

## **Beta-alanine did not improve high-intensity performance throughout simulated road cycling**

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## 1 **Abstract**

2 This study investigated the effect of beta-alanine supplementation on short-duration sprints and  
3 final 4-km simulated uphill cycling time-trial performance during a comprehensive and novel  
4 exercise protocol representative of the demands of road-race cycling, and determined if  
5 changes were related to increases in muscle carnosine content. Seventeen cyclists (age  $38\pm 9$  y,  
6 height  $1.76\pm 0.07$  m, body mass  $71.4\pm 8.8$  kg,  $\dot{V}O_{2\max}$   $52.4\pm 8.3$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) participated in  
7 this placebo-controlled, double-blind study. Cyclists undertook a prolonged intermittent  
8 cycling protocol lasting 125 minutes, with a 10-s sprint every 20 minutes, finishing with a 4-  
9 km time-trial at 5% simulated incline. Participants completed two familiarization and two main  
10 sessions pre-supplementation, and one post-supplementation session following 28 days of 6.4  
11 g·day<sup>-1</sup> of beta-alanine (N=11) or placebo (N=6; maltodextrin). Muscle biopsies obtained pre-  
12 and post-supplementation were analysed for muscle carnosine content. There were no main  
13 effects on sprint performance throughout the intermittent cycling test (all  $P>0.05$ ). There was  
14 no group ( $P=0.69$ ), time ( $P=0.50$ ) or group x time interaction ( $P=0.26$ ) on time-to-complete the  
15 4-km time-trial. Time-to-completion did not change from pre- to post-supplementation for BA  
16 ( $-19.2\pm 45.6$  s,  $P=0.43$ ) or PL ( $+2.8\pm 31.6$  s,  $P=0.99$ ). Beta-alanine did not influence blood  
17 lactate values or ratings of perceived exertion during the prolonged cycling test. Beta-alanine  
18 supplementation increased muscle carnosine content from pre- to post-supplementation  
19 ( $+9.4\pm 4.0$  mmol·kg<sup>-1</sup>dm;  $P<0.0001$ ) but was not related to performance changes. Chronic beta-  
20 alanine supplementation increased muscle carnosine content but did not improve short-duration  
21 sprint performance throughout simulated road race cycling, nor 4-km uphill time-trial  
22 performance conducted at the end of this cycling test.

23 **Key words:** buffering, endurance exercise, ergogenic, muscle carnosine, sprints,  
24 supplementation, time-trial

25 **Introduction**

26 Road cycling competitions are classified as endurance events (Jeukendrup, 2011), lasting from  
27 a few hours, up to three weeks (Mujika & Padilla, 2001). They are predominantly characterized  
28 by low- to moderate-intensity aerobic activity, although transient elements of these events are  
29 performed at high-intensities (Sanders & Heijboer, 2019; Vogt et al., 2006), such as short-  
30 duration intermediate sprints to gain category points or to make/catch a breakaway, or more  
31 sustained high-intensity efforts, such as those required to complete a hill/mountain climb.  
32 Performance during these prolonged events often depends on the ability to maintain an  
33 increased power output during these stages (Van Thienen et al., 2009). Thus, cyclists are likely  
34 heavily dependent on their ability to resist fatigue during these periods of high-intensity  
35 activity.

36

37 Performance during these critical, high-intensity phases of cycling road races requires  
38 considerable contribution from anaerobic energy pathways, which can result in hydrogen ion  
39 accumulation and metabolic acidosis (Abbiss & Laursen, 2005). Buffering of  $H^+$  is performed  
40 via intracellular and extracellular buffers, although their capacity to protect against pH changes  
41 can quickly become overwhelmed during high-intensity exercise. The subsequent  
42 accumulation of  $H^+$ , which results in a state of systemic acidosis, can directly limit muscle  
43 contractile machinery (Debold, Fitts, Sundberg, & Nosek, 2016; Jarvis, Woodward, Debold, &  
44 Walcott, 2018; Sundberg, Hunter, Trappe, Smith, & Fitts, 2018) and energy production  
45 (Jubrias, Crowther, Shankland, Gronka, & Conley, 2003; Spriet, Lindinger, McKelvie,  
46 Heigenhauser, & Jones, 1989), reducing force production and exercise performance. Increasing  
47 buffering capacity, therefore, could lead to improved performance in these higher-intensity  
48 efforts during a cycling race.

49

50 Carnosine is a histidine-containing dipeptide in skeletal muscle (Harris et al., 2006) that  
51 contributes to intracellular buffering capacity (Harris et al., 2006; Painelli et al., 2018). Beta-  
52 alanine supplementation is an effective way to increase muscle carnosine content (MCarn) with  
53 numerous studies demonstrating oral ingestion of  $\sim 6.4 \text{ g} \cdot \text{day}^{-1}$  for 4 weeks can significantly  
54 increase content in the *m. vastus lateralis* (Harris et al., 2006; Hill et al., 2007; Saunders,  
55 Painelli, et al., 2017). Meta-analytical data show beta-alanine to be an effective supplement to  
56 improve exercise outcomes on average, and most effective on high-intensity efforts with a  
57 duration of 0.5 to 10 min, with further meta-regressions showing it to be ineffective for longer  
58 duration exercise ( $>10 \text{ min}$ ) (Saunders, Elliott-Sale, et al., 2017). Indeed, individual studies  
59 have shown no effect of beta-alanine on 1-h (Chung, Baguet, Bex, Bishop, & Derave, 2014),  
60 10-km (Bellinger & Minahan, 2016) or 20-km (James et al., 2014) cycling time-trial  
61 performance. These exercise protocols required a more continuous, steady-state effort and so  
62 did not account for the dynamic nature of road race cycling, wherein cyclists are frequently  
63 and transiently required to increase their exercise intensity (Sanders & Heijboer, 2019). Beta-  
64 alanine improved final sprint performance following an intermittent cycling protocol, which  
65 more accurately reflected the fluctuating power outputs required during a road race (Van  
66 Thienen et al., 2009), demonstrating the ergogenic potential of beta-alanine at key high-  
67 intensity periods throughout endurance cycling. Beta-alanine might also positively influence  
68 other dynamic actions that are common during prolonged cycling stages with different profiles,  
69 such as short-duration intermittent sprints or mountain climbs (Sanders & Heijboer, 2019), by  
70 resisting severe intracellular pH changes and enhancing the capacity of the muscle to sustain  
71 these higher-intensity efforts, although no studies have investigated this.

72

73 This study investigated the effect of beta-alanine supplementation on short-duration sprints and  
74 a final 4-km simulated uphill cycle during a comprehensive exercise protocol representative of

75 the demands of road-race cycling and determined if changes were related to MCarn increases.  
76 We hypothesized that 4 weeks of beta-alanine supplementation would increase muscle  
77 carnosine content, subsequently improving intermittent sprint and 4-km time-trial  
78 performance.

## 79 **Materials and Methods**

### 80 *Participants*

81 An *a priori* power analysis performed using G\*Power (v.3.1, University of Düsseldorf,  
82 Germany) (Faul, Erdfelder, Lang, & Buchner, 2007), with  $\alpha=0.05$  and  $\beta=0.8$ , and using the  
83 change in 4-km time-trial performance with beta-alanine shown by Bellinger and Minahan  
84 (2016), indicated that eight participants per group was required. A call for participation was  
85 made requesting healthy male cyclists to partake in this randomized, double-blind, and  
86 placebo-controlled study. Athletes had to have a minimum of one year experience in cycling  
87 and a weekly training volume  $\geq 60$  km (De Pauw et al., 2013). Participants could not have used  
88 creatine- or beta-alanine-containing dietary supplements in the past 6 months. Fifty-three  
89 cyclists registered their interest, 38 of whom were assessed for eligibility, but 16 did not meet  
90 the criteria or declined to participate, leaving 22 who entered the randomization process  
91 (Supplemental File 1). Five individuals dropped out throughout the supplementation period  
92 (BA: N=1; PL: N=4) citing time difficulties, loss of interest or injuries unrelated to the study,  
93 meaning 17 recreationally trained cyclists (De Pauw et al., 2013) completed the study (Table  
94 1). The study was approved by the institution's Ethical Advisory Committee (CAAE:  
95 54253515.3.0000.5391) and all participants provided written informed consent prior to  
96 participation.

97

### 98 *Study Design*

99 Participants attended the laboratory on five separate occasions separated by a minimum of 7  
100 days. The first session comprised an incremental cycling test to exhaustion to determine  
101 maximal oxygen uptake ( $\dot{V}O_{2max}$ ). The following sessions comprised two familiarisations of  
102 the entire cycling protocol. Thereafter, two main trials of the simulated cycling protocol were  
103 performed pre and post a 4-week supplementation period (beta-alanine or placebo).

104 Participants underwent biopsies of the *m. vastus lateralis* one-hour after the exercise protocol  
105 for MCarn determination.

106

107 All familiarisation and main trials were performed at the same time of day to avoid effects of  
108 circadian variation (Atkinson & Reilly, 1996), and participants were requested to arrive a  
109 minimum of 2 h after their last food consumption. Participants recorded dietary intake in the  
110 24 h before the first main session and repeated this prior to the final main session. Strenuous  
111 exercise and alcohol were prohibited during the 24 h pre-test period, whereas caffeine intake  
112 was forbidden only on the day of the test. Any changes in training were monitored by obtaining  
113 distance covered during training in the 4-weeks prior to supplementation and during the  
114 supplementation period using each individual's global positioning system (GPS; e.g. Strava),  
115 although three individuals (two in BA, one in PL) did not use any GPS system.

116

## 117 *Materials and Methods*

### 118 *$\dot{V}O_{2max}$ test*

119 The incremental cycling test was performed on a cycle ergometer (Lode Excalibur, Lode, The  
120 Netherlands). Initial workload was 100 W and increased by 25 W in 3-min stages until  
121 exhaustion (De Pauw et al., 2013). Breath-by-breath gas measurements were continuously  
122 recorded using a calibrated gas analyser (Quark, Cosmed, Italy). The highest  $\dot{V}O_2$  value  
123 averaged over a 15-s period during the test was defined as  $\dot{V}O_{2max}$  and maximal power output  
124 ( $W_{max}$ ) was calculated as the last completed stage plus the fraction of time spent in the final  
125 non-completed stage multiplied by 25 W. The seat and handlebar positions of the cycle  
126 ergometer was determined before the incremental cycle session, recorded, and maintained for  
127 all subsequent trials. Participants chose their preferred pedal type to ensure they could use their

128 cycling shoes (with or without clips) but were required to repeat this choice in all subsequent  
129 sessions.

130

131 *Simulated cycling road race protocol and 4-km time-trial set at 5% incline*

132 The exercise protocol went through various stages of pilot testing with an elite road cyclist  
133 prior to the final version employed, and the varying power outputs and high-intensity efforts  
134 were chosen in accordance with data from stage racing in professional races (Sanders &  
135 Heijboer, 2019; Vogt et al., 2006). The intermittent section of the simulated cycling road race  
136 protocol was performed on a cycle ergometer (Lode Excalibur, Lode, The Netherlands).  
137 Following a 5-min warm-up at 1 W·kg<sup>-1</sup>BM, individuals cycled for 120 minutes at power  
138 outputs between 1.5 and 3 W·kg<sup>-1</sup>BM (Figure 1) at their self-selected cadence (range: 70-90  
139 rev·min<sup>-1</sup>). Corresponding power outputs were 107±13 W (38±6% W<sub>max</sub>), 143±18 W  
140 (51±7% W<sub>max</sub>), 178±22 W (64±9% W<sub>max</sub>) and 214±26 W (76±11% W<sub>max</sub>) for 1.5, 2, 2.5 and 3  
141 W·kg<sup>-1</sup>BM. A 10-s all-out sprint was performed every 19 min 50 s, totalling six sprints (Figure  
142 1). The volunteers reduced their cadence to 60 rpm in the 30 s prior so that each sprint was  
143 initiated with a starting cadence of ~60 rpm; the power was reduced to 75 W during this period  
144 so that the athletes could maintain this low cadence. The total duration of the prolonged  
145 simulated road race protocol including warm-up was 2 h and 5 min. Participants were required  
146 to ingest 200 ml of liquid containing 12 g of carbohydrate (CHO) every 20 min (totalling 1  
147 L·h<sup>-1</sup> containing 36 g·h<sup>-1</sup>) according to guidelines (Jeukendrup, 2014) to minimise any potential  
148 confounding effect of muscle glycogen depletion. Mean (MPO) and peak (PPO) power output  
149 during the six 10-s sprints were recorded.

150

151 Immediately following the simulated road race protocol, participants transferred to a road  
152 bicycle (Caloi, Brazil) attached to a roller (RacerMate, RacerMate Inc, USA) and performed

153 10 min of constant-load cycling ( $1 \text{ W} \cdot \text{kg}^{-1} \text{BM}$ ) to ensure the roller was sufficiently warmed-  
154 up. Thereafter, participants performed a simulated 4-km cycling time-trial with a resistance  
155 designed to simulate a 5% incline; the front fork was attached to an extension so that the  
156 position of the bike was on an incline of 5%. The starting gear was standardised after which  
157 participants could change gears freely to complete the climb as quickly as possible and were  
158 blinded to all performance information except distance covered. The time-trial was designed  
159 to simulate a mountain top finish and the test was terminated upon completion of the 4-km  
160 time-trial. Participants could rise from their seat to generate power throughout the test to  
161 simulate climbing. Time-to-completion was recorded as the performance measure during the  
162 time-trial. Blood lactate was determined from fingertip samples collected immediately pre- and  
163 post- the 4-km time-trial using a portable lactate analyser (Lactate Plus, Nova Biomedical,  
164 USA). Ratings of perceived exertion (Borg, 1974) were determined every 400 m during the 4-  
165 km time-trial and averaged over each session. The seat and handlebar positions of the bicycle  
166 was determined and recorded during the first familiarisation session and maintained for all  
167 subsequent trials. Similarly, participants could freely choose their preferred pedal type to  
168 ensure they could use their preferred cycling shoes.

169

#### 170 *Muscle biopsy and carnosine content*

171 Muscle biopsies (~100 mg) were taken from the mid-section of the *m. vastus lateralis* using a  
172 5-mm biopsy Allandale needle (Northern Hospital Supplies, Edinburgh, UK) (Neves et al.,  
173 2012). Samples were flash-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later analysis.  
174 Analysis of whole muscle carnosine content was subsequently performed by high-pressure  
175 liquid chromatography (Hitachi; Hitachi Ltd., Tokyo, Japan) coupled to a UV detector  
176 according to the method described by Mora et al. (Mora, Sentandreu, & Toldra, 2007). We

177 have previously reported the extraction and analysis methods to have a variability of 4.0 and  
178 2.5% (Saunders, Painelli, et al., 2017).

179

### 180 *Supplementation protocol*

181 Participants were randomly allocated from 2x2 blocks to a beta-alanine (BA; CarnoSyn,  
182 Natural Alternatives Inc., USA) or placebo (PL; maltodextrin, Natural Alternatives Inc., USA)  
183 group. Supplementation involved ingesting 6.4 g·day<sup>-1</sup> of sustained-release beta-alanine or  
184 placebo for 4 weeks, taken as 2 x 800 mg tablets four times per day at 3–4 h intervals. Diaries  
185 were maintained to ensure adherence to the supplementation protocol, with a high level of  
186 adherence in both groups (Table 1). A questionnaire was applied following the final session to  
187 extract information regarding supplementation including, i) what supplement they believe they  
188 had ingested (“beta-alanine”, “placebo”, “don’t know”), ii) any side-effects experienced and  
189 details thereof (Decombaz, Beaumont, Vuichoud, Bouisset, & Stellingwerff, 2012), and iii)  
190 whether they thought supplementation had improved their training.

191

### 192 *Data analysis*

193 Data were analysed using the SAS statistical package (SAS<sup>®</sup> University Edition, SAS Institute  
194 Inc., USA), and are presented as mean±1SD. Participant characteristics were analysed using  
195 an independent-samples t-test. Sprint performance was analysed using a mixed model  
196 assuming supplementation (2 levels; BA and PL), time (2 levels; Pre-supplementation and  
197 Post-supplementation) and sprint number (6 levels; 1-6) as fixed factors. Each individual sprint,  
198 4-km time-trial performance and MCarn were analysed using mixed model analysis with  
199 supplementation (2 levels; BA and PL) and time (2 levels; Pre-supplementation and Post-  
200 supplementation) assumed as fixed factors. Training volume (total distance) was analysed  
201 using mixed model analysis with supplementation (2 levels; BA and PL) and time (2 levels;

202 Pre-supplementation and Post-supplementation) assumed as fixed factors. Blood lactate was  
203 analysed using mixed model analysis with supplementation (2 levels; BA and PL), time (2  
204 levels; Pre-supplementation and Post-supplementation) and moment (2 levels; Pre-exercise and  
205 Post-exercise) assumed as fixed factors. Tukey–Kramer adjustments were performed when a  
206 significant F value was obtained. Individuals were assumed as a random factor for all mixed  
207 models. A Satterthwaite approximation was performed for all analyses to account for the  
208 unequal sample sizes. Performance data were analysed for the 17 complete data sets (BA,  
209 N=11; PL, N=6), but due to issues in extraction, two muscle samples were lost meaning that  
210 complete muscle data for 15 individuals (BA, N=10; PL, N=5) was included in that analysis.  
211 Hedges’ g effect sizes for repeated measures and small sample correction were calculated for  
212 the 10-s sprints, 4-km time-trial performance and muscle carnosine content, and interpreted  
213 according to <0.20 (trivial), 0.20–0.49 (small), 0.50–0.79 (moderate), and  $\geq 0.80$  (large)  
214 (Cohen, 1988). The pre- to post-supplementation change in 4-km time-trial performance  
215 ( $\Delta$ TTC; s) and muscle carnosine content ( $\Delta$ MCarn; mmol·kg<sup>-1</sup>dm) were calculated alongside  
216 95% confidence intervals (95%CI) and a Pearson product-moment correlation coefficient  
217 determined any relationship between the change in these measures in the beta-alanine group.  
218 A Fisher Exact Probability Test with Freeman-Halton extension for a 2 x 3 table was performed  
219 to determine differences between supplement identification, yielding two probability values  
220 ( $P_a$  and  $P_b$ ) (Freeman & Halton, 1951). Results were interpreted according to the statistical  
221 probabilities of rejecting the null hypothesis ( $H_0$ ) in the following categories:  $P > 0.1$ : no  
222 evidence against  $H_0$ ;  $0.05 < P < 0.1$ : weak evidence against  $H_0$ ;  $0.01 < P < 0.05$ : moderate evidence  
223 against  $H_0$ ;  $0.001 < P < 0.01$ : strong evidence against  $H_0$ ;  $P < 0.001$ : very strong evidence against  
224  $H_0$  (Amrhein, Korner-Nievergelt, & Roth, 2017; Bassinello et al., 2018).

225

## 226 **Results**

### 227 *Repeated 10-s sprints*

228 There was no evidence of a group (all  $P>0.1$ ) or time (all  $P>0.1$ ) effect for MPO or PPO,  
229 although there was an effect for sprint for all these measures (all  $P<0.0001$ ), reflecting a  
230 decrease in sprint performance with increasing sprint number (Table 2). Individual-sprint  
231 analysis showed no evidence of a group, time or group x time interaction effect for MPO or  
232 PPO for any sprint (all  $P>0.1$ ), except Sprint 6, which showed moderate evidence of a group x  
233 time interaction for MPO ( $P=0.014$ ); *post-hoc* adjustments did not indicate any significant  
234 differences. Pre- to post-supplementation effect sizes for MPO ranged from  $d=-0.12$  to  $d=0.30$   
235 for BA and  $d=-0.44$  to  $d=0.19$  for PL. Pre- to post-supplementation effect sizes for PPO ranged  
236 from  $d=-0.02$  to  $d=0.22$  for BA and  $d=-0.44$  to  $d=0.26$  for PL.

237

### 238 *4-km time-trial*

239 Time-to-completion was not different between BA and PL pre-supplementation (BA:  
240  $757.4\pm 86.0$  s, PL:  $724.3\pm 139.2$  s;  $P=0.43$ ,  $g=0.29$ ). There was no evidence of a group ( $P=0.69$ ),  
241 time ( $P=0.50$ ) or a group x time interaction ( $P=0.26$ ) on time-to-completion (Figure 2, Panel  
242 A). Time-to-completion did not change from pre- to post-supplementation for BA ( $\Delta$ TTC: -  
243  $19.2\pm 45.6$  s, 95%CI: -46.1 – 7.8,  $P=0.43$ ,  $g=0.22$ ) or PL ( $\Delta$ TTC:  $+2.8\pm 31.6$  s, 95%CI: -15.9 –  
244 21.5,  $P=0.99$ ,  $g=0.03$ ).

245

### 246 *Muscle carnosine content*

247 Muscle carnosine content was not different between BA and PL pre-supplementation (BA:  
248  $24.9\pm 2.5$  mmol·kg<sup>-1</sup>dm, PL:  $26.0\pm 6.1$  mmol·kg<sup>-1</sup>dm;  $P=0.97$ ,  $g=0.26$ ). There was no evidence  
249 of an effect of group ( $P=0.20$ ), but there was very strong evidence of an effect of time  
250 ( $P<0.0001$ ) and a group x time interaction ( $P=0.0008$ ). Post-hoc adjustments showed that

251 muscle carnosine content increased from pre- to post-supplementation in BA ( $\Delta\text{MCarn}$ :  
252  $+9.4\pm 4.0$  mmol·kg<sup>-1</sup>dm, 95%CI: 7.1 – 11.8,  $P<0.0001$ ,  $g=2.70$ ), but there was no evidence of  
253 a change in PL ( $\Delta\text{MCarn}$ :  $+1.4\pm 1.1$  mmol·kg<sup>-1</sup>dm, 95%CI: 0.8 – 2.1,  $P=0.78$ ,  $g=0.22$ ; Figure  
254 2, Panel B). There was no evidence of a correlation between  $\Delta\text{TTC}$  and  $\Delta\text{MCarn}$  in the BA  
255 group ( $r=0.320$ ,  $P=0.37$ ).

256

### 257 *Blood lactate and Ratings of Perceived Exertion*

258 There was no evidence of a group ( $P=0.61$ ) or time ( $P=0.76$ ) effect for blood lactate, but there  
259 was very strong evidence of an effect of moment ( $P<0.0001$ ), reflecting an increase from pre-  
260 to post-time-trial (Supplemental File 2). However, there was no evidence of any interaction  
261 effects (all  $P>0.1$ ). There was no evidence of an effect of group ( $P=0.83$ ), time ( $P=0.96$ ), or a  
262 group x time interaction ( $P=0.22$ ) for ratings of perceived exertion throughout the time-trial  
263 (BA, Pre-supplementation:  $17\pm 1$ , Post-supplementation:  $16\pm 1$ ; PL, Pre-supplementation:  
264  $17\pm 1$ , Post-supplementation:  $17\pm 2$ ).

265

### 266 *Supplementation and Training*

267 Two individuals in BA reported side-effects (both sensations of paraesthesia/pins and needles);  
268 one of these correctly identified supplementing with BA, the other did not know what he was  
269 taking. No one in PL reported any side-effects. There were no differences in supplement  
270 identification between groups ( $P_a=0.53$  and  $P_b=0.44$ ), with two individuals correctly  
271 identifying BA and three correctly identifying PL. Two individuals incorrectly believed they  
272 had ingested PL and two incorrectly believed they were on BA, while the remaining five (BA)  
273 and one (PL) did not know what they had taken. Four individuals in each group believed that  
274 the supplement improved some aspect of their training throughout the supplementation period.

275

276 Training volume in the 4-weeks pre-supplementation was  $516\pm 259$  km for BA and  $632\pm 392$   
277 km for PL, with no evidence of a difference between groups ( $P=0.54$ ). Training volume in the  
278 4-weeks throughout supplementation was  $499\pm 269$  km for BA and  $613\pm 420$  km for PL, There  
279 was no evidence of a group ( $P=0.52$ ), time ( $P=0.66$ ) or a group x time interaction ( $P=0.98$ ) for  
280 distance covered during training.

281 **Discussion**

282 Four weeks of beta-alanine supplementation did not improve short-duration sprint performance  
283 throughout simulated road cycling, nor final 4-km uphill time-trial performance, despite  
284 increases in muscle carnosine content.

285

286 Buffering capacity is an important determinant of sprint ability during repeated cycle sprints  
287 (Bishop, Edge, Davis, & Goodman, 2004; Bishop, Edge, & Goodman, 2004). Despite this, the  
288 current data did not show any improvement in 10-s sprints interspersed throughout intermittent  
289 cycling. It seems likely that the null effect shown here is due to the length of time available for  
290 pH recovery between each sprint. Previous data showing a relationship between repeated short-  
291 duration sprints and buffering capacity have commonly employed short recovery periods  
292 between sprints, resulting in insufficient recovery of acid-base balance (Bishop, Edge, Davis,  
293 et al., 2004; Bishop, Edge, & Goodman, 2004). The longer time between sprints in the current  
294 protocol may have allowed more complete recovery of muscle pH, meaning the increased  
295 buffering capacity provided by higher muscle carnosine content was irrelevant to performance.  
296 Although the current protocol required individuals to continue cycling at intermittent power  
297 outputs, the intensity thereof may have been too low to induce a metabolic acidosis that  
298 compromised sprint performance. It is also possible that the duration of these sprints may have  
299 been too short to induce sufficient acidosis to compromise power output (Saunders, Elliott-  
300 Sale, et al., 2017). These data suggest that beta-alanine supplementation is ineffective at  
301 improving short-duration sprints throughout simulated intermittent road cycling.

302

303 There was no effect of beta-alanine supplementation on 4-km cycling time-trial performance  
304 at a simulated 5% incline following prolonged intermittent cycling. Previous studies have  
305 shown beta-alanine to provide modest improvements on 4-km time-trial cycling (Bellinger &

306 Minahan, 2016), improving time-to-completion by an average of 6.5 s. Despite a mean -19.2 s  
307 ( $\pm 45.6$  s) change in 4-km time-trial performance herein, this difference was not statistically  
308 significant. The reason for the discrepancy in these results may be due to the simulated time-  
309 trial specifications and, ultimately, the duration of the exercise undertaken. Bellinger and  
310 Minahan (2016) employed a flat course profile, resulting in a performance time of  
311 approximately 6 min, whereas we simulated a hill-top finish using a 5% simulated resistance,  
312 with performance times closer to 13 min. This is in line with evidence showing beta-alanine to  
313 be most effective during exercise 0.5–10 min in duration (Saunders, Elliott-Sale, et al., 2017).  
314 Additionally, the athletes were in a fatigued state, since they had undergone 2 h of prior cycling,  
315 which may have meant that the exercise intensity during the time-trial was performed at a lower  
316 intensity given that prior intermittent exercise can compromise final power output following  
317 prolonged cycling (Etxebarria, Ingham, Ferguson, Bentley, & Pyne, 2019). Post-time-trial  
318 blood lactate values were far lower than those shown by Bellinger and Minahan (2016) ( $\sim 9$  vs.  
319  $15 \text{ mmol}\cdot\text{L}^{-1}$ ), supporting the notion that the 4-km time-trial undertaken herein was performed  
320 at a lower intensity. It cannot be ruled out that an uphill section earlier in a prolonged cycle  
321 stage, performed following less prior-fatigue, may be maintained at a higher intensity and  
322 might thus incur different results with beta-alanine supplementation. Similarly, since many  
323 sustained efforts during cycle racing are not self-paced and require maximal or supramaximal  
324 power output to maintain contact with the leaders, it might be of interest to determine the value  
325 of beta-alanine supplementation during this type of exertion.

326

327 As expected, 4-weeks of  $6.4 \text{ g}\cdot\text{day}^{-1}$  BA supplementation increased MCarn to a similar extent  
328 as previous studies employing similar doses (Harris et al., 2006; Hill et al., 2007; Saunders,  
329 Painelli, et al., 2017). These changes in MCarn were not associated with the changes in exercise  
330 performance during the 4-km time-trial. Muscle carnosine increases ranged from +3.1 to 14.8

331 mmol·kg<sup>-1</sup>dm, corroborating previous work showing large interindividual variability in MCarn  
332 increases with the same dose and duration (Saunders, Painelli, et al., 2017). The reason for this  
333 variability remains unclear, but may be related to several factors (Perim et al., 2019) including  
334 differences in training status (Bex et al., 2014), or interindividual differences in the activity of  
335 beta-alanine transaminases, the enzymes responsible for beta-alanine oxidation (Blancquaert  
336 et al., 2016). The efficiency of beta-alanine supplementation to increase MCarn appears low  
337 (3–6%; (Blancquaert, Everaert, & Derave, 2015)), although our data provide further evidence  
338 to support meta-analytical data showing that, in effect, all individuals respond to beta-alanine  
339 supplementation by increasing muscle carnosine (Rezende et al., 2020).

340

341 This study has some limitations. Five volunteers withdrew from the study following  
342 randomisation into supplementation groups, most dropouts coming from the placebo group. As  
343 a result, the sample number was lower than planned and may have lacked adequate statistical  
344 power to detect significant performance improvements. Nonetheless, the active intervention  
345 group was sufficiently powered but within-group pre- to post-supplementation effect sizes were  
346 small, suggesting that results would not have differed had we attained N=8 in each group. We  
347 recruited competitive cyclists with 8±4 years of training experience and monthly training  
348 volumes in excess of 500 km, although their  $\dot{V}O_{2max}$  categorised them as recreationally trained  
349 (De Pauw et al., 2013) while some had a  $\dot{V}O_{2max}$  that categorised them below this level. Higher  
350 level athletes, including professional female athletes who perform more high-intensity work  
351 during training and competition than their male counterparts (van Erp, Sanders, & de Koning,  
352 2019), might benefit differently from beta-alanine supplementation, particularly since they  
353 might complete the 4-km time-trial in under 10 min (Saunders, Elliott-Sale, et al., 2017).

354

355 **Conclusion**

356 Beta-alanine supplementation increased muscle carnosine content, but did not generate  
357 improvements in the performance of high-intensity cycling during a real-world simulated road  
358 race cycling protocol, namely repeated 10-s sprints and a final 4-km time-trial at a simulated  
359 5% incline. Our data suggest that short duration sprints ( $\leq 10$  s) and longer duration ( $> 10$  min)  
360 high-intensity activity throughout endurance cycling are not improved with beta-alanine  
361 supplementation despite increases in muscle carnosine content.

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366

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507

508 **Figure Legends**

509 Figure 1. Overview of the simulated cycling road race protocol and 4-km time-trial (TT) set at  
510 5% incline.

511

512 Figure 2. Panel A: Time-to-completion for the 4-km cycling time-trial (TT) in the beta-alanine  
513 (BA) and placebo (PL) groups pre- (Pre) and post- (Post) supplementation. Panel B: Muscle  
514 carnosine content in the beta-alanine (BA; N=10) and placebo (PL; N=5) groups pre- (Pre) and  
515 post- (Post) supplementation. \*P<0.0001 from Pre-supplementation. Data are means  $\pm$  1  
516 standard deviation.

517 **Supplemental Files**

518 Supplemental File 1. CONSORT Flow Diagram

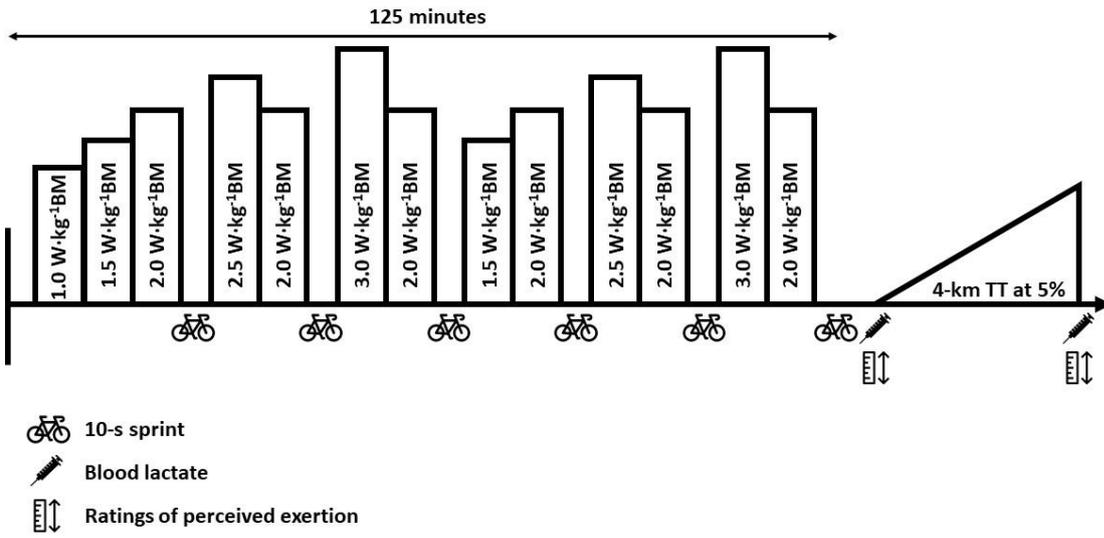
519

520 Supplemental File 2. Blood lactate concentration pre- and post- the 4-km cycling time-trial

521 (TT) in the beta-alanine (BA, Panel A) and placebo (PL, Panel B) groups pre- (Pre) and post-

522 (Post) supplementation. \* $P < 0.0001$  from Pre- 4-km TT. Data are means  $\pm$  1 standard deviation.

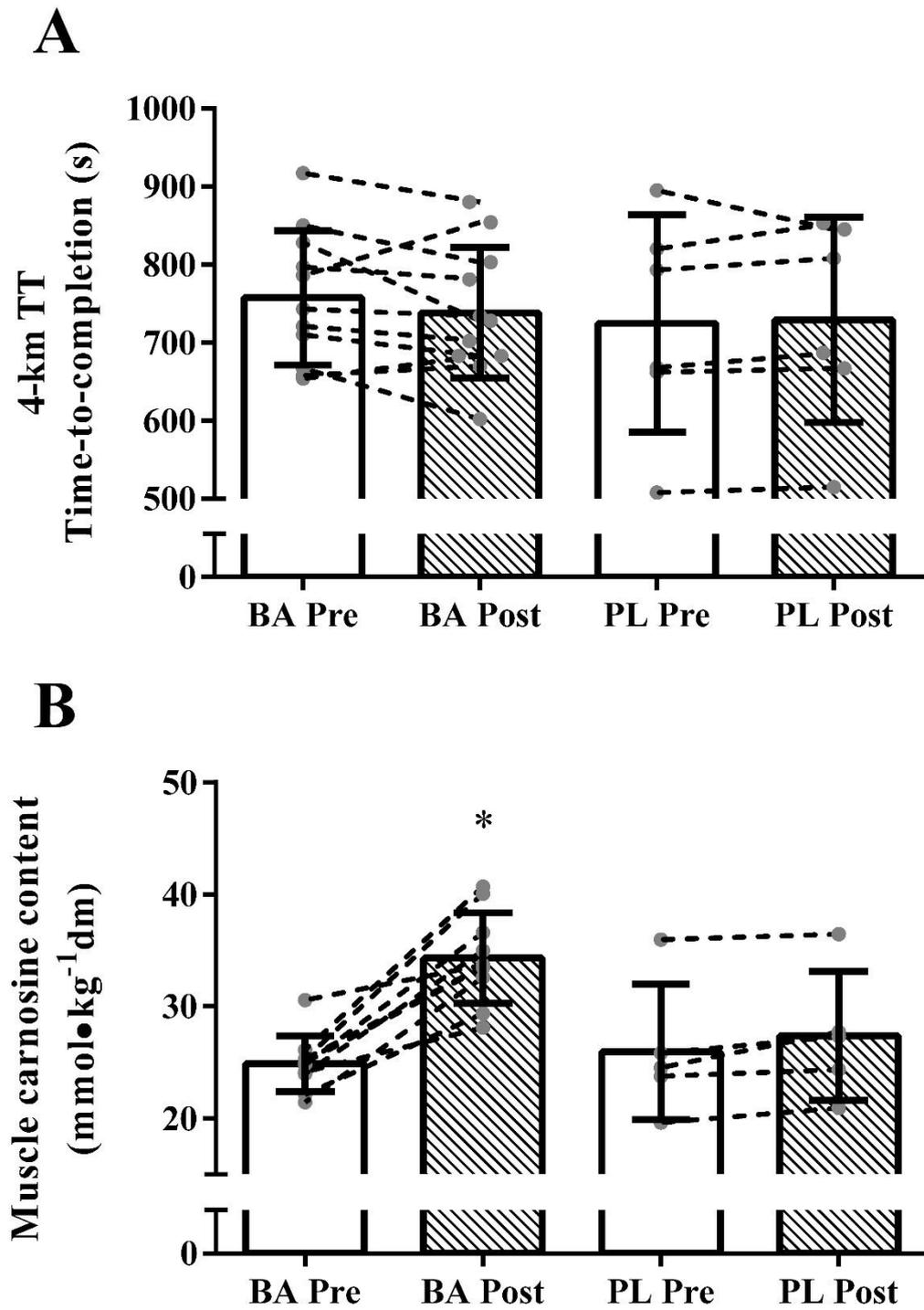
523



524

525 Figure 1.

526



527

528 Figure 2.

529

530 **Table 1. Participant characteristics in the beta-alanine (BA, N = 11) and placebo (PL, N = 6) groups.**

	<b>BA</b>	<b>PL</b>	<b>P value</b>
<b>Age (y)</b>	39 ± 8	37 ± 11	1.00
<b>Height (m)</b>	1.78 ± 0.07	1.71 ± 0.03	0.01
<b>Body mass (kg)</b>	71.9 ± 8.2	70.5 ± 10.6	0.79
<b>Cycling experience (y)</b>	8 ± 4	7 ± 4	0.63
<b>Weekly cycling load (km)</b>	129 ± 65	158 ± 98	0.54
<b>VO<sub>2max</sub> (mL·min<sup>-1</sup>·kg<sup>-1</sup>)</b>	52.4 ± 5.4	52.5 ± 14.1	0.99
<b>Maximal cycling power output (W)</b>	284 ± 28	299 ± 22	0.25
<b>Supplement compliance (%)</b>	97	99	0.14

531

532

**Table 2.** Mean power output (MPO) and peak power output (PPO) during each sprint throughout the prolonged exercise protocol pre- and post-supplementation.

		Beta-alanine		Placebo		Group x Time Interaction
		Pre	Post	Pre	Post	P
<b>Sprint 1</b>	<b>MPO (W)</b>	806 ± 115	792 ± 105	800 ± 96	811 ± 103	0.43
	<b>PPO (W)</b>	970 ± 165	996 ± 169	1007 ± 185	1003 ± 165	0.32
<b>Sprint 2</b>	<b>MPO (W)</b>	802 ± 119	808 ± 117	795 ± 96	783 ± 88	0.39
	<b>PPO (W)</b>	968 ± 185	980 ± 166	956 ± 155	963 ± 144	0.89
<b>Sprint 3</b>	<b>MPO (W)</b>	788 ± 131	793 ± 112	713 ± 65	723 ± 81	0.35
	<b>PPO (W)</b>	940 ± 185	946 ± 187	938 ± 186	859 ± 137	0.23
<b>Sprint 4</b>	<b>MPO (W)</b>	777 ± 121	788 ± 117	720 ± 129	743 ± 93	0.17
	<b>PPO (W)</b>	938 ± 174	934 ± 175	847 ± 159	889 ± 137	0.10
<b>Sprint 5</b>	<b>MPO (W)</b>	748 ± 137	772 ± 120	726 ± 103	700 ± 106	0.12
	<b>PPO (W)</b>	907 ± 173	923 ± 154	872 ± 151	838 ± 140	0.26
<b>Sprint 6</b>	<b>MPO (W)</b>	766 ± 116	801 ± 123	767 ± 117	712 ± 125	0.01
	<b>PPO (W)</b>	935 ± 183	976 ± 196	895 ± 174	894 ± 171	0.33