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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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1 **Abstract**

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

7 Methods: Electronic databases were searched from inception until May 2016. Specific
8 inclusion criteria were: 1) duration ≥ 1 week; 2) report of effects on systolic or diastolic BP or
9 both; 3) comparison of inorganic nitrate or beetroot juice supplementation with placebo
10 control groups. Random-effects models were used to calculate the pooled BP effect sizes.

11 Results: Thirteen trials met eligibility criteria. The trials included a total of 325 participants
12 with 7 to 65 participants per study. The duration of each intervention ranged from 1 week to 6
13 weeks. Ten trials assessed BP in resting clinic conditions whereas 24-hr ambulatory and daily
14 home monitoring were used in six and three trials, respectively. Overall, dietary nitrate was
15 associated with a significant decline in systolic [-4.1mmHg (95%CI: -6.1, -2.2) $P < 0.001$] and
16 diastolic BP [-2.0mmHg (95%CI: -3.0, -0.9) $P < 0.001$]. However, the effect was only
17 significant when measured in resting clinical settings since no significant changes in BP were
18 observed using 24-hr ambulatory and daily home BP monitoring.

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

22

23 **Background**

24 Blood pressure (BP) control is a global public health priority as hypertension contributes to
25 the burden of heart disease, stroke and kidney failure and premature death and disability[1].

26 The burden of hypertension is particularly important in developing countries where the
27 limited access to adequate treatments and health care and the concomitant occurrence of
28 communicable diseases and nutritional deficiencies increase the risk for disability and
29 cardiovascular fatal events[2].

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

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

46 nitrate-enriched and nitrate-depleted beetroot juice products, which has facilitated the
47 conduction of double-blind, placebo-controlled randomised nutritional interventions.

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

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

61 **Methods**

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

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

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

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

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

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

89 *Selection of studies:* Two investigators (MS, AA) assessed articles independently for
90 eligibility. The first screening phase was based on the analysis of titles and abstracts. When

91 full agreement had been reached, the article was either discarded or moved to the next phase.
92 In case of disagreement the article was moved to the next phase to increase the inclusiveness
93 level. Reference lists of included papers and relevant reviews were searched for articles
94 potentially missed during the electronic search. In the second phase, the full text of the
95 selected articles was assessed independently by two investigators. When full agreement had
96 been reached the article was either discarded or moved to the next phase for full data
97 extraction. In case of disagreement the article was evaluated by a third investigator (JL) and a
98 final decision was reached by consensus.

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

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

108 *Statistical Analysis:* A meta-analysis was conducted using Comprehensive Meta-Analysis 2
109 software (Biostat, Engelwood, New Jersey). Data are presented as mean differences of
110 systolic and diastolic BP (in mmHg) and 95% confidence intervals (95%CI). Four studies
111 [16-19] used more than one method to assess BP in each study. Each measurement was
112 considered as independent which resulted in a total of 19 BP measurements entered into the
113 model. In addition, pooled estimates were stratified by method of assessment of BP (resting
114 **clinical**, resting daily and 24-hr ABPM). The BP differences were combined across studies

115 using a weighted DerSimonian–Laird random-effects model. Forest plots were generated for
116 graphical presentations of the BP outcomes. Statistical heterogeneity across studies was
117 assessed using the I^2 and the Q tests according to specific categories (low=25%,
118 moderate=50%, high=75%) and significance level ($P<0.10$), respectively[20]. Funnel plots
119 and Egger's regression test were used to evaluate potential publication bias and selective
120 reporting bias. A random effects meta-regression model was conducted to evaluate whether
121 baseline systolic and diastolic BP, nitrate dose (mmol/day), sample size and changes in nitrite
122 concentrations (nmol/L) were associated with changes in BP.

123 **Results**

124 *Main Search:* A total of 16146 articles were identified by the primary search and, after the
125 removal of duplicates (N=10918), 5228 articles were screened for titles and abstracts. 143
126 articles were selected for a full-text review and 13 studies [7,16-19,21-28] were included in
127 the systematic review (qualitative analysis) after the exclusion of 130 articles. A flow chart of
128 the literature search is shown in Fig.1. Eight studies had a cross-over study design[7,16,21-
129 26]and the remaining five studies adopted a parallel study design[17-19,27,28]. Ten studies
130 used a double-blind placebo controlled design[7,16,18,22-28]. The trials were conducted
131 between 2010 and 2016 and included a total of 325 participants with 7 to 65 participants per
132 individual study. The duration of the interventions ranged from one to six weeks. The main
133 characteristics of the studies included in the analysis are presented in Table S1 of the online
134 supplementary material.

135 *Participant characteristics:* Five studies recruited young, non-smoking, healthy
136 participants[7,21,23,25,26], three studies were conducted in older healthy subjects[17,19,27],
137 two studies recruited patients with high BP[16,18], and three studies recruited patients with
138 type 2 diabetes[24], hypercholesterolemia[28] and heart failure[22]. In addition, there was a

139 slight over-representation of men (54%) and the mean BMI of the subjects ranged between 22
140 and 33 kg·m⁻². Five studies investigated the effects of dietary nitrate and beetroot juice on
141 exercise performance and recruited primarily young, physically active men[7,21,23,25,26].

142 *Nitrate supplementation:* Beetroot juice supplementation was tested in eleven studies [7,16-
143 19,21,23-26,28] whereas sodium nitrate was used in only one study[27], respectively. The
144 choice of the placebo varied between studies and included sodium chloride solutions (two
145 studies)[21,27], blackcurrant juice (two studies)[7,17], negative control (one study[19] and
146 nitrate-depleted beetroot juice (eight studies)[16,18,22-26,28]. All solutions were given
147 orally. Dietary nitrate intake was controlled in five studies[7,17,19,21,23]. Only one
148 study[25] did not measure plasma, salivary or urinary levels of nitrate and/or nitrite, which
149 was useful to provide information on the adherence to the dietary nitrate supplementation.

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

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

160 *BP qualitative results:* Eight studies showed a significant reduction in systolic BP
161 [7,17,18,21-23,26,27]whereas a significant change in diastolic BP was observed in four
162 studies[7,18,23,26]. Overall, studies reported a significant decline in resting clinical systolic

163 and diastolic BP but the beneficial effects were less consistent when ambulatory 24-hour or
164 daily home BP monitoring methods were used[16-19,24].

165 *Meta-Analysis:* Overall, dietary nitrate supplementation was associated with a significant
166 decline in systolic [-4.1mmHg (95%CI: -6.1, -2.2) $P<0.001$, Fig.2] and diastolic BP [-
167 2.0mmHg (95%CI: -3.0, -0.9) $P<0.001$], Fig. 3]. The stratification by BP method indicated a
168 greater effect on resting **clinical** measurements [7,17-19,21-23,25-28] compared to 24-hr
169 ambulatory [16-19,24] and daily home BP monitoring[16-18] for both systolic (Fig. 2) and
170 diastolic BP (Fig. 3). **The meta-regression analysis showed that the effect size for systolic and**
171 **diastolic BP was not significantly associated with their respective baseline BP values (Table**
172 **S3).** Interestingly, an increase in the amount of daily nitrate administered in each study was
173 associated **with a smaller effect** size for both diastolic and systolic BP (Table S3). Changes in
174 plasma nitrite concentrations and sample size were not associated with changes in diastolic
175 and systolic BP after nitrate supplementation (Table S3).

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

182 **Discussion**

183 Dietary nitrate **supplementation for more than one week** was overall associated with a
184 significant decrease in systolic and diastolic BP. The pooled effect for the two interventions
185 showed a reduction in systolic BP of -4.1 mmHg with a more modest decrease (-2.0 mmHg)
186 in diastolic BP. However, the significant effect size was mostly driven by BP measurements

187 performed in resting **clinical** settings whereas the effect became not significant when BP was
188 measured by 24-hour ambulatory and daily home BP monitoring. **In addition, trials were**
189 **generally characterised by a small sample size and relatively short duration, which demand**
190 **for a careful interpretation of the results in relation to their generalisability and robustness of**
191 **the current body of evidence.**

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

200 Four studies investigated the effects of dietary nitrate supplementation in older subjects
201 (mean age >60 y) with and without evidence of impaired cardiovascular health[17,19,24,27].

202 Overall, dietary nitrate appeared to be less effective in reducing systolic BP in older
203 populations since only one study showed a decrease in systolic BP[27]. Siervo et al have
204 recently showed that dietary nitrate supplementation had a lower effect on average nocturnal
205 systolic BP and dipping BP patterns in older subjects (mean age > 65y)[29]. It is conceivable
206 that ageing may be linked to reduced sensitivity of vascular components to the beneficial
207 effects of dietary nitrates, possibly mediated by reduced non-enzymatic conversion of nitrate
208 into NO and reduced responsiveness of the endothelium and vascular smooth muscular cells
209 to NO [30]. Ageing may also be associated with changes in oral microflora and gastric acid
210 production which may influence the efficiency of the conversion of nitrate into NO[31,32].

211 Whether greater doses of inorganic nitrate in older people are required to enhance NO

212 production and bioactivity is currently not known. It is also possible that dietary nitrate
213 interventions with longer duration might be required in older individuals to reach sufficiently
214 high and sustained concentrations of NO and induce beneficial effects on BP.

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

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

233 Our analyses also showed that the effect of dietary nitrate may be dependent on the method
234 used to measure BP. The results seem to indicate that the use of 24-hour ambulatory and
235 daily home BP monitoring may be associated with a lower efficacy of dietary nitrate on BP

236 outcomes. The choice of the most accurate and precise method for the measurement of BP is
237 a complicated and unresolved question for clinical and research monitoring of the effects of
238 specific dietary, lifestyle and pharmacological treatments[36]. While the use of clinical
239 resting BP is the most used method, its poor reliability is universally recognized in
240 consideration of measurement bias associated with white-coat syndrome, standardization of
241 protocol and operator bias [37]. Some of these issues are resolved by the recommended
242 adoption of 24-hour ambulatory and daily home monitoring for a more objective assessment
243 of BP [38,39].

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

260

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3 data from their studies.

4 **Authors' Contribution**

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

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13 **Conflicts of Interest**

14 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

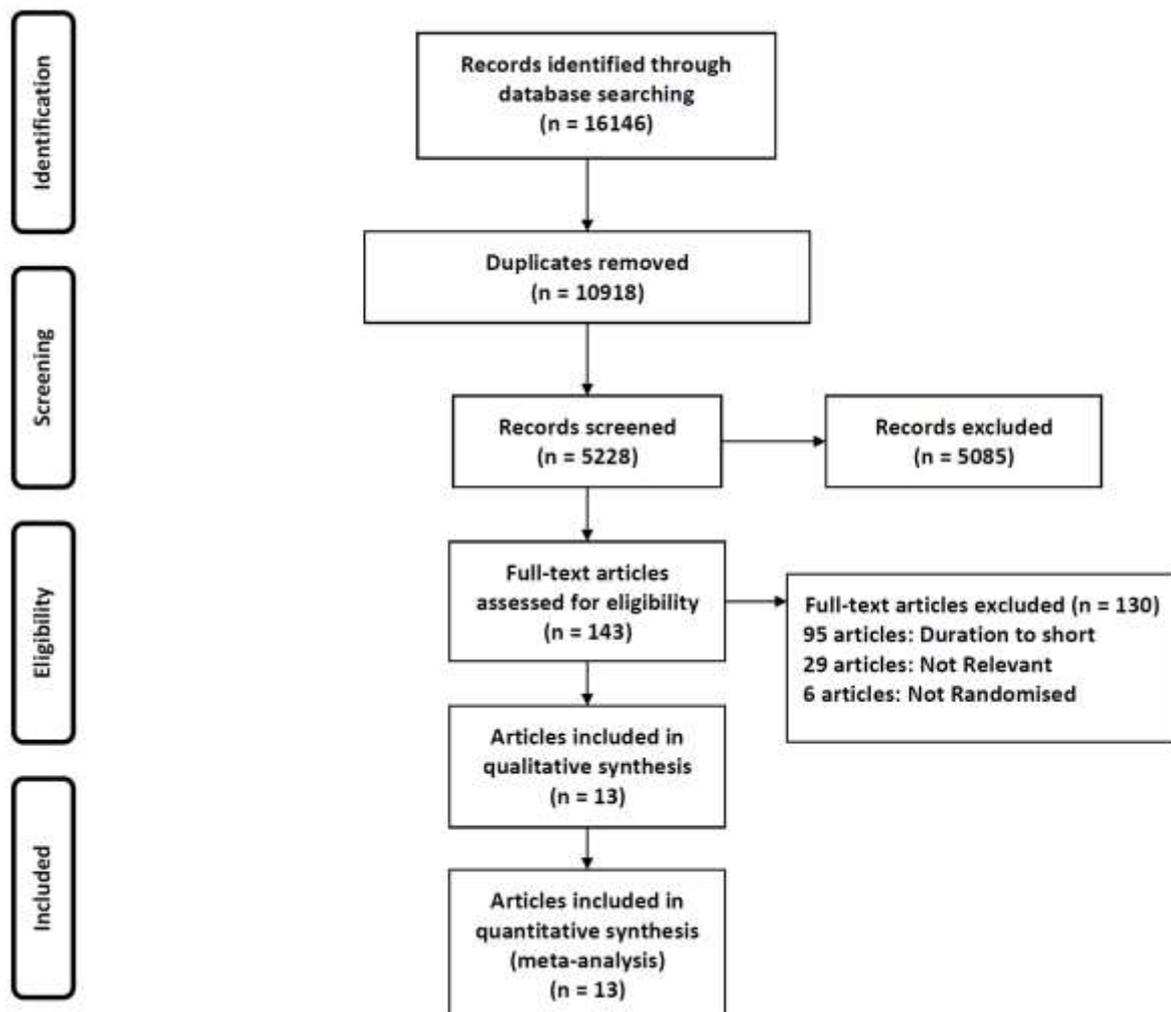


Figure 2

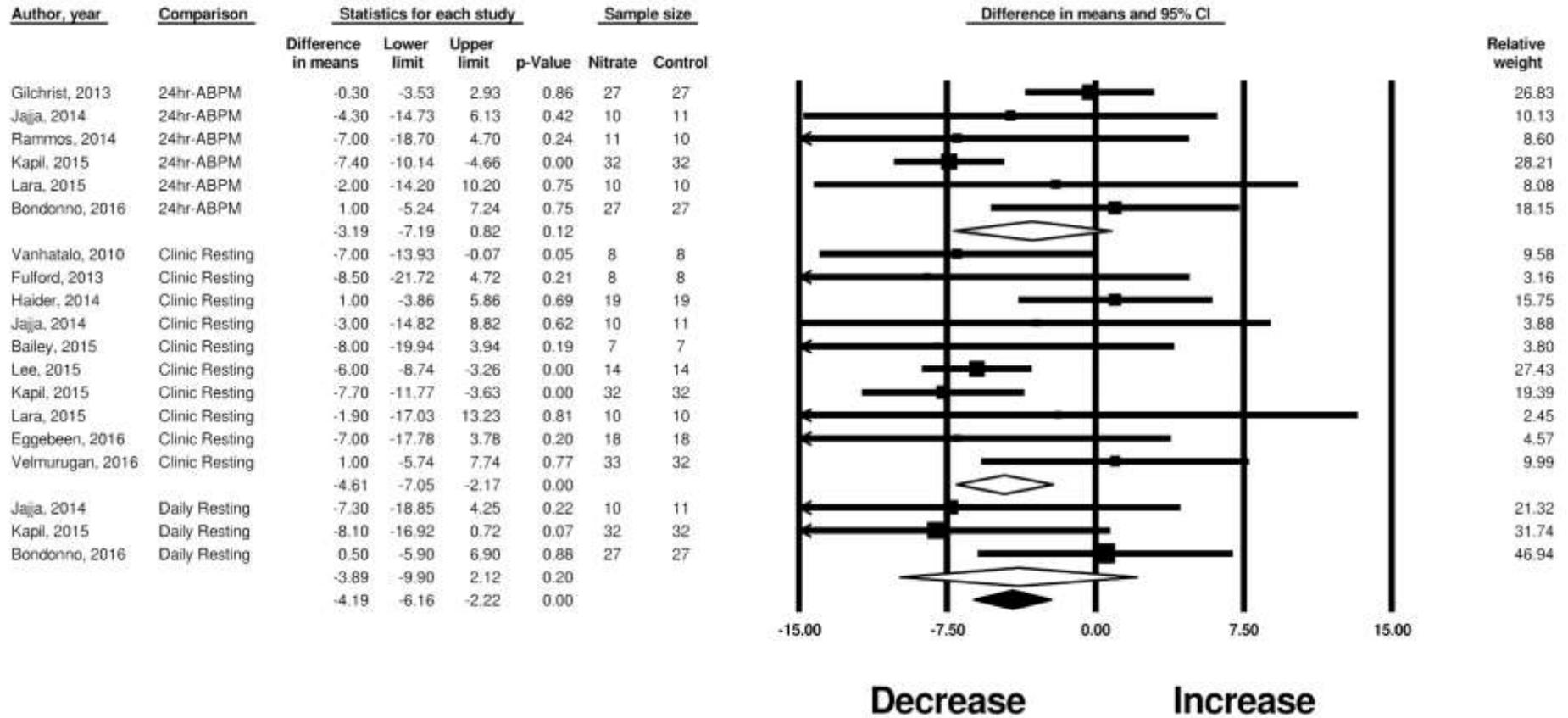
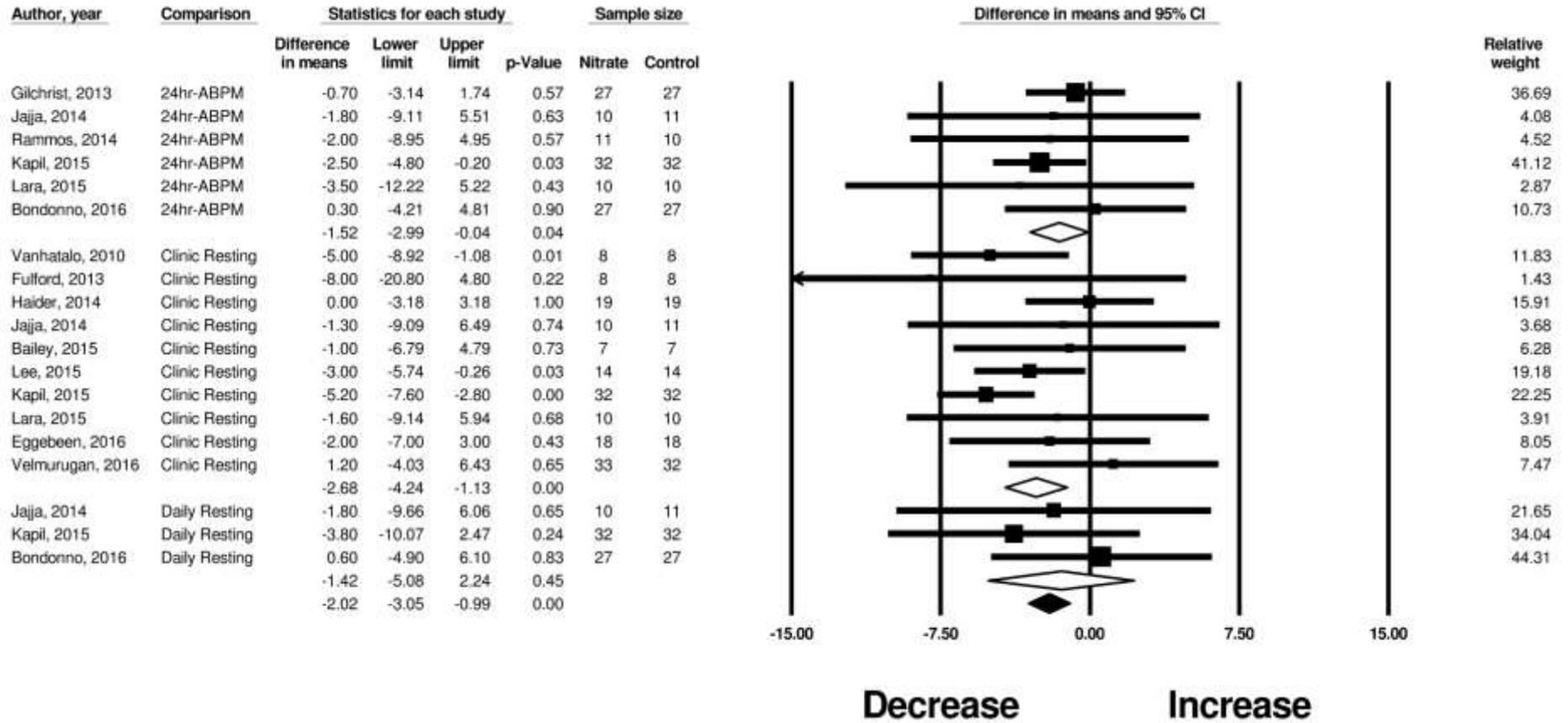


Figure 3



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-analysis¹							
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·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.

		<p>activities and consume similar meals in the first and second supplementation periods</p> <p>Plasma nitrate and nitrite concentrations were measured.</p>					<p>Subjects experienced beeturia during the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported.</p> <p>No conflicts of interest, financial or otherwise, were declared by the author(s).</p>
<p>Fulford et al², [2013]</p>	<p>8 physically active, healthy men. Age: 24y. BMI: ~22 kg·m⁻². None of the subjects was smoking or used dietary supplements.</p>	<p>Placebo controlled, cross-over, double blind randomised clinical trial.</p> <p>At the end of each intervention, measurement of resting clinic BP.</p> <p>Subjects were instructed to adhere to their normal exercise routine and diet throughout the experimentation.</p> <p>Plasma nitrate and nitrite</p>	<p>15 d (14 d)</p>	<p>250mL of beetroot juice</p>	<p>7.5mmol/d</p>	<p>250mL nitrate depleted beetroot juice</p>	<p>At 15 d, significant differences were found between beetroot and placebo groups for both systolic and diastolic BP.</p> <p>Plasma nitrite concentrations increased by 176nmol/L (175%)</p> <p>Primary outcome of the study was the effect of nitrate on exercise performance</p> <p>Subjects experienced beeturia during the BR supplementation. However, the supplementation regimen was well tolerated and no</p>

		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·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·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·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

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

Online Supporting Material

		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).

Online Supporting Material

<p>Bondonno et al⁸, [2015]</p>	<p>27 patients with diagnosed hypertension. Age: 63y. M/F: 17/ 10.</p> <p>BMI: 26.9 kg·m⁻²</p>	<p>Double blind, randomised, placebo-controlled crossover trial.</p> <p>Daily and 24-hour ambulatory blood pressure monitoring.</p> <p>Dietary nitrate intake was not restricted.</p> <p>Plasma nitrate and nitrite concentrations were measured.</p>	<p>1 week (1 week)</p>	<p>140mL of concentrated beetroot juice</p>	<p>7.5mmol/d</p>	<p>140mL nitrate depleted beetroot juice</p>	<p>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.</p> <p>Plasma nitrite concentrations increased by 3800nmol/L (290%)</p> <p>Adverse events: Not reported</p> <p>Funding source: National Health and Medical Research Council</p> <p>Conflicts of Interest: None to report</p>
<p>Kapil et al⁹, [2015]</p>	<p>68 drug-naïve and treated hypertensive patients. Age: 57y. M/F: 30/ 38.</p> <p>BMI: 26.5 kg·m⁻²</p>	<p>Randomised, placebo-controlled, double-blind parallel trial.</p> <p>Resting clinic, daily and 24-hour ambulatory blood pressure monitoring.</p>	<p>4 weeks</p>	<p>250mL of beetroot juice</p>	<p>~6mmol/d</p>	<p>250mL nitrate depleted beetroot juice</p>	<p>At the end of the intervention significant effect on resting, daily and 24-hour systolic and diastolic BP.</p> <p>Plasma nitrite concentrations increased by 470nmol/L (335%)</p> <p>Adverse events: Subjects experienced beeturia with the BR supplementation.</p>

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

Online Supporting Material

							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%)

		<p>Dietary nitrate intake was not restricted.</p> <p>Plasma nitrate and nitrite concentrations were measured.</p>					<p>Adverse events: two subjects excluded for unrelated events</p> <p>Funding source: National Institutes of Health</p> <p>Conflicts of Interest: Reported conflicts by some investigators</p>
<p>Velmurugan et al¹³, [2016]</p>	<p>67 untreated patients with high cholesterol. Age: 53y. M/F: 24/ 43.</p> <p>BMI: 26.8 kg·m⁻²</p>	<p>Randomised, placebo-controlled, double-blind parallel trial.</p> <p>Resting clinic pressure.</p> <p>Dietary nitrate intake: not reported controlled.</p> <p>Plasma and urine nitrate and nitrite concentrations were measured.</p>	<p>6 weeks</p>	<p>250mL of beetroot juice</p>	<p>~6mmol/d</p>	<p>250mL nitrate depleted beetroot juice</p>	<p>At the end of the intervention no significant effect on resting systolic and diastolic BP.</p> <p>Plasma nitrite concentrations increased by 400nmol/L (233%)</p> <p>Adverse events: Subjects experienced beeturia with the BR supplementation. However, the supplementation regimen was well tolerated and no harmful side effects were reported.</p> <p>Funding source: British Heart Foundation and Medical Research Council</p>

Online Supporting Material

							Conflicts of Interest: None to declare
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Acronyms are: BP: blood pressure; BMI: Body mass index; F: female; M: male; d: days; BR: beetroot; BW: body weight

