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Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity

C. A. Hill[1], **R. C. Harris**[1], **H. J. Kim**[2], **B. D. Harris**[1], **C. Sale**[3], **L. H. Boobis**[4], **C. K. Kim**[2], and **J. A. Wise**[5]

[1] School of Sports, Exercise & Health Sciences, University of Chichester, Chichester, U.K.
[2] Human Exercise Physiology, Korea National Sport University, Seoul, Korea
[3] Centre for Human Sciences, QinetiQ, Farnborough, U.K.
[4] Sunderland Royal Hospital, Sunderland, U.K.
[5] Natural Alternatives International, San Marcos, CA, U.S.A.

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Summary. Muscle carnosine synthesis is limited by the availability of β-alanine. Thirteen male subjects were supplemented with β-alanine (CarnoSyn™) for 4 wks, 8 of these for 10 wks. A biopsy of the *vastus lateralis* was obtained from 6 of the 8 at 0, 4 and 10 wks. Subjects undertook a cycle capacity test to determine total work done (TWD) at 110% ($CCT_{110\%}$) of their maximum power (W_{max}). Twelve matched subjects received a placebo. Eleven of these completed the $CCT_{110\%}$ at 0 and 4 wks, and 8, 10 wks. Muscle biopsies were obtained from 5 of the 8 and one additional subject. Muscle carnosine was significantly increased by +58.8% and +80.1% after 4 and 10 wks β-alanine supplementation. Carnosine, initially 1.71 times higher in type IIa fibres, increased equally in both type I and IIa fibres. No increase was seen in control subjects. Taurine was unchanged by 10 wks of supplementation. 4 wks β-alanine supplementation resulted in a significant increase in TWD (+13.0%); with a further +3.2% increase at 10 wks. TWD was unchanged at 4 and 10 wks in the control subjects. The increase in TWD with supplementation followed the increase in muscle carnosine.

Keywords: β-alanine – Muscle – Carnosine – Taurine – Exercise – Cycle performance

Introduction

Carnosine (β-alanyl-L-histidine) is a dipeptide found in high concentrations in both vertebrate and non-vertebrate skeletal muscle, and which is also present in cells of the CNS. Various physiological actions have been ascribed to carnosine in muscle including pH buffering (Bate-Smith, 1938; Harris et al., 1990), acting as an antioxidant (Boldyrev et al., 1993), the regulation of Ca^{2+} sensitivity and excitation-contraction (E-C) coupling (Lamont and Miller, 1992; Batrukova and Rubtsov, 1997), the protection of proteins against glycation by acting as a sacrificial peptide (Hipkiss et al., 1995) and in preventing the formation of protein-protein cross links by reacting with protein-carbonyl groups (Hipkiss, 2000). Of these, only that of pH buffering is undisputed resulting from a pKa of 6.83 of the imidazole ring of carnosine (Bate-Smith, 1938). In contrast free histidine (pKa of 5.83) is a relatively poor buffer over the physiological pH range.

The concentration of carnosine in muscle is particularly high in animals with a capacity for prolonged intense exercise, or in those exposed to frequent periods of increased hypoxia (Abe, 2000; Crush, 1970). In addition, carnosine is typically higher in fast-twitch (type II), compared to slow-twitch (type I), muscle fibres. In human *vastus lateralis* muscle, carnosine ranges from 10.5 $mmol \cdot kg^{-1}$ dry muscle (dm) in type I to 23.2 in type II fibres (Harris et al., 1998). A similar preferential distribution in type II fibres has also been shown in skeletal muscle of other species (Dunnett and Harris, 1995; Dunnett et al., 1997) where the concentration maybe 4 to 5 times higher than that found in type I fibres. The higher concentrations found in fast twitch muscle, particularly in those animals with enhanced capacity for intense exercise, is consistent with a role in pH buffering as well as a role in E-C coupling, but not with the other suggested roles of carnosine.

We have recently demonstrated (Harris et al., 2006) that carnosine in skeletal muscle may be increased by 60% and more by 2–4 weeks ingestion of β-alanine, a non-proteogenic amino acid. The intact dipeptide itself does not appear to be taken up into muscle and is readily

hydrolysed in plasma by the enzyme carnosinase (Asatoor et al., 1970; Perry et al., 1967). Studies indicate that β-alanine, rather than histidine, is limiting to synthesis (Bakardjiev and Bauer, 1984; Dunnett and Harris, 1999; Harris et al., 2006).

Increased H^+ concentration from dissociation of carboxylic acids, including lactic acid, formed from neutral glyco- or glucosyl units has been suggested as a cause of peripheral muscle fatigue since Meyerhoff (1920) but has never been unequivocally demonstrated as a limiting factor to performance in whole body exercise in humans. pH homeostasis in contracting muscle fibres is achieved firstly by physicochemical buffering mediated principally by organic and inorganic phosphates, bicarbonate anion (the contribution of this being determined by its concentration in muscle prior to the start of exercise) and by histidine residues in carnosine and (quantitatively to a much lesser extent) in proteins; and secondly by active and passive transport of H^+ into the surrounding interstitium (Juel et al., 2003). With the exception of carnosine, the concentrations of the other physicochemical buffers are constrained by their involvement in other reactions. Variations in buffering capacities between muscle fibre types (Sewell et al., 1992), and even between muscles of different species (Harris et al., 1990) largely depend upon differences in the histidine dipeptide content. In human *vastus lateralis* muscle, the most frequently cited range for the carnosine content is $17.5 \pm 4.8\,\text{mmol} \cdot \text{kg}^{-1}$ dm in females to $21.3 \pm 4.2\,\text{mmol} \cdot \text{kg}^{-1}$ dm in males (Mannion et al., 1992). A trend towards higher concentrations has been reported in athletes such as sprinters (Parkhouse et al., 1985) and body-builders (Tallon et al., 2005), while 8 weeks intensive training has been shown to almost double the carnosine content in the *vastus lateralis* (Suzuki et al., 2004). Suzuki et al. (2002) observed a significant relationship between the carnosine concentration in human skeletal muscle and high intensity exercise performance (30 s Wingate) further emphasizing the functional importance of carnosine.

Results from these studies suggest that high carnosine concentrations are important in attenuating the fall in intramuscular pH associated with high intensity exercise of short duration and by implication that this may be further enhanced by supplementation with β-alanine to increase the muscle concentration. However, it is by no means clear that this would contribute to improved exercise performance.

The aims of the study were to extend previous observations of the effect of 4 weeks β-alanine supplementation on muscle carnosine accumulation, to 10 weeks; to determine if changes were specific to type I or type II muscle fibres; and to determine the effect on a high-intensity cycling capacity test with an endurance time of around 150 s. The non-proteogenic amino acid taurine, which shares the same transporter as β-alanine (Jessen, 1994; Ramamoorthy et al., 1994) was also measured since administration of large (albeit non-physiological) doses of β-alanine have been shown in animal models to cause depletion of this in muscle (Dawson et al., 2002).

Materials and methods

Subjects

Twenty-five physically active male subjects, mainly undergraduate and postgraduate students at the University of Chichester, volunteered to participate in the study. None of the subjects were actively involved at the time in a structured training programme. Subject details are given in Table 1. Subjects were judged to be clinically healthy based on a health-history questionnaire. They were required not to have taken any form of sports supplement for at least 6 weeks prior to commencing the study and were requested not to change their current level of physical activity for the duration of the study or their average diet. None of the subjects were vegetarian and had estimated daily intakes of β-alanine from the digestion of histidine dipeptides in meat of 250–750 mg daily (estimates based on values of histidine dipeptide contents in Abe, 2000). The Ethics Review Committee of the University of Chichester approved the study and all subjects provided written informed consent prior to participation.

Experimental design

Each subject first completed a preliminary assessment to confirm that they met the selection criteria for the study after which they were allocated to one of two treatment groups. Following a cycle performance test subjects were supplemented for 4 or 10 weeks with either β-alanine (β-Ala, $n = 13$) or a matching placebo (P, $n = 12$). Allocation to treatments was randomized. β-alanine (CarnoSyn^{TM}) was obtained from Natural Alternatives International, San Marcos, USA. Details of the specific dosing strategies employed for both groups are provided in Table 2. As in a previous study (Harris et al., 2006), β-alanine was administered each day as 8 divided doses, with the dose increasing during the first 4 weeks. 800 mg β-alanine corresponds to the amount in dipeptide form available from 100 g of whale beef, 150 g turkey breast meat (Abe, 2000) or 100 g north-Atlantic sea-prawns (unpublished observations). Administration of the treatments was double-blind.

Muscle biopsy

A percutaneous muscle biopsy of the mid section of the *vastus lateralis* was obtained 1–2 days after the pre-supplementation cycle test, and 1–2 days after the 4 and 10 week tests from 6 β-Ala and 6 P subjects. Muscle biopsies were obtained using a 6 mm biopsy needle (Northern Hospital Supplies, Edinburgh, U.K.) by the method of Bergström (1962). Samples were freeze-dried and 2–3 mg dry muscle were powdered under a 10× microscope and visible connective tissue and blood was removed. Extracts of muscle were prepared using 0.1 M borate buffer, pH 9.65 and analysed by HPLC as previously described (Dunnett and Harris, 1997; Harris et al., 2006) using an APEX ODS 3 µm 150 mm × 4.6 mm I.D. analytical column (Grace Vydac, Hesperia, USA).

Muscle fibres

20–40 single muscle fibres were dissected from the 0 and 10 week biopsies of the subjects supplemented with β-alanine. From one end of each fibre, two 0.5–1.0 mm pieces were cut and dissolved, separately, overnight in 5 µl of 0.05 M tris-hydroxymethyl-aminomethane (pH 6.8); 1% w/v sodium dodecyl sulfate (SDS); 0.6% w/v EDTA; 18% w/v glycerol; 5% v/v mercaptoethanol, and with a trace of bromophenol blue, at +4 °C in a sealed capillary tube for subsequent characterisation of the myosin heavy chain isoform (MHC) profile by means of SDS-PAGE electrophoresis (Talmadge and Roy, 1993). The separating gel comprised 8% acrylamide; 0.08% Bis-acrylamide; 0.51% SDS; 0.2 M tris-hydroxymethyl-aminomethane (pH 8.8); 30% glycerol. Gels were run for at least 20 h on ice and proteins were visualised using a PlusOne™ Silver Staining Kit (Amersham Biosciences, UK). Most fibres were found to express just type I or IIa MHC. Very few fibres were found expressing IIx MHC and only the occasional hybrid fibre expressing both type I and IIa, and type IIa and IIx MHC. The remainder of each fibre was weighed on a quartz-fibre fish-pole balance to 0.01 µg calibrated using strands of DNA, the weights of which were determined after dissolving in 1 ml of water by comparison of the absorbance at 260 nm against a standard curve (absorbance versus weight) prepared using the same material. Carnosine and taurine were determined by HPLC, with fluorescence detection, in 1 ml water extracts, as described earlier. Carnosine and taurine concentrations in water extracts of muscle fibres were stable for one or more hours, with no change in either histidine or β-alanine, which would indicate hydrolysis of carnosine.

Frequency distribution graphs of single fibre contents were obtained by pooling data from type I or IIa fibres across subjects, after first normalisation to the mean of that fibre type within that muscle biopsy followed by adjustment to the mean of means for that fibre type across subjects. This was undertaken to minimise the between-subject variance. Consequently the variance of the data apparent in such plots is more akin to the variance in fibre contents within a single subject.

Cycle performance test

Each subject's maximum power output (W_{max}) was first determined on an SRM cycle ergometer (SRM, Germany). Prior to the measurement of W_{max}, subjects completed a 5 min progressive warm up starting at 120 W and increasing by 10 to 160 $W \cdot min^{-1}$. This was followed by 2 min of stretching. Subjects then began exercising at either 100 or 150 W, depending on their fitness, with the exercise intensity being increased by 12.5 watts every 30 s (ramp rate of 25 $W \cdot min^{-1}$) until volitional exhaustion. Subjects pedalled at a constant cadence throughout the test, and were given standardised verbal encouragement throughout. W_{max} is defined as the maximum power output averaged over a 60 s period, usually during the last 75 s of the W_{max} test.

Within 7 days, subjects undertook two cycle capacity tests at 110% W_{max} ($CCT_{110\%}$) for purposes of habituation, followed by a pre-supplementation $CCT_{110\%}$, a practice $CCT_{110\%}$ in the 4th week of supplementation and a 4 week post supplementation $CCT_{110\%}$. A further practice $CCT_{110\%}$ was undertaken in the 10th week of supplementation and a 10 week post supplementation $CCT_{110\%}$ was performed (β-Ala, n = 7; P, n = 8).

The $CCT_{110\%}$ test was conducted on the same SRM cycle ergometer as used for the W_{max} test. Prior to each test the SRM cycle ergometer was calibrated according to manufacturer's instructions. Riding position was customized for each subject by adjusting the vertical and horizontal position of the saddle and handlebars. Saddle height was determined with the subject sitting on the saddle with a straightened leg and with the bottom of their heal just able to touch the top of the pedal. Each subject's cycling position was recorded and maintained the same for subsequent tests. Once a comfortable riding position had been established, subjects performed the same standardised 5 min warm up as used for the W_{max} test.

Due to the intense nature of the test it was decided to increment the first 30 s of the test to allow subjects to become accustomed to the effort required. Thus, subjects exercised at power outputs corresponding to 80% W_{max} for the first 15 s, 95% W_{max} for the second 15 s and then 110% W_{max} until volitional exhaustion (or until power output dropped below 95% of the required power output). Real-time feedback of power-output was provided to subjects via the online SRM computer software and the ergometer was set-up such that if subjects maintained 90 ± 5 rpm, then their power output would automatically correspond to the required power output. Subjects were given standardised verbal encouragement throughout the test.

The time from the start of the test until subjects could no longer maintain 95% of 110% W_{max} was recorded, as was the average power output during the test. Total work done (TWD) was calculated as the product of the average power output during the test in watts and time in seconds.

Statistical analysis

Except where stated, all data are presented as means ± SEM, with statistical significance accepted at the $p < 0.05$ level. To examine intra-group differences between pre and post testing, paired samples *t*-tests were used. Delta values were calculated (post-pre) for muscle and performance data, with the significance of inter-group differences being identified by independent samples *t*-tests.

Results

Subject characteristics are shown in Table 1. The β-Ala and P groups were comparable and there was no effect of supplementation on body mass (Table 1). As far as could be determined subjects did not change their average diet during the course of the study. Subjects were mostly students with a low economy and meat ingestion, providing an estimated 250–750 mg β-alanine per day from the ingestion of histidine dipeptides, which was not particularly high in any one person. There was considerable variation in the β-alanine intake on different days, within subjects, particularly when the method of meat preparation was also taken into consideration.

Table 1. Age, height and body mass of the subjects at the start of the study, and body mass at the end of 4 and 10 weeks supplementation with β-alanine or placebo

	Age (yrs)	Height (cm)	Body mass (kg)		
			0 weeks	4 weeks	10 weeks
β-alanine (n = 13)					
Mean	25.4	184.1	79.9	80.1	80.7
SD	2.1	5.0	7.5	7.3	7.9
Placebo (n = 12)					
Mean	29.2	182.1	78.9	78.2	79.1
SD	6.9	7.9	7.8	8.0	9.2

Muscle biopsies were taken from 6 of the subjects in either group for analysis of carnosine and taurine

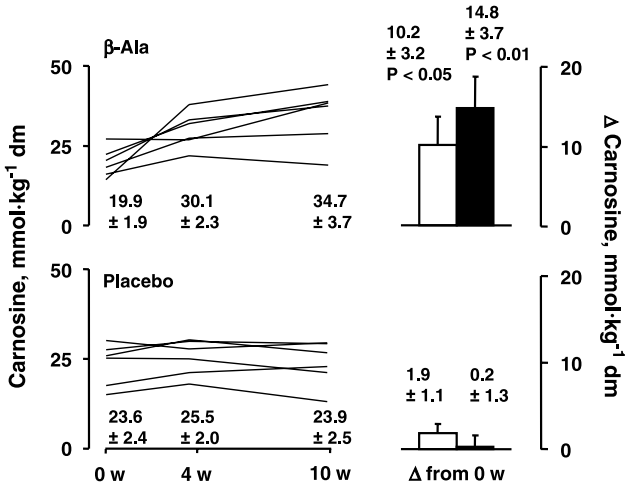

Fig. 1. Muscle carnosine in individual subjects, and the mean change (±SEM) in the carnosine content, prior to supplementation and after 4 and 10 weeks supplementation with β-alanine (n = 6) or placebo (n = 6)

Reports of symptoms of parathesia, noted previously (Harris et al., 2006) were infrequent and mild when they occurred.

Muscle concentrations

There was no significant difference in the pre-supplementation carnosine concentrations between the β-Ala and P groups, the mean of all being $21.8 \pm 1.6\,\text{mmol} \cdot \text{kg}^{-1}$ dm. The ingestion of β-alanine significantly increased skeletal whole muscle carnosine concentrations (Fig. 1) from 19.9 ± 1.9 to 30.1 ± 2.3 and $34.7 \pm 3.7\,\text{mmol} \cdot \text{kg}^{-1}$ dm after 4 and 10 weeks, with no corresponding change in the control group. The mean within-subject changes with β-alanine supplementation were $+10.2 \pm 3.2$ (+58.8%; range −1 to 161%)) and $+14.8 \pm 3.7$ (+80.1%; range 18 to 205%) $\text{mmol} \cdot \text{kg}^{-1}$ dm at 4 and 10 weeks, respectively. Changes at 4 and 10 weeks were significant ($p < 0.05$ and 0.01, respectively) whilst the change between 4 and 10 weeks ($+4.6 \pm 2.0$) was close to significance ($p \sim 0.07$). β-alanine in muscle extracts remained below the limit of detection and there appeared to be no change in histidine although, as the HPLC method is not optimised for the separation of this, this was not always separated from other amino acids. Muscle taurine did not change with supplementation averaging 35.8 ± 1.8, 29.2 ± 1.6 and $38.0 \pm 3.9\,\text{mmol} \cdot \text{kg}^{-1}$ dm at 0, 4 and 10 weeks in those subjects supplemented with β-alanine (Fig. 2).

Muscle fibre concentrations

Carnosine both before and following supplementation appeared normally distributed in both type I and IIa muscle fibres (Fig. 3). Prior to supplementation its content was 1.71 ± 0.11 times higher ($p < 0.01$ when compared to a ratio of 1) in type IIa compared to type I muscle fibres (Table 3). Carnosine increased in both fibre types ($p < 0.01$) and to the same extent with supplementation representing a doubling in the content in type I fibres and a 50% increase in type IIa fibres. At this time the mean content in IIa fibres was 1.43 ± 0.14 times higher than that in type I fibres ($p < 0.05$) (Table 3). In two of the subjects, a few IIx fibres were also classified and analysed. These had a concentration similar to IIa fibres from the same subject, both before and after supplementation. In one subject a few type I/IIa hybrid fibres were also obtained and these again had a concentration close to that in IIa fibres.

Taurine appeared normally distributed (Fig. 4) and, prior to supplementation, did not differ between fibre types (mean type IIa/I ratio: 0.96 ± 0.10 ($p > 0.05$) (Table 3). Absolute concentrations did not change with supplementation. However, the mean IIa/I ratio now indicated a significantly higher content in type I fibres (Table 3).

Table 2. Dosing strategies employed for the two treatment groups

Week	Dosing times								Per day
	9 am	10 am	11 am	12 am	3 pm	4 pm	5 pm	6 pm	
1	800 mg	400 mg	400 mg	400 mg	800 mg	400 mg	400 mg	400 mg	4.0 g
2	800 mg	400 mg	400 mg	800 mg	800 mg	400 mg	400 mg	800 mg	4.8 g
3	800 mg	400 mg	800 mg	800 mg	800 mg	400 mg	800 mg	800 mg	5.6 g
4	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	6.4 g
5–10	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	800 mg	6.4 g

Total: 146 g β-alanine (β-Ala) or maltodextrin (P)

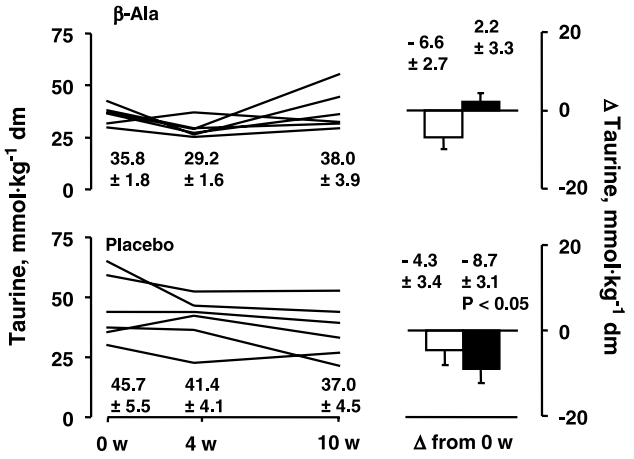

Fig. 2. Muscle taurine in individual subjects, and the mean change (±SEM) in the taurine content, prior to supplementation and after 4 and 10 weeks supplementation with β-alanine or placebo

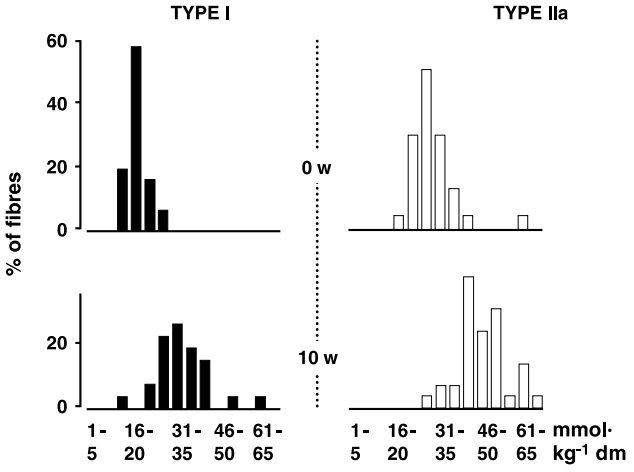

Fig. 3. Frequency distribution of carnosine in type I and IIa muscle fibres before and at the end of 10 weeks supplementation with β-alanine

Table 3. Mean carnosine and taurine concentrations in type I and IIa muscle fibres of subjects (n = 6) supplemented with β-alanine for 10 weeks

	Pre supplementation Fibre type			10 weeks Fibre type			Δ (10 w – Pre)	
	I	IIa	IIa/I	I	IIa	IIa/I	I	IIa
Carnosine								
Mean	17.8	29.6	1.71	34.3	46.6	1.43	16.5	17.0
SEM	1.9	2.1	0.11	4.7	3.4	0.14	2.8	2.6
P			<0.01[a]			<0.05[a]	<0.01	<0.01
Taurine								
Mean	33.4	31.2	0.96	37.4	24.4	0.67	4.0	−6.9
SD	3.3	3.1	0.10	3.3	3.1	0.10	3.0	4.0
P			NS[a]			<0.05[a]	NS	NS

[a] Results of test against a ratio of 1

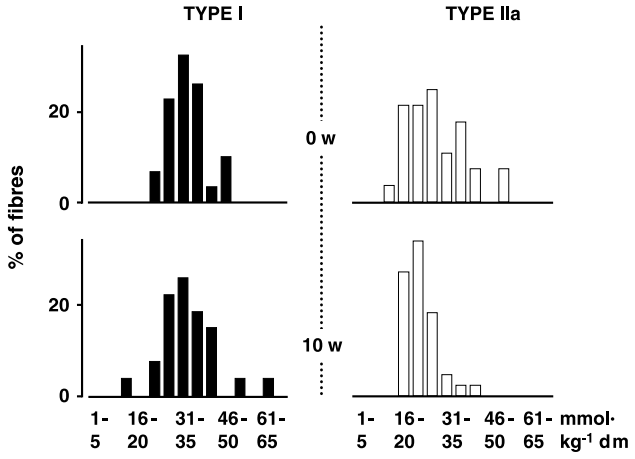

Fig. 4. Frequency distribution of taurine in type I and IIa muscle fibres before and at the end of 10 weeks supplementation with β-alanine

$CCT_{110\%}$

There was no significant difference between the two treatment groups in exercise time at the start of the study, the mean of all subjects being 156.5 ± 4.3 s, close to the expected time of 150 s. There was no significant difference between β-Ala and P in the total amount of work performed during the pre-supplementation test (β-Ala: 51.6 ± 3.2 kJ and P: 55.1 ± 2.8 kJ). The average coefficient of variation in TWD of the P subjects, based on the measurements at 0, 4 and 10 w, was 2.96%. TWD was significantly increased following 4 and 10 weeks of β-alanine supplementation (+7.3 ± 1.3 [+13.0%] and +8.6 ± 3.1 [+16.2%] kJ, respectively; Fig. 5) compared with no change in P (+1.1 ± 1.1 [+2.3%] and +1.7 ± 1.5 [+3.3%], respectively) [2-way rm-ANOVA: $p < 0.015$;

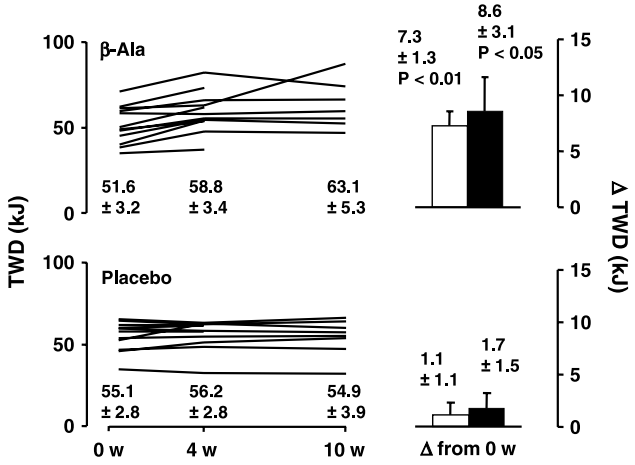

Fig. 5. TWD by individual subjects, and the mean change (±SEM) in TWD, prior to supplementation and after 4 and 10 weeks supplementation with β-alanine or placebo

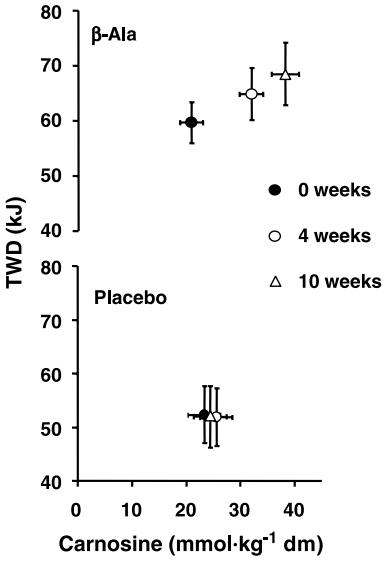

Fig. 6. Comparison of the mean (±SEM) TWD and mean (±SEM) muscle carnosine content prior to supplementation and after 4 and 10 weeks supplementation with β-alanine or placebo

$\eta_p^2 = 0.37$; observed power = 0.731]. The corresponding changes in exercise times from the initial mean of 156.5 s were $+11.8 \pm 3.2\%$ and $+15.9 \pm 5.1\%$, and, $-0.2 \pm 1.5\%$ and $+0.6 \pm 1.3\%$, respectively. Of the 12 subjects supplemented with β-alanine, only one showed a slight reduction in TWD during the 4 week-post $CCT_{110\%}$ (−0.6 kJ). Two other subjects showed only minor increases (+1.7 and +1.9 kJ). From one of these a biopsy was taken and this failed to show any increase in the skeletal muscle carnosine concentration.

Comparison of TWD with the muscle carnosine content in the 5 β-alanine supplemented subjects, where both were determined, indicated that the changes in TWD were related to the increase in carnosine (Fig. 6). However, the relationship was not significant, possibly because of the small numbers. No trend was evident when the same comparison was performed on the data from the P subjects.

Discussion

The changes in muscle carnosine following 4 weeks supplementation with β-alanine were comparable to those shown previously (Harris et al., 2006). The results in Fig. 1 suggest that a still greater increase may occur in some subjects following 10 weeks supplementation. By the end of 4 and 10 weeks supplementation the increases in muscle carnosine amounted to +58.8% and +80.1%, respectively. Analysis of single fibres indicated that the increase was approximately the same in type I and IIa fibres, and most probably in IIx fibres. This was despite the higher initial concentration of carnosine in type II fibres (Table 3), which we have reported previously for human (Harris et al., 1998), as well as for equine and camel muscle (Dunnett and Harris, 1995; Dunnett et al., 1997)

In agreement with a previous study (Harris et al., 2006), supplementation was not associated with any reduction in muscle taurine arising from increased competition between β-alanine and taurine for transport into muscle cells (Jessen, 1994; Ramamoorthy et al., 1994). The lack of any decrease in taurine was evident both at the whole muscle and single fibre level. Dawson et al. (2002) have previously reported that administration of 3% β-alanine in the drinking water of rats, may reduce the taurine content of muscle by up to 50%. However, assuming a water intake of around $200\,ml \cdot kg^{-1}$ (Wade et al., 2002) this would provide a β-alanine dose 70–80 times higher than that used in the present study (which at $4–6.4\,g \cdot d^{-1}$ we estimate to be 1–2 times greater than that found in some human diets).

The similar concentration of taurine measured in type I and IIa fibres from the biopsies taken prior to supplementation is in contrast to what we have observed previously in human muscle (Harris et al., 1998) where taurine was higher in type I fibres. In this respect, the results from the post-supplementation samples are more typical of those previously reported. Much higher type I/II ratios have been observed in other species (Dunnett and Harris, 1995; Dunnett et al., 1997) to the extent that in equine muscle taurine may be virtually absent from type II fibres.

Importantly, this study showed a significant effect of supplementation on exercise performance in a cycle test with a predicted exercise time (before supplementation) of 150 s. Furthermore, the change in TWD in those subjects where muscle data was also collected, appeared to follow the increase in the muscle carnosine content. In this regard the present results are consistent with the findings of Suzuki et al. (2002) who showed a relationship between greater muscle carnosine content in (non-supplemented) subjects and the increased ability to sustain power output in the final 10 seconds of a 30 second Wingate test. However, the present data suggest that there may be a limit to the increase in performance in the $CCT_{110\%}$ arising from the increase in muscle carnosine, this being reached at the end of 4 weeks of supplementation.

As carnosine has been implicated in a number of physiological actions, several explanations for the increase in

performance are possible. The most likely would seem to be a change in Ca^{2+} sensitivity, E-C coupling (Lamont and Miller, 1992; Batrukova and Rubtsov, 1997) and increase in intracellular buffering. Of these, we would favour the latter. An increase in muscle buffering capacity is inevitable given the observed change in carnosine, unless opposite and parallel changes occur in other compounds with pKa's within ±1 pH unit of ∼pH 7. The possible candidates here are largely limited to inorganic and organic phosphates, and the bicarbonate concentration at the start of contraction. However, changes in total phosphate would seem unlikely, being constrained by the participation of the cellular phosphate pool in reactions linked to ATP metabolism. The one time when an increase in phosphate buffering is likely is with creatine supplementation (Harris et al., 1992) although because of the low pKa of the phosphoryl group (pKa 4.58) an increase in buffering capacity will only take effect after a net conversion of phosphorylcreatine to creatine and phosphate. Parallel, opposite and quantitatively similar (in terms of H^+ buffering), changes in proteins containing histidine residues, or of other amino acids expressing a weaker buffering effect, would seem unlikely as this would almost certainly undermine cell integrity.

Carnosine has previously been dismissed as relatively unimportant as a physico-chemical buffer in human muscle contributing less than 10% (Hultman and Sahlin, 1980). If so, even a 50% change in its content in muscle might be too little to bring about a physiologically significant change in buffering affecting performance. However, estimates of the importance of carnosine have been derived from comparison of its buffering effect calculated from its pKa, and the buffering capacity of muscle (β) determined by titration of homogenates with acid. Homogenisation of muscle, even when performed with iodoacetate to inhibit glycolysis, changes its chemical composition including transfer of the phosphate bound as phosphorylcreatine (a weak buffer where the phosphoryl group has a pKa of 4.58) to inorganic and organic phosphates with pKa's between 6.1 and 6.8 (Harris et al., 1989). Where hydrolysis of phosphorylcreatine amounts to 100%, as with homogenisation, the buffering exerted by 20 $mmol \cdot kg^{-1}$ dm carnosine between pH 7.1 to 6.5 is ∼8% of β from sources other than carnosine (β non-carn), assuming this to be 74 $mmol\, H^+ \cdot kg^{-1}$ dm (Sewell et al., 1992). The value of 74 $mmol\, H^+ \cdot kg^{-1}$ dm is itself determined by homogenisation between the limits of pH 7.1 to 6.5. It can be shown using the Henderson-Hasselbach equation that the apparent importance of carnosine rises where the hydrolysis of phosphorylcreatine is less than 100%, to the extent that it is 13% of β non-carn when hydrolysis of phosphorylcreatine is zero. The corresponding values for a muscle of 40 $mmol \cdot kg^{-1}$ dm carnosine are ∼16% for 100% hydrolysis and ∼26% for 0% hydrolysis of phosphorylcreatine. Although changes in the status of phosphorylcreatine change the apparent importance of carnosine, the absolute contribution to buffering at any given concentration of carnosine will of course remain the same.

Estimates of β obtained from titration of muscle homogenates will also include contributions from both intra- and extra-cellular pools of pH active compounds. This will again tend to underestimate the contribution made by carnosine as well as the quantitative importance of any increase in this with β-alanine supplementation. Finally carnosine is not evenly distributed in muscle, occurring in higher concentrations in type II fibres (Harris et al., 1998). This again could raise the quantitative importance of carnosine as an intracellular buffer in type II fibres, as

Table 4. Estimates of the quantitative importance of carnosine before and after 4 and 10 weeks supplementation to intracellular physicochemical buffering capacity between the pH limits of 7.1 and 6.5

		0 weeks	4 weeks	10 weeks
Muscle	β non-carn	74	74	74
	β carn	6.6	10.0	11.5
	Total β	80.6	84.0	85.5
	% β carn of Total β	8.2	11.9	13.5
Type I	β non-carn	74	74	74
	β carn	5.9		11.4
	Total β	79.9		85.4
	% β carn of Total β	7.4		13.3
Type IIa	β non-carn	74	74	74
	β carn	9.8		15.5
	Total β	83.8		89.5
	% β carn of Total β	11.7		17.3

The calculations are based on the carnosine contents presented in Fig. 1 for mixed muscle and Table 3 for single fibres. The specific contribution of carnosine (β carn) was calculated from the Henderson-Hasselbach equation. The estimated contribution of 74 $mmol\, H^+ \cdot kg^{-1}$ dm from sources other than carnosine (β non-carn) was taken from Harris et al. (1990) and Sewell et al. (1992) and was determined by titration of homogenised freeze-dried muscle during which all phosphate included in phosphorylcreatine (with pKa 4.58) is converted to free phosphate (pKa 6.82) or sugar and nucleotide phosphates with pKa's between 6.1 to 6.8 (Harris et al., 1989). This estimate does not include any contribution from bicarbonate (Harris et al., 1989). Total β is the sum of β carn and β non-carn. Values: $mmol\, H^+ \cdot kg^{-1}$ dm

For the calculation of β it is assumed that β non-carn is numerically the same for type I and IIa fibres as suggested by Sewell et al. (1992)

well as the physiological significance of any increase with supplementation in these fibres. Using the cited estimate of 74 mmol $H^+ \cdot kg^{-1}$ dm for β non-carn (Sewell et al., 1992), the estimated β of muscle before supplementation was 80.6 mmol $H^+ \cdot kg^{-1}$ dm (Table 4). The contribution of carnosine is 8.2%. After 10 weeks supplementation β is estimated at 85.5 mmol $H^+ \cdot kg^{-1}$ dm, 13.5% from carnosine. As a result of the greater concentration of carnosine in type IIa fibres these estimates are increased to 83.8 mmol $H^+ \cdot kg^{-1}$ dm (11.7% from carnosine) and at 10 weeks, 89.5 mmol $H^+ \cdot kg^{-1}$ dm (17.3% from carnosine). As indicated, the estimate of β non-carn is almost certainly an over-estimate (and clearly so in the early stages of exercise when the net transformation of phosphorylcreatine to creatine and phosphate has only just begun). Consequently the true contribution of carnosine to β, and the effect of the increase in this with β-alanine supplementation, is likely to be considerably greater than the above estimates.

The formation and accumulation of hydrogen ions (H^+) in muscle with intense exercise has been shown to affect metabolic processes (Spriet et al., 1989), including disturbance of the creatine-phosphorylcreatine equilibrium limiting the resynthesis of phosphorylcreatine (Harris et al., 1976) and inhibition of glycolysis (Trivedi and Danforth, 1966), as well as the contractile process itself (Chin and Allen, 1998; Fabiato and Fabiato, 1978; Favero et al., 1995). While some have questioned the role of H^+ in the development of fatigue (Westerblad et al., 1997), a higher muscle buffering capacity in humans has been associated with an improvement in sprint exercise and cycle endurance performance (Bell and Wenger, 1988; Bishop et al., 2004; Edge et al., 2006; Nevill et al., 1989; Suzuki et al., 2002; Weston et al., 1997). At first sight this appears consistent with expectations of the effect of decreasing pH on exercise performance. However, because of the higher concentration of carnosine in type II fibres, higher levels of H^+ buffering may equally reflect a higher type II muscle fibre composition, and could be the cause of the differences in performance capacities. In the study of Edge et al. (2006), training increased both muscle buffering capacity and repeated-sprint ability but in this case the increases in performance, despite a positive correlation to buffering, may have been the result of other training induced adaptations. The present results are unique in that a dietary protocol has been used to directly affect the intracellular H^+ buffering capacity, without changing the training status of subjects.

Whilst the present results do not rule out that an increase in carnosine may express other physiological effects in muscle apart from buffering, or that the gains in H^+ buffering are offset by changes in other compounds, we believe that the present results are possibly the first demonstration of an improvement in human, whole body exercise capacity, linked to an increase in intracellular H^+ buffering content. Clearly this outcome would only hold true at exercise intensities where a decrease in muscle pH is exerting, in the first instance, a limiting effect on exercise duration.

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Authors' address: Roger C. Harris, Ph.D., School of Sport, Exercise and Health Sciences, University of Chichester, Chichester, West Sussex, PO19 6PE, U.K.,
Fax: +44 (0)1243 816080, E-mail: r.harris@chi.ac.uk