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Dietary nitrate and blood pressure: evolution of a new nutrient?

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Abstract

Dietary nitrate is mainly obtained from vegetables, especially green leafy vegetables and beetroot. As a result of early research, dietary nitrate is currently viewed as a contaminant linked to increased risks of stomach cancer and methaemoglobinaemia. Consequently, nitrate levels are restricted in certain vegetables and in water supplies to ensure exposure levels remain below an acceptable daily intake of 3·7 mg/kg per d. The average nitrate intake in the UK is approximately 70 mg/d, although some population groups, such as vegetarians, may consume three times that amount. However, recent studies in the last decade suggest that dietary nitrate can significantly reduce systolic blood pressure via the nitrate–nitrite–NO pathway. A small, downward shift in systolic blood pressure across the population could significantly reduce the incidence of hypertension and mortality from CVD such as stroke. Interestingly, vegetarians tend to have lower levels of blood pressure than omnivores and epidemiological studies suggest that vegetarians have lower risks of CVD. Recent evidence is mainly focused on the acute effects of dietary nitrate supplementation and there is a lack of data looking at the chronic effects of high nitrate consumption in humans. Nevertheless, due to potential health benefits, some authors are recommending that nitrate should be considered as a nutrient necessary for health, rather than as a contaminant which needs to be restricted. This review will discuss the emerging role of dietary nitrate in the control of blood pressure and whether there is sufficient evidence to state that nitrate is a 'new' nutrient.

Key words: **Dietary nitrate: Nitrite: Nitric oxide: Green leafy vegetables: Blood pressure: Stroke: CVD**

Introduction

Inorganic nitrate has traditionally been considered as an inert contaminant in food and water. This anion is formed as part of the N cycle by the action of N-fixing bacteria in plants and therefore vegetables are the main source of nitrate in our diet. However, the opinion of this natural compound has substantially changed over the last decade, as a result of new evidence linking dietary nitrate consumption with reduction of blood pressure and potential benefits to cardiovascular health. This is significant since hypertension affects 25 % of the world population and is considered a major risk factor for CVD, renal disease and dementia. In the UK, approximately 30 % of the adult population have high blood pressure[1]. A small reduction of blood pressure across the population in England alone could save 45 000 quality-adjusted life years over 10 years, in addition to £850 million not spent on health and social care every year[2]. Thus, new approaches to reduce the prevalence of high blood pressure across the population are urgently required. This review will summarise historical aspects and current understanding of the role of dietary nitrate as a potential nutrient in the diet to reduce blood pressure and promote cardiovascular health.

Inorganic nitrate in food and water

The circulatory levels of inorganic nitrate are determined by endogenous (NO synthase (NOS) enzymes) and exogenous (diet) sources. It has been estimated that up to 50 % of human plasma nitrate is derived from dietary sources[3]. Vegetables (defined as a plant or part of a plant used as food) are the main source of this anion although important variations can be found amongst different plants. For instance, green leafy vegetables such as rocket, spinach, kale, pak choy and certain types of lettuce have the highest levels of nitrate, with up to 480 mg/100 g[4]. In contrast, the nitrate content of legumes such as peas can be as low as 3 mg/100 g[4] (Table 1)[4–8]. Not only does nitrate content vary between species, but also within species, so that mature outer leaves have higher concentrations than younger inner leaves.

In addition to inter- and intra-species variations, environmental factors significantly affect nitrate content of vegetable crops. For example, nitrate levels increase if crops are grown in low light conditions, such as in dull or cloudy weather or when grown under cover. Therefore there are also seasonal differences, so that the nitrate content of vegetables harvested in autumn and winter can be significantly higher than those harvested in spring and summer[6]. Natural light activates the enzyme nitrate reductase, which converts nitrate to nitrite, so nitrate accumulates more in low light conditions[9]. Other environmental factors include the amount of water received by the vegetable crops: too little water restricts nitrate uptake whereas too much dilutes nitrate in the soil. The amount of organic matter such as animal manure in the soil, as well as the amount of N fertiliser applied also affects

Abbreviations: ADI, acceptable daily intake; NOS, NO synthase.

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Table 1. Nitrate content of vegetables, portion size and nitrate content per portion

Food (raw, unless stated)	Mean nitrate content* (mg/kg)	Average portion size (80 g)-equivalent household measure†	Nitrate content per portion (mg)
'High'-nitrate vegetables			
Lettuce (round)	3347	One cereal bowl	268
Rocket	3289	One cereal bowl	263
Swiss chard	2367	Three heaped tablespoons shredded	189
Spinach	1886	One cereal bowl	151
Celery	1390	One stick	111
Fennel	1024	One-third average bulb	82
Beetroot	1211	Two small whole	97
Beetroot (boiled)	906	Two small whole	73
Curly kale	537	Three heaped tablespoons shredded	43
Spinach (boiled)	468	Four heaped tablespoons	37
Leeks	345	One medium trimmed	28
Broccoli	279	Two spears	22
'Low'-nitrate vegetables			
Carrots	97	Three heaped tablespoons	8
Onion	48	One medium	7
Green pepper	92	Half medium pepper	7
Cucumber	79	5 cm piece	6
Cauliflower (boiled)	46	Eight florets	4
Peas	30	Three heaped tablespoons	2
Tomatoes	17	One medium	1

* Data obtained from the European Food Safety Authority[4], Ministry of Agriculture, Fisheries and Food[5], Santamaria *et al.*[6] and the Food Standards Agency[7].
† Data from NHS Choices[8].

nitrate content[4]. Vegetables produced from soil high in organic matter, even where no chemical fertiliser is applied, can have higher nitrate content than the same species grown in chemically fertilised soil[10].

Once harvested, nitrate content can be further affected by storage and cooking. For example, refrigeration or freezing of vegetables prevents nitrate loss, but storage at ambient temperature increases nitrate losses[4]. As nitrate is soluble, it can be lost by washing, peeling and boiling. For example, boiling vegetables can reduce nitrate content by up to 75 %[5], whereas steaming retains nitrate content[11].

To a lesser extent, water and food additives are also sources of nitrate in the diet. Levels of nitrate in drinking water supplies in the UK and other developed countries are strictly controlled by legislation at a maximum of 50 mg/l[12]. Due to the high solubility of nitrate, water supplies can be contaminated by overuse of nitrate-containing fertilisers, which are subsequently washed off agricultural land into streams, rivers and lakes. Excessive amounts of nitrate have been detected in water from 22 % of privately owned wells in the USA[13]. Guidelines for nitrate in drinking water were mainly promulgated to protect bottle-fed infants from developing methaemoglobinaemia, or cyanosis. Higher concentrations of nitrate in blood are associated with oxidation of Hb in erythrocytes and, if the level approaches 20 % of total Hb, methaemoglobinaemia can develop which interferes with blood transport of oxygen[10]. The risk of this condition in infants under 12 months old is higher than in adults, because of immaturity of their enzymic systems and the low acid production capacity of their stomachs. Higher gastric pH enables growth of intestinal flora which convert nitrate to nitrite, which is directly absorbed into the blood[14]. However, further analysis offers a more complex picture of the causes of infantile methaemoglobinaemia, suggesting that gastrointestinal infection and inflammation as well as overproduction of endogenous NO may be the cause of this condition[15]. Furthermore, other compounds such as vitamin C appear to be a protective factor against methaemoglobinaemia in infants[13]. Current opinion suggests that given the apparently low incidence of possible water-related methaemoglobinaemia, it is inappropriate to attempt to link the concentration of nitrate in drinking-water to methaemoglobinaemia[14].

Nitrate (and nitrite) salts are used as food additives due to their ability to inhibit the growth of *Clostridium botulinum* bacterial spores. For example, sodium and potassium nitrate, together with sodium chloride, are used in 'curing' solutions during the production of bacon and ham[16]. Nitrite in combination with salt and other curing factors may also control the growth of other pathogens such as *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*[3]. Other interesting properties of nitrite salts when added to meat products include the reduction and reaction with myoglobin to produce the characteristic reddish-pink colour, in addition to contribution to the cured flavour and inhibition of lipid oxidation. However, cured meats are minor contributors to total dietary nitrate intake compared with vegetables, as detailed below.

The prevailing opinion is that dietary nitrate from all sources consumed in excess of the acceptable daily intake (ADI) of 3·7 mg/kg per d is harmful to human health and this is reflected in UK[7] and European Community legislation[4]. An ADI is the 'amount of a food additive, expressed as mg/kg body weight that can be ingested daily over a lifetime without incurring any appreciable health risk'[17]. Although the toxicity of nitrate is

Table 2. European Commission regulations on maximum levels of nitrate in foods (adapted from Commission Regulation (EU) no. 1258/2011)[19]

Vegetable	Harvesting conditions	Maximum nitrate content (mg/100 g)
Fresh spinach		350
Frozen spinach		200
Lettuce (excluding 'iceberg' type)	Harvested 1 October to 31 March	
	Grown under cover	500
	Grown in the open air	400
	Harvested 1 April to 30 September	
	Grown under cover	400
	Grown in the open air	300
'Iceberg' type	Grown under cover	250
	Grown in the open air	200
Rocket	Harvested 1 October to 31 March	700
	Harvested 1 April to 30 September	600
Processed cereal-based foods and baby foods for infants and young children		20

low, the oral lethal dose of nitrate for humans has been reported at about 330 mg/kg per d (equivalent to about 23 100 mg for a 70 kg adult/d)[18]. Due to a lack of new data, the European Food Safety Authority accepted the previous ADI set by the Joint FAO/WHO Expert Committee on Food Additives in 2002[4]. To ensure that intakes remain below the ADI, maximum levels of nitrate in crops such as lettuce, spinach and rocket are defined by legislation[19] (Table 2). Similar restrictions apply to the addition of potassium and sodium nitrate to meat products, such as bacon and ham[20]. In addition, the agriculture industry must adhere to separate legislation to minimise the use of nitrate fertilisers in order to avoid contaminating the water supply, for example, by providing storage for animal waste[21]. Therefore, the view of nitrate as a harmful contaminant has significant implications for the food, agriculture and water industries today.

Dietary nitrate consumption

Given that humans consume the majority of nitrate from vegetables, assessment of vegetable intake has been used as an indicator of nitrate intake. For example, a recent Dutch prospective cohort study of men and women, aged 55–69 years, estimated nitrate intake by using a FFQ together with local nitrate data (Dutch State Institute for Quality Control of Agricultural Products)[22]. Taking into account seasonal differences and factoring in losses from cooking and preparation, these authors estimated the median nitrate intake from vegetables to be approximately 100 mg/d (range 74–124 mg/d). In contrast, the median intake from cured meats, such as bacon and ham, is approximately 5 mg/d[23] and from water is about 1 mg/d[22]. In the UK, the average consumption of vegetables (including potatoes, beans and pulses) by adults aged 19–64 years taking part in the National Diet and Nutrition Survey is about 220 g/d[24]. Taking a mean nitrate concentration of all vegetables, including potatoes, as 33·6 mg/100 g[4], the UK current nitrate intake from all vegetables can be estimated as approximately 70 mg/d. This is in line with previous figures reported by the UK Ministry of Agriculture, Fisheries and Food, who estimated the mean dietary nitrate intake (excluding water and beer) as 57 mg/d (upper limit 105 mg/d)[5] and 54 mg/d reported by Gangolli *et al.*[25]. In addition, it is consistent with estimated nitrate intake from vegetables reported from other countries, which range from 71 mg (Finland) to 134 mg/d (Italy)[6]. These estimates of nitrate intake are within the ADI for nitrate of 3·7 mg/kg per d or about 260 mg/d for a 70 kg adult.

Whilst worldwide production of fruits and vegetables has been increasing over recent years, inadequate consumption remains a problem in the UK, where only 30 % of adults achieve the '5-A-Day' target[24]. Bryan[26] noted that the typical Western diet will be low in nitrate as it is high in refined cereals, refined sugars, dairy products, refined vegetable oils, salt and fatty meats. Conversely, those with the highest vegetable intake such as vegetarians can potentially have the highest nitrate intakes, depending on food preferences. For instance, the nitrate intake of vegetarians in the UK has been estimated as three times greater than the rest of the population[25] and could be up to 268 mg/d[27]. Similar data have been reported from vegetarians in Poland showing an average consumption of nitrate nearly three times higher (mean 340 mg/d, range 37–2054 mg/d) than non-vegetarians (mean 125 mg/d, range 116–134 mg/d)[28]. However, the range of nitrate ingestion might differ substantially among vegetarians due to food choices and the wide variation of nitrate content in different plants and fruits. For instance, Hord *et al.*[3] quantified the nitrate concentration of two hypothetical diets that emphasised low-nitrate and high-nitrate vegetables based on the Dietary Approach to Stop Hypertesion (DASH) diet. They found that nitrate concentrations in these diets varied from 174 to 1222 mg, respectively[3]. Thus, these data suggest that different patterns in the consumption of vegetables can yield differences in nitrate intake of over 700 % and can be significantly greater than the ADI. It has also been suggested that the Mediterranean diet, well known for its cardioprotective properties, might provide substantial amounts of nitrate because of its high content of nitrate-rich vegetables[10,27]. However, no previous study has quantified the nitrate content of the Mediterranean diet to date. In addition, prospective cohort studies suggest that green leafy vegetables have protective effects against CVD[29] but have not assessed nitrate intake.

Metabolism of dietary nitrate

Traditionally, inorganic nitrate was associated with an increased risk of gastrointestinal cancer and methaemoglobinaemia in infants and so considered as a contaminant in vegetables and water. This link was supported by animal research performed between the 1960s and 1970s that suggested a link between dietary nitrate and carcinogenesis[30,31]. More specifically, it was reported that dietary nitrate consumption could lead to the formation of endogenous *N*-nitrosamines, most of which were shown to be carcinogens in rodents. Following these previous studies and the above data, it might be expected that the incidence of gastric cancer in vegetarians would be higher due to larger consumption of dietary nitrate. However, epidemiological studies in this population have shown the opposite effect, as vegetarian diets are associated with low mortality rates[32] and are protective against CVD and different types of cancer[33]. In accordance with this, Bryan *et al.*[31] indicated that the original nitrate/gastric cancer hypothesis was based on low-quality studies and has not been confirmed by more recent animal and epidemiological studies. Furthermore, a recently published meta-analysis on this issue found that a high nitrate intake was associated with a weak but statistically significant reduced risk of cancer in humans[34]. Therefore, current evidence for adverse effects of moderate dietary nitrate consumption above the ADI is weak.

On the other hand, research performed over the last decade has indicated that dietary nitrate could be viewed as a bioactive food component. Briefly, current evidence suggests that dietary nitrate is rapidly absorbed from the gastrointestinal tract into the circulation with plasma levels remaining high for 5–6 h after ingestion[35]. Subsequently, part of this nitrate is actively taken up by the salivary glands, concentrated up to twenty times higher than plasma levels and secreted into the oral cavity in the saliva[35]. Oral bacteria reduce salivary nitrate to nitrite which is swallowed and absorbed across the upper gastrointestinal tract and into the circulation[35]. It is not known how nitrite crosses the gut wall into the circulation, but there have been suggestions that anion exchanger 1 in erythrocytes may be involved[36]. When swallowed, the acidic environment of the stomach facilitates the reduction of nitrite to NO and other N forms[37]. This reaction is enhanced by the presence of vitamin C and polyphenols in the stomach[27], both of which can be obtained from vegetables. Circulatory nitrite can also be reduced to NO through the activity of a number of enzymes with nitrite reductase capability present in several cells such as Hb, myoglobin and mitochondria[35].

The key role of oral bacteria in the conversion of dietary nitrate to nitrite has been shown in recent studies using antiseptic mouthwash[38–41]. These studies found that disruption of the nitrate-reducing capacity of oral bacteria by oral mouthwash was associated with no change in plasma circulatory levels of nitrite after nitrate intake. In addition, this response was linked to a concomitant increase in blood pressure[39], demonstrating a correlation between oral bacteria, dietary nitrate ingestion, increased circulatory levels of nitrite and reduced blood pressure.

The identification of this bioactive role of dietary nitrate has substantially changed the view of NO metabolism. In the past it was thought that NO was only formed via an enzymic and oxygen -dependent pathway, where L-arginine is reduced to NO by several forms of NOS enzymes and the combination of several cofactors[42]. In addition, it was found that patients with hypertension have impaired NO bioavailability, caused by increased levels of reactive oxygen species which scavenge NO[43]. This reduced NO bioavailability is a major risk factor for both hypertension and CVD[43]. Consequently many studies in the last two decades have investigated the effects of L-arginine supplementation and NO bioavailability in patients with hypertension. A meta-analysis of these studies found that supplementation with L-arginine was effective in reducing blood pressure[44]. However, it must be noted that the amount of L-arginine provided by some studies was very high (>20 g/d) compared with the amount of L-arginine in the normal diet (about 5 g/d)[45]. This is important since large amounts of oral L-arginine have been reported to increase risk of mortality in both animals with septic shock and patients with acute myocardial infarction[46,47].

Thus, current research is now focusing on the effect of dietary nitrate on NO metabolism and vascular function. From this viewpoint, a study by Carlström *et al.*[48] found that nitrate administration was able to increase bioavailability and reverse cardiovascular dysfunction in mice with impaired function of the L-arginine–NOS pathway. However, these results have not been confirmed in longer-term studies[49] and there is a possibility of a cross-talk between the NOS-dependent and NOS-independent pathways in animals[50]. In addition there is a lack of long-term supplementation studies in human subjects and future studies should explore the effects of high intakes of dietary nitrate (>ADI) on the oral microbiome and blood pressure.

Is dietary nitrate a new nutrient?

Since the first human study showing a blood pressure-lowering effect of a dietary nitrate supplement was published in 2006[51], many studies have extended these findings. A systematic review and meta-analysis of such studies published from 2006 to 2013 found that nitrate supplements reduced systolic blood pressure by 4·4 mmHg and diastolic blood pressure by 1·1 mmHg[52]. Participants included a mixture of adults with and without health co-morbidities who were given pharmacological (sodium or potassium nitrate) or dietary (beetroot juice) supplements. Similarly, a review by Hobbs *et al.*[53] of studies up to 2012 found a significant inverse relationship between nitrate dose and systolic blood pressure. In agreement with these results, seventeen further intervention studies have been published[54–70] (Table 3). These studies found significant reductions in blood pressure after various forms of nitrate supplementation (mean dose about 500 mg nitrate) in a wide range of participants, including patients with hypertension, chronic obstructive pulmonary disease and heart failure. Together with previous reviews[52,53], these more recent studies support the hypothesis that dietary nitrate reduces blood pressure and therefore could improve cardiovascular health. It could be concluded that there are sufficient data to state that dietary nitrate, at least from vegetables, should be reconsidered as a new nutrient, rather than a harmful contaminant[3].

Accordingly, it has been suggested that the key element of the cardioprotective effect of diets rich in vegetables is dietary

Table 3. Summary of research studies on nitrate and health with significant ($P < 0{\cdot}05$) positive effects on systolic and diastolic blood pressure, 2013–2016

Supplement	Mean nitrate dose* (mg/d)	Mean reduction in SBP (mmHg)	Mean reduction in DBP (mmHg)	Duration (d)	Participant characteristics	Age (years)	Sample size	Other significant findings reported	Authors
Beetroot juice	217	11	10	1	Hypertension	53	15	Improved vascular compliance	Ghosh *et al.*[54]
Beetroot juice	595	5	3	2·5	Healthy	64	12	Improved V_{O2} kinetics during treadmill walking	Kelly *et al.*[55]
Beetroot juice (three doses)	260 521 1042	5 10 9	NS 3 4	1	Healthy	23	10	Reduced oxygen cost of moderate-intensity exercise	Wylie *et al.*[56]
Beetroot-enriched bread	139	NS	7·0	1	Healthy	31	23	Improved endothelium-independent microvascular vasodilation	Hobbs *et al.*[57]
250 g spinach	220	8	NS	1	Healthy	59	26	Improved arterial elasticity	Liu *et al.*[58]
Beetroot juice	165	7	NS	21	Overweight/ obese	62	21		Jajja *et al.*[59]
Sodium nitrate (150 μmol/kg)	About 7 mg/kg Mean dose not reported	8	NS	28	Moderate risk of CVD	63	21	Improved endothelial function	Rammos *et al.*[60]
High-nitrate vegetables	339	4	NS	7	Healthy	20	19		Ashworth *et al.*[61]
Beetroot juice	470	8	NS	1	COPD	70	15	Improved exercise performance	Berry *et al.*[62]
Spinach soup	845	3	3	7	Healthy	25	27	Reduced augmentation index, indicating decreased arterial stiffness	Jovanovski *et al.*[63]
Beetroot juice	400	8	2	28	Hypertension	57	64	Endothelial function improved by about 20 % and reduced arterial stiffness	Kapil *et al.*[64]
Beetroot juice	450	NS	12	3	Healthy	24	6	Improved NOS-independent vasodilatation	Keen *et al.*[65]
Beetroot juice	800	12	2	1	COPD	69	11	Increased exercise capacity	Kerley *et al.*[66]
Beetroot juice	800	NS	6	1	COPD	68	21	Reduced oxygen consumption	Curtis *et al.*[67]
Beetroot gel	397	6	5	1	Healthy	27	5		Silva *et al.*[68]
Beetroot juice	378	7	NS	7	Heart failure	69	19	Improved exercise endurance	Eggebeen *et al.*[69]
Beetroot juice Rocket drink Spinach drink	800 800 800	5 6 7	Reduced, absolute values not reported	1	Healthy	28	18		Jonvik *et al.*[70]

SBP, systolic blood pressure; DBP, diastolic blood pressure; COPD, chronic obstructive pulmonary disease; NO, NO synthase.
* Mean nitrate dose of all studies about 500 mg/d, range 139–1042 mg/d.

nitrate[3]. Interestingly, the prevalence of high blood pressure in vegetarians is significantly lower than in omnivores as shown by epidemiological studies[33,71]. Originally it was thought that the lower energy density, higher fibre and lower fat content of vegetarian diets induced low BMI and this could explain low values of blood pressure in vegetarians compared with omnivores[72]. However, weight differences do not fully explain the observed blood pressure differences between vegetarians and omnivores. Pettersen *et al.*[73] found that differences in systolic blood pressure (about 6–7 mmHg) between vegans and lacto-ovo vegetarians compared with omnivores remained after adjusting the data for age, sex and BMI. These authors also suggested that various factors such as the higher fibre and K intakes in vegetarians may be responsible for this effect on blood pressure, along with possible physiological mechanisms, such as improvement of glucose tolerance and lower blood viscosity[73]. However, positive effects of K or Mg supplements on blood pressure have not been confirmed and these are not recommended for treatment of hypertensive patients[74,75]. Low ingestion of Na can also be associated with low blood pressure as suggested by several studies in an attempt to explain the effects of the DASH diet[76,77]. There is still an intense debate on the main factors associated with low blood pressure, i.e. whether it is the reduced salt content associated with vegetable-rich diets, or other constituents of foods such as nitrate or polyphenols. From this viewpoint, we are currently undertaking a clinical trial to investigate the contribution of dietary nitrate in lowering blood pressure in individuals following a vegetarian diet for at least a year. This and further studies are needed to elucidate the potential therapeutic effect of dietary nitrate.

On the other hand, it should also be recognised that other studies have not found a significant effect of dietary nitrate on blood pressure in healthy subjects[78–81] or patients with cardiovascular risk factors[82], hypertension[83,84], type 2 diabetes[85], obesity[86] and chronic obstructive pulmonary disease[87] (Table 4). This lack of a significant effect of dietary nitrate supplementation on blood pressure could be related to several factors. For example, some studies included participants taking anti-hypertensive medications and/or hypoglycaemic medications[84,85,87], which could affect NO metabolism. Additionally, the recent use of antibiotics or antibacterial mouthwash was not reported in some studies[85,86]. These are important factors that can affect the response of blood pressure to dietary nitrate and which could explain negative findings. Also, it should be noted that some patients suffering from cardiovascular disorders, obesity and type 2 diabetes may have a lower response to dietary nitrate given the impaired NO metabolism associated with these conditions. Some negative studies reported significant correlations with baseline systolic blood pressure, inferring that healthy participants with the highest baseline experienced greatest reductions[79,80]. The conclusion that can be drawn from studies reporting non-significant changes to blood pressure is that the overall understanding of the efficacy of nitrate supplementation is still unclear, especially in patients with pre-existing clinical conditions. This view is supported by a recent meta-analysis and systematic review of dietary nitrate and endothelial

Table 4. Summary of research studies on nitrate and health with non-significant effects on systolic and diastolic blood pressure, 2006–2016

Supplement	Mean nitrate dose (mg/d)*	Mean reduction SBP (mmHg)	Mean reduction DBP (mmHg)	Duration (d)	Participant characteristics	Mean age (years)	Sample size	Other significant findings reported	Authors
High-nitrate diet with or without beetroot juice	682	NS	NS	3	Healthy	72	8	Increased plasma nitrite	Miller *et al.*[78]
Beetroot juice	600	NS	NS	7	Healthy	21	19	Increased contractile properties of skeletal muscle. Trend in SBP was significantly correlated with baseline SBP	Haider & Folland[79]
High-nitrate vegetables	420	3	3	14	Healthy	25	15	Trend in SBP was significantly correlated with changes in plasma nitrate and nitrite	Ashworth *et al.*[80]
Beetroot juice	800	NS	NS	1	Healthy	22	12	Reduced pulse wave velocity	Kim *et al.*[81]
'Neo40' lozenge of hawthorn and beetroot	Not reported	7	5	30	>/= Three CV risk factors	56	23	Reduced TAG	Zand *et al.*[82]
High-nitrate vegetables	345	NS	NS	7	High-normal SBP	61	38	Increased plasma and salivary nitrate and nitrite	Bondonno *et al.*[83]
Beetroot juice	434	NS	NS	7	Hypertension	63	27	Increased plasma, salivary and urinary nitrite and nitrate	Bondonno *et al.*[84]
Beetroot juice	480	NS	NS	14	Type 2 diabetes and hypertension	67	27	Increased plasma nitrite	Gilchrist *et al.*[85]
Beetroot juice	600	NS	NS	7	Overweight/obese	62	30		Lara *et al.*[86]
Beetroot juice	840	NS	NS	2·5	COPD	65	13		Shepherd *et al.*[87]

SBP, systolic blood pressure; DBP, diastolic blood pressure; CV, cardiovascular; COPD, chronic obstructive pulmonary disease.
* Mean nitrate dose of all studies about 600 mg/d, range 420–840 mg/d.

function, which found that increasing age, obesity and systolic blood pressure were associated with a reduced effect of nitrate supplementation[88]. Further research is needed to investigate whether dietary nitrate could improve cardiovascular health in the older population with cardiovascular risk factors[88].

Amount of dietary nitrate required to promote cardiovascular health

The systematic review and meta-analysis of nitrate supplementation trials concluded that inorganic nitrate and beetroot juice supplements are associated with significant reductions in systolic blood pressure[52]. A range of doses was reported, either in the form of beetroot juice or nitrate salts (mean dose about 500 mg nitrate, range 157–1395 mg nitrate)[52]. Similar variations in methodology can be seen in studies published in the last 3 years (Table 3). The average amount of nitrate used in these trials can be calculated as about 500 mg/d (range 139–1042 mg/d). However, a word of caution must be raised in regards to some studies using beetroot juice as a supplement. A recent study by Jajja *et al.*[59] found that 70 ml of concentrated beetroot juice, which was expected to provide between 300 and 400 mg of nitrate, in fact yielded only 165 mg. This could be due to environmental factors (season of the year, light, irrigation of the field), the use of fertilisers and storage conditions (temperature, humidity and light), as detailed earlier. Therefore, it is important that future studies using natural supplements such as beetroot juice should directly analyse the amount of nitrate in the supplement rather than reporting values provided by the manufacturer.

Using pharmacological salts (potassium nitrate), Kapil *et al.*[89] investigated the effect of two different doses providing 248 and 744 mg of nitrate, respectively. While both doses were similarly effective in reducing diastolic blood pressure, the lower dose did not show a significant effect in reducing systolic blood pressure. This suggests that a minimum amount of nitrate might be needed to induce changes in systolic blood pressure. In accord with current evidence, this threshold could be up to about 500 mg of nitrate (Table 3). This amount clearly exceeds the ADI of 3·7 mg/kg per d (about 260 mg/d for a 70 kg adult) as well as exceeding the average intake of vegetarians[27,28]. Furthermore, this relies on individual food choices and both omnivores and vegetarians could easily reach or exceed such levels of nitrate intake. Hord *et al.*[3] estimated that two servings per d of vegetables such as spinach and greens provide over 1000 mg of nitrate. However, Hord's estimates assumed a maximum nitrate content of those vegetables[3].

More precisely, Lidder & Webb[27] advocate daily consumption of at least one portion of high-nitrate vegetables to increase nitrate intake in order to lower blood pressure. Oyebode *et al.*[90] suggest that the current UK '5-a-day' message could be amended to specifically advise on numbers of portions of vegetables. This would be in line with Australian government recommendations, which advise Australians to eat five portions of vegetables and two portions of fruit daily[91]. For example, changing the UK message to 'at least 5-a-day + 1 green leafy veg' may help to remind consumers to include high-nitrate vegetables as part of their daily target.

However, it is important to note that there is a lack of human studies investigating the chronic effect of high doses of dietary nitrate on blood pressure. Previous studies have not analysed a period of nitrate supplementation beyond 1 month. Although several epidemiological studies have linked high consumption of vegetables (especially green leafy vegetables)[29,92] with a lower risk of CVD, it is not possible to state that nitrate is responsible for those effects to date. Furthermore, research using animal models has found conflicting results. Whilst a first study found that 8 weeks of nitrate (equivalent to a human dose of 350 mg/75 kg per d), reduced the mean arterial blood pressure in mice at the end of the lifespan[48], a second study from the same research group did not show any effect[49]. The authors suggested that long-term supplementation may have affected the nitrate homeostasis of the animals, which is supported by the lack of differences in final plasma levels of nitrite and nitrate[49]. The dose used in these studies was lower than the average given in human trials, but similar to the nitrate intake reported in vegetarians. The authors also suggested that the oral microbiome could be modified under extended nitrate treatment which affected the final results[49]. However, this hypothesis has not been confirmed in a recent study in human subjects, indicating that the oral microbiota of vegetarians did not differ from that of omnivores, despite significantly different dietary patterns[93]. In spite of the fact that these animal studies are difficult to replicate in human subjects, further research will be necessary to investigate the effects of chronic exposure to dietary nitrate and the extent to which long-term adaptations occur.

The UK Department of Health established dietary reference values (DRV) for essential nutrients to clearly define, where possible, the contexts in which intakes could be deficient, adequate or potentially excessive across different population groups[94]. No DRV has been established for nitrate as it is not currently considered as a nutrient with potential health benefits. The intake of excessive amounts of nitrate could be associated in specific contexts with an increased risk of negative health outcomes. However, the ADI for nitrate has been viewed as anachronistic and some authors have suggested it could be used to achieve an intake likely to derive cardiovascular benefits[27].

Implications both at population level and in population groups

Approximately 30 % of adults (about 15 million) in the UK have high blood pressure, usually defined as systolic blood pressure over 140 mmHg and diastolic blood pressure over 90 mmHg[1]. There is a strong relationship between blood pressure and mortality from CVD, so that an increase in adult systolic blood pressure by 20 mmHg is associated with doubling of death rate from CVD such as stroke[95]. Conversely, a reduction of 10 mmHg in systolic blood pressure is associated with about 40 % lower risk of stroke death throughout middle age[95]. A smaller reduction of just 5 mmHg across the population could reduce stroke mortality by 14 %[96]. This 5 mmHg reduction across the population in England could save 45 000

quality-adjusted life years over 10 years as well as £850 million spent on health and social care services[2]. In addition, stroke is one of the largest causes of disability and over one-third of survivors are dependent on others, such as family relatives, for their care[97]. This places an additional burden on carers as well as the economy, due to lost income. The total financial cost of stroke to UK society, including loss of productivity as well as cost of health care, is approximately £9 billion each year[98]. Prevention of high blood pressure has been identified as a key factor to improve the nation's health, by a combination of improvements in diet at a population level as well as encouraging individual behaviour change in diet, physical activity, alcohol and smoking[2]. Promoting consumption of high-nitrate vegetables could be used as a low-cost yet effective measure to improve cardiovascular health across the population[27,53,99,100] and this approach could be more effective than targeting high-risk individuals[101].

Certain groups in the population may be at risk from receiving insufficient nitrate due to their medical condition or interference with the nitrate–nitrite–NO pathway. For example, 3–7 d of antimicrobial mouthwash, which reduces the activity of nitrate-reducing commensal bacteria, has been shown to increase blood pressure[39,102]. This fact could be important in those at risk of CVD and future studies are needed to investigate the long-term effects of using antibacterial mouthwash in different population groups.

Hypertension is both a cause and consequence of chronic kidney disease. In the USA, it has been estimated that the prevalence of renal failure among subjects with prehypertension and undiagnosed hypertension could be 17 and 22 %, respectively[103]. Renal patients who require dialysis are another potentially 'at-risk' group, as haemodialysis effectively removes both nitrite and nitrate from blood and saliva which in turn could reduce NO bioavailability[104]. Consequently, this could be a cause of the increased risk of cardiovascular mortality in this group of patients.

Another potential 'at-risk' group are patients who are on intensive care units, who are often intubated so that their ability to swallow their saliva is affected. The formation of NO has been found to be almost abolished in this situation in the stomach, and nitrite supplementation has been shown to restore NO activity in this patient group[105]. However, critically ill patients often receive nutritional support as either enteral or parenteral nutrition, which contain little or no nitrate[26,106]. In this scenario, with reduced saliva production as well as the effect of broad-spectrum antibiotics on oral bacteria, NO production in the stomach can be limited. This could explain the high incidence of infection in these patients[106].

Finally, a recent review highlighted the protective effects of foods containing polyphenols, vitamin C and vitamin E against *N*-nitrosamine formation and reiterated the inverse association between dietary nitrate and stomach cancer[107]. However, it recommended the need for further research into nitrate consumption in specific populations. For example, the nitrate content of drinking water (especially from private wells in rural areas of the USA) should be recorded in prospective cohort studies to determine whether individuals with a high water nitrate intake are either at increased risk of developing cancer or benefit from improved cardiovascular health[107]. In addition, future cancer research should focus on population groups who may be at increased risk of *N*-nitrosamine formation such as smokers and those taking large quantities of nitrate or nitrite supplements which may not contain protective polyphenols and vitamins[107]. This review noted that dietary nitrate from vegetables should not be included in such cancer research studies[107].

Conclusion

The ADI for nitrate of 3·7 mg/kg body weight was set several decades ago due to fears of carcinogenicity arising from animal studies as well as the incidence in methaemoglobinaemia in babies. Subsequent legislation significantly affects the food, water and agriculture industries today. However, the findings of the original animal studies have been questioned and discounted in light of more recent research. In contrast, epidemiological studies suggest that green leafy vegetables, which are naturally high in nitrate, promote cardiovascular health. Vegetarians have been reported to have high intakes of nitrate and also have reduced risks of cancer, lower blood pressure, less CVD and lower mortality rates. There is increasing evidence from human intervention studies to support the hypothesis that dietary nitrate effectively reduces blood pressure, amongst other outcomes, in both healthy subjects and those with clinical conditions. Others studies have not confirmed these findings and this could be due to reduced nitrite and NO bioavailability, especially in clinical populations, amongst other methodological reasons. However, a small, population-wide downward shift in systolic blood pressure, as suggested by a systematic review and other more recent studies, could significantly reduce the incidence of CVD such as stroke. From this viewpoint, some authors have called for nitrate to be seen as a nutrient, rather than a contaminant. The amount of nitrate needed to reduce blood pressure could be over and above the ADI, although more studies are needed and the results of longer-term trials are awaited. This level of intake could be partly achieved by eating at least one portion of high-nitrate vegetables daily, in addition to other vegetables. This would be consistent with current UK Government advice to eat more fruit and vegetables.

The implication of the evidence presented and discussed is that the current classification of nitrate as a contaminant needs to be re-examined. Due to beneficial effects on systolic blood pressure, dietary nitrate may in future be recognised as a nutrient necessary for vascular health. If dietary nitrate is indeed a 'new' nutrient, then dietary strategies could be designed to reduce the risk of hypertension and CVD such as stroke.

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References

1. British Heart Foundation (2016) CVD Statistics – UK Fact Sheet. https://www.bhf.org.uk/research/heart-statistics (accessed April 2016).
2. Public Health England (2016) Tackling high blood pressure: from evidence into action. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/527916/Tackling_high_blood_pressure.pdf (accessed August 2016).
3. Hord NG, Tang Y & Bryan NS (2009) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* **90**, 1–10.
4. European Food Safety Authority (2008) Opinion of the Scientific Panel on Contaminants in the food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. *EFDA J* **689**, 1–79.
5. Ministry of Agriculture, Fisheries and Food (1998) Food surveillance information sheet number 158: nitrate in vegetables. http://webarchive.nationalarchives.gov.uk/20100927130941/http://archive.food.gov.uk/maff/archive/food/infsheet/1998/no158/158nitra.htm (accessed August 2016).
6. Santamaria P, Elia A, Serio F, *et al.* (1999) A survey of nitrate and oxalate content in fresh vegetables. *J Sci Food Agric* **79**, 1882–1888.
7. Food Standards Agency (2004) UK monitoring programme for nitrate in lettuce and spinach. http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/multimedia/pdfs/fsis7405.pdf (accessed April 2016).
8. NHS Choices (2016) Rough guide, fruit & vegetable portion sizes. https://www.nhs.uk/Livewell/5ADAY/Documents/Downloads/5ADAY_portion_guide.pdf (accessed August 2016).
9. Weightman RM, Hucklea AJ, Roquesa SE, *et al.* (2012) Factors influencing tissue nitrate concentration in field-grown wild rocket (*Diplotaxis tenuifolia*) in southern England. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **29**, 1425–1435.
10. Bryan NS & Loscalzo J (editors) (2011) *Nitrite and Nitrate in Human Health and Disease*. New York: Springer.
11. Mozolewski W & Smoczynski S (2004) Effect of culinary processes on the content of nitrates and nitrites in potatoe. *Pak J Nutr* **3**, 357–361.
12. World Health Organization (2004) Guidelines for Drinking-water Quality. http://www.who.int/water_sanitation_health/dwq/GDWQ2004web.pdf (accessed June 2016).
13. Ward MH, Kok TM, Levallois P, *et al.* (2005) Workgroup report: Drinking-water nitrate and health – recent findings and research needs. *Environ Health Perspect* **113**, 1607–1614.
14. Fewtrell L (2004) Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion. *Environ Health Perspect* **112**, 1371–1374.
15. Avery A (1999) Infantile methemoglobinemia: reexamining the role of drinking water nitrates. *Environ Health Perspect* **107**, 583–586.
16. European Commission (2016) Guidance document describing the food categories in Part E of Annex II to Regulation (EC) No 1333/2008 on Food Additives. http://ec.europa.eu/food/safety/docs/fs_food-improvement-agents_guidance_1333-2008_annex2.pdf (accessed June 2016).
17. European Commission (2001) Report from the commission on Dietary Food Additive Intake in the European Union. http://ec.europa.eu/food/fs/sfp/addit_flavor/flav15_en.pdf (accessed June 2016).
18. Walker R (1990) Nitrates, nitrites and *N*-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. *Food Addit Contam* **7**, 717–768.
19. European Commission (2011) Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs, https://www.fsai.ie/uploadedFiles/Reg1258_2011.pdf (accessed June 2016).
20. Food Standards Agency (2015) Food Additives Legislation Guidance to Compliance. http://www.food.gov.uk/sites/default/files/multimedia/pdfs/guidance/food-additives-legislation-guidance-to-compliance.pdf (accessed August 2016).
21. Council of the European Communities (1991) Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0676&from=EN (accessed August 2016).
22. Keszei AP, Schouten LJ, Driessen ALC, *et al.* (2014) Vegetable, fruit and nitrate intake in relation to the risk of Barrett's oesophagus in a large Dutch cohort. *Br J Nutr* **111**, 1452–1462.
23. Hsu J, Arcot J & Lee A (2009) Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. *Food Chem* **115**, 334–339.
24. Public Health England (2014) National Diet and Nutrition Survey Results from years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009–2011/2012). https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/310995/NDNS_Y1_to_4_UK_report.pdf (accessed August 2016).
25. Gangolli SD, van den Brandt PA, Feron VJ, *et al.* (1994) Nitrate, nitrite and *N*-nitroso compounds. *Eur J Pharmacol* **292**, 1–38.
26. Bryan NS (2012) Pharmacological therapies, lifestyle choices and nitric oxide deficiency: a perfect storm. *Pharmacol Res* **66**, 448–456.
27. Lidder S & Webb AJ (2013) Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate–nitrite–nitric oxide pathway. *Br J Clin Pharmacol* **75**, 677–696.
28. Mitek M, Anyzewska A & Wawrzyniak A (2013) Estimated dietary intakes of nitrates in vegetarians compared to a traditional diet in Poland and acceptable daily intakes: is there a risk? *Rocz Panstw Zakl Hig* **64**, 105–109.
29. Joshipura KJ, Hung HC, Li TY, *et al.* (2009) Intakes of fruits, vegetables and carbohydrate and the risk of CVD. *Public Health Nutr* **12**, 115–121.
30. Correa P, Haenszel W, Cuello C, *et al.* (1975) A model for gastric cancer epidemiology. *Lancet* **ii**, 58–60.
31. Bryan NS, Alexander DD, Coughlin JR, *et al.* (2012) Ingested nitrate and nitrite and stomach cancer risk: an updated review. *Food Chem Toxicol* **50**, 3646–3665.
32. Key TJ, Appleby PN, Spencer EA, *et al.* (2009) Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). *Am J Clin Nutr* **89**, 1613S–1619S.
33. Huang T, Yang B, Zheng J, *et al.* (2012) Cardiovascular disease mortality and cancer incidence in vegetarians: a meta-analysis and systematic review. *Ann Nutr Metab* **60**, 233–240.
34. Song P, Wu L & Guan W (2015) Dietary nitrates, nitrites, and nitrosamines intake and the risk of gastric cancer: a meta-analysis. *Nutrients* **7**, 9872–9895.

Nutrition Research Reviews

35. Lundberg JO, Weitzberg E & Gladwin MT (2008) The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* **7**, 156–167.
36. Vitturi DA, Teng X, Toledo JC, *et al.* (2009) Regulation of nitrite transport in red blood cells by hemoglobin oxygen fractional saturation. *Am J Physiol Heart Circ Physiol* **296**, H1398–H1407.
37. Lundberg JO & Govoni M (2004) Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic Biol Med* **37**, 395–400.
38. Govoni M, Jansson EA, Weitzberg E, *et al.* (2008) The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* **19**, 333–337.
39. Kapil V, Haydar SMA, Pearl V, *et al.* (2013) Physiological role for nitrate-reducing oral bacteria in blood pressure control. *Free Radic Biol Med* **55**, 93–100.
40. Woessner M, Smoliga JM, Tarzia B, *et al.* (2016) A stepwise reduction in plasma and salivary nitrite with increasing strengths of mouthwash following a dietary nitrate load. *Nitric Oxide* **54**, 1–7.
41. McDonagh STJ, Wylie LJ, Winyard PG, *et al.* (2015) The effects of chronic nitrate supplementation and the use of strong and weak antibacterial agents on plasma nitrite concentration and exercise blood pressure. *Int J Sports Med* **36**, 1177–1185.
42. Moncada S & Higgs A (1993) The l-arginine–nitric oxide pathway. *N Engl J Med* **329**, 2002–2012.
43. Hermann M, Flammer A & Lüscher TF (2006) Nitric oxide in hypertension. *J Clin Hypertens* **8**, 17–29.
44. Dong JY, Qin LQ, Zhang Z, *et al.* (2011) Effect of oral l-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Am Heart J* **162**, 959–965.
45. Venho B, Voutilainen S, Valkonen VP, *et al.* (2002) Arginine intake, blood pressure, and the incidence of acute coronary events in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am J Clin Nutr* **76**, 359–364.
46. Kalil AC, Sevransky JE, Myers DE, *et al.* (2006) Preclinical trial of l-arginine monotherapy alone or with *N*-acetylcysteine in septic shock. *Crit Care Med* **34**, 2719–2728.
47. Schulman SP, Becker LC, Kass DA, *et al.* (2006) l-Arginine therapy in acute myocardial infarction: the Vascular Interaction with Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA* 295, 58–64.
48. Carlström M, Persson AE, Larsson E, *et al.* (2011) Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovasc Res* **89**, 574–585.
49. Hezel MP, Liu M, Schiffer TA, *et al.* (2015) Effects of long-term dietary nitrate supplementation in mice. *Redox Biol* **5**, 234–242.
50. Carlström M, Liu M, Yang T, *et al.* (2015) Cross-talk between nitrate–nitrite–NO and NO synthase pathways in control of vascular NO homeostasis. *Antioxid Redox Signal* **23**, 295–306.
51. Larsen FJ, Ekblom B, Sahlin K, *et al.* (2006) Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med* **355**, 2792–2793.
52. Siervo M, Lara J, Ogbonmwan I, *et al.* (2013) Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J. Nutr* **143**, 818–826.
53. Hobbs DA, George TW & Lovegrove JA (2013) The effects of dietary nitrate on blood pressure and endothelial function: a review of human intervention studies. *Nutr Res Rev* **26**, 210–222.
54. Ghosh SM, Kapil V, Fuentes-Calvo I, *et al.* (2013) Enhanced vasodilator activity of nitrite in hypertension: critical role for erythrocytic xanthine oxidoreductase and translational potential. *Hypertension* **61**, 1091–1102.
55. Kelly J, Fulford J, Vanhatalo A, *et al.* (2013) Effects of short-term dietary nitrate supplementation on blood pressure, O_2 uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol Regul Integr Comp Physiol* **304**, R73–R83.
56. Wylie LJ, Kelly J, Bailey SJ, *et al.* (2013) Beetroot juice and exercise: pharmacodynamic and dose–response relationships. *J Appl Physiol* **115**, 325–336.
57. Hobbs DA, Goulding MG, Nguyen A, *et al.* (2013) Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers diastolic blood pressure in healthy men: a randomized controlled trial. *J Nutr* **143**, 1399–1405.
58. Liu AH, Bondonno CP, Croft KD, *et al.* (2013) Effects of a nitrate-rich meal on arterial stiffness and blood pressure in healthy volunteers. *Nitric Oxide* **35**, 123–130.
59. Jajja A, Sutyarjoko A, Lara J, *et al.* (2014) Beetroot supplementation lowers daily systolic blood pressure in older, overweight subjects. *Nutr Res* **34**, 868–875.
60. Rammos C, Hendgen-Cotta UB, Sobierajski J, *et al.* (2014) Dietary nitrate reverses vascular dysfunction in older adults with moderately increased cardiovascular risk. *J Am Coll Cardiol* **63**, 1584–1585.
61. Ashworth A, Mitchell K, Blackwell JR, *et al.* (2015) High-nitrate vegetable diet increases plasma nitrate and nitrite concentrations and reduces blood pressure in healthy women. *Public Health Nutr* **18**, 2669–2678.
62. Berry MJ, Justus NW, Hauser JI, *et al.* (2015) Dietary nitrate supplementation improves exercise performance and decreases blood pressure in COPD patients. *Nitric Oxide* **48**, 22–30.
63. Jovanovski E, Bosco L, Khan K, *et al.* (2015) 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* **4**, 160–167.
64. Kapil V, Khambata RS, Robertson A, *et al.* (2015) Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study. *Hypertension* **65**, 320–327.
65. Keen JT, Levitt EL, Hodges GJ, *et al.* (2015) Short-term dietary nitrate supplementation augments cutaneous vasodilatation and reduces mean arterial pressure in healthy humans. *Microvasc Res* **98**, 48–53.
66. Kerley CP, Cahill K, Bolger K, *et al.* (2015) Dietary nitrate supplementation in COPD: an acute, double-blind, randomized, placebo-controlled, crossover trial. *Nitric Oxide* **44**, 105–111.
67. Curtis KJ, O'Brien KA, Tanner RJ, *et al.* (2015) Acute dietary nitrate supplementation and exercise performance in COPD: a double-blind, placebo-controlled, randomised controlled pilot study. *PLOS ONE* **23**, e0144504.
68. Silva DVT, Oliveira Silva F, Perrone D, *et al.* (2016) Physicochemical, nutritional, and sensory analyses of a nitrate enriched beetroot gel and its effects on plasmatic nitric oxide and blood pressure. *Food Nutr Res* **60**, 10.3402/fnr.v60.29909.
69. Eggebeen J, Kim-Shapiro DB, Haykowsky M, *et al.* (2016) One week of daily dosing with beetroot juice improves submaximal endurance and blood pressure in older

patients with heart failure and preserved ejection fraction. *JACC Heart Fail* **4**, 428–437.
70. Jonvik KL, Nyakayiru J, Pinckaers P, *et al.* (2016) Nitrate-rich vegetables increase plasma nitrate and nitrite concentrations and lower blood pressure in healthy adults. *J Nutr* **146**, 986–993.
71. Key TJ, Thorogood M, Appleby PN, *et al.* (1996) Dietary habits and mortality in 11,000 vegetarians and health conscious people: results of a 17 year follow up. *BMJ* **313**, 775–779.
72. Drøyvold WB, Midthjell K, Nilsen TIL, *et al.* (2005) Change in body mass index and its impact on blood pressure: a prospective population study. *Int J Obes Relat Metab Disord* **29**, 650–655.
73. Pettersen BJ, Anousheh R, Fan J, *et al.* (2012) Vegetarian diets and blood pressure among white subjects: results from the Adventist Health Study-2 (AHS-2). *Public Health Nutr* **15**, 1909–1916.
74. Dickinson HO, Nicolson DJ, Campbell F, *et al.* (2006) Potassium supplementation for the management of primary hypertension in adults. *Cochrane Database of Systematic Reviews*, issue 3, CD004641.
75. Dickinson HO, Nicolson DJ, Campbell F, *et al.* (2006) Magnesium supplementation for the management of essential hypertension in adults. *Cochrane Database of Systematic Reviews*, issue 3, CD004640.
76. Sacks FM, Svetkey LP, Vollmer WM, *et al.* (2001) Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *N Engl J Med* **344**, 3–10.
77. Vollmer WM, Sacks FM, Ard J, *et al.* (2001) Effects of diet and sodium intake on blood pressure: subgroup analysis of the DASH-Sodium Trial. *Ann Intern Med* **135**, 1019–1028.
78. Miller GD, Marsh AP, Dove RW, *et al.* (2012) Plasma nitrate and nitrite are increased by a high-nitrate supplement but not by high-nitrate foods in older adults. *Nutr Res* **32**, 160–168.
79. Haider G & Folland JP (2014) Nitrate supplementation enhances the contractile properties of human skeletal muscle. *Med Sci Sports Exerc* **46**, 2234–2243.
80. Ashworth A, Bailey SJ, Hayward GM, *et al.* (2015) Dietary nitrate – an unrecognized nutrient. *Clinical Nutrition ESPEN* **10**, e201.
81. Kim J-K, Moore DJ, Maurer DG, *et al.* (2015) Acute dietary nitrate supplementation does not augment submaximal forearm exercise hyperemia in healthy young men. *Appl Physiol Nutr Metab* **40**, 122–128.
82. Zand J, Lanza F, Garg HK, *et al.* (2011) All-natural nitrite and nitrate supplement promotes nitric oxide production and reduces triglycerides in humans. *Nutr Res* **31**, 262–269.
83. Bondonno CP, Liu AH, Croft KD, *et al.* (2014) 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* **77**, 353–362.
84. Bondonno CP, Liu AH, Croft KD, *et al.* (2015) 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* **102**, 368–375.
85. Gilchrist M, Winyard PG, Aizawa K, *et al.* (2013) Effect of dietary nitrate on blood pressure, endothelial function, and insulin sensitivity in type 2 diabetes. *Free Radic Biol Med* **60**, 89–97.
86. Lara J, Ogbonmwana I, Oggioni C, *et al.* (2015) 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* **82**, 228–235.
87. Shepherd AI, Wilkerson DP, Dobson L, *et al.* (2015) The effect of dietary nitrate supplementation on the oxygen cost of cycling, walking performance and resting blood pressure in individuals with chronic obstructive pulmonary disease: a double blind placebo controlled, randomised control trial. *Nitric Oxide* **48**, 31–37.
88. Lara J, Ashor AW, Oggioni C, *et al.* (2016) Effects of inorganic nitrate and beetroot supplementation on endothelial function: a systematic review and meta-analysis. *Eur J Nutr* **55**, 451–459.
89. Kapil V, Milsom AB, Okorie M, *et al.* (2010) Inorganic nitrate supplementation lowers blood pressure in humans. *Hypertension* **56**, 274–281.
90. Oyebode O, Gordon-Dseagu V, Walker A, *et al.* (2014) Fruit and vegetable consumption and all-cause, cancer and CVD mortality: analysis of Health Survey for England data. *J Epidemiol Community Health* **68**, 856–862.
91. Australian Government Department of Health (2008) National Go for 2&5™ Campaign. http://www.healthyactive.gov.au/internet/healthyactive/publishing.nsf/Content/2and5 (accessed August 2016).
92. Joshipura KJ, Hu FB, Manson JE, *et al.* (2001) The effect of fruit and vegetable intake on risk for coronary heart disease. *J Ann Intern Med* **134**, 1106–1114.
93. Filippis F, Vannini L, Storia A, *et al.* (2014) The same microbiota and a potentially discriminant metabolome in the saliva of omnivore, ovo-lacto-vegetarian and vegan individuals. *PLOS ONE* **9**, e112373.
94. Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. Report on Health and Social Subjects no. 41. London: HMSO.
95. Prospective Studies Collaboration (2002) 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* **360**, 1903–1913.
96. Appel LJ, Brands MW, Daniels SR, *et al.* (2006) Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension* **47**, 296–308.
97. Stroke Association (2016) State of the Nation: Stroke statistics. https://www.stroke.org.uk/sites/default/files/state_of_the_nation_2016_110116_0.pdf (accessed August 2016).
98. Saka Ö, McGuire A & Wolfe C (2009) Cost of stroke in the United Kingdom. *Age Ageing* **38**, 27–32.
99. Gee LC & Ahluwalia A (2016) Dietary nitrate lowers blood pressure: epidemiological, pre-clinical experimental and clinical trial evidence. *Curr Hypertens Rep* **18**, 17.
100. Mills CE, Khatri J, Maskell P, *et al.* (2017) It is rocket science – why dietary nitrate is hard to Beet! Part II: further mechanisms and therapeutic potential of the nitrate–nitrite–NO pathway. *Br J Clin Pharmacol* **83**, 140–151.
101. Rose G (1985) Sick individuals and sick populations. *Int J Epidemiol* **14**, 32–38.
102. Bondonno CP, Liu AH, Croft KD, *et al.* (2014) Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. *Am J Hypertens* **28**, 572–575.
103. Crews DC, Planting LC, Miller ER, *et al.* (2010) Prevalence of chronic kidney disease in persons with undiagnosed or prehypertension in the United States. *Hypertension* **55**, 1102–1109.

Nutrition Research Reviews

104. Bryan NS, Torregrossa AC, Mian AI, *et al.* (2013) Acute effects of hemodialysis on nitrite and nitrate: potential cardiovascular implications in dialysis patients. *Free Radic Biol Med* **58**, 46–51.
105. Björne H, Govoni M, Törnberg DC, *et al.* (2005) Intragastric nitric oxide is abolished in intubated patients and restored by nitrite. *J Crit Care Med* **33**, 1722–1727.
106. Weitzberg E, Hezel M & Lundberg JO (2010) Nitrate–nitrite–nitric oxide pathway; implications for anethesiology and intensive care. *Anesthesiology* **113**, 1460–1475.
107. Ahluwalia A, Gladwin M, Coleman G, *et al.* (2016) Dietary nitrate and the epidemiology of cardiovascular disease: report from a National Heart, Lung, and Blood Institute Workshop. *J Am Heart Assoc* **5**, e003402.

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