The effect of beetroot juice (*Beta Vulgaris L.*) supplementation on ṼO_2max of youth soccer athletes

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Abstract

Soccer athletes' performance is influenced by perfect ṼO2max. However, athletes often receive rigorous exercise without being supported by proper nutrition which can lead to oxidative stress. It is necessary to maintain performance by supplementing beetroot juice which contains betalains and inorganic nitrates so that oxidative stress can be controlled. This research is aimed to determine the effect of beetroot juice on the ṼO2max of soccer athletes. By using true experimental with randomized pre-test post-test control group design, 16 healthy males aged 16-19 years old were divided into two groups, treatment, and control. Data were analyzed by independent sample t-test and paired t-test. There is a significant difference in weight and body mass index ($p<0.05$). There is no significant difference in age, height, pre-body fat, post-body fat, Δbody fat, nutrition intake, school hours, sleep hours, and physical exercise as well as ṼO2max before and after intervention ($p>0.05$). There is a significant difference in ṼO2max change in both groups ($p<0.05$). Thus, both beetroot juice and placebo significantly affected the increase of ṼO2max levels in soccer athletes.

Introduction

Soccer athletes are required to have excellent performance in every match which influenced by a perfect ṼO_2max. The average ṼO_2max of soccer athletes from PORPROV athletes in Batu City, East Java Province was 52.38 ml/kg/min.[1] The differences of ṼO_2max in each position such as forwarder, midfielder, and defender respectively were 45.69, 49.34, dan 42.54 ml/kg/min.[2] Then, the ṼO_2max of soccer athletes at PPLP South Sulawesi was 39.9 ml/kg/min.[3] ṼO_2max is the maximum amount of oxygen the body can consume during intense physical activity until the fatigue occurs.[1]

Several ways to improve soccer athlete's performance besides focusing on technique and tactic are by increasing physical fitness.[4] On the other hand, athletes generally receive physical training to improve technical skills, tactics, and physiological functions that can support performance which usually last from 3-4 months. In addition, several studies stated that rigorous physical exercise can cause oxidative stress.[5] Oxidative stress is associated with fatigue or tissue damage that can reduce athlete's performance and physical endurance.[6] The oxidative stress can be overcomed with proper nutrition management by giving exogenous antioxidants.

Beetroot (*Beta vulgaris L.)* contains *betalains* which have been shown to have anti-carcinogenic and anti-inflammatory effect.[7] Beetroot is one of the antioxidants and micronutrients sources including potassium, betain, sodium, magnesium, vitamin C, and nitrate.[8] In Indonesia, beetroots are widely cultivated in Java highlands especially Cipanas, Lembang, Pengalengan, and Batu.

Beside its antioxidant benefit, beetroot juice has been widely commercialized via internet with the aim of digestive health and improving blood circulation, increasing energy, natural "detox", and is claimed to increase nitric oxide levels which have an impact on blood flow. This is confirmed by previous research that there was a relationship between inorganic nitrate supplementation in the form of beetroot juice on physical performance seen from the increase in plasma nitrite concentration, decrease in blood pressure, and positively affect the physiological response after exercise.[9] In addition, other finding also found that there was an increase in *time trial*, *power output,* and ṼO_2max in group that consumed beetroot juice for six days.[10] Beetroot contains 250 mg NO_3^- per 100 grams net weight, which is relatively high compared with similar plants such as spinach, celery, lettuce, and carrot.[8]

In addition, it is also reported that linoleic peroxidation by cytochrome C could be inhibited by *betanin* from consumption of beetroot.[11] This explains that daily consumption of beetroot juice can provide protection against the production of oxidative stress and can create new strategies to speed up recovery process *after* training and during matches.

Therefore, intake of a diet high in antioxidants in the form of sport drinks to be given to athletes is still very wide open. This study is aimed to determine the effect of beetroot juice on ṼO_2max of youth soccer athletes, expected to be the basis for determining appropriate interventions to improve athlete's performance in Indonesia.

Materials and Methods

This research used a true experimental with randomized pre-test post-test control group design, conducted in Aji Santoso International Football Academy (ASIFA), Malang City. Subjects were healthy males aged 16-19 years old selected by consecutive sampling with total 16 subjects, consisting of 8 treatments and 8 controls that willing to sign the informed consent. Subjects were given the explanation regarding the objectives, courses, and benefits of

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the research. Informed consents were sent to subjects' parent whose age are less than 18 years old. Treatment groups were asked to consume 250 ml beetroot juice (~8.6875 mmol NO_3^-) while control groups were asked to consume 250 ml placebo (0 mmol NO_3^-). The intervention was carried out for 13 days. Multistage Fitness Test (MFT) were performed twice before and after the intervention to investigate the $\tilde{V}O_2$max of soccer athletes.

The control variables as confounding factors were nutrition intake, school hours, sleep hours, and physical exercise. In terms of nutrition intake, daily food recall was done to determine the daily nutrition adequacy and consumption of foods containing antioxidants. In terms of physical exercise, subjects were interviewed the daily training and outside of regular training by its duration, intensity, and frequency.

Data were analyzed by independent sample t-test and paired t-test. This study has received *Ethical Clearance* from *Medical and Health Research Ethics Committee* (MHREC) Faculty of Medicine, Nursing, and Public Health; Universitas Gadjah Mada, with reference number KE/FK/1043/EC/2017.

Results

Table 1 shows that there is no significant difference between age, height, pre body fat, post body fat, and Δbody fat in both groups (p>0.05). In addition, it is known that there is a difference in weight and body mass index (BMI) significantly between both groups (p<0.05).

Table 2 shows that there is no significant difference between energy, protein, fat, and carbohydrate intake of subjects in both groups (p>0.05). In term of micronutrients, there is also no significant difference between intake of sodium, potassium, vitamins A, C, and E of subjects in both groups (p>0.05). Besides, there is no significant difference between intake of iron, zinc, non-beet nitrates, and total nitrates of subjects in both groups as well (p > 0.05).

Table 3 shows that there is no significant difference between school hours, sleep hours, as well as frequency and duration of

Table 1. Characteristic of subjects.

Characteristics	Control (n = 8)			Treatment (n = 8)			p-value
	Mean±SD	Min	Max	Mean±SD	Min	Max	
Age (years old)	15.38±0.74	15	17	16.25±1.04	15	17	0.083**
Weight (kg)	52.39±5.42	42	58,1	64.78±4.74	59	72.9	0.000*
Height (cm)	165.69±4.96	159.5	174	166.8±6.18	158	175	0.69*
BMI (kg/m^2)	19.05±1.61	16.44	21.33	23.32±1.63	21.28	25.66	0.000*
Pre body fat (%)	18.58±2.65	13.5	21.3	20.08±2.69	17.9	25.5	0.28*
Post body fat (%)	18.61±2.4	15	22.5	21.38±2.76	18.8	26.7	0.51*
Δbody fat (%)	1.31±0.55	0.2	2.1	1.5±1.31	0.4	4.6	0.72*

*Independent sample t-test; **Mann-Whitney test.

Table 2. Nutrition intake of subjects.

Nutrition Intake	Control (n = 8)			Treatment (n = 8)			p-value
	Mean±SD	Min	Max	Mean±SD	Min	Max	
Energy (kcal)	2196.4±261	1834.7	2626.4	2149.1±391	1407.6	2614	0.78*
Protein (gr)	85.1±11,7	69.81	102.57	82.7±17.6	53.4	104.2	0.77*
Fat (gr)	115.5±16.31	90.02	138.8	114.4±18.64	84.35	130.5	0.9*
Carbohydrate (gr)	238.8±39.75	183.5	294.45	213.4±65.21	104.85	308.6	0.36*
Sodium (mg)	1234±284.5	616.58	1512.8	1266.9±352.6	764.25	1753.2	0.84*
Potassium (mg)	2070.7±449.73	1549.5	2787.2	1991.7±532.1	1362.8	2704.3	0.75*
Vitamin A (ug)	811.6±250.8	518.77	1265.3	674.6±202.15	363.8	924.6	0.25*
Vitamin C (mg)	54.4±36.15	23.9	122.4	197.4±420.16	12.24	1234.4	0.92**
Vitamin E (mg)	5.24±1.14	3.54	6.66	4.63±1.32	2.69	6.47	0.34*
Iron (mg)	12.31±4.46	8.59	22.24	11.17±3.37	6.95	15.63	0.57*
Zinc (mg)	7.76±1.59	5.85	10.19	7.27±1.71	5.21	9.29	0.56*
Non-beet Nitrates (mg)	272.54±108.49	125.97	423.05	239.41±93.24	157.81	416.63	0.52*
Total Nitrates (mg)	272.54±108.49	125.97	423.05	395.66±93.24	314.06	572.88	0.29*

*Independent sample-t test; **Mann-Whitney test.

Tabel 3. School hours, sleep duration, and physical exercise (PE) of subjects.

Conditioning	Control (n = 8)			Treatment (n = 8)			p-value
	Mean±SD	Min	Max	Mean±SD	Min	Max	
School Hours (hour)	3.56±0.68	2.5	4.5	3.88±0.64	3	4.5	0.36*
Sleep Hours (hour)	7.19±0.92	6	8,5	7.13±0.88	6	8.5	0.89*
PE Frequency (times/week)	3.63±0.74	3	5	4.88±2.23	3	9	0.39**
PE Duration (minutes)	84.28±32.9	45	135	112.5±52.6	30	210	0.22*

*Independent sample-t test; **Mann-Whitney test.

physical exercise in both groups ($p>0.05$). The types of PE usually done outside the routines include *ball games*, push up, jogging, *ball possession game*, *drilling*, weightlifting, swimming, *crossing-passing*, and rope skipping.

Table 4 shows that there is no significant difference between pre $\tilde{V}O_2$max, post $\tilde{V}O_2$max, and $\Delta\tilde{V}$O2max in both groups ($p>0.05$).

Table 5 shows that there is a significant difference in $\tilde{V}O_2$max change in both groups ($p<0.05$). It can be concluded that both placebo and beetroot juice consumption affect significantly to the increase of soccer athletes' $\tilde{V}O_2$max.

Discussion

The effect of beetroot juice on $\tilde{V}O_2$max

Descriptively, the average Multistage Fitness Test (MFT) results in treatment group showed a significant increase from 50.88 ml/kg/min to 54 ml/kg/min ($p<0.05$). Daily nitrate supplementation in the form of beetroot juice (BRJ) for thirteen days had a significant effect on athlete's $\tilde{V}O_2$max. This is in accordance with previous finding that nitrate supplementation for six days can improve physical performance seen from decreasing pulmonary O_2 uptake during submaximal exercise, increasing average tolerance for severe training, and increasing *time-trial* performance in cyclists.[12]

Increased levels of $\tilde{V}O_2$max in athletes can be caused by the vasodilator properties of nitrate. When consumed, it will be converted into nitrite by saliva and *xanthine oxidase* enzyme in oral cavity. Nitrite will then be reduced to nitric oxide in the endothelium by the endothelium nitric oxide synthase enzyme (eNOS) which will trigger blood vessels to vasodilate. The effect of BRJ supplementation on 14 trained swimmers who consumed 500 ml of BRJ (~5.5 mmol of NO_3^-) daily for six days showed that there was an increase in athlete's aerobic and anaerobic performance as seen from the decrease in aerobic energy cost (AEC) and an increase workload at anaerobic threshold (AT).[13] This proves that actually BRJ supplementation can positively affect the performance of athletes in aerobic sports, one of which is swimming.

This result is also in line with other findings that there was a significant increase in $\tilde{V}O_2$max after 15 days of 300 ml BRJ consumption, but there was no significant increase in $\tilde{V}O_2$max of control group and the 200 ml group.[14] Another research also investigates that BRJ supplementation daily can reduce malondialdehyde (MDA) levels and increase total antioxidant capacity (TAC) levels of athletes.[15] These decreasing MDA levels and increasing TAC levels are definitely related with athlete's performance and physical endurance.

In terms of anthropometry, the result showed that there was a significant difference in weight and body mass index (BMI) between both groups ($p<0.05$). It is defined that ectomorphic somatotype, BMI, pulse rate, and fluid intake are some of the main factors that affect $\tilde{V}O_2$max besides smoking, alcohol and caffeine consumption, also physical exercise.[16]

In addition, there was a negative relationship between BMI and levels of $\tilde{V}O_2$max.[17] The higher a person's BMI, the lower the person $\tilde{V}O_2$max. This occurs related to body fat which can affect cardiorespiratory endurance. Thus, treatment group which had a higher BMI and body fat percentage than control group did not experience a higher increase in $\tilde{V}O_2$max.

It is also shown that there was a negative relationship between body fat percentage and athlete's aerobic performance as seen from $\tilde{V}O_2$max and anaerobic performance seen from *vertical jump test* and *sprint test*.[18] The higher a person's body fat percentage, the lower the $\tilde{V}O_2$max achieved. This is, of course, the answer to why there was no higher increase in $\tilde{V}O_2$max in treatment group.

All athletes received regular training three times a week with the same duration and intensity of exercise. In addition, some athletes also receive additional training from the coach and/or perform additional exercises on their own. Research conducted in Semarang explained that aerobic exercise by running 400 meters for less than 130 seconds for 12 weeks 3 times per week significantly increased $\tilde{V}O_2$max.[19] So, this is one of the causes of $\tilde{V}O_2$max increase in both groups. Thus, regular physical exercise can improve athlete's cardiorespiratory performance and endurance. For professional soccer athletes, adequate nutrition intake is one of the main factors determining athlete performance. From the results of food recall 13 x 24 hours, the average energy intake in treatment group was 2149.1 kcal. It is explained that at least 3442–3824 kcal is needed for an athlete in their daily needs (daily training).[20] Most recently, the total energy expenditure of a professional soccer athlete is around 3566 kcal for a seven days period, including five days of routine training and two days of matches.[21] This explains that the fulfilment of athlete's energy intake in treatment group is still far from adequate.

Nitrates are mostly sourced from vegetables, fruit, and canned foods. In terms of non-beet nitrates intake, treatment group consumed with an average of 239.41 mg. Meanwhile, the average total nitrate intake was 395.66 mg. This result is classified as moderate which categorizes daily nitrate intake as low if 175 mg; moderate if > 175-700 mg; and high if > 700

Table 4. Pre $\tilde{V}O_2$max, post $\tilde{V}O_2$max, and $\Delta\tilde{V}O_2$max in both groups.

Measurement	Control (n = 8)			Treatment (n = 8)			p-value
	Mean±SD	Min	Max	Mean±SD	Min	Max	
Pre $\tilde{V}O_2$max (ml/kg/min)	51.94±4.88	47.1	60.3	50.88±5.84	44.2	62.2	0.69
Post $\tilde{V}O_2$max (ml/kg/min)	56.36±4.78	50.8	65.3	54.0±5.51	47.7	64	0.37
$\Delta\tilde{V}O_2$max (ml/kg/min)	4.43±3.47	0	8,1	3.13±2.76	0.6	7.7	0.42

*Independent sample-t test.

Table 5. Differences in $\tilde{V}O_2$max change after intervention in both groups.

Measurement	Control (n = 8)			p-value	Treatment (n = 8)			p-value
	Mean±SD	Min	Max		Mean±SD	Min	Max	
Pre $\tilde{V}O_2$max (ml/kg/min)	51.94±4.88	47.1	60.3	0.009	50.88±5.84	44.2	62.2	0.015
Post $\tilde{V}O_2$max (ml/kg/min)	56.36±4.78	50.8	65.3		54.0±5.51	47.7	64	

*Paired t-test.

mg.[22] The recommendation of nitrate consumption for soccer athletes are 6-8 mmol daily for 2-5 days before matches and 90 minutes before *kick-off*.[23]

The effect of placebo on $\tilde{V}O_2$max

Descriptively, $\tilde{V}O_2$max in control group showed a significant increase from 51.94 ml/kg/min to 56.36 ml/kg/min ($p< 0.05$). The increase of $\tilde{V}O_2$max in control group was not in accordance with the hypothesis that there should be no increase in $\tilde{V}O_2$max after the intervention. It could be due to the *placebo effect.*

The *placebo effect* related to carbohydrate supplementation and placebo on performance of 41 male cyclists and 2 female cyclists using *time trial test* showed that there was an increase in performance both in group that received placebo and in the treatment group.[24] It is speculated that "*treatment"* given to the athlete during intervention made the subject to perform better on the *post-test (make a greater effort)* than at the baseline. Besides, it was also found that this could happen because desire of the treatment group to improve performance at baseline was not as high as that of the control group.

The *placebo effect* was also described by another research about the effect of beverage supplementation by *5 km running test* and it showed that the group who received placebo performed better when told that *treatment* given contained an *"ergogenic aid"*.[25] Still about *placebo effect,* it is also found that the performance of professional runners who consumed "*super-oxygenated"* water and placebo and performed *5 km running test* showed that there was a significant increase in performance in control group who consumed plain water compared to treatment group that consumed intervention drink.[26]

Placebo effect has been a topic of special interest among researchers and clinical practitioners for many years and the mechanism of *placebo effect* have continued to evolve over the past two decades. *Placebo effect* is a phenomenon that occurs when an inert substance is given instead an active medication, but individuals are convinced that they had received the active substance.[27] Environmental and psychosocial determinants are two main factors for the *placebo effect*.[28] These two factors also include conditioning, verbal suggestions, and habits of health workers (in this case researchers).

The occurrence of changes in an individual related to *placebo effect* are not only caused by environmental and psychosocial factors but also due to expectations, desires, and natural history. Expectation means the magnitude of symptoms or conditions obtained in the outcome of intervention *(outcome)*. Desire is the hope to avoid a goal and/or the willingness to get a good *outcome*. Then, natural history is the magnitude of symptoms and conditions in the past that determine subject's knowledge specifically at the time before getting an intervention or *treatment*. These factors can play a role and influence both directly and indirectly on changes in the results of intervention given to subjects who take placebo.

Changes in $\tilde{V}O_2$max in both treatment and control group can occur due to the *placebo effect*.[29] *The placebo effect* could occur at the time of intervention because it coincided with soccer season so that all subjects received high-intensity exercise. Aerobic physical exercise can increase the capacity of blood to carry oxygen and reduce the pulse rate at rest and activities so as to increase cardiorespiratory endurance.[20] Therefore, the provision of high-intensity physical exercise can be an enabling factor in control group as well as changes in $\tilde{V}O_2$max.

Changes of $\tilde{V}O_2$max both in treatment and control group

The $\Delta\tilde{V}O_2$max in treatment and control group respectively were 4.43 ml/kg/min and 3.13 ml/kg/min. Statistically, the results showed p=0.42, so it can be concluded that there is no significant difference in $\Delta\tilde{V}O_2$max before and after intervention ($p>0.05$).

This is a new insight that no matter how good the supplementation given to athletes is, if the athlete's *self-maintenance* is still not good, it will not work. *Self-maintenance* that can support athletes' physical performance includes nutrition intake, physical exercise (adaptation), lifestyle (consumption of tobacco/smoking, caffeine, and alcohol), psychological factors (character, arousal, sleep quality, and stress), environmental factors (altitude, air pressure, temperature, humidity, air velocity, vibration, and pollution), somatic factors (health, gender, age, somatotype, nutrition status, and individual differences), as well as workload and exercise (intensity, duration, technique, position, rhythm, and exercise schedule).[30] All of these factors produce a *service function* and then affect the *energy yielding process* so that it can produce excellent physical performance of athletes. *Self-maintenance* will support the supplementation and intervention given during training or competition period.

During the process, this research certainly has some shortcomings, which are the lack of subjects' characteristic matching and no laboratory testing of nitrate levels and acceptability test of intervention products. Subjects *matching* need to be done to ensure that the intervention product given really has an influence on the dependent variable. In addition, laboratory testing of nitrate levels and acceptability test should be carried out in order to know the exact dose of nitrate and level of acceptance given to the research subjects.

Conclusions

There is a significant difference in weight, body mass index, and $\tilde{V}O_2$max change of youth soccer athletes. Besides, there is no significant difference in age, height, nutrition intake, school hours, sleep hours, and physical exercise as well as pre $\tilde{V}O_2$max, post $\tilde{V}O_2$max, and $\Delta\tilde{V}O_2$max. Thus, both beetroot juice and placebo significantly affected the increase of $\tilde{V}O_2$max in youth soccer athletes. $\tilde{V}O_2$max increase in soccer athletes who consumed beetroot juice were smaller although not significant compared to the placebo group.

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