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Journal article

Differences in lower limb strength and structure after 12 weeks of resistance, endurance, and concurrent training

Timmins, Ryan G., Shamim, Baubak, Tofari, Paul J., Hickey, Jack T. and Camera, Donny M.

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2 Differences in lower limb strength & structure after 12-weeks of resistance, endurance & concurrent
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6 **Authors:**

7 Ryan G Timmins¹, Baubak Shamim², Paul J Tofari¹, Donny M Camera^{2,3}

8 ¹School of Behavioural and Health Sciences, Australian Catholic University, Melbourne, Australia

9 ²Exercise and Nutrition Research Program, Mary MacKillop Institute for Health Research, Australian
10 Catholic University, Melbourne, VIC, Australia

11 ³Department of Health and Medical Sciences, Swinburne University of Technology, Hawthorn,
12 Australia.

13 **Corresponding author:**

14 **Ryan G. Timmins**

15 School of Behavioural and Health Sciences, Australian Catholic University, 115 Victoria Parade,
16 Fitzroy, 3065, Melbourne, Victoria, Australia

17 Ryan.Timmins@acu.edu.au

18 Telephone: +61 3 9953 3772

19 Fax: +61 3 9953 3095

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28 **ABSTRACT**

29 **Purpose:** Investigate strength & structural adaptations after 12-weeks of resistance, endurance cycling
30 and concurrent training.

31 **Methods:** Thirty-two healthy males undertook 12-weeks of resistance-only (RT; n=10), endurance-
32 only (END; n=10) or concurrent resistance and endurance training (CONC; n=12). Biceps femoris long
33 head (BF_{lh}) architecture, strength (three-lift 1RM) and body composition were assessed.

34 **Results:** Fascicle length of the BF_{lh} reduced 15±6% (p<0.001) and 9±6% (p<0.001) in the END and
35 CONC group post-intervention, with no change in the RT group (-4±11%, p=0.476). All groups
36 increased BF_{lh} pennation angle (CONC: 18±9%, RT: 14±8%, END: 18±10%). Thickness of the BF_{lh}
37 increased post-intervention by 7±6% (p=0.002) and 7±7% (p=0.003) in the CONC and RT groups,
38 respectively, but not in the END group (0±3%, p=0.994). Both the CONC and RT group significantly
39 increased by 27±11% (p<0.001) and 33±12% (p<0.001) in three-lift totals following the intervention,
40 with no changes in the END cohort (6±6%, p=0.166). No significant differences were found for total
41 body (CONC: 4±2%, RT: 4±2%, END: 3±2%) and leg (CONC: 5±3%, RT: 6±3%, END: 5±3%) fat
42 free mass.

43 **Conclusions:** 12-weeks of resistance-only, endurance-only or concurrent resistance and endurance
44 training significantly modified BF_{lh} architecture. This study suggests that conventional resistance
45 training may dampen BF_{lh} fascicle shortening from cycling training whilst increasing strength
46 simultaneously in concurrent training. Furthermore, the inclusion of a cycle endurance training stimulus
47 may result in alterations to hamstring architecture that increase the risk of future injury. Therefore, the
48 incorporation of endurance cycling training within concurrent training paradigms should be re-
49 evaluated when trying to modulate injury risk.

50

51 **Key words:** fascicle length, cycling, muscle injury, hamstring.

52 INTRODUCTION

53 Hamstring strain injuries (HSIs) are the most prevalent, non-contact injury in sports which involve high
54 speed running, with upwards of 80% of these occurring within the biceps femoris long head (BFH)¹.
55 These injuries also present a significant financial burden for the athlete and their sporting organisation².
56 However, despite the significant research effort over the last decade, HSI incidence has not declined³.
57 It has recently been shown that elite soccer players with short BFH fascicles are four times more likely
58 to suffer a HSI in a competitive season than those with longer fascicles⁴. It was identified that for every
59 0.5cm increase in fascicle length, there was a concomitant reduction in HSI risk of 74%⁴. Therefore,
60 understanding fascicle length adaptations in response to various training interventions will have
61 implications for HSI prevention programmes.

62 The architectural characteristics of the BFH can be modified with various forms of resistance-training
63 interventions⁵. However, the magnitude and direction of the adaptations are strongly influenced by the
64 contraction mode utilised during the training period. For example, interventions utilising the Nordic
65 hamstring exercise, a stimulus which only consists of an eccentric contraction of the knee flexors (and
66 is non-isokinetic in nature), have been shown to significantly increase fascicle length of the BFH by
67 30%^{6,7}. Conversely, concentric only training on an isokinetic dynamometer significantly reduces BFH
68 fascicle length by ~14%⁸. However, conventional resistance-training programs undertaken in elite
69 sporting environments consist of exercises that have both an eccentric and concentric phase⁹. Despite
70 the prevalence of these exercises in practical settings, there is a limited amount of evidence showing
71 the BFH architectural adaptations following these interventions. Therefore, more research into the BFH
72 architectural adaptations following conventional resistance-training interventions is needed.

73 In team sports, athletes are typically exposed to a range of stimuli that compete to promote various
74 adaptations in an attempt to balance performance improvement whilst minimising injury risk. With
75 these athletes, the prescription of resistance-training interventions is rarely undertaken independent of
76 aerobic conditioning¹⁰. For example, stationary cycling is used as part of an athlete's combined
77 concurrent training regime^{9,10}. Moreover, many team-sports incorporate concurrent training programs

78 to maximise adaptations to strength, power and/or endurance for optimal performance. Whilst the BFlh
79 architectural adaptations following various resistance-training interventions have been investigated^{6,8}
80¹¹, it is unclear what effect undertaking concurrent resistance and endurance cycling training (which
81 has a low level of hamstring activity¹² and in-series strain¹³) may have on these alterations.

82 The primary aim of this study was to investigate the BFlh architectural, strength and body composition
83 adaptations after a 12-week intervention of either conventional resistance only, endurance only or
84 concurrent resistance and endurance training in young recreationally active males. It was hypothesised
85 that the existence of a conventional resistance-training stimulus (in the concurrent and resistance-only
86 groups) would promote a lengthening of BFlh fascicle length with reductions in pennation angle and
87 improvements in lower limb strength. Whereas, it was hypothesized that the endurance-only stimulus
88 would result in a shortening of BFlh fascicles, increases in pennation angle and no changes in lower
89 limb strength.

90 **METHODS**

91 **Participants**

92 Thirty-two recreationally active males (age 24.6±1.1 years; height 1.79±0.02 metres; body mass
93 77.1±2.10 kilograms) with no history of lower limb injury in the previous 12 months were recruited to
94 participate in this study. All participants provided informed consent before testing and training, which
95 was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical approval was
96 granted by the Australian Catholic University Human Research Ethics Committee. The trial was also
97 registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617001229369).

98 **Study design**

99 This study was part of a larger project from which separate data has already been published¹⁴. This
100 training intervention was conducted between June 2016 and October 2017. The study utilised a parallel
101 groups design with participants being stratified according to their total lean body mass (LBM).
102 Participants were then allocated to either resistance only (RT; n=10), endurance only (END; n=10) or
103 concurrent resistance and endurance training (CONC; n=12) for the 12-week intervention. For the
104 duration of the study, participants consumed a high protein diet of 2g/kg/day, which was confirmed

105 through weekly food logs submitted to the researchers¹⁴. Participants underwent pre-intervention BFlh
106 architectural and body composition (by whole-body dual-energy X-ray absorptiometry (DXA))
107 assessments as well as $VO_{2\text{peak}}$, leg press, knee extension and bench press 1-repetition maximum (1RM)
108 testing. Participants met with a dietitian fortnightly for consultation regarding protein and energy
109 intakes for the duration of the study. Muscle architecture was re-assessed after weeks 2, 4 and 8 of
110 training and at the end of the intervention. After week 6 of training, $VO_{2\text{peak}}$ (END and CONC only)
111 and 1RM (RT and CONC only) testing was undertaken to adjust training prescription for subsequent
112 weeks. At the end of the intervention participants had all their pre-measures reassessed within seven
113 days of the final training session. All testing and training sessions were supervised by a member of the
114 research team at the Australian Catholic University.

115 **Methodology**

116 **BFlh architecture assessment**

117 The methods used by the research group to assess BFlh architecture has been previously reported¹⁵.
118 Briefly, muscle thickness, pennation angle and fascicle length of the BFlh was determined from
119 ultrasound images taken along the longitudinal axis of the muscle belly (Figure 1) utilising a two-
120 dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE
121 Healthcare Vivid-i, Wauwatosa, U.S.A). The scanning site was determined as the halfway point
122 between the ischial tuberosity and the knee joint fold, along the line of the BFlh. All architectural
123 assessments were performed with the participant prone on a massage plinth, after 5 min of inactivity.
124 The orientation of the probe was manipulated by the assessor whose reliability has been previously
125 reported¹⁵. The intraclass correlations for all variables ranged between 0.96 to 0.97. The typical error
126 as a percentage of coefficient of variation for all variables is less than 3.4% (range 2.1 to 3.4%).

127 Once the images were collected (Figure 1), analysis was undertaken off-line (MicroDicom, Version
128 0.7.8, Bulgaria). Muscle thickness was defined as the distance between the superficial and intermediate
129 aponeuroses of the BFlh. Pennation angle was defined as the angle between the inferior aponeurosis
130 and a fascicle of interest. The aponeurosis angle for both aponeuroses was determined as the angle
131 between the line marked as the aponeurosis and an intersecting horizontal reference line across the

132 captured image^{16,17}. As the entire fascicle was not visible in the field of view of the probe, its length
133 was estimated via the following equation^{16,17}:

$$134 \quad FL = \sin(AA + 90^\circ) \times MT / \sin(180^\circ - (AA + 180^\circ - PA)).$$

135 Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle.
136 Fascicle length was reported in absolute terms (cm). The same assessor collected and analysed all scans
137 and was blinded to participant identifiers during the analysis. The extrapolation technique and equation
138 has been validated against cadaveric tissues and as such is considered a robust way of estimating fascicle
139 lengths.^{17,18}

140 **VO_{2peak} Testing**

141 The assessment of VO_{2 peak} was performed on a Lode cycle ergometer (Excalibur sport, Lode, The
142 Netherlands) using an incremental test to volitional fatigue as previously described in detail¹⁹.
143 Assessments of VO_{2 peak} were undertaken pre, after week 6 of training (END and CONC only) and at
144 the completion of the intervention. The maximum aerobic power (MAP)²⁰ from the pre-training and
145 week 6 tests were used to determine training loads for the endurance program for the END and CONC
146 groups.

147 **Strength Testing**

148 **Maximal strength assessment**

149 Maximal strength was assessed by determining each participants 1RM plate-loaded 45° incline leg
150 press, seated knee extension and bench press, as previously described¹⁴. Briefly, following warm-ups
151 of each exercise at submaximal intensities, participants undertook a progressive series of near-maximal
152 single repetition efforts. The 1RM for each exercise was determined as the heaviest weight that was
153 lifted through a full range of motion. Assessments were undertaken prior to the training study, after
154 week 6 of training (CONC and RT only) and at the completion of the intervention. The 1RM's from the
155 pre-training and week 6 assessments were used to determine training loads for the resistance program
156 following these sessions for both the CONC and RT groups. For all analysis, a sum of the 3 lifts was
157 used to determine training-induced changes.

158 **Body composition**

159 Total body and leg fat free mass were determined using DXA (GE Lunar iDXA Pro, GE Healthcare;
160 software: Encore 2009, version 16). Assessments were undertaken prior to, after 4 and 8 weeks of
161 training as well as following the training intervention using best practice guidelines (e.g., provided
162 standardised meals the evening before)²¹.

163 **Training intervention**

164 Participants in the RT and END groups performed training 3 d/wk, separated by at least 24 hours. The
165 CONC group undertook the same training programs as the other two groups, however these were spread
166 across 6 d/wk in an alternating pattern (e.g. Resistance training: Monday, Wednesday, Friday;
167 Endurance training: Tuesday, Thursday and Saturday). This was done with the intent to maximise the
168 likelihood for lower body strength improvements²² and to lengthen the time between sessions to
169 minimize any potential inference between the RT and END programs²³.

170 The resistance training intervention utilised a range of exercises with the intent of improving hip and
171 knee extensor strength (45° leg press, Romanian deadlift, seated knee extensions, barbell step back
172 lunge, barbell hip thruster and stiff legged deadlift). Training volume was modified by manipulating
173 the number of sets, repetitions and relative intensity of each exercise to provide progressive overload
174 throughout the 12-week intervention. All training efforts of leg press or knee extension were performed
175 at a relative intensity between 60 to 97.5% of 1RM. For example, leg press was progressed from
176 participants doing 5 sets of 10-15 repetitions at 70% of 1RM in the first week of training, to 2 sets of 2
177 repetitions at 97.5% of 1RM in the final week. Additional exercises focussing on the hip and knee
178 extensors (Romanian deadlift, barbell step back lunge, barbell hip thruster and stiff legged deadlift)
179 were performed at an intensity eliciting an RPE of ~9-10 by the last set. The progression was similar to
180 the leg press, with participants undertaking 4 sets of 10 repetitions in the first week of training to doing
181 3 sets of 5 repetitions in the final week. All exercises consisted of a 3-minute between-set rest period
182 and were completed using conventional training methods, where the weight lifted in the concentric
183 phase is then lowered in the eccentric without any additional overload. Finally, all coaching cues were

184 provided with the intent to control the velocity of the weight/barbell throughout the range of motion as
185 best as possible.

186 The endurance training intervention was undertaken on a Lode cycle ergometer and was made up of a
187 range of simulations including undulating hill efforts (25-110% MAP), moderate-intensity continuous
188 training (50% MAP), moderate-intensity (70% MAP) and high-intensity interval training (100% MAP).
189 The moderate and high-intensity intervals were separated by recovery periods ranging between 20-60
190 seconds at 40% MAP. Training volume was modified by manipulating the number of intervals as well
191 as the relative intensity to provide progressive overload across the 12-week intervention.

192 Training volume and intensity were periodised throughout the 12-weeks to provide progressive
193 overload with the intent to improve lower body strength. Financial incentives were provided to
194 encourage participant compliance and effort during training with those having the largest pre- to post-
195 intervention increases in 1RM (CONC and RT) and $VO_{2\text{ peak}}$ (CONC and END) receiving these. All
196 training programs have previously been published and are available online¹⁴ and from the
197 supplementary documents 1 and 2.

198 **Statistical analyses**

199 All statistical analyses were performed using JMP version 11.0.0 (SAS Institute Inc., Cary, NC, USA).
200 Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and
201 homoscedasticity using Levene's test. Repeated measures linear mixed models fitted with the restricted
202 maximum likelihood (REML) method were used to assess changes in BFlh architecture, total body and
203 leg fat free mass, as well as the three-lift total across the duration of the study. For each measure, factors
204 were group (CONC, END or RT) and time (Pre, Weeks 2, 8, 12), with participant as the random factor.
205 For BFlh architecture and leg fat free mass, the left and right limbs were averaged, as they did not differ
206 at any time point. Additionally, all between group comparisons for all measures at baseline showed no
207 differences. Where significant main or interaction effects were detected, *post hoc* t tests with Tukey's
208 HSD were applied to determine where any differences occurred. Significance was set at $p < 0.05$ and
209 where possible Cohen's d^{24} was reported for the effect size of the comparisons, with the levels of effect

210 being deemed small ($d=0.20$), medium ($d=0.50$) or large ($d=0.80$). All data were expressed as mean \pm
211 SD.

212 **Sample Size**

213 Power analysis was undertaken *a priori* using G-Power²⁵. The analysis was based on the estimated
214 changes in BFlh fascicle length following the training intervention in the resistance training group.
215 Effect size estimates were determined using previous research looking at interventional changes in BFlh
216 fascicle length⁸. In this study, fascicle length changes had an effect size of 2.6. Therefore, in the name
217 of being conservative, an effect size of 1.3 was deemed a reasonable starting point. Power was set at
218 80%, with an alpha of 0.05 returning a calculated sample of 9 per group.

219 **RESULTS**

220 **Participant details**

221 The three groups were similar with respect to age, height and body mass at baseline (CONC age
222 26 \pm 4yrs, height 1.77 \pm 0.07m, body mass 76.4 \pm 10.2kg; RT age 24 \pm 6yrs, height 1.82 \pm 0.08m, body mass
223 75.5 \pm 10.3kg; END age 24 \pm 5yrs, height 1.79 \pm 0.07m, body mass 79.5 \pm 9.3kg). All groups increased body
224 mass after the intervention period (CONC: 3.9%, $p<0.001$, $d=0.39$; RT: 4.3%, $p<0.001$, $d=0.42$; END:
225 2.7%. $p=0.011$, $d=0.24$). Training compliance for strength training was 100% in both RT and CONC,
226 while compliance for cycling training was 99.4% and 99.5% in END and CONC, respectively.

227 **BFlh architectural characteristics**

228 A summary of the BFlh architectural alterations during this intervention can be found in Figure 2.

229 **Fascicle length**

230 A significant group x time interaction effect was found for fascicle length ($p=0.010$). *Post hoc* analyses
231 showed that fascicle length was significantly shorter after the training period, when compared to pre-
232 intervention measures, in both the CONC and END group but not the RT cohort (CONC: difference -
233 0.95cm, 95%CI -1.58 to -0.32cm; $p<0.001$, $d=-1.51$; END: difference -1.48cm, 95%CI -2.17 to -
234 0.79cm; $p<0.001$, $d=-1.87$; RT: difference -0.49cm, 95%CI -1.18 to 0.20cm; $p=0.476$, $d= -0.71$). The
235 fascicle lengths of the CONC and END group were also significantly shorter than pre-training values

236 after week 8 (CONC: difference -0.80cm, 95%CI -1.43 to -0.17cm; p=0.003, $d = -1.22$; END: difference
237 -1.08cm, 95%CI -1.77 to -0.39cm; p<0.001, $d = -1.34$). All other fascicle length comparisons to pre-
238 training values were not significant for all groups.

239 **Muscle thickness**

240 A significant group x time interaction effect was found for muscle thickness (p=0.013). *Post hoc*
241 analyses showed that muscle thickness was significantly increased after the training period, compared
242 to pre-intervention measures, in both the CONC and RT groups but not in the END cohort (CONC:
243 difference 0.16cm, 95%CI 0.03 to 0.28cm; p=0.002, $d = 0.62$; RT: difference 0.16cm, 95%CI 0.03 to
244 0.29cm; p=0.003, $d = 0.65$; END: difference -0.01cm, 95%CI -0.13 to 0.12cm; p=0.994, $d = -0.03$; Figure
245 2). In the CONC group, significant increases in muscle thickness were also found at week 4 (difference
246 0.16cm, 95%CI 0.03 to 0.28cm; p=0.001; $d = 0.58$) and week 8 (difference 0.20cm, 95%CI 0.08 to
247 0.32cm; p<0.001; $d = 0.76$) when compared to pre-intervention values. All other muscle thickness
248 comparisons to pre-training values were not significant for all groups.

249 **Pennation angle**

250 A significant group x time interaction effect was observed for pennation angle (p=0.015). *Post hoc*
251 analyses showed that pennation angle was significantly increased after the training period, compared to
252 pre-intervention, in all three groups (CONC: difference 2.74°, 95%CI 1.57 to 3.90; p<0.001; $d = 1.59$;
253 RT: difference 1.90°, 95% CI 0.51 to 3.30; p<0.001; $d = 1.17$; END: difference 2.65°, 95% CI 1.27 to
254 4.02; p<0.001; $d = 1.52$; Figure 2). Pennation angle was also significantly greater than pre-training values
255 after week 8 in all three groups (CONC: difference 2.62°, 95%CI 1.47 to 3.80; p<0.001; $d = 1.52$; RT:
256 difference 1.67°, 95% CI 0.27 to 3.06; p=0.020; $d = 1.03$; END: difference 2.30°, 95% CI 0.92 to 3.66;
257 p=0.002; $d = 1.31$). Whereas after 4 weeks of training, pennation angle was significantly greater than
258 pre-training values in only the CONC and END groups (CONC: difference 1.77°, 95%CI 0.62 to 2.94;
259 p=0.003; $d = 1.03$; END: difference 1.50°, 95% CI 0.13 to 2.87; p=0.033; $d = 0.85$) but not in the RT
260 cohort (RT: difference 1.35°, 95%CI -0.04 to 2.75; p=0.056; $d = 0.83$). All other pennation angle
261 comparisons to pre-training values were not significant for all groups.

262 **Strength testing**

263 **Three-lift total**

264 A significant group x time interaction effect was found for the three-lift total ($p < 0.001$). *Post hoc*
265 analyses showed that the three-lift total was significantly increased after the training period in both the
266 CONC and RT groups, but not in the END cohort (CONC: difference 109kg, 95%CI 82 to 137kg;
267 $p < 0.001$, $d = 1.00$; RT: difference 129kg, 95%CI 99 to 159kg; $p < 0.001$, $d = 1.31$; END: difference 25kg,
268 95%CI -6 to 57kg; $p = 0.166$, $d = 0.46$; Table 1).

269 **Body composition**

270 **Total body fat free mass**

271 A significant main effect for time ($p < 0.001$), but not group ($p = 0.901$) was found for total body fat free
272 mass. There was no significant group x time interaction ($p = 0.386$). All effect size comparisons showed
273 small to moderate effects for increases in total body fat free mass across the study (d range = 0.18 to
274 0.50; Table 1).

275 **Leg fat free mass**

276 A significant main effect for time ($p < 0.001$), but not group ($p = 0.995$) was found for leg fat free mass.
277 There was no significant group x time interaction ($p = 0.589$). All effect size comparisons showed small
278 to moderate effects for increases in leg fat free mass across the study (d range = 0.24 to 0.50; Table 1).

279 **DISCUSSION**

280 This study is the first to investigate the effects of conventional resistance-only, endurance cycling-only
281 and concurrent endurance and resistance training on BFlh architecture. The novel findings of this study
282 are: 1) 12-weeks of cycling only endurance training and concurrent endurance and resistance training
283 significantly increases pennation angle and shortens BFlh fascicles; 2) conventional resistance-only
284 training caused no changes in BFlh fascicle length yet significantly increases pennation angle; and 3)
285 concurrent training and resistance-only training can promote increases in BFlh muscle thickness.

286 It has been proposed that increasing BFlh fascicle length following eccentric training interventions may
287 be one of the beneficial adaptations that reduces the risk of a future HSI occurring²⁶. Prospectively, elite
288 soccer players who had short BFlh fascicles (< 10.56 cm – with longer fascicles being ≥ 10.56 cm) at the

289 start of pre-season were four times more likely to suffer a HSI in the subsequent season than those who
290 possessed longer fascicles⁴. In these athletes, for every 0.5cm increase in fascicle length, there was a
291 subsequent 74% reduction in HSI risk. While having longer fascicles is associated with a reduced risk
292 of injury, it is unknown if modifying BFlh fascicle lengths through training interventions specifically
293 alters the likelihood of future injury. In the current study, the participants in the concurrent and
294 endurance training groups reduced their fascicle lengths by 0.96cm and 1.48cm respectively, following
295 the intervention. Combining these findings with the prospective evidence presented, it is possible that
296 sustained periods of endurance cycling training, without any eccentric training stimuli, may have a
297 deleterious effect for HSI risk. Further to this, the level of eccentric stimuli provided by conventional
298 barbell training was not enough to offset the shortening effect that endurance cycling had on muscle
299 fascicle length. Therefore, if cycling training is part of an athletic performance program, in order to
300 address HSI prevention practices, practitioners should ensure the intensity of the eccentric resistance
301 training stimulus is sufficient²⁷.

302 This study provides further evidence that BFlh fascicle lengths respond differently following training
303 with dissimilar contractions modes. Concentric-only leg curl²⁸ and isokinetic dynamometry training⁸
304 have resulted in shortening of BFlh fascicles, whereas eccentric-only interventions have been an
305 effective stimulus for lengthening^{6,8,27}. The assumed addition of sarcomeres in-series after eccentric
306 training interventions is expected to be the mechanism for increasing fascicle length¹³. Interventions
307 using the *vastus intermedius* of rats support this assumption with significant reductions in the number
308 of in-series sarcomeres after undertaking uphill running training¹³. Comparably interventions utilising
309 downhill running (eccentric in nature) resulted in a significant increase in the number of in-series
310 sarcomeres¹³. As a result, Lynn and colleagues (1998) suggest that the large amount of in-series strain
311 experienced during eccentric contractions is a potent stimulus for increasing the number of sarcomeres
312 in-series¹³. Therefore the reduction of this stimulus (e.g during concentric-only efforts) may result in
313 the shedding of sarcomeres in-series¹³. In the current study, the endurance group was exposed to a
314 cycling-only stimulus for 12-weeks, where the hamstrings are mainly active concentrically, with

315 minimal eccentric activity^{12,29}. As such, the significant shortening of the BFlh fascicles may have been
316 driven by a reduced amount of in-series strain and the resultant shedding of sarcomeres in-series.

317 The concurrent training group was exposed to the same amount of cycling as the endurance cohort, and
318 the same resistance stimuli as the resistance-only group. Despite this, the concurrent group still saw a
319 significant shortening of BFlh fascicle length. This may be a result of the resistance training stimuli
320 only being comprised of conventional movements, where the limiting factor is the weight lifted in the
321 concentric phase. Therefore, all exercises were underloaded during the eccentric portion and as such
322 may have less in-series strain than those efforts with an overloaded lowering phase. As this in-series
323 strain from eccentric-only/overloaded interventions^{6,8}, or higher training volumes of conventional
324 exercises at long muscle length⁷ are needed to promote significant fascicle lengthening in the BFlh, it
325 is possible the concurrent training participants were not exposed to a potent enough eccentric stimulus
326 to counteract the shortening stimuli imposed by the cycling intervention. This may also partially explain
327 the lack of increases in BFlh fascicle length seen in the resistance training group, who did not have the
328 competing stimuli from the cycling intervention yet may have still lacked enough in-series strain to
329 promote fascicular lengthening. As a result, the resistance training participants did not see a change in
330 fascicle length. Additionally, the concurrent group (-0.96cm) saw a lesser reduction in fascicle length
331 than the endurance-only cohort (-1.48cm). It is conceivable that the conventional resistance training
332 intervention may have dampened some of the shortening stimuli provided by the cycling protocol, yet
333 it was not enough to overcome the gross shortening. Future research would benefit from investigating
334 whether a concurrent training program incorporating resistance exercise with more eccentric loading
335 would prevent fascicle length shortening induced by endurance cycling and potentially promote some
336 lengthening above baseline.

337 Another theory which may explain the shortening of fascicles after significant endurance training comes
338 from the evidence that distance runners possess shorter vastus lateralis and gastrocnemius fascicles than
339 sprinters³⁰. Abe and colleagues (2000) proposed that the large eccentric stimulus that sprinters
340 experience through their training may contribute to the adaptation of longer fascicles in the lower limb.
341 The authors also propose that it may have been a genetic pre-disposition where those with longer

342 fascicles were able to produce a greater amount of force during higher shortening velocities, and, as
343 such, became better sprinters than those with shorter fascicles³¹. However, another concept presented
344 by Abe and colleagues (2000) was that chronic endurance training may become more efficient with
345 lesser sarcomeres in-series³⁰. This theory centres around the proposition that having less sarcomeres in-
346 series would require less ATP to be used per shortening cycle. As an endurance athlete this may be a
347 beneficial training adaptation that allows them to be more efficient than those with a greater number of
348 sarcomeres in-series who may use more ATP with each step. Therefore, in the current study the
349 fascicular shortening could have been a beneficial adaptation in the endurance and concurrent training
350 cohorts which reduced the amount of ATP used per cycle stroke and as a result made them more efficient
351 during their training.

352 Following a period of training, changes in pennation angle have been suggested as a mechanism that
353 strongly influences changes in lower limb strength³². For example, Aagaard and colleagues (2001)
354 undertook 14 weeks of heavy-resistance training of the lower limbs, assessing maximal isometric knee
355 flexor torque and vastus lateralis pennation angle before and after the intervention. Following the
356 training program, there was a 16% increase in *vastus lateralis* pennation angle, which was strongly
357 associated ($r = 0.62$) with a 14% improvement in isometric knee extensor torque. The authors concluded
358 that the architectural arrangement of pennate muscles in the human body are strongly associated with
359 their function (represented by strength). In the current study, increases in BFlh pennation angle, muscle
360 thickness and the strength were seen following the concurrent and resistance training-only
361 interventions. Whilst this study was the first to determine the changes in BFlh architectural
362 characteristics in response to divergent training stimuli, it was not theoretically plausible to run a
363 correlational analysis of these changes against the improvements in the three-lift total. This is because
364 the BFlh, whilst being involved in the action of hip extension and subsequently the assessment of leg
365 press strength, was not isolated in a measure of single joint strength (e.g. maximal knee flexion).
366 However, when considered together with the findings of Aagaard and colleagues (2001), it is likely that
367 increase in BFlh pennation angle observed in the present study contributed to increases in maximal

368 lower body strength. Future studies are needed to directly assess the extent to which structural changes
369 in the hamstring muscles contribute to knee flexor strength through single- and multi-joint movements.

370 The results of the current study suggest that cycling endurance training may lead to significant BFlh
371 architectural alterations across a 12-week period. However, the time course of these changes (Figure 2)
372 may allow practitioners a ‘window of opportunity’ should they still wish to implement a cycling
373 stimulus as part of their program. Whilst there is still shortening within the first 8 weeks in both the
374 concurrent and endurance training groups, the lack of significance creates a small buffer period where,
375 as is commonplace in sporting programs^{9,10}, practitioners can prescribe ‘off-feet’ training on a bike to
376 stimulate improvements in cardiovascular performance. However, should this stimulus continue, there
377 is the possibility that fascicular shortening progresses, thus practitioners will need to begin
378 supplementing this with significant eccentric hamstring work.

379 The effect of nutrition on BFlh fascicle length is also unknown. While we were the first study to control
380 for and implemented a ‘high’ protein diet (2g/kg/day) throughout the 12-week intervention period to
381 maximise muscle remodelling processes, we did not compare this dietary intervention to a control
382 protein diet group to specifically ascertain if protein intake can positively or negatively alter BFlh
383 fascicle length. However it may not be feasible to tease out the impact that nutritional interventions
384 have on muscle architectural adaptations due to the malleability of these structural characteristics to the
385 various forms of interventions as well as periods of detraining^{6,8,27}. Therefore, controlling for protein
386 intake is the first and possibility most reasonable step in this process.

387 There are limitations in this study which should be considered. Firstly, the measure of fascicle length is
388 an estimation made from a validated equation. This is due to the small transducer field of view being
389 unable to capture an entire BFlh fascicle. However, whilst the results are still an estimation, the
390 methodology and equation employed has been validated against cadaveric samples and shows excellent
391 agreement between dissection and estimation methods^{17,18}. Secondly, we did not compare different
392 forms of endurance training (such as running). Whilst not the primary form of concurrent training within
393 sporting environments, cycling interventions still have a strong place in performance programs and are

394 often utilised for ‘off-feet’ sessions or as a cardiovascular stimulus during rehabilitation⁹. Finally, the
395 concurrent training intervention was undertaken on alternating days, which is different to elite,
396 professional sporting environments who may undertake two/ multiple sessions/day. However, the
397 requirement to have participants train twice a day (morning and afternoon) to mimic those elite
398 environments would have been too extensive a request for volunteers to undertake.

399 **PRACTICAL APPLICATIONS**

400 Possessing short BFlh fascicles has been associated with an increased risk of future hamstring injury.
401 Therefore, interventions which modify fascicle length are of interest to practitioners. However, these
402 interventions are typically investigated in isolation and without concurrent endurance training, which is
403 a common feature of elite sporting programs. This study investigated the effect of conventional
404 resistance, endurance and concurrent training on hamstring structural adaptations. A significant
405 shortening in BFlh fascicle length was evidenced following endurance-only and concurrent training
406 interventions, with no change after resistance-only training. These findings suggest that the inclusion
407 of a cycle endurance training stimulus may result in alterations to hamstring architecture that increase
408 the risk of future injury. Therefore, the incorporation of endurance cycling training within concurrent
409 training paradigms should be re-evaluated when trying to modulate injury risk.

410 **CONCLUSION**

411 In conclusion, this is the first study to investigate the effect of conventional resistance, endurance and
412 concurrent training on hamstring structural adaptations and strength. Following a 12-week progressive
413 overload training program, we observed a significant shortening in BFlh fascicle length following
414 endurance-only and concurrent training interventions, with no change after resistance-only training.
415 Further, we reported significant improvements in muscle thickness, pennation angle and maximal
416 strength following resistance-only and concurrent training interventions. These findings suggest that
417 the inclusion of a cycle endurance training stimulus may result in alterations to hamstring architecture
418 that increase the risk of future injury; thus, incorporation of cycling as a stimulus within concurrent
419 training paradigms should be re-evaluated.

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432

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- 517

518

519 **FIGURE LEGENDS**

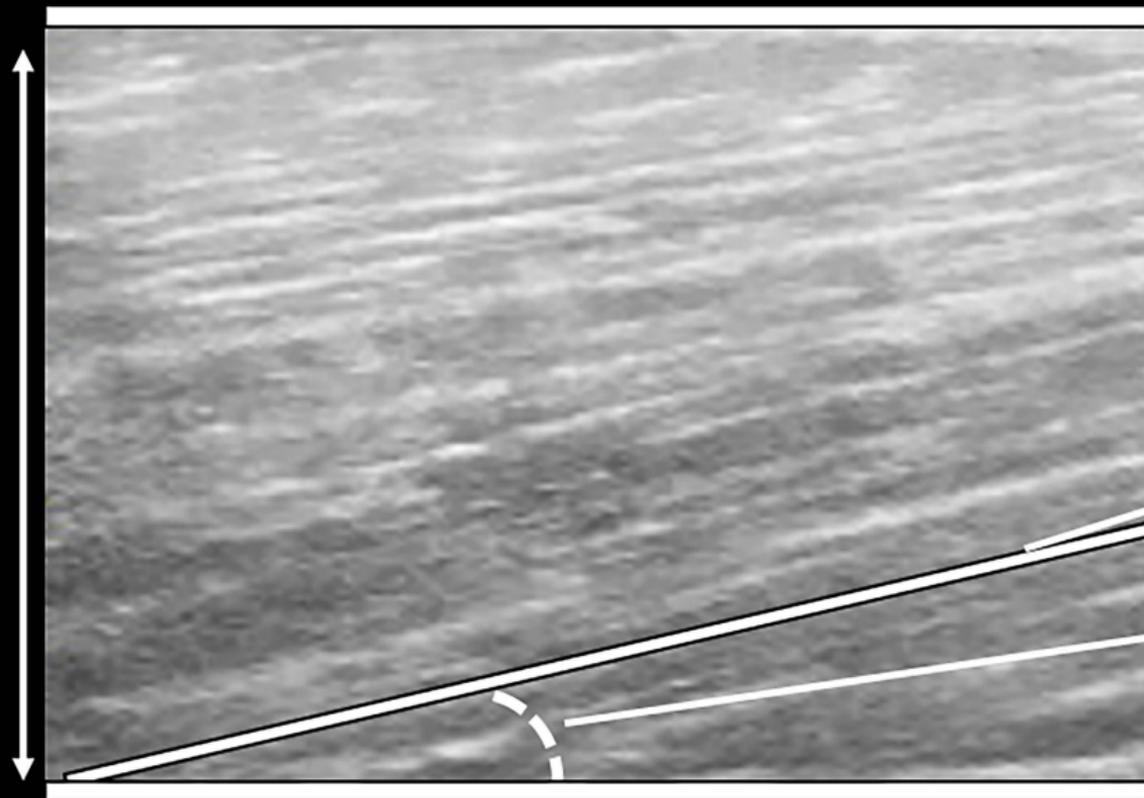
520 Figure 1: A two dimensional ultrasound image of the biceps femoris long head. This image of
521 the biceps femoris long head was taken along the longitudinal axis of the posterior thigh. From
522 these images it is possible to determine the superficial and intermediate aponeuroses, muscle
523 thickness,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

525 Figure 2: The change in biceps femoris muscle thickness (A), pennation angle (B) and fascicle length
526 (C) following 12-weeks of either resistance, endurance and concurrent training. ** = $p < 0.001$ compared
527 to baseline, * = $p < 0.05$ compared to baseline. CONC = concurrent training group, RT = resistance
528 training group, END = endurance training group, Δ = change compared to baseline.

529

530

Superficial aponeurosis



Muscle fascicle

Pennation angle

Intermediate aponeurosis

Muscle thickness

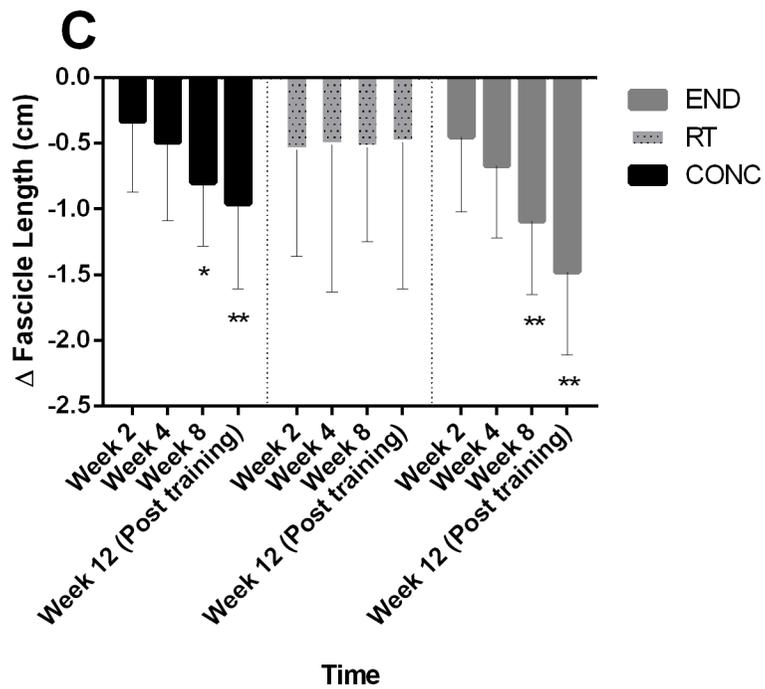
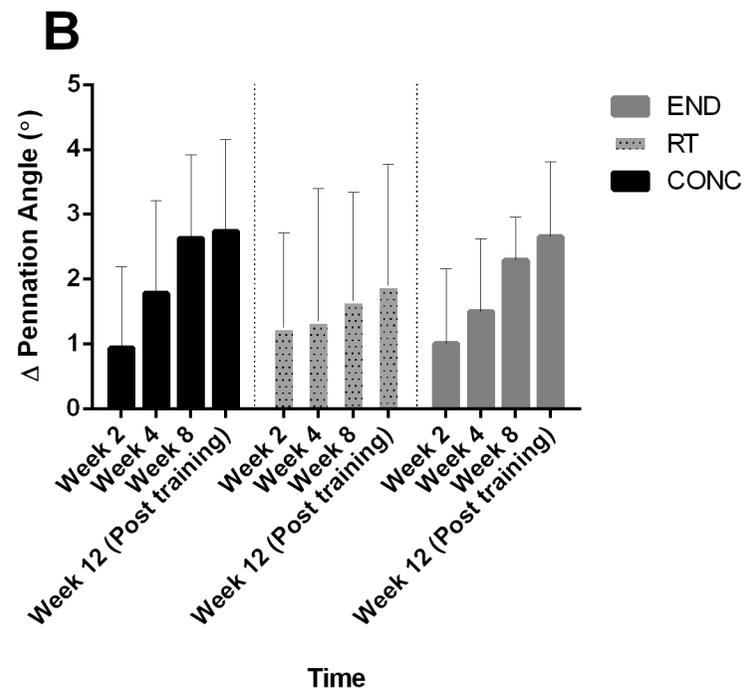
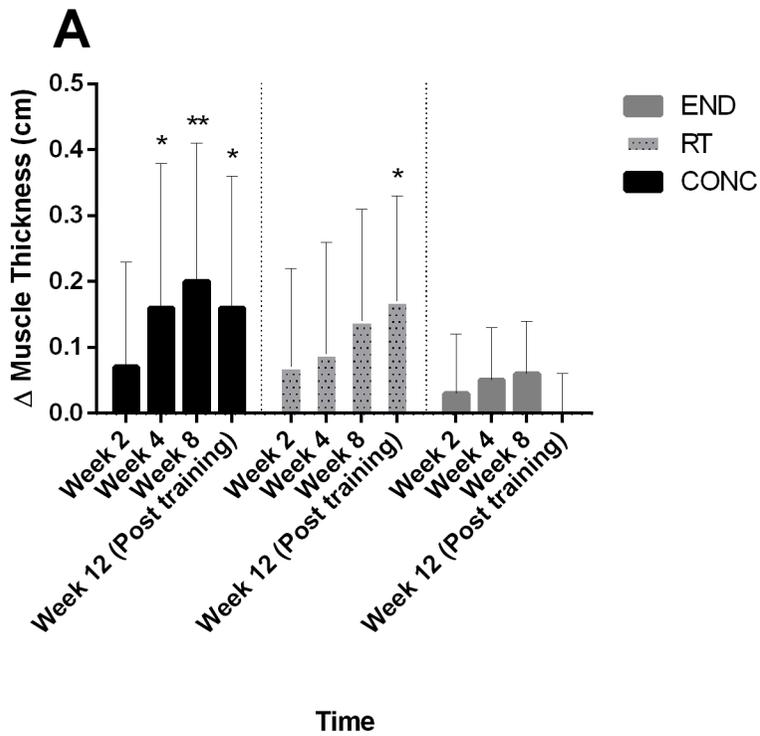


Table 1. The effect of conventional resistance, endurance and concurrent training on body composition and strength

	Resistance-only group			Endurance-only group			Concurrent group		
	Total FFM (kg)	Leg FFM (kg)	3-lift Total (kg)	Total FFM (kg)	Leg FFM (kg)	3-lift Total (kg)	Total FFM (kg)	Leg FFM (kg)	3-lift Total (kg)
Baseline	62.8	21.9	419	62.2	22.1	433	61.6	21.9	441
	±6.94	±2.47	±99	±5.77	±2.40	±58	±6.65	±2.96	±112
Week 4	64.1	22.7		63.2	22.9		62.9	22.7	
	±6.70	±2.40		±6.10	±2.60		±6.80	±2.96	
Week 8	64.8	22.8		63.3	22.7		63.8	23.0	
	±6.80	±2.40		±5.53	±2.38		±7.00	±2.95	
Week 12 (Post training)	65.1	23.1	548**	63.8	23.2	458	63.8	23.1	550**
	±6.84	±2.30	±99	±5.34	±2.50	±53	±6.76	±2.88	±105

Data is presented as mean ± standard deviation. **p<0.001 vs baseline, * p<0.05 vs baseline, FFM = fat free mass