VELOCITY
EQUINE CARNOSINE EMULGEL

VELOCITY INCREASES INTRAMUSCULAR CARNOSINE WITHIN ONE HOUR OF THE FIRST APPLICATION

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INTRODUCTION

Carnosine is a peptide that is found in the cells of almost all organisms and serves as one of the most important molecules in mammalian metabolism. Carnosine functions as the main intracellular buffer to maintain pH during exercise and also displays robust antioxidant and antiglycation properties. Due to the important nature of this molecule in metabolism, performance, and aging, carnosine has been widely studied for its ability to improve the overall health and physical work capacity of mammals.

Carnosine plays a critical role in the health and physical work capacity of horses and exists in much higher concentrations in horses than in humans. For example, intramuscular carnosine levels hover around 20-30 mmol • kg-1 in humans, and between 30 and 170 mmol•kg-1 in horses. Representing an up to 8-fold difference between these mammalian species. As such, increasing intramuscular concentrations of carnosine in horses would prove to be a substantial breakthrough for equine science.

Currently, increasing intramuscular carnosine has been a laborious and inefficient endeavor. Investigations in oral carnosine supplementation have been largely ineffective due to one key barrier preventing the oral carnosine from accumulating in muscle tissue: the presence of the enzyme carnosinase in blood. Orally ingested carnosine is quickly degraded by carnosinase that is present in the blood, preventing accumulation in skeletal muscle tissue from oral carnosine supplementation.[1] However, one can glean information regarding the potential of carnosine by looking at studies done on beta-alanine, the rate limiting precursor to carnosine. Beta-alanine is not broken down in the blood by carnosinase and can bioaccumulate in skeletal muscle. Indeed, several studies have shown that beta-alanine supplementation results in a modest increase in muscle carnosine (~20-40%) and concurrent improvements in buffering capacity and loss of force production during exercise.[2]

Due to the ability of transdermal carnosine (Velocity) to prevent declines in performance during sustained efforts and to aid in recovery from strenuous effort, this formulation is ideal for horses that engage in a wide variety of athletic endeavors (e.g. racing, show jumping, polo). Previous studies have shown the kinetics through which Velocity passes through the skin. However, no data exists to date quantifying the effect transdermal carnosine can increase skeletal muscle levels of carnosine. The present study is the first study to demonstrate a time course and a quantification of how much Velocity increases intramuscular concentrations of carnosine in horses.

METHODS

PROTOCOL OVERVIEW

The study was a randomized, placebo controlled, crossover study conducted in thoroughbred racehorses in collaboration with Dr. Warwick Bayly at Washington State University. The samples were collected over a period of two-days, 14 days apart (November 19th and December 3rd) to allow for washout. Horses were randomized to either the placebo arm or the transdermal carnosine arm through random number generation for the first collection period and then assigned to the other arm on the second collection period.

Horses were brought into the laboratory after an overnight fast. Height, weight, age, training status, and gender were recorded. A small area on one hindquarter was shaved, the area was numbed with a local anaesthetic, and a single baseline, pre-application biopsy was taken (T=0). Immediately following obtaining the first, baseline biopsy, 60 mL of either the placebo or the transdermal carnosine was applied. Biopsies were then taken at 30 (T=30), 60 (T=60), and 120 (T=120) minute intervals.

BIOPSY PROTOCOL

A total of 4 muscle biopsies were obtained from the middle gluteal muscle per horse. The muscle biopsy procedure required that the site be sterilized. After the site was cleaned, a small amount of local anesthesia (lidocaine) was injected just under the skin surface. After the area was treated with lidocaine (approximately 5 mL, 1% lidocaine), a small incision (approximately 1/4 inch long) was made through the skin and the outer covering (fascia) of the skeletal muscle muscle to a depth of approximately 3/4-1.5 inches. The biopsy needle was then inserted through the incision and the sample obtained. After the sample was obtained, the site was cleaned and closed with steri-strips and/or a single stitch and bandaid and wrapped with a compression bandage. The biopsy samples were snap frozen in liquid nitrogen, packaged in dry ice, and sent to a laboratory for biochemical analysis.

Figure 1. Graphical depiction of the study protocol.

QUANTIFICATION OF INTRAMUSCULAR CARNOSINE BY ELISA

Briefly, muscle biopsy samples were trimmed into ~31 mg pieces and aliquoted. One aliquot of the sample was homogenized in phosphate buffered saline and 1% protease inhibitor. Samples were then spun at 10,000 RPM for 10 min and the supernatant was removed and saved for use in the ELISA. Cellular debris was labeled and stored for potential future use. Carnosine levels were then assessed via a commercially available ELISA *(Novus Biological Sciences, CAT: NBP2-75013, www.novusbio.com)*.

STATISTICAL ANALYSIS

The data were analyzed for normality using the Shapiro-Wilk test. A two-way analysis of variance (ANOVA) was used to test for the main effects of time and condition (placebo or carnosine) as well as an interaction between time and condition. Independent F-tests were also conducted to examine the change in intramuscular carnosine levels from baseline to each time point (30, 60, and 120 minutes). Alpha values were set a priori at 0.05.

RESULTS

A total of 10 horses *(n=10)* were included in the study. There was no difference between the placebo or carnosine group at baseline for intramuscular levels of carnosine *(Placebo: 2646 [1502 -3231]; Carnosine: 1973 [1198 - 2760], p=0.29)*.

ANOVA showed a significant main effect of the treatment *(p=0.004)*, no significant main effect for time *(p=0.18)*, and a trend toward an interaction of treatment by time *(p=0.08)*.

There was a time-response curve present with relative changes in intramuscular levels of carnosine increasing from baseline through to two hours *(120 minutes) (Figure 2)*.

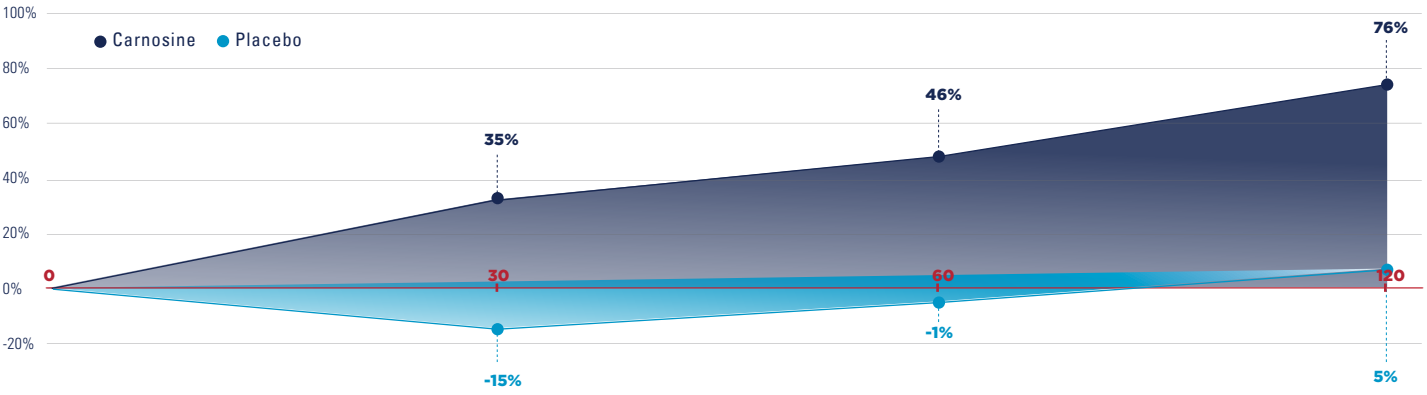

Figure 2. Effect of transdermal carnosine on muscle levels of carnosine over 120 minutes.

When comparing between carnosine and placebo, at 30 minutes, intramuscular levels of carnosine were increased by roughly 35% from placebo *(p=0.002)*. At 60 minutes, intramuscular levels of carnosine were increased to roughly 46% from placebo *(p=0.044)*. At 120 minutes muscle levels of carnosine increased 76%, however this was not statistically significant *(p=0.20) (Figure 3)*.

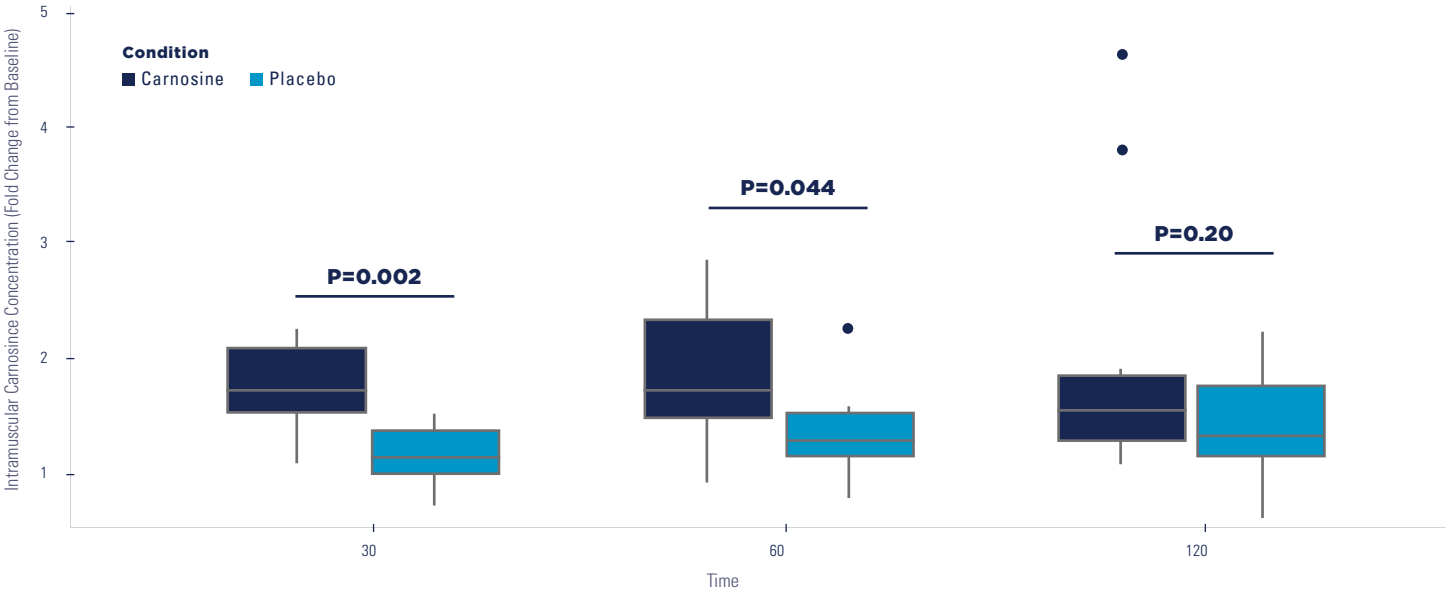

Figure 3. Effect of transdermal carnosine on intramuscular carnosine concentration.

DISCUSSION

Carnosine plays a critical role in maintaining intracellular pH during exercise and functions as the primary intracellular buffer in virtually all mammals. Until now, attempts to directly increase muscle levels of carnosine have been largely ineffective. These data present the first evidence for a transdermal delivery of carnosine into muscle tissue and demonstrate a roughly 50% increase in intramuscular carnosine levels in as little as 60 minutes, post application. These findings demonstrate that transdermal delivery of carnosine is efficacious and works within 30 minutes of application. These findings have substantial implications for the, the recovery health, and physical work capacity of horses in all equine sports.

When we examine the data on an individual horse level, we observed that horses showed an increase by roughly 100%, effectively doubling the amount of carnosine available in muscle tissue over a two hour time window. In a few horses, there were ~4-fold increases by 120 minutes. These results compare to previous attempts to raise intramuscular carnosine in horses using the precursor amino acids to carnosine, beta-alanine and histidine. In previous work, 30 days of beta-alanine *(100 mg/kg)* and histidine supplementation *(12.5 mg/kg)* showed a roughly 10-40% increase in intramuscular carnosine concentrations.[3] As such, the present study shows that a transdermal delivery is more efficacious and works in a single application within 60 minutes as opposed to 30 days of consistent feeding. This makes a transdermal approach substantially more efficacious and cost effective.

There are also important implications surrounding the general metabolism of carnosine based on these findings. First, the present study indicates that the single, acute application increases carnosine levels substantially and that when considering standard carnosine metabolism, the levels of carnosine should stay elevated above baseline for 2-24 hours after a single application of transdermal carnosine. Second, if we draw parallels from literature on beta-alanine, the chronic use of transdermal carnosine is likely to raise basal levels of carnosine substantially as well, with basal increases of 30-50% being reasonable targets to achieve.

The implications of the present results can be understood in the context of the role that carnosine plays in equine physiology from a health and recovery perspective as well as from a physical work capacity perspective. Carnosine has been well-studied as an antioxidant and exhibits robust free-radical scavenging ability in muscle tissue, as well as in brain tissue.[4] Additionally, it has been shown to reduce glycation in animal studies, suggesting it may play to attenuate some of the protein based mechanisms of aging.[5] From a work capacity aspect, increasing intracellular carnosine through a transdermal approach can reduce fatigue, improve recovery, and increase the work capacity of a horse. Studies in humans have shown that higher levels of muscle carnosine lead to lower levels of fatigue.[6] This reduction in fatigue may be, at least in part, due to the fact that increasing carnosine levels directly improves the buffering capacity of muscle tissue to handle physical work, which is also supported in horse models.[3] Improving the buffering capacity can allow a horse to produce more work in the glycolytic system as the system can handle more hydrogen ion accumulation. The reduced levels of fatigue and the improved buffering capacity may substantially improve the recovery capacity of the horse as the mechanical and biochemical strain placed on the system may be attenuated at the same given workload.

In addition, improving the intracellular buffering capacity within 60 minutes also presents an opportunity for attenuating metabolic related issues that may arise from injury or surgical interventions on the musculoskeletal system of horses. Anaerobic metabolism that can predominate at the sight of injury, trauma, or infection often leads to rapid drops in intracellular pH leading to either necrotic or apoptotic cell death. Increasing the intracellular buffering capacity in a short time window may provide substantial benefit to these scenarios.

Another aspect of this study that deserves consideration is the fact that the present study was conducted in the musculature of the horse that tend to already have the highest levels of carnosine, the gluteus muscles. These results demonstrate that even in a muscle group that has a naturally high level of carnosine present, these stores can be increased above baseline by nearly double. It may be that other muscle groups that have a naturally lower level *(e.g. the masseter and triceps brachii muscles*) may see even greater increases in intracellular carnosine levels.[7]

These results provide data that supports a transdermal delivery system for carnosine into the muscle tissue of horses within 60 minutes of the first application. These data have important implications for the health, recovery, and performance capacities of most equine disciplines.

CONCLUSIONS

These data are the first data to demonstrate that transdermal carnosine can substantially increase intramuscular levels of carnosine and do so within 30 minutes of application. Furthermore, these data provide kinetics and time-response data demonstrating that Velocity increases intramuscular levels of carnosine within one hour of the first application.

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