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Title: 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/d. The average nitrate intake in the United Kingdom is approximately 70 mg/d, although some population groups, such as vegetarians, may consume 3 times that amount. However, recent studies in the last decade suggest that dietary nitrate can significantly reduce systolic blood pressure via the nitrate-nitrite-nitric oxide pathway. A small, downward shift in systolic blood pressure across the population could significantly reduce the incidence of hypertension and mortality from cardiovascular disease 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 cardiovascular disease. Recent evidence is mainly focussed 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.

250 words

Introduction

Inorganic nitrate has traditionally been considered as an inert contaminant in food and water. This anion is formed as part of the nitrogen cycle by the action of nitrogen 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 cardiovascular disease, renal disease and dementia. In the United Kingdom (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 ten 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 (nitric oxide synthase 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 nitrate, with up to 480 mg/100g[(4)]. In contrast, the nitrate content of legumes such as peas can be as low as 3 mg/100g[(4)] (Table 1). 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 nitrogen fertilizer applied also affects nitrate content[4]. Vegetables produced from soil high in organic matter, even where no chemical fertilizer is applied, can have higher nitrate content than the same species grown in chemically fertilized 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/litre[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 United States of America[13]. Guidelines for nitrate in drinking water were mainly promulgated to protect bottle fed infants from developing methemoglobinemia, or cyanosis. Higher concentrations of nitrate in blood are associated with oxidation of haemoglobin in erythrocytes and, if the level approaches 20% of total haemoglobin, methemoglobinemia 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 enzymatic 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 methemoglobinemia, suggesting that gastrointestinal infection and inflammation as well as overproduction of endogenous nitric oxide (NO) may be the cause of this condition[15]. Furthermore, other compounds such as vitamin C appear to be a protective factor against methemoglobinemia in infants[13]. Current opinion suggests that given the apparently low incidence of possible water-related methemoglobinemia, 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 of 3.7 mg/kg/d is harmful to human health and this is reflected in UK[7] and EC legislation[4]. An Acceptable Daily Intake (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 low, the oral lethal dose of nitrate for humans has been reported at ~330/mg/kg/d (equivalent to ~23,100 mg /70 kg adult/d)[18]. Due to a lack of new data, the European Food Safety Authority accepted the previous Acceptable Daily Intake 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 minimize the use of nitrate fertilizers 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 Food Frequency Questionnaire 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 ~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 ~220 g/d[24]. Taking a mean nitrate concentration of all vegetables, including potatoes, as 33.6 mg/100g[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/d or ~260 mg/d/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 3 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 3 times higher (mean 340 mg/d, range 37-2054 mg/d) than non-vegetarians (mean 125mg/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 emphasized 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 cardiovascular disease[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 1960's and 1970's 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 cardiovascular disease and different types of cancer(33). In accordance with this, Bryan *et al.* 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(31). 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 hours after ingestion(35). Subsequently, part of this nitrate is actively taken up by the salivary glands, concentrated up to 20 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 red blood cells may be involved(36). When swallowed, the acidic environment of the stomach facilitates the reduction of nitrite to NO and other nitrogen 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 haemoglobin, 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 enzymatic and oxygen -dependent pathway, where L-arginine is reduced to NO by several forms of nitric oxide synthase (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 (ROS) which scavenge NO[43]. This reduced NO bioavailability is a major risk factor for both hypertension and cardiovascular disease[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 to the amount of L-arginine in the normal diet (~ 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 focussing on the effect of dietary nitrate on NO metabolism and vascular function. From this viewpoint, a study by Carlstrom *et al.* found that nitrate administration was able to increase bioavailability and reverse cardiovascular dysfunction in mice with impaired function of the L-arginine–NOS pathway[48]. 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 humans 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 2006-2013 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 comorbidities who were given pharmacological (sodium or potassium nitrate) or dietary (beetroot juice) supplements. Similarly, a review by Hobbs *et al.* of studies up to 2012 found a significant inverse relationship between nitrate dose and systolic blood pressure[53]. 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 ~500 mg nitrate) in a wide range of participants, including patients with hypertension, chronic obstructive pulmonary disease (COPD) 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 is sufficient data to state that dietary nitrate, at least from vegetables, should be re-considered as a new nutrient, rather than a harmful contaminant[3].

Accordingly, it is been suggested that the key element of the cardioprotective effect of diets rich in vegetables is dietary 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.* found that differences in systolic blood pressure (~6-7 mmHg) between vegans and lacto-ovo vegetarians compared with omnivores remained after adjusting the data for age, gender and BMI[73]. These authors also suggested that various factors such as the higher fibre and potassium 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 potassium or magnesium supplements on blood pressure have not been confirmed and these are not recommended for treatment of hypertensive patients[74,75]. Low ingestion of sodium 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 people 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 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 ~ 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 ~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.* found that 70 ml of concentrated beetroot juice, which was expected to provide between 300-400 mg of nitrate, in fact yielded only 165 mg(59). This could be due to environmental factors (season of the year, light, irrigation of the field), the use of fertilizers 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.* investigated the effect of two different doses providing 248 and 744 mg of nitrate, respectively(89). 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 ~500 mg of nitrate (Table 3). This amount clearly exceeds the

Acceptable Daily Intake of 3.7 mg/kg/d (~260 mg/d/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.* estimated that two servings a day of vegetables such as spinach and greens provide over 1000 mg of nitrate[3]. However Hord's estimates assumed a maximum nitrate content of those vegetables[3].

More precisely, Lidder & Webb advocate daily consumption of at least 1 portion of high nitrate vegetables to increase nitrate intake in order to lower blood pressure[27]. Oyebode *et al.* suggest that the current UK '5-a-day' message could be amended to specifically advise on numbers of portions of vegetables[90]. This would be in line with Australian government recommendations, which advise Australians to eat 5 portions of vegetables and 2 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 one month. Although several epidemiological studies have linked high consumption of vegetables (especially green leafy vegetables)[29,92], with a lower risk of cardiovascular disease, 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/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 humans 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 humans, 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 (~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 cardiovascular disease, so that an increase of adult systolic blood pressure by 20 mmHg is associated with doubling of death rate from cardiovascular disease such as stroke[95]. Conversely, a reduction of 10 mmHg in systolic blood pressure is associated with ~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 the 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-nitric oxide pathway. For example, 3-7 days 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 cardiovascular disease 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 US, 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[(104)]. However, critically ill patients often receive nutritional support as either enteral or parenteral nutrition, which contain little or no nitrate[(26,105)]. 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 United States of America) 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 Acceptable Daily Intake 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 cardiovascular disease 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 cardiovascular disease 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 implications 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 cardiovascular diseases such as stroke.

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Conflicts of interest.

None

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

Food (raw, unless stated)	Mean nitrate content[a] (mg/kg)	Average portion size (80g) equivalent household measure[b]	Nitrate content per portion (mg)
'High' nitrate vegetables			
Lettuce (round)	3347	1 cereal bowl	268
Rocket	3289	1 cereal bowl	263
Swiss chard	2367	3 heaped tablespoons shredded	189
Spinach	1886	1 cereal bowl	151
Celery	1390	1 stick	111
Fennel	1024	One third average bulb	82
Beetroot	1211	2 small whole	97
Beetroot (boiled)	906	2 small whole	73
Curly kale	537	3 heaped tablespoons shredded	43
Spinach (boiled)	468	4 heaped tablespoons	37
Leeks	345	1 medium trimmed	28
Broccoli	279	2 spears	22
'Low' nitrate vegetables			
Carrots	97	3 heaped tablespoons	8
Onion	48	1 medium	7
Green Pepper	92	Half medium pepper	7
Cucumber	79	5 cm piece	6
Cauliflower (boiled)	46	8 florets	4
Peas	30	3 heaped tablespoons	2
Tomatoes	17	1 medium	1

[a] Data obtained from European Food Safety Authority(4), Ministry Agriculture Fisheries and Food(5), Santamaria *et al.* (6) & Food Standards Agency(7)

[b] Data from NHS Choices(8).

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/100g)
Fresh spinach		350
Frozen spinach		200
Lettuce (excluding 'Iceberg' type)	Harvested 1st October to 31st March:	500
	Grown under cover	400
	Grown in the open air	
	Harvested 1st 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 1st October to 31st March:	700
	Harvested 1st April to 30 September:	600
Processed cereal- based foods and baby foods for infants and young children		20

Table 3. Summary of research studies on nitrate and health with significant ($P < 0.05$) positive effects on systolic and diastolic blood pressure, 2013-2016

Supplement	Mean nitrate dose* (mg/d)	Mean reduction SBP (mmHg)	Mean reduction 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 (3 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]
250g 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	~7mg/kg Mean dose not reported	8	NS	28	Moderate risk of cardiovascular disease	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 ~20% & 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	↓ Absolute values not reported.	1	Healthy	28	18		Jonvik *et al.*[70]

SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, non-significant; COPD, Chronic obstructive pulmonary disease.

*Mean nitrate dose of all studies ~ 500 mg/d, range 139-1042 mg/d.

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	>/= 3 CV risk factors	56	23	Reduced triglycerides	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; NS, non-significant; CV, cardiovascular; COPD, Chronic obstructive pulmonary disease.

*Mean nitrate dose of all studies ~600 mg/d, range 420-840 mg/d.