Effect of β-alanine supplementation on 20 km cycling time trial performance

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Abstract

The effects of β-alanine supplementation on high-intensity cycling performance and capacity have been evaluated, although the effects on longer duration cycling performance are unclear. Nineteen UK category 1 male cyclists completed four 20 km cycling time trials, two before and two after supplementation with either 6.4 g•d^{-1} β-alanine (n = 10; BA) or a matched placebo (n = 9; P). Performance time for the 20 km time trial and 1 km split times were recorded. There was no significant effect of β-alanine supplementation on 20 km time trial performance (BA-pre 1943 ± 129 s; BA-post 1950 ± 147 s; P-pre 1989 ± 106 s; P-post 1986 ± 115 s) or on the performance of each 1 km split. The effect of β-alanine on 20 km time trial performance was deemed unclear as determined by magnitude based inferences. Supplementation with 6.4 g•d^{-1} of β-alanine for 4 weeks did not affect 20 km cycling time trial performance in well trained male cyclists.

KEY WORDS: Carnosine; Endurance exercise; Exercise test reability.

Introduction

Carnosine (β-alanyl-L-histidine) has been shown to have an important role in the maintenance of homeostasis in the muscle cell due to the pH buffering capabilities of the imidazole ring. As such, the expansion of the imidazole ring content in human skeletal muscle through an increase in the muscle carnosine concentration offers a means to increase muscle buffering capacity and high intensity exercise performance and capacity[1-2]. Other potential physiological roles of carnosine, such as increased calcium sensitivity[3-4] and antioxidant capacity[5-6] might also contribute to a positive effect on exercise capacity and performance, although literature is inconclusive[7].

Carnosine is synthesised from the amino acids β-alanine and histidine, with β-alanine availability being rate limiting to the synthesis of the dipeptide within human skeletal muscle[8]. As a result, the dietary intake of β-alanine through supplementation is critical for the elevation in muscle carnosine content. Most studies have used doses of between 3.2 and 6.4 g•d^{-1} of β-alanine, with this resulting in increases of 40 to 80% in the muscle carnosine concentration, depending upon the duration of supplementation and the method of determining the muscle carnosine content.

Several reviews have concluded that there are significant effects of β-alanine supplementation on high-intensity exercise performance[9-10]. In particular, several studies have focussed on the effects of β-alanine supplementation on high intensity cycling capacity and have shown significant increases following supplementation[11-13]. The effects of β-alanine supplementation on cycling performance are more equivocal, with some showing no effect[14] and others a significant improvement[15].

Whilst there is general consensus in the literature for a beneficial effect of β-alanine supplementation on high intensity exercise performance[9-10,16-17] fewer

studies have examined the effects on longer duration exercise performance. However, a recent study examined the effects of β-alanine supplementation on cycling performance in a time trial lasting ~ 1 hr[18]. The findings suggest that despite marked increases in muscle carnosine concentration, β-alanine supplementation did not improve cycling performance[18].

However, Stout et al.[19] supplemented untrained males with β-alanine and showed a 14.5% increase in physical working capacity at fatigue threshold post supplementation, while those supplementing with placebo decreased their physical working capacity at fatigue threshold by 1.8%. Furthermore, in a graded exercise test lasting ~19 minutes using untrained female participants, Stout et al.[20] reported no effects of β-alanine on maximal oxygen consumption but did report an increase in the time to exhaustion, as well as a delay in the onset of neuromuscular fatigue and an increase in the ventilatory threshold at submaximal workloads. These findings indicate the potential for β-alanine supplementation to increase performance during endurance based exercise. It is important to note, however, that increases in power output at ventilatory threshold with β-alanine supplementation could also be caused by increased buffering capacity, rather than other purported mechanisms, since higher buffering capacity via carnosine could reduce the need for H+ buffering through hyperventilation and CO_2 elimination.

Interestingly, some evidence also exists to suggest that an increase in muscle carnosine concentration could influence muscle fibre function in humans[3]. Dutka et al.[3] mechanically skinned single muscle fibres taken from the "m. vastus lateralis" by muscle biopsy and subsequently characterised their calcium release and contractile properties. They showed that an increase in muscle carnosine content increased calcium sensitivity in slow and fast twitch muscle fibres and enhanced calcium release in slow twitch fibres. In addition Everaert et al.[4] showed that providing mice with water containing 1.2% β-alanine for 8 weeks resulted in a higher carnosine concentration in the "extensor digitorum longus" and the "tibialis anterior", and an increase in fatigue resistance in the soleus.

Taken together, the results of these investigations provide a potential mechanism by which an increase in the muscle carnosine content could confer benefits to exercise performances lasting longer than the 60-240 s mostly studied to date, for which increased muscle buffering capacity would seem the most likely mechanistic explanation. Therefore, we aimed to examine the effects of 4 weeks of β-alanine supplementation at 6.4 g·d^{-1} on 20 km cycling time trial performance in trained cyclists.

Method

Participants

Twenty-six UK category 1 male cyclists, completing at least 7 hours of cycle training per week, volunteered to participate in the study that was conducted from April 2012 to June 2013, meaning participants were recruited throughout all phases of an annual training cycle. They were randomly assigned to either a placebo (P) or β-alanine (BA) supplementation group; however, seven participants (4 from P and 3 from BA) withdrew, citing various reasons not associated with the study. As such, 19 participants completed all trials; participant characteristics are presented in TABLE 1. The study was approved by the Institution's Ethical Advisory Committee and conformed to the Declaration of Helsinki.

TABLE 1 - Participant characteristics.

	N	Age (y)	Height (m)	Body mass (kg)	Familiarisation(s)
β-alanine	10	37 ± 8	1.82 ± 0.06	78.7 ± 8.8	1975 ± 173
Placebo	9	32 ± 6	1.81 ± 0.04	80.4 ± 8.3	1993 ± 95
P value		0.211	0.902	0.671	0.786

Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing written informed consent. Participants had not taken any supplement in the three months prior to the study and had not taken β-alanine for at least six months

prior to the study due to the long washout period for muscle Carnosine[21]. Participants verbally agreed to maintain similar levels of physical activity and dietary intake for the duration of the study and compliance with this request was verbally confirmed with participants prior to each testing session. None of the cyclists were vegetarian and so would have ingested small amounts of dietary β-alanine from the hydrolysis of carnosine and methyl derivatives of this in meat. This would typically be expected to be around 50 to 400 mg per day.

Experimental design

This was a randomised, double-blind, placebo-controlled, parallel groups design experiment. Subsequent to preliminary testing, participants completed two baseline trials (separated by 48 h) and two follow-up trials (separated by 48 h), separated by four weeks of supplementation with either placebo or β-alanine. Participants in BA ingested two sustained-release tablets (CarnoSyn^{SR}, Natural Alternatives International, USA) each containing 800 mg of β-alanine (total dose per serving was 1.6 g) four times per day, separated by 3-4 h intervals, for a total daily dose of 6.4 g. The total amount of β-alanine ingested over the 4 week period was 179.2 g. Participants in P ingested a matched placebo containing maltodextrin. Double blinding was ensured as both beta-alanine and placebo tablets were given to participants in identical white tubs. An experimenter not directly involved in participant testing noted the lot number of each bottle and then removed the lot number prior to administration to participants. Once data collection was complete, the supplement corresponding to each lot number was revealed.

The sustained-release formulation of β-alanine used in the present study has been shown to reduce the symptoms of paraesthesia often associated with individual doses of β-alanine administered as free powder[22]. In this study, none of the participants reported any feelings of paraesthesia, and we are confident that the integrity of the double blinding was maintained. β-alanine tablets were tested by the manufacturer prior to release for the study and conformed to the label claim for β-alanine content. All supplements were independently tested by HFL Sports Science, UK, prior to use to ensure no contamination with steroids or stimulants according to ISO 17025 accredited tests.

Experimental protocol

Height (Seca, UK) and body mass (Seca, UK) were recorded before participants completed a full habituation test of the 20 km cycling time trial as described below. Following preliminary assessment, participants completed four identical main trials. All trials were completed at least 2 h postprandial following 24 h dietary standardisation, and participants had not completed vigorous exercise in the 24 h prior to each trial.

Upon arrival at the laboratory, a capillary finger prick blood sample was taken for the determination of baseline blood lactate concentration (2300 STAT Plus, YSI Ltd, UK). Participants then completed a self-paced five minute warm up on their bike followed by a three minute period of self-selected stretching before starting the 20 km time trial. The time trial was completed on a Cyclus2 (RBM Electronic, Germany) ergometer on which the participants mounted their own bike frames. The settings on the Cyclus2 were adjusted for each individual bike by entering the size of the front and back cog, bike weight and the crank length, ensuring an accurate measurement of distance and performance. During the exercise trial, the participant was deprived of any information regarding performance (e.g., time, speed, cadence) other than distance covered and gear selection and no feedback or encouragement was provided by experimenters. Participants were allowed to consume water ad libitum during exercise. Heart rate (T31 system, Polar, Finland) was recorded after every 1 km interval and RPE (using the Borg 6-20 scale[23]) after every 5 km interval. Cycling performance data (e.g., time, distance, speed and cadence) was recorded by the Cyclus2 every 0.5 seconds. Immediately upon completion of the 20 km time trial and then again following a self-paced cool down, capillary finger prick blood samples were taken for the determination of blood lactate concentration. Participants also performed three cognitive function tests (Stroop test, Sternberg paradigm and Rapid Visual information Processing (RVIP) task) before and after each 20 km time trial to assess selective attention, working memory, and sustained attention. However, these data are presented elsewhere and only the exercise performance aspects of the study are presented here.

In order to determine the reliability of the 20 km time trial, we used the data from trials 1 and 2 on 21 participants (age 35 ± 8 y, height 1.82 ± 0.05

m, body mass 80.7 ± 9.0 kg) who completed the 20 km time trial as described above. There was no significant difference in the time taken to complete the 20 km between trials 1 and 2 (trial 1: 1969 ± 112 s; trial 2: 1975 ± 119 s; p = 0.222). The intra-class correlation between trials 1 and 2 was r = 0.99, with the coefficient of variation being 0.7%.

Statistical methods

All data were checked for normality of distribution using the Shapiro-Wilk test. Data were analysed using unpaired t-tests between groups where there was only one time point. Mixed model ANOVA was employed in SPSS v20 to examine differences in 20 km time trial performance, 1 km split times, blood lactate, heart rate and rating of perceived exertion between groups and across time and trials. Differences between trials 1 and 2 and trials 3 and 4 within groups were examined using paired t-tests. Where no differences were shown, the data from trials 1 and 2 were combined and the mean used as the pre-supplementation value and the data from trials 3 and 4 were combined and the mean used as the post-supplementation value. Statistical significance was set at $p \leq 0.05$ and data are presented as mean ± 1SD unless otherwise stated.

Time trial performance data were also analysed using a contemporary magnitude-based inferences approach[24] in order to detect small effects of practical importance in an applied setting, a technique which is becoming increasingly common in exercise performance research. This technique establishes the likelihood of an intervention having a positive/ trivial/ negative effect. A Cohen's unit of 0.2 was employed as the smallest meaningful change in performance. Where the chance of both a positive and negative effect is > 5%, the effect is deemed unclear. Qualitative descriptors were assigned to the quantitative percentile scores as follows: 25-75% possible; 75-95% likely; > 99% almost certain[25-26]. For determination of the magnitude based effects, we chose to average trials 1 and 2 and trials 3 and 4 within each group. We then calculated the difference between pre- and post- supplementation in each group, with the magnitude of the effect of β-alanine supplementation being determined as the difference between the two groups.

Results

Performance data

There was no main effect of trial across the four trials (p = 0.649) or of supplementation between the BA and P groups (p = 0.484) for 20 km time trial performance. There was also no interaction effect (p = 0.624).

Using paired t-tests and magnitude based inferences there were no differences between trials 1 and 2 and trials 3 and 4 in BA (p = 0.463, *unclear*; p = 0.130, *unclear*) or P (p=0.537, *unclear*; p = 0.967, *unclear*). Therefore, the data from trials 1 and 2 were combined and the mean used as the pre-supplementation value and trials 3 and 4 were combined and the mean used as the post-supplementation value.

There was no main effect of trial across the pre- and post- supplementation trials (p = 0.795) or of supplementation between the BA and P groups (p = 0.484) for 20 km time trial performance. The mean performance time for the BA group was 1943 ± 129 s pre-supplementation and 1950 ± 147 s post-supplementation, while for the P group this was 1989 ± 106 s pre-supplementation and 1986 ± 115 s post supplementation. There was also no interaction effect (p = 0.575).

The magnitude of the effect of supplementation in the BA group was *unclear* (p = 0.556), as was the effect of supplementation in the P group (p = 0.847). Finally, the difference between the two groups, and therefore the magnitude of the effect of β-alanine supplementation, was also deemed *unclear* (p = 0.575).

With regard to individual responses to supplementation, in the BA group five participants improved with supplementation, one stayed the same and four became slower. In the P group, four participants became slower and five participants improved (FIGURE 1).

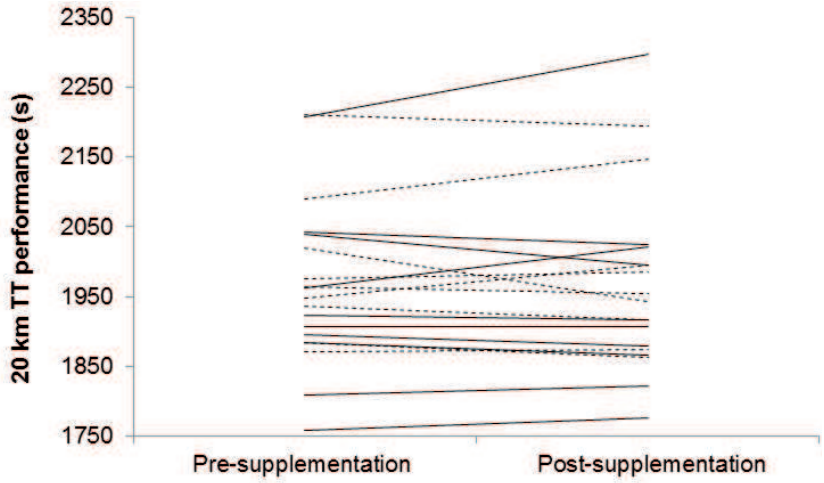

FIGURE 1 - Individual 20 km time trial performances pre- and post- supplementation in β-alanine (BA) and Placebo (P).

Supporting data

There was a significant effect of exercise on heart rate ($p < 0.001$) and rating of perceived exertion ($p < 0.001$), which both increased over the 20 km time trial. However, there was no effect of trial and no difference between the groups for either heart rate ($p = 0.520$; $p = 0.958$) or rating of perceived exertion ($p = 0.240$; $p = 0.396$; TABLE 2).

TABLE 2 - Heart rate (beats·min^{-1}) and rating of perceived exertion (6-20) at 5 km intervals during the 20 km time trial and lactate concentration before and after the 20 km time trial (mmol·L^{-1}).

	Distance (km)	Pre-supplementation				Post-supplementation			
		5	10	15	20	5	10	15	20
Heart rate	β-alanine	154 ± 8	157 ± 10	160 ± 11	171 ± 8	155 ± 6	159 ± 8	163 ± 10	173 ± 10
	Placebo	153 ± 10	159 ± 7	162 ± 8	175 ± 7	156 ± 12	161 ± 9	162 ± 10	177 ± 8
RPE	β-alanine	15 ± 2	16 ± 1	17 ± 1	19 ± 1	15 ± 2	16 ± 1	17 ± 1	18 ± 1
	Placebo	14 ± 1	16 ± 1	17 ± 1	19 ± 1	14 ± 1	16 ± 1	17 ± 1	19 ± 1
		Pre-supplementation		Post-supplementation					
		Pre-TT	Post-TT	Pre-TT	Post-TT				
Lactate	β-alanine	1.32 ± 0.49	10.04 ± 1.90	1.55 ± 0.56	9.49 ± 2.32				
	Placebo	1.26 ± 0.60	9.81 ± 2.24	1.39 ± 0.67	9.64 ± 1.80				

Due to difficulties with the Cyclus2 saving split data in some trials, 1 km splits are given for n = 7 in BA and n = 7 in P. There was a significant effect of time on 1 km splits, with split times becoming faster towards the end of the 20 km time trial. There was no difference across the four trials or between the two groups for 1 km split times ($p = 0.630$; $p = 0.760$; FIGURE 2). Finally, blood lactate concentrations increased due to the exercise ($p < 0.001$) but again there was no effect of trial or group ($p = 0.093$; $p = 0.869$; TABLE 2).

The effects of β-alanine supplementation on high-intensity cycling performance and capacity have been evaluated, although the effects on longer duration cycling performance are unclear. Nineteen UK category 1 male cyclists completed four 20 km cycling time trials, two before and two after supplementation with either 6.4 g•d^{-1} β-alanine (n = 10; BA) or a matched placebo (n = 9; P). Performance time for the 20 km time trial and 1 km split times were recorded. There was no significant effect of β-alanine supplementation on 20 km time trial performance (BA-pre 1943 ± 129 s; BA-post 1950 ± 147 s; P-pre 1989 ± 106 s; P-post 1986 ± 115 s) or on the performance of each 1 km split. The effect of β-alanine on 20 km time trial performance was deemed unclear as determined by magnitude based inferences. Supplementation with 6.4 g•d^{-1} of β-alanine for 4 weeks did not affect 20 km cycling time trial performance in well trained male cyclists.

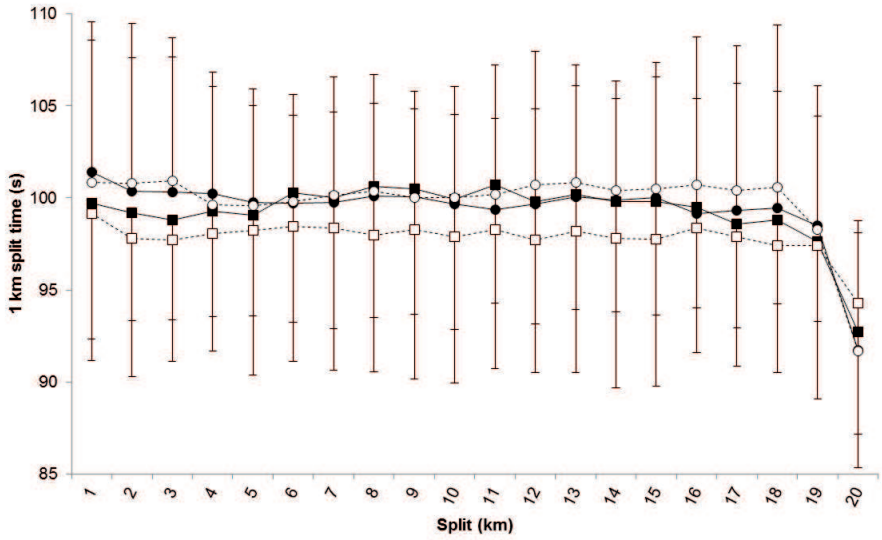

FIGURE 2 - 1 km split times for the mean pre- (■) and post- (□) supplementation trials in β-alanine (BA) and mean pre- (●) and post- (□) supplementation trials in Placebo (P).

Discussion

This is the first study to examine the effects of β-alanine supplementation on 20 km time trial performance in well trained male cyclists, demonstrating no significant effect of β-alanine supplementation on this model of exercise performance. The individual data is also supportive of a lack of β-alanine supplementation on time trial performance, with equal numbers of participants showing increased and decreased performance; with all changes being relatively marginal in any case. In an attempt to detect small effects of practical importance we also determined the magnitude based inferences. In line with the finding of no significant effect, the magnitude of the effect of β-alanine supplementation on 20 km time trial performance was *unclear*. As such, and given the high degree of reliability of the performance test used in this study (test-retest data having a CV of 0.7%), we can be confident in the lack of an effect of β-alanine on this performance test. These findings are also in accordance with those of Chung et al.[18], demonstrating no effect of β-alanine supplementation on ~ 1 hour cycling time trial performance. Thus, these findings suggest that despite the beneficial effects of β-alanine supplementation on high intensity exercise capacity[9-10], β-alanine supplementation does not influence longer duration (30 min - 1 hr), lower intensity, cycling performance.

An inherent problem of time trial tests in well trained participants who are familiar with the exercise protocol is that they are influenced by pacing strategies[27] and sometimes these pacing strategies do not produce optimal performance[28]. There is also a variety, possibly an infinite number, of pacing strategies available to an individual[29]. However, if pacing strategies were employed by the athletes in the present study variables such as heart rate, blood lactate and particularly the RPE responses to exercise would have been lower in the group supplemented with β-alanine. Although, as expected, exercise increased heart rate, lactate and RPE, β-alanine supplementation exerted no effect on any parameter during exercise, indicating that this is unlikely to have been the case.

We hypothesised that β-alanine supplementation would improve time trial performance given the previously reported influence of β-alanine on the ventilatory threshold[20] and the potential for an increase in muscle carnosine to increase the calcium sensitivity of slow and fast twitch muscle fibres and enhance calcium release in slow twitch fibres[3]. However, the strength of the currently available evidence to support the effects of carnosine on calcium handling is limited[2,9], with only one study[3] being conducted on human muscle fibres. If calcium handling were the main mechanism for an effect of

elevated muscle carnosine on exercise performance, significant effects of β-alanine supplementation would be expected across a wider range of exercise performance tests than have currently been shown[2,9]. Whilst the present study was not designed to examine the mechanisms related to increased exercise performance as a result of elevated muscle carnosine levels, the lack of an effect of β-alanine would be consistent with the lack of an effect of carnosine on calcium handling. Either that or alterations to calcium sensitivity or release do not influence 20 km time trial performance.

Taking the existing evidence as a whole, the most likely mechanism for an effect of increased muscle carnosine on exercise performance and capacity remains the effect it has on intracellular pH buffering. Lending support to this assertion, studies have examined the effects of β-alanine on exercise capacity, using tests designed to induce large increases in intramuscular hydrogen cation levels and reductions in pH[11-12,30], and have shown significant increases in exercise capacity. In relation to cycling, studies have shown significant effects of β-alanine supplementation on high-intensity cycling capacity, as determined by increases in total work done[11-12]. The cycling capacity test at 110% of powermax used by both of these studies is one of the few tests to be independently repeated with β-alanine supplementation. The increase in cycling capacity reported following 4 weeks of supplementation was similar in both studies; Hill et al.[11] reported an increase of 13% and Sale et al.[12] an increase of 15% using a slightly higher total intake of β-alanine. The fact that reductions in intracellular pH are unlikely to be limiting to 20 km time trial performance might thus help to explain the lack of a significant effect in the present study. However, during competitive cycling inclines in the road surface will frequently be encountered, with trials typically ending in a sprint, both of which might be expected to reduce intramuscular pH. It is therefore possible that increased muscle carnosine levels brought about by β-alanine supplementation may improve endurance cycling performance in real-life competitive races.

That said, not all studies with regards to high-intensity cycling performance tests have shown significant effects of β-alanine supplementation. Bellinger et al.[14] reported no effect of β-alanine supplementation on maximal 4 minute cycling performance, although van Thienen et al.15 reported a significant effect of β-alanine on 30 s isokinetic sprint cycling, performed at the end of a 110 min simulated endurance cycle race. Due to the findings of van Thienen et al.[15], we also determined the 1 km split times in the current study to ascertain whether there was any effect of β-alanine supplementation on exercise performance towards the end of the 20 km time trial. Split times were relatively consistent throughout but did become faster towards the end of the 20 km time trial in both groups, particularly in the final kilometre. However, there was no effect of β-alanine on the performance of any 1 km split.

The present study is not without its limitations; it should be noted that we did not determine the influence of β-alanine supplementation on muscle carnosine concentrations. This was primarily due to the lack of availability of a non-invasive method of determining muscle carnosine content in these well-trained participants. As such, we cannot confirm in the present study that muscle carnosine contents were significantly increased and, if so, what the magnitude of this increase was. However, supplementation with β-alanine at this level has consistently been shown to increase muscle carnosine concentrations by over 60%[8,11], with others reporting no non-responders to β-alanine supplementation[11,21,31]. Indeed, only one participant from the series of muscle studies to-date has failed to respond to β-alanine supplementation with an increase in muscle carnosine[8]. Overall increases have been shown to be between 40 and 80% depending upon dose (between 3.2 and 6.4 $g \cdot d^{-1}$) and duration of administration (between 4 and 10 weeks). Furthermore, based on the dose-response relationship shown by Stellingwerff et al.[32], our supplementation regimen would be expected to result in a 3.6 $mmol \cdot kg_{ww}^{-1}$ increase in muscle carnosine concentration.

Practical applications

These data suggest that endurance cyclists competing in events lasting around 30 minutes will not benefit from β-alanine supplementation. However, it may be that when the intensity of the cycling is increased, for example during a hill climb or a sprint finish, enhanced intramuscular H^+ buffering, through β-alanine supplementation, may be beneficial to performance. Future research should investigate this possibility.

In conclusion, supplementation with 6.4 $g \cdot d^{-1}$ of β-alanine for 4 weeks did not affect 20 km cycling time trial performance in well trained male cyclists. This might suggest that a mechanism to support an effect for β-alanine supplementation on longer

duration exercise does not exist and that the most likely effects are to be seen with short duration high-intensity exercise performance as the result of improved intracellular pH buffering.

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