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Transdermal carnosine gel fails to improve repeated Wingate performance in trained male cyclists: A randomized controlled cross-over trial

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Abstract
Objective: The purpose of this study was to investigate whether a transdermal carnosine (TC) gel improved repeated Wingate sprint performance in trained cyclists.
Methods: 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.
Results: 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, with five showing a significant (p=0.0380) decrease in total and significantly (p=0.0127) higher BLC after TC treatment.
Conclusions: A single recommended dose of TC gel did not improve repeated Wingate sprint performance in trained male cyclists. Even after elimination of nocebo subjects, performance improvement was still negligible.

Keywords: 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 performance in competitive cycling is largely determined 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.

Materials and Methods

Participants and Ethics Approval

Prior to initiating this research, all methodology was reviewed and approved by the Shenandoah University Institutional Review Board (IRB). The initial sample size was determined based on unpublished normative lab Wingate data of trained cyclists, published values [18], and reported improvements using TC [8]. Our calculations yielded a power of 80% at an alpha level of 0.05 using 14 subjects. To ensure we would achieve the target power a goal of 18 subjects was set for recruitment. All participants were recruited from the local cycling community via social media and word of mouth. 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 recruited between November 1, 2018, and February 28, 2019. Each was 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. Recruitment was ended after the target sample size was exceeded.

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 research assistant supervising test session was 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 with principal investigator (PI- CRH) the 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 by PI. 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.

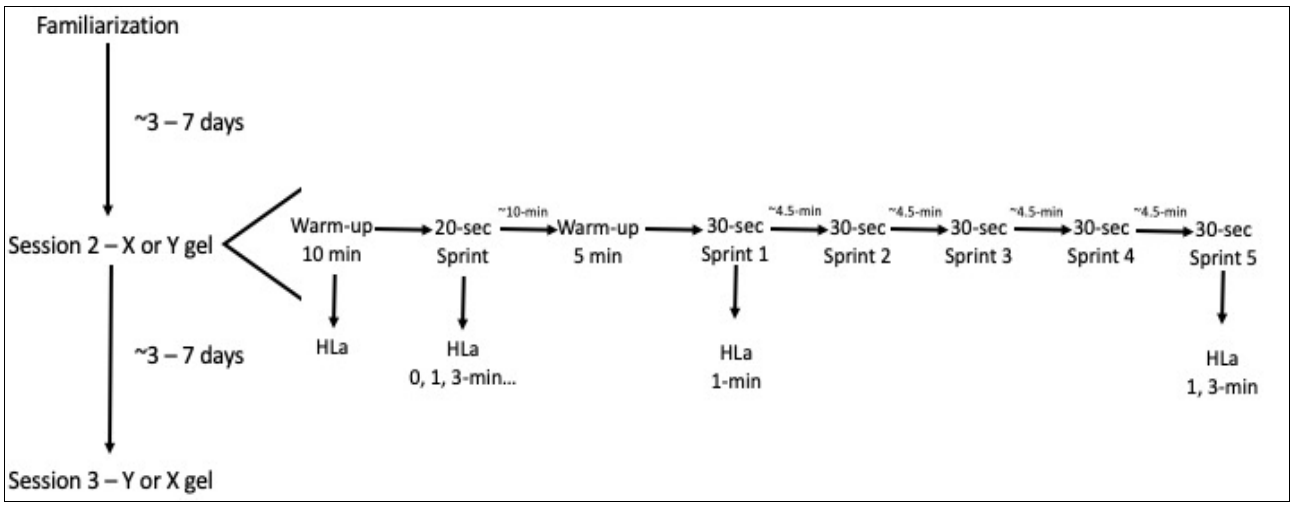

Fig 1: General study flow for transdermal carnosine supplement trial. N = 16 subjects were enrolled and N = 15 completed all trials; one subject self-withdrew after session 1.

Transdermal Carnosine Supplement and Placebo

The supplement used in the study was a topical gel (LactiGo™, Outplay Inc., Las Vegas, NV, USA) 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 (CRH) following ACSM Guidelines with body fat estimated using the Siri equation [19].

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 [20]. 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). Where applicable, Kruskal-Wallis non-parametric analysis was used to compare subsets within the data and Cramer's V effect size (φ_c) calculated and noted.

Results

Sixteen male cyclists volunteered for the study, with fifteen completing all trials and one withdrawing after the first familiarization trial due to other commitments. Participants included road, mountain bike, and cyclocross cyclists of USA category ranking 1-4, as well as three elite triathletes; their characteristics are summarized in table 1. All subjects received both the placebo and TC per the randomized assignment, applying the gels as directed. There were no statistical differences (p=0.545) for application time of the placebo (72.3 ± 5.3) min and treatment gels (74 ± 9.1 min). Data for all trials are summarized in Table 2 and show that there were no significant differences in any measured variables across all trials. Group averages for individual sprints for each session are illustrated in Figure 2. While there appeared to be a small non-statistical learning improvement from the initial familiarization trial, there were no significant differences for any sprint or trial.

Table 1: Baseline subject characteristics for N = 15 male cyclists.

	Mean ± SD
Age (yrs)	34.9 ± 9.0
Height (cm)	179.4 ± 5.5
Weight (kg)	73.4 ± 11.8
% Body Fat	10.5 ± 4.7
VLa_{Max} ($mM \cdot sec^{-1} \cdot L^{-1}$)	0.74 ± 0.31
15-sec power (W)	755.1 ± 153.6

Table 2. 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 with individual effect sizes reported below.

Table 2: Summary data for N = 15 cyclists performing a 15-sec sprint with ~15-min recovery followed by five Wingate sprints.

	Familiarization	**Placebo Gel**	**Carnosine Gel**	**P**	**ω^2**
Mean 15-sec Power (W)	755.1 ± 153.6	741.2 ± 139.0	749.4 ± 123.1	0.9729	<0.01
Peak BLC (mM)	10.4 ± 2.5	9.4 ± 1.7	9.2 ± 2.5	0.2207	0.05
Time to Peak BLC (sec)	3.2 ± 2.1	3.4 ± 1.7	2.8 ± 2.4	0.9382	<0.01
Repeated Wingate Sprints					
Peak Power 1-sec (W)	796.9 ± 138.0	783.8 ± 141.6	765.4 ± 143.1	0.8193	<0.01
Mean 30-sec Power (W)	566.7 ± 78.2	593.8 ± 83.8	593.6 ± 83.1	0.5629	0.01
Total Work (kJ)	85.0 ± 11.7	89.3 ± 12.5	84.3 ± 22.8	0.5448	0.01

Peak BLC (mM)	17.3 ± 1.9	16.0 ± 2.3	17.0 ± 2.2	0.4139	0.02
Rest (sec)	275.1 ± 12.9	280.9 ± 9.8	280.1 ± 10.5	0.2974	0.03

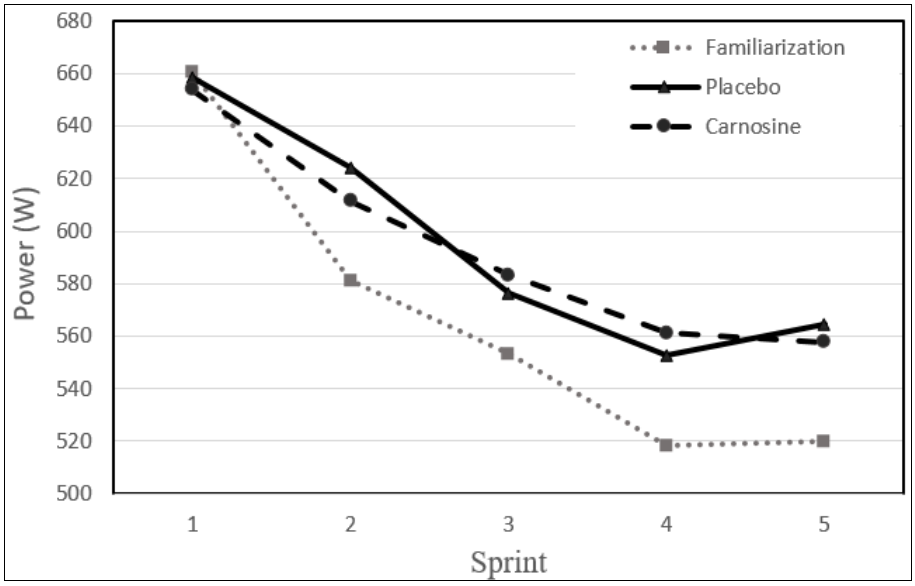

Fig 2: Comparison of individual sprints by SIT session. There were no significant differences between any sprints.

Taken together, the TC treatment had no discernible impact on any performance measure or on BLC. However, five of fifteen subjects performed noticeably worse after treatment with four of the five indicating a strong dislike toward menthol. Kruskal-Wallace post-hoc analysis indicated a non-significant (p=0.0539) decline in performance for the five "nocebos". The 3.0% drop in average session power from placebo trials might be linked to the significantly (p=0.0127, φ_c = 0.50) higher BLC than the other subjects; Table 3 summarizes select performance data for the two group responses.

Table 3. Selected data from sprint interval sessions comparing participants who showed even marginal improvement (N=9) with those who exhibited a negative response (N = 5); one subject showed no change across all trials. "Nocebo" subjects saw a significant drop in total kJ and exhibited significantly higher (p=0.0127, φ_c = 0.50) peak BLC in the TC trial; denoted by *****.

Table 3: Selected data from sprint interval sessions comparing participants who showed even marginal improvement (N=9) with those who exhibited a negative response (N = 5);

		Familiarization	Placebo Gel	Carnosine Gel	P	ω²
Response						
Peak Power 1-sec (W)	Positive	810.9 ± 137.9	811.3 ± 156.1	780.6 ± 151.5	0.8933	0.05
	Negative	797.4 ± 149.3	773.4 ± 74.7	750.6 ± 137.6	0.6907	0.17
Mean Power 30-sec (W)	Positive	577.6 ± 92.8	597.4 ± 100.2	607.1 ± 98.3	0.7757	0.08
	Negative	562.9 ± 30.1	605.8 ± 27.7	587.8 ± 24.8	0.0539	0.48
Total Work (kJ)	Positive	86.6 ±13.9	89.6 ± 15.0	91.1 ± 14.7	0.7757	0.08
	Negative	84.4 ± 4.5	91.5 ± 3.0	88.2 ± 3.7	0.0380*	0.51
Peak BLC (mM)	Positive	16.4 ± 2.2	15.5 ± 2.6	15.6 ± 2.2	0.6074	0.11
	Negative	18.8 ± 1.9	16.8 ± 3.7	18.2 ± 1.0*	0.3667	0.28

Discussion

More than a thousand new supplements enter a largely unregulated market each year [21]. 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, our data indicates that there is no meaningful improvement from this supplement, with one-third of our participants exhibiting 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, 22]. While there is substantial evidence indicating that increased carnosine levels in the muscle, typically through BA loading [5, 6, 9-12, 23, 24], 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

conclusions 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 [25, 26], but is also familiar to trained cyclists; all of the subjects reported a post-SIT rating of perceived exertion of at least 9 out of 10.

In the present study, the TC gel had no meaningful 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, five of the cyclists showed a non-significant negative trend in performance, performing significantly less work (kJ) and exhibiting a significantly higher BLC. As noted, our TC gel contained menthol and four of the five "nocebos" expressed negative feelings toward menthol. This menthol aversion could have exacerbated the normal day-to-day variation or induced a greater sympathetic nervous system response, which would result in a catecholamine release dependent stimulation of glycolysis. Nonetheless, considering the small sample, such speculation must be viewed with making any conclusions 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 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. Finally, this study was funded to study just men, without available funds to study women, making application of the results to women tenuous, but unlikely to benefit them as well.

In conclusion, manufacturer recommended single dose of TC gel does not improve repeated Wingate sprint performance in trained male cyclists. Additionally, among the cyclists studied, there were individuals who exhibited a potential negative response to the supplement, possibly related to the menthol in the product. More research, particularly using multiple doses prior to performance and more endurance specific trials in both men and women 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.

Ethical Declaration and Consent to Participate

This study was reviewed and approved by the Shenandoah University Institutional Review Board. All participants freely volunteered to complete the study and consented to results publication.

Author Contributions

All authors contributed to the design and preparation of this manuscript.

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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