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Muscle Carnosine Metabolism and β-Alanine Supplementation in Relation to Exercise and Training — Source link

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Muscle carnosine metabolism and β-alanine supplementation in relation to exercise and training

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Figure 1 : Gaussian distribution of the carnosine content of the gastrocnemius and soleus muscle in an adult male population (age range : 19-49 years; n= 93). Data are compiled from subjects of previous studies from our laboratory. Carnosine concentration is measured by means of proton MRS, as previously described [1], and expressed relative to the water signal.

Figure 2: Putative determinants of the human muscle carnosine content.

Figure 3: Correlation between the carnosine content in the soleus and in the gastrocnemius in 93 male subjects as measured by proton MRS. Similar correlations were also found for tibialis anterior vs. gastrocnemius and tibialis anterior vs. soleus (data not shown). In almost all subjects, carnosine concentrations are higher in gastrocnemius than soleus. Data are the same as in figure 1.

Figure 4: Muscle carnosine content before (0w) and after (5w) 5 weeks of oral supplementation (4.8 $g \cdot day^{-1}$) of beta-alanine or placebo in physically active students or in trained 400m runners. Data are extracted from previous studies from our laboratory [1;2].

Abstract:

Carnosine is a dipeptide with a high concentration in mammalian skeletal muscle. It is synthesized by carnosine synthetase from the amino acids L-histidine and β-alanine, of which the latter is the rate-limiting precursor, and degraded by carnosinase. Recent studies have shown that the chronic oral ingestion of β-alanine can substantially (up to 80%) elevate the carnosine content of human skeletal muscle. Interestingly, muscle carnosine loading leads to improved performance in high-intensity exercise in both untrained and trained individuals. Although carnosine is not involved in the classic ATP-generating metabolic pathways, this suggests an important role of the dipeptide in the homeostasis of contracting muscle cells, especially during high rates of anaerobic energy delivery. Carnosine may attenuate acidosis by acting as a pH buffer, but improved contractile performance may also be obtained by improved excitation-contraction coupling and defence against reactive oxygen species. High carnosine concentrations are found in individuals with a high proportion of fast-twitch fibers, because these fibers are enriched with the dipeptide. Muscle carnosine content is lower in women, declines with age and is probably lower in vegetarians, whose diets are deprived from beta-alanine. Sprint-trained athletes display markedly high muscular carnosine, but the acute effect of several weeks of training on muscle carnosine is limited. High carnosine levels in elite sprinters are therefore either an important genetically-determined talent selection criterion or a result of slow adaptation to years of training. Beta-alanine is rapidly developing as a popular ergogenic nutritional supplement for athletes worldwide, and the currently available scientific literature suggests that its use is evidence-based. However, many aspects of the supplement, such as the potential side-effects and the mechanism of action, require additional and thorough investigation by the sports science community.

Text pages:

Until recently, relatively little was known about the physiological role of carnosine in skeletal muscle, despite the fact that the molecule was discovered more than a century ago and that it is one of the most abundant metabolites in muscle cells. The objective of this review is to provide a state-of-the-art overview of the science on carnosine's role in muscle. The recent progress in this area originates from the discovery in 2006 by Roger Harris and co-workers that oral beta-alanine supplementation can increase the muscle carnosine content and thereby the performance during high-intensity exercise [3;4]. This review aims to discuss the potential mechanisms, based on the known biochemical properties of the dipeptide, that may underlie the ergogenic effects of carnosine loading. Second, muscle carnosine concentration displays a high interindividual variation in humans and in this review we describe the possible determinants of this variability. The interaction of carnosine and training is discussed. Both the effect of exercise training on the muscle carnosine content, as well as the value of beta-alanine as a training aid remain a matter of debate. This review contains a number of practical implications for athletes who seek improved exercise performance by using beta-alanine.

In order to provide a comprehensive summary of the currently available knowledge on muscle carnosine and beta-alanine supplementation with respect to exercise performance and training, a literature search was performed on PubMed and Web of Science using the search terms 'carnosine' or 'beta-alanine' in combination with 'muscle', 'exercise' or 'performance'. This literature overview is based on more than 100 published articles from 1950 to August 2009 on this topic.

Metabolic pathways of carnosine

Carnosine (beta-alynyl-L-histidine) is a dipeptide, combining the proteinogenic amino acid histidine with the non-proteinogenic beta-amino acid beta-alanine. Carnosine was first identified by the Russian biochemist Vladimir Gulevich in 1900, when he was looking for unidentified nitrogen-containing compounds in meat extract. Accordingly, he named the discovered molecule carnosine (*carnis* is Latin for *meat/flesh*). This name appeared to be accurately chosen as carnosine is predominantly present in skeletal muscle tissue of mammals and virtually absent from most other organs, except from its heterogeneous presence in brain regions. A concise review on carnosine's discovery and identification is written by Alexander A. Boldyrev [5].

Carnosine is absent from plants (and therefore from vegetarian food) and invertebrates, whereas in the animal kingdom it appears in high but varying quantities in muscles from different vertebrates [6]. In humans, the muscle concentration of carnosine is 5-8 mM in wet weight (or 20-30 mmol/kg dry weight), which is comparable to the concentrations of ATP, carnitine or taurine and lower than (phospho)creatine. Carnosine is the only histidine-containing dipeptide (HCD) found in human muscle, whereas muscles of other animals/mammals may also contain methylated analogues of carnosine, namely anserine (beta-alanyl-N_1-methylhistidine) and balenine/ophidine (beta-alanyl-N_3-methylhistidine). When comparing the total HCD content in muscles of different animals, humans are somewhere in the middle. Some endurance exercise type animals like pigeons and migrating birds have lower concentrations (< 5 mM), while other animals that are involved in more

burst-like and sprint exercise (chicken, grey-hound dogs, thoroughbred horse) have markedly higher concentrations (20-50 mM) [7]. Some of the highest HCD concentrations, higher than the concentrations of ATP and CrP combined, have been observed in whale, whose exercise profile is characterized by extremely prolonged hypoxic dives and anaerobic energy delivery [6]. The specific evolutionary drive of high HCD content in muscles of animals involved in anaerobic work, may be of particular importance when trying to understand in what exercise conditions carnosine plays a pivotal role.

The major pathways involved in carnosine metabolism are synthesis and hydrolysis, respectively from and to its constituent amino acids [8]. The enzymatic condensation of beta-alanine and histidine is catalysed by carnosine synthase (or synthetase), but the enzyme is poorly identified and its genetic origin unknown [9]. High activities of carnosine synthase have been found in skeletal muscle tissue [6] and in specific regions of the brain, such as the olfactory bulb [9]. In humans and horses, β-alanine is considered to be the rate-limiting precursor of carnosine synthesis. β-alanine can be obtained through the hydrolysis of dipeptides extracted from dietary meat or fish and through the degradation of uracil in the liver [10]. Bakardjiev and Bauer [11] have shown that the transsarcolemmal transport of one molecule of β-alanine requires two sodium ions and one chloride ion.

The enzymatic hydrolysis of carnosine is catalyzed by carnosinase, of which two forms exist in the human body [12]. Serum carnosinase (CN1) is highly active in humans, resulting in the mere absence of carnosine from human blood, in contrast to other mammals such as rodents who lack serum carnosinase and whose blood contains considerable amounts of carnosine [13]. Tissue carnosinase (CN2), also known as cytosolic nonspecific dipeptidase, is quantitatively less important for the degradation of carnosine in humans.

Proposed role of skeletal muscle in whole-body carnosine metabolism

Literature suggests that skeletal muscle is the major production site and storage site for carnosine in the human body. With respect to its storage function, probably more than 99% of the body's carnosine is present in the skeletal muscles, because of the size of the musculature (constituting 40-50% of the total body weight) and the high muscle carnosine concentration (5-8 mM) compared to its concentration in other carnosine containing tissues, such as brain (0.1 mM on average) [5]. The support for the production function comes from several lines of evidence. First, the highest rates of carnosine synthase activity are found in skeletal muscle tissue, along with the olfactory bulb [5;14]. Second, there is reason to believe that very little of the synthesized carnosine in the muscle cells is subsequently hydrolyzed again, since carnosinase is virtually absent from muscle [14;15] and there is no non-enzymatic degradation process for carnosine. Instead, synthesized carnosine either remains present in the muscle cells for long periods, or it is secreted into the circulation through a regulated transport process. Evidence for the former was recently shown by Baguet et al. [2], who showed that a highly increased concentration of carnosine in human calf muscles following beta-alanine supplementation can remain present for >9 weeks following cessation of supplementation. Evidence for the regulated carnosine release from muscle into the circulation is fragmentary at present. Carnosine can be transported as an intact dipeptide across the plasma membrane through proton-coupled oligopeptide transporters (PEPT1, PEPT2, PHT) [16-18]. Kamal et al. [19] have recently shown that the genetic knockout of PEPT2 in mice results in a decreased tissue carnosine content in spleen, kidney and olfactory bulb. Interestingly, the muscle carnosine content was increased rather

than decreased in PEPT2 knockout mice, suggesting that release of carnosine from muscle was inhibited by PEPT2 deficiency [19].

There is some evidence to believe that carnosine metabolism (synthesis, degradation, release) increases upon muscle contractile activity and exercise. Nagai et al. [20] observed elevated circulating carnosine levels and increased carnosine synthase activity in gastrocnemius muscle when rats were exercising in a running wheel. Also in humans, carnosine concentration markedly increases in the skeletal muscle interstitium during leg extension exercise at 20W, as determined by microdialysis [21]. Although some researchers believe that the contraction-induced release of carnosine by muscles is a measure of muscle damage and exertional rhabdomyolysis [21-23], Nagai et al. [20] have proposed that carnosine release from muscle is a regulated process. The fact that proton accumulation (acidosis) can stimulate the proton-driven dipeptide transporter (PEPT2) provides one possible mechanism as to why muscles would release/secrete more carnosine during contractions than at rest. The observation that the histidine concentration increases in parallel with carnosine in human muscle interstitium [23] suggests that at least a portion of the contraction-induced release of carnosine from muscle is immediately hydrolyzed by the serum carnosinase present in the interstitium/circulation in humans. In vitro animal experiments have suggested that the muscle carnosine concentration decreases following a period of contractions [24], further supporting the hypothesis of contraction-induced carnosine release. Finally, it is suggested that exercise training increases the activity of serum carnosinase in rats [20] and humans [25;26], although the latter studies are based on a limited number of subjects and are not well-controlled.

The physiological function and importance of exercise-induced carnosine release by skeletal muscle remains to be determined, but could be diverse. Hypothetically, released carnosine by muscle could have a paracrine or endocrine hemodynamic function, as carnosine is shown to have vasodilatory [27] and venoconstrictive potential [28]. Carnosine has also been proposed to modulate the sympathetic nervous system, thereby affecting the autonomic control of the pancreas [20], kidney [29], adipose tissue [30] and blood pressure [29]. Possibly, for some of these actions, carnosine can exert its effect by serving as a precursor of histamine through histidine [31]. The carnosine-histidine-histamine pathway may be involved in the therapeutic effects of carnosine [32]. However, it could also be that the rapid hydrolysis of circulating carnosine is a disadvantage, as certain tissues need intact carnosine for protective purposes. It is in this context that we should interpret the findings of Janssen et al. [33]. They observed that a short allelic form of the carnosinase gene CNDP1 is associated with a lower serum carnosinase activity and with a decreased risk of diabetic nephropathy (Janssen et al. 2005). This suggests that the slower carnosine is hydrolyzed in the blood, the longer it can exert a protective effect in diabetes [34].

Carnosine is predominantly present in skeletal muscle. Its abundance probably serves two functions, 1) to contribute to homeostatic control in other organs through carnosine release, and 2) to support local homeostasis in the muscle cells during contractions. The latter will be discussed in the following part.

Role of carnosine in myocellular homeostasis

Since most metabolites (ATP, phosphocreatine, glycogen, glutamine, carnitine,…) that have a high abundance in skeletal muscle cells are directly involved in energy transport and delivery,

initial searches for the role of carnosine in muscle were focussed on its possible role as a phosphagen system. Goodall [35] proposed that monophospho- and diphosphocarnosine function as a phosphate donor for ATP resynthesis during contractions. However, neither the phosphorylated forms of carnosine [36] nor a 'carnosine kinase' [37] have ever been detected in muscle tissue, and it is therefore unlikely that phosphocarnosine/carnosine plays a similar role as the phosphocreatine/creatine kinase system in energy delivery and storage in skeletal muscle [38]. Instead, a number of other biochemical properties of carnosine, mostly of the imidazole moiety of the histidine residue, have been described that can be of considerable value for contracting muscle cells.

pH buffer

The total intramyocellular buffering capacity is composed by the buffer actions of proteins, phosphates (Pi, CrP), ammonia, bicarbonate and histidine-containing dipeptides. Of all the amino acid side-chains in proteins, only the imidazole ring of histidine (pKa 6.1) has a good pKa to function as a pH buffer in the physiological range. The pKa is the acid dissociation constant and the pKa is ideal when it lies within the physiological pH range (6.5-7.1) of myocytes, so it can dynamically accept protons during contraction-induced acidosis. HCD are thought to be a way to up concentrate the content of histidine in muscle, as in proteins only 1 out of 20 amino acids (on average) would be histidine, whereas in HCD this ratio is 1 out of 2. Moreover, when bound to beta-alanine the pKa of histidine increases slightly to 6.83, which is right within the range of pH values that exist in contracting and fatigued myocytes. The role of carnosine as a physiologically relevant pH buffer was the first function of the dipeptide to be discovered [39;40]. Some fish species have no HCD in their muscles but instead display a very high concentration of free histidine (up to 100 mM in pelamyd or 'histidine fishes', [5]), which suggests that one reason why carnosine is so abundant in muscles is to increase the histidine and imidazole content.

Anti-oxidative potential, metal chelation and anti-glycation

Carnosine can contribute to the defence against oxidative stress of tissues by directly interacting with reactive oxygen species (ROS). At physiological concentrations in muscle, carnosine can interact with singlet oxygen and scavenge peroxyl radicals [41] and superoxide radicals [42]. Therefore, carnosine can reduce the products of lipid peroxidation (TBARS, malondialdehyde) and act as a natural hydrophylic antioxidant [5;43;44].

The mechanism for the anti-oxidative capacity may in part relate to the fact that carnosine can chelate ferrous ions and other transition metals [41]. Transition metals are known to promote the production of free radicals such as hydroxyl radical ($OH^{\cdot}$) formation through the Fenton reaction. Carnosine can also form complexes with other divalent cations like copper and zinc. The importance of this property in muscle homeostasis remains to be determined, whereas in brain it has been shown that carnosine can protect against copper- and zinc-induced neurotoxicity [45].

Glycation or non-enzymatic glycosylation describes the reaction of sugar aldehydes with amino groups of proteins which eventually leads to protein cross-linking and formation of advanced glycation end-products (AGEs). The latter may be involved in the aetiology of aging and diabetic complications [34]. In vitro studies have shown that carnosine can inhibit glycation and protein cross-linking in a sacrificial process of aldehyde scavenging [46].

Carnosine has been implicated as a potential therapeutic adjuvant in a number of pathologies, such as cataract [47], aging [48] and diabetic complications [33], which may relate to carnosine's biochemical properties of anti-glycation, anti-oxidation or metal chelation or a combination of these.

Several other chemical properties of carnosine have been described, among others as an activator of carbonic anhydrase [49] and an inhibitor of angiotensin converting enzyme (ACE) [50]. The physiological relevance of these effects is not intensively studied and lies beyond the scope of this review. More extensive reviews on the chemical properties and therapeutical benefit of carnosine have previously been published [5;34;47;51;52].

Determinants of muscle carnosine content

The concentration of carnosine in human muscle follows a Gaussian distribution (Figure 1) and is characterized by a high variation coefficient in soleus (27.5%) and gastrocnemius (27.6%). The currently available methods for quantification include invasive procedures, where dipeptides in muscle biopsy homogenates or fibers are separated and quantified by high-performance liquid chromatography (HPLC) [53;54], and a non-invasive procedure based on proton magnetic resonance spectroscopy (proton MRS) [1;55;56]. Both methods show similar effects of gender, beta-alanine supplementation, etc. on muscle carnosine content. The currently identified determinants of muscle carnosine content are graphically summarized in figure 2, and are described below.

Fiber type

In humans, fast-twitch muscle fibers have markedly higher carnosine content compared to slow-twitch fibers. The reported fast/slow concentration ratio, measured by HPLC-based single fiber analysis, varies from 1.3 to 2.0 [4;57;58]. As anaerobic energy delivery is quantitatively and qualitatively more important in glycolytic fibers, this pattern is in accordance with the supposed role of carnosine as a pH buffer. In most animal species a same fiber-type specific carnosine content in favour of fast-twitch fibers is observed [59;60]. Human skeletal muscles with a known high proportion of fast-twitch muscle fibers, such as gastrocnemius, have higher carnosine content than muscles with a typical slow-twitch profile, like soleus (figure 3). The strong correlation in figure 3 indicates that subjects with high carnosine content in one muscle will also have high values in the other skeletal muscles, and vice versa.

Age and gender

Mannion et al. [61] have compared the carnosine content of vastus lateralis muscles across gender and showed that men have approximately 20-25% higher muscle carnosine content than women, which is in line with their superior anaerobic performance capacity [62]. This could partly be caused by a higher proportion of fast-twitch fibers in male muscles [63], although there is no consensus regarding the latter [64]. Sexual dimorphism with respect to muscle carnosine and HCD content is species dependent, as it appears more pronounced in rodents [65], but is absent in the horse [66].

Rodent skeletal muscle carnosine content markedly declines with advancing age [67;68]. A similar pattern is expected in humans although the current evidence is mainly based on cross-sectional comparisons with elderly with a specific pathology, such as osteoarthritis [69], neuromuscular disease [70] and glucose intolerance [71]. Advancing age is associated with a

gradual transition towards a slower muscle type, which could relate to the lower carnosine levels in the old.

However, a more slow-twitch fibre type profile in the female and in the old can probably only account for a small portion of the gender and age effects on muscle carnosine content. More likely, androgens have a stimulating effect on muscle carnosine synthesis. Indeed, experiments in mice show that males have a 3-4 fold higher carnosine content than females and that in both genders the carnosine content is reduced by ~40% following gonadectomy [65]. Interestingly, the female muscle carnosine content can be elevated up to the level of males by exogenous testosterone administration. Although the extragonadal sexual dimorphism in mice is more pronounced than in humans, there is indirect evidence supporting a role for androgens in carnosine synthesis. In a descriptive study on body-builders, where substance abuse with anabolic steroids is not unusual, very high muscle carnosine concentrations (approximately twice as high as in a control population) have been reported [72]. Also the well-described decline in androgen concentration with advancing age may be a determinant of the lower carnosine content of old muscle. If indeed androgens play an important role in muscle carnosine content, then it could be expected that a substantial elevation takes place in the maturation from boy to man. Data on humans are lacking, yet male but not female rodents show a doubling of muscle carnosine content during puberty [65].

Training status

Parkhouse et al. [73] have cross-sectionally compared the carnosine concentration of the vastus lateralis of sprinters and rowers with marathoners and untrained. Sprinters (4.93 ± 0.76 µmol/g) and rowers (5.04 ± 0.72) showed a markedly higher content than marathoners (2.80 ± 0.74) and untrained (3.75 ± 0.86) ($p<0.01$). Likewise, as shown in figure 4, trained 400m-runners display higher carnosine concentrations than physically active students. These differences probably relate to both selection/genetic factors (such as fiber-type distribution) and to training-induced alterations in muscle carnosine content.

Training intervention studies show differing results, depending on the training mode, as shown in table 1. Suzuki et al. [74] reported that the carnosine content of the vastus lateralis dramatically increased after 8 weeks of sprint training (1 or 2 Wingate cycling sprints per session, 2 sessions per week) from 5.17 ± 1.69 to 11.01 ± 3.05 mmol/kg wet muscle. Most other studies, however, do not report an increase in muscle carnosine content following different types of training. According to Kendrick et al. [75], 10 weeks (4 sessions per week) of resistance training did not change the carnosine content of the vastus lateralis. Also in response to isokinetic knee extensor training [58;76], no carnosine loading is observed. Although not intended as a training intervention study, Derave et al. [1] observed that the carnosine content in the gastrocnemius increased with 16% ($p<0.05$) in the placebo group, consisting of seven male trained 400m runners during a 5 week period while in preparation for the indoor competition season. In summary, the limited amount of training intervention studies available to date is equivocal with respect to the effects of exercise training on muscle carnosine content, but the effect of short-term training is probably small.

The mechanism for the potential effects of chronic training on the carnosine content is to be elucidated. Hirakoba [77] proposes that the conditions of hypoxia and acidosis during high-intensity exercise can be responsible for increased carnosine concentration in skeletal muscle. This point of view is nevertheless inconsistent with the research of Edge et al. [78].

Their high-intensity interval training (6-12, 2-min intervals at 100% VO2max, with 1 min of rest between sets) performed 3 sessions per week for five weeks, resulted in a decrease of buffering capacity despite large exercise-induced decreases in muscle pH (pH=6.81). Moreover, the vision of Hirakoba [77] can not explain the differences in training effects, since both resistance training and isokinetic training can result in acidosis.

Nutrition

Carnosine can enter the circulation by intact absorption from the gut. The circulating carnosine concentrations are elevated for some time following the ingestion of HCD containing meat [25;79], but most of the dipeptide is hydrolysed by serum carnosinase within minutes to hours. The synthesis of carnosine in skeletal muscle is limited by the availability of beta-alanine rather than histidine [3;80]. Despite its designation as an essential amino acid, histidine occurs in sufficient concentrations in the circulation and will only limit carnosine synthesis in situations where a specific histidine-free diet is applied [81]. Beta-alanine is not present in proteins and its main endogenous source is from the irreversible degradation of the pyrimidines uracil and thymidine. Postabsorptive circulating beta-alanine concentrations are therefore low. The transsarcolemmal transport of histidine and beta-alanine provides the precursors for carnosine synthesis in skeletal muscle.

The HCD content in the nutrition will have an impact on the availability of beta-alanine and therefore possibly on the muscle carnosine content. A vegetarian diet is free of HCD and an abstract reports that vegetarians have low muscle carnosine contents [82]. On the other hand, regular ingestion of chicken breast extract (CBEX), high in HCD content [83], is thought to elevate muscle carnosine content [84]. Between these extremes (vegetarianism on the one hand and systematic large intakes of CBEX on the other), it remains to be determined to what degree variation in the amount and type of daily meat intake influences the variation in muscle carnosine content between individuals.

It was recently demonstrated that 15 weeks of oral creatine supplementation can substantially elevate muscle carnosine content in mice [68]. The mechanism for this phenomenon remains elusive at present. In humans, however, a carnosine loading effect of a short (1 week) period of oral creatine supplementation was not observed (Harris RC, personal communication).

Beta-alanine supplementation

Beta-alanine supplementation is probably one of the most powerful means to elevate muscle carnosine content (figure 2 and 4). The development of beta-alanine as a useful nutritional supplement has emerged from the elegant work of Roger C. Harris and co-workers. They demonstrated, first in the horse [80] and later in humans [3], that the ingestion of large daily amounts (~100 mg/kg body weight) of beta-alanine is enough to elevate muscle carnosine content. Daily doses of 4.8 to 6.4 g of beta-alanine can elevate human muscle carnosine content with 60% in 4 weeks and 80% in 10 weeks [3;4]. Equimolar carnosine ingestion does not elevate muscle carnosine more than beta-alanine alone. Beta-alanine supplementation increases muscle carnosine content in both type I and type II fibres [3]. When comparing between individuals, high initial carnosine levels do not seem to impair the effectiveness of muscle carnosine loading [1]. Likewise, sprint-trained athletes who have high initial carnosine levels respond equally well to beta-alanine supplementation, see figure 4. Following cessation of beta-alanine supplementation, carnosine washout occurs at a slow

rate of 0.03 mM per day. An increase of 55% in muscle carnosine content was calculated to require a washout period of 15 weeks [2].

Fasting circulating beta-alanine concentrations are very low (<0.5 µM). When ingested in pure form, peak concentrations of beta-alanine appear in the blood within the first hour and rapidly decline within the second hour [3]. Doses of more than 10 mg/kg body weight are to be avoided, since circulating beta-alanine concentrations above 100 µM give rise to parasthesia symptoms, that probably relate to a sensitization of nociceptive neurons in the skin involved in neuropathic pain [85]. A daily dose of 4.8 to 6.4g therefore requires 6-8 servings, separated by at least 2h. However, new controlled-release formulations of beta-alanine are currently investigated, that display reduced peak concentrations and reduced parasthesia [86]. Apart from parasthesia, no other side effects of beta-alanine have been described so far [3]. Unlike creatine supplementation, beta-alanine does not induce body weight gain. Further research on the possible side effects and safety of beta-alanine as a nutritional supplement is warranted.

Ergogenic effects of elevated muscle carnosine content

Exercise types that benefit from beta-alanine supplementation

Based on the observations that animals and humans that are performing well in sprint-type exercise have higher muscle carnosine content than those that excel in endurance exercise, it can be expected that high muscle carnosine content is ergogenic in anaerobic exercise, as first proposed by Parkhouse and McKenzie [87]. In 2002, Suzuki et al. [88] indicated that in a group of healthy men, high muscle carnosine contents correlated positively with the mean power per body mass ($r=0.785$, $p<0.01$) during a 30-s all-out cycling sprint, and especially in the latter phase of the exercise bout.

Several recent investigations have explored the potential ergogenic effect of chronic beta-alanine supplementation on different types of exercise performance, summarized in table 2 . Beta-alanine doses vary from 2 to 6.4 $g \cdot day^{-1}$ and durations from 3 to 13 weeks.

In one of the first studies, Hill et al. [4] supplemented men for 10 weeks with 6.4 $g \cdot day^{-1}$ beta-alanine. The total work done (TWD) during a cycle capacity test at 110% of their maximal power (duration of ~2.5 min) increased following 4 and 10 weeks of beta-alanine supplementation (+13% and +16.2% respectively). During an incremental cycling exercise test (starting at 40W and increasing with 20W every 3 min until exhaustion) in untrained women, Stout et al [89] found that the ventilatory threshold (+13.9%) and the time to exhaustion (+2.5%) significantly increased after 28 days of beta-alanine supplementation (6.4 $g \cdot day^{-1}$), although the VO_2-max did not change. Also the physical working capacity at the fatigue threshold, determined by EMG of the vastus lateralis during cycling, increased significantly by beta-alanine supplementation in young [89] and aged [90] population. These studies were all performed in untrained subjects.

In trained 400m-runners, 4-5 weeks of beta-alanine supplementation (4.8 $g \cdot day^{-1}$) did not improve 400m running performance more than in a placebo group despite a marked increase in muscle carnosine content [1]. However, during repeated isokinetic knee extensions (5x30 contractions with 1min rest intervals) beta-alanine reduced fatigue in the latter 2 bouts in this trained population. Whether beta-alanine is ergogenic in aerobic endurance exercise remains to be established. However, beta-alanine supplementation was

shown to improve the 30-s sprint capacity by 11% at the end of a 2-h simulated cycling race in moderately- to well-trained cyclists [91].

The effect of beta-alanine supplementation on isometric knee extensor performance is equivocal. Ponte et al. [92] observed a 10-15% (8 seconds) improvement in isometric endurance at 45-50% of maximal voluntary contraction (MVC) of the knee extensors, whereas Derave et al. [1] could not identify an effect of oral beta-alanine supplementation on isometric endurance of the knee extensors at 45% of MVC.

A single acute pre-exercise administration of a chicken breast extract (CBEX) soup (containing 1.5g of carnosine and anserine) does not improve performance during intermittent exercise that consisted of 10 x 5-s maximal cycle ergometer sprints with a 25-s recovery period between each sprint [84]. This finding supports the notion that the ergogenic effects of beta-alanine supplementation results from an increase in muscle carnosine content, which cannot be achieved by a single dose, but only following several weeks of supplementation.

It can be concluded that chronic beta-alanine supplementation can have ergogenic effects during single or repeated bouts of high-intensity exercise or maximal contractions. Although the scientific evidence in untrained populations is substantial, more studies need to be conducted in trained populations and in various sport disciplines in order to fully understand the value of beta-alanine supplementation in performance enhancement in elite sports.

Possible mechanisms of performance improvement

The observed improvement in anaerobic exercise performance following beta-alanine supplementation is most likely related to the increased muscle carnosine content, inducing an attenuation of peripheral (rather than central) fatigue. This is supported by studies with isolated muscle preparations of frogs and rodents, that show reduced contractile fatigue when muscles are exposed to increased extracellular carnosine (a process termed Severin's phenomenon [5;93;94]) or increased muscle carnosine content [68].

Increased availability of carnosine in myocytes can improve contractile behaviour and reduce fatigue in several possible ways. Carnosine is indisputably a functional pH buffer. This is supported in a recent study by Derave et al. [95] on the effect of 4-5 week beta-alanine supplementation on exercise-induced acidosis in physically active students. The decline in circulating pH was significantly attenuated during a 6-min cycling exercise bout at an intensity of 50% of the difference between ventilatory threshold (VT) and VO_2peak. The role of acidosis in muscular fatigue remains a matter of debate [96]. However, since both acute oral bicarbonate ingestion [97] and chronic beta-alanine supplementation have been shown to improve performance in exercise modes of similar duration and intensity, it is probably correct to conclude that at least part of the ergogenic effect of beta-alanine supplementation is related to improved physicochemical buffer capacity. However, the quantitative contribution of carnosine to total buffer capacity is limited, so it is likely that additional underlying mechanisms are at play. The initial estimates of carnosine's contribution to total muscle buffer capacity were as high as 60% [39]. Later studies [61;98], however, estimated the contribution of carnosine to the total buffering capacity to be only 7%.

A second mechanism that is involved in muscle fatigue is the reduction in Ca^{2+} release from the sarcoplasmic reticulum (SR) [99]. Russian studies [100;101] have proposed that the

Severin's phenomenon is explained by the modulation of SR Ca^{2+} release channel activity by carnosine. Specifically, carnosine could increase the sensitivity of Ca^{2+} release channels to their well-known activators (caffeine, AMP and Ca^{2+}) and decrease the inhibitory effect of low concentrations of Mg^{+} [102]. However, Dutka and Lamb [103] could not support these findings. They demonstrated that the positive effect of carnosine on the contractile fatigue is due to increased Ca^{2+} sensitivity of the contractile machinery and not to facilitated Ca^{2+}release. This effect was observed in chemically skinned fiber preparations of frog [104] as well as in mechanically skinned rat muscle fibers [103]. Thus, the increase of the Ca^{2+} sensitivity of the contractile apparatus by carnosine could aid in maintaining a higher level of force during the later stages of fatigue when Ca^{2+} release declines [103]. Mishima et al. [105] examined whether this carnosine-induced increase in Ca^{2+} sensitivity has any ergogenic effect on high-intensity exercise in rats. Despite an attenuation of the exercise-induced reduction in SR Ca^{2+} handling following 5 weeks of dietary chicken breast extract, the performance remained unchanged during high-intensity running for 2.5 minutes. It has to be noted that the evidence for the carnosine-stimulated increase in Ca^{2+} sensitivity is only based on *in vitro* and on rodent experiments and that the physiological *in vivo* evidence in human myofibers remains to be determined.

A third possible mechanism of performance improvement evoked by carnosine could be related to its anti-oxidative potential. Skeletal muscle fibers continually generate reactive oxygen species (ROS) at a slow rate that increases during muscle contraction, which contributes to fatigue of skeletal muscle during intense and prolonged exercise [106]. Due to its anti-oxidative potential, an increased carnosine content could, theoretically, diminish this ROS accumulation. Indeed, the acute oral ingestion of 450mg carnosine can, after 1h but not after 2h, elevate the serum total antioxidant capacity in humans (expressed as µmol/l Trolox equivalents, by chemiluminescent assay) [107]. Another mechanism by which carnosine could diminish this ROS-induced fatigue could be related to its potential to increase the Ca^{2+} sensitivity, since reactive oxygen species can reduce the myofibrillar Ca^{2+} sensitivity in fatiguing mouse skeletal muscle [108]. However, it is clear that further research is recommended to verify the effects of prolonged ingestion of beta-alanine on oxidative stress evoked by muscle contractions in humans.

Beta-alanine as a training aid

Nutritional supplementation for athletes is not only useful in competition, but it can also be functional to optimize the training effects, e.g. by improving recovery, maintaining higher energy levels, optimizing the training adaptations, etc. [109]. In the literature there is disagreement whether the training-induced adaptations could be stimulated by beta-alanine supplementation.

Several studies have investigated the effects of combined β-alanine supplementation and training on muscle and exercise performance, with conflicting results. According to Smith et al. [110], the effects of six weeks of high-intensity interval training on EMG-based neuromuscular fatigue in recreationally active men were not different between a control and a beta-alanine (3-6 $g.day^{-1}$) supplemented group. In physical education students, the supplementation of 6.4 $g \cdot day^{-1}$ beta-alanine (10 weeks) did not have any additive effect compared to training alone on whole body strength, isokinetic force production, muscular endurance and body composition after 10 weeks of resistance training [75]. On the other hand Hoffman and colleagues [111] reported that collegiate football players, who are used

to strength and power training, improved strength and body composition after 10 weeks of resistance training in a group consuming both beta-alanine and creatine compared to the placebo and/or creatine group. Hofmann and colleagues [112] subsequently showed that the training volume of collegiate football players, during their preparation for the season, can be improved by the supplementation of beta-alanine (30 days, 4.5 $g \cdot day^{-1}$). The experimental group reached a significantly higher training volume in the bench press exercise and a trend for all resistance exercise sessions, combined with a better subjective feeling. In addition to the differences in initial training levels of the subjects, the higher volume and the possible synergistic effect of beta-alanine with creatine in the study of Hoffman et al. [111] could explain the inconsistency between these two comparable studies [75].

However, it seems that the performance enhancement of combined beta-alanine supplementation and training is smaller than the potential of creatine to stimulate training-induced muscle hypertrophy. Nevertheless, it should be mentioned that training can stimulate the responsiveness to creatine supplementation [113;114], which is not the case with carnosine loading [58].

Practical implications for athletes

There is a relatively high interindividual variation in skeletal muscle carnosine content between humans and carnosine is able to improve high-intensity exercise. Therefore, low muscle carnosine content in athletes may be disadvantageous for sprint performance. The carnosine content is determined by several factors, as depicted in figure 3. Oral beta-alanine supplementation is probably the most efficient way to increase the skeletal muscle carnosine content. Still, it must be noted that the effects of beta-alanine supplementation on the performance are small and probably only relevant to athletes who have already optimized the other training modalities and who are seeking a minor improvement in performance.

The carnosine loading in muscle differs in several ways from the supplementation of the well-known creatine. Firstly, the loading of carnosine takes at least several weeks in contrast to the initial loading phase of one week for creatine. Conversely, also the washout rate is faster for creatine than for carnosine, indicating that the elevated muscle carnosine content is more stable [2]. A second difference with creatine supplementation is that individuals with a high initial muscle carnosine content, like sprint-trained athletes, respond equally well to beta-alanine supplementation as persons with a low initial content. In line with this apparent absence of a ceiling effect for carnosine, the highest reported supplementation-induced muscle loading is markedly higher for carnosine [4] than for creatine [115]. Finally, the supplementation of beta-alanine, in contrast to creatine, does not result in increased body mass which has important implications for athletes in weight-baring exercise types or weight class sports.

Although both beta-alanine and bicarbonate have been suggested to attenuate exercise-induced acidosis, there are marked differences between both ergogenic supplements [97;116]. First, sodium bicarbonate or citrate should be taken as an single pre-exercise dose, inducing acute metabolic alkalosis [117], whereas beta-alanine requires chronic supplementation for weeks, but does not affect the blood pH at rest. Beta-alanine is thought to work as a first-line defense as carnosine is elevated in the muscle cells where the protons

are produced during contractions, whereas bicarbonate resides in the circulation and only buffers once protons have entered the blood (second-line buffer). Additionally, bicarbonate elicits in many athletes a degree of gastro-intestinal discomfort, that does not occur with beta-alanine.

Currently, no health-related side effects of the oral chronic supplementation of beta-alanine have been reported, except from the acute parasthesia that occurs when the prescribed maximum dose of 1g per 2h-period is exceeded (see 'Beta-alanine supplementation'). A standard supplementation advice is to supplement 4 to 6.4 $g.day^{-1}$ (divided over 0.8 to 1g servings) for at least 4 weeks. However, the effects on health of continuous beta-alanine supplementation beyond 10 weeks, as well as of its combination with other supplements remain to be established.

Conclusions and future directions for research

In summary, it seems that the high interindividual variation in muscle carnosine content between humans is related to several determinants like muscle fiber type, age, gender, nutrition and training status. The underlying physiological mechanisms for this variation have to be elucidated. Another important question to be clarified is whether the high muscle carnosine levels of sprint-trained athletes are the result of the chronic effect of years of training or of selection effects and genetic factors. There are a number of indications that an elevated muscle carnosine content can delay fatigue during high-intensity exercise. Even though there is a shortage of available literature concerning the underlying mechanisms, especially in humans, it seems reasonable to assume that the performance improvement by beta-alanine supplementation is the result of carnosine's potential to act as pH buffer, as a stimulator of the Ca^{2+} sensitivity and/or as antioxidant. Beside the ergogenic effects, carnosine probably contributes to homeostatic control in organs other than the muscle and to the susceptibility to certain diseases, but this research area is virtually unexplored at present.

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Table 1: Effect of a training intervention on the carnosine content in the vastus lateralis.

Reference	Training type	Training duration	Subjects	Muscle carnosine content (mmol/kg dm)
Kendrick et al. 2008 [75]	Resistance training (4 days/week)	10 weeks	13 male physical education students	PRE: 29.2 ± 9.82 POST: 27.3 ± 9.52
Kendrick et al. 2009 [58]	One-legged isokinetic training (4 days/week)	4 weeks	7 physical education students	PRE: 22.6 ± 2.1 POST: 24.7 ± 3.7
Mannion et al. 1994 [76]	Isokinetic knee extensor training (3 days/week)	16 weeks	13	No statistical difference
Suzuki et al. 2004 [74]	Wingate sprint training (2days/week)	8 weeks	6 untrained males	PRE: 5.17±1.69 mM POST: 11.01±3.05 mM°

°$p<0.05$

Table 2: Ergogenic effects of beta-alanine supplementation

Reference	Exercise	Supplementation	Performance improved?	Subjects
Suzuki et al. 2006 [84]	10x 5s maximal cycle ergometer sprints, with a recovery of 25s	CBEX (1.5g carnosine and anserine) Acute: 30min before exercise	Total/ mean power: No	Untrained males
Ponte et al. 2006 [92]	Isometric knee extension at 45-50% MVC until exhaustion	6.4 $g \cdot day^{-1}$ β-alanine 4 weeks	Isometric: Yes	Untrained males
Derave et al. 2007 [1]	5 bouts of 30 maximal isokinetic knee extensions Isometric knee extension at 45% MVC until exhaustion 400m race	4.8 $g \cdot day^{-1}$ β-alanine 4 weeks	Isokinetic: Yes Isometric: No 400m race: No	Trained 400m athletes (males)
Hill et al. 2007 [4]	Cycle capacity test at 110% maximum power output	6.4 $g \cdot day^{-1}$ β-alanine 4-10 weeks	Total work done: Yes	Untrained males
Stout et al. 2007 [89]	Continuous incremental cycle ergometry test to exhaustion Physical working capacity at fatigue threshold	6.4 $g \cdot day^{-1}$ β-alanine 4 weeks	Time to exhaustion: Yes Physical working capacity: Yes	Untrained females
Stout et al. 2008 [90]	Physical working capacity at fatigue threshold	2.4 $g \cdot day^{-1}$ β-alanine 90 days	Physical working capacity: Yes	Aged men and women (73 ± 11y)
Van Thienen et al. 2009 [91]	30s all out cycling at the end of simulated cycling race	2 – 4 $g \cdot day^{-1}$ β-alanine 8 weeks	Peak and mean power output: Yes	Moderate to well-trained cyclists (males)