(1). It is possible that this limited

333 activation may result in a reduced amount of strain within the tissue and limit the stimulus  
334 required to increase fascicle length (4, 13). However, from a mechanistic perspective, this  
335 phenomenon requires further investigation. No study has investigated whether individuals with  
336 and without a prior history of HSI respond differently to controlled interventions aimed at  
337 increasing eccentric strength and fascicle length. Should differences exist, further exploration as  
338 to whether inhibition manifests at the spinal or supraspinal level would be necessary to guide  
339 interventions targeted at restoring voluntary activation capacity after injury.

340

341 The authors acknowledge there are limitations in the current study. First, there are  
342 methodological limitations with the use of two-dimensional ultrasound to estimate BFlh fascicle  
343 length. As the fascicles which were measured are longer than the field of view which was  
344 utilised, the entire fascicle was not captured. Therefore, estimation was required to determine  
345 BFlh fascicle length. The estimation process used has been previously validated against  
346 cadaveric samples(5, 16). However, it must be recognised that there is still error associated with  
347 the determination of BFlh fascicle length (in this assessment typical error is approximately  
348 0.30cm). Secondly, there was no concurrent collection of match and training exposure, internal  
349 and external training load and resistance training programming variables. As several factors are  
350 likely modulators of fascicle length, examining the interaction between previous injury status  
351 and the aforementioned variables needs to be the focus of the next series of studies in this area.

352



353 **CONCLUSION**

354 **Paragraph**

355 Elite Australian footballers with a history of HSI display shorter BF<sub>lh</sub> fascicles at the completion  
356 of the season compared to the start, in both their injured and uninjured limbs. In contrast, athletes  
357 without a history of HSI finish the season with similar fascicle lengths to what they started with.  
358 Yet they do experience lengthening shortly after the commencement of the season which is then  
359 succeeded by a sustained period of shortening for the rest of the season. The impact of injury  
360 history on the structural and functional adaptations of the hamstrings requires further  
361 examination, as practitioners and clinicians search for novel strategies to mitigate the risk of  
362 recurrent HSI in their athletes.

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366 **CONFLICT OF INTEREST**

367 The authors wish to disclose that there were no conflicts of interest associated with professional  
368 relationships, that the study does not constitute endorsement by ACSM and that the results of the  
369 study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data  
370 manipulation.

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472 Figure 1

473 A two-dimensional ultrasound image of the biceps femoris long head. The image was along the  
474 longitudinal axis of the posterior thigh. From these images, it is possible to determine the  
475 superficial and intermediate aponeuroses, muscle thickness and angle of the fascicle in relation to  
476 the aponeurosis. Estimates of fascicle length can then be made via trigonometry using an  
477 equation validated against cadaveric tissue (5).

478 Figure 2

479 Fascicle length changes of the biceps femoris long head in previously hamstring strain injured  
480 limbs, the contralateral uninjured limb and two-limb average of the control group without a  
481 history of hamstring strain injury from elite Australian footballers. The weeks are each separated  
482 by ~28 days and all data were collected during the in-season period. Error bars represent  
483 standard deviations.

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