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

Pedro Perim[1], Nathan Gobbi[1], Breno Duarte[1], Luana Farias de Oliveira[1], Luiz Augusto Riani Costa[1], Craig Sale[2], Bruno Gualano[1,3], Eimear Dolan[1], Bryan Saunders[1,4]

1 – Applied Physiology and Nutrition Research Group, School of Physical Education and Sport; Rheumatology Division; Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, BR, University of São Paulo, SP, Brazil.

2 – Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement Research Centre, Nottingham Trent University, United Kingdom.

3 – Food Research Center, University of São Paulo, São Paulo, Brazil.

4 – Institute of Orthopaedics and Traumatology, Faculty of Medicine FMUSP, University of São Paulo, Brazil.

Correspondence:

Dr Bryan Saunders

Applied Physiology & Nutrition Research Group,

Rheumatology Division, Faculty of Medicine FMUSP,

Av. Dr. Arnaldo, 455 - Cerqueira César - CEP: 01246903

University of São Paulo,

São Paulo, SP, Brazil.

E-mail: drbryansaunders@outlook.com

Phone: +55 11 3061-8789

Fax: +55 11 3813-5921

Abstract

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

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

Introduction

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

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

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

This study investigated the effect of beta-alanine supplementation on short-duration sprints and 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.

Materials and Methods

Participants

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

Study Design

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

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

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

Materials and Methods

$\dot{V}O_{2max}$ test

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

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

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

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

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

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

Muscle biopsy and carnosine content

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

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

Supplementation protocol

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

Data analysis

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

Pre-supplementation and Post-supplementation) assumed as fixed factors. Blood lactate was analysed using mixed model analysis with supplementation (2 levels; BA and PL), time (2 levels; Pre-supplementation and Post-supplementation) and moment (2 levels; Pre-exercise and Post-exercise) assumed as fixed factors. Tukey–Kramer adjustments were performed when a significant F value was obtained. Individuals were assumed as a random factor for all mixed models. A Satterthwaite approximation was performed for all analyses to account for the unequal sample sizes. Performance data were analysed for the 17 complete data sets (BA, N=11; PL, N=6), but due to issues in extraction, two muscle samples were lost meaning that complete muscle data for 15 individuals (BA, N=10; PL, N=5) was included in that analysis. Hedges' g effect sizes for repeated measures and small sample correction were calculated for the 10-s sprints, 4-km time-trial performance and muscle carnosine content, and interpreted according to <0.20 (trivial), 0.20–0.49 (small), 0.50–0.79 (moderate), and ≥0.80 (large) (Cohen, 1988). The pre- to post-supplementation change in 4-km time-trial performance (ΔTTC; s) and muscle carnosine content (ΔMCarn; $mmol \cdot kg^{-1}dm$) were calculated alongside 95% confidence intervals (95%CI) and a Pearson product-moment correlation coefficient determined any relationship between the change in these measures in the beta-alanine group. A Fisher Exact Probability Test with Freeman-Halton extension for a 2 x 3 table was performed to determine differences between supplement identification, yielding two probability values (Pa and P_b) (Freeman & Halton, 1951). Results were interpreted according to the statistical probabilities of rejecting the null hypothesis (H0) in the following categories: P>0.1: no evidence against H0; 0.05<P<0.1: weak evidence against H0; 0.01<P<0.05: moderate evidence against H0; 0.001<P<0.01: strong evidence against H0; P<0.001: very strong evidence against H0 (Amrhein, Korner-Nievergelt, & Roth, 2017; Bassinello et al., 2018).

Results

Repeated 10-s sprints

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

4-km time-trial

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

Muscle carnosine content

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

muscle carnosine content increased from pre- to post-supplementation in BA (ΔMCarn: +9.4±4.0 mmol·kg^{-1}dm, 95%CI: 7.1 – 11.8, P<0.0001, g=2.70), but there was no evidence of a change in PL (ΔMCarn: +1.4±1.1 mmol·kg^{-1}dm, 95%CI: 0.8 – 2.1, P=0.78, g=0.22; Figure 2, Panel B). There was no evidence of a correlation between ΔTTC and ΔMCarn in the BA group (r=0.320, P=0.37).

Blood lactate and Ratings of Perceived Exertion

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

Supplementation and Training

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

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

Discussion

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

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

There was no effect of beta-alanine supplementation on 4-km cycling time-trial performance at a simulated 5% incline following prolonged intermittent cycling. Previous studies have 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 (±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) (~9 vs.
319 15 $mmol \cdot L^{-1}$), 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 $g \cdot day^{-1}$ 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

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

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

Conclusion

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

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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.

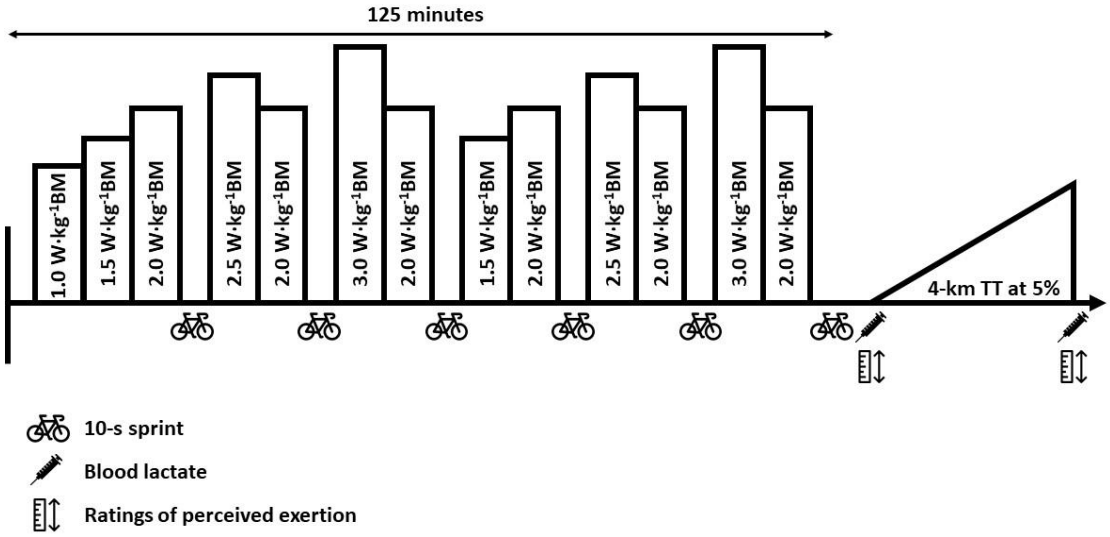
511

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 ± 1
516 standard deviation.

Supplemental Files

Supplemental File 1. CONSORT Flow Diagram

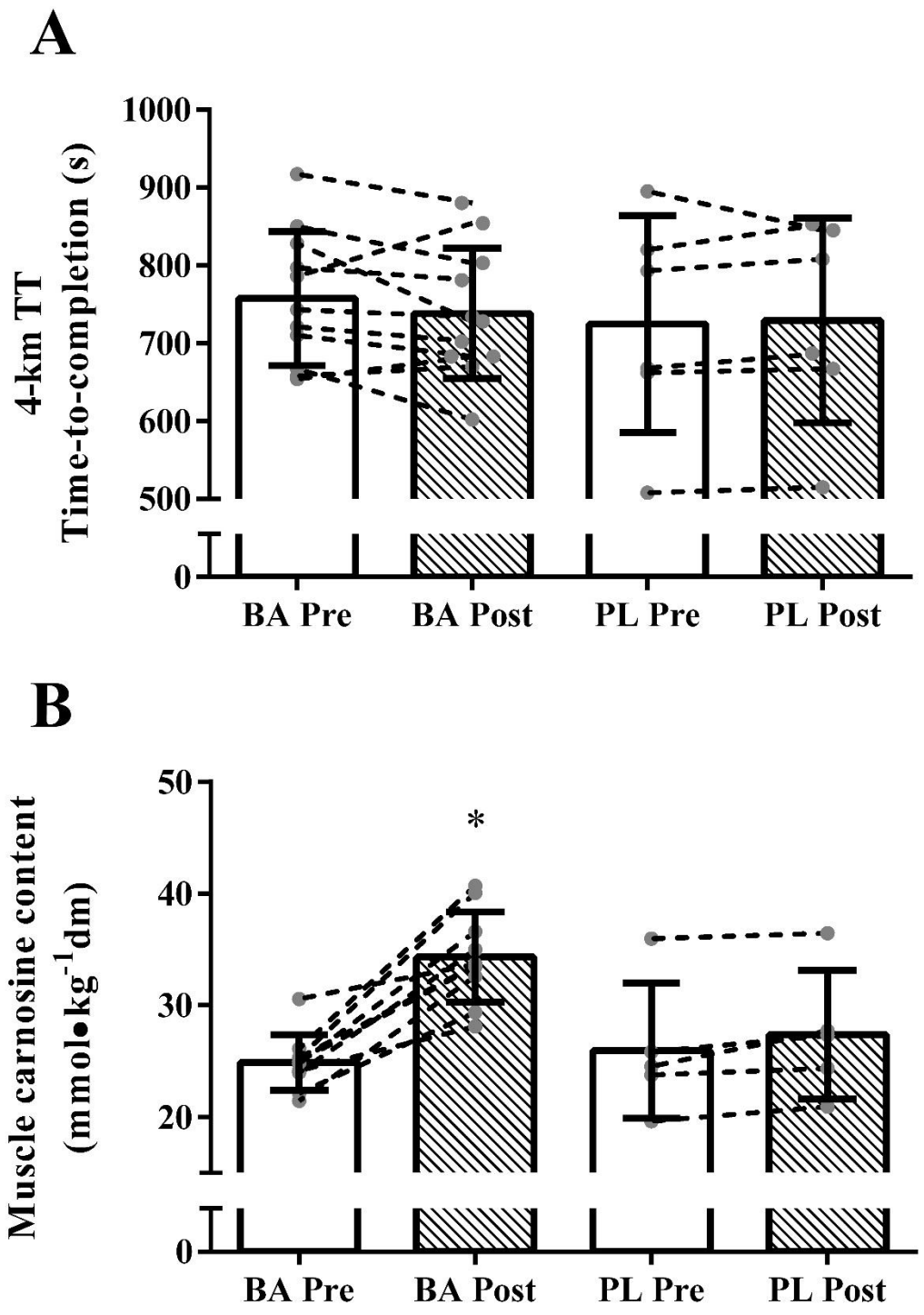
Supplemental File 2. Blood lactate concentration pre- and post- the 4-km cycling time-trial (TT) in the beta-alanine (BA, Panel A) and placebo (PL, Panel B) groups pre- (Pre) and post- (Post) supplementation. *P<0.0001 from Pre- 4-km TT. Data are means ± 1 standard deviation.



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.**

	BA	PL	P value
Age (y)	39 ± 8	37 ± 11	1.00
Height (m)	1.78 ± 0.07	1.71 ± 0.03	0.01
Body mass (kg)	71.9 ± 8.2	70.5 ± 10.6	0.79
Cycling experience (y)	8 ± 4	7 ± 4	0.63
Weekly cycling load (km)	129 ± 65	158 ± 98	0.54
VO_{2max} ($mL \cdot min^{-1} \cdot kg^{-1}$)	52.4 ± 5.4	52.5 ± 14.1	0.99
Maximal cycling power output (W)	284 ± 28	299 ± 22	0.25
Supplement compliance (%)	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
Sprint 1	MPO (W)	806 ± 115	792 ± 105	800 ± 96	811 ± 103	0.43
	PPO (W)	970 ± 165	996 ± 169	1007 ± 185	1003 ± 165	0.32
Sprint 2	MPO (W)	802 ± 119	808 ± 117	795 ± 96	783 ± 88	0.39
	PPO (W)	968 ± 185	980 ± 166	956 ± 155	963 ± 144	0.89
Sprint 3	MPO (W)	788 ± 131	793 ± 112	713 ± 65	723 ± 81	0.35
	PPO (W)	940 ± 185	946 ± 187	938 ± 186	859 ± 137	0.23
Sprint 4	MPO (W)	777 ± 121	788 ± 117	720 ± 129	743 ± 93	0.17
	PPO (W)	938 ± 174	934 ± 175	847 ± 159	889 ± 137	0.10
Sprint 5	MPO (W)	748 ± 137	772 ± 120	726 ± 103	700 ± 106	0.12
	PPO (W)	907 ± 173	923 ± 154	872 ± 151	838 ± 140	0.26
Sprint 6	MPO (W)	766 ± 116	801 ± 123	767 ± 117	712 ± 125	0.01
	PPO (W)	935 ± 183	976 ± 196	895 ± 174	894 ± 171	0.33