Carnosine-based supplement*

Preliminary clinical studies

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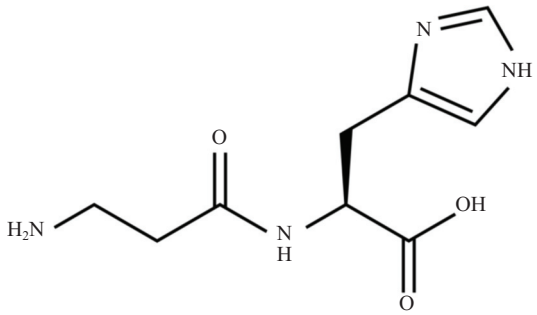
SUMMARY

Carnosine (β-alanyl-L-histidine) (***Fig 1***) is a cytoplasmic dipeptide synthesized from the precursors L-histidine and β-alanine by carnosine synthetase and degraded by carnosinase which regulates its cellular level. Carnosine is present in relatively high concentrations in skeletal muscle (5-10 mM) and other excitable tissues, such as nervous tissue. Given that the pKa of the imidazole ring is 6.83, carnosine acts as a physicochemical buffer in myocytes. This function is consistent with the fact that carnosine is present in higher concentrations in glycolytic than in oxidative muscle fibers, with the highest levels found in animals that perform frequent sprint exercises (e.g., greyhounds, thoroughbred horses), explosive flight behaviors (e.g., pheasants), and prolonged hypoxic dives (e.g., whales) (**1**). Recent studies have described other biological roles for carnosine such as antioxidant, anti-diabetic (reduction of glycation), anti-aging (lengthening of chromatid telomere), anti-cataract (when administered as eye-drops) and anti-autism (**2**). The present paper reviews some recent studies on the role of carnosine in athletes and in the elderly and adds some preliminary results from athletes in acute and stressed conditions where lactic acid is produced.

Figure 1 *Chemical structure of carnosine (β-alanyl-L-histidine)*



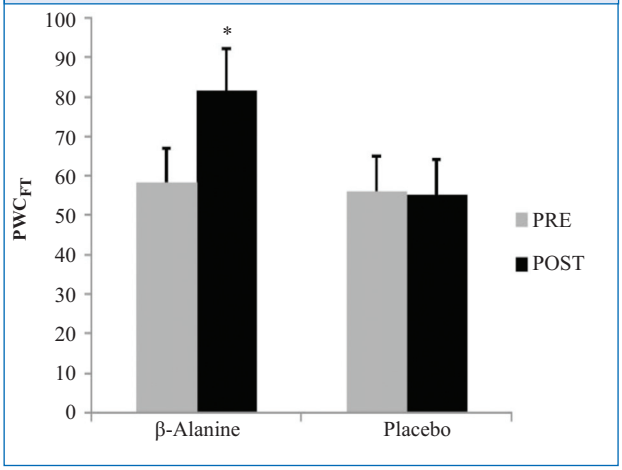
INTRODUCTION

Carnosine in the elderly

Carnosine is an efficient hydrogen ion (H^+) buffer over the physiological pH range (**3**). In muscle, where its concentration is highest, carnosine makes an important contribution to the maintenance of intracellular pH, which is vital for normal muscle function during intense exercise (**1**). While the dipeptide is found in both Type I and Type II muscle, its concentration is highest in Type II muscle. Studies in humans and rats have demonstrated an inverse relationship between age and muscle carnosine content (**4,5**). Sarcopenia, the loss in muscle mass with age, is associated with significant reductions in strength, power, and the ability to resist fatigue in elderly men and women (**5,6**). Significant decreases in skeletal muscle and decline in muscle function are clearly evident after the age of fifty (**6,7**). Deterioration of motor coordination, as a result of loss in strength and/or fatigue, is related to an increase in the frequency of falls (**8,9**) which repeatedly lead to injury and even death among the elderly (**10**). Recently, Hill *et al* (**11**) have shown that β-alanine (the rate-limiting precursor in carnosine synthesis) supplementation can significantly increase skeletal muscle carnosine levels, and that the increase is correlated to improvements in exercise performance. This finding can be considered important because carnosine, likely due to

*DDM Carnosina manufactured by Farmaceutici Procemsa Nichelino (TO) Italy; distributed by PharmExtracta/Omeopiacenza Pharma Group, Pontenure (PC), Italy (www.pharmextracta.com)

Figure 2 *Effect of treatment with beta-alanine or placebo on physical working capacity at fatigue threshold (PWCft) in elderly subjects. β-Alanine at 800 mg x 3 per day for 90 days (n = 12); placebo (n = 14). Before (pre) and after (post) the supplementation period, participants performed a discontinuous cycle ergometry test to determine the PWC_{FT}. *p < 0.05. Figure taken from (**13**)*



its dipeptidic structure, is not completely absorbed when administered as such (**12**).

The role played by carnosine in 26 elderly subjects (9 males, 17 females, 55-92 years) has been recently investigated after administering 800 mg β-alanine (BA) three times daily for 90 days and determining the muscle content of carnosine, in a placebo controlled trial (**13**). Before (pre) and after (post) the supplementation period, participants performed a discontinuous cycle ergometry test. A significant increase in physical working capacity at fatigue threshold (PWC_{FT}) (28.6%) from pre- to post-supplementation were found for the BA treatment group ($p < 0.05$), but no change was observed with placebo treatment. These findings suggest that ninety days of BA supplementation may increase physical working capacity by delaying the onset of neuromuscular fatigue in elderly men and women. The extent of the H^+ buffering capacity of carnosine generated in muscle cells by the β-alanine supplementation may explain the increase in the physical working capacity at the fatigue threshold (***Fig 2***).

Absorption and excretion

Despite the fact that carnosine is present in high concentrations in human skeletal muscle, and the oral ingestion of β-alanine is known to raise the muscle carnosine content in trained and in untrained subjects, little human data exist about the dynamics of the muscle carnosine content, its metabolic regulation, and its dependence on muscle fiber type.

Baguet *et al* (**14**) recently investigated in three skeletal muscle types the supplementation-induced amplitude of carnosine synthesis and its subsequent elimination on cessation of supplementation (washout). Fifteen untrained males participated in a placebo-controlled double-blind study. They were supplemented for 5-6 wk with either 4.8 g β-alanine/day or placebo. Muscle carnosine was quantified in soleus, tibialis anterior, and medial head of the gastrocnemius by proton magnetic resonance spectroscopy (MRS), before and after supplementation and 3 and 9 week into washout. The β-alanine supplementation significantly increased the carnosine content in soleus by 39% (***Fig 3***), in tibialis by 27%, and in gastrocnemius by 23% and declined post-supplementation at a rate of 2-4%/week. Average muscle carnosine remained increased compared with baseline after 3 weeks of washout (only one-third of the supplementation-induced increase had disappeared) and returned to baseline values within 9 weeks (***Fig 3***). Following subdivision into high responders (+55%) and low responders (+15%), the washout period was 15 weeks and 6 weeks, respectively (***Fig 4***). In the placebo group, carnosine remained relatively constant with variation coefficients of 9-15% over a 3-month period.

Figure 3 *Muscle carnosine content in soleus before and after 6 week supplementation with placebo or β-alanine and 9 week washout in elderly subjects. *p < 0.05 versus pre-supplementation. Figure taken from (**14**)*

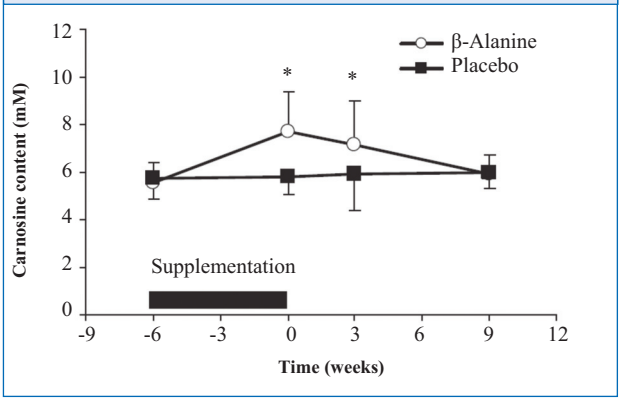
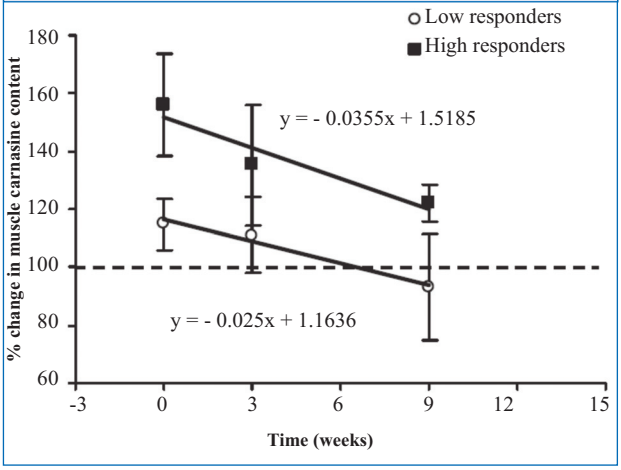


Figure 4 *Whey proteins and peptides exhibit various properties that can have positive effects on human health (Information compiled from 1-7*



Carnosine in athletes

Besides the biological role played in the elderly, carnosine is an important substance for athletes. Several recent studies have suggested that muscle carnosine content is a determining factor in high-intensity dynamic and isometric exercise performance (**15-17**). Suzuki *et al* (**17**) observed a positive correlation between the vastus lateralis carnosine content and the power output generated at the end of a 30-sec all-out cycling sprint (Wingate-test) in untrained men. Additionally, Hill *et al* (**15**) supplemented untrained men with β-alanine for 10 weeks, which resulted not only in a substantial increase in the carnosine content of the vastus lateralis, but also in an increased time to exhaustion in a cycling test performed at 110% of power output at maximal heart rate, where the expected endurance time is approximately 2.5 min. The role played by carnosine or β-alanine is not only of interest in untrained subjects, but they also have a positive effect the endurance of trained athletes, especially sprinters, body-builders and oarsmen (**18,19**). Nevertheless, as shown in *Figure 5*, supplementation with of β-alanine in trained sprinters, aimed to increase muscle carnosine content, did not affect either their sprint time (**20**) or the sport results.

SUSTAINED RELEASE CARNOSINE-BASED FORMULA

Galenic Development

Taking into consideration the limited oral bioavailability of carnosine, and the role of β-alanine as the rate limiting step in the endogenous synthesis of carnosine, an oral dosage form containing 250 mg pure carnosine and 250 mg pure β-alanine has been developed (DDM Carnosina, PharmExtracta/Omeopiacenza Pharma Group, Pontenure (PC), Italy). The galenic form of DDM-Carnosina (DDM-C) has been designed to time-dependently release the actives within 8 h (***Fig 6***) with the aim of improving the pharmacokinetic properties of carnosine in primis and its rate and extent of oral bioavailability. A standard dissolution test was performed according to European Pharmacopoeia where in the first 2 h the tablet is placed in 0.1N HCl solution followed by 6 h in phosphate buffer at pH 6.8.

Preliminary clinical study in athletes

The study was supervised by the medical director (A. Bressan) and the medical nutritionist (A. Bertuccioli) of a professional European volley ball team (Scavolini, Pesaro, Italy; Italian Champions in 2008, 2009 and 2010). To determine the role played by a single oral treatment of a product containing both carnosine (250 mg) and β-alanine (250 mg), 6 male athletes, after signing the informed consent and the established protocol, were enrolled for an open trial. All 6 of the athletes were subjected to a bout of severe training consisting of: 600 meters walking (30% of fcMAX) followed by 1,200 meters running (60% of fcMAX), followed by 5 squats and 5 sumo squats, then 5 lateral lounges followed by 25 meters frog walking and 25 meters frog jumps, 50 meters sprint running, 5 wide push ups, 5 simple push ups, 5 narrow push ups and finally 50 abdominals. Fifteen min before training, blood samples were drawn from each athlete for the determination of lactic acid. Two hours before training, the athletes were given either placebo (4 tablets, containing only the excipient formula) or DDM-C (4 tablets, each tablet containing carnosine, 250 mg and β-alanine, 250 mg). At the end of training, all athletes were subjected to a second blood withdrawal for lactic acid determination. The trial with DDM-C was done on the same 6 athletes, 4 days after the test with placebo to allow a good recovery time.

As shown in *Figure 7*, all the athletes showed a net negative difference in terms of lactate production indicating a beneficial effect. In terms of absolute values, the delta lactate between post-training and pre-training in the placebo group was much greater than the post- to pre-training delta in the athletes treated with the carnosine-based product.

Figure 5 *Time (sec) to complete a 400-m run before and after 4 week of placebo or β-alanine supplementation. Individual values (shaded symbols) and group averages (open symbols) Figure taken from (**20**)*

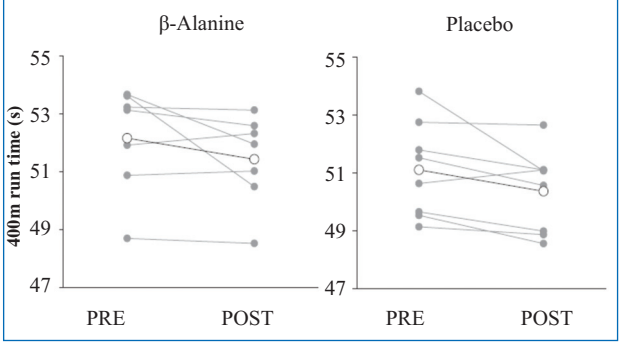


Figure 6 *Time course, in vitro, of the release of carnosine from DDM-C. A similar time course was observed for β-alanine*

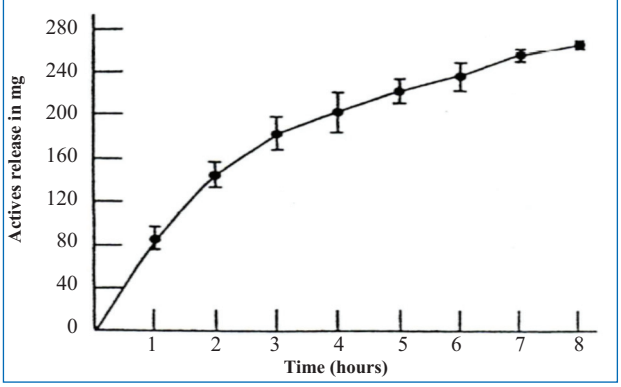
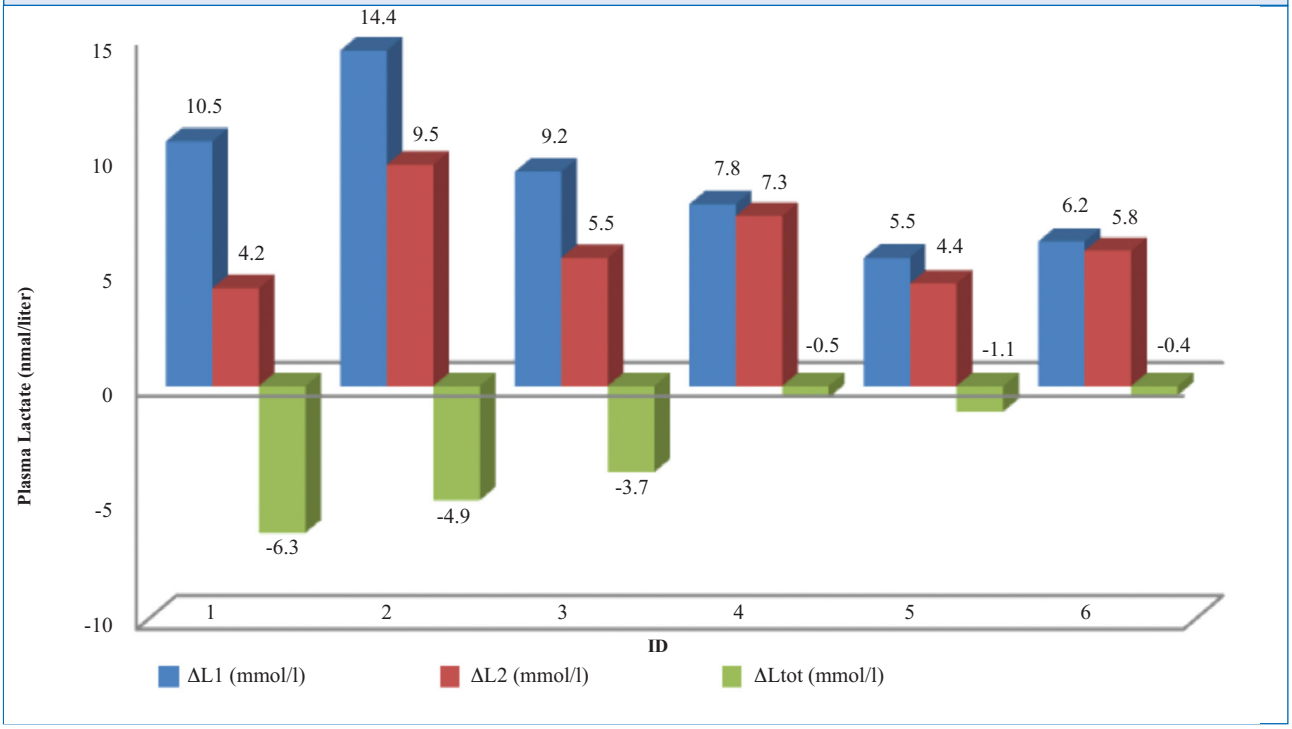


Figure 7 *Effect of a combination of carnosine (250 mg) and β-alanine (250 mg) on lactate production pre-training and post-training in the same athlete. For protocol see text. Placebo (blue bars). Treated (red bars). Delta per single athlete (green bars). ΔL, Lactate is expressed as mmol /liter. ID (1-6) refers to the athlete's identification*



DISCUSSION AND CONCLUSION

Carnosine is a widely studied molecule starting from the time of the initial investigations having been performed almost a century ago (**1**). According to the data available, carnosine possesses a wide range of potential actions, especially in the elderly and in athletes. This is mainly due to the physicochemical properties of the compound which permit it to buffer the excess acidity formed by excessive catabolism during exercise and at the same time to strengthen muscles. These properties are documented in the elderly, where carnosine content (inversely related to age) is reduced, and in athletes, where carnosine demand is increased.

To verify the hypothesis formulated by some authors, a sustained-release tablet form containing carnosine (250 mg) and β-alanine (250 mg) has been developed and tested in volunteers. Our previuslly unpublished data showed that DDM-C reduced pain in subjects suffering from diuretic-induced muscle spasms and returned creatine phosphokinase (CPK) levels to normal in patients with elevated CPK after one month of treatment (2 tablets/day were used in two trials).
DDM-C was thereafter studied in a model of lactic acid production in athletes. The administration of 4 tablets/*day* reduced lactate production in all the athletes, albeit the reduction was greater in three of the athletes (athletes 1, 2 and 3) with respect to athletes 4, 5 and 6 (***Fig 7***).

The lactate production induced by the training in the 3 high responders might be due to an initial lower level of muscle carnosine or, conversely, the 3 low responders could have had initial higher levels so that the treatment could not have induced any significant reduction in lactate.
The study confirmed the benefits resulting from carnosine supplementation; it should be underlined that the effect was obtained by a single administration of a galenic formulation permitting the release of moderate amounts of the active principals in a sustained manner. The previously suggested dosage for elderly and athletes was 2.8 and 4.8 g of carnosine and β-alanine, respectively, while 2 g of the active principals in 4 divided doses of 500 mg each (250 mg of carnosine and 250 mg of β-alanine) were used in this study.
In addition, a reduction of lactic acid production after training has been observed even after the administration of 2 tablets/day of DDM-C (data not shown). However the reduction was only present in 50% of the athletes.
In conclusion, carnosine is an agent that can reinforce muscle cells by different mechanisms of action. Athletes and elderly may use carnosine to improve their strength and resistance (athletes) and to counteract the weakness and fatigue syndrome reducing the risk of bone fracture (elderly). A sustained release formula containing both carnosine and its precursor and limiting step β-alanine may be effective even after a single administration at reduced dosages. Other studies are currently ongoing on larger number of subjects.

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