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The influence of amaranth (*Amaranthus hypochondriacus*) dietary nitrates on the aerobic capacity of physically active young persons

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Abstract

Background: Recent evidence indicates that elevating plasma nitrites through dietary nitrates (NO_3^-) supplementation is associated with enhanced muscle efficiency, fatigue resistance and performance. Beetroot (in various forms) is the dominant source of dietary NO_3^- primarily due to its vast availability and the simple form of preparation suitable for final consumption. After a few years of research and experimentation, our scientific team identified alternative source rich with dietary NO_3^- as possible nitric oxide precursor, amaranth (*Amaranthus hypochondriacus*) with a standardized concentration 9–11% of NO_3^-. This study aimed to evaluate the effect of single-dose (±400 mg of dietary NO_3^-) and long-term (6 days) supplementation of amaranth concentrate derived dietary NO_3^- on aerobic capacity in physically active young people.

Methods: We conducted a randomized, double-blind, placebo-controlled human study. Thirteen healthy and physically active young male participants were randomized into experimental and placebo groups. The aerobic capacity was tested during increasing cycling exercise (ICE) with pulmonary gas exchange recording and analysis.

Results: The peak power of the ICE, the maximum oxygen consumption and the first ventilatory threshold were significantly increased after long-term consumption of dietary amaranth (from 4.44 ± 0.50 to 4.55 ± 0.43 W/kg; from 37.7 ± 2.7 to 41.2 ± 5.4 mL/kg/min and from 178.6 ± 30.3 to 188.6 ± 35.2 W, $p < 0.05$; respectively) in experimental group.

Conclusions: Long-term (6 days) use of dietary NO_3^- from amaranth may improve the aerobic capacity during ICE in young physically active male persons. It can be recommended as the nutritional supplement during last week of preparation for competition in endurance events.

Keywords: Aerobic capacity, Dietary nitrates, Amaranth, Cycling, Young persons

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Introduction

Green leafy vegetables and roots are the main source of dietary nitrates (NO_3^-) [1–3]. NO_3^- is a naturally occurring compound as well as an approved food additive [1, 2]. A number of studies have already confirmed the benefits of dietary nitrates to human health [4]: their consumption reduces blood pressure, suppresses platelet aggregation, protects against ischemic diseases, and improves endothelial function [1]. Nitric oxide and nitrites, both NO_3^- products, affect vasodilatation by increasing blood flow [5], thus increasing the oxygen uptake and oxidative processes in the working muscles [6]. Additionally, nitrates show to increase the bioavailability of blood plasma, which is important for the exogenous pathway of nitrates-nitrite-NO and acts as a regulator of hypoxic signals and NO-induced vasodilatation [7].

The effects of nitrate/nitrite/NO on the muscle circulatory system and mitochondrial and contractile efficacy [8, 9] may increase muscle blood flow circulation and improve the metabolic response to physical activity [10]. The evidence supports that even the concentration of plasma nitrites is an independent factor of physical performance [5, 11]. Nevertheless, studies on the effects of nitrates on work capacity indicators are highly controversial so far.

Studies have shown nitrates to have a positive effect on work efficiency and oxygen expenditure [10, 12–17], but other studies have not found visible and conclusive changes in given performance [14, 18–22].

A large number of researchers found that 300–500 mg of beetroot nitrates have a single and long-lasting positive effect on the aerobic performance of physically active individuals [10, 12, 16–19, 23]. Recently became popular and actively researched beetroot (in various forms) is the dominant source of dietary NO_3^-, primarily due to its vast availability and the simple form of preparation suitable for final consumption. Remarkably, limited studies have evaluated NO_3 rich leafy vegetables and, more specifically, amaranth on exercise performance. Importantly, amaranth is not only rich in NO_3, potassium (> 10% by weight) and antioxidant polyphenols (e.g. amaranthine), but also devoid of sugar and oxalates. It has recently been reported that red spinach extract as a nutritional supplement can elicit an ergogenic response by delaying the ventilatory threshold during graded treadmill exercise testing [24]. After a few years of research and experimentation, our scientific team identified alternative source rich in dietary NO_3^- as possible and alternative nitric oxide precursor, amaranth (*Amaranthus hypochondriacus*) with a standardized concentration 9–11% of NO_3^-. Since NO_3^- supplementation increases plasma NO_2^-, this intervention may therefore have the potential to improve muscle blood flow [5], thus increasing the oxygen uptake and oxidative processes in the working muscles [6] and exercise tolerance. Thus, based on research data available we formulated the hypothesis that 400 mg of dietary NO_3^- from amaranth (dietary amaranth) will increase the aerobic capacity of physically active young people.

In this study we aimed to evaluate the effect of single and long-term doses of dietary amaranth on the aerobic capacity of physically active young persons.

Materials and methods

Participants

The study recruited 13 volunteering graduate students (all males) from Lithuanian Sports University. Every participant was informed about the research objectives and methods and signed an Informed Consent form for participation. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Kaunas Regional Ethics Committee, Nr. BE-2-11, 21 March 2017. The anthropometric data and age of the participants are presented in Table 1.

Measurements

Anthropometry

Electronic weighing scales (Body Composition Analyzer TBF-300, Tanita, Japan). were used to measure the weight and relative fat mass of the participants. Height was measured using a stadiometer (Leicester height measure, UK).

Increasing cycling exercise (ICE)

For the assessment of aerobic capacity, an ICE was performed on a cycle ergometer (Ergoline-800, Denmark). The seat and handlebar positions on the cycle ergometer were adjusted for each subject prior to the initial exercise test and maintained in that position for the subsequent exercise tests. Prior to the ICE, a 5-min warm-up was performed. The ICE consisted of 3 min of cycling at 40 W, then the ramp protocol was applied, and the workload was continuously increased by 30 W per min. The cadence was 70 rpm. The participants were encouraged to exercise until voluntary fatigue, and the test was stopped when the participant was not able to maintain a cadence above 65 rpm.

Table 1 Characteristics of study participants

Variable	Placebo group[1]	Experimental group[2]	*p*-Value
Age (years)	21.9 ± 1.9	21.3 ± 0.8	0.940
Height (cm)	182.1 ± 5.9	180.5 ± 8.3	0.829
Body weight (kg)	88.1 ± 12.5	84.3 ± 23.7	0.668
Body mass index (BMI)	26.5 ± 2.8	25.6 ± 5.8	0.568
Relative fat mass (%)	13.1 ± 3.8	18.9 ± 5.6	0.063

Values are reported as the mean ± standard deviation (SD). [1] $n = 6$; [2] $n = 7$

Pulmonary gas exchange recording and analysis

The subjects breathed through a face mask, and pulmonary gas exchange parameters were measured breath-by-breath using a wireless, portable spirometric system "Oxycon Mobile" (Viasys Healthcare; California, USA). Prior to each exercise session, the spirometric system was calibrated according to the recommendations of manufacturers. The maximum value of oxygen uptake (VO_2) over the 20 s of cycling was referred to as VO_2 max, and the first and second ventilatory thresholds (VT1 and VT2) were determined from the data of the incremental cycling exercise. The determination was based on the analysis of the relationship between ventilatory equivalents of oxygen or carbon dioxide and cycling power. The VT1 was identified as the first point at which the ventilatory equivalent for O_2 increased without a concurrent increase in the ventilatory equivalent for CO_2. The VT2 was identified as the point of constant increase of ventilatory equivalent of CO_2. A least squares method was used to fit two lines representing ventilatory equivalents versus load plots. The intersection point of the two regression lines was assigned to VT1 and VT2. Heart rate (HR) was recorded continuously with a wireless Polar monitoring system (Polar, Finland).

Biochemical analysis of blood

Blood samples for the measurement of blood lactate concentration [La] (Lactate Pro2, Japan) were taken from fingertips at the end of the 5th min of recovery after the ICE.

Protocol

A randomized double-blinded design was used in this study. During the first visit, the participants had their anthropometric measurements taken and performed the ICE (1 T) (Fig. 1).

After 2 days of rest, participants repeated the ICE (2 T) 1 h after consumption of supplement with (experimental group) or without (placebo group) *Amaranthus hypochondriacus*. Then, participants in both groups consumed supplement for 4 days during breakfast and, on the next day, performed a third ICE (3 T) after consuming supplements 1 h before the test.

The experimental group consumed a hand-made oat bar supplement (OB) 60 g total weight - made of oats, honey, vanilla, containing 4 g standardized *Amaranthus hypochondriacus* concentrate (9–11% equivalent to ± 400 mg of active ingredient (NO_3^-). The placebo group consumed visually and flavory identical oat bar (OB) - 60 g, containing oats, honey and vanilla (excluding active ingredient - ie. *Amaranthus hypochondriacus* concentrate). Participants were asked not to change their nutritional habits during the period of study.

Statistical analysis

The statistical analysis was carried out with SPSS (Statistical Package for Social Sciences, version 19.0) and Microsoft Office Excel 2007. The normal distribution of variables was checked using the Kolmogorov-Smirnov test. Non-parametric data analysis methods were used to assess the effect of dietary amaranth on aerobic capacity. The significance of the difference between the independent samples was evaluated using the Mann-Whitney test.

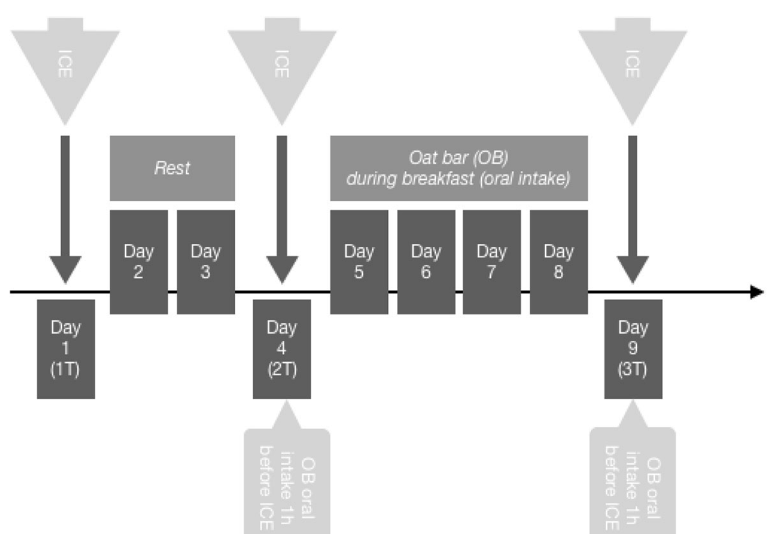

Fig. 1 Study protocol. Note: ICE – increasing cycling exercise test; OB - handmade oat bar; 1–9 –days of study. (1 T) - test Nr. 1 executed at day 1 of the study; (2 T) - test Nr. 2 executed at day 4 of the study; (3 T) - test Nr. 3 executed at day 9 of the study

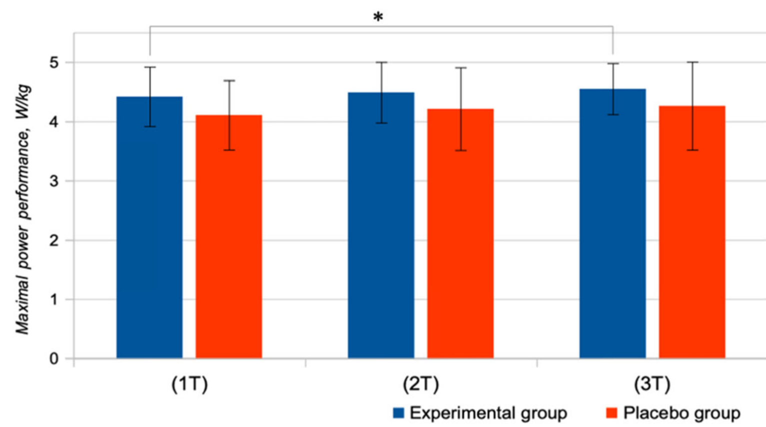

Fig. 2 Peak power of increasing cycling exercise (ICE) following single-dose (2 T) and long-term (3 T) doses of supplements in the experimental and placebo groups. * $p < 0.05$ - statistically significant difference compared to the 1 T

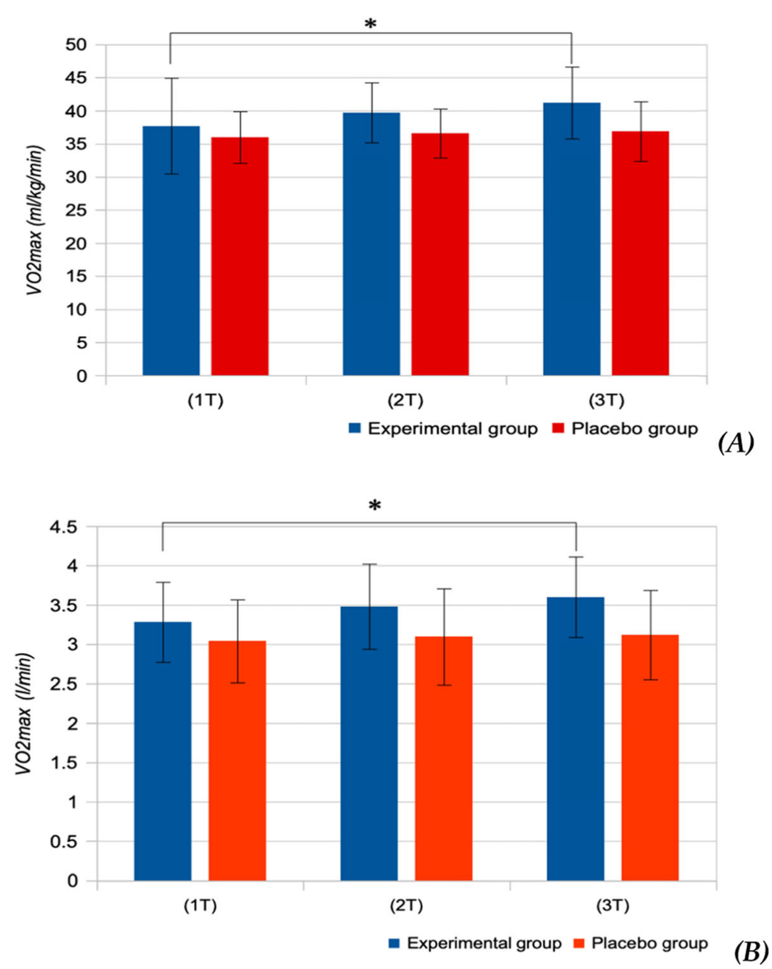

Fig. 3 Maximal oxygen uptake (VO2max) following single-dose (2 T) and long-term (3 T) doses of supplements in the experimental and placebo groups. * *$p < 0.05$ - statistically significant difference compared to the 1 T*

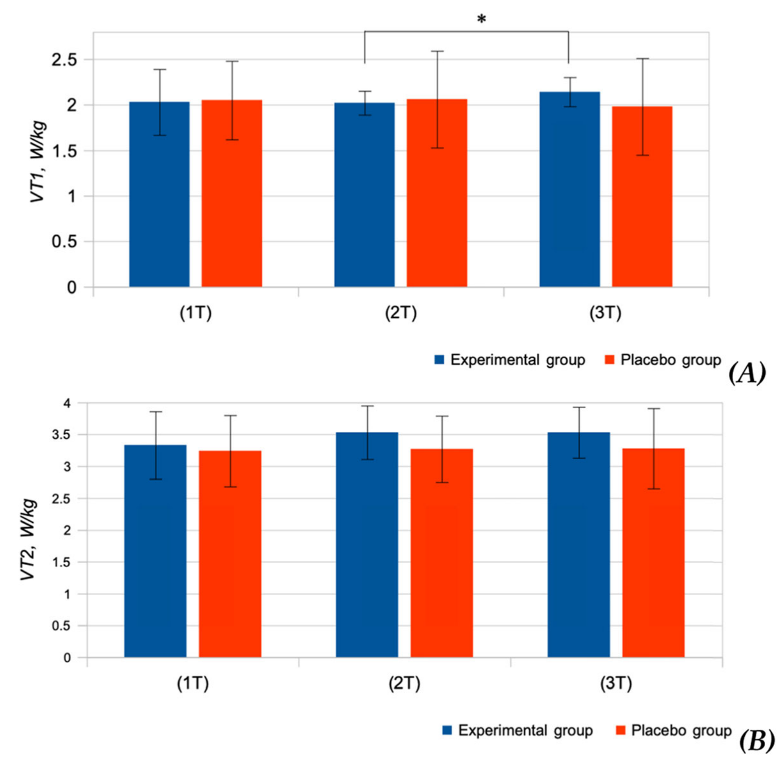

Fig. 4 First ventilatory threshold (VT1) and second ventilatory threshold (VT2) following single-dose (2 T) and long-term (3 T) doses of supplements in the experimental and placebo groups. ** p < 0.05- statistically significant difference between the 2 T and 3 T*

The difference between dependent samples was assessed by the Wilcoxon test. Statistical significance was accepted when $p < 0.05$. All data are reported as the mean ± standard deviation (SD).

Results and discussion

Results

No significant changes of the parameters of aerobic capacity were observed in Placebo group (Figs. 2, 3, 4). Peak power of ICE has increased significantly in the experimental group, from 4.42 ± 0.50 W/kg during the first testing to 4.55 ± 0.43 W/kg during the third testing ($P = 0.043$; Fig. 2). The single dose of supplements did not have any significant effect on the VO_2max in the experimental group. After long-term use of dietary amaranth, absolute and relative values of VO_2max demonstrated a significant increase in the experimental group (from 3.282 ± 0.51 l/min and 37.7 ± 2.7 mL/kg/min during the first test to 3.599 ± 0.51 l/min ($p = 0.028$) and 41.2 ± 5.4 mL/kg/min ($p = 0.043$) during the third test, respectively (Fig. 3).

VT1 did not change after a single dose of dietary amaranth, but a significant difference was observed in the experimental group after long-term supplementation (third testing). After a single dose dietary amaranth VT1 was 2.02 ± 0.13 W/kg and after long-term use of dietary amaranth the value of VT1 increased significantly to 2.14 ± 0.16 W/kg ($p = 0.028$; Fig. 4a). The VT2 did not change significantly in any of the groups ($p > 0.05$, Fig. 4b).

Other physiological variables measured during ICE did not change significantly in any group of participants (Table 2).

Table 2 Maximal physiological responses during increasing cycling exercise following single-dose (2 T) and long-term (3 T) doses of supplements in the experimental and placebo groups (HR - heart rate; RER - respiratory exchange ratio; [La] - blood lactate concentration)

Indexes	Experimental group		
	1 T	2 T	3 T
HR, beats/min	186.1 ± 8.2	183.8 ± 9.1	184.2 ± 7.4
RER	1.18 ± 0.07	1.16 ± 0.04	1.24 ± 0.06
[La] at 5th min after ICE, mmol/l	14.3 ± 2.9	15.2 ± 4.8	12.4 ± 1.6
Indexes	Placebo group		
	1 T	2 T	3 T
HR, beats/min	189.2 ± 5.7	188.2 ± 8.1	187.0 ± 7.6
RER	1.20 ± 0.07	1.16 ± 0.04	1.22 ± 0.07
[La] at 5th min after ICE, mmol/l	13.0 ± 2.1	13.6 ± 4.6	13.9 ± 4.2

Key: Values are expressed as mean ± SD

Table 3 Effects of single and long-term use of nitrates on the indicators of work capacity of healthy and physically active persons

References	Participants	Supplemen-tation	Concentration of nitrates mmol/day	Duration of consumption	Protocol	Findings
[12]	Healthy, physically active people, *n* = 8; placebo (P) and experimental groups (E)	500 ml beetroot juice /day	~ 340 mg or 11 mmol	6 days	Time trial at 70% between the ventilation threshold and VO_2max	The increase in pulmonary O_2 uptake following the onset of moderate exercise was reduced by 19% in the beetroot juice condition. During severe exercise, the O_2 uptake slow component was reduced, and the time-to-exhaustion was extended. Trial duration: E: 675 ± 203 s P: 583 ± 145 s ($p < 0{,}05$)
[19]	Healthy people, *n* = 9;	$NaNO_3$	0.033 mmol $NaNO_3$ kg/ body WT three times daily	Randomized and double-blinded; the washout period between the two trials was at least 7 days	Maximal combined arm and leg exercise tests.	Dietary nitrate reduced VO_2max from 3.72+/−0.33 to 3.62+/− 0.31 L/min, $p < 0.05$. Despite the reduction in VO_2max the time-to-exhaustion trended towards an increase after nitrate supplementation (524+/− 31 vs 563+/− 30 s, $p = 0.13$).
[10]	Healthy, physically active people; *n* = 7	500 ml beetroot juice /day	300 mg or ~ 5,1 mmol	6 days	Leg extension until fatigue at 30% of MVC	20% reduced O_2 cost of exercise following dietary NO_3- supplementation appears to be due to a reduced ATP cost of muscle force production. Trial duration: E: 734 ± 109 s P: 586 ± 80 s ($p < 0{,}05$)
[18]	Healthy, physically active people; *n* = 9	500 ml beetroot juice /day	300 mg or ~ 5,1 mmol	A. 2,5 h before B. 5 days C. 15 days	Increasing exercise test	**A**.E: 325 ± 71 Wmax P: 322 ± 68 Wmax **B**. E:328 ± 68 Wmax P: 323 ± 67 Wmax **C**. E:331 ± 68 Wmax P: 323 ± 68 Wmax After 15 days maximal power was increased by 4%
[16]	Healthy, physically active people; *n* = 9	500 ml of beetroot juice (BR)	380 mg or ~ 6,1 mmol	6 days	Subjects completed treadmill exercise tests and knee-extension exercise tests for estimation of Q_{max}.	The O_2 cost of walking, moderate-intensity running, and severe-intensity running was reduced by BR; time-to-exhaustion during severe-intensity running was increased by 15%. Trial time: E: 8,7 ± 1,8 min P: 7,6 ± 1,5 min ($p < 0{,}05$)
[17]	Healthy, physically active men; *n* = 15	Beetroot juice	4,4 mg or 0.07 mmol nitrate/kg body WT/day	6 days	Increased exercise test simulating a 5000-m altitude	Short-term dietary nitrate supplementation improves arterial and muscle oxygenation status but not cerebral oxygenation status during exercise in severe hypoxia. Trial duration: E: 597 ± 22 s P: 568 ± 23 s ($p < 0{,}05$)
[11]	Healthy, recreationally active men; *n* = 10	70, 140, or 280 ml of beetroot juice or placebo (PL) 70, 140, or 280 ml	~ 4.2, ~ 8.4, or ~ 16.8 mmol nitrates or placebo containing ~ 0.04, ~ 0.08, or ~ 0.12 mmol nitrates	4–5 weeks	Two, 5-min bouts of moderate-intensity exercise and one bout of severe-intensity exercise that was continued until task failure as a measure of exercise tolerance, 2.5 h post-ingestion	8.4 and 16.8 mmol of nitrates significantly improved the time-to-task failure by 14 and 12%, respectively, during severe-intensity exercise. End-exercise VO_2 during

Table 3 Effects of single and long-term use of nitrates on the indicators of work capacity of healthy and physically active persons *(Continued)*

References	Participants	Supplemen-tation	Concentration of nitrates mmol/day	Duration of consumption	Protocol	Findings
					of beetroot juice or placebo.	moderate-intensity exercise was reduced significantly following the ingestion of 280 ml of BR ($p < 0.05$).
[23]	Healthy, physically active men; $n = 16$	500 ml beetroot juice	5 mmol	1.5 h before physical load	A continuous cycle exercise test involving 20-min stages at 50 and 70% VO_2max and a final stage at 90% VO_2 max until volitional exhaustion.	Dietary nitrate reduced VO_2max by 15,63% and increased the time-to-exhaustion by 16%. Trial duration: E: 185 ± 122 s P: 160 ± 109 s ($p < 0,05$)
[25]	Healthy, recreationally active participants $n = 15$ (males = 8, females = 7)	1000 mg red spinach extract powder (RSE) or placebo (PBO) (maltodextrin) in gelatin capsules	~ 90 mg	One single dose	At one occasion 65–75-min post-RSE/PBO ingestion, venipuncture was performed (PRE), after which graded exercise test - the Bruce protocol - was performed.	Significantly increased plasma NO_3-. A large effect on the ventilatory threshold compared to the placebo (+ 0.12 ± 0.14 L/min) with the ventilatory threshold occurred at a significantly higher relative VO_2 (+ 3.6 ± 5.2%, $p < 0.05$).

Discussion

In the present study we analyzed the effect of single dose (equivalent to ±400 mg) and long term consumption of dietary nitrates obtained from amaranth (*Amaranthus hypochondriacus)* on aerobic capacity of healthy physically active young people. Our principal findings are that the VO_2max, VT1 and peak power measured during gradually increasing cycling exercise increased significantly only after 6 days of supplementation, but demonstrated no significant effect after a single dose.

Our data coincides with other studies of a similar nature carried out by other authors (Table 3). The researchers cited used beetroot juice with varying amounts of NO_3. The most popular use of the fixed duration of the dietary NO_3 is 4–6 days, and the amount varies from 5 mmol up to 9 mmol. Positive and significant effect for VO_2max was found by Bailey [10, 12], Lansley [16], Vonhatalo [18], and Wylie [11]. Their studies involved healthy, physically active people and tested physical performance. Vanhatalo [18] explored the long-term effects of nitrates with 5 and 15 days of 500 ml of 5.2 mmol nitrate from beetroot juice and found that the 15-day period significantly improved the maximum aerobic power (Table 3). Most previous studies have used beetroot juice as a source of dietary NO3–, but only a few have investigated the effect of other sources. In one study, the immediate ergogenic effect (delaying the ventilatory threshold) of red spinach extract was demonstrated [24]. The authors reported that after acute ingestion of 1000 mg of red spinach extract, VO2 at the ventilatory threshold was significantly higher, although no significant changes were seen in the time-to-exhaustion or maximal aerobic power. By contrast, in our study, significant changes were observed after 6 days of dietary amaranth consumption. Our dose of NO3– was lower (400 mg) than that in the report by More et al. [25], and this could be why a single dose had no effect.

The mechanisms by which NO_3 supplementation might influence oxygen uptake and utilization could include a reduction of NO_3 to NO, directly influencing mitochondrial efficiency, vascular tone and/or tissue oxygenation. However, the evidence for these mechanisms depends on the measurement of NO_2 in the plasma [9].

Amaranth is a leafy vegetable, and its leaves and grains contain large amounts of NO_3 as well as other nutrients [26]. The NO_3 content of amaranth may range from 965 to 4259 mg/kg [1] or from 1800 to 9200 mg/kg [27]. There is scientific evidence suggesting that nitrate-rich spinach can augment nitric oxide levels, enhance endothelial function, and lower blood pressure acutely [13]. Moore and co-authors [25] reported that acute ingestion of 1000 mg of an amaranth extract substantially increased only the plasma NO_3 level and not the NO_2 level. Subramanian and Gupta [28] reported that an acute 2000 mg dose of amaranth extract, delivering ~ 180 mg (~ 2.9 mmol) of NO_3, increased plasma NO_3 and NO_2. This increase is similar to, or exceeds that observed with acute ingestion of relatively higher NO_3 doses from beetroot juice [11, 18].

There were some limitations to our study. We did not measure the plasma contents of NO_3 or NO_2; thus, based on the results of other studies, we can only assume that it increased after 6 days of amaranth consumption. Further studies are needed to measure acute and long-term changes in plasma NO_3 and NO_2 after the consumption of given amaranth supplement. Secondly, the number of study participants was rather small, so the statistical power was moderate (49.9% for the absolute VO2 max in the experimental group). We did not utilize a crossover type of experiment design in this particular study which is a particularly useful option in evaluating the safe and effective use of tested substances when participants switch from one substance to another. It has both advantages and disadvantages as compared to parallel study. Usually the order effect and carry-over effects are discussed among limitations. In our case the study involved repeated graded exercise tests so often testing might have some training effect. The washout period of ingesting dietary nitrates is unknown, so the experiment could be rather long with increased influences of different covariates. In this case a randomized double-blinded was used as a way of carrying out an experiment in an attempt to minimize subjective biases on the part of the experimenter and on the part of the participant.

Conclusion

Long-term (6 days) supplementation with dietary NO_3 from amaranth may improve aerobic capacity during ICE in young physically active male persons. It can be recommended as the nutritional supplement during last week of preparation for competition in endurance events.

Abbreviations

ICE: Increased cycling exercise; OB: Oat bar; VT1: First ventilatory threshold; VT2: Second ventilatory threshold; 1 T: Test Nr. 1 executed at day 1 of the study; 2 T: Test Nr. 2 executed at day 4 of the study; 3 T: Test Nr. 3 executed at day 9 of the study; HR: Heart rate; RER: Respiratory exchange ratio; La: Blood lactate concentration; SD: Standard deviation; NO_3^-: Nitrate; NO: Nitric oxide; W: Watts; VO_2max: The maximum rate of oxygen consumption measured during incremental exercise

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Authors’ contributions

The study was designed by T.L., R.K. and A.S.; the active components were extracted, fractionated and prepared for supplementation by P.V. and D.U.; the subjects were recruited and hosted the informative session by R.K.; L.S., A.S., S.C., checked that subjects followed the diet guidelines and the timing of supplement ingestion; data were collected and analyzed by A.S., L.S., S.C., T.L., P.V.; L.S., S.C., T.L. and J.V. conducted the statistical analysis; and data interpretation and manuscript preparation were undertaken by T.L., R.K., L.S., S.C., A.S., P.V. J.V. and D.U. The author(s) read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding Tomas Liubertas, T.L. The data are not publicly available due to General Data Protection Regulation (GRDP) of European Union (restrictions containing information that could compromise the privacy of research participants);

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Kaunas Regional Ethics Committee, Nr. BE-2-11, 21 March 2017.

Consent for publication

All authors approved the final version of the paper.

Competing interests

The authors declare no conflict of interest.

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