

Architectural, functional, and molecular responses to concentric and eccentric loading in human skeletal muscle

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Authors Contribution

Conceived and designed the experiments: MVN PJA NDR MF. Performed the experiments: MVF NDR WKM. Analysed the data: MVF AS RMBV. Contributed reagents/materials/analysis tool: MVN PJA MF JW. Wrote the paper: MVF MVN PJA NDR MF.

Running head: Muscular responses to concentric vs. eccentric training in young men

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1 **ABSTRACT**

2

3 **Aim:** We investigated architectural, functional, and molecular responses of human
4 skeletal muscle to concentric (CON) or eccentric (ECC) resistance training (RT).

5 **Methods:** Twelve young males performed 10 weeks of concentric (CON) or eccentric
6 (ECC) resistance training (RT) (n = 6 CON, 6 ECC). An additional 14 males were recruited
7 to evaluate acute muscle fascicle behaviour and molecular signalling in biopsies
8 collected from vastus lateralis (VL) after 30 min of single bouts of CON or ECC exercise.
9 VL volume was measured by magnetic resonance imaging. Muscle architecture (fascicle
10 length, Lf; pennation angle, PA) was evaluated by ultrasonography. Muscle remodelling
11 signals to CON or ECC loading (MAPK/AKT-mammalian target of rapamycin (mTOR)
12 signalling) and inflammatory pathway (TNF α /Murf-1-MAFbx) were evaluated by
13 immunoblotting.

14 **Results:** Despite the ~1.2 fold greater load of the ECC group, similar increases in muscle
15 volume (+8% CON and +6% ECC) and in maximal voluntary isometric contraction (+9 %
16 CON and +11 % ECC) were found after RT. However, increases in Lf were greater after
17 ECC than CON (+12 vs. +5%) while increases in PA were greater in CON than ECC (+30
18 vs. +5%). Distinct architectural adaptations were associated with preferential growth in
19 the distal regions of VL for ECC (+ECC +8% vs. +CON +2) and mid-belly for CON (ECC +7
20 vs. CON +11%). While MAPK activation (p38MAPK, ERK1/2, p90RSK) was specific to
21 ECC, neither mode affected AKT-mTOR or inflammatory signalling 30 min after exercise.

22 **Conclusion:** Muscle growth with CON and ECC RT occurs with different morphological
23 adaptations reflecting distinct fibre fascicle behaviour and molecular responses.

24

25 **Introduction**

26 Skeletal muscles can contract by shortening (concentric) or lengthening (eccentric)
27 (Joyce et al. 1969; Joyce & Rack 1969). “Conventional” resistance exercise training, using
28 commercial exercise machines is the most common form of resistance-exercise,
29 consisting of lifting and lowering a constant external load. Thus, conventional resistance
30 exercise training combines CON (lifting-phase) and ECC (lowering-phase) actions.
31 According to the force-velocity (F-V) relationship, each value of force and velocity on a
32 given curve should belong to the same level of neural activation (Bigland & Lippold,
33 1954; Camilleri & Hull, 2005; Chow & Darling, 1999). Yet, this requirement is not met by
34 conventional RT as the same external load is displaced during both lifting and lowering
35 phases. Thus, motor units must be de-recruited in the ECC part to enable the load to be
36 lowered (Reeves et al. 2009); as such the load used for conventional training is limited
37 by the CON muscle action. Therefore, to ensure that the ECC component of resistance
38 training is not under-loaded, it would be necessary that both shortening and lengthening
39 phases follow the physiological force-velocity curve i.e., the absolute load should be
40 greater for the ECC than the CON contraction (Katz 1939), theoretically involving the
41 same level of neural activation between contraction modes. Nonetheless, to our
42 knowledge comparisons of pure CON to ECC exercise with such matching to equalize the
43 relative loading stimulus, meeting a fundamental premise of the F-V relation, have not
44 yet been made.

45

46 A recent investigation (Reeves et al. 2009) provided evidence that distinct loading
47 patterns also lead to distinct architectural adaptations to exercise training, as suggested
48 by Hortobágyi *et al.* (1996). In this previous study (Reeves et al., 2009), the architectural
49 responses to muscle loading in older-aged individuals undergoing conventional vs. ECC

50 only training regimes were compared. After 14-wk of training the authors noted a
51 greater increase in muscle fibre (fascicle; Lf) length in the ECC only group compared to
52 the conventional RT group. Conversely increases in pennation angle (PA) were only
53 evident following conventional RT, but not after ECC only exercise. Furthermore, since
54 conventional RT involves mixtures of both CON and ECC contractions, the architectural
55 responses to “pure” ECC or CON contractions performed on standard isotonic machines
56 with such matching for relative loading stimulus are unknown.

57

58 Distinct architectural adaptations to ECC vs. CON contractions also raise the question as
59 to what could be the molecular basis of this phenomenon. Since both human and pre-
60 clinical work has provided evidence of distinct molecular responses to e.g. CON vs. ECC
61 contractions, it is likely that similar mechanisms underlie the different architectural
62 adaptations. This hypothesis seems supported by the recent observation that ECC vs.
63 CON growth of cardiomyocytes is regulated via ERK1/2 MAPK signalling (Kehat et al.
64 2011), demonstrating that acute signalling differences in response ECC vs. CON exercise
65 could underlie the ensuing distinct architectural adaptations. In another investigation,
66 using isolated rat muscle Wretman et al. (Wretman et al. 2001) reported greater
67 increases in phosphorylation of ERK 1/2 and p38 MAPKs induced by ECC vs. CON
68 contractions. In addition Martineau et al. (Martineau & Gardiner 2001), observed that
69 activation of MAPKs activation was quantitatively related to muscular tension with ECC
70 contraction providing the greater stimulus. Finally, microarray analyses in young men
71 (Kostek et al. 2007) demonstrated distinct responses to *acute* CON vs. ECC contractions,
72 suggesting that contraction-specific muscle remodelling results both from distinct
73 signalling and genomic responses to CON vs. ECC exercise. Nonetheless, the
74 relationships between MAPK (or other) signals and that of the distinct architectural

75 basis of skeletal muscle hypertrophy in response to CON vs. ECC exercise, remains
76 unknown.

77 Therefore, the aim of the present study was to compare the effects of pure CON vs. ECC
78 exercise training in terms of architecture, morphology and functional outcomes, and
79 relate this to muscle cell signalling responses potentially ascribing the distinct structural
80 and functional adaptations to CON vs. ECC training. The hypothesis put forward was
81 that different mechanical stimulus (shortening vs. lengthening), chronically applied and
82 matched to balance the relative loading inducement, would result in distinct adaptations
83 in muscle morphology, function and architecture: possible underlying mechanical and
84 biochemical mechanisms may be involved in these distinct remodelling processes.

85

86 **METHODS**

87 We recruited 12 young men (25 ± 3 y, height = 182 ± 8.5 cm, mass = 71.9 ± 8.5 Kg; means \pm
88 SD) not partaking in resistance exercise training to undergo a 10-week resistance
89 exercise-training program. Based on their maximum isometric knee extension torque,
90 they were divided (matched for baseline strength) into two training groups: EG (ECC,
91 $n=6$, 25 ± 3 y) or CG (CON, $n=6$, 25 ± 3 y). Resistance exercise training was carried out with
92 a leg-press machine (Technogym, Gambettola Italy) modified to enable performance of
93 either an ECC only (EG) or CON only (CG) contractions. This was achieved using an
94 electric engine attached to the back of the leg-press (Fig 1): in the EG, the chair was
95 pulled back with a cable that connected the electric winch to the weight stack via a steel
96 cable, ensuring that subjects did not exert any force with their quadriceps to perform
97 what would otherwise have been the concentric component of the exercise. When the
98 chair was released, it enabled the subject to lower the training load under control

99 through an ECC contraction of the quadriceps. Conversely the CG performed a CON-only
100 movement consisting of lifting the load. In this case the engine operated only during
101 lowering of the load, ensuring that subjects did not exert any force with their quadriceps
102 to perform what would have otherwise been the eccentric component of the exercise.
103 The timing of the contraction was slightly different for the two groups: the CG were
104 asked to complete the contraction in ~ 2 s, whereas this time period was ~ 3 s for the EG.
105 This time difference (~ 2 s CG vs ~ 3 s EG) was necessary to ensure that the load was
106 indeed lowered under control in the EG. The training period for the first study was
107 performed, after a familiarisation session, three times per week for 10-wk and people
108 trained both legs but unilaterally. Both training and acute exercise bouts on the leg press
109 machine involved the main extensor muscles of the lower limbs. The training load used
110 was of 80% of the concentric (CG) or 80% of the eccentric (EG) 1RM, with 4 series of a
111 minimum of 8 to a maximum of 10 repetitions with one-minute rest in between the sets.
112 The 1RM was assessed unilaterally after a warm-up program performed on the leg press
113 machine using a very light weight that allowed the subject to easily perform 8 to 10
114 repetitions (concentrically or eccentrically: lifting or lowering phase, respectively).
115 Then, the protocol followed for both contraction phases was the one suggested by
116 Baechle & Earle (1994). This study was approved by the ethical committee of the health
117 care science faculty of the Manchester Metropolitan University and conformed to the
118 requirements of the Declaration of Helsinki. Volunteers were informed of the purpose of
119 the study, the experimental design and procedures involved and all the potential risks
120 involved before giving their written consent

121 *Measurement of electromyographic (EMG) activity*

122 VL integrated EMG was measured as representative of the knee extensors to provide an
123 indication of neural drive to this muscle group during the tests performed on the Cybex
124 dynamometer. Two surface electrodes (10 mm diameter) were placed next to each other
125 on the lower third of the VL muscle with a 20 mm centre-to-centre electrode distance.
126 These two electrodes were arranged in a “bi-polar” configuration with a third electrode,
127 the “ground”, placed on a bone area (the patella bone in this case). The skin was shaved
128 and conditioned using a special skin preparation gel (Nuprep™) to reduce skin
129 impedance (using an electrode impedance tester - Oxford medical ltd, Medilog, UK)
130 below 5,000 Ohms. In order to reproduce the same electrode positioning in the
131 successive recording sessions, measurements were taken and anatomical spots (bone
132 processes, tendon and muscle insertions) were used to know exactly the right portion of
133 VL for the surface electrodes to be placed. Acquisition of the surface EMG signal was
134 obtained through the Biopac A/D acquisition system at a sampling frequency of 2000 Hz
135 –and filtered through a bandwidth of 10-500 Hz. The root mean square (RMS) was
136 calculated from the raw EMG over a 200 ms time frame where the peak of torque was
137 expressed during the isometric MVC trials. During the 1RM assessment, EMG was
138 monitored in order to support our assumption that CON and ECC 1RMs would have
139 resulted in similar neural drive: the RMS was calculated over a 200 ms time frame
140 during the mid-portion of the contraction phase.

141

142 *Magnetic Resonance images (MRI)*

143

144 Axial plane scans of the thigh were taken before (1 week) and post-training (4-5 days)
145 using a 0.25 Tesla magnetic resonance imaging (MRI) scanner (Esaote G-scan, Italy). A
146 T1-weighted Spin Echo protocol was used (repetition time 900 ms, echo time, 26 ms,
147 number of excitation 2, Field of View 200x200 mm, slice thickness 10 mm, gap between

148 slices, 1.0 mm). Participants were asked to lie supine on the MRI bed and to insert their
149 leg into a circular coil. Due to the scanning area of the coil, the thigh was imaged in 3-4
150 separate sections. Markers were placed on the thigh from the patella to the hip to denote
151 different sections and avoid overlap. Axial plane scans along the entire length of the VL
152 were collected; on average, the number of axial scans obtained in each subject was the
153 same for the baseline and post-training periods (~34). From these scans the contours of
154 the VL muscle of each MRI scan were digitized using the Osirix image analysis software
155 and, subsequently, VL Muscle Volume was calculated as follows:

156

157 $\text{Volume}_{\text{VL}} (\text{cm}^3) = \sum \text{ACSA} \cdot (\text{slice thickness} + \text{gap between slices}).$

158

159 Regional VL hypertrophy was calculated after training by obtaining the baseline and
160 post-exercise average values of the first 5 axial scans where the VL muscle was visible
161 starting from the hip/knee joint (proximal and distal portions respectively) and the 5
162 scans around the peak of ACSA (muscle mid portion): from these mean values, the
163 percentage increase in ACSA was calculated for the 3 different regions of the VL muscle.

164

165 *Muscle (VL) Architecture*

166 Before (1 week) and after training (4-5 days), VL muscle architecture i.e., Lf and PA were
167 measured (by the same investigator) from images obtained *in vivo* at rest using B-mode
168 ultrasonography (Mylab 70, Esaote Biomedica, Italy), with a 100 mm, 10-15 MHz, linear-
169 array probe. Resting ultrasound images were taken at a specific joint angle (150°),
170 corresponding almost to full knee extension (180°), while the participant was seated on
171 the Cybex Norm dynamometer chair; the transducer was aligned in the fascicle plane in

172 order to be able to visualize an optimal portion of fascicles on the ultrasound screen.
173 The muscle architectural parameters were quantified from the ultrasound scans using
174 the image analysis software, ImageJ 1.42q (National Institutes of Health, USA). The
175 visible portion of the fascicle length was directly measured using this software. In some
176 instances, a small portion of the fascicle extended off the ultrasound window and it was
177 necessary to estimate this non-visible portion using a linear extrapolation of fibres and
178 aponeuroses (Erskine et al. 2009). Pennation angle was measured as the intersection
179 between fascicles and the deep tendon aponeurosis (Fig 2). The reliability of these
180 ultrasound techniques has been published (Intra Class Correlation value = 0.99)(Reeves
181 et al. 2004) ; images were collected and digitally analysed by the same operator.

182 *Muscle function*

183 Participants were familiarized with all the devices and procedures involved in the study
184 before the actual test sessions: the exercise-training participants were asked to perform
185 contractions in a seated position on the reclining chair of the Cybex Norm dynamometer
186 (hip angle = 85°, hip angle at supine position = 0°). The lower leg was strapped to a pad
187 situated at the end of the Cybex lever arm and the knee joint center of rotation was
188 aligned with the dynamometer fulcrum. The torque produced on the Cybex
189 dynamometer was sampled into an analogue to digital acquisition system (Biopac
190 System, Inc. California) at a frequency of 200 Hz and displayed on the screen of an Apple
191 computer (Mac. G4). Maximum isometric torque of the knee extensor muscle group was
192 evaluated by participants performing an isometric maximum voluntary contraction
193 (MVC) at every 10° (0.175 rad) from 90° to 150° (from 1.57 to 2.62 rad) of knee joint
194 angle (180° = full extension). Two MVCs were recorded at each joint angle with 2 min

195 separating each contraction and the highest torque produced was used to assess MVC
196 changes from pre to post training.

197 *Acute behavioural and molecular responses to CON and ECC contraction*

198 An additional untrained 14 men (25 ± 4 y, height = 184 ± 7 cm, mass = 74 ± 4 Kg) were
199 recruited and divided into two groups (CG acute, $n=7$, 26 ± 4 y and EG acute, $n=7$, 25 ± 4 y)
200 to perform a single bout of ECC or CON exercise, adopting the same design of the
201 training study (same load-repetitions-sets combination). Vastus lateralis (VL) muscle
202 biopsies were collected in these additional volunteers before and 30 min after exercise
203 for signalling purpose: this time was specifically chosen as MAPK activation appears to
204 be transient (Nader & Esser 2001). Ultrasound scans were also acquired during a single
205 CON or ECC contraction performed on the leg-press device. Measures of fascicle length
206 and pennation angle were recorded from screen captures during contractions and
207 analysed in an identical fashion to in the training study.

208 *Immunoblotting*

209 Post exercise biopsies were processed in a similar fashion to as previously described
210 (Atherton et al, 2010). Briefly, ~ 20 mg of muscle was snipped in ice-cold buffer (50 mM
211 Tris-HCl (pH 7.4), 50 mM NaF, 10 mM β -Glycerophosphate disodium salt, 1 mM EDTA, 1
212 mM EGTA, 1 mM activated Na_3VO_4 (all Sigma-Aldrich, Poole, UK)) and a complete
213 protease inhibitor cocktail tablet (Roche, West Sussex, UK) at $10 \mu\text{l}\cdot\mu\text{g}^{-1}$ of tissue.
214 Homogenates were rotated for 10 min and the supernatant collected by centrifugation
215 at $13,000 \times g$ for 5 min at 4°C . The supernatant (sarcoplasmic fraction) was used for
216 immunoblot analysis: protein concentrations were determined using a NanoDrop
217 ND1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE-US) and
218 adjusted to $1\mu\text{g}\cdot\mu\text{l}^{-1}$ in $3\times$ laemmli. Each sample was loaded onto pre-cast 12% Bis-Tris

219 Criterion XT gels (BioRad, Hemel Hempstead, UK) at 15 µg per lane and separated
220 electrophoretically at 200 V for 1 h. Proteins were then wet-transferred at 100 V for 1 h
221 onto polyvinylidene difluoride (PVDF) membranes (0.22 µm), blocked for 1 hour in
222 2.5% skimmed milk in 1× Tris-buffered saline/ Tween-20 (TBS-T), and then incubated
223 in 1° antibodies (1:2000 dilution in 2.5% BSA in TBS-T) rocking overnight at 4°C. For
224 phosphorylation of MAPK p38 (Ser189/207), p90RSK (Thr359/Ser363), ERK1/2
225 (Thr202/Tyr204), p70S6K (Thr389), Akt (Ser473), p65 (Ser536) and pan-actin
226 antibodies were obtained from Cell Signaling Technology, Inc. (MA, US), 4E-BP1
227 (Ser65/Thr70) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA-US) and MAFbx,
228 Murf-1 (C-terminal region) from ECM Bioscience (KY, US). For total amount of TNFα,
229 p65 and IκBα antibodies were obtained from Cell Signaling Technology, Inc. (MA, US)
230 The next day, membranes were washed 3×5 min in TBS-T, incubated in HRP-conjugated
231 2° antibody (New England Biolabs, Hertfordshire, UK; 1:2000 in 2.5% BSA in TBS-T) at
232 room temperature for 1 h, before 3×5 min washes in TBS-T. Membranes were exposed
233 to chemiluminescent HRP Substrate (Millipore Corporation, Billerica, MA-US) for 5 min
234 and bands quantified by Chemidoc XRS (BioRad, Hertfordshire, UK). Software measures
235 were taken to prevent pixel saturation; loading anomalies were corrected to Pan-Actin.

236

237 *Statistical analysis*

238 Differences for group (CG vs. EG, the training groups and CG1 vs. EG1, the acute study
239 groups) and time (baseline vs. post-training / baseline vs. post-exercise) were analyzed
240 using a two-way factorial analysis of variance test using GraphPad PRISM software
241 (version 5.0d; GraphPad software Inc. San Diego, CA). Significant interactions between
242 groups and time were located by Bonferroni post-hoc test. The delta (Δ) training values

243 (percentage increases) were statistically tested between groups using an independent t-
244 test, that was also used to compare baseline differences between CG and EG for
245 physiological parameters. A power calculation was performed: our current sample size
246 has a beta level of 0.8 (i.e., power of 80%) for the training study (12 participants) using
247 the parameter of pennation angle and a beta level of 0.9 (i.e., power of 90%) for the
248 acute study (14 participants) using the parameter of p38 MAPK.

249 **RESULTS**

250 *EMG of CON-ECC 1-RM, maximum lifting or lowering ability (1-RM) and training load*

251 As mentioned, to test our assumption that both CON and ECC contractions belonged to
252 the same Force-Velocity curve, EMG activity was measured during performance of a
253 single concentric and eccentric 1-RM in each subject to evaluate if the two phases
254 correspond to a similar level of neural activation. The means of baseline EMG values for
255 CON and ECC group 1-RM are presented in Table 1. These values represent the mean of
256 the individual rectified EMG activity measured during the entire CON or ECC 1RM and
257 were collected just prior to the training period. In support of our assumption, no
258 significant difference existed in neural activation during the performance of the CON or
259 ECC only exercise. As expected, regarding the maximum lifting or lowering ability data,
260 the baseline and post-training 1-RM was higher in the ECC than the CON group (Table
261 1), resulting in a higher ECC training load and consequently higher training volume,
262 (132592 vs. 105120 Kg, $P < 0.01$, calculated as number of sets X number of repetitions X
263 training load for ECC compared to CON exercise in the 10-wk period). The pre-to-post
264 training increase in 1-RM was statistically significant in both groups, but with no
265 significant difference in the percentage increase between the ECC and CON group.

266

267 *Muscle morphology and architecture and maximum voluntary contraction*

268 After training, both groups showed an increase in VL muscle volume but the change was
269 similar between the EG ($6 \pm 0.4\%$, mean \pm SEM, $P < 0.0001$) and CG ($8 \pm 0.5\%$, $P <$
270 0.0001). However, Lf increased significantly more ($P < 0.01$) in the EG ($12 \pm 2\%$, $P <$
271 0.0001) compared to the CG ($5 \pm 1\%$, $P < 0.01$); conversely PA increased significantly
272 less ($P < 0.01$) in the EG ($5 \pm 1\%$, $P > 0.05$) than the CG ($30 \pm 0.5\%$, $P < 0.0001$) group.
273 Maximum voluntary contraction (MVC) peak amplitude changed in both groups
274 similarly (significant pre-to-post difference, $P < 0.05$) (EG = $11 \pm 8\%$, $P < 0.05$, CG = $9 \pm$
275 6% increase, $P < 0.05$; Figure 3).

276 *Regional hypertrophy of VL muscle in response CON or ECC training*

277 Differences in localized hypertrophy were observed in response to 10-wk of either CON
278 or ECC resistance exercise (Figure 4). While both loading modalities induced similar
279 effects on ACSA % increase/decrease in the proximal area (EG = $-1 \pm 1\%$, mean \pm SEM,
280 and CG = $-0.5 \pm 1\%$) a significant difference was found in both mid portion (EG = $7 \pm 1\%$,
281 and CG = $11 \pm 1\%$, $P < 0.01$) and distal part of vastus lateralis (EG = $+8 \pm 2\%$ versus CG =
282 $+2 \pm 1.5\%$, $P < 0.05$) between the two types of training.

283 *Architectural behaviour of VL muscle during performance of CON vs. ECC contractions*

284 Following discovery of such distinct architectural adaptations, we recruited a second
285 cohort to interrogate possible mechanical reasons for these findings, with the aim of
286 determining fascicle behaviour during CON and ECC contractions. Differential behaviour
287 was observed in Lf and PA during CON and ECC resistance exercise performed with leg-
288 press (Figure 5). During ECC, fibres lengthened during performance of ECC exercise (Lf =

289 +19 ± 2%, mean ± SEM, from start to end of contraction, P < 0.0001) whereas during
290 CON there was a substantial fascicle shortening in Lf (Lf = -19 ± 2%, P < 0.0001).
291 Similarly, while PA remained similar from the start to the end of ECC (PA = -3 ± 1%, P >
292 0.05), it showed a substantial increase during CON (PA = +28 ± 1%, P < 0.0001).

293 *Acute MAPK, AKT-mTOR and Inflammatory/breakdown signaling responses to a single* 294 *acute bout of CON vs. ECC exercise*

295 By taking biopsies 30 min following this single bout of CON or ECC we were also able to
296 interrogate intramuscular signalling purported to be involved in exercise adaptations.
297 Significant increases in phosphorylation of mitogen activated protein kinases (MAPKs)
298 i.e., p-38MAPK, ERK1/2 and p90RSK (Figure 6) were found 30 min after ECC resistance
299 exercise (p38MAPK = 20 ± 4-fold, ERK1/2 = 2 ± 0.3-fold, p90RSK = 3 ± 1-fold) but not
300 after CON resistance exercise. In contrast, there was no modulation in the
301 phosphorylation of Akt (Ser473) and mammalian target of rapamycin (mTOR) substrate
302 p70S6K 30 min after CON or ECC exercise, although a significant suppression (P < 0.05)
303 in activation of 4-EBP1 was found only after CON exercise (Figure 7). Non-significant
304 changes in the activation of the TNF α /Murf-1-MAFbx pathway (TNF α , p-p65, p65, I κ B α ,
305 p-MurF-1, p-MAFbx) were found 30 min after CON or ECC exercise.

306

307 **DISCUSSION**

308 In the present study we compared, for the first time, the structural remodelling of
309 human skeletal muscle in response to pure CON and ECC loading while attempting to
310 link these with the molecular signalling pathways implicated in muscle remodelling
311 (MAPKs, mTOR etc). Furthermore, whilst previous investigations focused on

312 morphological and architectural responses to CON and ECC resistance training matched
313 for work-load (Blazevich et al. 2007; Higbie et al. 1996; Moore et al. 2012), no study has
314 yet, to the best of our knowledge, matched the CON and ECC phases for the same relative
315 load while monitoring neural drive in order to meet one of the fundamental
316 requirements of the F-V relationship (Bigland & Lippold 1954; Camilleri & Hull, 2005;
317 Chow & Darling, 1999). Hence, our training loads were matched to the same percentage
318 of the CON and ECC repetition maximum (i.e., CON 1RM and ECC 1RM) and EMG values
319 revealed similar levels of neural activation for both CON and ECC 1-RM (Table 1). The
320 ECC group/CON group training load ratio remained between the 1.21 to 1.29 range
321 (Table 1), this confirms previous observations of the greater forces associated with ECC
322 than CON *in vivo* (Aagaard et al. 2000; Westing et al. 1988). These findings support our
323 contention that both shortening and lengthening phases of our resistance exercise
324 paradigms belonged to the same F-V curve. Although 1RM assessment is a sort of an
325 “unrefined” method, the fact remains that 1RM is still recognised as ‘gold standard’ in
326 training studies. Furthermore, the best available technique to assess neural drive *in vivo*
327 is still EMG. In this investigation, integrated EMG (i.e. result of recruitment and rate
328 coding) was similar in the two different conditions. The authors would like to emphasise
329 that in the present study the load was not matched for neural activation but rather, EMG
330 was recorded in relatively equal external loads and similar EMG values were found.

331

332 Muscle hypertrophy *per se*, was an expected consequence of our resistance training
333 protocols. However, muscle volume showed similar changes after both training modes
334 (ECC = +6 and CON = + 8%, non significant difference between groups). Although both
335 concentric and eccentric exercise programs have shown to induce gains in muscle mass
336 there seems to be insufficient evidence of the superiority of either these two types of

337 contraction (Wernbom et al. 2007). Nevertheless, the similar changes in muscle volume
338 in the present study were considered unexpected, as not only ECC training load was
339 higher, but also because ECC training has been suggested to produce greater
340 hypertrophy and strength than CON training (increase in muscle fibre size, Hortobágyi
341 et al. 1996) and associated trends towards whole muscle greater CSA (Roig et al. 2009).
342 If the predominant promoter of muscle hypertrophy were the mechanical stimulus, one
343 would expect to find a greater hypertrophy in the ECC group due to the higher training
344 load (i.e. higher mechanical stimulus). However, this was not the case, indicating that the
345 intensity of mechanical stimulus may not be the sole determinant for muscle
346 hypertrophy; rather, this might be governed by the type of contraction performed
347 (ECC/CON) and also that other factors blunting muscle hypertrophy may be at play in
348 ECC contractions.

349
350 Interesting differences in muscle architecture using ultrasound were also found as result
351 of the different training regimens. Although Lf increased in both groups, the ECC group
352 showed a significantly greater gain in Lf compared to the CON one, while training
353 produced an increase in PA after both types of training but the increase in pennation
354 angle in the ECC group was much lower than in the CON one (Fig. 3). These findings
355 suggest that addition of serial sarcomeres occurs in response to muscle lengthening
356 scenarios (e.g. Holly et al. 1980; Reeves et al. 2009; Seynnes et al. 2007), and herein
357 mainly, as a result of the ECC component. Instead, increases in PA occur to bundle more
358 contractile units along the tendon aponeurosis (Gans & Bock 1965; Kawakami et al.
359 1993) primarily reflecting muscle shortening, principally as a result of the CON
360 component. Finally, while these findings of distinct architectural responses are allied
361 with those reported by Reeves et al. (Reeves et al. 2009) in older humans after ECC vs.

362 conventional training, our current evidence for distinct architectural adaptations to *pure*
363 CON vs. ECC in younger individuals is the first report of its kind and reveals that
364 adaptations following conventional RT are dominated by the concentric component (at
365 least in older men), perhaps reflecting the greater loading stimulus of the CON phase
366 compared to the ECC one, when applied using standard gym equipment.

367

368 Intriguingly, while both groups showed a similar overall increase in VL muscle volume,
369 the regional morphological patterns of muscle hypertrophy induced by the two loading
370 modes differed substantially. For instance, while ECC exercise promoted greater muscle
371 hypertrophy (as measured by changes in ACSA by MRI) in the distal portion compared
372 to CON, increases in the mid VL muscle were greater for CON than ECC (Fig. 4). We
373 contend that evidence of these differences in the regional distribution of hypertrophy
374 along the muscle belly, reflect a differential addition of sarcomeres in series and in
375 parallel. Pennation of muscle fibres allows greater packing of sarcomeres in parallel
376 along the tendon aponeurosis (Gans & Bock 1965). Hence, the finding that CON training
377 promoted a large increase in pennation (30%), with little increase in fascicle length
378 (5%), strongly suggests that CON training leads to hypertrophy mainly through addition
379 of sarcomeres in-parallel. As indicated by the increase in ACSA, this phenomenon seems
380 to mainly occur in the central region of the VL, which, because of the bell-shaped
381 distribution of muscle ACSA, comprises a large portion (~ 60%) of the whole VL volume.
382 Instead, when training involved muscle stretch, i.e. with ECC training, hypertrophy
383 occurred mainly through an elongation of fascicles (12%) and with little increase in
384 pennation angle (5%), suggesting preferential addition of sarcomeres in-series. The
385 increase in ACSA over a larger portion (about 2/3) of the muscle belly (central and distal
386 regions) associated with preferential increase in fascicle length suggests that the

387 addition of new sarcomeres in series occurred over a large portion of the muscle belly. It
388 remains to be established where along muscle fibres sarcomere were added but it is
389 probable that this occurred at the periphery since early (Williams & Goldspink 1971) as
390 well as recent (Allouh et al. 2008) observations showed (directly or indirectly),
391 preferential addition of sarcomeres in series at the periphery of muscle fibres in
392 response to stretch overload, and in response to developmental growth, as satellite cell
393 frequency and concentration seems particularly high at the ends of muscle fibres
394 (Allouh et al. 2008). Although this accordance between architectural and morphological
395 adaptations to training seems reasonable, a limitation of the present study is that
396 ultrasound scans were taken just from the middle of the muscle belly with the
397 assumption that changes in architecture observed in this region would be
398 representative of changes along the whole muscle. This may not be the case, as
399 pennation angle might have increased more closer to the myotendineous junction after
400 ECC exercise (i.e. causing the greater VL distal hypertrophy). However, although in
401 principle it could be argued that limiting the ultrasound scans to a single muscle site
402 may not also be representative of other changes occurring in other muscles, it must be
403 acknowledge that Vastus Lateralis presents a more uniform architecture throughout its
404 length compared to other heads of the quadriceps (i.e. Vastus intermedius (VI) presents
405 inhomogeneous architecture, Blazevich et al. 2006). Moreover, a very recent publication
406 investigating the changes in muscle architecture between different sites of the four
407 heads of the quadriceps, observed how the adaptations in muscle CSA, thickness and PA
408 were quite consistent between the Vasti and only significantly different if compared to
409 Rectus Femoris (RF) changes size and architecture (Ema et al. 2013). These results
410 could be explained by the fact that RF is a bi-articular muscle, differing from the vasti
411 anatomically and biomechanically. The region of VL investigated in the present study

412 (VL mid length) coincides with the site in which the largest CSA value was observed.
413 Furthermore, our aim was to show different responses brought by the two different
414 loading paradigms: the choice of the muscle site is supported by the study by Ema et al.
415 (2013) in which VL is the muscle that showed less inhomogeneous changes between
416 CSA and architecture throughout the muscle hence we have reason to believe that this
417 site could still be the best representative of the whole quadriceps.

418

419 Blazeovich and colleagues (2007) similarly reported an increase of Lf in response to ECC
420 exercise in the first 5 weeks of training but it could be argued whether the architectural
421 adaptations of the present study do continue overtime, as it appears that in Blazeovich's
422 study these adaptations did not occur beyond the 5 weeks period. Thus, whilst Blazeovich
423 and colleagues confirmed the early architectural adaptations phenomenon previously
424 reported by Seynnes et al. (2007), our present work suggests that these changes are still
425 detectable after 10 weeks of RET. Further investigation is needed in order to assess if
426 these architectural responses will be observed of different magnitude (i.e. similar or
427 milder) after 5 weeks of RET.

428

429 Despite the distinct global (i.e., whole-muscle volume) hypertrophy responses between
430 CON and ECC training groups, functional (strength) adaptations revealed similar
431 increases in isometric MVC for both groups (CON 9%; ECC 11%). Although this
432 similarity in the strength increase seems paralleled by the changes in muscle volume, it
433 does raise the question of why, despite the greater training load (1.2 fold) of the ECC
434 group, VL hypertrophy was similar. Possible causes of this finding and of the different
435 architectural adaptations to ECC and CON training may be linked to muscle damage

436 caused by ECC contractions and to distinct signalling pathways involved in ECC and
437 CON.

438 Following such intriguing findings in the adaptive features of VL muscle after 10-wk of
439 CON vs. ECC, we chose to investigate the acute changes of muscle architecture in
440 response to single CON or ECC exercise bouts by recruiting a second subject group. As
441 expected, based upon the behaviour of the muscle-tendon unit, during CON contractions
442 fascicles shortened (by -19%) and lengthened (+19%) during ECC contractions. We
443 speculate that the contraction-specific VL fascicle length change (i.e., marked fascicle
444 shortening during CON and marked fascicle lengthening during ECC) is a primary cause
445 of the differential architectural adaptations and that such adaptations start from the first
446 training session after CON and ECC bouts, as suggested by Seynnes *et al.* (Seynnes et al.
447 2007) who showed that such differences in muscle architecture can be detected at very
448 early stages of training.

449

450 Although distinct cell signalling responses to CON and ECC in humans have yet to be
451 established, in the present study we observed increases in phosphorylated MAPK e.g. p-
452 38 MAPK, ERK 1/2 and p90RSK in the ECC but not CON. Similarly, reputed differences in
453 the signalling response of muscle cells have been observed in animal models (Martineau
454 & Gardiner 2001; Wretman et al. 2001). Furthermore, ERK1/2 expression was
455 previously shown to regulate CON vs. ECC growth pathways in cardiomyocytes,
456 suggesting MAPKs are involved in regulating architectural remodelling processes in
457 muscle tissue (Kehat et al. 2011). In this latter study, cardiomyocytes isolated from mice
458 lacking ERK showed an increase in length of the cardiomyocytes (ECC growth) whereas
459 cardiac cells isolated from mice over-expressing MEK1 (a MAPK-Kinase, ERK1/2 up-

460 regulator) showed a preferential growth in myocyte thickness (CON growth).
461 Recruitment of this second study group allowed us to interrogate the effects of an acute
462 bout of CON or ECC upon intramuscular signalling proteins associated with adaptation
463 to exercise, with particular focus on MAPK's. In doing this, we observed a specific MAPK
464 activation only in response to ECC (which lead to a preferential increase in Lf), although,
465 a similar and simple relationship between ERK1/2 and determination of architectural
466 adaptation in human skeletal muscle could not be confirmed, unless it is the reverse of
467 the mechanisms occurring in cardiac muscle. Further work is needed to define this.

468 Another clue as to why these architectural differences may exist is that ECC leads to a
469 greater degree of damage than CON (Byrne et al. 2004), with greater myofibrillar
470 disruption occurring with ECC (Schoenfeld 2012). However, we found no significant
471 increases in TNF α /Murf-1-MAFbx pathway (p-TNF α , p-p65, p65, I κ B, p-MurF-1, p-
472 MAFbx) 30 min after exercise. This supports the notion that activation of MAPK in
473 response to ECC occurred independently of muscle damage/inflammation, acting
474 through MAPK (Kramer & Goodyear 2007; Murton et al. 2008) and more likely through
475 mechano-transduction mechanisms (although we cannot exclude the likely onset of
476 muscle damage/inflammation phenomena after our single biopsy time point).
477 Furthermore, despite the fact that we observed no differences in whole-muscle
478 hypertrophy between ECC and CON (despite the marked architectural adaptive
479 differences), we measured activation of growth signalling (mTOR substrates). Apart
480 from suppression of 4EBP1 30 min post CON only (Atherton et al. 2005), no other
481 signals were modulated by 30 min post CON or ECC. Clearly, the energy stress after
482 exercise, which governs the latency in muscle protein synthesis responses (Cuthbertson
483 et al. 2006), may have prevented us from evaluating MAPK and AKT-mTOR cross-talk.

484 However, no further biopsies were taken, which represents a study limitation. It must be
485 acknowledge that performing acute and chronic studies in different groups precluded
486 interrogation of correlative links e.g. between MAPK phosphorylation and architectural
487 adaptations. Nonetheless, the acute exercise data revealing substantial contraction-
488 dependent divergence in mechanical and molecular responses and the chronic data
489 revealing divergent architectural/ morphological adaptations are highly robust. It could
490 also be argued that one limitation of this study is the different time under tension curve
491 found in the two types of contractions: as stated, the greater time under tension in the
492 eccentric mode was specifically chosen to enable to perform the lengthening/lowering
493 of the load phase in safety. Nevertheless, as previous studies have shown (Burd et al.
494 2012) the greater time under tension curve is associated to increased anabolic response.
495 If this was the case in this study, we should have observed differences in hypertrophic
496 response (i.e. ECC Vol > CON Vol), which did not occur. Burd and colleagues investigated
497 the anabolic responses to different time under tension comparing 1 second contractions
498 vs. 6 seconds ones (6-fold greater) whereas the present investigation used 2 sec vs. 3 sec
499 (0.5-fold difference): it is likely that this relatively smaller difference in time under
500 tension was not sufficient to trigger different hypertrophic adaptations, as the
501 morphological data are much more reflecting the findings presented by Adams et al.
502 (2004) that showed same increase in muscle size to ECC, CON and isometric training of
503 the same duration. Moreover, although the greater time under tension could
504 reflect/suggest a likely increased physiological blood flow restriction occurring during
505 the ECC phase, which should therefore result in a higher stimulation of hypertrophic
506 signalling and/or greater muscle volume (Meyer et al. 2006), in this study no differences
507 have been found in terms of anabolic signalling between the two training regimes.

508 Hence we may conclude that this difference in time under tension was not sufficient to
509 modulate a differential anabolic response.

510

511 **Conclusions**

512 This study has shown that CON and ECC training paradigms lead to divergent structural
513 adaptations, supported by different myogenic responses. ECC training leads to a marked
514 increase in fascicle length (~1.5 fold) with no significant change in pennation angle
515 while CON training induces a 3-fold increase in pennation angle, with little (<1-fold)
516 change in fascicle length. These results suggest that ECC training seems to promote the
517 addition of sarcomere in series whereas CON training favours the addition of sarcomere
518 in parallel. This differential pattern of sarcomere addition induced by the two types of
519 training, as inferred by the increase in fascicle length and pennation angle, seems also
520 reflected by the distribution of muscle hypertrophy along the VL muscle belly,
521 predominant in the mid to distal regions for ECC training and predominant in the mid-
522 belly region for CON training. The different muscle remodelling induced by CON and ECC
523 training may be associated with distinct MAPK responses to the two contraction modes.
524 The similar hypertrophy with ECC and CON RT may be explained by the greater
525 myofibrillar disruption caused by ECC loading, followed by possible activation of
526 inflammatory pathways likely antagonizing muscle hypertrophy.

527

528

529

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FIGURES

Fig 1. Subject on the Technogym leg press modified ad hoc with the special electric engine visible on the right corner indicated by the arrow (Fig 1 A). The electric winch attached to the chair via steel cable; Fig 1B shows how the winch was connected to the chair (the red arrow indicates a counterweight that prevented the cable from becoming too slack and getting damaged); Fig 1C presents the site where the engine was placed

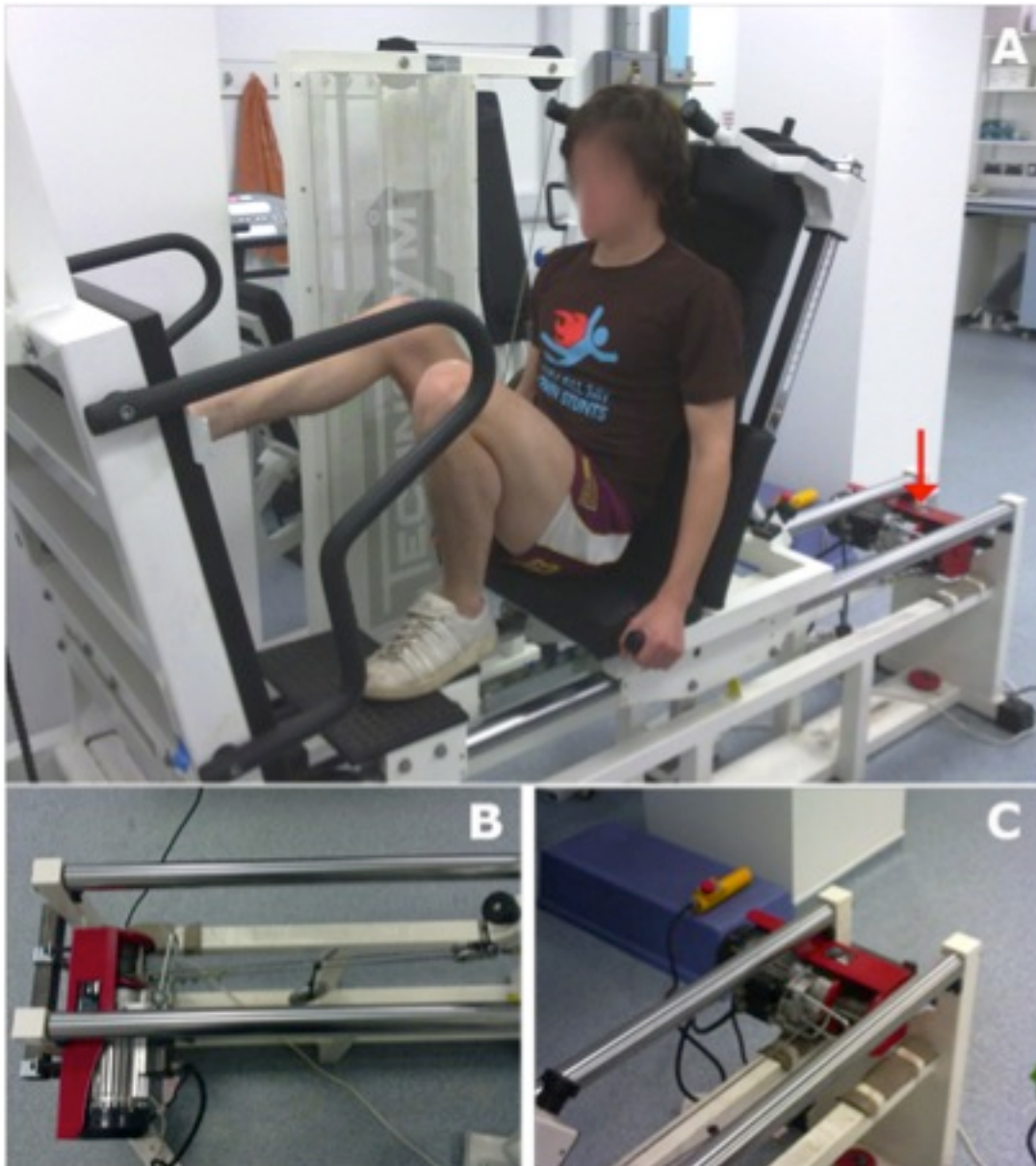


Fig 2. VL ultrasound image captured at rest: pennation angle and the visible part of a muscle fascicle is shown.

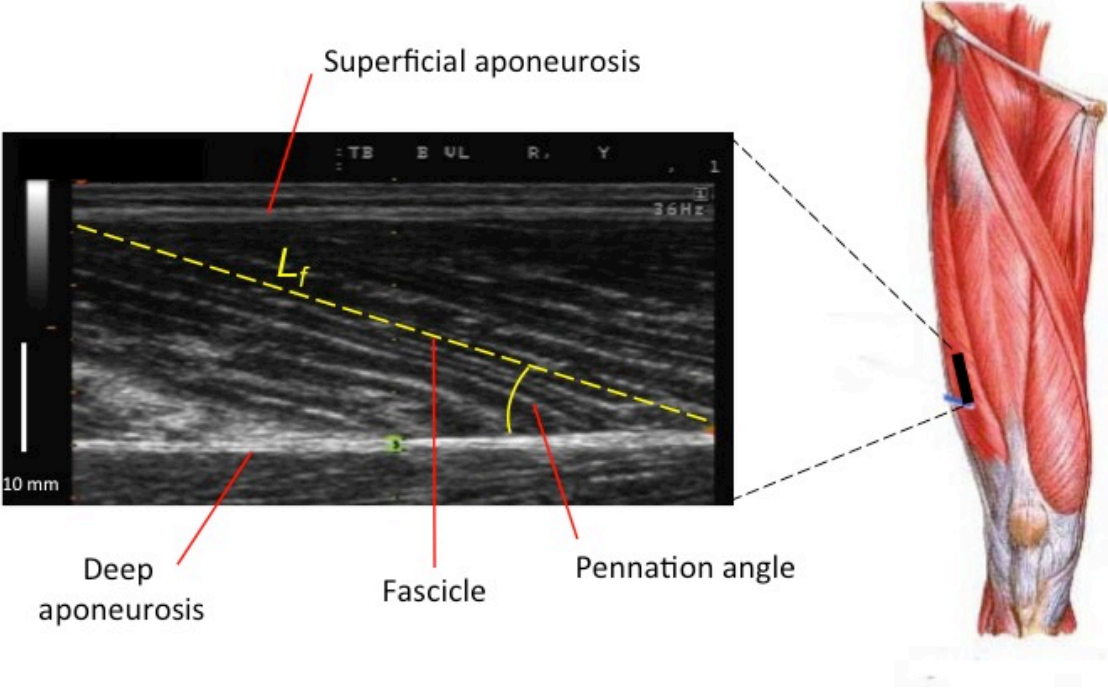


Fig 3. Post/Pre training ratios of muscle volume, isometric MVC and muscle architecture in the concentric and eccentric exercise groups. Y = 1 represent the baseline value. Data normalized to pre values; means \pm SEM (* $P < 0.05$ ** $P < 0.001$ *** $P < 0.0001$ - ^^, ^^^ = significantly different between groups: $P < 0.01$ and $P < 0.001$, respectively).

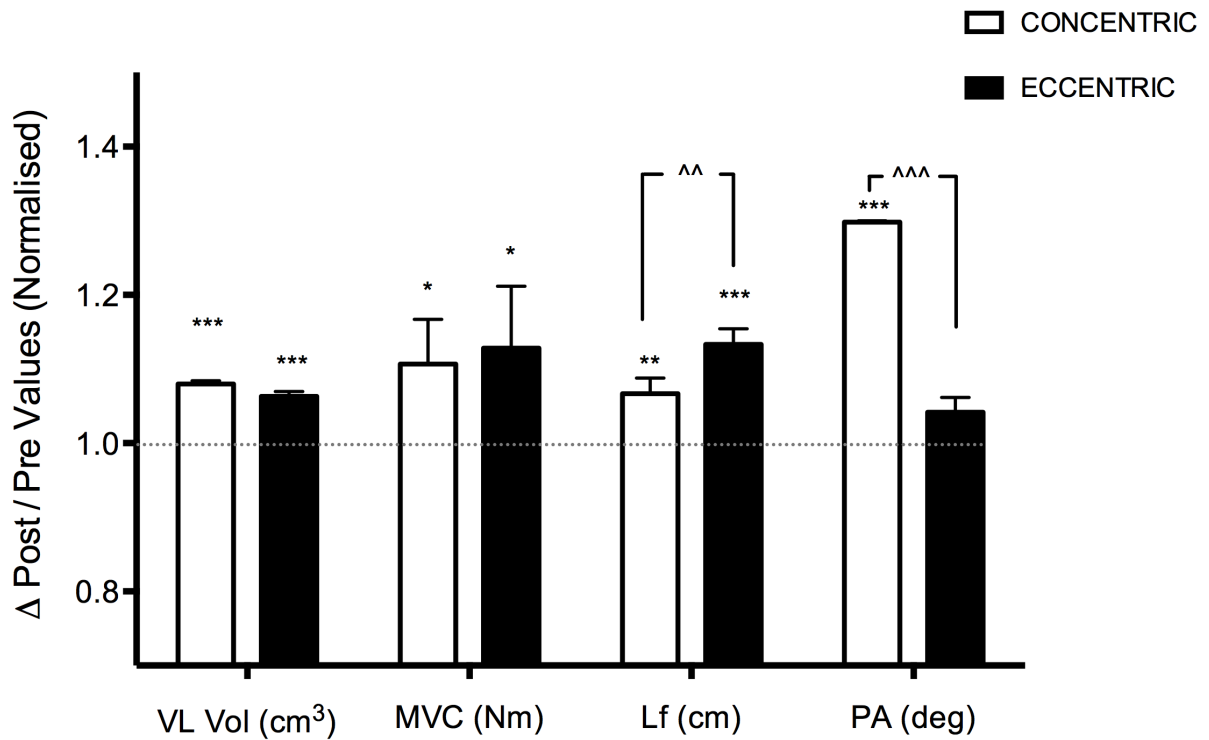


Fig 4. Regional Hypertrophy of VL muscle (ACSA = Anatomical Cross Sectional Area) after concentric and eccentric training. Data are means \pm SEM (** $P < 0.01$ *** $P < 0.001$ - ^, ^^ = significantly different between groups: $P < 0.05$, $P < 0.01$).

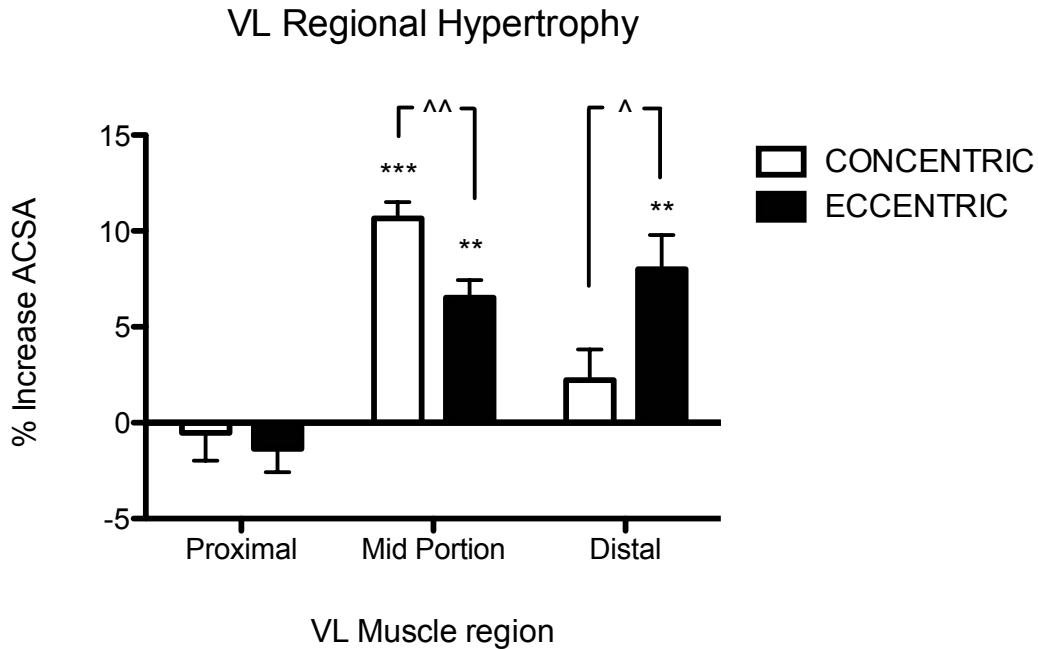


Fig 5. Muscle architectural behavior during a concentric and eccentric contraction performed on the leg-press device (90° to 170° knee joint angle, 180° = anatomical zero). Y = 1 represent the baseline value. Data normalized to pre values; means \pm SEM (*** $P < 0.0001$ - ^^^ = significantly different between groups $P < 0.0001$).

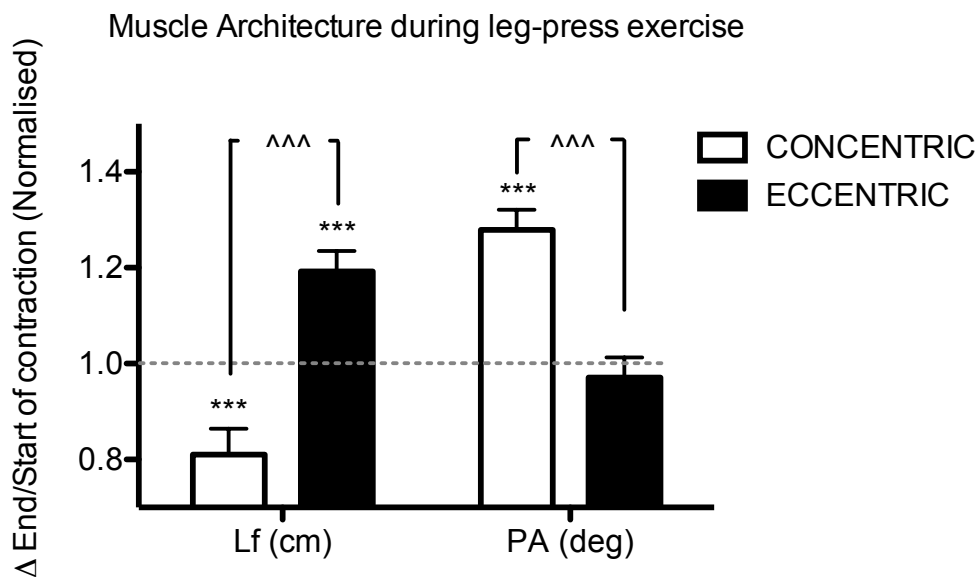
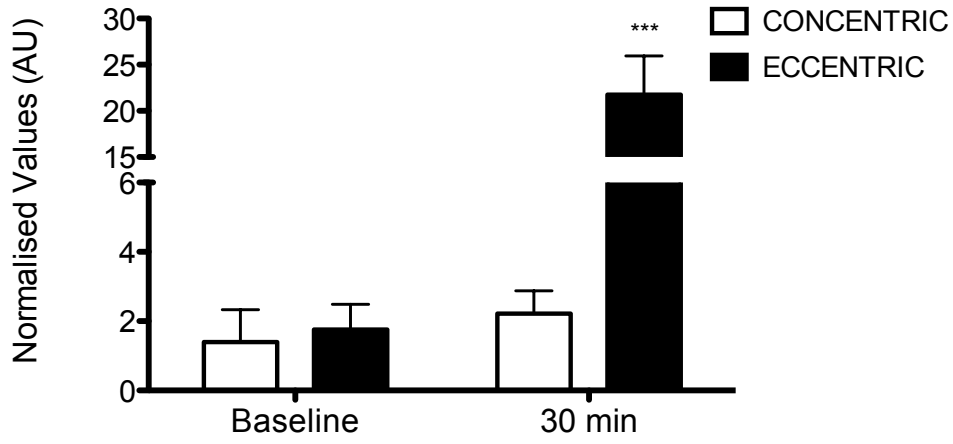
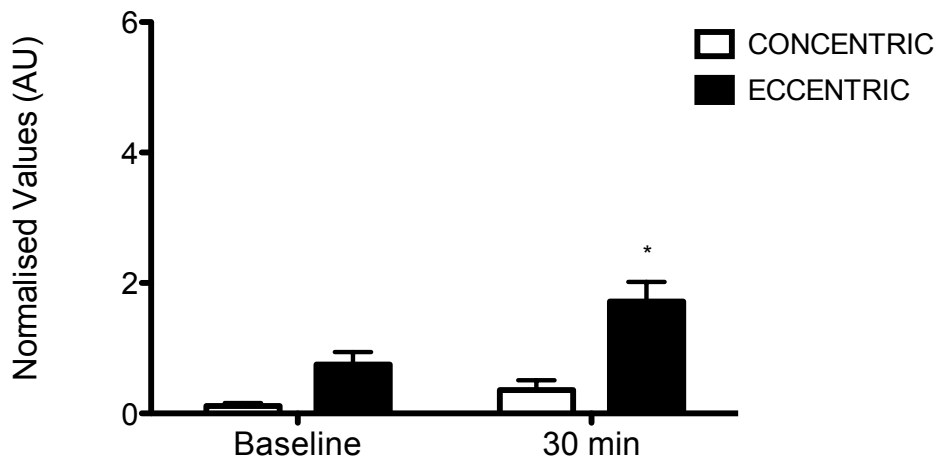


Fig 6. MAPK Molecular responses (phosphorylation) at 30 minutes after either a single concentric or eccentric training session. Data are means \pm SEM. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

p-p38 MAPKinase



p-ERK 1/2



p-p90RSK

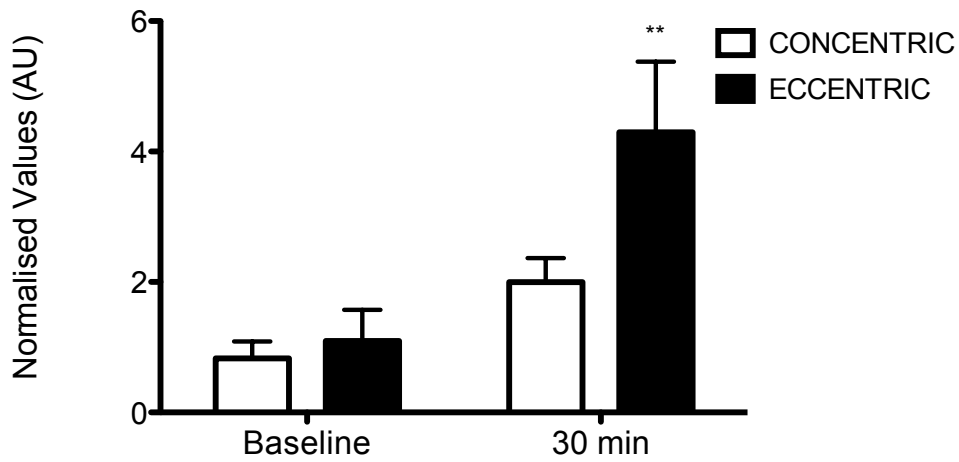
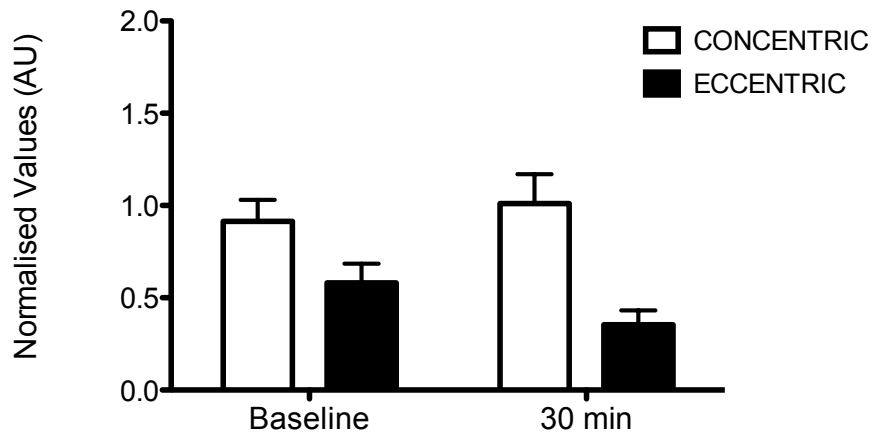
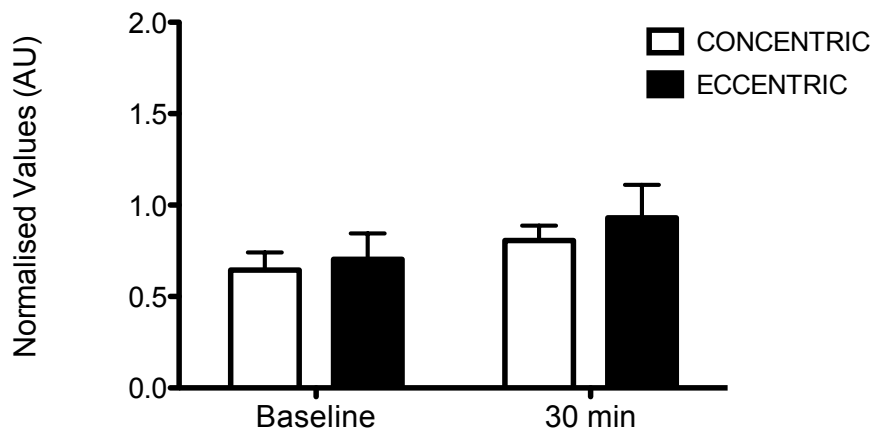


Fig 7. Akt, p70S6K and 4EBP1 molecular responses (phosphorylation) at 30 minutes after either a single concentric or eccentric training session. Data are means \pm SEM (* $P<0.05$).

p-AKT Ser 473



p-p70S6K



p-4EBP1

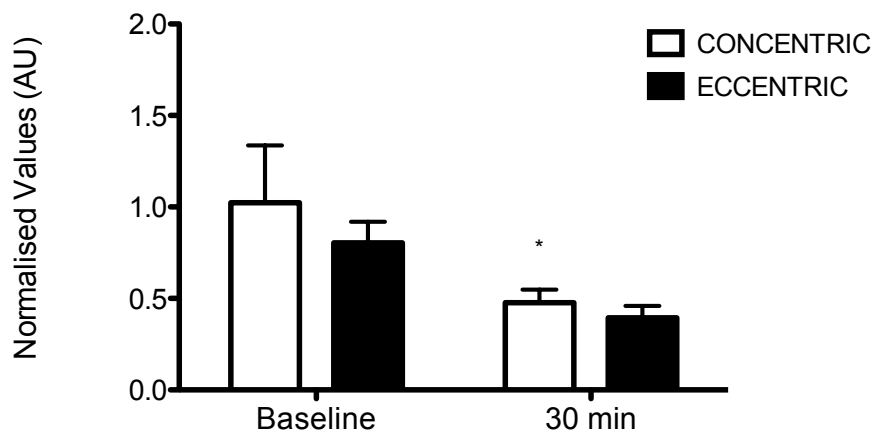


Table 1. Maximum lifting or lowering ability changes for the CON Group (CON) and the ECC one (ECC). EMG values were recorded only at baseline during 1RM leg-press for concentric and eccentric phases. Load ratio is also showed and calculated as the ratio of pre and post ECC/CON training loads.

CON 1RM (Kg)			ECC 1RM (Kg)			Load ratio	
Pre	Post	Δ%	Pre	Post	Δ%	Pre	Post
192 ± 16	262 ± 30	36*	233 ± 13	337 ± 9	44*	1.21	1.29
EMG (mV)			EMG (mV)				
0.33± 0.1			0.31± 0.1				

(Pre = baseline, Post = Post-training) values are means ± SEM (* $P < 0.05$, pre-to-post difference).