

Role of β -Alanine Supplementation on Muscle Carnosine and Exercise Performance

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ABSTRACT

ARTIOLI, G. G., B. GUALANO, A. SMITH, J. STOUT, and A. H. LANCHI, JR. Role of β -Alanine Supplementation on Muscle Carnosine and Exercise Performance. *Med. Sci. Sports Exerc.*, Vol. 42, No. 6, pp. 1162–1173, 2010. In this narrative review, we present and discuss the current knowledge available on carnosine and β -alanine metabolism as well as the effects of β -alanine supplementation on exercise performance. Intramuscular acidosis has been attributed to be one of the main causes of fatigue during intense exercise. Carnosine has been shown to play a significant role in muscle pH regulation. Carnosine is synthesized in skeletal muscle from the amino acids L-histidine and β -alanine. The rate-limiting factor of carnosine synthesis is β -alanine availability. Supplementation with β -alanine has been shown to increase muscle carnosine content and therefore total muscle buffer capacity, with the potential to elicit improvements in physical performance during high-intensity exercise. Studies on β -alanine supplementation and exercise performance have demonstrated improvements in performance during multiple bouts of high-intensity exercise and in single bouts of exercise lasting more than 60 s. Similarly, β -alanine supplementation has been shown to delay the onset of neuromuscular fatigue. Although β -alanine does not improve maximal strength or $\dot{V}O_{2\max}$, some aspects of endurance performance, such as anaerobic threshold and time to exhaustion, can be enhanced. Symptoms of paresthesia may be observed if a single dose higher than 800 mg is ingested. The symptoms, however, are transient and related to the increase in plasma concentration. They can be prevented by using controlled release capsules and smaller dosing strategies. No important side effect was related to the use of this amino acid so far. In conclusion, β -alanine supplementation seems to be a safe nutritional strategy capable of improving high-intensity anaerobic performance. **Key Words:** BUFFER CAPACITY, FATIGUE, PHYSICAL CAPACITY, SKELETAL MUSCLE

Muscular fatigue is a complex multifactorial phenomenon that is still not completely understood. Some of the putative causes of fatigue include inhibition of energy production enzymes, decreased calcium (Ca^{++}) sensitivity of the contractile apparatus, decreased Ca^{++} release and/or reuptake from the sarcoplasmic reticulum, and depletion of energy substrate (3,60,61). During high-intensity short-term exercise, intramuscular accumu-

lation of several metabolites, such as adenosine diphosphate, inorganic phosphate, lactate, and hydrogen ions (H^+) (16,21,41), occurs. At present, the specific role of these metabolites on the onset of muscular fatigue is in debate (14,47,62). Although early evidence has indicated a negative association between lactate concentration and force production during muscular fatigue (28), others have suggested that lactate *per se* does not have a direct negative effect on contractile function or energy provision (17,27). It is argued that H^+ accumulation, which results from carboxylic group formation through glycolytic pathway rather than lactate, may inhibit glycolytic enzymes and impair several steps of contractile process (27). Furthermore, other authors minimize the role of intramuscular acidosis on the onset of muscular fatigue and propose an alternative mechanism in which inorganic phosphate accumulation may be the primary cause of decreased performance in high-intensity exercise (75).

Although some studies examining isolated muscle have suggested little detrimental effects of acidosis on muscle

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TABLE 1. pK_a values of imidazole-containing compounds, bicarbonate, and inorganic phosphate according to Abe (1) and Bate-Smith (9).

Compound	pK _a
Histidine in proteins	6.5
Free L-histidine	6.21
Carnosine	6.83–7.01
Anserine	7.15
Balenine	6.93
Bicarbonate	6.37
Inorganic phosphate	6.88–7.20

fatigue (4,17,75), several other studies have demonstrated that induced acidosis can exacerbate fatigue during whole-body dynamic exercise and induced increases in buffering capacity may be capable of improving short-term high-intensity exercise performance (5,12,15,19,22,23,31,36,41,50,51,58,67,68,76). Thus, it is generally assumed that decreased intracellular pH decisively contributes, directly or indirectly, to the failure of maintaining performance during anaerobic exercises, and therefore any strategy that could enhance acid–base balance would be potentially ergogenic.

Mammals have a well-regulated system that keeps intracellular and extracellular pH within the normal physiological range. This system includes chemical blood buffers, respiratory, and renal acid–base regulations. The blood buffering system is constituted with bicarbonate, phosphates, proteins, and amino acids. Bicarbonate is the most important extracellular buffer, whereas phosphates (especially H₂PO₄⁻ and HPO₄²⁻) exert their buffering function inside the cells,

where the pH is near the phosphates pK_a. The active transport of H⁺ outside the cells plays another relevant role in the maintenance of acid–base homeostasis. Finally, the buffering activity of proteins, peptides, and amino acids occurs inside the cell and is undertaken only by the molecules that display imidazole groups (1).

The imidazole chemical group is found in histidine residues of proteins, in free L-histidine molecules, and in the dipeptides containing L-histidine such as carnosine (β-alanylhistidine), anserine (β-alanyl-L-methylhistidine), and balenine (β-alanyl-3-methylhistidine) (1). All compounds containing an imidazole group may potentially act as intracellular buffers because the pK_a of imidazole group is near the physiological pH range and the nitrogen of imidazole rings can be protonated at physiological pH (1,9). However, a more careful examination of the pK_a of these compounds (Table 1) indicates that only three of them are within the intramuscular pH range. Among them, only carnosine can be found in humans (35), and, for the majority of mammals, it is found in greater abundance than anserine and balenine (1). Considering that bicarbonate and phosphate concentrations are constrained by their involvement in other intracellular reactions, increasing carnosine content becomes a promising strategy to enhance total buffering capacity in skeletal muscle (1,35).

It is well established that bicarbonate buffering capacity is strongly dependent on the initial bicarbonate concentration before muscle contractions (35). In parallel, it has been shown

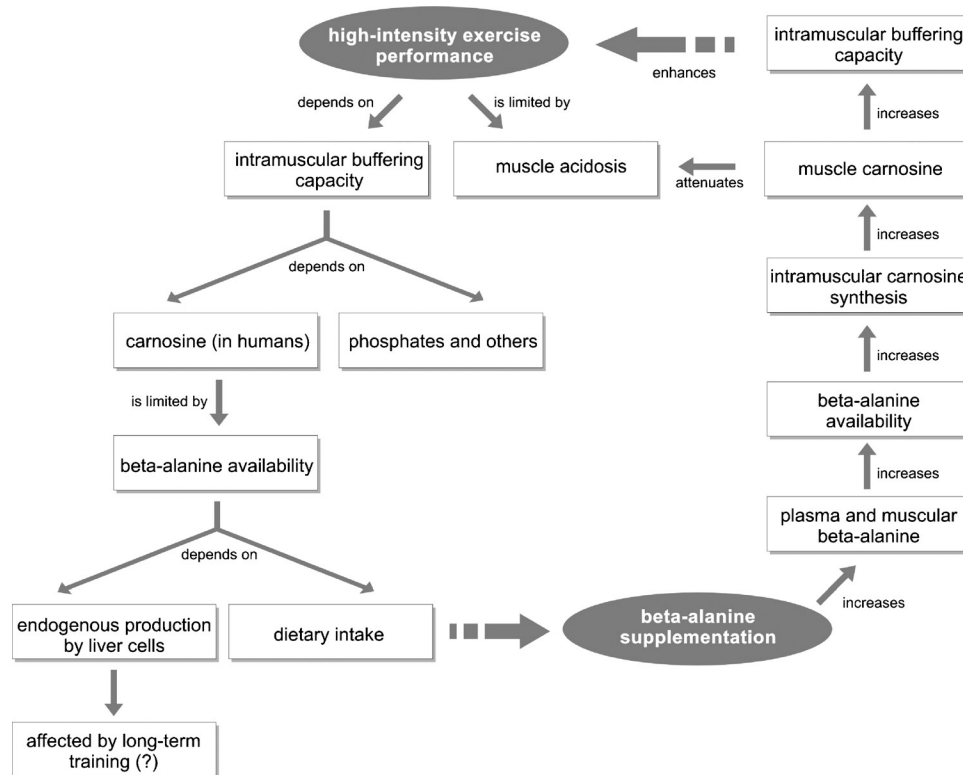


FIGURE 1—The role of β-alanine supplementation on high-intensity exercise performance: an overview.

that histidine dipeptides can be stored in large amounts without any apparent impairment to the cell function (1). Therefore, nutritional strategies capable of improving intracellular levels of carnosine and extracellular levels of bicarbonate are potentially ergogenic, especially along acidosis-limited conditions. In fact, recent investigations have demonstrated that augmentation of muscle carnosine levels can be achieved through supplementation with the nonessential amino acid β -alanine (22,35,36) (for an overview, see Fig. 1). Because the effects of sodium bicarbonate and sodium citrate supplementation were extensively discussed by others (52,59), the present narrative review aims to summarize the current knowledge on carnosine and β -alanine metabolism as well as the effects of β -alanine supplementation on muscle carnosine content, buffering capacity, and exercise performance. We initially describe the role of carnosine as an intracellular buffer. Afterward, the role of exercise training and β -alanine supplementation in increasing muscle carnosine is discussed. A critical discussion of all the studies evaluating the effects of increased muscle carnosine on exercise performance is presented, including the possible proposed side effects. We searched on MEDLINE database, crossing the terms “ β -alanine” and “carnosine” with “skeletal muscle,” “exercise,” “performance,” and “metabolism.” All retrieved articles evaluating the effects of increased muscle carnosine content on physical performance were included in the review. The retrieved articles regarding the other subtopics of this review were selected according to their relevance to

the subject. Related articles as well as articles cited by the retrieved articles were also considered.

CARNOSINE CHARACTERISTICS AND ITS ROLE ON INTRACELLULAR ACID-BASE HOMEOSTASIS

Carnosine (β -alanyl-L-histidine) is a cytoplasmatic intracellular dipeptide found in high concentrations of vertebrate and invertebrate skeletal muscle (35). The utmost documented physiological role of carnosine is the maintenance of acid-base homeostasis (35). However, because carnosine is present in tissues other than skeletal muscle, particularly in excitable tissues such as brain and heart, it likely has additional physiological roles. In fact, some studies suggest that carnosine may be neuroprotective, possibly via γ -aminobutyric acid-homocarnosine interaction in the nervous system and that carnosine supplementation can enhance neurological function in patients with autism (18). Similarly, some studies attribute other effects to carnosine, including protection of proteins against glycation (38), antioxidant activity (13), antiaging activity (37), and the capability to improve Ca^{++} sensitivity in the contractile apparatus (26).

Carnosine is synthesized in skeletal muscle from L-histidine and β -alanine amino acids in a reaction catalyzed by carnosine synthetase (Fig. 2). In addition, carnosine, which is naturally found in some meats, can be obtained

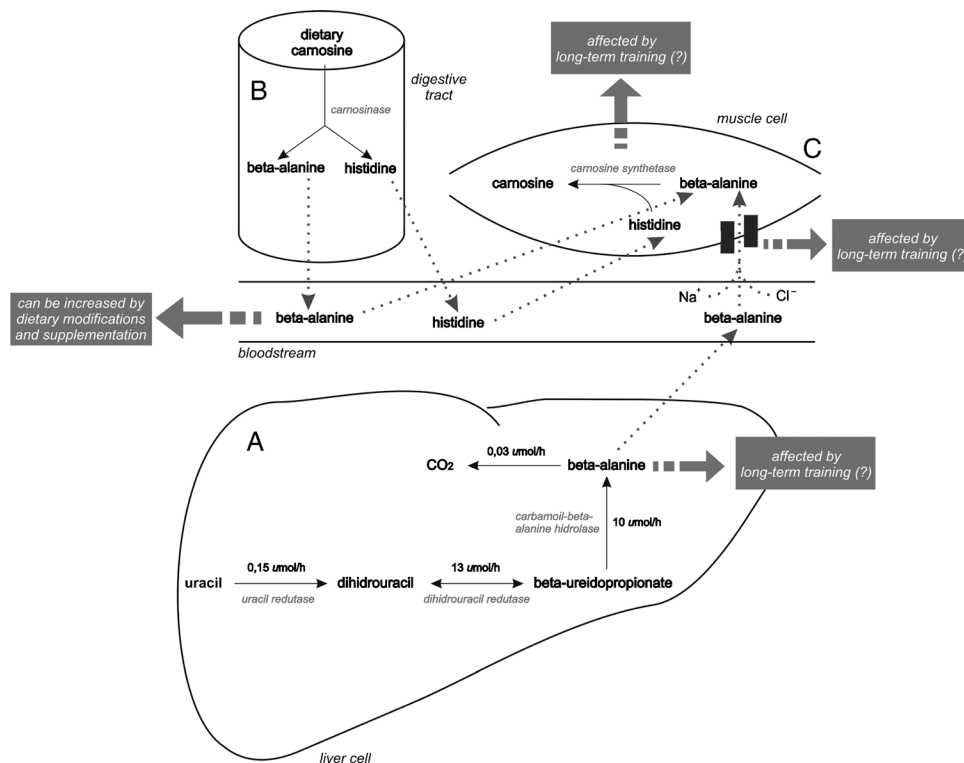


FIGURE 2—Endogenous synthesis of β -alanine by the liver (A); absorption of dietary carnosine by the digestive tract (B); and β -alanine uptake and carnosine synthesis by skeletal muscle (C) (based on the data by Asatoor et al. (6), Bakardjiev and Bauer (8), and Bauer and Schulz (10) and adapted from a figure kindly provided by Dr. Roger C. Harris).

through the diet, and it is subjected to hydrolysis by the carnosinase enzyme, which is present in the gastrointestinal tract (Fig. 2). Although skeletal muscle is able to synthesize carnosine, these cells cannot take up carnosine from the bloodstream (10). Moreover, skeletal muscle does not produce either L-histidine (an essential amino acid) or β -alanine, whose production is confined to the liver cells (49). Therefore, endogenous synthesis of carnosine is primarily dependent on the uptake of β -alanine and L-histidine by the muscle cells from blood. Also, plasma concentration of L-histidine is greater than β -alanine (35), and the affinity of carnosine synthetase for L-histidine ($K_m \sim 16.8 \mu\text{M}$) (42) is greater than the affinity for β -alanine ($K_m \sim 1\text{--}2.3 \text{ mM}$) (53). Consequently, the rate-limiting point of endogenous synthesis of carnosine in humans is the availability of β -alanine within the muscle.

A large body of evidence confirms the relevant role of carnosine in maintaining pH homeostasis of muscle cells. Earlier evidence comes from comparative studies with different species of animals exhibiting different levels of muscle buffering capacity. Studies with fish, for example, demonstrated that species living in deep ocean, where O_2 availability is low and therefore muscle acidosis is high, exhibit a greater concentration of intramuscular carnosine than the nondeepwater fish (1). Moreover, fish with a higher intramuscular carnosine content also exhibit a higher total buffering capacity in muscle and higher LDH enzyme activity (1). It is known that the *Balaenoptera acutorostrata* whale, which is able to hunt for as long as 30 min in deepwater without taking a breath, is the animal with the highest known intramuscular concentration of histidine-related dipeptides, reaching $400 \text{ mmol}\cdot\text{kg}^{-1}$ of dry muscle. The conditions in which this whale hunts suggest an extremely acidic intramuscular environment and, in parallel, an extremely efficient buffering system. Such conditions match well with the high concentration of intramuscular histidine dipeptides found in this species. Similarly, the intramuscular carnosine and anserine concentrations are especially elevated in naturally anaerobic mammals, such as racing dogs (e.g., greyhound dog) and horses (e.g., thoroughbred horse), and reduced in “naturally” sedentary mammals, such as the human being (see Table 2) (1,34).

Further evidence that histidine dipeptides act as intracellular buffers comes from studies demonstrating greater intramuscular content of carnosine and anserine in type II

fibers compared with type I fibers. Of interest, this pattern is observed in several animal species (24,34,63), including humans (36). Knowingly, type II fibers are much more prone and resistant to acidotic environments and hence demonstrate greater buffering capacity in comparison with type I fibers. For example, in racing horses, the carnosine content is 1.7 times greater in type IIa than that in type I fibers and 2.3 times in type IIb compared with type I fibers (25). In type II fibers, the carnosine contribution for total buffering capacity can reach 45% (1). This considerable contribution reinforces the relevant role of histidine dipeptides on muscular pH regulation during acidosis.

It has been shown that highly anaerobic trained athletes (e.g., 800-m runners and rowers) have greater buffering capacity and simultaneously greater intramuscular carnosine content than endurance athletes (e.g., marathon runners) and sedentary people (55). Of note, these athletes were able to perform better on an anaerobic task, and their blood lactate concentrations after exercise were greater in comparison with endurance athletes and sedentary individuals. Similarly, Suzuki et al. (69) demonstrated a strong and positive correlation between intramuscular carnosine concentrations and performance in the anaerobic Wingate test, especially during the final seconds of the test, when acidosis is more evident. Tallon et al. (72) showed that experienced bodybuilders present up to two times higher carnosine concentration than untrained subjects, reaching approximately $40 \text{ mmol}\cdot\text{kg}^{-1}$ of dry muscle. In these athletes, the contribution of carnosine for total buffering capacity was estimated to be approximately 20%, whereas in the control group the contribution was only 10%. The authors estimate that in type II fibers, the contribution can reach approximately 40% or more. Given that bodybuilding training elicits a relatively high muscular acidosis, elevated intramuscular carnosine concentration because of long-term training adaptations has been proposed. Clearly, these data corroborate and strengthen the role of carnosine as an intracellular buffer. Finally, the most important evidence about the buffering role of carnosine in humans arises from studies that supplemented β -alanine and observed positive effects on performance and a delay in fatigue (22,36,65,67,68).

EFFECTS OF TRAINING ON MUSCULAR CARNOSINE CONCENTRATION

Although evidence from cross-sectional studies supports the hypothesis of increased muscular carnosine as an adaptation of long-term high-intensity exercise training (55,72), most of the longitudinal prospective studies have not supported this idea (43,44,46,48). Exceptionally, Suzuki et al. (70) have shown that 8 wk of high-intensity training elicited approximately 100% carnosine increase in sedentary males. Four other investigations, however, have failed to demonstrate any increase in muscle carnosine content after 10 (43,46) or 16 wk (48) of intensive training. Hence, it can be assumed that intensive training up to 16 wk does not

TABLE 2. Carnosine and anserine content ($\text{mmol}\cdot\text{kg}^{-1}$ of dry muscle), total buffering capacity (TBC), and percent contribution of histidine-related dipeptides to total buffering capacity (%D-TBC) in racing horses, racing dogs, and humans (according to Harris et al. [34] and Abe [1]).

	Racing Horse	Racing Dog	Humans
Carnosine	108	33	16
Anserine	—	48	—
TBC	117	105	79
%D-TBC	30	25	7

TBC is expressed in units of slyke, which is defined as the amount of sodium hydroxide or hydrogen chloride (μmol) necessary to change the pH of 1 g tissue by 1 U (e.g., from pH 6 to 7).

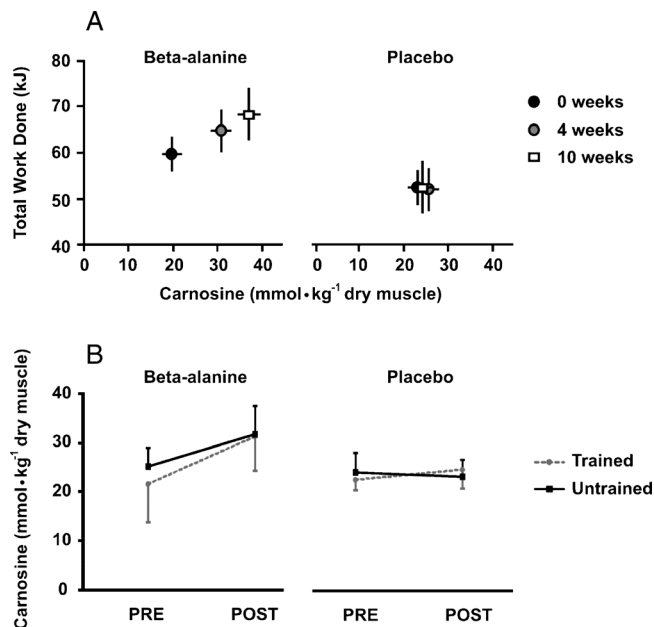


FIGURE 3—A, Effects of β -alanine supplementation on muscle carnosine content and total work done on a cycle ergometer test at 110% of maximum power. This study illustrates that the increase on performance is closely related to the increase in muscle carnosine (adapted from Hill et al. [36]). **B,** The role of β -alanine supplementation and high-intensity training on muscle carnosine. In this study, subjects underwent a 4-wk, single-leg, isokinetic training (the contralateral leg served as control) and received β -alanine or placebo. The results indicate that β -alanine supplementation but not short-term training elicits a marked increase in intramuscular carnosine (data from Kendrick et al. [44]).

affect carnosine content in muscle. Figure 3B illustrates the results recently obtained by Kendrick et al. (44), showing the lack of effect of anaerobic training on muscle carnosine and the positive effect of β -alanine supplementation on this parameter.

As mentioned before, data from Parkhouse et al. (55) and Tallon et al. (72) have indicated that athletes engaged in high-intensity sports (i.e., 800-m runners, rowers, and bodybuilders) display greater intramuscular carnosine concentrations compared with endurance athletes or with untrained subjects. However, it is worthy to highlight that the diet was not controlled in the study of Tallon et al. (72). In addition, the bodybuilders reported steroids use, which is certainly an important bias when considering a previous study that showed mice receiving testosterone demonstrated a 250% increase in intramuscular carnosine content (56). Therefore, it is impossible to affirm whether the high carnosine level found in the bodybuilders occurred because of long-term training adaptations, use of steroids, ingestion of histidine dipeptide-rich food, or a combination of these factors.

Collectively, these findings indicate that short-term training does not seem to affect intramuscular carnosine, whereas long-term training may possibly result in increased muscle carnosine concentrations, although this in turn would suggest an increase in β -alanine synthesis by the liver, an increase in β -alanine uptake by muscle cells, an increase in

carnosine synthetase activity, and/or an increase in dietary intake of β -alanine (Fig. 2). Future studies should confirm this hypothesis and elucidate the underlying mechanisms.

β -ALANINE SUPPLEMENTATION INCREASES INTRAMUSCULAR CARNOSINE

As previously mentioned, carnosine is synthesized in muscle and in other tissues from the amino acids β -alanine and L-histidine by carnosine synthetase. Because this enzyme has more affinity to L-histidine than to β -alanine and L-histidine concentration is higher than β -alanine, it is clear that endogenous synthesis of carnosine *in vivo* is limited by β -alanine availability (35). Consequently, the increase of intramuscular availability of β -alanine is the more effective way to increase endogenous synthesis of carnosine (Fig. 3).

In view of this, Harris et al. (35) investigated whether β -alanine supplementation could increase intramuscular carnosine content in humans. The authors tested three different doses: 40, 20, and 10 mg·kg⁻¹ of body weight. It was verified that the highest dose elicited a peak in serum β -alanine levels, which was related to intense and unpleasant symptoms of paresthesia starting approximately 20 min after the capsule ingestion and ending approximately 60 min after. The intermediate dose resulted in a lower peak in serum β -alanine but was also accompanied by symptoms of paresthesia, which were less frequent and intense. On the other hand, 10 mg·kg⁻¹ resulted in no significant symptoms of paresthesia and a discrete peak of serum β -alanine. This demonstrates that the maximum tolerated single dose is 10 mg·kg⁻¹, which corresponds to an average of 800 mg of β -alanine. Regarding the kinetics of blood β -alanine responses, the time to peak was approximately 30–40 min after capsule ingestion, the half-life time was roughly 25 min after the peak, and the return to baseline values (i.e., undetectable) occurred 3 h after capsule ingestion. This suggests that it is possible to increase the total daily dose if a minimum of 3-h interval between single doses is adopted (35).

The same research group investigated different supplementation protocols: 3.2 g·d⁻¹ in four daily doses of 800 mg and 4 g·d⁻¹ in the first week followed by 6.4 g·d⁻¹ in eight daily doses of 800 mg. The results showed that both protocols were effective in increasing intramuscular carnosine content, but the highest total dose protocol was more effective for augmenting muscle carnosine levels (~65% vs ~40%) (35). Later studies have also confirmed the efficacy of this supplementation protocol (36,43), and more recently, data suggest that it is possible to reach the same total daily dose of 6.4 g using a twofold higher single dose when using controlled release capsules (1600 mg instead of 800 mg) (22,40,68,77). The controlled release capsules eliminated all symptoms of paresthesia and augmented muscle carnosine concentration by 40% after 4 wk (33). These results demonstrate that four daily doses of 1600 mg may be more practical than eight daily doses and may also ensure greater

compliance. Moreover, the controlled release capsules allow a higher dose intake, with a lower peak and a longer half-life time of serum β -alanine, without significant symptoms of paresthesia.

It is estimated that the contribution of carnosine to the total buffering capacity in muscle is approximately 10% (35). Because β -alanine supplementation increases muscle carnosine content by approximately 65%, the contribution of carnosine to total buffering capacity after supplementation may reach approximately 15%, and if only type II fibers are considered, the estimated contribution may be up to 25% or higher (35). This may be decisive in sports whose performance is limited by muscle acidosis. Although type II fibers naturally exhibit higher carnosine concentrations than type I fibers, β -alanine supplementation yields similar absolute increases in muscle carnosine content in both fiber types (35), suggesting that the efficiency of the β -alanine uptake mechanism is nearly identical in both fiber types.

Recently, Baguet et al. (7) have characterized the time course of muscle carnosine washout after β -alanine supplementation. These authors hypothesized that muscle carnosine would be stable in skeletal muscle and therefore display a slow washout profile in this tissue. The rationale for this relies on the virtual absence of expression of the enzyme tissue carnosinase (CN2, which can hydrolyze carnosine in several tissues). In fact, data from this study (7) showed that at 3 wk of postsupplementation, muscle carnosine has decreased only 30%. Although after 9 wk the average muscle carnosine had returned to baseline values, the subjects who displayed a more marked increase in muscle carnosine (i.e., high responders) still presented an elevated carnosine concentration after 9 wk, whereas the low responders presented baseline values at the ninth washout week. The authors estimated that the low responders would reach baseline carnosine concentration at the 6th week and the high responders at the 15th week (7).

β -ALANINE METABOLISM

β -Alanine is a nonproteogenic amino acid (i.e., it does not take part in proteins structure) endogenously produced by the liver (49). β -Alanine is produced from the degradation of uracil, as shown in Figure 2 (29). *In vitro* studies show that despite complete degradation of β -alanine in liver cells, only a small fraction of β -alanine is converted to CO_2 because the conversion rate of β -alanine- CO_2 is low. Therefore, it can be assumed that the major role of this pathway is the β -alanine production rather than the complete uracil degradation (30).

Once produced in the liver, β -alanine is taken up by several tissues, including skeletal muscle. Considering that the rate of β -alanine production is relatively low, serum β -alanine concentration is undetectable in normal conditions (35). Experiments involving β -alanine transporter kinetics performed with cell cultures of embryonic chicken muscle elucidated relevant properties of the β -alanine transporter. It

was demonstrated that β -alanine uptake by muscle cells involves a single protein, whose K_m for β -alanine is approximately 40 μM . Also, β -alanine uptake occurs through stoichiometric transport involving this amino acid, chloride, and sodium inside the cells in a proportion of 1:1:2, respectively (8). Indeed, caution should be exercised because these data, obtained from embryonic chicken muscle cell cultures, perhaps cannot be extrapolated to humans. Future studies should confirm the mechanisms underlying β -alanine transport in human myocytes.

Evidence from whole-body studies have suggested that synthesis of carnosine inside muscle cells is limited by the availability of β -alanine whenever its muscular concentration is below 40 μM (35), which is the saturation point of carnosine synthetase (8). In normal physiological conditions, intramuscular β -alanine is below 40 μM , and therefore β -alanine availability is the limiting factor for carnosine synthesis.

EFFECTS OF β -ALANINE SUPPLEMENTATION ON PERFORMANCE AND FATIGUE

The role of carnosine in intramuscular pH regulation and the ability of β -alanine supplementation to increase the intramuscular carnosine content have become a recent avenue of exploration. More so, the ergogenic potential of such supplementation strategy in high-intensity exercise is promising. In fact, several studies have evaluated the effects of β -alanine supplementation on performance using different exercise protocols. Despite some conflicting results, literature has shown that β -alanine supplementation is an effective performance enhancer (Fig. 4).

The use of β -alanine supplementation does not seem to improve maximal strength (39,40,43). Kendrick et al. (43) evaluated the effects of this supplement on whole-body strength (box squat, bench press, and dead lift) as well as on isokinetic strength (90° knee extensions at 180°s⁻¹ in an isokinetic dynamometer). The results of this study showed no contribution of β -alanine to the measured parameters. Similarly, Hoffman et al. (40) also showed no improvements on maximal strength measured by a one-repetition maximum (1RM) free weight squat exercise after supplementation. These findings are not surprising, considering that β -alanine supplementation increases buffering capacity and maximal strength performance is not limited by acidosis. Previous studies with other buffering agents such as sodium bicarbonate also failed in demonstrating any ergogenic effects on strength performance (57,74).

Regarding the effects of β -alanine on maximum aerobic power, two studies have shown no positive effect of the supplementation on $\dot{V}\text{O}_{2\text{peak}}$ measured in graded exercise test (GXT) (40,43). Despite the lack of positive effects on $\dot{V}\text{O}_{2\text{max}}$, β -alanine supplementation has been reported to improve anaerobic threshold, shifting the curve to the right (68,77). Zoeller et al. (77), investigating the effects of 28 d

Variable	Improve?	Ref	Study design	Exercise performance tests
Max. strength	yes	{ -	-	-
	no	{ (41) (38)	26 active males submitted to resistance training; β -Al and PL groups 8 resistance trained males; β -Al and PL groups in cross-over design (4 wk wash-out)	- 1 RM; isokinetic - 1 RM
Intermittent	yes	{ (20)	15 track and field male athletes; β -Al and PL groups	- 5x 30s max isokinetic contractions
	no	{ (68)	8 males; acute ingestion of CBEX or PL	- 10x 5s max cycloergometry with 25s recovery
Neuro-muscular fatigue	yes	{ (64) (65)	51 recreationally active males; PL, β -Al, Cr and β -Al+Cr groups 22 untrained females; β -Al and PL groups	- PWC_{FT} (neither Cr alone nor combined with β -Al had effect) - PWC_{FT}
	no	{ -	-	-
High-intensity	yes	{ (38)	8 resistance trained males; β -Al and PL groups in cross-over design (4 wk wash-out)	- total work done in strength training sessions
		{ (34)	25 active males; β -Al and PL groups	- total work done in cycloergometry at 110% of max power
		{ (70)	17 moderately well-trained male cyclists; β -Al and PL groups	- 30s isokinetic sprint cycling after 110min simulated cycling race
	no	{ (20)	15 track and field male athletes; β -Al and PL groups	- max isometric contraction until exhaustion (knee at 45°) - 400m running time trial
		{ (21) (22) (23)	26 active males submitted to resistance training; β -Al and PL groups 8 resistance trained males; β -Al and PL groups in cross-over design (4 wk wash-out) 17 moderately well-trained male cyclists; β -Al and PL groups	- upper arm curl fatigue test (approx. 20-40 RM) - mean and peak power during squat sets - 10min cycling time trial after 110min simulated cycling race
Anaerobic threshold	yes	{ (65) (74)	22 untrained females; β -Al and PL groups 55 recreationally active males; PL, β -Al, Cr and β -Al+Cr groups	- ventilatory threshold - lactate threshold
	no	{ (74)	55 recreationally active males; PL, β -Al, Cr and β -Al+Cr groups	- ventilatory threshold
Endurance	yes	{ (65)	22 untrained females; β -Al and PL groups	- time to exhaustion in graded exercise test
	no	{ (74)	55 recreationally active males; PL, β -Al, Cr and β -Al+Cr groups	- time to exhaustion in graded exercise test
Aerobic power	yes	{ -	-	-
	no	{ (65) (74)	22 untrained females; β -Al and PL groups 55 recreationally active males; PL, β -Al, Cr and β -Al+Cr groups	- $VO_{2\ peak}$ in graded exercise test - $VO_{2\ peak}$ in graded exercise test

FIGURE 4—A summary of research assessing the ergogenic effects of β -alanine supplementation on exercise performance. β -Al, β -alanine; PL, placebo; RM, repetition maximum; CBEX, chicken breast extract; Cr, creatine; PWC_{FT} , physical working capacity at neuromuscular fatigue threshold.

of β -alanine and/or creatine supplementation on endurance performance, demonstrated a significant increase in the power attained at the lactate threshold in subjects supplemented with β -alanine. In line with this result, Stout et al. (68) showed a significant increase of 13% on ventilatory threshold in women supplemented with β -alanine. The most attractive explanation for these results is the less acidic environment inside muscle cells caused by a greater buffering capacity, which allowed subjects to attain a higher power output with a smaller lactate concentration. These two studies have also evaluated the effects of β -alanine supplementation on endurance performance, measured by time to exhaustion (TTE) during a GXT. Although increased buffer capacity is not expected to enhance long-term endurance performance because muscle acidosis does not play a significant role in fatigue during this kind of exercise (66), Stout et al. (68) have reported a slight but significant 2.5% increase in TTE during a GXT on a cycle ergometer. This result can be explained by the anaerobic component observed in the final stages of a GXT, which may be accounted for from improvements in intracellular buffer capacity. In contrast, Zoeller et al. (77) demonstrated no positive effect of β -alanine supplementation on TTE. Together, these data suggest that β -alanine supplementation has a discrete or nonergogenic effect in endurance performance, although anaerobic threshold can be shifted after supplementation.

The major putative effects of increased buffer capacity are likely to be observed in exercises that elicit a high intracellular acidosis. In fact, studies with sodium bicarbonate ingestion have shown greater benefits in exercises lasting from approximately 60 s to 5 min (for a review, see McNaughton et al. [52]). In particular, increased buffer capacity has been especially ergogenic in multiple bouts of high-intensity short-term exercises interspersed by short recovery intervals (5,11,12). Studies on β -alanine supplementation have shown a similar pattern. Only two studies have evaluated the effects of β -alanine on intermittent performance (22,71). Derave et al. (22) demonstrated a significant improvement on 5×30 -s maximal torque in isokinetic knee extension. In contrast, Suzuki et al. (71) failed to demonstrate an ergogenic effect of β -alanine and anserine ingestion on 10×5 -s maximal cycle ergometry with a 25-s interval among bouts. However, it is important to highlight that Suzuki et al. (71) did not use a chronic supplementation protocol. Rather, the authors tested an acute ingestion of chicken breast extract, which is a rich source of histidine-related dipeptides. The lack of positive effects may be explained by the use of acute ingestion instead of chronic supplementation that knowingly increases muscle carnosine content. Although β -alanine probably possesses a relevant ergogenic effect on anaerobic intermittent performance, more studies are needed to confirm these results.

Studies evaluating the effects of β -alanine supplementation on the onset of neuromuscular fatigue have been

unanimous in demonstrating its ability in delaying fatigue (67,68). According to the data by Stout et al. (67), 28 d of β -alanine supplementation was able to significantly increase the physical working capacity at fatigue threshold (PWC_{FT}), which suggests a delay in the onset of neuromuscular fatigue. These authors also tested the effects of creatine alone and β -alanine combined with creatine on PWC_{FT} . Interestingly, creatine did not affect PWC_{FT} and also did not exert any additive effect over β -alanine supplementation alone. Confirming these findings, another study by Stout et al. (68) demonstrated a significant improvement in PWC_{FT} in women after 28 d of β -alanine supplementation.

Available literature has been somewhat conflicting regarding the effects of β -alanine on high-intensity performance. However, the characteristics of exercises used to evaluate performance might explain the equivocal data. When an exercise protocol eliciting an extreme acidosis was used, a significant improvement in performance was observed (36). Hill et al. (36) investigated the effects of 4 and 10 wk of β -alanine supplementation on cycling performance at 110% $\dot{V}O_{2peak}$ and muscle carnosine content. At the end of the fourth week, muscle carnosine was significantly increased by approximately 60%, which was accompanied by a significant 13% increase in total work done during cycling test. An additional 6 wk of supplementation yielded a nearly significant ($P = 0.07$) further 20% increase in muscle carnosine, which was followed by a 16.2% increase in total work done when compared with presupplementation values (Fig. 3A). These results clearly indicate the ergogenic effect of β -alanine supplementation on high-intensity performance. In accordance, Hoffman et al. (40) showed that β -alanine supplementation, despite not enhancing maximal strength performance, resulted in an approximately 20% increase in total work volume in strength training sessions in well-trained resistance athletes.

On the other hand, when exercise protocols elicit a less extreme muscular acidosis (i.e., a single bout of exercise lasting less than 60 s or a single bout of exercises using small muscle groups), no effects of β -alanine have been observed (22,40,43). Derave et al. (22) investigated the effects of 4 wk of supplementation on 400-m running time trials and on maximal isometric knee extension to exhaustion (knee at 45°). The authors did not observe any beneficial effects of β -alanine on these performance parameters. Although 400-m running, which lasts approximately 45 s, has a considerably high anaerobic contribution and consequently leads to intramuscular acidosis, evidence has indicated that the ergogenic effects of an increased buffer capacity are evident in exercises with an extremely high anaerobic demand lasting more than 60 s (51,52). In the same study, β -alanine did not improve TTE during a maximal isometric knee extension at 45° and at 45% of the maximal voluntary contraction. According to the authors, the angle of 45° might not sufficiently obstruct blood flow to the working muscles, which could be demonstrated by

the much longer TTE (~200 s) observed in this study when compared with approximately 80 s predicted by the Rohmert curve (2). Thus, it is believed that in the study by Derave et al. (22), the isometric contractions were undertaken with little to no occlusion of blood flow, and consequently the acidosis observed may not have been great enough to limit performance. This likely explains the lack of ergogenic effect of β -alanine supplementation on this performance test. Similarly, Kendrick et al. (43), using an upper arm-curl resistance test (with ~20–40 RM), and Hoffman et al. (40), measuring the power output during a set of squat exercise, failed to demonstrate positive effects of β -alanine on performance. Other studies investigating metabolic-induced alkalosis also showed no effects of an increased buffer capacity on exercises with similar characteristics (57,74). Recently, Van Thienen et al. (73) showed that 8 wk of orally administered β -alanine can improve mean power output by 5% and peak power output by 11.5% in a 30-s sprint cycling performance after 110-min simulated cycling racing. In the same study, however, no differences were observed in a 10-min time trial performance. Taken together, these data suggest that β -alanine supplementation is capable of improving performance in exercises resulting in an extreme intramuscular acidotic environment, such as multiple bouts of high-intensity short-term exercises, single bouts of high-intensity exercises lasting more than 60 s, and single bouts undertaken when fatigue is already present. Exercises with a lower level of acidosis are unlikely to benefit from β -alanine supplementation.

Some interesting studies have combined β -alanine with creatine (39) and β -alanine with high-intensity exercise training (64,65). Hoffman et al. (39) tested the effects of creatine alone and creatine plus β -alanine on 1RM performance, 2 \times 30-s Wingate performance, and 20-s vertical jump. A significant increase in 1RM strength in both experimental groups was observed, which was likely due to the effects of creatine, because the addition of β -alanine did not elicit further improvements to 1RM. Performance on Wingate bouts and on vertical jump tests was also not affected by supplementation. As discussed above, the characteristics of a single bout of 20-s vertical jump may not be affected by increases in muscle buffer capacity. However, the lack of effects on 2 \times 30-s Wingate bouts is somewhat unexpected. Nonetheless, the study of Artioli et al. (5), investigating the effects of sodium bicarbonate ingestion, has shown that ergogenic effects of an increased buffer capacity were only evident at the third and fourth bouts of 30-s Wingate tests, which may explain the lack of positive effects on 2 \times 30-s Wingate bouts reported by Hoffman et al. (39). In the study of Hoffman et al. (39), however, the addition on β -alanine resulted in greater total volume trained in resistance training sessions, which resulted in further accruals in body composition than creatine alone.

Smith et al. (64) have evaluated the effects of combining β -alanine with high-intensity training on neuromuscular fatigue, demonstrating that 3 wk of training elicits signif-

icant improvements in electromyographic fatigue threshold and electrical activity efficiency. In addition, the combination of β -alanine with high-intensity training resulted in discrete enhancements in TTE at 110% of $\dot{V}O_{2\max}$ greater than training alone (65). The same authors also verified that β -alanine plus training has more beneficial effects on training volume and lean body mass in comparison with training alone. These data indicate the potential application of β -alanine supplementation in athletic setting.

ADVERSE EFFECTS OF β -ALANINE SUPPLEMENTATION

Currently, the only known adverse effect of β -alanine supplementation are the symptoms of paresthesia, which are triggered by a high and acute single dose and disappear within approximately 1 h after the ingestion (35). These symptoms can be avoided by the use of controlled release capsules and by smaller dosing strategies. No studies have evaluated the effects of β -alanine supplementation for more than 10 wk, and hence the possibility of long-term adverse effects cannot be ruled out. However, considering that β -alanine is an amino acid that naturally plays a relevant role in the human body and that doses studied so far are quite similar to that found in diet, it is likely that this supplement is safe.

β -Alanine and taurine share the same transporter that uptakes them inside the cell (35). Consequently, the increase in plasma β -alanine concentration could, at least theoretically, decrease taurine intracellular concentration due a competitive inhibition of taurine uptake. Moreover, it has been shown that because of the role of taurine on intracellular osmoregulation (20), there is a moderate and a negative association between intramuscular concentrations of taurine and carnosine (45). Hence, increases in carnosine content are expected to result in decreases in taurine content as a result of osmotic compensation. In fact, studies with rodents showed that the addition of 3% of β -alanine on the drinking water resulted in a dramatic decrease in intracellular taurine concentration in several tissues, especially in liver, heart, and brain (54). Although taurine has demonstrated to act as an antioxidant (32), Parildar-Karpuzoglu et al. (54) showed no evidence of increased susceptibility of the cells neither to lipid peroxidation nor to oxidative stress after β -alanine supplementation, despite the severe depletion on intracellular taurine concentration. The same research group, however, showed that old mice are more susceptible to the damage caused by β -alanine-mediated taurine depletion. It is worthy to note that the total amount of β -alanine provided through addition of 3% in drinking water is substantially greater than the dose typically used in humans (35). Furthermore, the typical β -alanine dose used in humans is quite similar to that available in a histidine-related peptide-rich diet. Most importantly, the typical human dose does not elicit any change in intracellular taurine (36). Therefore, the safety of this supplement can be assumed because there

“Take-home” messages

- ✓ carnosine is a relevant intramuscular buffer;
- ✓ carnosine is synthesized in muscle cells from L-histidine and beta-alanine;
- ✓ endogenous synthesis of carnosine is limited by beta-alanine availability;
- ✓ 4 weeks of beta-alanine supplementation is capable to increase muscle carnosine and buffering capacity;
- ✓ high-intensity training of up to 16 weeks does not affect muscle carnosine;
- ✓ the maximum tolerated single dose of beta-alanine is 800 mg. Higher doses can result in symptoms of paresthesia, unless controlled release capsules are used. No other beta-alanine-related side effect was reported;
- ✓ Multiple doses should be used to reach the desired daily dose;
- ✓ beta-alanine supplementation can enhance short-term high-intensity performance.

FIGURE 5—“Take-home” messages presented and discussed in this article.

is no evidence of harmful effects when physiological doses are used.

CONCLUSIONS AND PERSPECTIVES

The take-home messages are depicted in Figure 5. In short, carnosine plays a relevant role in skeletal muscle buffering capacity, especially in type II fibers. Carnosine is synthesized in muscle cells by L-histidine and β -alanine; the availability of β -alanine is the rate-limiting point of carnosine

synthesis. Thus, the most effective way to increase intramuscular carnosine concentration is through β -alanine supplementation. Although short-term high-intensity training does not increase muscle carnosine content, the possibility of increased muscle carnosine as an adaptation to chronic long-term high-intensity training cannot be discarded. Longitudinal follow-up studies should address whether long-term intensive training can augment carnosine levels in muscle. The ergogenic effects of β -alanine supplementation are evident in activities that elicit a strong intramuscular acidotic condition and therefore are limited by intramuscular pH decreases such as high-intensity intermittent exercises and high-intensity exercises lasting more than 60 s. In addition, β -alanine supplementation has proven to delay the onset of neuromuscular fatigue and anaerobic threshold. All evidence so far has provided support that β -alanine supplementation is free of important side effects. Recent studies have demonstrated the potential use of β -alanine as an ergogenic aid in athletic settings. However, how this novel nutritional strategy could enhance performance in specific sport modalities is a subject for further investigations. Finally, future studies should explore the metabolism of β -alanine and carnosine through experimental models more similar to human's physiological conditions to confirm β -alanine metabolism data obtained from experimental studies with animal models.

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