



Vegetables with High-Nitrate Content Significantly Increase Plasma Nitrate and Nitrite Concentrations but Do Not Significantly Reduce Systolic Blood Pressure in Young Healthy Men

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Authors' contributions

This work was carried out in collaboration among all authors. Author AA was responsible for study design, recruitment, data collection, statistical analysis, data interpretation and drafting of the manuscript. Author GMH was responsible for data collection. Author JRB was responsible for plasma analysis. Authors AV and AMJ were responsible for study design, recruitment, data interpretation, study supervision and approval of the final manuscript.

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Study Protocol

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ABSTRACT

Aims: To investigate the effects of supplementation with high-nitrate and low-nitrate vegetables on plasma nitrate and nitrite concentrations, blood pressure and the oxygen demand of moderate-intensity exercise.

Study Design: A randomized, cross-over design.

Place and Duration of Study: Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, between January 2011 and March 2012.

Methodology: 15 non-smoking, physically active healthy men (age 25 ± 6 years, BMI 24 ± 4 kg/m²) were randomized to receive a 2-week supply of high-nitrate or low-nitrate vegetables, with a 2-week 'wash-out' period in between. Clinic blood pressure, plasma nitrate and nitrite concentrations and physiological responses to moderate-intensity exercise tests were measured before and after each 2-week intervention. Nitrate intake was calculated using nutritional analysis of reported vegetables consumed.

Results: Participants consumed significantly more dietary nitrate on the high-nitrate diet (417 ± 139 mg/day) than the low-nitrate diet (26 ± 11 mg/day). The high-nitrate diet supplied 5.5 mg nitrate/kg body weight, exceeding the Acceptable Daily Intake (ADI) of 3.7 mg nitrate/kg body weight. Supplementation with high-nitrate vegetables significantly increased plasma nitrate concentrations (baseline; 30 ± 20 μ M, after high-nitrate vegetables; 129 ± 87 μ M) and plasma nitrite concentrations (baseline; 119 ± 35 nM, after high-nitrate vegetables; 227 ± 89 nM) but did not significantly change systolic blood pressure or the physiological response to moderate exercise. There were significant correlations between diastolic blood pressure and plasma nitrite concentrations (low-nitrate diet; $r = 0.63$, high-nitrate diet, $r = 0.56$).

Conclusion: Supplementation with high-nitrate vegetables above the ADI significantly increased plasma nitrate and nitrate concentrations but did not significantly reduce systolic blood pressure or the physiological response to moderate exercise. Plasma nitrite concentrations significantly correlated with diastolic blood pressure after high-nitrate and low-nitrate diets.

Keywords: High-nitrate vegetables; plasma nitrate; nitrite; blood pressure.

1. INTRODUCTION

Dietary nitrate is not currently viewed as a nutrient necessary for health. Previous research both in animals and humans linked dietary nitrate to the formation of carcinogenic N-nitrosamines [1-3]. Furthermore, high nitrate levels in contaminated well water was associated with methaemoglobinaemia (blue baby syndrome) [4]. Subsequent evaluations of the potential toxicity of nitrate were carried out and an Acceptable Daily Intake (ADI) of 0 to 3.7 mg nitrate/kg bodyweight was set [5,6]. To ensure the ADI is met, current legislation limits the nitrate content of drinking water [7], cooked meats [8] and high nitrate vegetables such as spinach, lettuce and rocket, [6,9]. The ADI equates to 260 mg nitrate/70 kg person/day and remains in place today despite subsequent risk assessments [10].

The important discovery of nitric oxide (NO) in the 1980's has led to a re-evaluation of the metabolism of dietary nitrate. When NO was discovered, it was believed that nitrate was an inert end product from the oxygen-dependent L-arginine-NO pathway [11]. However, it is now recognized that NO can be derived from dietary nitrate via the nitrate-nitrite-NO pathway which is facilitated in hypoxic conditions [12]. After oral consumption, nitrate is effectively absorbed with an absolute bioavailability of close to 100% [13], and plasma nitrate rapidly increases and remains high for several hours [14]. The salivary glands actively take up and concentrate nitrate from

plasma, so that nitrate concentration in saliva is at least 10 times higher than plasma nitrate [15]. The key step of this entero-salivary pathway is the action of commensal bacteria in the mouth, which reduce concentrated salivary nitrate to nitrite [14] and the role of the oral microbiome is becoming increasingly recognized [16]. When swallowed, some nitrite is reduced to NO in the acidic environment of the stomach, but the remaining nitrite is absorbed into the circulation, elevating plasma nitrite levels [14]. Plasma nitrite acts as a major pool of NO, a powerful hypoxic vasodilator associated with blood pressure (BP) regulation [17]. NO is also important in situations such as myocardial infarction and stroke as it protects against tissue injury associated with hypoxia and ischaemic stress [18].

Since the first human intervention study demonstrating that a nitrate supplement (~430 mg nitrate/day) significantly reduced BP compared to placebo [19], a substantial body of research has been published, including several systematic reviews [20-25]. The primary driver of this research has been a desire to find the optimum dietary approach to control elevated BP, one of the leading risk factors for global mortality [26]. For example, one systematic review estimated that dietary nitrate reduces systolic BP by ~5 mmHg, which in turn could reduce the prevalence of hypertension and mortality from stroke by 20% and 14% respectively [21]. Most studies have used vegetable juices or soups, beetroot-enriched

foods or nitrate supplements to deliver acute doses of ~500 mg nitrate/day [27-56], approximately twice the ADI. A few studies have used fresh, frozen or cooked vegetables [31,57-62]. Beneficial outcomes of supplementation include a reduction in BP [28,33,36,41-43,45-49, 57,59,63-67], improvements in chronic obstructive pulmonary disease [27,35,36,38,39, 68], cerebral blood flow [32,69], vascular function [27,30,31,34,37,40,58], renal function [44] and exercise performance in patients with peripheral arterial disease [68]. Nitrate supplementation has also been reported to improve exercise efficiency and time to exhaustion [29,51,67,70-79]. Although the precise mechanisms are not fully understood, nitrate supplementation may reduce the ATP cost of muscle force production [73] and increase mitochondrial efficiency [80].

On the basis of reductions in BP reported in intervention studies and subsequent systematic reviews, it has been suggested that dietary nitrate could change from being regarded as a food contaminant to being recognized as a nutrient with a potential role in improving cardiovascular health outcomes [10,61,81-86]. Animal studies suggest that long-term dietary nitrate deficiency causes metabolic syndrome, endothelial dysfunction and premature death from cardio-vascular disease [87]. In addition, epidemiological data suggests that a higher intake of nitrate-rich vegetables is associated with reduced cardiovascular disease [88]. However, the risk-benefit assessment of dietary nitrate is subject to debate and it has been stated that further research is required to establish the effects of dietary nitrate on long-term health [84, 89-91].

This study aimed to test the hypothesis that diets supplemented for 2 weeks with fresh high-nitrate vegetables commonly available in the UK would increase plasma nitrate and nitrite concentrations, would be associated with lower BP and reduce the oxygen demand of moderate-intensity exercise in healthy men compared to diets supplemented for 2 weeks with fresh low-nitrate vegetables.

2. METHODOLOGY

2.1 Participants

Healthy, physically active, male participants aged 18-40 years were recruited from the University of Exeter and the surrounding area. All participants

who responded were provided with written information and those who were subsequently recruited provided written, informed consent to participate. Participants were healthy, non-smoking and free from pathological or physiological impairment or any other limitation that would restrict their ability to carry out both moderate and incremental cycling tests.

The study used a randomized, repeated measures cross-over design. Participants were randomized to receive a 2-week supply of either high- or low-nitrate vegetables followed by a 2-week 'wash out' period where they returned to their normal diet, with no restrictions. This was followed by a further 2-week supply of vegetables so that each participant received both dietary interventions over a 6-week time period. Before and after each 2-week supplementation period, participants underwent tests to assess plasma nitrate and nitrite, BP and physiological responses to moderate-intensity exercise. Participants were not informed about the study hypotheses and which diets were high or low in nitrate until after data collection was complete. A laboratory technician who was blinded to the test conditions carried out the analysis of plasma nitrate and nitrite.

Participants were invited to attend the Exercise Physiology Laboratory at Sport and Health Sciences, University of Exeter. Prior to each visit, participants were requested to avoid strenuous physical activity, caffeine and alcohol during the previous 24 hours and to have eaten their last meal or snack three hours beforehand. They were requested to avoid using mouthwashes during the study as chlorhexidine-containing, antibacterial mouthwash has been shown to attenuate the conversion of nitrate to nitrite [92]. During the first visit, the timeline of the study was explained by a registered dietitian who obtained a diet history by 24-hour recall, during a face-to-face interview, to ensure that participants were able to eat a variety of vegetables. Participants were weighed on digital scales sensitive to 100 g (XWM-105K, Hampel Electronic Co. Ltd, Taiwan), their height measured (Harpender Portable Stadiometer, Holtain Ltd, UK) and their BMI calculated as weight/height^2 (kg/m^2). They completed a Physical Activity Readiness questionnaire (PAR-Q, Canadian Society for Exercise Physiology, 2002). Participants then had their BP measured and performed an incremental exercise test to determine the maximum oxygen consumption (VO_2max).

2.2 Exercise Protocol

An incremental exercise test was performed using an electronically braked cycle ergometer (Lode Excalibur Sport V2, Lode BV, Groningen, The Netherlands). Pulmonary gas exchange and ventilation were continuously measured using a portable metabolic cart (Cortex Metalyzer 3B, Cortex Biophysik gmbh, Leipzig, Germany). Participants wore a nose clip and breathed through a mouthpiece securely attached to a turbine. Data from the test was used to determine the gas exchange threshold (GET) which has been described in detail elsewhere [93] and the maximum oxygen uptake (VO_2 max).

Before and after each 2-week supplementation period, participants attended the exercise physiology laboratory for exercise tests on the cycle ergometer. Having completed a 4 min baseline cycle (20 W), the work rate was increased stepwise to a level consistent with 80% of their individual GET, for 3 bouts of 6 min duration. In between each 6 min bout, participants rested on the bike for 7 min, followed by a 3 min baseline cycle at 20 W. The mean pulmonary oxygen consumption (VO_2), carbon dioxide output (VCO_2) and respiratory exchange ratio (RER) from the final 60 seconds of the baseline and moderate intensity exercise periods were calculated.

Participants were requested to continue with their normal training/exercise routine during the study. They were requested to complete a simple exercise diary to obtain a measure of their physical activity levels throughout.

2.3 Blood Pressure

Before and after each 2-week supplementation period and before exercise testing, participants were seated, undisturbed for a minimum of 5 min before BP was measured, according to current guidelines [94]. Four readings of systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure (MAP) were obtained using an automated sphygmomanometer (Dinamap PRO 100V2, GE Medical Systems Information Technology, Tampa, USA). The first reading was discarded and the mean of the final three readings was taken.

2.4 Dietary Protocol

Data on the nitrate content of readily available vegetables was obtained from literature sources

[6,95] and used to design high- and low-nitrate vegetable boxes. High-nitrate vegetable boxes were designed to provide approximately 300-500 mg nitrate daily, using a range of vegetables, in line with previous studies using beetroot juice and sodium nitrate [19,71]. This amount of nitrate is achievable in a normal diet by eating 2 or 3 portions of green leafy vegetables daily [83]. Fruit was included to equate the carbohydrate content of the boxes (fruit contains little dietary nitrate, apart from rhubarb). Vegetable boxes were delivered to participants via a delivery company (Riverford Organic Farms Ltd, Buckfastleigh, Devon, UK). Participants were requested not to change their normal diet, apart from the vegetable component. Participants were given a list of vegetables to avoid whilst on the low-nitrate vegetable diet but were not informed that these were high in nitrate.

During each 2-week supplementation period, participants were asked to record their dietary intake using a daily food diary. In addition, after each supplementation period they were asked to recall if they had eaten all of the vegetables and if not, to recall the quantity uneaten, or wasted. Quantitative nutritional assessments of the edible portions of fruit and vegetables eaten from the vegetable boxes were carried out using a nutritional analysis software programme, (Microdiet, Downlee Systems, Chapel-en-le-Frith, UK) with nitrate (mg/100 g) added as an additional nutrient. Similarly, total polyphenol and quercetin content was assessed using figures from an on-line database, Phenol-Explorer [96].

2.5 Plasma Nitrite and Nitrate Analysis

Before and after each 2-week supplementation period, venous blood samples were collected into a lithium-heparin tube (BD Vacutainer Plasma Tube, 6 ml, Becton Dickinson, Plymouth, UK). The sample was immediately placed into a refrigerated centrifuge (Sorvall ST16R Thermo Scientific, Hemel Hempstead, UK) and spun at 4000 rpm for 10 min. Plasma was subsequently withdrawn into 3 separate micro centrifuge tubes, (Fisher brand FB74031, Fisher Scientific, Loughborough, UK) and frozen at -80°C for later analysis of nitrate and nitrite, using a modified chemiluminescence technique described elsewhere [71,97].

2.6 Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences version 19 (IBM

Corporation, Armonk, NY, USA), with statistical significance accepted when $P < 0.05$. Results are reported as means (\pm SD), unless stated otherwise. Paired sample t-tests were conducted to compare differences in body mass, reported exercise and nutritional analyses of vegetables consumed. A two-way repeated-measures ANOVA (diet by time) was used to assess the impact of the high- and low-nitrate diets on plasma nitrate, nitrite, BP and exercise performance variables. Post hoc (Fisher's LSD) tests were carried out to identify significant differences. Relationships between changes in the variables were assessed using Pearson's product-moment correlation coefficients, where correlation was accepted as significant at the 0.05 level (one-tailed). A power calculation was performed (G*Power statistical analysis programme) using results from a previous study where systolic BP was significantly reduced [73]; assuming a power of 80%, at the 5% level of significance and a standard deviation of 5 mmHg, to detect a difference of 5 mmHg between the two groups resulted in an estimated sample size of 18 subjects.

3. RESULTS

3.1 Participants

Twenty-one participants were recruited from January 2011 to March 2012. Five participants withdrew for various reasons including illness and pressures of work. Data from one participant was subsequently excluded due to non-adherence to the study protocol, resulting in high plasma nitrite concentrations (Fig. 1). The main factors affecting participation included: remaining healthy throughout the six weeks of the study, availability to attend all 5 laboratory visits at the same time of day and ability to follow the study protocol. Every attempt was made to replace participants lost to achieve the target sample size, by continually advertising and running the study for over 12 months. Data were analysed on the remaining 15 participants, age 25 ± 6 years, BMI 24 ± 4 kg/m². Three participants had a BMI of >25 kg/m² but actively participated in weight training or rugby and therefore were deemed to be 'healthy'. There was no significant difference between the mean body mass of participants at the beginning compared to the end of the study (beginning: 75.3 ± 11.4 kg; end: 75.6 ± 11.6 kg, $P = .25$). There was no significant difference between mean reported physical activity during the two-week supplementation periods (first: 12.8 ± 7.5 hours; second: 11.1 ± 6.6 hours, $P = .20$).

3.2 Dietary Analysis

The nutritional analysis of the edible content of the vegetable boxes reported as consumed is shown in Table 1.

Based on mean body-weight at the end of the study, (75.6 kg), participants consumed ~ 5.5 mg dietary nitrate/kg body weight on the high-nitrate diet, whereas on the low-nitrate diet, they consumed ~ 0.3 mg dietary nitrate/kg body weight.

3.3 Plasma Nitrate and Nitrite

Plasma nitrate and nitrite concentrations are shown in Figs. 2 and 3, respectively. The ANOVA revealed significant main effects by diet and time and an interaction effect of dietary supplementation on plasma nitrate (Fig. 2; all ANOVA $P = .001$).

Similarly, there was a significant main effect of dietary supplementation on mean plasma nitrite ($P = .037$) and there was a significant main effect for time on plasma nitrite ($P = .007$). There was a significant interaction effect for supplementation and time on plasma nitrite ($P < .001$) (Fig. 3; all $P < .05$).

3.4 Blood Pressure

The ANOVA suggested no significant main or interaction effects between diet (low-nitrate and high-nitrate) and time (baseline and post-supplementation) (Table 2; all $P > .05$).

To determine whether there was any association between BP and plasma nitrate or nitrite concentrations, Pearson's product-moment correlation coefficient, r , was used. A moderate but significant negative correlation was found between change in SBP and change in plasma nitrate after supplementation with high-nitrate vegetables, indicating that increased plasma nitrate was associated with reduced SBP ($r = -0.49$, $P = .03$). A similar effect was found between change in MAP and changes in plasma nitrate ($r = -0.44$, $P = .049$) and plasma nitrite ($r = -0.50$, $P = .03$) after supplementation with high-nitrate vegetables. In addition, there were significant correlations between change in DBP and change in plasma nitrite after supplementation with both low-nitrate and high-nitrate vegetables indicating that increased plasma nitrite was associated with reduced DBP (Fig. 4).

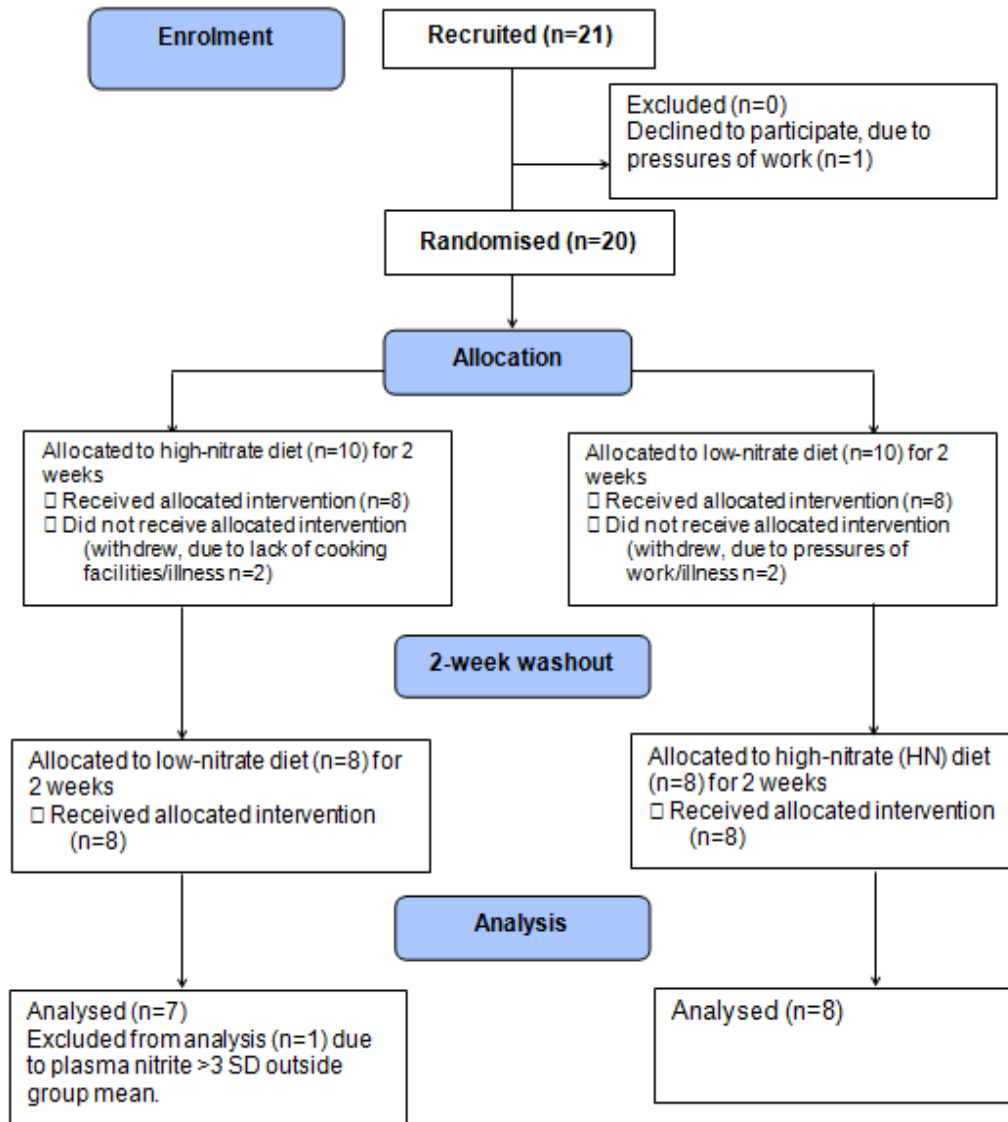


Fig. 1. CONSORT diagram

Table 1. Nutritional analysis of edible portion of high-nitrate and low-nitrate vegetable boxes with mean (\pm SD) daily intake during 2-week supplementation period

Intake per day	Low-nitrate diet	High-nitrate diet	P value*
Vegetables consumed (g)	350 \pm 100	266 \pm 58	.005
Nitrate (mg)	26 \pm 11	417 \pm 139	< .001
Energy (kcal)	136 \pm 54	146 \pm 47	.17
Energy (kJ)	574 \pm 226	620 \pm 199	.14
Carbohydrate (g)	28 \pm 10	28 \pm 10	.48
Total polyphenols (mg)	463 \pm 256	718 \pm 368	.001
Quercetin (mg)	6 \pm 2	6 \pm 3	.43

*P values are for paired sample t-tests (2-tailed)

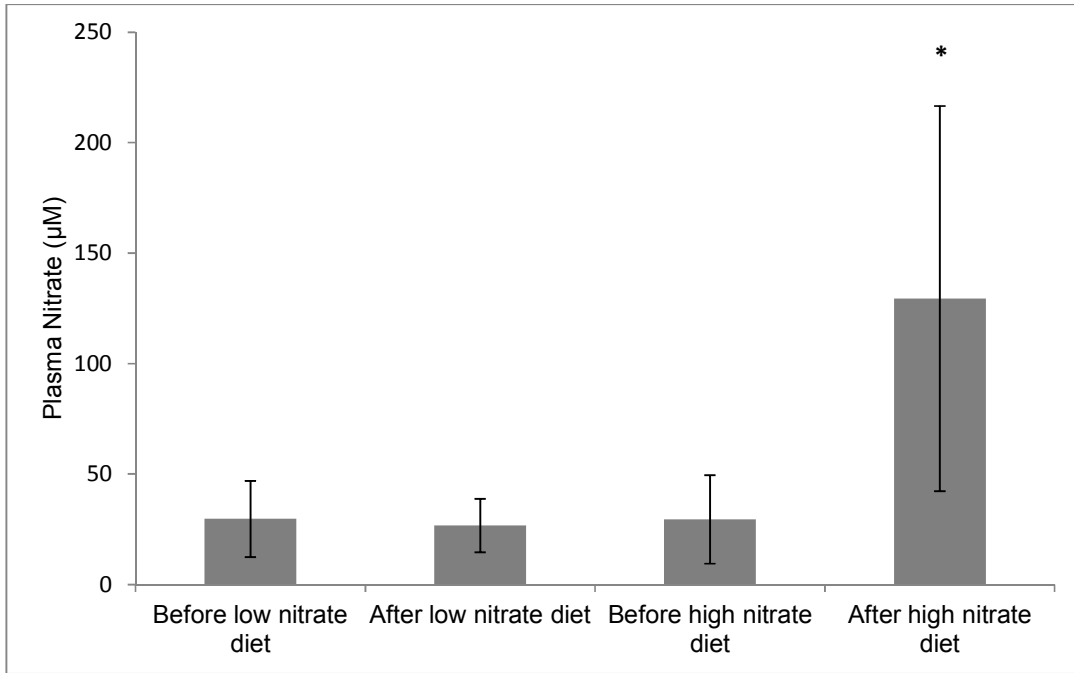


Fig. 2. Effects of two weeks supplementation with high and low-nitrate vegetables on mean plasma nitrate in 15 participants. Plasma nitrate was significantly higher after eating high-nitrate vegetables

**ANOVA P = .001, error bars indicate SD*

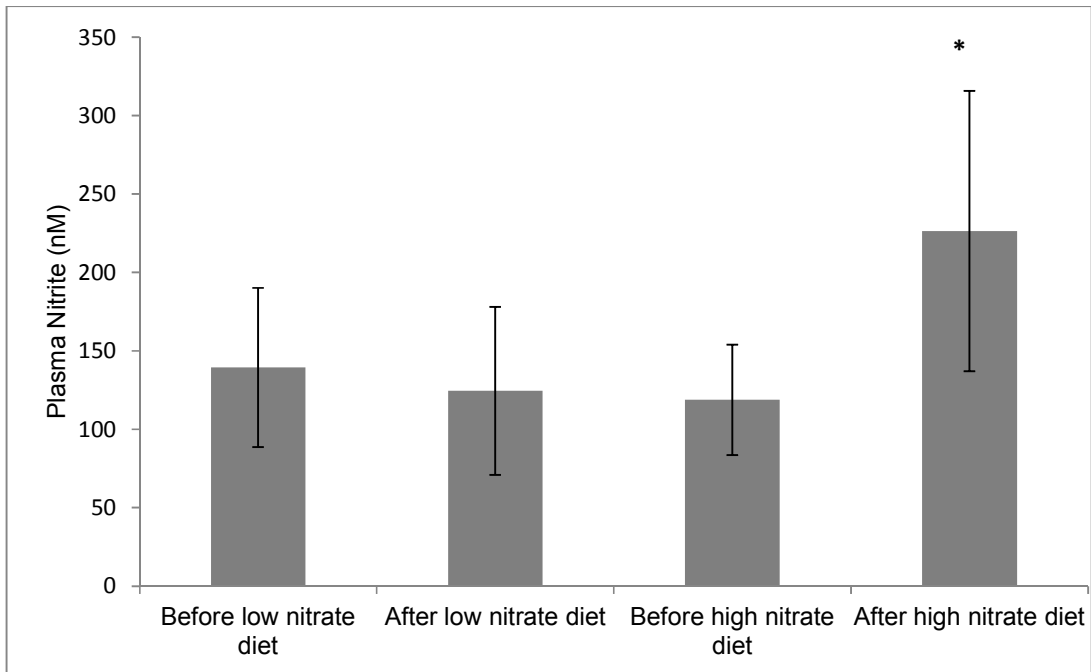


Fig. 3. Effects of high and low-nitrate vegetables on mean plasma nitrite in 15 participants. Plasma nitrite was significantly higher after eating high-nitrate vegetables

**ANOVA P < .001, error bars indicate SD*

Table 2. Blood pressure (systolic, diastolic and mean arterial pressure) at baseline and post-supplementation with low-nitrate diet and high-nitrate diets (mean ± SD)

BP (mmHg)	Low-nitrate diet			High-nitrate diet		
	Baseline	Post-supplementation	Δ	Baseline	Post-supplementation	Δ
SBP	118 ± 9	118 ± 10	0	121 ± 9	118 ± 9	-3
DBP	65 ± 9	66 ± 9	1	68 ± 8	65 ± 8	-3
MAP	86 ± 6	87 ± 7	1	89 ± 5	86 ± 7	-3

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure

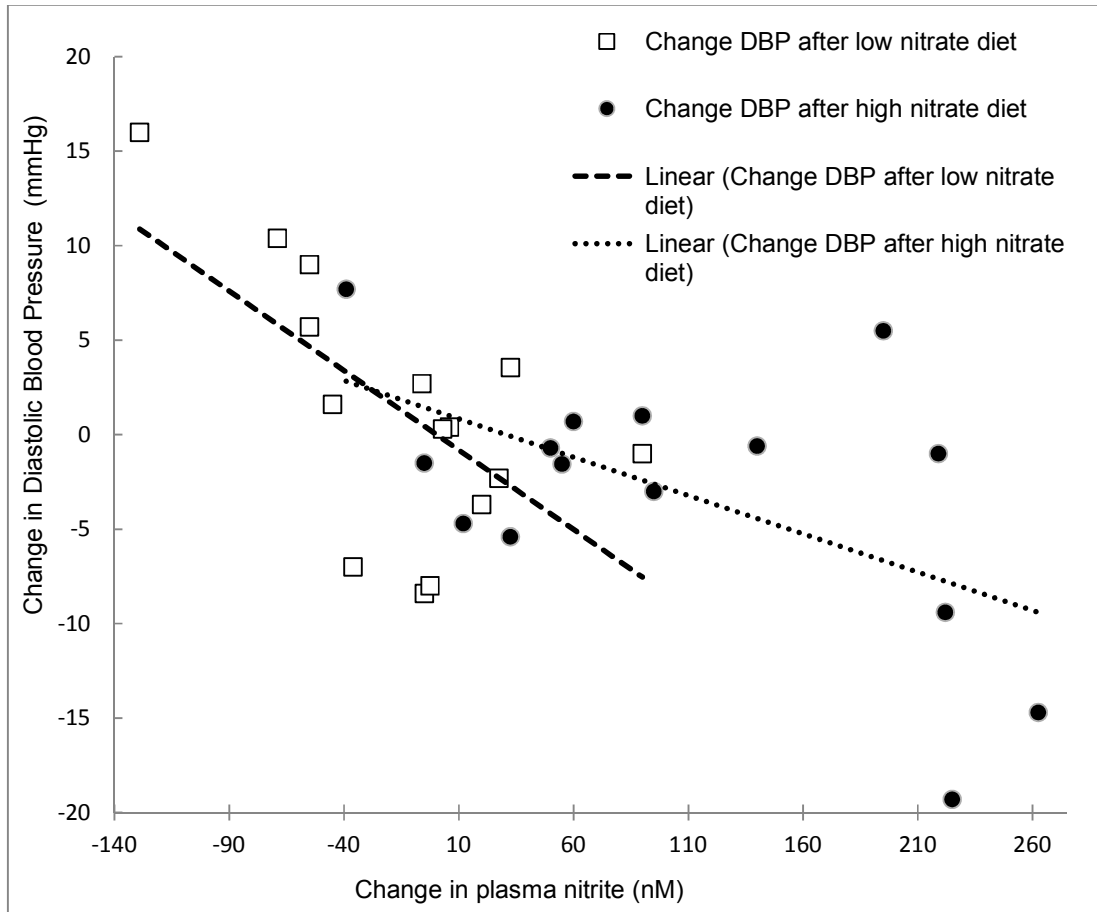


Fig. 4. Correlation between diastolic blood pressure and plasma nitrite following two-week supplementation with low-nitrate (dashed line) and high-nitrate diets (dotted line)
 Low-nitrate diet; Pearson's product-moment correlation, $r = -0.63$, $P = .006$, high-nitrate diet, $r = -0.56$, $P = .015$

Table 3. Gas exchange variables after moderate exercise measured at baseline and post-supplementation with low-nitrate diet and high-nitrate diets (mean ± SD)

	Low-nitrate diet		High-nitrate diet	
	Baseline	Post-supplementation	Baseline	Post-supplementation
(VO ₂)(L/min)	1.36 ± 0.24	1.34 ± 0.20	1.38 ± 0.24	1.36 ± 0.21
(VCO ₂)(L/min)	1.21 ± 0.22	1.20 ± 0.21	1.20 ± 0.20	1.21 ± 0.19
RER	0.90 ± 0.04	0.89 ± 0.05	0.88 ± 0.03	0.89 ± 0.04

VO₂; oxygen consumption, VCO₂; carbon dioxide production, RER; respiratory exchange ratio

3.5 Gas Exchange during Moderate Exercise

There were no significant main or interaction effects of either high- or low-nitrate vegetables on VO_2 , VCO_2 or RER during the moderate exercise tests (80% GET) (Table 3).

4. DISCUSSION

This study suggests that eating high-nitrate vegetables may increase dietary nitrate consumption over the ADI of 3.7 mg nitrate/kg/day. This is relevant, as it has been suggested that a minimum dose of ~500 mg nitrate, approximately double the ADI for a 70 kg person, is required to significantly reduce BP [21, 43]. In addition, a recent systematic review and meta-analysis noted that nitrate doses of ≥ 650 mg led to the greatest reductions in SBP (-10.5 mmHg; 95%CI, -15.4 to -5.5; $P < .0001$) and DBP (-6.3 mmHg; 95%CI, -12.5 to -0.1; $P = .045$) [98]. The ADI was set to protect consumers from a perceived risk of cancer from the formation of N-nitroso compounds. In 2010, the World Health Organization International Agency for Research on Cancer Working Group recognised that nitrate consumed in vegetables is less likely to form N-nitroso compounds due to the inhibition of this process by vitamin C and antioxidants abundant in vegetables [99]. More recently, Mills et al. [100] have suggested that according to data from the World Cancer Research Fund, high nitrate vegetables such as spinach and lettuce are associated with decreased risks of risks of cancer of the mouth, pharynx, larynx, oesophagus and stomach. Furthermore, epidemiological studies suggest that green leafy vegetables and salad (high in dietary nitrate) are protective against cardiovascular disease and stroke [101-103]. There is therefore a need to examine more recent data to explore whether dietary nitrate from vegetables presents a risk to human health or whether it might instead be protective.

The increases in plasma nitrate and nitrite reported are consistent with other randomised controlled crossover trials using high-nitrate vegetables [57,61,65]. Despite these increases, there was no significant reduction in SBP, in contrast to recent research suggesting inorganic nitrate can significantly reduce blood pressure via the nitrate-nitrite-NO pathway [19,27,31,33, 34,43,57-59,61,64,65,67,71,104,105]. However, these findings are in agreement with studies which reported using high-nitrate vegetables, but

no subsequent significant reduction in SBP [60-62,106], although these studies included older participants and some with elevated BP. Many factors could explain the discrepancy between non-significant reductions in BP after nitrate supplementation. Firstly, individuals with higher baseline SBP values have been reported to experience a greater decrease after nitrate supplementation [57,64] and baseline SBP in this study was lower than the population average for men of a similar age (125 mmHg) [107]. Other factors include: the possibility of a down-regulation of NO synthase [108], nitrate dose, age, gender and previous health status [21,25, 109]. In addition, the role of the oral microbiome and the physiological effects of nitrate stores are now emerging [16,48,110,111] and these are important factors which should be included in future research.

However, the findings that DBP correlated with changes in plasma nitrite on both high- and low-nitrate diets supports previous findings by Sobko et al. [65], who reported that supplementing diets of 15 women and 10 men with high-nitrate, Japanese vegetables significantly reduced DBP but not SBP. Likewise, a study supplementing diets of 23 men with beetroot bread also significantly reduced DBP but not SBP [30]. Similarly, Kapil et al. [64] suggest that plasma nitrite, rather than plasma nitrate concentrations correlate with baseline BP. These changes in plasma nitrite concentrations may reflect endothelial NO synthase activity, NO oxidation, nitrate reduction or all three at once, with different effects noted according to gender [64]. These findings suggest further research is required into the effects of dietary nitrate supplementation on plasma nitrite concentrations and BP, focussing on gender-specific responses.

With respect to the physiological response to moderate exercise, the findings of this study contrast with previous studies where supplementation with sodium nitrate and beetroot juice significantly reduced the oxygen cost of exercise [112]. Some studies have noted reduced physiological effects of dietary nitrate supplementation in highly trained athletes [113-116]. In an attempt to explain these lesser effects, it has been suggested that well-trained subjects have enhanced NO bioavailability compared to less trained individuals [112]. The participants in this study included triathletes, rowers and a cross-country skier who were training 1-2 hours/day, which could explain the lack of significant effects on the physiological response to moderate exercise. Furthermore,

there is a lack of studies on the effects of supplementing diets with fresh, high-nitrate vegetables on exercise performance. Further research is required to determine whether the effects of supplements can be replicated by using fresh, whole vegetables as part of a normal diet.

This study adds to the limited body of literature which includes fresh, whole high-nitrate vegetables as part of the methodology, instead of supplements such as beetroot juice or nitrate salts and therefore has ecological validity. Limitations of this study included a high drop-out rate (20%). Consequently, this study was underpowered to detect statistically significant changes in BP and is therefore subject to Type II error. Recruitment was difficult, possibly due to the duration of the study and the inclusion of maximal exercise tests. This was reflected in the fact that it took 15 months to complete. In addition, supplementation was over a comparatively short period in a small group of young healthy men. Therefore, extrapolation of the main results of this study to the general population is limited. A further limitation includes the lack of direct estimation of the nitrate content of the vegetable boxes, as the nitrate content of vegetables varies significantly according to environmental conditions during growth and subsequent storage [6]. Therefore, direct estimation of nitrate content of vegetables should be included in future studies.

5. CONCLUSION

The main findings of this study are that diets of young, healthy men supplemented with high-nitrate vegetables resulted in significant increases in plasma nitrate and nitrite concentrations compared to supplementation with low-nitrate vegetables. Furthermore, changes in plasma nitrite concentrations correlated with changes in DBP after supplementation with both high-nitrate and low-nitrate vegetables. Further research is required to elucidate the physiological role of dietary nitrate obtained from vegetables.

CONSENT

All participants who responded were provided with written information and those who were subsequently recruited provided written, informed consent to participate.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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