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An acute dose of inorganic dietary nitrate does not improve high-intensity, intermittent exercise performance in temperate or hot and humid conditions

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1 **An acute dose of inorganic dietary nitrate does not improve high-intensity, intermittent**
2 **exercise performance in temperate or hot and humid conditions**

3

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16

17 **Running Title:** Dietary nitrate does not improve high intensity, intermittent exercise
18 performance in the heat

19 **Key words:** Nitrate, exercise, heat, high-intensity, beetroot juice, heat, humidity

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29 **Abbreviations:**

- 30 BRJ Beetroot Juice
31 HR Heart Rate
32 IST Intermittent Sprint Test
33 NO Nitric Oxide
34 NO_2^- Nitrite
35 NO_3^- Nitrate
36 PLA Placebo
37 RER Respiratory Exchange Ratio
38 RPE Rating of Perceived Exertion
39 T_C Core Temperature
40 T_{sk} Skin Temperature
41 TT Time Trial
42 T_{TYMP} Tympanic Temperature

43

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53

54 **Abstract**

55 **Purpose:** Dietary nitrate (NO_3^-) has repeatedly been shown to improve endurance and
56 intermittent, high-intensity events in temperate conditions. However, the ergogenic effects of
57 dietary NO_3^- on intermittent exercise performance in hot conditions has yet to be investigated.

58 **Methods:** In a randomised, counterbalanced, double-blind crossover study, twelve
59 recreationally trained males ingested a nitrate-rich beetroot juice shot (BRJ) (6.2 mmol NO_3^-)
60 or a nitrate-depleted placebo (PLA) ($<0.004 \text{ mmol NO}_3^-$) 3h prior to an intermittent sprint test
61 (IST) in temperate (22°C , 35% RH) and hot conditions (30°C , 70% RH). The cycle ergometer
62 IST consisted of twenty maximal 6s sprints interspersed by 114s of active recovery. Work
63 done, power output, heart rate and RPE were measured throughout; tympanic temperature was
64 measured prior to and upon completion.

65 **Results:** There were no significant effects of supplement on sprint performance in either
66 temperate or hot, humid conditions ($p>0.05$). There was a reduced peak (BRJ: $659\pm 100\text{W}$ vs.
67 PLA: $693\pm 139\text{W}$; $p=0.056$) and mean power (BRJ: $543\pm 29\text{W}$ vs PLA: $575\pm 38\text{W}$; $p=0.081$)
68 following BRJ compared to PLA in the hot and humid condition, but this was not statistically
69 significant. There was no effect of supplement on total work done irrespective of environmental
70 condition. However, $\sim 75\%$ of participants experienced performance decreases following BRJ
71 in the hot and humid environment. No differences were observed between trials for tympanic
72 temperature measured at the conclusion of the exercise trial.

73 **Conclusion:** In conclusion, an acute dose of inorganic dietary NO_3^- does not improve repeated
74 sprint performance in either temperate, or hot and humid conditions.

75

76

77

78

79 **Introduction**

80 Nitric oxide (NO) is a gaseous signalling compound associated with a plethora of physiological
81 effects including modulating contractile properties of skeletal muscle (Ferguson et al. 2013),
82 mitochondrial efficiency (Clerc et al. 2007; Heinonen et al. 2011) and peripheral/cutaneous
83 blood flow (Lundberg et al. 2008). Circulating NO in the blood is short-lived and rapidly
84 oxidised to nitrite (NO_2^-) and nitrate (NO_3^-). NO_3^- is also known to be stored within skeletal
85 muscle (Piknova et al. 2015) and the skin. Collectively they may act as a reservoir to ensure
86 NO bioactivity is available during conditions of low pO_2 (Lundberg et al. 2008), such as during
87 intense physical exercise.

88

89 Dietary NO_3^- has been shown to be effective at increasing circulating plasma NO_2^- and NO_3^-
90 that coincides with improvement in indices of performance during cycling time trials (TT)
91 (Cermak et al. 2012a; Lansley et al. 2011; Muggeridge et al. 2014), supra-maximal intensity
92 cycling (Aucouturier et al. 2015) and explosive running (Sandbakk et al. 2015). This has been
93 attributed to a reduced ATP cost during muscular contractions (Bailey et al. 2010) and
94 potentially reduced $\dot{V}\text{O}_2$ for mitochondrial ATP resynthesis, although the latter has failed to be
95 confirmed more recently (Whitfield et al. 2015). However, some studies show that inorganic
96 dietary NO_3^- has been ineffective at improving performance (Cuenca et al. 2018; Sandbakk et
97 al. 2015; Cermak et al. 2012b), which could be attributed to altered oral microbiota important
98 for the initial conversion of NO_3^- to NO_2^- (Burleigh et al. 2018), chronic versus acute dosages
99 (Vanhatalo et al. 2010; Boorsma et al. 2014) and the level of athlete investigated, with those
100 towards elite showing less of an ergogenic aid of nitrate than less trained individuals (Porcelli
101 et al. 2015).

102

103 NO-mediated physiological signalling following NO_3^- supplementation is potentiated as the O_2
104 (Castello et al. 2006) and pH (Modin et al. 2001) tension declines, therefore NO_3^- should in
105 theory be more effective in high intensity exercise as it creates favourable physiological
106 conditions for NO production (Richardson et al. 1995). Dietary NO_3^- supplementation has been
107 reported to elevate skeletal muscle O_2 delivery (Ferguson et al. 2013) and enhance
108 sarcoplasmic calcium handling in fast twitch type II muscle fibres (Hernandez et al. 2012)
109 translating to increased force production (Coggan et al. 2015). As such, high-intensity physical
110 activities are more likely to increase NO synthesis from stored NO_3^- reservoirs, and thus,
111 improve performance (Wylie et al. 2016).

112

113 Exercise in the heat poses a formidable challenge to the body's ability to control its internal
114 environment through heat gain from external temperatures and high rates of metabolic heat
115 production (Maughan and Shirreffs 2004). Given that cutaneous vasodilation is critical for the
116 maintenance of a stable core temperature (T_c), the role of dietary NO_3^- supplementation in the
117 heat warrants investigation. Indeed, the effect of dietary NO_3^- supplementation on exercise
118 performance in heat has recently been investigated in one non-athletic population (Kuennen et
119 al. 2015) and in three studies of well-trained cyclists (Kent et al. 2018b; Kent et al. 2018a;
120 McQuillan et al. 2018). Following a moderate dose of inorganic dietary NO_3^- (8.3 mmol NO_3^-
121 $\cdot\text{d}^{-1}$) for 6d, Kuennen et al. (2015) observed a reduced O_2 cost of a 45 minute loaded march in
122 a hot and humid environment compared to a PLA. Interestingly, it was shown that dietary NO_3^-
123 supplementation increased subject's T_c , a finding that was later replicated during a 4km cycling
124 TT in hot conditions (McQuillan et al. 2018). This may be due to elevated gastrointestinal
125 blood perfusion, which may enhance thermal transfer during exercise in the heat. Additionally,
126 the improved workload of the skeletal muscles could cause a subsequent 'overspill' of
127 metabolic heat. However, this has most recently been disputed, where dietary NO_3^- regimens

128 have not influenced cycling TT performance (Kent et al. 2018b) or thermoregulatory responses
129 in young adults (Amano et al. 2018) and elite cyclists (Kent et al. 2018a).

130

131 The prospective notion that dietary NO_3^- supplementation alters heat tolerance is yet to be fully
132 understood, where its effect on intermittent, sporting performance in trained individuals is yet
133 to be investigated in hot conditions. As such, this investigation aimed to investigate whether
134 an acute dose of inorganic dietary NO_3^- would elicit performance benefits in recreationally
135 trained males during an intermittent high-intensity exercise cycling protocol in temperate as
136 well as in hot and humid conditions, with a potential improvement in performance resulting
137 from an enhanced tissue and skin perfusion, resulting in enhanced O_2 delivery, and heat
138 dissipation. It was hypothesised that high-intensity, intermittent performance (mean and peak
139 power; total work done) in the heat would improve following dietary NO_3^- supplementation
140 compared to a placebo in both conditions.

141

142 **Materials and Methods**

143

144 *Participants*

145 Twelve recreationally trained male university students (22 ± 4 years, $1.81 \pm 0.06\text{m}$, $80.43 \pm$
146 5.84kg , $46.11 \pm 6.42\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$) volunteered to participate in the study. All participants had
147 a history of competing at a high standard of team sports and had been training ≥ 2 times per
148 week for at least 1 year. Participants gave their written consent prior to participation and all
149 risks and potential benefits were fully explained prior to. The procedures employed in this
150 study and risks were accepted in adherence to Edinburgh Napier University's ethical committee
151 and conformed to the code of ethics of the Declaration of Helsinki.

152

153 *Experimental Design*

154 Participants reported to the laboratory on 5 separate occasions. During the first visit,
155 participants performed a ramp incremental test for assessment of $\dot{V}O_{2peak}$ (see *Assessment of*
156 *Peak Oxygen Uptake*). After 20 minutes of recovery, participants then performed 10 minutes
157 of the intermittent sports test (IST) in temperate conditions for individual gear calibration for
158 the subsequent incremental exercise tests using a magnetically-braked cycle ergometer
159 (Velotron Pro, RacerMate Inc, USA). The 10 minute IST required participants to perform five
160 2 minute blocks (114s of active recovery cycling at 100W maintaining 60rpm and 6s maximal
161 sprint). Participants were asked during this session if they felt they could replicate this intensity
162 for the full 40 minute IST, following their response amendments were made to their gearing
163 for the active recovery and maximal effort bouts.

164

165 Following completion of the preliminary testing, participants were assigned in a randomised,
166 counterbalanced, double-blind, crossover experimental design to receive either an acute dose
167 of NO_3^- -rich beetroot juice shot (BRJ: 6.2 mmol NO_3^-) or a NO_3^- -depleted placebo (PLA:
168 <0.004 mmol NO_3^-), which they would ingest 3h prior to the IST in temperate (22°C, 35% RH)
169 and hot conditions (30°C, 70% RH). This dose of BRJ has been shown previously to improve
170 exercise performance if ingested 2.5-3h prior to exercise (Thompson et al. 2014; Hoon et al.
171 2014; Lansley et al. 2011). Randomisation was performed using an online programme, blinded
172 to the researchers. At least 4-7d separated each IST allowing for optimal recovery and
173 supplement washout for circulating plasma NO_3^- ($[NO_3^-]$) and $[NO_2^-]$ levels to return to
174 baseline (Wylie et al. 2013).

175

176 Prior to participation, all participants were instructed to fill out a food screening questionnaire,
177 detailing how often they ate certain foods and in what portion size. Participants were also asked

178 to record their food intake 24h prior to testing and were instructed to try and replicate this
179 before subsequent sessions. All participants were given information regarding what foods
180 contain the highest amount of $\text{NO}_3^- \cdot \text{g}^{-1}$ and to avoid consuming in high doses for the duration
181 of the testing period. Participants were instructed to arrive to the laboratory in a fully rested,
182 hydrated state at least 3h postprandial and were advised to avoid any strenuous activity in the
183 24h preceding each testing sessions. Caffeine and alcohol were to be refrained from
184 consumption 6h and 24h, respectively, before each laboratory visit. Participants were also
185 asked to abstain from antibacterial mouthwash and chewing gum use around supplement
186 ingestion and experimental trials as these products have been previously shown to blunt the
187 reduction of NO_3^- to NO_2^- in the oral cavity (Govoni et al. 2008). Testing all took place at the
188 same time of day ($\pm 3\text{h}$).

189

190 *Assessment of Peak Oxygen Uptake*

191 A $\dot{V}\text{O}_2$ peak test to volitional exhaustion was performed on a Velotron Pro (RacerMate Inc,
192 USA) cycle ergometer using a breath-by-breath gas analyser (CPX Jaeger, Germany), which
193 monitored $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, and respiratory exchange ratio (RER). Participants warmed up for 5
194 minutes, cycling at an initial power output of 60W at 60-80rpm. Following the warm up, in
195 one-minute increments, resistance was increased by 30W until participants could no longer
196 complete the 1-minute step at 60-80rpm or when they felt they could go on no further. $\dot{V}\text{O}_2$ peak
197 was taken as the highest mean-value attained during the final 30s of exercise. HR was
198 monitored throughout (Polar RS400 Heart Rate Monitors, Polar, Finland).

199

200 *Intermittent Sport Test (IST)*

201 The IST was based on a motion analysis study of international field hockey players (Spencer
202 et al. 2004) and is an abstract of the protocol previously described by Bishop and Claudius

203 (Bishop and Claudius 2005). The IST, like the familiarisation and $\dot{V}O_2$ peak session was
204 performed on a Velotron Pro (Racer Mate, USA) cycle ergometer. All IST sessions took place
205 in an environmental chamber (Weiss Gallenkamp, UK) in both temperate (22°C, 35% RH) and
206 hot and humid conditions (30°C, 70% RH). Mean and peak power, work done, HR, and RPE
207 were recorded after every sprint of the IST. Fatigue index per sprint was determined as:
208 (maximum power – minimum power)/maximum power. Participant tympanic temperature
209 (T_{TYMP}) was measured upon commencement and immediately upon completion of the IST
210 using a thermometer placed in the cavity of the ear (Braun IRT 4520, Braun ThermoScan,
211 Germany).

212

213 Before the onset of the IST, a standardised warm-up was completed comprising of cycling for
214 5-minutes at 100W at 60rpm followed by a 2 minute practice block of the IST. The 40 minute
215 IST replicates the duration of ‘one half’ of a rugby or hockey match, which was broken down
216 into twenty x 2 minute blocks consisting of a maximal 6s sprint followed by 114s active
217 recovery. Participants were able to drink water *ad libitum*. The fixed resistance during the
218 active recovery and maximum effort sprints were individually determined during the
219 familiarisation session.

220

221 *Supplementation*

222 Participants were randomly allocated in a crossover manor to consume either NO_3^- -rich BRJ
223 (6.2 mmol NO_3^- per 70ml; Beet it, James White Drinks Ltd, United Kingdom) or a nitrate-
224 depleted PLA (<0.004 mmol NO_3^- per 70ml; Beet it, James White Drinks Ltd) shot identical
225 in appearance and taste, administered in a double-blind fashion. Participants consumed their
226 supplements 3h prior to either the IST. Three hours prior to testing was chosen as

227 pharmacokinetic data suggests that $[\text{NO}_2^-]$ will be at its peak after a single dose of BRJ (Webb
228 et al. 2008).

229

230 *Statistical Analysis*

231 All data were assessed for normal distribution. Data that were not normally distributed were
232 logarithmically transformed (Log10). Paired sample T-tests were performed to compare the
233 means of HR, delta T_{TYMP} , peak power, mean power and mean work done per sprint and total
234 work done during the IST between supplements (BRJ vs PLA). The effect of inorganic dietary
235 NO_3^- on work done, power output, HR, RPE over the duration of the IST were analysed by a
236 two-way repeated measures analysis of variance (ANOVA; time/sprint x condition). Cohen's
237 effect size (d) was calculated and expressed as: small effect > 0.2 ; medium effect > 0.5 ; large
238 effect > 0.8 . Inferential statistical analysis was conducted using the software package IBM
239 SPSS Statistics (IBM Corp, USA). Data are presented as mean \pm standard deviation (SD) unless
240 stated otherwise. Significance was set at $\alpha \leq 0.05$.

241

242 **Results**

243

244 *Physiological and Perceptual Responses*

245 Upon termination of the IST, there were no differences in T_{TYMP} between BRJ and PLA in both
246 temperate (BRJ: $35.8 \pm 0.8^\circ\text{C}$ vs. PLA: $35.9 \pm 0.5^\circ\text{C}$, $p = 0.78$) and in the heat (BRJ: $37.3 \pm$
247 0.6°C vs. PLA: $37.2 \pm 0.6^\circ\text{C}$, $p = 0.93$). Similarly, the increase in T_{TYMP} following the IST was
248 not different between supplements (temperate: ΔBRJ : $0.57 \pm 1.1^\circ\text{C}$ vs ΔPLA : $0.68 \pm 0.33^\circ\text{C}$; p
249 $= 0.74$; heat: ΔBRJ : $1.49 \pm 0.61^\circ\text{C}$ vs ΔPLA : $1.38 \pm 0.7^\circ\text{C}$; $p = 0.37$). There were also no
250 differences in HR or RPE between supplements during the IST temperate (HR- BRJ: 151 ± 14
251 bpm vs PLA: 151 ± 12 bpm; $p = 0.94$; RPE- BRJ: 14 ± 1 vs. PLA: 14 ± 2 , $p = 0.99$). and in

252 hot, humid conditions (HR- BRJ: 152 ± 17 bpm vs PLA: 152 ± 16 bpm; $p = 0.41$; RPE- BRJ:
253 14 ± 1 vs. PLA: 14 ± 1 , $p = 0.74$).

254

255 *Intermittent Exercise Performance*

256 There was no effect of dietary NO_3^- ingestion on IST performance measures in temperate
257 conditions (mean power production; BRJ: $562 \pm 120\text{W}$, PLA: $571 \pm 124\text{W}$, $p = 0.433$; total
258 work done: BRJ: 67.44 ± 14.39 kJ, PLA: 68.46 ± 15.07 kJ, $p = 0.447$; Figure 1). Mean power
259 produced per sprint and total work done was reduced in BRJ than PLA in the heat, but these
260 were not statistically significant differences (mean power production; BRJ: $543 \pm 29\text{W}$, PLA:
261 $575 \pm 39\text{W}$, $p = 0.081$; total work done: BRJ: 66.07 ± 10.84 kJ, PLA: 69.74 ± 15.13 kJ, $p =$
262 0.101 ; Figure 2). There was a trend for dietary NO_3^- supplementation to reduce mean peak
263 power production during the IST in the heat which neared statistical significance ($p = 0.056$; d
264 $= 0.28$) compared to the PLA (Figure 2). On average, peak power production in the heat was
265 $\sim 6\%$ lower following BRJ ($659 \pm 100\text{W}$) compared to PLA ($683 \pm 139\text{W}$) (Figure 2A & 2B).

266

267 There were no significant condition and sprint interactions for mean power production in both
268 temperate ($F_{(19, 209)} = 0.476$, $p = 0.971$; Figure 3A) and hot ($F_{(19, 209)} = 1.147$, $p = 0.306$; Figure
269 3B) conditions. There was a trend for a lower mean power production per sprint following the
270 BRJ ($543 \pm 29\text{W}$) supplement compared to the PLA in the hot condition ($575 \pm 38\text{W}$; $p =$
271 0.081 ; $d = 0.34$) (Figure 2B).

272

273 Likewise, no condition x sprint interaction effect was shown within mean work done for both
274 temperate ($F_{(19, 209)} = 0.498$, $p = 0.963$; Figure 4A) and hot conditions ($F_{(19, 209)} = 1.062$, $p =$
275 0.392 ; Figure 4B). Mean work done per sprint was not different between supplements in either
276 temperate ($p = 0.45$, $d = 0.07$; Figure 1C) or hot conditions ($p = 0.12$, $d = 0.26$; Figure 2C).

277 BRJ did not influence total work done completed over the IST (temperate- BRJ: 67.44 ± 14.39
278 kJ vs. PLA: 68.46 ± 15.07 kJ) ($p = 0.447$, $d = 0.07$; Figure 1D; hot- BRJ: 66.07 ± 10.84 kJ vs.
279 PLA: 69.74 ± 15.13 kJ) ($p = 0.101$, $d = 0.28$; Figure 2D). In addition, there was no difference
280 in fatigue index between supplements (temperate- BRJ: $48.14 \pm 9.77\%$ vs. PLA: $49.89 \pm$
281 10.67% ; $p = 0.38$ Figure 1E ; hot- BRJ: $50.51 \pm 9.20\%$ vs. PLA: $50.49 \pm 13.51\%$; $p = 0.99$
282 Figure 2E).

283

284 **Discussion**

285 This is the first study to investigate the effect of dietary NO_3^- supplementation on intermittent,
286 high-intensity performance in both temperate as well as hot and humid conditions. Dietary
287 NO_3^- did not influence cardiovascular, perceptual or thermoregulatory responses to the exercise
288 protocol, and appeared to impair some indices of performance, however this was only in the
289 hot and humid condition, and was not statistically significant. This contrasts with previous
290 research in temperate conditions which typically demonstrates that NO_3^- is ergogenic for high
291 intensity intermittent exercise performance (Thompson et al. 2016; Thompson et al. 2015;
292 Wylie et al. 2013; Wylie et al. 2016), with only one other study showing no effect of an acute
293 NO_3^- dose on intermittent exercise performance (Martin et al. 2014).

294

295 The present investigation included 12 recreationally trained males, where following the
296 ingestion of ~ 6 mmol NO_3^- , there was a trend for lower peak power ($p = 0.056$) and mean
297 power production per sprint ($p = 0.081$) compared to the PLA trial in the hot condition only,
298 with no such trend in temperate conditions. The reduction in power output with nitrate in the
299 heat was observed in 8 out of the 12 participants, with the remaining 4 showing either no
300 change, or slight improvement in power output (example figure provided; Figure 5A-D). This
301 is the first investigation to reveal such potential negative results following dietary NO_3^-

302 supplementation within a recreationally trained population, and appears to be only present in
303 hot and humid conditions. In fact, lower doses of NO_3^- (5-6 mmol NO_3^-) have produced
304 favourable improvements in mean and peak power production during a 30s Wingate test
305 (Cuenca et al. 2018; Dominguez et al. 2017) and in cycling TT performances in both simulated
306 altitude (Muggeridge et al. 2014) and normoxic conditions (Lansley et al. 2011). Speculatively,
307 disparities between studies may be explained by the environmental conditions, where exercise
308 in heat increases sympathetic nervous activity (Drust et al. 2005) influencing muscle
309 metabolism (Febbraio et al. 1994) and vascular control (Johnson 2010).

310

311 Increases in muscle temperature can improve cross-bridge cycling rates (Karatzaferi et al.
312 2004) and sprint performance through enhancements in muscle fibre conductance (Girard et al.
313 2013; Gray et al. 2006). When recovery periods are long enough to allow for complete recovery
314 between short duration sprints and in the absence of hyperthermia, there is little evidence to
315 suggest hyperthermic conditions are detrimental to repeated sprint performance compared to
316 temperate conditions (Almudehki et al. 2012; Girard et al. 2013). Interestingly, we show that
317 peak power production was lower following the BRJ supplement compared to PLA, nearing
318 statistical significance ($p = 0.056$). Given type II muscle fibres are extensively recruited during
319 shorter sprints compared to longer maximal efforts (Casey et al. 1996; Gray et al. 2008) and
320 the known preferential NO_3^- -treatment fibre effects (Jones et al. 2016), such as preferential
321 increases in blood flow to type II fibres (Ferguson et al. 2013), our findings are in stark contrast
322 to previous literature within temperate environments (Thompson et al. 2016; Thompson et al.
323 2015; Wylie et al. 2013; Wylie et al. 2016), but the addition of a heat stress, as provided in our
324 study, may compromise the ergogenic impact of inorganic NO_3^- on performance.

325

326 It has been reported that dietary NO_3^- supplementation augments an increase in T_c during
327 exercise in the heat (Kuennen et al. 2015; McQuillan et al. 2018). The authors postulate that
328 these effects may be specifically induced in metabolically active muscles, overriding the
329 sympathetic vascular response in the skin that allows redistribution of blood flow to dissipate
330 heat from the body (Crandall and Gonzalez-Alonso 2010). Whilst we displayed that T_{TYMP} rose
331 to a similar extent in the BRJ and PLA conditions, it is plausible this may not fully represent
332 the thermoregulatory responses our subjects may have experienced. Indeed, T_{TYMP} has been
333 revealed to underestimate T_c during exercise in heat (Huggins et al. 2012). As such, T_{TYMP}
334 measurements in this study may not accurately reflect any changes in T_c in our experiment.
335 Increases in T_c during hyperthermic exercise creates a simultaneous demand for blood flow
336 between active skeletal tissues, the skin and vital organs (Kent et al. 2018a); thus, influencing
337 muscle metabolism and oxidative function (Febbraio et al. 1994), and subsequently limiting
338 exercise performance (Drust et al. 2005).

339

340 Following local and whole-body heating, BRJ increases cutaneous vasodilation through NO-
341 induced vasodilation despite not influencing skin blood flow suggesting no improved
342 thermoregulatory benefit (Keen et al. 2015; Levitt et al. 2015). However, it has been reported
343 that NO-dependent cutaneous vasodilation is diminished during high-intensity exercise in heat
344 (Fujii et al. 2014). Given power output and total work done was lower in 19 out of the 20 sprints
345 during the BRJ trial compared to the PLA in the heat (Figures 3B & 4B), blood flow may have
346 been preferentially distributed to other surrounding tissues or other neural thermoregulatory
347 factors were at work (Drust et al. 2005; Febbraio et al. 1994). However, neither T_c , T_{sk} ,
348 peripheral nor muscle blood flow were measured in the present investigation leaving this open
349 for future debate.

350

351 *Considerations*

352 Larger or loaded dosages of inorganic dietary NO_3^- have been consistently shown to improve
353 repeated sprint performance of short durations (Thompson et al. 2015; Wylie et al. 2016; Wylie
354 et al. 2013) - a hallmark of invasion team sports (Mohr et al. 2003; Spencer et al. 2004).
355 However, we showed that the ingestion of dietary NO_3^- 3h prior to an IST in heat non-
356 significantly reduced performance by 4 – 6%, which may represent a substantial performance
357 detriment on the field of play. Despite this, our lack of benefit in the temperate conditions may
358 be due to insufficient dose for this exercise mode, however, this dose did still correspond to a
359 small reduction in performance indices in intermittent sprint activity in hot and humid
360 conditions, as seen in this study. While these data seem highly relevant for competitive team
361 sport athletes, they must be interpreted with caution. Our analysis was conducted on a small
362 sample ($n = 12$) and differences between BRJ and PLA conditions were small and did not reach
363 statistical significance, despite observing trends for impaired performance in the heat with BRJ
364 supplementation. However, our findings of a potential negative impact of BRJ on performance
365 in the heat, along with an absence of such negative impacts in ambient conditions, we can
366 suggest that dietary NO_3^- may impair high intensity exercise performance in a recreationally
367 trained population.

368

369 In addition, our measures of thermoregulation (T_{TYMP}) are insufficient to fully understand the
370 impact of nitrate on thermoregulation in hot and humid environmental conditions. Therefore
371 future studies should employ more accurate measures of thermoregulatory strain, such as T_c ,
372 T_{sk} , sweat rate, muscle and skin blood flow. As a result of the small sample size, and the
373 insufficient thermoregulatory measures, we are unable to specifically determine the
374 physiological mechanisms that underpin this potential negative impact of BRJ on high
375 intensity exercise in the heat.

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Conclusions

This study demonstrated that relative to the PLA, BRJ does not offer any beneficial aid to high-intensity, repeated-sprint performance in both ambient and hot conditions, but may be detrimental in the heat as demonstrated in our performance indices. However, with the trend of an acute dose potentially being ergolytic in hot and humid environments, the more common dietary NO₃⁻ supplementary regimes of loading are postulated to be detrimental to repeated sprint performance in heat through alterations in thermoregulatory responses and/or reductions in skeletal muscle blood flow. As such, we do not recommend athletes ingest dietary NO₃⁻ supplements prior to high-intensity exercise in the heat.

Author Contributions

KS, MR designed the study. KS undertook data collection. KS, MR, DM analysed the data. KS, MR wrote the manuscript. KS, MR, DM, CE reviewed the data and the manuscript. All authors read and approved of the manuscript.

Conflicts of Interest

The authors report no conflicts of interest

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600 **Figure Legends**

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602 **Figure 1.** Mean peak power output (A), mean power production (B) and mean work done (C)
603 produced per sprint during the intermittent sprint test (IST) in temperate conditions after
604 ingesting either nitrate-rich beetroot juice (BRJ) or placebo (PLA). (D) Illustrates total work
605 done across the twenty 6s sprints during the IST and (E) represents fatigue index between trials.
606 Dashed lines represent individual participant response. Data is presented as mean \pm SEM.

607

608 **Figure 2.** Mean Peak Power Output (A), Mean Power Production (B) and Mean Work Done
609 (C) produced per sprint during the intermittent sprint test (IST) in the heat after ingesting either
610 nitrate-rich beetroot juice (BRJ) or placebo (PLA). (D) Illustrates Total Work Done across the
611 twenty 6s sprints during the IST and (E) represents Fatigue Index between trials. Dashed lines
612 represent individual participant response. Data is presented as mean \pm SEM.

613

614 **Figure 3.** Mean Power Output during the intermittent sprint test (IST) following the nitrate-
615 rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in temperate (A) and
616 hot and humid (B) conditions. Data is presented as mean \pm SEM.

617

618 **Figure 4.** Mean Work Done per Sprint during the intermittent sprint test (IST) following the
619 nitrate-rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in temperate
620 (A) and hot and humid (B) conditions. Data is presented as mean \pm SEM.

621

622 **Figure 5.** Mean Power Output during the intermittent sprint test (IST) following the nitrate-
623 rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in different
624 participants. Example of one of 4 participants who showed little or no change in performance

625 indices in the IST in temperate (A) and hot, humid conditions (C), and one example of the 8
626 participants who displayed decrements in performance in IST with BRJ supplementation
627 (temperate: B, hot, humid: D).

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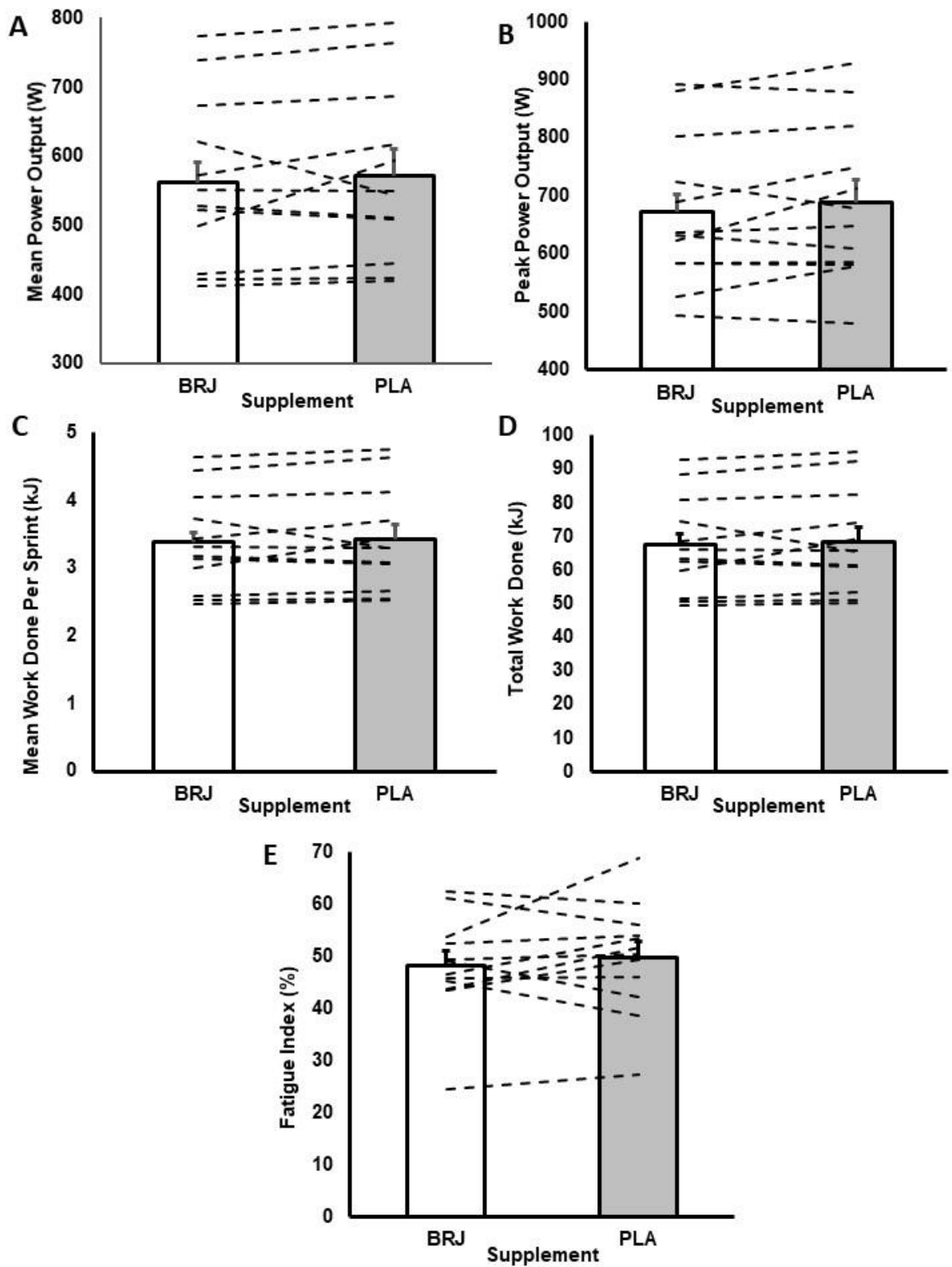
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650 **Figure 1.**

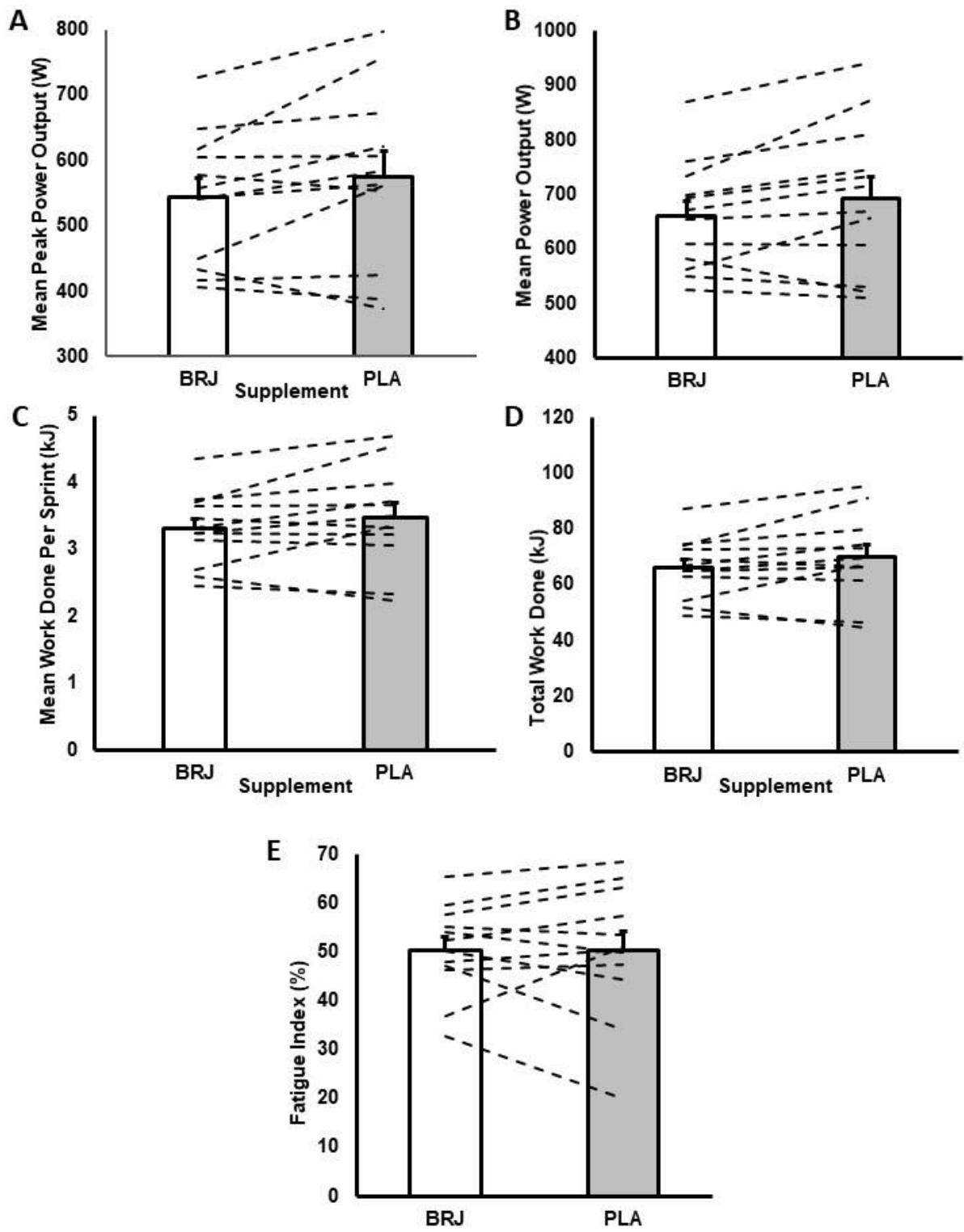


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654 **Figure 2.**

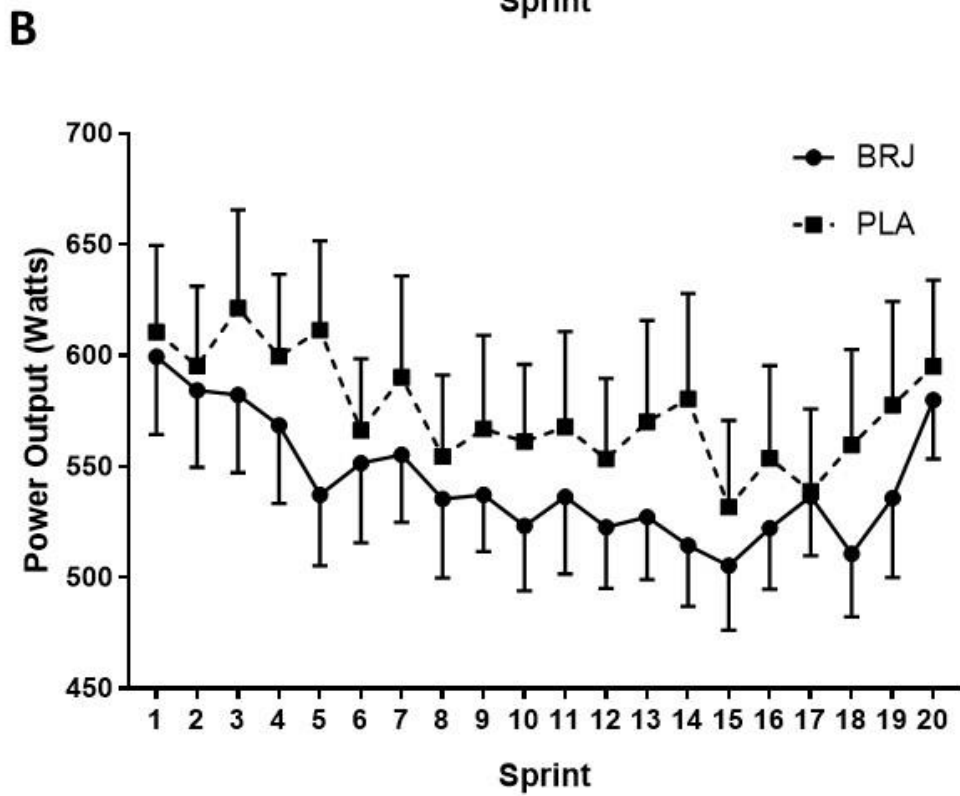
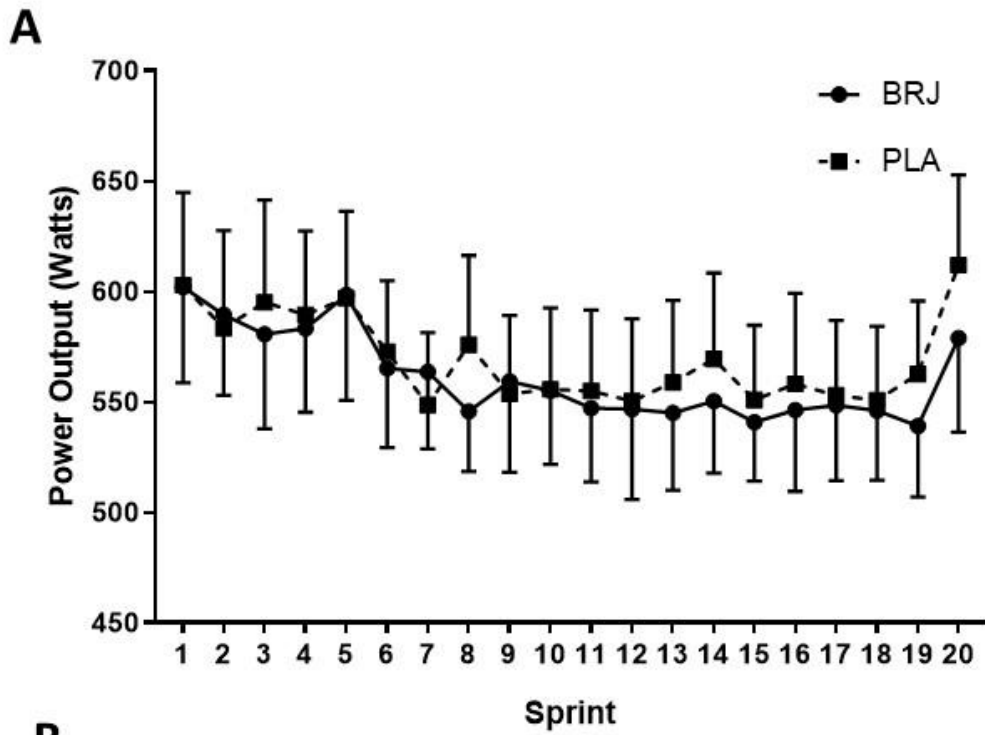


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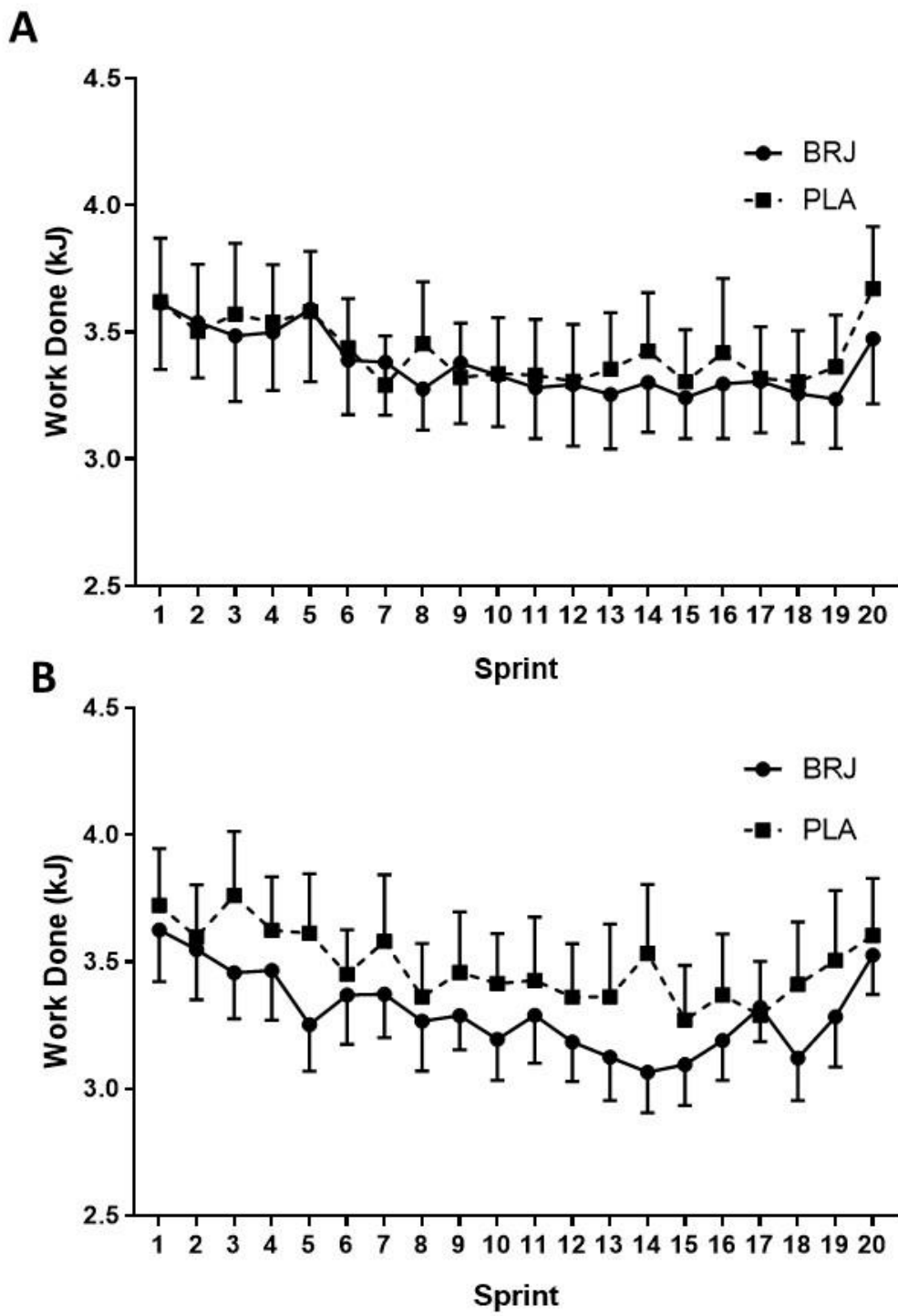


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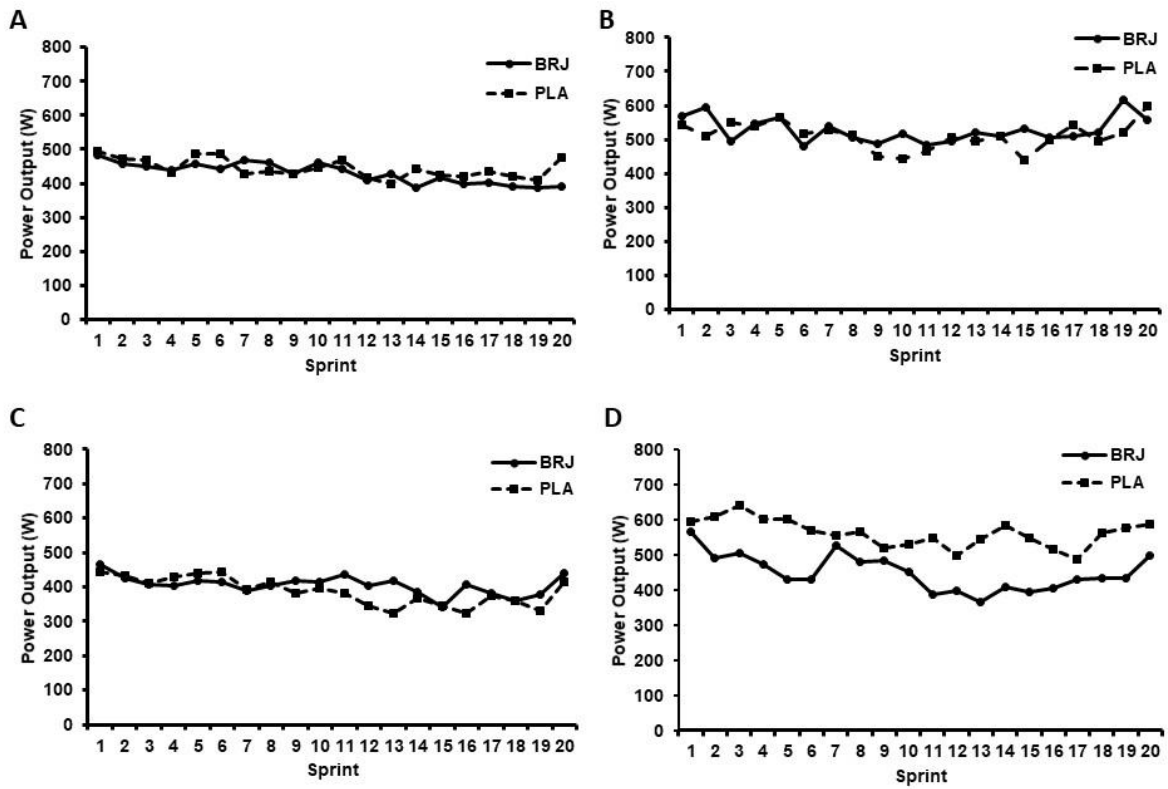


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666 **Figure 5.**



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