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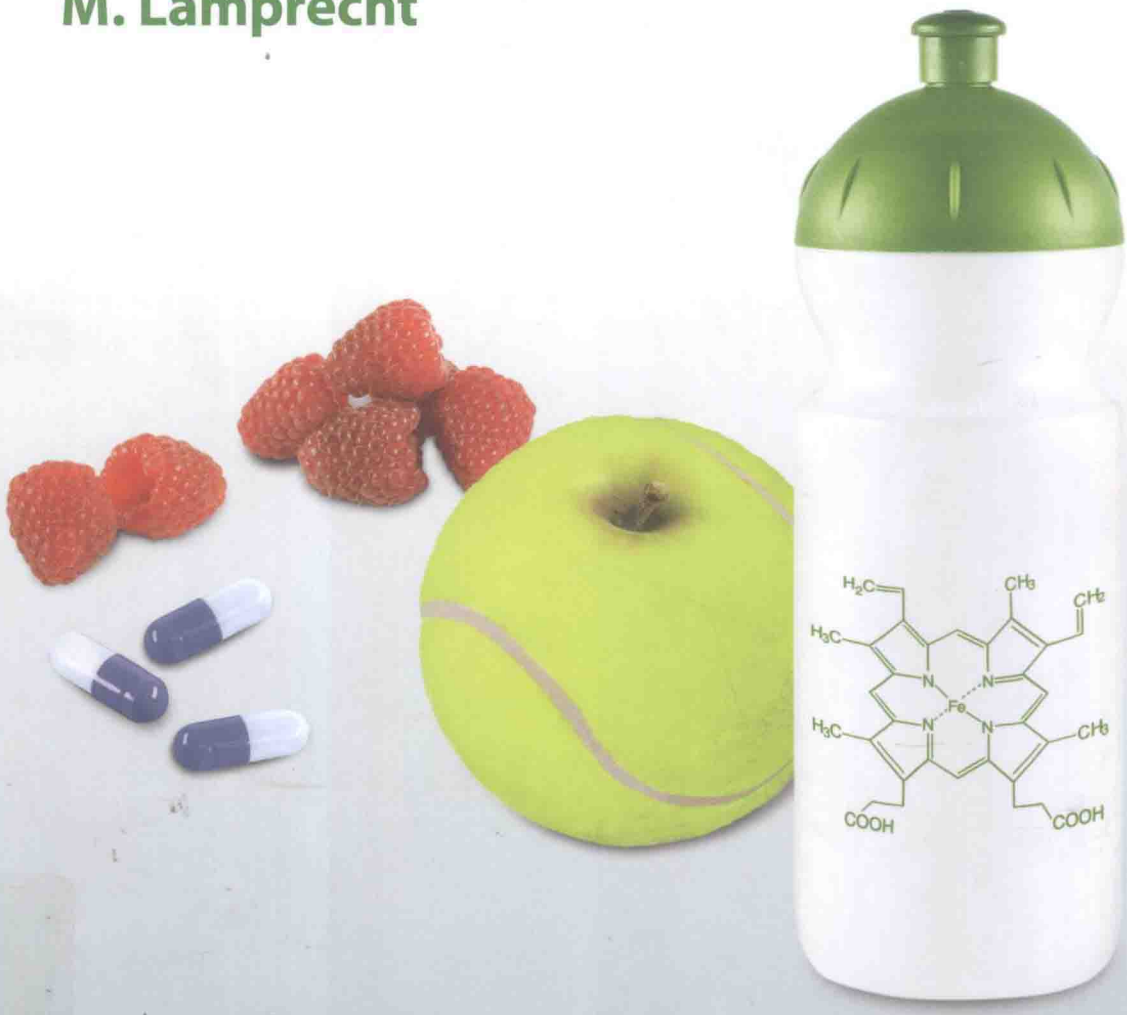

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Acute Topics in Sport Nutrition

Volume Editor

Manfred Lamprecht Graz

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Preface

In many high-performance sports, far less than one percent difference in maximum power density decides between victory and defeat. An optimal diet and nutritional interventions can make the difference between winners and losers. Hence it is plausible that athletes and their carers have also realized the importance of optimal nutrition to achieve at least a minor advantage when it comes to competitive sports. In recent years, sport nutrition research has increased, and scientific journals, university courses, advanced education, scientific societies, etc., in the field have received more and more attention. The sport nutrition trade and related industries reacted and sales and marketing for sport nutrition products increased hand in hand with the interest of the consumers.

Many scientific publications exist about the classic themes of sport nutrition, like carbohydrate, protein or vitamin intake. On the other hand, scientific articles on topics that are not mainstream are still scarce although these issues are of importance; for example, more information is needed about specific sport supplements and dietary approaches to minimize the conflicting information circulating among carers and their athletes.

This volume of *Medicine and Sport Science* is devoted to 'Acute Topics in Sport Nutrition' and provides scientifically-based information with regard to the bioefficacy of trendy sport supplements and dietary approaches away from the mainstream. Experts in specific fields have attempted to inform and clarify under which circumstances the application of certain supplements and nutritional interventions could be beneficial, either for the performance or health of the athletes. At this juncture, I would like to thank all the contributors for taking the time to provide their expertise via excellent and scientifically-based articles.

The sixteen chapters of this book refer to a broad spectrum of current topics in sport nutrition: four chapters are dedicated to selected sport supplements off the mainstream claimed to influence lactate accumulation (β-alanine; R. Harris & C. Sale), blood flow (arginine, citrulline; A. Sureda & A. Pons), oxygen consumption and mitochondrial respiration (dietary nitrate; A.M. Jones, S.J. Bailey, A. Vanhatalo), and growth hormone response (γ-aminobutyric acid, M. Powers). The chapters about probiotics (M. Lamprecht & A. Frauwallner), immunoglukan (J. Majtan), bovine

colostrum (G. Davison), fruit and vegetable concentrates (M. Lamprecht), cherry juice (K.S. Kuehl), and milk consumption plus resistance training (A.R. Josse & S.M. Phillips) refer to athletes' gut health, immune function, antioxidant potential, pain relief, and females' body composition and skeletal health. Hydration, hyperhydration and fluid balance/loading are covered by three chapters about glycerol use (S.P. van Rosendal & J.S. Coombes), salt and fluid loading (R. Mora-Rodriguez & N. Hamouti), and milk protein consumption (L. James). The chapters about chocolate milk (K. Pritchett & R. Pritchett) and L-carnitine (A. Huang & K. Owen) treat the important issue about exercise recovery. Finally, there is one special chapter – apart from certain ingredients, substances or dietary interventions – that informs about over-the-counter sport supplements and inadvertent doping (C. Judkins & P. Prock).

Sport nutrition advisors, sport physicians and scientists as well as coaches and interested athletes will benefit from the current information provided by this volume. The sport nutrition industry could draw benefits from the expert remarks in this book to create innovative ideas for the development of new and effective products. And it would also be desirable that this volume of *Medicine and Sport Science* stimulates research collaborations between sport nutrition scientists and companies in the field to improve the nutritional support to the target group that should benefit the most – the adult athlete.

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Beta-Alanine Supplementation in High-Intensity Exercise

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Abstract

Glycolysis involves the oxidation of two neutral hydroxyl groups on each glycosyl (or glucosyl) unit metabolised, yielding two carboxylic acid groups. During low-intensity exercise these, along with the remainder of the carbon skeleton, are further oxidised to CO_2 and water. But during high-intensity exercise a major portion (and where blood flow is impaired, then most) is accumulated as lactate anions and H^+. The accumulation of H^+ has deleterious effects on muscle function, ultimately impairing force production and contributing to fatigue. Regulation of intracellular pH is achieved over time by export of H^+ out of the muscle, although physicochemical buffers in the muscle provide the first line of defence against H^+ accumulation. In order to be effective during high-intensity exercise, buffers need to be present in high concentrations in muscle and have pK_as within the intracellular exercise pH transit range. Carnosine (β-alanyl-L-histidine) is ideal for this role given that it occurs in millimolar concentrations within the skeletal muscle and has a pK_a of 6.83. Carnosine is a cytoplasmic dipeptide formed by bonding histidine and β-alanine in a reaction catalysed by carnosine synthase, although it is the availability of β-alanine, obtained in small amounts from hepatic synthesis and potentially in greater amounts from the diet that is limiting to synthesis. Increasing muscle carnosine through increased dietary intake of β-alanine will increase the intracellular buffering capacity, which in turn might be expected to increase high-intensity exercise capacity and performance where this is pH limited. In this study we review the role of muscle carnosine as an H^+ buffer, the regulation of muscle carnosine by β-alanine, and the available evidence relating to the effects of β-alanine supplementation on muscle carnosine synthesis and the subsequent effects of this on high-intensity exercise capacity and performance.

Dedication

This paper is dedicated to the memory of Dr. John Wise, of Natural Alternatives International, San Marcos, California, USA, who was initially invited by the editor to be a co-author of this review, but who sadly passed away in November 2011. Dr. Wise played a key role in the early investigations of the effects of β-alanine supplementation on muscle carnosine synthesis and performance.

Abbreviations Used

$CCT_{110\%}$	cycle capacity test and 100% of power max
b.w.	body weight
dm	dry muscle
E-C	excitation-contraction coupling
G-protein	guanine nucleotide-binding proteins
^{1}H-MRS	proton magnetic resonance spectroscopy
HCD	histidine-containing dipeptide
K_m	Michaelis constant (the substrate concentration supporting a reaction rate half of maximum, in an enzyme-catalysed reaction)
Lac^-	lactate anion
H^+	hydrogen cation
M-Carn	muscle carnosine
Mrg	Mas-related gene receptors (Mrgs form a large family of G-protein-coupled receptors expressed in dorsal root ganglia and trigeminal ganglia associated with sensory neurons that detect painful stimuli)
MVIC	maximal voluntary isometric contraction
PCr	phosphorylcreatine
pH_i	intracellular pH
pK_a	acid dissociation constant
SR	sustained release
$t_{1/2}$	half-life
Td_1	defines time-delay 1 for the first monoexponential term computing respiratory oxygen uptake
$Vo_{2\,max}$	maximal oxygen uptake

Background

β-Alanine is found in muscle in combination with L-histidine forming the dipeptide, carnosine (β-alanyl-L-histidine, abbreviated in the context of the muscle content as M-Carn). Carnosine is a member of a family of three related HCDs, the others being anserine (β-alanyl-L-(1-methyl)-histidine) and balenine (β-alanyl-L-(3-methyl)-histidine. Carnosine and related HCDs are found in high concentrations in skeletal muscle of both vertebrates and non-vertebrates, as well as in the central nervous system.

Carnosine was first measured in human muscle by Bergstrom et al. [1] where it is the only HCD present. In human muscle, for instance m. vastus lateralis, the molar concentration of M-Carn varies from 4 to 20 mM (12–60 $mmol \cdot kg^{-1}$ dry muscle) making it one of most abundant small molecular weight compounds present in resting muscle after PCr (75 $mmol \cdot kg^{-1}$ dm), creatine (49 $mmol \cdot kg^{-1}$ dm) and ATP (24 $mmol \cdot kg^{-1}$ dm) [2]. Factors determining the concentration of M-Carn in humans include muscle fibre composition, with a 1.3–2 times higher concentration in type II compared to type I muscle fibres [3–6] and dietary intake of β-alanine [7]. Age and gender may also influence the carnosine content in muscle [8, 9] although the evidence for this is somewhat tenuous, at least in humans, as the

changes reported could be secondary to changes in fibre composition (including changes in mean fibre area, as a determinant of the volume occupied by each fibre type in the muscle sampled) and diet [7]. Suzuki et al. [10] reported a doubling in M-Carn with 8 weeks of training, consisting of 28 bouts of multiple 30-s Wingate tests performed on a cycle ergometer (a total exercise time over the 8 weeks of 14 min). Other studies using 4–16 weeks of intensive sprint training [6, 11], 12 weeks of whole-body training [12] and 4 weeks of unilateral cycle training [13] have, however, shown no effect on M-Carn. Chronic training, however, is associated with an increase in M-Carn [5, 14], although again this may be secondary to changes in fibre composition (and mean fibre area) and diet.

Role of M-Carn as Buffer of H^+ during High-Intensity Exercise

High-intensity exercise is associated with the formation of two carboxylic acid groups arising from the oxidation of neutral hydroxyl groups within each glucose or glycosyl (from glycogen) unit metabolised. Both carboxylic acid groups are fully dissociated over the physiological pH range, with most accumulated as Lac^- and H^+ in muscle. In reality, H^+ exist mostly in the hydrated state, bound to one or more molecules of water for instance as the hydronium ion (H_3O^+). However, for convenience the term H^+ is used to define all such states.

Lac^- production accounts for the generation of 94% of the H^+ produced in skeletal muscle during high-intensity exercise [15] causing a decline in intracellular pH (pH_i) from around 7.0 at rest [16] to as low as 6.0 [17]. H^+ accumulation may contribute to fatigue by interfering with several metabolic processes affecting force production [18]. More specifically, the accumulation of H^+ in skeletal muscle disrupts the recovery of PCr [19] and its role as a temporal buffer of ADP accumulation [20–22], inhibits glycolysis [23] and disrupts functioning of the muscle contractile machinery [24, 25]. Regulation of pH_i is achieved in the long term by export of H^+, but in the short term by a limited range of intracellular buffers which include carnosine and its methylated derivatives.

To be effective, buffers need to occur in high concentrations and to have a pK_a within the exercise pH_i transit range. Carnosine satisfies both requirements occurring in the low millimolar range in human muscle while the imidazole ring of the histidine residue exhibits a pK_a of 6.83 [26]. Other potential buffers in skeletal muscle with pK_as in or close to the pH_i transit range include proteins, inorganic and organic phosphates, and bicarbonate present in cells at the start of contraction. The contribution of proteins is limited to their respective histidine contents as no other amino acid has a side chain with an effective pK_a. Organic phosphates include nucleotide phosphates and hexose phosphates, principally glucose 6-phosphate, fructose 6-phosphate, and glycerol 1-phosphate, which accumulate with an increased glycolytic rate during intense exercise. PCr, one of the largest metabolic pools of phosphate, has a pK_a of

4.58 and does not itself contribute to buffering in the resting state. However, with the commencement of exercise, net decline in the PCr pool, matched by increases in the concentration of inorganic and organic phosphates, will increase its contribution. As a result of the changes in the PCr pool, the physicochemical buffering capacity of muscle increases with exercise, reaching a theoretical peak when all PCr has been hydrolysed.

Estimates of the importance of M-Carn to intracellular physicochemical buffering determined by acid titration of muscle homogenates [27] or by calculation [15] both indicate a contribution in human skeletal muscle (with an M-Carn concentration of ~20 mmol·kg^{-1} dm) of between 5 and 10%. However, implicit in these estimates (as a result of the methods used) is the assumption that during exercise, (1) all muscle-located proteins, both intracellular and extracellular, are available to contribute to H^+ buffering, (2) that hydrolysis of PCr is complete, and (3) that the distribution of carnosine in muscle is uniform. Point 1 is incorrect and will result in an overestimation of the contribution of non-carnosine buffers, lessening the apparent relative importance of M-Carn. In the absence of modelling for the partial hydrolysis of PCr during muscle contraction, point 2 will similarly overestimate non-carnosine buffering. With respect to point 3, the distribution of carnosine in human muscle is not uniform with the content in fast-twitch fibres being 1.3–2 times higher than in slow twitch [3, 4, 6, 28]. This will underestimate the importance of carnosine in type II fibres where its role as a pH buffer would be expected to be greatest; though equally it would overestimate its importance in type I muscle fibres.

The combination of β-alanine, a non-proteogenic amino acid (not to be confused with α-alanine found in most proteins), with histidine raises the pK_a of the imidazole ring from pH ~6.0 to ~6.8, improving its effectiveness in buffering H^+ over the exercise pH_i transit range. More importantly, combination with β-alanine renders the histidine inert to participation in proteogenesis, enabling high concentrations to be accumulated in muscle cells. This affords a more efficient means to vary the intracellular physicochemical buffering capacity than by alteration of the protein content, where histidine is only 1 of 20 amino acids. When considered along with the other HCDs, this has provided a highly efficient means for species to vary the intracellular buffering capacity of muscle by evolution, matching the exercise demands imposed by escape, combat or hunting in the wild.

Other Suggested Physiological Roles of M-Carn

Other physiological roles have been ascribed to carnosine in muscle; including protection of proteins against glycation by acting as a sacrificial peptide [29, 30], the prevention of protein-protein cross-links through reactions with protein-carbonyl groups [29, 31], acting as an antioxidant (for reviews, see Boldyrev et al. [32] and Boldyrev [33]) and increasing calcium sensitivity in muscle fibres augmenting force production

and total work done [34–38]. However, few of the ascribed physiological roles for carnosine, other than its role as a pH buffer, have been shown to occur in vivo and in humans. Indeed the majority of the work cited above has been conducted in vitro.

Arguably, the potentiation of calcium sensitivity of E-C coupling in both type I and II human muscle fibres could have relevance to muscle function in vivo but needs further characterisation in terms of field performance (strength or endurance). Lamont and Miller [34] 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 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. Dutka and Lamb [37] and Dutka et al. [38] showed that the increase in Ca^{2+} ion sensitivity on E-C coupling occurred in a concentration-dependent manner and suggested that higher concentrations of M-Carn could, by this mechanism, delay the onset of fatigue. However, it is by no means certain that Ca^{2+} ion sensitivity, and changes in this, are factors integral to fatigue in whole-body exercise[1].

Notwithstanding any additional physiological role, M-Carn and the HCDs in general remain important contributors to pH_i regulation in muscle and in practice the only means to vary the buffering capacity within and across species.

Regulation of M-Carn by β-Alanine

Carnosine in muscle is synthesised in situ by the action of carnosine synthase

ATP + L-histidine + β-alanine → AMP + diphosphate + carnosine

Synthesis of carnosine appears limited by the low concentration in muscle cells of β-alanine in comparison to the high K_m (1.0–2.3 mM) that β-alanine has for carnosine synthase [40, 41]. In contrast, histidine is present in much higher concentrations in muscle, has a much lower K_m (16.8 μM) for the synthase [42], and is unlikely to be limiting to carnosine synthesis. β-Alanine is synthesised in the liver as the final metabolite of uracil and thymine degradation [43, 44] before being transported via the blood to muscle and other tissues. To this will be added β-alanine available from carnosine and other HCDs absorbed with the ingestion of muscle meat [45–48], and

[1] Whereas there is a rationale for pH_i decrease causing muscle fatigue, and there is extensive evidence from laboratory studies in support of this, again there is a lack of evidence at the level of whole-body exercise. A possible exception to this is the study of Hultman et al. [39] where ingestion of ammonium chloride (0.3 $g \cdot kg^{-1}$ b.w.) was used to induce a moderate acidosis prior to in situ percutaneous electrical stimulation of muscle. As a result, pH_i was lower at the end of 75 s stimulation (pH 6.54 as opposed to 6.70) as was also the final force sustained (44.6% of initial compared with 55.4% without ammonium chloride.)

hydrolysed to their constituent amino acids by the action of carnosinase present in intestinal mucosa and serum [49–51]. 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 [52, 53] and exhibits a K_m of ~40 μM with respect to β-alanine [54].

In vegetarians, M-Carn is limited by hepatic synthesis of β-alanine resulting in comparatively low contents of the order of 13 mmol·kg^{-1} dm determined in muscle biopsies of m. vastus lateralis by HPLC from a subject group mostly comprising aerobically trained female UK athletes [55, 56]. Similarly, a reduced level of M-Carn in vegetarians was reported by Everaert et al. [57] with reductions of 17–26% seen in m. soleus, m. gastrocnemius and m. tibialis anterior, compared with Belgian subjects eating a mixed diet.

In omnivores, de novo hepatic β-alanine synthesis may be augmented by the hydrolysis of dietary-supplied HCDs from muscle meat resulting in M-Carn levels two or more times higher than in vegetarians [55]. Hydrolysis of dietary-supplied HCD from, for instance, the ingestion of chicken broth, has been shown to supply close to the theoretical level of the amount of β-alanine present in the bound HCD form [48]. Direct supplementation of the diet with β-alanine over a period of weeks will similarly increase M-Carn. In the first of a series of studies [48] where multiple doses of 800 mg β-alanine were given per day over 4 weeks in gelatine capsules, the mean increase in M-Carn in m. vastus lateralis was 60%. When extended to 10 weeks, the increase was 80% with absolute values now close to 40 mmol·kg^{-1} dm [4]. A maximal single dose of 800 mg, on average 10 mg·kg^{-1} b.w., was used in these studies to avoid symptoms of paraesthesia, and is equivalent to the amount of β-alanine available from the ingestion of 150 g of chicken breast meat, assuming hydrolysis of the HCDs present. A maximum of 8 such doses was given in a single day without any negative effects such as increased feelings of paraesthesia, clinical chemistry or ECG. Supplementation with L-carnosine itself resulted in the same increase in muscle over 4 weeks of supplementation as an isomolar dose of β-alanine, showing no additional effect of the histidine also released on hydrolysis [48].

With curtailment of supplementation, M-Carn returns to the presupplementation level in m. vastus lateralis with an estimated $t_{1/2}$ of ~9 weeks [58, 59]. Estimates of $t_{1/2}$ following elevation of M-Carn in m. anterior tibialis, m. gastrocnemius and m. soleus range from 5 to 8 weeks [59–61]. No information is available on the rates of decline of M-Carn specifically in types I and II fibres; a faster rate of decline could possibly explain the lower M-Carn content observed in type I fibres. The mechanism governing the loss of M-Carn accumulated during supplementation is unknown, but the possibilities are a slow release from muscle fibres or destruction within the fibres due to reaction with free radicals or carbonyl groups [62, 63].

In equines there is no measureable loss of M-Carn with acute exercise [64]. However, exercise-induced muscle damage may result in temporarily raised plasma

concentrations in equines where plasma carnosinase is absent [65] but the increases are of an order which would be barely measureable as a change in M-Carn in muscle. No comparable measurements have been performed in humans, although it is clear that there is no progressive loss of M-Carn with chronic training [6, 11].

Single doses of β-alanine in excess of 10 $mg \cdot kg^{-1}$ b.w., or the equivalent molar dose of L-canosine, administered in solution as a drink or in gelatine capsules cause symptoms of flushing together with a prickly sensation affecting (in approximate order of occurrence) the face and ears, neck and shoulders, arms, hands and upper trunk, and lower trunk [48]. Symptoms, also termed paraesthesia, last 15–60 min and in terms of severity appear dose-dependent [66]. At 10 $mg \cdot kg^{-1}$ b.w., only very mild symptoms may be experienced by a small percentage of subjects but at 40 $mg \cdot kg^{-1}$ b.w. severe and uncomfortable symptoms are experienced by most.

Several possible mechanisms exist to account for the symptoms of paraesthesia, including β-alanine activation of strychnine-sensitive glycine receptor sites, associated with glutamate sensitive N-methyl-D-aspartate receptors in the brain and central nervous system [67–69] and activation of the Mrg (mas-related gene) family of G-protein-coupled receptors, which are triggered by interactions with specific ligands, such as β-alanine [70]. MrgD-containing dorsal route ganglia neurons terminate in the skin, but not in blood vessels, muscle, or other major internal organs and participate in the modulation of neuropathic pain. To paraphrase Crozier et al. [70], 'neuropathic pain is qualitatively different from ordinary pain and is usually perceived as a steady burning, pins and needles and/or electric shock sensation'. This is an accurate description of the symptoms experienced by subjects reporting sensations of paraesthesia after taking high doses of β-alanine or L-carnosine.

To circumvent symptoms of paraesthesia, early β-alanine supplementation studies used a maximum single dose of 800 mg, corresponding to ~10 $mg \cdot kg^{-1}$ b.w., and administered up to 8 times a day to give a total dose of 6.4 g [4, 48]. More recent supplementation studies have used a sustained release formulation (CarnoSyn™ SR, from Natural Alternatives International, San Marcos, Calif., USA, available to the public as High Intensity™, from Power Bar, Florham Park, N.J., USA) enabling two 800-mg SR tablets to be given simultaneously without symptoms of paraesthesia [59, 71–74].

Ergogenic Effect of Raised M-Carn

As reviewed by Hobson et al. [75], a number of studies have demonstrated a significant effect of β-alanine-induced M-Carn elevation on exercise performance. In this meta-analysis, 15 published peer-reviewed studies were assessed, from which it was concluded that exercise lasting 60–240 s was improved ($p = 0.001$) with β-alanine supplementation, as was exercise of >240 s ($p = 0.046$). In contrast, there was no benefit of β-alanine supplementation on sprint exercise lasting <60 s ($p = 0.312$), consistent with the mechanism for the improvement in performance being linked to an

increase in H^+ buffering capacity, as opposed to an effect of increased Ca^{2+} ion sensitivity on E-C coupling.

The median effect of β-alanine supplementation on exercise >60 s was a 2.85% (range –0.37 to 10.49%) improvement in exercise capacity. Of particular note was the study of Hill et al. [4] which used a $CCT_{110\%}$ output with an expected endurance time of 150 s, where 4 weeks of β-alanine supplementation resulted in a ~60% increase in M-Carn and a 11.8% improvement in endurance time. This was subsequently repeated by Sale et al. [71], using the same exercise protocol, when an increase of 12.1% in endurance time was recorded. Evidence that the effect of β-alanine supplementation was due to an increase in H^+ buffering capacity was suggested by a positive gain in performance (+6.5%) when subjects were supplemented with sodium bicarbonate (0.3 $g \cdot kg^{-1}$ b.w.), and with the combination of β-alanine and sodium bicarbonate resulting in a 16.2% increase in cycling capacity.

In three studies where both performance measures have been made along with measurements of M-Carn, improvement in exercise capacity was positively correlated with the increase in M-Carn. The exercise modalities included cycling with an expected pre-supplementation endurance time of 150 s [4], rowing over a distance of 2,000 m [76], and time-to-exhaustion in a constant-load submaximal test and incremental test in 60- to 80-year-old subjects [72]. The demonstration of a positive cause-and-effect relationship between increased M-Carn and performance, in these three studies, is almost unique in studies of dietary supplements.

A positive effect of β-alanine supplementation on isometric endurance of the knee extensors contracting at 45% of MVIC force has recently been reported by Sale et al. [77]. Lac^- plus pyruvate accumulation by the time of fatigue varies with the % MVIC sustained, reaching a peak at 45% MVIC [78]. As the circulation to the contracting muscles is largely occluded at this intensity by the increase in intramuscular pressure, Lac^- and H^+ loss from the contracting muscle is minimal. Based upon this, the expected endurance time at 45% MVIC is around 78 s [78], with baseline data from Sale et al. [77] being within 3–4 s of this. Endurance time after 4 weeks of β-alanine supplementation at 6.4 $g \cdot day^{-1}$ was increased by a mean of 9.7 s (13.2%) with the estimated increase in H^+ production (estimated from the known rate of Lac^- plus pyruvate production at this intensity [78] closely matched to the increase in H^+ buffering capacity from the estimated increase in M-Carn (estimated from the changes observed in Hill et al. [4] and Kendrick et al. [6]). Whilst isometric exercise is involved in a number of sports (e.g., rock climbing and dingy sailing), it is more often encountered in everyday manual activities involving the lifting and carrying of heavy weights.

Effect on Maximal/Supramaximal Exercise

Since completion of the meta-analysis by Hobson et al. [75], three further studies have been published on the effects of β-alanine supplementation on maximal/