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Physiological Role of Carnosine in Contracting Muscle

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High-intensity exercise leads to reductions in muscle substrates (ATP, PCr, and glycogen) and a subsequent accumulation of metabolites (ADP, Pi, H^+, and Mg^{2+}) with a possible increase in free radical production. These factors independently and collectively have deleterious effects on muscle, with significant repercussions on high-intensity performance or training sessions. The effect of carnosine on overcoming muscle fatigue appears to be related to its ability to buffer the increased H^+ concentration following high-intensity work. Carnosine, however, has other roles such as an antioxidant, a metal chelator, a Ca^{2+} and enzyme regulator, an inhibitor of protein glycosylation and protein-protein cross-linking. To date, only 1 study has investigated the effects of carnosine supplementation (not in pure form) on exercise performance in human subjects and found no improvement in repetitive high-intensity work. Much data has come from in vitro work on animal skeletal muscle fibers or other components of muscle contractile mechanisms. Thus further research needs to be carried out on humans to provide additional understanding on the effects of carnosine in vivo.

Key Words: pluripotent dipeptide, antioxidant, buffering agent, calcium regulation, sports performance

Skeletal muscles are proficient at generating enormous force and power outputs when appropriately activated (44). Continued attempts to reproduce the same output are consistently met with failure, however, which is characterized by progressive deterioration in performance. It is this decline in performance that we identify as fatigue (57). A wealth of research shows that muscle fatigue can occur with all types of muscular activity, both short-term intense or long-term moderate exercise (2, 4, 9, 43). High-intensity (HI) exercise is involved in many sporting activities especially sprint events and can only be maintained for short periods of time (119). In addition, team sports such as football, rugby, and basketball are all characterized by HI exercise with episodic periods of reduced-intensity exercise or rest (104).

A number of possible sites/processes might be involved in the development of fatigue during HI exercise: disruption of contractile mechanisms, depletion of fuel, accumulation of metabolites (59, 73), and oxidative stress (Figure 1). Thus

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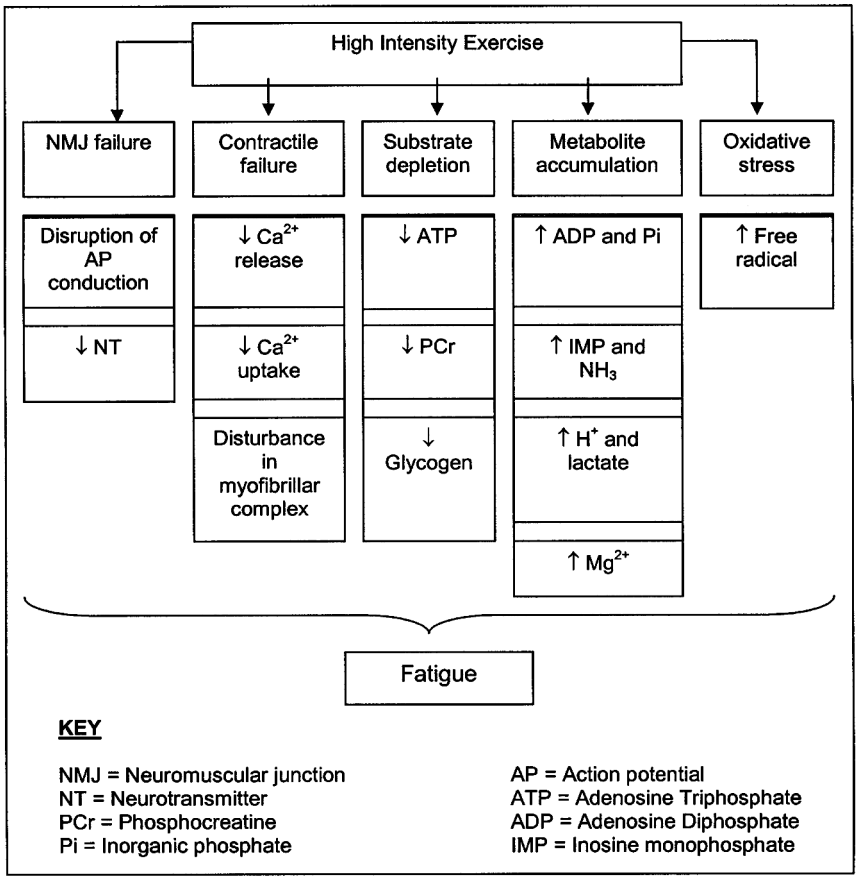

Figure 1—Sites/mechanisms of fatigue during high-intensity exercise.

the literature presented herein aims to highlight the principal sites of fatigue that could be influenced by carnosine, a pluripotent dipeptide.

Carnosine—Multifunctional Dipeptide

Carnosine is a naturally occurring histidine containing dipeptide also known as β-alanyl-Lhistidine (50, 96). It is found in many tissues, most notably in muscle cells (22) and nerve cells (96) at levels of up to 20 mM (49). Currently there is considerable interest in carnosine due to an abundance of research into its inhibitory effects on protein glycosylation and protein-protein cross-linking and suggestions regarding possible anti-senescence roles (15, 40, 48, 120). Carnosine, however, is a multifunctional dipeptide and has a number of other roles such as buffering (24, 28), quenching free radicals (19, 76), enzyme regulation (56), and sarcoplasmic reticulum (SR) calcium regulation (12, 16).

Absorption, Uptake, and Distribution

Carnosine makes up a significant fraction of the water soluble (42) nitrogen containing compounds in the body (96). As mentioned, it is a component of skeletal muscle (101), which also contains the enzyme carnosine synthetase (16). Carnosine breakdown is catalyzed by carnosinase (92), which is present in many tissues (kidney, liver, plasma) with the exceptions of skeletal musculature (16) (Figure 2).

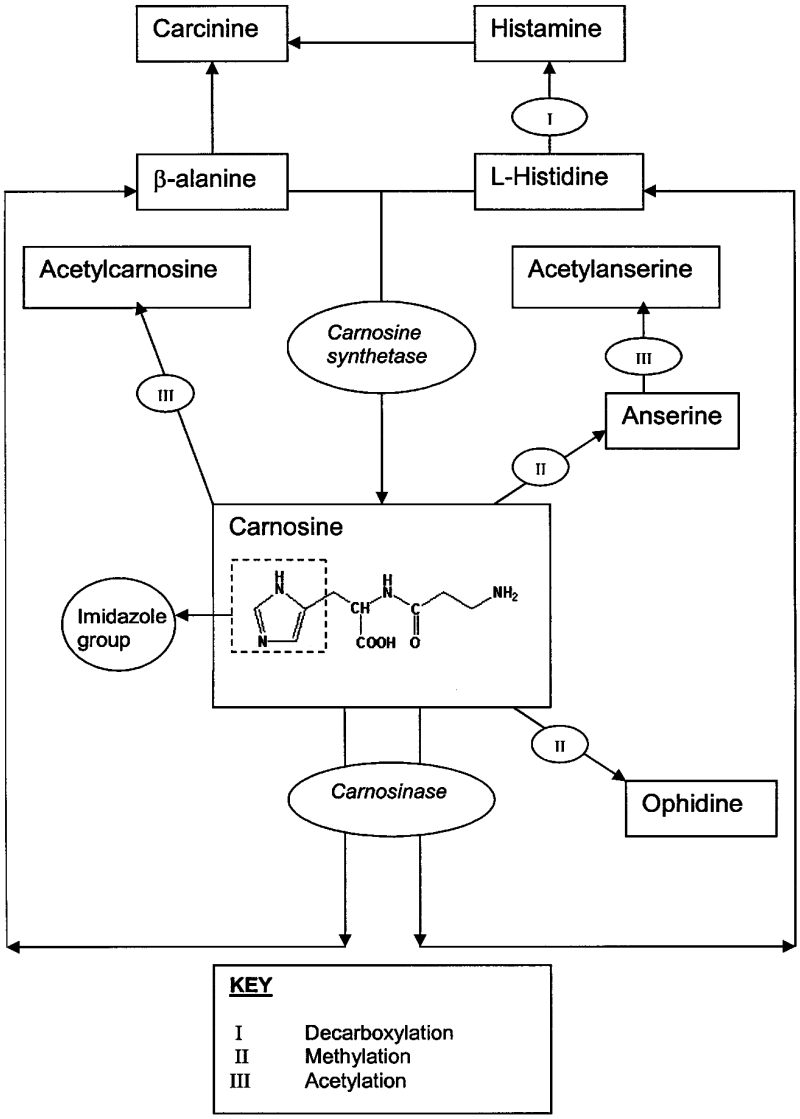

Figure 2—Metabolism of carnosine [adapted from (16)].

Much research has been carried out on the muscle carnosine content of vertebrate species (horses, fishes, rats, mice) (1, 29, 30, 80, 94). There are, however, only a few reports of normal values in human skeletal muscle (46, 77, 90). Gardener et al. (41) investigated the absorption of carnosine supplements in healthy humans by measuring oral tolerance, plasma levels, and urinary outputs over 5 h post-ingestion. The authors reported no adverse effects of carnosine ingestion, although a few subjects experienced mild and momentary digital paresthesia within the first hour. In their initial experiments, Gardner and colleagues were unable to detect carnosine in plasma after ingestion. Further experiments suggested that the inability to detect carnosine in plasma was due to carnosinase activity in plasma. Gardener et al. did however, recover up to 14% of the ingested carnosine in urine and suggested that this was as a result of intact carnosine being absorbed across the gastrointestinal tract (41). Furthermore, Ferraris et al. (35) found that the small intestine in mice contained a system responsible for carnosine transport from the intestine to the blood flow. Ferraris et al. also assessed the intestinal transport of carnosine by feeding varying levels of protein (72% protein/l18% protein) to mice. Carnosine uptake by isolated mouse small intestine increased with dietary protein level (possibly due to increases in the carnosine transporter). The proposed site for maximal uptake of carnosine (in vitro) is the jejunum. In vivo studies on the transport of other dipeptides also suggest a proximal site for uptake, which corresponds to that for active glucose uptake. Conversely, some researchers purpose that carnosine is principally hydrolyzed in the small intestine thus the constituent amino acids enter the blood (113).

Dunnett and Harris (28) found that β-alanine bioavailability influenced the concentration of carnosine in muscle. These investigators examined the effect of sustained dietary β-alanine and L-histidine supplementation on horse muscle carnosine concentrations (in type I, IIA, and IIB muscle fibers). They reported an adaptive response to sustained β-alanine supplementation, thought to be due to increased β-amino acid transport across the gastrointestinal tract. The same was not seen for L-histidine, however. Administration of β-alanine lead to increases in carnosine in type IIA and type IIB fibers and, according to Dunnett and Harris, such increases would produce a corresponding increase in skeletal muscle nonbicarbonate intracellular physicochemical buffering capacity (β). On the other hand, Tamaki et al. (112) found that deficiency of dietary histidine reduces carnosine concentrations in skeletal muscles of rats, while supplementation of histidine increased carnosine levels. Furthermore, dietary carnosine also increased muscle concentrations. Contradictory to Tamaki et al.'s finding, Chan et al. (18) reported that dietary carnosine supplementation did not increase carnosine concentrations in heart, liver, and skeletal muscle. Dietary supplementation with both carnosine and α-tocopherol (vitamin E), however, resulted in greater increases of carnosine concentrations in liver and heart. This implies a possible in vivo inter-relationship between carnosine and α-tocopherol.

The short-term increases in tissue levels of carnosine (established by intervention studies) is followed by its decrease due to carnosinase action (16). In addition, Gardener et al. (41) found individual variability in plasma carnosinase activity to be associated with subjects' exercise status (exercisers had increased carnosinase activity and decreased carnosine excretion). Other investigators reported that carnosinase is involved in muscle turnover and could explain the different excretion levels in urine in exercisers and non-exercisers (8, 27).

In summary, the literature on carnosine absorption is somewhat equivocal. While some studies report carnosine supplementation having an effect on muscle carnosine content, others report that supplementation of the monomers β-alanine and l-histidine affect carnosine levels. Furthermore, hydrolysis of the dipeptide by carnosinase in plasma might mean that carnosine may not be taken up by skeletal muscles. Gardener et al. (41), however, found that by the fourth hour post-carnosine ingestion, carnosine was still being excreted in urine. They argued that the absorbed carnosine might be rapidly cleared from the plasma and sequestered in other compartments (possibly including muscle), the remainder being excreted by kidneys. In addition, there have been no reports on any toxic effects of carnosine or its derivatives (96).

Accumulation of H^+ and Lactate—Carnosine as a Component of Muscle-Buffering Capacity

During relatively brief, high-intensity (> 50 to 60% of VO_{2max}) muscular effort, lactic acid accumulates (within the muscle fibers) as a result of the anaerobic breakdown of glycogen (52, 75). The build up of lactic acid has long been recognized as a potential contributor to muscle fatigue. Lactic acid, however, which has a pK of 3.86, dissociates at intracellular pH to lactate and H^+ (88). It is now thought that the reduction in force occurs through the negative effect on cross-bridge cycling of increased H^+ (21, 67, 85, 123). Thus the capacity of a muscle to neutralize excess H^+ could be important during high-intensity exercise performance.

Lamb et al. (67) examined the effect of pH on excitation-contraction-coupling (ECC) in skinned fiber preparation from toad skeletal muscle. Depolarization-induced responses were examined at different pH levels (7.1, 7.6, 8.0, 6.6, and 6.1). At all pH levels the first depolarization elicited a large response. Subsequent depolarizations became progressively small except with pH 7.1 (similar responses between repeated depolarizations). The authors concluded that at low pH the Ca^{2+}-ATPase operated poorly and this can account for the successively smaller depolarization-induced response at acidic pH. In alkaline pH the SR lost far more Ca^{2+} than in pH 7.1, indicating a leaky SR due to the pH dependence of Ca^{2+}-activated opening of ryanodine receptors (RyR).

As a defense mechanism against changes in intracellular pH, cells have evolved proton buffering systems. The intracellular non-bicarbonate buffering of muscle is dominated by the imidazole group (Figure 2) which exists in histidine residues of proteins, in free histidine, and in histidine-containing dipeptides such as carnosine and anserine (1).

The imidazole groups are potent proton buffering constituents as they have pK values close to intracellular pH, thus one of the two nitrogens of the imadazole ring can be protonated in the physiological range of pH (1). The regulatory process that keeps intracellular pH close to pK values of imidazole groups is called "alphastat regulation" (11). Its role is to maintain α-imidazole [in other words, the ratio of non-protonated imidazole/(non-protonated imidazole + protonated imidazole)] relatively constant (1).

In 1953, Severin et al. first described a clear function of carnosine as a pH buffer in muscle. Using isolated frog muscle, they demonstrated that in the presence of carnosine, the muscle could accumulate large amounts of lactate without hindrance but in the absence of carnosine, lactate caused significant acidification of

the tissue (107). The proton buffering capacity of carnosine and anserine, however, differ markedly between animal species and muscle types (depending on the ability for anaerobic exercise) (1, 11). With respect to human muscle, studies have found small amounts of carnosine while anserine was undetectable.

Mannion et al. (77) examined carnosine content in the lateral portion of the quadriceps femoris muscle of 50 human volunteers and found the average muscle carnosine content was 20 mmol/kg (± 4.7 standard deviation) of dry muscle mass. In addition, the level of carnosine was significantly higher in male subjects than in female subjects of a similar age and training status. In another study by Harris et al. (46) carnosine was found to be twice as high in type II fibers of human vastus lateralis muscle compared with type I.

In 1992, Mannion et al. (77) estimated that carnosine could contribute, on average, approximately 7% to the total muscle buffering. This could suggest that in humans (in contrast to many other species) carnosine is of only limited importance in preventing the reduction in pH observed during high-intensity exercise. Davey (24) has argued that anserine and carnosine can contribute as much as 40% to the buffering in the physiological range (between 6.5 and 7.5). In addition, a number of studies (although in equine research) corroborate that increased muscle carnosine concentrations lead to increased intramuscular H^+ buffering capacity (23, 28, 29). Furthermore, Suzuki et al. (111) investigated the relationship between carnosine concentration and high-intensity exercise performance (30 s Wingate test) in 11 healthy men. They reported a significant correlation between carnosine concentration and proportion of type IIB fibers (important in explosive movements), similar to findings from studies in horses and camels (29, 103). There was also a significant correlation between carnosine concentration and mean power per kilogram of body weight in particular during the final stages of the test (21 to 30 s). This study demonstrated that athletes in anaerobic sports have a potentially greater requirement for skeletal muscle carnosine stores and this might be a factor in determining performance during high-intensity exercise (111).

To our knowledge, there is only one study to date which has investigated the effects of carnosine supplementation on high-intensity performance. Kraemer et al. (62) tested 10 trained (cyclists) and 10 untrained male subjects. Subjects carried out 2 test sessions post 3.5 d intervention of either PhosFuel (1000 mg dibasic sodium phosphate, 240 mg potassium bicarbonate, and 12.5 mg carnosine) or placebo capsules (containing similar quantities in sodium and potassium). The test sessions consisted of four consecutive 30 s Wingate tests separated by 2 min, with blood samples being analyzed at baseline and post each Wingate test. The data from this intervention study found no effect of PhosFuel on acid-base status or on performance during repetitive Wingate tests. It is possible that the small dose of carnosine ingested in Kraemer et al.'s study would scarcely affect intramuscular carnosine concentrations (41, 114). In animal studies where carnosine has been reported to be efficacious (with respect to immunomodulating activity), researchers used doses of between 50 to 100 mg/kg/d of body weight (64) while others have reported using doses of up to 200 mg/kg/d (102). Experiments with rats, however, have shown that carnosine injections of as little as 2 to 20 mg/kg produce anti-inflammatory and antihistaminic effects [see review by Quinn et al. (96)].

With respect to sports performance, fiber composition of various skeletal muscles determines the capacity to perform glycolytic and oxidative processes.

Therefore, the role of carnosine in skeletal muscle could depend in part on the fiber composition of the skeletal muscle in which carnosine is found. According to Maynard et al. (80) high intake of dietary carnosine (1.8%) over 8 wk, appears to preferentially increase carnosine concentrations in rat soleus muscle and lateral gastrocnemius, while low levels of dietary carnosine (0.1%) were found to be ineffective. A wide range of carnosine dosages have been used in animal and human studies with equivocal findings. A definitive dosage or dosage range for humans has yet to be elucidated.

Overall, carnosine appears to be an important component of muscle buffering capacity. In addition, intramuscular levels show adaptive increases in response to physiochemical stressors and in humans at least, gender differences in concentrations as measured. Although Mannion et al. (77) suggested that the quantity of carnosine in human muscles might not be as effective in buffering as in other animals, it is possible that there is potential for increasing this content through supplementation. No studies to date have investigated the effects of pure carnosine supplementation on high-intensity performance in humans or a specific role for carnosine in pH homeostasis during muscle contractions.

Oxidative Stress—Carnosine As An Antioxidant

Exercise is associated with oxidative stress as muscle contractions increase oxidative metabolism (55, 78, 115). Adaptations to habitual exercise, however, appear to have an antioxidant protective effect (71, 82, 128). Investigations over the years have generated inconsistent results with respect to the relative importance of these effects. This is possibly due to the different modes of exercise that have been studied or the diverse biomarkers which have been used for detecting oxidative stress (20, 117).

A number of explanations for how exercise increases the levels of reactive oxygen species (ROS) have been put forward:

1. Increased respiration with increased flow of electrons in the electron transport chain might elevate the levels of ROS (78).
2. Depletion of ATP pools leads to higher intracellular levels of ADP, triggering catabolism of ADP and conversion of xanthine dehydrogenase to the superoxide generating enzyme xanthine oxidase (82).
3. Decreases in pH promote oxygen release from hemoglobin and increase $p0_2$ in tissues (31).

Many investigators have proposed that ROS are a principal cause of exercise-induced disturbances in muscle homeostasis associated with muscle fatigue (7, 93, 118). In addition, ROS could contribute to the late phase of exercise-induced muscle injury (95). It also appears that oxidative damage can inhibit the function of the SR Ca^{2+}-ATPase (83) thus affecting calcium uptake following contraction. According to Xu et al. (126), ROS directly damage the Ca^{2+}-ATPase by attacking the ATP binding site. Such detriments to the function of the Ca-pump can affect muscle relaxation and might even cause a decrease in the availability of calcium for release during muscle contraction.

To prevent or delay the accumulation of oxidized products, several endogenous antioxidant systems are found in muscle tissue. These include α-tocopherol

(vitamin E) (105), carnosine and anserine (histidine-containing dipeptides) (96) and antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase (117). Antioxidants such as α-tocopherol and glutathione peroxidase are largely influenced by diet. Carnosine and anserine seem to be less affected by diet (17), though some supplementation studies have found levels of carnosine to be affected by intake (18, 112).

Carnosine is proposed to have antioxidant properties due to its biological function of quenching singlet molecular oxygen and scavenging hydroxyl and superoxide radicals (42, 61, 91). In addition, carnosine could reduce oxidative stress by a combination of free-radical scavenging and metal chelation.

According to Boldyrev et al. (14), the antioxidative function of carnosine is one of the most important manifestations of its biological role. They investigated the effect of carnosine on dog eyes with senile cataract (which is caused by lipid peroxidation) and demonstrated carnosine's ability to interact directly with lipid peroxidation products (both in vitro and in vivo). Similarly, Nagasawa et al. (84) examined (in vitro and in vivo) the antioxidant properties of carnosine. They took muscle tissue from rats (hind limb homogenates) and exposed it in vitro to free radicals and then examined the effects of carnosine on muscle lipid peroxidation and oxidative alteration of protein. The authors reported the minimum effective concentrations of carnosine to inhibit lipid and protein oxidation to be 2.5 and 1 mM, respectively. When the individual amino acid constituents of carnosine (histidine and β-alanine) were tested, however, no such inhibitory effects were seen, an outcome also reported in other studies whereby the amino acid constituents of carnosine were not as effective at inhibiting oxidation (6, 13). Another aspect of Nagasawa et al.'s study (84) was to supplement the diet of rats with histidine for a period of 13 d while subjecting them to Fe-nitrilotriacetate administration (which produces free radicals). They found a marked increase in carnosine with a simultaneous reduction in muscle lipid peroxidation and protein carbonyl content in the skeletal muscle of the rats.

In a study by Klebanov et al. (60), carnosine and its constituent amino acids were investigated using several model systems such as glutathione-horseradish peroxidase-luminol (GSH-HRP-luminol) among others. Unlike the findings of Nagasawa et al. (84) they found that both carnosine and histidine caused a 50% suppression of free radical reactions in the GSH-HRP-luminol system. They did find, however, that β-alanine displayed no activity. Overall, it was found that carnosine had the ability to scavenge different radicals as antioxidant activity was seen in all the systems studied. Interestingly, the effective carnosine concentrations correspond to those found in the human brain and muscles. In addition, a number of studies found not only carnosine but also other histidine derivatives (homocarnosine, anserine) demonstrating antioxidant activity (58, 61).

In contrast, Chan et al. (18) investigated the effects of dietary supplementation of carnosine and α-tocopherol, either separately or in combination, on the formation of thiobarbituric acid reactive substances (TBARS) in rat skeletal muscles. The data obtained indicated that all supplementation methods were effective in decreasing TBARS, with carnosine plus α-tocopherol and α-tocopherol alone being most effective. Thus from this study, it appears that carnosine's antioxidant role, while measurable, is not as significant as that of α-tocopherol.

Research in this area has also examined carnosine's chelating properties in combination with its antioxidant properties (10, 81, 96, 129). The term "chelate" refers to the ability of a substance to coalesce with surplus metals in the cells and bloodstream, so the liver and kidney can excrete them. Carnosine and its analogues have been shown to be efficient chelating agents for copper (Cu^{2+}) and other transition metals. Human skeletal muscle contains one-third of the total copper in the body. In addition, the concentration of carnosine in skeletal muscle is relatively high, thus the chelation of Cu^{2+} might be of biological significance. Kohen et al. (61) investigated the complex of carnosine:copper and other carnosine derivatives. Their results indicated that the carnosine:copper compounds can scavenge superoxide radicals released by neutrophils and this was also shown by complexes of homocarnosine:copper. Copper:anserine complexes, however, failed to have the same effectiveness. In contrast, Kang et al. (58) found that carnosine, homocarnosine, and anserine can all act as copper chelators. They examined the effects of all three histidine-related compounds against oxidative damage to human copper, zinc-superoxide dismutase (Cu,Zn-SOD) by peroxyl radicals. The oxidative damage leads to protein fragmentation, which is associated with the inactivation of the enzyme. All three compounds, however, significantly inhibited fragmentation, hence inactivation of Cu,Zn-SOD. In addition, all compounds inhibited the release of copper ions from the enzyme and thus these results suggest that carnosine and related compounds are copper chelators and peroxyl radical scavengers.

A recent finding by Decker et al. (25), however, places caution on the results obtained from a number of past studies on carnosine's antioxidant capabilities. They found that some sources of synthetic carnosine were contaminated by hydrazine concentrations between 0.01 to 0.2%. Hydrazine is a powerful reducing agent capable of inactivating free radicals, among other activities. Thus, the hydrazine might be directly inhibiting lipid oxidation rather than carnosine. Decker et al. (25), however, also report that purified carnosine was capable of scavenging peroxyl radicals.

Oxidative stress is an integral product of exercise and has been associated with muscle fatigue (7) or injury (95). Along with other antioxidants and enzymes, carnosine appears to function as an endogenous defense mechanism attenuating progressive damage during exercise. To date, however, there is no evidence to prove that intake of oral carnosine (or its precursors) can prevent oxidative damage during exercise in humans, thus work in this area is desired.

Energy Production and Use—Carnosine As An Enzyme Regulator

Transition from rest to exercise can increase energy demand by more than 100-fold and is a major challenge to cellular energetics (63). A physiological model often used to explain fatigue during high-intensity exercise is the inability to supply ATP at rates sufficiently fast to sustain exercise (87, 100). ATP is closely involved in cross-bridge function, with the binding of ATP to myosin causing dissociation of the actomyosin complex.

It is generally agreed that it is impossible to deplete ATP stores by more than 20 to 25% in voluntary exercise (regardless of the type of task) and as the affinity of

the ATPase for ATP is so high, depletions in this range do not affect the saturation of the enzyme (44). Even in muscles forced to contract under ischemic conditions, ATP levels do not drop below 60% of resting values (51, 109), indicating that muscle ATP concentrations are in some way safeguarded to prevent the development of skeletal muscle rigor (36). Therefore the overriding evidence suggests that ATP levels are not likely to contribute to the cause of fatigue, rather other factors reduce ATP utilization before ATP becomes limiting. The free energy available from the hydrolysis of ATP depends, however, on changes in relative concentrations of ADP, Pi, and H^+ (reaction products). Hence, it is possible that even a small change in ATP during fatigue could affect the capacity for force generation (57).

Carnosine (and anserine) appears to regulate a variety of enzymes (53, 56) and has also been proposed as a physiological activator of myosin ATPase (89). According to Nagradeva (1959), in Quinn et al.'s review (96) on histidine dipeptides, carnosine has been suggested to have an activating effect on glyceraldehyde-3-phosphate dehdrogenase which catalyses the central reaction in glycolysis. In addition, histidine was found to have a similar effect. However, in the presence of EDTA (ethylene diamine tetra-acetic acid) neither of these compounds had an effect, thus chelation of heavy metal ions in the incubation medium might play a key role in the observed effects (96).

Contractile Mechanisms—Carnosine As a Regulator of Skeletal Muscle Function

A growing body of evidence implicates alterations in sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release (65) or SR Ca^{2+} uptake (79) having an impact on mechanical performance and muscular fatigue (3, 44, 72, 124) (Figure 3). Control of cytoplasmic [Ca^{2+}] by the SR depends on three separate functions (Table 1). Failure of the SR to maintain Ca^{2+} homeostasis could arise directly from impairment to the SR itself or as a consequence of a disturbance in external control (44). Reductions in Ca^{2+}, as found in single fiber preparations subjected to repeated stimulation, could be due to direct disturbances in either the Ca^{2+}-releasing function or the Ca^{2+} sequestering function of the SR (5).

Calcium Release. Repetitive tetanic contractions of isolated muscles cause a gradual decline of force, which is closely associated with a decline in sarcoplasmic [Ca^{2+}]. Favero et al. (33) demonstrated inhibition of SR Ca^{2+} release in rat skeletal muscle after a run to exhaustion. They reported a decline in ATPase activity and found a concomitant decrease in muscle glycogen. Studies have shown that glycogen is tightly associated with SR membrane in skeletal muscle thus it has been postulated that glycogen plays a role in Ca^{2+} regulation.

Decreased Ca^{2+} release for binding to troponin will reduce the number of actomyosin complexes and hence reduce the force output by the muscle. This can be as a result of 1) impaired coupling between the voltage sensors (dihydropyridine receptors—DHPR) in the T-tubule membrane and calcium channels (ryanodine receptors—RyR) (110) (Figure 4) or 2) reduced Ca^{2+} release due to reductions in or modification of the SR Ca^{2+} content or 3) due to transient modification of the RyR (reducing its opening probability after activation) (45, 122).

Reduction in Ca^{2+}, mediated by alterations to the RyR (or its regulatory proteins), has clearly been the most studied and well supported (34, 69, 70, 121). A

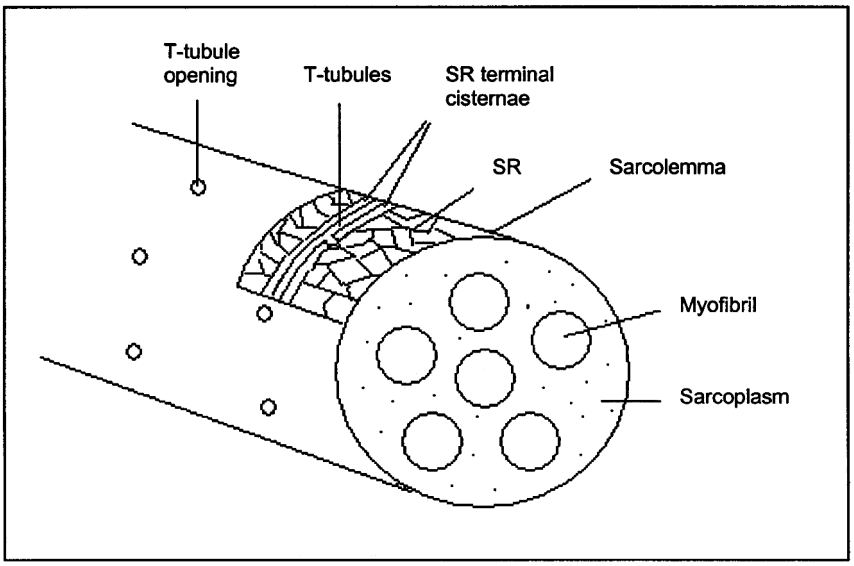

Figure 3—Diagram showing a single muscle fiber and location of sarcoplasmic reticulum (SR) and T-tubules [adapted from (125)].

Table 1 Major Functions of the Sarcoplasmic Reticulum (SR)

	Functions of SR	Details
1	Storage of Ca^{2+}	Proteins such as calmodulin exist in the lumen of the SR and are believed to maintain Ca^{2+} storage pending excitation (116).
2	Release of Ca^{2+}	Specialized Ca^{2+} channel proteins exist primarily in the terminal cisternae (TC), also known as ryanodine receptors (RyR). Different isoforms exist (108)—however, the same isoform (RyR1) is expressed in both FT and ST fibers (26).
3	Uptake of Ca^{2+}	Sequestration via Ca^{2+}-ATPase (Ca^{2+}-pump) primarily in longitudinal tubules (LT). Obligatory for relaxation (47).

great variety of compounds that activate or inhibit RyR (Figure 5) have been discovered both endogenous and exogenous (38, 39, 68, 98) and carnosine is thought to be an activator of RyR .

Batrukova and Rubtsov (12) examined the effect of carnosine on rabbit skeletal muscles (predominantly of hind legs) via in vitro tests using isolated heavy fraction of the SR. It was found that increasing carnosine concentrations progres-

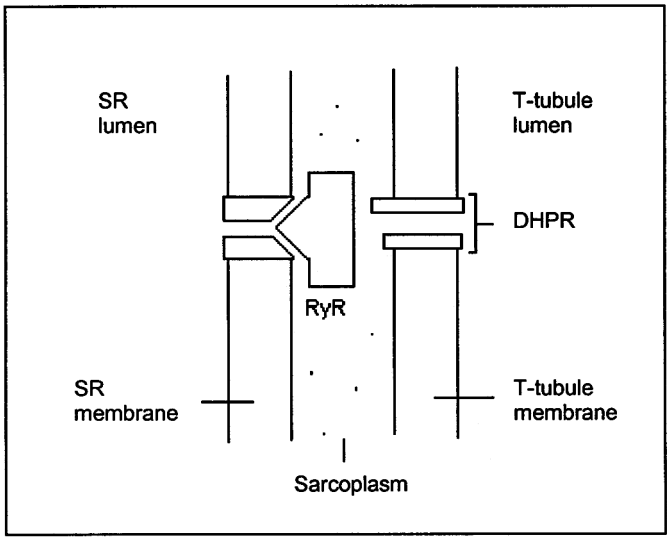

Figure 4—Location of ryanodine receptors (RyR) in terminal cisternae of sarcoplasmic reticulum (SR) and dihydropyridine receptors (DHPR) in T-tubule [adapted from (66)].

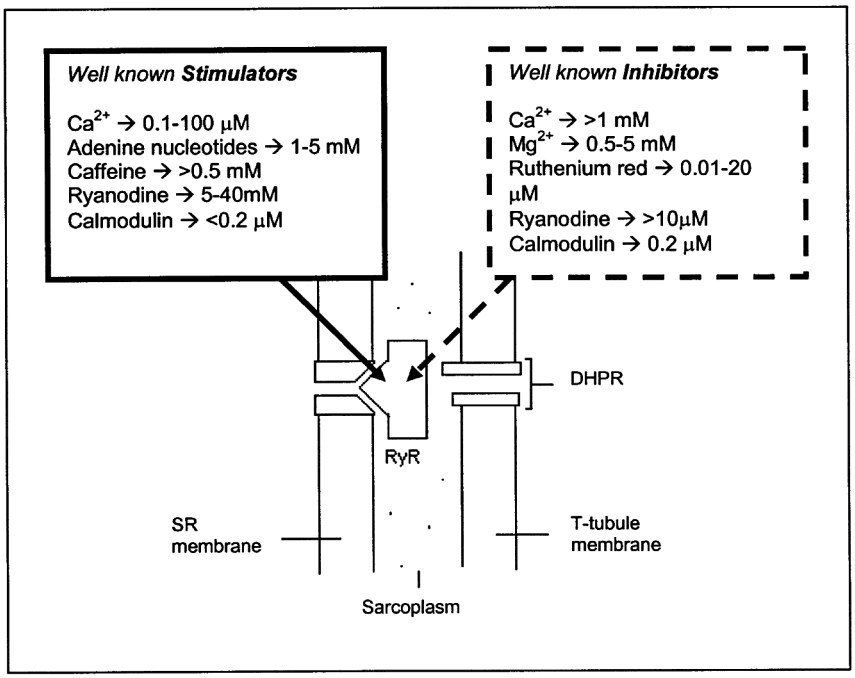

Figure 5—Modulators of ryanodine receptors (RyR) [adapted from (32)].

sively decreased the rate of Ca^{2+} accumulation by SR vesicles. They concluded that this was as a result of increased Ca^{2+} leakage from the vesicles, as carnosine has previously been shown not to inhibit the Ca^{2+}-pump (99). The leakage of Ca^{2+} was therefore attributed to the activation of Ca-release channels. Batrukova and Rubtsov (12) also demonstrated that carnosine (and anserine) induced rapid Ca^{2+} release from SR. The amount released depended on the concentration of carnosine (or anserine), thus SR Ca^{2+}-channels probably have saturable binding site(s) for these dipeptides. Furthermore, components of the carnosine molecule, L-histidine and β-alanine were tested either separately or together in equimolar concentrations and were found not to activate Ca^{2+}-channels (99). Therefore, the effect of carnosine appears to be provided by the whole molecule rather than by any functional group of the molecule. Furthermore, in vitro studies found carnosine to potentiate the effect of other activators of Ca^{2+}-channels in particular caffeine (by increasing the affinity of binding sites in RyR for the activating ligand) and decrease the influence of inhibitors such as Mg^{2+} (up to a concentration of 1 mM Mg^{2+}) (12).

Experiments on isolated rat hearts (when perfused with carnosine) also demonstrated increased contractility (97). In chemically skinned cardiac cells, carnosine releases calcium thus causing a rise in myoplasmic Ca^{2+} concentration, produces contracture, and alters the contractile protein's tension response to Ca^{2+}. Carnosine also acts directly on the RyR producing large increases in open state probability and dwelltime. Thus studies on carnosine (and derivatives) and effects on contractile mechanisms have shown that such compounds are efficient activators of RyR and can induce Ca^{2+} release.

Calcium Uptake. Depression of Ca^{2+}-ATPase has a critical role in Ca^{2+} sequestration and muscle relaxation. Westerblad et al. (123) suggest that reductions in Ca^{2+} uptake during repeated stimulation can in turn impair Ca^{2+} release as a result of decreased SR intraluminal Ca^{2+} content. Green (44) stated that this might not be the case, however, as Ca^{2+} released would build up in sarcoplasm (if Ca^{2+} uptake was impaired) and if high values did not reduce release, it was unlikely for impairment to occur. If Ca^{2+} buffering were increased, however, by sarcoplasmic binding proteins such as parvalbumin, or if some of the Ca^{2+} is sequestered to sites other than SR (mitochondria), Ca^{2+} uptake could potentially be a limiting factor in Ca^{2+} release (44).

Inashima et al. (54) investigated the impact of high-intensity running on Ca^{2+}-ATPase and found decreased activity. In a follow up study, Inashima and colleagues (127) found that acute high-intensity muscular activity (100% of VO_{2max}) increased the affinity of Ca^{2+}-ATPase for ATP and Ca^{2+} thus leading to an activation of Ca^{2+} transport function (though this might not be observed in all fiber types (e.g., fast-twitch fibers). They also found, however, that exhaustive exercise (at 75% of VO_{2max}) resulted in decreased activity of Ca^{2+}-ATPase (127).

Dupin et al. (30) investigated the action of carnosine on frog skeletal muscles which had been destroyed by ascorbic acid-dependent lipid peroxidation. It was established that 25 mM of carnosine in the incubation medium slowed down the inactivation of Ca^{2+}-ATPase of SR membranes at the same time maintaining the coupling of hydrolyzing and transport functions of the Ca-pump.

Transmembrane Potential. In addition to the above, it is now well documented that during repeated contractions of a muscle fiber, there is pronounced K^+ release

from HI exercising muscle (decreased intracellular and increased extracellular K^+) (37, 106). This results from an inability to maintain Na^+ and K^+ homeostasis by the exchange pumps, which could either be low in numbers or have slow Na^+-K^+ ATPase activity (74, 86). The disturbance of the membrane potential in the T-tubules can potentially inhibit the spread of the action potential and further, excitation of the SR to release Ca^{2+} into the sarcoplasm (37).

The classic study by Severin et al. found that at the point of fatigue, addition of carnosine into solution bathing isolated frog muscle preparations, quickly and efficiently augmented the force of muscle contraction and the total work produced by such preparations (Severin's phenomenon) (99). A possible clarification of this effect is provided by Petukhov et al. (94) who studied the effects of carnosine on a neuromuscular preparation under fatigue and found that dipeptides such as carnosine significantly restored muscle contraction. Analysis of this event showed that neither synaptic processes nor contractile mechanisms were involved in the effect. They found, however, that carnosine (and anserine) restored the value of the transmembrane potential depolarized by exhaustion, thus enabling the continuation of muscle contraction.

Conclusion

High-intensity endurance exercise leads to reductions in muscle substrates (ATP, PCr, and glycogen) and a subsequent accumulation of metabolites (ADP, Pi, H^+, and Mg^{2+}) with accompanying increases in free radical production. All these factors independently and collectively have deleterious effects on muscle function and have been reported to have significant repercussions on high-intensity performance or training sessions. Many of the consequences of substrate depletion and metabolite accumulation have been purported to affect the contractile mechanisms, in particular RyR and possibly even Ca^{2+} ATPase. Any reductions in the activity of either will hamper the excitation-contraction-coupling process and hence cross-bridge formation and muscle contraction.

With respect to its functions, carnosine has potentially important roles during high-intensity exercise (Figure 6). Much data in relation to this dipeptide, however, has been obtained from in vitro work on animal skeletal muscle fibers (frogs, mice, rats, horses) or other components of muscle contractile mechanisms (12, 30, 94) and therefore it might not be appropriate to extrapolate such findings to humans. There have been no reports on any toxic effects of carnosine or its derivatives, thus further research in humans is warranted to provide better understanding of the effects of carnosine in vivo in man. Moreover, does pure carnosine supplementation actually improve high-intensity exercise performance? In particular, how does it affect muscle function—voluntary and involuntary? Furthermore, with some data suggesting carnosine's action in potentiating the effects of caffeine (another activator of Ca^{2+}-channels) (12), is there a basis to investigate the use of carnosine in combination with caffeine? Indeed, as well as performance studies, much work needs to be carried out on the effects of supplementation (both short- and long-term) on the levels of carnosine in muscle and in the different fiber types. Overall, it seems that an interesting theoretical base exists for conducting well-controlled studies into any effects carnosine could have on performance.

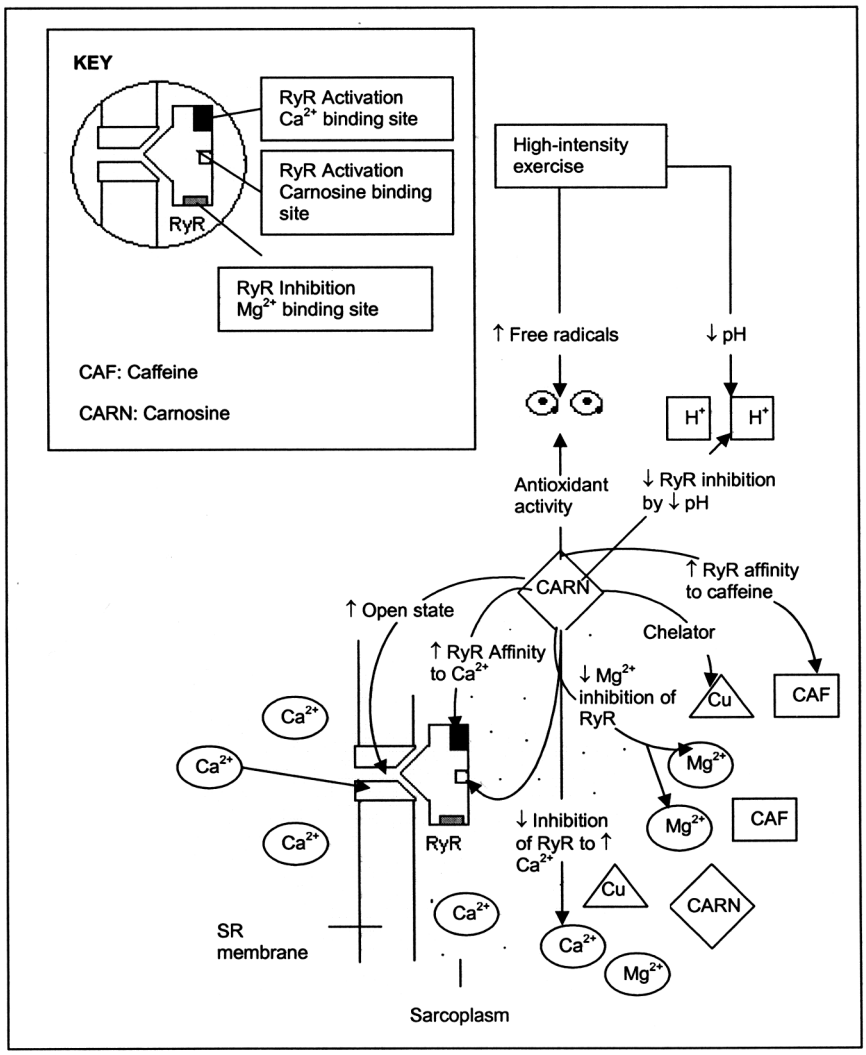

Figure 6—Pluripotent carnosine and its possible actions during high-intensity exercise.

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