Caffeinated Nitric Oxide-releasing Lozenge Improves Cycling Time Trial Performance

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Key words
- beetroot juice
- nitrate
- oxygen efficiency
- nitrite
- steady-state exercise

Abstract

Boosting nitric oxide production during exercise by various means has been found to improve exercise performance. We investigated the effects of a nitric oxide releasing lozenge with added caffeine (70 mg) on oxygen consumption during steady-state exercise and cycling time trial performance using a double-blinded randomized, crossover experimental design. 15 moderately trained cyclists (7 females and 8 males) were randomly assigned to ingest the caffeinated nitric oxide lozenge or placebo 5 min before exercise. Oxygen consumption and blood lactate were assessed at rest and at 50%, 65% and 75% maximal oxygen consumption. Exercise performance was assessed by time to complete a simulated 20.15 km cycling time-trial course. No significant treatment effects for oxygen consumption or blood lactate at rest or during steady-state exercise were observed. However, time-trial performance was improved by 2.1% ($p < 0.01$) when participants consumed the nitric oxide lozenge (2424 ± 69 s) compared to placebo (2476 ± 78 s) and without a significant difference in rating of perceived exertion. These results suggest that acute supplementation with a caffeinated nitric oxide releasing lozenge may be a practical and effective means of improving aerobic exercise performance.

Introduction

Researchers have found that nitric oxide (NO) increases during both acute aerobic and resistance exercise. It also has been observed that NO levels at rest and during exercise are highest in those individuals who are the most fit, and that the rate of NO production is positively related to exercise performance [1,9,29]. Moreover, mild aerobic and resistance exercise training has been observed to increase blood markers of NO production and detraining results in their decline [24–26].

Research indicates that raising the nitrate and nitrite levels in the body prior to exercise through consumption of dietary nitrate can increase NO production and lead to an increased oxygen efficiency, i.e., increased ATP production per unit oxygen consumed, and exercise performance [2,3,17,31]. For example, Lansley et al. [18] found that consumption of 500 ml of beetroot juice, which is high in dietary nitrate, 2.5 h before exercise improved the power output and performance of 9 club-level competitive male cyclists during a 4-km and 16.1-km cycling time trial compared to placebo. Oxygen consumption was similar during the stages of the time trials, suggesting that NO improves cycling economy. Furthermore, Bailey et al. (2) reported that elevated NO production reduced the ATP cost of muscle contraction and increased exercise performance without an increase in perceived effort. Improvements in exercise performance following beetroot juice consumption has typically been found to range between 2–16% and demonstrated in such exercise modalities as running, cycling, rowing and resistance exercise [4,5,19,27].

Caffeine has also been found to significantly improve exercise performance without an increase in perceived effort [7,12,28] and appears to have a positive effect when consumed prior to exercise as well as during exercise [7,12,17,28]. Ingestion of as low as 3 mg/kg body weight 1 h before exercise has been found to be effective at increasing exercise time to exhaustion at exercise intensities of 70–80% maximal oxygen consumption (VO_2max) [17,28]. However, energy drinks containing much lower amounts of caffeine have also been found to have effective ergogenic properties [16,21]. The reasons behind the ergogenic effects of caffeine, however, are not clear, but have been associated

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with a shift in substrate utilization and sparing of endogenous carbohydrate stores [7, 10, 15, 30] and reduced perceived effort through the blocking of adenosine receptors in the nervous system [8], and increasing plasma endorphin levels [22].

Consumption of beetroot juice or a beetroot concentrate has been found to be an effective ergogenic aid for various modes of exercise and exercise intensities. However, its taste is objectionable and may cause gastric discomfort and nausea. It also has to be consumed several hours before exercise or competition to be effective. In the current study, we evaluated the effects of a novel lozenge composed of dried beetroot crystals and a low dose of caffeine along with a patented formulation designed to release nitric oxide immediately upon dissolution in the oral cavity (US patents 8,298,589 8,303,995 & 8,435,570) on exercise performance. We hypothesized that this lozenge, provided within a few minutes of exercise, would lower VO_2 during steady state exercise and improve performance in a 21.15 km cycling time trial. We found the lozenge significantly improved cycling time trial performance without an increase in perceived effort, but we could not attribute this improvement to an increase in oxygen efficiency.

Methods

Experimental protocol and subjects

The experimental protocol was a double-blinded, randomized, placebo-controlled, two-period, within-subjects crossover study. The study was conducted in accordance with international ethics standards as outlined by Harris and Atkinson [13] and approved by The University of Texas at Austin Institutional Review Board (ClinicalTrials.gov number NCT 01710761). Subjects completed 4 visits approximately 1 week apart, consisting of a visit for screening, familiarization, and 2 experimental trials. The familiarization visit protocol was identical to the experimental trials except that no study product was consumed and no blood was drawn.

16 (8 male, 8 female) moderately trained cyclists between 21 and 50 years of age were recruited from the Austin, Texas area, but one female subject did not comply with the study protocol and was excluded. The VO_2max of the final 15 (8 male, 7 female) participants was 43.7 ± 1.7 ml/kg/min and fell between the 50th and 90th percentile for age and gender. Participants had a mean age of 37.3 ± 2.5 years and a mean body mass of 73.8 ± 2.9 kg. The mean age for male participants was 34.0 ± 4.3 years, and they had a mean body mass of 80.7 ± 2.8 kg and mean VO_2max of 46.7 ± 2.5 ml/kg/min. The mean age for female participants was 41.1 ± 1.4 years, and they had a mean body mass of 65.8 ± 3.3 kg and mean VO_2max of 40.2 ± 1.5 ml/kg/min. After being advised of the purpose and potential risks of the study, all subjects provided written, informed consent.

Study supplement

The 2 test products were Neo40^{TM}, a 420 mg nitric oxide blend with 70 mg caffeine, and a non-caloric placebo (PLA). Neogenis Labs (Austin, TX) provided the Neo40^{TM} and the PLA in lozenge form. Neo40^{TM} is a patented (US patents 8,298,589 & 8,303,995) technology developed out of the University of Texas Health Science Center at Houston that delivers bioactive NO and is registered as a dietary supplement with the US Food and Drug Administration (reg # 3008524085). It contains small amounts of vitamin C and B12, and a proprietary blend of beetroot powder, Hawthorn berry extract, L-citrulline and sodium nitrite. The lozenge (NO-L) rapidly generates NO as it dissolves. This results in a significant increase in plasma nitrite and nitrate, which plateaus in 20 min and is maintained for more than 60 min [34]. The placebo consisted primarily of cellulose, silica, sucralose natural flavors and vitamin C, and had the same color, taste and texture as the NO-L. The NO-L and placebo were randomly distributed by a laboratory technician who did not participate in the testing of the subjects. The randomization procedure resulted in 7 subjects receiving the product at the first experimental trial, while the other 8 subjects received it at the second experimental trial. The subjects were instructed to refrain from using any antibacterial mouthwash during the 24-h period before each experimental trial in order to preserve commensal oral bacteria that reduce nitrate to nitrite.

Screening visit

After each subject signed the consent form and completed the health screening, height, weight and blood pressure were collected. Acceptable subjects then completed a VO_2max test on a Velotron cycle ergometer controlled by a computer-controlled CompuTrainer (Racermate, Seattle, WA) that was also used in the practice and experimental trials. The protocol for establishing VO_2max consisted of a 5 min warm-up followed by 2-min stages of increasing difficulty until fatigue. 4 separate VO_2max protocols were developed to accommodate low and high strength levels for both genders. Briefly, workload increased every 2 min for the first 10 min by 50 W for men and 25 W for women. After 10 min, the workload increased every minute by 25 W for both genders. Starting workload was based on the subject's gender, exercise frequency and body size and type. During the test, the participants breathed through a Hans Rudolph valve, with expired gases directed to a mixing chamber for analysis of oxygen (O_2) and carbon dioxide (CO_2). Inspired volumes were measured and analyzed using a computer-controlled metabolic cart (True One 2400 Metabolic Measurement System, Parvo Medics, Sandy, UT). The criterion used to establish VO_2max was the achieving of a plateau in VO_2 with increasing exercise intensity and a respiratory exchange ratio (RER) > 1.10.

Once accepted for the study, subjects were instructed to maintain a training and dietary log for the 2 and 3 days, respectively, before the familiarization trial and to keep training and diet consistent with that recorded for the remaining experimental trials. The subjects provided a copy of their training and dietary logs on the day of each trial. An investigator reviewed and verified the entries in the logs with the subjects at each session in order to verify that consistency with the previous logs was maintained. The data from the logs were entered into Nutribase Clinical Nutrition Manager 7.17 (CyberSoft, Inc., Phoenix, AZ) for nutritional analysis and compliance verification. All subjects complied with the diet and exercise requirements. Diets were not standardized across all subjects, as each subject served as his or her own control.

Experimental trials

The familiarization and experimental trials consisted of cycling at 3 steady state exercise intensities followed by a cycling time trial. The familiarization visit was identical to the experimental trial visits except that no experimental product was consumed and no blood samples were collected. The subjects reported to the laboratory in the morning after fasting for 12 h. Upon arriving each subject was weighed and a heart rate monitor (Polar

Beat, Polar Electro, Oy, Finland) was secured in place around his or her chest. Next, a catheter fitted with a 3-way stopcock with catheter-extension tubing was inserted into a forearm vein. After the subject sat quietly for 10 min, his or her respiratory gases were collected for 5 min. During this time resting heart rate and blood pressure were recorded, and an initial blood sample drawn. Following the resting respiratory gas collection, the subject consumed the caffeinated NO-L or PLA and rested for an additional 5 min while respiratory gases were collected for an additional 5 min. Following the last resting respiratory gas collection, resting heart rate and blood pressure were again recorded and a blood sample drawn.

Following the resting data collection, the subject then mounted the cycle ergometer and commenced cycling. The subject cycled at 50%, 65% and 75% of VO_{2max} for 8, 6 and 6 min, respectively, while expired respiratory gases were collected. Ventilation, VO_2, VCO_2 production and RER were recorded with the respiratory gas analysis system used during the VO_2max test. Measurements collected during the last 3 min of each period were averaged to compute VO_2 and substrate utilization. Fat and carbohydrate oxidation rates were calculated from the VO_2 and VCO_2 values using the equation of Frayn [11]. Exercise blood samples were collected at 7, 13 and 19 min during the 20-min steady-state ride. HR and rating of perceived exertion (RPE) using the Borg scale were recorded during steady state exercise at the time of blood collection. Once the steady state exercise protocol was completed, the subject dismounted the cycle ergometer for a 5-min break, during which time the catheter was removed from the arm of the subject, and the Velotron reprogramed for the 21.15 km cycling time trial.

The time trial course was programmed using Velotron 3D software (Version 3) that coordinated with the cycle ergometer (Racemate, Seattle, WA). The resistance was self-selected. Time to complete 6 km, 10 km, 19.5 km and 20.15 km was recorded for each time-trial ride. Heart rate and RPE were also collected throughout the time-trial ride at 6 km, 10 km and 19.5 km. After completing the time trial, the subject then began a cool down ride at a self-selected resistance and continued to spin until his or her heart rate was below 120 bpm. Including the steady-state ride and cool down, the total exercise time ranged from 55 to 75 min. Subject were then provided with a light meal. The heart rate monitor was retrieved, and instructions for the next trial were given to the subjects.

Blood collection and lactate analysis

Blood samples (1.5 ml) were dispensed into a microfuge tube containing 0.35 ml of EDTA (24 mg/ml, pH 7.4). A 0.5 ml sample of this anticoagulated blood was immediately transferred to another tube cooled over ice containing 1 ml 10% perchloric acid (PCA). All tubes were centrifuged for 15 min at 3000 rpm with a JS-7.5 rotor in a J2-21 centrifuge (Beckman Coulter, Inc., Fullerton, CA). PCA extracts were transferred and stored at −80 °C until analyzed for blood lactate by enzymatic analysis [14]. After each blood sample was taken, the catheter line was immediately flushed with a sterile saline solution to prevent clotting.

Statistical analysis

RPE, RER, blood lactate and heart rate were analyzed using repeated-measures ANOVA. Time trial performance data were analyzed using a one-tailed matched paired t-test. Post hoc analysis was performed using a Tukey's HSD test. A Levene test was used to analyze variance. Differences were considered significant at $p < 0.05$. Data are expressed as means ± SEM.

Results

Steady state exercise

Steady state exercise data were collected at exercise intensities of 50%, 65% and 75% of VO_2max. VO_2, respiratory exchange ratio (RER), heart rate (HR), RPE and blood lactate concentration were also determined (**Table 1**). Carbohydrate and fat utilization as percentages were also analyzed during steady state exercise.

VO_2 increased as exercise intensity increased during both experimental trials. However, there was no significant difference in VO_2 between trials at rest before or after providing treatments. Additionally, there were no treatment differences in VO_2 at exercise intensities of 50%, 65% or 75% of VO_2max.

HR did not differ between treatments at rest. With increasing exercise intensity, HR increased significantly, but again there was no treatment effect noted. Likewise, RPE determined during steady state exercise did not differ between treatments.

There were no treatment or treatment-by-time effects for substrate utilization during steady state exercise (**Table 2**). Carbohydrate and fat each accounted for about 50% of substrate utilized at rest. At an exercise intensity of 50% of VO_{2max}, carbohydrate utilization rose to approximately 80%, while fat utilization declined to 20%. When exercise intensity was increased to 75% VO_{2max}, carbohydrate oxidation accounted for 90% of substrate utilization.

Blood lactate increased significantly as exercise intensity increased. Although blood lactate was slightly less for NO-L than PLA, there was no significant difference (**Table 1**). At each exercise intensity female participants tended to produce less lactate than male participants (data not shown).

Cycling time trial

There were significant treatment and treatment-by-time effects of NO-L on time trial completion compared to PLA (**Fig. 1**). The average time to completion for PLA was 2477 ± 78 s and for NO-L

Table 1 Physiological measurements and blood lactate during steady state exercise (N = 15).

	Treatment	Rest	Pre-Ex	50% VO_2max	65% VO_2max	75% VO_2max
VO_2	PLA	0.25 ± 0.10	0.24 ± 0.10	1.61 ± 0.09	1.98 ± 0.11	2.32 ± 0.15
(L/min)	NO-L	0.26 ± 0.01	0.25 ± 0.01	1.60 ± 0.08	2.00 ± 0.11	2.27 ± 0.14
HR	PLA	59.1 ± 2.5	60.1 ± 3.0	117.1 ± 2.7	134.8 ± 3.3	149.9 ± 3.9
(Beat/min)	NO-L	58.3 ± 2.2	60.3 ± 2.4	116.6 ± 2.6	133.9 ± 3.1	147.3 ± 3.8
RPE	PLA	N/A	N/A	10.6 ± 0.2	12.1 ± 0.2	13.7 ± 0.3
Borg Scale	NO-L	N/A	N/A	10.6 ± 0.3	12.2 ± 0.2	13.6 ± 0.3
Lactate	PLA	1.39 ± 0.10	1.27 ± 0.08	1.67 ± 0.14	2.28 ± 0.25	3.46 ± 0.45
(mmol/L)	NO-L	1.33 ± 0.12	1.31 ± 0.13	1.46 ± 0.09	2.10 ± 0.17	3.27 ± 0.35

Values are means ± SEM. There were no significant differences between treatments for any of the physiological measures evaluated

	Treatment	Rest	Pre-ex	50% VO_2max	65% VO_2max	75% VO_2max
RER	PLA	0.85 ± 0.02	0.84 ± 0.02	0.94 ± 0.01	0.98 ± 0.02	0.99 ± 0.02
	Neo40™	0.85 ± 0.02	0.86 ± 0.02	0.94 ± 0.01	0.98 ± 0.01	0.98 ± 0.02
CHO (%)	PLA	49.7 ± 6.0	48.0 ± 5.8	80.1 ± 4.2	87.0 ± 3.7	89.5 ± 3.4
	Neo40™	50.4 ± 6.7	51.7 ± 6.4	80.0 ± 3.8	87.9 ± 3.3	90.2 ± 3.0
FAT (%)	PLA	50.8 ± 5.9	52.5 ± 5.8	20.5 ± 4.2	13.4 ± 3.7	10.9 ± 3.4
	Neo40™	50.0 ± 6.6	50.0 ± 6.6	20.6 ± 3.9	12.6 ± 3.4	10.2 ± 3.1

Values are means ± SD. There were no significant differences found for substrate utilization between treatments

Table 2 Substrate utilization during steady state exercise (N = 15).

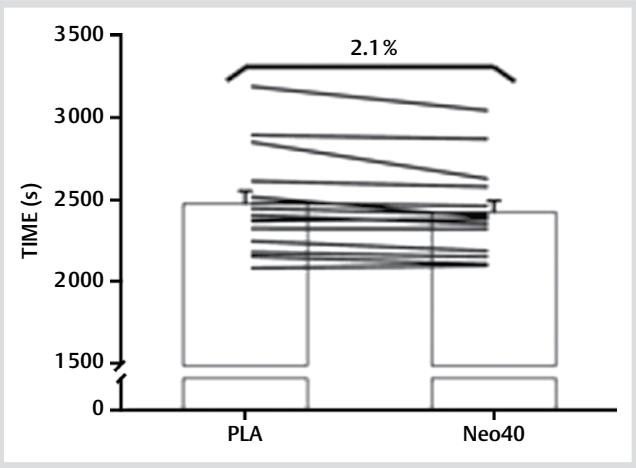

Fig. 1 Mean time to complete a 20.15 km cycling time trail, with individual data depicted by single lines. There was significant difference between treatments (N = 15, $P = 0.01$). The percent improvement of the time trial was 2.1%. Error bar indicates SEM. * indicates there was a significant difference between treatments.

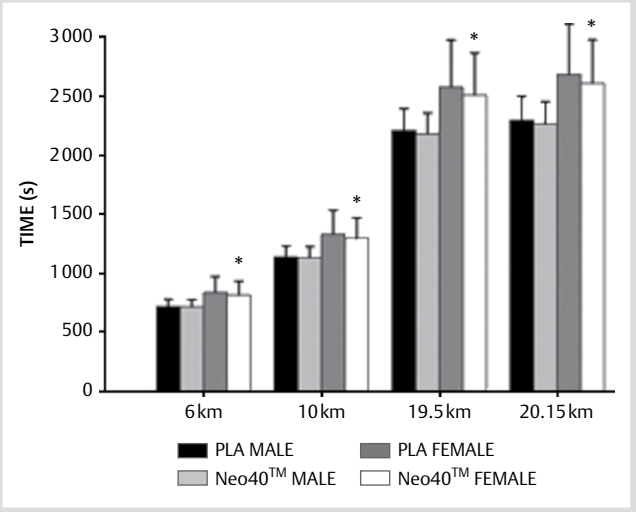

Fig. 2 Cycling time trial completion time separated by gender at different stage of cycling. There was a significant treatment difference for female subjects (N = 7, $P = 0.03$). No treatment difference was observed for male subjects (N = 8, $P = 0.11$). Error bars indicate SEM. * indicates significantly different between treatments for female subjects.

it was 2424 ± 69 s ($p < 0.01$), with 11 of 15 subjects having faster times to completion when treated with NO-L, 2 subjects with faster times to completion when treated with the placebo, and 2 subjects with the same time for both treatments (**Fig. 2**). The percent improvement of time trial performance was 2.1%. In addition, when subjects received NO-L they reached 19.5 km sooner than when they received PLA (**Table 3**). We also analyzed the effect of treatment based on gender (**Fig. 2**). We found a treatment effect in female subjects (N = 7, $p = 0.03$). However, there was no significant treatment effect detected for male subjects, although significance was approached (N = 8, $p = 0.11$). Although subjects worked harder when supplemented with NO-L, there was no significant difference in HR or RPE by treatment (**Table 3**). However, both RPE and HR increased significantly with time during the course of the time trial.

Discussion

Previous studies have reported longer time to exhaustion during exercise after 4–6 days of dietary nitrate supplementation [2,3,19]. However, such time-to-exhaustion protocols do not necessarily imply improvements in exercise performance in a more practical setting that simulates normal athletic competition. In this regard, Lansley and colleagues [18] adopted a more practical time trial design to investigate the acute ergogenic effect of nitrate after only one dose (6.2 mmol NO_3^-) ingested 2.5 h before completion on a 4- and 16.1-km time trial. Performance improved in both distances after nitrate ingestion, suggesting that the ergogenic effects of nitrate are acutely attainable. After nitrate ingestion, subjects improved 2.8% and 2.7% in the 4- and 16.1-km time trials, respectively. Recently, Cermak et al. [5] tested the effects of 6 days of beetroot concentrate (8 mmol/d nitrate) consumption on cycling time trial performance in trained cyclists. Subjects cycled for 30 min at 45% and 65% VO_2max and then completed a 10 km time trial. Results indicated that time trial performance and power output improved significantly following beetroot concentrate consumption compared with placebo. Although a modest 1.2%, improvement with beetroot concentrate was significant, and 11 of the 12 subjects did show improvement.

In the current study, 7 moderately trained female and 8 moderately trained male subjects exercised for 8 min at 50%, 6 min at 65% and 6 min at 75% VO_2max and then completed a 20.15 km time trial after consuming a placebo or NO-generating lozenge containing caffeine approximately 5 min before commencing cycling. Time trial performance was significantly improved following the Neo40™ lozenge. The improvement in performance averaged 2.1%, which relates favorably to the improvements demonstrated by Cermak et al. [5] and Lansley et al. [18]. However, in a more recent study, Cermak and colleagues [6] reported that a single bolus of beetroot concentrate (8.7 mmol NO_3^-) with breakfast 2.5 h before a 1 h cycling time trial had no effect on performance in highly trained cyclists. The difference in time trial performance between the most recent study by Cermak et al. [6] and the current study may be due to differences in technology involved in delivery of nitrite and nitrate and the training

	Treatment	6.00 km	10.00 km	19.50 km	21.15 km
heart rate (beats/min)	PLA	162.9 ± 2.8	162.3 ± 3.0	167.0 ± 3.7	N/A
	Neo40™	162.1 ± 2.9	163.4 ± 2.9	167.4 ± 3.3	N/A
RPE (Borg scale)	PLA	14.3 ± 0.3	15.4 ± 0.3	16.9 ± 0.4	N/A
	Neo40™	14.7 ± 0.2	15.2 ± 0.3	16.7 ± 0.4	N/A
time (s)	PLA	773 ± 24	1 228 ± 38	* 2 381 ± 73	* 2 478 ± 78
	Neo40™	762 ± 21	1 208 ± 31	2 334 ± 66	2 425 ± 68

Values are means ± SD. * Indicates significant difference between treatment ($p < 0.05$)

Table 3 Time-trial performance data (N = 15).

status of the subjects. It may also be possible that the addition of caffeine in the NO-L helped to enhance exercise performance.

Caffeine has been demonstrated to be a powerful ergogenic aid for aerobic exercise performance. A number of studies have demonstrated that consumption of caffeine 45–60 min prior to exercise will increase time to exhaustion when exercising at intensities of 60–75 % VO_{2max} and cycling time trial performance [7, 12, 15, 28]. Caffeine alone has been found to be effective at levels as low as 3 mg/kg body wt [17, 28]. However, the NO-L contained only 70 mg of caffeine, which would translate to an average of 1.2 mg/kg body wt for the female subjects and 1.0 mg/kg body wt for the male subjects. Therefore, the amount of caffeine provided by the NO-L is about one-third that typically seen to have an ergogenic effect, and therefore its contributions to improved performance is questionable. However, there is the possibility that providing the caffeine with a NO source improved the efficacy of the caffeine. Previous research with energy drinks containing relatively low levels of caffeine have been found to improve cycling time trial performance without an increase in perceived effort [16, 21].

Improvements in aerobic performance by increasing blood nitrate levels either by consumption of sodium nitrate or beetroot juice has been associated with an increase in oxygen efficiency [2, 3, 19, 20]. This increase in oxygen efficiency has been found to be highly correlated with improved exercise performance. In the current study, we did not see a significant reduction in the oxygen cost of exercise following our NO-L treatment. Oxygen consumption attributed to exercise alone (exercise VO_2-resting VO_2) at 50 %, 65 % or 75 % of VO_2max was not different between treatments. However, VO_2 at 75 % VO_2max was 2.9 % lower after the NO-L treatment compared to PLA and approached statistical significance ($p = 0.08$).

The reason that an improvement in exercise oxygen efficiency was not observed is not evident, but could be related to a temporal response of the lozenge. The NO-L is designed to generate NO immediately upon dissolving. However, blood nitrite levels do not peak for 20 min [34]. Additionally, for NO to have an effective ergogenic response nitrite levels must be increased in the muscle as well as the blood, and it is likely that additional time is required for the active muscles to increase their nitrite and NO concentrations to levels that will significantly affect mitochondrial function. Therefore, it is conceivable that providing the lozenge only 5 min before the onset of exercise testing could account for our failure to find an increase in exercise oxygen efficiency. Our finding that the oxygen cost of exercise appeared to be declining during the last stage of steady-state exercise lends some support to this hypothesis. Regardless, the finding that just 20 min after taking the NO-L exercise performance was improved demonstrates its rapid bioactivity and practicality.

We also found that blood lactate increased in proportion to exercise intensity. However, the NO-L had no effect on blood lactate accumulation. This finding is also in agreement with those of Bailey et al. [3] and Cermak et al. [5]. Although NO has been found to increase oxygen efficiency, it apparently has no effect on blood lactate kinetics.

An interesting observation was that the NO-L improved time trial performance without increasing perceived effort. This decrease in perceived effort during exercise may be related to an increase in muscle contractile efficiency as demonstrated by Bailey et al. [2]. Using a high and low intensity exercise paradigms, Bailey et al. [2] found that NO reduced the ATP cost of muscle force production and slowed the rate of creatine phosphate breakdown. This raises the possibility that NO improves actin/myosin cross-bridge cycling efficiency or calcium kinetics. Caffeine is also known to lower perceived effort [7, 12, 15]. This has been attributed to the blocking of adenosine receptors [8] and increased plasma endorphin levels [22], but also to a reduced level of neuronal activity required for muscle contraction [32] as a result of altered intracellular Ca^{++} kinetics [23] and myofibril Ca^{++} sensitivity [33]. It is therefore possible that this improved efficiency of muscle contractile function made muscle fiber recruitment easier, thereby reducing perceived effort.

It is of interest to note that only the female subjects had a significant response to the NO-L. Of the 7 female subjects, 6 had a positive response as compared to 5 of 8 male subjects. The reason for this difference in gender response is not clear. It could simply be due to a type II statistical error as the study was not designed to evaluate gender differences. It could also be that more of our male subjects, although randomly selected, were unable to convert nitrate to nitrite. For nitrate to be converted to NO, it must first be converted to nitrite by commensal bacteria found in the saliva glands and under the tongue. Approximately 25–30 % of the population lacks the appropriate bacteria. Also, the bacteria can be destroyed by antibacterial mouthwash. While we instructed the subjects to refrain from using mouthwash while participating in the study, prior use could have lowered the bacterial count and limited the effectiveness of the NO-L.

There are several limitations to this study. First, the independent effects of nitric oxide and caffeine could not be determined with the experimental design employed. However, the NO-L was found to improve exercise performance and in a relatively short period of time. Second, the time of administering the lozenge did not appear to be ideal. Although NO-L generates nitric oxide almost immediately as it dissolves in the mouth, it was evident more time was needed for it to produce its physiological effects. Third, the population tested was comprised of moderately trained subjects, and it is therefore not possible to conclude that the dosage of NO-L used would benefit highly trained athletes. Finally, this study did not take into consideration or try to control for the menstrual cycle phase of the female subjects when tested.

In summary, it was found that the NO-L caffeinated lozenge taken just before exercise improved cycling time trial performance in moderately trained cyclists. Effects were more favorable in women than men. The reason for the improved performance could not be attributed to a reduction in the oxygen cost of exercise or changes in substrate metabolism. However, the results suggest that the caffeinated NO-L is a practical, fast-acing and effective pre-exercise ergogenic aid for aerobic exercise training and competitions.

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Conflict of interest: Dr. Ivy is currently a consultant for Neogenis Labs. At the time the study was conducted, he was not involved with this company. During the course of the study, the everyday laboratory testing was conducted by Lynne Kammer. Randomization of treatments and dispensing of supplements were controlled by a laboratory technician not involved directly in the study.

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