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Effects of Neo40™ with caffeine on cycling time trial performance

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by

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Abstract

Effects of Neo40^{TM} with caffeine on cycling time trial performance

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The vasodilatory effects of nitric oxide (NO) have attracted a lot of attention from many investigators who are interested in treatment for cardiovascular disease or hypertension. Recently, however, NO has drawn the attention of people who are looking for new avenues to improve their health, as well as effective ways to enhance exercise performance. In particular, NO, a potent vasodilator, is known to regulate blood flow to active muscles and improve muscle contractile efficiency during exercise, allowing participants to exercise much longer with less fatigue. Neo40^{TM} contains 420mg of a nitric oxide blend with 75mg of caffeine. Therefore, the purpose of the current study was to investigate the effects of Neo40^{TM} on cycling time trial performance and exercise efficiency in 15 moderately trained cyclists. The protocol was a double-blind, randomized, placebo-controlled, two-period, within-subjects crossover study. The treatments were Neo40^{TM}, and a non-caloric similarly favored placebo (PLA). Fifteen participants were randomly assigned to ingest a Neo40 ^{TM} or PLA in lozenge form. Exercise performance was assessed by time to complete a simulated 20.15km time-trial

course. Exercise efficiency was also measured by VO_2 and lactate accumulation at standardized submaximal steady-state exercise intensities. Time-trial performance was enhanced by 2.1% when participants consumed Neo40^{TM} compared to a PLA without a significant difference in rating of perceived exertion (RPE). Time to complete 6km, 10km, 19.5km and 20.15km of cycling was analyzed by gender. A significant difference was found in female subjects at all time points, but not in male subjects. We did not find significant treatment effects for VO_2, respiratory exchange ratio (RER), RPE, heart rate (HR) and lactate concentration during steady state exercise. In conclusion, acute supplementation with Neo40^{TM} improved time-trial performance by an average of 2.1% although there were no treatment effects in regards to factors related to work efficiency.

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INTRODUCTION

Recently, people have been looking at new avenues to improve their health, as well as effective ways to enhance exercise performance to obtain instant results. Often, they find supplements to reach their goals. Nitric oxide (NO) is a powerful vasodilator, but also has effects on muscle force production and mitochondrial function. NO production during exercise is stimulated naturally by the activation of NO synthase, which is increased by shear stress within the arterioles and capillary beds of skeletal muscle. Clearly, increasing NO production during exercise by training, consuming nitrate or nitrite compounds, or consuming vegetables that contain high levels of nitrate and nitrites may improve oxygen efficiency and exercise performance. Neo40^{TM} is a neutraceutical/botanical supplement that increases the body's ability to generate NO by reducing nitrates and nitrites to NO, an alternative process for NO production. Investigators have discovered that dietary nitrate and nitrite also can serve as sources for production of a diverse group of nitrogen metabolites, including nitric oxide, via nitrate/nitrite reductases in tissues (Hord, 2011). Inorganic nitrate from dietary sources is converted in vivo to nitrite, which along with dietary and other sources of nitrite, is reduced in vivo to NO and other bioactive nitrogen oxides (Hord et al, 2009; Carlström et al., 2011).

There are a number of recent studies that support the effects of nitric oxide on exercise performance. Lansley et al. (2011A) found that consumption of beetroot juice, which is a natural source of nitrate, 2.5 hours before testing 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 trial, suggesting beetroot juice improves cycling economy (Lansley et al., 2011A). Similar to the finding of Lansley et al . (2011A, Cermak et al. (2012) found that power output and time-trial performance improved in 10 trained cyclists following consumption of

concentrated beetroot juice although the performance difference between the beetroot juice and placebo trials was relatively small (Cermak et al., 2012). As a measure of exercise performance, many studies use tests that involve exercise to exhaustion, where the subject can no longer continue to exercise at a given rate or they stop because of complete fatigue. Using such protocols, investigators also have reported significant improvement in exercise tests following beetroot supplementation. Lansley and others (2011B) reported improved treadmill running time to exhaustion in a high-intensity treadmill test after 4 to 5 days of supplementation. Bailey and others (2010B; 2009) utilized various protocols involving high-intensity exhaustive knee-extension and cycling tests and found that 4 to 6 days of beetroot jucie supplementation improved exercise time to exhaustion. Vanhatalo and others (2011) found that one day after beetroot supplementation, performance in a knee-extension test to exhaustion under hypoxic conditions was restored to values observed in normoxia.

Aside from nitrate supplementation, 75mg of caffeine is included in Neo40^{TM}. Caffeine has also been found to significantly improve exercise performance (Costill et al., 1978; Graham et al., 1995; Pasman et al., 1995) and appears to have a positive effect when consumed prior to exercise as well as during exercise (Costill et al., 1978; Graham et al., 1995; Pasman et al., 1995; Kovacs et al., 1998). Traditionally, however, doses of at least of 3mg caffeine per kg of body weight have been used to elicit the ergogenic effect (Costill et al., 1978). However, the effectiveness of the caffeine may be enhanced by the increase in nitric oxide availability.

As mentioned above, many researchers have investigated NO with both acute and chronic supplementation and with subjects of different fitness levels. However, the acute effect of NO within 1 hour of exercise has not been investigated. The purpose of this study

was to investigate the acute effects of a novel nitric oxide synthesizer, Neo40^{TM} on exercise performance and work efficiency. Specifically, we had the following hypothesis:

Hypothesis 1: Neo40^{TM} in lozenge form would enhance cycling time-trial performance.

Hypothesis 2: Neo40^{TM} in lozenge form have a positive effect on work efficiency by reducing O_2 uptake at standardized submaximal steady-state exercise intensities.

METHODS

Protocol

The experimental protocol was a double-blind, randomized, placebo-controlled, two-period, within-subjects crossover study. The test exercise protocol was made up of 20 min of steady-state cycling based on percent of VO_2 max and a 20.15 km time trial. Completion times at standardized time points were gathered to measure exercise performance. Rating of perceived exertion (RPE), respiratory exchange ratio (RER), oxygen consumption (VO_2) and blood lactate were collected to measure work efficiency during the steady-state cycling. Blood pressure was also measured before and after providing Noe40^{TM} and placebo (PLA). Furthermore, height, weight, blood pressure and VO_{2max} were collected during a screening visit.

Participants

Sixteen (8 male, 8 female) moderately trained cyclists between 21 and 50 years of age were recruited from the Austin, TX area, but one female subject did not comply with the study protocol and was excluded. The VO_2max of the final fifteen (8 male, 7 female) participants was 43.7 ± 1.72 ml/kg/min and fell between the 50th and 90th percentile for age and gender. Participants had a mean age of 37.3 ± 2.49 years and a mean body mass of 73.8 ± 2.86 kg. The mean age for male participants was 34.0 ± 4.28 years,and they had a mean body mass of 80.7 ± 2.78kg and mean VO_2max of 46.7 ± 2.53 ml/kg/min. The mean age for female subjects was 41.1 ± 1.39 years, and they had a mean body mass of 65.8 ± 3.30kg and mean VO_2max of 40.2 ± 1.53ml/kg/min. After being advised of the purpose and potential risks of

the study, all subjects provided written, informed consent. The experimental protocol was approved by The University of Texas at Austin Institutional Review Board.

Study Supplement

The two test products were Neo40^{TM}, a 420mg nitric oxide blend with 75mg caffeine, and a non-caloric similarly flavored placebo (PLA). Neogenis Labs (Austin, TX) provided the Neo40^{TM} and the placebo in lozenge form. These study products were given in a random order. The randomization procedure resulted in 6 subjects receiving the product at the first experimental trial, while the other 9 subjects received it at the second experimental trial. The subjects were instructed to refrain from using any antibacterial mouthwash during the 24-hr period before each experimental trial in order to preserve commensal oral bacteria which reduces nitrate to nitrite.

Food, Exercise Logs

All subjects received a log to record their food intake for the 2 days before the exercise visits (familiarization visit and two experimental trials). The participants recorded all food ingested in the 24 hours before the exercise visits and were instructed to refrain from alcohol and caffeine. Furthermore, subjects logged their exercise routines for the 48 hours prior to each exercise trial. The participants completed the logs prior to the familiarization visit in order to prove correct completion of the records. For 24 h before the first trial, the subjects were instructed to maintain food and exercise habits based on what they reported in the familiarization visit and were instructed to replicate these in the 24 h before the subsequent trial. If the subject reported a significantly different level of caloric intake or exercise routines compared to the previous trial, the experiment was rescheduled.

Screening Visit

After signing the consent form and completing the health screener, 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 was comprised of a warm-up followed by short stages of increasing difficulty until fatigue. The subject breathed expired gases into 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 reached was a plateau in VO_2 with increasing exercise intensity and respiratory exchange ratio (RER) > 1.10.

Familiarization Visit

The familiarization visit was identical to the experimental trial visits except that no experimental product was consumed and blood was collected. The subjects received a copy of an exercise and food log to replicate before the experimental trials.

Experimental Trials

The subjects reported to the lab in the morning. A heart rate monitor (Polar Beat, Polar Electro, Oy, Finland) was secured in place around the subject's chest. The subjects' weight was collected, and then the subject sat quietly in a chair for 5 min. The subjects' resting heart rate was collected. A catheter fitted with a three-way stopcock with catheter-extension tubing was inserted into a forearm vein and a 1.5ml blood sample was drawn, with

1ml of blood transferred to a tube containing 2ml of 10% perchloric acid (PCA). The catheter remained in the subject's forearm for the duration of the steady state exercise. The subject sat quietly on a chair for 5 min while respiratory gases were collected. The subject then consumed the study product and rested for an additional 5 min while respiratory gases were collected.

Steady-State Ride

The steady-state ride consists of several time points (Rest, Pre-EX, 50%, 65% and 75% of VO_{2max}). Sitting blood pressure (Omron Healthcare Inc, Bannockburn, IL, USA) was measured in the dominant arm before and after the providing Neo40^{TM} and placebo (PLA). The subject rode at 50% VO_{2max} for 8 min, 65% VO_{2max} for 6 min then 75% VO_{2max} for 6 min while expired gases were collected. Ventilation, VO_2, CO_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. Blood was collected prior to consuming the study product, 4 min after the study product, and at 7, 13 and 19 min during the 20 min steady-state ride. HR was recorded at all time points. Subjective rating of perceived exertion (RPE) on a Borg scale (unit-less scale ranging from 6 to 20) was obtained at exercise intensities of 50%, 65% and 75% of VO_{2max}.

Time-Trial Ride

During a 5 min break, the catheter was removed from the subject's arm, the Velotron program was changed, and the ventilation equipment removed. The time trial course consisting of a 20.15km distance 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 at each time points (6km, 10km, 19.5km and 20.15km) was obtained during the time-trial ride. The heart rate and RPE were also collected throughout the time-trial ride at several time points (6km, 10km and 19.5km). After completion of 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. The subject was then provided with a light meal. The heart rate monitor was retrieved and instructions for the next trial were given to the subject.

Sample Collection and Lactate Analysis

PCA tubes were centrifuged for 15 minutes at 3,000 rpm with a JS-7.5 rotor in a Beckman J2-21 centrifuge. PCA extracts were transferred and stored at -80˚C until used to measure blood lactate. Lactate was analyzed from the perchloric acid extracts for all blood draws (Rest, Pre-Ex, 50%, 65% and 75% of VO_2max). Blood lactate was determined by enzymatic analysis according to Hohorst (1963). Samples and standards reacted with nicotinamide adenine dinucleotide (NAD) in the presence of lactate dehydrogenase (LDH) to form pyruvate and NADH. The pyruvate reacted with hydrazine irreversibly to form pyruvate hydrazone. The NADH was visualized at 340 nm by a Beckman DU 640 spectrophotometer. When duplicates were tested, they were tested within the same assay (intra-assay).

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 $p<.05$. Data were expressed as means ± SEM.

RESULTS

Steady State Trial

Steady state exercise data was collected during 20 minutes exercise consisting of 50%, 65% and 75% of $VO_{2max.}$ VO_2, respiratory exchange ratio (RER), heart rate (HR), rating of the perceived exertion (RPE) and lactate concentration were measured during steady state exercise. The percent carbohydrate and fat utilization was also analyzed during steady state exercise trial.

Table 1 A. Physiological and Blood Lactate During Steady State Exercise in PLA

	Treatment	Rest	Pre-EX	50%	65%	75%
VO_2 (L/min)	PLA	0.25±0.098	0.24±0.098	1.61±0.090	1.98±0.114	2.32±0.152
	Neo40™	0.26±0.010	0.25±0.092	1.60±0.085	2.00±0.113	2.28±0.137
HR (Beat/min)	PLA	59.1±2.54	60.1±2.96	117.1±2.75	134.8±3.32	149.9±3.93
	Neo40™	58.3±2.23	60.3±2.44	116.6±2.64	133.9±3.15	147.3±3.81
RPE	PLA	N/A	N/A	10.60±0.255	12.13±0.192	13.77±0.262
	Neo40™	N/A	N/A	10.60±0.255	12.23±0.233	13.60±0.255
Lactate (mmol/L)	PLA	1.39±0.103	1.27±0.083	1.67±0.146	2.28±0.249	3.46±0.446
	Neo40™	1.33±0.119	1.31±0.132	1.46±0.087	2.10±0.167	3.27±0.351

Table 1 B. Substrate Utilization During Steady State Exercise in Neo40™

	Treatment	Rest	Pre-EX	50%	65%	75%
RER	PLA	0.85±0.017	0.84±0.017	0.94±0.014	0.98±0.017	1.00±0.020
	Neo40™	0.85±0.022	0.86±0.019	0.94±0.012	0.98±0.015	0.99±0.015
CHO (%)	PLA	49.68±5.871	47.98±5.731	80.08±4.127	87.01±3.589	89.49±3.292
	Neo40™	50.45±6.527	51.68±6.188	79.96±3.736	87.86±3.229	90.20±2.942
FAT (%)	PLA	50.80±5.829	52.48±5.690	20.50±4.136	13.45±3.636	10.88±3.358
	Neo40™	49.96±6.506	48.76±6.174	20.57±3.773	12.61±3.281	10.23±3.001

Note that there were no significant differences in any of the variables tested between treatments during steady state exercise (N=15).

VO_2

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

RER (Respiratory Exchange Ratio)

RER was determined during steady state exercise at 5 time points. There was no significant difference by treatment or treatment-time interaction between trials.

Heart Rate (HR)

Neo40^{TM} reduced heart rate during exercise although there was no significant difference by treatment or treatment by time interaction between trials.

Rating of Perceived Exertion (RPE)

RPE was collected throughout steady state exercise. There was no significant difference between trials. RPE increased throughout the trial regardless of treatment.

Lactate Concentration

There was no significant difference by treatment or treatment-time interaction for lactate concentration throughout both trials. Lactate concentration increased as exercise intensity increased regardless of treatment during exercise. The lactate concentration was higher in male subjects than female subjects (Figure 1). The treatment effect was also more pronounced in male subjects (N=8, P=. 079) than female subjects (N=7, P=. 399).

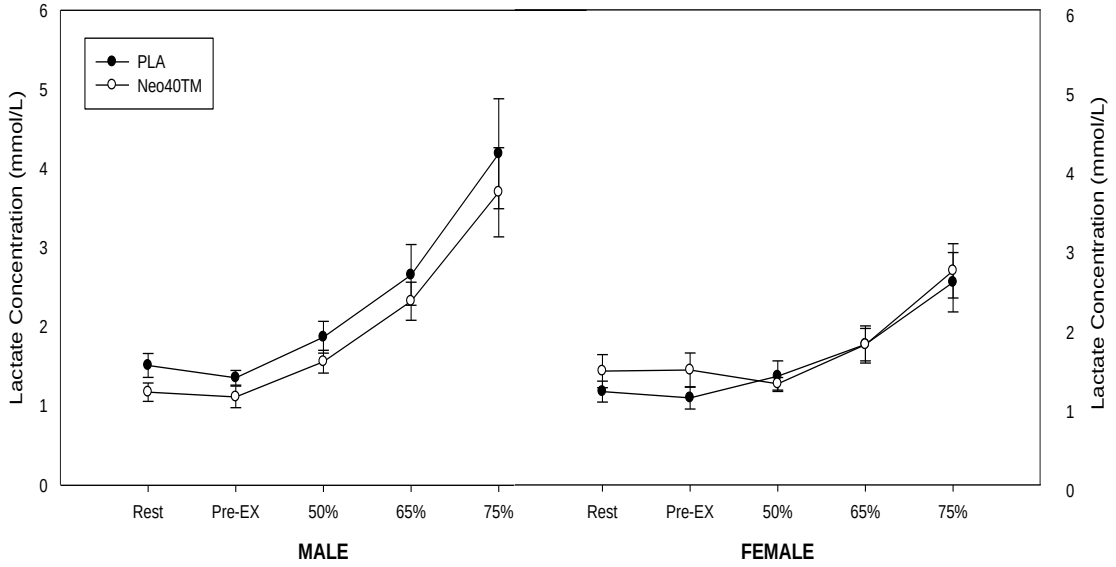

Figure1. Lactate concentration separated by gender during steady state exercise between PLA and Neo40^{TM}. Error bar indicates SEM. No significant difference was found between trial in both male and female subjects (P=. 05).

Time-Trial Performance

Heart rate (HR), rating of perceived exertion (RPE) and completion time were measured at 6km, 10km, and 19.5km through 20.15km of cycling time trial

Table 2.Time-Trial Performance Data (N=15)

	Treatment	6km	10km	19.5km	20.15km
HR (Beat/min)	PLA	162.93	162.33	167.00	N/A
	Neo40^{TM}	162.13	163.40	167.47	N/A
RPE	PLA	14.3	15.4	16.9	N/A
	Neo40^{TM}	14.7	15.2	16.7	N/A
TIME (sec)	PLA	773.47	1228.33	2381.33	2476.88
	Neo40^{TM}	762.00	1208.53	2334.53	2426.37

Note that there was significant difference in time at 20.15km (P=. 010) between PLA and Neo40^{TM}. *Different from PLA, P < 0.05.Values are means ± S.E. You need to indicate where the differences are in the table. I see no asterisk

Time to Complete (TTC)

There were significant treatment and treatment by time effects of Neo40^{TM} on TTC compared to PLA. The average TTC for PLA was 2476.88±78.430 sec and for Neo40^{TM} it was 2424.37±69.167 sec. The percent improvement of TTC was 2.1% (Figure 2). We also analyzed the effect of treatment based on gender (Figure 3). We found a treatment effect in female subjects (N=7.P= .037). Time to complete 6km, 10km, 19.5km and 20.15km was significantly improved by Neo40 among female subjects. However, there was no significant treatment effect detected for male subjects (N=8, P= .123) although the completion time at all time points was reduced when they consumed Neo40^{TM} prior to the cycling time trial.

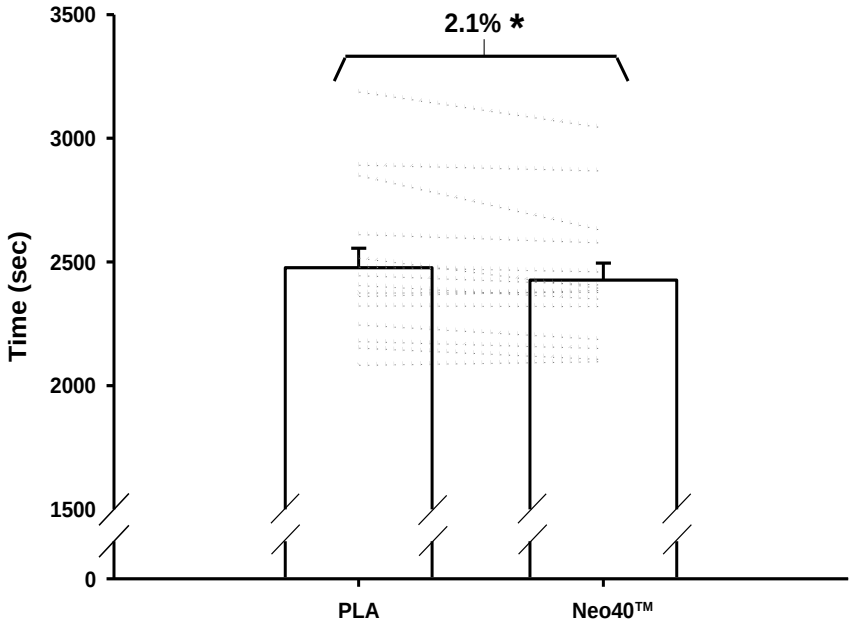

Figure 2. 20.15km time to complete (TTC) with individual data. There was significant difference between treatments (N=15, P= .011). The percent improvement of TTC was 2.1%. Error bar indicates SEM. * indicates significant difference between treatments ($P < 0.05$).

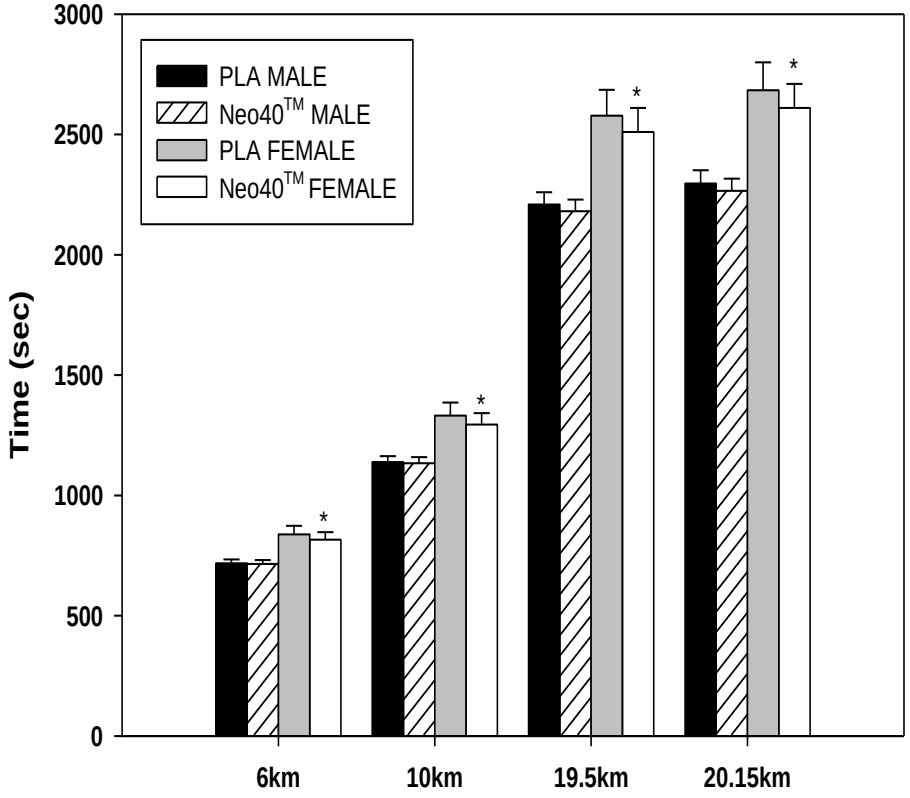

Figure 3.Completion time separated by gender at each time points between treatments. There was significant difference in female subjects (N=7, P =. 037). No difference was observed in male subjects (N=8, P= .123). Error bar indicates SEM. * indicates significantly different between treatments for female subjects.

Rating of Perceived Exertion (RPE)

RPE was collected throughout time trial performance. Although subjects performed better when taking Neo40, there was no significant difference in RPE by treatment. RPE increased throughout the trial regardless of treatment.

Heart Rate (HR)

Heart rate was measured at 6km, 10km and 19.5km during the time trial performance. There was no significant difference by treatment or treatment by time interaction.

Blood Pressure

Blood pressure was measured before and after the study products were given to the subjects. We compared the delta value of PLA and Neo40^{TM} treatment for both systolic and diastolic blood pressure. There were no treatment effects on either systolic or diastolic blood pressure.

DISCUSSION

The effects of Neo40™ on time trial performance

The current study assessed the effect of Neo40™ on physical performance and metabolism at rest and during exercise. The primary finding of this study was that the acute dietary supplementation with Neo40™ enhanced cycling time trial performance among the subjects, which consists of 20.15km compared to placebo (PLA). The average time to complete cycling time trial improved by 2.1% when the subjects consumed Neo40™ about 30 minutes before the time trial. In addition, the participants reported that they experienced improved exertion when they consumed Neo40™ before time trial riding compared to PLA although there was no significant difference in RPE between trials. These results point out that when participants received Neo40™ prior to exercise, they showed enhanced performance during the 20.15km time trial.

Previous studies have reported a longer time to exhaustion during exercise following 4-6 days of dietary nitrate supplementation (Bailey et al., 2010, Lansely et al., 2011). However, it is known that such time to exhaustion protocols do not always equate to improvements in exercise performance in sports that have a set distance to cover. Therefore, to assess the latent ergogenic effects in a more practical setting, we investigated the acute effects on time trial performance about 30 minutes after Neo40™ ingestion among moderately trained cyclists. The current findings are in line with previous research studies that have measured the effects of sodium nitrate or beetroot juice supplementation on exercise performance. Lansley et al. (2011) found that acute dietary nitrate supplementation enhanced 4 and 16.1km TT (cycling time trial) performance in well trained cyclists by 2.8% and 2.7%, respectively when it was measured 2.5 h after intake of a single dose of beetroot

juice (BR) compared with a nitrate-depleted beetroot-juice placebo. They also suggested that the improved TT performance after ingestion of nitrate-rich beetroot juice was due to an improved power output (PO) for the same VO_2 (increased O_2 efficiency). It was also speculated that nitric oxide (NO) improved muscle contractile efficiency as suggested by a lower total ATP turnover and a reduced muscle metabolic perturbation at the same P:O ratio following BR supplementation compared with placebo. Furthermore, Murphy et al. (2011) reported that running velocity for a 5km run was slightly improved after beetroot ingestion as compared to a placebo (12.3±2.7 vs. 11.9±2.6 km/h; P=0.06). This 0.4 km/h (3%) improved velocity resulted in a 41 second faster finishing time. In this study, 11 participants completed two 5km treadmill time trials in random order, once 75 minutes after consuming baked beetroot (200 g with ≥500 mg nitrate) or cranberry relish as a placebo. The average rating of perceived exertion (RPE) for the 5km was not significantly different between trials (beetroot 15.2±1.7; placebo 15.5±1.5; P=0.21). Interestingly, they also found that the treatment effect of beetroot occurred only late in the 5km running trial. This result is fairly consistent with our current study. In our study, the time was determined to reach four distances (5km, 10km, 19.5km and 20.15km). Similarly, to the findings of Murphy et al. (2011), the time difference between trials increased as the cycling distance increased.

There is no clear explanation for this result for the greater response at the end of the time trial. It is possible that nitrite or NO levels continued to rise to the point at which ergogenic effects became more pronounced late in the time trial. Castell et al. (2006) also reported that the reduction of nitrite to nitric oxide was more powerful in a hypoxic and acidic (Modin et al., 2001) environment, when the NO production was restricted by the impaired NOS pathway. Recently Cermak et al. (2012. A) found that 6 days of concentrated dietary nitrate supplementation (140 ml, ~8 mmol/day NO_3^-) enhanced 10 km time-trial

performance in well trained cyclists. In this study, they demonstrated that consuming a small volume of concentrated BR containing 8 mmol of nitrate could induce a similarly effective ergogenic effects compared to a daily consumption of 0.5L of BR. The authors also reported that 0.5 L of BR is not practical for athletes to consume a few hours before the beginning of competition. From this point of view, Neo40^{TM} in a lozenge form is an attractive alternative because the lozenge is simple, does not result in stomach distress, and demonstrates ergogenic effects within 30 minutes.

Even though many researchers have proven the effect of nitrate supplementation on exercise performance, Cermak et al. (2012. B) found that the acute intake of 140 ml of concentrated nitrate-rich (~8.7 mmol NO_3^-) beetroot juice did not induce ergogenic effects during a 1-hr time-trial in well-trained male cyclists. In this study, 140 ml of concentrated BR or a placebo with breakfast was administrated to participants 2.5 h before cycling time trial. The authors assumed that the acute, one-time consumption of concentrated BR was ineffective in raising nitrite levels fast enough to have a significant effect on NO production. Additionally, Wilkerson et al. (2012) found that acute BR juice supplementation did not induce an ergogenic effect during a 50-mile time trial in eight well-trained cyclists. In this study, the authors claimed that less intense time trial might not induce the development of hypoxic and acidic environment in the skeletal muscle and potentially reduce level of nitrite leading reduced NO production. More recently, Bescos et al. (2012) also found that sodium nitrate supplementation did not enhance a 40-minute distance-trial in endurance athletes. The authors suggest that the results can be consequent to well-trained participants who might already have a higher basal concentration of NO or higher nitric oxide synthase (NOS) activity than the moderately trained or untrained participants. However, this explanation is

refuted by other investigators (Lansely et al., 2011, Cermak et al., 2012 A) who found an ergogenic effect of supplementation with a nitric oxide donor on well-trained participants.

In present study, we started the time-trial exercise about 30 minutes after they consumed Neo40^{TM}. In previous studies, nitrate supplementation occurred after 75 minutes of consumption. With Neo40^{TM} the effect on time-trial exercise occurred in 30 minutes or twice as fast as in previous studies. Of the research conducted so far, the current study is showing the fastest acute effect on time-trial performance. It is possible, however, that other micronutrients that are included in Neo40^{TM} could help increase the effectiveness of the product. Of the other ingredients, except for the 420 mg nitric oxide blend consisting mainly of beetroot powder, the 75mg of caffeine added could have had an effect on time-trial performance. In this regard, Pasman et al. (1995) reported that intake of as low as 3 mg/kg body weight provided 1 hour before exercise was found to be helpful in increasing exercise time to exhaustion at exercise intensities of 70 to 80% VO_{2MAX}. Additionally, Desbrow et al. (2011) reported that 3 mg/kg of caffeine ingested 90 minutes before time trial riding showed a 4.2% improvement on cycling performance in well trained cyclists. Given that the average body weight of the 15 participants was 73.8 kg, the addition of 75mg of caffeine is not likely to induce an ergogenic effects by itself within a shorter time (30min). However, this does not rule out the possibility that 75 mg of caffeine might act synergistically with Neo40^{TM} to positively affect the physiological responses to exercise after Neo40^{TM} supplementation.

We also analyzed the effects of Neo40^{TM} on time to complete by gender (Figure 2) and the treatment effect was significant in female subjects at all time points (P=0.037), but not in male subjects (P=0.123). Although we did not measure the level of nitrite or NO concentration, it is possible that the increase in a level of nitrite or NO concentration after Neo40^{TM} supplementation might be higher in female subjects than in male subjects allowing

completion time in females to be faster. It is widely accepted that estrogen regulates vascular sensitivity to stimuli evoking flow-induced vasodilation, through improved release of vasodilatory signaling molecules such as nitric oxide, prostacyclin and endothelial-derived hyperpolarizing factors from the vascular endothelium (Huang et al., 1998; Jokela et al., 2003; Mazmudar et al., 2000). However, it is unclear that the estrogen has a lasting vasodilatory effect during exercise because numerous vascular regulatory mechanisms are utilized at the same time during exercise to maintain adequate O_2 delivery to working muscles. In addition Kapil et al. (2010) also reported that plasma nitrite concentration was notably higher in the females although baseline plasma nitrate concentration was comparable between genders after nitrate supplementation. In this study, about 2-fold higher plasma nitrite was found despite a similar dietary nitrate amount. The authors also assumed that different amount of lingual bacteria or species resulting in nitrate reduction to nitrite may lead to the difference in nitrite level between genders. However, the gender differences in colonization of the tongue or the activity of nitrate reductase are currently unknown.

The effect of Neo40^{TM} on steady state exercise

Previous studies indicate that 3-15 days of dietary nitrate supplementation lowers average VO_2 during submaximal exercise (Bailey et al., 2010, Lansely et al., 2011, Larsen et al., 2007, Vanhatalo et al., 2010). However, we did not find this effect of Neo40^{TM} on O_2 cost during 20 minutes of steady state exercise. It is possible that there was insufficient time before exercise to induce reduction of nitrate to nitrite and consequently to bioactive NO. That is, the nitrate loading time in current study was likely too short to stimulate ergogenic effects of Neo40^{TM} compared to previous studies that loaded nitrate for 3-15 days before testing.

We also did not find a significant difference in blood lactate concentration between treatments. Interestingly, however, the average of lactate concentration was lower in female subjects than in male subjects although it was not significantly different. It is well established that the blood lactate accumulation is normally linked to reduced fat utilization (Issekuts et al., 1975). Tarnopolsky et al. (1990) reported that during a 90 minute run at 65% of VO_{2max}, females had extensively lower RER values compared with males, representing an increased fat utilization as fuel. In addition, Horton et al. (1998) confirmed a greater fat oxidation in females than in males at relatively moderate intensities of exercise. Ruby et al. (1997) also reported that estrogen plays a significant role in increasing growth hormone concentration, which is known to stimulate lipoysis and to suppress glycogenolyic activity by reducing plasma epinephrine secretion. In the current study, we also found a lower average RER in female subjects compared to male subjects during both submaximal exercise intensities (0.89±0.017 VS 0.95±0.019 in PLA, 0.92±0.027 VS 0.93±0.017 in Neo40^{TM}). These results also suggest the female subjects showed greater fat oxidation than in male subjects. Therefore, the lower lactate concentration found in female subjects in this study is likely due to the greater fat utilization of female subjects as fuel during steady state exercise.

The effect of Neo40^{TM} on Blood Pressure

Blood pressure measurements were made before consumption of supplements and 5 minutes after consumption. However, unlike the previous study (Vanhatalo et al., 2010) that reported nitrate supplementation lowers blood pressure, we did not find a treatment effect. However, given that the previous studies showed blood pressure lowering effects occur after several days of nitrate loading (Larsen et al., 2007) or did not observe treatment effects until

2.5 to 3.0 hours after acute nitrate loading (Webb et al., 2008), it is possible that there was insufficient time for the Neo40^{TM} to induce a blood pressure lowering effect.

Summary

In conclusion, dietary supplementation with Neo40^{TM} enhanced time-trial performance by 2.1% in 15 moderately trained cyclists when consumed Neo40^{TM} about 30 minute before the start of the time trial. This result may be attributed to increased level of nitrite or NO after Neo40^{TM} supplementation. To the best of our knowledge, this represents the fastest acute effects on time trial performance for a nitric oxide synthesizer. Unlike previous studies, however, Neo40^{TM} supplementation had no effects on blood pressure or VO_2 during rest or steady state exercise, respectively. It is possible that the nitrate loading time in the current study was too short to elicit early effects on blood pressure and VO_2 compared as previous studies loaded nitrate for 2.5 h before testing to observe these effects. In addition, ingestion of 0.5 L BR seems to be impractical for athletes to consume a few hours before the beginning of competition. From this point of view, Neo40^{TM} in a lozenge form is an attractive alternative because the lozenge is simple, does not result in stomach distress, and demonstrates ergogenic effects within 30 minutes.

LITERATURE REVIEW

Introduction

In Humans, nitric oxide is a very important cellular signaling molecule involved in many physiological and pathological processes. Since three scientists won the Nobel Peace Prize in 1998 for discovering nitric oxide's (NO) role in cell signaling, many researchers have investigated its various roles in the body. One major mechanism of nitric oxide formation is the metabolism of the amino acid L-arginine, which is catalyzed by nitric oxide synthase (NOS). There are three types of NOS known: nNOS, used for neuronal communication in both the peripheral and central nervous systems, iNOS, found in macrophage and generating NO for cellular defense, and eNOS, located in vascular endothelial cells and generating NO for maintenance of vascular tone. Each of these types of NOS produce NO from arginine with the aid of molecular oxygen and nicotinamide adenine dinucleotide phosphate. (NADPH) Dietary nitrate and nitrite also can be sources for production of NO via nitrate/nitrite reductase in tissues. Inorganic nitrate from dietary sources can be converted into nitrite in vivo and nitrite can be reduced in vivo to NO and other bioactive nitrogen compounds.

Aside from its effects on the nervous, immune and vascular systems, NO is known to interacts with mitochondria to regulate cell respiration and to augment the generation of reactive oxygen species, thus triggering mechanisms of cell survival or death. There is also information linking NO to stimulation of muscle glucose uptake that appears to work independently of insulin. This review will focus on these roles of the NO in the human body.

Effects of NO in Human Body

Vasodilator

NO initiates and maintains vasodilation through a cascade of biological events that end in the relaxation of smooth muscle cells that line arteries, veins, and lymphatics. While somewhat complicated, the sequence of biological events that are caused by NO are largely divided into 4 steps. First, NO gas released from nitrosothiols in hemoglobin or from endothelial cells, diffuses into smooth muscle cells that line small blood vessels. Once inside the smooth muscle cell, NO binds to the enzyme guanylate cyclase (GC) and this results in GC activation. Activated GC is able to split two phosphate groups from another compound guanosine triphosphate (GTP), which results in the formation of cyclic guanosine monophosphate (cGMP).

cGMP induces smooth muscle relaxation by multiple mechanisms.

A rise in cGMP causes the Ca2+ pump (SERCA) to sequester intracellular Ca2+ stores. This reduced Ca2+ concentration in the cytoplasm leads to vasodilation in the smooth muscle. In addition, cGMP stimulates cGMP dependent protein kinase, which stimulates myosin light chain phosphatase that dephosphorylates myosin light chains. This then causes smooth muscle relaxation (Cohen et al., 1998). However, only a limited number of GC enzymes are present in any one smooth muscle cell and once all the GC enzymes have been activated, additional NO will not initiate any further vasodilation. Any "extra" NO is simply sequestered as a nitrosothiol bound to hemoglobin in red blood cells for future use. To return the blood vessels to their normal diameter the phosphate groups bound to myosin in the smooth muscle cells must be removed.

Yu et al (2002) claim that the vasodilator response to NO in rat middle cerebral artery (MCA) is mediated by activation of Ca^{2+}-activated K^{+} channels via a cGMP-

independent pathway and that cGMP also contributes to the vasodilator response to NO by decreasing the contractile response to elevations in $[Ca^{2+}]_{i.}$ in various other isolated preparations and animal models. NO has been shown to have potentially profound effects on local and systemic circulatory control mechanisms (Dinerman et al.1997). However, human studies performed to date suggest a more modest role for NO in the overall circulatory control scheme and in the vasodilator responses to various forms of physical and mental stress (Halliwill et al. 1996). These modest responses reinforce the general concept that multiple redundant regulatory systems operate at a variety of levels to control the circulatory system in humans. In this context, NO is a new piece of the puzzle for those attempting to gain an integrative understanding of human cardiovascular regulation.

Blood Flow

NO relaxes the smooth muscle in the walls of the arterioles. At each systole, the endothelial cells that line the blood vessels release a puff of NO. This diffuses into the underlying smooth muscle cells causing them to relax and thus permit the surge of blood to pass through easily. For decades, NO was known to be involved in the microvascular response to muscle contraction, hypoxia, and the reflex sympathetic discharge in both resting and exercising muscle. Low-intensity electrical stimulation of the lumbar chain produced a striking hindlimb vasodilation that was markedly diminished by pretreatment with the specific inhibitor of nNOS, 7- nitroindazole, thus implicating NO in this microvascular response (Davisson et al. 1997).

Another study found that inhibition of NOS reduced the hyperemic responses to a brief mechanical stimulus and to a single muscle contraction. (Brock et al.1998). In addition to these studies, Thomas and co-workers showed that nNOS mediates the metabolic

inhibition of sympathetic vasoconstriction. In particular, they revealed that α-2 but not α-1 adrenergic vasoconstriction is inhibited during contraction of fast twitch gastrocnemius muscle that express nNOS, but not in the slow twitch muscle that are NOS deficient. In conclusion, these results demonstrate that NO-mediated inhibition of sympathetic vasoconstriction is one important mechanism underlying functional sympatholysis, which can cause increase in blood flow.

Mitochondria Respiration

For the past two decades, many researchers have investigated NO and its effects on mitochondria respiration. In general, NO and its derivative peroxynitrite (ONOO-) inhibit mitochondrial respiration by distinct mechanisms. Low (nanomolar) concentrations of NO specifically inhibit cytochrome oxidase in competition with oxygen, and this inhibition is fully reversible when NO is removed. Higher concentrations of NO can inhibit the other respiratory chain complexes, probably by nitrosylating or oxidizing protein thiols and removing iron from the iron-sulphur centers (Brown, 1999).

Mason et al (2005) found that at least in vitro, both forms of inhibition my occur simultaneously, but the binding to reduced cytochrome α-3 is favored at high levels of cytochrome reduction and low oxygen, whereas the interaction of NO with oxidized CuB2+ is favored by the opposite condition. However, the inhibition observed in cells in response to NO or expression of inducible NO synthase (iNOS or NOS2) appears to be largely competitive with oxygen and reversible by light, suggesting that inhibition due to reversible binding to heme a_3^{2+} may be more important in cells.

In addition to NO effects on mitochondria respiration, NO also plays an important role in antiapoptotic reactions. Brooks et al. (2000) investigated the relationship between

cytochrome C release, PTP (permeability transition pore) opening and the effects of NO, and found that NO reversibly inhibited PTP opening at physiological levels by mitochondrial membrane depolarization and inhibition of Ca2+ accumulation. Furthermore NO exposure resulted in significantly lower cytochrome C release for the same degree of PTP opening. With regard to the above effects of NO, mitochondria contains their own nitric oxide synthase (mtNOS) and can therefore produce is own NO. The mtNOS enzyme has the same cofactor and substrate requirements as other constitutive nitric-oxide synthases. Even though this type of NO is known to modulate oxygen gradients in tissues, apoptosis, and protein nitrative/oxidative stress, other researchers still question the effects of mtNOS.

Immune response

The role of nitric oxide in immunology was first investigated between 1985 and 1990. Then, the role of NO was simply defined as "a product of macrophages activated by cytokines, microbial compounds or both, derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS or NOS2), and that functions as a tumoricidal and antimicrobial molecule in vitro and in vivo." (Nathan. 1992).

However, more recent findings added a few new facts to the role of NO on immune response. First, it was discovered that a lot more immune-system cells produce and respond to NO other than macrophages. Second, all known isoforms - nNOS, iNOS, and eNOS- of NO synthase (NOS) were found to operate in the immune system, contrary to previous belief. Third, the activity of NO is not restricted to the site that it is produced. Fourth, unlike cytokines, NO interaction is not restricted to a single receptor, but rather can react with lots of other inorganic molecules, such as oxygen or transition metals, structures in DNA, prosthetic groups (heme), or proteins (Bogdan. 2001). It is now known that NO plays a much more

diverse role in infection than was first suggested. Previously accepted facts that NO is produced at high levels from iNOS and that it has host-protective effects and tissue damaging effects are evidently oversimplifications. Now it is known that iNOS is detrimental in some infectious diseases and it counteracts immune d-signaling molecules shaping the immune response.

Furthermore, iNOS, nNOS and eNOS are now believed to be involved in immunological processes such as apoptosis, cell adhesion, autoimmunity and antimicrobial defense. Despite the important role NO plays in human immune response, the regulation, expression, and function of the NOS isoforms are so complex that therapies using NO against infectious, autoimmune or malignant diseases are not easy to design.

Although NO frequently is an important mediator in intracellular inhibition or viral replication, which in turn produces lower viral yields and more efficient host clearance of infection, there is no clear way of telling whether NO will have a role in viral clearance or pathogenesis. (Staeheli.1990) Furthermore, many pathogens are not inhibited by NO, and NO may be harmful to certain tissues if substantial amounts of macrophages are activated. A result of such detrimental events could lead to Borna disease (Akaike. 1995) or HSV-1 pneumonia (Adler et al. 1997). However, in the case of viral encephalitis "due to infection with picornaviruses, rhabdoviruses, HSV-1, or JEV, for instance," activation of NOS-1 may be lifesaving. (Reiss et al. 1998)

Glucose Transport

In the skeletal muscle, in particular, two out of the three members of the NOS families are expressed: nNOS and eNOS. (Kobzic et al. 1994) There are two distinct pathways that stimulate glucose transport in muscle. One pathway is stimulated by insulin

and insulin like growth factor-1 and requires the activation of phosphoinositide 3-kinase (PI3K) for activation of glucose transport. Another pathway is stimulated by contraction/hypoxia, and is PI3K independent. In 1997, Balon and Nadler (1997) suggested NO as a potential mediator of the exercise-induced glucose transport. In their research, they found that the effects of sodium nitroprusside (SNP), an exogenous NO donor, and insulin had an additive effect on the transport of 2-deoxyglucose (2-DG). However, the mechanism by which NO increases glucose transport has not been elucidated.

Higaki et al (2001) found two important discoveries related to NO and glucose transport. First, the NO-stimulated glucose uptake is independent of the mechanism in which insulin and muscle contraction increase glucose transport. In this study, they observed an additive effect of SNP plus insulin on skeletal muscle glucose uptake and found that NO stimulated transport is partially blocked by the PI3K inhibitor, wortmannin. In addition, they found that there was full additivity on skeletal muscle glucose uptake when they combined SNP with electrical contraction. Together, these findings demonstrated the NO signaling pathway is insulin and contraction independent.

Second, NO-stimulated glucose transport is associated with the activation of 5'AMP-activated protein kinase (AMPK). However, they also reported activation of the α-2 catalytic subunit of AMPK, which is required for contraction induced glucose transport was not required for activation by NO. This finding also supports the argument that NO-glucose transport signaling pathway has a distinct mechanism.

More recently, research has suggested an increase in intracellular calcium, increases in reactive oxygen species (ROS), the activation of AMPK, or the combination of any of the above factors as a possible cause for the activation of NOS during exercise. It has also been suggested that the cGMP second messenger system is involved. This is supported by the

finding that NO production and the skeletal muscle glucose transport are decreased in the presence of LY83583, an inhibitor of soluble guanylate cyclase, which decreases skeletal muscle cGMP content. However, the exact mechanism by which NO increases skeletal muscle glucose uptake has not been clearly defined (McCornell et al. 2006).

NO not only has an effect on skeletal muscle glucose uptake, but also appears to increase smooth muscle glucose uptake. Bergandi et al (2003) revealed that human vascular smooth muscle cell glucose transport is mediated by NO. Using the knowledge that insulin increases GLUT-4 translocation to membranes, researchers investigated whether an increased level of NO had effects on the glucose uptake when insulin-stimulated GLUT-4 recruitment was blocked. In this study, they found that insulin stimulated glucose uptake was blocked by an inhibitor of NO synthesis and mimicked by NO releasing drugs. Glucose uptake elicited by combination of insulin and NO was blocked by inhibitors of soluble guanylate cyclase and cGMP dependent protein kinase.

In addition, glucose transport was stimulated by an analog of cGMP. Therefore, these findings provide strong evidence that insulin induced glucose transport in human vascular smooth muscle cells is mediated by an increased synthesis of NO, which stimulates the production of cGMP and the subsequent activation of a cGMP dependent protein kinase. In conclusion, NO seems to be involved in glucose transport in both skeletal muscles and smooth muscle.

The role of NO during exercise

Accumulating data suggests that nitric oxide (NO) has an important role in both coronary and peripheral hemodynamic control and metabolic regulation during exercise. Although there is controversy surrounding the mechanisms controlling coronary vasodilation

during exercise, it is well established that hyperpolarization of vascular smooth muscle cell membranes brought about by opening of K^+ATP channels is very important for metabolic coronary vasodilation. (Nichols et al. 1991) Blockade of vascular smooth muscle K^+ATP channels in chronically instrumented dogs, however, did not attenuate the coronary blood flow response to exercise (Duncker et al. 1993)), although both adenosine and K^+ATP channel blockade reduced the exercise-induced increase in coronary flow by half. Ishibashi et al. (1998) also found that coronary flow was reduced to levels below that of resting control conditions when NOS blockade was added to adenosine and K+ ATP channel blockade.

However, NOS inhibition alone or combined with adenosine receptor blockade did not influence either resting or exercise coronary flow, indicating that when K^+ATP channels are intact, neither NO nor adenosine-dependent mechanisms are obligatory for maintaining coronary flow. Thus, all three systems are important for coronary vasodilation during exercise. Therefore, like metabolic skeletal muscle, vasodilation in the coronary system has substantial redundancy. In addition, the mechanisms controlling skeletal muscle blood flow during exercise are complex and involve neural, metabolic, endothelial, myogenic, and muscle pump control. These mechanisms control blood flow via effects on perfusion pressure and the caliber of resistance vessels.

Traditionally, vessel caliber has been thought to indicate a balance between vasodilation mediated directly by production of metabolites from the exercising muscle and sympathetic activation via muscle metabo- and mechanoreceptor stimulation. NO derived from both the endothelium (endothelial NOS, type III) and skeletal muscle (neuronal NOS, type I) may, however, play an important role in matching tissue perfusion to demand. In support of this argument, Roberts et al. have recently shown that a 45 min exhaustive exercise bout increases both neuronal and endothelial NOS activity in rats. (Roberts et al.

1999) This activated NOS in active skeletal muscle appears to regulate muscle contraction and metabolism during exercise. In particular, recent human data indicate that NO plays a significant role in muscle glucose uptake during exercise independently of blood flow. The study using rat isolated muscle and sarcolemmal vesicular preparations also indicate that NO-mediated glucose uptake occurs during exercise. (Bradley et al. 1999)

The effect of NO on metabolism

It is known that NO is released from skeletal muscles and a great body of evidence has shown that NO plays a critical role in glucose metabolism. The administration of NO synthase (NOS) inhibitors induces remarkable insulin resistance (Baron et al. 1995). NO donors stimulate glucose transport in skeletal muscles (Young et al. 1997), and endothelial nitric oxide synthase (eNOS) knockout mice have insulin resistance (Duplain et al. 2001). Thus, NO seems to be an important modulator of glucose metabolism. The NO-stimulated glucose uptake is not inhibited by wortmannin, an inhibitor of phophatidylinositol 3 kinase (PI3K). PI3K is a downstream effector for the insulin receptor, and this effect of NO appears to be independent of the insulin-signaling pathway. It has been proposed that NO mediates contraction-stimulate glucose transport in skeletal muscles. (Etgen et al. 1997)

However, in a recent study, NOS inhibitors fail to affect contraction stimulated glucose transport in isolated muscle, suggesting that NO is not involved in the signaling pathway of contraction stimulated glucose uptake and that NO increases skeletal muscle glucose uptake through a mechanism distinct from the insulin and contraction signaling pathways. Bradley et al. (1999) have provided evidence that NO-mediated glucose uptake during exercise is important in humans. These investigators infused L-NMMA, the NOS inhibitor, into the femoral artery during cycling. They found glucose uptake was reduced by

48% compared with a control saline infusion. Importantly, this effect occurred in the absence of any changes in blood flow, suggesting that l-NMMA directly affected the ability of skeletal muscle to extract glucose from the blood. However, the exact mechanism for this effect has not been fully elucidated.

The effect of NO on Exercise performance

NO has been suggested to play an important role in acute adaptation to physical exercise by modulating blood flow, muscular contraction, glucose uptake, glycolysis, and cellular respiration. It has been assumed that NO is involved in endothelium mediated vasodilatation and that this is one of the regulatory mechanisms by which substrate supply to working muscles is increased, thus allowing prolonged exercise.

Conventionally, many researchers have investigated the effects of NO on exercise in terms of blood flow. Hirai et al. (1994) found that NO release plays an important role in regulating blood flow to working skeletal muscle in the conscious exercise rat. In this study, they administered L-NAME to rats and found that NOS inhibition by L-NAME resulted in a relative vasoconstriction of the resistance vessels in skeletal muscles that normally dilate during exercise. In addition, they also found the magnitude of the exercise induced hyperemia in skeletal muscle is greatly influenced by NO synthesis and release, in particular, muscles containing a high percentage of oxidative fibers. These blood flow differences among muscles during exercise may relate to differences in microvascular architecture proximal and distal to the capillary bed, in addition to dissimilar vasomotor tone elicited by L-NAME.

Aside from the effects of NO on blood flow during exercise, Chowdhary et al. (2004) observed that NO exerts a powerful facilitating influence on indirect but not direct human cardiac parasympathetic control. Stimulation of the nitric oxide pathway might thus enhance

parasympathetic protection against the adverse influences of cardiac sympathetic over activity. This seems to support the effect of NO on reduction in heart rate during exercise in human subjects. Decreased plasma lactate levels after L- arginine supplementation, a NO precursor, also has positive effects on exercise performance leading heart failure subjects to lower muscular peripheral stress. This decreased plasma lactate level appears to be attributed to improved muscular vasodilation, secretion of endogenous insulin and better O_2 delivery by L-arginine supplementation. However, this last effect may be questioned since oxygen consumption is not increased for standardized exercise intensity.

Recently, many researchers have focused on the effects of NO on specific exercise performance. They have tried to find the effects of NO donors on exercise performance from various perspectives. Since the positive effects of NO on exercise performance were first observed, there have been a number of studies on the acute and chronic effects of NO on exercise performance conducted . Vanhatalo et al. (2010) studied the acute (2.5 h) and chronic (up to 15 days) effects of dietary nitrate supplementation on the O_2 cost of moderate-intensity constant-work rate cycle exercise, and on the GET (Gas Exchange Threshold), VO_2max, and peak work rate measured during a ramp incremental test to exhaustion. They found a lower O_2 cost of moderate-intensity exercise measured 2.5 h after the first ingestion of organic beetroot juice (BJ) and that this was maintained when supplementation was continued for 15 days. The significantly elevated plasma [NO_2^-] throughout the 15 day dietary nitrate supplementation period was accompanied by reduced systolic and diastolic BP, and these effects tended to be particularly pronounced after 12 days of supplementation. The mechanistic basis for reduced O_2 cost of exercise observed following dietary nitrate supplementation remains unclear.

However, these investigators recently reported that dietary nitrate supplementation

reduced the muscle ATP turnover rate for the same external work rate, and that nitrate supplementation resulted in reduced phosphocreatine degradation and reduced accumulation of adenosine diphosphate and inorganic phosphate for a standardized submaximal work rate. These changes would be expected to reduce the stimuli to oxidative phosphorylation and also to reduce the rate of muscle fatigue development.

In contrast to the acute effects of nitrate supplementation on VO_2 and blood pressure, they found that the peak power output achieved at the limit of tolerance in the incremental test was not significantly altered at 2.5 h post-ingestion or after 5 days of supplementation, but was elevated after 15 days. Additionally, relative to baseline, the VO_{2max} was increased by 140 ml/min after 15 days of nitrate supplementation. The gradual increase in VO_{2max} over the 15-day nitrate supplementation period could be linked to increased mitochondrial mass as a consequence of elevated NO availability (Clementi, et al, 2005). Chronic exposure of mammalian cells to NO has been shown to result in cGMP-mediated activation of regulatory protein sirtuin (SIRT1), which upregulates transcriptional factors and nuclear respiratory factors involved in the coordination of mitochondrial fusion and fission events (Kelly et al, 2004). However, whether NO-cGMP-induced mitochondrial biogenesis is manifested in human skeletal muscle in vivo following dietary nitrate intervention remains to be determined.

Lansley et al. (2011) found that acute dietary nitrate supplementation improved 4- and 16.1-km cycling time trial (TT) performance in competitive cyclists by 2.8% and 2.7%, respectively. The improved TT performance after ingestion of nitrate-rich BJ was consequent to a significantly greater power output (PO) for the same $\dot{V}O_2$(increased O_2 efficiency). Even though the precise mechanism is unclear, the authors suggested the possibility that NO induced improvement in muscle contractile efficiency as evidenced by a reduced total ATP turnover and a reduced muscle metabolic perturbation for the same P:O ratio after BR

supplementation. In this study, the authors found a reduced total ATP turnover and a reduced muscle metabolic perturbation for the same P:O after BR supplementation.

Moreover, Cermak et al. (2012) found that 6 days of chronic dietary nitrate supplementation in the form of BJ (~0.5 L/d) reduced pulmonary oxygen uptake during submaximal exercise and increased tolerance to high-intensity work rates, suggesting that nitrate can be a potent ergogenic aid. They also suggested that the mechanism responsible for a lower VO_2 during exercise is likely related to the role of nitrite and NO as regulators of cellular O_2 utilization. It has been suggested that NO may improve the efficiency of oxidative phosphorylation by reducing failure of the mitochondrial proton pumps or attenuating the expression of uncoupling proteins, reducing the total ATP cost of muscle force production, thereby improving metabolic efficiency.

In general, it is likely that both acute or chronic nitric oxide supplementation have positive effects on exercise performance based on the recent trend in NO donor research. However, more studies are required to elucidate the mechanistic basis for both acute and chronic effects of NO donors on exercise performance.

Researchers have also focused their attention on the effects of nitrate supplementation on exercise performance in terms of the training status of subjects. Currently, it is known that the exercise response is different in highly trained athletes compared with the untrained population. Chronic exercise training induces improvements in vascular structures, muscle tissues, and the metabolism of NO. In this context, training level may alter the physiological response to NO possible due to changes in mitochondrial volume and aerobic capacity in type II fibers, which are increased greatly in endurance athletes.

Bescos et al (2011) suggested that the effects of acute nitrate supplementation at low to moderate intensities of exercise might be more limited in endurance-trained athletes than

in moderately trained subjects. This is supported by the fact that decreases in submaximal oxygen uptake after endurance training may be due to changes in the working muscle's oxidative capacity and metabolic processes, represented by an increase in the activity of the mitochondrial enzymes. They also found that the VO_2 peak was significantly reduced when athletes ingested nitrate supplement at maximal intensity of exercise. These in vivo results were found without any changes in cardiorespiratory and performance parameters, which suggests that nitrate and its reaction products could play an important role in oxygen consumption at maximal intensity of exercise in well-trained athletes.

It is known that the nitrate–nitrite–NO pathway is gradually activated as the oxygen supply is limited and nitrite is converted to NO under hypoxic and acidic conditions. Interestingly, in this study, plasma nitrite levels decreased significantly just after finishing maximal workload, suggesting activation of nitrate–nitrite–NO pathway. However, more recently, Bescos et al. (2012) found that sodium nitrate supplementation did not improve a 40-minute distance-trial performance in endurance athletes. In addition, the concentration of plasma endothelin-1 increased significantly after exercise following supplementation with sodium nitrate. It has been reported that the NO production pathway and the ET-1 production pathway cross talk. NO inhibits the production of ET-1 in the vascular endothelium. Therefore, this result suggests nitrate supplement does not have any positive effect on exercise performance in highly trained athletes.

Moreover, Tsung-Han Liu et al. (2008) found that there was no significant difference between an arginine trial (6g/day arginine) and CON trial in plasma nitrate, nitrite, lactate and ammonia concentrations and peak and average power during a standardized exercise. The results of this study suggested that short-term arginine supplementation had no effect on nitric oxide production, lactate nor ammonia metabolism and performance in intermittent

anaerobic exercise in well-trained male athletes. According to the author, the results can be attributed to well-trained subjects who might already have higher basal concentration of NO than the general population. Aside from the concentration of NO, the concentration of lactate and ammonia were not altered in this study. It is also suggested that the large amounts of lactate and ammonia generated during the relatively short period of time by athletes may exceed the potential effect of arginine in removing these two metabolites.

Summary

In conclusion, NO has been investigated extensively since its positive effect on exercise were first disclosed. However, the results have shown disparities due to different experimental methods, fitness level of subjects and mechanisms to elevate NO in the body. In addition, the mechanistic bases for improved exercise efficiency and performance have not been clearly revealed to date. For the potential use of dietary nitrate in enhancing training adaptations in athletic populations and improving cardiovascular health within the wider population, future studies are required.

APPENDIX A: DESCRIPTIVE AND INFERENTIAL STATISTIC

Table 1: participant Characteristics

	Female(N=7)		Male(N=8)		Total(N=15)	
	Mean	SEM	Mean	SEM	Mean	SEM
Age	41.14	1.388	34.00	4.280	37.33	2.486
Height(cm)	164.84	2.388	181.75	2.128	173.86	2.726
VO2max(ml/min)	2640.00	146.986	3722.50	149.173	3217.33	176.315
VO2max(ml/kg/min)	40.16	1.527	46.71	2.533	43.65	1.715
Mass(kg)	65.84	3.299	80.73	2.775	73.78	2.858

Table 2: Time to Complete (Seconds)

N=15	Mean	SEM
Neo40^{TM}	2426.37*	78.430
PLA	2476.88	69.317
* Effect found for treatment (P<.05)		

Table 3: Time to complete by Gender (Second)

	Female		Male	
	Mean	SEM	Mean	SEM
Time_6km_PLA	837.57	35.878	717.375	16.437
Time_10km_PLA	1331.14	54.341	1138.375	24.599
Time_19km_PLA	2578.14	107.680	2209.125	50.330
TTC_PLA	2684.06	115.352	2295.596	55.024
Time_6km_ Neo40™	815.57	31.480	715.125	16.429
Time_10km_Neo40™	1294.57	47.059	1133.25	25.211
Time_19km_Neo40™	2509.86	95.964	2181.125	47.731
TTC_ Neo40™	2610.09	99.672	2265.615	50.778

Table 4: Delta VO2 Data (L/min)

Delta Value	Mean	SEM
VO2 50% - Rest PLA	1.36	0.083
VO2 50% - Rest Neo40™	1.35	0.078
VO2 65% - Rest PLA	1.73	0.107
VO2 65% - Rest Neo40™	1.74	0.107
VO2 75% - Rest PLA	2.07	0.145
VO2 75% - Rest Neo40™	2.03	0.131

Table 5: Lactate Concentration by Gender (mmol/L)

	Female(N=7)		Male(N=8)		Total(N=15)	
Rest_PLA	1.25	0.132	1.51	0.150	1.39	0.103
Rest_Neo40™	1.17	0.141	1.36	0.093	1.27	0.083
PreEx_PLA	1.45	0.193	1.87	0.201	1.67	0.146
PreEx_Neo40™	1.85	0.235	2.66	0.384	2.28	0.249
50_PLA	2.63	0.375	4.19	0.694	3.46	0.447
50_Neo40™	1.51	0.209	1.18	0.116	1.33	0.119
65_PLA	1.52	0.216	1.12	0.137	1.31	0.132
65_Neo40™	1.35	0.080	1.56	0.144	1.46	0.087
75_PLA	1.84	0.205	2.32	0.240	2.10	0.167
75_Neo40™	2.78	0.343	3.70	0.563	3.27	0.351

Table 6: Blood Pressure (mmHg)

	Mean	SEM
BP_Rest_Sys_PLA	116.07	4.312
BP_PreEx_Sys_PLA	115.00	3.006
BP_Rest_Sys_ Neo40™	114.07	3.371
BP_PreEx_Sys_Neo40™	113.20	2.569

APPENDIX B: RAW DATA

Table 7: Participant Characteristics

Participant	Age	Height(cm)	Mass(kg)	VO2max(ml/min)	VO2max(ml/kg/min)
F02	42	169.4	72.1	2930	40.7
F03	39	166.0	62.5	2850	45.5
F04	45	159.0	61.0	2370	38.8
F05	34	164.0	74.1	3170	42.7
F06	42	176.0	77.9	2710	34.7
F07	44	158.0	57.3	2030	35.5
F08	42	161.5	56.0	2420	43.2
M01	50	185.0	91.6	4270	46.6
M02	48	173.0	85.3	3060	35.8
M03	27	175.0	67.5	3440	51.9
M04	47	184.0	86.8	3360	38.8
M05	21	183.0	82.7	3800	46.6
M06	25	183.0	80.9	3800	46.9
M08	29	179.0	78.8	3800	48.2
M09	25	192.0	72.2	4250	58.9

Table 8: Time to complete (second)

Participant	Neo40™	PLA
F02	2629.32	2851.91
F03	2352.45	2408.58
F04	3045.12	3190.80
F05	2381.16	2378.58
F06	2410.74	2448.66
F07	2871.30	2895.00
F08	2580.54	2614.89
M01	2189.58	2249.04
M02	2323.92	2326.14
M03	2389.26	2364.06
M04	2395.38	2519.34
M05	2463.00	2483.04
M06	2153.64	2181.87
M08	2100.78	2085.90
M09	2109.36	2155.38

Table 9: Completion Time during Time-Trial (second)

	6km		10km		19.5km		20.15km	
	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™
F02	886	837	1400	1306	2723	2524	2723	2524
F03	744	726	1208	1176	2324	2268	2324	2268
F04	1001	953	1567	1491	3052	2930	3052	2930
F05	741	728	1188	1169	2290	2286	2290	2286
F06	775	769	1206	1200	2359	2314	2359	2314
F07	891	882	1440	1419	2783	2760	2783	2760
F08	825	814	1309	1301	2516	2487	2516	2487
M01	720	705	1130	1102	2167	2114	2167	2114
M02	730	730	1160	1153	2246	2234	2246	2234
M03	750	766	1188	1211	2274	2298	2274	2298
M04	750	762	1193	1197	2404	2302	2404	2302
M05	788	762	1242	1221	2384	2368	2384	2368
M06	670	676	1080	1078	2101	2068	2101	2068
M08	655	670	1041	1061	2012	2025	2012	2025
M09	676	650	1073	1043	2085	2040	2085	2040

Table 10: VO2 Data (L/min)

	Rest		Pre-EX		50%		65%		75%	
	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™
F02	0.2493	0.2458	0.2659	0.2439	1.3335	1.502	1.7538	1.8094	1.9898	2.0298
F03	0.206	0.2241	0.2314	0.2129	1.395	1.3617	1.7541	1.6808	1.9488	1.9773
F04	0.2008	0.1842	0.199	0.2147	1.309	1.2703	1.4766	1.6118	1.722	1.8172
F05	0.2233	0.2399	0.1754	0.228	1.6721	1.6675	2.0444	2.0083	2.3357	2.2789
F06	0.2297	0.292	0.2247	0.2407	1.2856	1.3186	1.6156	1.6578	1.8385	1.8424
F07	0.2363	0.2391	0.2138	0.2347	1.0668	1.0744	1.2654	1.2761	1.453	1.4351
F08	0.2043	0.211	0.1996	0.2134	1.2506	1.1968	1.5089	1.4416	1.6933	1.5924
M01	0.2955	0.311	0.2838	0.3129	2.295	2.2216	2.8523	2.8327	3.3712	3.2884
M02	0.2908	0.241	0.2904	0.2656	1.5648	1.5293	1.881	1.9327	2.1544	2.1188
M03	0.2224	0.2309	0.2229	0.2182	1.705	1.6522	2.1586	2.1031	2.4869	2.4671
M04	0.284	0.2575	0.2512	0.2612	1.6515	1.6692	2.0507	2.0441	2.3535	2.3128
M05	0.3174	0.3076	0.3015	0.3023	2.0816	1.8703	2.4093	2.3634	2.9405	2.8671
M06	0.2773	0.2857	0.2805	0.2841	1.7383	1.7578	2.2091	2.1947	2.5708	2.5053
M08	0.2748	0.3167	0.2651	0.3096	1.7166	1.8639	2.1366	2.3081	2.4953	2.6349
M09	0.2812	0.2555	0.2536	0.2741	2.0914	2.0881	2.636	2.6938	3.4308	3.0489

Table 11: RER DATA (CO_2/VO_2)

	Rest		Pre-EX		50%		65%		75%	
	PLA	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**
F02	0.918	1.0544	0.8585	1.02	0.9766	1.0428	1.0127	1.0548	1.0468	1.0713
F03	0.8449	0.9085	0.7833	0.8557	0.8998	0.9122	0.9224	0.9201	0.9191	0.9341
F04	0.8332	0.8127	0.8125	0.8116	0.9112	0.9255	0.9209	0.9732	0.9399	0.9514
F05	0.9394	0.8621	0.9854	0.8879	0.8917	0.9236	0.9113	0.9509	0.945	0.9907
F06	0.7479	0.7553	0.7771	0.7731	0.8714	0.8717	0.8851	0.8844	0.8794	0.8946
F07	0.7117	0.6984	0.7559	0.7459	0.8892	0.904	0.9301	0.9469	0.954	0.9481
F08	0.8451	0.9292	0.8464	0.9589	0.9182	0.9574	0.9377	0.9691	0.9443	0.9767
M01	0.8943	0.8602	0.9105	0.8702	1.024	0.9676	1.0687	1.0255	1.0985	1.0472
M02	0.7515	0.7633	0.7461	0.7729	0.8586	0.8706	0.9052	0.9078	0.9225	0.9227
M03	0.8961	0.8542	0.8768	0.8756	1.0225	0.9989	1.064	1.0348	1.1039	1.0553
M04	0.8415	0.8491	0.8186	0.8131	0.9426	0.9281	0.9905	0.9613	1.0088	0.9818
M05	0.8556	0.8681	0.8341	0.848	0.9882	0.9691	1.0734	1.0321	1.0834	1.0727
M06	0.8511	0.8256	0.8348	0.8196	0.9672	0.9391	0.997	0.988	1.0022	1.007
M08	0.8832	0.8411	0.9194	0.8414	0.9576	0.8861	0.9807	0.9171	0.9941	0.9275
M09	0.899	0.9152	0.8846	0.9314	0.9873	1.0042	1.0372	1.0627	1.1068	1.0387

Table 12: Heart Rate (Beat/min) During Steady state exercise

	Rest		**Pre-EX**		**50%**		**65%**		**75%**	
	PLA	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**
F02	62	58	62	56	126	122	147	138	160	149
F03	64	54	65	57	110	108	124	121	140	136
F04	59	58	58	60	125	121	138	142	151	152
F05	55	64	47	65	121	115	137	130	147	144
F06	40	43	42	46	88	89	99	101	109	108
F07	68	67	74	68	129	134	150	149	162	160
F08	65	68	72	70	126	124	136	135	145	142
M01	54	50	51	50	108	110	131	132	150	147
M02	67	64	69	68	110	113	125	128	136	134
M03	48	48	50	51	121	121	147	148	166	168
M04	49	48	49	47	113	109	131	125	145	142
M05	65	70	62	76	125	122	147	137	165	160
M06	77	67	80	72	116	118	141	140	162	161
M08	48	52	51	56	113	120	129	138	143	147
M09	65	63	70	63	125	123	140	145	167	160

Table 13: Heart Rate (Beat/min) During Time-Trial

	6km		10km		19.5km	
	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™
F02	163	158	163	158	161	162
F03	162	168	151	162	163	168
F04	156	157	155	154	147	160
F05	158	159	158	155	172	158
F06	134	130	133	134	137	137
F07	178	172	171	169	180	177
F08	167	164	163	163	170	167
M01	153	151	153	155	164	159
M02	166	162	168	168	178	172
M03	170	172	178	179	181	186
M04	158	159	163	167	147	156
M05	164	164	167	169	161	170
M06	179	178	174	177	184	183
M08	169	167	171	171	177	177
M09	167	171	167	170	183	180

Table 14: RPE during Steady state exercise

	50%		65%		75%	
	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™
F02	10	9	11	11	13	12
F03	10	10	12	12	13	13
F04	11	11	12	12	13	13
F05	11	12	13	13.5	14	15
F06	9	10	11	11	12	12
F07	9	9	12	11	14	13
F08	11	11	12	12	13	13
M01	9	9	11	12	15	14
M02	11	12	12	14	13	14
M03	12	11	13	12	15	13
M04	11	11	13	13	14	15
M05	11	11	13	13	15	15
M06	11	11	13	13	15.5	14
M08	12	11	12	12	13	14
M09	11	11	12	12	14	14

Table 15: RPE during Time-Trial

	6km		10km		19.5km	
	PLA	**Neo40™**	**PLA**	**Neo40™**	**PLA**	**Neo40™**
F02	14	14	15	15	17	16
F03	16	16	15	16	16	17
F04	13	13	14	13	15	14
F05	14	15	15	17	19	19
F06	13	14	14	13	15	14
F07	15	15	16.5	15	18	17
F08	14	15	15	16	17	18
M01	13	14	17	15	19	19
M02	14	16	15	15	17	17
M03	16	14	17	16	18	18
M04	13	14	15	15	16	15
M05	14	14	15	16	16	16
M06	17.5	16.5	18	17	19	18.5
M08	15	15	16	15.5	17	17
M09	13	15	14	14	15	15

Table 16: Lactate Concentration (mmol/L)

	Rest		Pre-EX		50%		65%		75%	
	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™	PLA	Neo40™
F02	1.1548	0.9158	0.9578	0.86	1.2171	1.1391	1.8325	1.5367	2.8658	2.34
F03	1.7945	2.1693	1.8639	2.4525	2.4033	1.2055	2.6272	1.3574	2.8632	2.4003
F04	0.9537	1.5448	0.8084	1.4648	1.4709	1.1872	2.3497	2.2114	2.6242	4.1315
F05	0.9001	0.9497	0.9431	1.131	0.9391	1.4825	1.0859	2.3502	2.2225	3.2583
F06	1.1568	1.0991	1.2556	1.2115	1.5914	1.4668	1.5631	1.4461	1.6796	1.6294
F07	1.6801	2.2468	1.4243	2.1491	1.5879	1.7155	2.3512	2.6525	4.5504	3.6144
F08	1.1082	1.6345	0.9461	1.3954	0.9056	1.246	1.1128	1.3554	1.6097	2.0609
M01	1.0976	0.9629	1.516	0.898	2.0295	1.5818	3.3885	2.7792	4.8016	4.5144
M02	1.5889	1.2014	1.4942	0.7739	1.6249	0.8438	1.3372	1.4754	1.8102	1.3858
M03	2.2524	0.7922	1.0343	1.211	1.4815	2.0929	2.4337	2.8881	4.5767	4.6512
M04	1.1867	1.7256	1.1649	1.6451	1.4	1.479	1.5281	1.3574	2.2185	2.2043
M05	1.5934	0.8048	1.5788	0.622	3.0983	1.6441	4.676	2.7928	6.9887	5.5345
M06	1.5175	1.3934	1.2946	1.4967	2.0103	2.0346	2.7042	3.0127	4.1695	4.2799
M08	1.8822	1.4304	1.7398	1.4643	1.9556	1.6107	2.0665	1.7717	2.3507	1.9683
M09	0.9826	1.1011	1.0383	0.82	1.3605	1.2004	3.119	2.5183	6.5764	5.067

Table 17: Blood Pressure

	Rest				Pre-EX			
	PLA (Sys/Dia)		Neo40™ (Sys/Dia)		PLA (Sys/Dia)		Neo40™ (Sys/Dia)	
F02	134	87	121	91	120	93	121	91
F03	100	59	92	59	107	75	107	60
F04	89	62	93	63	97	69	89	64
F05	117	72	111	70	114	82	120	70
F06	126	67	130	83	113	64	113	75
F07	107	79	112	79	111	79	117	74
F08	102	66	108	74	109	70	102	72
M01	114	79	108	73	110	76	118	72
M02	116	90	134	86	125	94	118	87
M03	94	58	95	62	96	62	99	69
M04	156	84	118	70	142	89	115	79
M05	122	72	124	70	122	81	116	72
M06	123	79	121	74	113	72	124	87
M08	119	72	125	73	127	72	125	65
M09	122	77	119	77	119	75	114	78

APPENDIX C: INFORMED CONSENT FORM

Consent for Participation in Research

Title: Effects of Neo40^{TM} with caffeine on cycling time trial performance

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

You have been asked to participate in a research study about the effect a dietary supplement on exercise performance. The purpose of this study is to determine if the supplement will enable you to ride faster and feel better during the ride compared to a flavored lozenge.

What will you to be asked to do?

If you agree to participate in this study, there will be 4 study visits where you will be asked to spend approximately 2 hours per visit in our lab. We will ask you to:

- Complete health, exercise schedule and availability forms and allow researcher to measure your blood pressure (30 minutes) then complete a VO_{2MAX} test (1 hour)
- Complete 1 Familiarization trial (2 hours)
- Complete 2 Experimental trials (2 hours each)

Each trial will be scheduled one week apart in the morning. Each trial we will ask you to:

- Fast (do not eat or drink except water) for 12 hours prior to your appointment
- Wear a heart rate monitor on a thin elastic belt around your chest
- Warm your hand with a heating pad for 5 minutes (2 times)
- Consume a lozenge containing the test product (1 time)
- Ride our lab bicycle for approximately 1 hour at varying intensities
- Have your finger pricked to obtain a drop of blood (5 times)
- Answer questions about how you feel (6 times)

We are recruiting 30 subjects (15 male, 15 female) for this study.

Blood will not be collected during the Familiarization Trial.

Blood Flow Testing: We will select 6 people out of the 30 who perform our study for additional testing. You may choose to participate in the primary study without participating in the additional testing. If selected, we will ask if you want to participate during the screening visit. If you participate, during each trial we will ask you to:

- Walk down the hall to another lab (2 times)
- Sit in a reclining chair while a technician places a device on the skin near your groin at rest and after performing leg extension exercise with low resistance (1 time each)

What are the risks involved in this study?

This dietary supplement contains ingredients found in foods and caffeine but may involve risks that are currently unforeseeable. Possible risks associated with this study are:

- Feeling fatigued at the end of of the VO_{2MAX} test and after each trial
- Elevated heart rate, headache or feeling of anxiety

What are the possible benefits of this study?

The possible benefits of participation are receipt of your VO_{2MAX} results, which will provide information about your cardiovascular fitness level. If you are asked and choose to participate in the blood flow testing, you will receive information about your blood flow to your leg before and during exercise.

You will also provide information to the study researchers about the effects of a possible exercise supplement that may improve exercise performance.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in any way.

Will there be any compensation?

You will receive $50 for one experimental trial or $200 for completion of the study (two experimental trials). You will not receive payment if you only complete the VO_{2MAX} test or VO_{2MAX} test and familiarization trial. If you are asked and choose to participate in the blood flow testing, you will not receive additional compensation. Payment will typically occur 2-4 weeks after your last study visit. Payment will be directly deposited if you are or were a UT employee, otherwise, a check will be mailed to you**.** You will be responsible for any taxes assessed on the compensation.

What if you are injured because of the study?

The risk of injury from participating in this study is low. The University has no program or plan to provide treatment for research related injury or payment in the event of a medical problem. In the event of a research related injury, please contact the principal investigator. If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center, but the University has no program or plan to provide payment in the event of a medical problem.

What are my confidentiality or privacy protections when participating in this research study?

This study is confidential. You will be assigned a subject ID that will be used throughout the study. All information, including this Informed Consent Form will be retained in a locked office and will only be available to key study personnel. We will also collect your name, address and social security number for payment purposes. Once the payment information is entered into UT's system, it will be shredded; no copies will be retained.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

Whom to contact with questions about the study?

Prior, during or after your participation you can contact the researcher, John Ivy, Ph.D. at 512-471-8599 or send an email to johnivy@mail.utexas.edu. This study has been

reviewed and approved by The University Institutional Review Board and the study number is 2012-05-0033.

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate, please sign below. We will give you a copy of your signed informed consent form.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

_____

Printed Name

_____ _____

Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____

Print Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX D: PARTICIPATION HEALTH RESEARCH SCREENING FORM

Answer the questions below by checking the appropriate box.

Yes	No	
☐	☐	1. Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month have you had chest pain when you were not doing physical activity?
☐	☐	4. Have you ever lost your balance because of dizziness or have you ever had a loss of consciousness? If yes explain: _____
☐	☐	5. Have you ever had racing of your heart or skipped heartbeats?
☐	☐	6. Has any family member or relative died of heart problems or sudden death before the age of 50?
☐	☐	7. Have you been told you have high blood pressure?
☐	☐	8. Have you been told you have a heart murmur?
☐	☐	9. Have you been told that you have kidney disease?
☐	☐	10. Have you been told that you have Type 1 or Type 2 diabetes?
☐	☐	11. Have you received an organ transplant?
☐	☐	12. Have you had or do you currently have a malignancy (cancer)?
☐	☐	13. Have you had a severe viral infection within the past month?
☐	☐	14. Have you been told that you have a chronic contagious, infectious disease such as tuberculosis, hepatitis B, hepatitis C or HIV?
☐	☐	15. Do you have any other medical problems or illnesses such as osteoporosis, diabetes, kidney or liver disease, or other chronic contagious or infectious disease? If yes, please describe: _____
☐	☐	16. Do you smoke?
☐	☐	17. Are you currently taking/using non-prescription illicit drugs?
☐	☐	18. If you are female, are you pregnant or are you trying to become pregnant in the next 2 months?

☐	☐	19. Are you aware through your own experience, a doctor's advice or any other physical reason that would prohibit you from engaging in physical activity?

Yes	No	
☐	☐	20. Are you taking any performance enhancing drugs or supplements other than a multivitamin. If so, what? _____
☐	☐	21. Are you currently on any regular medication including non-prescription medications? If your answer is "yes," please list your regular medication(s) and how long you have been taking it (them): _____ _____
☐	☐	22. Do you have any known allergies? If yes please specify: _____
☐	☐	23. Are you currently in another study? If yes, please specify: _____
		24. When was the last time you had a physical? _____

_____ _____

Signature Date

APPENDIX E: SAMPLE FOOD LOG

Your Test Schedule:

	Visit Detail	Date	Time
Familiarization	****BRING FOOD AND EXERCISE LOG!** Visit will last approximately 2 hours 12 hour fast (water okay) 24 hour no alcohol No caffeinated drinks, including diet drinks OR decaffeinated coffee 12 hours prior to appointment		
Exercise Trial 1			
Exercise Trial 2			

1. You will report to the lab for one familiarization ride and two experimental trials. Your finger will be pricked to obtain drops of blood during the experimental trials but not during the familiarization. Each trial will last approximately 2 hours.

2. Consume similar foods before each of your experimental trials and record everything that you eat and drink for the specified days that we have labeled for you in this packet. Include portion size, brand or restaurant, and method of preparation. For items that are available in different flavors, include the flavor. If you think that an item on your Food Log is unusual, please cut out the *Nutrition Facts* panel from the packaged food and attach to your Food Log. If you consume a mixture of foods, such as a casserole, estimate the amount of each ingredient in your serving. Also list any medications/dietary supplements you take on the Medication Log. If you need additional space, feel free to record on a separate piece of paper.

3. DO NOT participate in strenuous exercise the day before your visit.

4. Do not eat or drink anything, other than water during your 12 hour fast including the morning of your trial. Do not drink anything that contains caffeine including coffee, tea, diet sodas or decaffeinated beverages, including decaf coffee.

5. Take all of your prescribed medications as directed by your physician at the same time daily while you are enrolled in the study. If there is any change in your medication intake, you must discuss this change with the study coordinator.

FOOD LOG INSTRUCTIONS

Include all information about the foods that you have eaten

<u>If in doubt, bring the package label!</u>

FOOD	PREPARATIONS AND ADDITIONS	AMOUNT
Beverages	Coffee- regular, decaffeinated, cream, sugar Soft drinks- sugar sweetened, diet Juices- sweetened, unsweetened Alcohol- type	ounces
Bread, Cereal Rice, Pasta	Cereal - presweetened, sugar added, hot cereal, oatmeal (instant, quick or whole) Type – Rice (brown or white), pasta (white or wheat), dinner rolls (white or wheat), biscuits, etc.	slice, cups, dimensions
Fruits, Vegetables	Fresh, cooked, dried, canned: packed in water, light or heavy syrup Fat (butter, olive oil, canola oil, etc.) used in processing or cooking	cups, pieces
Dairy Products	Milk-nonfat, low fat, whole Yogurt - plain, flavored, low fat, whole, sugar- sweetened, artificially sweetened Cottage cheese - regular (creamed), low fat, nonfat, dry curd Cheese- type Non-dairy cream substitute- liquid, powder	ounces, cups tsp., Tbsp., dimensions for hard cheese
Eggs	Fried, boiled, scrambled, omelet, deviled, etc. Additions - milk, cheese, oil, butter, mayonnaise	number eaten, ounces

Meat, Poultry, Fish	Baked, broiled, fried, BBQ, boiled, stewed Cut of meat and did it have a bone? Is weight given for raw or cooked meat? Was fat trimmed or not? Poultry- light, dark, was skin eaten? Tuna- oil or water packed, drained?	ounces, dimensions: Length x Width x Height
Fats: butter, margarine, oil, shortening, salad dressing gravy, sauces	Margarine - soft (tub), stick, diet Oil - brand name, kind Real mayonnaise, Miracle Whip, imitation, low-calorie, fat-free Gravy sauce - type of fat used	tsp., Tbsp., cups, ounces,
Pizza	Toppings	diameter of whole plus fraction eaten
Snacks	Anything eaten between meals- candy, crackers, pretzels, chips, etc.	cups, ounces, tsp., Tbsp., number of pieces
Dessert, Pastries, Ice Cream	Homemade (mix from scratch), commercial Topping, filling, nuts, raisins, etc. Cake - layer, sheet, tube, bundt, loaf - chocolate, yellow, white, angel food, etc., - frosting type Pie - crust (type, single, double, fat used)	cups, dimensions, scoops, pie-width of wedge, portion of whole

Example Food Log			**Date:**
	Amount	***Brand***	***Food***
Breakfast	**8 oz**		**Nonfat milk**
	12 oz		**Black coffee**
	1.5 Cups	**Nature's Path**	**Heritage Heirloom Whole Grains Cereal**
	1 T	**Sun-Maid**	**Fruit bits**
Lunch	**1.3 Cups**	**Homemade**	**Chili: ½ Cup ground beef, 1 T onion, 2 T garbanzo beans, 2T black beans, 2 T red sweet pepper**
	3 T		**Grated cheddar/jack cheese**
	½ Cup		**Fresh strawberries**
	½ Cup	**Stoneyfield**	**Lowfat vanilla yogurt**
	2 T		**Raw almonds**
Dinner	**5 oz**		**Grilled chicken breast**
	¾ Cup		**Slaw: ¼ cup cabbage, ¼ grated carrots, ¼ broccoli, 1 tsp olive oil, 1 tsp cider vinegar**
	1 piece	**Kirkland Signature**	**Multigrain bread**
	2 tsp		**honey**
	½ tsp		**butter**
	¾ Cup		**Grilled vegetables: ¼ cup yellow squash, ¼ red pepper, ¼ cup eggplant**

Snacks	**1**	**Clif**	**Chocolate Builder's Bar**
	1 medium		**Cara Cara navel orange**

Familiarization

Exercise Log (Familiarization)

Please use the following intensities when you record your exercise. Please include the exercise trials that you are performing as part of this study.

Low: My heart rate did not rise; I did not sweat or sweated very little
Medium: My heart rate rose somewhat, but I was able to easily talk
High: My heart rate was very high and I was unable to carry on a conversation

Two days before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

One day before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

Two days before Familiarization **Date:**

	Amount	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

Day before Familiarization	**Date:**
Time last food:	

	Amount	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

Experimental Trial 1

Exercise Log (Experimental Trial 1)

Please use the following intensities when you record your exercise. Please include the exercise trials that you are performing as part of this study.

Low: My heart rate did not rise very much, I did not sweat or sweated very little
Medium: My heart rate rose somewhat, but I was able to talk easily
High: My heart rate was very high and I was unable to carry on a conversation

Two days before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

One day before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

Two days before Experimental Trial 1 Date:

	Amount	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

Day before Experimental Trial 1 **Date:**

Time last food:

	Amount	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

Experimental Trial 2

Exercise Log (Experimental Trial 2)

Please use the following intensities when you record your exercise. Please include the exercise trials that you are performing as part of this study.

Low: My heart rate did not rise very much, I did not sweat or sweated very little
Medium: My heart rate rose somewhat, but I was able to talk easily
High: My heart rate was very high and I was unable to carry on a conversation

Two days before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

One day before (Date:)

Type of Exercise		Low Intensity	Medium Intensity	High Intensity
	#Minutes:			
	#Minutes:			
	#Minutes:			

Comments:_____

_____

_____

Two days before Experimental Trial 2 Date:

	Amount	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

Day before Experimental Trial 2 **Date:**

Time last food:			
	Amount	***Brand***	***Food***
Breakfast			
Lunch			
Dinner			
Snacks			

REFERENCES

Abe, K., Hayashi, N., & Terada, H. (1999). Effect of endogenous nitric oxide on energy metabolism of rat heart mitochondria during ischemia and reperfusion. *Free Radic Biol Med, 26*(3-4), 379-387.

Adler, H., Beland, J. L., Del-Pan, N. C., Kobzik, L., Brewer, J. P., Martin, T. R., & Rimm, I. J. (1997). Suppression of herpes simplex virus type 1 (HSV-1)-induced pneumonia in mice by inhibition of inducible nitric oxide synthase (iNOS, NOS2). *J Exp Med, 185*(9), 1533-1540.

Akaike, T., Weihe, E., Schaefer, M., Fu, Z. F., Zheng, Y. M., Vogel, W., . . . Dietzschold, B. (1995). Effect of neurotropic virus infection on neuronal and inducible nitric oxide synthase activity in rat brain. *J Neurovirol, 1*(1), 118-125.

Bailey, S. J., Winyard, P. G., Vanhatalo, A., Blackwell, J. R., DiMenna, F. J., Wilkerson, D. P., & Jones, A. M. (2010). Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. [Randomized Controlled Trial]. *J Appl Physiol, 109*(5), 1394-1403. doi: 10.1152/japplphysiol.00503.2010

Balon, T. W., & Nadler, J. L. (1997). Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol, 82*(1), 359-363.

Baron, A. D., Zhu, J. S., Marshall, S., Irsula, O., Brechtel, G., & Keech, C. (1995). Insulin resistance after hypertension induced by the nitric oxide synthesis inhibitor L-NMMA in rats. *Am J Physiol, 269*(4 Pt 1), E709-715.

Bergandi, L., Silvagno, F., Russo, I., Riganti, C., Anfossi, G., Aldieri, E., . . . Bosia, A. (2003). Insulin stimulates glucose transport via nitric oxide/cyclic GMP pathway in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol, 23*(12), 2215-2221. doi: 10.1161/01.ATV.0000107028.20478.8e

Bescos, R., Rodriguez, F. A., Iglesias, X., Ferrer, M. D., Iborra, E., & Pons, A. (2011). Acute administration of inorganic nitrate reduces VO(2peak) in endurance athletes. *Med Sci Sports Exerc, 43*(10), 1979-1986. doi: 10.1249/MSS.0b013e318217d439

Bescos, R., Sureda, A., Tur, J. A., & Pons, A. (2012). The effect of nitric-oxide-related supplements on human performance. *Sports Med, 42*(2), 99-117. doi: 10.2165/11596860-000000000-00000

Bloomer, R. J., Farney, T. M., Trepanowski, J. F., McCarthy, C. G., & Canale, R. E. (2011). Effect of betaine supplementation on plasma nitrate/nitrite in exercise-trained men. *J Int Soc Sports Nutr, 8*, 5. doi: 10.1186/1550-2783-8-5

Bogdan, C. (2001). Nitric oxide and the immune response. *Nat Immunol, 2*(10), 907-916. doi: 10.1038/ni1001-907

Bond, H., Morton, L., & Braakhuis, A. J. (2012). Dietary nitrate supplementation improves rowing performance in well-trained rowers. *Int J Sport Nutr Exerc Metab, 22*(4), 251-256.

Bradley, S. J., Kingwell, B. A., & McConell, G. K. (1999). Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes, 48*(9), 1815-1821.

Brock, R. W., Tschakovsky, M. E., Shoemaker, J. K., Halliwill, J. R., Joyner, M. J., & Hughson, R. L. (1998). Effects of acetylcholine and nitric oxide on forearm blood flow at rest and after a single muscle contraction. *J Appl Physiol, 85*(6), 2249-2254.

Brooks, K. J., Hargreaves, I. P., & Bates, T. E. (2000). Nitric-oxide-induced inhibition of mitochondrial complexes following aglycaemic hypoxia in neonatal cortical rat brain slices. *Dev Neurosci, 22*(5-6), 359-365. doi: 17461

Brown, G. C., & Borutaite, V. (1999). Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp, 66*, 17-25.

Carlstrom, M., Persson, A. E., Larsson, E., Hezel, M., Scheffer, P. G., Teerlink, T., . . . Lundberg, J. O. (2011). Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovasc Res, 89*(3), 574-585. doi: 10.1093/cvr/cvq366

Ceneviva, G. D., Tzeng, E., Hoyt, D. G., Yee, E., Gallagher, A., Engelhardt, J. F., . . . Pitt, B. R. (1998). Nitric oxide inhibits lipopolysaccharide-induced apoptosis in pulmonary artery endothelial cells. *Am J Physiol, 275*(4 Pt 1), L717-728.

Cermak, N. M., Gibala, M. J., & van Loon, L. J. (2012). Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab, 22*(1), 64-71.

Cermak, N. M., Res, P., Stinkens, R., Lundberg, J. O., Gibala, M. J., & van Loon, L. J. (2012). No improvement in endurance performance after a single dose of beetroot juice. *Int J Sport Nutr Exerc Metab, 22*(6), 470-478.

Chowdhary, S., Marsh, A. M., Coote, J. H., & Townend, J. N. (2004). Nitric oxide and cardiac muscarinic control in humans. *Hypertension, 43*(5), 1023-1028. doi: 10.1161/01.HYP.0000126171.46645.f9

Clementi, E., & Nisoli, E. (2005). Nitric oxide and mitochondrial biogenesis: a key to long-term regulation of cellular metabolism. *Comp Biochem Physiol A Mol Integr Physiol, 142*(2), 102-110. doi: 10.1016/j.cbpb.2005.04.022

Cohen, J., Evans, T. J., & Spink, J. (1998). Cytokine regulation of inducible nitric oxide synthase in vascular smooth muscle cells. *Prog Clin Biol Res, 397*, 169-177.

Coleman, J. W. (2001). Nitric oxide in immunity and inflammation. *Int Immunopharmacol, 1*(8), 1397-1406.

Costill, D. L., Dalsky, G. P., & Fink, W. J. (1978). Effects of caffeine ingestion on metabolism and exercise performance. *Med Sci Sports, 10*(3), 155-158.

Davidson, S. M., & Duchen, M. R. (2006). Effects of NO on mitochondrial function in cardiomyocytes: Pathophysiological relevance. *Cardiovasc Res, 71*(1), 10-21. doi: 10.1016/j.cardiores.2006.01.019

Davidson, S. M., & Yellon, D. M. (2005). The role of nitric oxide in mitochondria. Focus on "Modulation of mitochondrial Ca2+ by nitric oxide in cultured bovine vascular endothelial cells". *Am J Physiol Cell Physiol, 289*(4), C775-777. doi: 10.1152/ajpcell.00277.2005

Davisson, R. L., Possas, O. S., Murphy, S. P., & Lewis, S. J. (1997). Neurogenically derived nitrosyl factors mediate sympathetic vasodilation in the hindlimb of the rat. *Am J Physiol, 272*(5 Pt 2), H2369-2376.

Desbrow, B., Biddulph, C., Devlin, B., Grant, G. D., Anoopkumar-Dukie, S., & Leveritt, M. D. (2012). The effects of different doses of caffeine on endurance cycling time trial performance. *J Sports Sci, 30*(2), 115-120. doi: 10.1080/02640414.2011.632431

Dreissigacker, U., Wendt, M., Wittke, T., Tsikas, D., & Maassen, N. (2010). Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men. *Nitric Oxide, 23*(2), 128-135. doi: 10.1016/j.niox.2010.05.003

Duncker, D. J., Van Zon, N. S., Altman, J. D., Pavek, T. J., & Bache, R. J. (1993). Role of K+ATP channels in coronary vasodilation during exercise. *Circulation, 88*(3), 1245-1253.

Duplain, H., Burcelin, R., Sartori, C., Cook, S., Egli, M., Lepori, M., . . . Scherrer, U. (2001). Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation, 104*(3), 342-345.

Engelke, K. A., Halliwill, J. R., Proctor, D. N., Dietz, N. M., & Joyner, M. J. (1996). Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol, 81*(4), 1807-1814.

Etgen, G. J., Jr., Fryburg, D. A., & Gibbs, E. M. (1997). Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. *Diabetes, 46*(11), 1915-1919.

Graham, T. E., & Spriet, L. L. (1995). Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *J Appl Physiol, 78*(3), 867-874.

Higaki, Y., Hirshman, M. F., Fujii, N., & Goodyear, L. J. (2001). Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. *Diabetes, 50*(2), 241-247.

Hord, N. G. (2011). Dietary nitrates, nitrites, and cardiovascular disease. *Curr Atheroscler Rep, 13*(6), 484-492. doi: 10.1007/s11883-011-0209-9

Hord, N. G., Ghannam, J. S., Garg, H. K., Berens, P. D., & Bryan, N. S. (2011). Nitrate and nitrite content of human, formula, bovine, and soy milks: implications for dietary nitrite and nitrate recommendations. *Breastfeed Med, 6*(6), 393-399. doi: 10.1089/bfm.2010.0070

Hord, N. G., Tang, Y., & Bryan, N. S. (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr, 90*(1), 1-10. doi: 10.3945/ajcn.2008.27131

Huang, A., Sun, D., Kaley, G., & Koller, A. (1998). Superoxide released to high intra-arteriolar pressure reduces nitric oxide-mediated shear stress- and agonist-induced dilations. *Circ Res, 83*(9), 960-965.

Ishibashi, Y., Quebbeman, B. B., Duncker, D. J., Klassen, C., & Bache, R. J. (1998). Acadesine increases blood flow in the collateralized heart during exercise. *J Cardiovasc Pharmacol, 32*(4), 552-561.

Issekutz, B., Jr., Shaw, W. A., & Issekutz, T. B. (1975). Effect of lactate on FFA and glycerol turnover in resting and exercising dogs. *J Appl Physiol, 39*(3), 349-353.

Jokela, H., Dastidar, P., Rontu, R., Salomaki, A., Teisala, K., Lehtimaki, T., & Punnonen, R. (2003). Effects of long-term estrogen replacement therapy versus combined hormone replacement therapy on nitric oxide-dependent vasomotor function. *J Clin Endocrinol Metab, 88*(9), 4348-4354.

Jones, A. M., Bailey, S. J., & Vanhatalo, A. (2012). Dietary nitrate and O(2) consumption during exercise. *Med Sport Sci, 59*, 29-35. doi: 10.1159/000342062

Kapil, V. (2010). Inorganic Nitrate Supplementation Lowers Blood Pressure in Humans : Role for Nitrite-Derived NO. *Hypertension*.

Kelly, L. K., Wedgwood, S., Steinhorn, R. H., & Black, S. M. (2004). Nitric oxide decreases endothelin-1 secretion through the activation of soluble guanylate cyclase. *Am J Physiol Lung Cell Mol Physiol, 286*(5), L984-991. doi: 10.1152/ajplung.00224.2003

Kenjale, A. A., Ham, K. L., Stabler, T., Robbins, J. L., Johnson, J. L., Vanbruggen, M., . . . Allen, J. D. (2011). Dietary nitrate supplementation enhances exercise performance in

peripheral arterial disease. *J Appl Physiol, 110*(6), 1582-1591. doi: 10.1152/japplphysiol.00071.2011

Kingwell, B. A. (2000). Nitric oxide as a metabolic regulator during exercise: effects of training in health and disease. *Clin Exp Pharmacol Physiol, 27*(4), 239-250.

Kobzik, L., Reid, M. B., Bredt, D. S., & Stamler, J. S. (1994). Nitric oxide in skeletal muscle. *Nature, 372*(6506), 546-548. doi: 10.1038/372546a0

Kovacs, E. M., Stegen, J., & Brouns, F. (1998). Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *J Appl Physiol, 85*(2), 709-715.

Lacerda, A. C., Marubayashi, U., Balthazar, C. H., & Coimbra, C. C. (2006). Evidence that brain nitric oxide inhibition increases metabolic cost of exercise, reducing running performance in rats. *Neurosci Lett, 393*(2-3), 260-263. doi: 10.1016/j.neulet.2005.09.076

Lansley, K. E., Winyard, P. G., Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Blackwell, J. R., . . . Jones, A. M. (2011). Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc, 43*(6), 1125-1131. doi: 10.1249/MSS.0b013e31821597b4

Lansley, K. E., Winyard, P. G., Fulford, J., Vanhatalo, A., Bailey, S. J., Blackwell, J. R., . . . Jones, A. M. (2011). Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. *J Appl Physiol, 110*(3), 591-600. doi: 10.1152/japplphysiol.01070.2010

Larsen, F. J., Schiffer, T. A., Borniquel, S., Sahlin, K., Ekblom, B., Lundberg, J. O., & Weitzberg, E. (2011). Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab, 13*(2), 149-159. doi: 10.1016/j.cmet.2011.01.004

Larsen, F. J., Weitzberg, E., Lundberg, J. O., & Ekblom, B. (2007). Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol (Oxf), 191*(1), 59-66. doi: 10.1111/j.1748-1716.2007.01713.x

Larsen, F. J., Weitzberg, E., Lundberg, J. O., & Ekblom, B. (2010). Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radic Biol Med, 48*(2), 342-347. doi: 10.1016/j.freeradbiomed.2009.11.006

Liu, T. H., Wu, C. L., Chiang, C. W., Lo, Y. W., Tseng, H. F., & Chang, C. K. (2009). No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. *J Nutr Biochem, 20*(6), 462-468. doi: 10.1016/j.jnutbio.2008.05.005

Machha, A., & Schechter, A. N. (2011). Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. *Eur J Nutr, 50*(5), 293-303. doi: 10.1007/s00394-011-0192-5

Mason, R. P., Kalinowski, L., Jacob, R. F., Jacoby, A. M., & Malinski, T. (2005). Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. *Circulation, 112*(24), 3795-3801. doi: 10.1161/circulationaha.105.556233

McConell, G. K., & Kingwell, B. A. (2006). Does nitric oxide regulate skeletal muscle glucose uptake during exercise? *Exerc Sport Sci Rev, 34*(1), 36-41.

Modin, H., Dai, Y., Masterman, T., Svejgaard, A., Sorensen, P. S., Oturai, A., . . . Hillert, J. (2001). No linkage or association of the nitric oxide synthase genes to multiple sclerosis. *J Neuroimmunol, 119*(1), 95-100.

Murphy, M., Eliot, K., Heuertz, R. M., & Weiss, E. (2012). Whole beetroot consumption acutely improves running performance. *J Acad Nutr Diet, 112*(4), 548-552. doi: 10.1016/j.jand.2011.12.002

Nathan, C. (1992). Nitric oxide as a secretory product of mammalian cells. *FASEB J, 6*(12), 3051-3064.

Nichols, A. J., Gellai, M., & Ruffolo, R. R., Jr. (1991). Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol. *Fundam Clin Pharmacol, 5*(1), 25-38.

Pasman, W. J., van Baak, M. A., Jeukendrup, A. E., & de Haan, A. (1995). The effect of different dosages of caffeine on endurance performance time. *Int J Sports Med, 16*(4), 225-230. doi: 10.1055/s-2007-972996

Ragoobirsingh, D., McGrowder, D., Dasgupta, T., & Brown, P. (2004). The effect of nitric oxide on glucose metabolism. *Mol Cell Biochem, 263*(1-2), 29-34.

Rassaf, T., Lauer, T., Heiss, C., Balzer, J., Mangold, S., Leyendecker, T., . . . Kelm, M. (2007). Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. *Br J Sports Med, 41*(10), 669-673; discussion 673. doi: 10.1136/bjsm.2007.035758

Reiss, C. S., & Komatsu, T. (1998). Does nitric oxide play a critical role in viral infections? *J Virol, 72*(6), 4547-4551.

Roberts, C. K., Barnard, R. J., Jasman, A., & Balon, T. W. (1999). Acute exercise increases nitric oxide synthase activity in skeletal muscle. *Am J Physiol, 277*(2 Pt 1), E390-394.

Ruby, B. C., Robergs, R. A., Waters, D. L., Burge, M., Mermier, C., & Stolarczyk, L. (1997). Effects of estradiol on substrate turnover during exercise in amenorrheic females. *Med Sci Sports Exerc, 29*(9), 1160-1169.

Stamler, J. S., & Meissner, G. (2001). Physiology of nitric oxide in skeletal muscle. *Physiol Rev, 81*(1), 209-237.

Tarnopolsky, L. J., MacDougall, J. D., Atkinson, S. A., Tarnopolsky, M. A., & Sutton, J. R. (1990). Gender differences in substrate for endurance exercise. *J Appl Physiol, 68*(1), 302-308.

Thomas, G. D., & Victor, R. G. (1998). Nitric oxide mediates contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. *J Physiol, 506 (Pt 3)*, 817-826.

Umans, J. G., & Levi, R. (1995). Nitric oxide in the regulation of blood flow and arterial pressure. *Annu Rev Physiol, 57*, 771-790. doi: 10.1146/annurev.ph.57.030195.004011

Vanhatalo, A., Bailey, S. J., Blackwell, J. R., DiMenna, F. J., Pavey, T. G., Wilkerson, D. P., . . . Jones, A. M. (2010). Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol Regul Integr Comp Physiol, 299*(4), R1121-1131. doi: 10.1152/ajpregu.00206.2010

Vanhatalo, A., Fulford, J., Bailey, S. J., Blackwell, J. R., Winyard, P. G., & Jones, A. M. (2011). Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J Physiol, 589*(Pt 22), 5517-5528. doi: 10.1113/jphysiol.2011.216341

Webb, A. J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., . . . Ahluwalia, A. (2008). Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension, 51*(3), 784-790. doi: 10.1161/hypertensionaha.107.103523

Wilkerson, D. P., Hayward, G. M., Bailey, S. J., Vanhatalo, A., Blackwell, J. R., & Jones, A. M. (2012). Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. *Eur J Appl Physiol, 112*(12), 4127-4134. doi: 10.1007/s00421-012-2397-6

Young, M. E., Radda, G. K., & Leighton, B. (1997). Nitric oxide stimulates glucose transport and metabolism in rat skeletal muscle in vitro. *Biochem J, 322 (Pt 1)*, 223-228.

Yu, M., Sun, C. W., Maier, K. G., Harder, D. R., & Roman, R. J. (2002). Mechanism of cGMP contribution to the vasodilator response to NO in rat middle cerebral arteries. *Am J Physiol Heart Circ Physiol, 282*(5), H1724-1731. doi: 10.1152/ajpheart.00699.2001