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CHAPTER FIVE

Effects of β-Alanine Supplementation on Carnosine Elevation and Physiological Performance

Jay R. Hoffman[1], **Alyssa Varanoske, Jeffrey R. Stout**
Institute of Exercise Physiology and Wellness, University of Central Florida, Orlando, FL, United States
[1]Corresponding author: e-mail address: jay.hoffman@ucf.edu

Contents

Abstract

β-Alanine is one of the more popular sport supplements used by strength/power athletes today. The popularity of β-alanine stems from its ability to enhance intracellular muscle-buffering capacity thereby delaying fatigue during high-intensity exercise by increasing muscle carnosine content. Recent evidence also suggests that elevated carnosine levels may enhance cognitive performance and increase resiliency to stress. These benefits are thought to result from carnosine's potential role as an antioxidant. This review will discuss these new findings including recent investigations examining β-alanine supplementation and increased resiliency to posttraumatic stress and mild

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traumatic brain injury. This review will focus on the physiology of carnosine, the effect of β-alanine ingestion on carnosine elevations, and the potential ergogenic benefits it has for competitive and tactical athletes.

1. INTRODUCTION

β-Alanine is considered to be one of the more popular supplements being used by competitive athletes to improve athletic performance (Blancquaert, Everaert, & Derave, 2015; Maughan, Greenhaff, & Hespel, 2011). Its efficacy has been summarized in a number of recent reviews (Blancquaert et al., 2015; Saunders, Elliott-Sale, et al., 2017; Trexler et al., 2015), and recent evidence has also reported the potential efficacy of β-alanine supplementation for the tactical athlete as well (Hoffman et al., 2014, Hoffman, Landau, et al., 2015). β-Alanine is a nonproteogenic amino acid and does not appear to have any ergogenic potential by itself. Once ingested, it combines with histidine within skeletal muscle and other organs to form carnosine. When β-alanine combines with histidine, the pK_a of the imidazole ring of the histidine residue is increased to 6.83, enabling it to act as a highly effective intracellular pH buffer (Hoffman, Stout, Harris, & Moran, 2015). β-Alanine is considered to be the rate-limiting step in muscle carnosine synthesis (Bauer & Sculz, 1994; Harris et al., 2006). Thus, the primary goal of β-alanine supplementation is to increase carnosine content in skeletal muscle thereby enhancing intracellular buffering capacity, enabling a greater tolerance of sustained anaerobic activity. There are also additional physiological benefits associated with elevated carnosine content, which will be discussed within this chapter.

2. PHYSIOLOGICAL ROLES OF CARNOSINE

Carnosine is part of a family of histidine-containing dipeptides that are found in the skeletal muscle of all vertebrates (Harris, Marlin, Dunnett, Snow, & Hultman, 1990), but are especially high in mammals that rely on anaerobic metabolism to fuel their activity. Carnosine (β-alanyl-L-histidine), homocarnosine (γ-aminobutyryl-L-histidine), anserine (*N*-β-alanyl-3-methyl-L-histidine), and balenine (*N*-β-alanyl-1-methyl-L-histidine) are all widely distributed in mammalian tissue, but carnosine is found to be the primary histidine dipeptide in humans (Boldyrev, Aldini, & Derave, 2013). There is a strong positive relationship between carnosine content

and muscle-buffering capacity across all species (Abe, 2000; Harris et al., 1990; Parkhouse, McKenzie, Hochachka, & Ovalle, 1985), with a significantly greater concentration of carnosine found in Type II compared to Type I muscle fibers (Harris, Dunnett, & Greenhaff, 1998; Hill et al., 2007; Kendrick et al., 2009). Carnosine's primary physiological role in skeletal muscle appears to be in its role as a pH buffer (Abe, 2000; Harris et al., 2006). Its ability to bind muscle H^+ during intense exercise moderates the decline in intracellular pH, allowing exercise to continue for longer duration. High concentrations of muscle carnosine are reported to delay the onset of peripheral fatigue during intense exercise lasting 1–5 min (Hobson, Saunders, Ball, Harris, & Sale, 2012). Recent investigations have suggested that intramuscular carnosine may also act as a diffusible Ca^{2+}/H^+ exchanger at the level of the sarcomere, augmenting skeletal muscle force production (Swietach, Leem, Spitzer, & Vaughan-Jones, 2014; Swietach et al., 2013). Because carnosine can bind both H^+ and Ca^{2+}, increase of H^+ binding to carnosine may induce Ca^{2+} unloading at the sarcomere, subsequently increasing cross-bridge formation and force production.

Besides serving as an intracellular buffer, additional physiological roles have been associated with carnosine. Several studies have suggested that carnosine may serve as a neuroprotector (Boldyrev, Stvolinsky, Fedorova, & Suslina, 2010; Hoffman, Stout, Harris, & Moran, 2015; Hoffman et al., 2017). This is supported by evidence demonstrating carnosine's biological role as an antioxidant and antiglycating and ion-chelating agent (Boldyrev et al., 2004; Hipkiss, Worthington, Himsworth, & Herwig, 1998; Kohen, Yamamoto, Cundy, & Ames, 1988; Trombley, Horning, & Blakemore, 2000). Carnosine's antioxidant properties have been demonstrated through its ability to scavenge reactive oxygen species and react directly with superoxide anions and peroxyl radicals in vitro (Boldyrev et al., 2013). The ability of carnosine to act as an antioxidant is primarily due to its histidine component, while β-alanine has been shown to be ineffective as an antioxidant itself (Decker, Livisay, & Zhou, 2000). High-intensity exercise elicits a significant oxidative stress response, causing inflammation and muscle damage (Packer, 1997), and attenuation of oxidative stress may be beneficial to the recovery process and subsequent exercise performance. However, the examination of carnosine as an antioxidant in vivo has been limited to animal models, where carnosine has been shown to have different physiological roles (Aydin, Kucukgergin, Ozdemirler-Erata, Kocak-Toker, & Uysal, 2010; Kim, Kim, Kim, Choi, & Lee, 2011). Therefore, the effectiveness of carnosine as an antioxidant in humans remains to be explored. Carnosine has also been

shown to act as an ion-chelating agent, preventing ions such as copper and zinc from excessive accumulation that may lead to lipid peroxidation and further cellular damage (Trombley et al., 2000). Additionally, carnosine has been shown to act as an antiglycating agent, preventing the formation of advanced lipoxidation end-products and advanced glycoxidating end-products, delaying the aging process and preventing various diseases (Boldyrev et al., 2013). Thus, carnosine's physiological role goes far beyond those of muscle-buffering capacity.

2.1 Effect of Training on Changes in Muscle Carnosine

Research examining the influence of exercise on changes in muscle carnosine is limited. A study conducted by Suzuki, Ito, Takahashi, and Takamatsu (2004) is, to the best of our knowledge, the only investigation which has examined the effect of high-intensity training on changes in muscle carnosine content. The results of their investigation resulted in a doubling of muscle carnosine content following 8 weeks of sprint training. Their total training time during the duration of the study was 14 min (i.e., single 30-s sprints performed twice per week for the first 2 weeks and two 30-s sprints performed twice per week for the next 6 weeks with 20-min recovery between sprints). To increase muscle carnosine content, this training stimulus would have required an increase in hepatic β-alanine synthesis and/or an increased rate of β-alanine transport into muscle fibers. Other studies examining similar training intensities but with greater training volume for longer durations (4–16 weeks) have been unable to confirm these results (Kendrick et al., 2008, 2009; Mannion, Jakeman, & Willan, 1994). However, recently Bex et al. (2014) suggested that the effect of β-alanine supplementation may be increased with training, although a similar effect was not observed by Kendrick et al. (2009) when measured in different muscle fiber types. Additional research is warranted in this area.

2.2 Effect of Diet on Changes in Muscle Carnosine

Diet appears to have a significant effect on muscle carnosine content. Carnosine levels in skeletal muscle of omnivores are significantly higher than those seen in vegetarians (Everaert et al., 2011; Harris et al., 2007). In vegetarians the only source of β-alanine for muscle synthesis is from uracil degradation in the liver. This process is either too slow or the availability of uracil for degradation is very limited to maintain normal levels of carnosine synthesis in muscle. As a result, dietary intake of β-alanine through red meat,

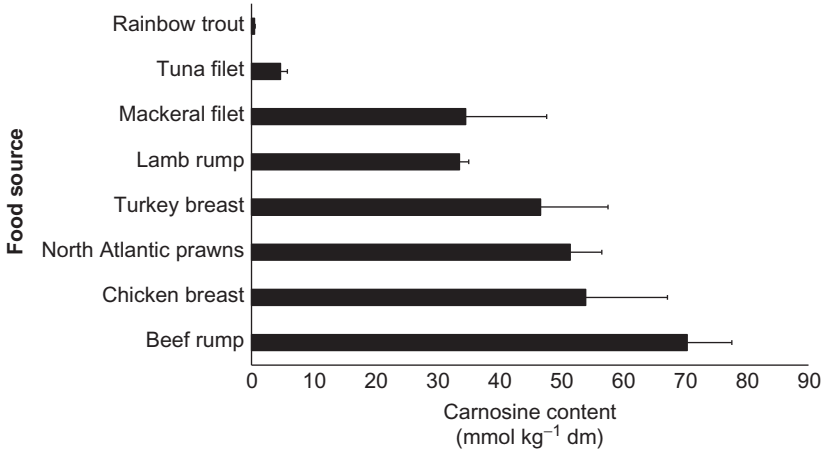

Fig. 1 Carnosine content from various food sources. *Data from Jones, G., Smith, M., & Harris, R.C. (2011). Imidazole dipeptide content of dietary sources commonly consumed within the British diet. Proceedings of the Nutrition Society, 70 (OCE6), E363.*

poultry, and fish consumption is required if the muscle carnosine concentration is to be further elevated (Harris et al., 2007). Fig. 1 provides the carnosine content of various food sources. Ingestion of ~200 g of chicken breast meat, or 150 g of turkey breast meat, has been suggested to increase the plasma bioavailability of β-alanine equivalent to an 800 mg β-alanine supplement (Harris et al., 2006). Considering that the daily dose range in supplementary β-alanine is reported to range from 1.6 to 6.4 g (Hoffman, Stout, et al., 2015), this would require the consumption of between 400 and 1600 g of chicken breast or 300 and 1200 g of turkey breast per day. Furthermore, Everaert et al. (2011) did not observe a significant relationship between dietary β-alanine consumption and muscle carnosine content. Thus, supplementing with β-alanine may be the most effective means to increase muscle carnosine content.

3. KINETICS OF β-ALANINE INGESTION

Ingesting 10 mg kg^{-1} of β-alanine (equivalent to an 800 mg dose for an 80 kg individual) is reported to result in an increase of β-alanine concentrations in plasma that will peak between 30 and 40 min following ingestion (Harris et al., 2006). Its reported half-life (time at which there is a 50% reduction of peak concentration) is 25 min but will remain in the circulation for about 3 h following ingestion (Harris et al., 2006). The use of a sustained-release formulation of β-alanine will slow its release into circulation and may

increase retention with a reduction in the amount lost via the urine. In one study, a 1.6-g ingestion of sustained-release β-alanine tablet resulted in a smaller peak plasma concentration than a similar dose of a regular release capsule (82 vs 248 μmol L^{-1}, $P < 0.001$) and a delayed time to peak concentration (1.0 vs 0.5 h, $P < 0.01$) (Décombaz, Beaumont, Vuichoud, Bouisset, & Stellingwerff, 2012). However, no differences were noted in plasma bioavailability when comparing area under the curves (AUC) plasma concentrations. However, in consideration of the greater retention of β-alanine (decrease loss in urine) in sustained-release formulations, the percentage of β-alanine available for carnosine synthesis in the muscle may be increased (Harris, Jones, & Wise, 2008).

4. DOSE–RESPONSE FOR β-ALANINE INGESTION AND MUSCLE CARNOSINE SYNTHESIS

A β-alanine supplementation regimen of 1.6–6.4 g day^{-1} has been demonstrated to result in significant increases in muscle carnosine (Hoffman, Landau, et al., 2015; Kendrick et al., 2008, 2009; Stellingwerff, Anwander, et al., 2012). Doses higher than 6.4 g day^{-1} have generally not been examined due to the greater risk of symptoms of paresthesia occurring. However, most of these studies were conducted prior to the availability of a sustained-release formulation of β-alanine, which permits greater doses without the risk of paresthesia (Harris & Stellingwerff, 2013), a side effect later described (see Section 8). A recent study compared two dosing protocols (6 g vs 12 g day^{-1}) of sustained-release formulation on muscle carnosine content (Church et al., 2017). A total of 30 participants (18 men and 12 women) were randomized into three groups: placebo, 6 g day^{-1} of β-alanine, or 12-g day^{-1} of β-alanine. The placebo and 6-g day^{-1} groups supplemented for 4 weeks, while the 12-g day^{-1} group supplemented for 2 weeks. Both the 12-g day^{-1} ($P = 0.026$) and 6-g day^{-1} ($P = 0.004$) groups significantly increased muscle carnosine compared to the placebo group, but no differences were noted between the β-alanine ingestion groups. This study presented evidence that doubling the daily dose can achieve similar amounts of muscle carnosine in half the time. However, it would likely be a mistake to extrapolate these results to longer duration of high daily ingestion of β-alanine. Increases in muscle carnosine content with β-alanine supplementation appear to be dependent upon several factors including training history, supplement dose, and duration of supplement use. In an investigation comparing multiple dosing strategies, Stellingwerff, Anwander, et al. (2012)

demonstrated that 4 weeks of 3.2 g d^{-1} of β-alanine supplementation resulted in a greater increase in carnosine content in both fast-twitch (predominant in the *gastrocnemius* muscle) and slow-twitch (abundant in the *tibialis anterior* muscle) muscle fibers than a lower dose (1.6 g d^{-1}). An additional study provided 4.8 g day^{-1} of β-alanine for 5–6 weeks and reported a 23% increase in carnosine content in the *gastrocnemius* muscle of recreationally trained men (Baguet et al., 2009). This was similar to another study investigating elite rowers ingesting a slightly higher dose (5.0 g day^{-1}) for 7 weeks and reported a 28% increase in carnosine content in the *gastrocnemius* muscle (Baguet, Bourgois, Vanhee, Achten, & Derave, 2010). A study examining β-alanine supplementation (4.8 g day^{-1} for 4–5 weeks) in track and field athletes reported a 37% increase in carnosine content in the *gastrocnemius* muscle (Derave et al., 2007). Interestingly, Hoffman et al. (2014) examining soldiers in an elite combat unit ingesting 6.4 g day^{-1} of sustained-release tablets for 4 weeks reported a 28% increase in the carnosine content of the *gastrocnemius* muscle. Thus, doses ranging in amounts from 4.8 to 6.0 g day^{-1} for 4–7 weeks will result in an increase in carnosine content in the *gastrocnemius* muscle ranging from 23% to 28% in recreationally trained individuals, but possibly higher levels in competitive athletes, suggesting a potential a synergistic effect from training.

Increases in muscle carnosine have been suggested to be linearly related with β-alanine ingestion (Stellingwerff, Decombaz, Harris, & Boesch, 2012). However, this is believed to be based upon an empirical interpretation of collected data, and not on any kinetic model (Hoffman, Stout, et al., 2015). Synthesis of muscle carnosine is thought to follow zero-order kinetics with respect to the availability of β-alanine (Harris et al., 2006; Stellingwerff, Anwander, et al., 2012), while the decay in muscle carnosine back to the presupplementation level follows first-order kinetics (Harris et al., 2009; Stellingwerff, Decombaz, et al., 2012). In this potential model in which carnosine synthesis follows zero-order kinetics with respect to β-alanine availability and is opposed by a first-order decline back to the presupplementation levels, a steady-state plateau (synthesis matches decay) will eventually be reached with any dose provided (Hoffman, Stout, et al., 2015). This is highlighted in a study by Hill et al. (2007). These investigators examined 25 physically active men using an initial dosing protocol of 4.0 g day^{-1} for the first week which was then increased by 800 mg $week^{-1}$ until week 4 and then maintained at 6.4 g day^{-1} for an additional 6 weeks. The dose administered over the first 4 weeks resulted in a 58.8% increase in the muscle carnosine concentration, while an additional 21% increase was seen by week 10.

Increases in muscle carnosine were rapid during the initial stages of ingestion, but the rate of carnosine elevation began to slow as supplementation was continued. Based upon these findings and those of other studies, it is apparent that increases in muscle carnosine above the presupplementation level will be directly related to the β-alanine dose (i.e., the absolute increase with 6 g day^{-1} will be twice that with 3 g day^{-1}) and not to the total dose given. This was recently supported by Church et al. (2017) that demonstrated that 12 g of β-alanine provided daily for 2 weeks resulted in a similar increase in muscle carnosine as was observed from daily 6 g feedings over 4 weeks.

A recent study examined 24 weeks of β-alanine supplementation (6.4 g day^{-1}) in recreationally active men (Saunders, de Salles Painelli, et al., 2017). Results indicated that muscle carnosine content was increased 25.7 ± 7.6 mmol kg^{-1} dry muscle (dm), although these changes were quite variable (ranging from +17 to +41 mmol kg^{-1} dm). This range of increase was 59%–200% from baseline levels. In addition, variability was also noted in the time to achieve maximal carnosine content. Improvements in exercise capacity (time to exhaustion during a graded cycling exercise test) were shown with supplementation that mirrored changes in muscle carnosine, although some degree of variability was demonstrated. This was the first study to indicate that there is large variability in changes in muscle carnosine between individuals and that maximal elevation during prolonged supplementation may not necessarily occur during the final stages of supplementation for all individuals. These results further suggest that determination of muscle carnosine content may not be limited to only the availability of β-alanine ingestion and that various other factors may be involved. Fig. 2 provides the relationship between total β-alanine consumed and the increase in muscle carnosine from studies measuring muscle biopsy measures in dry mass.

4.1 Effect of β-Alanine Supplementation and Training on Muscle Carnosine Elevations

There is some evidence to suggest that the combination of β-alanine ingestion and exercise can enhance muscle carnosine levels to a greater extent than supplementation alone. As previously discussed, Bex and colleagues (2014) provided competitive swimmers 6.4 g of β-alanine per day for 23 days and compared changes in muscle carnosine elevations to a group of nonathletes consuming the same amount β-alanine ingestion for the same duration. Greater increases in muscle carnosine were observed in the exercised

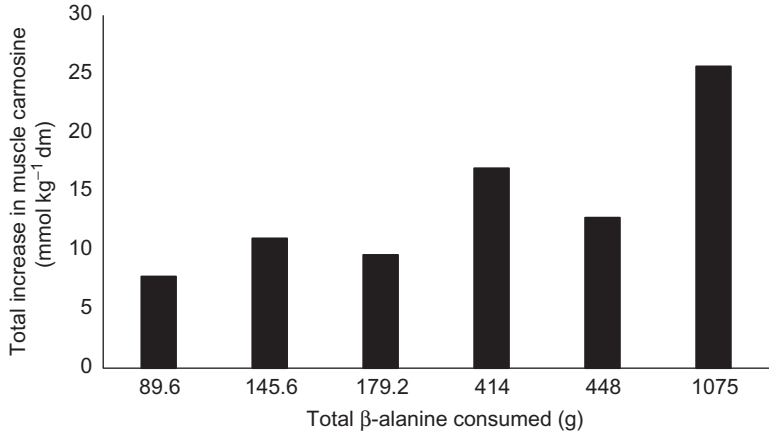

Fig. 2 Relationship between β-alanine consumed and increase in muscle carnosine content. *Data from Harris, R. C., Tallon, M. J., Dunnett, M., Boobis, L., Coakley, J., Kim, H. J., et al. (2006). The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis.* Amino Acids, 30, *279–289; Hill, C. A., Harris, R. C., Kim, H. J., Harris, B. D., Sale, C., Boobis, L. H., et al. (2007). Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity.* Amino Acids, 32, *225–233; Kendrick, I. P., Harris, R. C., Kim, H. J., Kim, C. K., Dang, V. H., Lam, T. Q., et al. (2008). The effects of 10 weeks of resistance training combined with beta-alanine supplementation on whole body strength, force production, muscular endurance and body composition.* Amino Acids, 34, *547–54; Kendrick, I. P., Kim, H. J., Harris, R. C., Kim, C. K., Dang, V. H., Lam, T. Q., et al. (2009). The effect of 4 weeks beta-alanine supplementation and isokinetic training on carnosine concentrations in type I and II human skeletal muscle fibres.* European Journal of Applied Physiology, 106, *131–138; Saunders, B., de Salles Painelli, V., de Oliveira, L. F., da Eira Silva, V., da Silva, R. P., Riani, L., et al. (2017). Twenty-four weeks of β-alanine supplementation on carnosine content, related genes, and exercise.* Medicine and Science in Sports and Exercise, 49, *896–906; Saunders, B., Elliott-Sale, K., Artioli, G. G., Swinton, P. A., Dolan, E., Roschel, H., et al. (2017). β-Alanine supplementation to improve exercise capacity and performance: A systematic review and meta-analysis.* British Journal of Sports Medicine, 51, *658–669.*

muscles of athletes (82% increase in the deltoid muscle) compared to non-exercised muscle in nonathletes (63% increase). However, this was not supported by the work of Kendrick et al. (2009). They provided 6.4 g day^{-1} for 4 weeks to subjects that served as their own controls (i.e., one leg was exercised, while the other remained sedentary). No differences in carnosine elevations were noted between the trained and untrained muscle (*vastus lateralis*).

Stellingwerff, Anwander, et al. (2012), using an empirically based model, suggested that a total of ~230 g of β-alanine consumed over several weeks, using dosing schedules of 1.6–6.4 g day^{-1}, would result in a ~50% increase

in muscle carnosine. This suggested a linear dependency ($R^2 = 0.921$) between the increase in muscle carnosine content and the total amount of β-alanine consumed, independent of muscle type. Others have indicated (using a meta-analysis) that an accumulated ingestion of 179 g of β-alanine (the median dose across all studies) will result in a median performance improvement of 2.85% compared with a placebo (Hobson et al., 2012).

4.2 β-Alanine Supplementation and Muscle Fiber Type

A large variability in carnosine content has been reported between Type II and Type I fibers (Harris et al., 1998; Hill et al., 2007; Kendrick et al., 2009). Carnosine content of Type II fibers has been reported to be up to two times higher than carnosine levels in Type I fibers (Everaert et al., 2011; Hill et al., 2007; Kendrick et al., 2009). Regardless of initial difference, supplementation with β-alanine has been demonstrated to result in similar absolute increases in both Type I and Type II muscle fibers (Hill et al., 2007; Kendrick et al., 2009). However, these baseline differences will result in different relative changes. Derave et al. (2007) provided 4.8 g day^{-1} for 4–5 weeks in track and field athletes and reported a 47% increase in the carnosine content of the *soleus* muscle, a predominantly slow twitch muscle, but only a 37% increase in the *gastrocnemius* muscle, a predominantly fast twitch muscle. Considering that the presupplementation level in the *gastrocnemius* was higher than that of the *soleus* muscle, it is likely that the difference in the absolute change was similar.

5. CARNOSINE WASHOUT

When β-alanine supplementation is stopped, a gradual return of muscle carnosine concentrations to baseline levels are observed. Baguet et al. (2009) reported significant increases in muscle carnosine levels following 6 weeks of 4.8 g day^{-1} of β-alanine supplementation. Following 3 weeks after supplement cessation, the mean carnosine concentrations in three muscles were reported to have decreased by 31.8%. Following 9 weeks of supplement cessation, muscle carnosine returned to baseline levels. Harris et al. (2009) suggested an exponential rate of decay back to the presupplementation level with a half-life ($t_{1/2}$) of 8.6 weeks. Others have suggested that the rate of return of muscle carnosine to its baseline levels is a function of the initial increase above the presupplementation level (Stellingwerff, Decombaz, et al., 2012). Participants who were reported

to be high responders (i.e., a greater accumulation of muscle carnosine content) required a greater washout time to return to baseline levels (~15 weeks) than participants that were reported to be low responders (~6 weeks). In an additional study, Stellingwerff, Anwander, et al. (2012) reported an increase in absolute muscle carnosine content by an average of 2.01 mmol kg^{-1} ww in untrained men supplementing with β-alanine (1.6 or 3.2 g day^{-1}) for 8 weeks. This absolute change was equivalent to a relative ~30%–45% increase (depending upon fiber type) in muscle carnosine content. Supplement cessation resulted in a slow washout time (~15–20 weeks) with a decay rate of approximately 2% per week, which was slower than that reported by Baguet et al. (2009), and may have been related to the higher baseline levels. Such a dependency of the decay rate on degree of elevation above baseline is consistent with the decay following first-order kinetics (Hoffman, Stout, et al., 2015).

6. ERGOGENIC PROPERTIES OF β-ALANINE SUPPLEMENTATION

The ergogenic effects associated with β-alanine supplementation are not the result of any direct action of the amino acid itself but from β-alanine's role in combining with histidine within skeletal muscle to form carnosine. For the past 10 years, a large body of evidence has been accumulated supporting the ergogenic benefit of elevating muscle carnosine (Blancquaert et al., 2015; Hobson et al., 2012; Hoffman, Stout, et al., 2015; Saunders, Elliott-Sale, et al., 2017; Trexler et al., 2015). In their meta-analysis, Hobson et al. (2012) reported that the greatest ergogenic potential from elevated carnosine levels occurs during high-intensity exercise lasting 60–240 s in duration. The overall analysis included 360 subjects (174 with β-alanine supplementation and 186 with placebo) from 15 published manuscripts. No significant benefit in β-alanine ingestion was noted in performance durations lasting <60 s compared to placebo ingestion.

The ergogenic effect of β-alanine supplementation has been consistently reported in both recreational and competitive athletic populations performing high-intensity activity (Hill et al., 2007; Hoffman, Ratamess, Faigenbaum, et al., 2008; Hoffman et al., 2006; Hoffman, Ratamess, Ross, et al., 2008; Kendrick et al., 2008; Stout et al., 2006, 2007). Studies in previously untrained men and women have also been demonstrated to improve performance (Stout et al., 2006, 2007). In both cases, participants consumed 6.4 g day^{-1} of β-alanine for the first 6 days of the study and then

3.2 g day^{-1} thereafter (days 7–28). In the study examining previously untrained men, investigators reported a 14.5% improvement in physical working capacity at fatigue threshold (PWC_{FT}) in the β-alanine group, which was significantly better than the placebo group (Stout et al., 2006). In previously untrained women, the results were similar; a 12.6% improvement in the PWC_{FT} and a 2.5% increase in time to exhaustion during a graded exercise cycle ergometry test in participants supplementing with β-alanine compared to the placebo supplemented group (Stout et al., 2007).

Examinations on competitive athletes supplementing with β-alanine have produced similar improvements in performance. In a study on 400-m sprinters, 4 weeks of β-alanine supplementation (4.8 g day^{-1}) significantly ($P < 0.05$) delayed fatigue during repeated bouts of isokinetic exercise (5 sets of 30 maximal voluntary knee extensions) (Derave et al., 2007). In a study on resistance-trained strength/power athletes, 4.8 g day^{-1} of β-alanine for 4 weeks resulted in significant increase in training volume in the athletes supplementing with β-alanine ($+9.0 \pm 4.1$ repetitions in the squat exercise) compared to the athletes consuming the placebo ($+0.3 \pm 7.8$ repetitions) (Hoffman, Ratamess, Ross, et al., 2008). In addition, the average mean power output per repetition for each set was significantly ($P < 0.05$) higher in the athletes supplementing with β-alanine than those given the placebo (Hoffman, Ratamess, Ross, et al., 2008). In another study on strength/power athletes, Hoffman, Ratamess, Faigenbaum, et al. (2008) provided 4.5 g day^{-1} of β-alanine for 4 weeks to college football players. Initial performance testing, occurring after 2 weeks of supplementation, revealed no significant differences in sprint times or fatigue rates during repeated (total of three) shuttle runs (30–35 s per run with a 2-min rest between each sprint). However, a trend ($P = 0.07$) was noted in fatigue rate during a 60-s Wingate anaerobic power test. Following the full 4-week supplementation period, evaluation of the player's resistance-training logbooks revealed a trend ($P = 0.09$) toward a higher (9.2%) training volume (load × repetitions performed during their workouts) in those athletes supplementing with β-alanine compared to those consuming the placebo. In addition, the lack of any significant difference in repeated sprints (duration of each sprint was approximately 30–35 s) appears to be consistent with the results of Hobson et al. (2012). These results also suggest that the 2-week supplementation period (only 63 g of β-alanine ingested) in trained athletes was likely not sufficient to increase muscle carnosine content by an amount producing a measurable change in performance. However, the trend toward an improved rate of fatigue observed in the 60-s Wingate anaerobic power test was

consistent with the physiological role that β-alanine supplementation has on buffering capacity and high-intensity exercise (Hobson et al., 2012).

Additional studies examining β-alanine supplementation in competitive athletes for a longer duration of time have also reported significant benefits. In one investigation, elite competitive rowers were provided 5 g day^{-1} of β-alanine for 7 weeks (Baguet et al., 2010). Athletes ingesting β-alanine were 2.8 ± 4.8 s faster during a 2000-m rowing time trial performance than their presupplement times, while the placebo group was 1.8 ± 6.8 s slower than their presupplement times. Although these differences were not statistically significant ($P = 0.07$), this trend toward a difference among an elite group of athletes takes on much practical significance. Furthermore, muscle carnosine content in the experimental group was significantly ($P < 0.05$) higher by 45% and 28% in the *soleus* and *gastrocnemius* muscles, respectively, and the changes in muscle carnosine were significantly correlated ($r = 0.498$, $P = 0.042$) to performance improvement in the rowing time trial. These results were supported by others that showed positive benefits following 4 weeks of β-alanine supplementation (~80 mg kg^{-1}) on 2000 m rowing performance with similar gains in each case (Ducker, Dawson, & Wallman, 2013; Hobson et al., 2013). Based on these investigations, it is apparent that performance in this type of sustained high-intensity exercise lasting 6–7 min is improved by β-alanine-facilitated increases in muscle carnosine (Hoffman, Stout, et al., 2015).

Others have demonstrated that β-alanine supplementation may benefit high-intensity exercise performed immediately following a prolonged, fatiguing bout of endurance exercise. Van Thienen et al. (2009) examining trained cyclists provided them with β-alanine or placebo in gradated doses from 2 to 4 g day^{-1} for 8 weeks. Following 110 min of a varied intensity (50%–90% of their maximal lactate steady state [MLSS]) cycle ergometer trial, athletes performed an additional 10-min time trial at 100% of their MLSS, which was followed by a 30-s sprint. The athletes consuming β-alanine showed significant improvements in both peak (11.4%; $P < 0.0001$) and mean power (5.0%; $P < 0.005$), respectively, during the 30-s sprint performance. These improvements were significantly greater than those seen in the athletes that consumed the placebo.

6.1 Benefits of β-Alanine Supplementation on Endurance Performance

There have only been a limited number of studies examining the effects of β-alanine ingestion on endurance performance. One study provided

6 g day^{-1} of β-alanine for 4 weeks to college students who were not previously trained and performed no training during the supplement period (Jordan, Lukaszuk, Misic, & Umoren, 2010). The investigators reported a delay in blood lactate accumulation, but a decrease in aerobic capacity was also noted. These results were not surprising considering that the physiological role of carnosine in muscle does not provide a strong mechanism for enhancing endurance performance. However, Smith et al. (2009) reported significant ($P < 0.05$) improvements in VO_2 peak, time to fatigue, and total work performed during endurance performance in recreationally active males supplementing with β-alanine (6.0 g day^{-1}for 21 days followed by 3.0 g day^{-1}for another 21 days) and performing high-intensity interval training (5–7 sets of 2-min intervals at 90% max power output, with 1-min rest between each interval). Although performance improvements were noted in both the supplement and placebo groups following 3 weeks of training, only the β-alanine group showed significant ($P < 0.05$) aerobic improvements after 6 weeks of training. These results were supported by a subsequent study on recreationally active women (Walter, Smith, Kendall, Stout, & Cramer, 2010). Although there are no direct physiological benefits associated with β-alanine supplementation for endurance and aerobic performance, the combination of anaerobic high-intensity intervals training and β-alanine is likely to improve the quality of the high-intensity sprints performed, indirectly affecting aerobic capacity and cardiovascular fitness.

6.2 Benefits of β-Alanine Supplementation on Tactical Performance

The benefits observed in supplementing β-alanine in competitive athletes have led scientists to explore its potential use in tactical athletes (e.g., soldiers). Improvements in muscle buffering and potential antioxidative effects may provide benefits for soldiers during sustained, high-intensity military operations. The first study examining the efficacy of β-alanine supplementation and tactical athletes investigated the effects of 4 weeks of β-alanine supplementation (6 g day^{-1}) in elite combat soldiers performing high-intensity military activity that required maintaining high levels of physical performance, focus, and decision making under fatiguing conditions (Hoffman et al., 2014). During the 4-week study period, all participants took part in advanced military training tasks that included combat skill development, physical work under pressure, navigational training, self-defense/hand-to-hand combat, and conditioning. Results of the study indicated that 4 weeks of β-alanine supplementation were effective in

maintaining lower body power and psychomotor performance. Peak jump power, target engagement speed, and shooting accuracy were significantly improved for the β-alanine group in comparison with the placebo group ($P < 0.034$, $P < 0.039$, and $P < 0.012$, respectively). Improvements in marksmanship and target engagement speed were an interesting finding from the study in which improvements in cognitive function (recognizing and fixing a misfire and continue with directing fire on target) were either related to improvements in fatigue reduction or possibly decision-making ability. Enhancing fatigue resistance in muscle fibers responsible for maintaining both standing and kneeling shooting positions may assist the soldier in keeping the weapon steady during target acquisition and marksmanship. However, these improvements may also have been related to a possible reduction in oxidative stress associated with intense military training.

A subsequent study again provided the same supplement protocol (6.0 g day^{-1} of β-alanine for 30 days) in a group of elite combat soldiers (Hoffman, Landau, et al., 2015). During this study, changes in both muscle (*gastrocnemius*) and brain carnosine content were assessed by magnetic resonance spectroscopy. Following the 30-day supplement period, significant elevations in muscle carnosine were observed in the β-alanine group, but no changes were seen in brain carnosine levels. Significant performance improvements were also reported in the β-alanine group in the 50-m casualty carry task and in cognitive function, but no changes were noted in the 2.5-km run, 1-min sprint, or repeated sprints (5×30 m). Cognitive function was assessed from the Serial Sevens Test (Hayman, 1942), which consists of a 2-min timed written test in which soldiers were required to subtract the number 7 from a randomly generated four digit number, in order to measure how quickly and accurately they can compute a simple mathematical problem provided. Soldiers ingesting the supplement had a significantly ($P = 0.022$) greater number of correct answers on the 2-min test than soldiers that consumed the placebo. Cognitive function assessments were also performed in the shooting range, while continuous live fire was being directed at targets. This required the soldiers to maintain their focus despite the loud noise from the active firing line. This type of environment has been reported to increase levels of anxiety, making it difficult to solve mathematical problems solving and significantly decrease cognitive performance in soldiers (Nibbeling, Oudejans, Ubink, & Daanen, 2014). The results indicate that 30 days of β-alanine ingestion may enhance cognitive function to a greater extent than placebo in a stressful environment.

7. ADDITIONAL PHYSIOLOGICAL EFFECTS OF β-ALANINE SUPPLEMENTATION

One of the more interesting physiological benefits of elevated carnosine levels is associated with carnosine's role as a potential antioxidant (Boldyrev et al., 2004, 2010; Kohen et al., 1988). However, investigations examining the role of β-alanine supplementation and oxidative stress have been limited. One study examined the effect of a daily 4.8 g dose of β-alanine (sustained release form) for 28 days on markers of oxidative stress during a 40-min treadmill run in moderately trained young women (Smith, Stout, Kendal, Fukuda, & Cramer, 2012). No differences were noted between the treatment and placebo groups in any of the antioxidant markers leading the authors to conclude that β-alanine supplementation using this specific protocol design did attenuate the oxidative stress response. A subsequent study by the same research group, using the same dosing and training scheme albeit in men, confirmed their previous results indicating β-alanine ingestion had no significant influence on reducing exercise-induced oxidative stress (Smith-Ryan, Fukuda, Stout, & Kendall, 2014). However, subsequent analysis using 95% confidence intervals did suggest a reduction in lipid peroxidation (Smith-Ryan et al., 2014). It is possible that blood markers of oxidative stress or antioxidant activity may not be sufficient to provide a complete picture of the antioxidative benefits of elevated carnosine levels.

In the past 7 years, there have been a number of investigations that have demonstrated that β-alanine may have a significant role as an antioxidant in the brain. One of the initial studies examining β-alanine supplementation and brain function was performed by Murakami and Furuse (2010). They provided 22.5 mmol kg^{-1} feedings of β-alanine for approximately 5 weeks to mice and observed significant increase in the carnosine content in the cerebral cortex and hypothalamus. These increases were associated with an increase in brain-derived neurotrophic factor (BDNF), and a decrease in 5-hydroxyindoleacetic acid concentrations, a metabolite of serotonin, despite the rodents' exposure to stressful conditions (anxiolytic compounds). These biochemical changes also corresponded to an improved time in a maze. Although the investigators did not measure any oxidative stress, the results did suggest that β-alanine ingestion may reduce anxiety during stressful activities. A subsequent study examined the effects of β-alanine ingestion on posttraumatic stress disorder (PTSD)-like behavioral changes in rodents

exposed to a predator-scent stress (PSS) (Hoffman, Ostfeld, Stout, Kaplan, & Cohen, 2015). The validity of this model has been previously reported by Cohen and Zohar (2004) and has been shown to be an effective approach to determine the effects of various interventions on the behavioral response to stress (Cohen, Kozlovsky, Cramer, Matar, & Zohar, 2012). Animals were provided with 100 mg of a β-alanine/glucomannan blend per kg of body mass in a powder form for 30 days. A total of 30 mg of powder (80:20 blend) was dissolved in 25 mL of water and provided daily. This blend provided a sustained-release-like effect, and none of the rats provided the supplement demonstrated any supplement-related discomfort. Following 30 days of β-alanine ingestion, the animals were exposed to the PSS. Results indicated that β-alanine ingestion in animals exposed to the PSS was effective in increasing the resiliency to the PSS. Animals provided β-alanine and exposed to the PSS were recorded as spending greater time in the open arms of an elevated plus maze, more entries into the open arms of the maze, and a lower anxiety index than animals fed a normal diet and exposed to the PSS. Animals exposed to the PSS and fed a normal diet were reported to be significantly less active in the elevated maze and had a greater anxiety level compared to animals that were either unexposed or animals that were exposed and supplemented with β-alanine. Rats supplemented with β-alanine experienced elevations in carnosine in all compartments of the brain. In addition, BDNF expression in the hippocampus was maintained in animals that were supplemented with β-alanine. Elevations in brain carnosine levels were inversely associated with anxiety index (r's ranging from -0.471 to -0.550, P's < 0.002) and positively associated with improved time spent in the open arms (r's ranging from 0.453 to 0.521, P's < 0.003). The protective effects associated with elevations in brain carnosine appeared to be related to a protection of BDNF expression in the hippocampus.

The mechanism associated with elevated brain carnosine and maintenance of BDNF expression in the hippocampus is not well understood, but it is likely related to carnosine's role as a neural protectant through its action as an antioxidant (Kohen et al., 1988). Oxidative stress and inflammation in the brain have been suggested to be part of the sequelae of physiological events contributing to PTSD (Wilson et al., 2013) but may also contribute to the cognitive and neurodegeneration associated with mild traumatic brain injury (mTBI) (Aungst, Kabadi, Thompson, Stoica, & Faden, 2014). A recent study by Hoffman et al. (2017) investigated the benefit of β-alanine supplementation on behavioral and cognitive responses

relating to mTBI. In addition, the effects of β-alanine ingestion on inflammatory, neurotrophin, and tau protein expression in the hippocampus were also examined. A low-pressure blast wave, which was previously demonstrated to be an effective model to elicit distinct behavioral and morphological changes that simulate mTBI-like, PTSD-like, and comorbid mTBI–PTSD-like responses (Zuckerman et al., 2017), was used as the experimental model. The results of the study indicated that 30 days of β-alanine ingestion in rats were effective in reducing the incidence of mTBI-like phenotype following exposure to a low-pressure blast wave (46% of the animals exposed to the blast and fed a normal diet exhibited mTBI symptoms, while only 26.5% of the animals fed β-alanine and were exposed to the blast exhibited mTBI symptoms), and that these differences were significant ($P = 0.044$). In addition, animals supplemented with β-alanine and exposed to the blast wave also appeared to have a reduced inflammatory response (an attenuation of the glial fibrillary acidic protein) response and a higher BDNF expression in specific regions of the hippocampus compared to rats that were exposed, but fed a normal diet. The results of this study provided initial evidence that 30 days of β-alanine supplementation may increase resiliency to mTBI-like responses in animals exposed to a low-pressure blast wave and may provide further support to a potential antioxidant role from elevated carnosine levels. Fig. 3 depicts the potential effects of β-alanine supplementation on brain function when exposed to stress.

8. SAFETY

The only side effect that is associated with β-alanine supplementation is paresthesia. Paresthesia is a sensation of numbing or tingling in the skin. Symptoms of paresthesia generally disappear within 60–90 min following supplementation (Stellingwerff, Decombaz, et al., 2012). Paresthesia is commonly reported among individuals when they are consuming β-alanine (>800 mg kg^{-1}) in a nonsustained-release form (Harris et al., 2006). Reports of paresthesia have not been reported in studies that use sustained-release formulations. Recently, Church et al. (2017) reported no differences in symptoms of paresthesia between participants consuming the placebo and participants consuming a daily dose of 12 g sustained-release β-alanine (four doses of 3 g day^{-1}). The mechanism associated with symptoms of paresthesia isthought to involve the binding of β-alanine to Mas-related G-protein-coupled receptor D (MrgprD). MrgprD isexpressed in cutaneous sensory neurons in the skin, and the binding of β-alanine to

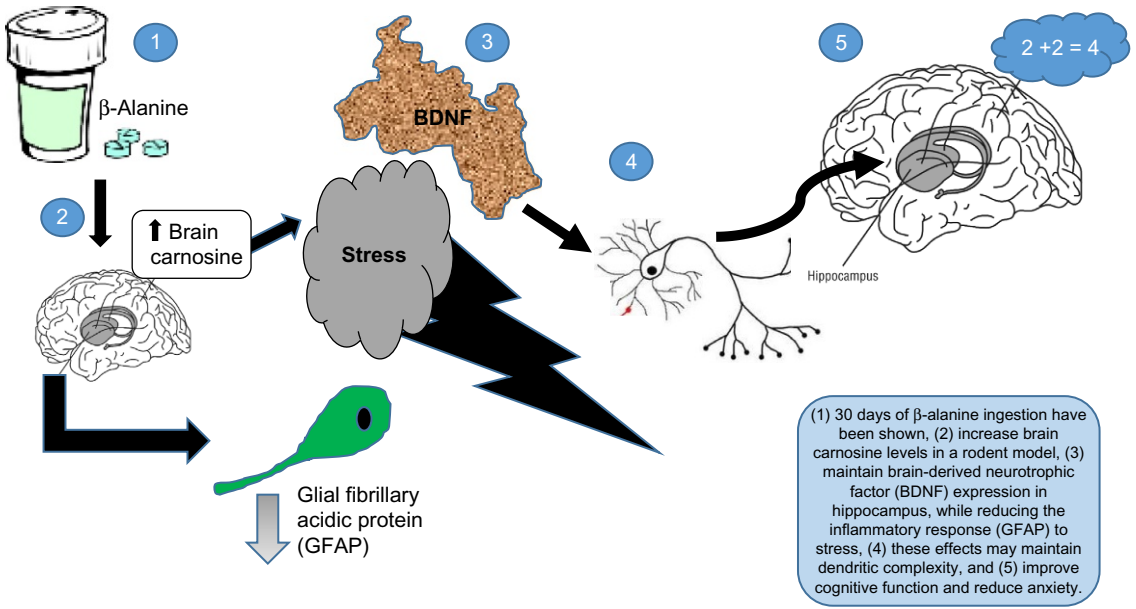

Fig. 3 Potential effects of β-alanine supplementation on brain function when exposed to stress.

MrgprD may be responsible for the associated symptoms of paresthesia (Liu et al., 2012; MacPhee, Weaver, & Weaver, 2013).

Considering that β-alanine is a naturally occurring amino acid with an important physiological role in the body, it is likely a very safe supplement to use. A recent study examining 12 g day^{-1} of β-alanine ingestion for 2 weeks reported no changes in any hematological variables, and values for all blood count variables remained within references norms during the supplementation period (Church et al., 2017). Additionally, Harris et al. (2006) reported no changes on 12-lead electrocardiogram and blood chemistry and hematological safety data following 4 weeks of 3.2 g day^{-1} of β-alanine ingestion, while others have reported similar findings during longer supplementation protocols (8–12 weeks) using doses ranging between 0.8 and 3.2 g day^{-1}, in both young (Stellingwerff, Anwander, et al., 2012) and older adult populations (del Favero et al., 2012; McCormack et al., 2013). Despite these favorable health profiles, the long-term effect of β-alanine supplementation and the combinations with other supplements are unknown. Further studies are needed to understand the long-term benefits and safety of β-alanine.

9. SUMMARY

When β-alanine is ingested, whether through food or as a dietary supplement, it combines with histidine to form carnosine in various tissues in the body. Physiologically, elevations in muscle carnosine will increase intracellular buffering capacity. The efficacy of β-alanine supplementation has been supported through several studies examining sustained, high-intensity exercise in competitive and recreational athletes. Based on current research, β-alanine supplementation appears to be efficacious during high-intensity activity lasting 60–360 s. In addition, recent evidence has also suggested that β-alanine may provide some benefits toward enhancing cognitive function during stressful conditions and improved resiliency to stress. Further research, however, is warranted in this area.

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