



Original Research

The Effect of Betaine on Nitrate and Cardiovascular Response to Exercise

J. LUKE PRYOR^{#1,2}, S. TONY WOLF ^{†1}, GARY SFORZO^{#2}, AND TOM SWENSEN^{#2}

¹Department of Kinesiology, California State University Fresno, Fresno, CA, USA; ²Department of Exercise and Sport Sciences, Ithaca College, Ithaca, NY, USA

[†]Denotes graduate student author, [#]Denotes professional author

ABSTRACT

International Journal of Exercise Science 10(4): 550-559, 2017. Betaine (BT) supplementation improves selected markers of physical performance, however, the mechanism(s) by which this change occurs remains largely unknown. Some speculate that BT may increase circulating nitrate concentrations, improving physical performance by augmentation of endothelial nitric oxide production. The purpose of this study was to investigate the effects of acute BT supplementation and exercise on plasma nitrate levels and related cardiovascular response (CVR). Placebo and BT trials were administered in a cross-over, randomized, double-blind, and counterbalanced fashion. Ten healthy college-aged volunteers consumed either a 250 ml placebo (carbohydrate-electrolyte beverage, CHO) or 250 ml CHO + 2.5 g BT. Subjects rested for 45 min, then cycled for 30 min at 60 rpm with a resistance of 2.5% body weight. Blood was drawn before and 45 min after BT supplementation, and immediately post exercise to assess plasma nitrate levels. Repeated measures ANOVA across treatments and times assessed differences in plasma nitrate and CVR variables with an alpha level set at 0.05. No significant interactions nor differences between groups were found for plasma nitrate levels or CVR variables with acute BT supplementation. A significant time effect ($p \leq 0.013$) for all CVR variables was found and expected due to the effect of exercise. Acute BT supplementation did not increase plasma nitrate levels nor alter CVR at rest or during light to moderate cycling.

KEY WORDS: Aerobic exercise, metabolism, vasodilation, ergogenic aid

INTRODUCTION

There is growing interest in investigating nitrate containing supplements given the promising health and physical performance findings (16). Nitrate can be recycled to bioactive nitric oxide (NO) under certain physiological conditions and through the action of NO, modulate skeletal muscle function, contraction, mitochondrial respiration and biogenesis, and blood flow (16).

Betaine (BT) is a naturally occurring compound found in many foods including beets and spinach, which are rich in nitrate (32, 40).

BT supplementation improves athletic performance by enhancing metabolism when consumed with a carbohydrate-electrolyte fluid replacement drink. For example, our laboratory (31) and others (4, 13, 22) showed BT supplementation improved select physical performance measures, likely due to its ability to donate methyl groups thereby upregulating creatine synthesis in skeletal muscle (3, 7). This mechanism, however, was recently questioned (8). Alternatively, it may be that BT's ergogenicity is related to its ability to upregulate gene expression of NO synthase resulting in increased NO availability (15). It may also be that BT supplementation increases circulating nitrate concentrations, resulting in improved performance through the nitrate-NO pathway.

Two studies evaluated whether BT supplementation affected the nitrate-NO pathway at rest. BT supplementation increased resting plasma NO in older adults from $28.8 \pm 3.4 \mu\text{mol}\cdot\text{L}^{-1}$ to $82.3 \pm 13.2 \mu\text{mol}\cdot\text{L}^{-1}$ after $6 \text{ g}\cdot\text{d}^{-1}$ for one week (14). More recently, it was shown that resting concentrations of nitrite and nitrate, precursors to NO (18), were unaffected by acute or chronic BT supplementation ($1.25 - 6 \text{ g}$ for up to 14 days) in young exercise trained men (2). The discordance among studies examining the effects of BT supplementation at rest on nitrate and NO necessitates additional research. Furthermore, BT is sourced from nitrate rich foods and remains a common ingredient in many pre-exercise supplements. Although BT may or may not affect the nitrate-NO pathway at rest, it remains unknown whether the combination of exercise plus BT consumption may have a synergistic effect on the nitrate-NO pathway.

It is possible that exercise and BT may work synergistically to elevate nitrate levels and subsequently NO. Accordingly, BT supplementation could enhance exercise performance (e.g., greater hyperemia in working muscles), recovery (e.g., anti-inflammatory), and cardiovascular health (e.g., inhibition of platelet aggregation and adhesion) through the association of nitrate with NO (5, 6, 9, 16, 19, 39). Considering the emerging interest in nitrate as an ergogenic aid and the dissonance of previous studies evaluating the effect of BT supplementation at rest on measures of nitrate, further investigation is needed. Therefore, the aim of this investigation was to determine if acute BT supplementation combined with moderate intensity exercise affects nitrate levels and cardiovascular responses to exercise.

METHODS

Participants

Ten healthy college aged males volunteered to participate in the study. All subjects were recreationally active (a population likely to use such a supplement), participating in light to moderate exercise ≥ 3 days per week and not engaged in a collegiate sport or periodized endurance or resistance training program. Subjects were excluded if they reported high blood pressure, history of diabetes, renal disease, current orthopedic injuries that would prevent cycling, allergies, illness or any other medical reason that may endanger them or affect the quality of the study. Subjects who chose to participate were made aware of risks, benefits, and

protocols of the study before providing informed consent. Subjects did not consume any caffeine, alcohol, or any other drugs within 24 h of testing sessions and maintained current physical activity and dietary habits throughout the course of the study. Additionally, subjects discontinued any nitrate/NO boosting supplements at least 14 d prior to data collection and were not permitted concomitant supplementation during this study. The methods of this study were approved by the college Human Subjects Review Board.

Protocol

This study utilized a placebo controlled, double-blind, repeated measures design. To limit the influence of diet and relative cycling workload on BT and NO levels, a crossover design. Placebo and BT (BetaPower™ Danisco A/S, White Plains, NY) trials were administered in a partially randomized, counterbalanced fashion to reduce order effects. Subjects completed a 12-hour fast prior to testing. Subjects consumed either a 250 ml placebo (carbohydrate-electrolyte beverage, CHO) or a 250 ml carbohydrate-electrolyte beverage with 2.5g BT (CHO + BT). To ensure beverage anonymity, all subjects received uniform opaque sport drink bottles with cap ties broken. Monark (Series 818e, Varberg, Sweden) cycle ergometers were calibrated each day prior to data collection by one of two researchers to ensure accurate resistance levels.

All data collection sessions were time-matched between visits and completed between the hours of 8:00 and 11:00 AM in a temperature controlled laboratory (72° F). Upon subject arrival, 3.5 ml of blood was drawn to assess baseline plasma nitrate levels (-45 min). Subjects then consumed either the CHO or CHO + BT beverage and sat for 45 min. Based upon human digestion pharmacokinetics of BT, 45 min allows BT to reach peak levels in the plasma (0.65 - 1.3 hrs post-digestion; Schwahn, Hafner, Hohlfeld, Balkenhol, Laryea, et al., 2003). During this time, subjects sat quietly and were free to read, study, or use a computer. After this 45 min period, another resting blood sample was taken (0 min). On a cycle ergometer with seat height adjusted to 10-15° knee flexion, subjects completed a 30 min cycling bout at 60 rpm with resistance set at 2.5% body weight (kg). The calculated workload was analogous to light to moderate intensity exercise. Blood was drawn immediately after the 30 min cycling bout and the subjects then cooled down for 5 min against light resistance. Subjects completed a second trial in the alternate condition after waiting 6 to 8 d to allow for adequate BT wash out.

To ensure hydration, subjects drank 12 oz of water prior to sleeping the night before and again on the morning of data collection. Upon subject arrival, euhydration ($U_{sg} \leq 1.025 \mu\text{G}$) was verified using a digital refractometer (KS-0050; Kernco Instruments, El Paso, TX). The subject was then fitted with a Polar HR monitor (Model S610i; Polar Electro, Kempele, Finland), and HR data were measured during rest (-45, -15, and 0 min) and cycling (15 and 30 min). In addition, blood pressure data were collected at the same time intervals as HR data using auscultation and sphygmomanometer.

Venous blood from an antecubital vein was drawn at rest (-45 min, 0 min) and immediately following cycling (30 min). Blood was collected in citrate containing vials and immediately centrifuged at 3500 rpm for 10 min at 4° C. Plasma was collected, aliquoted and stored at -80°

C until analysis. Due to the extremely short half-life of NO and the high degree of measurement variability, nitrate and/or nitrite concentrations are widely accepted surrogate markers of NO (18, 23). Therefore, the concentration of inorganic nitrate, a stable end product of NO oxidation, was assayed by a colorimetric method using the Greiss method in a total NO Nitrate/Nitrite Assay Kit (Assay Designs Inc., Ann Arbor, MI). The mean minimum detectable dose for this assay kit was $0.625 \mu\text{mol}\cdot\text{L}^{-1}$. Samples were ultra-filtrated with 10,000 MWCO filter. All samples were run in duplicate with the average value reported (mean intra-assay coefficient of variance = 3.9%).

Statistical Analysis

A 2×3 repeated measures ANOVA across treatments (CHO + BT and CHO) and times (-45, 0 and 30 min) assessed differences in plasma nitrate. A 2×5 repeated measures ANOVA across treatments and times (-45, -15, 0, 15 and 30 min) assessed differences in cardiovascular response variables (CVR; SBP, DBP, MAP and HR). Significant results were further analyzed using pairwise comparisons with Bonferroni adjustments to control alpha inflation and minimize the chance of Type 1 error. When sphericity was violated, a Greenhouse-Geisser analysis was performed. Effect sizes (Cohen's *d*) were calculated to assess the magnitude of difference in nitrate levels between groups. All statistics were performed on PASW (v. 17.0) with an alpha set at 0.05.

RESULTS

Means \pm SD for pertinent anthropometric data were in the expected range (height = 170.0 ± 12.4 cm, weight = 78.7 ± 11.0 kg, and age = 20.2 ± 3.6 y). Relative BT dosage averaged $0.03 \text{ g}\cdot\text{kg}^{-1}$ ranging from $0.02\text{-}0.04 \text{ g}\cdot\text{kg}^{-1}$.

No significant interactions ($p = 0.23$) nor differences between groups ($p = 0.31$) or time ($p = 0.12$) were noted for plasma nitrate levels. Examining descriptive data (means \pm SD) in Table 1, nitrate was lowest 45 min after BT supplementation. Nitrate also returned to pre-BT supplementation levels after 30 min of exercise. Effect sizes at -45 min, 0 min, and 30 min were 0.249, -0.075, and 0.742, respectively.

No significant interactions (HR, $p = 0.74$; MAP, $p = 0.68$; SBP, $p = 0.80$; DBP, $p = 0.46$) nor differences between groups (HR, $p = 0.40$; MAP, $p = 0.47$; SBP, $p = 0.87$; DBP, $p = 0.32$) were noted for any CVR. There was, however, a significant main effect for time ($p \leq 0.013$) on CVR variables (Figure 1) which was expected due to the 30 min resisted cycling bout.

Table 1. Plasma nitrate levels pre- and post-exercise.

Time (min)	Group	Nitrate ($\mu\text{mol L}^{-1}$)	Average ($\mu\text{mol L}^{-1}$)
-45	Control	55.1 ± 14.0	53.5 ± 12.8
	Betaine	51.9 ± 11.6	
0	Control	46.3 ± 11.7	46.8 ± 12.0
	Betaine	47.2 ± 12.2	
30 [#]	Control	60.8 ± 21.1	54.5 ± 16.8
	Betaine	$48.2 \pm 11.5^*$	

Note. Values are mean \pm SD, * n = 10 except this data point where n = 9, # = post-exercise

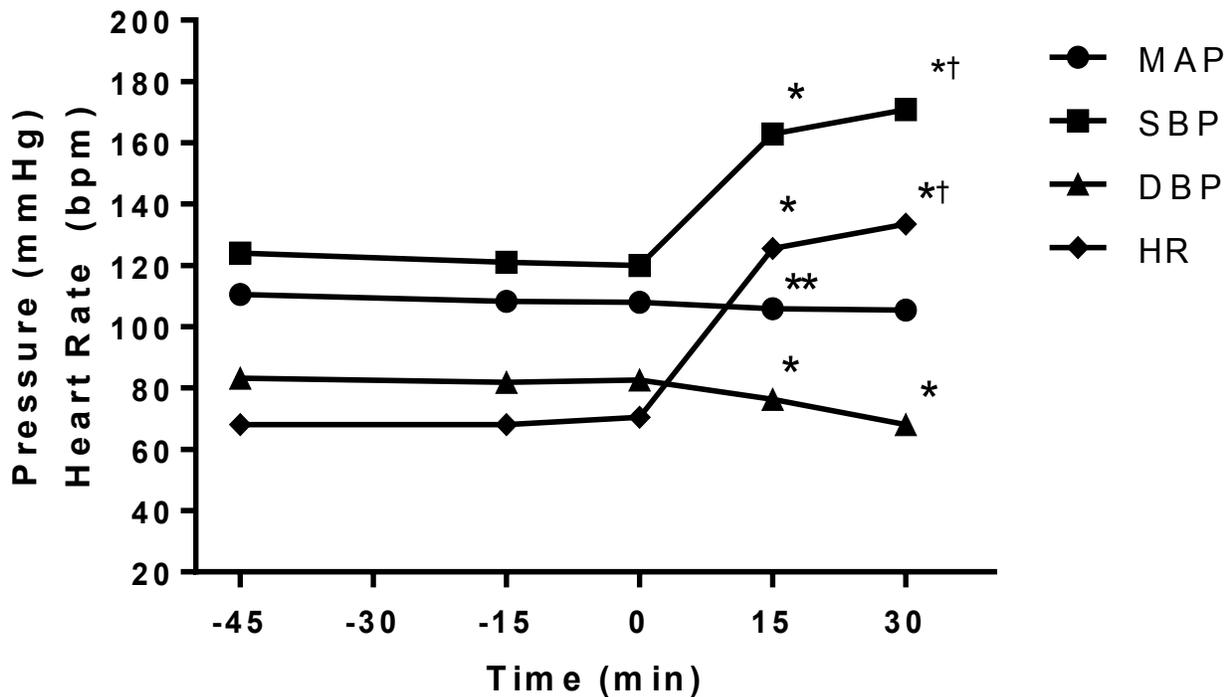


Figure 1. Cardiovascular response to BT supplementation at rest and during aerobic exercise. *Significantly different from rest times ($P \leq 0.013$), **Significantly less than -15 min ($P \leq 0.013$), †Significantly greater than 15 min ($P \leq 0.013$).

DISCUSSION

The purpose of this study was to investigate the effects of acute BT supplementation and moderate intensity exercise on plasma nitrate levels. The present study found that the manufacturer's recommended BT dose of 2.5 g did not significantly increase plasma nitrate levels at rest in young fit males, supporting observations from Bloomer et al. (2) but not Iqbal et al. (14). The conflicting results are most likely due to some combination of the following factors: amount of BT titration (a function of supplementation dosage), subject population, and/or time. Upon ingestion, BT is metabolized and stored in the liver and kidneys with excess BT circulating in the blood (3, 10). With chronic or mega doses, as seen in Iqbal et al., BT may overcome the effect of titration and reach the target tissue (smooth muscle vasculature) enabling upregulation of NO eliciting vasodilation (25) and CVR effects, at least in older individuals. Again, in the present study BT did not affect resting plasma nitrate concentrations in young fit males.

Differences across age in the ability to generate NO and the consequential blood flow implications are documented (12, 24). This relationship may explain the divergent results regarding BT supplementation, NO levels, and age. In short, older individuals with lower basal nitrate and nitrite concentrations may respond more favorably to BT supplementation

than a young population (2). However, Konstantinova et al. (21) observed higher BT levels for older compared to younger subjects confounding this theory. Regardless, it does not appear that BT ingestion improves resting nitrate concentrations in young males. Although NO was not directly measured in this study, it appears that additional research is required to assess the notions of Iqbal et al. (14) and Jallel-Messadek (15).

Moderate intensity exercise was selected because Goto et al. (11) compared the effects of training at three exercise intensities for 30 min in healthy young men and found moderate intensity (50% $\text{VO}_{2\text{max}}$) training enhanced endothelium-dependent vasodilation while low (25% $\text{VO}_{2\text{max}}$) and high (75% $\text{VO}_{2\text{max}}$) intensities did not. The added oxidative stress associated with high intensity training may reduce NO bioavailability, diminishing any vascular effect seen as a result of NO production (25). Additionally, low intensity exercise may not provide a vasodilatory stimulus strong enough to reach a threshold to promote NO release and affect CVRs. Despite selecting an exercise intensity likely to affect the nitrate-NO pathway, plasma nitrate did not increase after the exercise protocol suggesting that BT supplementation combined with moderate intensity exercise has no synergistic effect immediately post-exercise. Although plasma nitrate was not different between groups at any time point, the large effect size ($d = 0.742$) at 30 min suggests CHO + exercise alone has a greater effect on plasma nitrate levels than CHO+BT. However, the standard deviation in the control group at 30 min was twice that of all other measures suggesting this increased plasma nitrate level was an artifact of the assay or biological variation within our sample population and likely not indicative of any effect of CHO or exercise on plasma nitrate levels.

If BT increases nitrate concentration subsequently enhancing NO production, an obligatory vasodilation induced effect on CVRs would be observed. However, we observed BT supplementation in combination with moderate intensity exercise did not impact CVR responses to exercise. This finding is mirrored by previous investigations showing no effect of BT ingestion on HR (1, 28) and blood pressure (33). Similar to the results of this study, Schwab et al. (33) found no significant differences in resting SBP and DBP between a control and BT group after a 12 week hypoenergetic diet in 42 obese subjects. Conversely, Konstantinova et al. (21) demonstrated BT concentration in plasma is inversely related to components of metabolic syndrome. Specifically, SBP and DBP were identified as having an inverse relationship when BT is consumed habitually (-0.73, 95% CI: [-1.03, -0.43] and -0.86, 95% CI: [-1.13, -0.59], $P < 0.0001$, respectively). However, the impact of the small yet statistically significant improvements in SBP and DBP resulting from BT supplementation may not be clinically relevant.

The presented data should be analyzed within the limitations of our work. NO was not directly measured in this study due to its extremely short half-life (ranging from milliseconds to <1 s in circulating blood (18, 22). Typically, NO, in a bidirectional biochemical pathway, is quickly oxidized to the stable end products nitrite and nitrate, which are then typically measured. Similar to previous research, we only evaluated nitrate levels (36) which is the primary NO oxidation metabolite. According to Lundberg and Weitzberg (23), basal plasma nitrite levels are usually in the range of 0.1-0.5 $\mu\text{mol}\cdot\text{L}^{-1}$, as a consequence the combination of

nitrite and nitrate measures are almost identical to nitrate levels. However, other research has shown significant increases in nitrate after a maximal effort test in chronic fatigue syndrome subjects (37). The effects of acute BT supplementation on nitrate or NO concentrations in other tissues (e.g., muscle) or exhaled NO concentrations were not determined in this study. BT supplementation may increase nitrate or NO levels when assessed using these other methods.

The large variance between subject plasma variables (Table 1) diminished the ability to detect statistical significance. Although our sample size was similar to other ergogenic aid/exercise performance cross-over studies, we contend that increasing power (via increasing sample size) would be futile as our main outcome variable was far from significant ($p = 0.23$). Further, the percent change scores were extremely small and in some cases negative. To achieve statistical power, a post-hoc power analysis was performed and revealed up to 1388 additional subjects would be required to find significant differences between groups in plasma nitrate concentrations suggesting the risk of Type 2 error was negligible.

We measured plasma nitrate immediately after the cycling bout instead of during exercise. It is difficult to ascertain whether this methodological factor significantly affected measures of nitrate and theoretically endothelial derived NO. Exercise intensity and duration is correlated with NO production during exercise, however, studies measuring NO post-exercise have shown both significant (29) and non-significant (27, 36) results. Given the variability and difficulty of directly measuring NO and the half-life of its metabolites, perhaps concomitant venous sampling using a catheter would be prudent. Finally, local (quadriceps muscle) increases in NO metabolites may not be sufficient to overcome the diluting effect when circulating throughout the body to be detected at peripheral (antecubital space) sampling sites, a theory originally purported by St. Croix et al (36).

Within the limitations of this study, an acute dose (2.5 g) of BT had no CVR effect at rest or during 30 min of moderate cycling. Additionally, acute BT supplementation did not increase resting or post-exercise plasma nitrate levels. While other ergogenic effects may occur with acute BT supplementation, benefits sought with elevated nitrate or NO production does not appear to occur in young individuals (our data; 2) but may occur in other populations such as older individuals (14, 15), females or untrained populations. Manipulation of acute program variables such as resistance and endurance training modes, intensities, and durations may exhibit differential effects on nitrate and NO production in combination with chronic BT supplementation and merits future investigation. Chronic BT supplementation may produce different NO levels during exercise and should also be explored.

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