Review

Repeated administration of inorganic nitrate on blood pressure and arterial stiffness: a systematic review and meta-analysis of randomized controlled trials

Dandan Li[a,b], Stephanie K. Nishi[a,b], Elena Jovanovski[a,b], Andreea Zurbau[a,b,c], Allison Komishon[a], Sonia Blanco Mejia[a,b,c], Tauseef A. Khan[b,c], John L. Sievenpiper[a,b,c,d,e,f], Davor Milicic[g], Alexandra Jenkins[a], and Vladimir Vuksan[a,b,d,e,f]

Objective: We aim to synthesize effects of repeated administration (≥3 days) of inorganic nitrate on blood pressure and arterial stiffness measures.

Methods: We conducted a systematic review and meta-analysis of randomized controlled trials with at least 3 days treatment of inorganic nitrate on blood pressure and arterial stiffness in individuals with or without elevated cardiovascular disease risk. MEDLINE, EMBASE and the Cochrane Library were searched through 2 July 2019. Two independent reviewers extracted relevant study data. Data were pooled using the generic inverse variance method with random-effects model, and expressed as mean differences with 95% confidence intervals. Certainty in the evidence was assessed using GRADE.

Results: Forty-seven trials were included ($n = 1101$). Administration of inorganic nitrate significantly lowered SBP [mean difference: −2.91 mmHg, 95% confidence interval (95% CI): −3.92 to −1.89, $I^2 = 76\%$], DBP (mean difference: −1.45 mmHg, 95% CI: −2.22 to −0.68, $I^2 = 78\%$], central SBP (mean difference: −1.56 mmHg, 95% CI: −2.62 to −0.50, $I^2 = 30\%$) and central DBP (mean difference: −1.99 mmHg, 95% CI: −2.37 to −1.60, $I^2 = 0\%$). There was no effect on 24-h blood pressure, augmentation index or pulse wave velocity. Certainty in the evidence was graded moderate for central blood pressure, pulse wave velocity and low for peripheral blood pressure, 24-h blood pressure and augmentation index.

Conclusion: Repeated administration (≥3 days) of inorganic nitrate lower peripheral and central blood pressure. Results appear to be driven by beneficial effects in healthy and hypertensive individuals. More studies are required to increase certainty in the evidence.

Keywords: blood pressure, cardiovascular, diet, hypertension, nutrition

Abbreviations: ADI, adequate daily intake; AIx, augmentation index; BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; DASH, dietary approach to stop hypertension; eNOS, endothelial nitric oxide synthase; GRADE, Grading of Recommendations Assessment, Development and Evaluation; MID, minimally important difference; PRIMSA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PWV, pulse wave velocity; RCT, randomized controlled trial; SD, standard deviation; SE, standard error; T2DM, type 2 diabetes

INTRODUCTION

Elevated blood pressure (BP) remains the leading attributable cause of mortality on the global scale [1], and is estimated to increase in prevalence over the next several years [2,3]. As such, there is a pressing need to establish effective dietary strategies for hypertension management, as a cornerstone in primary prevention or an adjunct to pharmacotherapy [4–7]. Currently, dietary advice for those with hypertension or those at risk of developing hypertension consists of modifications to micronutrient intake including sodium and potassium, and an emphasis on the Dietary Approach to Stop Hypertension (DASH) diet [6,8]. Although the BP-lowering effects of DASH are likely attributed to the combination of dietary components, including high fruit and vegetables, fibre-rich whole grains and reduced saturated/total fat [9,10], emerging evidence suggests the importance of improved nitric oxide bioavailability as one of its key cardioprotective effects [11].

It was not until the recent decades that dietary sources of inorganic nitrate (NO_3^-) were appreciated for its link to nitric oxide bioavailability and potential cardiovascular

Journal of Hypertension 2020, 38:2122–2140

[a]Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, Unity Health, [b]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, [c]Toronto 3D Knowledge Synthesis & Clinical Trials Unit, [d]Division of Endocrinology and Metabolism, Department of Medicine, [e]Li Ka Shing Knowledge Institute, St. Michael's Hospital, [f]Department of Medicine, University of Toronto, Toronto, Ontario, Canada and [g]Internal Medicine and Cardiology, Rebro Clinic-Hospital, Faculty of Medicine, University of Zagreb, Zagreb, Croatia

Correspondence to Vladimir Vuksan, PhD, Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, 30 Bond Street, Toronto, ON M5B1X1, Canada. Tel: +1 416 864 5525; fax: +1 416 864 5538; e-mail: v.vuksan@utoronto.ca

Received 10 April 2020 **Revised** 1 May 2020 **Accepted** 3 May 2020

J Hypertens 38:2122–2140 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

DOI:10.1097/HJH.0000000000002524

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

benefit [12,13]. Given the wide availability of nitrate through dietary sources, particularly in green leafy vegetables and beetroot, various clinical studies have tested the acute vascular effects of nitrate administration in healthy volunteers through concentrated beetroot juice, specific vegetable diets, and through nitrate solutions [14,15]. To date, accumulating evidence from pilot trials in healthy groups appears to suggest a range of beneficial effects including lowering BP and improving endothelial function [14–16]. However, the effects of repeated administration and the long-term therapeutic potential of nitrate especially in hypertensive and higher cardiovascular disease (CVD) risk groups are not well understood. Particularly, questions have been raised with regards to the development of tolerance and diminished efficacy, as has been observed with the organic nitrate counterparts [17].

Given the vital role of nitric oxide in the vascular system, sources of inorganic nitrate may represent an avenue to restore diminished nitric oxide availability and endothelial dysfunction characteristic of hypertension and other CVDs [18]. Thus, a systematic review and meta-analysis of randomized controlled trials (RCTs) was conducted to provide an effect estimate of repeated administration of inorganic nitrate on BP and arterial stiffness in individuals with or without elevated CVD risk. For the first time, the certainty of the evidence for this research question was assessed by the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach and differences between healthy and patient populations were explored.

MATERIALS AND METHODS

Design

This systematic review and meta-analysis followed the Cochrane Handbook for Systematic Reviews of Intervention [19]. Results were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20]. The review protocol was registered on PROSPERO (CRD42019141215).

Search strategy and data sources

MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials were searched through 2 July 2019. Manual search of references of included studies supplemented the electronic search. RCTs were identified using search terms that identify the intervention and outcome of interest. Limits were applied at the end of the search to further narrow the number of hits retrieved [21] (Table S1, http://links.lww.com/HJH/B379).

Study selection

RCTs were considered for analysis if they investigated the oral administration of inorganic nitrate compared with an appropriate control (placebo, active or standard care) on at least one BP or arterial stiffness outcome. We decided to assess the effects of repeated exposure of nitrate, therefore only trials with an intervention of at least 3 days were included. No restrictions were placed on the source of inorganic nitrate. This included studies providing nitrate-concentrated beetroot juice, nitrate-rich vegetable diets and nitrate salts in solution or capsules (KNO_3 or $NaNO_3$). Populations of interest were healthy, CVD or groups with elevated CVD risk (as defined by the presence of one or more established risk factors such as hypertension, diabetes or hyperlipidaemia) [22]. No restrictions were placed on the language of the article. Exclusion criteria included animal studies, observational studies and combination therapies in which the effect of nitrate could not be distinguished. Trials that investigated pharmacological organic nitrates were excluded. Interventions with a primary focus on exercise capacity were included if the BP effect can be isolated from the effect of exercise. Trials with environmental manipulation (temperature control, high-altitude/hypoxic conditions) were excluded.

Data extraction

Two independent reviewers (D.L. and S.K.N.) extracted data from included articles using a standardized proforma. Relevant data included information on authorship, publication year, study design, duration, blinding, sample size, participant characteristics (age, sex, health status, baseline BP, arterial stiffness and BMI), compliance, funding source and setting. Outcome variables were SBP, DBP, 24-h ambulatory SBP and DBP, central (aortic) SBP and DBP, augmentation index (AIx) or pulse wave velocity (PWV). Office BP, home BP and daytime BP were pooled in one meta-analysis. If a study reported multiple BP outcomes for the same sample population, office BP was extracted in preference, followed by home BP and daytime BP. Central BP and 24-h ambulatory BP each composed of its own analysis. Mean differences (MD) ± standard error (SE) between treatment and control were extracted, or when provided, means ± standard deviations (SD) at baseline and follow-up or the mean changes ± SD from baseline were extracted for intervention and control. When SDs were not reported, it was derived from available data [95% confidence interval (95% CI), P values, t or F statistics, SEM] using standard formulas [19]. Authors were contacted for outcome data that were measured but not reported. Data represented through graphs without numerical values were extracted using Plot Digitizer, version 2.5.1. All discrepancies and disagreements were resolved through discussion until consensus, and if needed, by consulting a third reviewer (VV).

Risk of bias assessment

Each study was assessed for risk of bias using the Cochrane Risk of Bias Assessment tool. Domains of assessment include sequence generation, allocation concealment, blinding, outcome data and reporting [19]. Trials were considered 'high risk' if they contained methodological flaws that may have affected the results, 'low risk' if the flaw was deemed inconsequential and 'unclear risk' when insufficient information was provided to determine risk of bias. Disagreements were resolved by consensus between coextractors.

Data synthesis and analysis

Review Manager (RevMan) version 5.3 (Copenhagen, Denmark) was used for primary analyses and Stata (version 13; College Station Texas, USA) was used for subgroup, dose–response and publication bias analyses. If available, the MD ± SEs in change-from-baseline values for BP, AIx

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

and PWV were preferred and calculated if needed. When change values were not available, the difference in posttreatment values were used. Paired analyses were applied to all crossover trials assuming a within-individual correlation coefficient of 0.5 [23,24]. For studies with multiple intervention arms, the SE(MD) was adjusted to take into account the number of comparisons extracted per control group [19]. Results were reported as MDs with 95% CI. When at least two studies provided data for an outcome, data were pooled using the generic inverse-variance method with DerSimonian and Laird random effects model, which yields conservative CIs around effect estimates in the presence of heterogeneity. Heterogeneity was assessed by the Cochran Q test (significant at $P < 0.10$) and quantified by the I^2 statistic [25], where I^2 more than 50% was considered evidence of substantial heterogeneity. Sources of heterogeneity were explored through sensitivity and subgroup analyses. Sensitivity analyses were carried out by systematically removing individual studies and recalculating summary effect sizes with the remaining studies. A study was considered influential if its removal changed the significance of the effect estimate or the evidence of heterogeneity.

If at least 10 studies per outcome were available, then a-priori subgroup analyses were conducted using meta-regression. Categorical subgroup analyses were carried out for study duration (median), study design (parallel vs. crossover), baseline values (median), baseline BMI (median), health status [healthy, hypertension, type 2 diabetes (T2DM), hypercholesterolemia, overweight/obese, heart failure, peripheral artery disease], funding (agency vs. industry), BP measurement (automated vs. manual), nitrate form of administration (nitrate salt, vegetable powder, beetroot juice, other vegetables), age (median) and dose (median). Continuous subgroup analyses were conducted for baseline values, duration (days), change in plasma nitrite (nmol/l), BMI (kg/m^2) and age (years). Linear (continuous) and nonlinear (cubic spline) dose–response analyses were performed using meta-regression in Stata.

If at least 10 studies per outcome were available [26], then publication bias was assessed by visual inspection of funnel plots and formal testing using the Egger and Begg tests [19]. If there was evidence of publication bias ($P < 0.1$), the Duval and Tweedie Trim and Fill method was used to adjust for funnel plot asymmetry by imputing missing study data [27].

Grading the evidence

The GRADE approach was used to assess the overall certainty of the evidence [28]. Evidence was rated as 'very low', 'low', 'moderate' or 'high' quality. RCTs receive an initial grade of 'high', and could be downgraded based on a series of prespecified criteria, including risk of bias (weight of studies show high risk of bias assessed by the Cochrane Risk of Bias tool), inconsistency (substantial unexplained heterogeneity, $I^2 > 50\%$, $P < 0.1$), indirectness (presence of factors that limited the generalizability of the results), imprecision (the 95% CI for effect estimates were crossed a minimally important difference [MID] for benefit or harm for SBP [± 2 mmHg] and DBP [± 1 mmHg] [29], AIx [±2%] [30], PWV [±1 m/s] [31,32]) and publication bias (evidence of small-study effects).

RESULTS

Search results

The PRISMA flow is illustrated in Fig. 1. The search identified 3381 reports, of which 1357 reports were excluded as duplicates and 1779 were excluded based on title and/or abstract. Of 245 reports reviewed in full, 199 reports were excluded for not meeting the eligibility criteria, and 46 reports (47 trials, $n = 1101$) were included in the meta-analysis. Forty-five trials [33–76] (48 comparisons) reported on SBP, 44 trials [33–45,47–77] (48 comparisons) on DBP, nine trials [59–61,64,66,68,70,74,76] (nine comparisons) on 24-h ambulatory SBP and DBP, three trials (three comparisons) on central SBP [45,60,78], two trials (two comparisons) on central DBP [45,60], seven trials [45,52,59,60,64,65,73] (seven comparisons) on AIx and eight trials [58–60,64,65,73,74,78] (nine comparisons) on PWV.

Study characteristics

Characteristics of the included trials are presented in Table 1. Of the 47 trials, 27 were in healthy, three in overweight, nine in hypertensive, four in T2DM, one in peripheral artery disease, one in hypercholesterolemic and two in heart failure patients. Results for each outcome were presented in subgroups of population health status. All of the trials were conducted in an outpatient setting, with 29 trials conducted in European countries, nine in the USA, three in Canada, three in Australia, two in Asia and one in New Zealand. Of the included trials, 34 were crossover and 13 were parallel design. Across all trials, the median age of participants was 55 years, (range: 17–74) and the median BMI was 26.7 kg/m^2 (range: 21.8–33.6). The median dose of nitrate across all trials was 456 mg/day (range: 150–1000) and median treatment duration of trials was 7 days (range: 3–168). Across all trials, 12 recruited only men, 19 recruited both men and women, one recruited only women and the remaining 15 did not report on sex. Method of BP measurement was reported to be automatic in 35 of trials, manual in three and unreported in nine. Twenty-six trials received funding by agency (government, not-for-profit health agency or academic institution), seven were funded by industry, three reported no funding and 11 did not report on funding sources.

Risk of bias

The risk of bias assessment for all included studies is shown in Figure S1, http://links.lww.com/HJH/B379. Due to the lack of standardized and detailed reporting, most studies were assessed as having an unclear risk of bias. Only one study [74] was assigned a high risk of bias for blinding and selective outcome reporting [54]. The remaining studies were either assessed as low or unclear risk of bias across all domains: sequence generation, allocation concealment, blinding, incomplete outcome data and selective outcome reporting.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

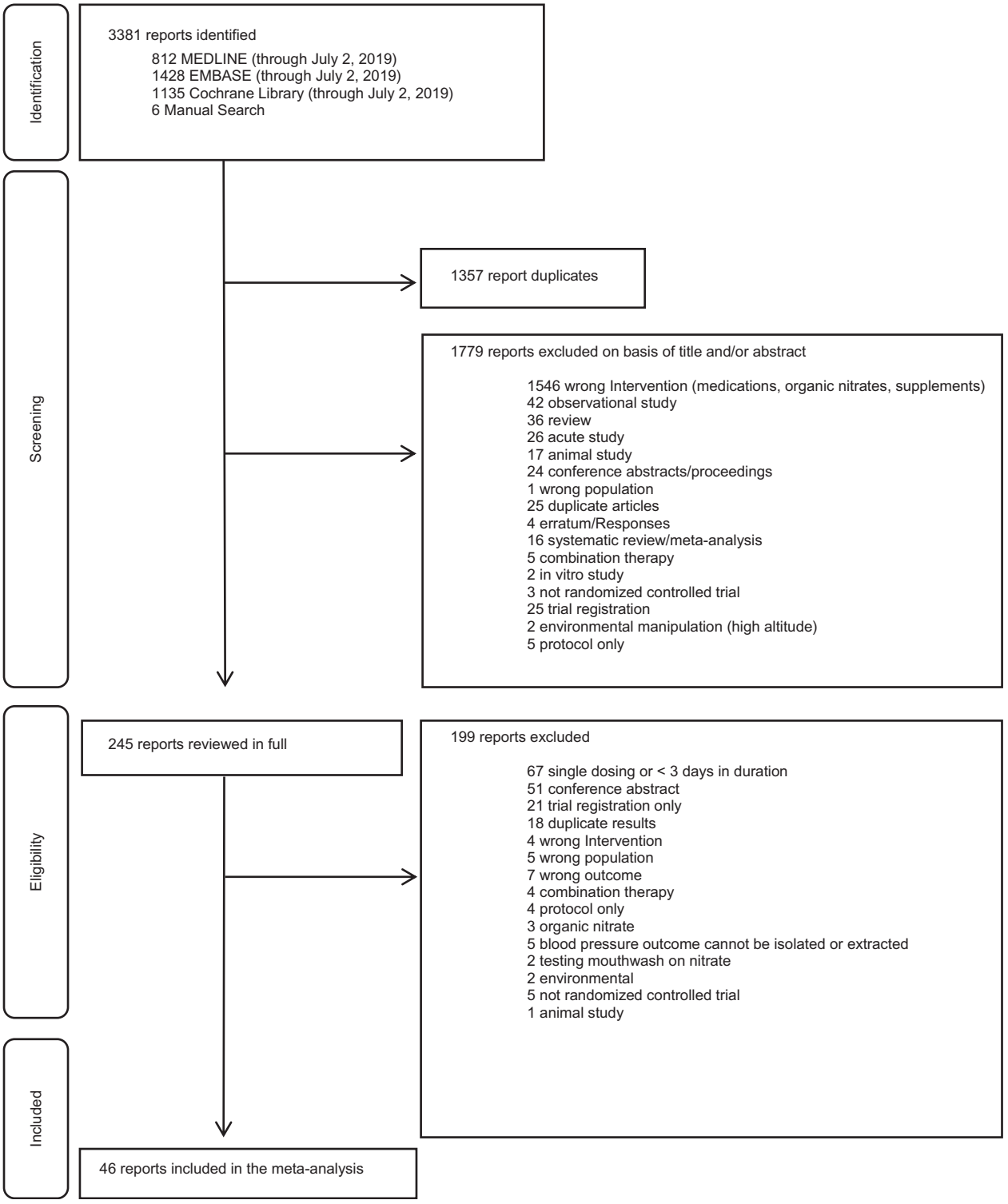


FIGURE 1 Summary of the search and article selection process for the effect of nitrate-rich interventions on blood pressure and arterial stiffness outcomes.

Outcomes

SBP and DBP

The effect of repeated administration of inorganic nitrate on SBP is presented in Fig. 2, showing a lowering effect in SBP (MD: −2.91 mmHg, 95% CI: −3.92 to −1.89, $P < 0.001$) and evidence of substantial heterogeneity ($I^2 = 76\%$, $P < 0.001$). Data were pooled from 45 trials with a median dose of 456 mg nitrate per day and median treatment duration of 7 days. There were no significant subgroup differences by health status ($P = 0.07$). Overall, removal of each individual study in sensitivity analyses did not change the significance of the effects or heterogeneity (*data not shown)*. A-priori subgroup analyses using continuous and categorical meta-

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 1. Characteristics of included studies

Study	Individual status	Design	Intervention (per day)	No. of individuals[a]	Age[b] (years)	BMI[b] (kg/m^2)	Dose (mg/day)	BP measure	Follow-up	Wash-out	Funding	Country
Aamand *et al.* [33]	Healthy	CO, DB	$NaNO_3$ solution	18 (18M: 0F)	NR	NR	6.2/kg BW	A	3 days	9–11 days	Agency	Denmark
Ashor *et al.* [75]	Overweight	P, O	70 ml beetroot juice	21 (12M: 9F)	55-70	29.9 (4.2)	300-400	A	28 days	NA	NR	England
Ashworth *et al.* [34]	Healthy, premenopausal women	CO, O	Vegetables	19 (0M:19F)	NR	22.5 (3.8)	339	A	7 days	3 weeks	None	England
Aucouturier *et al.* [35]	Healthy	CO, SB	500 ml high nitrate beetroot juice	12 (12M:0F)	22.8 (3.1)	NR	340	A	3 days	1–2 weeks	None	France
Bailey *et al.* [37]	Healthy	CO, DB	140 ml beetroot juice	Nonsmokers: 8 (4M:4F) Smokers: 9 (5M: 4F)	Nonsmokers: 24 (5) smokers:24 (7)	Nonsmokers: 23 (4) smokers:23 (2)	520	A	6 days	7–10 days	NR	England
Bailey *et al.* [36]	Healthy	CO, DB	500 ml beetroot juice	8 (8M:0F)	19-38	NR	341	A	6 days	10 days	NR	England
Blekkenhorst *et al.* [60]	Mildly hypertensive	CO, O	200 g vegetables	30	63 (55.5–70.5)	25.9 (3.4)	150	A	4 weeks	4 weeks	Agency	Australia
Bock *et al.* [67]	Peripheral artery disease	P, DB	1 g $NaNO_3$ tablets	21	72 (10)	28.8	527	NR	8 weeks	NA	Agency	USA
Bock *et al.* [38]	Healthy	CO, DB	10 g beetroot powder	11 (7M:4F)	67 (3)	26 (3)	250	A	28 days	$\geq$ 28 days	Industry	USA
Bond *et al.* [39]	Healthy, trained athletes	CO, DB	500 ml beetroot juice	14 (14 M:0F)	16.7 (0.5)	NR	341	Manual	6 days	7 days	Agency	New Zealand
Bondonno *et al.* [61]	Treated hypertensive	CO, DB	140 ml beetroot juice	27	53-70	26.9 (3.2)	434	A	4 weeks	4 weeks	Agency	Australia
Bondonno *et al.* [59]	Mildly hypertensive	CO, O	Green leafy vegetables	38	38-70	27.1 (3.8)	400	A	7 days	7 days	Agency	Australia
Bourdillon *et al.* [40]	Healthy, trained athletes	CO, DB	$NaNO_3$ gelatin capsules	12 (12M:0F)	31 (7)	NR	456	A	3 days	$\geq$ 5 days	Agency	Switzerland
Burleigh *et al.* [41]	Healthy	CO, SB	140 ml nitrate-rich beetroot juice	11 (11 M: 0 F)	30 (7)	NR	769	A	7 days	4 weeks	Agency	Scotland
Cermak *et al.* [42]	Healthy, athletes	CO, DB	140 ml beetroot juice	12 (12M: 0F)	31 (3)	NR	496	A	6 days	14 days	Agency	Canada
Cherukuri *et al.* [62]	Hypertensive	P, DB	NO_3 tablets[c]	67 (26M:41F)	59.3 (9)	NR	314	NR	12 weeks	NR	NR	USA
Esen *et al.* [43]	Healthy, athletes	CO, DB	140 ml beetroot juice	10 (5 M: 5 F)	22 (6)	NR	496	A	3 days	$\geq$ 72 h	NR	England
Faconti *et al.* [69]	T2DM or at risk of T2DM	P, DB	Beetroot juice	87 (60M:27F)	NR	32.3 (5.3)	Phase 1: 279 Phase 2: 682	NR	6 months	NR	Industry	England
Gilchrist *et al.* [70]	T2DM	CO, DB	250 ml beetroot juice	27 (18 M: 9 F)	67.2 (4.9)	30.8 (3.2)	465	A	14 days	4 weeks	Agency	England
Haider *et al.* [44]	Healthy	CO, DB	300g diluted beetroot juice	19 (19M:0F)	21 (3)	NR	600	A	7 days	9 days	Agency	England
Henrohn *et al.* [63]	Pulmonary arterial hypertension	CO, DB	140 ml beetroot juice	15 (7M:8F)	31-82	NR	1000	NR	7 days	4–9 days	NR	Sweden
Hirai *et al.* [72]	Heart failure with reduced ejection fraction	CO, DB	140 ml beetroot juice	10 (10M:0F)	63 (15.8)	31.0 (6.95)	800	Manual	9 days	$\geq$ 5 days	Agency	Canada
Jajja *et al.* [76]	Overweight	P, O	70 ml beetroot juice	21 (12M: 9F)	55-70	29.92 (1.3)	165	A	21 days	NA	NR	England
Jovanovksi *et al.* [45]	Healthy	CO, SB	Spinach soup	27	24.5 (11)	22.8 (3.7)	845	A	7 days	1 week	Agency	Canada
Kapil *et al.* [64]	Hypertensive	P, DB	250 ml beetroot juice	64 (26M:38F)	18-85	26.7	397	A	4 weeks	2 weeks	Agency	England
Kelly *et al.* [47]	Healthy	CO, DB	140 ml beetroot juice	10	60-70	NR	595	A	3 days	$\geq$ 3 days	NR	England
Kerley *et al.* [68]	Hypertensive	CO, DB	140 ml beetroot juice	20	62.5 (13.1)	30.7 (5.8)	800	A	7 days	None	Agency	Ireland
Lansley *et al.* [48]	Healthy, physically active	CO, DB	500 ml beetroot juice	9 (9M:0F)	22 (4)	NR	384	A	6 days	10 days	NR	England
Lara *et al.* [74]	Overweight or obese	P, O	140 ml beetroot juice	20 (9M:11F)	55-70	29.9 (4.5)	600	A	7 days	NA	none	England
Larsen *et al.* [15]	Healthy, physically active	CO, DB	$NaNO_3$ solution	17 (15M:2F)	24	NR	0.62/kg BW	NR	3 days	10 days	Agency	Sweden

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 1 (*Continued*)

Study	Individual status	Design	Intervention (per day)	No. of individuals[a]	Age[b] (years)	BMI[b] (kg/m^2)	Dose (mg/day)	BP measure	Follow-up	Wash-out	Funding	Country
Lee *et al*. [49]	Healthy	CO, DB	70 ml beetroot juice	14 (14M:0F)	22 (3.74)	23.4 (3)	400	NR	15 days	2 weeks	NR	South Korea
Miller *et al*. [50]	Healthy	CO, O	Diet or 500 ml beetroot juice	8 (3M:5F)	72.5 (4.7)	28.5 (3.9)	45, 155, 570 or 682	A	3 days	3–12 days	Agency	USA
Mills *et al*. [78]	T2DM or at risk of T2DM	P, DB	Beetroot juice	126	57 (12)	NR	580	NR	6 months	NA	Industry	England
Nyakayiru *et al*. [51]	Healthy, trained athletes	CO, DB	140 ml $NaNO_3$ solution	17 (17M: 0F)	25 (4)	21.8 (1.8)	800	A	6 days	8 days	Industry	Nether- lands
Oggioni *et al*. [52]	Healthy	CO, DB	140 ml beetroot juice	20	60-75	25.6 (3.4)	744	A	7 days	$\geq$7 days	Agency	England
Perez *et al*. [53]	Healthy	CO, DB	70 ml beetroot juice	20 (20M:0F)	21.8 (2.4)	NR	397	A	7 days	10 days	Agency	USA
Rammos *et al*. [65]	Mildly hypertensive	P, DB	$NaNO_3$ solution	21	63 (5)	25 (3.6)	9.3/kg BW	NR	4 weeks	NA	Agency	Germany
Shaltout *et al*. [66]	Hypertensive, controlled	P, DB	Beetroot juice	26 (13M:13F)	65 (5)	33.6 (5.7)	500	A	6 weeks	NA	Agency	USA
Shaltout *et al*. [66]	Heart Failure with Preserved Ejection Fraction	P, DB	70 ml beetroot juice	18	69.2 (6.8)	32.6 (5.6)	380 three times/week	NR	4 weeks	NA	Agency	USA
Shepherd *et al*. [71]	T2DM	CO, DB	70 ml beetroot juice	48	63.35 (7.27)	30.17 (2.93)	398	A	4 days	10–14 days	Agency	England
Sobko *et al*. [77]	Healthy	CO, O	Fresh vegetables and staple Japanese foods	25 (10M:15F)	36 (10)	<18.5	1103[d]	Manual	10 Days	None	Agency	Japan
Schneider *et al*. [54]	Healthy	CO, DB	Beetroot powder	13 (6F:7M)	67 (1)	25.5	250	A	4 weeks	$\geq$ 4 weeks	Industry	USA
Sweazea *et al*. [55]	Healthy	P, DB	60 ml fruit & vegetable liquid	45	18–40	25.4 (3.5)	248	A	14 days	NA	Industry	USA
Thompson *et al*. [56]	Healthy, physically active	CO, DB	70 ml beetroot juice	36	24 (4)	NR	396	A	5 days	7 days	Industry	England
Vanhatalo *et al*. [57]	Healthy	CO, DB	500 ml beetroot juice	8	29 (6)	NR	322	A	15 days	10 days	NR	England
Vanhatalo *et al*. [58]	Healthy	CO, DB	140 ml beetroot juice	Younger: 9 (4M:5F) Older: 9 (3M:6F)	Young: 19–22 Old: 70–79	Young: 24.2 (3.5) Older: 24.4 (4.7)	768	A	10 days	3–47	Agency	England
Velmurugan *et al*. [73]	Hypercholesterolemic, untreated	P, DB	250 ml beetroot juice	65	18–80	26.7 (5.0)	372	A	6 weeks	NA	Agency	England

A, automatic; BW, body weight; CO, cross-over; DB, double-blind; F, female; M, male; NA, not applicable; $NaNO_3$, sodium nitrate; NR, not reported; O, open-label; P, parallel; SB, single-blind; T2DM, type 2 diabetes mellitus.
[a]No. of individuals reflect number included in final analysis if reported, or number randomized if unreported.
[b]Values for age and BMI are reported as mean (SD) or (range).
[c]NO_3 tablet contained beetroot extract, thiamin nitrate, potassium nitrate in the presence of ascorbic acid.
[d]Dose estimated based on provided dose/kg body weight and mean body weight.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

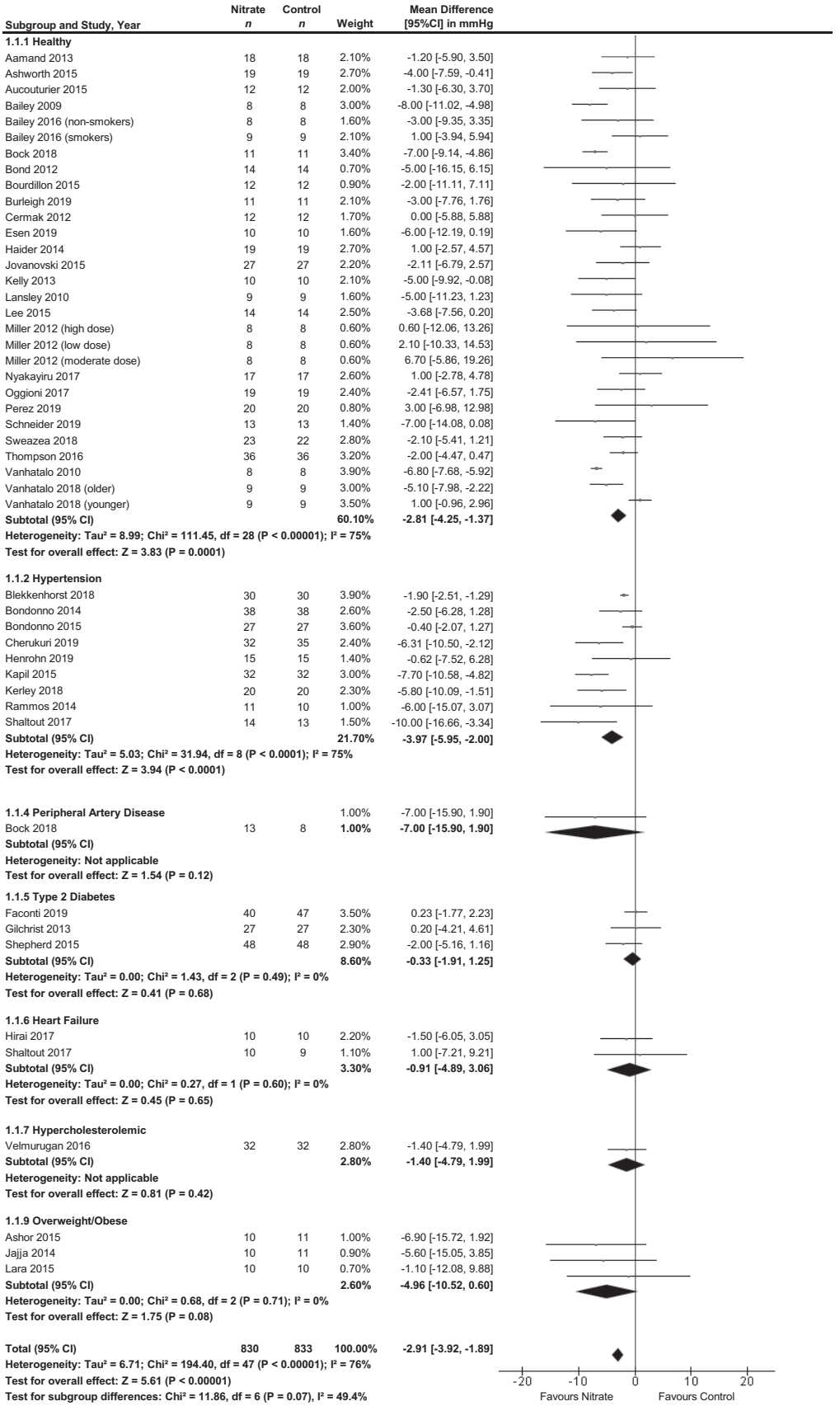


FIGURE 2 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on systolic blood pressure. n = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

regression for the effect of nitrate-rich interventions on SBP are shown in Table S2A, http://links.lww.com/HJH/B379 and Figure S2, http://links.lww.com/HJH/B379, respectively. There was no evidence of a subgroup effect in continuous meta-regression. In categorical meta-regression, there was a significant effect modification by dose ($P = 0.04$). Pairwise comparisons showed that studies that included doses of nitrate at less than 445 mg/day had a greater lowering effect on SBP (MD: −3.82 mmHg, 95% CI:−5.09 to −2.56) than those that included doses ≥445 mg/day (MD:−1.8 mmHg, 95% CI: −3.22 to −0.39). There was no evidence of a linear or nonlinear dose response (Figures S4A & S5A, http://links.lww.com/HJH/B379).

The effect of repeated administration of inorganic nitrate on DBP is presented in Fig. 3, showing a lowering effect in DBP (MD: −1.45 mmHg, 95% CI: −2.22 to −0.68, $P < 0.001$) and evidence of substantial heterogeneity ($I^2 = 78\%$, $P < 0.001$). Data were pooled from 44 trials with a median dose of 461 mg nitrate per day and a median duration of 7 days. There were no significant subgroup differences by health status ($P = 0.41$). Sensitivity analyses did not change the heterogeneity or the significance of the effects (*data not shown)*. A-priori subgroup analyses using continuous and categorical meta-regression for the effect of nitrate-rich interventions on DBP are shown in Table S2B, http://links.lww.com/HJH/B379 and Figure S3, http://links.lww.com/HJH/B379, respectively. In continuous subgroup analysis, change in plasma nitrite ($P < 0.05$) were observed to be a significant effect modifier, with every unit increase associated with a smaller lowering effect on DBP. In categorical analysis, there was significant effect modification by dose ($P = 0.04$). Pairwise comparisons showed that studies that included doses of nitrate less than 460.5 mg/day had a greater lowering effect on DBP (MD: −2.11 mmHg, 95% CI: −3.16 to −1.06) than those that included doses at least 460.5 mg/day (MD: −0.45 mmHg, 95% CI: −1.59 to 0.69). Evidence was also observed for effect modification by form of administration ($P < 0.01$). Administration by vegetable powder showed a greater lowering effect on DBP (MD: −7.00 mmHg, 95% CI: −10.02 to −3.98), compared with administration by beetroot juice (MD: −0.99 mmHg, 95% CI:−1.83 to −0.15), nitrate salt (MD: −1.81 mmHg, 95% CI: −3.83 to 0.21) or other vegetables (MD: −1.38 mmHg, 95% CI: −3.04 to 0.27). A linear dose response was observed ($P = 0.01$) where increasing doses appeared to have a smaller lowering effect on DBP (Figure S4B, http://links.lww.com/HJH/B379). There was no evidence of a nonlinear response ($P > 0.05$) (Figure S5B, http://links.lww.com/HJH/B379).

Twenty-four-hour ambulatory SBP and DBP

The effect of repeated administration of inorganic nitrate on 24-h ambulatory SBP is presented in Fig. 4, showing no significant effect on 24-h ambulatory SBP (MD: −1.40 mmHg, 95% CI: −2.98 to 0.19, $P = 0.08$) and evidence for substantial heterogeneity ($I^2 = 83\%$, $P < 0.001$). Data were pooled from nine trials with a median dose of 434 mg nitrate per day and a median duration 14 days. In sensitivity analysis, (Table S3, http://links.lww.com/HJH/B379), removal of the study by Kapil *et al.* [64] explained the heterogeneity ($I^2 = 27\%$, $P = 0.22$) and changed the significance of the effect to near significance (MD: −0.80 mmHg, 95% CI: −1.60 to −0.01, $P = 0.05$). Removal of the study by Lara *et al.* [74] or Bondonno *et al.* [59] each changed the significance of the effect to near significance ($P = 0.05$). There was no evidence of a linear dose response (Figure S4c, http://links.lww.com/HJH/B379). The effect of repeated administration of inorganic nitrate on 24-h ambulatory DBP is presented in Fig. 5, showing no significant effect 24 h ambulatory DBP (MD: −1.04 mmHg, 95% CI: −2.11 to 0.02, $P = 0.06$) and evidence of substantial heterogeneity ($I^2 = 78\%$, $P < 0.001$). Data were pooled from nine trials with a median dose was 434 mg nitrate per day and a median duration of 14 days. Individual removal of studies by Lara *et al.* [74] Bondonno *et al.* [59] or Shaltout *et al.* [66] changed the significance of the effect ($P < 0.05$) without altering the heterogeneity (Table S3, http://links.lww.com/HJH/B379). There was no evidence of a linear dose response (Figure S4d, http://links.lww.com/HJH/B379). For 24-h SBP and 24-h DBP, subgroup analyses were not conducted because fewer than 10 studies were available. Nonlinear dose–response analyses could not be conducted due to insufficient number of studies for cubic spline procedure.

Central SBP and DBP

The effect of repeated administration of inorganic nitrate on central SBP is shown in Fig. 6, based on pooled evidence from two study comparisons. Trials reporting on central SBP had a median dose of 580 mg nitrate/day and a median duration of 28 days. Repeated administration of inorganic nitrate significantly lowered central SBP (MD: −1.56 mmHg, 95% CI: −2.62 to −0.50, $P < 0.01$) without evidence of substantial heterogeneity ($I^2 = 30\%$, $P = 0.24$). There was no evidence of a linear dose response (Figure S4e, http://links.lww.com/HJH/B379). The effect of repeated administration of inorganic nitrate on central DBP is presented in Fig. 7, showing a significant lowering effect on central DBP (MD: −1.99 mmHg, 95% CI: −2.37 to −1.60, $P < 0.001$) without evidence of heterogeneity ($I^2 = 0\%$, $P = 0.60$). The two studies on central DBP had a median dose of 498 mg nitrate per day and a median duration of 18 days. Linear dose response on central DBP could not be conducted due to insufficient number of observations. For central SBP and DBP, removal of each study in sensitivity analysis did not change the heterogeneity or the significance of the effects. Subgroup analyses were not conducted because fewer than 10 studies were available. Nonlinear dose–response analyses could not be conducted due to insufficient number of observations for cubic spline procedure.

Arterial stiffness

The effect of repeated administration of inorganic nitrate on AIx is presented in Fig. 8, showing no significant effect on AIx (MD: −2.23%, 95% CI: −4.92 to 0.47, $P = 0.11$) and evidence of substantial heterogeneity ($I^2 = 90\%$, $P < 0.001$). Data were pooled from seven trials with a median dose of 299 mg nitrate per day and a median treatment duration of 18 days. There was no evidence of a linear dose response (Figure S4f, http://links.lww.com/HJH/B379). The effect of

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

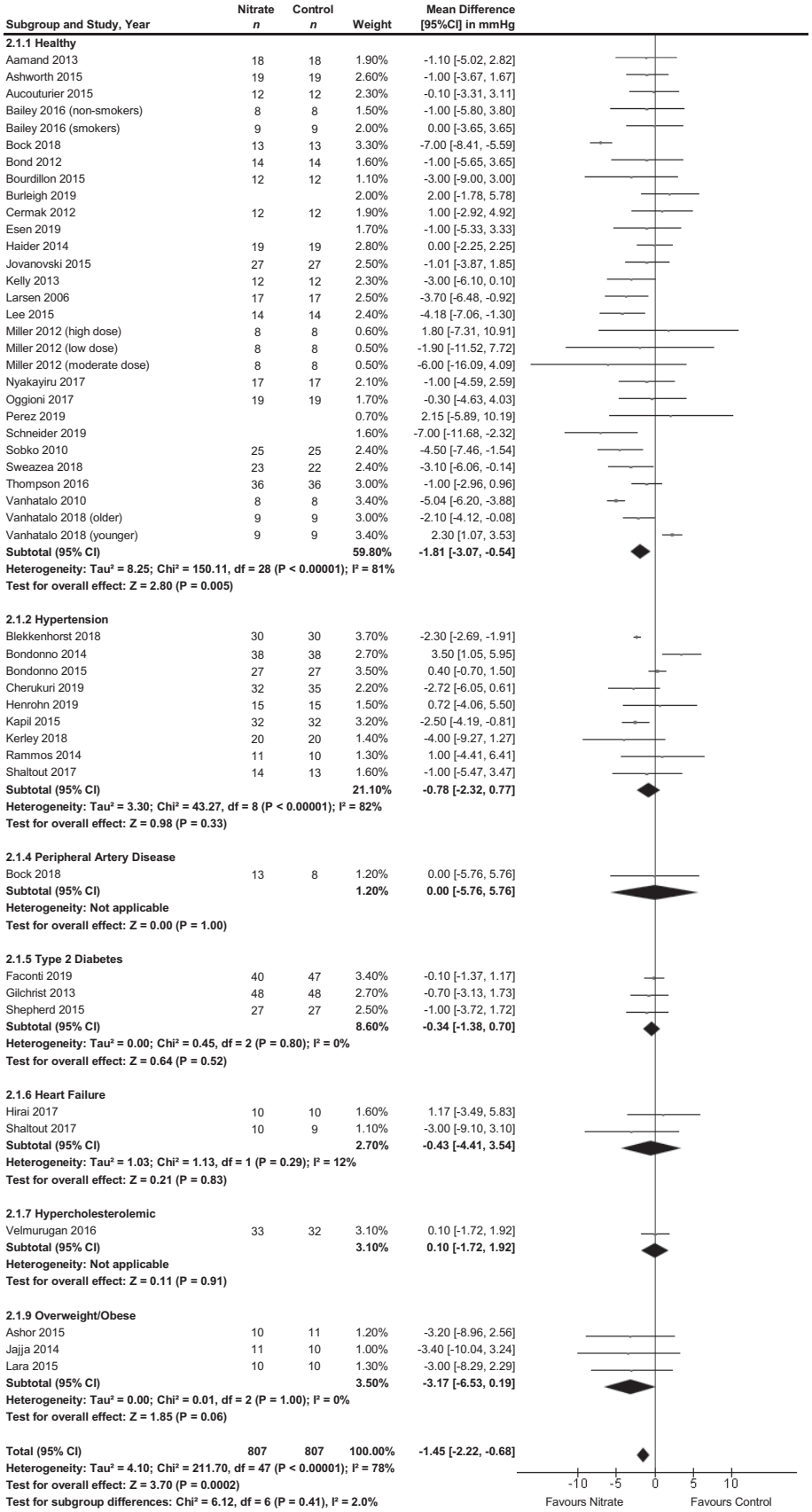


FIGURE 3 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on diastolic blood pressure. n = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

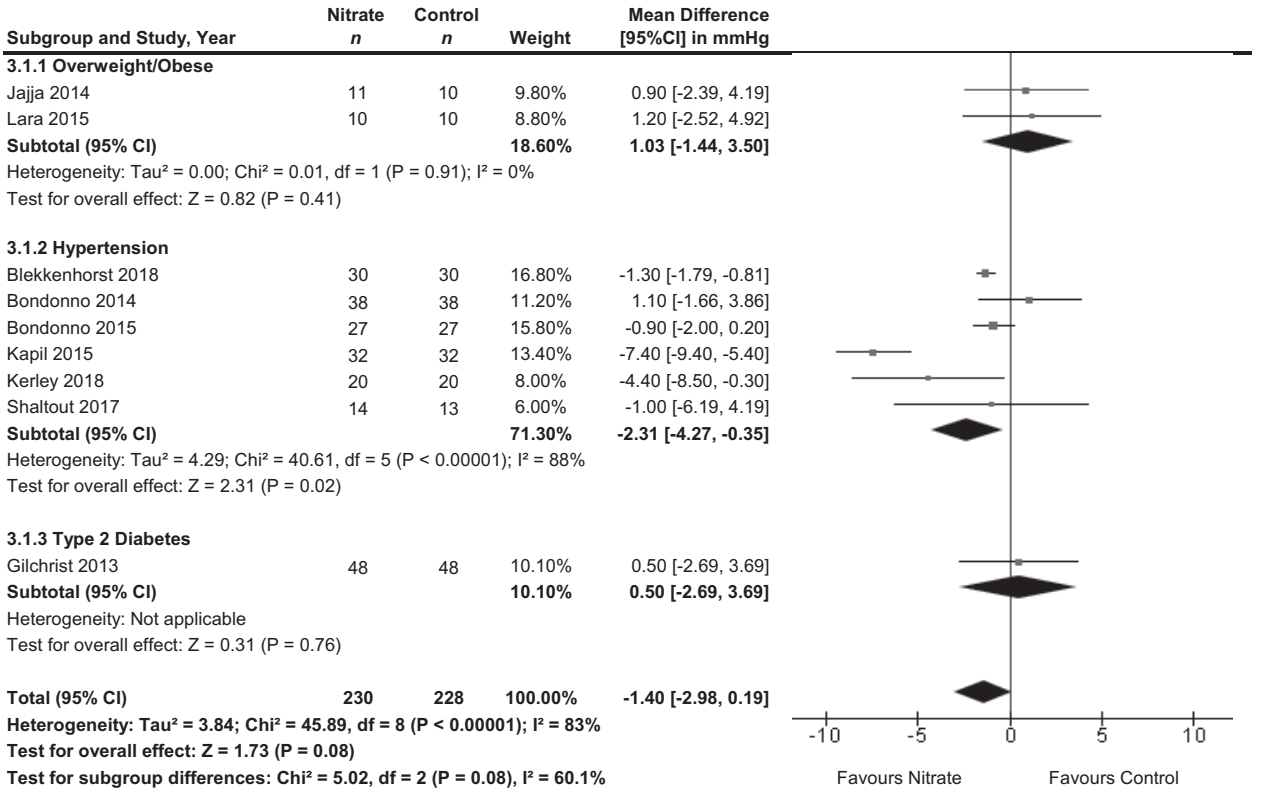


FIGURE 4 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on 24-h ambulatory SBP. *n* = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

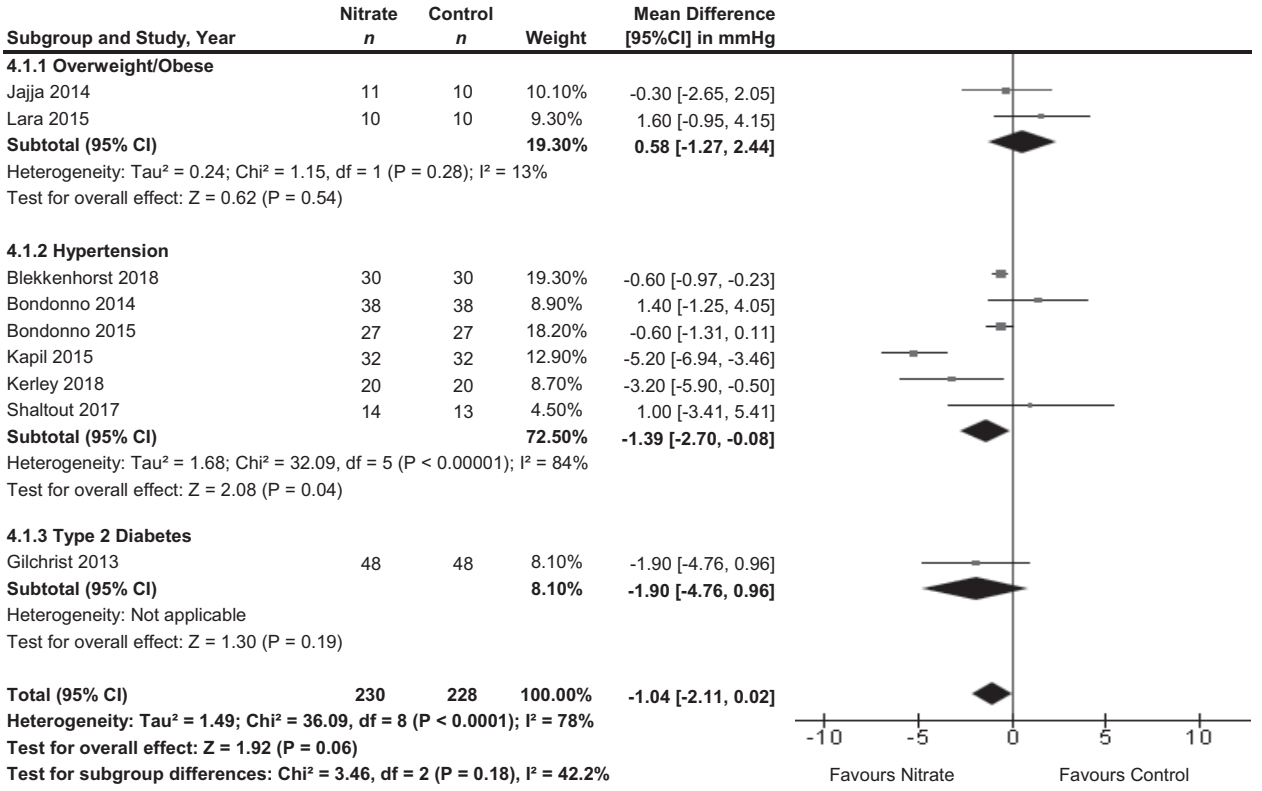


FIGURE 5 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on 24-h ambulatory DBP. *n* = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

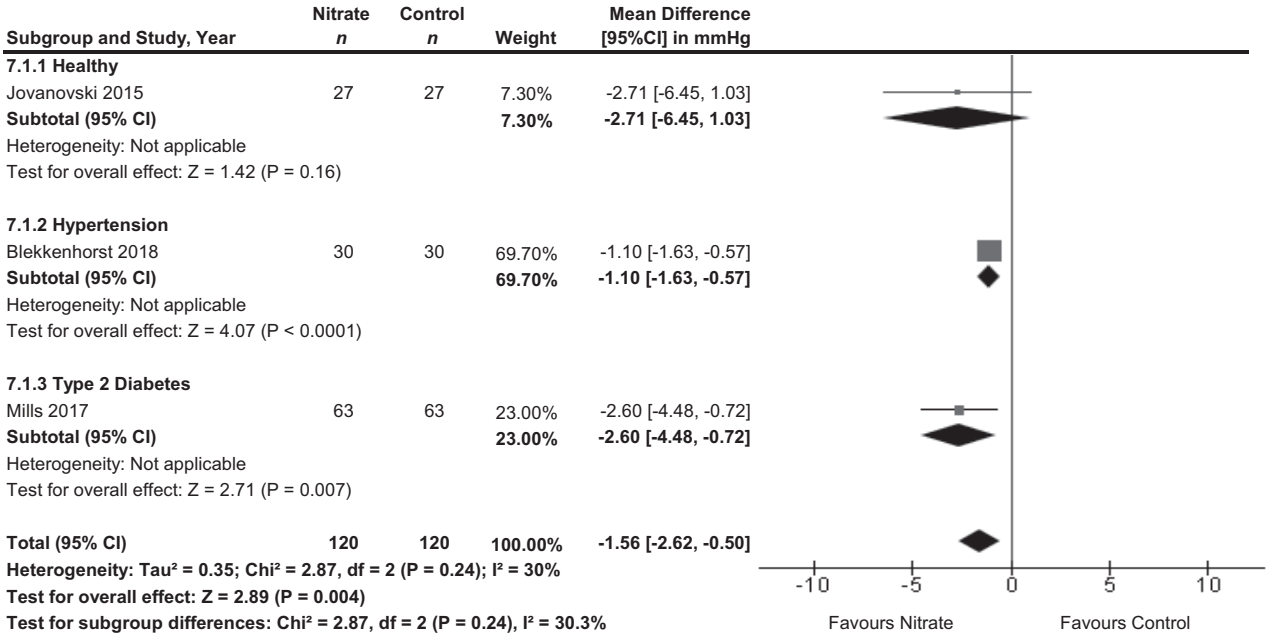


FIGURE 6 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on central SBP. n = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

repeated administration of inorganic nitrate on PWV is presented in Fig. 9, showing no significant effect on PWV (MD: −0.24 m/s, 95% CI: −0.50 to 0.01, $P = 0.06$) and evidence of substantial heterogeneity ($I^2 = 71\%$, $P < 0.001$). Data were pooled from eight trials with a median dose of 490 mg nitrate per day and a median duration of 19 days. There was no evidence of a linear dose response (Figure S4 g, http://links.lww.com/HJH/B379). For both AIx and PWV, systematic removal of individual studies did not change the heterogeneity or the significance of the effects (*data not shown*). Subgroup analyses were not conducted because fewer than 10 studies were available. Nonlinear dose–response analyses could not be conducted due to insufficient number of observations for cubic spline procedure.

Publication bias

Publication bias for the effect of repeated administration of inorganic nitrate on SBP and DBP is shown in Figure S6, http://links.lww.com/HJH/B379. There was no evidence of publication bias for either outcomes as assessed by visual inspection of the funnel plots and Egger and Begg tests ($P > 0.05$). Publication bias was not conducted for other outcomes because fewer than 10 studies were available for analyses.

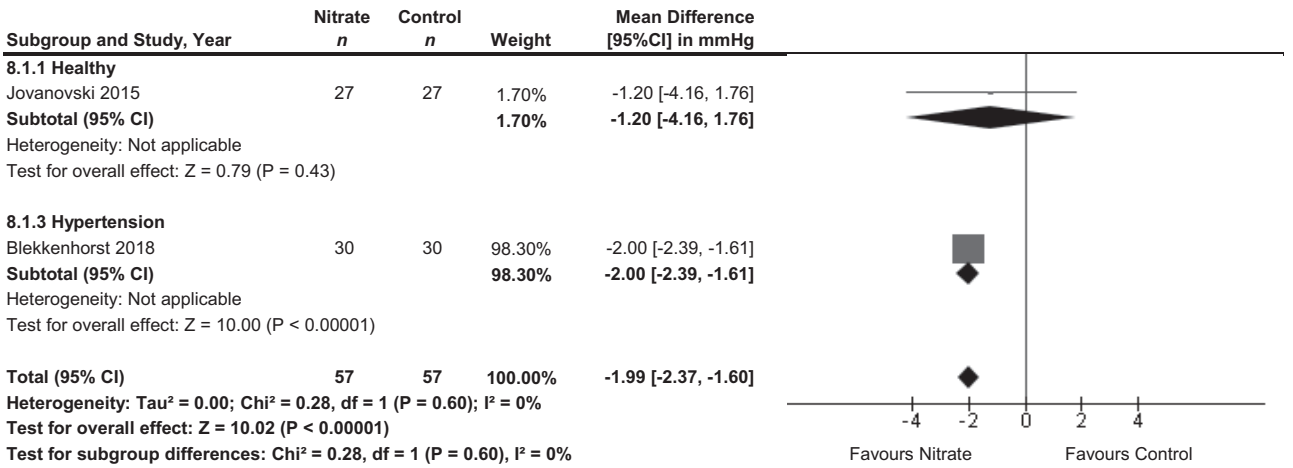


FIGURE 7 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on central DBP. n = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

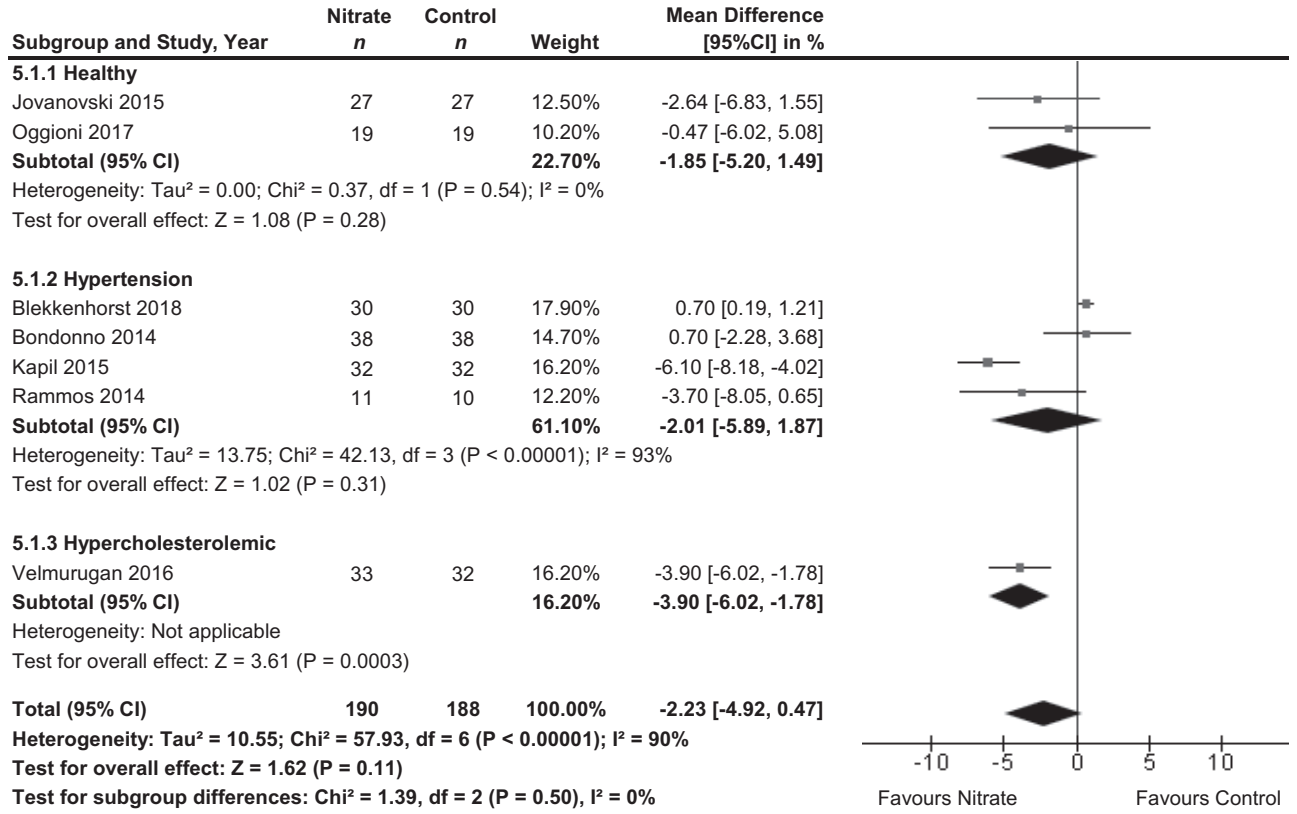


FIGURE 8 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on augmentation index. *n* = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

GRADE assessment

The GRADE assessment for the effect of repeated administration of inorganic nitrate on BP and arterial stiffness outcomes is presented in Table S4, http://links.lww.com/HJH/B379. The certainty of the evidence was graded as moderate for central BP, PWV and low for SBP, DBP, 24-h ambulatory BP and AIx. Evidence for SBP, DBP, 24-h ambulatory BP, AIx and PWV were downgraded for inconsistency, based on the presence of substantial heterogeneity ($I^2 > 50\%$, $P < 0.1$) that remained largely unexplained. Evidence for central DBP was downgraded for indirectness due to the limited generalizability of the results from the small number of studies that were available. Outcomes SBP, DBP, 24-h ambulatory BP, central SBP and AIx were also downgraded for imprecision due to a wide 95% CI that overlaps with MID for the respective outcome.

DISCUSSION

The present systematic review and meta-analysis of 47 trials including 1101 participants assessed repeated administration (≥3 days) of inorganic nitrate at doses ranging from 150 to 1000 mg, over a treatment duration range of 7–168 days. Our results showed an overall modest beneficial lowering effect of inorganic nitrate on BP. Although there was no evidence of significant subgroup differences by health status, the pooled estimate appears to be driven by effects in the healthy and hypertensive subgroups. Considering the small number of studies available in individuals with additional CVD risk factors, the analysis was likely underpowered to detect significant differences and more studies are required to draw reliable conclusions in these patient groups.

Repeated administration of nitrate showed a similar lowering effect in SBP than the lowering effect of DASH interventions [9,79]. A considerable amount of the DASH effect could be attributed to the ingestion of nitrate-rich vegetables, considering that DASH dietary patterns could provide nitrate levels ranging from 174 to 1222 mg/day depending on the vegetables selected [80]. Effect of nitrate is also comparable to other recommended dietary BP-lowering strategies, including reducing sodium intake [10], following the Mediterranean diet [81] and increasing potassium intake [82]. However, on the basis of available evidence, the lowering effect on peripheral BP does not seem to extend to more progressed atherosclerotic disease phenotypes, as observed in our analysis, including T2DM, hypercholesterolemia, heart failure and peripheral artery disease in which endothelial dysfunction and diminished nitric oxide bioactivity are predominant features. Despite evidence for elevated levels of plasma nitrite concentrations and thus elevated nitric oxide stores [67,71–73], lack of BP-lowering effect may be related to the enhanced nitric oxide inactivation by reactive oxygen species and other

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

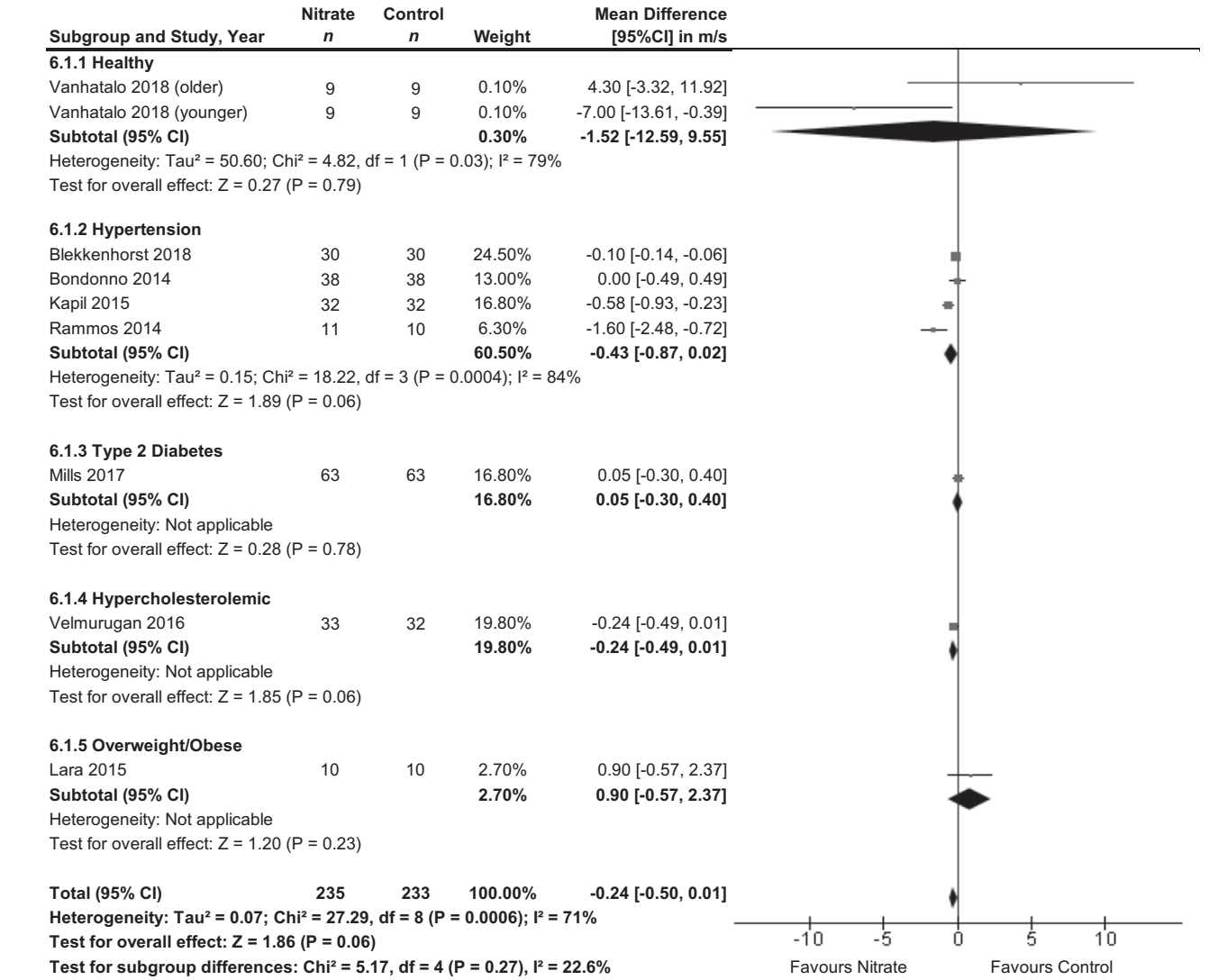


FIGURE 9 Forest plot of randomized controlled trials investigating repeated administration of inorganic nitrate on pulse wave velocity. n = number of participants in each treatment group. Individual study effects are represented by the red boxes and 95% confidence intervals (CIs). Pooled effect estimates for each subgroup and overall effect are represented by the black diamonds.

mechanisms typical of a pro-oxidant environment [83–87]. Absence of a BP-lowering effect may also possibly indicate a lack of further additive effect of nitrate in combination with existing antihypertensive medications for participants in these trials [66,69–73]. Interestingly, dietary nitrate was found to lower central BP independently of brachial BP in a large study conducted in participants with T2DM [78], suggesting possible selectivity for large conduit arteries over resistance arterioles. However, this trial implemented a 2×2 factorial design in which nitrate-rich beetroot juice was compared with placebo juice on top of spironolactone or doxazosin. There exists the possibility of additive effects from the combined therapy of nitrate and the diuretic, spironolactone. The clinical application of nitrate in different pathophysiological conditions is not yet well understood and will be of interest to explore further. In contrast to the large number of studies that reported on BP outcomes, results for the other vascular outcomes should be interpreted with caution given the smaller number of studies.

In exploring sources of heterogeneity, sensitivity analysis did not reveal any particular study to have an influence on the overall estimate of peripheral and central BP, or arterial stiffness. In 24-h BP measures, however, removal of studies by Lara *et al.* [74], Bondonno *et al.* [59] and Shaltout *et al.* [66] (DBP only) altered the significance of the effect, changing it from nonsignificant to significant. Furthermore, removal of the study by Kapil *et al.* [64] explained the heterogeneity ($I^2 = 27\%$, $P = 0.22$). A comparatively higher baseline 24-h SBP (148.2 ± 10.0 mmHg) together with the relatively larger sample size ($n = 68$) in this trial likely yielded the observed larger and more precise magnitude of effect. Given the influence of these single studies on the significance of the results, more studies may be needed to improve our confidence in the effect estimate.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

In subgroup analysis, dose (mg/day) was observed to be an effect modifier of SBP and DBP, wherein a greater lowering effect was observed in doses below approximately 450 mg/day compared with higher doses, and a linear dose response was detected for DBP, again favouring lower doses. The relevance of these observations is unclear and raises the question of a J-shaped relationship between dose and effect on BP, and the potential crosstalk between the classical endothelial NO synthase (eNOS)-dependent pathway and the alternative pathway for nitric oxide generation. Repeated exposure to high doses of nitrate supplying the alternative pathway may result in the downregulation of eNOS and a subsequent rise in BP [88]. This paradoxical effect on BP from higher doses may be more prominent in younger healthy groups and less so in elderly or high CVD risk groups in which eNOS-dependent NO production may already be diminished. Our meta-analysis is comprised largely of the healthy population; thus, our subgroup analysis could possibly be reflecting this phenomenon. It may be worthwhile to consider that lower doses, which can be achieved daily through the diet, may be sufficient to elicit a therapeutic effect and may be a practical option. Selecting nitrate-rich vegetables, such as green leafy vegetables and beetroot that can provide up to 120 mg nitrate per serving (70 g) [89], may serve to optimize benefits on BP. Four servings of nitrate-rich vegetables may easily meet targets up to 450 mg per day and coincides with healthy eating recommendations to consume five servings of vegetables daily [90].

Furthermore, subgroup analysis showed the form of nitrate administration to be an effect modifier, with nitrate ingested through vegetable powder showing a larger lowering effect than beetroot juice, nitrate salts and other vegetables. Dehydration of vegetables has been shown to limit growth of microorganisms and preserve stability of nutrients [91], possibly limiting the reduction of nitrate to nitrite by bacterial nitrate reductases, and preserving vitamin C content, which facilitates nitrite reduction to nitric oxide in circulation [92,93]. Differences in nutrient stability between various forms of vegetables could exist if fresh vegetables and juice were not stored properly or stored for prolonged periods before ingestion [94,95]. The majority of the trials did not report on the timing of the provision of vegetable interventions throughout the study duration and whether proper storage instructions were given. Considering only two comparisons were available for vegetable powder, more studies are required to better analyse the effect of different delivery methods.

In the context of previous reviews, our results are consistent with the latest meta-analysis demonstrating a beneficial lowering effect from inorganic nitrate interventions in SBP and DBP [96]. By excluding acute trials and only pooling data for at least 3 days of inorganic nitrate administration, our pooled estimate in healthy participants continue to show a similar lowering of at least 4 mmHg/2 mmHg in SBP/DBP with the inclusion of 21 new studies in healthy individuals (contributing 65% of studies to this subgroup). To our knowledge, our meta-analysis is the first to pool data from individuals with elevated CVD risk, providing the addition of 34 new studies (contributing 65% of studies to the overall analysis).

The mechanisms underlying the lowering effect of inorganic nitrate on peripheral BP have been elucidated given the improved understanding of nitric oxide formation in the alternative pathway independent of eNOS [97]. In this pathway, ingested nitrate is absorbed with nearly 100% bioavailability, after which an estimated nearly 25% of ingested nitrate is secreted into saliva [98,99]. A critical step in this pathway occurs at the oral cavity site, where a proportion of the secreted nitrate (~20%) is reduced to nitrite on surface of the tongue by the action of bacterial reductases [99–102]. Upon swallowing, bio-converted nitrite enters the gastrointestinal tract and reaches peak plasma concentrations at nearly 2.5 h following a nitrate load [98]. Circulating nitrite is sequentially reduced to nitric oxide, a systemic process that can be mediated by a variety of endogenous nitrate reductases [103] including deoxyhemogoblin and ascorbic acid [103–105]. This final step in its bio-activation to nitric oxide is pH and oxygen pressure dependent, thus the reaction is greatly enhanced when oxygen levels are reduced, such as in ischemic tissue [106,107]. The characterization of the nitrate-nitrite-nitric oxide pathway has reshaped the view of diet-derived nitrate as a potential candidate for restoring nitric oxide signalling, and thus a mediator of BP regulation and haemodynamics. Although nitrite-derived nitric oxide has been shown to be a potent vasodilator of resistance arterioles [103,108], further corroborated by our data showing consistent lowering effects in peripheral BP, the effect on the large elastic artery and consequently aortic stiffness is less well understood as the trials to date are of insufficient duration [109]. Nonetheless, from our findings, nitrate appears to also improve aortic BP, findings that were paralleled by a near-significant improvement in PWV, suggesting potential for modification of aortic stiffness over time.

Although there are concerns regarding unsafe levels of nitrate and nitrite in the food and water supply, consensus statements from regulatory guidelines indicate that it may not be appropriate to apply the adequate daily intake (ADI) derived for meats/drinking water to vegetable matrices [110]. Vegetables are typically a rich source of nitrate, at which a proportion (~5%) of ingested nitrate is eventually converted to nitrite [102]. Ingested nitrite has been observed to have positive associations with gastrointestinal cancer risk in certain case–control studies; however, most pronounced effects were seen in instances of very high levels of nitrite intake with low vitamin C [111]. Although nitrite ingested with amines or amides from cured meats may result in endogenous nitrosation and thus carcinogenic compounds, vegetables provide a rich source of vitamin C among other antioxidants that inhibit the formation of n-nitroso compounds by favouring conversion of nitrite to nitric oxide [112]. With regards to nitrate ingestion *per se*, epidemiological studies have not provided convincing evidence for increased risk of carcinogenicity from nitrate ingested through food or water [111,113].

This systematic review and meta-analysis has several strengths. First, our search and selection process represented an unbiased and reproducible methodology that allowed for a comprehensive evaluation of the effect of inorganic nitrate on BP. Second, we imposed eligibility criteria to pool evidence only from repeated administration

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

of inorganic nitrate. This allowed for an evaluation of potential physiological adaptations to the intervention not otherwise captured by acute feeding trials. Particularly, questions of tolerance and paradoxical effects on BP from high doses were better assessed. Third, our meta-analysis was the first to assess the quality of the evidence for the effect of nitrate on BP using GRADE, which represents a transparent and systematic framework for presenting summaries of evidence and is increasingly utilized in the development of guidelines.

Limitations of our analyses should also be recognized. First, our meta-analysis included several nitrate-rich vegetable interventions that were compared with control interventions providing low-nitrate vegetables or no vegetables. In these comparisons, the effects of other nutrients on BP cannot entirely be isolated from the effects of nitrate. The inclusion of these trials in our analysis remains useful for the assessment of inorganic nitrate and increases the generalizability of results considering vegetables represent the most common and widely accessible form of inorganic nitrate consumption. Interestingly, in our subgroup analysis, we observe no differences between the effect of nitrate-rich vegetable interventions and interventions that isolated effects of nitrate (beetroot juice, vegetable powder and nitrate salts that can be compared with nitrate-depleted equivalents). Second, there was evidence of substantial and unexplained heterogeneity for SBP, DBP, 24-h DBP, AIx and PWV. For SBP and DBP, heterogeneity was not explained by the effects of different subgroups, including population subgroups. Furthermore, sensitivity analysis did not explain heterogeneity and thus we downgraded the quality of the evidence for these outcomes. Although substantial heterogeneity was present in 24-h SBP, heterogeneity was explained in sensitivity analysis with the removal of the study by Kapil *et al.* [64], and therefore we did not downgrade for inconsistency. A third limitation was the presence of imprecise estimates for DBP, 24-h BP, central SBP and AIx, where the quality of the evidence was downgraded due to 95% CIs that crossed the MID for each outcome. A possible reason for the observed imprecision is the small number of comparisons available. Conversely, the estimate for SBP remained precise. Lastly, the quality of evidence for central DBP was downgraded for indirectness due to the small number of studies available ($n = 57$), therefore reducing the generalizability of the results. Weighing the strengths and limitations in our GRADE assessment, the certainty of the evidence was graded as moderate for central BP, and PWV; and low for SBP, DBP, 24-h BP and AIx.

In light of our findings demonstrating clinically relevant benefits of inorganic nitrate on BP and our certainty in the evidence graded as low to moderate, more long-term RCTs with larger sample sizes are required in both healthy and elevated CVD risk groups. Although future studies should consider the dose–response findings in our analysis, the dose-effects still remain unclear, particularly in patient groups for which data are currently limited. On the basis of our findings, selecting a diet providing modest doses of inorganic nitrate may be relevant as a preventive front-line dietary approach for healthy and hypertensive individuals. Nitrate-concentrated supplementation derived from whole vegetables could be adopted as a strategy on top of habitual diets. Alternatively, recognizing nitrate-rich vegetables as a key component of heart-healthy dietary patterns may further increase the efficacy of these recommendations. Ultimately, current hypertension guidelines are broad with respect to vegetable types, as they do not recommend which particular vegetables may better target high BP [6,8]. Going forward to establish the long-term benefits of nitrate-rich vegetable diets may have significant implications for hypertension management.

In conclusion, the results from our systematic review and meta-analysis show repeated administration ($\geq$3 days) of inorganic nitrate lower peripheral and central BP, without affecting 24-h BP and arterial stiffness measures. On the basis of available evidence, dietary intervention with modest doses of inorganic nitrate from vegetables may be beneficial for healthy and hypertensive individuals. Given the low to moderate certainty in the evidence, more trials are needed in both healthy individuals and individuals with additional CVD risk factors.

ACKNOWLEDGEMENTS

Aspects of this work were presented at the Banting and Best Diabetes Centre Annual Scientific Day, Toronto, Canada, 10 May 2019, and at the 37th International Symposium on Diabetes & Nutrition, Kerkrade, The Netherlands, 12–15 June 2019.

D.L. was supported by the Ontario Graduate Scholarship, Banting and Best Diabetes Centre Novo-Nordisk Graduate Studentship, and Loblaws Food as Medicine Graduate Award. J.L.S. was supported by a Diabetes Canada Clinician Scientist Award.

Conflicts of interest

D.L., E.J., A.K., S.B.M., D.M. have no conflicts to declare. S.K.N. was an employee at GI Labs (now INQUIS Clinical Research, Inc.), a contract research organisation from 2014 to 2016, and is currently employed part-time by the Diabetes Heart Research Centre coordinating trials sponsored by Novo Nordisk, Sanofi, AstraZeneca, Eli Lilly and Boehringer Ingelheim. A.Z. is a part-time research associate at INQUIS Clinical Research Ltd, a contract research organization. T.A.K. has received research support from the Canadian Institutes of Health Research (CIHR) and an unrestricted travel donation from Bee Maid Honey Ltd. He was an invited speaker at a Calorie Control Council annual general meeting for which he received an honorarium. V.V. received research support from CIHR for a clinical trial project investigating dietary nitrate. He was a consultant for and received conference travel grants from Dicofarm (Rome, Italy) and Kellogg Co (Battle Creek, USA). He previously held an American (No. 7,326,404 B2) and Canadian (No. 2,410,556) patent for use of viscous fibre blend in diabetes, IGT/metabolic syndrome and cholesterol lowering; was a consultant for and received conference travel grant from InovoBiologic Inc (Calgary, Alberta, Canada) the producer of the viscous Fibre blend, PGXTM that is developed based on his work on viscous fibre blend. He was Vice President and partial owner of Glycemic Index Laboratories, Inc. (Toronto, Ontario, Canada) from 2004 to 2016.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

A.L.J. was a consultant for InovoBiologic, Inc and is Director of Research and partial owner of INQUIS Clinical Research (Toronto, Ontario, Canada). J.L.S. has received research support from the Canadian Foundation for Innovation, Ontario Research Fund, Province of Ontario Ministry of Research and Innovation and Science, CIHR, Diabetes Canada, PSI Foundation, Banting and Best Diabetes Centre (BBDC), American Society for Nutrition (ASN), INC International Nut and Dried Fruit Council Foundation, National Dried Fruit Trade Association, The Tate and Lyle Nutritional Research Fund at the University of Toronto, The Glycemic Control and Cardiovascular Disease in Type 2 Diabetes Fund at the University of Toronto (a fund established by the Alberta Pulse Growers) and the Nutrition Trialists Fund at the University of Toronto (a fund established by an inaugural donation from the Calorie Control Council). He has received in-kind food donations to support a RCT from the Almond Board of California, California Walnut Commission, American Peanut Council, Barilla, Unilever, Unico/Primo, Loblaw Companies, Quaker, Kellogg Canada and WhiteWave Foods. He has received travel support, speaker fees and/or honoraria from Diabetes Canada, Dairy Farmers of Canada, FoodMinds LLC, International Sweeteners Association, Nestlé, Pulse Canada, Canadian Society for Endocrinology and Metabolism (CSEM), GI Foundation, Abbott, Biofortis, ASN, Northern Ontario School of Medicine, INC Nutrition Research & Education Foundation, European Food Safety Authority (EFSA), Comité Européen des Fabricants de Sucre (CEFS) and Physicians Committee for Responsible Medicine. He has or has had ad hoc consulting arrangements with Perkins Coie LLP, Tate & Lyle, and Wirtschaftliche Vereinigung Zucker e.V. He is a member of the European Fruit Juice Association Scientific Expert Panel and Soy Nutrition Institute (SNI) Scientific Advisory Committee. He is on the Clinical Practice Guidelines Expert Committees of Diabetes Canada, European Association for the study of Diabetes (EASD), Canadian Cardiovascular Society (CCS) and Obesity Canada. He serves or has served as an unpaid scientific advisor for the Food, Nutrition, and Safety Program (FNSP) and the Technical Committee on Carbohydrates of the International Life Science Institute (ILSI) North America. He is a member of the International Carbohydrate Quality Consortium (ICQC), Executive Board Member of the Diabetes and Nutrition Study Group (DNSG) of the EASD and Director of the Toronto 3D Knowledge Synthesis and Clinical Trials foundation. His wife is an employee of AB InBev.

REFERENCES

1. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388:1659–1724.
2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet (London, England)* 2005; 365:217–223.
3. Poulter NR, Prabhakaran D, Caulfield M. Hypertension. *Lancet (London, England)* 2015; 386:801–812.
4. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, *et al.* Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation* 2017; 135:e146–e603.
5. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, *et al.* 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014; 63:2960–2984.
6. Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018; 71:1269–1324.
7. Leung AA, Daskalopoulou SS, Dasgupta K, McBrien K, Butalia S, Zarnke KB, *et al.* Hypertension Canada's 2017 guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults. *Can J Cardiol* 2017; 33:557–576.
8. Nerenberg KA, Zarnke KB, Leung AA, Dasgupta K, Butalia S, McBrien K, *et al.* Hypertension Canada's 2018 guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults and children. *Can J Cardiol* 2018; 34:506–525.
9. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, *et al.* A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med* 1997; 336:1117–1124.
10. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, *et al.* Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (dash) diet. *N Engl J Med* 2001; 344:3–10.
11. Lin PH, Allen JD, Li YJ, Yu M, Lien LF, Svetkey LP. Blood pressure-lowering mechanisms of the DASH Dietary Pattern. *J Nutr Metab* 2012; 2012:472396.
12. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Disc* 2008; 7:156–167.
13. Lundberg JO, Carlstrom M, Larsen FJ, Weitzberg E. Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovasc Res* 2011; 89:525–532.
14. Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, *et al.* Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 2008; 51:784–790.
15. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med* 2006; 355:2792–2793.
16. Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, *et al.* Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. *Hypertension* 2010; 56:274–281.
17. Elkayam U. Tolerance to organic nitrates: evidence, mechanisms, clinical relevance, and strategies for prevention. *Ann Intern Med* 1991; 114:667–677.
18. Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol* 2004; 15:1983–1992.
19. Higgins J, Green S. Cochrane handbook for systematic reviews of interventions. Version 5.1.0. March 2011.
20. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; 6:e1000097.
21. BMJ best practice: study design search filters. https://bestpractice.bmj.com/info/toolkit/learn-ebm/study-design-search-filters/.
22. Cardiovascular diseases (CVDs). World Health Organization; 2017. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
23. Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. *Int J Epidemiol* 2002; 31:140–149.
24. Follmann D, Elliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. *J Clin Epidemiol* 1992; 45:769–773.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327:557–560.
26. Sterne JA, Gavaghan D, Egger M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol* 2000; 53:1119–1129.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

27. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; 56:455–463.
28. Schünemann H, Brożek J, Guyatt G, Oxman A. Grade handbook. October 2013.
29. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; 360:1903–1913.
30. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; 55:1318–1327.
31. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010; 31:1865–1871.
32. Zhong Q, Hu MJ, Cui YJ, Liang L, Zhou MM, Yang YW, *et al.* Carotid-femoral pulse wave velocity in the prediction of cardiovascular events and mortality: an updated systematic review and meta-analysis. *Angiology* 2018; 69:617–629.
33. Aamand R, Dalsgaard T, Ho YC, Moller A, Roepstorff A, Lund TE. A NO way to BOLD? Dietary nitrate alters the hemodynamic response to visual stimulation. *NeuroImage* 2013; 83:397–407.
34. Ashworth A, Mitchell K, Blackwell JR, Vanhatalo A, Jones AM. High-nitrate vegetable diet increases plasma nitrate and nitrite concentrations and reduces blood pressure in healthy women. *Public Health Nutr* 2015; 18:2669–2678.
35. Aucouturier J, Boissière J, Pawlak-Chaouch M, Cuvelier G, Gamelin FX. Effect of dietary nitrate supplementation on tolerance to supramaximal intensity intermittent exercise. *Nitric Oxide* 2015; 49: 16–25.
36. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, *et al.* Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol (1985)* 2009; 107:1144–1155.
37. Bailey SJB, Wylie JR, Holland LJ, Winyard T, Jones PG. Improvement in blood pressure after short-term inorganic nitrate supplementation is attenuated in cigarette smokers compared to nonsmoking controls. *Nitric Oxide Biol Chem* 2016; 61:29–37.
38. Bock JMU, Schneider K, Hughes AC, Limberg WE, Bryan JK, Casey NS. Inorganic nitrate supplementation attenuates peripheral chemoreflex sensitivity but does not improve cardiovagal baroreflex sensitivity in older adults. *Am J Physiol Heart Circ Physiol* 2018; 314:H45–H51.
39. Bond H, Morton L, Braakhuis AJ. Dietary nitrate supplementation improves rowing performance in well trained rowers. *Int J Sport Nutr Exerc Metab* 2012; 22:251–256.
40. Bourdillon N, Fan JL, Uva B, Müller H, Meyer P, Kayser B. Effect of oral nitrate supplementation on pulmonary hemodynamics during exercise and time trial performance in normoxia and hypoxia: a randomized controlled trial. *Front Physiol* 2015; 6: 288.
41. Burleigh ML, Muggeridge L, Monaghan DJ, Sculthorpe C, Butcher N, Henriquez J, *et al.* Dietary nitrate supplementation alters the oral microbiome but does not improve the vascular responses to an acute nitrate dose. *Nitric Oxide* 2019; 89:54–63.
42. Cermak NM, Gibala MJ, van Loon LJ. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab* 2012; 22:64–71.
43. Esen O, Nicholas C, Morris M, Bailey SJ. No effect of beetroot juice supplementation on 100-m and 200-m swimming performance in moderately trained swimmers. *Int J Sports Physiol Perform* 2019; 14:706–710.
44. Haider G, Folland JP. Nitrate supplementation enhances the contractile properties of human skeletal muscle. *Med Sci Sports Exerc* 2014; 46:2234–2243.
45. Jovanovski E, Bosco L, Khan K, Au-Yeung F, Ho H, Zurbau A, *et al.* Effect of spinach, a high dietary nitrate source, on arterial stiffness and related hemodynamic measures: a randomized, controlled trial in healthy adults. *Clin Nutr Res* 2015; 4:160–167.
46. Mortality, summary list of causes 2009. Statistics Canada; 2012 Statistics Canada.
47. Kelly JF, Vanhatalo J, Blackwell A, French JR, Bailey O, Gilchrist SJ, *et al.* Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol Regul Integr Comp Physiol* 2013; 304:R73–83.
48. Lansley KEW, Fulford PG, Vanhatalo J, Bailey A, Blackwell SJ, DiMenna JR, *et al.* Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. *J Appl Physiol* 2011; 110:591–600.
49. Lee JSS, Jung CL, Nho E, Kim H, Chang JK, Choi MJ. Effects of chronic dietary nitrate supplementation on the hemodynamic response to dynamic exercise. *Am J Physiol Regul Integr Comp Physiol* 2015; 309:R459–R466.
50. Miller GDM, Dove AP, Beavers RW, Presley D, Helms T, Bechtold C, *et al.* Plasma nitrate and nitrite are increased by a high-nitrate supplement but not by high-nitrate foods in older adults. *Nutr Res* 2012; 32:160–168.
51. Nyakayiru JMJ, Pinckaers KL, Senden PJ, van Loon J, Verdijk LJ. No effect of acute and 6-day nitrate supplementation on VO_2 and time-trial performance in highly trained cyclists. *Int J Sport Nutr Exerc Metab* 2017; 27:11–17.
52. Oggioni CJ, Klonizakis DG, Ashor M, Ruddock AW, Ranchordas A, Williams M, *et al.* Dietary nitrate does not modify blood pressure and cardiac output at rest and during exercise in older adults: a randomised cross-over study. *Int J Food Sci Nutr* 2018; 69:74–83.
53. Perez JMD, Ryan JL, Riggs GA. The effects of beetroot juice on VO_2 max and blood pressure during submaximal exercise. *Int* 2019; 12:332–342.
54. Schneider AC, Hughes WE, Ueda K, Bock JM, Casey DP. Reduced blood pressure responsiveness to skeletal muscle metaboreflex activation in older adults following inorganic nitrate supplementation. *Nitric Oxide* 2018; 78:81–88.
55. Sweazea KL, Johnston CS, Miller B, Gumpricht E. Nitrate-rich fruit and vegetable supplement reduces blood pressure in normotensive healthy young males without significantly altering flow-mediated vasodilation: a randomized, double-blinded, controlled trial. *J Nutr Metab* 2018; 2018:1729653.
56. Thompson CV, Jell A, Fulford H, Carter J, Nyman J, Bailey L, *et al.* Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance. *Nitric Oxide* 2016; 61:55–61.
57. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, *et al.* Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol Regul Integr Comp Physiol* 2010; 299:R1121–R1131.
58. Vanhatalo AB, L'Heureux JR, Williams JE, Smith DW, van der Giezen A, Winyard M, *et al.* Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. *Free Radic Biol Med* 2018; 124:21–30.
59. Bondonno CP, Liu AH, Croft KD, Ward NC, Yang X, Considine MJ, *et al.* Short-term effects of nitrate-rich green leafy vegetables on blood pressure and arterial stiffness in individuals with high-normal blood pressure. *Free Radic Biol Med* 2014; 77:353–362.
60. Blekkenhorst LC, Lewis JR, Prince RL, Devine A, Bondonno NP, Bondonno CP, *et al.* Nitrate-rich vegetables do not lower blood pressure in individuals with mildly elevated blood pressure: a 4-wk randomized controlled crossover trial. *Am J Clin Nutr* 2018; 107:894–908.
61. Bondonno CP, Liu AH, Croft KD, Ward NC, Shinde S, Moodley Y, *et al.* Absence of an effect of high nitrate intake from beetroot juice on blood pressure in treated hypertensive individuals: a randomized controlled trial. *Am J Clin Nutr* 2015; 102:368–375.
62. Cherukuri LS, Li C, Birudaraju D, Hamal D, Flores S, Roy F, *et al.* Acute and sustained effects of plant-based bioequivalent inorganic nitrate supplement tablets on blood pressure reduction in pre-hypertensives men and women. *J Am Coll Cardiol* 2019;1729.
63. Henrohn DB, Lundberg K, Granstam JO, Baron SO, Ingimarsdottir T, Hedenstrom IJ, *et al.* Effects of oral supplementation with nitrate-rich beetroot juice in patients with pulmonary arterial hypertension-results from BEET-PAH, an exploratory randomized, double-blind, placebo-controlled, crossover study. *J Card Fail* 2018; 24:640–653.
64. Kapil V, Khambata RS, Robertson A, Caulfield MJ, Ahluwalia A. Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension* 2015; 65:320–327.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

65. Rammos C, Hendgen-Cotta UB, Sobierajski J, Bernard A, Kelm M, Rassaf T. Dietary nitrate reverses vascular dysfunction in older adults with moderately increased cardiovascular risk. *J Am Coll Cardiol* 2014; 63:1584–1585.
66. Shaltout HA, Eggebeen J, Marsh AP, Brubaker PH, Laurienti PJ, Burdette JH, *et al.* Effects of supervised exercise and dietary nitrate in older adults with controlled hypertension and/or heart failure with preserved ejection fraction. *Nitric Oxide* 2017; 69:78–90.
67. Bock JM, Treichler DP, Norton SL, Ueda K, Hughes WE, Casey DP. Inorganic nitrate supplementation enhances functional capacity and lower-limb microvascular reactivity in patients with peripheral artery disease. *Nitric Oxide* 2018; 80:45–51.
68. Kerley CP, Dolan E, James PE, Cormican L. Dietary nitrate lowers ambulatory blood pressure in treated, uncontrolled hypertension: a 7-d, double-blind, randomised, placebo-controlled, cross-over trial. *Br J Nutr* 2018; 119:658–663.
69. Faconti L, Mills CE, Govoni V, Gu H, Morant S, Jiang B, *et al.* Cardiac effects of 6 months' dietary nitrate and spironolactone in patients with hypertension and with/at risk of type 2 diabetes, in the factorial design, double-blind, randomized controlled VaSera trial. *Br J Clin Pharmacol* 2019; 85:169–180.
70. Gilchrist M, Winyard PG, Aizawa K, Anning C, Shore A, Benjamin N. Effect of dietary nitrate on blood pressure, endothelial function, and insulin sensitivity in type 2 diabetes. *Free Radic Biol Med* 2013; 60:89–97.
71. Shepherd AI, Gilchrist M, Winyard PG, Jones AM, Hallmann E, Kazimierczak R, *et al.* Effects of dietary nitrate supplementation on the oxygen cost of exercise and walking performance in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled crossover trial. *Free Radic Biol Med* 2015; 86:200–208.
72. Hirai DM, Zelt JT, Jones JH, Castanhas LG, Bentley RF, Earle W, *et al.* Dietary nitrate supplementation and exercise tolerance in patients with heart failure with reduced ejection fraction. *Am J Physiol Regul Integr Comp Physiol* 2017; 312:R13–r22.
73. Velmurugan S, Gan JM, Rathod KS, Khambata RS, Ghosh SM, Hartley A, *et al.* Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. *Am J Clin Nutr* 2016; 103:25–38.
74. Lara J, Ogbonmwan I, Oggioni C, Zheng D, Qadir O, Ashor A, *et al.* Effects of handgrip exercise or inorganic nitrate supplementation on 24-h ambulatory blood pressure and peripheral arterial function in overweight and obese middle age and older adults: a pilot RCT. *Maturitas* 2015; 82:228–235.
75. Ashor AW, Jajja A, Sutyarjoko A, Brandt K, Qadir O, Lara J, *et al.* Effects of beetroot juice supplementation on microvascular blood flow in older overweight and obese subjects: a pilot randomised controlled study. *J Hum Hypertens* 2015; 29:511–513.
76. Jajja A, Sutyarjoko A, Lara J, Rennie K, Brandt K, Qadir O, *et al.* Beetroot supplementation lowers daily systolic blood pressure in older, overweight subjects. *Nutr Res* 2014; 34:868–875.
77. Sobko T, Marcus C, Govoni M, Kamiya S. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* 2010; 22:136–140.
78. Mills CE, Govoni V, Faconti L, Casagrande ML, Morant SV, Webb AJ, *et al.* Reducing arterial stiffness independently of blood pressure: the VaSera trial. *J Am Coll Cardiol* 2017; 70:1683–1684.
79. Siervo M, Lara J, Chowdhury S, Ashor A, Oggioni C, Mathers JC. Effects of the Dietary Approach to Stop Hypertension (DASH) diet on cardiovascular risk factors: a systematic review and meta-analysis. *Br J Nutr* 2015; 113:1–15.
80. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* 2009; 90:1–10.
81. Estruch R, Martinez-Gonz lez MA, Corella D, Salas-Salvad¢ J, Ruiz-Gutierrez V, Covas MI, *et al.* Effects of a Mediterranean-style diet on cardiovascular risk factors a randomized trial. *Ann Intern Med* 2006; 145:1–11.
82. Poorolajal J, Zeraati F, Soltanian AR, Sheikh V, Hooshmand E, Maleki A. Oral potassium supplementation for management of essential hypertension: a meta-analysis of randomized controlled trials. *PLoS One* 2017; 12:e0174967.
83. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997; 96:25–28.
84. Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG. Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest* 1990; 86:2109–2116.
85. Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, *et al.* Increased oxidative stress in patients with congestive heart failure. *J Am Coll Cardiol* 1998; 31:1352–1356.
86. Kojda G, Harrison D. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovasc Res* 1999; 43:562–571.
87. Ismaeel A, Brumberg RS, Kirk JS, Papoutsi E, Farmer PJ, Bohannon WT, *et al.* Oxidative stress and arterial dysfunction in peripheral artery disease. *Antioxidants (Basel)* 2018; 7:pii: E145.
88. Carlstrom M, Liu M, Yang T, Zollbrecht C, Huang L, Peleli M, *et al.* Cross-talk between nitrate-nitrite-NO and NO synthase pathways in control of vascular NO homeostasis. *Antioxid Redox Signal* 2015; 23:295–306.
89. Blekkenhorst LC, Prince RL, Ward NC, Croft KD, Lewis JR, Devine A, *et al.* Development of a reference database for assessing dietary nitrate in vegetables. *Mol Nutr Food Res* 2017; 61:1–13.
90. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, *et al.* Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006; 114:82–96.
91. Karam M, Petit J, Zimmer D, Djantou E, Scher J. Effects of drying and grinding in production of fruit and vegetable powders: a review. *J Food Eng* 2016; 188:32–49.
92. Weitzberg E, Lundberg JO. Nonenzymatic nitric oxide production in humans. *Nitric Oxide* 1998; 2:1–7.
93. Sibmooh N, Piknova B, Rizzatti F, Schechter AN. Oxidation of iron-nitrosyl-hemoglobin by dehydroascorbic acid releases nitric oxide to form nitrite in human erythrocytes. *Biochemistry* 2008; 47:2989–2996.
94. Chung JC, Chou SS, Hwang DF. Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. *Food Addit Contam* 2004; 21:317–322.
95. Galani JHY, Patel JS, Patel NJ, Talati JG. Storage of fruits and vegetables in refrigerator increases their phenolic acids but decreases the total phenolics, anthocyanins and vitamin C with subsequent loss of their antioxidant capacity. *Antioxidants (Basel)* 2017; 6:pii: E59.
96. Jackson JK, Patterson AJ, MacDonald-Wicks LK, Oldmeadow C, McEvoy MA. The role of inorganic nitrate and nitrite in cardiovascular disease risk factors: a systematic review and meta-analysis of human evidence. *Nutr Rev* 2018; 76:348–371.
97. Machha A, Schechter AN. Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. *Eur J Nutr* 2011; 50:293–303.
98. Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic Biol Med* 2004; 37:395–400.
99. Spiegelhalder B, Eisenbrand G, Preussmann R. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. *Food Cosmet Toxicol* 1976; 14:545–548.
100. Duncan C, Dougall H, Johnston P, Green S, Brogan R, Leifert C, *et al.* Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nat Med* 1995; 1:546–551.
101. Doel JJ, Benjamin N, Hector MP, Rogers M, Allaker RP. Evaluation of bacterial nitrate reduction in the human oral cavity. *Eur J Oral Sci* 2005; 113:14–19.
102. WHO. *Nitrate and nitrite in drinking-water. Background document for development of WHO guidelines for drinking-water quality.* Geneva: World Health Organization; 2011.
103. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, *et al.* Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* 2003; 9:1498–1505.
104. Modin A, Bjorne H, Herulf M, Alving K, Weitzberg E, Lundberg JO. Nitrite-derived nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol Scand* 2001; 171:9–16.
105. Carlsson S, Wiklund NP, Engstrand L, Weitzberg E, Lundberg JO. Effects of pH, nitrite, and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* 2001; 5:580–586.
106. Vitturi DA, Patel RP. Current perspectives and challenges in understanding the role of nitrite as an integral player in nitric oxide biology and therapy. *Free Radic Biol Med* 2011; 51:805–812.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

107. Zweier JL, Wang P, Samouilov A, Kuppusamy P. Enzyme-independent formation of nitric oxide in biological tissues. *Nature Med* 1995; 1:804–809.
108. Maher AR, Milsom AB, Gunaruwan P, Abozguia K, Ahmed I, Weaver RA, *et al*. Hypoxic modulation of exogenous nitrite-induced vasodilation in humans. *Circulation* 2008; 117:670–677.
109. Stewart AD, Millasseau SC, Kearney MT, Ritter JM, Chowienczyk PJ. Effects of inhibition of basal nitric oxide synthesis on carotid-femoral pulse wave velocity and augmentation index in humans. *Hypertension* 2003; 42:915–918.
110. *Nitrate (WHO Food Additives Series 35) [Internet].* WHO INCHEM; 1995
111. Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, Cogliano V. Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *Lancet Oncol* 2006; 7:628–629.
112. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. *IARC Monogr Eval Carcinog Risks Hum* 2010; 94:v–vii; 1–412.
113. Speijers GJA, van den Brandt PA, WHO food additives series: 50 nitrate (and potential endogenous formation of N-nitroso compounds) [Internet]. INCHEM. 2002.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.