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REVIEW ARTICLE

Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance

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Abstract High-intensity exercise results in reduced substrate levels and accumulation of metabolites in the skeletal muscle. The accumulation of these metabolites (e.g. ADP, Pi and H^+) can have deleterious effects on skeletal muscle function and force generation, thus contributing to fatigue. Clearly this is a challenge to sport and exercise performance and, as such, any intervention capable of reducing the negative impact of these metabolites would be of use. Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide found in high concentrations in the skeletal muscle of both vertebrates and non-vertebrates and is formed by bonding histidine and β-alanine in a reaction catalysed by carnosine synthase. Due to the pKa of its imidazole ring (6.83) and its location within skeletal muscle, carnosine has a key role to play in intracellular pH buffering over the physiological pH range, although other physiological roles for carnosine have also been suggested. The concentration of histidine in muscle and plasma is high relative to its K_m with muscle carnosine synthase, whereas β-alanine exists in low concentration in muscle and has a higher K_m with muscle carnosine synthase, which indicates that it is the availability of β-alanine that is limiting to the synthesis of carnosine in skeletal muscle. Thus, the elevation of muscle carnosine concentrations through the dietary intake of carnosine, or chemically related dipeptides that release β-alanine on absorption, or supplementation with β-alanine directly could provide a method of increasing intracellular buffering capacity during exercise, which could provide a means of increasing high-intensity exercise capacity and performance. This paper reviews the available evidence relating to the effects of β-alanine supplementation on muscle carnosine synthesis and the subsequent effects on exercise performance. In addition, the effects of training, with or without β-alanine supplementation, on muscle carnosine concentrations are also reviewed.

Keywords Beta-alanine · Carnosine · Exercise performance and capacity · Muscle buffering

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Introduction

Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide found in high concentrations in the skeletal muscle of both vertebrates and non-vertebrates, as well as in the central nervous system. It was first isolated by Gulewitsch and Amiradzhibi (1900) and was subsequently classified as a histidine containing dipeptide by Krimberg (1906, 1908), who demonstrated the hydrolysis of carnosine to its constituent amino acids (β-alanine and histidine). It is now known that carnosine is formed, mainly in muscle and brain tissue, by bonding histidine and β-alanine in a reaction catalysed by carnosine synthase.

Initial studies examining a potential physiological role for carnosine demonstrated that it had a side chain pKa of 6.83 making it a suitable buffer over the physiological pH range (Bate-Smith 1938), although other physiological roles have also been ascribed to carnosine (see below). Tanokura et al. (1976) used nuclear magnetic resonance to show that carnosine has pKa's of 2.77 (carboxyl group),

9.66 (amino group) and 6.83 (imidazole ring), supporting the earlier work of Bate-Smith (1938).

In contrast to other cellular buffers (e.g. phosphate and bicarbonate), the concentration of carnosine exhibits large variations between muscles from different species, and even between different muscle fibre types within the same muscle (Davey 1960). In humans, mean concentrations are 17.5 ± 4.8 mmol kg^{-1} dm in females and 21.3 ± 4.2 mmol kg^{-1} dm in males (Mannion et al. 1992), with the highest concentrations identified in fast-twitch fibres (Harris et al. 1998). Sewell et al. (1992) also reported that carnosine concentrations were higher in the fast-twitch muscle fibres of equine, with Dunnett and Harris (1997) confirming these observations in both equine and camel single fibres. The species having the highest skeletal muscle histidine containing dipeptide concentrations are those whose muscles are exposed to frequent bouts of hypoxia, such as diving whales, or those who depend upon anaerobic exercise for survival, such as hunting or escaping animals (Abe 2000). High histidine containing dipeptide contents have been shown in several species involved in athletic competition, such as horses, greyhounds, camels and, indeed, humans (Harris et al. 1990; Dunnett and Harris 1997).

Taken together, these findings suggest that pH buffering is a key physiological function of histidine containing dipeptides in skeletal muscle and that increasing muscle concentrations of carnosine would increase the buffering capacity of muscle, thus contributing to the regulation of intracellular pH. Parkhouse et al. (1985) showed that sprinters and other athletes performing high-intensity exercise possessed higher muscle buffering capabilities and skeletal muscle carnosine levels than marathon runners and untrained subjects. As such, the elevation of muscle carnosine concentrations could provide a method of increasing intracellular buffering capacity during exercise, thus potentially providing a means of increasing high-intensity exercise capacity and performance. This paper reviews the available evidence relating to the effect of dietary supplementation with β-alanine on muscle carnosine concentrations and exercise performance. In addition, studies investigating the effects of training on muscle carnosine concentrations, with or without β-alanine supplementation, are reviewed.

The physiological roles of carnosine

One undisputable physiological role for carnosine is that of pH buffering, since this is determined by its molecular structure and is simply a case of carnosine following the fundamental laws of chemistry. As highlighted above, the distribution of carnosine in skeletal muscle across species and between different muscle fibre types in the same species suggests that evolution has made best use of carnosine as an effective H^+ buffer. There was some early experimental support for this assertion as Severin et al. (1953) demonstrated that, when in the presence of carnosine, electrically stimulated frog sartorius muscle was more resistant to fatigue. They showed that the accumulation of high concentrations of lactate in the muscle could occur without deterring muscle action, provided carnosine was present. In the absence of carnosine, lactate appeared to result in acidification of the muscle tissue, resulting in fatigue of the isolated muscle.

However, the clear role for carnosine in pH buffering does not exclude other physiological roles. Other physiological roles have been ascribed to carnosine in muscle; including the protection of proteins against glycation by acting as a sacrificial peptide (Hipkiss et al. 1993, 1995), prevention of the formation of protein-protein cross-links through reactions with protein-carbonyl groups (Hipkiss et al. 1993, 2001), acting as an anti-oxidant (for reviews see Boldyrev et al. 1987; Boldyrev 1993) and increasing calcium sensitivity in muscle fibres augmenting force production and total work done (Lamont and Miller 1992; Batrukova and Rubtsov 1997; Dutka and Lamb 2004; for a review see Rubtsov 2001).

However, few of the ascribed physiological roles for carnosine, other than its role as a pH buffer, have been shown in humans. Indeed, the majority of the work cited above has been conducted in vitro. Hipkiss et al. (2001) examined the possible anti-glycating effect of carnosine in vivo, reporting a trend towards suppression of the diabetes associated rise in blood pressure in fructose fed rats. The decreased blood pressure response was attributed to reduced formation of protein–protein cross-links, resulting in enhanced elasticity of blood vessels.

If occurring in vivo in human muscle, the effects of carnosine on Ca^{2+} ion sensitivity could lead to an enhancement of force production and a reduction in fatigue. This might suggest a role for carnosine, in addition to pH buffering, which could be important for exercise capacity and performance. Lamont and Miller (1992) showed that the presence of carnosine reduced the amount of Ca^{2+} ions required to produce half-maximum tension in chemically skinned cardiac and skeletal muscle. They also reported an increase in maximal force production by different muscle types and suggested that the higher concentrations of carnosine shown in fast-twitch fibres might relate to an effect of enhanced Ca^{2+} ion sensitivity on muscle contractility in muscle fibres that are capable of producing greater force. Dutka and Lamb (2004) examined the effect of carnosine on processes affecting excitation–contraction coupling in skeletal muscle fibres. They showed an increased Ca^{2+} ion sensitivity of the contractile apparatus, in a concentration-dependent manner,

with the addition of carnosine to the cytoplasmic environment. In addition, the increased force produced by caffeine induced Ca^{2+} ion release from the sarcoplasmic reticulum was also potentiated in the presence of carnosine. The authors concluded that carnosine potentiates force production as a result of sensitising the contractile apparatus to Ca^{2+} ions, without causing additional release from the sarcoplasmic reticulum.

The current evidence base for increased Ca^{2+} ion sensitivity in muscle fibres is restricted to in vitro work, although it would be of interest to investigate any possible effect in vivo. If increased muscle carnosine concentrations were to result in the enhancement of exercise capacity over a range of exercise intensities, as opposed to those only limited by reduced pH in the muscle, then a potential mechanism for this effect might be through increased Ca^{2+} ion sensitivity.

In addition to its high concentration in muscle, carnosine is also present in other tissues, including lenticular tissue and nerve tissue (including in the brain). Carnosine concentrations are especially high in the olfactory lobe (Sassoe-Pognetto et al. 1993; Bakardjiev 1997; Bonfanti et al. 1999). The high concentrations of carnosine in these tissues is not consistent with a primary role as a pH buffer and possible functions in these tissues might include, as in muscle, the protection of proteins against glycation by acting as a sacrificial peptide, prevention of the formation of protein–protein cross-links through reactions with protein-carbonyl groups and acting as an anti-oxidant. Others have suggested that the presence of carnosine in neural tissue might be to act as a histidine reservoir for histamine synthesis (Otani et al. 2008). Further possible functions in these tissues include a role as a chelator of Ca^{2+}, Zn^{2+}, Cu^{2+} and Fe^{2+} ions or as a neurotransmitter.

β-Alanine supplementation and muscle carnosine concentrations

In human blood, carnosine is rapidly hydrolysed to its constituent amino acids due to the presence of carnosinase, a specific hydrolysing enzyme (Asatoor et al. 1970; Perry et al. 1967). Following this, β-alanine and histidine can then be transported to other organs and tissues. Whilst the carnosinase enzyme has been identified in several tissues including the blood, liver and kidney; critically it is not found in skeletal muscle (Lenney et al. 1985). The transport of β-alanine into muscle is mediated by a specific β-amino acid transport protein that is dependent upon stoichiometric concentrations of both Na^+ and Cl^- in a 2:1:1 (Na^+:Cl^-:β-amino acid) ratio (Miyamoto et al. 1990; Ramamoorthy et al. 1994) and has a K_m of ~40 μM (Bakardjiev and Bauer 1994). It is apparent that the resynthesis of carnosine in muscle tissue is limited by the very low concentration of β-alanine and the high K_m (1.0–2.3 mM) that β-alanine has for carnosine synthase (Ng and Marshall 1978; Skaper et al. 1973), which causes its rapid conversion to carnosine. By comparison, histidine is shown in much higher concentrations in muscle and has a much lower K_m of 16.8 μM for carnosine synthase (Horinishi et al. 1978). Excesses in both β-alanine (Harris et al. 2006a) and carnosine (Perry et al. 1967) are excreted in urine.

In humans it would therefore seem sensible to suggest that the rate of carnosine synthesis in skeletal muscle is limited by the availability of β-alanine from the diet. However, this might not be the case in other animals, since early studies on rats have focused mainly on the administration of carnosine (Chan et al. 1994; Hama et al. 1976; Maynard et al. 2001) or histidine (Tamaki et al. 1977, 1985). Dunnett and Harris (1999) performed one of the earliest studies examining the effect of β-alanine supplementation on muscle carnosine contents. They observed a significant increase in the carnosine content of horse mid-gluteal muscle following 30 days supplementation with both β-alanine and histidine.

In the first studies to examine the influence of β-alanine supplementation on carnosine concentrations in human skeletal muscle, Harris et al. (2006a) reported upon the outcome of three studies examining dietary supplementation with β-alanine and the effects on concentrations of β-alanine, taurine and carnosine in plasma, urine and skeletal muscle (Harris et al. 2006a). The intention of the first of these studies was to compare the ingestion of β-alanine in free form with an equivalent dose (40 mg kg^{-1} BM) contained within food (in this case a chicken broth). However, upon administration of this dose of free β-alanine, several subjects began to complain of symptoms of flushing. These symptoms of flushing (also termed paraesthesia) were described as an unpleasant prickly sensation on the skin around the body that lasted for ~60 min post-ingestion. It became apparent that these symptoms were dose dependent and were uncomfortable enough in some subjects to cause them to stop taking the supplement. Interestingly, no subjects complained of these symptoms when ingesting 40 mg kg^{-1} BM β-alanine in the chicken broth.

Several possible mechanisms exist for the parasthesia, including β-alanine activated strychnine-sensitive glycine receptor sites, associated with glutamate sensitive *N*-methyl-D-aspartate receptors in the brain and central nervous system (Mori et al. 2002; Tokutomi et al. 1989; Wang et al. 2003) and the mas-related gene family of G protein coupled receptors, which are triggered by interactions with specific ligands, such as β-alanine (Crozier et al. 2007).

As a result of these initial findings, two further doses of β-alanine were used, with some subjects still reporting symptoms of parasthesia after taking the 20 mg kg^{-1} BM

dose. In addition, some subjects also reported mild symptoms following the ingestion of 10 mg kg^{-1} BM (Harris et al. 2006a). The times to peak concentration with the ingestion of 10, 20 and 40 mg kg^{-1} BM were between 30 and 40 min, with the half-life of disappearance being ~25 min for each dose. As a result, Harris et al. (2006a) conducted a second study to examine the effects of 2 weeks of supplementation with β-alanine at 10 mg kg^{-1} BM, taken three times per day. The peak elevation in plasma β-alanine concentrations and the time to peak concentration were the same as in the first study for the corresponding dose. Neither was affected over the 2 weeks of supplementation.

Following these investigations Harris et al. (2006a) conducted a third study to examine the effects of 4 weeks of supplementation with either β-alanine or carnosine on muscle carnosine concentrations. In this study, 21 male subjects were split into four groups: a low-dose β-alanine group (5 subjects ingested 800 mg of β-alanine four times per day, an average daily dose of 3.2 g), a high-dose β-alanine group (5 subjects ingested a total of 145.6 g β-alanine, with subjects ingesting a total of 4.0 g day^{-1} in week 1, increasing to 6.4 g day^{-1} in week 4), a carnosine supplemented group (5 subjects ingested a total of 364 g of L-carnosine, equating to 143.3 g of β-alanine) and a placebo group (6 subjects ingested placebo capsules in the same dosing strategy as employed in the high-dose β-alanine group).

The placebo group showed an increase in muscle carnosine concentrations of 1.87 ± 1.73 mmol kg^{-1} dm (~10%). Increases in muscle carnosine were shown in all groups ingesting β-alanine, with the increases in the low-dose β-alanine group being 7.80 ± 0.36 mmol kg^{-1} dm, which compares with an increase of 11.04 ± 2.68 mmol kg^{-1} dm in the high-dose β-alanine group and an increase of 16.37 ± 3.03 mmol kg^{-1} dm in the L-carnosine group (Fig. 1).

Thus, the L-carnosine group showed a greater increase in muscle carnosine concentrations than the high- and low-dose β-alanine groups. However, one subject in the high-dose β-alanine group showed no increase in muscle carnosine concentrations with supplementation (subject J in Fig. 1). Interestingly, this subject had the highest pre-exercise muscle carnosine concentration, which was as high as some of the other subjects post-supplementation carnosine levels. This subject is the only subject from the studies that we have conducted thus far who has shown no increase in muscle carnosine concentrations with supplementation. In contrast, there are reports suggesting that around 30% of subjects do not respond to creatine supplementation (Syrotuik and Bell 2004), which does not compare favourably to the response of muscle carnosine to β-alanine supplementation. It is possible that this subject did not adhere to the supplementation regimen or that there

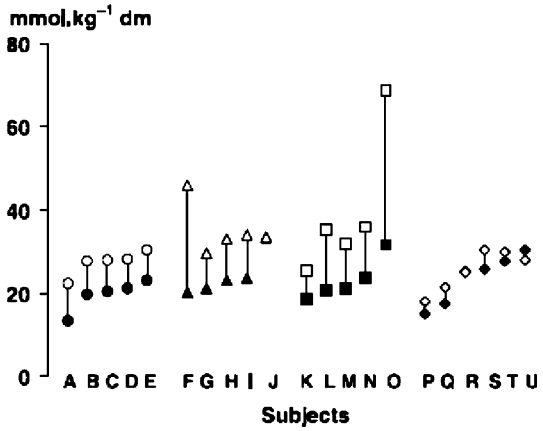

Fig. 1 Muscle carnosine before (*filled symbols*) and at the end (*open symbols*) of 4 weeks supplementation with β-alanine (group 1, *subjects A–E*; group 2, *subjects F–J*), with L-carnosine (group 3, *subjects K–O*) or placebo (group 4, *subjects P–U*). Groups 2 and 3 treatments were isomolar with respect to β-alanine (reproduced from Harris et al. 2006a with permission)

was an error in the analysis of the pre-exercise muscle sample, which may explain the non-response. With the exclusion of this subject, based upon the assertion that there was some error in analysis or subject adherence to the protocol, the percentage increases in the high-dose β-alanine group and the L-carnosine group were of a similar magnitude (64.2 and 65.8%).

Subsequently, Hill et al. (2007) investigated the effects of 10 weeks of β-alanine supplementation on muscle carnosine concentrations and cycling capacity at 110% of powermax. In addition to the effects of supplementation on high-intensity cycling capacity (the results of which will be discussed in the next section), biopsies were taken from a sub-section of the subjects at 4 and 10 weeks. Skeletal muscle carnosine concentrations were significantly elevated from pre-supplementation levels by 60% after 4 weeks and by a further 20% (an 80% increase from pre-supplementation levels) after 10 weeks (Fig. 2). As such, the changes in muscle carnosine concentrations reported by Hill et al. (2007) were of a similar magnitude to those previously shown by Harris et al. (2006a). Interestingly, Hill et al. (2007) provided evidence to suggest that further supplementation with β-alanine over the subsequent 6 weeks resulted in an additional increase in muscle carnosine levels. This would indicate that 4 weeks of β-alanine supplementation, at 6.4 g day^{-1}, is not sufficient to reach a threshold level for carnosine storage in the skeletal muscle.

Hill et al. (2007) also analysed the single fibre content of carnosine following 10 weeks of β-alanine supplementation. Whilst the concentrations of carnosine remained higher in fast-twitch fibres than in slow-twitch fibres following supplementation, the magnitude of the increase in both fibre types was approximately the same. The higher

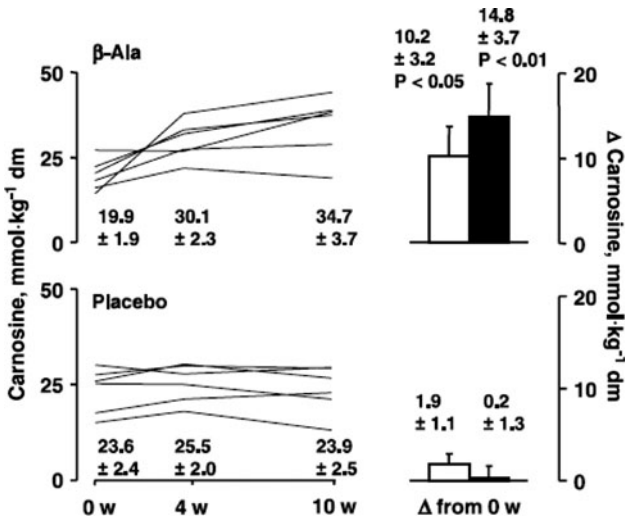

Fig. 2 Muscle carnosine in individual subjects, and the mean change (±SEM) in the carnosine content, prior to supplementation and after 4 and 10 weeks supplementation with β-alanine ($n = 6$) or placebo ($n = 6$) (reproduced from Hill et al. 2007 with permission)

concentration of carnosine in fast-twitch fibres was shown previously in human (Harris et al. 1998) and in camel and equine (Dunnett and Harris 1992, 1997) muscle fibres.

Following on from the studies by Harris and co-workers (Harris et al. 2006a; Hill et al. 2007), who reported significant elevations in the carnosine concentrations of whole skeletal muscle and muscle fibres taken from the *m vastus lateralis* following β-alanine supplementation, Derave et al. (2007) used proton magnetic resonance spectroscopy to examine the effects of 4 weeks of β-alanine supplementation on the carnosine concentrations in the soleus and gastrocnemius muscles of trained 400 m sprint athletes. This study showed that proton magnetic resonance spectroscopy could be used effectively to non-invasively determine muscle carnosine concentrations in humans and provided some agreement with the findings from the previous muscle biopsy studies conducted by Harris et al. (2006a; Hill et al. 2007). Derave et al. (2007) reported that muscle carnosine concentrations increased by 47% in the soleus and 37% in the gastrocnemius muscles following supplementation, which was slightly lower than the 60 and 64% increases shown by Harris et al. (2006a) and Hill et al. (2007).

Several possible reasons exist for these differences, including the fact the two studies examined carnosine concentrations using different techniques and in different muscle groups. Another possible explanation is that the dosing strategy employed by Derave et al. (2007) provided slightly less β-alanine than the dosing strategies previously used by Harris and co-workers. Derave et al. (2007) provided an average daily dose of 4.8 g day^{-1} β-alanine over the 4 weeks of supplementation, whereas both Harris et al. (2006a) and Hill et al. (2007) provided an average daily dose of 5.2 g day^{-1}. One further possible explanation is the fact that Harris and co-workers examined recreationally active subjects for their investigations due to the invasive nature of muscle biopsies, whereas Derave et al. examined trained sprint athletes.

It is interesting to note that Derave et al. (2007) showed increases in the concentrations of carnosine in the skeletal muscles of trained sprinters with β-alanine supplementation. Previous reports have suggested that the highest concentrations of muscle carnosine are shown in those individuals whose muscles are subjected to frequent bouts of hypoxia through resistance training (Tallon et al. 2005). Derave et al. (2007) showed that even in those trained sprinters with initially high muscle carnosine concentrations (>12 mmol l^{-1}), there was still an increase following β-alanine supplementation by as much as 4–5 mmol l^{-1}. This finding provides some further support for the notion that there is no currently known threshold level for the storage of carnosine in skeletal muscle. In contrast, creatine is known to reach threshold levels following around 7 days of dietary supplementation (Harris et al. 1992).

Baguet et al. (2009) were the first to examine the washout period for carnosine concentrations in the skeletal muscle following β-alanine supplementation. Fifteen untrained subjects were supplemented with 4.8 g day^{-1} β-alanine or a placebo over a 5- to 6-week period. Carnosine content was determined, in the soleus, tibialis anterior and gastrocnemius muscles using proton magnetic resonance spectroscopy. Measurements were taken before and after supplementation, as well as after a 3- and 9-week washout period. β-alanine supplementation resulted in an increase in the carnosine content of the soleus (39%), tibialis anterior (27%) and gastrocnemius (23%) muscles, which was in line with the previous findings of Derave et al. (2007). However, the magnitude of the increases in skeletal muscle carnosine levels were somewhat lower than shown by Derave et al. (2007) and lower still than the increases observed by Harris et al. (2006a) and Hill et al. (2007).

Baguet et al. (2009) reported that, after the cessation of β-alanine supplementation, muscle carnosine concentrations declined at a rate of 2–4% per week on average. Given this, mean muscle carnosine concentrations remained significantly elevated from baseline after 3 weeks of washout, but not following 9 weeks. However, the authors also separated subjects into high responders and low responders to β-alanine supplementation. High responders were classified as those subjects whose muscle carnosine concentrations increased by over 30% (with the mean increase being 55%) with low responders being those subjects whose muscle carnosine concentrations increased by less than 30% (with the mean increase being 15%). In high responders, the washout period was increased to 14.6 weeks, whereas the washout period in the low

responders was 6.5 weeks. This study indicates that the use of a cross-over design in studies examining the exercise performance effects of β-alanine supplementation is not practical. There is a similar issue with creatine monohydrate supplementation, since previous studies have shown that a washout period of around 30 days was not sufficient to return skeletal muscle concentrations of creatine to pre-supplementation values (Rawson et al. 2004). However, it is generally accepted that an appropriate washout period following creatine monohydrate supplementation is between 6 and 9 weeks making cross-over studies practically difficult, but still feasible.

Given the increases in muscle carnosine concentrations observed following β-alanine supplementation reported above, an increase in muscle buffering capacity will have occurred. The only way in which an increase in muscle buffering capacity would not have occurred with these increased levels of skeletal muscle carnosine is if other compounds with pKa's within 1 pH unit of pH 7 showed a concomitant decrease. However, other compounds involved in muscle buffering (e.g. organic phosphate or bicarbonate), demonstrate minimal variation in human skeletal muscle (Harris et al. 1990). This makes physiological sense, since reductions in total phosphate, at an equivalent level to the increases in carnosine content, would almost certainly have negative connotations for metabolic processes, given that there is a requirement for phosphate in the reactions of ATP resynthesis (Harris et al. 1992).

Despite the fact that it is inevitable that increases in muscle buffering will have occurred following significant increases in muscle carnosine concentration, there remains some debate about the magnitude of the increase in muscle buffering capacity that would occur with any given elevation in muscle carnosine content. Early opinions relating to the effect of carnosine as an intracellular buffer were not positive; with Mannion et al. (1995) reporting that it only contributed around 7% to total buffering capacity. Calculations relating to the importance of carnosine have come from a comparison of its buffering effect, derived from its pKa, against calculations of total muscle buffering capacity. Muscle buffering capacity is usually determined by the titration of skeletal muscle homogenates (Harris et al. 1990; Sewell et al. 1991; Mannion et al. 1994; Bishop et al. 2004). The homogenisation of muscle causes changes to the chemical composition of the intracellular environment, even with the inhibition of glycolysis by iodoacetate (Bueding and Goldfarb 1941). Included within the homogenised tissue will be intracellular and extracellular pools of pH active compounds from the mitochondria and external membranes, which, in the normal cell, may not contribute to physico-chemical buffering. In addition, the homogenisation process will also expose lipid-bound phosphate groups, which would again not be involved in intracellular pH control. Furthermore, titration releases bound phosphates contained within phosphorylcreatine, which again would contribute to an over estimation of muscle buffering capacity and equally an underestimation of the contribution made by carnosine. In truth, total muscle buffering capacity is constantly changing; being lowest at rest (at which point the relative contribution of carnosine might be calculated to be three to four times higher than commonly held) and increasing with exercise. The estimate of 7% made by Mannion et al. (1995) represents a minimum estimate and even then in a muscle with a metabolic composition close to that of rigor mortis. Recently, Baguet et al. (in press) examined the effect of β-alanine supplementation on acidosis and oxygen uptake kinetics during high-intensity cycling. Their data indicated that supplementation with β-alanine, and thus an increase in muscle carnosine, attenuated the reduction in blood pH during 6 min of high-intensity cycling exercise, when compared with placebo supplementation.

Effects of beta-alanine supplementation on acute exercise performance

Suzuki et al. (2002) examined the relationship between skeletal muscle carnosine concentrations and exercise performance during 30 s of maximal cycling in 11 healthy male subjects. Muscle biopsies were withdrawn from the *m vastus lateralis* at rest and were analysed for carnosine concentration. Subjects then performed 30 s of maximal sprint cycling, during which the authors calculated mean power output in each of six 5-s periods. The results showed a significant positive correlation between carnosine concentration and power output during the last two 5-s periods. The authors concluded that muscle carnosine concentration could be an important factor in high-intensity exercise performance. However, Bogdanis et al. (1998) indicated that reduced pH did not affect performance of a single bout of 30 s cycling, although suggested that a reduced intramuscular pH might be the cause of reduced performance in a follow-up 30 s maximal cycle, performed after 2 min of passive recovery. Previously, the same group had also indicated that there was no effect of intramuscular pH on the restoration of power output following two 30-s maximal sprint cycles (Bogdanis et al. 1995).

As such, Hill (2007, unpublished thesis) examined the effects of 4 weeks of β-alanine supplementation on the performance of three repeated bouts of 30 s sprint cycling. In contrast to the results of Suzuki et al. (2002), Hill (2007, unpublished thesis) did not show any significant effect of β-alanine supplementation on peak power output, mean power output or fatigue index during any of the 30-s

maximal sprint cycles. One possible explanation for the difference in results from these two investigations is that the relationship shown by Suzuki et al. (2002) between carnosine concentrations and exercise performance followed from the significant correlation also observed between carnosine and muscle fibre type. It is possible that the area occupied by type II muscle fibres was more important to 30 s maximal sprint cycling than pH regulation directly, thus explaining why Hill (2007, unpublished thesis) showed no effect of supplementation on repeated sprint performance. It is also possible that the 30-s timeframe is not long enough to be limited by reductions in pH, as previously indicated by Bogdanis et al. 1995, 1998).

In line with the results presented by Hill (2007, unpublished thesis), Hoffman et al. (2008) also observed no significant effects of β-alanine supplementation on fatigue rates in 26 collegiate football players during repeated line drills. However, a trend ($P = 0.07$) was observed for a lower rate of fatigue during a modified Wingate anaerobic power test (lasting 60 s), which was coupled with significantly lower feelings of fatigue communicated by players supplemented with β-alanine. The exercise duration of the high-intensity protocols in this study (up to 60 s) is lower than in previous investigations showing a positive effect of β-alanine supplementation on exercise performance (Hill et al. 2007; Stout et al. 2006, 2007, 2008). As previously mentioned, it might be that the length of the exercise protocol explains the lack of significant findings, with the exercise duration being insufficient to induce a significant H^+ ion accumulation and consequent drop in intramuscular pH of sufficient magnitude to influence exercise performance. Another possible reason for the lack of an effect of β-alanine supplementation was that the length and dose of the supplementation protocol employed by Hoffman et al. (2008) was shorter and smaller than previously used (3 weeks at 4.5 g day^{-1}).

Hill et al. (2007) investigated the effect of β-alanine supplementation on a 110% cycle capacity test ($CCT_{110\%}$), which usually lasts between 2 and 4 min. The $CCT_{110\%}$ is a high-intensity exercise protocol designed to induce a large accumulation of H^+ ions and a resultant drop in intramuscular pH. Therefore, this type of exercise test would directly focus on the ability of carnosine to improve exercise capacity as a result of an increased muscle buffering capacity.

Twenty-four subjects completed the study, with 13 subjects being supplemented with β-alanine and 11 with a maltodextrin placebo. Subjects were supplemented with an incremental dosage scheme from 4.0 g day^{-1} in the first week to 6.4 g day^{-1} in the fourth week, which then continued for a further 6 weeks, meaning that subjects were supplemented for 10 weeks in total. As highlighted above, increases in muscle carnosine concentration were ~60 and ~80% at 4 and 10 weeks. As a result of these increases in muscle carnosine content, total work done during the $CCT_{110\%}$ was increased by 13.0% following 4 weeks of β-alanine supplementation, with further improvements to 16.2% being shown when supplementation was extended to 10 weeks (Fig. 3). These results provide some support for the suggestion that β-alanine supplementation increases muscle carnosine content and, as a consequence, muscle buffering capacity, which allows an increase in high-intensity cycling capacity through a reduction in the impact of H^+ accumulation on muscle function and fatigue.

The potential for an increased muscle buffering capacity to enhance exercise capacity was also shown by Harris et al. (2006b, abstract) during fixed exercise of the knee extensors at 45–50% of maximal voluntary contraction. This exercise protocol was designed specifically to result in peak intramuscular lactate accumulation and a corresponding pH decrease, although neither was directly measured in this study. Subjects were supplemented with either 4×1.6 g day^{-1} β-alanine or a matching maltodextrin placebo. Each dose, in both groups, was co-ingested with 45–65 g of glucose in an attempt to increase the rate of transport and uptake of β-alanine into the skeletal muscle, thus reducing the supplementation period from 4 to 2 weeks. These results showed that isometric muscle endurance at 45–50% of maximal voluntary contraction

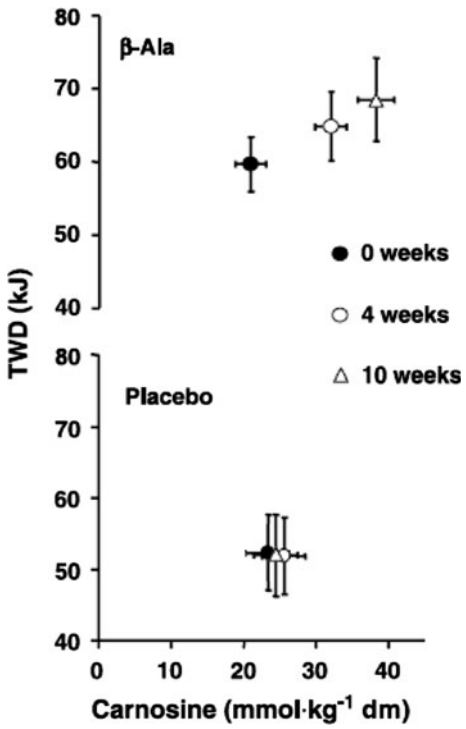

Fig. 3 Comparison of the mean (±SEM) total work done and mean (±SEM) muscle carnosine content prior to supplementation and after 4 and 10 weeks supplementation with β-alanine or placebo (reproduced from Hill et al. 2007 with permission)

was significantly improved, by around 11%, in the β-alanine group compared with the placebo group. Derave et al. (2007) also examined the effects of β-alanine supplementation on isokinetic knee extension exercise and isometric muscle endurance of the knee extensors at 45% maximal voluntary contraction in trained 400 m runners. In contrast to the results of Harris et al. (2006b), Derave et al. (2007) reported no significant effect of 4 weeks β-alanine supplementation at 4.8 g day^{-1} on isometric endurance, although a significant reduction in fatigue during repeated bouts of maximal dynamic exercise was shown.

In addition to examining the effects of β-alanine supplementation on isometric and isokinetic knee extensor performance, Derave et al. (2007) also examined 400 m running performance on an indoor athletic track. There was an improved run time following supplementation in both the β-alanine and placebo groups, but there was no additional benefit of β-alanine on performance, with both groups showing a similar level of improvement (~0.7 s). Currently this study represents one of the few studies to examine the effects of β-alanine supplementation on exercise performance in a sport specific setting. Further sport specific performance studies are needed to confirm the full range of the effect that β-alanine supplementation might have on sport performance.

The subsequent delay in H^+ ion removal from the cell, as the direct result of increased intracellular buffering, following an elevation in skeletal muscle carnosine content, might also delay CO_2 by-production due to a reduced requirement for extracellular buffering. As such, it could be hypothesised that β-alanine supplementation would have an impact upon the ventilatory threshold in man. Indeed, Zoeller et al. (2007) investigated both the individual and combined effects of β-alanine and creatine monohydrate supplementation on indices of endurance performance, including markers of the ventilatory threshold and lactate threshold. Participants were allocated to one of four supplementation groups; placebo, creatine monohydrate (21 g day^{-1} for 6 days and then 10.5 g day^{-1} for 22 days), β-alanine (6.4 g day^{-1} for 6 days and then 3.2 g day^{-1} for 22 days) or a combination of β-alanine and creatine (creatine: 21 g day^{-1} for 6 days and then 10.5 g day^{-1} for 22 days plus β-alanine: 6.4 g day^{-1} for 6 days and then 3.2 g day^{-1} for 22 days). No significant between-group differences were observed, indicating that there was no effect of β-alanine or β-alanine plus creatine monohydrate on ventilatory and lactate thresholds. However, the authors did report some significant within-group differences following combined β-alanine plus creatine monohydrate supplementation, indicating increases in V_{O_2} (+5.7%) and power output (+9%) at the lactate threshold and V_{O_2} (+7.9%), $\%V_{O_2\,peak}$ (+7.9%) and power output (+10.9%) at the ventilatory threshold. However, the true significance of these within-group differences is questionable given the lack of any significant between-group effects and the fact that significant decreases in percentage $V_{O_2\,peak}$ and power output at the lactate threshold, as well as an increase in time to exhaustion, were observed with placebo supplementation. This might suggest that subjects lacked familiarisation with the exercise protocols.

Prior to the investigation by Zoeller et al. (2007), Hill (2007, unpublished thesis) investigated the effects of β-alanine supplementation, with and without creatine monohydrate supplementation, on $CCT_{110\%}$ (as described previously). Muscle carnosine was increased following β-alanine and β-alanine plus creatine monohydrate supplementation, whereas total muscle creatine was increased with creatine monohydrate and β-alanine plus creatine monohydrate supplementation. Total work done during the $CCT_{110\%}$ was significantly increased in all three supplementation groups, while the placebo group showed no changes. Interestingly, this study showed that β-alanine and creatine supplementation improved high-intensity exercise capacity, although there was no additional benefit from the co-ingestion of β-alanine and creatine monohydrate.

Stout et al. observed a positive effect of β-alanine supplementation on neuromuscular fatigue in men (Stout et al. 2006), in women (Stout et al. 2007) and in the elderly (Stout et al. 2008). Exercise consisted of incremental cycle stages until exhaustion, during which electromyography was used to determine the onset of neuromuscular fatigue using the physical working capacity at the fatigue threshold (PWC_{FT}). This protocol was developed by de Vries et al. (1987) and utilises the relationship between electromyography amplitude and fatigue in order to identify the power output that corresponds to the onset of neuromuscular fatigue. Stout et al. (2006) suggested that the accumulation of metabolic by-products in muscle, including lactate and H^+ ions, was a potential mechanism for increased electromyography amplitude during exhaustive exercise. Stout et al. (2006), using the same participants and supplementation protocol as Zoeller et al. (2007), showed that PWC_{FT} improved by 14.5% following 28 days of β-alanine supplementation, while combined β-alanine plus creatine monohydrate supplementation resulted in an observed 11% increase. No significant effects of creatine monohydrate supplementation were shown, suggesting that the changes that were observed were due to an effect of β-alanine supplementation.

Stout et al. (2007) examined the effects of β-alanine supplementation (although not in combination with creatine monohydrate supplementation) on neuromuscular fatigue in women, using the same exercise protocol as Stout et al. (2006). Similar to the findings previously observed in men, Stout et al. (2007) showed a significant 12.6% increase in the PWC_{FT} following β-alanine

supplementation. This improvement increased to 28.6% when the β-alanine supplemented population were elderly (55–92 year) (Stout et al. 2008).

The series of studies by Stout and colleagues advocated the potential of β-alanine to enhance exercise performance and delay the onset of neuromuscular fatigue, as demonstrated by an improvement in PWC_{FT}. The likely cause of the lengthened time to neuromuscular fatigue is an improved intramuscular buffering of H^+ ions, owing to increased carnosine concentration by means of β-alanine supplementation (Harris et al. 2006a). However, the precise physiological mechanisms by which improved H^+ ion regulation would affect neuromuscular fatigue are as yet unclear (Stout et al. 2006).

Recently, van Thienen et al. (2009) observed that an 8 week β-alanine supplementation programme (2–4 g day^{-1}) could enhance sprint power output at the end of a simulated endurance cycle race. Twenty-one participants performed a 110-min intermittent endurance exercise protocol, varying between 50 and 90% (10-min stages) of their previously estimated maximal lactate steady state. A time trial at 100% maximal lactate steady state proceeded immediately afterwards, followed by a 5-min active recovery period at 50% of maximal lactate steady state. A 30-s all-out sprint was performed to conclude the exercise. Following the supplementation period, participants on β-alanine improved their peak (+11.2%), mean (+4.9%) and final (+10.9%) power output during the 30-s sprint. The high blood lactate concentrations observed ($\sim$7 mmol l^{-1}) highlight the anaerobic nature of the sprint exercise performed, and give credence to the argument that increased H^+ ion buffering results in improved performance during high-intensity anaerobic exercise.

These studies provide some evidence to suggest that β-alanine supplementation can sufficiently increase intramuscular carnosine concentration, such that exercise performance is enhanced. However, not all studies have shown a positive effect of β-alanine supplementation. Some of the inconsistent findings reported might be due to a variety of factors, including supplementation duration, dosage, training status of the subjects and protocol familiarisation.

Effects of beta-alanine supplementation and training on muscle carnosine content

Carnosine concentrations range from 17.5 ± 4.8 mmol kg^{-1} dm in females to 21.3 ± 4.2 mmol kg^{-1} dm in males (Mannion et al. 1992). However, several authors have observed higher contents in physically active and highly trained individuals. Parkhouse et al. (1985) showed that sprinters and rowers had elevated levels of carnosine in comparison to marathon runners and untrained individuals. Bodybuilders have also been shown to have carnosine concentrations as high as 50.87 mmol kg^{-1} dm, with average values being as high as $\sim$43 mmol kg^{-1} dm (Tallon et al. 2005). It could be suggested, that due to the fact that these types of athlete (e.g. sprinters, rowers and bodybuilders) are routinely subjected to repeated bouts of high-intensity exercise, the elevated carnosine concentrations observed are resultant of a training effect in relation to repeated exposure of the tissues to acidosis and hypoxia. However, despite the logic of this hypothesis, the effect of high-intensity exercise training on muscle carnosine concentrations remains unclear. That said, particularly in the case of the bodybuilders, the potential role of anabolic steroid use in elevating muscle carnosine concentrations should not be overlooked. Indeed, previous studies have reported higher carnosine contents in the skeletal muscles of male mice compared with female mice, which was hypothesised to be due to the anabolic effects of androgens on the skeletal muscle (Penafiel et al. 2004). It is possible that this might also, at least in part, explain differences in the muscle carnosine concentrations between males and females in humans (Mannion et al. 1992).

Mannion et al. (1994) investigated the effect of 16 weeks of isokinetic knee extension exercise training in 33 physically active subjects. Training groups performed either six sets of 25 maximal repetitions with 30 s of rest between sets or five sets of 15 maximal repetitions with 40 s of rest between sets. This protocol was used due to the expected contribution of anaerobic glycolysis towards this type of high-intensity, repetitive exercise with short recovery periods. Results indicated that 16 weeks of training had no effect on the carnosine concentrations of the quadriceps muscles. Similarly, Kendrick et al. (2008, 2009) observed no effect of 10 weeks of strength training and 4 weeks of isokinetic training on muscle carnosine content in Vietnamese sports science students.

Suzuki et al. (2004) disagree with the results observed in other studies, particularly those findings of Mannion et al. (1994) and Kendrick et al. (2009). Following an 8 week sprint training programme, Suzuki et al. (2004) observed a doubling of muscle carnosine concentration in six Japanese participants. All participants showed increases in muscle carnosine concentrations, with the mean post-training value of 47.4 mmol kg^{-1} dm being significantly higher than the concentrations previously observed even in bodybuilders (43.0 mmol kg^{-1} dm) (Tallon et al. 2005). The sprint training programme used by Suzuki et al. (2004) consisted of twenty-eight 30-s Wingate cycle sprints over the 8-week period. Somewhat surprisingly, the total training volume related to just 14 min of high-intensity maximal exercise. The training protocol used in this study does not suggest sufficient training volume to increase muscle carnosine concentrations by the magnitude observed. Alternative

explanations of these findings are that subjects drastically increased their consumption of foods containing histidine dipeptides from the onset of training, or that an error was made in the analysis of muscle samples.

Kendrick et al. (2009) investigated the effects of combined training with β-alanine supplementation on muscle carnosine concentration. Seven subjects were supplemented with β-alanine at 6.4 g day^{-1} and seven were supplemented with a matching placebo. Each subject undertook 4 weeks of isokinetic training of the right leg, whilst also serving as their own control by leaving their left leg untrained. Observations showed no effect of 4 weeks of isokinetic training on muscle carnosine content in the placebo group. However, β-alanine supplementation did increase muscle carnosine concentrations in both the trained and the untrained leg, although there was no significant difference in the magnitude of the change between legs. Despite this, it is of interest to note that the increase in muscle carnosine content in the trained leg, following β-alanine supplementation, was larger (+52.2%) than the increase in the untrained leg (+28.3%). As such, one possible explanation for the lack of a significant finding here is the reasonably small number of subjects, which suggests the need for further equally well controlled studies utilising a larger population.

These results suggest that training does not increase the synthesis of carnosine in muscle even with β-alanine supplementation. In order for training to result in an increase in muscle carnosine synthesis directly (without the use of β-alanine supplementation) there would need to be an increase in the liver synthesis of β-alanine from uracil degradation. In the case of the increase in muscle carnosine with training reported by Suzuki et al. (2004), it is clear that this would have represented (in the absence of an increased dietary source of β-alanine) a major increase in the degradation of the pyrimidine base, uracil, in the liver.

Smith et al. (2009a) investigated the effects of high-intensity interval training combined with β-alanine supplementation. Forty-six recreationally active men completed 6 weeks of training and ingested either β-alanine (at 6 g day^{-1} with 1 day on and 1 day off during the final 3 weeks), a matched placebo or no supplement (control group). Exercise performance was assessed pre-training, mid-training and post-training using four incrementally ascending workloads (70–300 W) on an electronically braked cycle ergometer. Electromyography was used to determine fatigue (EMG_{FT}) and efficiency of electrical activity (EEA), which quantifies the functional state of the muscle (de Vries 1968). EMG_{FT} has been closely related to steady state lactate metabolism (Moritani et al. 1993) and, thus, as suggested from training, should reflect improvements in lactate and H^+ clearance and muscle buffering capacity.

High-intensity interval training significantly improved absolute values of EEA and EMG_{FT} from pre- to mid-training in both the β-alanine and placebo groups, although there was no significant difference between groups. Interestingly, more subjects improved with training alone (56% of individuals) than those also supplemented with β-alanine (39% of individuals). The results of Smith et al. (2009a) do not support the suggestion that the effectiveness of high-intensity interval training can be further improved with β-alanine supplementation. However, the training protocol used may have been a superior stimulus to the untrained population, rendering any changes in muscle carnosine ineffective.

Smith et al. (2009b) further examined the combined effects of 6 weeks high-intensity interval training with β-alanine supplementation. Subjects were randomly allocated to either a β-alanine group (6 g day^{-1}; $n = 18$) or a matching placebo group (PL; $n = 18$). $V_{O_2\,peak}$, time to exhaustion and ventilatory threshold were assessed pre-training, mid-training and post-training using a continuous graded exercise test on an electronically braked cycle ergometer. Similarly, total work done was recorded during a 110% $V_{O_2\,peak}$ workload test to exhaustion. $V_{O_2\,peak}$ and time to exhaustion improved significantly from pre- to mid-training in both supplementation groups, although a further increase from mid- to post-training was only observed in those subjects also supplemented with β-alanine. In addition, there was a significant improvement in total work done during the 110% $V_{O_2\,peak}$ test from pre- to mid-training and from mid- to post-training in both groups. However, there was no effect of β-alanine supplementation or training on ventilatory threshold. These results suggest some potential for β-alanine supplementation to further enhance the benefits of high-intensity interval training. During training, the β-alanine group consistently trained at higher workloads for longer periods of time than the placebo group, although these differences did not reach the assigned level of significance. The ability of the β-alanine group to train at higher intensities without fatigue, although not statistically significant, could be a key element in explaining the further improvements seen in certain performance markers.

Hoffman et al. (2008) observed a similar trend in collegiate football players. Players supplemented with β-alanine had a higher (9.2%) training volume than those consuming placebo. The delay in fatigue caused by increased buffering of muscle H^+ ions during training could explain this trend and suggests that further longer-term training studies of a sports specific nature are required.

Athletes involved in high-intensity, anaerobic based sports have been shown to have increased levels of muscle carnosine (Parkhouse et al. 1985; Tallon et al. 2005). It has been suggested that this is due to the nature of their training, where muscle acidosis occurs consistently, and

the elevated carnosine concentrations observed are due to a training effect. However, few studies have shown the potential to increase muscle carnosine through high-intensity training, with the results of Suzuki et al. (2004) standing alone in showing a significant effect, although others have reported a trend towards higher muscle carnosine content (Kendrick et al. 2009), higher training volumes and periods (Hoffman et al. 2008; Smith et al. 2009b), and subsequent performance improvements (Smith et al. 2009b). As such, there is a need to further examine the effects of exercise training, with and without β-alanine supplementation, on muscle carnosine concentrations and exercise performance.

Summary

β-Alanine supplementation has consistently been shown to augment muscle carnosine concentrations in man (Harris et al. 2006a; Derave et al. 2007; Hill et al. 2007; Baguet et al. 2009). Carnosine's role as an intracellular buffer is undisputed given its location within the skeletal muscle of humans and its chemical structure (Harris et al. 1990). Consequently, there is potential for supplementation with β-alanine to result in improved exercise performance, especially during high-intensity exercise. There is now a growing body of evidence to show that β-alanine supplementation of 4 weeks or longer evokes significant improvements to exercise capacity, especially when that performance is likely to be limited by the accumulation of H^+ ions in the skeletal muscle (i.e. in high-intensity exercise tests lasting between 1.5 and 4 min). However, not all studies examining the influence of β-alanine supplementation on exercise performance have shown positive results. In addition, few studies have shown the potential to increase muscle carnosine through high-intensity training, even when combined with β-alanine supplementation.

Future directions

- Currently it is unknown whether or not there is a ceiling concentration beyond which further β-alanine supplementation will not confer any additional increase in carnosine levels in the muscle. Further long-term supplementation studies are required to determine whether there is a limit to the accumulation of carnosine in skeletal muscle.
- It is currently unknown to what extent increases in muscle carnosine concentration induce a concomitant increase in muscle buffering capacity. One problem is the current limitation with regards to the determination of muscle buffering capacity using titration techniques. Further studies are required to improve the accuracy of the determination of muscle buffering capacity so that the effect of any given increase in muscle carnosine concentrations on muscle buffering capacity might be accurately determined.
- Further studies are required to confirm the full range of the effect that β-alanine supplementation might have on sport and exercise performance. For example, the majority of studies showing an improvement in exercise performance with β-alanine supplementation to-date have used high-intensity exercise capacity tests and further information is required on the effects of β-alanine supplementation on sports performance.
- Further studies examining the impact of β-alanine in combination with other supplements would be of interest.

References

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

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

Baguet A, Reyngoudt H, Pottier A, Everaert I, Callens S, Achten E, Derave W (2009) Carnosine loading and washout in human skeletal muscles. J Appl Physiol 106:837–842

Baguet A, Koppo K, Pottier A, Derave W (in press) β-alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. Eur J Appl Physiol

Bakardjiev A (1997) Biosynthesis of carnosine in primary cultures of rat olfactory bulb. Neurosci Lett 227:115–118

Bakardjiev A, Bauer WJ (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 rigour: 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. Biochim Biophys Acta 1324:142–150

Bishop D, Edge J, Goodman C (2004) Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women. Eur J Appl Physiol 92:540–547

Bogdanis GC, Nevill ME, Boobis LH, Lakomy HKA, Nevill AM (1995) Recovery of power output and muscle metabolites following 30s of maximal sprint cycling in man. J Physiol 482:467–480

Bogdanis GC, Nevill ME, Lakomy HKA, Boobis LH (1998) Power output and muscle metabolism during and following recovery from 10 and 20s of maximal sprint exercise in humans. Acta Physiol Scand 163:261–272

Boldyrev AA (1993) Does carnosine possess direct antioxidant activity? Int J Biochem 25:1101–1107

Boldyrev AA, Dupin AM, Bunin AY, Babizhaev MA, Severin SE (1987) The antioxidative properties of carnosine, a natural histidine containing dipeptide. Biochem Int 15:1105–1113

Bonfanti L, Peretto P, de Marchis S, Fasolo A (1999) Carnosine related dipeptides in the mammalian brain. Prog Neurobiol 59:333–353

Bueding E, Goldfarb W (1941) The effect of sodium fluoride and sodium iodoacetate on glycolysis in human blood. J Biol Chem 141:539–544

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

Crozier RA, Ajit SK, Kaftan EJ, Pausch MH (2007) MrgD activation inhibits KCNQ/M-currents and contributes to enhanced neuronal excitability. J Neurosci 27:4492–4496

Davey CL (1960) The effects of carnosine and anserine on glycolytic reactions in skeletal muscle. Arch Biochem Biophys 98:296–302

de Vries HA (1968) Method for evaluation of muscle fatigue and endurance from electromyographic fatigue curves. Am J Phys Med 47:125–135

de Vries HA, Tichy MW, Housh TJ, Smyth KD, Tichy AM, Housh DJ (1987) A method for estimating physical working capacity at the fatigue threshold (PWCFT). Ergonomics 30:1195–1204

Derave W, Ozdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, Achten E (2007) β-alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. J Appl Physiol 103:1736–1743

Dunnett M, Harris RC (1992) Determination of carnosine and other biogenic imidazoles in equine plasma by isocratic reversed-phase ion-pair high-performance liquid chromatography. J Chromatogr 579:45–53

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 Biomed Appl 688:47–55

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 30:499–504

Dutka TL, Lamb GD (2004) Effect of carnosine on excitation-contraction coupling in mechanically-skinned rat skeletal muscle. J Muscle Res Cell Motil 25:203–213

Gulewitsch WS, Amiradzhibi S (1900) Uber der carnosin, eine neue organische base des fleischextrakten. Ber Dtsch Chem Ges 33:1902–1903

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

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

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

Harris RC, Tallon MJ, Dunnett M, Boobis LH, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, Wise JA (2006a) The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. Amino Acids 30:279–289

Harris RC, Ponte J, Hill CA, Sale C, Jones GA, Kim HJ, Wise JA, Kraemer WJ (2006b) Effect of 14 days β-alanine supplementation on isometric strength of the knee extensors. Med Sci Sports Exerc 38:S125–S126

Hill CA (2007) β-Alanine supplementation and high-intensity exercise. Unpublished doctoral thesis, University of Southampton

Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK, Wise JA (2007) Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. Amino Acids 32:225–233

Hipkiss AR, Carmichael PL, Zimmermann B (1993) Metabolism of crystallin fragments in cell-free extracts of bovine lens: effects of ageing and oxygen free-radicals. Acta Biol Hung 42:243–263

Hipkiss AR, Michaelis J, Syrris P (1995) Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent. FEBS Lett 28:81–85

Hipkiss AR, Brownson C, Carrier MJ (2001) Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. Mech Ageing Dev 15:1431–1445

Hoffman JR, Ratamess NA, Faigenbaum AD, Ross R, Kang J, Stout JR, Wise JA (2008) Short duration β-alanine supplementation increases training volume and reduces subjective feelings of fatigue in college football players. Nutr Res 28:31–35

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

Kendrick IP, Harris RC, Kim HJ, Kim CK, Dang VH, Lam TQ, Bui TT, Smith M, Wise JA (2008) The effects of 10 weeks of resistance training combined with beta-alanine supplementation on whole body strength, force production, muscular endurance and body composition. Amino Acids 34:547–554

Kendrick IP, Kim HJ, Harris RC, Kim CK, Dang VH, Lam TQ, Bui TT, Wise JA (2009) The effect of 4 weeks β-alanine supplementation and isokinetic training on carnosine concentrations in type I and II human skeletal muscle fibres. Eur J Appl Physiol 106:131–138

Krimberg R (1906) Zur Kenntnis der Extraktivstoffe der muskelin. IV. Mutterlung. Uberdas vorkommen des carnosins, carnitins und methylguanidins im fleisch. Hoppe Seylers Z Physiol Chem 48:412

Krimberg R (1908) Zur Kenntnis der Extraktivstoffe der muskelin. X. Mitteilung. Uber die identitat des novsains mit dem carnitin. Hoppe Seylers Z Physiol Chem 55:466

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

Lenney JF, Peppers SC, Kucera-Orallo CM, George RP (1985) Characterization of human tissue carnosinase. Biochem J 228:653–660

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

Mannion AF, Jakeman PM, Willan PLT (1994) Effects of isokinetic training of the knee extensors on high-intensity exercise performance and skeletal muscle buffering. Eur J Appl Physiol 68:356–361

Mannion AF, Jakeman PM, Willan PL (1995) Skeletal muscle buffer value, fibre type distribution and high intensity exercise performance in man. Exp Physiol 80:89–101

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

Miyamoto Y, Nakamura H, Hoshi T, Ganapathy V, Leibach FH (1990) Uphill transport of β-alanine in intestinal brush-border membrane vesicles. Am J Physiol 259:G372–G379

Mori M, Gahwiler BH, Gerber U (2002) Beta-alanine and taurine as endogenous agonists at glycine receptors in rat hippocampus in vitro. J Physiol 15:191–200

Moritani T, Takaishi T, Matsumoto T (1993) Determination of maximal power output at neuromuscular fatigue threshold. J Appl Physiol 74:1729–1734

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

Otani H, Okumura A, Nagai K, Okumura N (2008) Colocalization of a carnosine-splitting enzyme, tissue carnosinase (CN2)/cytosolic non-specific dipeptidase 2 (CNDP2), with histidine decarboxylase in the tuberomammillary nucleus of the hypothalamus. Neurosci Lett 445:166–169

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

Penafiel R, Ruzafa C, Monserrat F, Cremades A (2004) Gender-related differences in carnosine, anserine and lysine content of murine skeletal muscle. Amino Acids 26:53–58

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

Ramamoorthy S, Leibach FH, Mahesh VB, Han H, Yang-Feng T, Blakely RD, Ganapathy V (1994) Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. Biochem J 300:893–900

Rawson ES, Persky AM, Price TB, Clarkson PM (2004) Effects of repeated creatine supplementation on muscle, plasma and urine creatine levels. J Strength Cond Res 18:162–167

Rubtsov AM (2001) Molecular mechanisms of regulation of the activity of sarcoplasmic reticulum Ca-release channels (ryanodine receptors), muscle fatigue, and Severin's phenomenon. Biochemistry 66:1132–1143

Sassoe-Pognetto MM, Cantino D, Panzanelli P, Verdun di Cantogno L, Giustetto M, Margolis FL, De Biasi S, Fasolo A (1993) Presynaptic colocalization of carnosine and glutamate in olfactory neurones. Neuroreport 5:7–10

Severin SE, Kirzon MV, Kaftanova TM (1953) Effect of carnosine and anserine on action of isolated frog muscles. Dokl Akad Nauk SSSR 91:691–701

Sewell DA, Harris RC, Dunnett M (1991) Carnosine accounts for most of the variation in physico-chemical buffering in equine muscle. Equine Exerc Physiol 3:276–280

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

Smith AE, Moon JR, Kendall KL, Graef JL, Lockwood CM, Walter AA, Beck TW, Cramer JT, Stout JR (2009a) The effects of beta-alanine supplementation and high-intensity interval training on neuromuscular fatigue and muscle function. Eur J Appl Physiol 105:357–363

Smith AE, Walter AA, Graef JL, Kendall KL, Moon JR, Lockwood CM, Fakuda DH, Beck TW, Cramer JT, Stout JR (2009b) Effects of β-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. J Int Soc Sports Nutr 6:5

Stout JR, Cramer JT, Mielke M, O'Kroy J, Torok DJ, Zoeller RF (2006) Effects of twenty-eight days of beta-alanine and creatine monohydrate supplementation on the physical working capacity at neuromuscular fatigue threshold. J Strength Cond Res 20:928–931

Stout JR, Cramer JT, Zoeller RF, Torok D, Costa P, Hoffman JR, Harris RC, O'Kroy J (2007) Effects of β-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. Amino Acids 32:381–386

Stout JR, Graves BS, Smith AE, Hartman MJ, Cramer JT, Beck TW, Harris RC (2008) The effect of beta-alanine supplementation on neuromuscular fatigue in elderly (55–92 years): a double-blind randomized study. J Int Soc Sports Nutr 5:21–26

Suzuki Y, Ito O, Mukai N, Takahashi H, Takamatsu K (2002) High levels of skeletal muscle carnosine contributes to the latter half of exercise performance during 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

Syrotuik DG, Bell GJ (2004) Acute creatine monohydrate supplementation: a descriptive physiological profile of responder vs nonresponders. J Strength Cond Res 18:610–617

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

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

Tamaki N, Ikeda T, Fujimoto S, Mizutani N (1985) Carnosine as a histidine source: transport and hydrolysis of exogenous carnosine by rat intestine. J Nutr Sci Vitaminol 31:607–618

Tanokura M, Tasumi M, Miyazawa T (1976) 1H nuclear magnetic resonance studies of histidine containing di and tripeptides. Estimation of the effects of charged groups on the pK_a value of the imidiazole ring. Biopolymers 15:393–401

Tokutomi N, Kaneda M, Akaike N (1989) What confers specificity on glycine for its receptor site? Br J Pharmacol 97:353–360

van Thienen R, van Proeyen K, vanden Eynde B, Puype J, Lefere T, Hespel P (2009) β-alanine improves sprint performance in endurance cycling. Med Sci Sports Exerc 41:898–903

Wang DS, Zhu HL, Li JS (2003) Beta-alanine acts on glycine receptors in the rat sacral dorsal commissural neurons. Int J Neurosci 113:293–305

Zoeller RF, Stout JR, O'Kroy J, Torok D, Mielke M (2007) Effects of 28 days of beta-alanine and creatine monohydrate supplementation on aerobic power, ventilatory and lactate thresholds and time to exhaustion. Amino Acids 33:505–510