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Effect of transdermal carnosine on repeated sprint performance in trained cyclists: A randomized controlled cross-over trial.

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ABSTRACT:

The purpose of this study was to investigate whether a transdermal carnosine (TC) gel improved repeated Wingate sprint performance in trained cyclists. Fifteen trained male cyclists completed three cycling sessions that included a 15-sec sprint to estimate glycolytic capacity (VLa_{Max}) followed by five Wingate sprints with 4 to 5-min of active recovery. Session 1 served as a final familiarization trial, while sessions 2 and 3 utilized a randomized application of either 10 ml of placebo or 10 ml of a mentholated TC gel to the legs at least 60-min prior to the session. Blood lactate concentration (BLC) and power output were measured during the session. A 3 X 5 crossover design with a repeated measures Analysis of Variance (ANOVA) was used to explore statistical differences ($\alpha = 0.05$) in two main effects. Mean VLa_{Max} and 15-sec power were 0.74 $\pm$ 0.31 $mM \cdot sec^{-1} \cdot L^{-1}$ and 748.6 $\pm$ 135.2 W, respectively, while mean Wingate peak BLC and 30-sec power of 16.8 $\pm$ 2.6 mM and 584.7 $\pm$ 78.3 W, respectively, and mean rest time between sprints was 278.7 $\pm$ 11.2 sec. There were no statistically significant improvements in any performance measure between familiarization, placebo, or TC gel sessions. Among subjects, five showed a non-statistical 3% decline in performance after treatment. A single recommended dose of TC gel did not improve repeated sprint performance in trained cyclists. Even after elimination of nocebo subjects, performance improvement was still negligible.

KEY WORDS Beta-alanine, cycling, wingate, transdermal, blood lactate

INTRODUCTION

There are numerous factors associated with or potentially mitigating fatigue during exercise. The generation of lactic acid and its subsequent rapid dissociation to lactate and H^+ has long been associated with fatigue during very high intensity exercise (1,2). While lactate itself has not only been ruled out as a "fatigue villain"(3), evidence does suggest that increased H^+ and a subsequent drop in pH may negatively impact several aspects of muscle power output by inhibiting phosphofructokinase (PFK) activity, oxidative phosphorylation, or Ca^{2+} release from the sarcoplasmic reticulum (2). Therefore, ingestion of buffering agents like bicarbonate have long been used to improve performance(4), while more recently supplements like carnosine and its precursor beta-alanine have garnered a great deal of interest as performance aids (5,6).

Carnosine, aka, beta-alanyl-L-histidine, is a naturally occurring histidine containing dipeptide and is found in high levels in the muscle and is known to play many roles in the body (7). Of particular interest to exercise physiology is carnosine's role as an intracellular buffer (7,8). While oral carnosine supplementation fails to elevate plasma or muscle carnosine levels adequately, beta-alanine (BA), a non-essential amino acid produced in the liver, has been shown to be safe and effective at increasing muscle carnosine levels and improving specific high-intensity activities (5,6,9–12). For example, Saunders et al. (12) showed that 12-weeks of BA supplementation improved repeated sprint running performance. Similarly, both 4-min cycling time trial and repeated 30-sec sprint performance has been shown to improve with as little as 4-weeks of BA supplementation (9,10). There is limited evidence, however, on the acute ingestion of BA or other routes of administration (8,13).

In addition to ingestion, carnosine can be absorbed across the skin. A transdermal carnosine (TC) formulation could overcome the limitations of ingested carnosine and BA absorption and/or storage (7,8,14). A TC formulation has been shown to be effective at increasing intramuscular carnosine levels by 46% within 60-min of application (15). Prior research using this topical gel suggested it was effective at improving repeated sprint and repeated 1000 m run trials in elite soccer players (15). While sport of competitive cycling is typically dominated by an individual's $VO_{2\ max}$, lactate threshold, and economy (16), the ability to perform repeated sprint efforts lasting between 15 – 30-sec, as well as the anaerobic energy contribution and buffering ability can play an important role in the outcome of a competition. Therefore, an easily administered supplement to improve repeated sprints would be of great benefit.

The sports supplement industry was valued at $42.9 billion in 2022 and is expected to grow 7.4% from 2023 to 2030 (17), however, the efficacy and regulation of most supplements is relatively nonexistent. This makes empirical testing of manufacturer claims essential. Therefore, the purpose of this study was to investigate the effect that TC gel had on repeated Wingate sprints in trained cyclists. Based on the available research, we hypothesized that overall average sprint power and total work would be 5% higher after using a commercially available carnosine gel.

METHODS

Participants and Ethics Approval

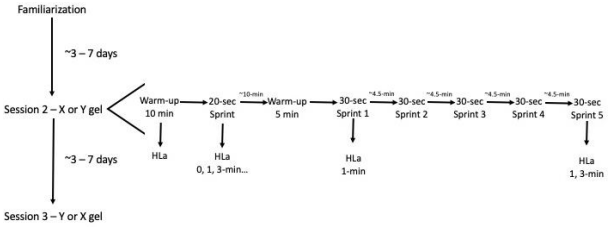
Prior to initiating this research, all methodology was reviewed and approved by the Shenandoah University Institutional Review Board (IRB). All participants were self-reported trained cyclists recruited from the local area and met the following *Inclusion Criteria:* apparently healthy male cyclists between the ages of 18 – 50 years, actively training 8 or more hours each week, and who reported significant bicycle and/or triathlon racing experience. *Exclusion Criteria included:* individuals outside the age range, who did not meet the experience level needed, and those with any known medical condition that would preclude participation. All volunteers were informed of the purposes and requirements of the study and provided consent.

In return for their participation, subjects received a monetary reward at the completion of the study. Prior to their participation, participants were asked to complete two sprint trials on their own during training.

Study Design

The study was designed as a randomized control double-blind placebo cross-over study. However, during the allocation of supplement gel to new containers it was noted that the supplement included menthol, while the identical placebo gel did not. Thus, a protocol deviation was submitted and approved to the IRB and the study was revised to a single blind deception study; subjects were informed they were receiving two different supplement formulation dosages. The researchers supervising test sessions were only aware of which supplement was received after the subjects arrived and provided no specific commentary about either during any session. Participants completed three sprint interval training (SIT) sessions over a period of 2-weeks with no less than 3-days between exercise/supplementation sessions to allow a significant washout period (8). Each subject completed their consent forms prior to anthropometric measurements. They then performed a third familiarization sprint session in the lab identical to the two supplement trials. Following this trial, participants were given two 10-ml plastic containers marked X or Y and instructed which container to apply prior to the next session. Figure 1 depicts the general study flow. Randomization was achieved using a standard coin toss for subject 1 with following subject completing the opposite order.

Figure 1. General study flow for transdermal carnosine supplement trial.



Transdermal Carnosine Supplement and Placebo

The supplement (LactiGo™, Outplay Inc., Las Vegas, NV, USA) used in the study was a topical gel consisting of water, glycerin, magnesium sulphate, a proprietary Carnosine-Complex, and 1.25% menthol). The placebo was an identical gel that did not contain either carnosine or menthol. Participants were instructed to apply the entire contents of the supplement container (10 ml) to both legs and gluteus maximus no less than 1-hr prior to their appointment with the application time recorded upon arrival.

Anthropometric Measurements

Following informed consent participants height and weight were measured without shoes and shoes and just cycling shorts Health o meter 402LB Mechanical Beam Scale (Sunbeam, Inc. Boca Raton, FL USA). Skinfold measurements were taken by the same certified technician following ACSM Guidelines with body fat estimated using the Siri equation (18).

Repeated Sprint Sessions

All exercise sessions were completed using a participant's own bike attached to a Wahoo Kickr direct drive trainer (Wahoo Fitness, Atlanta, USA); prior research has shown this trainer to be valid and reliable (19). A high-powered fan was provided for cooling and subjects were encouraged to drink to thirst. Participants completed three repeated sprint sessions consisting of a standard 10-min easy warm-up at ~100 W. A 1-min rest period was then provided and a 3 µl blood lactate sample from the fingertip and analyzed for blood lactate using a Lactate Plus analyzer (Nova Biomedical Corporation, Waltham, MA, USA). The cyclists then performed a single maximal 15-sec sprint, at which point they dismounted and sat in a chair to rest passively while blood lactate samples were taken 1-min, 3min, 5-min, and so on until levels peaked and then dropped at least 1-mM. Following the final blood sample (~15-min), participants warmed-up again for approximately

5-min before completing five 30-sec *Wingate* sprints with approximately 5-min very light active recovery. Participants received strong verbal encouragement throughout each sprint. Blood lactate samples were taken 1 and 3-min *after* the first, third, and fifth sprint, as well as 5 and 7-min after the fifth sprint. A rating of perceived exertion (10-point RPE) was taken 5-min after the final sprint. Subjects were allowed to use the bathroom during rest periods, if needed. Before, during, and after each trial subjects could report any opinions or sensations about the products they received.

Statistical Analysis

This study used a 3 X 5 crossover design employing the use of Repeated Measures Analysis of Variance (ANOVA) to explore statistical differences in two main effects that include independent peak power, average power, kJ, and lactate across five time periods and three trials and the interaction between time and trial. All statistical analyses were performed using JASP v16 with statistical significance set at $\alpha \leq 0.05$. Levene's test of Homogeneity of Error Variances with statistical significance indicative of an assumption violation. The Mauchly's Test of Sphericity was employed to explore the assumption of sphericity with statistical significance indicative of an assumption violation. In the case of a violation, the Greenhouse-Geisser degrees of freedom correction were employed. Effect sizes are reported as the Omega Squared Values (ω^2).

RESULTS

Sixteen male cyclists volunteered for the study, with N = 15 completing all trials, and one withdrawing after the first familiarization trial due to other commitments. Participants included road, mountain bike, cyclocross cyclists of USA category ranking 1 – 4, as well as three elite triathletes. They were 34.9 ± 9.0 yo, 179.4 ± 5.5 cm tall, 73.4 ± 11.8 kg, and 10.5 ± 4.7% body fat. Across all trials the mean VLaMax and 15-sec power were 0.74 ± 0.31 $mM \cdot sec^{-1} \cdot L^{-1}$ and 748.6 ± 135.2 W, respectively, compared to Wingate peak BLC and 30-sec power of 16.8 ± 2.6 mM and 584.7 ± 78.3 W, respectively. Mean rest time between sprints was 278.7 ± 11.2 sec. Data for all trials are summarized in Table 1, with individual sprints for each session illustrated in Figure 1. Across trials, application time for the placebo and treatment were 72.3 ± 5.3 min and 74 ± 9.1 min, respectively, and not statistically different (p = 0.545).

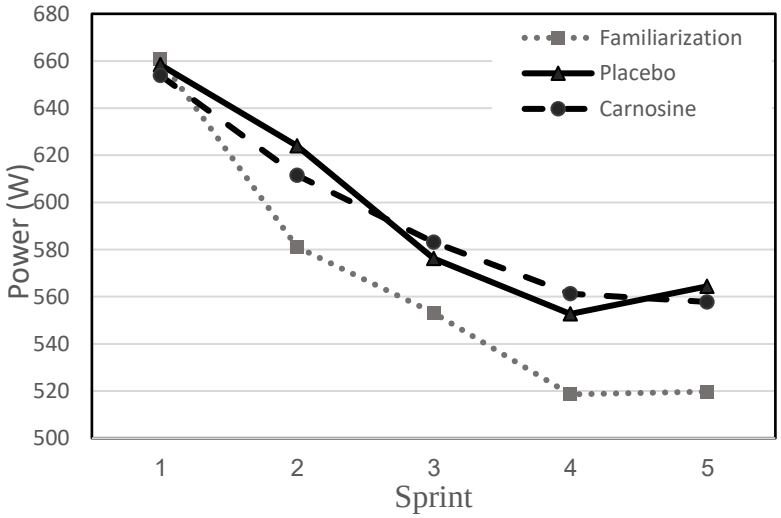
Table 1. Summary data for N = 15 cyclists performing a 15-sec sprint with ~15-min recovery followed by five Wingate sprints. Each cyclist performed a familiarization session followed by randomly assigned sessions using either a placebo or transdermal carnosine gel. No significant differences were seen between any variable.

	Familiarization	Placebo Gel	Carnosine Gel
Mean 15-sec Power (W)	755.1 ± 153.6	741.2 ± 139.0	749.4 ± 123.1
Peak BLC (mM)	10.4 ± 2.5	9.4 ± 1.7	9.2 ± 2.5
Time to Peak BLC (sec)	3.2 ± 2.1	3.4 ± 1.7	2.8 ± 2.4
Repeated Wingate Sprints			
Peak Power 1-sec (W)	796.9 ± 138.0	783.8 ± 141.6	765.4 ± 143.1
Mean 30-sec Power (W)	566.7 ± 78.2	593.8 ± 83.8	593.6 ± 83.1
Total Work (kJ)	85.0 ± 11.7	89.3 ± 12.5	84.3 ± 22.8
Peak BLC (mM)	17.3 ± 1.9	16.0 ± 2.3	17.0 ± 2.2
Rest (sec)	275.1 ± 12.9	280.9 ± 9.8	280.2 ± 10.5

Table 2. Selected data from sprint interval sessions comparing participants who showed even marginal improvement (N=10) with those who exhibited a negative response (N = 5). Despite the negative trends, no significant differences were seen between groups.

		Familiarization	Placebo Gel	Carnosine Gel
	Response			
Peak Power 1-sec (W)	Positive	796.7 ± 137.6	789.0 ± 163.1	772.8 ± 145.0
	Negative	797.4 ± 149.3	773.4 ± 74.7	750.3 ± 137.6
Mean Power 30-sec (W)	Positive	568.6 ± 92.0	587.8 ± 99.2	596.5 ± 98.6
	Negative	562.9 ± 30.1	605.8 ± 27.7	587.8 ± 24.8
Total Work (kJ)	Positive	85.3 ±13.8	88.2 ± 14.9	89.5 ± 14.8
	Negative	84.4 ± 4.5	91.5 ±3.0	74.0 ± 31.7
Peak BLC (mM)	Positive	16.7 ± 2.2	15.7 ± 2.5	16.4 ± 3.1
	Negative	18.8 ± 1.9	16.8 ± 3.7	18.2 ± 1.0

Fig. 2 Comparison of individual sprints by SIT session. There were no significant differences between any sprints.



DISCUSSION

More than a thousand new supplements enter a largely unregulated market each year (20). This makes testing the safety and efficacy a nearly insurmountable but essential responsibility of exercise and nutrition researchers. The purpose of this study was to ascertain whether a single application of a TC gel would improve repeated maximal sprint performance in trained cyclists. Based on the available research (8), we hypothesized that overall average sprint power and total work would be 5% higher in the treatment group. However, as our data indicate, there does not appear to be meaningful improvement from this supplement, with one-third of our participants exhibited a nocebo response, while ten subjects showed varying degrees of insignificant response.

The prevailing hypothesis for improved performance from carnosine/BA is an improvement in intracellular buffering (5,7,14,21). While there is substantial evidence

indicating that increased carnosine levels in the muscle, typically through BA loading (5,6,9–12,22,23), research using a TC gel has been limited but positive (8,15). Sharpe and Macias (8) suggested the TC gel used in the present study significantly improved repeated sprint and 1000 m running performance in elite male soccer players. However, the design of their study was sequential and unblinded, where the TC trial was performed last, allowing for a significant learning effect. Our own data indicated that even after two home sessions and one lab familiarization trial, subjects still showed a consistent, albeit non-significant improvement in Wingate performance. This coupled with the unblinded study design would account for the small performance gains seen in that earlier study. Other methodological analysis issues with the study make application difficult at best. Nonetheless, we believed that an easy to apply acute TC gel would be useful for trained cyclists. Thus, we chose SIT, which is regarded as both highly glycolytic and a powerful training stimulus (24,25), but is also familiar to trained cyclists.

In the present study, the TC gel had no significant effect on SIT; even among the seven "best responders", performance improved just over 2% and three showed no improvement. As illustrated in Figure 2, individual sprints during each session were also very similar. Interestingly, and though not statistically significant, five cyclists performed worse after treatment. However, as noted, our TC gel contained menthol and four of the five "nocebos" expressed negative feelings toward menthol. We also cannot discount the normal data to variation in performance which could have exacerbated the aversion to menthol. And while it would be interesting to speculate about the higher BLC measured in the nocebo group, the small sample makes any conclusion tenuous at best.

As already noted, a major limitation of this study was the lack of blinding and use of a treatment that some viewed negatively, making definitive conclusions difficult. Nonetheless, we believe our results, as well as those of earlier work (8) do not support the efficacy of a single application of TC gel. However, one particular area that should be studied is the effect of multiple applications over at least a week, as it is possible a single application is insufficient to raise muscle carnosine levels sufficient for performance enhancement. Nonetheless, if manufacturers are recommending a single application a product, it may also be worth testing the efficacy of topical products during more high intensity endurance sessions, as it is incumbent on exercise scientists to continue to independently examine these products to help guide the public.

In conclusion, it appears that a single recommended dose of TC gel does not improve repeated sprint performance in trained cyclists. Additionally, among the cyclists studied, there were individuals who exhibited a potential negative response to the supplement provided. More research, particularly using either multiple doses prior to performance, or more endurance specific trials should be conducted to better test the claims of the manufacturer.

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COMPETING INTERESTS

The authors declare no financial interests related to this publication.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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