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INVITED REVIEW

Carnosine: from exercise performance to health

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Abstract Carnosine was first discovered in skeletal muscle, where its concentration is higher than in any other tissue. This, along with an understanding of its role as an intracellular pH buffer has made it a dipeptide of interest for the athletic population with its potential to increase high-intensity exercise performance and capacity. The ability to increase muscle carnosine levels via β-alanine supplementation has spawned a new area of research into its use as an ergogenic aid. The current evidence base relating to the use of β-alanine as an ergogenic aid is reviewed here, alongside our current thoughts on the potential mechanism(s) to support any effect. There is also some emerging evidence for a potential therapeutic role for carnosine, with this potential being, at least theoretically, shown in ageing, neurological diseases, diabetes and cancer. The currently available evidence to support this potential therapeutic role is also reviewed here, as are the potential limitations of its use for these purposes, which mainly focusses on issues surrounding carnosine bioavailability.

Keywords ß-alanine · Carnosine · Exercise performance · Health

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Introduction

Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide synthesised from β-alanine and histidine and is found at highest concentrations in the skeletal muscle of both vertebrates and non-vertebrates. It can also be found in relatively high concentrations in the central nervous system. Gulewitsch and Amiradzhibi (1900) were the first to isolate carnosine, before subsequently classifying it as a histidine-containing dipeptide, having demonstrated the hydrolysis of carnosine to its constituent amino acids (β-alanine and histidine). In humans, carnosine is the only histidine-containing dipeptide present, with concentrations ranging from 12 to 60 mmol kg^{-1} dm in the vastus lateralis muscle. This makes carnosine one of the most abundant small-molecular compounds in human skeletal muscle with concentrations having a similar order of magnitude to phosphorylcreatine, creatine and ATP (Harris et al. 1974).

A complete understanding of the biological role of carnosine requires further work, although research interest in this molecule has expanded significantly in recent years. Initial studies examining a potential physiological role for carnosine demonstrated that it had an imidazole side chain with a pKa of 6.83 making it a suitable buffer over the physiological pH range (Bate-Smith 1938). This, combined with the findings that power athletes have higher carnosine levels than untrained individuals and endurance athletes (Parkhouse et al. 1985), prompted the exploration of a potential role for dietary supplementation to increase muscle carnosine (M-Carn) concentration and to delay fatigue during high-intensity exercise. There are numerous determinants of the M-Carn concentration, including species, gender, age, muscle fibre type, diet, supplementation, exercise and training; with this being the subject of a recent review (Harris et al. 2012). The predominant source of

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dietary carnosine in humans is via meat and fish consumption (Abe 2000), although it should be noted that cooking practices significantly influence the amount available. Given the diversity of the human diet, the potential range of dietary carnosine intakes are relatively broad and might range from 50 to 500 mg d^{-1} in the omnivorous diet (Baguet et al. 2009). In high meat and fish consumers, such as those in the US, South America and Asia, dietary intake is likely to be higher than this. Conversely, vegetarians have been shown to have significantly lower M-Carn levels than their meat-eating counterparts (Everaert et al. 2011). For the majority of humans, dietary supplementation is likely to be the best means of attaining and maintaining higher M-Carn levels.

Carnosine can be ingested through the diet, but the presence of carnosinase in the enterocytes of humans, especially in the jejunal mucosa (Sadikali et al. 1975), suggests that some carnosine is cleaved into β-alanine and L-histidine before reaching the blood stream. However, since the jejunal activity is low (Sadikali et al. 1975), it is very likely that part of the ingested carnosine reaches the blood stream where it is rapidly hydrolysed in plasma due to the high activity of the enzyme carnosinase (Asatoor et al. 1970; Fig. 1), meaning that only negligible levels of carnosine are detectable in the blood (Park et al. 2005). Intact carnosine in the blood cannot be taken up by muscle cells, as they appear to lack the relevant dipeptide transporter, and although the highly active serum carnosinase cleaves most of the absorbed carnosine (Harris et al. 2006), small amounts of intact carnosine can be found in urine 4 h after carnosine ingestion (Gardner et al. 1991).

Carnosine in the muscle is synthesised in situ and depends upon the activity of the enzyme carnosine synthase (Drozak et al. 2010), while the magnitude of synthesis is dependent upon the availability of β-alanine from the diet, as demonstrated by Harris et al. (2006) who showed that the ingestion of free β-alanine resulted in an increase in the plasma level, with the peak concentration being seen after 30–45 min.

In humans, carnosine is present in some brain regions (e.g. olfactory lobe; Gaunitz and Hipkiss 2012), suggesting that some neuronal cells express the carnosine transporter PepT2, which has already been shown in rats (Shu et al. 2002; Wang et al. 1998), thereby overcoming the putative inability of carnosine to transfer across the blood–brain barrier. β-Alanine is readily transported throughout the brain by Na^+ dependent β-amino acid transport system(s) and might, in theory, either form carnosine or act as

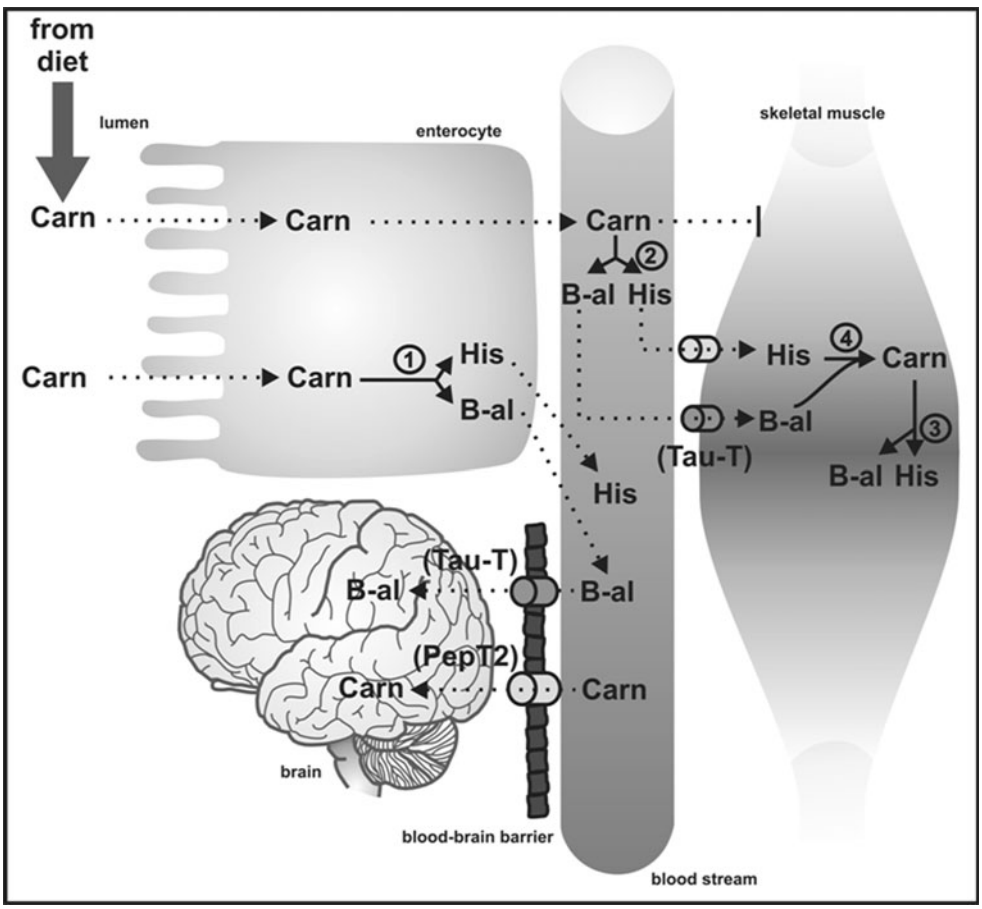


Fig. 1 A schematic illustration of carnosine bioavailability and metabolism. *1* Jejunal carnosinase; *2* serum carnosinase; *3* tissue carnosinase; *4* carnosine synthase; *Tau-T* taurine/β-alanine transporter; *PepT2* carnosine transporter; *Carn* carnosine; *B-al* β-alanine; *His* histidine

a neuromodulator/neurotransmitter itself (Tiedje et al. 2010).

β-Alanine is also produced endogenously in the liver from the degradation of uracil (Fig. 1). Alternative synthesis pathways in the gut (Sadikali et al. 1975) and kidney (Hayaishi et al. 1961) might also account for some endogenous production of β-alanine, although research in humans in this area is scarce and is beyond the scope of this review. Degradation of β-alanine to CO_2 in the liver is possible, although the conversion rate is relatively slow. As a result, only a small fraction of β-alanine is converted to CO_2 in liver and consequently this affords the major pathway of β-alanine synthesis in the body (Artioli et al. 2010). Figure 1 illustrates carnosine bioavailability and metabolism.

Harris et al. (2006) also demonstrated that supplementation of the diet with 6.4 g d^{-1} of free β-alanine over a 4-week period was capable of increasing M-Carn concentrations by ~65 % (typically about 13 mmol kg^{-1} dm). Subsequent to this study, a similar level of increase (~60 %) was confirmed following 4 weeks of supplementation at an average of 5.2 g d^{-1}, with the magnitude of the increase in M-Carn being ~80 % (typically about 16 mmol kg^{-1} dm) when a further 6 weeks of supplementation was provided at 6.4 g d^{-1} (Hill et al. 2007). The increase of ~13 mmol kg^{-1} dm during 4 weeks of supplementation is estimated, in an 80-kg individual, to correspond to retention in muscle of 5–6 % of the administered dose, and the increase of ~16 mmol kg^{-1} dm to an average retention of 2.5–3 % over the 10 weeks.

As well as being confirmed in muscle biopsy studies, other studies using proton magnetic resonance spectroscopy (^{1}H-MRS) concur (e.g. Baguet et al. 2009; Derave et al. 2007). Overall, increases in M-Carn are between 40 and 80 % depending upon the dose given and the duration of supplementation. In addition, the level of increase reported is somewhat dependent on the muscle measured and the technique used to determine the muscle content, with muscle biopsies generally showing higher concentrations than ^{1}H-MRS. The absolute increase in M-Carn is a function of the total amount of β-alanine consumed, as determined by the dose multiplied by the duration of supplementation (Stellingwerff et al. 2012), although this could be fortuitous, reflecting the short duration of most studies to date and the slow turnover of carnosine in muscle. Although the M-Carn content is higher in fast twitch muscle fibres (Hill et al. 2007; Tallon et al. 2007), the increases observed in single muscle fibres of both types with supplementation are of the same order of magnitude (Hill et al. 2007; Kendrick et al. 2009).

Initial studies using free β-alanine in powder form (Harris et al. 2006), usually at a single dose in excess of 10 mg kg^{-1} BM, reported that participants experienced symptoms of paraesthesia, which were broadly described as a prickly sensation causing irritation to the skin. Symptoms are most commonly felt on the face and head or the arms and hands, but sometimes they are also recorded at the base of the spine and the buttocks. Symptoms typically appear within 10–20 min of consuming the supplement but can last for around 60 min or longer, suggesting that they are related to the peak concentration of β-alanine in blood. In order to reduce the incidence of paraesthesia, Harris et al. (2006) split the amounts given daily into multiple doses of 800 mg (maximum ~10 mg kg^{-1} BM) consumed throughout the day. This approach was successful in reducing the incidence of symptoms in some participants and the severity of symptoms overall. As such, it was employed in numerous future supplementation studies (e.g. Kendrick et al. 2008, 2009; Stout et al. 2007; van Thienen et al. 2009). However, more recently, a sustained release formulation has been developed (Carnosyn SR™, Natural Alternatives International, San Marcos, California, USA), which imposes a physical restraint on the rate of release of β-alanine from the gut. This means that the peak plasma concentration from a single dose is reduced, while release into blood is maintained over 6 h (Decombaz et al. 2012). Initial studies using the sustained release tablets have shown that symptoms of paraesthesia are prevented even with single doses of 20 mg kg^{-1} BM (Sale et al. 2011), with this being confirmed by several subsequent studies (e.g. Saunders et al. 2012a, b). In a systematic investigation of the effects of sustained release β-alanine tablets on the experience of paraesthesia by participants, Decombaz et al. (2012) showed that ingesting 1.6 g of β-alanine resulted in sensory side-effects that could not be differentiated from the placebo.

Whether there is a ceiling to the amount of carnosine that can be stored in skeletal muscle is currently unknown. However, we do know that M-Carn concentrations, once elevated by supplementation, are very stable, indicating that although tissue carnosinase is present in muscle, its activity seems to be very low in humans. Baguet et al. (2009) were the first to report on the decline in M-Carn from human skeletal muscle in 15 untrained men following 5–6 weeks' supplementation with 4.8 g d^{-1} β-alanine. M-Carn was determined using ^{1}H-MRS before and after supplementation, as well as in the following 3 and 6 weeks, in the soleus, tibialis anterior and gastrocnemius muscles. M-Carn concentrations were increased by 39 % in the soleus, 27 % in the tibialis anterior and by 23 % in the gastrocnemius. The rate of washout for M-Carn was slow, occurring at 2–4 % per week, meaning that 3 weeks after the cessation of supplementation M-Carn remained significantly higher than baseline concentrations. It was estimated that M-Carn would have returned to baseline 9 weeks following the end of supplementation, although the M-Carn levels of some individuals likely remained

above baseline until 15 weeks after supplementation. Stellingwerff et al. (2012), again using ^{1}H-MRS, reported a half-life of ~6 weeks for the decline in M-Carn concentrations in the tibialis anterior and gastrocnemius muscles, and 9 weeks in vastus lateralis (in this case measured in muscle biopsy samples). Taken together, the results from both of these studies (Baguet et al. 2009; Stellingwerff et al. 2012) suggest that M-Carn has a very low rate of turnover in skeletal muscle tissue, making crossover designs practically impossible in β-alanine supplementation studies.

β-Alanine supplementation has been the subject of several recent reviews (e.g. Artioli et al. 2010; Sale et al. 2010; Harris et al. 2012) and a meta-analysis (Hobson et al. 2012) in relation to the effects shown on exercise performance and capacity. However, given the speed of current publications relating to the effects of β-alanine on exercise performance, there remains a need to continually update the summaries of this topic area. To date, the performance improvements shown following β-alanine supplementation have largely been ascribed to increases in intracellular buffering as a result of the increased M-Carn concentrations, although there are other potential mechanisms that might also explain a performance effect, including improved calcium (Ca^{2+}) handling and potential antioxidant effects. Furthermore, it is becoming clear that there might be additional therapeutic effects of carnosine, as highlighted at the recent International Congress on Carnosine in Exercise and Disease (Derave and Sale 2012). Some of these potential effects might also be explained by the other purported physiological roles of carnosine, which include the protection of proteins against glycation by acting as a sacrificial peptide (Hipkiss et al. 1995) and attenuating the formation of protein–protein cross-links through reactions with protein-carbonyl groups (Hipkiss et al. 2001). As such, we intend to update the review of exercise performance studies following β-alanine supplementation, review the current understanding of the biological role of carnosine in humans as it relates to exercise performance and highlight the potential for therapeutic benefit from supplementation with either carnosine or β-alanine.

β-Alanine and exercise performance update

Since increased M-Carn undoubtedly results in increased muscle buffering capacity, there has been much interest in determining the effects of β-alanine supplementation on exercise performance and capacity limited by the accumulation of hydrogen cations (H^{+}). Despite this, not all research has shown a beneficial effect of β-alanine supplementation (Fig. 2), although this may be due to the variety and durations of the exercise tests involved (Hobson et al. 2012). Furthermore, in the meta-analysis of Hobson et al. (2012), results indicated significant improvements in exercise capacity tests ($P = 0.013$), but not exercise performance tests ($P = 0.204$), although it was noted that there were fewer studies using performance measures. However, there have been a significant number of studies conducted since this meta-analysis that have measured the effect of β-alanine supplementation on both performance and capacity.

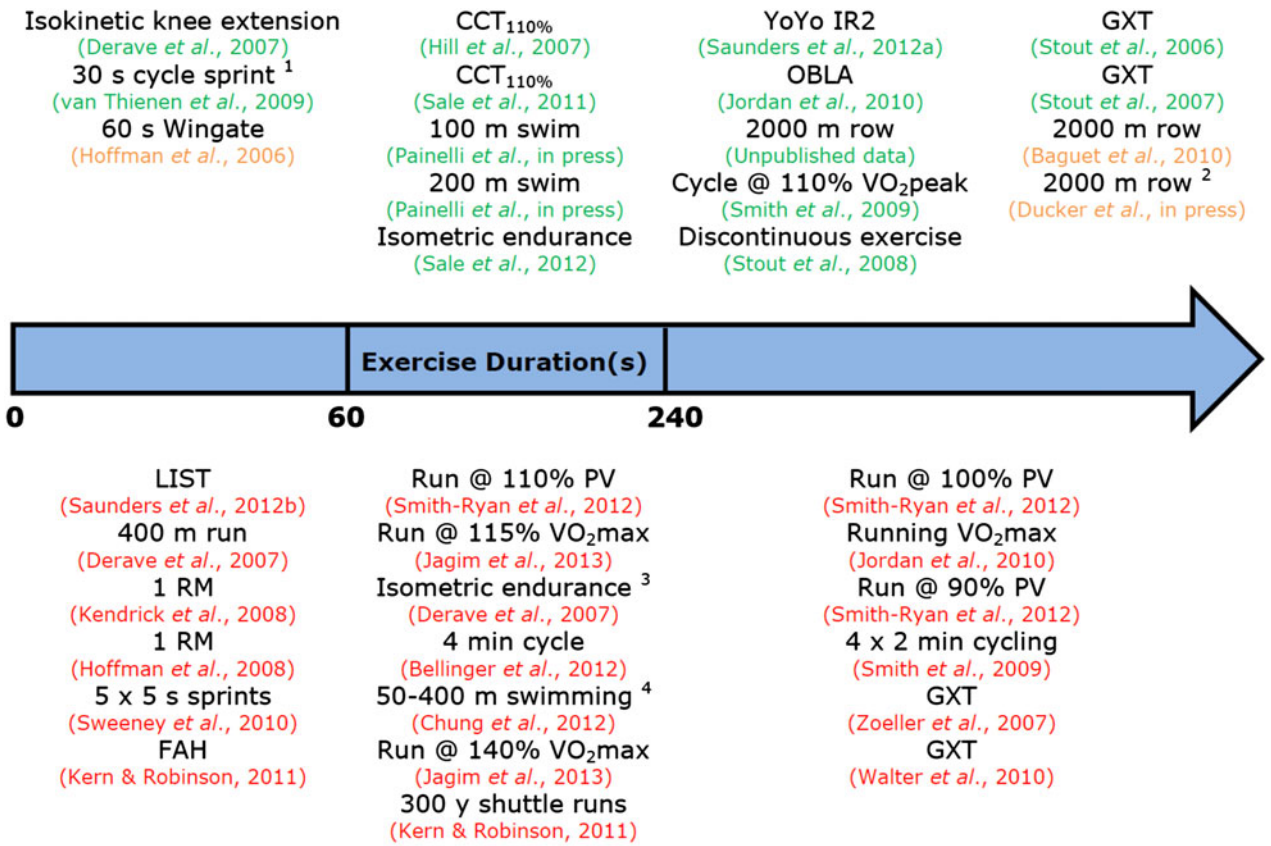
Exercise ≤60 s in duration

Bogdanis et al. (1998) indicated that reduced intramuscular pH (pH_i) did not affect a single exercise bout of 30 s, suggesting that increased muscle buffering capacity through increased M-Carn is unlikely to have an effect on exercise of this duration. Indeed, the majority of studies investigating the effects of β-alanine supplementation on exercise of less than 60 s in duration have shown no beneficial effect (Hoffman et al. 2008b; Kern and Robinson 2011), although Hoffman et al. (2008a) showed a trend ($P = 0.07$) for lower rates of fatigue during a modified Wingate power test (lasting 60 s) and reduced feelings of fatigue, following 30 days of β-alanine supplementation in football players.

van Thienen et al. (2009) are the only authors to report a beneficial effect of β-alanine supplementation on exercise of less than 60 s in duration. Pre and post 8 weeks of supplementation, 17 cyclists performed a 110-min simulated endurance cycle race, followed by a 10-min time trial and 30-s isokinetic sprint (100 rpm). Following supplementation, those on β-alanine improved their peak (+11.2 %), mean (+4.9 %) and final (+10.9 %) power output during the isokinetic sprint. Although seemingly counter to other studies on exercise of <60 s duration, these findings may be explained by the preceding exercise resulting in a decrease in pH_i prior to the start of the sprint.

Exercise between 60 and 240 s in duration

Hill et al. (2007) investigated the effect of β-alanine supplementation on a cycling capacity test at 110 % of previously determined Powermax ($CCT_{110\%}$), a test designed to last between 120 and 240 s. The $CCT_{110\%}$ is designed to induce a large accumulation of H^{+} and a resultant drop in pH_i, and the test has been shown to be highly reliable with a CV of 4.93 % for total work done (TWD; Saunders et al. 2013). Hill et al. (2007) showed that TWD during the $CCT_{110\%}$ was increased by 13.0 % alongside a 58.8 % increase in M-Carn following 4 weeks of β-alanine supplementation; when supplementation was extended to 10 weeks, M-Carn was increased by 80.1 % and TWD by 16.2 % Sale et al. (2011) confirmed these findings



Footnotes

1. The 30 s cycle sprint followed 5 min after a 10 min time trial which itself followed immediately after a 110 min simulated cycle race.
2. As discussed in the text, comparison of treatment groups with Students t-test for unpaired means showed a significant effect of β-alanine supplementation (P=0.034).
3. Isometric exercise was claimed to have been performed at 45% MVIC but with pre-supplementation endurance times of 173±55 s and 201±48 s the recalculates MVIC is circa 25-30%, at which level little or no occlusion of blood flow will occur (Ahlborg *et al.*, 1972).
4. Analysis was carried out on combined results from athletes habitually training over different distances, which may have affected the statistical outcome.

Fig. 2 A schematic showing the studies to date examining the effect of β-alanine supplementation on exercise performance and capacity. Studies have been separated by the duration of the exercise test employed, grouped as those lasting <60, 60–240 and >240 s. Those in *red* are where no effect of β-alanine supplementation was shown compared with placebo, those in *amber* showed a trend towards a positive effect, and those in *green* showed a significant beneficial effect of β-alanine supplementation

following 4 weeks of supplementation and also showed improvements in TWD with β-alanine (14.6 %), sodium bicarbonate (6.9 %), and co-supplementation of β-alanine and sodium bicarbonate (18.9 %), which gives credence to the suggestion that the improvements were due to gains in H^+ buffering capacity.

Derave et al. (2007) showed no effect of increased M-Carn on isometric endurance of the knee extensors contracting at 45 % of maximal voluntary isometric contraction (MVIC) force in trained sprinters. However, baseline contraction times were 173 ± 55 and 201 ± 48 s for the placebo and β-alanine groups, compared with a predicted time of ~78 s (Ahlborg et al. 1972). At 45 % MVIC, circulation to the contracting muscles is largely occluded due to the increase in intramuscular pressure with minimal efflux of lactate and H^+ from the contracting muscle (Ahlborg et al. 1972). This suggests that MVIC sustained by participants in the Derave et al. (2007) study

was very much lower than 45 %, resulting in minimal lactate accumulation. More recently, Sale et al. (2012) repeated this exercise test with recreationally active participants and in this case showed baseline data within 3–4 s of the expected endurance time of 78 s. Endurance time was increased by 9.7 s (13.2 %) following 4 weeks of supplementation at 6.4 g d^{-1}, likely due to an increase in muscle buffering capacity based upon the expected increase in muscle H^+ formation and increase in M-Carn.

Hobson et al. (2012) in their meta-analysis concluded that exercise lasting between 60 and 240 s was likely to be positively influenced by an increase in muscle buffering capacity, although not all agree. Bellinger et al. (2012) showed no effect of β-alanine on 4 min all-out cycle performance in trained athletes, despite individuals improving their performance with sodium bicarbonate. Similarly, there was no effect on two runs to exhaustion at 115 and 140 % VO_{2max} lasting approximately 70 and 150 s (Jagim et al. 2013). Recently, Smith-Ryan et al. (2012) evaluated the effect of β-alanine supplementation on aerobic and anaerobic exercise capacity using three runs to exhaustion at 110, 100 and 90 % of peak velocity obtained during an incremental running test. Fifty participants (24 men and 26 women) were recruited and supplemented for 4 weeks with either β-alanine (4.8 g d^{-1}) or placebo. There was no effect of β-alanine on aerobic or anaerobic exercise capacity or on TTE during any of the runs. However, despite the authors reporting a good reliability of the test, results may have been affected by the fact that the determination of critical velocity and anaerobic running capacity was based upon exercise bouts conducted on the same day with only 15-min recovery between bouts, compared with the more appropriate and accurate method of determining this model based upon exercise bouts performed on discrete days.

Exercise >240 s in duration

Several recent studies have investigated the effect of β-alanine supplementation on 2,000 m rowing ergometry in well-trained male rowers, where the exercise duration is typically between 6 and 7 min and with an estimated 12 % of energy sources from non-oxidative glycolysis (Stellingwerff et al. 2011). Baguet et al. (2010) recruited 18 elite Belgian rowers who performed a 2,000-m rowing test pre and post 7 weeks of supplementation with either 5 g d^{-1} β-alanine or placebo. Results showed a 2.7 ± 4.8 s improvement in performance with β-alanine, which only just failed to reach statistical significance ($P = 0.07$), although the individual increases in rowing speed were positively correlated to the increases in M-Carn. Ducker et al. (2013) showed an improvement of 2.9 ± 4.1 s in well-trained rowers ($N = 7$) supplemented with 80 mg kg^{-1} BM d^{-1}, compared with placebo-supplemented participants ($N = 9$) who were 1.2 ± 2.9 s slower, and again this just failed to reach significance ($P = 0.06$), although $P = 0.034$ when just the two performance times were compared using an unpaired t test. Unpublished observations from our research group (Hobson et al.) used magnitude-based inferences to determine small effects of β-alanine supplementation of practical importance in the applied setting. A 6.4 ± 8.1 s improvement in rowing performance with β-alanine over placebo was *very likely* to have been due to β-alanine. Taken together, these data highlight the need for greater participant numbers and the potential for differences in outcome depending upon the statistical approach taken.

High-intensity intermittent and repeated sprint exercise

Several studies have investigated the effect of β-alanine supplementation on intermittent repeated bouts of exercise, due to increasing interest in repeated sprint activities, such as team sports. Most of this research has shown no effect on repeated sprints alone (Hoffman et al. 2008a; Sweeney et al. 2010), or repeated sprint performance throughout simulated games play (Saunders et al. 2012b). However, the exercise durations of these protocols may have been insufficient to be positively influenced by increased concentrations of M-Carn. Further to this, Saunders et al. (2012a) employed the YoYo Intermittent Recovery Test Level 2 (YoYo IR2) which determines an individual's ability to repeatedly perform and recover from high-intensity exercise. Following a 12-week supplementation period of 3.2 g d^{-1} β-alanine or placebo, male amateur footballers supplemented with β-alanine showed a 34.4 % improvement in performance contrasting with the 7.3 % reduction in performance in the placebo group. Likewise, unpublished observations from our group have demonstrated that 4 weeks of β-alanine supplementation (6.4 g d^{-1}) was capable of inducing a significant 7 % improvement in high-intensity intermittent performance (four bouts of 30 s Wingate test for the upper body with 3 min recovery between bouts) in highly trained combat athletes.

Applied exercise

Derave et al. (2007) supplemented sprint trained athletes for up to 5 weeks with β-alanine and showed no effect on 400 m running performance (lasting ~52 s). However, recently Painelli et al. (2013) have shown performance improvements of 1–2 % in highly trained Brazilian junior-standard swimmers in 100 and 200 m freestyle swimming following supplementation with 3.2 g d^{-1} for 1 week followed by 6.4 g d^{-1} for 3 weeks of β-alanine. This is in contrast to Chung et al. (2012) who supplemented trained

swimmers (50–400 m) throughout training and competition with unclear effects on both competition and training performance. Overall, these studies suggest that the effect of β-alanine supplementation on performance for highly trained individuals at competitive events remains equivocal and requires further research.

Exercise and training

It has been suggested that training might increase M-Carn through repeated exposure to acidosis and hypoxia. However, Mannion et al. (1994) failed to show any effect of 16 weeks of isokinetic knee extension training on M-Carn, and similarly, Kendrick et al. (2008) showed no effect of 10 weeks of strength training or 4 weeks of isokinetic training (Kendrick et al. 2009) on M-Carn in Vietnamese sports science students. An apparent increase in M-Carn, however, would be expected if training increased the proportion of type 2 fibres, or even the cross-sectional area of these (denoting the volume occupied by such fibres in muscle biopsies or voxel sample volumes in ^{1}H-MRS scans), since type 2 fibres have close to twice the M-Carn content of type 1 fibres. An actual increase in M-Carn with training alone would almost certainly require an increase in hepatic β-alanine synthesis since this is clearly the limiting factor for M-Carn synthesis in the absence of supplementation (Kendrick et al. 2008, 2009). This emphasises the need to measure M-Carn in different fibre types in training studies.

More recently, Baguet et al. (2011) had 20 non-vegetarian students complete a sprint training protocol while following a vegetarian or mixed diet for 5 weeks. M-Carn remained stable in the gastrocnemius lateralis and tibialis anterior over the 5-week training period, although a non-significant increase in M-Carn in the soleus with the mixed diet (+11 %) and a non-significant decrease with the vegetarian diet (−9 %) suggested that changes in M-Carn were due to the dietary intake of β-alanine as opposed to the sprint training programme. However, this study is weakened by the lack of single fibre determinations of type and M-Carn.

High-intensity interval training (HIIT) results in a large accumulation of metabolites, some of which may provide the mechanism for training adaptation, including the ability to delay acidosis (Weston et al. 1997). Therefore, combining HIIT and β-alanine supplementation may result in additive gains, although Smith et al. (2009a) showed no additional effect of β-alanine on fatigue and efficiency of electrical activity over a placebo. Similarly, Walter et al. (2010) showed no added benefit of β-alanine supplementation to HIIT on VO_{2peak} during a graded cycling exercise. Without knowing the extent of the changes in M-Carn with β-alanine supplementation and/or HIIT, it is impossible to explain these findings, although the training protocol used in these studies may have provided a superior stimulus, given the untrained nature of the participants. Smith et al. (2009b) showed VO_{2peak} and time to exhaustion were improved during graded cycling exercise at 3 weeks in both β-alanine and placebo groups, although further increases from 3–6 weeks were only observed in the β-alanine group. In addition, both groups showed improvements in total work done during a 110 % VO_{2peak} test over the study. These results suggest the potential for β-alanine supplementation to further enhance the benefits of HIIT, although further research is undoubtedly required.

Mechanisms for increase in performance

Currently, the most likely explanation for the potential ergogenic effects of β-alanine supplementation during exercise relates to the role of M-Carn in pH_i buffering. The effect of M-Carn on intracellular buffering is undisputable, as we have previously discussed (Sale et al. 2010). Further indirect evidence for this role comes from comparative physiology across species where M-Carn levels (or muscle histidine containing dipeptide levels) are shown to be highest in animals that depend upon their ability to sustain high-intensity exercise for survival (e.g. deer or hunting dogs) or those surviving in conditions where their muscles are exposed to significant hypoxia (e.g. diving mammals) and where variations in the β-alanyl-histidine content account for most of the variation in muscle buffering capacity (Harris et al. 1990). Similar indirect evidence is also provided by current β-alanine supplementation studies, as indicated above, that have predominantly shown significant effects of supplementation in exercise tests lasting 60–240 s where H^+ accumulation is likely to be at its highest and where this is a more likely cause of fatigue than with shorter or much longer exercise durations.

Despite this, there remains some question over the extent to which H^+ production is the cause of muscle fatigue during high-intensity exercise (Allen et al. 2008), with some even suggesting that H^+ production during high-intensity exercise might be protective against fatigue. Overgaard et al. (2010) have recently indicated that acidification can hinder dynamic contractile function, but only in non-fatigued muscle. When muscles were exposed to high extracellular potassium (K^+), acidification seemed to have a protective effect against the K^+ induced loss of dynamic muscle function. However, it should be noted that this was shown after 30-min exposure of the muscle to extracellular K^+ while the exposure during short-term high-intensity exercise is likely to result in less depolarisation than that observed in the study by Overgaard et al.

(2010). As such, increased extracellular K^+ is likely to be less important during short duration high-intensity exercise (Overgaard et al. 2010). This study, taken together with other studies showing similar effects (please see Overgaard et al. 2010 for references), would appear to suggest that moderate acidification during exercise does not have a significant effect on muscle fatigue. Estimates of pH_i following high-intensity exercise obtained using the muscle biopsy technique have recorded pH values around 6.8 (Mohr et al. 2004) indicating moderate acidification of the muscle; although others have reported values as low as 6.5 (Costill et al. 1984) using muscle biopsy and 6.0 using ^{1}H-MRS (Pan et al. 1991). It might be that pH is limiting to high-intensity exercise performance but only when intracellular acidification is more pronounced. Either way, the interaction between increased extracellular K^+ levels and intracellular acidification is a worthy area for further investigation. It might be of interest to examine the influence of β-alanine supplementation on exercise performance when extracellular K^+ concentrations are elevated, since in this case, following the theory outlined above, performance should actually be reduced under conditions of an elevated M-Carn content.

There also remains some level of uncertainty as to the contribution of carnosine to muscle buffering capacity, and consequently the relative increase in this with a given increase in M-Carn. Mannion et al. (1995) suggested that M-Carn only contributed 7 % to total buffering capacity, although this is likely a minimum estimate based upon muscle with a metabolic composition close to that of rigor mortis. The relative contribution from M-Carn is usually based upon 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; Mannion et al. 1994), although this technique remains fundamentally flawed with the result that the contribution of M-Carn is significantly underestimated. This flaw relates to the hydrolysis of phosphorylcreatine upon homogenisation and the measurement of muscle buffering capacity without complimenting this with measurements of inorganic phosphate, phosphorylcreatine, adenosine triphosphate, glucose-6-phosphate, fructose-6-phosphate and glycerol-1-phosphate to determine the effect of the altered phosphate profile in homogenates. Homogenisation of muscle by the procedure used causes a total loss of phosphorylcreatine (pKa of the phosphate 4.58) and adenosine triphosphate (pKa 6.1), with the phosphate reappearing as inorganic phosphate (pKa 6.8), hexose-monophosphates (pKa's $\sim$6.1) and glycerol-1-phosphate (pKa $\sim$6.5). As such, it is likely that this method drastically overestimates the muscle buffering capacity and, thus, underestimates the potential contribution of M-Carn.

Although we believe at the current time that the most likely explanation for the potential ergogenic effects of β-alanine is the effect of the increased M-Carn on muscle buffering, this does not exclude the possibility of other potential mechanisms. Indeed, some (Dutka and Lamb 2004) have suggested a potential role for carnosine in increasing the sensitivity of the muscle fibres to calcium (Ca^{2+}), which could potentially maintain or even increase force production and exercise capacity and performance. More recently, Everaert et al. (2013) examined the effect of longer term supplementation (8–12 weeks) with increasing doses of β-alanine and carnosine on muscle contractility in mice. β-alanine, but not carnosine, supplementation resulted in a leftward shift of the force–frequency curve in the predominantly fast-twitch extensor digitorum longus muscle but not in the predominantly slow-twitch soleus muscle. This might indicate, at least in the extensor digitorum longus, an effect mediated by improved Ca^{2+} handling, although it is unclear as to whether this was the result of improved Ca^{2+} sensitivity or enhanced Ca^{2+} release from the sarcoplasmic reticulum (Everaert et al. 2013). It is likely that the differences in results shown following β-alanine and carnosine supplementation related to the higher histidine-containing dipeptide levels achieved following β-alanine supplementation, although it should be noted that there was no carnosine dose provided that was matched to an isomolar dose of β-alanine. Interestingly, β-alanine supplementation attenuated fatigue during repeated tetani in the soleus, but not in the extensor digitorum longus. Given that the response in the soleus occurred in a well-buffered environment, the authors suggested that a mechanism other than muscle buffering might explain the findings. However, the reasons for the differences in findings between the two muscles is unclear and the results from the soleus are further confounded by the fact that the histidine-containing dipeptide content could not be determined as it was below the limit of detection.

To date, only one study has determined the effects of increasing M-Carn content on muscle fibre function in humans (Dutka et al. 2012). In this study, muscle fibres, taken from biopsies of the vastus lateralis, were mechanically skinned, with their Ca^{2+} release and contractile properties being subsequently characterised. Results indicated that increased M-Carn improves muscle performance through increased Ca^{2+} sensitivity in both fast and slow twitch muscle fibres and also through enhanced Ca^{2+} release in slow twitch fibres.

However, despite these studies there remain questions as to the extent to which the ergogenic effect of increased M-Carn is explained by improved Ca^{2+} handling. First, if Ca^{2+} handling were the main mechanism supporting an effect, positive effects of β-alanine supplementation on exercise performance and capacity might be expected

across a much wider range of exercise durations than has currently been shown. Second, H^+ can compete with Ca^{2+} at the troponin-binding site, thereby limiting the ability of the muscle contractile machinery to operate effectively (Donaldson and Hermansen 1978). As such, it is possible that any improvement in Ca^{2+} handling could occur subsequent to an improvement in pH buffering, rather than being an alternative to it. Third, and perhaps most importantly, the biochemical mechanism by which elevated M-Carn mediates improvements in Ca^{2+} handling is yet to be elucidated.

A further possible mechanism for the ergogenic properties of increased M-Carn is the protection against the exercise-induced increases in reactive oxygen species production within the muscle. However, there are limited data to support this hypothesis, particularly in humans. Dawson et al. (2002) reported that 4 weeks of β-alanine supplementation reduced lipid peroxidation in the extensor digitorum longus muscle of rats following 90 min of downhill running, although there was no effect in the gastrocnemius. In one of the few human exercise studies on this topic to date, Smith et al. (2012) examined the effects of supplementing 4.8 g d^{-1} of β-alanine over 4 weeks on markers of oxidative stress before and after 40 min of treadmill running in women. β-alanine supplementation at this dose did not confer any real protection against exercise-induced reactive oxygen species production. As with the suggested mechanism involving improved Ca^{2+} handling, the potential antioxidant function of carnosine has been well established in vitro, but not so much in vivo. As such, the current biochemical explanation of how carnosine might act as an antioxidant in vivo is missing and any effect following high-intensity exercise might also be explained as a secondary effect to pH buffering, resulting from a reduction in uric acid formation. In addition, there is some debate as to the true relevance of reactive oxygen species production during exercise, particularly if this does not overwhelm the antioxidant defences and given that Reid (2001) has suggested that the accumulation of low levels of reactive oxygen species might have positive effects on muscle force output.

Therapeutic effects of carnosine

In addition to the potential role in improving exercise performance, more recently attention has spread to the potential therapeutic benefits of carnosine. Despite studies dating back over two decades (Boldyrev 1992), there remains a paucity of clinical data supporting the use of carnosine in medicine, evidencing a large gap between the experimental and clinical findings. Carnosine has been considered a natural scavenger/suppressor of reactive oxygen species, advanced glycation end products and reactive aldehydes (Boldyrev 1993; Hipkiss et al. 1995; Babizhayev et al. 1994). Such properties may confer therapeutic effects, particularly in those conditions characterised by exacerbated oxidative stress, including neurodegenerative diseases, cancer, diabetes and senescence.

It has been shown that carnosine can increase the lifespan of mice (Yuneva et al. 1999) and fruit flies (Yuneva et al. 2002) and protects rats and Mongolian gerbils against the implications of brain ischaemia (Dobrota et al. 2005). In humans, however, data are still scarce to support the protective role of carnosine in the prevention of neurodegeneration.

Fedorova et al. (2008) performed a double-blind, placebo-controlled trial to examine the therapeutic role of carnosine in patients with chronic discirculatory encephalopathy. Patients received carnosine (0.75 or 2 g d^{-1}) along with the conservative pharmacological therapy. Cognitive function was characterised using a functional brain imaging method that measures brain's electrical response to a specific sensory stimulus (i.e. P300 spikes, which typically occurs approximately 300 ms after the stimulus). After 21 days of supplementation, only the high-dose regimen was effective in significantly reducing the latency of the cognitive spikes and the number of responses with low amplitude, both being indicative of improvements in cognitive aspects of information processing. In addition, it was observed that blood lipoproteins from the patients treated with carnosine were protected from Fe^{2+}-induced oxidation, suggesting attenuated oxidative stress. However, neither blood nor tissue carnosine or in vivo oxidative stress products were measured in the study, precluding any definitive conclusion on the mechanism of action for carnosine in this disease.

Boldyrev et al. (2008) tested the efficacy of carnosine supplementation in Parkinson's disease. In an open-label trial, patients on dihydroxyphenylalanine (DOPA)-containing drugs were given carnosine supplementation (1.5 g d^{-1}) or no additive treatment for 30 days. Patients receiving carnosine in addition to their regular pharmacological treatment showed a clinical improvement of 36 % (vs. 16 % in controls), as assessed by the Unified Parkinson's Disease Rating Scale. The carnosine-treated patients also experienced improvements in physical symptoms (e.g. rigidity of extremities and upper-limb movements) by up to 38 %. The decrease in the neurological symptoms correlated with the decrease in blood serum carbonyl levels, the increase in resistance to oxidation of blood lipoproteins and the increase in superoxide dismutase activity of red blood cells. Based on their findings, the authors concluded that the "combination of carnosine with basic therapy of Parkinson's patients (…) might be a reasonable way to improve the results of Parkinson's treatment and to

decrease possible toxic effects of overloading of DOPA-containing drugs" (Boldyrev et al. 2008). However, these results must be carefully interpreted in light of the absence of a placebo-controlled double-blind design. In spite of the increasing experimental evidence showing that carnosine (and its derivatives) can ameliorate aspects of Alzheimer's disease (Hipkiss 2005; Boldyrev et al. 2003; Preston et al. 1998), no clinical trial has yet assessed the potential therapeutic role of this dipeptide in Alzheimer's patients.

Evidence also exists suggesting that carnosine can significantly inhibit tumour growth. Nagai and Suda (1986) were the first to demonstrate that carnosine administration (50 mg kg^{-1} BM d^{-1}) for 2 days was able to delay tumour growth and reduce mortality in ddY mice inoculated with Sarcoma-180 tumour cells. Supporting these findings, Renner et al. (2008) showed that carnosine inhibited the growth of cultured tumour cells isolated from human glioblastoma. A few years later, the same research team showed reduced tumour growth and mitotic replication in tumour cells of carnosine-treated mice implanted with cells expressing the human epidermal growth factor receptor 2 (Her2/neu) (Renner et al. 2010a). These data led to the speculation that carnosine could exert "anti-cancer" effects. The inhibitory effects of carnosine on cancer cell growth have been attributed to an interference with tumour glycolysis (Renner et al. 2010b), although the exact underlying mechanisms remain largely unknown. While the anti-neoplastic actions of carnosine seem to be promising (Gaunitz and Hipkiss 2012), to the best of our knowledge no clinical trial has so far been conducted. The complete understanding of the mechanism underlying carnosine-induced anti-neoplastic activity constitutes the first-step in determining the possible therapeutic role of this dipeptide in the clinical setting.

Carnosine, which has been recognized as potential glycation inhibitor, could be also useful in the treatment of diabetes. According to Hipkiss (2006), this hypothesis relies on the following observations: (i) diabetic rats have lower plasma carnosine concentrations than healthy rats; (ii) human diabetics have lower erythrocyte carnosine levels than their healthy peers; (iii) carnosine protects against acidic haemolysis in diabetic rat erythrocytes; and (iv) carnosine exerts regulatory effects on blood glucose levels in rats. Furthermore, it has been suggested that carnosine is implicated in the alleviation of some diabetic complications. For instance, it was recently shown that intra-peritoneal (100 mg kg^{-1} BM) and topically applied carnosine (100 μL) can accelerate wound healing in db/db mice, a murine model of type 2 diabetes mellitus (Ansurudeen et al. 2012). Also, it was demonstrated that carnosine (administered as eye drops) was able to prevent cataract formation in streptozotocin-induced diabetic rats (Shi et al. 2009). Moreover, diabetic nephropathy has been associated with the (CTG)n polymorphism in the carnosine dipeptidase-1 (CNDP1), which encodes the serum carnosinase enzyme, affecting serum carnosinase secretion (Janssen et al. 2005; Riedl et al. 2007). In this regard, homozygosity for the (CTG) allele has been related to lower plasma carnosinase activity and reduced risk of developing diabetic nephropathy (Janssen et al. 2005). Corroborating this finding, we recently demonstrated that type 2 diabetic patients, but not type 1 diabetic patients, have a lower M-Carn concentration as compared with their healthy counterparts (Gualano et al. 2012). Collectively, these data suggest that tissue and/or blood carnosine concentrations may have a role in diabetes physiopathology and/or diabetes complications. Whether carnosine ingestion is able to counteract diabetes symptoms in humans remains to be determined in future clinical trials.

Importantly, recent evidence has suggested that a carnosine-rich diet may have therapeutic benefits for elderly individuals. In this respect, it has been demonstrated that intramuscular carnosine content may be reduced in elderly subjects (Tallon et al. 2007), although conflicting results exist (Kim 2009). In a randomised, double-blind, placebo-controlled study, Stout et al. (2008) showed that β-alanine supplementation elicited a 28.6 % increase in physical working capacity at the neuromuscular fatigue threshold after 90 days of β-alanine supplementation (3 × 800 mg d^{-1}) in men and women aged 55–92 years. However, the authors did not assess M-Carn content. Recently, we showed that that β-alanine supplementation increased M-Carn content by ~85 % in healthy older individuals (60–80 years) with improvements in physical exercise capacity (del Favero et al. 2012). Benefits in maintaining a high M-Carn content may be both immediate and long-term, since this may encourage individuals to maintain a more active lifestyle. Taken together, these studies suggest that β-alanine supplementation represents one of the few evidenced-based dietary interventions that may help to delay the decline in muscle function with ageing. Further studies should examine the role of carnosine and its derivatives in preventing sarcopenia (e.g. muscle dysfunction and poor physical conditioning) in frail elderly individuals.

Other recent studies have suggested that carnosine might also exert anti-nociceptive (Ohsawa et al. 2012), anti-lipaemic (Aldini et al. 2011) and anti-hypertensive effects (Aldini et al. 2011), although none of these actions have been confirmed in humans.

Carnosine in medicine: the bioavailability issue and a word of caution

Even though more than a century of basic and experimental research has attributed several therapeutic properties to carnosine, it appears that some of the message has been

"lost in translation" when it comes to the actual clinical benefits of this dipeptide. For instance, a variety of in vitro and experimental studies have suggested that carnosine has strong antioxidant properties. In contrast, there is weak evidence, if any, in support of this effect in humans, decreasing the enthusiasm for the therapeutic application of this compound. Certainly, carnosine bioavailability is a major issue that must be resolved in human studies. In this regard, several studies have failed to detect any increase in plasma carnosine following carnosine, β-alanine, beef, chicken broth or chicken breast ingestion (Harris et al. 2006; Yeum et al. 2009). Conversely, Everaert et al. (2012) showed that eight out of 25 participants displayed a measurable increase in plasma carnosine up to 1 h after acute carnosine supplementation (60 mg kg^{-1} BM). Interestingly, participants with no measurable increment in plasma carnosine had twofold higher plasma carnosinase protein content and 1.5-fold higher activity when compared with those who had detectable increases in plasma carnosine. The authors speculated that higher carnosinase activity could impede the ability of carnosine to exert its protective effects against cytotoxic agents (e.g. glycation, reactive oxygen species, advanced glycation end products), whilst lower carnosinase activity and, thus, higher plasma carnosine levels could confer protection against oxidative stress and hyperglycemia. However, a question remains as to whether such a small (range in "responders": 30.7–195 μmol L^{-1}, in contrast to mmol kg^{-1} levels in skeletal muscle) and short-term increase in plasma carnosine levels in response to an oral carnosine challenge would be sufficient to promote any significant biological effect. In contrast, there is evidence supporting the concept that carnosine may not have such a short half-life in blood. Park et al. (2005) showed in humans that peak plasma carnosine (32.7 g L^{-1} or 144 μmol L^{-1}) was attained 2.5 h after the ingestion of 200 g of beef and that only 5.5 h after beef ingestion could carnosine no longer be detected in plasma. In addition, Gardner et al. (1991) showed that up to 14 % of the ingested carnosine could be recovered intact in urine during the first 5 h following ingestion.

In order to circumvent the bioavailability problem (i.e. the presence of serum carnosinase and the blood–brain barrier), there have been efforts to develop carnosine derivatives, which are comprehensively reviewed by Bellia et al. (2012). Carnosinase resistance has been reported for most of the carnosine conjugates. Moreover, some of these derivatives are capable of exerting antioxidant effects comparable or superior to the natural dipeptide (Bellia et al. 2012). Nonetheless, it is currently unknown whether these carnosine derivatives possess any biological effects or therapeutic benefits to humans.

Another question remains as to whether supplementary carnosine (or its natural constituents and derivatives) can penetrate the blood–brain barrier and, hence, promote neurologic effects in humans. However, ^{1}H-MRS techniques can detect the homocarnosine signal (c-aminobutyryl-L-histidine), with regional concentrations in human brain ranging from 0.3 to 0.6 mmol kg^{-1} ww (Perry et al. 1979, 1981). Immunohistological studies for homocarnosine suggest a neuronal localization in human brain, most likely in subclasses of GABAergic neurons. Like its β-alanine analogue, carnosine, the histidine part of the dipeptide is sensitive to pH with a pKa in the physiological range (6.86; Rothman et al. 1997). Our research group is currently examining whether brain homocarnosine levels are sensitive to dietary carnosine. The search for a marker of brain carnosine in humans, even if indirect, is of utmost relevance for the advancement of applications for carnosine in neurology.

Conclusion

Today, the global production of β-alanine for use as a dietary supplement is believed to amount to 100,000 kg annually (figures supplied by Natural Alternatives International, San Marcos, California, US). The effect of β-alanine supplementation on exercise performance and capacity remains a worthy area of investigation, highlighted by the dramatic increase in the number of recent studies. Overall, the balance of available evidence supports a positive effect of β-alanine supplementation on exercise capacity, with more work still required in relation to exercise performance effects. In a meta-analysis of the available literature, Hobson et al. (2012) showed that the median overall effect of β-alanine supplementation was a 2.85 % (−0.37 to 10.49 %) improvement in the outcome of an exercise test, when 179 g of β-alanine was supplemented. It was also concluded that β-alanine supplementation was effective in improving high-intensity exercise of durations between 60 and 240 s ($P = 0.001$) and in excess of 240 s ($P = 0.05$), but not less than 60 s ($P = 0.3$).

The most likely mechanism for the ergogenic effect of β-alanine supplementation is the increase in muscle buffering capacity conferred by an increase in M-Carn. However, other potential mechanisms exist, including improved Ca^{2+} handling and an increased antioxidant capacity with increased M-Carn. Clearly there is far more work needed to precisely determine the mechanism(s) by which increased M-Carn concentrations, particularly following chronic supplementation with β-alanine, might influence high-intensity exercise performance and capacity. It is of course possible that the true mechanism behind the ergogenic potential of increased M-Carn involves a complex interaction between muscle buffering, improved Ca^{2+} handling and reduced reactive oxygen species production.

Well conducted in vivo studies in humans are required to determine the true mechanism and the extent of these potential interactions.

There is some encouraging evidence to suggest that carnosine might have some therapeutic benefits, although this line of investigation is very much in its infancy. Whilst preliminary studies in humans have suggested that the increase in M-Carn results in clinical benefits for specific populations (e.g. the elderly), other claims of therapeutic potential, particularly regarding antioxidant effects, remain to be proven in humans. The few existing studies are flawed by very small sample sizes, lack of a placebo-control, a short-term follow-up, and absence of mechanistic evaluations. Indeed, further high-quality large-scale randomised clinical trials are imperative to narrow the gap between experimental and clinical research in this area.

Conflict of interest The authors declare that they have no conflict of interest.

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