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Efficacy on performance of athletes on supplementation of beetroot juice

Sri Vidhya NAC, * Radhai Sri S
Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Abstract
The nitrate in beet juice enhance nitric oxide bioavailability through supplementation to lower blood pressure, to reduce the oxygen (O2) cost of physical exertion and improve athletic performance. Hence, an effort has been taken to test the efficacy of beetroot juice on performance of athletes in 12 healthy subjects (Age: 19±1.54; BMI: 20±1.9). Following baseline tests, subjects were assigned to a randomized single blind cross over design to receive Beetroot juice (BR) 0.5L for 6 consecutive days. Performance of beneficiaries (experiment group n = 6) were assessed by physical parameters including speed test, endurance test and physiological measures such as blood pressure, pulse rate and compared with control (CT) non-supplement group (n=6). Speed test showed a significant difference between control and experimental group in time trail performance of 50mt dash (mean ± SD, CT 6 ± 0.37 vs. BR 5 ± 0.19 sec. µsec; $p<0.05$). Similarly, experimental group showed a reduction in time to complete the task (mean ± SD, I 6 ±0.37 vs. F 5 ± 0.19 sec. µsec). In endurance test BR increased the number of counts in experimental group from 40 ± 6.2 to 55 ± 6.8 counts / min (mean ± SD) and showed a significant increment in number of counts in comparison with control group (mean ± SD, CT 43 ± 4.8 vs. BR 55 ± 6.8 counts/min; $p< 0.05$). A significant reduction in systolic blood pressure was observed in experimental group (mean ± SD, I 123 ± 10.42mmHg vs. F 116 ± 7.7mmHg). However, no alteration / change was observed in pulse rate. These results indicate that positive effects of 6 days of beetroot juice supplementation on performance of athletes can be ascribed to the high nitrate content per se.

Keywords: nutrition, sport, exercise, nitric oxide, physical activity

1. Introduction

Beetroot (*Beta vulgaris* L.ssp.culgaris,) is a member of the *chenopodiaceae* family which include silver beet, sugar beet and fodder beet (Grubben and Denton, 2004) [1]. Beetroot has been considered as a nutritional food since early Roman and Greek times for reduction of fevers and for the blood. Beetroot should be obtained fresh and grated or juice for maximum benefits (Manfred Urs Koch, 2011). Beetroot juice is a rich source of dietary nitrate as well as other metabolically active compounds including antioxidants and polyphenols (Ferreira and Behnke, 2009; Bailey *et al.* 2009) [3, 12]

It is widely accepted that increased intake of the preservatives sodium nitrate found in processed meats such as deli metas, bacon and hot dogs is associated with negative health outcomes for certain subgroups of the population (Gilchrist, 2009) [4]. These association have resulted in dietary nitrates and nitrites receiving a "bad name". However scientist have been quick to point out diets proven to be beneficial for cardiovascular health contain high amount of fruits and vegetables, are low in sodium and contain polyphenols, potassium, fibre and nitrate (Hord *et al.* 2009) [5].

Nitrate is one of the family of compounds containing nitrogen and oxygen that is both found in our diets and produced within our bodies. Our main dietary nitrate sources are vegetables, processed meats and the water supply (Australian sports commission, 2011) [6]. Nitrate (NO3) and nitrite (NO2) have been known predominantly as undesired molecules in the food chain with potentially harmful effects, or as inert oxidative end products of endogenous nitric oxide (NO) metabolism (Lundberg *et al*, 2008) [24]. However, research carried out over the last decade has shown that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides (Lundberg and Giovoni, 2004) [25].

Dietary nitrate has recently emerged as a potential modulator of resting blood pressure (Kapil *et al.* 2010; Vanhatalo *et al.* 2010 and Webb *et al.* 2008) [7-9, 11] and muscle energy metabolism (Larsen *et al.* 2007 and Bailey *et al.* 2010) [10, 11] and a possible "natural" ergogenic aid to exercise performance (Bailey *et al.* 2009; Bailey *et al.* 2010 and Lansley *et al.* 2011) [10-12].

Strikingly, dietary supplementation either with pharamacological sodium nitrate or nitrate rich beetroot juice has been shown to result in a significantly reduction in the oxygen cost of submaximal cycling (Larsen *et al.* 2007 and Bailey *et al.* 2009) [10, 12], knee extensor exercise (Bailey *et al.* 2010) [11] and treadmill walking and running (Lansley and Winyard, 2011) [14]. This enhanced exercise efficiency is likely to be mediated through the metabolic conversion of inorganic nitrate to bioactive nitrite and subsequently, nitric acid (Benjamin *et al.* and Cosby *et al.* 2003) [16].

Dietary nitrate supplementation has received much attention in the literature due to its proposed ergogenic properties (Cermak *et al.* 2012) [17]. Considering the most beneficial aspect of beetroot, an effort was made to develop beetroot juice and test its efficacy on performance of athletes.

2. Methods and materials

2.1 Preparation of beetroot juice

Beetroots were washed with clean water to remove dust, dirt and outer material. The weight of beetroot was taken using electrical weighing balance (digital balance). Sorting and grading was done by manual methods. The beetroot was cut into slices of about of 2-3 mm thick and subjected to extraction using juice pulper. Optimum quantity of sugar and citric acid were added to juice and then mixture was filtered through muslin cloth, further pasteurized at 85°C to destroy enzymes and reduce the microbial load and filled in 200ml hot filling pet bottle.

2.2 Selection of subjects and sample size

Stephen *et al.* (2011) [22] conducted a double blind, crossover study. Seven men consumed 500ml/day of nitrate rich beetroot juice resulted in a reduction in muscle concentration amplitude of the slow components and improved time to exhaustion. Many studies reveals a small sample size was more self-sufficient to give an accurate data in dietary nitrate supplementation. Twelve well trained athletes (Mean ± SD: age = 19 ± 1.5, height =176 ± 8.6, weight = 63 ± 7.6, Body mass = 20 ± 1.9) of PSG College of Arts and Science volunteered to participate in this study. All subjects were healthy and free of disease. The subjects also abstained from using antibacterial mouthwash and chewing gum during the supplementation period because these are known to eradicate the oral bacteria that are necessary for the conversion of nitrate to nitrite. None of the subjects consumed dietary supplements. Before testing, subjects gave their oral informed consent to participate after the experimental procedure, associated risks and the benefits of participation were explained. Base line survey was conducted to record the details of the subjects and pre participation sports questionnaire was administered to collect necessary details.

2.3 Pre supplementation tests

The subjects were randomly assigned in a single blind crossover design to one of two condition: Beetroot (BR) or Placebo (PL) (Hannah *et al.* 2010) [18]. Subjects were required to report at 4.00 pm to the physical education department of PSG CAS on the day before the intervention begins. Blood pressure and pulse rate were taken initially for both the groups before the physical fitness test was conducted so that there was no difference marked in pressure by the test conducted. After an hour subjects were required to perform 50mt dash as a parameter of speed test. Endurance test was marked by number of sit ups performed by subjects in a minute. There was an interval of about half an hour between the speed test and endurance test.

2.4 Supplementation

Lansley *et al.* (2011) [14] reported improved treadmill run time to exhaustion in a severe-intensity treadmill test after 4 and 5 days of supplementation. Bailey *et al.* (2010) [11] utilized various protocols involving high intensity exhaustion knee extension and cycling tests and found that 4-6 days of supplementation improved exercise time to exhaustion. 500ml of beetroot juice was supplemented to the test group and juice was not given to the control group. 500ml of beetroot juice contains 6.2mmol of nitrate.

In the present study, a 6 day supplementation period was implemented where subjects required to consume 500ml of their designated beverage each day. Placebo group did not receive any juice. 24 hour before the supplementation, subjects were asked to record their food and fluid intake and physical activity. The subjects were instructed to arrive at the physical education department in a rested and fully hydrated state, at least 1 hour postprandial and to avoid strenuous activity in 24 hour preceding the test session. Subjects were also asked to refrain from caffeine and alcohol 6 and 24 hour before the test.

During the supplementation period, physiological measures such as blood pressure and pulse rate were measured for experimental group before the ingestion of beetroot juice. Subjects were administered with 500ml of beetroot juice. After ingestion of the beetroot juice subjects were given a period of one hour which they were allowed to leave the department but were asked to refrain from strenuous physical activity. Subjects were also asked to fast during this time, although water was permitted adlibitum. Subjects returned after an hour to the department and blood pressure and pulse rate measurements were repeated. Consecutively the test was repeated for three days.

2.5 Post supplementation test

On day 6 of the supplementation period, subjects of control and experimental groups reported to the physical education department. The instruction followed for pre-test was again repeated for post supplementation test. Blood pressure and pulse rate were monitored for both the groups. During the measurement, subjects were informed to be in a relaxed mind, so that there is no alternative in pressure measurements. After an hour, subjects performed 50mt dash speed test and endurance test. Time interval of half an hour was left between the speed test and endurance test.

3. Analysis of data

Data on quality attributes of the developed beetroot juice, physical and physiological measures were analysed statistically using the following statistical tools namely standard deviation and 't' test.

4. Results and Discussion

4.1 Anthropometric measurements of athletes

Athletes studying at PSG College of Arts and Science, Coimbatore were included for the study. The selected male athletes were in the age group of 15 – 20 (58 per cent) and 20-25 (42 per cent) years. Anthropometric data showed that 33 and 66 per cent had the mean height of 150-160 and 160-170cm respectively. Similarly, 58 and 42 per cent of their weight ranged between 45-60 and 60-75kg respectively. On computing the Body Mass Index, the mean value was 20.48 indicating that they come under normal category.

4.2 Nutrient intake

Nutrient intake of the subjects based on 24 hour recall method is given in Table -1

4.3 Efficacy of developed beetroot juice on performance of athletes

Nitrate supplementation had favourable effects on phosphocreatine recovery time and muscle pH, factors that could contribute to enhance exercise performance compared to placebo group under hypoxia condition. Dietary nitrate supplementation enhance muscle energy efficiency during exercise, which could lead to a reduction in oxygen consumption (Vanhatalo *et al.* 2011) [13, 22]. Fitness can be gauged through conducting various tests i.e., performing tasks (Shubhangini, 2007) [23]. Parameters including speed and strength were assessed. Speed and strength tests were performed before initiation of supplementation for both control and experimental groups and during post intervention period. Blood pressure and pulse rate were measured for both the groups on pre and post supplementation period.

4.3.1 Physical parameters

Fitness can be gauged through conducting various tests i.e., performing tasks (Shubhangini, 2007) [23]. Parameters including speed and strength were assessed. Speed and strength tests were performed before initiation of supplementation for both control and experimental groups and during post intervention period.

Physiological measures Blood pressure and pulse rate were measured for both the groups on pre and post supplementation period.

4.3.1.1 Speed Test

Initially athletes under control group covered 50mt dash between 6.30 to 6.71 sec. microsec whereas it ranged between 6.25 to 6.84 sec. microsec for experimental group of athletes. After intervention period reduction in time taken to complete the task was relatively low 5.55 to 6.12 by experimental group of athletes whereas it was similar to initial level in the case of (5.76 to 6.46 sec. microsec) of control group of athletes. This could be attributed to supplementation of beetroot juice. Dietary nitrate supplementation with 5 – 7 mmol nitrate results in a significant increase in plasma nitrate and associated physiological effects including a lower resting blood pressure, reduced pulmonary oxygen uptake during submaximal exercise and enhanced exercise tolerance or performance (Bailey *et al.* 2009; Vanhatalo *et al.* 2010; Lansley *et al.* 2011 and Lansley K.E, 2011) [8, 11-14]. There was no significant difference observed between the groups (control and experimental) in time trail to complete the 50mt dash before initiating the supplementation. Extreme statistically significant difference in time trail was observed within the experimental group between pre and post supplementation (P value: 0.0033, 't' Value: 5.2507). Control group showed no statistical significant difference between the time trail recorded before and after the study. Significant difference with a 't' value of 3.1188 was observed between the groups on post-test trail.

4.3.1.2 Endurance test

Initially control group of athletes recorded 41 to 50 counts per min whereas it ranged between 31 to 51 counts by experimental group of athletes. Considering the post-test trial, it ranged from 39 to 50 and 42 to 60 counts by control and experimental groups respectively. There was no significant difference between control and experimental group of athletes in number of counts per minute during initial stage, yet significant difference was exhibited between the groups at the final stage as well as within the experimental group (P value: 0.0032, 't' Value: 5.3047) (I vs. F). The results are concurrent with the study of Stephen *et al.* (2011) [22] who mentioned that during low intensity exercise, BR attenuated the reduction in muscle phosphocreatine concentration (PL 8.1 ± 1.2 vs. BR 5.2 ± 0.8mM; P< 0.05) and the increase in VO_2 (PL 484 ± 41 vs. BR 362 ± 30ml/min; P< 0.05). During high intensity exercise, BR reduced the amplitudes of phosphocreatine concentration (PL3.9 ± 1.1 vs. BR 1.6 ± 0.7mM; P< 0.05) and VO_2 (PL 209 ± 30vs. BR 100 ± 26ml/min; P< 0.05) slow components and improved time to exhaustion (PL 586 ± 80 vs. BR 734 ± 109s; P< 0.01). The reduced muscle metabolic perturbation with nitrate supplementation allowed high intensity exercise to be tolerated for a greater period of time.

Table – 2 contains the changes in performance level on supplementation of juice.

4.3.2 Blood Pressure

Efficacy of developed beetroot juice on physiological measures Most health related research with nitrate supplementation, either sodium nitrate or dietary source of nitrate, has focused on vascular aspects. Research has shown that dietary approaches to stop hypertension (DASH) diet, which may be rich in vegetables and nitrates, is an effective means to lower blood pressure (Frisoli *et al.* 2011) [20].

Table – 3 Mean blood Pressure of athletes before and an hour after supplementation

Systolic blood pressure recorded for athletes showed no significant difference between the groups during pre and post-test periods. Likewise no significant difference exist between the initial and final stage in control group however experimental group showed a significant difference between the blood pressure recorded before and after supplementation period with the p value of 0.0137. This was confirmed by Coles and Clifton, (2012) [21] who found that there was trend (P = 0.064) to lower systolic blood pressure at 6-h after drinking beetroot juice relative to placebo. On Diastolic blood pressure, Significant difference in blood pressure was observed between the groups on post-test ('t' value: 3.7471; P value: 0.0038) and within the experimental group (I vs. F) ('t' value: 4.1582; P value: 0.0088). No significant difference was found between the groups on pre-test ('t' value: 0.2796; P value: 0.7855) and within the control group ('t' value: 0.2997; P value: 0.7765). This is in accordance with the studies of Larsen *et al.* (2006) [19], who found that sodium nitrate supplementation, in amounts equivalent to 150-250 g of nitrate rich vegetables as found in the DASH diet, significantly reduce diastolic blood pressure in young, normotensive subjects. No significant difference was found between and within the group at any point of study (before and after supplementation) in pulse rate.

5. Summary and Conclusion

Dietary supplementation with beetroot juice had exhibited a significant reduction in systolic blood pressure and improved the performance specially enhancing the athletic performance,

which would involve subjects completing a given distance (50mt dash) in the fastest possible time.

6. Tables

Table 1: Nutrient intake of the subjects (24 Hour Recall)

Nutrients	Mean ± SD
Energy	4203
Carbohydrate	812
Protein	106 ± 37
Fat	58
Fibre	10 ± 4.3
Minerals	14 ± 4.5

Table 2: Changes in performance level on supplementation

Group	Speed test Time (Sec. microsec)		Endurance Test Situps /min	
	Initial	Final	Initial	Final
Control group	7 ± 0.18	6 ± 0.37	44 ± 3.9	43 ± 4.8
Experimental Group	7 ± 0.26	5 ± 0.19	40 ± 6.2	55 ± 6.8

Table 3: Mean blood Pressure of athletes before and an hour after supplementation

Group	Control	Experimental
Initial		
Systolic (mm HG)	132 ± 6.1	127 ± 4.7
Diastolic (mm HG)	71 ± 7.5	72 ± 8.1
Pulse Rate	71 ± 8.4	73 ± 6.0
Final		
Systolic (mm HG)	123 ± 10.42	116 ± 7.7
Diastolic (mm HG)	70 ± 4.4	60 ± 4.7
Pulse Rate	84 ± 11.4	76 ± 5.34

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