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ORIGINAL ARTICLE

The effect of β -alanine supplementation on cycling time trials of different length

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Abstract

The varying results reported in response to β -alanine supplementation may be related to the duration and nature of the exercise protocol employed. We investigated the effects of β -alanine supplementation on a wide range of cycling performance tests in order to produce a clear concise set of criteria for its efficacy. Fourteen trained cyclists (Age = 24.8 ± 6.7 years; VO_{2max} = 65.4 ± 10.2 mL·kg·min⁻¹) participated in this placebo-controlled, double-blind study. Prior to supplementation, subjects completed two (familiarization and baseline) supramaximal cycling bouts until exhaustion (120% pre-supplementation VO_{2max}) and two 1-, 4- and 10-km cycling time trial (TT). Subjects then supplemented orally for 4 weeks with 6.4 g/d placebo or β -alanine and repeated the battery of performance tests. Blood lactate was measured pre-exercise, post-exercise and 5 min post-exercise. β -alanine supplementation elicited significant increases in time to exhaustion (TTE) (17.6 ± 11.5 s; p = 0.013, effect compared with placebo) and was *likely* to be beneficial to 4-km TT performance time (-7.8 ± 8.1 s; 94% likelihood), despite not being statistically different (p = 0.060). Performance times in the 1- and 10-km TT were not affected by treatment. For the highly trained cyclists in the current study, β -alanine supplementation significantly extended supramaximal cycling TTE and may have provided a worthwhile improvement to 4-km TT performance. However, 1- and 10-km cycling TT performance appears to be unaffected by β -alanine supplementation.

Keywords: Carnosine, buffer, supplement, cycling

Introduction

 β -alanine supplementation is one nutritional strategy that acts to raise carnosine concentrations in human muscle, resulting in improved buffering capacity (Baguet, Koppo, Pottier, & Derave, 2010b). Furthermore, β -alanine supplementation has been suggested to increase the sensitivity of the muscle fibres to calcium in vitro (Dutka & Lamb, 2004) and may play an important role in enhancing the reuptake of Ca²⁺ into the sarcoplasmic reticulum in human muscle (Hannah et al., 2015), which has been speculated to improve muscle contraction efficiency (Hannah et al., 2015). A large number of athletes report using β -alanine (Kelly, Jenkins, Leveritt, Brennan, & Slater, 2013) and recent studies have examined the potential ergogenic effects of chronic β -alanine supplementation on a variety of exercise protocols [see review Bellinger (2014)].

The ergogenic effect of β -alanine is not always observed when athletes are tested under conditions that closely simulate competitive performance, for example, swimming (50-400 m competition swim times) (Chung et al., 2012), 1-h cycling time trial (TT) performance (Chung, Baguet, Bex, Bishop, & Derave, 2014) and 400-m running TT performance (Derave et al., 2007). A recent meta-analysis by Hobson, Saunders, Ball, Harris, and Sale (2012) suggests that β -alanine supplementation could significantly increase time to exhaustion (TTE) during supramaximal exercise but not decrease exercise time (i.e. improve performance) during fixed distance tests (p = 0.204). Nonetheless, Hobson et al. (2013) demonstrated that β -alanine supplementation was very likely to be beneficial to 2000-m rowing TT performance $(6.4 \pm 8.1 \text{ s effect})$

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compared with placebo) which is consistent with other studies reporting meaningful improvements in rowing performance (Baguet, Bourgois, Vanhee, Achten, & Derave, 2010a; Ducker, Dawson, & Wallman, 2013), while de Salles Painelli et al. (2013) reported a significant improvement in 100and 200-m swimming TT performance by 2.1% and 2.0%, respectively. Thus, it is reasonable to suggest that the nature and duration of the exercise task may dictate the ergogenic potential that β alanine supplementation provides on exercise performance. According to Hobson et al. (2012), exercise tasks that are less than 60 s in duration are not likely to be improved by β -alanine supplementation, while exercise tasks of 60-240 s are generally improved, and exercise tasks over 240 s are also improved, but to a lesser extent. Although these guidelines seem appropriate, it remains unclear to what extent trained individuals performing TTs of different durations within these time frames can benefit from supplementation.

The diversity of exercise protocols, employed in studies investigating β -alanine supplementation, highlights the importance of a comprehensive study that examines different protocols in order to deliver a clear set of criteria. The main purpose of the current study was to assess the efficacy of β -alanine supplementation on cycling TTs of different length in the same group of trained cyclists and to contrast the effects of β -alanine supplementation on a supramaximal time to fatigue test and practical, performance-based tests that closely simulate athletic competition.

Methods

Subjects

Trained male cyclists (n = 14; mean \pm SD: age = 24.8 ± 6.7 years, mass = 71.1 ± 7.1 kg, VO_{2max} = 65.4 ± 10.2 mL·kg·min⁻¹) accustomed to high-intensity exercise and familiar with laboratory testing participated in the present study; eight subjects had previously participated in cycling trials in the present laboratory (Bellinger & Minahan, 2014; Bellinger & Minahan, 2015). Subjects were presently cycling 250–600 km·week⁻¹ and competing in local A-grade criterion racing (n = 10) and the national-road series (n=4). Cyclists had not taken any nutritional supplements in the 3 months before the study with the exception of seven cyclists who were consuming a multi-vitamin supplement and six cyclists who were consuming a fish-oil supplement during the duration of the study. All cyclists consumed carbohydrate and whey protein beverages during training rides and all cyclists were naive to chronic β -alanine

supplementation before the commencement of the study. All cyclists gave their written informed consent to participate and the study was approved by the institution's ethics committee. Of the initial 16 cyclists that completed medical screening, familiarization and pre-experimental tests, one cyclist withdrew due to lack of interest while another withdrew following a road-cycling injury not associated with performance testing in the current research study; these data are not included in the study. Both cyclists withdrew from the study during the supplementation period.

Experimental design

A randomised, placebo-controlled study was performed, which consisted of a supramaximal cycling bout until exhaustion (120% of the power output achieved at VO_{2max}) and a 1-, 4- and 10-km cycling TT both before and after 28 days of either β -alanine or placebo supplementation. Preliminary testing consisted of a long-graded exercise test for the determination of VO_{2max} and peak aerobic power output and a familiarization of the battery of performance tests on separate days (>48 h). More than 72 h later, cyclists completed their baseline performance tests over a 2-week period. All performance tests were performed at the same time of day (± 2 h) and laboratory conditions were maintained at 22.5– 23.0°C and 50–55% humidity.

Subjects recorded training type (e.g. cycling, resistance training), duration and intensity [using a CR10 Borg Scale (Borg, 1982)] in a training diary for 2 weeks before testing and we used the session RPE method to quantify training load (Foster et al., 2001). Subjects were matched for training load in the 2 weeks before preliminary testing and randomly assigned to receive either 6.4 g day⁻¹ of β -alanine (Carnosyn® slow-release, Collegiate Sport Nutrition, San Marcos, California, USA, n = 7) or a placebo (dextrose monohydrate, n = 7) ingested in four equal daily doses for 28 days. In a recent study, Chung et al. (2014) demonstrated that a similar supplementation regimen increased muscle carnosine concentration in trained athletes. All supplements were distributed to the cyclists on a weekly basis in order to regularly monitor compliance.

Subjects were instructed to continue their normal dietary and training regimen throughout the 28days supplementation period while continuing to record their training sessions in a diary. Subjects were asked to abstain from caffeine and alcohol for a period of 12 h and avoid strenuous exercise for 24 h before each performance test. Each subject recorded a 24-h diet diary leading up to the first performance test which was then replicated for each subsequent performance test. Following the 28-days supplementation period, subjects repeated the battery of performance tests over a 2-week period with each test being separated by at least 72 h. During the post-supplementation testing period, subjects continued to ingest a maintenance dose of β -alanine (1.2 g·d⁻¹). Recent evidence has suggested that a maintenance dose of ~1.2 g·d⁻¹ is adequate to maintain already elevated muscle carnosine content (Stegen et al., 2014).

Preliminary testing

The subjects were required to report to the laboratory on at least five occasions during a 3-week period before the baseline performance tests. Subjects initially completed an incremental exercise test to exhaustion on the Velotron Pro cycle ergometer (RacerMate Inc., Seattle, WA, USA) to determine VO_{2max} and peak power output. The test comprised a starting power output of 100 W, followed by a step protocol in which power output increased by 50 W every 5 min until volitional exhaustion. Peak power output was determined as the power output reached in the last completed stage. If the subject finished part way through a 5-min stage, peak power was calculated in a pro-rata manner. Respiratory variables were measured and recorded every 30 s (Parvomedics Trueone 2400, Utah, USA). The subjects then performed a habituation trial for 1-, 4- and 10-km TT and the supramaximal cycling test to exhaustion on separate occasions (>48 h apart).

1-km TT

Subjects commenced a standardized 20-min warmup comprising 5 min of cycling at 150 W, 8 min of cycling at 60% peak aerobic power output, 2 min of cycling at a self-selected power output, 2 min of cycling to include three 6-s maximal sprints and finished with 3 min of cycling at a selfselected power output. Subjects were then instructed to sit passively for a period of 8 min before starting the cycling TT. During each 1-km TT, subjects were required to complete the TT in the quickest time possible and they had access to the distance remaining. The gearing was self-selected by the cyclists on the Wattbike ergometer during their familiarization trial and then replicated during each subsequent TT. A blood sample was taken from the earlobe for determination of blood lactate concentration using the Lactate ProTM (Arkray KDK, Japan) pre- (Pre-E), immediately post- (Post-E) and 5 min post-exercise (Post-E5+) while the cyclists cycled at 100 W.

4- and 10-km TT

A flat course profile was created using the Velotron Coaching Software (RacerMate Inc., Seattle, USA) for each distance. Subjects commenced the standardized warm-up before beginning each TT. During the TT, subjects were able to see the distance remaining and the gear ratio. The gear ratio (53×17) was the same at the start of each TT, but subjects were permitted to adjust the gear ratio throughout the trial to reflect their preferred cadence. Blood samples were obtained for measurement of blood lactate concentration Pre-E, Post-E and Post-E5+ while the cyclists cycled at 100 W.

Supramaximal cycling bout

Subjects commenced a standardized warm-up period and were then given 10 s of unloaded cycling to allow subject to reach a pedalling cadence of approximately 110 rev·min⁻¹ before the predetermined power output equivalent to 120% VO_{2max} was applied. To determine the TTE, the test was terminated when each cyclist could not maintain a pedalling cadence of above 60 rev·min⁻¹ for a 3-s period. Blood samples were obtained for measurement of blood lactate concentration Pre-E, Post-E and Post-E5+ while the cyclist cycled at 100 W.

Statistical analysis

Results are presented as mean \pm SD unless otherwise stated. Performance data were analysed using a twoway (group × trial) analysis of variance (ANOVA) and blood lactate concentration was analysed using a three-way ANOVA (group × trial × time) using the SPSS-statistics program (PASW statistics 20, Windows XP). Tukey tests were used for *post hoc* analyses. Significant interaction effects were followed-up by independent sample *t*-tests on the individual percentage change values for each group. Independent *t*-tests were used to compare any differences between the two groups in training load during the 28-days supplementation period. Significance was assumed at p < 0.05.

We also applied a more contemporary analytical approach, calculating the probability of practical significance (Hopkins, Marshall, Batterham, & Hanin, 2009). This approach uses the smallest worthwhile change to establish the likelihood (in percentage terms) of the experimental condition having a positive, trivial or negative effect which were determined from a Microsoft Excel spreadsheet designed for sport science research (Batterham & Hopkins, 2005). When clear interpretation was able to be made, a qualitative descriptor was assigned to the

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following quantitative chances of benefit: 0-25%; unclear; 25–75%, benefit possible; 75–95%, benefit likely; 95–99%, benefit very likely; > 99%, benefit almost certain. Measures of reliability known as coefficient of variation (identified as the difference between the familiarization and pre-supplementation tests) were halved and used as the smallest worthwhile change for each of the variables (Hopkins, 2004). The CVs for performance time (0.6%) and mean power for the 1-km TT (1.2%) have previously been determined in our laboratory (Bellinger & Minahan, 2014). The CVs for performance time for the 4- and 10-km TT and supramaximal cycling bout were 1.1, 1.3 and 6.9%. The CVs for mean power for the 4- and 10-km TT were 2.3 and 2.4%.

Results

There was no beneficial effect of β -alanine on performance time during the 1- and 10-km TT as indicated by the lack of interaction effects for each TT (Table I). The mean time to complete the 4-km TT performance times were not statistically different (group × trial, p = 0.060), despite the β -alanine group improving by 6.5 s and the placebo group slowing by 1.3 s. Despite not being statistically significant, the effect of β -alanine on 4-km TT performance time was likely (94% likelihood of a beneficial effect) to have been beneficial compared with the effect of a placebo (a difference of 7.8 \pm 8.1 s; 2.1 \pm 2.4% between the Δ β -alanine – Δ Placebo) (Figure 1). In the supramaximal cycling bout, TTE was significantly improved (+15.2 \pm 14.1 s; group × trial interaction, p = 0.029, 94% likelihood of a beneficial effect) in the β -alanine group compared with the placebo group (Figure 2).

No significant differences were present at any time point between β -alanine and placebo groups during any of the post-supplementation TT for blood lactate concentration (Table II). Analysis of the completed training diaries showed that there was no significant difference (p = 0.860) between the mean weekly training load between the β -alanine and placebo groups during the 2 weeks before preliminary testing, as identified by the session RPE method (β -alanine = 4898 ± 629 arbitrary units (AU), placebo = $4977 \pm$ 742 AU) or between average weekly training load during the 28-days supplementation period (β alanine = 4768 ± 325 AU, placebo = 4946 ± 520 AU).

Compliance with the supplementation protocol was verbally confirmed from all subjects. One subject reported mild symptoms of paraesthesia (i.e. burning, pins and needles, prickling, and/or stinging sensation) during the supplementation period and three out of the seven subjects in each group correctly guessed which group they were in.

	Plac	Placebo	β -alanine	nine		-	Likelihood of β -alanine
Variable	pre	Post	pre	Post	ANOVA group × time interaction, <i>p</i> value	Δp -alanine – Δp_{1acebo} raw difference (90%CL)	being positive/trivial/negative (compared with placebo)
1-km TT mean power (W)	532.6 ± 63.8	530.6 ± 48.2	548.0 ± 35.1	545.0 ± 32.1	0.520	-1.0 (-3.8 to 1.8)	1 / 98 / 1
1-km TT performance time (s)	68.9 ± 2.2	69.1 ± 3.6	68.9 ± 2.3	69.0 ± 3.5	0.770	-0.1 (-0.5 to 0.74)	28 / 58 / 13
4-km TT mean power (W)	344.4 ± 39.0	341.3 ± 37.7	335.0 ± 61.5	346.5 ± 62.9	0.062	14.6 (-0.2 to 0.6)	91/7/2
4-km TT performance time (s)	356.5 ± 15.8	357.8 ± 15.4	363.1 ± 24.9	356.6 ± 24.0	0.060	-7.8 (-14.4 to - 1.2)	94 / 4 / 2
10-km TT mean power (W)	303.2 ± 30.4	302.2 ± 33.0	295.6 ± 51.1	296.9 ± 51.1	0.384	2.5 (-2.5 to 7.5)	34 / 63 / 3
10-km TT performance time (s)	928.1 ± 33.4	929.9 ± 36.7	940.1 ± 58.3	938.1 ± 57.6	0.297	-2.5 (-10.1 to 2.6)	34 / 64 / 2
Supramaximal cycling TTE (s)	191.3 ± 21.9	188.9 ± 27.7	194.4 ± 37.4	209.6 ± 34.6	0.013	17.6 (7.8 to 27.4)	96/4/0

*Indicates significant differences between β -alanine and placebo groups ($\rho < 0.05$)

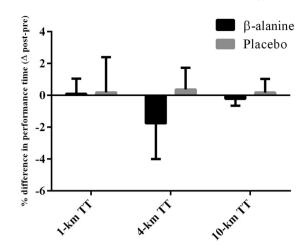


Figure 1. Change in performance time from pre- to post-supplementation in 1-, 4- and 10-km cycling TT performance in each treatment group.

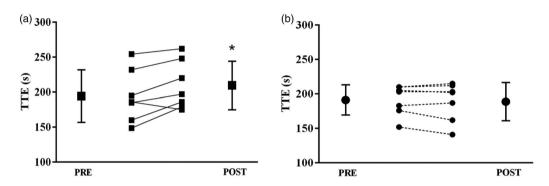


Figure 2. Change in TTE in the supramaximal cycling bout pre- and post-supplementation. The left panel (A) shows the individual data points of the β -alanine group with mean \pm SD data indicated by the large symbols. The right panel (B) is for the placebo group. *Indicates significant differences between β -alanine and placebo groups (p < 0.05).

Table II. Blood lactate concentration (mean ± SD) at pre-exercise (Pre-E), immediately post-exercise (Post-E) and 5 min post-exercise
(Post-E5+) in the β -alanine and placebo groups during the 1-, 4- and 10-km cycling TT and supramaximal cycling bout.

		Placebo			β -alanine	
	pre-E	Post-E	Post-E5+	Pre-E	Post-E	Post-E5+
1-km TT						
Pre-supplementation	2.6 ± 0.4	15.7 ± 4.5	17.1 ± 6.4	2.1 ± 0.2	15.6 ± 5.5	16.1 ± 5.6
Post-supplementation	2.7 ± 0.9	15.9 ± 4.3	16.9 ± 3.9	2.4 ± 0.4	15.8 ± 5.4	16.4 ± 5.2
4-km TT						
Pre-supplementation	2.1 ± 0.5	15.0 ± 2.7	10.4 ± 3.3	2.8 ± 1.1	13.3 ± 3.5	9.9 ± 3.4
Post-supplementation	2.4 ± 0.2	15.4 ± 1.8	11.7 ± 2.4	2.5 ± 0.7	15.3 ± 2.3	12.0 ± 3.2
10-km TT						
Pre-supplementation	1.7 ± 0.3	13.0 ± 3.0	10.2 ± 2.6	2.4 ± 0.3	10.5 ± 3.8	7.7 ± 2.4
Post-supplementation	1.6 ± 0.4	12.9 ± 2.7	9.6 ± 2.7	1.3 ± 0.5	11.0 ± 2.9	7.7 ± 2.6
Supramaximal cycling bout						
Pre-supplementation	1.6 ± 0.4	14.4 ± 2.9	13.2 ± 2.6	1.6 ± 0.7	12.9 ± 4.0	10.9 ± 2.3
Post-supplementation	1.8 ± 0.8	14.5 ± 2.6	13.5 ± 2.2	1.6 ± 0.3	13.9 ± 3.9	11.7 ± 1.8

Discussion

Our results indicate that 28 days of β -alanine supplementation significantly extends supramaximal

cycling TTE and may provide a worthwhile enhancement of 4-km TT performance in trained male cyclists. In contrast, β -alanine supplementation does not provide a detectable benefit to 1-km or 10-km TT performance.

 β -alanine supplementation significantly improved cycling TTE by 8.6% during a supramaximal cycling bout performed at an intensity equal to 120% of the power output achieved at VO_{2max}. This compares favourably with earlier work from Hill et al. (2007) who reported an improvement in cycling TTE of 11.8%, and has since been replicated in similarly untrained populations in the study of Sale et al. (2011) (+12.1%) and Danaher, Gerber, Wellard, and Stathis (2014) (+14%). The smaller magnitude of improvement in TTE in the current study may have been due to the intensity of the test (120% VO_{2max} vs. 110% VO_{2max}) (Weber & Schneider, 2000) or the characteristics of the study participants. However, despite differences in training status between the participants in the current study (trained cyclists) and that of others (untrained males) (Danaher et al., 2014; Hill et al., 2007; Sale et al., 2011), it has been suggested that β -alanine can act as an effective ergogenic aid for high-intensity exercise regardless of the training status of the individual (de Salles Painelli et al., 2014).

The data in the current study were examined both in relation to the statistical probability of an effect of β -alanine supplementation on cycling performance and in terms of the potential meaningfulness of the magnitude of the effect of β -alanine supplementation. The current study did not show a statistically significant effect of β -alanine supplementation on 4-km cycling TT time in trained cyclists. Indeed, the small sample size in the current study (n = 14) may have been insufficient to provide adequate statistical power to detect a significant performance improvement with traditional null hypothesis testing. However, β -alanine supplementation did improve 4km cycling time by an average of $1.8 \pm 2.1\%$ (-6.5) ± 8.6 s), resulting in a 93% likelihood that this reduction in cycling time represents a worthwhile improvement in performance. This finding is in line with previous work from Bellinger, Howe, Shing, and Fell (2012) who reported a $1.6\% \pm 1.7\%$ improvement in a 4-min cycling performance trial and the study of Hobson et al. (2013) who reported that β -alanine supplementation was very likely to be beneficial to 2000-m rowing performance (6.4 ± 8.1) s effect compared with placebo). Thus, it is possible that our results may be of practical relevance to athletes, despite the statistically non-significant overall finding. Indeed, Paton and Hopkins (2006) reported that the 'smallest worthwhile change' for top athletes lies in the region of 0.4-0.7% of the typical withinathlete CV in performance between events (i.e. 1.8% group mean change in our study). The typical within-athlete CV for track cycling is 1-2% for good

competitors and often <1% for the best international competitors (Paton & Hopkins, 2006). Thus a 1.8% improvement in performance is likely to be worth-while to a well-trained cyclist.

The mechanism supporting the ergogenic effects of β -alanine supplementation during supramaximal cycling and 4-km TT performance may be explained by enhanced muscle contractile properties as a result of altered Ca²⁺ handling (Dutka, Lamboley, McKenna, Murphy, & Lamb, 2012; Everaert, Stegen, Vanheel, Taes, & Derave, 2013), an increase in intracellular buffering capacity (Baguet et al., 2010b) or a combination of these mechanisms (Swietach, Leem, Spitzer, & Vaughan-Jones, 2014; Swietach et al., 2013). Despite Dutka et al. (2012) demonstrating that elevated carnosine content could increase the Ca2+ sensitivity of isolated skeletal muscle fibres, Hannah et al. (2015) reported that β alanine supplementation had no effect on the electrically evoked force-frequency relationship, which is analogous to the force-calcium concentration relationship. In support of augmented buffering capacity being the primary mechanism to support the ergogenic benefits of β -alanine supplementation, Baguet et al. (2010b) reported an attenuation in the decline in blood pH during a 6-min high-intensity cycling bout following 4–5 weeks of β -alanine supplementation. Additionally, Chung et al. (2014) reported an increase in the lactate/proton concentration ratio following β alanine supplementation, indicating that a similar lactate concentration was accompanied by an attenuated degree of systemic acidosis. This indicates that carnosine may act as a physiologically meaningful physicochemical buffer in human skeletal muscle and may provide, at least a part of, the explanation for the ergogenic effect of β -alanine supplementation found in some exercise modes.

 β -alanine supplementation did not affect 1-km TT performance in the current study. One previous study has investigated the effect of β -alanine supplementation on sprint cycling performance reporting a significant improvement in peak (+11.4%) and mean power output (+5.0%) during a 30 s cycling sprint following 2 h of intermittent-endurance cycling and a 10 min TT (Van Thienen et al., 2009). Indeed, the preceding bouts of exercise may have led to a degree of fatigue in the cyclists evidenced by the high blood lactate concentrations (~7 mmol·L⁻¹) at the end of the 10-min TT preceding the final sprint. This may have exacerbated the ergogenic potential of β -alanine supplementation during the 30 s cycling sprint. In addition, induced metabolic alkalosis, albeit through oral pre-exercise ingestion of sodium bicarbonate, has been shown to be ergogenic to isolated sprint cycling performance of a similar duration (~60 s) (McNaughton, 1992). McNaughton (1992) reported an increase in peak power and the amount of work completed during a 60 s cycling performance trial. In the present study, the presumed increase in muscle carnosine content in the β -alanine group will inevitably give rise to an increase in the intramyocellular buffer capacity (Baguet et al., 2010b). The apparent contradiction with earlier data may, in part, relate to differences in training status, since the male subjects in the study by McNaughton (1992) were untrained males, or the difference in contribution of the extracellular (i.e. blood bicarbonate) and intracellular buffering mechanisms (i.e. carnosine) during maximal ~60 s cycling exercise.

The present study showed no clear benefit of β alanine supplementation on 10-km TT performance. The lack of difference in 10-km TT time following β alanine supplementation may suggest that muscle acidosis is not a limiting factor to 10-km TT performance in trained individuals. Furthermore, endurance exercise is likely to be limited relatively more by central or motivational factors than by peripheral fatigue mechanisms. These findings are also in accordance with other studies demonstrating no effect of β -alanine supplementation on longer duration (30 min-1 h) cycling TT performance (Chung et al., 2014; James et al., 2014). Despite the lack of direct benefit on extended cycling TT performance (15-60 min), the endurance athlete may still benefit from β -alanine supplementation as a way to augment the sprint capacity during or at the end of an endurance race (Van Thienen et al., 2009).

For the trained cyclists in the current study, β alanine supplementation significantly improved TTE at a supramaximal intensity which may have implications for improving high-intensity training sets that maximally stress exercise capacity. Furthermore, athletes competing in high-intensity events that are ~6 min in duration may receive worthwhile improvements in performance, but there appears to be limited benefit of β -alanine supplementation in very short (~1 min) and longer TT (~15 min).

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Both authors were responsible for the conception, design, data collection, data analysis and data interpretation of the study and writing the manuscript. The authors declared no conflict of interest. Authors thank the cyclists for their participation in this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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