SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/SEMOJ-3-151

Research

*Corresponding author
Judy R. Wilson, PhD
Department of Kinesiology
The University of Texas at Arlington
Box 19259, Arlington
Texas 76019, USA
Tel. 817-272-3128
Fax: 817-272-3233
E-mail: jrwilson@uta.edu

Volume 3 : Issue 3
Article Ref. #: 1000SEMOJ3151

Article History
Received: May 2nd, 2017
Accepted: August 4th, 2017
Published: August 4th, 2017

Citation
Almubiadin M, Awadi D, Wilson JR. Effects of dietary nitrate supplementation over four weeks on maximum oxygen consumption in recreational runners: A pilot study. *Sport Exerc Med Open J*. 2017; 3(3): 68-73. doi: 10.17140/SEMOJ-3-151

Effects of Dietary Nitrate Supplementation Over Four Weeks on Maximum Oxygen Consumption in Recreational Runners: A Pilot Study

Mohamed Almubiadin, BS; Daneyah Awadi, BS; Judy R. Wilson, PhD*

Department of Kinesiology, The University of Texas at Arlington, Arlington, Texas 76019, USA

ABSTRACT

Introduction: Nutritional aids to improve exercise performance have become popular. One of the newest is dietary nitrate, often administered as a drink known as beetroot juice (BR). This has led many athletes, both elite and recreational, to consume BR prior to competition or physical activity. However, the results have been inconsistent and indicate that several factors need to be considered.
Purpose: The purpose of this study was to evaluate the effects of BR consumption or placebo in two groups of recreational runners running a minimum of 15 miles per week.
Methods: Ten women and three men volunteered to participate in this four week study. Each participant completed a maximal oxygen consumption test (VO_{2max}). Data from the exercise test used for analysis included VO_{2max}, maximum heart rate (HR_{max}) and time of the maximal exercise test (T). Participants were then randomly assigned to either the Beetroot Juice Group (BRJ, n=7) or the Placebo Group (P, n=6). Each participant was given 16-four ounce bottles prefilled with the juice for their respective group. Participants were instructed to consume the juice 30 min prior to their exercise bouts (4/week) over the next four weeks, then return for follow-up testing.
Results: Thirteen subjects completed the four week study (BRJ=7; P=6) and returned for post-testing. Statistical analyses were conducted using a two factor, repeated measures ANOVA to determine differences between the two groups for the initial and final VO_{2max} tests, as well as within the groups from pre- to post-testing after the four weeks. The initial values for VO_{2max}, (P=44.7±5.0 ml/kg/min; BRJ=47.6±8.4 ml/kg/min) HR_{max} (P=174.7±15.6 bpm; BRJ=171.7±11.2 bpm), and T (P=12:06±1:26 min:sec; BRJ=12:22±0:08 min:sec) were not significantly different between the two groups ($p>0.05$). At the conclusion of the four week study, the results for the post maximal exercise test were VO_{2max} (P=45.0±6.3 ml/kg/min; BRJ=49.3±8.8 ml/kg/min), HR_{max} (P=171.7±16.6 bpm; BRJ=172.3±8.7 bpm), and T (P=12:06±1.36 min:sec; BRJ=12.43±0.07 min:sec) were not significantly different ($p>0.05$). However, there was a significant increase in the average time of the VO_{2max} test for the BRJ of 20.7 sec ($p=0.02$). None of the other variables measured over the four week training session were significantly different ($p>0.05$).
Conclusion: These results indicate that dietary nitrate supplementation (16 oz/week) did not increase exercise performance with the exception of a significant increase in the average time of the VO_{2max} test for the BRJ group. This may be the result of learning to run on the treadmill or a reduction in the O_2 cost of exercise due to possible ergogenic effects of dietary nitrate supplementation. These findings may be further attributed to the varying training regimens, diets, and time of consumption.

KEY WORDS: Beetroot juice; Maximum oxygen uptake; Dietary nitrate; Nitric oxide.

ABBREVIATIONS: RPE: Rated Perceived Exertion; HR_{max}: maximum Heart Rate; VO_{2max}: maximum oxygen uptake; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; NO_3^-: Nitrate; NO_2^-: Nitrite; BR: Beetroot juice; BRJ: Beetroot Juice Group; O_2: Oxygen.

ISSN 2379-6391 http://dx.doi.org/10.17140/SEMOJ-3-151

INTRODUCTION

Elite athletes, their coaches as well as exercise physiologists and sports scientists are constantly looking for ways to enhance performance. But even recreational "athletes" who may simply want to finish a race[1] are often seeking ways to improve training and recovery. Nutritional aids have become popular and one of the newest ones is dietary nitrate, often administered as a drink known as beetroot juice (BR). The high nitrate concentration of beetroot juice is thought to serve as a precursor for nitric oxide (NO) production. This would be an additional source to the endogenous pathway during which L-arginine is oxidized in a reaction catalyzed by the nitric oxide synthase (NOS) family.[2] Nitrate (NO_3^-) is found in all vegetables and is especially abundant in leafy greens and beetroot.[3] After ingestion the NO_3^- is reduced to nitrite (NO_2^-) by anaerobic bacteria in the oral cavity by the action of nitrate reductase enzymes,[4] then to nitric oxide (NO) in the stomach.[5] It is well established that nitrate or BR consumption can significantly increase plasma nitrite concentration (both as a substrate for and a biomarker of nitric oxide production).[6]

Nitric oxide (NO) is an important signaling molecule in many physiological processes including muscle contractility, mitochondrial respiration and biogenesis, and the regulation of tissue blood flow.[7] Physiological mechanisms for NO_2^- reduction are facilitated by hypoxic conditions; therefore, NO as a vasodilator is produced in the parts of the muscle that are consuming or in need of more oxygen (O_2).[8]

These findings suggest that supplementation with NO_3^- to increase the bioavailability of NO to influence muscle function should improve exercise performance, primarily in aerobic metabolism.[9] This has led many athletes, both elite and recreational, to consume BR prior to competition in an effort to improve performance.[10]

However, the results have been inconsistent and indicate that several factors need to be considered. These include the duration of supplementation (acute *vs*. more chronic), the dose of nitrate consumed, the training status of the participants tested, habitual nitrate intake, and the duration and intensity of the exercise.[10] Well-trained individuals will typically have higher baseline plasma nitrite and nitrate values than those who are less physically active.[11] If athletes are already consuming a greater amount of dietary nitrates then the addition of a nitrate supplement may have no effect.[12] Therefore, the purpose of this study was to evaluate the effects of BR consumption or a placebo (Sugar-free Cherry-flavored Kool-Aid) in two groups of recreational runners who had not been consuming beetroot juice as part of their training regimen.

METHODS

Participants

Approval for this study was obtained from the University's Institutional Review Board (IRB) and 13 participants (males and females) gave written informed consent to participate. The inclusion criteria required that participants be willing to consume their given drink four times per week for four weeks and that they were willing to run a minimum of 15 miles per week. Participants were excluded if they were unwilling to consume the drink provided, were allergic to the placebo or beetroot juice or were unable to run a minimum of 15 miles/week for the next four weeks. The 13 participants who completed the program (10 females and 3 males) had an average age of 41.9±14.9 years.

Maximal Exercise Test

The participants reported to the Exercise Science Research Laboratory for an initial maximal exercise test. Participants were asked to dress in exercise clothes for the initial assessment and asked not to eat for 2-3 hours before testing. Water prior to exercise test was allowed and recommended. Upon arriving at the lab, a Polar heart rate monitor was strapped around the chest and the headgear fitted to hold the Rudolph mouthpiece in place during the Bruce protocol. A Parvo TrueOne® 2400 Metabolic Cart was used to assess the oxygen consumption on a breath-by-breath basis while the participants walked/ran on a Quinton Q Stress treadmill. Maximal oxygen uptake (VO_{2max}) and maximum heart rate (HR_{max}) were assessed along with the duration (T) of the maximal exercise test for each participant.

Supplement

Upon completion of the VO_{2max} test, the 13 participants were randomly assigned to either the placebo (sugar-free Cherry-flavored Kool Aid, n=6) group or to the group that would consume beetroot (BRJ, n=7) juice. Lakewood Farms PURE Beet Juice containing organic beetroot juice and organic lemon juice (1%) was used. Each participant was given 16 prefilled bottles and instructed to consume 4 bottles per week. Each bottle contained 4 oz (120 ml) of either the placebo (P) or BRJ and the participants were instructed to consume one bottle 15-30 min prior to their workout, at least 3 times per week. The 4th bottle could be consumed prior to a 4th workout or sometime during the week if they routinely worked out 3 days per week. Participants were then asked to continue their regular exercise routine, minimum of 15 miles/week and to log the day the liquid was consumed along with the distance in miles they ran. The 4 oz or 120 ml of concentrated BRJ contained 9.7 mmol NO_3^-.

At the completion of four weeks each participant returned to the Exercise Science Research Laboratory and repeated the maximal exercise test utilizing the Bruce protocol. No additional beetroot juice was consumed prior to the final exercise test.

Statistical Analysis

Analyses were conducted using SPSS (Version 23.0; IBM SPSS Inc., Chicago, IL, USA) to conduct a two factor repeated mea-

ISSN 2379-6391 http://dx.doi.org/10.17140/SEMOJ-3-151

sures ANOVA with one within subjects factor time (pre, post) and one between subjects factor group (placebo, beetroot juice). The following variables were analyzed: VO_{2max}, HR_{max}, and duration of maximal exercise test (in minutes and seconds). The alpha level for significance was set at $p \leq 0.05$.

RESULTS

Thirteen participants completed the study (P, n=6; BRJ, n=7). The demographic information for the two groups is provided in Table 1. Differences in the variables measured during the initial maximal oxygen consumption test are found in Table 2 and those same variables measured at the end of the four weeks of consuming either the sugar-free Kool-Aid (P) or the beetroot juice (BRJ) are found in Table 3. There were no significant differences found in the initial maximal exercise test results for VO_{2max}, the HR_{max}, or the time (T) of the maximal exercise test between the two groups at the beginning of training session or for the final maximal exercise results ($p>0.05$). However, analysis of the changes within each group, over the course of the four weeks, indicated a significant increase ($p=0.02$) in the time of the VO_{2max} test (20.7±16.9 sec) for the BRJ group (Table 5) but not for the other variables for the BRJ or P groups (Table 4; $p>0.05$).

The changes (Δ) for each variable were also analyzed using ANOVA to determine if the two groups changed differently over the four week time period (Table 6). However, there were no significant interactions between the two groups for any of the variables ($p>0.05$).

From the exercise logs it was determined that the runners for the BRJ averaged 22.5±9.7 miles/wk while the P group averaged 21.7±5.6 miles/wk.

Table 1: Subject Demographics.

P (n=6)	Mean	SD	BRJ (n=7)	Mean	SD	*p* values
Age (yrs)	44.7	18.7		39.6	12.0	0.56
Height (cm)	166.1	4.54		170.4	7.3	0.24
Weight (kg)	62.4	6.2		60.6	16.2	0.80

Table 2: Pre 4 Week Training Test Results – Between Groups.

	Placebo (n=8)		Beet Juice (n=8)		
	Mean	SD	Mean	SD	*p* values
VO_{2max} mL/kg/min	44.7	5.0	47.6	8.4	0.47
HR_{max} bpm	174.6	15.6	171.7	11.2	0.69
Time Max Min:sec	12:06	1:26	12:22	0.08	0.64

Table 3: POST 4 Week Training Test Results – Between Groups.

	Placebo (n=6)		Beet Juice (n=7)		
	Mean	SD	Mean	SD	*p* values
VO_{2max} mL/kg/min	45.0	6.3	49.3	8.8	0.34
HR_{max} bpm	171.7	16.6	172.3	8.7	0.93
Time Max Min:sec	12:06	1:36	12:43	0.07	0.52

Table 4: Within Group (Placebo).

	Pre		Post		
	Mean	SD	Mean	SD	*p* values
VO_{2max} mL/kg/min	44.7	5.0	45.0	6.3	0.82
HR_{max} bpm	174.6	15.6	171.7	16.6	0.11
Time Max Min:sec	12:06	1:26	12:06	1.36	0.30

ISSN 2379-6391 http://dx.doi.org/10.17140/SEMOJ-3-151

Table 5: Within Group (Beetroot Juice).

	Pre		Post		
	Mean	**SD**	**Mean**	**SD**	***p* values**
VO_{2max} mL/kg/min	47.63	8.4	49.3	8.8	0.21
HR_{max} bpm	171.7	11.2	172.3	8.7	0.85
Time Max Min:sec	12:22	0.08	12:43	0.07	0.02

Table 6: 4-Week Changes (Δ).

	Placebo (n = 6)		Beet Juice (n=7)		
	Mean	**SD**	**Mean**	**SD**	***p*-values**
Δ VO_{2max} mL/kg/min	0.3	3.1	1.7	3.0	0.44
Δ HR_{max} bpm	-3.0	3.8	0.6	7.5	0.32
Δ Time Max sec	8.33	18.18	20.71	16.9	0.23

DISCUSSION

The results of our study of comparing the effects of BRJ to a placebo over the course of four weeks did not show any significant improvement in VO_{2max} or HR_{max}. However, the BRJ group had an increase in the time of exercise (20.7±16.9 sec) that was significantly different (p=0.02) from the increase of the P group (8.3±18.1 sec). Bailey et al[13] hypothesized that dietary BR supplementation would reduce the O_2 cost of moderate-intensity exercise and increase exercise tolerance. Their definition of increased exercise tolerance was assessed as an increase in the "time to task failure" and results showed an increased time to task failure of 16%, suggesting that dietary nitrate supplementation might enhance high intensity exercise performance. Our results appear to be in agreement that the beetroot juice supplementation increased the time to exhaustion (task failure) during the maximal exercise test for the BRJ as there were no significant increases in either the VO_{2max} and HR_{max}.

There are other reasons that may account for this lack of change in exercise performance. Participants were asked to continue their individual training regimens and only asked to complete a minimum number of miles/week (15 miles) and to consume the BR 15-30 min prior to at least three workouts per week. The 4th bottle could be consumed prior to a workout or at anytime during the week. No instruction was given as to the time of day of their workouts. For those who prefer to conduct their workouts in the morning, it is possible that the BR may have been consumed following the use of an antibacterial mouthwash. An early study showed that the increase in plasma nitrite after consuming dietary nitrates is the result of nitrate accumulation in saliva and reduction to nitrite by oral bacteria. The activity of oral bacteria is stopped by antibacterial mouthwash activity thus attenuating any rise in plasma nitrate.[6]

Training status has also been shown to have an impact on whether dietary nitrate supplementation will improve performance. Endurance-trained athletes have higher eNOS (endothelial NO synthase situated in the capillary walls) and nNOS (neuronal NO synthase situated in muscle fibers) activity[14,15] which will likely lead to an increased NO production *via* the L-arginine pathway and may explain why the majority of studies with trained individuals (VO_{2max}>60 ml/kg/min) report no effects on exercise performance[16,17]. The runners in our study withVO_{2max}- levels in the mid-40s's range (45 ml/kg/min P; 46 ml/kg/min BR) would not be considered "elite" and it was anticipated that there would be some benefit from consumption of the dietary nitrate supplementation.

The dosage or mmol/L of dietary nitrates consumed has been shown to impact exercise performance. Our dosage of 9.7 mmol NO_3^- per dose was similar to that found to elicit responses in exercise performance to acute consumption (8.4 mmol).[12] However, when the effects of long-term consumption of dietary supplementation (15 days) was investigated, participants consumed the supplement on a daily basis.[18] Our study extended the time over which the supplement was consumed (4 weeks) however, the total days of consumption would have been less (12 days *vs*. 15 days). It would seem that four doses per week of even a high concentration of BR were not sufficient to improve exercise performance.

Due to the large daily energy expenditure of athletes, it is likely that these individuals are already consuming greater amounts of dietary nitrates suggesting that additional intake may not be effective.[10] Upon questioning participants as to their daily vegetable intake, the majority indicated that they did not consume the daily recommended vegetable servings and that it was closer to 1-3 servings/day. One subject in the BRJ stated that they consumed a vegetarian diet, and, in fact, her VO_{2max} was the only one to show a decrease within the BRJ group. In a previous investigation, supplementation of dietary ingestion of nitrate ap-

ISSN 2379-6391 http://dx.doi.org/10.17140/SEMOJ-3-151

peared to have actually reduced VO_{2max}.[19]

CONCLUSION

Four weeks of consumption of a high dose of dietary nitrate supplementation did not improve maximal exercise performance when compared to a placebo. Despite a high concentration of the dietary nitrate, four doses per week did not appear to be sufficient to impact maximal exercise. Another recommendation that should be made is for the timing of the consumption of the BR prior to exercise. In a previous study, the acute effects of BR consumption on plasma [NO_2^-] were assessed and it was found that levels peaked 3 h post-ingestion and remained close to peak values until 5 h post-ingestion.[20] Consumption by our participants 30 min prior to exercise may have resulted in the plasma levels peaking when they had completed their exercise bout. Future studies should include charting dietary intake to determine the nitrate consumption from the daily diet to determine the total intake along with any supplementation.

The one significant finding of an increased time of exercise by BRJ group (p=0.02) supports the findings by Bailey et al,[13] while their study found a considerable reduction in the O_2 cost during submaximal cycle exercise, it was also suggested that increased dietary NO_3^- intake has the potential to enhance exercise tolerance during high intensity exercise performance. Therefore, the possible ergogenic benefit of dietary nitrate supplementation should also be an area of further research.[13]

ACKNOWLEDGEMENTS

The authors wish to thank Brian Prejean, M.S. for his help with the statistical analysis.

AUTHORS CONTRIBUTIONS

All authors have contributed to the data collection and writing of this manuscript.

CONFLICTS OF INTEREST

The authors indicate that they have no conflicts of interest to declare.

PARTICIPANTS CONSENT

The Informed Consent Document was approved by the Institutional Review Board for the Protection of Human Subjects at our University.

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