Title: Medium-term effects of dietary nitrate supplementation on systolic and diastolic blood pressure in adults. A systematic review and meta-analysis

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Abstract

Objectives: Dietary nitrate supplementation has been shown to lower blood pressure (BP) particularly in short-term clinical trials. Whether these effects are sustained in the long-term remains to be established. The objective was to conduct a meta-analysis of randomised controlled trials that examined whether dietary nitrate supplementation for more than one week has beneficial effects on systolic and diastolic BP.

Methods: Electronic databases were searched from inception until May 2016. Specific inclusion criteria were: 1) duration ≥1 week; 2) report of effects on systolic or diastolic BP or both; 3) comparison of inorganic nitrate or beetroot juice supplementation with placebo control groups. Random-effects models were used to calculate the pooled BP effect sizes. Results: Thirteen trials met eligibility criteria. The trials included a total of 325 participants with 7 to 65 participants per study. The duration of each intervention ranged from 1 week to 6 weeks. Ten trials assessed BP in resting clinic conditions whereas 24-hr ambulatory and daily home monitoring were used in six and three trials, respectively. Overall, dietary nitrate was associated with a significant decline in systolic [-4.1mmHg (95%CI: -6.1, -2.2) $P<0.001$] and diastolic BP [-2.0mmHg (95%CI: -3.0, -0.9) $P<0.001$]. However, the effect was only significant when measured in resting clinical settings since no significant changes in BP were observed using 24-hr ambulatory and daily home BP monitoring.

Conclusions: Positive effects of medium-term dietary nitrate supplementation on BP were only observed in clinical settings, which were not corroborated by more accurate methods such as 24-hr ambulatory and daily home monitoring.

Background

Blood pressure (BP) control is a global public health priority as hypertension contributes to the burden of heart disease, stroke and kidney failure and premature death and disability[1]. The burden of hypertension is particularly important in developing countries where the limited access to adequate treatments and health care and the concomitant occurrence of communicable diseases and nutritional deficiencies increase the risk for disability and cardiovascular fatal events[2].

A correct anti-hypertensive drug therapy combined with dietary and lifestyle modifications attenuate the adverse effects of BP on cardiovascular health; however adequate and sustained control of BP using these combined approaches is only achieved in ~60-70% of hypertensive patients [3]. Therefore, the primary prevention of hypertension becomes a priority to minimise the population burden of hypertension and nutritional and lifestyle approaches are unanimously recognised as fundamental components of primary prevention programmes [4].

Larsen et al.[5] tested for the first time in a double-blind crossover study the effects of sodium nitrate on BP in healthy volunteers and reported a significant reduction in DBP (-3.7 mmHg). This was followed by a growing interest in the effects of dietary nitrate on cardiovascular health and several studies have confirmed the lowering effects of dietary nitrate on systolic BP[6-8]. The effect size of these interventions was quantified in a meta-analysis of randomised clinical trials (RCTs) as a decrease of -4.4mmHg for systolic BP. However, the majority of the studies included in that meta-analysis were acute and the longest intervention had a duration of two weeks[9]. In addition, the majority of these studies have used beetroot juice to increase nitrate intake. Beetroot was specifically chosen because of its high nitrate content (~300mg per 100gr) as well as for the commercial availability of nitrate-enriched and nitrate-depleted beetroot juice products, which has facilitated the conduction of double-blind, placebo-controlled randomised nutritional interventions.

Reduced nitric oxide (NO) bioavailability has been associated with impairment of endothelial function and increased risk of hypertension and cardiovascular diseases[10,11]. The BP-lowering effects of dietary nitrate may derived from increased generation of nitric oxide (NO) via a non-enzymatic pathway (nitrate-nitrite-nitric oxide pathway)[12]. Dietary and endogenous nitrate molecules may be reduced by facultative anaerobic bacteria on the dorsal surface of the tongue to nitrite which can be chemically (low pH) and enzymatically (xanthine oxidoreductase, myoglobin, cytochrome P450, complexes of the mitochondrial electron transport chain) further reduced to NO[12].

Here we conducted a systematic review and meta-analysis of the evidence from RCTs investigating the medium- and long-term ($\geqslant$ 1 week) efficacy of dietary nitrate and beetroot supplementation on BP in humans. The results will inform whether longer-term dietary nitrate supplementation could be considered as an effective nutritional strategy for the prevention and treatment of hypertension.

Methods

The present systematic review was conducted according to established guidelines and it is reported according to PRISMA guidelines[13] (Table S1).

Types of studies: RCT's on human subjects were included and the specific characteristics and designs of the trials (type of placebo, parallel or cross-over design, blinding of the interventions and duration) were assessed.

Subjects: Adult male and female subjects (age>18 y) with or without health comorbidities were included. Studies reporting data from subjects with different body mass index (BMI), ethnic background and physical activity level were not excluded.

Types of interventions: RCTs investigating the effects of dietary nitrate or beetroot juice supplementation and providing information on the type of nitrate salt (potassium or sodium nitrate), volume, formulation, frequency and route of administration were included. Studies that delivered the nitrate or beetroot supplementation alongside another intervention (e.g. exercise, pharmacological agent or dietary supplement) were excluded if the interventions were different between groups. A combined meta-analysis model was derived for inorganic nitrate solutions and beetroot juice on BP. This approach was based on the evidence that inorganic nitrate is absorbed rapidly from the stomach and proximal small intestine with high bioavailability[14].

Outcome measures: The primary outcomes of the analyses were changes in diastolic and systolic BP after dietary nitrate supplementation measured in resting clinical setting, 24-hr ambulatory and home daily BP monitoring.

Sources: A literature search of the PubMed, Embase and Scopus databases was undertaken from inception until May 2016. The systematic review was restricted to articles published in English. The search was conducted based on pre-defined search terms (dietary, inorganic, nitrate, beetroot, beet root, blood pressure, hypertension, vascular, nitric oxide, endothelial) and using specific building blocks (Boolean terms, truncation) to create the algorithms entered in each database. The full details of the algorithms are reported in the Supplemental Methods (Box 1).

Selection of studies: Two investigators (MS, AA) assessed articles independently for eligibility. The first screening phase was based on the analysis of titles and abstracts. When full agreement had been reached, the article was either discarded or moved to the next phase. In case of disagreement the article was moved to the next phase to increase the inclusiveness level. Reference lists of included papers and relevant reviews were searched for articles potentially missed during the electronic search. In the second phase, the full text of the selected articles was assessed independently by two investigators. When full agreement had been reached the article was either discarded or moved to the next phase for full data extraction. In case of disagreement the article was evaluated by a third investigator (JL) and a final decision was reached by consensus.

Data extraction and study quality: Two investigators extracted the data using a standardised data collection form. A list of the extracted variables is provided in the Supplemental Methods (Box 2). When BP measurements were incomplete, the corresponding authors were contacted to request the missing data. The quality of each study was assessed using the Cochrane Risk Assessment Tool [15].

Measurement of treatment effect: For matched study design (cross-over studies) and parallel studies, the effect of dietary supplementation (inorganic nitrate or beetroot juice) on systolic and diastolic BP was calculated as the difference between the supplementation and placebo groups at the end of each intervention.

Statistical Analysis: A meta-analysis was conducted using Comprehensive Meta-Analysis 2 software (Biostat, Engelwood, New Jersey). Data are presented as mean differences of systolic and diastolic BP (in mmHg) and 95% confidence intervals (95%CI). Four studies [16-19] used more than one method to assess BP in each study. Each measurement was considered as independent which resulted in a total of 19 BP measurements entered into the model. In addition, pooled estimates were stratified by method of assessment of BP (resting clinical, resting daily and 24-hr ABPM). The BP differences were combined across studies using a weighted DerSimonian–Laird random-effects model. Forest plots were generated for graphical presentations of the BP outcomes. Statistical heterogeneity across studies was assessed using the I^2 and the Q tests according to specific categories (low=25%, moderate=50%, high=75%) and significance level (P<0.10), respectively[20]. Funnel plots and Egger's regression test were used to evaluate potential publication bias and selective reporting bias. A random effects meta-regression model was conducted to evaluate whether baseline systolic and diastolic BP, nitrate dose (mmol/day), sample size and changes in nitrite concentrations (nmol/L) were associated with changes in BP.

Results

Main Search: A total of 16146 articles were identified by the primary search and, after the removal of duplicates (N=10918), 5228 articles were screened for titles and abstracts. 143 articles were selected for a full-text review and 13 studies [7,16-19,21-28] were included in the systematic review (qualitative analysis) after the exclusion of 130 articles. A flow chart of the literature search is shown in Fig.1. Eight studies had a cross-over study design[7,16,21-26]and the remaining five studies adopted a parallel study design[17-19,27,28]. Ten studies used a double-blind placebo controlled design[7,16,18,22-28]. The trials were conducted between 2010 and 2016 and included a total of 325 participants with 7 to 65 participants per individual study. The duration of the interventions ranged from one to six weeks. The main characteristics of the studies included in the analysis are presented in Table S1 of the online supplementary material.

Participant characteristics: Five studies recruited young, non-smoking, healthy participants[7,21,23,25,26], three studies were conducted in older healthy subjects[17,19,27], two studies recruited patients with high BP[16,18], and three studies recruited patients with type 2 diabetes[24], hypercholesterolemia[28] and heart failure[22]. In addition, there was a slight over-representation of men (54%) and the mean BMI of the subjects ranged between 22 and 33 kg·m^{-2}. Five studies investigated the effects of dietary nitrate and beetroot juice on exercise performance and recruited primarily young, physically active men[7,21,23,25,26].

Nitrate supplementation: Beetroot juice supplementation was tested in eleven studies [7,16-19,21,23-26,28] whereas sodium nitrate was used in only one study[27], respectively. The choice of the placebo varied between studies and included sodium chloride solutions (two studies)[21,27], blackcurrant juice (two studies)[7,17], negative control (one study[19] and nitrate-depleted beetroot juice (eight studies)[16,18,22-26,28]. All solutions were given orally. Dietary nitrate intake was controlled in five studies[7,17,19,21,23]. Only one study[25] did not measure plasma, salivary or urinary levels of nitrate and/or nitrite, which was useful to provide information on the adherence to the dietary nitrate supplementation.

BP measurement: Resting BP was measured in eleven trials[7,17-19,21-23,25-28], five studies measured ambulatory 24-h BP[16-19,24] and three studies used daily home BP monitoring [16-18]. Two studies used all three methods to assess changes in BP[17,18].

Study Quality and Adverse Events: The majority of the studies were rated as having a high quality design. Five studies [7,17,19,21,25] were characterised by a higher risk of bias, which was related to uncertainties around allocation concealment and blinding of the interventions (Fig. S1). The exclusion of these studies from the analysis however did not modify the results for both systolic and diastolic BP (Table S2). Information on adverse events occurred during the study was reported in nine studies [7,17-19,21-24,28]. The most common side effect reported in the beetroot juice trials was beeturia (red urine) and red stools.

BP qualitative results: Eight studies showed a significant reduction in systolic BP [7,17,18,21-23,26,27]whereas a significant change in diastolic BP was observed in four studies[7,18,23,26]. Overall, studies reported a significant decline in resting clinical systolic and diastolic BP but the beneficial effects were less consistent when ambulatory 24-hour or daily home BP monitoring methods were used[16-19,24].

Meta-Analysis: Overall, dietary nitrate supplementation was associated with a significant decline in systolic [-4.1mmHg (95%CI: -6.1, -2.2) P<0.001, Fig.2] and diastolic BP [-2.0mmHg (95%CI: -3.0, -0.9) P<0.001], Fig. 3]. The stratification by BP method indicated a greater effect on resting clinical measurements [7,17-19,21-23,25-28] compared to 24-hr ambulatory [16-19,24] and daily home BP monitoring[16-18] for both systolic (Fig. 2) and diastolic BP (Fig. 3). The meta-regression analysis showed that the effect size for systolic and diastolic BP was not significantly associated with their respective baseline BP values (Table S3). Interestingly, an increase in the amount of daily nitrate administered in each study was associated with a smaller effect size for both diastolic and systolic BP (Table S3). Changes in plasma nitrite concentrations and sample size were not associated with changes in diastolic and systolic BP after nitrate supplementation (Table S3).

Publication bias and heterogeneity: Funnel plots for systolic and diastolic BP revealed an overall symmetric distribution of the studies around the mean effect size indicating a low risk for publication bias (Fig. S6 and Fig. S7). Egger's regression test confirmed the non-significant publication bias for both systolic and diastolic BP outcomes (P=0.68 and P=0.56, respectively). We observed a low heterogeneity for both systolic (I^2=39%, Q=29.8, P=0.04) and diastolic BP (I^2=0%, Q=16.8, P=0.53) meta-analysis models.

Discussion

Dietary nitrate supplementation for more than one week was overall associated with a significant decrease in systolic and diastolic BP. The pooled effect for the two interventions showed a reduction in systolic BP of -4.1 mmHg with a more modest decrease (-2.0 mmHg) in diastolic BP. However, the significant effect size was mostly driven by BP measurements performed in resting clinical settings whereas the effect became not significant when BP was measured by 24-hour ambulatory and daily home BP monitoring. In addition, trials were generally characterised by a small sample size and relatively short duration, which demand for a careful interpretation of the results in relation to their generalisability and robustness of the current body of evidence.

The general quality of the studies was high. All studies were randomised and the majority of them (75%) were double-blind placebo controlled. Five studies had a parallel study design[17-19,27,28]. The studies reported a high compliance with the interventions which may supported by the reported rise in nitrate concentrations in plasma or urine. Five trials recruited active, healthy individuals as primary outcome was focussed on effects on exercise performance[7,21,23,25,26]. The exclusion of these trials from the meta-analysis did significantly affect the results as they became not significant for both systolic and diastolic BP (data not showed).

Four studies investigated the effects of dietary nitrate supplementation in older subjects (mean age >60 y) with and without evidence of impaired cardiovascular health[17,19,24,27]. Overall, dietary nitrate appeared to be less effective in reducing systolic BP in older populations since only one study showed a decrease in systolic BP[27]. Siervo et al have recently showed that dietary nitrate supplementation had a lower effect on average nocturnal systolic BP and dipping BP patterns in older subjects (mean age > 65y)[29]. It is conceivable that ageing may be linked to reduced sensitivity of vascular components to the beneficial effects of dietary nitrates, possibly mediated by reduced non-enzymatic conversion of nitrate into NO and reduced responsiveness of the endothelium and vascular smooth muscular cells to NO [30]. Ageing may also be associated with changes in oral microflora and gastric acid production which may influence the efficiency of the conversion of nitrate into NO[31,32]. Whether greater doses of inorganic nitrate in older people are required to enhance NO production and bioactivity is currently not known. It is also possible that dietary nitrate interventions with longer duration might be required in older individuals to reach sufficiently high and sustained concentrations of NO and induce beneficial effects on BP.

Our results also appears to indicate that a higher daily dose of dietary nitrate does not necessarily produce a greater effect on BP; rather, we found a significant smaller effect size for both systolic and diastolic BP. These findings are contrary to what was previously reported in a similar meta-analysis, which indicated a higher reduction in BP with higher doses of inorganic nitrate [9]. However, the discrepant results may be explained by the very short study duration of the trials included in the first meta-analysis. These new results suggest that the long term administration of higher doses of dietary nitrate may not be necessarily associated with greater vascular benefits. This could be linked to the development of nitrate-specific tolerance possibly related to a declined efficiency of the conversion of nitrate into nitrite and NO, downregulation of the l-arginine-nitric oxide synthase pathway and/or reduced sensitivity of cellular targets to NO [33-35]. These results should be considered as preliminary and the hypotheses require confirmation in appropriately designed studies.

There is currently a lack of strong evidence on the effects of prolonged dietary nitrate supplementation in individuals with greater cardiovascular risk. One study reported positive effects of beetroot juice in patients with HF[22], whereas contrasting results have been found in patients with hypertension, type 2 diabetes or hypercholestelomia[16,18,24,28]. Several factors may account for these discrepancies including differences in sample size, duration, recruitment criteria and measurement protocols of BP.

Our analyses also showed that the effect of dietary nitrate may be dependent on the method used to measure BP. The results seem to indicate that the use of 24-hour ambulatory and daily home BP monitoring may be associated with a lower efficacy of dietary nitrate on BP outcomes. The choice of the most accurate and precise method for the measurement of BP is a complicated and unresolved question for clinical and research monitoring of the effects of specific dietary, lifestyle and pharmacological treatments[36]. While the use of clinical resting BP is the most used method, its poor reliability is universally recognized in consideration of measurement bias associated with white-coat syndrome, standardization of protocol and operator bias [37]. Some of these issues are resolved by the recommended adoption of 24-hour ambulatory and daily home monitoring for a more objective assessment of BP [38,39].

This meta-analysis showed that medium-term dietary nitrate supplementation was overall linked to a significant decline in systolic and diastolic BP, but these effects were mostly driven by studies measuring BP in resting clinical settings and recruiting young healthy individuals. Sample size of the trials was generally small (range: 7- 69 participants) with only two studies enrolling more than 50 participants. Studies employing more accurate methods, such as 24-hr ABPM and daily home monitoring, produced non-significant results. In addition, a limited efficacy has been also observed in older individuals at greater CVD risk, which may introduce some uncertainty around the efficacy of prolonged dietary nitrate supplementation for the control of BP in adults. Dietary nitrate supplementation may represent a promising nutritional therapy for the control of BP. However, the evidence on the long term effects of dietary nitrate on blood pressure in patients with an increased cardiovascular risk is inconclusive at this point, as completed studies are characterised by a short duration and small sample size. Hence, larger clinical trials (> 100 participants) with more prolonged supplementation period (≥ 6 months) and employing accurate methods for the assessment of BP outcomes are a research priority to verify the efficacy of inorganic nitrate as a dietary approach to prevent and treat hypertension.

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Authors' Contribution

The systematic review was conceived by MS. AA and MS searched, collected and analysed the data and co-wrote the manuscript. All authors contributed to subsequent analyses and interpretation. All authors contributed to the final revision of the manuscript. The corresponding author (MS) is the guarantor for the manuscript and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the paper.

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Conflicts of Interest

None to declare

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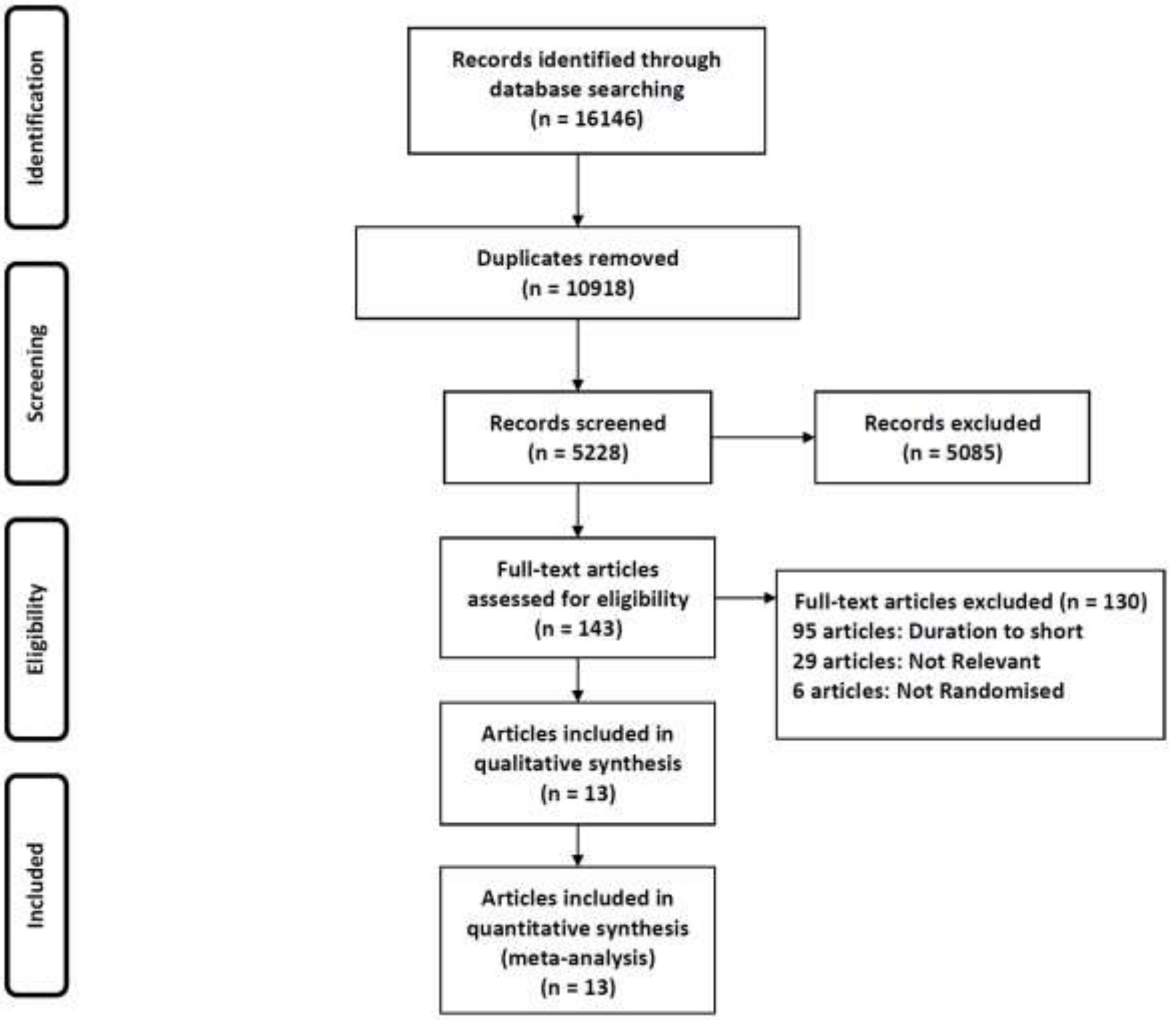
Figure Legends

Figure 1: Flow chart of the literature search

Figure 2: Forest-plot of randomized clinical trials investigating the effects of beetroot juice and inorganic nitrate supplementation on systolic blood pressure (BP, mmHg) measured by resting clinic, 24-hr ambulatory (24-hr ABPM) and daily resting BP monitoring.

Figure 3: Forest-plot of randomized clinical trials investigating the effects of beetroot juice and inorganic nitrate supplementation on diastolic blood pressure (BP, mmHg) measured by resting clinic, 24-hr ambulatory (24-hr ABPM) and daily resting BP monitoring.

Figure 1



Identification
Screening
Eligibility
Included
Records identified through database searching (n = 16146)
Duplicates removed (n = 10918)
Records screened (n = 5228)
Records excluded (n = 5085)
Full-text articles assessed for eligibility (n = 143)
Full-text articles excluded (n = 130)
95 articles: Duration to short
29 articles: Not Relevant
6 articles: Not Randomised
Articles included in qualitative synthesis (n = 13)
Articles included in quantitative synthesis (meta-analysis) (n = 13)

Figure 2

Author, year	Comparison	Difference in means	Lower limit	Upper limit	p-Value	Nitrate	Control	Relative weight
Gilchrist, 2013	24hr-ABPM	-0.30	-3.53	2.93	0.86	27	27	26.83
Jajja, 2014	24hr-ABPM	-4.30	-14.73	6.13	0.42	10	11	10.13
Rammos, 2014	24hr-ABPM	-7.00	-18.70	4.70	0.24	11	10	8.60
Kapil, 2015	24hr-ABPM	-7.40	-10.14	-4.66	0.00	32	32	28.21
Lara, 2015	24hr-ABPM	-2.00	-14.20	10.20	0.75	10	10	8.08
Bondonno, 2016	24hr-ABPM	1.00	-5.24	7.24	0.75	27	27	18.15
		-3.19	-7.19	0.82	0.12			
Vanhatalo, 2010	Clinic Resting	-7.00	-13.93	-0.07	0.05	8	8	9.58
Fulford, 2013	Clinic Resting	-8.50	-21.72	4.72	0.21	8	8	3.16
Haider, 2014	Clinic Resting	1.00	-3.86	5.86	0.69	19	19	15.75
Jajja, 2014	Clinic Resting	-3.00	-14.82	8.82	0.62	10	11	3.88
Bailey, 2015	Clinic Resting	-8.00	-19.94	3.94	0.19	7	7	3.80
Lee, 2015	Clinic Resting	-6.00	-8.74	-3.26	0.00	14	14	27.43
Kapil, 2015	Clinic Resting	-7.70	-11.77	-3.63	0.00	32	32	19.39
Lara, 2015	Clinic Resting	-1.90	-17.03	13.23	0.81	10	10	2.45
Eggebeen, 2016	Clinic Resting	-7.00	-17.78	3.78	0.20	18	18	4.57
Velmurugan, 2016	Clinic Resting	1.00	-5.74	7.74	0.77	33	32	9.99
		-4.61	-7.05	-2.17	0.00			
Jajja, 2014	Daily Resting	-7.30	-18.85	4.25	0.22	10	11	21.32
Kapil, 2015	Daily Resting	-8.10	-16.92	0.72	0.07	32	32	31.74
Bondonno, 2016	Daily Resting	0.50	-5.90	6.90	0.88	27	27	46.94
		-3.89	-9.90	2.12	0.20			
		-4.19	-6.16	-2.22	0.00			

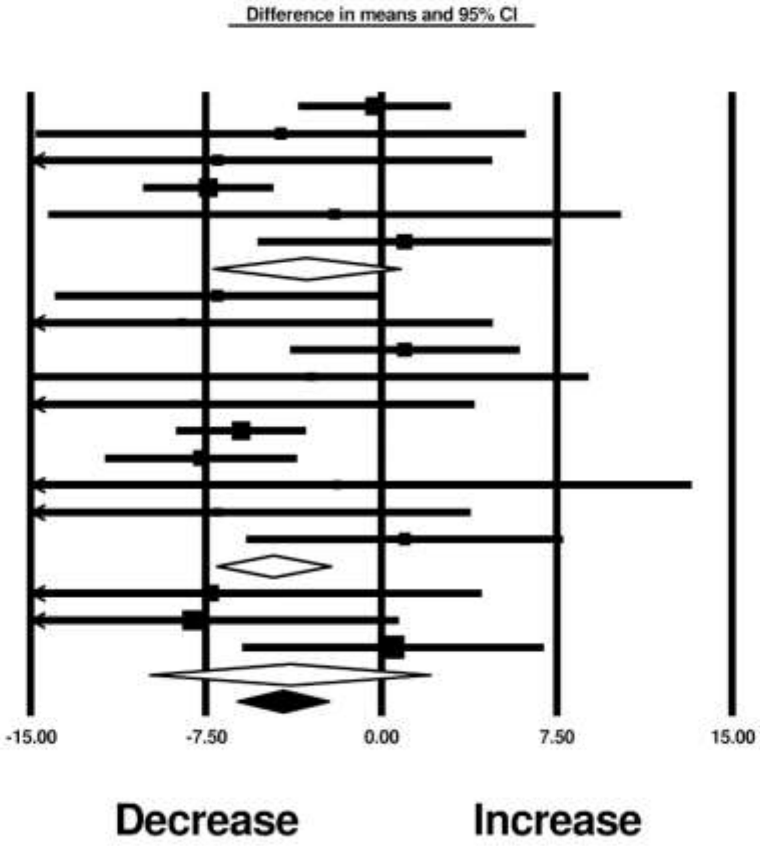
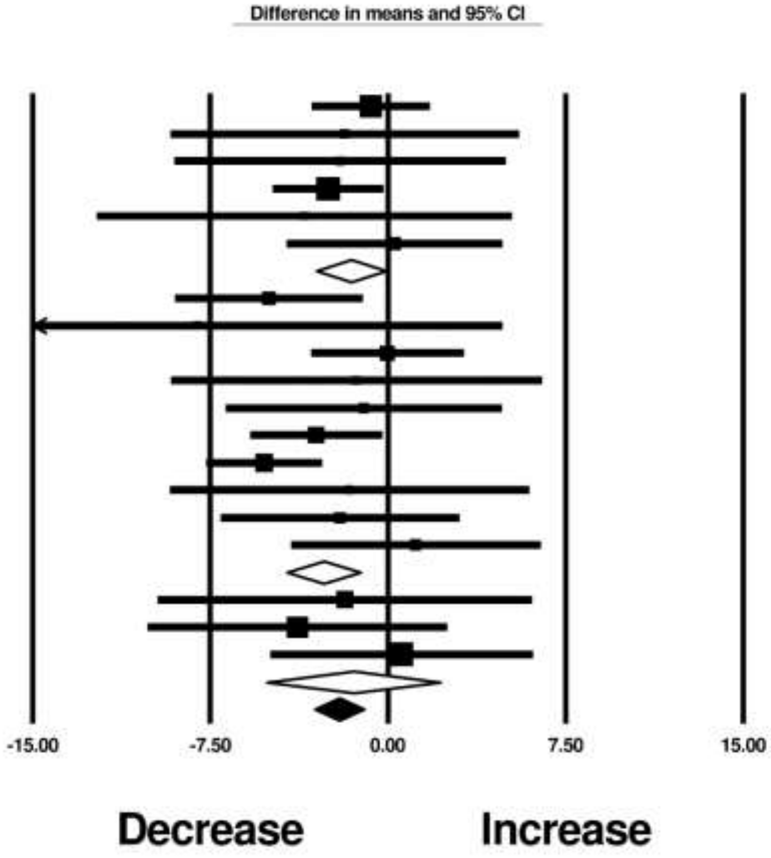


Figure 3

Author, year	Comparison	Statistics for each study: Difference in means	Lower limit	Upper limit	p-Value	Sample size: Nitrate	Control	Relative weight
Gilchrist, 2013	24hr-ABPM	-0.70	-3.14	1.74	0.57	27	27	36.69
Jajja, 2014	24hr-ABPM	-1.80	-9.11	5.51	0.63	10	11	4.08
Rammos, 2014	24hr-ABPM	-2.00	-8.95	4.95	0.57	11	10	4.52
Kapil, 2015	24hr-ABPM	-2.50	-4.80	-0.20	0.03	32	32	41.12
Lara, 2015	24hr-ABPM	-3.50	-12.22	5.22	0.43	10	10	2.87
Bondonno, 2016	24hr-ABPM	0.30	-4.21	4.81	0.90	27	27	10.73
		-1.52	-2.99	-0.04	0.04			
Vanhatalo, 2010	Clinic Resting	-5.00	-8.92	-1.08	0.01	8	8	11.83
Fulford, 2013	Clinic Resting	-8.00	-20.80	4.80	0.22	8	8	1.43
Haider, 2014	Clinic Resting	0.00	-3.18	3.18	1.00	19	19	15.91
Jajja, 2014	Clinic Resting	-1.30	-9.09	6.49	0.74	10	11	3.68
Bailey, 2015	Clinic Resting	-1.00	-6.79	4.79	0.73	7	7	6.28
Lee, 2015	Clinic Resting	-3.00	-5.74	-0.26	0.03	14	14	19.18
Kapil, 2015	Clinic Resting	-5.20	-7.60	-2.80	0.00	32	32	22.25
Lara, 2015	Clinic Resting	-1.60	-9.14	5.94	0.68	10	10	3.91
Eggebeen, 2016	Clinic Resting	-2.00	-7.00	3.00	0.43	18	18	8.05
Velmurugan, 2016	Clinic Resting	1.20	-4.03	6.43	0.65	33	32	7.47
		-2.68	-4.24	-1.13	0.00			
Jajja, 2014	Daily Resting	-1.80	-9.66	6.06	0.65	10	11	21.65
Kapil, 2015	Daily Resting	-3.80	-10.07	2.47	0.24	32	32	34.04
Bondonno, 2016	Daily Resting	0.60	-4.90	6.10	0.83	27	27	44.31
		-1.42	-5.08	2.24	0.45			
		-2.02	-3.05	-0.99	0.00			



Online Supplementary material

Box 1: Algorithms used in the main search

(i) inorganic AND nitrate* AND blood pressure/hypertension/vascular/nitric oxide/endotheli*

(ii) beet root OR beetroot AND blood pressure/hypertension/vascular/nitric oxide/endotheli*

(iii) diet* OR dietary AND nitrate* AND blood pressure/hypertension/vascular/nitric oxide/endotheli*

Box 2: Variables extracted during the full-text phase

The variables extracted were: year of publication, study design, inclusion and exclusion criteria, dietary and lifestyle requirements (e.g. low nitrate diet, caffeine intake and physical activity, compliance to dietary interventions), study duration, duration of washout period in cross-over studies, sample size, number of participants at follow up, type of intervention (beetroot or nitrate) and placebo, measurement protocol of BP, statistical analysis, age, gender, body mass index (BMI), baseline and post-intervention measurements of BP readings (systolic, diastolic), measurements of nitrate and nitrite concentrations in biological fluids (urine, plasma, saliva), conflicts of interest, funding resources.

Supplemental Table 1: Main characteristics of the studies included in the systematic review and meta-analysis[1]							
First Author, Reference [year of publication]	**Participants**	**Study Design**	**Duration of intervention (washout period)**	**Type of Intervention**	**Dose of Inorganic Nitrate**	**Placebo**	**Main Findings**
Vanhatalo et al,[1] [2010]	8 physically active, healthy individuals. Age: 29±6y. M/F: 5/3. BMI: 24 $kg \cdot m^{-2}$.None of the subjects was smoking or used dietary supplements.	Placebo controlled, cross-over randomised clinical trial. At the end of each intervention, measurement of resting clinic BP. Subjects were instructed to adhere to their normal exercise routine and diet throughout the experimentation. The subjects kept a physical activity and dietary diary and were asked to perform similar	15 d (10 d)	500mL of beetroot juice	5.2mmol/d	500mL of low calorie blackcurrant juice	At 15 d, significant differences were found between beetroot and placebo groups for both systolic and diastolic BP. Changes in systolic BP relative to baseline were significantly different at 15 d but the effect was not observed for diastolic BP. Plasma nitrite concentrations increased by 185nmol/L (192%) Primary outcome of the study was the effect of nitrate on exercise performance.

		activities and consume similar meals in the first and second supplementation periods Plasma nitrate and nitrite concentrations were measured.					Subjects experienced beeturia during the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported. No conflicts of interest, financial or otherwise, were declared by the author(s).
Fulford et al[2], [2013]	8 physically active, healthy men. Age: 24y. BMI: ~22 $kg{\cdot}m^{-2}$. None of the subjects was smoking or used dietary supplements.	Placebo controlled, cross-over, double blind randomised clinical trial. At the end of each intervention, measurement of resting clinic BP. Subjects were instructed to adhere to their normal exercise routine and diet throughout the experimentation. Plasma nitrate and nitrite	15 d (14 d)	250mL of beetroot juice	7.5mmol/d	250mL nitrate depleted beetroot juice	At 15 d, significant differences were found between beetroot and placebo groups for both systolic and diastolic BP. Plasma nitrite concentrations increased by 176nmol/L (175%) Primary outcome of the study was the effect of nitrate on exercise performance Subjects experienced beeturia during the BR supplementation. However, the supplementation regimen was well tolerated and no

		concentrations were measured.					harmful side effects were reported. No conflicts of interest, financial or otherwise, were declared by the author(s).
Gilchrist et al[3], [2013]	27 patients with type 2 diabetes. Age: 67±5y. M/F: 15/ 8. BMI: 30.8 $kg \cdot m^{-2}$	Double blind, randomised, placebo-controlled crossover trial. At the conclusion of each intervention period 24-hour ambulatory blood pressure monitoring. Dietary nitrate intake was not restricted. Plasma nitrate and nitrite concentrations were measured.	2 weeks (4 weeks)	250mL of beetroot juice	7.5mmol/d	250mL nitrate depleted beetroot juice	At the end of the two weeks no significant differences were found in systolic and diastolic BP between the beetroot and placebo groups. Mean 24-hr systolic and diastolic BP were included in the meta-analysis. Plasma nitrite concentrations increased by 158nmol/L (168%) Adverse events: There were no adverse events reported in response to intervention products, apart from beeturia (red urine). Funding source: NIHR Exeter Clinical Research Facility. Conflicts of Interest: James White Drinks

							Limited and David Upson of Stoke Farm Orchards for beetroot juice and placebo juice donations and production, respectively.
Haider et al[4], [2014]	19 physically active, healthy men. Age: 21y. BMI: ~22 $kg{\cdot}m^{-2}$.	Placebo controlled, cross-over, double blind randomised clinical trial. At the end of each intervention, measurement of resting clinic BP. Subjects were instructed to adhere to their normal exercise routine and diet throughout the experimentation. Plasma nitrate and nitrite concentrations were not reported.	7 d (9 d)	120mL of concentrated beetroot juice	9.7mmol/d	120mL nitrate depleted beetroot juice	At 15 d, no significant differences were found between beetroot and placebo groups for both systolic and diastolic BP. Primary outcome of the study was the effect of nitrate on exercise performance Adverse events: not reported Conflicts of interest: Gatorade Sports Science Institute
Jajja et al[5], [2014]	21 healthy older and overweight subjects. Age: 62y. M/F: 12/ 9. BMI: 30.1 $kg{\cdot}m^{-2}$	Randomised, parallel trial. Resting clinic, daily and 24-hour ambulatory blood	3 weeks	70mL of concentrated beetroot juice	~6mmol/d	100mL of blackcurrant juice	At the end of the intervention no significant effect on resting and 24-hour systolic and diastolic BP. A significant difference

		pressure monitoring. Dietary nitrate intake: controlled Plasma, salivary and urine nitrate concentrations were measured.					was found for daily systolic BP. Salivary nitrate concentrations increased by 5mmol/L (600%) Adverse events: Subjects experienced beeturia with the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported. Funding source: Newcastle University Core Budget Conflicts of Interest: None to declare
Rammos et al[6], [2014]	21 healthy older subjects. Age: 63y. M/F: 13/8. BMI: 24 $kg \cdot m^{-2}$	Randomised, placebo controlled, double-blind parallel trial. Resting clinic blood pressure. Dietary nitrate intake: not reported	4 weeks	Sodium nitrate (0.15mmol*kg BW) dissolved in drinking water	-	Sodium chloride (0.15mmol* kg BW)	At the end of the intervention significant effect on resting systolic BP. Plasma nitrite concentrations increased by 219nmol/L (297%) Adverse events: not reported.

		Plasma nitrate and nitrite concentrations were measured.					Funding source: German Research Foundation Conflicts of Interest: None to declare
Bailey et al[7], [2015]	7 physically active, healthy men. Age: 21y. BMI: ~22 kg·m^{-2}. None of the subjects was smoking or used dietary supplements.	Placebo controlled, cross-over randomised clinical trial. At the end of each intervention, measurement of resting clinic BP. Subjects were instructed to adhere to their normal exercise routine and diet throughout the experimentation. Plasma nitrate and nitrite concentrations were measured.	9 d (10 d)	70mL of beetroot juice	6.2mmol/d	Sodium chloride (0.1 mmol*kg BW)	At 9 d, significant differences were found between beetroot and placebo groups for systolic BP. Plasma nitrite concentrations increased by 160nmol/L (260%) Primary outcome of the study was the effect of nitrate on exercise performance Subjects experienced beeturia during the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported. No conflicts of interest, financial or otherwise, were declared by the author(s).

Bondonno et al[8], [2015]	27 patients with diagnosed hypertension. Age: 63y. M/F: 17/ 10. BMI: 26.9 kg·m^{-2}	Double blind, randomised, placebo-controlled crossover trial. Daily and 24-hour ambulatory blood pressure monitoring. Dietary nitrate intake was not restricted. Plasma nitrate and nitrite concentrations were measured.	1 week (1 week)	140mL of concentrated beetroot juice	7.5mmol/d	140mL nitrate depleted beetroot juice	At the end of the one week no significant differences were found in systolic and diastolic BP between the beetroot and placebo groups measured with both methods. Plasma nitrite concentrations increased by 3800nmol/L (290%) Adverse events: Not reported Funding source: National Health and Medical Research Council Conflicts of Interest: None to report
Kapil et al[9], [2015]	68 drug-naïve and treated hypertensive patients. Age: 57y. M/F: 30/ 38. BMI: 26.5 kg·m^{-2}	Randomised, placebo-controlled, double-blind parallel trial. Resting clinic, daily and 24-hour ambulatory blood pressure monitoring.	4 weeks	250mL of beetroot juice	~6mmol/d	250mL nitrate depleted beetroot juice	At the end of the intervention significant effect on resting, daily and 24-hour systolic and diastolic BP. Plasma nitrite concentrations increased by 470nmol/L (335%) Adverse events: Subjects experienced beeturia with the BR supplementation.

		Dietary nitrate intake: not reported. Plasma and urine nitrate and nitrite concentrations were measured.					However, the supplementation regimen was well tolerated and no harmful side effects were reported. Funding source: British Heart Foundation Conflicts of Interest: None to declare
Lara et al[10], [2015]	20 healthy older and overweight subjects. Age: 63y. M/F: 9/ 11. BMI: 29.9 $kg{\cdot}m^{-2}$	Randomised, parallel trial. Resting clinic and 24-hour ambulatory blood pressure monitoring. Dietary nitrate intake controlled. Plasma and urine nitrate concentrations were measured.	1 week (1 week)	140mL of concentrated beetroot juice	~10mmol/d	Negative control (only diet)	At the end of the intervention no significant effect on resting and 24-hour BP. Plasma nitrate concentrations increased by 100μmol/L (176%) Subjects experienced beeturia with the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported. Funding source: Newcastle University core budget

							Conflicts of Interest: None to declare
Lee et al[11], [2015]	14 physically active, healthy men. Age: 22y. BMI: 23 kg·m^{-2}. None of the subjects was smoking or used dietary supplements.	Placebo controlled, cross-over, double blind randomised clinical trial. At the end of each intervention, measurement of resting clinic BP. Control of dietary nitrate intake: not reported Plasma nitrate concentrations were measured.	15 d (14 d)	70mL of beetroot juice	6.2mmol/d	70mL nitrate depleted beetroot juice	At 15 d, significant differences were found between beetroot and placebo groups for systolic and diastolic BP. Plasma nitrate concentrations increased by 69nmol/L (170%) Primary outcome of the study was the effect of nitrate on exercise performance Adverse events: not reported No conflicts of interest, financial or otherwise, were declared by the author(s).
Eggebeen et al[12], [2016]	18 patients with diagnosed heart failure with preserved ejection fraction. Age: 69y. M/F: 2/ 17. BMI: 32.9 kg·m^{-2}	Double blind, randomised, placebo-controlled crossover trial. Resting clinic blood pressure.	1 week (1 week)	70mL of concentrated beetroot juice	6.1mmol/d	70mL nitrate depleted beetroot juice	At the end of the one week a significant decline was found for systolic BP between the beetroot and placebo groups. Plasma nitrite concentrations increased by 440nmol/L (229%)

		Dietary nitrate intake was not restricted. Plasma nitrate and nitrite concentrations were measured.					Adverse events: two subjects excluded for unrelated events Funding source: National Institutes of Health Conflicts of Interest: Reported conflicts by some investigators
Velmurugan et al[13], [2016]	67 untreated patients with high cholesterol. Age: 53y. M/F: 24/ 43. BMI: 26.8 $kg{\cdot}m^{-2}$	Randomised, placebo-controlled, double-blind parallel trial. Resting clinic pressure. Dietary nitrate intake: not reported controlled. Plasma and urine nitrate and nitrite concentrations were measured.	6 weeks	250mL of beetroot juice	~6mmol/d	250mL nitrate depleted beetroot juice	At the end of the intervention no significant effect on resting systolic and diastolic BP. Plasma nitrite concentrations increased by 400nmol/L (233%) Adverse events: Subjects experienced beeturia with the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported. Funding source: British Heart Foundation and Medical Research Council

							Conflicts of Interest: None to declare

Acronyms are: BP: blood pressure; BMI: Body mass index; F: female; M: male; d: days; BR: beetroot; BW: body weight