doi:10.20944/preprints201911.0336.v1

Article

The Influence of Amaranth (*Amaranthus hypochondriacus*) Dietary Nitrates on the Aerobic Capacity of Physically Active Young Persons

Tomas Liubertas [1,*], Ramutis Kairaitis [2], Loreta Stasiule [1], Sandrija Capkauskiene[1], Arvydas Stasiulis [1], Pranas Viskelis [3], Jonas Viškelis [3], Dalia Urbonaviciene [3]

[1] Department of Applied Biology and Rehabilitation, Lithuanian Sports University, 44221 Kaunas, Lithuania; loreta.stasiule@lsu.lt (L.S.); sandrija.capkauskiene@lsu.lt (S.C.); arvydas.stasiulis@lsu.lt (A.S.)
[2] Department of Coaching Science, Lithuanian Sports University, 44221 Kaunas, Lithuania; ramutis.kairaitis@lsu.lt (R.K.)
[3] Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, 54333 Babtai, Lithuania; biochem@lsdi.lt (P.V.); j.viskelis@lsdi.lt (J.V.); d.urbonav@gmail.com (D.U.)
* Correspondence: kamazinas@gmail.com (T.L.); Tel.: +370-612-66664

Abstract: Over the past five years, the popularity of dietary nitrates as an ergogenic device among athletes has increased significantly. Hypoxic and acidic conditions that occur during exercise facilitate the conversion of nitrite to nitric oxide (NO) and increase the physiological efficiency of exogenously produced nitrite. After a few years of our team predicted experiments, as a nitric oxide precursor, amaranth (*Amaranthus hypochondriacus*) was identified as a source of dietary nitrates (concentrations 9-11%). The aim of this study was to evaluate the effect of single-dose and long-term doses of dietary nitrates from amaranth concentrate on the aerobic capacity of physically active young persons. Thirteen healthy and physically active young participants were randomized to experimental and placebo groups using a increasing cycling exercise (ICE) and placebo-controlled design. Pulmonary gas exchange recording (oxygen uptake (VO_2), pulmonary ventilation (VE), respiratory exchange ratio (RER)) and analysis of blood lactate samples were obtained. Our findings indicate that the single dose of dietary amaranth (400 mg) significantly improved only the power of the test performance. Long-term (6 days) intake significantly increased the power of the test performance, the maximum oxygen consumption and the power of the test for the first ventilation threshold value (from 37.7±2.7 mL/kg/min during the first test to 41.2±5.4 mL/kg/min during the third test, $p < 0.05$).

Keywords: aerobic capacity, dietary nitrates from amaranth, young persons

---

1. Introduction

In the rapidly growing market for sports supplements, researchers and traders are increasingly focusing on botanical/organic substances, which are increasingly being used in the manufacture of preparations. According to forecasts, the market for botanical supplements, which as of 2013 was estimated at 54.6 billion US dollars, should reach 90.2 billion US dollars by the end of 2020 [1]. Green leafy vegetables and roots are the main sources of nitrates [2,3]. Nitrate is a naturally occurring compound as well as an approved food additive [2]. Recently, amaranth and beetroot (in various forms) are the dominant source of dietary nitrates, primarily due to their easy availability and the simple preparation required for final consumption. Some researchers think of amaranth as a plant that accumulates toxic nitrate concentrations and as a poisonous plant affecting animal and human health [4]. Depending on the conditions of cultivation, soil acidity, harvesting, processing, storage conditions and other factors, the concentration of nitrates in amaranth leaves can range from 1.8 to 9.2 g/kg [5], which complicates the standardization of this biologically active substance. Therefore, a few years ago, the strong group of professionals (chemists, food technologist and engineer, trainers, nutritionist, athletes) began testing the effects of dietary nitrates on the physical performance of athletes and physically active people. With the use of active substances (in various forms) for a longer period of time, the characteristics of the beet taste and the required amount of consumption have become major factors; therefore, searches for optimization and taste improvement have begun. During the last 2 years, with the participation of colleagues from the Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, more than fifty sources of nitrate from organic sources have been checked for the purpose of finding a treatment method and/or for the substance itself, which would be superior to the amount and/or effect of the current, most widely used source of dietary NO_3^-, e.g., amaranth.

Dietary nitrates are currently one of the most consumed nutritional supplements among athletes. A number of studies have already confirmed the benefits of dietary nitrates to human health: their consumption reduces blood pressure, suppresses platelet aggregation, protects against ischemic diseases, and improves endothelial function [6]. Nitric oxide and nitrites, both nitrate products, affect vasodilatation by increasing blood flow [7], thus increasing the oxygen uptake and oxidative processes in the working muscles [8]. Additionally, the use of nutrient nitrates increases 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 [9].

Over the past five years, the popularity of dietary nitrates as an ergogenic device has increased significantly. Hypoxic and acidic conditions that occur during exercise facilitate the conversion of nitrite to NO and increase the physiological efficiency of exogenously produced nitrite [7]. The effects of nitrate/nitrite/NO on the muscle circulatory system and mitochondrial and contractile efficacy [10,11] may increase muscle circulation and improve the metabolic response to physical activity [12]. There is evidence that even the concentration of plasma nitrites is an independent factor of physical performance [7,13]. Nevertheless, studies on the effects of nitrates on work capacity indicators are highly controversial thus far.

Some studies have shown the benefits of nitrates for work efficiency and oxygen expenditure [12,14-19], but other studies have not found visible and positive changes in performance [16,21-25].

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 [14,12,21-22,18-19,26]. Thus, based on these studies, we formulated the hypothesis that 400 mg of nitrates from amaranth will increase the aerobic capacity of physically active young people after 6 days of use.

The aim of the study was to evaluate the effect of single and long-term doses of dietary nitrates from amaranth on the aerobic capacity of physically active young persons.

2. Materials and Methods

3.1. Participants

Thirteen healthy and physically active young people agreed to participate in the study. All participants were randomized to experimental and placebo groups. The anthropometric data and age of the participants are presented in Table 1.

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 (m)	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	0.063
Relative fat mass (%)	13.1±3.8	18.9±5.6	0.568

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

3.2. Measurements

Anthropometry. Electronic weighing scales were used to measure the weight of the subjects (Body Composition Analyzer TBF-300, Tanita, Japan). These scales showed the age (years), height (m), body weight (kg), body mass index (BMI), relative fat mass (%), (kg) 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 cycling ergometer (Ergoline-800, Denmark). Prior to the ICE, a 5-min warn-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.

Pulmonary gas exchange recording and analysis. The subjects breathed through a face mask, and pulmonary gas exchange (oxygen uptake (VO_2), pulmonary ventilation (VE), respiratory exchange ratio (RER)) was measured breath-by-breath using a wireless, portable spirometric system called the "Oxycon mobile" (Viasys Healthcare; California, USA). Prior to each exercise session, the spirometric system was calibrated. The peak value of VO_2 over the 20 s of cycling was referred to as VO_2 peak, 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 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. Heart rate (HR) was calculated continuously with a wireless Polar monitoring system (S810 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.

3.4. 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.

The experimental group consumed a hand-made oat bar (60 g weight). It contained oats, honey, vanilla and 400 mg of active ingredient *Amaranthus hypochondriacus* concentrate. The placebo group consumed a similar meal weighing 60 g, containing oats, honey and vanilla. A nutritional supplement of 1 portion/day was used in the morning, right after breakfast.

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

3.5. 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 nitrates on aerobic capacity. The significance of the difference between the independent samples was evaluated using the Mann-Whitney test. 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).

doi:10.20944/preprints201911.0336.v1

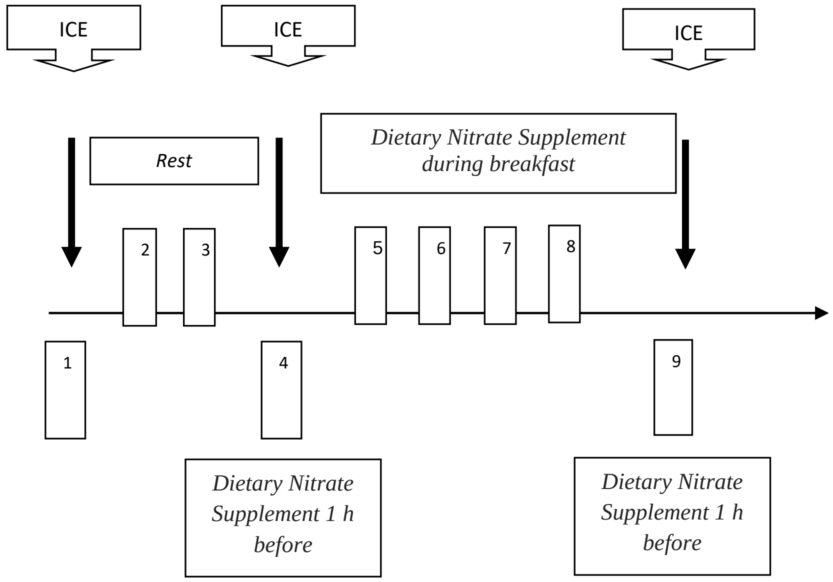

Figure 1. Study protocol

Note: ICE – increasing cycling exercise test; 1-9 –days of study

3. Results

The single dose of supplements did not have any significant effect on the relative VO_2 peak in either the experimental or placebo group. After long-term use of the supplements, the VO_2 peak was significantly increased in the experimental group (from 37.7±2.7 mL/kg/min during the first test to 41.2±5.4 mL/kg/min during the third test, $p < 0.05$) (Figure 2).

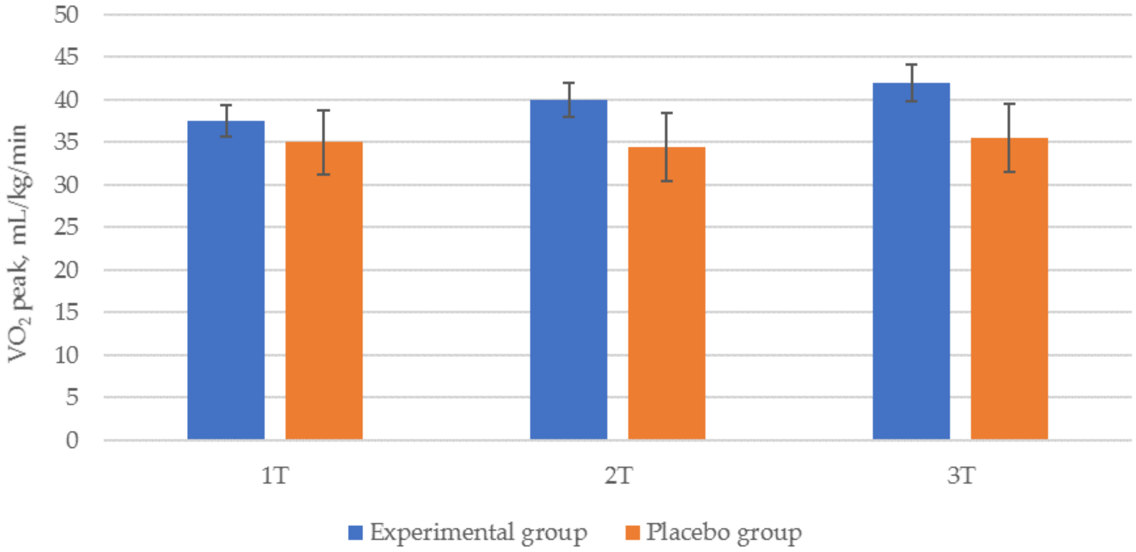

Figure 2. The effect of single-dose (2T) and long-term (3T) doses of nitrates on the maximal oxygen consumption (VO_2peak) in the experimental and placebo groups. * p - statistically significant difference compared to the first test.

HR_{max} did not change significantly in any group of participants ($p > 0.05$) (Figure 3).

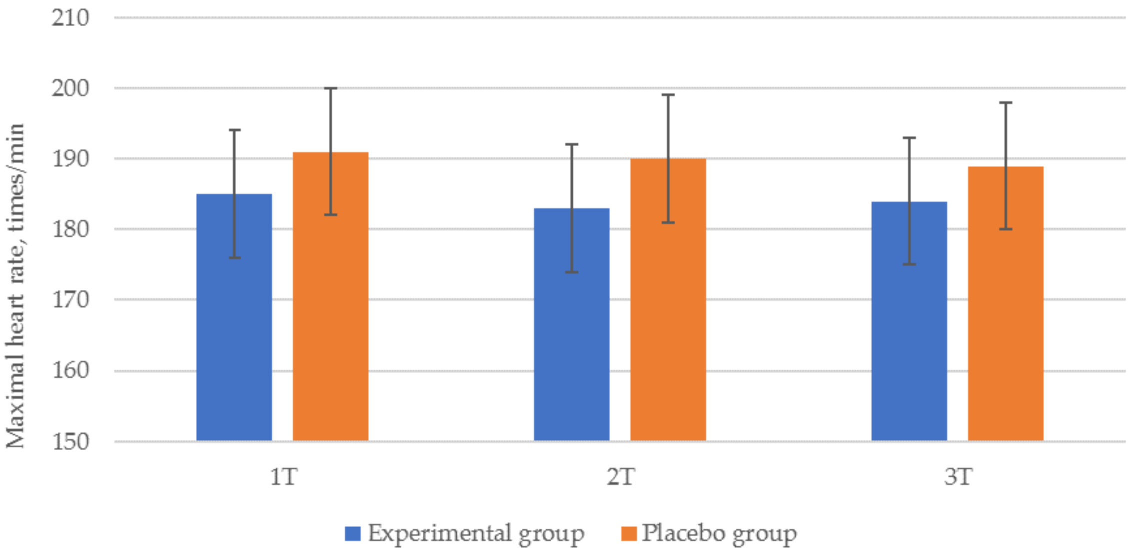

Figure 3. The effect of single-dose (2T) and long-term (3T) doses of supplements on the HR_{max} in the experimental and placebo groups.

VT1 did not change after a single dose of nitrates, but a significant difference was recorded in the experimental group between the first ventilatory thresholds after the second (2T) and third (3T) tests. After a single dose of nitrates, VeS1 was 178.6 ± 30.3 W, and after long-term use of nitrates, the value of VT1 increased significantly to 188.6 ± 35.2 W ($p < 0.05$; Figure 4)

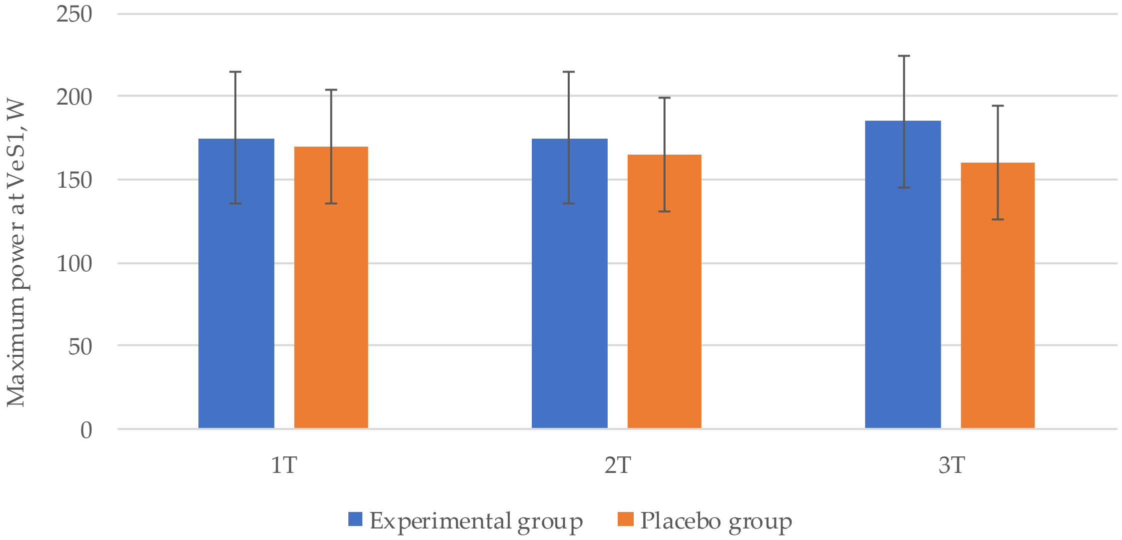

Figure 4. First ventilatory threshold (VeS1) after single-dose (2T) and long-term (3T) doses of supplements. * $p < 0.05$ - statistically significant difference between the 2nd and 3rd tests.

The second ventilation threshold (Figure 5) did not change significantly in any of the groups ($p > 0.05$).

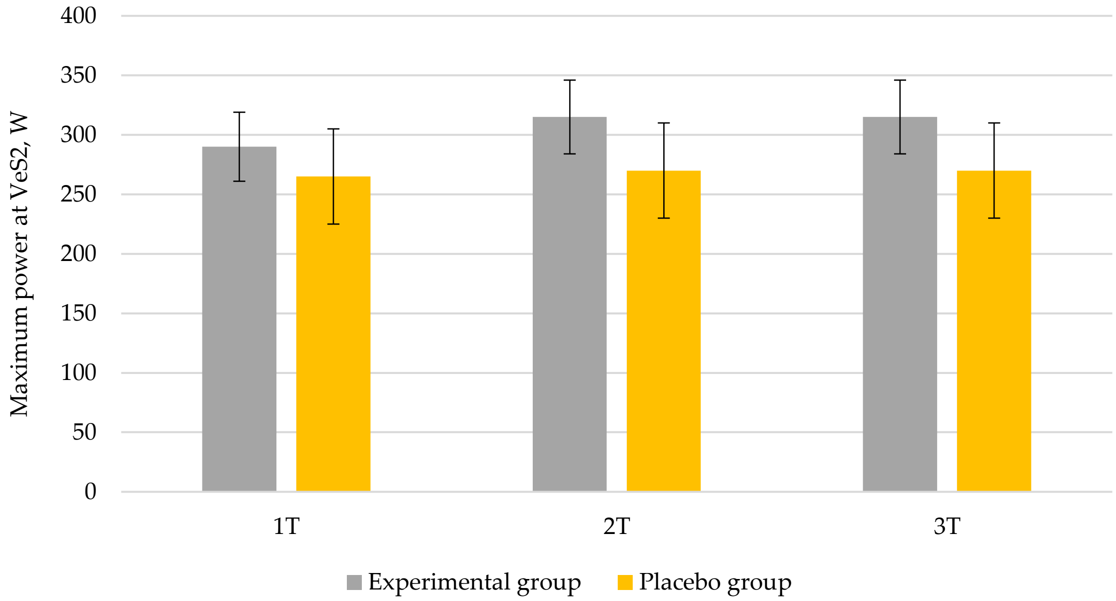

Figure 5. Second ventilatory threshold (VeS2) after single-dose (2T) and long-term (3T) doses of supplements in the experimental and placebo groups.

4. Discussion

Amaranth is a leafy vegetable, and its leaves and grains contain large amounts of nitrates as well as other nutrients [27]. The nitrate content of amaranth may range from 965 to 4259 mg/kg [6] or from 1,800 to 9,200 mg/kg [5]. There is scientific evidence suggesting that nitrate-rich spinach can augment nitric oxide status, enhance endothelial function, and lower blood pressure acutely [15]. 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 [13,21].

Based on this scientific evidence, we studied the effect of a single dose (400 mg) of dietary nitrates from amaranth on the aerobic capacity of young physically active persons. Our principal findings were that the VO_2 peak, which was measured both after a single consumption of 400 mg (or 6.5 mmol nitrate) of amaranth and after 6 days of consumption of this source of nitrate, was significantly increased only after six days of amaranth consumption. Our data coincide with other studies of a similar nature carried out by the authors (Table 2). The researchers cited used beetroot juice with varying amounts of nitrate. The most popular use of the fixed duration of the nitrates is 4-6 days, and the amount varies from 5 mmol up to 9 mmol. Significant differences between the groups were found in VO_2 peak by Bailey and colleagues [14,12], Lansley and colleagues [18], Vonhatalo and colleagues [21], and Wylie and colleagues [13]. Their studies involved healthy, physically active people and tested physical performance. Vanhatalo and colleagues [21] 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 2). Additionally, the first ventilatory threshold was significantly increased after the long-term use of nitrates in our study ($p< 0,05$). Therefore, we can argue that the long-term (6 days) consumption of 400 mg of nitrate in amaranth has a positive impact on the aerobic capacity of young people.

Table 2. Effects of single and long-term use of nitrates on the indicators of work capacity of healthy and physically active persons.

References	Participants	Supplementation	Concentration of nitrates	Duration of consumption	Protocol	Findings

			mmol/day			
[14]	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)
[22]	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).
[12]	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)
[21]	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%
[18]	Healthy, physically active people; n=9	500 ml 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 ($p<0,05$)
[19]	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 5,000-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 ($p<0,05$)
[13]	Healthy, recreationally active men; n=10	70, 140, or 280 ml of beet root 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	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 moderate-intensity exercise was reduced significantly following the

					measure of exercise tolerance, 2.5 h post-ingestion of beet root juice or placebo.	ingestion of 280 ml of BR ($p<0.05$).
[26]	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_2peak and a final stage at 90% VO_2 peak 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 ($p<0,05$)
[29]	Healthy, recreationally active participants n = 15 (males = 8, females = 7)	1000 mg red spinach extractus 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 exercising 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 occurring at a significantly higher relative VO_2 (+3.6±5.2%, $p<0.05$).

Moore and co-authors [29] 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. Additionally, the scientists observed a large effect on the ventilatory threshold compared to the placebo group, with the ventilatory threshold occurring at a significantly higher relative VO_2 (+3.6±5.2%, $p<0.05$).

5. Conclusions

A single dose of dietary nitrates from amaranth significantly improved only the power of the test performance. Long-term intake significantly increased the power of the test performance, the maximum oxygen consumption and the power of the test for the first ventilation threshold value.

Author 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 was 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. All authors approved the final version of the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thomas, A. Global Botanical Supplements Market Will Reach $90.2 Billion in 2020. **2014**. Available online: http://www.persistencemarketresearch.com/mediarelease/botanical-supplements-market.asp (accessed on 25 September 2019)
2. European Food Safety Authority (EFSA). Nitrate in vegetables-scientific opinion of the panel on contaminants in the food chain. *EFSA Journal* **2008**, *6(6)*, 689.
3. Viškelis, J., Rubinskienė, M., Urbonavičienė, D., Bobinaitė, R., Viškelis, P. Optimal postharvest storage parameters and shelf life of baby spinach (Spinacia oleracea L.), Proceedings of the 7th International Scientific Conference Rural Development 2015, Aleksandras Stulginskis University, Lithuania, November 19–20, 2015; Raupelienė A. Eds.; Kaunas, Lithuania, 2015; 1-4.
4. Stegelmeier, B.L., Field, R., Panter, K.E., Hall, J.O., Welch, K.D., Pfister, J.A., Gardner, D.R., Lee, S.T., Colegate, S., Davis, T.Z., Green, B.T., Cook D. *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, 3rd ed.; Academic Press, USA, 2013; pp. 1259-1314.
5. Prakash, D., Pal, M. Nutritional and antinutritional composition of vegetable and grain amaranth leaves. *Journal of the Science of Food and Agriculture* **1991**, *57(4)*, 573-583.
6. Lidder S., Webb A.J. Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. *British Journal of Clinical Pharmacology* **2013**, *75(3)*, 677-696.
7. Totzeck, M., Hendgen-Cotta, U.B., Luedike, P., Berenbrink, M., Klare, J.P., Steinhoff, H.-J., Semmler, D., Shiva, S., Williams, D., Kipar, A., Gladwin, M.T., Schrader, J., Kelm, M., Cossins, A.R., Rassaf, T. Nitrite regulates hypoxic vasodilation via myoglobin-dependent nitric oxide generation. *Circulation* **2012,** *17;126(3)*, 325-34.
8. Dreißigacker, U., Wendt, M., Wittke, T., Tsikas, D., Maassen, N. Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men. *Nitric Oxide* **2010**, *23(2)*, 128-135.
9. Lundberg, J.O., Carlstrom, M., Larsen, F.J., Weitzberg, E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovascular Research* **2011**, *89(3), 525-532.*
10. Larsen, F.J., Schiffer, T.A., Borniquel, S., Kent Sahlin, K., Ekblom, B., Lundberg, J.O., Weitzberg, E. Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metabolism* **2011**, *13*(2), 149–159.
11. Larsen, F.J., Schiffer. T.A., Weitzberg, E., Lundberg, J.O. Regulation of mitochondrial function and energetics by reactive nitrogen oxides. *Free Radical Biology and Medicine* **2012**, *53(10)*, 1919-1928.
12. Bailey, S.J., Fulford, J., Vanhatalo, A., Winyard, P.G., Blackwell, J.R., DiMenna, F.J., Wilkerson, D.P., Benjami N., Jones, A.M. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *Journal of Applied Physiology* **2010**, *109*, 135–148.
13. Wylie, L.J., Kelly, J., Bailey, S.J., Blackwell, J.R., Skiba, P.F., Winyard, P.G., Jeukendrup, A.E., Vanhatalo, A., Jones, A.M. Beetroot juice and exercise: pharmacodynamic and dose-response relationships. *Journal of Applied Physiology* **2013**, *115(3)*, 325-336.
14. Bailey, S.J., Winyard, P., Vanhatalo, A., Blackwell J.R., DiMenna F.J., Wilkerson D.P., Tarr, J., Benjamin, N., Jones, A.M. Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology* **2009**, *107(4)*, 1144-1155.
15. Bond, H., Morton, L., Braakhuis, A.J. Dietary nitrate supplementation improves rowing performance in well-trained rowers. *International Journal of Sport Nutrition and Exercise Metabolism* **2012**, 22, 251–256.

16. Cermak, N.M., Gibala, M.J., van Loon, L.J. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *International Journal of Sport Nutrition & Exercise Metabolism* **2012**, *22(1)*, 64-71.
17. Lansley, K.E., Winyard, P.G., Bailey, S.J., Vanhatalo, A., Wilkerson, D.P., Blackwell, J.R., Gilchrist, M., Benjamin, N. Acute dietary nitrate supplementation improves cycling time trial performance. *Medicine & Science in Sports & Exercise* **2011a**, *43(6)*, 1125-1131.
18. Lansley, K.E., Winyard, P.G., Fulford, J., Vanhatalo. A., Bailey, S.J., Blackwel, J.R., DiMenna, F.J., Gilchrist, M., Benjamin, N., Jones, A.M. Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. *Journal of Applied Physiology* **2011b**, *110(3)*, 591-600.
19. Masschelein, E., Van Thienen, R., Wang, X., Van Schepdael, A., Thomis, M., Hespel, P. Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *Journal of Applied Physiology* **2012,** *113(5)*, 736-745.
20. Larsen, F.J., Weitzberg, E., Lundberg, J.O., Ekblom, B. Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiologica* **2007**, *191(1)*, 59-66.
21. Vanhatalo, A., Bailey, S.J., Blackwell, J., DiMenna, F.J., Pavey, T.G., Wilkerson, D.P., Benjamin, N., Winyard, P.G., Jones, A.M. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. [Randomized Controlled Trial]. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2010**, *299(4)*, R1121-R1131.
22. Larsen, F.J., Weitzberg, E., Lundberg, J.O., Ekblom, B. Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free Radical Biology and Medicine* **2010**, *48(2)*, 342-347.
23. Murphy, M., Eliot, K., Heuertz, R.M., Weiss, E. Whole beetroot consumption acutely improves running performance. *Journal of the Academy of Nutrition and Dietetics* **2012**, *112(4)*, 548-552.
24. Peacock, O., Tjonna, A.E., James, P., Wisløff, U., Welde, B., Böhlke, N., Smith, A., Stokes, K., Cook, C., Sandbakk, O. Dietary nitrate does not enhance running performance in elite cross-country skiers. *Medicine and Science in Sports and Exercise* **2012**, *44(11)*, 2213-2219.
25. Wilkerson, D.P., Hayward, G.M., Bailey, S.J., Vanhatalo, A., Blackwell, J.R., Jones, A.M. Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. *European Journal of Applied Physiology* **2012**, *112(12)*, 4127-4134.
26. Thompson, K.G., Turner, L., Prichard, J., et al. Influence of dietary nitrate supplementation on physiological and cognitive responses to incremental cycle exercise. *Respiratory Physiology and Neurobiology* **2014**, *193*, 11–20.
27. Fasuyi, A.O., Dairo, F.A.S., Adeniji, A.O. Tropical vegetable (Amaranthus cruentus) leaf meal as alternative protein supplement in broiler starter diets: Bionutritional evaluation. *Journal of Central European Agriculture* **2008**, *9(1)*, 23-34.
28. Subramanian, D., Gupta, S. Pharmacokinetic study of amaranth extract in healthy humans: A randomized trial. *Nutrition* **2016**, *32(7-8)*, 748-753.
29. Moore, A.N., Haun, C.T., Kephart, W.C., Holland, A.M., Mobley, C.B., Pascoe, D.D., Roberts M.D., Martin, J. S. Red spinach extract increases ventilatory threshold during graded exercise testing. *Sports* **2017**, *5(4).*