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Ergogenic effects of dietary nitrates in female swimmers

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Abstract

Introduction. **A diet enriched with inorganic nitrates by increasing nitrite plasma levels, has been described to benefit exercise performance, blood pressure, endothelial function, and other body functions. It was also proven that additional dietary polyphenols, e.g. red wine or fruits such as strawberries, currants and berries, significantly stimulate the NO_3^- – NO_2^- – NO metabolic pathway.** Aim of Study. **A double-blind crossover study was performed to evaluate the influence of 8-day nitrate-rich (5.1 mmol NO_3^-) juice supplementation (C – carrot juice versus B/A – beetroot juice with chokeberry addition) on blood pressure and exercise tolerance in female swimmers.** Material and Methods. **Eleven female swimmers volunteered for the study (age 20.9 ± 1.3 years). A two-part freestyle swimming exercise test was performed: the anaerobic part consisting of six 50 meter maximal sprints, and the endurance part consisting of an 800 meter continuous swim. Blood Pressure (BP) and Heart Rate (HR) at rest, performance time of all the distances, and peak HR after both test parts were assessed.** Results. **There were no significant differences in blood pressure and heart rate at rest between two measurement terms. Improvements in 50 meter sprint performance were noted in later repetitions (3-6) of the anaerobic part of the test, and they were more pronounced after B/A juice supplementation. The significant increase in the 800 meter freestyle swim performance was obtained after consuming both juices. However, during C juice supplementation this improvement was accompanied by a significant increment in peak heart rate.** Conclusions. **8-day supplementation with beetroot juice with chokeberry addition increases exercise tolerance in both, repeated, maximal and endurance swims. Ergogenic effects of carrot juice with a high nitrate content seem to be possible in young female swimmers, but they still require further research.**

KEYWORDS: high-nitrate diet, beetroot, carrot, exercise tolerance.

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What is already known on this topic?
Among the multiple biologically active dietary compounds inorganic nitrates are of particular significance. The possibility of their anaerobic reduction to nitric oxide is a kind of nitric oxide sourcing security system in the state of ischemia or hypoxia. Nitrate supplementation induces a wide range of biological effects e.g. antibacterial, cytoprotective, and ergogenic. Nitrate rich foods are known to decrease blood pressure and stomach cancer risk, and improve endothelial function, peripheral circulation, and mitochondrial metabolism.

Introduction

Biological effects of fruit and vegetable juice consumption have been the subject of numerous studies. Scientists have selected vast amounts of biologically active compounds such as anthocyanins, polyphenols, carotenoids, etc. and demonstrated their positive impact on the human body [1, 2, 3].

Dietary nitrates have attracted a great research interest since the discovery of the alternative biochemical pathway of endogenous nitric oxide production: nitrate (NO_3^-) → nitrite (NO_2^-) → nitric oxide (NO) [4, 5]. The classic pathway originates from L-arginine and requires the NOS enzymes family, which is active in aerobic conditions [6]. Therefore, the metabolism of inorganic nitrates, mainly dietary, is a kind of nitric oxide sourcing security system in the state of ischemia or hypoxia. Moreover, due to rapid oxidation of very unstable NO to NO_2^- , and later to NO_3^- , nitrite and nitrate are the natural storage pools of nitric oxide molecules.

Over the last two decades many authors have proven that nitrate supplementation (regardless of its form, e.g. beetroot juice, baked whole beetroot, or nitrate salts) reduces blood pressure [7, 8, 9, 10, 11] and stomach cancer risk [12, 13, 14], and improves endothelial function [10, 15], peripheral circulation [16], and mitochondrial metabolism [17, 18]. Its antibacterial [19, 20] and cytoprotective properties are also well documented [18, 21, 22]. Several research teams analyzed the potential ergogenic effects of dietary nitrates in a wide range of sports. It appears that the extent of biological effects of nitrates depends on athletes' baseline physical fitness, i.e. it is smaller in highly trained athletes [23, 24, 25, 26, 27]. Some authors confirm that nitrates improve aerobic or anaerobic performance in some athletes (e.g. decreased VO_2 consumption while performing submaximal or maximal exercise; increased time-to-exhaustion, shorter time on specific distance time trials) [7, 8, 28, 29, 30, 31, 32, 33], but not in all of them [23, 24, 25, 34, 35]. Only one study has been performed on swimmers. Pinna et al. [33] showed the positive effects of 6-day beetroot juice supplementation (5.5 mmol of NO_3^-) on the workload and aerobic energy at the anaerobic threshold but not during the maximal output in an incremental swimming test. In interval exercise the effects of nitrate supplementation are more exposed in later repetitions. A study of Bond et al. [36] on elite rowers after 6-day nitrate supplementation shows that improvement in the rowing time in 500 meter ergometer sprints is more distinct in the later stages of exercise (repetitions 4-6).

We considered it interesting to conduct a complex study regarding inorganic nitrate supplementation in swimmers. Swimming training and major competitions are highly repetitive and, at the same time, require both aerobic and anaerobic performance. No such studies have been conducted to date.

Gago et al. [37] and Rocha et al. [38] observed that the addition of polyphenolic dietary compounds (red wine or fruits such as strawberries, currants and berries) exhibits strong reducing properties that significantly stimulate nitric oxide production in the mentioned metabolic pathway. Despite this knowledge there have been no studies examining the influence of beetroot juice with a polyphenolic addition on exercise performance. Moreover, in previous papers many vegetables, rich in nitrates, were only mentioned, but apart from beetroot none of them was an object of exercise physiology research. It was interesting to ascertain whether carrot juice (rich in nitrates and for many consumers better tasting) cause comparable effects to beetroot juice.

That is why we conducted a double-blind crossover study to evaluate the influence of 8-day juice supplementation (carrot versus beetroot with chokeberry addition) on the levels of blood pressure and exercise tolerance in female swimmers. Our hypothesis was that: 1) beetroot juice with the addition of chokeberry increases exercise tolerance in swimmers; and that 2) carrot juice exerts an ergogenic effect.

Materials and Methods

Eleven healthy, nonsmoking female students volunteered for the study (age 20.9 ± 1.3 years; body mass 64.4 ± 8.62 kg; body height 167.4 ± 4.76 cm). All procedures were approved by the Ethics Committee of the Poznań University of Medical Sciences. All the girls were university league swimmers, and they trained regularly during their entire study period (three training sessions per week) and participated in competitions. Before obtaining written informed consent the participants were precisely instructed about the protocol of the study.

The intervention covered two 8-day supplementation periods separated with three weeks of washout time. Participants were asked to drink 0.5 l of given juice each day, between 7 and 12 a.m. On testing days the time of consumption was stricter: immediately after exercise tests, and three hours before exercise tests (on day 1 and on day 8, respectively). The two juices used in the study were reconstituted from concentrate, with equal amounts of inorganic nitrates (V), i.e. 10.2 mmol/L (5.1 mmol per serving). The description of both supplements is shown in Table 1. The B/A juice was a mixture of beetroot (*Beta Vulgaris*) and chokeberry

Table 1. Biochemical characteristics of beetroot and chokeberry juice and carrot juice (means ± SD)

Juice		B/A	C
Extract	%	10.5 ± 0.2	10.5 ± 0.2
Nitrate	mmol/l	10.2 ± 0.2	10.2 ± 0.2
ABTS	micromol Trolox/l	24.5 ± 1.1	0.6 ± 0.3
Titratable acidity	%	2.5 ± 0.03	0.65 ± 0.05
Polyphenols	mg/l	3231 ± 5.4	354 ± 12
Karotenoids	mg/l	–	177
Anthocyanins	mg/l	298 ± 6.0	–
Red pigments	mg/l	524 ± 4.1	–
Yellow pigments	mg/l	237 ± 3.2	–
Betaine	mg/l	1629 ± 12.2	–

B/A – beetroot and chokeberry juice; C – carrot juice

(*Aronia Melanocarpa*) juices in a ratio of 7:3. The C juice was a carrot juice with the initial level of nitrates of 4.5 mmol. To equalize the nitrate content in both supplements, a slight addition of inorganic nitrate salts (KNO_3^-) to carrot juice was essential. When the salts were added, they did not change the sensory characteristics of the juice. In the beginning of the cross-over design study volunteers were randomly assigned into two subgroups. One subgroup started the program with the B/A juice, while the other subgroup with the C juice. After a three-week wash-out period the swimmers drank the alternative juice for the following 8-days.

In the week prior to the study participants underwent a regular sport medical examination, where among other indices their body mass and body height were measured. During each supplementation period participants were instructed to arrive for the exercise tests on two consecutive Tuesdays at 10:00 a.m., after a light standardized breakfast (roll with butter and cheese, tea without sugar). They were asked to avoid exhausting training as well as alcohol and caffeine intake on days prior to the tests. After arriving at the swimming pool the girls were asked to sit and rest for at least 15 minutes. After that time, still in the sitting position, their blood pressure and heart rate at rest were measured three times with the use of a semiautomatic sphygmomanometer (Omron M2, Poland). The mean of three measurements was taken as a final result.

After the measurements a freestyle swimming exercise test was performed consisting of two parts: anaerobic – comprising six 50-meter maximal sprints, and endurance – involving an 800-meter continuous swim. The two parts of the swimming test were separated with a 10-minute passive recovery. Immediately after each part peak heart rate was measured.

One week before and during the entire study period the participants were asked to maintain their regular dietary habits. Their nutritional approach was verified by keeping a nutritional diary for three days prior to and for seven days during the supplementation period. All swimmers throughout the study period were nutritionally guided not to change their nitrate and polyphenolic intake. All data were expressed as means ± SD. All statistical analyses were performed with the STATISTICA v. 10.0 software package. The differences between paired variables were investigated with the Wilcoxon test. A $p \leq 0.05$ was considered statistically significant.

Results

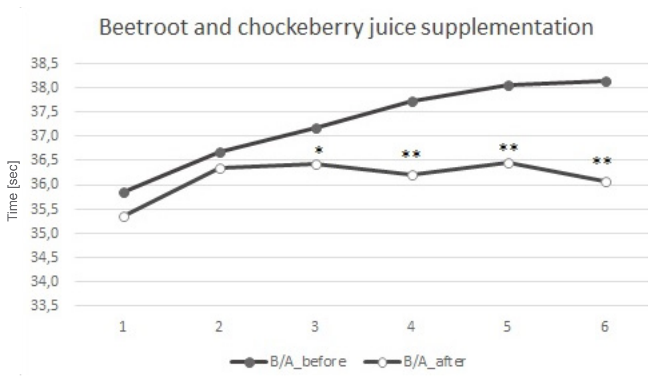
The levels of blood pressure and heart rate at rest before and after each supplementation period are shown in Table 2. There were no significant differences in these variables between the measurement terms.

Table 3 as well as Figures 1 and 2 present the results of the anaerobic part of the swimming tests. The mean benefit of using B/A juice was –3.13%, while of carrot juice only 2.09% (Table 3). The improvements of all the repetitions were greater after B/A juice supplementation compared to C juice supplementation (Table 3). Independently of supplementation the results obtained during the first two repetitions did not differ significantly

Table 2. Effects of beetroot and chokeberry juice compared to carrot juice on resting blood pressure and heart rate in female swimmers (means ± SD)

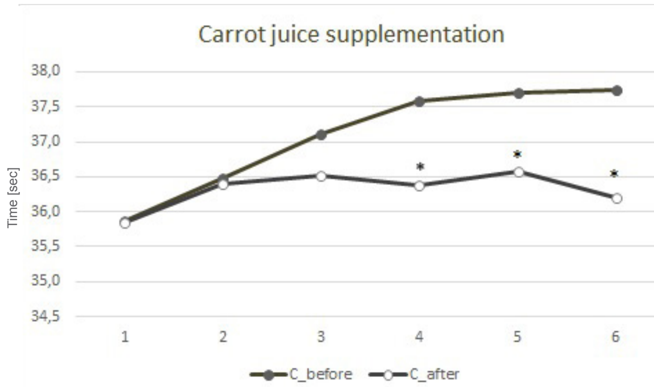
	BPsys [mmHg]	BPdia [mmHg]	HR [1× min^{-1}]
B/A_before	114 ± 9.16	64 ± 7.38	76 ± 13.11
B/A_after	109 ± 7.12	62 ± 7.06	74 ± 7.30
C_before	108 ± 9.55	60 ± 7.45	74 ± 8.63
C_after	108 ± 8.04	60 ± 9.71	72 ± 9.25

B/A – beetroot and chokeberry juice; C – carrot juice



1, 2, 3, 4, 5, 6 – consecutive sprints of the sprinting part of exercise test
Difference between study terms * $p \leq 0.05$; ** $p \leq 0.01$

Figure 1. The results of the sprinting part of the swimming test after B/A juice supplementation



1, 2, 3, 4, 5, 6 – consecutive sprints of the sprinting part of exercise test
Difference between study terms * $p \leq 0.05$

Figure 2. The results of the sprinting part of the swimming test after C juice supplementation

Table 3. Comparison of anaerobic performance improvements after supplementations (%)

	Repetitions						
	Mean (1-6)	1	2	3	4	5	6
B/A	–3.13%	–1.36%	–0.96%	–2.08%	–4.19%	–4.39%	–5.79%
C	–2.09%	–0.02%	–0.19%	–1.62%	–3.34%	–3.10%	–4.28%

B/A – beetroot and chokeberry juice; C – carrot juice

between the measurement terms. Both juices brought about improvements in time of sprint swimming in repetitions 3-6. The difference in the third repetition of carrot juice supplementation was still not big enough to reach the confidence limit, while the difference after B/A juice supplementation was significant at $p \leq 0.05$. Significant improvements were noted in repetitions 4-6 after both supplementations (Figure 1, 2). Yet, the level of statistical significance was greater after B/A juice supplementation ($p \leq 0.01$) compared to carrot juice supplementation ($p \leq 0.05$).

The peak heart rate achieved in both parts of exercise tests and times of endurance parts is shown in Table 4. Drinking beetroot and chokeberry juice caused a significant ($p \leq 0.05$) decrease in the 800 meter freestyle swim time. The peak heart rate after both physical exercises did not change after drinking B/A juice. The time of the endurance part also decreased significantly after carrot juice supplementation ($p \leq 0.01$). The peak heart rate measured after both parts of exercise tests was much higher after the C juice supplementation period; however, only after the endurance part was the difference reach statistically significant ($p \leq 0.05$).

Discussion

The present study (Table 2) revealed no significant differences in the resting level of blood pressure and heart rate, either after beetroot and chokeberry juice or carrot juice supplementation. There was a trend towards decreasing systolic blood pressure after the B/A juice supplementation period. This insignificance may be in part due to high standard deviations achieved in both study terms, which generally result from diverse individual reactions to nitrate supplementation.

No effect of nitrates on the resting heart rate is consistent with previous studies [25, 32, 33, 39]. Yet, the influence of inorganic nitrates on blood pressure is still uncertain. A meta-analysis of randomized clinical trials reported an association between beetroot juice supplementation and a significant reduction in systolic BP [11]. Recently, a placebo-controlled trial [40] in 27 individuals with

Table 4. Comparison of peak heart rates achieved after both parts of exercise test and times obtained in endurance parts after both supplementations (mean ± SD)

	HR sprint [$1 \cdot min^{-1}$]	800 m distance [sec]	HR endurance [$1 \cdot min^{-1}$]
B/A_before	180.64 ± 18.48	697.84 ± 50.56	175.27 ± 13.00
B/A_after	179.18 ± 12.45	690.41 ± 50.16 *	174.70 ± 10.87
C_before	169.45 ± 16.42	696.38 ± 43.61	165.55 ± 13.20
C_after	180.91 ± 11.57	681.64 ± 43.05 **	176.64 ± 12.38 *

Difference between study terms * $p \leq 0.05$; ** $p \leq 0.01$; B/A – beetroot and chokeberry juice; C – carrot juice

treated hypertension has revealed no effects of one-week nitrate-rich beetroot juice intake on the level of blood pressure. Studies on active individuals or athletes are not coherent either. Larsen et al. [41] after a 3-day intake of sodium nitrate water solution (0.1 mmol per kg of body weight per day) in nine healthy, well-trained males observed a significant reduction in the resting values of both systolic and diastolic blood pressure ($p \leq 0.01$) compared with placebo (sodium chloride). It is rather unlikely that there will be more research undertaken in young healthy volunteers, which showed the reduction only in the level of systolic blood pressure following the ingestion of nitrate rich beverages [7, 9, 29, 31, 39], or did not demonstrate any blood pressure changes [25]. There are studies indicating the occurrence of dose-related reduction of systolic and diastolic blood pressure under nitrate treatment [39, 42], but the above mentioned studies do not fully confirm such findings. Interestingly, most research has been conducted on male groups, and very few studies on mixed groups. Kapil et al. [39] showed that nitrate-induced reduction in systolic and diastolic blood pressure is significantly greater in males compared with females, and negatively correlated with baseline BP ($p \leq 0.001$).

The group in our study was homogenous in terms of gender, and therefore less explicit reaction may be proper. The beetroot juice used in our study contained a relatively small amount of inorganic nitrates and – what seems to be more relevant – the baseline blood pressure was in the lower normal range: 114 ± 9.16 mmHg before B/A and 108 ± 9.55 mmHg before C juice supplementation.

An important finding is that 8-day supplementation with both juices, rich in inorganic nitrates, resulted in the improvement of both anaerobic and aerobic swimming performance. The extent of these improvements is much more pronounced in anaerobic conditions, especially in the later stages of exercise. During C juice supplementation, repetition 3 only trended to improve exercise performance, while the next three repetitions showed, in fact, significant changes. B/A juice caused significant improvements in the time of sprint swimming in repetitions 3 to 6. The nitrate influence was negligible during the first two 50-meter distances. No effect of nitrates on a single maximal swimming performance is consistent with the results of another study where maximal effort in the incremental swimming test was not affected by six days of 0.5 l of beetroot juice consumption (5.5 mmol NO_3^-) [33]. The importance of nitrate influence increases with subsequent repetitions, probably due to lowering the partial pressure of oxygen in active muscle tissues causing the dysfunction of NOS enzymes, and through stimulating the nitrate-nitrite-NO pathway. The reason for improving anaerobic performance is probably due to NO positive interference in mitochondrial metabolism causing its more economical functioning [17, 18]. Many study designs conducted in diverse sports confirm improvements in the basal mitochondrial function under ischemic conditions, e.g. lowering oxygen consumption during maximal exercise [8, 29, 43]. The finding on sport performance increments in repetitive maximal exercise is consistent with the results of Bond at al. [36], who demonstrated an improvement in repeated maximal rowing-ergometer performance under beetroot juice supplementation. In fourteen junior rowers their performance increased for 0.4% across all repetitions, and 1.7% in repetitions 4, 5 and 6. In our study the improvements were even higher (3.13% in B/A, and 2.09% in C; Table 3), with a relevant trend of changes, which can be explained by diverse sports, sex and performance levels of participants of both studies.

Comparing the two supplementation strategies, it is clear that the effectiveness of B/A juice was more profound than carrot juice. In all six repetitions the extent of performance improvements was greater in B/A juice compared to C juice (Table 3). The concentration of inorganic nitrates in both supplements were even thus, in such a situation, as predicted, it could be the result of polyphenolic compound addition known to improve the nitrate-nitrite-NO pathway [28, 38]. But, it is very essential that there were great differences in peak heart rate between the study terms after C juice supplementation (Table 2). Like in other studies using beetroot juice, in our study under beetroot and chokeberry supplementation there were no changes in peak heart rates before and after the intervention [27, 29, 32, 41, 44]. Yet, following carrot juice ingestion the mean peak HR increased by 11 beats per minute, which directly suggests a higher physiological cost of that exercise. The significantly shorter time of 50 meter swims is appropriate, but the subsequent increment of peak HR negates the anticipated ergogenic effect of 8-day carrot juice supplementation with 5.1 mmol concentration of inorganic nitrates (V). Therefore, this study provides arguments that ergogenic effect in repeated maximal swimming is achieved only after 8-day supplementation with beetroot juice with chokeberry addition.

Increments in aerobic performance under nitrate influence were proven in various sports, especially in running and cycling, and in various kinds of exercise tests [29, 41, 44]. There were time-trials (TT) performed under nitrate conditions, but to the best of our knowledge, not in swimming. No effect of beetroot juice consumption was indicated in a 4-min high-intensity cycle ergometer TT [26] and in a 50-mile ergometer TT [25], as well as in a 5-km self-paced treadmill TT [32] and in a 10-km treadmill TT at 2500 m simulated altitude [27]. However, in 9 noncompetitive cyclists, a single 0.5 l dose of beetroot juice caused a significant improvement in 4 km and 16.1 km time-trial performance [31]. In all those studies the level of peak heart rates did not differ significantly between the compared measurement terms. In the studied swimmers both supplementation strategies caused a significant decrease in 800 meter swimming time-trials. After B/A supplementation the improvement in time of the distance coverage was 1.08% with no impact on the peak HR, which implies the favorable increment of aerobic performance after this treatment. On the contrary, the carrot juice treatment caused a two-fold higher decrease in time of 800 meter swim (–2.16%), but at the same time a significant rise in peak heart rate was observed ($p \leq 0.05$). Such reaction is, in fact, argumentative and does not undoubtedly indicate whether carrot juice supplementation should be proposed to swimmers as an ergogenic aid.

In most of the aforementioned studies, with no improvements noted in the time-trials, the nitrate concentration ranged between 6.2 and 7 mmol of NO_3^-. On the contrary, in the present study, it was only 5.1 mmol. This fact suggests that the dose-dependent influence of inorganic nitrate on exercise performance in the case of time-trials, proposed by Wylie et al. [42], is probably not relevant. There is also no convincing data to state that participants' sex influences the results. No previous study has covered only women.

The increased aerobic swimming performance under nitrate conditions found in our study (shorter 800 meter swim timne) is consistent with the study by Pinna et al. [33] where 14 male swimmers, during an incremental swimming test, improved the workload and reduced the aerobic energy cost at the anaerobic threshold. These observations show that inorganic nitrates do promote the ergogenic effect in endurance swimming.

Conclusions

8-day supplementation with beetroot juice with chokeberry addition increases the physical tolerance in swimmers in both repeated maximal sprints and endurance swims. Drinking 0.5 l of carrot juice per day – even when it is enriched with inorganic nitrates – seems to cause minor effects in terms of increasing exercise tolerance in young female swimmers, irrespectively of chosen distance. Given this context we believe that there is a need to carry out further research in order to state clearly that carrot juice (even with high nitrate content) does not induce ergogenic effects.

What does this study add?

This study shows that 8-day supplementation with beetroot juice with chokeberry addition increases swimmers' exercise tolerance in both repeated maximal sprints and in endurance swims. High-nitrate carrot juice does not induce similar ergogenic effects to beetroot juice with chokeberry addition.

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