Amino Acids (2006) 30: 279–289
DOI 10.1007/s00726-006-0299-9

Amino Acids
Printed in Austria

The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis

R. C. Harris[1], **M. J. Tallon**[1], **M. Dunnett**[1], **L. Boobis**[2], **J. Coakley**[3], **H. J. Kim**[4], **J. L. Fallowfield**[1], **C. A. Hill**[1], **C. Sale**[1], and **J. A. Wise**[5]

[1] School of Sports, Exercise and Health Sciences, University College Chichester, Chichester, U.K.
[2] Sunderland Royal Hospital, Sunderland, U.K.
[3] Intensive Care Unit, Homerton Hospital, London, U.K.
[4] Human Exercise Physiology, Korea National Sport University, Seoul, Korea
[5] Natural Alternatives International, San Marcos, CA, U.S.A.

Received October 10, 2005
Accepted October 18, 2005
Published online March 24, 2006;

Summary. β-Alanine in blood-plasma when administered as A) histidine dipeptides (equivalent to $40\,mg \cdot kg^{-1}$ bwt of β-alanine) in chicken broth, or B) 10, C) 20 and D) $40\,mg \cdot kg^{-1}$ bwt β-alanine (CarnoSyn™, NAI, USA), peaked at $428 \pm$ SE 66, 47 ± 13, 374 ± 68 and $833 \pm 43\,\mu M$. Concentrations regained baseline at 2 h. Carnosine was not detected in plasma with A) although traces of this and anserine were found in urine. Loss of β-alanine in urine with B) to D) was <5%. Plasma taurine was increased by β-alanine ingestion but this did not result in any increased loss via urine. Pharmacodynamics were further investigated with $3 \times$ B) per day given for 15 d. Dietary supplementation with I) 3.2 and II) $6.4\,g \cdot d^{-1}$ β-alanine (as multiple doses of 400 or 800 mg) or III) L-carnosine (isomolar to II) for 4 w resulted in significant increases in muscle carnosine estimated at 42.1, 64.2 and 65.8%.

Keywords: Carnosine – β-Alanine – Muscle – Buffering – Intracellular pH

Introduction

Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide found in high concentrations in both vertebrate and non-vertebrate skeletal muscle, and is present also 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 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 as it does from a pKa of 6.83 of the imidazole ring (Bate-Smith, 1938; Tanokura et al., 1976). Two methyl derivatives of carnosine (anserine: β-alanyl-1-methylhistidine and balenine: β-alanyl-3-methylhistidine), with similar pKa's to carnosine, are found in some animal muscles but not in human. In contrast to carnosine, 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 mammals, birds and fish with a capacity for prolonged intense or sprint exercise, and still higher in aquatic species including whales (Abe, 2000). In addition carnosine is typically higher in fast-twitch (type II) muscle fibres compared to slow-twitch (type I) muscle fibres (Harris et al., 1998, 2005). In human vastus lateralis muscle carnosine ranges from $10.5 \pm SD_{ind}$ $7.6\,mmol \cdot kg^{-1}$ dry muscle (dm) in type I and $23.2 \pm SD_{ind}$ $17.8\,mmol \cdot kg^{-1}$ dm in type II fibres (Harris et al., 1998), where SD_{ind} is the estimated standard deviation in fibre content within an individual. A similar preferential distribution into type II fibres has also been shown in skeletal muscle of horse and camel (Dunnett and Harris, 1995; Dunnett et al., 1997) where the histidine dipeptide concentration may be 4 to 5 times that in type I fibres. The higher concentrations found in fast twitch muscle, and 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.

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. However, the concentrations of the physicochemical buffers other than carnosine 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 10 days to 8 weeks intensive training has been shown to almost double the carnosine content in vastus lateralis (Kim et al., 2005; 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.

Carnosine is synthesized in muscle and cells of the CNS (Bakardjiev and Bauer, 1984) although other cell types express a dipeptide transporter enabling the molecule to be taken up intact (Hoffmann et al., 1996; Dieck et al., 1999). Dunnett and Harris (1999) observed a significant increase in the carnosine content of type II fibres of horse middle gluteal muscle following a 30 day supplementation period with β-alanine and histidine, as precursors of carnosine. As the concentration of histidine in muscle (and plasma) is high relative to its Km with muscle carnosine synthetase (CS) (Horinishi et al., 1978), in contrast to the low concentration of β-alanine in muscle which exhibits a much higher Km with CS (Skaper et al., 1978), it was concluded that β-alanine was probably limiting to carnosine synthesis in equine muscle. Other studies in rats have focused mainly on the administration of carnosine itself (Chan et al., 1994; Hama et al., 1976; Maynard et al., 2001) or histidine (Tamaki et al., 1977, 1984). In humans, however, the intact dipeptide is rapidly hydrolysed in plasma due to the presence of the hydrolyzing enzyme, carnosinase (Asatoor et al., 1970; Perry et al., 1967). β-Alanine, a non-proteinogenic amino acid, is synthesized in the liver as the final metabolite of uracil and thymine degradation (Matthews and Traut, 1987), but in meat eating animals quantitatively the greatest source may be from the hydrolysis of dipeptides in the diet. The aim of this study was to investigate the availability of β-alanine supplied orally and the effect of supplementation on muscle carnosine synthesis.

Material and methods

All parts of the investigation were first approved by the Ethics Review Committee of the University College Chichester. The study was described in detail to all subjects prior to obtaining their written consent to participate. All were questioned about their general state of health and only subjects free of any apparent infection and without any history of any metabolic, muscular, cardiovascular or other organ disease, were recruited to the study. Subjects were staff and students of University College Chichester, non-smokers and had not taken any dietary supplements 4 to 6 weeks prior to the start of the relevant study. None of the subjects recruited to the various studies were vegetarians.

Study 1: Administration of 0–40 mg · kg^{-1} bwt β-alanine

Six healthy male subjects ($33.5 \pm$ SD 9.9 yrs; $80.2 \pm$ SD 17.1 kg) were recruited to the study. Each experimental session commenced after an overnight fast (a minimum of 12 h being allowed after consumption of the last meat containing meal). Subjects were given the option to consume a small quantity of warm water prior to the start of the study. Catheterization was begun at 8.30 am and the study started at 9 am. Each subject underwent four treatment tests out of a possible five:

Treatment A (all subjects): The treatment involved ingestion of $8\,\text{ml} \cdot \text{kg}^{-1}$ bwt of chicken broth containing in total approximately $40\,\text{mg} \cdot \text{kg}^{-1}$ bwt β-alanine in the form of anserine and carnosine. For a subject of 80 kg bwt this amounted to the ingestion of 640 ml of broth containing 3.2 g of β-alanine in dipeptide form.

Treatments B, C, D and E (3 of the possible 4 treatments being taken by each subject): Ingestion of $3\,\text{ml} \cdot \text{kg}^{-1}$ bwt of a drink containing B) 0 (control), C) 10, D) 20 or E) $40\,\text{mg} \cdot \text{kg}^{-1}$ bwt β-alanine (CarnoSyn™, NAI, San Marcos, California, USA) followed by ingestion of a further $5\,\text{ml} \cdot \text{kg}^{-1}$ bwt of water. For a subject of 80 kg bwt the dose of $40\,\text{mg} \cdot \text{kg}^{-1}$ bwt amounted to the ingestion of 3.2 g of β-alanine. The order of the tests was randomised within and between subjects (treatments taken are identified in Table 1).

Table 1. Subjects reporting symptoms of flushing with treatments A) to E)

Subject	A Broth 40 mg/kg bwt[a]	B β-ala 0 mg/kg bwt	C β-ala 10 mg/kg bwt	D β-ala 20 mg/kg bwt	E β-ala 40 mg/kg bwt
1	X			SS	SS*
2	X			X	SS*
3	X	X	X	SS	
4	X	X	MS		SS*
5	X	X	X		SS*
6	X	X	MS	SS	

X No symptoms reported, *MS* mild symptoms of flushing recorded, *SS* significant symptoms, *SS** symptoms recorded as unpleasant
[a] Equivalent content of β-alanine

In all sessions subjects additionally consumed a further 8 ml · kg^{-1} bwt of water (in 50 ml portions) during the period of 1 to 2 h after ingestion. A vegetarian pizza was provided at 6 h. Normal food was allowed from 8 h onwards.

2.5 ml venous blood samples were drawn through an indwelling catheter, inserted into a forearm vein, at 10 min intervals for the first 90 min and thereafter at 120, 180, 240 and 360 min. Blood was dispensed into tubes containing lithium-heparin as anti-coagulant, centrifuged and harvested for plasma within 5 min. The patency of the catheter was maintained by flushing with non-heparinised saline. 1 ml plasma was deproteinised with 200 ul 30% w/v 5′-sulphosalicylic acid, centrifuged and made alkaline by diluting 20 times with 0.1 M borate buffer, pH 9.65. Alkaline extracts were derivitised with ortho-pthaldehyde/mercaptoproprionic acid reagent and analysed for titled compounds by HPLC with fluorescence detection, with comparison to standards of 1 to 100 μM (Dunnett and Harris, 1997). Areas under the plasma concentration curves (AUC) were calculated by the trapezoidal rule.

Urine was collected over 9 h following ingestion of the test dose and following centrifugation and 50 fold dilution of a portion of the supernatant with 0.1 M borate buffer, pH 9.65 was analysed by HPLC as before to determine the excretion of β-alanine and taurine.

Preparation of chicken broth (treatment A)

Fresh chicken breast (skinned and boned) was finely chopped and boiled for 15 min with water (1 litre for every 1.5 kg of chicken). Residual chicken meat was removed by course filtration. The filtrate was flavoured by the addition of carrot, onion, celery, salt, pepper, basil, parsley and tomato puree, and re-boiled for a further 15 min and then cooled before final filtration through fine muslin at 4 °C. The yield from 1.5 kg chicken + 1 litre of water was 870 ml of stock. A portion of the stock was assayed for total β-alanyl-dipeptides (carnosine and anserine) and β-alanine. Typical analyses were: total β-alanyl-dipeptides, 74.5 mmol · l^{-1}; free β-alanine, 5.7 mmol · l^{-1}. From this a dose estimated to yield 40 mg · kg^{-1} bwt of β-alanine was calculated and was provided hot to each subject.

Study 2: Two weeks administration of 10 mg · kg^{-1} bwt β-alanine, 3× per day

Six male subjects aged 28.3 ± SD 2.7 yrs; 83.2 ± SD 14.3 kg) were recruited to the study. On day 1 the subjects reported to the blood-sampling laboratory at 8 am following an overnight fast and a minimum of 12 h since the last meat containing meal. Following catheterisation of a forearm vein and a basal sample, each subject was given three doses of 10 mg · kg^{-1} bwt β-alanine at 0 (9 am), 3 and 6 h. Five ml blood samples were collected using lithium heparin as anticoagulant at 0.5, 1, 2, 3, 3.5, 4, 5, 6, 7, 8 and 9 h. Between days 2–15 subjects continued to take 10 mg · kg^{-1} bwt β-alanine 3 times per day. On day 15 the protocol followed on day 1 was repeated with blood samples collected before the first supplementation at 9 am and between 0.5 and 9 h thereafter. Plasma samples were treated and analysed as before for β-alanine and taurine.

Study 3: The effect of 4 weeks dietary supplementation with β-alanine or carnosine on the muscle carnosine content

The main study was preceded by an investigation of 16 male subjects (19.4 ± SD 1.6 yrs; 79.5 ± SD 9.3 kg) to assess the effects of repeated administration of β-alanine on blood biochemistry and haematology. Subjects first completed a medical questionnaire and underwent a 12-lead ECG at the start and end of the study to establish normal cardiac function. Venous blood samples were taken at St. Richard's Hospital, Chichester, at the start and end of the 4 weeks supplementation with 4 doses per day of 800 mg of β-alanine (n = 8) or a matching placebo (n = 8). Both treatments were provided as 2 × 400 mg contained in gelatin capsules. Subjects were instructed to take the 4 doses at approximately 9 am, 12 am, 3 pm and 6 pm.

To study the effects on muscle, twenty-one male subjects (26.1 ± SD 5.6 yrs; 79.5 ± SD 10.5 kg) were recruited to the study. Subjects were physically active and regularly ate meat. Subjects completed a medical questionnaire and underwent a 12-lead ECG before being recruited into the study. Subjects were weighed at the start and end of the study and completed a weekly health questionnaire.

Subjects ingested β-alanine daily using one of two regimens (I and II, both n = 5), L-carnosine (III, n = 5) or placebo (IV, n = 6) for 4 weeks:

I) 800 mg of β-alanine was given 4 times per day (approximately 9 am, 12 am, 3 pm and 6 pm) to give an average daily dose of 3.2 g and total 4 week dose of 89.6 g.

II) used a more frequent dosage strategy in order to increase the dose but not to exceed 800 mg in any one dose. In week 1 subjects consumed 800, 400, 400, 400, 800, 400, 400 and 400 mg at 9, 10, 11 and 12 am, and, 3, 4, 5, and 6 pm to give an average daily dose of 4 g. In week 2 the 11 am and 5 pm doses were increased to 800 mg; in week the 10 am and 4 pm doses were similarly increased and in week 4 also the 12 am and 6 pm doses. Thus in week 4 the average daily dose was 6.4 g. The total dose over the 4 weeks was 145.6 g.

III) subjects ingested L-carnosine using a dosing strategy identical to II) and where each individual dose was approximately isomolar with respect to β-alanine. Thus where 400 and 800 mg of β-alanine were given in II), 1000 and 2000 mg of L-carnosine were given in III). The total given over the 4 weeks was 364 g of L-carnosine corresponding to 143.3 g of β-alanine.

IV) subjects were given capsules containing maltodextrin to match those of II) and at the same frequency as given also in III).

Subjects were aware of differences in the frequency of doses (group I contrasted with groups II, III and IV) and the size of capsules (group III contrasted with II and IV) but were not aware of the nature of the treatments themselves. A single muscle biopsy from the mid belly of the vastus lateralis was taken at the start and end of the 4 week study using a one-handed NHS 6 mm muscle biopsy needle (Northern Hospital Supplies, Edinburgh, UK) with suction, following the procedure of Bergström (1962). Muscle samples were frozen in liquid nitrogen and stored at −85 °C until freeze-dried. One to two mg of freeze-dried muscle was dissected free of fat, blood and connective tissue, powdered using an agate pestle and mortar and extracted by vortexing for 5 min with 1 ml 0.4 M borate buffer, pH 9.65. Extracts were further diluted 5 or 10 times with 0.4 M borate buffer, pH 9.65. Diluted extracts were analysed for β-alanine, histidine, carnosine and taurine as previously described.

Results are means and standard error (SE). The effect of 4 weeks supplementation on muscle contents was determined by ANOVA or Students t-test applied to the mean within-subject differences.

Results

Study 1

The original aim was to compare the administration of 40 mg · kg^{-1} bwt free β-alanine with an equivalent dose contained in dipeptide form in the chicken broth, and against a control of 0 mg · kg^{-1} bwt. However, the first subjects given 40 mg · kg^{-1} bwt β-alanine quickly complained of symptoms of flushing (described variously as an irritation of the skin and prickly sensation) which began within 20 min and lasted up to one hour. This first affected the ears, forehead and scalp, followed by the upper trunk including the arms and the back of the hands,

and finally the base of the spine and buttocks. At $40\,mg \cdot kg^{-1}$ bwt the experience was considered unpleasant and 2 subjects refused this dose. As a result of this two lower doses of 10 and $20\,mg \cdot kg^{-1}$ bwt were introduced. Symptoms were again evident at $20\,mg \cdot kg^{-1}$ bwt and followed a similar time course but were judged to be less intense. Only mild symptoms were experienced by 2 of the 4 subjects taking $10\,mg \cdot kg^{-1}$ bwt (Table 1). (In subsequent studies involving more than 50 subjects given a fixed dose of 800 mg β-alanine, corresponding to approximately $10\,mg \cdot kg^{-1}$ bwt, an incidence of nearer 25% made up of mild to very mild symptoms was generally recorded.) None of the 6 subjects given the chicken broth, which contained the equivalent of $40\,mg \cdot kg^{-1}$ bwt β-alanine, reported symptoms of flushing or did any in the control group.

β-Alanine was below the limit of detection in plasma (<0.5 μM) prior to all treatments and at all time points with $0\,mg \cdot kg^{-1}$ bwt. β-Alanine increased in plasma following ingestion of the chicken broth. The increase was approximately half that with ingestion of a solution of $40\,mg \cdot kg^{-1}$ bwt β-alanine and exhibited a later time to peak, i.e. $427.9 \pm 66.1\,\mu mol \cdot l^{-1}$ at 90 min versus $833.5 \pm 42.8\,\mu mol \cdot l^{-1}$ at 40 min (Fig. 1). Based on the 4 subjects given both the broth and solution the mean area-under-the-curve (AUC), however, did not differ significantly between treatments, i.e. $44{,}500 \pm 2{,}200$ and $46{,}900 \pm 8{,}300\,[\mu mol \cdot l^{-1}] \cdot min$ ($P > 0.05$). Carnosine, itself, was not detected in plasma following any of the treatments including administration of the chicken broth. However, traces of anserine in plasma were found following ingestion of the broth.

Times to the peak plasma concentration with ingestion of 10, 20 and $40\,mg \cdot kg^{-1}$ bwt β-alanine lay between

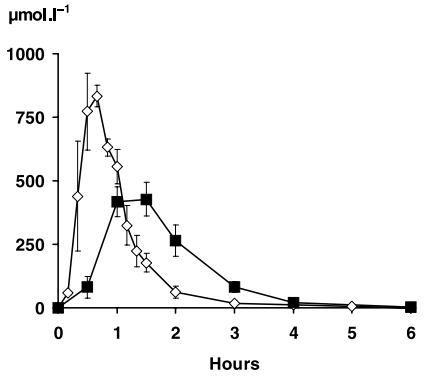

Fig. 1. Mean plasma β-alanine concentration with time following ingestion of 40 (◇) $mg \cdot kg^{-1}$ bwt β-alanine or a chicken broth (■) containing the equivalent of $40\,mg \cdot kg^{-1}$ bwt β-alanine in the form of anserine and carnosine. The SE of the means is shown for both sets of values

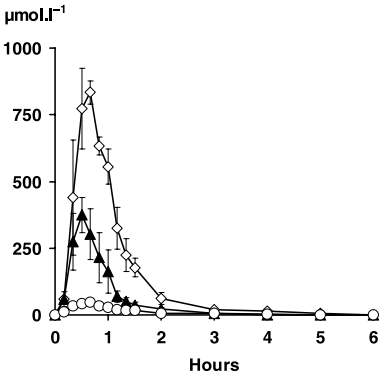

Fig. 2. Mean plasma β-alanine concentration with time following ingestion of 10 (○), 20 (▲) or 40 (◇) $mg \cdot kg^{-1}$ bwt β-alanine. For reasons of clarity the SE of the means is shown only for measurements following 20 and $40\,mg \cdot kg^{-1}$ bwt

30–40 min (Fig. 2). However, the increases in peak concentration and AUC were out of proportion to the increases in amounts administered. Between 10 to $20\,mg \cdot kg^{-1}$ bwt a 6–8 fold increase occurred although from 20 to $40\,mg \cdot kg^{-1}$ bwt the increase was 2.2 fold. Following the peak in concentration, the half-life of disappearance was approximately 25 min with each of the three doses. Loss of β-alanine via the urine following 10, 20 and $40\,mg \cdot kg^{-1}$ bwt was $0.60 \pm 0.09\%$, $1.50 \pm 0.40\%$ and $3.64 \pm 0.47\%$ respectively, of the administered dose (Fig. 3). Administration of β-alanine resulted in an increase in the plasma taurine concentration, the increase being dose dependent between 10 and $40\,mg \cdot kg^{-1}$ bwt (Fig. 4). However, this did not result in any significant increase in taurine loss in the urine (Fig. 5).

Study 2

With a 3 h gap between successive ingestions of β-alanine ($10\,mg \cdot kg^{-1}$ bwt) the plasma concentration was able to

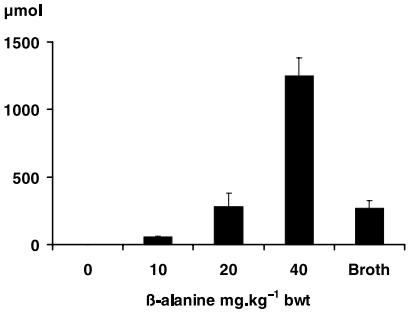

Fig. 3. Mean (+SE) loss of β-alanine in the urine during 9 h following ingestion of 10, 20 or $40\,mg \cdot kg^{-1}$ bwt β-alanine, i.e. 112, 224 and $449\,\mu mol \cdot kg^{-1}$ bwt, or chicken broth containing the equivalent of $40\,mg \cdot kg^{-1}$ bwt β-alanine in the form of anserine and carnosine

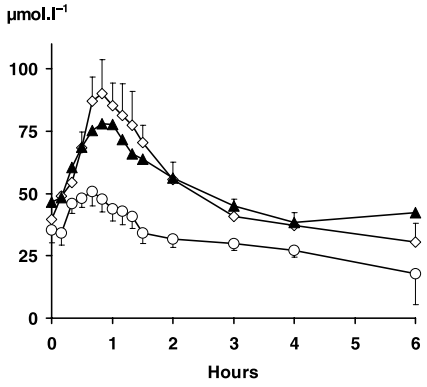

Fig. 4. Plasma taurine concentration with time following ingestion of 10 (○), 20 (▲) or 40 (◇) mg · kg^{-1} bwt β-alanine. For reasons of clarity the SE of the means is shown only for measurements following 10 (negative SE) and 40 (positive SE) mg · kg^{-1} bwt

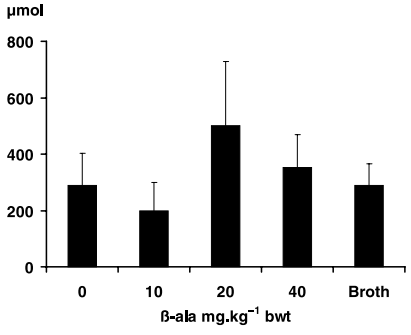

Fig. 5. Mean (+SE) loss of taurine in the urine during 9 h following ingestion of 10, 20 or 40 mg · kg^{-1} bwt β-alanine, i.e. 112, 224 and 449 µmol · kg^{-1} bwt, or chicken broth containing the equivalent of 40 mg · kg^{-1} bwt β-alanine in the form of anserine and carnosine

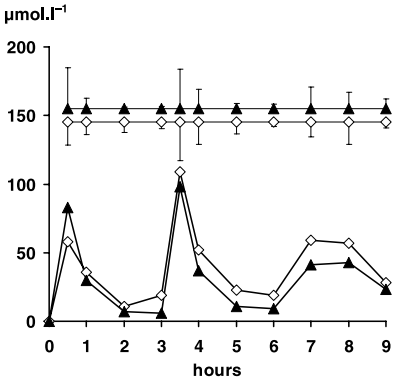

Fig. 6. Plasma β-alanine over 9 h following the oral ingestion of 10 mg · kg^{-1} bwt of β-alanine at 0, 3 and 6 h on days 1 (▲) and 15 (◇) whilst dosing at 3 × 10 mg · kg^{-1} bwt per day. For reasons of clarity the SE of the means are shown separately above the main trend line

return to baseline before the next dose (Fig. 6). The peak increase in plasma β-alanine, and time to peak, were as in study 1 and neither was changed by 15 days of supplementation. Repeated dosing did not give rise to any problems apart from the occasional report of mild symptoms of flushing. The increase in plasma β-alanine was associated with a small but non-significant increase in the plasma taurine concentration following both doses on day 0. The increase, however, was significant following the second dose on day 15. On both days 0 and 15, taurine had regained the baseline concentration before administration of the second dose (Fig. 7).

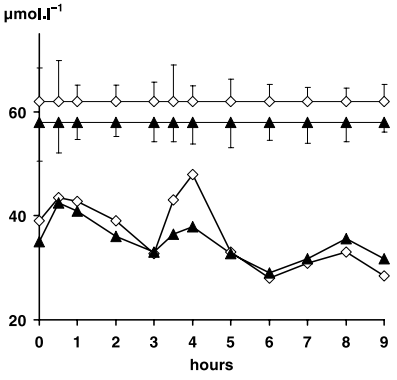

Fig. 7. Plasma taurine over 9 h following the oral ingestion of 10 mg · kg^{-1} bwt of β-alanine at 0, 3 and 6 h on days 1 (▲) and 15 (◇) whilst dosing at 3 × 10 mg · kg^{-1} bwt per day. For reasons of clarity the SE of the means are shown separately above the main trend line

Study 3

There were no clinically significant changes by the end of 4 weeks supplementation with 4 × 800 mg β-alanine in any of the biochemical or haematological variables measured (Table 2). Over the 4 weeks only one instance of a sore throat was recorded, this being by a subject in group III. No other incidences indicating an adverse effect on health of β-alanine or carnosine supplementation were recorded. Mild symptoms of flushing were reported in week 2 by 4 of the subjects given β-alanine (with fewer subjects in the other weeks) and 3 subjects in week 4 given carnosine. One subject given the placebo also recorded mild symptoms of flushing.

β-Alanine was below the limit of detection of 0.1–0.2 mmol · kg^{-1} dm in most muscle extracts both before and following supplementation, and maximally 0.4 mmol · kg^{-1} dm where a peak in an individual chromatogram could be detected. The HPLC method is not optimised for histidine, which is not fully separated from another amino acid which can contribute up to 30% of the integrated peak area. Separation was particularly poor when analysing samples from I, II and III. The apparent means before supplementation for I–III were 5.76 ± 0.26,

Table 2. Blood biochemical and haematological markers before and at the end of 4 weeks supplementation with 800 mg β-alanine 4 times per day

	Placebo				β-Alanine			
	Pre		Post		Pre		Post	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Na	139.6	0.5	138.4	0.7	138.0	0.29	138.57	0.35
K	4.23	0.07	4.20	0.16	4.24	0.14	4.29	0.07
Bicarbonate	31.3	0.6	30.9	0.5	31.1	0.7	31.3	0.4
Creatinine	95.3	4.2	86.9	3.0	96.4	3.4	87.6	3.7
Urea	3.98	0.21	4.53	0.29	4.71	0.31	4.81	0.40
Total protein	71.6	0.9	72.6	0.7	73.1	1.9	72.6	1.0
Albumin	44.3	0.7	46.1	0.6	45.3	0.8	45.4	0.9
Globulin	27.4	0.7	26.4	0.9	27.9	1.6	27.1	1.0
Total bilirubin	19.6	3.1	20.0	2.4	19.4	2.2	15.6	1.3
Alk phosphatase	84.4	6.0	84.9	6.2	84.9	8.9	77.9	10.0
AST	40.4	8.3	28.6	1.7	43.3	5.7	29.4	3.9
Hb	15.8	0.3	16.3	0.3	15.1	0.3	15.4	0.2
Platelets	238.5	9.6	251.1	12.8	240.9	14.3	225.9	17.5
RBCs	5.06	0.12	5.29	0.11	4.87	0.07	4.96	0.07
PCV	0.46	0.01	0.47	0.01	0.44	0.01	0.46	0.01
MCV	91.0	1.4	89.7	0.8	90.9	0.9	91.4	0.9
MCH	31.3	0.4	30.8	0.3	31.0	0.3	31.1	0.3
MCHC	34.3	0.2	34.4	0.2	34.1	0.2	34.0	0.1
WBC	7.39	0.73	7.27	0.36	6.54	0.64	6.24	0.33
Neutrophils	4.56	0.70	4.16	0.38	3.59	0.60	3.49	0.36
Lymphocytes	1.84	0.18	2.19	0.18	1.97	0.22	1.89	0.10
Monocytes	0.63	0.07	0.60	0.04	0.57	0.07	0.54	0.04
Eosinophils	0.31	0.09	0.27	0.08	0.39	0.11	0.33	0.10
Basinophils	0.04	0.02	0.04	0.02	0.04	0.02	0.03	0.02
CS troponin	<0.15		<0.15		<0.15		<0.15	

5.56 ± 0.26 and $7.01 \pm 1.16\,\text{mmol} \cdot \text{kg}^{-1}$ dm, respectively, almost certainly contained a contribution from this other source. The apparent mean for IV was $2.95 \pm 0.39\,\text{mmol} \cdot \text{kg}^{-1}$ dm. There was no change in the apparent mean after the four weeks, the treatment group means being: I: 5.12 ± 0.52, II: 5.52 ± 0.39, III: 5.38 ± 0.62 and IV: $2.92 \pm 0.28\,\text{mmol} \cdot \text{kg}^{-1}$ dm.

The mean carnosine content of all subjects prior to treatment was $22.69 \pm 1.11\,\text{mmol} \cdot \text{kg}^{-1}$ dm and the mean change over the 4 weeks in the four groups was: I: $+7.80 \pm 0.36$ ($P < 0.05$), II: $+11.04 \pm 2.68$ ($P < 0.05$), III: $+16.37 \pm 3.03$ ($P < 0.05$), IV: $+1.87 \pm 1.73$ ($P > 0.05$) $\text{mmol} \cdot \text{kg}^{-1}$ dm (Table 3). Whilst these results would seem to indicate a greater increase in the subjects supplemented with

Table 3. Muscle carnosine and taurine contents ($\text{mmol} \cdot \text{kg}^{-1}$ dm), and the ratio of carnosine to taurine (C/T) before and after 4 weeks supplementation in groups I to IV

Treatment		Carnosine		Taurine		C/T	
		Pre	4 weeks	Pre	4 weeks	Pre	4 weeks
I	Mean	19.58	27.38	36.52	33.70	0.551	0.836
	SE	1.66	1.33	3.47	3.57	0.065	0.057
II	Mean	24.23	35.27	28.68	27.54	0.878	1.372
	SE	2.36	2.76	2.36	3.92	0.130	0.191
III	Mean	23.15	39.52	35.40	32.32	0.706	1.493
	SE	2.27	7.58	3.99	6.79	0.130	0.453
IV	Mean	23.63	25.49	45.72	41.38	0.563	0.680
	SE	2.43	2.03	5.53	4.08	0.094	0.133

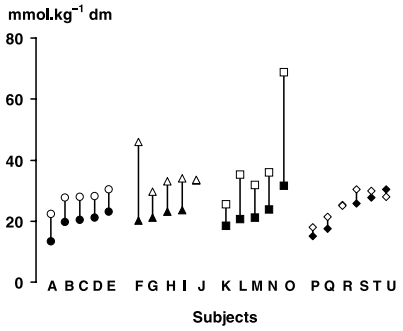

Fig. 8. Muscle carnosine before (◆) and at the end (△) of 4 weeks supplementation with β-alanine (I: subjects A–E; II: subjects F–J), with L-carnosine (III: subjects K–O) or placebo (IV: subjects P–U). Groups II and III treatments were isomolar with respect to β-alanine

carnosine, inspection of the individual data (Fig. 8) showed that subject J in group II, who began with the highest pre-supplementation muscle content of all subjects, failed to show any increase in their muscle carnosine content. The changes in the remaining subjects in this group were of the same order as the range in individual changes in III (mean of subjects F–I in group II: $13.72 \pm 3.40\,\text{mmol} \cdot \text{kg}^{-1}$ dm ($P < 0.05$)). The mean percent changes in the four groups (after exclusion of subject J from group II) were: I: +42.1%, II: +64.2%, III: +65.8%, IV: +9.9%. With the small numbers of subjects in the different groups there was no significant difference between the changes in groups I to III despite the trend towards a greater muscle carnosine content in the two higher dose groups (II and III). (As there is the possibility that the result obtained in subject J resulted from his failure to keep to the treatment schedule, further results for group II are presented with and without subject J.)

The mean taurine content of all subjects prior to treatment was $37.02 \pm 2.39\,\text{mmol} \cdot \text{kg}^{-1}$ dm and the mean change over the 4 weeks in the four groups was: I: -2.82 ± 3.56, II: $+0.86 \pm 2.09$ (-1.15 ± 2.70 without subject J), III: -3.08 ± 6.09, IV: $+4.35 \pm 3.41\,\text{mmol} \cdot \text{kg}^{-1}$ dm. There was no significant effect of treatment between groups or within treatment groups (see Table 3).

There were no significant changes in body mass over the 28 days, the mean changes in the four treatment groups being: I: -0.11 ± 0.24, II: -0.03 ± 0.80 (-0.66 ± 0.56 without subject J), III: 0.21 ± 0.61 and IV: -0.43 ± 0.40 kg.

Discussion

Carnosine in muscle is synthesised *in situ* (Bakardjiev and Bauer, 1994) by the action of CS from its two constituent amino acids, β-alanine and histidine. In contrast other cell types are equipped with a dipeptide transporter enabling carnosine to be taken up intact (Hoffmann et al., 1996; Dieck et al., 1999). Ingestion of meat containing carnosine and its methyl derivatives, reportedly lead to modest amounts of carnosine appearing in blood particularly when given in large amounts (Gardner et al., 1991; Park et al., 2005). However, in humans carnosine and related dipeptides are rapidly hydrolysed by the action of carnosinase. In the present study, carnosine remained below the limit of detection following ingestion of the chicken broth although traces of anserine were detected. To one of the subjects L-carnosine corresponding to $20\,\text{mg} \cdot \text{kg}^{-1}$ bwt of β-alanine was also given as a drink. This resulted in an increase in plasma β-alanine which was almost identical to that following administration of the same dose of β-alanine itself. Once again carnosine was not detected in plasma. In contrast herbivorous animals, such as the horse, lack a carnosinase in plasma and show a much greater and prolonged increase in carnosine when supplemented with the intact dipeptide (Dunnett, 1995; Dunnett et al., 2002). Carnosine is normally present in equine plasma from 10–13 μM in mature horses and 5–7 μM in foals and yearlings (Dunnett et al., 2002).

In previous studies on equines, administered repeated doses of $100\,\text{mg} \cdot \text{kg}^{-1}$ bwt of β-alanine (Dunnett and Harris, 1999) the AUC increased during the supplementation period of 30 days although the half-life of disappearance following the peak concentration remained essentially the same. This suggested an increase with time in the absorption of β-alanine from the GIT. However, this was in a species that would never normally encounter β-alanine or carnosine in the diet, in contrast to meat eating humans. No evidence was seen in study 2 with 15 days thrice-daily administration of any change in either β-alanine absorption, or the rate of disappearance following the peak concentration.

Transport of β-alanine into muscle cells has been shown in cultures of embryonic chick pectoral muscle to be sodium and chloride dependent and to show Michaelis-Menten kinetics with a Km of approximately 40 μM (Bakardjiev and Bauer, 1994). Prior to administration of β-alanine the plasma concentration was <0.5 μM in all subjects but increased to 50 to 100 μM within 30 min of ingestion of $10\,\text{mg} \cdot \text{kg}^{-1}$ bwt (Figs. 2 and 6). This, however, subsequently fell 60% within 30 min and 95% to an average of 12.8 μM within 90 min. Within this range transport of β-alanine will be concentration dependent fluctuating between 10 and 70% of Vmax. Nonetheless, $10\,\text{mg} \cdot \text{kg}^{-1}$ bwt must be close to the upper practical dose if symptoms of flushing are to be avoided. Consequently

in study 3 supplementation was provided 4 (group I) or 8 (groups II and III) times per day in order to provide a more even concentration profile in plasma with a maximum concentration of 125 to 250% of the assumed Km of the transporter.

Increases in β-alanine in plasma were associated with increases in the plasma taurine concentration. β-Alanine shares the same transporter as taurine, a β-sulfonic amino acid, and acts as an antagonist of taurine uptake into tissues. Three to 4 weeks administration of β-alanine (3% in the drinking water) has been shown to halve the taurine content of myocardium and skeletal muscle of rats (Allo et al., 1997; Harada et al., 1988; Mozaffari et al., 1986; Dawson et al., 2002) whilst 22 mmol · kg^{-1} bwt administered twice per day for 5 days decreased the taurine concentration in both muscle and brain of chickens (Tomonaga et al., 2005). However, the amounts of β-alanine given in these studies are typically more than 100 times higher than the doses used in the present investigation, which were based on amounts obtained from the diet. Thus 22 mmol · kg^{-1} bwt would correspond to 156,000 mg in an 80 kg individual whereas the highest single dose used in the investigation of 4 weeks supplementation (study 3) was 800 mg β-alanine. This corresponds to approximately 10 mg · kg^{-1} bwt (0.112 mmol · kg^{-1} bwt), and is the amount in dipeptide form in 100 g of whale beef or 150 g turkey breast meat (Abe, 2000), and 100 g north-Atlantic sea-prawns (unpublished observations). Despite the increase in plasma taurine following 10–40 mg kg^{-1} bwt β-alanine, no significant loss of taurine was detected in urine. Four weeks of β-alanine supplementation in the free form, or as L-carnosine, did not result in any change in the muscle content. However, this does not preclude the possibility of selective taurine loss in one or other muscle fibre type, given that type I muscle fibres contain higher concentrations than type II (Harris et al., 1998).

Administration of β-alanine above 10 mg · kg^{-1} bwt was associated with symptoms of flushing, beginning within 20 min. The incidence and severity of the symptoms appeared dose dependent. Symptoms of flushing have also been recorded, in our laboratory, where 3000 mg L-carnosine, corresponding to approximately 15 mg · kg^{-1} bwt β-alanine, have been given. However, it was notable that the chicken broth which resulted in a maximum concentration of β-alanine in plasma close to that given by 20 mg · kg^{-1} bwt of the free amino acid (where significant symptoms were recorded in 3 of 4 subjects), did not give rise to symptoms in any of the subjects. At 40 mg · kg^{-1} bwt symptoms were sufficiently uncomfortable that the treatment was refused by two of the volunteers. This together with the potential loss of taurine with high β-alanine (and L-carnosine) concentrations emphasises the need to keep individual doses and plasma concentrations of β-alanine close to those regularly obtained through the normal diet.

L-Carnosine supplied to cultures of skeletal muscle cells must first be hydrolysed to its constituent amino acids, histidine and β-alanine, before these can be used for carnosine synthesis *in situ* (Bakardjiev and Bauer, 1994). Based on the affinity of CS for its two substrates – the Km for β-alanine is 1.0–2.3 mM (Ng and Marshall 1978; Skaper et al., 1973), and the Km for histidine is 16.8 μM (Horinishi et al., 1978) – compared with the relative abundance of histidine in muscle and plasma and the very much lower concentrations of β-alanine, it is probable that muscle carnosine synthesis is limited only by the intracellular availability of β-alanine. Where β-alanine is in abundance then synthesis may be further limited by the activity of CS itself. In study 3 there was no evidence of any decrease in the muscle concentration of histidine even with the highest β-alanine dose used (group II), although as noted chromatograms for this amino acid were not fully resolved. Supplementation with an isomolar dose of L-carnosine, where there is the possibility of some of the dipeptide being absorbed into the blood stream intact and where hydrolysis in plasma will provide histidine isomolar with β-alanine, was no more effective in increasing the muscle carnosine concentration during 4 weeks. This, together with the absence of any change in the muscle histidine concentration, further confirms the limiting role of β-alanine.

The greatest increases in muscle carnosine occurred in subjects F (treatment II) and O (treatment III isomolar with II). In the case of subject F it is known that he was a cyclist and in active training at the time of the study, this was not the case with subject O. However, it is clear that training alone can increase the muscle carnosine content (Kim et al., 2005; Suzuki et al., 2004) and it is possible that this may have contributed to the greater increases in subject F.

The specific contribution of carnosine to muscle buffering between the pH limits of 7.1 (rest) and 6.5 (assumed post-exercise) (β carn) was estimated from the Henderson-Hasselbach equation (assuming a pK_a of 6.83 for carnosine) to be 7.5 mmol $H^+ \cdot kg^{-1}$ dm prior to supplementation, and 10.7, 12.4 and 12.5 mmol $H^+ \cdot kg^{-1}$ dm following 4 weeks supplementation in I, II and III (Table 4). Assuming an estimate of non-carnosine buffering capacity (β non-carn) between pH 6.5 and 7.1 of 74 mmol $H^+ \cdot kg^{-1}$ dm (Harris et al., 1990; Sewell et al., 1992),

Table 4. Estimates of the quantitative importance of carnosine before and after supplementation to intracellular physicochemical buffering capacity (β) between pH's 7.1 and 6.5

		Pre	4 weeks		
			I	II	III
Mixed muscle	carnosine [a]	22.7	32.2	37.3	37.6
	β carn [b]	7.5	10.7	12.4	12.5
	β non-carn [b]	74	74	74	74
	Total β [b]	81.5	84.7	86.4	86.5
	β carn as % of total β	9.2	12.6%	14.3%	14.4%
	% change in total β		3.7%	5.6%	5.7%
Type I	carnosine [a]	15.1	21.5	24.8	25.1
	β carn [b]	5.0	7.1	8.2	8.3
	β non-carn [b]	74	74	74	74
	Total β [b]	79.0	81.1	82.2	82.3
	β carn as % of total β	6.4	8.8%	10.0%	10.1%
	% change in total β		2.6%	3.9%	4.0%
Type II	carnosine [a]	30.3	43.0	49.7	50.2
	β carn [b]	10.0	14.3	16.5	16.6
	β non-carn [b]	74	74	74	74
	Total β [b]	84.0	88.3	90.5	90.6
	β carn as % of total β	11.9	16.2%	18.2%	18.4%
	% change in total β		4.8%	7.1%	7.3%

[a] $\text{mmol} \cdot \text{kg}^{-1}$ dm
[b] $\text{mmol H}^{+} \cdot \text{kg}^{-1}$ dm
The model assumes at its starting point a muscle carnosine content of 22.7 $\text{mmol} \cdot \text{kg}^{-1}$ dm (the mean of all the subjects in the present study) and percentage increases of this after 4 weeks supplementation, i.e. 42.1, 64.2 and 65.8% in groups I, II and III. The specific contribution of carnosine (β carn) was calculated from the Henderson Hasselbach equation. The estimated contribution of 74 $\text{mmol H}^{+} \cdot \text{kg}^{-1}$ 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 (pKa's 6.1 to 6.8). This estimate does not include any contribution from bicarbonate. Total β is the sum of β carn and β non-carn. In addition an estimate has been made of the probable carnosine contents in type I and II muscle fibres assuming a muscle with 50% type I and 50% type II fibres, and a 1:2 ratio of carnosine between types I and II fibres (Harris et al., 1998, 2005)

carnosine would account in this case for 9.2%, and, 12.6, 14.3 and 14.3% of total muscle buffering capacity before and after 4 weeks supplementation in I–III. The estimate of 74 $\text{mmol H}^{+} \cdot \text{kg}^{-1}$ dm was derived from titration of homogenates of freeze-dried muscle resulting in the total breakdown of phosphorylcreatine (a weak buffer with a pKa of 4.58) with formation of hexosemonophosphates and inorganic phosphate (stronger phosphate buffers with pKa's of 6.1 to 6.82) (Harris et al., 1989). Although the estimate of 74 $\text{mmol H}^{+} \cdot \text{kg}^{-1}$ dm does not include buffering from bicarbonate it is, nonetheless, almost certainly an overestimate of β non-carn in muscle. Consequently the estimates given in Table 4 are most probably an underestimate of the true contribution from carnosine.

As carnosine is preferentially distributed into type II fibres both before (Harris et al., 1998) and following supplementation (Harris et al., 2005) an estimate has also been made of its probable content in different fibre types, and the contribution of carnosine in these to intracellular buffering. Following supplementation in II and III we estimate that carnosine contributes minimally 10 and 18% to total physico-chemical buffering in types I and II fibres, compared to 6.4 and 11.9% before supplementation. However, after allowing for the imprecision of the homogenate method in determining β non-carn, the true contribution of carnosine to buffering in all cases is likely to be much higher. The estimated changes in physicochemical buffering appear relatively modest ranging from +3.7% in A to +5.7% in III but may well have been nearer to +5% and +7.5% specifically in type II fibres.

In the case of subjects F and O, with the largest increases in muscle carnosine, the estimated changes in buffering capacity were much greater. In these two subjects the increase at the whole muscle level amounted to +9.6% (subject F) and +12.8% (subject O) after 4 weeks

of supplementation. In these two cases the calculated increase in carnosine in different fibre types would account for 17.1% and 23.6% of the total physicochemical buffering capacity, respectively and specifically in type II fibres nearer to 21.5 and 29.0%. Once again, however, these estimates are probably underestimates of the actual contribution made by carnosine.

Intense exercise results in the accumulation of lactate and H^+ in muscle, with decreases in pH from 7.1 at rest to 6.5 or lower at fatigue. It has long been assumed that pH plays a role in fatigue. However, to date no means, apart from increasing with creatine supplementation the phosphate accumulated in muscle as phosphorylcreatine (Harris et al., 1992), has been available in humans to increase intracellular buffering capacity allowing this to be tested. But in the case of phosphorylcreatine elevation this has other effects apart from its potential to increase physicochemical buffering, notably in maintaining adenine nucleotide homeostasis. The increases in muscle carnosine reported here are of an order as to permit such studies to be undertaken to test the importance of intracellular acid-base regulation as a limiting factor to performance with different exercise modalities.

Acknowledgement

The authors acknowledge the long term support provided by Natural Alternatives International, San Marcos, CA, U.S.A.

References

Abe H (2000) Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. Biochemistry (Mosc) 65: 757–765

Asatoor AM, Bardon JK, Lant AF, Milne MD, Navab F (1970) Intestinal absorption of carnosine and its constituent amino acids in man. Gut 11: 250–254

Bakardjiev A, Bauer K (1994) Transport of β-alanine and biosynthesis of carnosine by skeletal muscle cells in primary culture. Eur J Biochem 225: 617–623

Bate-Smith EC (1938) The buffering of muscle in rigor: protein, phosphate and carnosine. J Physiol 92: 336–343

Batrukova MA, Rubtsov AM (1997) Histidine-containing dipeptides as endogenous regulators of the activity of sarcoplasmic reticulum Ca-release channels. Biochem Biophys Acta 1324: 142–150

Bergström J (1962) Muscle electrolytes in man. Determined by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients and patients with chronic diarrhoea. Scand J Clin Lab Invest 14: 1–110

Boldyrev AA, Koldobski A, Kurella E, Maltseva V, Stvolinski S (1993) Natural histidine-containing dipeptide carnosine as a potent hydrophilic antioxidant with membrane stabilizing function. A biomedical aspect. Mol Chem Neuropathol 19: 185–192

Chan WK, Decker EA, Chow CK, Boissonneault GA (1994) Effect of dietary carnosine on plasma and tissue antioxidant concentrations and on lipid oxidation in rat skeletal muscle. Lipids 29: 461–466

Dawson R Jr, Biasetti M, Messina S, Dominy J (2002) The cytoprotective role of taurine in exercise-induced muscle injury. Amino Acids 22: 309–324

Dieck ST, Heuer H, Ehrchen J, Otto C, Bauer K (1999) The peptide transporter PepT2 is expressed in rat brain and mediates the accumulation of the fluorescent dipeptide derivative beta-Ala-Lys-Nepsilon-AMCA in astrocytes. Glia 25: 10–20

Dunnett M (1995) Carnosine metabolism and function in the thoroughbred horse. PhD thesis, Open University

Dunnett M, Harris RC (1995) Carnosine and taurine contents of different fibre types in the middle gluteal muscle of the Thoroughbred horse. Equine Vet J [Suppl] 18: 214–217

Dunnett M, Harris RC (1997) High-performance liquid chromatographic determination of imidazole dipeptides, histidine, 1-methylhistidine and 3-methylhistidine in equine and camel muscle and individual muscle fibres. J Chromatogr B 688: 47–55

Dunnett M, Harris RC, Soliman MZ, Suwar AAS (1997) Carnosine, anserine and taurine contents in individual fibres from the middle gluteal muscle of the camel. Res Vet Sci 62: 213–216

Dunnett M, Harris RC (1999) Influence of oral β-alanine and L-histidine supplementation on the carnosine content of the gluteus medius. Equine Vet J [Suppl] 30: 499–504

Dunnett M, Harris RC, Dunnett CE, Harris PA (2002) Plasma carnosine concentration: diurnal variation and effects of age, exercise and muscle damage. Equine Vet J [Suppl] 34: 283–287

Gardner MLG, Illingworth KM, Kelleher J, Wood D (1991) Intestinal absorption of the intact peptide carnosine in man, and comparison with intestinal permeability to lactulose. J Physiol 439: 411–422

Hama T, Tamaki N, Miyamoto F, Kita M, Tsunemori F (1976) Intestinal absorption of β-alanine, anserine and carnosine in rats. J Nutr Sci Vitaminol 22: 147–157

Harada H, Allo SN, Viyuoh J, Takahashi AK, Schaffer SW (1988) Regulation of calcium transport in drug-induced taurine-depleted hearts. Biochim Biophys Acta 944: 273–278

Harris RC, Kim HJ, Harris DB, Hill CA, Kim CK, Boobis L, Sale C, Wise JA (2005) The distribution of carnosine and taurine in different muscle fibre types from human v lateralis and the effects of beta-alanine supplementation. Amino Acids 29: 13

Harris RC, Dunnett M, Greenhaff PL (1998) Carnosine and taurine contents in individual fibres in human vastus lateralis muscle. J Sports Science 16: 639–643

Harris RC, Katz A, Sahlin K, Snow DH (1989) Effect of freeze drying on measurements of pH in biopsy samples of the middle gluteal muscle of the horse: Comparison of muscle pH to the pyruvate and lactate content. Equine Vet J 21: 45–47

Harris RC, Marlin DJ, Dunnett M, Snow DH, Hultman E (1990) Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. Comp Biochem Physiol 97A: 249–251

Harris RC, Söderlund K, Hultman E (1992) Elevation of creatine in resting and exercised muscle in normal subjects by creatine supplementation. Clin Sci 83: 367–374

Hipkiss AR (2000) Carnosine and protein carbonyl groups: a possible relationship. Biochemistry (Mosc) 65: 771–778

Hipkiss AR, Michaelis J, Syrris P (1995) Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent. FEBS Lett 371: 81–85

Hoffmann AM, Bakardjiev A, Bauer K (1996) Carnosine-synthesis in cultures of rat glial cells is restricted to oligodendrocytes and carnosine uptake to astrocytes. Neurosci Lett 215: 29–32

Horinishi H, Grillo M, Margolis FL (1978) Purification and characterization of carnosine synthetase from mouse olfactory bulbs. J Neurochem 31: 909–919

Kim HJ, Cho J, Kim CK, Harris RC, Harris DB, Sale C, Wise JA (2005) Effect on muscle fibre morphology and carnosine content after 12 days

training of Korean speed skaters. Med Sci Sports Exerc 37 [Suppl 5]: S192

Lamont C, Miller DJ (1992) Calcium sensitizing action of carnosine and other endogenous imidazoles in chemically skinned striated muscle. J Physiol 454: 421–434

Mannion AF, Jakeman PM, Dunnett M, Harris RC, Willan PLT (1992) Carnosine and anserine concentrations in the quadriceps femoris muscle of healthy humans. Eur J Appl Physiol 64: 47–50

Matthews MM, Traut TW (1987) Regulation of N-carbamoyl-beta-alanine amidohydrolase, the terminal enzyme in pyrimidine catabolism, by ligand-induced change in polymerization. J Biol Chem 262: 7232–7237

Maynard LM, Boissonneault GA, Chow CK, Bruckner CG (2001) High level of dietary carnosine are associated with increased concentrations of carnosine and histidine in rat soleus muscle. J Nutr 131: 287–290

Ng RH, Marshall FD (1978) Regional and subcellular distribution of homocarnosine-carnosine synthetase in the central nervous system of rats. J Neurochem 30: 187–190

Park YJ, Volpe SL, Decker EA (2005) Quantitation of carnosine in humans plasma after dietary consumption of beef. J Agric Food Chem 53: 4736–4739

Parkhouse WS, McKenzie DC, Hochachka PW, Ovalle WK (1985) Buffering capacity of deproteinized human vastus lateralis muscle. J Appl Physiol 58: 14–17

Perry TL, Hansen S, Tischler B, Bunting R, Berry K (1967) Carnosinemia: a new metabolic disorder associated with neurologic disease and mental defect. N Engl J Med 277: 1219–1226

Sewell DA, Harris RC, Marlin DJ, Dunnett M (1992) Estimation of the carnosine content of different fibre types in the middle gluteal muscle of the thoroughbred horse. J Physiol 455: 447–453

Skaper SD, Das S, Marshall FD (1973) Some properties of a homocarnosine-carnosine synthetase isolated from rat brain. J Neurochem 21: 1429–1445

Suzuki Y, Ito O, Mukai N, Takahashi H, Takamatsu K (2002) High level of skeletal muscle carnosine contributes to the latter half of exercise performance during 30-s maximal cycle ergometer sprinting. Jpn J Physiol 52: 199–205

Suzuki Y, Ito O, Takahashi H, Takamatsu K (2004) The effect of sprint training on skeletal muscle carnosine in humans. Int J Sport Health Sci 2: 105–110

Tallon MJ, Harris RC, Boobis L, Fallowfield J, Wise JA (2005) The carnosine content of vastus lateralis is elevated in resistance trained bodybuilders. J Strength Condit Res 19: 725–729

Tamaki N, Funatsuka A, Fujimoto S, Hama T (1984) The utilization of carnosine in rats fed on a histidine-free diet and its effect on the levels of tissue histidine and carnosine. J Nutr Sci Vitaminol 30: 545–551

Tamaki N, Funatsuka A, Wakabayashi M, Hama T (1977) Effects of histidine-free and -excess diets on anserine and carnosine contents in rat gastrocnemius muscle. J Nutr Sci Vitaminol 23: 331–340

Tomonaga S, Kaji Y, Tachibana T, Denbow D, Furuse M (2005) Oral administration of β-alanine modifies carnosine concentrations in the muscles and brains of chickens. Anim Sci J 76: 249–254

Authors' address: Professor R. C. Harris, School of Sports, Exercise and Health Sciences, University College Chichester, Chichester, West Sussex, P019 6PE, U.K.,

Fax: +44(0)-1243-816080, E-mail: r.harris@ucc.ac.uk