

1 **β -alanine supplementation enhances human skeletal muscle relaxation**
2 **speed but not force production capacity**

3

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23 **Running head:** β -alanine supplementation and muscle contractile properties

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33 **ABSTRACT**

34 **PURPOSE:** β -alanine (BA) supplementation improves human exercise performance. One
35 possible explanation for this is an enhancement of muscle contractile properties, occurring
36 via elevated intramuscular carnosine resulting in improved calcium sensitivity and handling.
37 This study investigated the effect of BA supplementation on *in vivo* contractile properties and
38 voluntary neuromuscular performance. **METHODS:** Twenty-three men completed two
39 experimental sessions, pre- and post-28 days supplementation with $6.4 \text{ g}\cdot\text{d}^{-1}$ of BA ($n = 12$)
40 or placebo (PLA; $n = 11$). During each session, force was recorded during a series of knee
41 extensor contractions: resting and potentiated twitches and octet (8 pulses, 300 Hz)
42 contractions elicited via femoral nerve stimulation; tetanic contractions (1 s, 1 – 100 Hz) via
43 superficial muscle stimulation; and maximum and explosive voluntary contractions.
44 **RESULTS:** BA supplementation had no effect on the force-frequency relationship, or the
45 force responses (force at 25 ms and 50 ms from onset, peak force) of resting or potentiated
46 twitches, and octet contractions ($P > 0.05$). Resting and potentiated twitch electromechanical
47 delay and time-to-peak tension were unaffected by BA supplementation ($P > 0.05$), although
48 half-relaxation time declined by 7-12% ($P < 0.05$). Maximum and explosive voluntary forces
49 were unchanged after BA supplementation. **CONCLUSION:** BA supplementation had no
50 effect on evoked force responses, implying that altered calcium sensitivity and/or release are
51 not the mechanisms by which BA supplementation influences exercise performance. The
52 reduced half-relaxation time with BA supplementation might, however, be explained by
53 enhanced reuptake of calcium, which has implications for the efficiency of muscle
54 contraction following BA supplementation.

55 **Key words:** beta-alanine; muscle contractile properties; electrical stimulation; force-
56 frequency relationship

57

58 INTRODUCTION

59 Carnosine (β -alanyl-L-histidine) is a cytoplasmic dipeptide synthesised from β -alanine (BA)
60 and histidine, and is found in high concentrations within mammalian skeletal muscle.
61 Carnosine is formed, primarily in skeletal and brain tissue, by bonding histidine and BA in a
62 reaction catalysed by carnosine synthase (23; 40). The availability of BA in the human diet is
63 the rate-limiting factor for carnosine synthesis in human skeletal muscle [for a brief review
64 see (20)]. Long-term (4-10 weeks) dietary supplementation with BA significantly increases
65 human skeletal muscle carnosine content (19; 21; 24). Interest in elevating carnosine levels
66 through BA supplementation has dramatically increased since it was first shown that doing so
67 increased high-intensity cycling capacity (21). Since then, it has been well established that
68 BA supplementation can improve high-intensity exercise performance (e.g. 2000 m rowing
69 performance and 100-200 m swimming performance) and capacity during exercise of ~1-6
70 minutes [see reviews: (22; 34)]. However, the physiological mechanisms for these ergogenic
71 effects remain poorly understood.

72

73 Carnosine is suggested to have several physiological roles in muscle, which are pertinent to
74 muscle function and performance. For example, its molecular structure makes it well suited
75 to act as a pH buffer (36). The pKa of its imidazole ring is 6.83, placing it right in the middle
76 of the pH transit range of exercising muscle. This means that an increase in carnosine
77 content within the skeletal muscles also results in an expansion of the imidazole ring content,
78 concomitantly increasing the muscle buffering capacity. As a result, performance
79 improvements in high-intensity exercise (particularly when hydrogen cation accumulation is
80 likely to limit performance) have largely been ascribed to increases in intracellular buffering
81 [see review: (34; 35)].

82

83 Alternative mechanisms for the enhancement of exercise performance following BA
84 supplementation have been proposed. For example, previous work in rat skeletal muscle
85 suggested a role for carnosine in increasing the sensitivity of the contractile apparatus to
86 calcium ions (Ca^{2+}) (10). More recent work in skinned human *m. vastus lateralis* fibre
87 preparations showed a similar increase in Ca^{2+} sensitivity (11). Although only slight changes
88 were shown in the maximum Ca^{2+} activated force ($\leq 3\%$), a significant leftward shift in the

89 force-calcium concentration relationship was shown, indicating that force for a given
90 submaximal Ca^{2+} concentration was increased in the presence of higher carnosine levels in
91 both fibre types. Elevated carnosine levels also increased Ca^{2+} release from the sarcoplasmic
92 reticulum of type I fibres, whereby carnosine appeared to enhance the Ca^{2+} sensitivity of
93 ryanodine receptors and potentiated Ca^{2+} induced Ca^{2+} release (11). Thus, it was suggested
94 that elevated carnosine after BA supplementation could alleviate the decline in contractile
95 performance during fatiguing contractions by countering factors that might cause reduced
96 calcium sensitivity and release (11).

97

98 A recent study provided the first evidence that dietary BA supplementation may influence the
99 muscle contractile properties of mice (13), potentially via elevated intramuscular carnosine
100 and its effect on calcium sensitivity and handling (11). BA supplementation was associated
101 with a leftward shift in the electrically-evoked force-frequency relationship of excised
102 muscle, which is analogous to the force-calcium concentration relationship (5; 27), eliciting a
103 10-30% increase in the force produced at low stimulation frequencies (13). However, the
104 possibility that dietary BA supplementation might change *in vivo* human muscle contractile
105 properties, and thus voluntary muscle performance, has not been investigated. There is a
106 need to examine this possibility given that we would expect a wider range of performance
107 effects of carnosine than have currently been shown if improved calcium handling were the
108 major physiological role of carnosine in human skeletal muscle (34).

109

110 As such, we examined the effects of 28 d BA supplementation on the intrinsic contractile
111 properties of human skeletal muscle *in vivo*, as well as on voluntary muscle function. Intrinsic
112 contractile properties were assessed via the force-frequency relationship in response to
113 muscle stimulation, and the evoked twitch and octet [8 pulses at 300 Hz, which drives the
114 muscle at its maximum capacity for rapid or “explosive” force production; (8)] responses to
115 supramaximal nerve stimulation. We hypothesised that BA supplementation would enhance
116 intrinsic contractile properties; producing a left-ward shift in the force-frequency relationship;
117 increasing the peak and explosive force responses to twitch and octet stimulation and
118 increasing explosive voluntary force production. In addition, we hypothesised that the altered
119 contractile properties would lead to changes in motor control, reflected as a shift in the force-
120 electromyography (EMG) relationship towards lower EMG levels for a given level of force.

121 **METHODS**

122 **Participants**

123 Twenty-six participants were recruited to the study and were stratified and allocated to the
124 two supplement groups [placebo (PLA) or β -alanine (BA)] on the basis of maximum knee
125 extensor strength (maximum voluntary force, MVF; see below) values recorded during the
126 familiarisation session, such that the two groups were matched for knee extensor strength.
127 However, three participants withdrew from the study (two from PLA and one from BA), one
128 during familiarisation due to a lack of tolerance of electrical stimulation and two following
129 baseline testing with no reason provided. As such, twenty-three participants completed all
130 aspects of the study (PLA group: $n = 11$; age, 25.6 ± 5.6 y; body mass, 79.1 ± 13.0 kg; height,
131 1.80 ± 0.07 m; BA group: $n = 12$; age, 26.1 ± 7.4 y; body mass, 90.1 ± 32.1 kg; height, $1.79 \pm$
132 0.06 m). All participants provided written informed consent, and completed this study, which
133 was approved by the Institutional Human Ethical Review Committee. None of the
134 participants had taken any nutritional supplements in the previous 6 months. Participants had
135 no injuries to the lower limbs, were not involved in any systematic physical training, and
136 were categorised as having moderate habitual levels of physical activity using the
137 International Physical Activity Questionnaire Short Format [<http://www.ipaq.ki.se/ipaq.htm>;
138 (7)]. Throughout the study participants were requested to maintain similar levels of physical
139 activity and dietary intake; this was verbally confirmed at the start of each session. None of
140 the subjects were vegetarian or vegan, and therefore they would likely have encountered
141 small amounts of BA in their diet (1).

142

143 **Study design**

144 This was a double-blind placebo controlled experiment. Participants completed three
145 experimental sessions over a five week period: a familiarisation session, which preceded a
146 baseline session by at least 7 days, and a follow-up session after 28 days of supplementation
147 with either BA or PLA. Participants were instructed to abstain from alcohol and
148 strenuous/unaccustomed exercise for 36 hours prior to measurement sessions, with caffeine
149 prohibited on the day of measurement sessions. Compliance with these requests was
150 confirmed verbally with each participant prior to them commencing each session.
151 Measurement sessions were completed at a consistent time of day, with recordings of force

152 and surface EMG during a series of voluntary and involuntary (electrically evoked) isometric
153 contractions of the knee extensors of the dominant leg. The familiarisation session involved
154 all the voluntary and evoked contractions, except the evoked octet contractions. The baseline
155 and follow-up sessions involved an identical protocol performed according to a strict
156 schedule. All raw data, exclusions and statistical analyses were completed blind to
157 supplement group.

158

159 **Supplementation**

160 Participants received 6.4 g·d⁻¹ of either BA (sustained-release Carnosyn™) or a matched
161 placebo (PLA, maltodextrin) for 28 days (2 × 800mg tablets, ingested 4 times per day). The
162 sustained-release formulation used in this study has been shown to reduce or remove the
163 paraesthesia often experienced by participants following doses of free BA powder (9). We
164 would expect the increase in muscle carnosine content to be close to 15 mmol·kg⁻¹ dry muscle
165 (an increase of circa 65% in a participant eating a mixed diet), given that Harris et al. (2006)
166 reported this level of increase following a similar but slightly lower total dose of BA. None of
167 the participants reported any feelings of paraesthesia during the study. Throughout
168 supplementation participants completed a log to verify supplement compliance, with similar
169 compliance reported at 91 ± 7% in the BA group and 88 ± 10% in the PLA group
170 (independent sample *t*-test, *P* = 0.60).

171

172 Supplements were provided to each participant in identical white tubs by an individual not
173 directly involved in testing or data analysis, in order to maintain the double-blind. BA tablets
174 were tested by the manufacturer prior to release for the study and conformed to the label
175 claim for BA content. In addition, BA and PLA supplements were independently tested by
176 HFL Sports Science, UK, prior to use to ensure no contamination with steroids or stimulants
177 according to ISO 17025 accredited tests.

178

179 **Experimental set-up**

180 *Knee extension force*

181 Participants were seated in a rigid, custom-built dynamometer with hip and knee joint angles
182 of approximately 95° and 100° (180° = full extension), as adapted from previous studies (17;
183 18). Adjustable strapping across the pelvis and shoulders prevented extraneous movement
184 during muscle activation. An ankle cuff was attached to the dominant leg of the participant
185 ~2 cm proximal to the medial malleolus and was in series with a linear strain gauge (615,
186 Tedeo-Huntleigh, Herzliya, Israel) oriented perpendicular to the tibia. Dynamometer
187 configuration was established during the familiarisation session and replicated thereafter.
188 The force signal was amplified ($\times 1000$) in the frequency range of 0 – 500 Hz, and sampled at
189 2000 Hz using an external A/D converter (1401; CED, Cambridge, UK), interfaced with a
190 personal computer (PC) using Spike 2 software (CED, Cambridge, UK). Force data were
191 low-pass filtered in both directions at 450 Hz using a fourth-order zero-lag Butterworth filter
192 prior to analysis. Baseline resting force was subtracted from all force recordings to correct for
193 the effects of gravity.

194

195 *Electromyography (EMG)*

196 EMG signals were recorded from the superficial quadriceps: *m. rectus femoris* (RF), *m.*
197 *vastus medialis* (VM) and *m. vastus lateralis* (VL). After preparation of the skin by shaving,
198 light abrasion and cleaning with alcohol, bipolar surface electrodes (2.5 cm inter-electrode
199 distance; silver/silver chloride, 95 mm² area, Ambu Blue Sensor, Ambu, Ballerup, Denmark)
200 were attached over each muscle at standardised percentages of thigh length, as measured
201 from the knee joint space to the greater trochanter: RF, 55%; VM, 25% and VL, 45%. These
202 sites were selected to avoid the innervation zones of each of the assessed muscles (32). A
203 reference electrode was placed on the patella of the same limb. EMG signals were pre-
204 amplified by active EMG leads (input impedance 100 M Ω , CMMR > 100 dB, base gain 500,
205 1st order high pass filter set to 10 Hz; Noraxon, Scottsdale, U.S.A) connected in series to a
206 custom-built junction box and subsequently to the same A/D converter and PC software that
207 enabled synchronisation with the force data. The signals were sampled at 2000 Hz. EMG
208 data were band-pass filtered in both directions between 20 and 450 Hz using a fourth-order
209 zero-lag Butterworth filter prior to analysis.

210

211 *Electrical stimulation*

212 A constant current variable voltage stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City,
213 UK) was used to assess knee extensor contractile properties whilst the participant was
214 voluntarily passive. Square-wave pulses (0.2 ms duration) were delivered via: (i)
215 supramaximal femoral nerve stimulation to evoke maximal resting twitch, potentiated twitch
216 and octet contractions; (ii) percutaneous sub-maximal muscle stimulation to evoke
217 contractions at a range of frequencies (1 to 100 Hz) to assess the force-frequency
218 relationship. Femoral nerve stimulation involved a cathode stimulation probe (1 cm diameter,
219 Electro-Medical Supplies Ltd, Wantage, UK) firmly pressed into the skin over the femoral
220 nerve in the femoral triangle, and an anode (7×10 cm carbon rubber electrode; Electro-
221 Medical Supplies Ltd, Wantage, UK) coated with electrode gel and taped to the skin over the
222 greater trochanter. The precise location of the cathode was determined as the position that
223 evoked the greatest twitch response for a particular submaximal electrical current (typically
224 30–50 mA). For percutaneous stimulation, the surfaces of two carbon rubber electrodes (14 ×
225 10 cm; Electro-Medical Supplies Ltd, Wantage, UK) were coated with electrode gel and
226 secured over the proximal and distal surface of quadriceps at standardised percentages of
227 thigh length, as measured from the patella to the anterior superior iliac spine (ASIS):
228 proximal electrode placed 20% distal to the ASIS; distal electrode placed 10% proximal to
229 the patella.

230

231 **Protocol and measurements**

232 Measurements were completed in the following order, according to a consistent time
233 schedule including ≥ 3 minutes rest between successive measurements.

234 Force and EMG onsets for all evoked and voluntary contractions were identified manually
235 using visual identification by the same investigator, in accordance with a previously
236 published method (16; 37). This approach is considered more valid than the use of automated
237 methods of identification (38).

238

239 *Resting twitches*

240 Resting twitches were evoked following ≥ 15 min passive sitting, in order to remove any
241 lingering potentiation, which incorporated the time for securing the participant in the

242 dynamometer, and preparing them for EMG and electrical stimulation. Single electrical
243 impulses were delivered with stepwise increments in the current, separated by 10 s to allow
244 for neuromuscular recovery, until a plateau in the amplitude of twitch force and compound
245 muscle action potentials (M-waves) were reached. The stimulus intensity was then increased
246 by 25% above the value required to elicit a plateau to ensure supramaximal stimulation, and
247 three discrete supramaximal stimuli separated by 10 s were then delivered to elicit maximal
248 twitch responses and M-waves.

249

250 The time difference between M-wave onset (first electrode site to be activated) and twitch
251 force onset was defined as the electromechanical delay (EMD). Twitch force was measured at
252 25 and 50 ms from onset, as markers of the explosive force production during the rising
253 slope, and at the peak of the force response. The time-to-peak tension (TPT) and half-
254 relaxation time (HRT) were also recorded. All measurements were averaged across the three
255 maximal twitch contractions. The M-wave response for the three quadriceps electrodes was
256 measured for M-wave area, from EMG onset to the point where the signal returned to
257 baseline, and averaged across the three sites. The mean M-wave area of the three
258 supramaximal stimuli was defined as the maximal M-wave area (M_{max}) and was used for
259 normalisation of voluntary quadriceps EMG (6).

260

261 *Maximum voluntary contractions and potentiated twitches*

262 A brief warm-up of 3 sub-maximal knee extension contractions at 50%, 75% and 90% of the
263 participants' perceived maximal force were performed; contractions lasted ~3 s each and
264 were separated by ~20 s. Participants then completed 4 maximum voluntary contractions
265 (MVCs) of the knee extensors ≥ 60 s apart, during which they were instructed to contract "as
266 hard as possible" for 3-4 s. During and after each contraction they received strong verbal
267 encouragement reiterating the instructions, together with online feedback of the force signal
268 and a marker of their maximum force during that session displayed onscreen. Supramaximal
269 stimulation of the femoral nerve, using the same configuration and stimulus intensity as for
270 resting twitches, was used to elicit a maximal potentiated twitch ~1 s after each of the MVCs.
271 The greatest instantaneous force during either the knee extensor MVCs or explosive
272 voluntary contractions (see below) of that trial was defined as MVF. The root mean square

273 (RMS) of the EMG signal for each muscle (RF, VM, VL) was calculated over a 500 ms
274 epoch surrounding MVF (250 ms either side) and normalised to the corresponding M_{\max} (6),
275 before averaging across all 3 sites to calculate a mean quadriceps value. The EMD, force at
276 25 and 50 ms from onset, peak twitch force, TPT and HRT were averaged across the four
277 maximal potentiated twitch contractions.

278

279 *Explosive voluntary contractions*

280 The protocol followed previously published procedures (6; 16). Participants completed ≥ 10
281 isometric explosive voluntary knee extensions, each separated by ~ 20 s. Starting from a
282 completely relaxed state, they were instructed to respond to an auditory signal by extending
283 their knee “as fast and hard as possible” for ~ 1 s, with an emphasis on “fast”. An on-screen
284 cursor was used to provide online feedback on their explosive performance, displaying the
285 maximum rate of force development (2 ms time constant) of their best attempt. Strong verbal
286 encouragement was provided to participants to exceed this target during each subsequent
287 contraction. A second visual marker on the screen depicted 80% of the peak force recorded
288 during MVCs, which participants were expected to achieve or exceed during each explosive
289 contraction. Resting force was also displayed on a sensitive scale during all explosive
290 contractions to aid the detection of pretension or countermovement. The explosive
291 contractions were performed until 10 contractions, with no prior countermovement or pre-
292 tension, been had been recorded.

293

294 The three contractions with the greatest maximum rate of force development, meeting the
295 following criteria, were used for analysis: (i) no prior countermovement or pre-tension, and
296 (ii) peak force $\geq 80\%$ MVF. Analyses involved measurement of the force–time and EMG–
297 time traces in short periods after their onsets. Explosive force was measured at 25 ms
298 intervals up to 150 ms after force onset. The RMS of the EMG signal from each muscle was
299 measured over three consecutive 50 ms time periods from EMG onset of the first agonist
300 muscle to be activated (*i.e.*, 0-50, 50-100 and 100-150 ms). Thereafter, RMS EMG at each
301 EMG site was normalized to M_{\max} and averaged to provide a mean quadriceps value. All
302 measurements were averaged across the three selected contractions.

303

304 *Force-EMG relationship (via voluntary incremental knee extension contractions)*

305 A series of submaximal knee extension contractions were performed at 15% increments of
306 MVF, in ascending order, up to 90%. Horizontal cursors on the screen in front of participants
307 depicted the target levels of force. Participants were instructed to reach the target quickly and
308 maintain the level of force as accurately as possible for ~3 s. Contractions were separated by
309 ~20 s. The RMS of the EMG and average force over a stable 500 ms part of the force trace
310 (minimal standard deviation of the force trace for that contraction) were analysed at each of
311 the contraction intensities. The EMG RMS values were normalised to M_{\max} and plotted
312 against the respective force values. Linear regression was used to evaluate the slope and
313 intercept of the force-EMG relationship incorporating all data between 15 – 90% MVF.

314

315 *Octet contractions*

316 Octet contractions [8 impulses at 300 Hz; (8)] were evoked via supramaximal stimulation of
317 the femoral nerve. First, a brief series of single stimuli were administered, and twitch force
318 and M-wave amplitudes were monitored to confirm that the stimuli were supramaximal. The
319 current was increased if necessary to ensure supramaximal stimulation. Then 3 discrete pulse
320 trains (≥ 15 s apart) were delivered with a supramaximal current (+25%) to evoke maximal
321 octet contractions. The current increased by ~5% after each pulse train in order to confirm a
322 plateau in both the peak force and maximum rate of force development. On some occasions,
323 where the first pulse train elicited a submaximal response, a 4th pulse train was delivered to
324 ensure 3 maximal responses. The octet force response was measured at 25 and 50 ms from
325 force onset, as well as at the peak. All measurements were averaged across the 3 analysed
326 contractions.

327

328 *Force-frequency relationship*

329 Surface EMG electrodes were removed and carbon rubber electrodes attached over the
330 quadriceps, taking ~5 minutes. The force-frequency relationship was then evaluated during
331 tetanic contractions elicited via submaximal percutaneous electrical stimulation (3; 15).

332 Initially, 100 Hz contractions were evoked at increasing current intensities, ≥ 30 s apart, to
333 determine the current that elicited 50% of baseline MVF. This current (typically 110 – 200

334 mA) was then used for the following force-frequency measurements. The final calibration
335 contraction at 100 Hz and the subsequent measured contractions were separated by ≥ 60 s.
336 The force-frequency relationship contractions consisted of two twitch contractions (1 Hz),
337 followed by single contractions of 1 s duration at each of 9 different frequencies (5, 10, 15,
338 20, 30, 40, 50, 80, 100 Hz) performed in ascending order with ~ 30 s between contractions.
339 Peak force was defined as the greatest instantaneous force. Thereafter, the force values at
340 each stimulation frequency were normalized to 100 Hz force. The force-frequency
341 relationship was fitted with a Hill curve and evaluated for frequency at 50% of the maximum
342 force response (11).

343

344 **Statistical Analysis**

345 Dependent variables measured over several time points/periods (force and EMG during
346 explosive voluntary contractions, evoked twitch and octet force) were analysed using a three-
347 way (group \times session \times time point) analysis of variance (ANOVA). Similarly, the force-
348 frequency relationship was assessed by a three-way (group \times session \times frequency) ANOVA.
349 Other dependent variables (MVF, HRT, TPT, slope and intercept of force-EMG relationship,
350 frequency at 50% of force response for the force-frequency relationship) were evaluated
351 using two-way ANOVA (group \times session). A Greenhouse-Geisser correction was applied
352 when the ANOVA assumption of sphericity was violated, and significant interaction effects
353 were followed-up by independent sample *t*-tests on the individual percentage change values
354 for each condition. The change in group mean values was used to calculate the percentage
355 change values presented. Intra-individual variability was assessed using the mean intra-
356 individual coefficient of variation (CV) across the two measurement session for the PLA
357 group [(mean \div standard deviation) \times 100]. Statistical analyses were completed using SPSS
358 version 21 (SPSS Inc, Chicago, USA) and statistical significance was accepted at $P \leq 0.05$.
359 Data are presented as mean \pm one standard deviation (1SD).

360

361 **RESULTS**

362 **Electrically-evoked contractile properties**

363 *Resting twitches*

364 There was no influence of supplementation on resting twitch force ($P = 0.46$ and 0.70 , Figure
365 1A), EMD ($P = 0.63$, Fig 2A) or TPT ($P = 0.29$; Fig 2B), although there was a group \times
366 session interaction for HRT ($P = 0.018$; Fig 2C). *Post hoc* analysis showed that the change in
367 HRT was greater for the BA group ($-12 \pm 10\%$) compared to the PLA group ($+2 \pm 11\%$; $P <$
368 0.01). Mean CV values for the PLA group were: force at 25, 50 ms and peak were 14%, 9%
369 and 8%; EMD 7%; TPT 3%; HRT 7%.

370

371 *Potentiated twitches*

372 There was no influence of supplementation on potentiated twitch force ($P = 0.44$ and 0.52 ,
373 Figure 1B), EMD ($P = 0.48$, Fig 2D) or TPT ($P = 0.32$; Fig 2E). However, there was a group
374 \times session interaction for HRT ($P = 0.041$; Fig 2F) and *post hoc* analysis showed that the
375 change in HRT was greater for the BA group ($-7 \pm 11\%$) compared to the PLA group ($+1 \pm 8$
376 $\%$; $P = 0.050$). Mean CV values for the PLA group were: force at 25, 50 ms and peak were
377 6%, 3% and 3%; EMD 6%; TPT 3%; HRT 4%.

378

379 *Octet contractions*

380 Supplementation did not influence resting octet force at any time point (Figure 1C). Mean
381 CV values for the PLA group were: force at 25, 50 ms and peak were 10%, 3% and 4%.

382

383 *Force-frequency relationship*

384 The peak force at each frequency of stimulation (Fig 3) and the frequency at 50% of the force
385 response (Table 1) were both unaffected by supplementation. Mean CV values for relative
386 force (% maximum at 100 Hz) in PLA group were 6-8% at 1 - 10 Hz, 1-3% at 15 - 80 Hz,
387 and 6% for the frequency at 50% of force response.

388

389 **Maximum and explosive voluntary force production**

390 There was affect of supplementation on MVF (Fig 4). The mean CV for MVF in the PLA
391 group was 3%. Similarly, there was no influence of supplementation on force measured at 25
392 ms intervals during explosive voluntary contractions (Fig 4). The mean CV values for
393 voluntary force production in the PLA group were: 13-17% at 25 – 50 ms, 4-7% from 75 –
394 150 ms and 3% at MVF.

395

396 **Neuromuscular activation**

397 *Agonist neuromuscular activation during maximum voluntary and explosive voluntary*
398 *contractions*

399 Agonist EMG normalised to M_{max} during MVCs and explosive contractions was not affected
400 by supplementation (Fig 5), indicating that neuromuscular activation was consistent across
401 measurement sessions. The mean CV values for agonist EMG in the PLA group were: 26%,
402 23% and 9% in the 0-50, 50-100 and 100-150 ms time windows, and 13% at MVF.

403

404 *Force-EMG relationship*

405 The slope and y-intercept of the force-EMG relationship were unaffected by supplementation
406 (Fig 5, Table 1). The mean CV value for slope of the force-EMG relationship in the PLA
407 group was 15%. Although the CV was very high for the intercept of the relationship (80%) as
408 a consequence of intercept values being close to zero, the mean difference between sessions
409 was actually very low when expressed as a percentage of maximal EMG at MVF (4%).

410

411 **DISCUSSION**

412 The present study is the first to comprehensively examine the influence of BA
413 supplementation on the electrically-evoked contractile properties of human skeletal muscle *in*
414 *vivo*. BA supplementation had no effect on the force-frequency relationship, evaluated during
415 submaximal muscle stimulation. Similarly, BA did not influence the EMD, explosive force

416 (at 25 and 50 ms), peak force or TPT of resting twitch, potentiated twitch or octet
417 contractions elicited by supramaximal stimulation of the femoral nerve. In line with these
418 findings, there were no changes in maximum or explosive voluntary force production
419 following BA supplementation. The only significant effect of BA was a 12% and 7%
420 reduction in HRT during resting and potentiated twitch contractions.

421

422 *Knee extensor intrinsic contractile properties*

423 The force-frequency relationship of the knee extensors was evaluated during submaximal
424 muscle stimulation at a range of frequencies (1-100 Hz) in order to evaluate potential effects
425 of BA supplementation on calcium handling and sensitivity, since an association between
426 intracellular calcium levels and force production in response to different stimulation
427 frequencies has previously been shown (5). BA supplementation, however, had no effect on
428 knee extensor force production at relatively low (1-15 Hz) or high (20-80 Hz) frequencies of
429 muscle stimulation, corresponding to relatively low (19 – 53% force at 100 Hz) and high (63
430 – 95% force at 100 Hz) levels of force. Previous *in vitro* research showed that increasing
431 cytoplasmic carnosine levels from those normally present to levels approaching those
432 attained after supplementation produced a marked enhancement in Ca^{2+} sensitivity (*i.e.*, an
433 increased force response to submaximal Ca^{2+} levels) of fibres from human *m. vastus lateralis*,
434 as well as enhanced Ca^{2+} release in type I fibres (11). Thus the present data showing no effect
435 of BA supplementation on the force-frequency relationship, the *in vivo* analogue of the force-
436 calcium concentration relationship (5; 27), responses might therefore be taken to imply that
437 supplementation did not grossly influence Ca^{2+} sensitivity (10; 11) or Ca^{2+} release (11; 33).

438

439 The present force-frequency data are supported by the findings that force and contraction
440 time responses to supramaximal nerve stimulation at low- (resting and potentiated twitch) and
441 high-frequencies (300 Hz, octet) were not affected by BA supplementation. Improved Ca^{2+}
442 sensitivity or release would be expected to be particularly beneficial in situations where
443 calcium saturation is submaximal; during resting twitch contractions evoked by a single nerve
444 impulse, for example. Combined evaluation of resting and potentiated twitch responses might
445 have been expected to reveal any influence of BA supplementation on these processes, since
446 the mechanisms for potentiation include the phosphorylation of myosin, which increases the

447 sensitivity of the contractile elements to Ca^{2+} , as well as altered Ca^{2+} handling (39). However,
448 neither peak force, TPT nor the rate of force development (force at 25 and 50 ms) of resting
449 and potentiated twitches were affected by BA supplementation. Similarly, the resting and
450 potentiated twitch EMD, which reflects the time for excitation-contraction coupling processes
451 and for muscle shortening to remove slack from the muscle tendon unit (29; 31), was
452 unaltered following BA supplementation. These data further imply that BA supplementation
453 had little influence on Ca^{2+} sensitivity or release.

454

455 The current data appear at odds with the human single fibre data mentioned above (11), and
456 recent findings in mouse muscle where 10-31% increases in force were shown at frequencies
457 between 25-125 Hz, but not at 1 Hz, following BA supplementation (13). Several factors
458 could explain the present results and apparent contrast with the previous data. Firstly, there
459 are obvious differences with the study of Dutka et al. (2012), including the manner by which
460 they increased carnosine levels (acute exposure to a carnosine containing solution), the
461 conditions of the muscle (skinned fibres devoid of connective tissue and not attached to bone)
462 and the manner in which it was activated (exposure to Ca^{2+} buffered solutions), all of which
463 bear little resemblance to the present *in vivo* study. Secondly, although enhanced Ca^{2+} -
464 induced Ca^{2+} release was observed following exposure to carnosine in single fibre
465 preparations (11), the authors concede that this may not occur *in vivo*, since this mechanism
466 might have limited relevance to the control of Ca^{2+} release through ryanodine receptors by
467 the dihydropyridine receptors (12; 25). Thirdly, species differences in carnosine metabolism
468 and histidine-containing dipeptide content (4; 13) could explain the discrepancy between the
469 data of Everaert et al. (2013) in mice and the data from the present study. The potential for
470 inter-species differences is suggested by the fact that previous human data showed no fibre-
471 type differences in the carnosine-related changes in Ca^{2+} sensitivity (11), whilst there was
472 some suggestion of fibre type differences in mice (*i.e.*, differences in the response of “slow”
473 *soleus* versus “fast” *extensor digitorum longus* muscles to BA supplementation) (13). It
474 should be noted that we did not measure muscle carnosine content in the present study and so
475 we cannot confirm the actual change due to BA supplementation or whether this directly
476 relates to the individual responses in muscle contractile properties. It is likely, given the
477 previous data on the topic [e.g. (19)], that the increase in muscle carnosine would be around
478 15 mmol.kg^{-1} dry muscle or +65% in these participants, with this supplementation regimen.

479

480 Whilst the majority of the evoked contractile properties showed no change in response to BA
481 supplementation, HRT decreased by 7-12% during resting and potentiated twitch
482 contractions. Muscle relaxation is initiated by a reduction in sarcoplasmic reticulum Ca^{2+}
483 concentration. The rate of relaxation may be influenced by: (i) the rate of dissociation of Ca^{2+}
484 from troponin (26); (ii) the rate of translocation of Ca^{2+} to a site close to the sarcoplasmic
485 reticulum (28); and (iii) the rate of re-uptake of Ca^{2+} into the sarcoplasmic reticulum by
486 ATPase driven Ca^{2+} pumps (30). At present, there do not appear to be any reports of
487 carnosine influencing these aspects of excitation-contraction coupling. Interestingly,
488 however, Everearts et al. (2013) reported an attenuation of the fatigue-related increases in
489 relaxation times after BA supplementation in murine soleus muscle. Whilst their finding in
490 this case could be a consequence of enhanced buffering capacity, during the repeated
491 contractions, since BA supplementation had no influence on resting rate of relaxation, their
492 report further highlight the functional implications of the present data. During fatigue, the
493 rate of muscle relaxation slows as a consequence of a reduced rate of cross-bridge
494 dissociation or impaired Ca^{2+} pumping into the sarcoplasmic reticulum (2). The latter is
495 energetically costly (30) and, as such, any improvements in Ca^{2+} handling with BA
496 supplementation could reduce the total energy expenditure during high-intensity cyclic joint
497 movements by reducing that energy cost, and also by improving the efficiency of joint
498 movements by reducing co-contraction. Future research should attempt to confirm the
499 present findings and extend them by investigating the changes in evoked contractile
500 properties during fatigue in order to better understand the influence of BA supplementation
501 on muscle contractility and implications for metabolic and movement efficiency during
502 exercise.

503

504 *Voluntary force production and motor control*

505 BA supplementation had no effect on MVF, a finding consistent with the lack of changes in
506 electrically-evoked twitch or tetanic (octet) peak force in the present study. Maximum
507 isometric force is not affected by either increased Ca^{2+} sensitivity or increased myoplasmic
508 Ca^{2+} concentration (27), and previous studies reported minimal effects of carnosine on
509 maximum calcium activated force (0-3% increase) (11) and of BA supplementation on
510 maximal twitch and tetanic force (13). Improved Ca^{2+} sensitivity or release would be

511 expected to be beneficial for force production in situations where calcium saturation is
512 submaximal [*e.g.*, during the rising phase of voluntary force production where neuromuscular
513 activation is submaximal, (14)] and during sustained submaximal contractions. Thus, one
514 might have expected improvements in explosive voluntary force and/or alterations in the
515 force-EMG relationship, indicative of the change in neuromuscular activation required to
516 produce a given change in force, if BA supplementation had influenced these Ca²⁺ related
517 functions. However, in accordance with the lack of changes in the force-frequency
518 relationship, as well as the force responses during twitch and octet contractions, BA
519 supplementation did not influence voluntary explosive force or the force-EMG relationship.
520 The similar neural drive during both the maximum voluntary contractions and explosive
521 voluntary contractions confirm that the force results were not confounded by changes in
522 neuromuscular activation over time.

523

524 *Conclusions*

525 The results of the present study showed that BA supplementation had no effect on the force-
526 frequency relationship, implying a lack of any effect on muscle Ca²⁺ sensitivity or release. In
527 support of these data, there was no effect of BA supplementation on force responses to
528 resting and potentiated twitches and octet contractions. As such, the study findings do not
529 support the idea that exercise performance and capacity improvements after BA
530 supplementation are due to enhanced Ca²⁺ sensitivity or release. We do, however, show a
531 reduction in HRT with BA supplementation, which might possibly be explained by enhanced
532 reuptake of Ca²⁺ into the sarcoplasmic reticulum. This has potentially important implications
533 for the efficiency of muscle contraction following BA that should be explored in future
534 studies, since this could conceivably contribute to the ergogenic potential of BA
535 supplementation during high-intensity exercise involving rapid muscle contractions.

536

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545

546

Reference List

547

548 1. **Abe H.** Role of histidine-related compounds as intracellular proton buffering
549 constituents in vertebrate muscle. *Biochemistry (Mosc)* 65: 757-765, 2000.

550 2. **Allen DG, Lamb GD and Westerblad H.** Impaired calcium release during fatigue. *J*
551 *Appl Physiol (1985)* 104: 296-305, 2008.

552 3. **Allman BL and Rice CL.** An age-related shift in the force-frequency relationship
553 affects quadriceps fatigability in old adults. *J Appl Physiol (1985)* 96: 1026-1032,
554 2004.

555 4. **Baguet A, Everaert I, De NH, Reyngoudt H, Stegen S, Beeckman S, Achten E,**
556 **Vanhee L, Volkaert A, Petrovic M, Taes Y and Derave W.** Effects of sprint training
557 combined with vegetarian or mixed diet on muscle carnosine content and buffering
558 capacity. *Eur J Appl Physiol* 111: 2571-2580, 2011.

559 5. **Balnave CD and Allen DG.** The effect of muscle length on intracellular calcium and
560 force in single fibres from mouse skeletal muscle. *J Physiol* 492 (Pt 3): 705-713, 1996.

561 6. **Buckthorpe MW, Hannah R, Pain TG and Folland JP.** Reliability of neuromuscular
562 measurements during explosive isometric contractions, with special reference to
563 electromyography normalization techniques. *Muscle Nerve* 46: 566-576, 2012.

564 7. **Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE,**
565 **Pratt M, Ekelund U, Yngve A, Sallis JF and Oja P.** International physical activity

- 566 questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381-1395,
567 2003.
- 568 8. **de Ruiter CJ, Kooistra RD, Paalman MI and de Haan A.** Initial phase of maximal
569 voluntary and electrically stimulated knee extension torque development at different
570 knee angles. *J Appl Physiol* 97: 1693-1701, 2004.
- 571 9. **Decombaz J, Beaumont M, Vuichoud J, Bouisset F and Stellingwerff T.** Effect of
572 slow-release beta-alanine tablets on absorption kinetics and paresthesia. *Amino Acids*
573 43: 67-76, 2012.
- 574 10. **Dutka TL and Lamb GD.** Effect of carnosine on excitation-contraction coupling in
575 mechanically-skinned rat skeletal muscle. *J Muscle Res Cell Motil* 25: 203-213, 2004.
- 576 11. **Dutka TL, Lambolely CR, McKenna MJ, Murphy RM and Lamb GD.** Effects of
577 carnosine on contractile apparatus Ca(2)(+) sensitivity and sarcoplasmic reticulum
578 Ca(2)(+) release in human skeletal muscle fibers. *J Appl Physiol (1985)* 112: 728-736,
579 2012.
- 580 12. **Endo M.** Calcium-induced calcium release in skeletal muscle. *Physiol Rev* 89: 1153-
581 1176, 2009.
- 582 13. **Everaert I, Stegen S, Vanheel B, Taes Y and Derave W.** Effect of beta-alanine and
583 carnosine supplementation on muscle contractility in mice. *Med Sci Sports Exerc* 45:
584 43-51, 2013.

- 585 14. **Folland JP, Buckthorpe MW and Hannah R.** Human capacity for explosive force
586 production: Neural and contractile determinants. *Scand J Med Sci Sports* 2013.
- 587 15. **Haider G and Folland JP.** Nitrate Supplementation Enhances the Contractile
588 Properties of Human Skeletal Muscle. *Med Sci Sports Exerc* 2014.
- 589 16. **Hannah R, Minshull C, Buckthorpe MW and Folland JP.** Explosive neuromuscular
590 performance of males versus females. *Exp Physiol* 97: 618-629, 2012.
- 591 17. **Hannah R, Minshull C and Folland JP.** Whole-body vibration does not influence
592 knee joint neuromuscular function or proprioception. *Scand J Med Sci Sports* 23: 96-
593 104, 2013.
- 594 18. **Hannah R, Minshull C, Smith SL and Folland JP.** Longer Electromechanical Delay
595 Impairs Hamstrings Explosive Force versus Quadriceps. *Med Sci Sports Exerc* 46: 963-
596 972, 2014.
- 597 19. **Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL,**
598 **Hill CA, Sale C and Wise JA.** The absorption of orally supplied beta-alanine and its
599 effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids* 30: 279-
600 289, 2006.
- 601 20. **Harris RC, Wise JA, Price KA, Kim HJ, Kim CK and Sale C.** Determinants of
602 muscle carnosine content. *Amino Acids* 43: 5-12, 2012.

- 603 21. **Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK and Wise**
604 **JA.** Influence of beta-alanine supplementation on skeletal muscle carnosine
605 concentrations and high intensity cycling capacity. *Amino Acids* 32: 225-233, 2007.
- 606 22. **Hobson RM, Saunders B, Ball G, Harris RC and Sale C.** Effects of beta-alanine
607 supplementation on exercise performance: a meta-analysis. *Amino Acids* 43: 25-37,
608 2012.
- 609 23. **KALYANKAR GD and MEISTER A.** Enzymatic synthesis of carnosine and related
610 beta-alanyl and gamma-aminobutyryl peptides. *J Biol Chem* 234: 3210-3218, 1959.
- 611 24. **Kendrick IP, Kim HJ, Harris RC, Kim CK, Dang VH, Lam TQ, Bui TT and Wise**
612 **JA.** The effect of 4 weeks beta-alanine supplementation and isokinetic training on
613 carnosine concentrations in type I and II human skeletal muscle fibres. *Eur J Appl*
614 *Physiol* 106: 131-138, 2009.
- 615 25. **Lamb GD, Cellini MA and Stephenson DG.** Different Ca²⁺ releasing action of
616 caffeine and depolarisation in skeletal muscle fibres of the rat. *J Physiol* 531: 715-728,
617 2001.
- 618 26. **Little SC, Tikunova SB, Norman C, Swartz DR and Davis JP.** Measurement of
619 calcium dissociation rates from troponin C in rigor skeletal myofibrils. *Front Physiol* 2:
620 70, 2011.
- 621 27. **MacIntosh BR and Willis JC.** Force-frequency relationship and potentiation in
622 mammalian skeletal muscle. *J Appl Physiol (1985)* 88: 2088-2096, 2000.

- 623 28. **Muntener M, Kaser L, Weber J and Berchtold MW.** Increase of skeletal muscle
624 relaxation speed by direct injection of parvalbumin cDNA. *Proc Natl Acad Sci U S A*
625 92: 6504-6508, 1995.
- 626 29. **Muraoka T, Muramatsu T, Fukunaga T and Kanehisa H.** Influence of tendon slack
627 on electromechanical delay in the human medial gastrocnemius in vivo. *J Appl Physiol*
628 96: 540-544, 2004.
- 629 30. **Nogueira L, Shiah AA, Gandra PG and Hogan MC.** Ca²⁺-pumping impairment
630 during repetitive fatiguing contractions in single myofibers: role of cross-bridge
631 cycling. *Am J Physiol Regul Integr Comp Physiol* 305: R118-R125, 2013.
- 632 31. **Nordez A, Gallot T, Catheline S, Guevel A, Cornu C and Hug F.** Electromechanical
633 delay revisited using very high frame rate ultrasound. *J Appl Physiol (1985)* 106: 1970-
634 1975, 2009.
- 635 32. **Rainoldi A, Melchiorri G and Caruso I.** A method for positioning electrodes during
636 surface EMG recordings in lower limb muscles. *J Neurosci Methods* 134: 37-43, 2004.
- 637 33. **Rubtsov AM.** Molecular mechanisms of regulation of the activity of sarcoplasmic
638 reticulum Ca-release channels (ryanodine receptors), muscle fatigue, and Severin's
639 phenomenon. *Biochemistry (Mosc)* 66: 1132-1143, 2001.
- 640 34. **Sale C, Artioli GG, Gualano B, Saunders B, Hobson RM and Harris RC.**
641 Carnosine: from exercise performance to health. *Amino Acids* 44: 1477-1491, 2013.

- 642 35. **Sale C, Saunders B and Harris RC.** Effect of beta-alanine supplementation on muscle
643 carnosine concentrations and exercise performance. *Amino Acids* 39: 321-333, 2010.
- 644 36. **Smith EC.** The buffering of muscle in rigor; protein, phosphate and carnosine. *J*
645 *Physiol* 92: 336-343, 1938.
- 646 37. **Tillin NA, Jimenez-Reyes P, Pain MT and Folland JP.** Neuromuscular performance
647 of explosive power athletes versus untrained individuals. *Med Sci Sports Exerc* 42: 781-
648 790, 2010.
- 649 38. **Tillin NA, Pain MT and Folland JP.** Identification of contraction onset during
650 explosive contractions. Response to Thompson et al. "Consistency of rapid muscle
651 force characteristics: influence of muscle contraction onset detection methodology" [*J*
652 *Electromyogr Kinesiol* 2012;22(6):893-900]. *J Electromyogr Kinesiol* 23: 991-994,
653 2013.
- 654 39. **Vandenboom R, Gittings W, Smith IC, Grange RW and Stull JT.** Myosin
655 phosphorylation and force potentiation in skeletal muscle: evidence from animal
656 models. *J Muscle Res Cell Motil* 34: 317-332, 2013.
- 657 40. **WINNICK T and WINNICK RE.** Pathways and the physiological site of anserine
658 formation. *Nature* 183: 1466-1468, 1959.

659

660 **Figure legends**

661

662 **Figure 1.** Electrically-evoked force of BA and PLA groups pre- and post-supplementation:
663 (A), resting twitch force; (B), potentiated twitch force; (C), octet force. Data are mean \pm 1SD.

664 **Figure 2.** Contraction times during twitch contractions evoked via femoral nerve stimulation
665 for BA and PLA groups pre- and post-supplementation. Resting twitch: EMD (A); TPT (B);
666 and HRT (C). Potentiated twitch: EMD (D); TPT (E); and HRT (F). Data are mean \pm 1SD.
667 $**P \leq 0.01$ and $*P \leq 0.05$ for *post-hoc* independent *t*-test on % change values in BA and PLA
668 groups.

669 **Figure 3.** Force-frequency relationship assessed during submaximal percutaneous stimulation
670 for BA and PLA groups pre- and post-supplementation. Data are mean \pm 1SD.

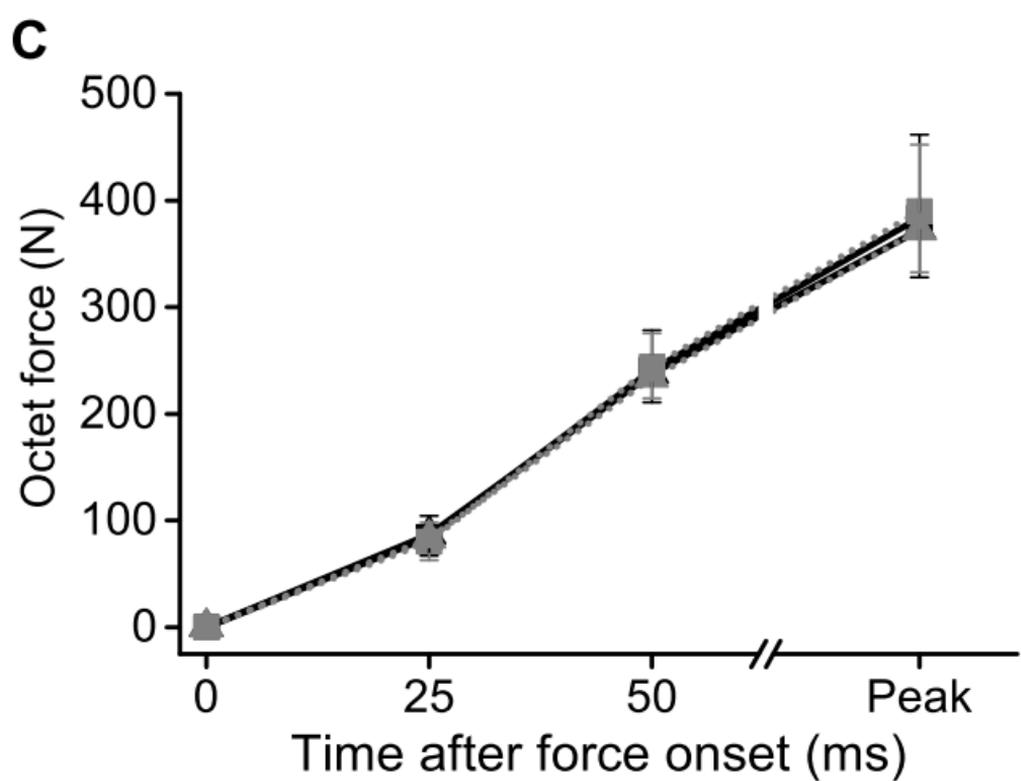
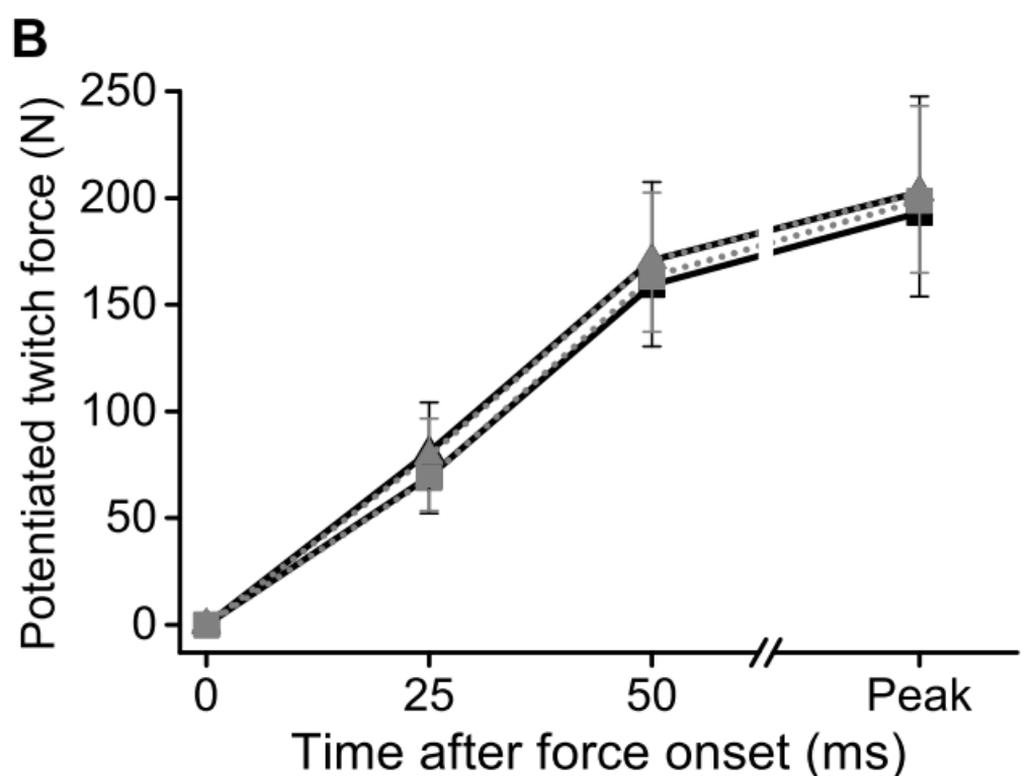
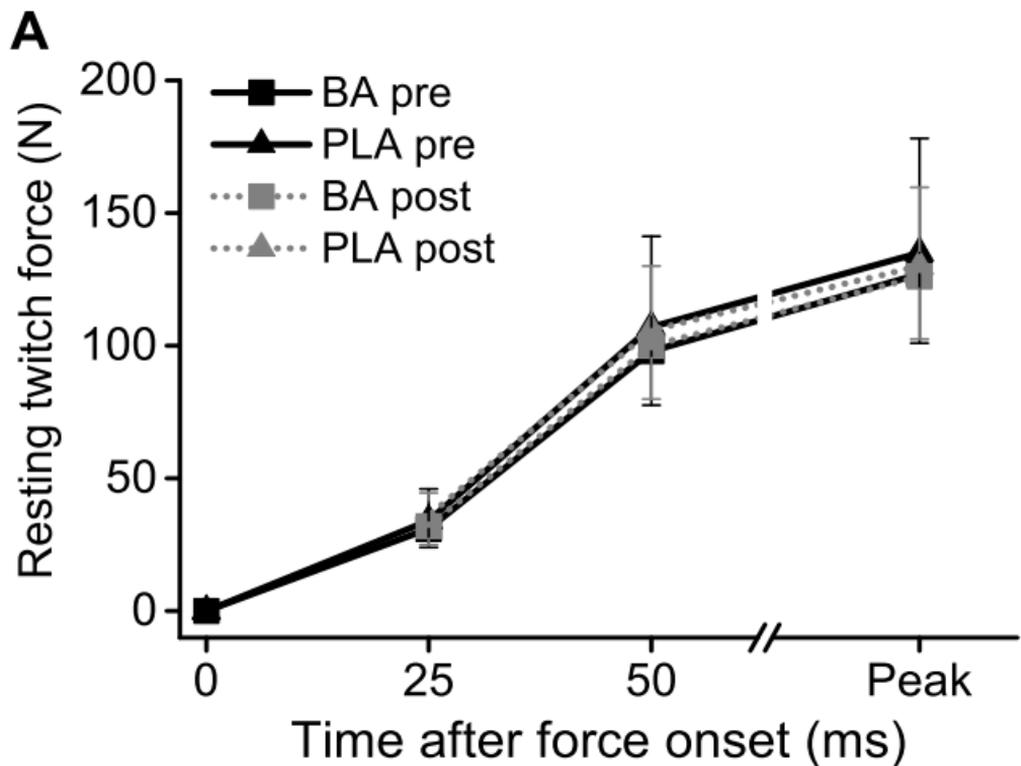
671 **Figure 4.** Explosive and maximum voluntary force of BA and PLA groups pre- and post-
672 supplementation (A). Agonist EMG normalised to M_{\max} during explosive contractions (0-50,
673 50-100 and 100-150 ms from onset) and at MVF for the BA and PLA groups pre- and post-
674 supplementation (B). Data are mean \pm 1SD.

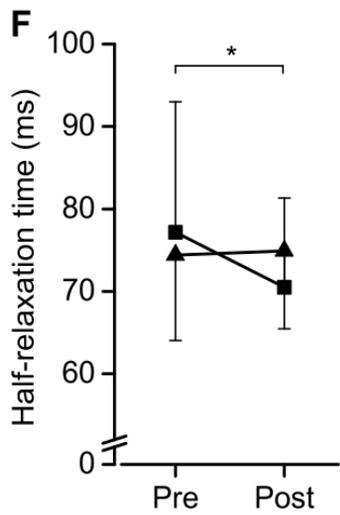
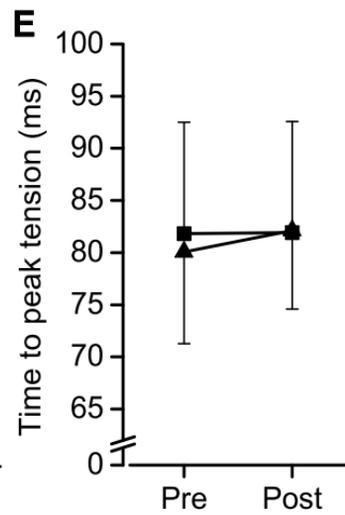
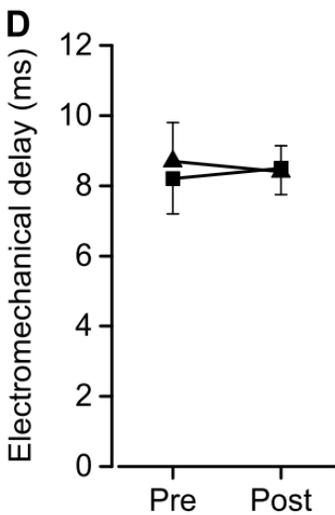
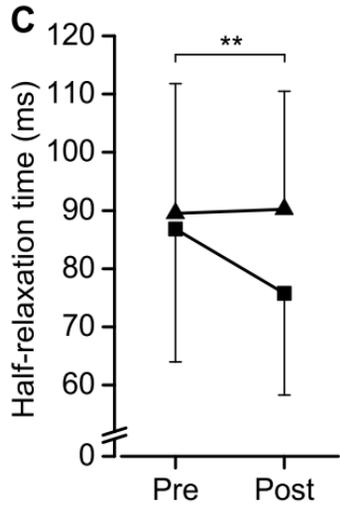
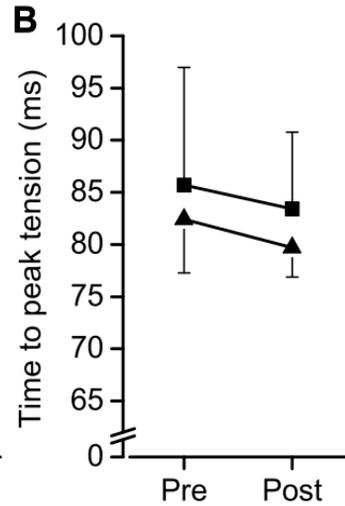
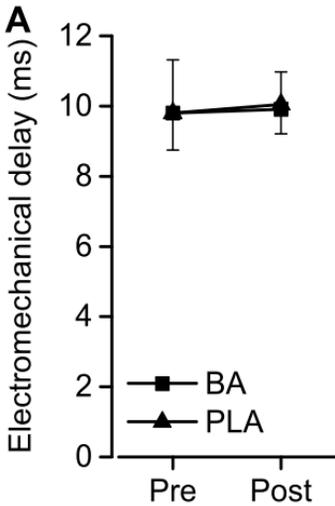
675 **Figure 5.** Force-EMG relationship measured during submaximal voluntary contractions (15 –
676 90% MVF) for BA and PLA groups pre- and post-supplementation. Data are mean \pm 1SD.

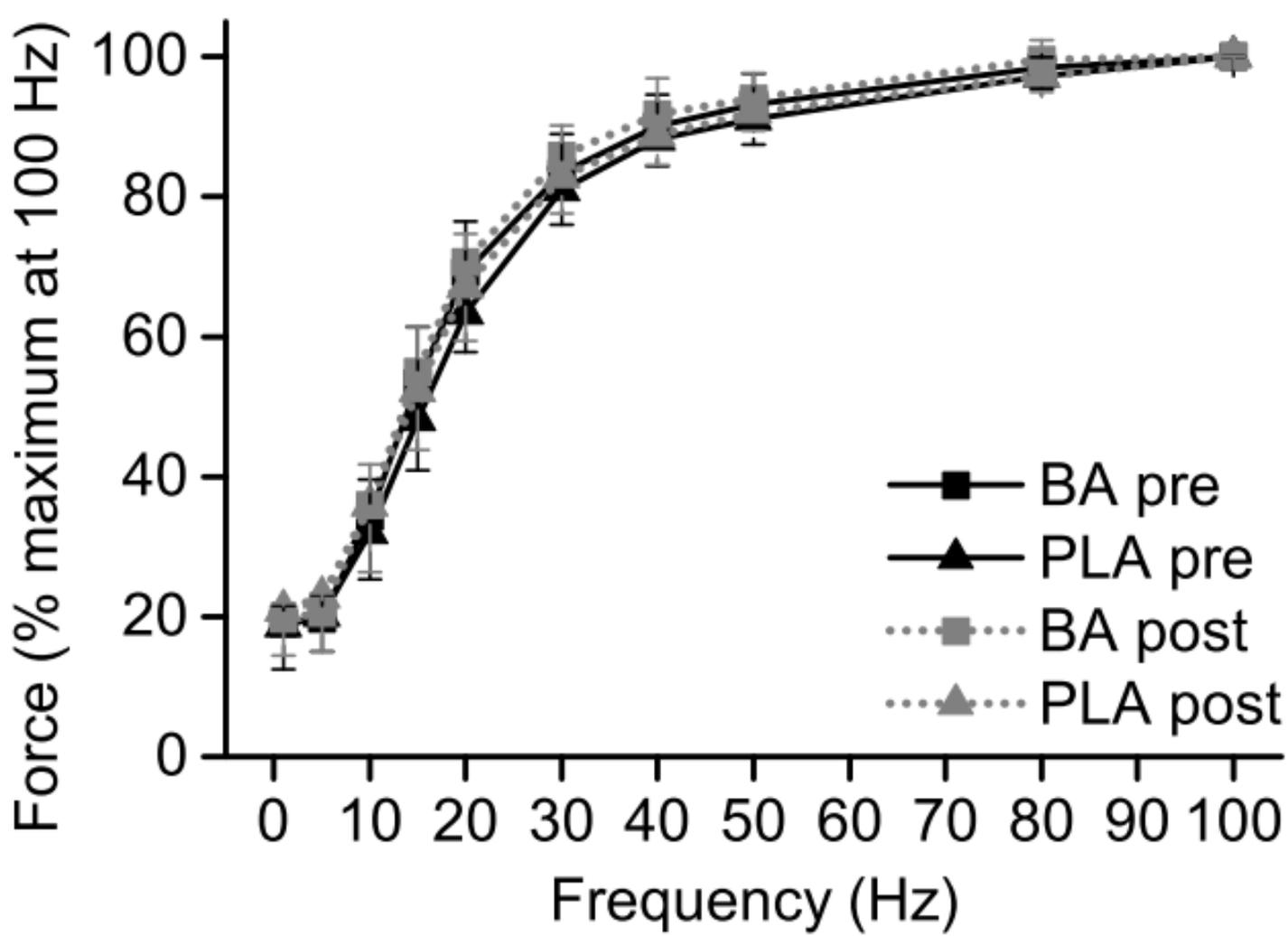
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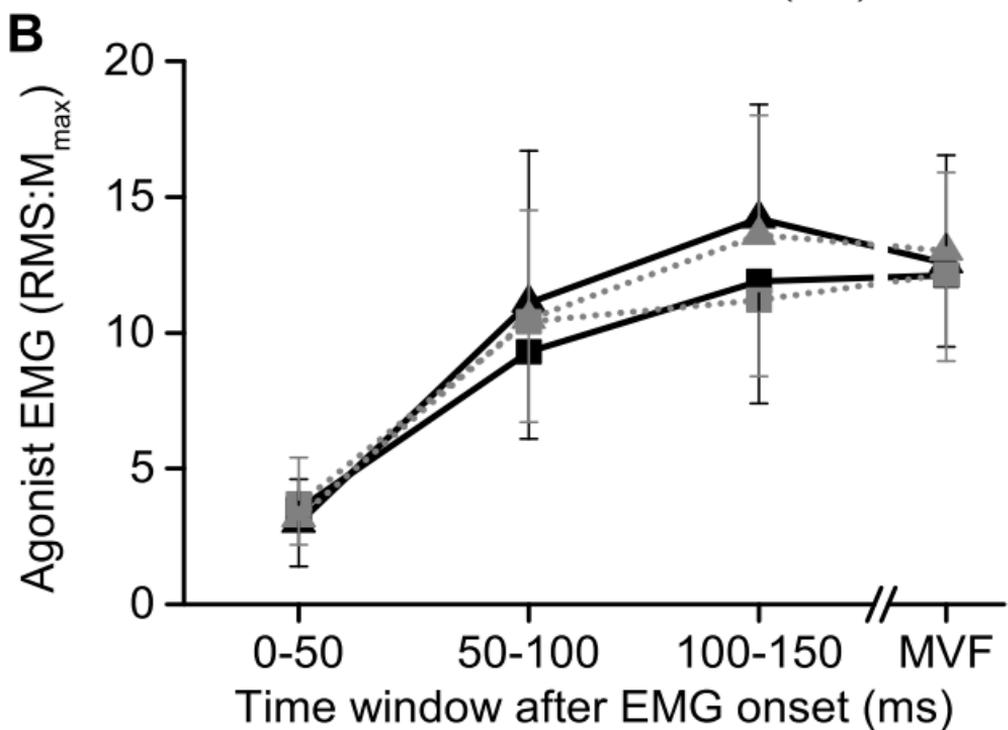
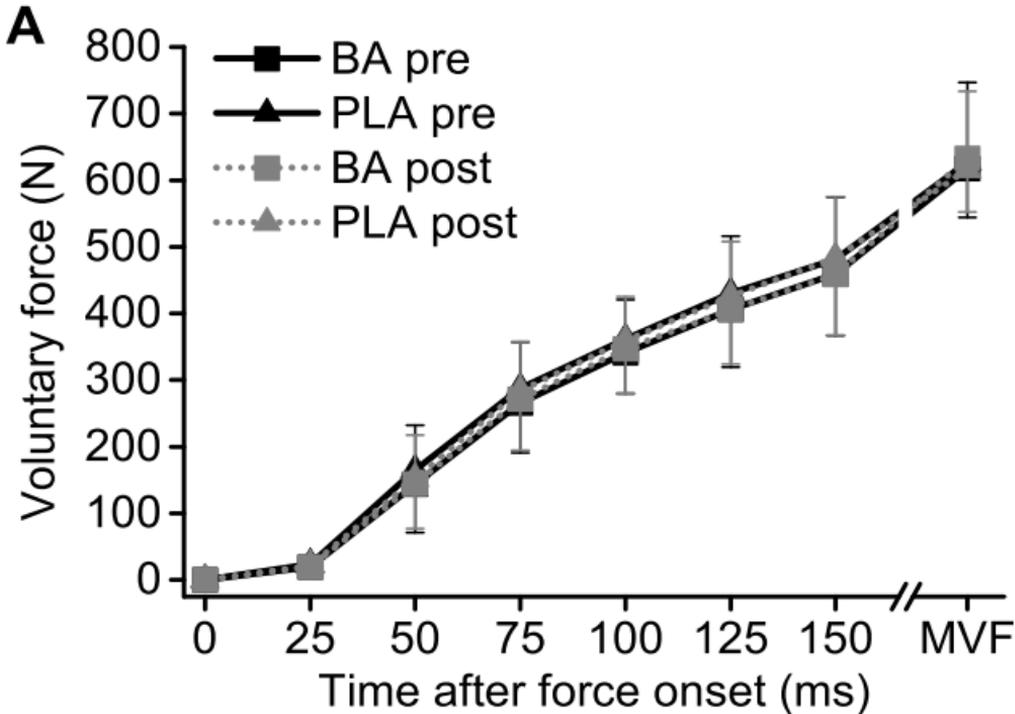
Table 1. Characteristics of the force-frequency and force-EMG relationships of BA and PLA groups pre- and post-supplementation. Data are mean \pm 1SD.

	Pre		Post	
	BA	PLA	BA	PLA
<i>Force-frequency relationship</i>				
Frequency at 50% of force response (Hz)	17.3 \pm 2.4	18.8 \pm 1.5	16.8 \pm 1.7	18.0 \pm 1.9
<i>Force-EMG relationship</i>				
Intercept (RMS: M_{\max})	-0.49 \pm 0.74	-0.70 \pm 0.47	-0.53 \pm 0.91	-0.56 \pm 0.43
Slope (RMS: $M_{\max} \cdot N^{-1}$)	0.0175 \pm 0.0054	0.0180 \pm 0.0035	0.0174 \pm 0.0037	0.0170 \pm 0.0037









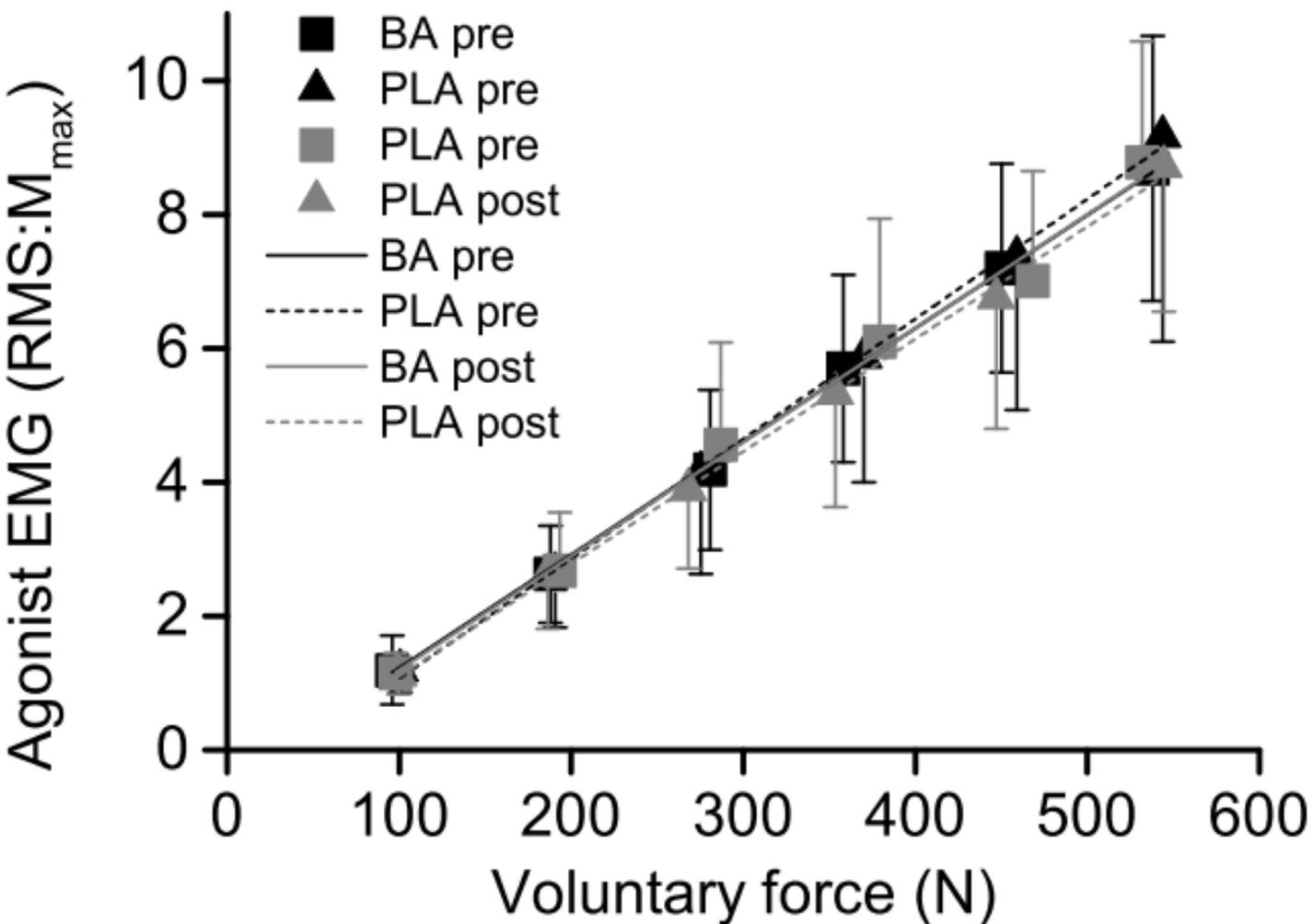


Table 1.

	Pre		Post	
	BA	PLA	BA	PLA
<i>Force-frequency relationship</i>				
Frequency at 50% of force response (Hz)	17.3 ± 2.4	18.8 ± 1.5	16.8 ± 1.7	18.0 ± 1.9
<i>Force-EMG relationship</i>				
Intercept (RMS:M _{max})	-0.49 ± 0.74	-0.70 ± 0.47	-0.53 ± 0.91	-0.56 ± 0.43
Slope (RMS:M _{max} ·N ⁻¹)	0.0175 ± 0.0054	0.0180 ± 0.0035	0.0174 ± 0.0037	0.0170 ± 0.0037