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A combination of amino acids and caffeine enhances sprint running capacity in a hot, hypoxic environment

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A combination of amino acids and caffeine enhances sprint running capacity in a hot,

hypoxic environment

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Abstract

Heat and hypoxia exacerbate central nervous system (CNS) fatigue. We therefore investigated whether essential-amino-acid (EAA) and caffeine ingestion attenuates CNS fatigue in a simulated team-sport specific running protocol in a hot and hypoxic environment. Sub-elite male team-sport athletes (n=8) performed a repeat-sprint running protocol on a non-motorized treadmill in an extreme environment on four separate occasions. Participants ingested one of four supplements: a double placebo, 3 mg.kg^{-1} body mass of caffeine+placebo, 2x7 g EAA (Musashi Create™)+placebo, or caffeine+EAA prior to each exercise session using a randomized, double-blind crossover design. Electromyography (EMG) activity and quadriceps evoked responses to magnetic stimulation were assessed from the dominant leg at pre-, half-time, and post-exercise. Central activation ratio (CAR) was used to quantify completeness of quadriceps activation. Oxygenation of the pre-frontal cortex was measured via near-infrared spectroscopy. Mean sprint work was higher (+174J, 23–24J; mean, 95% confidence interval, d=0.30; effect size, likely beneficial) in the caffeine+EAA condition versus EAAs alone, but not caffeine or a placebo. The decline in EMG activity was less (13%, 0–26%; d=0.58, likely) in caffeine+EAA versus EAA alone. Similarly, the pre-to-post exercise decrement in CAR was significantly less (-2.7%, 0.4–5.4%; d=0.50, likely) when caffeine+EAA were ingested compared to placebo. Cerebral oxygenation was lower (-5.6%, 1.0–10.1%; d=0.60, very likely) in the caffeine+EAA condition compared to LNAA alone. Co-ingestion of caffeine and EAA appears to maintain muscle activation and central drive, with a small improvement in running performance relative to EAA alone in hot, hypoxic environments.

Key words – Repeated sprint exercise, Central fatigue, Hypoxia, Heat, Amino acids, Caffeine

Introduction

Fatigue is characterized by the inability of skeletal muscle to maintain the required power output

(Allen et al. 2008), and may occur at a muscular level (peripheral fatigue) or in the central

nervous system (CNS, central fatigue). Central fatigue manifests as the inadequacy to voluntarily

activate muscles (Gandevia 2001), alterations in cognitive function, perception of effort, and

pain (Meeusen et al. 2006). The Central Governor theory highlights the importance of the CNS

and the potential role of accumulation and/or depletion of neurotransmitters in fatigue (Noakes

2011). Because neurotransmitter synthesis and function can be manipulated using nutritional

strategies, it may be possible to use these strategies to reduce central fatigue (Pardridge 1998;

Meeusen et al. 2006).

Caffeine is considered a legitimate nutritional supplement in competitive sport to delay the onset

of fatigue. Research on the impact of EAA on fatigue, however, has been equivocal (Davis et al.

1999; Watson et al. 2004; Stepto et al. 2011). EAA supplementation did not improve

performance in endurance exercise (Madsen et al. 1996; Davis et al. 1999) in normal conditions

(thermoneutral, normoxic environment), but may do so in the heat when production of dopamine

is augmented. (Mittleman et al. 1998; Tumilty et al. 2011) Reducing dopamine synthesis is

associated with a quicker onset of fatigue (Bailey et al. 1993). To our knowledge no studies have

examined the effect of EAA supplementation on exercise performance in hypoxia.

Caffeine may enhance performance in endurance exercise, (Doherty & Smith 2004; Ganio et al.

2009), high-intensity running, and sprint activity (Glaister et al. 2008; Astorino & Roberson

2010) in normal conditions. Caffeine ingestion prior to exercise can reduce ratings of perceived

70 exertion (RPE) (Doherty & Smith 2005), improve running power (Schneiker et al. 2006), and

71 decrease sprint times (Glaister et al. 2008; Gant et al. 2010). Evidence for an ergogenic effect of

72 caffeine in hypoxia is limited or equivocal when exercise is performed in the heat (Del Coso et

73 al. 2008; Roelands et al. 2011).

74

75 Exercise in the heat and/or at altitude represents a situation of maximal environmental stress that

76 could accelerate the development of central fatigue. Voluntary muscle activation is reduced after

77 exercise in the heat (Skein et al. 2012), possibly related to altered dopamine activity and

78 inhibitory signals from thermoreceptors in the hypothalamus (Nybo 2008). Similarly, voluntary

79 activation is reduced after exercising in hypoxia (Verges et al. 2012; Billaut et al. 2013) as a

80 result of cerebral deoxygenation (Smith & Billaut 2010; Billaut et al. 2013). Therefore,

81 manipulating environmental conditions in combination with nutritional interventions can provide

82 insights into novel supplementation strategies and the mechanisms of CNS fatigue.

83

84 The aim of this study was to investigate whether nutritional supplementation can attenuate

85 central fatigue and reduce performance decrements under conditions of environmental stress

86 (simulated heat and hypoxia). Although these conditions are uncommon, it provides an

87 opportunity to observe the potentially beneficial effects of nutritional manipulations under

88 standardized conditions. Nutritional supplementation, in combination with assessing markers of

89 central fatigue (e.g., muscle recruitment, central drive), will clarify the viability of invoking

90 changes in neurotransmitter concentration in relation to central fatigue. We hypothesized that

91 central fatigue and decrements in intermittent-exercise performance induced by environmental

92 stress would be attenuated by targeted nutritional supplementation strategies utilizing a

93 combination of both caffeine and EAAs.

94

95 **Materials and Methods**

96 **Participants**

97 Ten male, sub-elite football players regularly competing and training (~60 min, 3 times per

98 week) volunteered for the study (Table 1). Two athletes were unable to complete the study, due

99 to issues and/or injuries unrelated to the study. Prior to participation, each subject provided

100 written informed consent for their voluntary participation in the study. The study was approved

101 by the local Human Research Ethics Committee.

102

103 **Participant characterisation/familiarisation**

104 Participants reported to the laboratory 10-14 days prior to the experimental protocols on two

105 occasions. On the first visit, age, height and body mass were recorded, body composition

106 assessed by a whole body light scanner ([TC]^2, Cary, U.S.A., (Ryder & Ball 2012)), and

107 participants were familiarized with the exercise protocol. On the second visit, participants

108 performed a 20-m sprint followed, 30 min later, by the Yo-Yo intermittent recovery test (Mohr et

109 al. 2003).

110

111 **Diet and exercise control**

112 Two days prior to each experimental trial participants consumed a moderate-high carbohydrate

113 diet (4 g.kg^{-1} body mass) that provided ~106 kJ.kg^{-1} body mass per day (70% CHO, 15% protein

114 and 15% fat), while controlling protein intake in line with Australian dietary recommendations.

115   Participants were also instructed to abstain from any caffeinated products, alcohol and formal

116   exercise training 24 h prior to testing.

117

118   **Study design**

119   In each session, after voiding, and being weighed, athletes consumed the initial supplement

120   (EAA or Placebo) and remained in the laboratory at normoxic and thermoneutral conditions for

121   the first 2 hours prior to exercise (Figure 1). At the end of this period, one hour before exercise,

122   athletes consumed the second dose of supplements (caffeine, placebo and/or EAA) and rested in

123   the environmental exercise laboratory (15% O_2 [~2700m above sea level], 30°C and 20% relative

124   humidity). Although this combination of conditions is uncommon, in research studies it provides

125   a unique opportunity to invoke central fatigue under standardized conditions and observe the

126   potentially-beneficial effects of nutritional manipulations.

127

| Table 1: Participant characteristics	
Age	22 ± 2
Height (cm)	181 ± 6
Body mass (kg)	77 ± 11
Estimated body fat (%)	14.7 ± 4.4
20m sprint time (sec)	3.1 ± 0.1
Yo-Yo IR1 distance (m)	1320 ± 528
Estimated VO2 max (ml/kg.min^{-1})	47.5 ± 4.4

128

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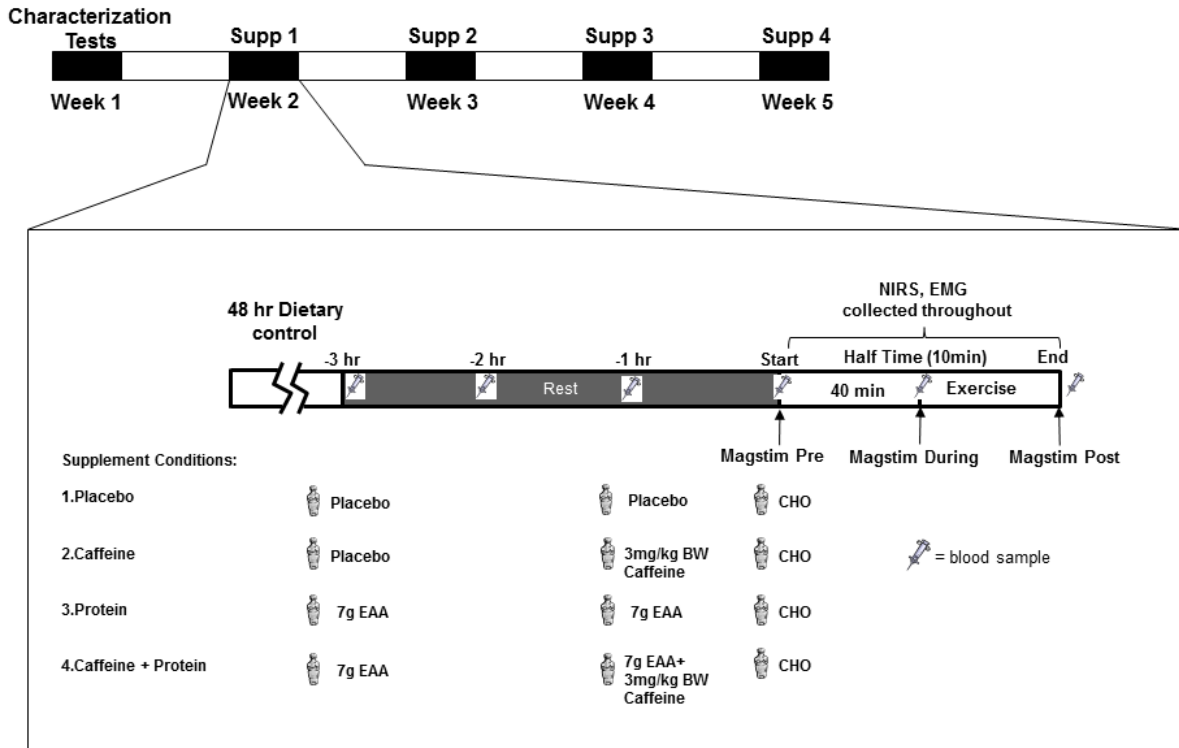
130
131 **Figure 1:** Schematic diagram of the study design. Supp = Supplement, Magstim = Central

132 activation and MVC assessment, BW = Body Weight.

133

134 **Exercise Protocol**

135 The exercise protocol (Figure 2) was designed to mimic the running profile of team-sport

136 athletes (Sirotic & Coutts 2008; Zois et al. 2013). During each session, participants completed a

137 total of 6 sets of 4 s sprints with 8 s of rest between each sprint on a non-motorized treadmill

138 (Woodway Force, Waukesha, U.S.A.). The time between each sprint set was interspersed with

139 standing (22% of total time), walking (27%), jogging (22%), running (12%) and fast running

140 (9%). Mean power, peak power and peak velocities during the exercise protocol were recorded

141   by the treadmill force transducer at 25 Hz. Performance data were stored and analyzed as

142   described previously (Serpiello et al. 2011).
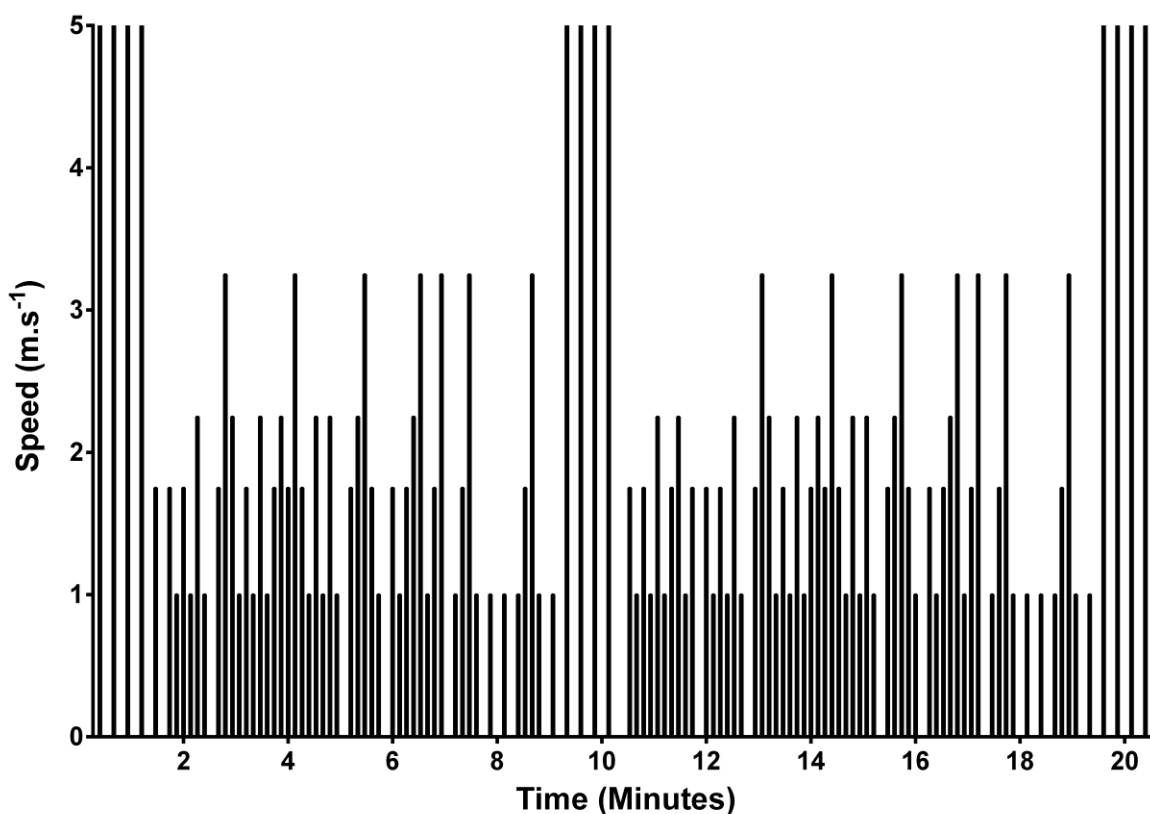
143

144   **Figure 2:** Graphical representation of one half of the exercise protocol, including 3 x 4 x 4

145   second sprints and intermittent activity for participants with a maximal sprint speed of 4.8 – 5.2

146   m.s^{-1}. This protocol was completed twice with a 10 minute break in-between to simulate half-

147   time.

148

149   **Supplementation regimens**

150   Four supplement protocols were undertaken in a counterbalanced, randomized and double-blind

151   fashion to provide the participants with a placebo, caffeine only, EAA only, or caffeine + EAA

152   (Figure 1). The EAA was provided in two 7 g doses of Musashi Create[TM] powder, dissolved in

153 250 ml of water containing; arginine (854 mg), leucine (837 mg), phenylalanine (794 mg), lysine

154 (709 mg), valine (598 mg), histidine (555 mg), isoleucine (512 mg), threonine (418 mg) and

155 methionine (162 mg), to reduce serotonin, and increase dopamine production within the CNS.

156 Musashi also provided an inactive placebo that was indistinguishable in color and taste.

157 Anecdotally, participants were unable to correctly identify any of the treatments. The EAAs or

158 placebos were given to the participants 3 h and 1 h prior to commencing exercise trials. Caffeine

159 (to block the action of adenosine; No-Doz^{TM}; 3 mg.kg^{-1} body mass) or placebo ($CaCO_3$) was

160 given to participants as a capsule (blinded) 1 h before exercise. Immediately before the warm-up,

161 participants also consumed a carbohydrate gel (27 g; Powerbar Gel™, Musashi Performance

162 Nutrition, Nestle, Australia) to ensure adequate provision of carbohydrate (Figure 1).

163

164 **Blood sampling**

165 Six 5ml blood samples were taken at regular intervals in each trial (Figure 1). Baseline blood

166 samples (3 h pre-) were taken prior to the ingestion of the initial supplement. Blood samples

167 were analyzed immediately for partial pressure of oxygen (pO_2), partial pressure of carbon

168 dioxide (pCO_2), oxygen saturation (sO_2) (Siemens, Henkestrasse, Germany) and lactate and

169 glucose concentrations (YSI, Yellow Springs, U.S.A).

170

171 **Central fatigue assessment**

172 The knee extension protocol used has been described in detail elsewhere (Billaut et al. 2013).

173 Before, at half-time, and immediately after exercise, participants were instructed to perform a

174 maximum voluntary contraction (MVC) of the quadriceps (dominant leg) against the strain

175 gauge. In this position, a magnetic stimulator (Magstim RAPID; JLM Accutek Healthcare,

176 NSW) and a double 70-mm coil (producing two overlapping circular fields) were used to

177 stimulate each participant's quadriceps during the MVC (Amann et al. 2007; Billaut et al. 2013).

178 The central activation ratio (CAR) was calculated as described previously, where

179 CAR=MVC/(MVC + superimposed twitch) (Gandevia 2001).

180

181 **Surface electromyography (EMG) acquisition and analysis**

182 EMG signals were recorded via skin surface electrodes over the vastus lateralis, rectus femoris,

183 tibialis anterior, biceps femoris, gluteus maximus and lateral gastrocnemius of the dominant

184 lower limb. In each trial, electrodes were secured to the skin with tape and an elastic tubing to

185 prevent cable movements. In the first trial, electrode placement was marked with a permanent

186 marker to ensure that the position of the electrodes was replicated on subsequent visits.

187 EMG signals were collected using the Delsys system (Delsys Inc., Boston, MA) at 2 kHz after

188 being filtered with a 50-Hz line filter. Muscle activity was quantified as the integrated EMG of

189 the signal between the onset and offset of activation of each burst in the last 2 s of every 4 s

190 sprint. EMG signal from all muscles were added together to provide a value representative of

191 overall muscle activation in the lower limb. Data were normalized to the first sprint set to

192 provide a percentage change for total electrical activity to account for a potential

193 disproportionate effect of the first sprint.

194

195 **Near-Infrared Spectroscopy (NIRS) measurement and analysis**

196 Brain oxygenation was measured using NIRS (Artinis Medical Systems, AS Zetten,

197 Netherlands). The protocol allowed the calculation of the tissue saturation index (TSI) for

198 cerebral tissue (Smith & Billaut 2010; Billaut et al. 2013).

199 **Plasma amino acid analysis**

200 Concentrations of the free amino acids histidine, arginine, threonine, lysine, methionine, valine,

201 isoleucine, leucine, phenylalanine, and tryptophanin in plasma were determined using reversed-

202 phase high-performance liquid chromatography (RP-HPLC) as previously reported (Stepto et al.

203 2011). The limit of quantitation for this study was $0.5\mu mol.L^{-1}$ with intra- and inter-assay

204 variability of 4.5% and 5.1%, respectively, for tryptophan, and <2.3% and 3.8%, respectively, for

205 the other EAAs.

206

207 **Caffeine analysis**

208 Plasma caffeine concentrations were assessed via RP-HPLC (GBC Scientific HPLC system,

209 GBC Scientific, Victoria, Australia) according to the method of Alvi & Hammami (2011). The

210 internal standard was antipyrine in 30% $HClO_4$ [40 µg/ml]) A 5µm, 4.6x150mm RP C18 column

211 and a 5µm, 4.6x20mm Guard Cartridge insert (Water Atlantis$^{®}$ T3) were used for the separation.

212 The mobile phase consisted of 15 mM potassium phosphate (pH 3.5) and acetonitrile (83:17,

213 v/v), The analysis was performed under isocratic conditions using a flow rate of 1.0 $mL.min^{-1}$.

214 Chromatograms were recorded at 274 nm with a run time of 10 min. The data were collected

215 with a personal computer using EZChrom Elite Chromatography Manager Software and plasma

216 caffeine concentration determined using peak area ratio (caffeine to antipyrine). The assays limit

217 of quantification in plasma was 0.05 $mg.L^{-1}$ with an intra- and inter- assay variability of 2.5%

218 and 5.6% respectively.

219

220

221

222   **Statistical analysis**

223   Data for the 8 participants are expressed as mean ± SD (see Supplementary Tables 1-4). All data

224   were analyzed using a linear mixed model of the estimated true difference between

225   supplementation protocols with Bonferroni *post hoc* adjustments used where appropriate.

226   Estimated mean differences for the fixed effects of time and supplementation strategy, and the

227   interaction of both (time × supplement) were determined for each parameter. Precision of

228   estimation was indicated with 95% confidence intervals (95%CI). Magnitudes of difference

229   between treatments were determined using the standardized mean difference (effect size), the

230   within-subject placebo standard deviation, and are expressed as Cohen's *d*. Chances of the true

231   value of the effect being possibly (≥75%), likely (≥95%), very likely (≥99.5%), or most likely

232   (=100%), were calculated according to established criteria (Hopkins et al. 2009), with clinical

233   chances of benefit or harm considered for running performance variables. An n=8 subjects

234   provided adequate statistical power in a post-only cross-over design assuming a reference

235   difference (between treatments) in work done of 7%, a typical error in repeat sprints of 4.5%,

236   and Type I and II errors of 5% and 20% respectively.

237

238   **Results**

239   **Running performance**: Participants ingesting a combination of caffeine + EAAs completed

240   more mean sprint work (+174 J, 23–324 J; mean difference, 95%CI; *d*=0.30, likely beneficial ;

241   Table 2) and mean sprint power (43 W, 6–81 W; *d*=0.30, likely beneficial), and had higher peak

242   sprint velocity (0.17 m.s^{-1}, 0.04–0.31 m.s^{-1}; *d*=0.50, very likely beneficial) compared to EAA's

243   alone (Table 2). Mean sprint work, power and velocity were greater in set 1 compared to all

244   other sets (Supplementary Table 1). There was no interaction effect between time and

245   supplement for any running performance variables. Total work performed excluding the sprints

246   showed no significant differences between supplementation protocols.

247

Table 2: Comparison of mechanical data between supplementation strategies. Data are mean ± SD

	Placebo	Caffeine	LNAA	Caffeine + LNAA
Mean Sprint Work (J)	2705 ± 581	2791 ± 604	2678 ± 664	2858 ± 763*
Mean Sprint Power (W)	676 ± 145	698 ± 151	669 ± 166	715 ± 191*
Peak Sprint Velocity (m.s^{-1})	5.78 ± 0.34	5.83 ± 0.35	5.76 ± 0.44	5.94 ± 0.37*
Total Work (Without Sprints) (J)	104894 ± 18359	108638 ± 15593	105047 ± 14985	103086 ± 18698

* denotes statistical significant difference from LNAA where p ≤ 0.05

248

249   **Blood parameters**: All supplementation strategies were effective in elevating protein and

250   caffeine concentration (Figure 3, Supplementary table 2).

251

252   **EAA**: In particular, supplementing with EAAs reduced the plasma free tryptophan:LNAA ratio

253   in the caffeine + EAA condition compared to placebo (0.0009, 0.0005–0.0013;d=0.76, most

254   likely) and caffeine alone (0.0014, 0.0010–0.0018; d=1.21, most likely). There was an effect of

255   time on the plasma free tryptophan:LNAA ratio consistent with ingestion timing.

256

257   **Caffeine**: As expected, plasma caffeine was increased in the caffeine only condition when

258   compared to placebo (4.3 µmol.L^{-1}, 3.2–5.4 µmol.L^{-1}; d=2.65, most likely), and EAA only (4.4

259   µmol.L^{-1}, 3.4–5.5 µmol.L^{-1}; d=2.74, most likely). Plasma caffeine was also higher in the caffeine

260   + EAA condition compared to placebo (4.7 µmol.L^{-1}, 3.6–5.7 µmol.L^{-1}; d=2.88, most likely) and

261   EAA alone (4.8 µmol.L^{-1}, 3.7-5.9 µmol.L^{-1}; $d=2.97$, most likely; Figure 3B) There was also an

262   effect of time on caffeine concentration, relevant to when caffeine was ingested.

263

264   **pO$_2$ & SO$_2$:** Venous blood pO$_2$ was substantially higher in the caffeine + EAA treatment

265   compared to caffeine alone (7.0 mmHg, 1.7–12.3mmHg; $d=0.39$, likely) and placebo (6.2

266   mmHg, 1.1–11.2 mmHg; 0.35, likely) across the exercise protocol (Supplementary Table 2).

267   Oxygen saturation, as indicated by venous blood SO$_2$, increased from pre- to post-exercise by

268   37% (23-50%; $d=2.28$, most likely) across treatments (Supplementary Table 2).

269

270   **Lactate**: Blood lactate was also substantially higher (0.55 mmol L^{-1}, 0.15–0.95; $d=0.17$,

271   possibly; Supplementary Table 2) in the caffeine + EAA group compared to a placebo across all
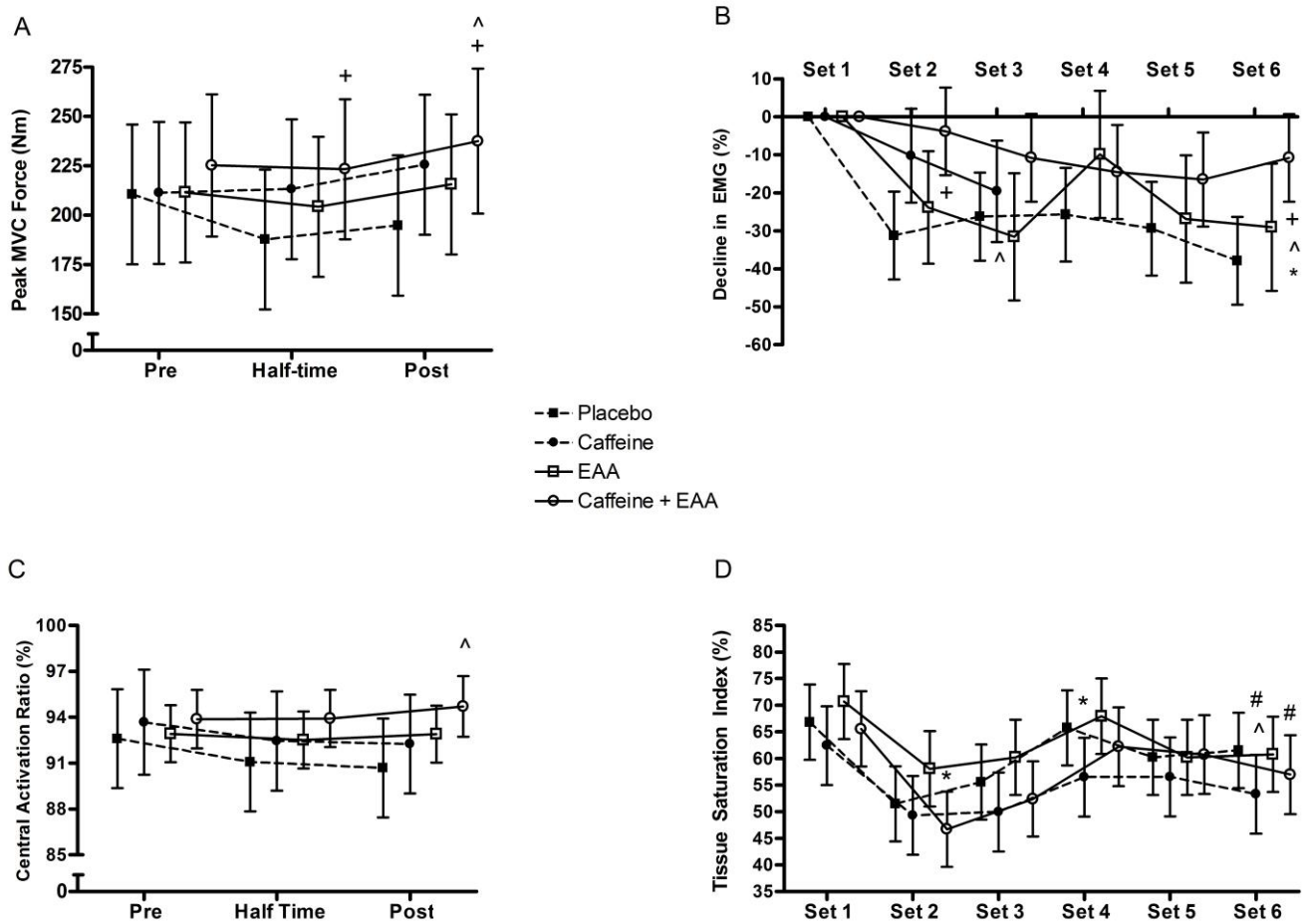
272   time points.

273

274

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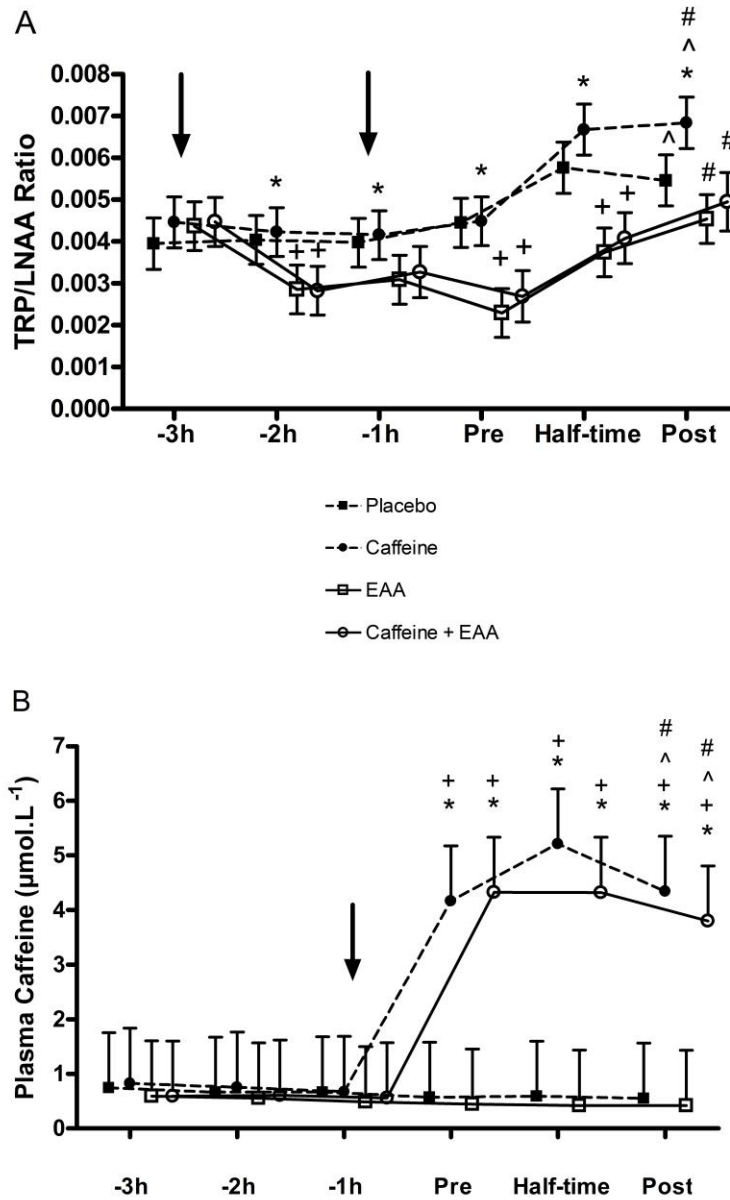
Figure 3: Comparison of Free Tryptophan:LNAA ratio and caffeine concentrations between supplementation strategies. Data are estimated mean ± 95% CI. * denotes significant difference from EAA at time point where $p \leq 0.05$. + denotes significant difference from placebo at time point where $p \leq 0.05$. ^ denotes significant difference from EAA overall where $p \leq 0.05$. # denotes significant difference from placebo overall where $p \leq 0.05$. ↓ indicates supplement ingestion time.

284   **MVC**: Overall, MVC force was higher (31nM, 8–54nM; d=0.62, likely beneficial) when caffeine

285   + EAA were ingested compared to a placebo (Figure 4A).

286

287   **Peripheral Twitch**: Peripheral twitch force was not different between supplementation protocols

288   across all time points.

289

290   **Central Activation Ratio and EMG**: The decline in normalized EMG activity was attenuated

291   with caffeine + EAA compared with placebo (19%, 9–29%; d=0.84, most likely beneficial) and

292   EAA alone (13%, 0–26%; d=0.58, likely beneficial). Supplementing with 'caffeine only'

293   resulted in a lower reduction in normalized EMG compared to placebo (15%, 1–29%; d=0.67,

294   likely beneficial; Figure 4B). There was no effect of time and no interaction between time and

295   supplement for decline in EMG activity (Supplementary Table 1). Ingesting a combination of

296   caffeine + EAA resulted in a higher overall CAR (2.7%, -5.4 to -0.4%; d=0.5, likely beneficial;

297   Figure 4C) compared to a placebo. There was no effect of time, and no interaction between time

298   and supplement.

299

300   **NIRS**: Greater mean declines in TSI across the exercise protocol were observed when caffeine (-

301   8.3%, 3.7–13.0%; d=0.9, most likely beneficial) and caffeine + EAA (-5.6%, 1.0–10.1%; d=0.6,

302   very likely beneficial) were ingested (the two conditions that produced the highest amount of

303   work), compared with EAAs alone (Figure 4D, Supplementary Table 1).

304

305

306

Figure 4: Comparison of central fatigue markers and total work (J) between supplementation strategies. Data are estimated mean ± 95%CI. * denotes significant difference from EAA at time point where $p \leq 0.05$. + denotes significant difference from placebo at time point where $p \leq 0.05$. ^ denotes significant difference from EAA overall where $p \leq 0.05$. # denotes significant difference from placebo overall where $p \leq 0.05$.

317 **Discussion**

318 This study demonstrated, for the first time, that nutritional supplementation with a combination

319 of caffeine and EAAs attenuated the development of central fatigue in response to simulated

320 team-sport exercise in hot and hypoxic environmental conditions. Using EMG activity and CAR

321 as markers of muscle recruitment and central activation, respectively, supplementation with

322 caffeine + EAA delayed the onset of central fatigue in team-sport players, but this effect did not

323 translate into clear performance benefits.

324

325 Our supplementation protocol was designed to target two different neurotransmitter pathways to

326 reduce central fatigue, based on previous literature (Pardridge 1998). Due to the challenges of

327 measuring neurotransmitter concentrations *in vivo* in humans, we can only use a conceptual

328 framework based on prior literature using EAA's containing LNAA's to manipulate

329 neurotransmitters (Pardridge 1998). Our supplementation protocol used EAA's (including

330 LNAA's) to inhibit serotonin synthesis as indicated by a lower free tryptophan:LNAA ratio

331 (Figure 4A; (Pardridge 1998). Additionally, we aimed to promoting dopamine production in

332 the CNS via phenylalanine ingestion (Pardridge 1998). We believe this was achieved, as

333 indicated by the lower ratio of free tryptophan:LNAA and increased phenylalanine

334 concentrations in the plasma when EAAs were ingested with or without caffeine (Figure 4A,

335 Supplementary Table 3). We believe this effectively lowered the serotonin:dopamine ratio,

336 which is considered important in attenuating central fatigue (Fernstrom 2013). Secondly, we

337 aimed to antagonise adenosine receptors via caffeine supplementation (Figure 4, Supplementary

338 Table 3). Isolated EAA and caffeine supplementation are known to effect central drive and motor

339 unit recruitment in thermoneutral (Meeusen et al. 2006) and hot environments (Del Coso et al.

340   2008; Meeusen & Roelands 2010). Antagonizing adenosine or increasing dopamine synthesis

341   has been linked to maintenance of motor unit recruitment and frequency of activation concurrent

342   with improved exercise performance (Del Coso et al. 2008). It is surprising that only caffeine

343   and caffeine + EAA had a clear influence on central fatigue variables. We consider that changes

344   in markers of central drive (CAR and EMG) are likely due to caffeine, and a minor contribution

345   from the EAA's in our combined supplementation protocol.

346

347   Physiological changes in central fatigue variables induced by caffeine and/or EAA, did not

348   translate into a substantial performance benefit. Participants who ingested a combination of

349   caffeine and EAAs performed more work during sprints than with EAA alone, but not

350   significantly more than with a placebo. This is surprising given that supplementation with 3–

351    $6mg.kg^{-1}$ of caffeine has previously shown to improve sprint times and repeated-sprint

352   performance (Glaister et al. 2008; Gant et al. 2010) compared to a placebo. While EAA

353   supplementation may not improve performance in normal conditions, there is evidence it is

354   beneficial when exercise is performed in the heat (Mittleman et al. 1998; Tumilty et al. 2011).

355   However, the effect of EAA supplementation on high-intensity exercise performance is

356   unknown. Our findings provide new insight into the influence of isolated and combined

357   supplementation protocols on repeat-sprint performance.

358

359   Cerebral oxygenation impairs central drive and motor unit recruitment during repeated-sprint

360   performance in hypoxic conditions (Smith & Billaut 2010; Billaut et al. 2013), and other forms

361   of high-intensity exercise (Rooks et al. 2010). Caffeine ingestion may reduce cerebral blood flow

362   and oxygen availability (Kennedy & Haskell 2011), which could explain the observed reductions

363   in cerebral oxygenation in the final sets of sprints. However, our data appear to dissociate

364   cerebral oxygenation as a mechanism for central fatigue in hot and hypoxic conditions. We

365   consider that reduced cerebral oxygenation observed in the caffeine only and caffeine + EAA

366   trials is a likely consequence of caffeine ingestion and/or the somewhat greater mechanical work

367   produced.

368

369   To our knowledge, this is the first study to measure central fatigue via EMG and magnetic

370   stimulation in conjunction with nutritional supplementation. Although we used a simulated

371   exercise protocol and unique environmental conditions, we believe our data provide novel

372   insights into central fatigue and match performance. This study highlights the potential for

373   nutritional interventions to sustain or enhance exercise performance in challenging

374   environmental conditions. We observed large standard deviations in our performance data, which

375   may be responsible for the lack of significant difference between caffeine + EAA and placebo

376   supplementation protocols. Furthermore, the dose of caffeine used in the study is at the bottom of

377   the recommended range for ergogenic benefit, which may explain why we observed no

378   performance improvements with caffeine supplementation alone. We acknowledge that there

379   may also have been an unaccounted for interaction between pre-exercise carbohydrate feeding

380   and AA supplement on performance. Finally, it is plausible that the environmental conditions

381   chosen for the study induced a level of fatigue that was too great for supplementation alone to

382   overcome, again contributing to the lack of running performance improvements. The observed

383   changes in central fatigue variables may have been too small to enhance performance and these

384   relationships warrant further investigation.

385

386 In conclusion, team-sport athletes who ingest a combination of caffeine and EAA may attenuate

387 central fatigue and perform more work than when ingesting EAA's alone during repeated sprint

388 exercise in hot, hypoxic conditions. The mechanisms explaining this improved performance

389 appear to be associated with central drive and muscle activation, and, possibly, altered

390 neurotransmitter production or function influencing the motivation to exercise. Further work

391 should evaluate similar supplementation and exercise protocols in thermoneutral and normoxic

392 conditions. This line of investigation would help establish the effectiveness of combining

393 supplements for team-sport athletes in more common match and training environments.

394

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Supplementary Table 1. Individual time point data for performance outcome variables (6 time points) Data are mean ± SD

Variable	Condition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Mean sprint work (J)	**Placebo**	2860 ± 458	2719 ± 466	2510 ± 766	2819 ± 567	2599 ± 638	2722 ± 657
	Caffeine	2940 ± 484	2775 ± 687	2829 ± 664	2811 ± 610	2686 ± 671	2708 ± 660
	LNAA	2911 ± 530	2620 ± 798	2513 ± 826	2820 ± 595	2525 ± 680	2679 ± 634
	Caffeine + LNAA	2974 ± 677	2888 ± 860	2837 ± 814	2858 ± 781	2742 ± 821	2867 ± 868
Mean sprint power (W)	**Placebo**	715 ± 115	680 ± 117	628 ± 191	705 ± 142	650 ± 159	681 ± 164
	Caffeine	735 ± 121	694 ± 172	707 ± 166	703 ± 152	672 ± 167	677 ± 165
	LNAA	728 ± 132	655 ± 199	628 ± 206	705 ± 149	631 ± 170	670 ± 158
	Caffeine + LNAA	743 ± 169	722 ± 215	709 ± 203	715 ± 195	686 ± 205	717 ± 217
Peak sprint velocity (m.s^{-1})	**Placebo**	5.71 ± 0.35	5.78 ± 0.29	5.79 ± 0.41	5.74 ± 0.35	5.69 ± 0.40	5.97 ± 0.34
	Caffeine	5.81 ± 0.26	5.77 ± 0.32	6.00 ± 0.30	5.64 ± 0.44	5.75 ± 0.39	6.02 ± 0.29
	LNAA	5.79 ± 0.32	5.73 ± 0.47	5.79 ± 0.50	5.68 ± 0.47	5.63 ± 0.50	5.92 ± 0.45
	Caffeine + LNAA	5.93 ± 0.29	5.91 ± 0.30	6.00 ± 0.39	5.79 ± 0.30	5.87 ± 0.44	6.13 ± 0.46
Mean EMG decline (%)	**Placebo**	100 ± 0	31.5 ± 31.1	26.5 ± 9.2	26.7 ± 21.4	30.0 ± 17.7	38.2 ± 30.2
	Caffeine	100 ± 0	9.9 ± 9.7	20.9 ± 6.6			
	LNAA	100 ± 0	25.2 ± 7.1	27.7 ± 4.3	11.8 ± 9.1	28.6 ± 4.0	30.2 ± 10.2
	Caffeine + LNAA	100 ± 0	4.9 ± 4.9	11.8 ± 5.8	15.4 ± 10.5	17.0 ± 8.5	11.9 ± 10.4
Tissue saturation index (%)	**Placebo**	66.8 ± 6.2	51.5 ± 4.9	55.6 ± 9.2	65.8 ± 7.1	60.2 ± 9.2	61.5 ± 9.8
	Caffeine	62.4 ± 9.3	49.1 ± 10.8	49.8 ± 11.8	56.4 ± 9.7	56.5 ± 10.5	53.2 ± 14.5
	LNAA	70.7 ± 8.3	58.1 ± 10.0	60.2 ± 9.3	67.9 ± 6.9	60.2 ± 9.4	60.8 ± 9.6
	Caffeine + LNAA	65.6 ± 9.5	46.7 ± 12.7	52.4 ± 7.9	62.3 ± 11.5	60.8 ± 16.9	57.1 ± 11.6

Supplementary Table 2. Venous pCO_2, pO_2, sO_2, lactate, and glucose concentrations between supplementation strategies. Data are mean ± SD

Variable	Condition	3hr pre	2hr pre	1hr pre	Pre	Half time	Post
pCO₂ (mmHg)	Placebo	57 ± 7	59 ± 4	62 ± 5	54 ± 9	33 ± 4	30 ± 5
	Caffeine	53 ± 9	55 ± 9	60 ± 5	56 ± 9	32 ± 7	30 ± 6
	LNAA	56 ± 7	57 ± 5	59 ± 4	50 ± 7	31 ± 6	31 ± 6
	Caffeine + LNAA*	59 ± 8	56 ± 8	57 ± 9	51 ± 8	28 ± 4	26 ± 3
pO₂ (mmHg)	Placebo	31 ± 4	24 ± 3	20 ± 4	27 ± 10	53 ± 14	63 ± 9
	Caffeine	34 ± 8	27 ± 12	20 ± 4	24 ± 8	55 ± 17	54 ± 17
	LNAA	33 ± 11	24 ± 4	20 ± 4	33 ± 9	64 ± 13	60 ± 10
	Caffeine + LNAA*^	33 ± 9	29 ± 8	24 ± 9	30 ± 10	65 ± 4	71 ± 3
sO₂ (%)	Placebo	52 ± 11	39 ± 10	34 ± 12	46 ± 23	82 ± 12	89 ± 8
	Caffeine	65 ± 18	45 ± 23	31 ± 10	43 ± 19	80 ± 16	79 ± 16
	LNAA	58 ± 20	40 ± 12	30 ± 12	60 ± 19	88 ± 10	87 ± 8
	Caffeine + LNAA*^	60 ± 13	51 ± 17	41 ± 20	53 ± 21	90 ± 1	92 ± 1
Lactate (mmol/L)	Placebo	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	7.1 ± 1.9	7.3 ± 1.9
	Caffeine	1.1 ± 0.5	0.9 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	7.4 ± 2.0	8.5 ± 1.8
	LNAA	1.2 ± 0.7	0.9 ± 0.3	0.9 ± 0.6	1.2 ± 0.5	7.5 ± 1.9	7.1 ± 1.5
	Caffeine + LNAA*	0.9 ± 0.3	0.8 ± 0.1	0.9 ± 0.5	1.3 ± 0.9	8.2 ± 2.4	9.4 ± 2.4
Glucose (mmol/L)	Placebo	4.0 ± 0.9	4.1 ± 0.6	4.0 ± 0.4	4.7 ± 0.8	5.9 ± 0.9	5.6 ± 1.7
	Caffeine	4.5 ± 0.5	4.5 ± 0.8	4.5 ± 1.1	4.6 ± 0.7	6.1 ± 1.9	6.8 ± 1.8
	LNAA^	4.5 ± 1.0	3.7 ± 0.3	4.4 ± 0.8	4.3 ± 0.5	6.3 ± 1.5	5.7 ± 1.3
	Caffeine + LNAA	3.9 ± 1.0	3.7 ± 0.3	4.2 ± 1.1	4.6 ± 0.9	6.8 ± 2.2	6.3 ± 1.8

* denotes significant difference from placebo treatment over all time points where $p \leq 0.05$

^ denotes significant difference from caffeine treatment over all time points where $p \leq 0.05$

Supplementary Table 3. Change in individual LNAA concentrations between supplementation strategies. Data are mean ± SD

Variable	Condition	3hr pre	2hr pre	1hr pre	Pre	Half time	Post
Histidine (μmol/L)	Placebo*^	82 ± 6	78 ± 6	78 ± 5	75 ± 8	82 ± 10	77 ±112
	Caffeine	80 ± 5	83 ± 7	81 ± 9	76 ± 10	77 ± 10	85 ± 9
	LNAA#	80 ± 9	104 ± 11	84 ± 9	109 ± 21	87 ± 9	85 ± 7
	Caffeine + LNAA	84 ± 12	119 ± 23	90 ± 11	116 ± 17	94 ± 14	91 ± 13
Arginine (μmol/L)	Placebo*	92 ± 9	84 ± 11	84 ± 9	76 ± 11	83 ± 15	81 ± 19
	Caffeine*	91 ± 14	90 ± 14	87 ± 13	77 ± 11	74 ± 12	82 ± 11
	LNAA	90 ± 19	124 ± 18	105 ± 14	139 ± 39	103 ± 15	96 ± 16
	Caffeine + LNAA	95 ± 16	145 ± 24	110 ± 11	138 ± 23	105 ± 9	91 ± 6
Threonine (μmol/L)	Placebo*^	129 ± 23	122 ± 22	116 ± 15	108 ± 18	110 ± 17	105 ± 24
	Caffeine*^	132 ± 29	131 ± 26	126 ± 22	116 ± 28	105 ± 20	115 ± 25
	LNAA	128 ± 32	141 ± 23	125 ± 19	153 ± 37	122 ± 18	121 ± 15
	Caffeine + LNAA*	136 ± 22	163 ± 24	137 ± 17	157 ± 32	129 ± 20	122 ± 16
Lysine (μmol/L)	Placebo*^	167 ± 26	151 ± 21	146 ± 17	134 ± 22	147 ± 22	132 ± 27
	Caffeine*^	170 ± 40	166 ± 30	158 ± 27	139 ± 33	126 ± 20	136 ± 16
	LNAA*	165 ± 51	196 ± 36	165 ± 31	200 ± 52	150 ± 28	136 ± 24
	Caffeine + LNAA	178 ± 48	238 ± 57	179 ± 22	211 ± 40	159 ± 24	143 ± 22

Methionine (µmol/L)	**Placebo***	28 ± 6	25 ± 5	23 ± 4	22 ± 4	24 ± 4	26 ± 6
	Caffeine	32 ± 6	29 ± 4	27 ± 4	24 ± 6	24 ± 5	28 ± 4
	LNAA	28 ± 7	34 ± 6	28 ± 4	34 ± 10	28 ± 4	28 ± 4
	Caffeine + LNAA	32 ± 7	40 ± 7	28 ± 5	34 ± 8	28 ± 5	27 ± 3
Valine (µmol/L)	**Placebo*^**	255 ± 33	249 ± 62	241 ± 46	237 ± 33	240 ± 36	237 ± 49
	Caffeine*^	257 ± 25	245 ± 28	240 ± 44	220 ± 21	211 ± 26	224 ± 43
	LNAA*	250 ± 46	320 ± 41	284 ± 22	372 ± 72	305 ± 40	285 ± 36
	Caffeine + LNAA	280 ± 45	391 ± 67	320 ± 50	402 ± 47	324 ± 38	301 ± 47
Isoleucine (µmol/L)	**Placebo*^**	65 ± 15	60 ± 25	58 ± 17	62 ± 11	62 ± 14	65 ± 24
	Caffeine*^	72 ± 13	62 ± 12	63 ± 21	55 ± 10	55 ± 8	61 ± 12
	LNAA	65 ± 17	100 ± 17	82 ± 19	122 ± 30	87 ± 14	77 ± 20
	Caffeine + LNAA	78 ± 15	125 ± 32	87 ± 24	121 ± 24	89 ± 21	69 ± 20
Leucine (µmol/L)	**Placebo*^**	129 ± 23	118 ± 34	113 ± 25	116 ± 17	118 ± 19	120 ± 37
	Caffeine*^	140 ± 25	125 ± 21	123 ± 35	108 ± 18	106 ± 13	116 ± 22
	LNAA	126 ± 29	179 ± 25	151 ± 25	213 ± 50	160 ± 23	142 ± 29
	Caffeine + LNAA	148 ± 8	222 ± 52	162 ± 38	220 ± 38	161 ± 27	136 ± 33

Phenylalanine (µmol/L)	**Placebo*^**	57 ± 7	52 ± 7	51 ± 8	52 ± 7	55 ± 7	57 ± 10
	Caffeine*^	60 ± 10	58 ± 9	55 ± 10	52 ± 7	54 ± 6	60 ± 9
	LNAA*	55 ± 11	81 ± 14	73 ± 15	102 ± 23	88 ± 18	86 ± 12
	Caffeine + LNAA	62 ± 13	99 ± 24	75 ± 4	112 ± 22	96 ± 18	93 ± 14
Tryptophan (µmol/L)	**Placebo^+**	4.0 ± 0.7	3.8 ± 0.8	3.6 ± 0.8	3.9 ± 0.6	5.1 ± 1.8	5.4 ± 1.0
	Caffeine^	4.6 ± 0.4	4.2 ± 0.7	4.0 ± 1.0	3.9 ± 0.4	5.5 ± 1.2	6.2 ± 0.8
	LNAA*	4.3 ± 0.7	3.6 ± 0.5	3.4 ± 0.9	3.3 ± 1.1	4.2 ± 1.1	4.8 ± 1.2
	Caffeine + LNAA*	4.9 ± 0.8	4.3 ± 0.8	3.9 ± 0.7	4.1 ± 1.4	4.9 ± 0.8	5.2 ± 0.8

* denotes significant difference between treatment and caffeine + LNAA treatment over all time points where $p \leq 0.05$

^ denotes significant difference between treatment and LNAA treatment over all time points where $p \leq 0.05$

+ denotes significant difference between treatment and caffeine treatment over all time points where $p \leq 0.05$

# denotes significant difference between treatment and placebo treatment over all time points where $p \leq 0.05$

Supplementary Table 4. Individual time point data (3 time points) for main outcome variables. Data are mean ± SD

Variable	Condition	Pre	Half-Time	Post
Mean MVC force (nM)	**Placebo**	211 ± 52	188 ± 48	195 ± 53
	Caffeine	218 ± 43	213 ± 44	226 ± 62
	LNAA	212 ± 46	204 ± 36	216 ± 40
	Caffeine + LNAA	215 ± 35	223 ± 41	231 ± 33
Mean twitch force (nM)	**Placebo**	68.5 ± 21.6	62.7 ± 22.9	71.2 ± 24.5
	Caffeine	68.1 ± 19.3	65.8 ± 22.9	69.5 ± 15.6
	LNAA	68.9 ± 20.9	65.0 ± 17.5	66.1 ± 14.5
	Caffeine + LNAA	62.8 ± 17.4	59.5 ± 12.2	71.0 ± 16.3
Central activation ratio (%)	**Placebo**	92.6 ± 2.9	91.1 ± 5.3	90.7 ± 7.6
	Caffeine	93.5 ± 1.5	92.5 ± 2.5	92.3 ± 5.5
	LNAA	92.9 ± 4.2	92.5 ± 4.7	92.9 ± 4.5
	Caffeine + LNAA	94.5 ± 2.0	93.9 ± 3.8	96.1 ± 2.2