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The role of inorganic nitrate and nitrite in cardiovascular disease

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Abstract

Cardiovascular disease is the leading cause of death worldwide, a consequence of mostly poor lifestyle and dietary behaviours. Although whole fruit and vegetable consumption has been consistently shown to reduce cardiovascular disease risk, the exact protective constituents of these foods are yet to be clearly identified. A recent and biologically plausible hypothesis supporting the cardio-protective effects of vegetables has been linked to their inorganic nitrate content. Approximately 60-80% inorganic nitrate exposure in the human diet is contributed from vegetable consumption. Although inorganic nitrate is a relatively stable molecule, under specific conditions it can be metabolised in the body to produce nitric oxide via the newly discovered nitrate-nitrite-nitric oxide pathway. Nitric oxide is a major signalling molecule in the human body, and has a key role in maintaining vascular tone, smooth muscle cell proliferation, platelet activity and inflammation. Currently, there is accumulating evidence demonstrating that inorganic nitrate can lead to lower blood pressure and improved vascular compliance in humans. The aim of this review is to present an informative, balanced and critical review of the current evidence investigating the role of inorganic nitrate and nitrite in the development, prevention and/or treatment of cardiovascular disease. Although there is evidence supporting short term inorganic nitrate intakes for reduced blood pressure, there is a severe lack of research examining the role of long-term nitrate intakes in the treatment and/or prevention of hard cardiovascular disease outcomes, such as myocardial infarction and cardiovascular mortality. Epidemiological evidence is needed in this field to justify continued research efforts.

Introduction

Despite major medical research advancements over the past 50 years, cardiovascular disease (CVD) remains the leading cause of death worldwide and is responsible for 39% of non-communicable disease (NCD) deaths in populations aged under 70 years old[1]. The leading NCD risk factor is hypertension, which is responsible for 13% of global deaths each year and is a major risk factor for coronary artery disease (CAD), ischemic heart disease (IHD) and stroke[1].

The pathogenesis of CVD is influenced by a variety of risk factors that can be broadly categorised as either modifiable or non-modifiable[2]. Non-modifiable risk factors cannot be controlled through intervention and include advancing age, gender (men at greater risk than pre-menopausal women; post-menopausal women at greater risk than men), ethnicity and family history of CVD[2]. Modifiable risk factors on the other hand, have the ability to be manipulated through intervention in order to control, treat or modify the risk factor[2]. Established modifiable risk factors for CVD include hypertension, tobacco use, raised blood glucose, physical inactivity, unhealthy diet, raised blood cholesterol/lipids and overweight and obesity[2].

Implementation of various lifestyle strategies which target specific modifiable risk factors can reduce the risk of CVD by up to 80%[1; 2]. Thus indicating that CVD is a chronic and mostly lifestyle induced disease, to which the majority of current mortality is the consequence of previous exposures to behavioural risk factors such as inappropriate nutrition, insufficient physical activity and tobacco exposure [2; 3; 4; 5]. In addition, excess weight and central obesity, increased blood pressure, dyslipidaemia, diabetes and low cardiorespiratory fitness are among the factors contributing principally to CVD risk[2; 6].

Given the scope and prevalence of CVD within our current food and lifestyle environment, it is clear that preventative measures are the most appropriate to deal with this global health issue in order to reduce the costs to both the community (through improved quality of life) and governments through a reduction in hospitalizations, medication use and rehabilitation[2]. Although behavioural factors such as smoking cessation and increased physical activity appear relatively straight forward targets for public health preventative interventions, the definition of a perceived "healthy" diet has changed over time leading to a general sense of public confusion and uncertainty surrounding the topic [7; 8].

Currently, the most compelling dietary evidence for CVD prevention is linked to whole diet approaches such as the Mediterranean and DASH (Dietary Approaches to Stop Hypertension) diets [7; 9]. Although the cardio-protective effects of these diets may be credited to a whole diet/whole food effect, some individual nutritive components of these foods have also been extensively investigated.

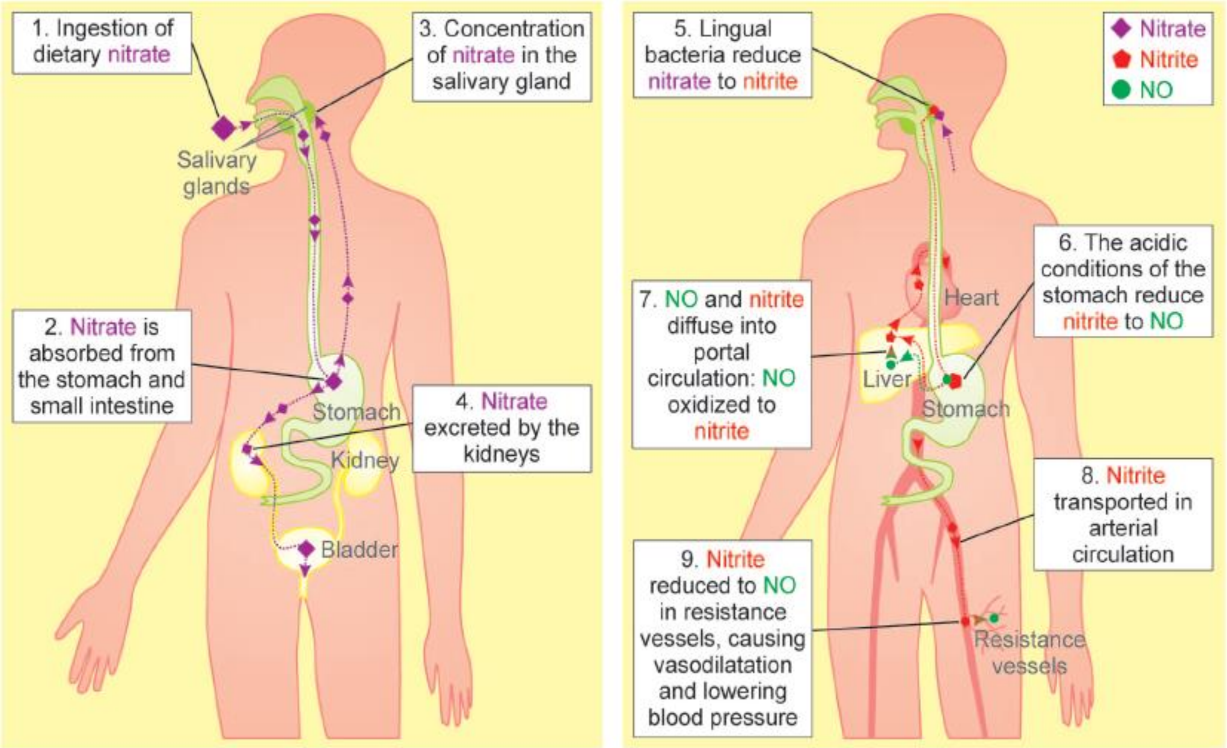
The investigation of single nutritive components demonstrates the evidence is less clear, this is especially noticeable for fruit and vegetable constituents. While whole fruit and vegetable consumption has been consistently shown to reduce CVD risk, as evidenced by various prospective studies showing a direct inverse association between fruit and vegetable intakes and the development of CVD events such as myocardial infarction (MI) and stroke[10; 11; 12; 13], the various constituents of fruits and vegetables such as vitamin C, polyphenols, fibre and antioxidants are yet to clearly demonstrate a beneficial link or a physiological pathway for their individual effect[14; 15; 16; 17; 18].

A recent and biologically plausible hypothesis for the cardio-protective and blood pressure lowering effect of vegetables has been linked to their inorganic nitrate (NO_3^-)/nitrite (NO_2^-) content [19]. Support for this hypothesis has been implied in studies indicating that nitrate rich green leafy vegetables and vitamin C rich fruits and vegetables contribute most to the apparent cardiovascular protective effect of total fruit and vegetable intake [20; 21]. Additionally, cardio-protective diets including the DASH, Mediterranean and Traditional Japanese diets have been shown to naturally contain high quantities of inorganic nitrate (147-1222 mg/d) relative to a typical Western style diet (~75mg/d) [22; 23; 24].

Within the human body, inorganic nitrate/nitrite (NO_x) can be metabolised to produce nitric oxide (NO) (Figure 1.) [25; 26]. NO is a highly valuable signalling molecule and has been demonstrated to mediate favourable effects on blood pressure control, platelet function, vascular health and exercise performance [27; 28; 29; 30]. In addition, the utility of inorganic NO_x as a NO donor may be of particular relevance given that one serving of nitrate rich vegetables (such as beetroot) has been estimated to produce more NO under specific conditions than can be endogenously formed by the classical L-arginine-Nitric-Oxide-Synthases pathway each day[19; 31; 32].

Currently, the true effect dietary/inorganic NO_x may have on CVD risk factors and outcomes is poorly understood, but it is a highly worthwhile line of investigation given that an increased daily consumption of nitrate intake represents a potential low cost and simple treatment option for reducing CVD burden.

Figure 1. The fate of dietary nitrate. Nitrate is systematically absorbed becoming concentrated in the salivary glands and part of the salivary circulation. Salivary nitrate is reduced to nitrite by oral bacteria. In the stomach nitrite may produce NO. Nitrite transported in arterial circulation can be reduced to NO in low oxygen concentrations which can lead to vasodilation and reductions in blood pressure (from Webb et al. 2008 [33]).



Production of nitric oxide in the body

Endogenous production via the L-arginine nitric oxide synthase pathway

The notion that NO_x could be produced endogenously in the body was first considered in the early 1980s, upon finding that NO_x excretion was exceeding quantities of ingestion in animal and human models [34; 35]. Later it was demonstrated that L-arginine was the substrate for synthesizing nitrogen oxides endogenously via the action of NO synthase (NOS) enzymes [36].

In healthy individuals the L-arginine-NOS pathway can produce sufficient quantities of NO to maintain health (approximately 1.7 mmol/day)[31; 32]. However, conditions such as diabetes mellitus, aging, hypercholesterolemia and tobacco exposure have been found to impact the bioactivity of endogenously produced NO via one or more of the following functions [37; 38; 39; 40; 41; 42]:

- Increased degradation of NO [38; 42; 43]
- Altered phosphorylation and activation of NOS [38; 43]
- Increased production of NOS inhibitors (eg. Asymmetric Dimethylarginine (ADMA)), leading to disruption of NOS activation [38; 39; 41; 42; 43]
- Deficiency of NOS substrate, L-arginine [34; 38; 41]
- Reduced availability of one or more cofactors essential for NOS function [34; 38]

While appropriate medical management, consumption of a healthy diet and moderate exercise can somewhat reverse these effects, it has been postulated that supplementing portions of the NOS pathway may enhance NOS activity and NO production [38; 41; 43]. This has been of particular importance given that increased ADMA levels inhibit NOS function and has been cited as the strongest risk predictor of cardiovascular events, and all cause and cardiovascular mortality in people with CAD[44]. Although it remains unclear whether a change in ADMA can alter CVD risk, interventions such as L-arginine supplementation have been shown to improve endothelial-mediated vasodilation in people with elevated ADMA levels[41; 44].

As a result, the effect of L-arginine supplementation has been investigated and short term supplementation was shown to improve endothelial function and relieve symptoms in patients with coronary heart disease[45]. Long-term (6 months) supplementation however, demonstrated no beneficial effect[46]. In fact the long-term L-arginine supplementation lead to increased rates of death and less cardiovascular improvements compared to the placebo due to the development of arginine toxicity and hyperkalemia (abnormally high serum potassium)[47; 48]. In addition, the utility of supplementing arginine is questionable given that arginine is classified as a “semi essential” or “conditionally essential” amino acid, depending on the developmental stage or health status of the individual[49]. However, it is generally accepted that healthy adults should not need to supplement with arginine as their bodies produce physiologically sufficient amounts[48]. Arginine is also highly abundant in the diet, as rich dietary sources include meat, dairy, vegetables, legumes and wholegrains[48; 49].

The "arginine paradox" appears to address this notion, as it refers to the phenomenon that exogenous arginine causes NO mediated biological effects, despite the fact that NOS are theoretically saturated in the substrate L-arginine [49]. A recently published cross-sectional study including 2771 men and women investigated whether regular dietary intakes of L-arginine were associated with serum NOx, as an indicator of systemic NO production [50]. This study found that increased dietary L-arginine intakes were strongly associated with serum NOx, which was independent of the overall dietary patterns of the study participants and other dietary factors, including intakes of high nitrate containing foods (likely due to collection of fasting blood samples) [50]. Therefore, although there may be some utility in consuming adequate amounts of arginine, which is readily achieved by consumption of a healthy balanced diet, there appears to be no great benefit for the general population to be using arginine supplements. However, dietary intervention to also consume nitrate rich foods holds much promise for supplementing the NOS pathway via the alternative nitrate-nitrite-NO pathway.

The nitrate-nitrite-NO pathway:

Up until the early 1990s, plasma NO_x were considered to be biologically inactive end products of NO production in the human body. However it is now clear that under specific conditions nitrate and nitrite anions can be recycled in vivo back to NO[26; 27; 51; 52].

With a bioavailability of 100%, ingested inorganic nitrate is swiftly absorbed in the proximal small intestine leading to significantly raised plasma nitrate concentrations for a period of up to 5-6 hours post nitrate ingestion [27; 33; 53; 54; 55]. About 75% of this nitrate is excreted at the kidneys, however the other 25% of plasma nitrate is actively extracted by the salivary glands, leading to salivary nitrate concentrations which are 10-20 times higher than plasma nitrate concentrations [27; 43; 55; 56; 57]. Salivary nitrate accumulation must occur in order for nitrate to be reduced to nitrite, as anaerobic bacteria in the oral cavity use nitrate as an alternative electron acceptor to oxygen during respiration [27; 55; 56; 58]. When this nitrite rich saliva is swallowed it is reduced in the acidic stomach to produce nitrogen oxides including NO [26; 27; 52; 59]. Today, this process is widely known as the nitrate-nitrite-NO pathway, and is thought to be one of the body's major sources of NO generation, especially in situations when NO bioavailability via the conventional L-arginine-NOS pathway is compromised. In addition it has been suggested that the nitrate-nitrite-NO pathway may play a significant role in maintaining levels of bioactive NO and may be critical for maintaining cardiovascular homeostasis in the body [27; 53; 60].

Noteworthy factors other than inorganic nitrate and nitrite consumption which have been shown to facilitate the nitrate-nitrite-NO pathway include:

- **The entero-salivary nitrate cycling:** Approximately 25% of plasma nitrate is actively taken up by the salivary glands leading to significant nitrate accumulation in the saliva. Within the oral cavity, anaerobic bacteria reduce nitrate to nitrite via the action of nitrate reductive enzymes. Nitrite rich saliva must be swallowed to produce NO in the acidic stomach.

  The importance of this salivary nitrate cycling has been demonstrated in studies where subjects spat after a dietary load of inorganic nitrate, preventing the opportunity for nitrate to accumulate in the saliva and be reduced to nitrite, therefore preventing NO production and any beneficial effects [25; 33; 61].
- **Presence of anaerobic bacteria:** Mammalian bacteria can utilise nitrate as an alternative electron acceptor to oxygen during respiration, and is a vital component of the nitrate-nitrite-NO pathway as human cells lack the required nitrate reductase enzymes [61]. The importance of these bacteria has been further established in studies of germ free rats, in which gastric NO formation was negligible post dietary nitrate load [62]. Additionally, human studies have demonstrated that the use of commercial antibacterial mouthwash in humans abolished any blood pressure lowering effects of a dietary nitrate load indicating that the mouthwash killed off the commensal facultative bacteria in the mouth, thus preventing the production of nitrite and NO leading to a loss of beneficial health effects [63; 64; 65].
- **Hypoxic conditions:** The rate in which nitrate is reduced to nitrite is 30 times greater during conditions of low oxygen tension, as the oral bacteria use salivary nitrate as an alternative electron acceptor to oxygen during respiration[65]. Xanthine oxidoreductase (XOR) has also been shown to catalyse the reduction of nitrite to NO in hypoxic conditions [66; 67; 68].

  This could also account for the increased production and utility of NO seen in exercising skeletal muscle or during myocardial ischemia [52; 61; 69].

  It is also important to note that plasma nitrite can be reduced to NO along the physiological oxygen gradient of the circulatory system [70]. Specifically, deoxygenated haemoglobin in the peripheral circulation can act as a nitrite reductase for NO production, as it has been revealed that as haemoglobin deoxygenation increases, more NO is produced [71; 72; 73]. This provides an explanation for how various human studies have observed vasodilation post a NO_x load, in healthy subjects at rest [33; 74].

- **Acidic conditions:** Nitrite in the acidic stomach has been shown to spontaneously decompose to NO, a reaction that appears to increase in conditions of reduced pH (increased acidity)[26]. The importance of an acidic stomach for this reaction has been demonstrated in a study, showing that NO production via nitrite protonation was inhibited in individuals using proton pump inhibitors (medications which reduce the acidity of gastric juices) [75].
- **Presence of reducing agents including vitamin C and polyphenols:** Both vitamin C and polyphenols are abundant in a vegetable rich diet, and their presence in the diet has been shown to favour the formation of NO via the nitrate-nitrite-NO pathway and prolong the half-life of NO in the stomach [76; 77].

Sources of dietary inorganic nitrate and nitrite:

Nitrogen is vital to life on Earth and can undergo many chemical and biological changes in order to be amalgamated into living and non-living material. An essential form of environmental nitrogen includes inorganic nitrate, as an adequate nitrate supply in the soil is essential for plant growth [43; 78].

The two major determining factors of the nitrate content of vegetables and fruit, include their species and the amount of available nitrate in the soil [43]. Some species of vegetables such as green leafy vegetables (mean nitrate ~ 975-3624 mg/kg) and beetroot (mean nitrate ~ 1992 mg/kg) are naturally high in nitrate, however environmental factors can lead to great variation among samples [22]. These factors include seasonal differences and disruption to normal plant growth, leading to nitrate accumulation in the plant leaves, stems and stalks, due to changes in the photosynthetic conversion of plant nitrate to amino acids [78; 79; 80]. Therefore, established factors shown to effect the normal growth of plants include drought conditions, high temperatures, shady and cloudy conditions, deficiency of soil nutrients, and excessive soil nitrogen [43]. Additionally, farming practices leading to damaged produce, early harvest, storage and transport conditions, processing and cooking practices will also result in significant variation in vegetable and fruit nitrate content [43].

European based studies have demonstrated that organically grown vegetables have a lower nitrate content than conventionally grown crops, despite the fact that organic fertilizers may cause high nitrate levels in vegetables, depending on the types and amount of organic fertilizers applied [81]. A California based study by Muramoto et al (1999) reiterated this notion, as it found spinach grown and harvested during the same season and under the same farming practices had a wide range of nitrate contents. This range appeared greatest in organic spinach, in which the maximum nitrate content measured was 3000 mg/kg, which was five times higher than the minimum (600 mg/kg) [81].

However, this study also demonstrated that conventionally grown spinach contained on average 30% more nitrate than spinach grown organically, a result most likely explained due to the wide use of nitrogen containing fertilizers in conventional farming [81].

Muramoto et al. also found a statistically significant seasonal difference in the nitrate content of iceberg lettuce, as winter samples were found to have on average 52% more nitrate than summer samples [81]. This finding is consistent with Ekart et al (2013), which found lettuce harvested during summer had a statistically significant lower nitrate content than lettuce harvested during winter (summer harvest:1209 mg/kg, winter harvest: 2164 mg/kg) [82]. In addition, Ekart et al found that washing leafy greens reduced the nitrate content of foods on average by 19%. Other processing such as boiling, blanching and sautéing, were found to significantly reduce the nitrate content of spinach by 53%, 36% and 30% respectively [82]. A finding which could be partly explained due to the water soluble nature of inorganic nitrate [83].

Due to the high variability of nitrate within plant species, accurate and reliable nitrate intake measured from fruit and vegetable consumption is difficult to predict. Despite this, combined vegetable and fruit intake is the major source of exogenous inorganic nitrate exposure and are predicted to constitute 30-90% of total nitrate intake [84]. Other sources of nitrate intake include drinking water and meat products, however their nitrate content is highly regulated to comply with strict government limits [85; 86; 87; 88; 89].

Nitrate occurs naturally in the water supply, however in most developed countries water nitrate is generally present in concentrations much lower than allowed in the water guidelines (≤50 mg/L) [85; 86; 88]. Therefore, nitrate from the water supply is unlikely to contribute significantly to total nitrate intake in comparison to food sources.

Nitrate and nitrite salts (e.g. potassium nitrite/ sodium nitrate) have been used as food additives in cured meats for many years due to its effectiveness in ensuring microbial safety and its ability to enhance the flavour and appearance of the product [43]. The maximum levels of nitrate and nitrite allowed as a food additive have been defined (Table 1) [85; 90; 91; 92].

It has been estimated that approximately 60-80% of dietary nitrates are derived from vegetables (mainly green leafy and root vegetables) indicating that vegetable intake tends to contribute the greatest quantities of dietary nitrate (Table 2) [22; 93]. This has been further implied by dietary patterns such as the DASH diet, Mediterranean, vegetarian and traditional Japanese diets which tend to include high quantities of vegetables (5 or more serves/d) and provide approximately 147-1222 mg nitrate per day [22; 23; 24]. This is a relatively high nitrate intake compared with the typical Western style diets which tends to be low in vegetables (1-3 serves/d) and provides around 60-75 mg nitrate per day [24]. In addition, processed and cured meats are frequently cited as the major dietary source of nitrite (Table 3) [22; 25; 84; 94], followed by various fruits and vegetables (Table 2, 4 and 5), which have been physically damaged or poorly stored as enzymes present in the plant tissues and/or contaminating bacteria facilitate the reduction of nitrate to nitrite [43; 85] .

Nitric oxide in the cardiovascular system:

Within the cardiovascular system, basal endothelial NO has a critical role in maintaining cardiovascular health as it controls vascular tone, smooth muscle cell proliferation and growth, platelet activity and aggregation, leukocyte trafficking, expression of adhesion molecules, and inflammation [34; 94; 95; 96; 97; 98; 99]. However, when the bioavailability of NO is compromised, the beneficial effects of NO are lost and endothelial dysfunction predominates due to the imbalance created between the release of vasoconstrictors and vasodilators (such as NO) [53; 100; 101]. This idea has been supported in a study conducted by Kleinbongard et al (2005) which found that plasma nitrite levels are a reliable indicator of endothelial dysfunction and correlate with cardiovascular risk factors in humans [102]. Additionally, endothelial dysfunction has been strongly linked with atherosclerosis development and a number of cardiovascular disorders such as hypertension, coronary artery disease, congestive heart failure and peripheral artery disease in multiple longitudinal studies [53; 101; 103; 104; 105; 106; 107].

While in the past most of the evidence suggesting a relationship between endothelial dysfunction and clinical events from atherosclerosis development were considered “circumstantial”, more recently conducted cross-sectional studies have indicated that severe endothelial dysfunction of the arteries can trigger events of unstable angina and myocardial infarction [108; 109]. Al Suwaidi et al (2000) studied 157 patients with mild coronary artery disease for 2.3 years, and found an increased incidence of cardiovascular events in patients with impaired endothelium-dependent vasodilation (NO production of endothelium) of the coronary arteries [104]. In another study by Katz et al (2005), 259 subjects with chronic heart failure were assessed prospectively, to which endothelial dysfunction in chronic heart failure was found to significantly increase risk of mortality [110]. Thus supporting the notion that coronary endothelial dysfunction plays a role in the pathogenesis of coronary atherosclerosis, risk of cardiac events and death [104; 110].

Many factors are known to predispose endothelial dysfunction, due to reductions in NO concentrations and bioavailability in humans [34; 111; 112]. These factors are consistent with the modifiable and non-modifiable risk factors for CVD, including hypertension, hypercholesterolemia, diabetes, tobacco use, physical inactivity, consumption of unhealthy diets and increased age and gender (NO bioavailability is reduced in post-menopausal women, a period in which CVD risk is drastically increased in women) [34; 112; 113; 114; 115; 116; 117; 118; 119; 120]. Interestingly, improved endothelial function is a common feature of experimental intervention studies, which have been shown to reduce cardiovascular risk and improve endothelial dependent vasodilation in the coronary and peripheral circulation [108]. Such interventions commonly include use of lipid and blood pressure lowering medications, smoking cessation and increased physical activity [108; 117; 121; 122; 123; 124]. However, the notion that inorganic nitrate and nitrite either consumed from dietary sources such as green leafy vegetables or supplement is relatively new, and their therapeutic potential as a NO donor via the nitrate-nitrite-NO pathway remains unclear [112; 125].

Cardiovascular protective actions of nitric oxide:

Nitric oxide is non-polar and can diffuse freely across cell plasma membranes and is a key signalling molecule capable of many important functions acting primarily by stimulating intra-cellular receptors within the target cell [126].

Within the vasculature of the cardiovascular system, the primary role for NO's action is for the regulation of vascular function and blood pressure, a notion which has been clearly demonstrated in animal models in which synthesis of NO was blocked leading to persistently elevated blood pressure [112; 127]. In addition, this interaction has been demonstrated in some recently conducted short-term dietary nitrate trials in humans, which showed that peak blood pressure lowering effects were achieved in synchronization with peak plasma concentrations of nitric oxides (NOx) post a dietary nitrate load [28; 33; 128].

The cellular pathway in which NO exerts this vasodilatory action is well established. Nitric oxide rapidly diffuses across vascular smooth muscle cell membranes. Within the smooth muscle cells, NO binds to and activates guanylyl cyclase to produce cyclic guanosine monophosphate (cGMP) [126]. Once produced, cGMP can have a number of effects in the cells, but many of these effects are mediated thought the activation of protein kinase G (PKG). Activation of PKG via cGMP leads to the activation of myosin phosphatase which in turn leads to smooth muscle cell relaxation and vasodilation [126; 127].

In addition to regulating vascular tone, NO can facilitate many other important functions preventing the development of atherosclerosis, which include antiplatelet effects, anti-proliferative effects, anti-inflammatory, and anti-oxidant effects [127; 129; 130]. Although the cellular pathways for these actions are yet to be clearly defined, it is clear that NO is capable of binding to or reacting with a variety of chemical modalities within the cellular environment, including metal containing proteins, membrane receptors, ion channels, enzymes, transcription factors and oxygen species [127; 131].

Other Nitric Oxides and Possible Mechanisms in the Cardiovascular System:

While NO is the most widely cited bioactive metabolite underpinning the cardiovascular therapeutic benefits of dietary inorganic nitrates and nitrites, it has been suggested that other nitric oxides also play a role [25; 93]. This may be expected, given that dietary constituents in the stomach may react with each other in order to form a variety of bioactive compounds [25]. Examples of such compounds include; nitrated fatty acids, nitrosothiols and ethyl nitrite [25].

While the biological significance of these compounds are yet to be made clear, the following actions have been suggested:

- **Ethyl Nitrite:** Rat models have shown that ethanol from alcoholic drinks can interact with salivary-derived nitrite in the acidic stomach leading to the production of ethyl-nitrite [25; 132]. Ethyl-nitrate is a potent smooth muscle relaxant and may have a vasodilatory role in the cardiovascular system [132].
- **Nitrosothiols:** In the stomach, nitrite has been shown to induce S-nitrosation within the gastric compartment. S-nitrosothiols are thought to represent a circulating endogenous reservoir of NO acting as a NO donor [25].
- **Nitrated Fatty Acids (nitroalkenes):** Nitrogen oxides can react with unsaturated fatty acids to produce nitroalkenes. Analysis of synthetic nitroalkenes derivatives of oleic, linoleic and arachidonic acid reveals that these species possess unique chemical reactions which may support multiple cell signalling events such as vasodilation and reduced inflammation [25]. Such events may be mediated through their NO donor capabilities.

Currently the systemic capabilities of these bioactive nitrogen compounds remain uncertain, however it highlights a possible whole diet effect for exerting a beneficial effect on NO and other relevant cardiovascular signalling molecules. This notion is highlighted by Lundberg and Weitzberg (2010), indicating that various dietary constituents of the Mediterranean diet may interact in the stomach to produce these potentially therapeutic compounds, and may provide an additional explanation for the cardiovascular health benefits/protection seen with this dietary pattern [25; 93].

Inorganic versus organic nitrate and nitrite

Organic nitrates such as glyceryl trinitrate (GTN) and isosorbide mononitrate represent the first class of NO donors to reach the clinical setting and have been used extensively in the treatment of various cardiovascular conditions including angina, coronary artery disease and heart failure [(83)].

Unlike inorganic nitrates which are relatively simple molecules and naturally occurring in fruits and vegetables, organic nitrates are synthetic compounds produced by a reaction between nitric acid and an alcohol group [(83)]. Organic nitrates are complex, non-polar hydrocarbon chains attached to a nitrooxy-radical (-ONO_2), which is responsible for its biological effects (Table 6.) [(83)].

Once organic nitrates are introduced to the blood system, levels rise quickly leading to the rapid onset of their action [(83)]. At low doses (≤1.25mg/kg body weight) organic nitrate has been demonstrated to dilate large conductance veins and large arteries. While at high doses (2.5-5mg/kg body weight) organic nitrates can also induce dilation of the arterioles of the microcirculation [(83)]. These vasodilatory effects of organic nitrates have been shown to reduce cardiac work and lower myocardial oxygen requirements, which may alleviate or even prevent cases of myocardial infarction [(133)]. In addition, it has been suggested that organic nitrates have anti-aggregatory properties in patients with stable and unstable angina [(133)].

Today in clinical practice short acting organic nitrates most notably in the form of GTN are administered during the symptomatic treatment of myocardial infarction and angina [(83; 133)]. Glyceryl tri-nitrates are generally administered in the form of either a mouth spray or intravenous infusion, to which onset of action is rapid (2-3 minutes) [(133)]. Although short term treatment with organic nitrates has some positive impact on endothelial function, acute side-effects of their use include hypotension, dizziness, nausea and headache [(83)]. Also, despite the high potency of organic nitrates and their long history as being used to treat various cardiovascular diseases, nitrate tolerance is a huge limitation and an undesirable side effect of their use [(83; 133)].

Nitrate tolerance is a complex phenomenon and is poorly understood, however it is clearly a result of chronic organic nitrate use to which nitrovasodilator-responsiveness is lost [(83)]. Nitrate tolerance has been reported to occur within 1-3 days of continuous GTN treatment in patients with myocardial infarction, stable angina and chronic congestive heart failure [(133)]. Further, chronic organic nitrate use has also been linked to endothelial dysfunction, increased production of free radicals and development of vascular tolerance to other endothelium dependent vasodilators [(83)]. Although this phenomenon is poorly understood, recent animal and human studies indicate that increased vascular production of the superoxide anion (O_2^-) underlies the mechanism for tolerance [(133)]. This oxidative stress hypothesis of nitrate tolerance is supported by numerous reports demonstrating that the tolerance is prevented by co-administration of antioxidants (eg. vitamin C, vitamin E and folic acid) and interventions which inhibit reactive oxygen species (ROS) formation (lipid and blood pressure lowering medications) [133; 134; 135; 136].

It is interesting to note that the phenomenon of tolerance is not exhibited with the consumption of inorganic nitrates/nitrites, however despite showing promise in preventing or treating certain cardiovascular conditions, such as hypertension, they have received little attention by the medical community [27].

Inorganic nitrate and nitrite: From dietary contaminant to potential therapeutic nutrient

Throughout history, cases of accidental toxic exposure to nitrate and nitrite have been documented, however the health risk of excessive inorganic nitrate and nitrite consumption appears specific to population subgroups [22]. One of these subgroups includes infants aged less than 6 months, to which excessive nitrite exposure has been linked to cases of methemoglobinaemia (blue baby syndrome) [137]. As a result, strict regulatory limits have been established to govern the nitrate/nitrite content of the drinking water supply and their use as an additive to processed and cured meats in order to limit exposure to the population [85; 86].

Methemoglobinaemia can occur when nitrite oxidises ferrous iron (Fe^{2+}) in haemoglobin to the ferric state (Fe^{3+}), resulting in methemoglobin. Methemoglobin is incapable of binding molecular oxygen, and impairs oxygen delivery to the tissues causing hypoxia and cyanosis [137]. While most cases of methomeoglobinaemia have been attributed to the consumption of well water (prone to high nitrate accumulation) used for the preparation of infant formula, there have been reported cases of nitrate poisoning in infants from the ingestion of plant nitrates [86; 137]. While Martinez et al found that the use of certain high nitrate vegetables (herbs and green leafy vegetables) in infant homemade vegetable pureé increased methemoglobinemia in infants (herbs: OR 5.2; 95% CI 1.1-24.6; and green leafy vegetables: OR 2.0; 95% CI 0.4-8.7), the most important factor increasing methemoglobinemia was the time lapse between vegetable pureé preparation and consumption (OR 17.4, 95% CI 3.5-86.3 if pureé was prepared 24-48 hrs before and OR 24.9; 95% CI 3.3-187.6 if prepared >48 hours before) [138].

To date human nitrate and nitrite exposure studies have failed to prove a direct link with methemoglobinaemia, suggesting that nitrate/nitrite exposure alone may not be responsible for methemoglobinaemia development [139; 140].

Another population subgroup that is thought to be at health risk due to excessive nitrate/nitrite exposure are high consumers of cured and processed meats [22; 141]. It has been theorised that nitrates and nitrites from processed meats generate N-nitroso compounds which can be carcinogenic [142].

In October 2015 the International Agency for Research on Cancer (IARC) summarized more than 800 studies conducted globally, and determined that 50 grams of processed meat each day increased the risk of colorectal cancer by 18%, and therefore concluded that processed meats are carcinogenic [141]. In animal studies N-nitrosamines and related N-nitrosamides have been shown to be carcinogenic in a variety of molecular structures [143; 144]. However, such direct evidence demonstrating nitrate and nitrite as human carcinogens is severely lacking. This has been reflected in the conclusions of the Food and Agriculture Organisation expert committee who found no consistent increased risk of cancer with increasing consumption of nitrate, as available epidemiological studies did not provide evidence that nitrate is carcinogenic to humans [145].

Currently, researchers are interested in understanding whether the health risks associated with inorganic nitrates/nitrites outweigh the recently discovered health benefits, however there is a growing consensus that any weak and inconclusive data on inorganic nitrate/nitrite and cancer associations are far outweighed by the potential health benefits of restoring NO homeostasis [22; 84; 139; 143]. In particular this has been demonstrated in various animal and human experimental studies, in which inorganic NO_x has been shown to improve outcomes such as blood pressure, endothelial function, platelet function, ischemia reperfusion injury, exercise performance and host defence [143; 146; 147; 148; 149; 150; 151].

Evidence of cardiovascular benefit from animal studies:

Intakes of dietary inorganic nitrate have been shown to be strongly cardio protective in animal studies. Carlström et al (2011) indicated this in a four arm dietary intervention trial in rats [152]. The rats were placed on either a normal salt diet (control); a high salt diet; a high salt diet supplemented with a nutritional (low) dose of nitrate; and a high salt diet supplemented with a pharmacological (high) dose of nitrate for 8-11 weeks [152]. As expected, results demonstrated that chronic consumption of a high salt diet develops hypertension, however when combined with a low nitrate dose, blood pressure was non-statistically significantly lower [152]. On the other hand, the higher nitrate dose lowered blood pressure by a significant 24 mm Hg compared with the plain high salt diet, a magnitude of blood pressure reduction considerably magnified compared with blood pressure reductions observed in another study of healthy normotensive rats using the same nitrate dose [152; 153]. Similar results were reported by Kanematsu et al. finding that in hypertensive rats, antihypertensive effects were only apparent with the highest dose of nitrate, yet there was a strong tissue protective effect seen with lower doses equivalent to modest dietary intakes [154]. Ferguson et al. demonstrated clinically significant reductions in mean arterial pressure with beetroot juice (BJ) supplementation in exercising rats (Control: 137 ± 3; BJ: 127 ± 4 mm Hg, $P<0.05$), indicating that clinically significant blood pressure reductions may be achievable in doses attained from dietary sources [155].

In addition to significant blood pressure control, Carlström el at (2011) found dietary nitrate supplementation can partly prevent the development of cardiac hypertrophy and high nitrate doses significantly reduced the fibrotic changes which were observed in the high salt group, two factors which are major predictors of heart failure [152]. Two other studies found mice ingesting inorganic nitrate lead to a significantly reduced infarct size during myocardial ischemia, an important finding given that reduced infarct size is associated with lower heart failure risk post myocardial infarction and mortality [156; 157; 158].

When Baker et al. treated rats with an intra-venous bolus of sodium nitrite across various doses (0.04, 0.4, 1.0, 4.0, 7.0 and 10.0 mg/kg), prior to initializing a blockage of the coronary artery, there was a clear doses dependent effect of nitrite on infarct size[149]. However, it was intriguing to note protection was only found in doses up to 4.0 mg/kg, an effect which was absent at higher doses[149]. Rats administered with 4.0 mg/kg nitrite exhibited a significant 32% reduction in infarct size compared to controls [149]. Nitrite was also found most effective when administered before and/or during the ischemic event, but not at the onset of reperfusion[149]. Further, equivalent doses of sodium nitrate had no effect on infarct size[149]. Indicating that administration timing and doses are key considerations for nitrite protection from MI[149].

Thrombosis is largely a result of platelet adhesion, activation and aggregation, and is a common pathology underlying ischemic heart disease and ischemic stroke [159; 160]. Nitric oxide plays a key role in preventing thrombosis development[161]. Park et al. demonstrates this notion upon discovering an inverse correlation between NO_x levels and platelet activity/aggregation in mice [161]. In addition, Apostoli et al. examined the effect of inorganic nitrite on platelet aggregation in eNOS deficient mice [162]. This study found that inorganic nitrite exerts an antiplatelet effect during eNOS deficiency and suggest that dietary nitrate may reduce platelet hyperactivity during endothelial dysfunction [162].

Pulmonary hypertension can lead to the remodelling of the artery wall causing abnormalities of elastic fibres, intimal fibrosis and medial hypertrophy[163]. This can result in vascular stiffness and is a condition linked to the development of chronic heart failure[163]. Sodium nitrite interventions in lamb and mice models have shown reductions in pulmonary hypertension specifically during hypoxic conditions [164; 165]. However, Casey et al. found intravenous injections of sodium nitrite during normoxic-conditions could lead to reductions of pulmonary and systemic arterial pressure and increased cardiac outputs in adult male rats [166]. This suggests that sodium nitrite may have a role in reducing the workload of the heart during pulmonary hypertension and thus protects the heart and vascular system from associated damage and dysfunction [166].

Hendgen-Cotta et al. pre-treated mice with nitrate before inducing chronic limb ischemia, and nitrate supplementation was found to enhance revascularization and increased mobilization of circulating angiogenic cells (CACs), which are important for the recovery and maintenance of healthy endothelial function [167]. Heiss et al. on the other hand injected inorganic nitrite into healthy mice, and found that nitrite significantly increased CACs at 1 hour compared with controls [168]. It is interesting to note however that when this test was repeated in eNOS deficient mice, no CAC mobilization was observed, indicating that NOS may be required to take part in nitrate-mediated CAC mobilization [168].

In a study conducted by Sindler et al. the effect of nitrite in aged, but healthy mice was investigated and high dietary nitrite doses were found to reverse age-related vascular dysfunction, arterial stiffness and reduce levels of oxidative stress [169]. This is in line with Carlström et al (2011) which found key plasma and urinary oxidative stress markers (MDA, iPF2α-VI and 8-OHdG) were significantly reduced (despite co-consumption of a high salt diet) with both low (0.1 mmol nitrate/d) and high (1.0 mmol nitrate/d) dose dietary nitrate supplementation, which may be useful in preventing NO degradation and endothelial dysfunction [152; 170]. An interesting finding, given that oxidative stress is directly linked with an inflammatory response which is thought to have a central role in the development of atherosclerosis [93].

Stokes et al. found that mice fed cholesterol-enriched diets for three weeks tend to develop clear signs of vascular disease pathology, including elevated leukocyte adhesion and endothelial dysfunction, an effect which was prevented with nitrite supplementation in the drinking water [171]. In another study by Carlström et al (2010) it was demonstrated that several features of metabolic syndrome (including visceral fat and circulating triglycerides, which are strong risk factors for cardiovascular disease) can be reversed by dietary nitrate supplementation, in amounts which correspond to those derived from eNOS under normal healthy conditions or a vegetable rich diet [172].

Evidence of cardiovascular benefit from human studies:

In 2003 Cosby et al. conducted one of the first studies demonstrating a relationship between inorganic nitrite supplementation and blood pressure reductions in healthy human subjects [71]. This study chose to use sodium nitrite ($NaNO_2^-$) infusions providing approximately 75 mg $NaNO_2^-$ over two 15 minute periods, a dose which was found to significantly reduce mean blood pressure by 7 mm Hg ($P<0.01$) [71]. Similar findings were later established using sodium nitrate ($NaNO_3^-$) in a study conducted by Larsen et al. [173]. In this study healthy subjects consumed $NaNO_3^-$ (8.5 mg/kg/day for 3 days) as a dietary supplement, and although systolic blood pressure was not changed during this time compared with placebo (sodium chloride), diastolic blood pressure was significantly reduced on average by 3.7 mm Hg (P<0.02) and mean arterial pressure was lowered by 3.2 mm Hg (P<0.03) [173]. Soon after, Webb et al. investigated this topic further using beetroot juice (containing approximately 1400 mg inorganic nitrate) [33]. Results from Webb et al. showed a peak reduction in systolic blood pressure of 10.4 ± 3 mm Hg (P<0.01), a reduction in diastolic blood pressure of 8.1 ± 2.1 mm Hg (P<0.01) and mean arterial pressure reduction of 8.0 ± 2.1 mm Hg (P<0.01), thus indicating that significant blood pressure reductions are possible with the acute consumption of dietary inorganic nitrate in healthy subjects [33]. A notion which has been further supported by a recently conducted systematic review and meta-analysis which found inorganic nitrate and beetroot juice consumption were associated with greater changes in systolic blood pressure (-4.4 mm Hg (95% CI: -5.9, -2.8); P<0.001) than diastolic blood pressure (-1.1 mm Hg (95% CI: -2.2, 0.1); P=0.06) [174]. However it is important to note that these findings have not been consistent across the literature, as a few recently conducted randomised controlled trials have found inorganic nitrate consumption from either beetroot juice or from a high nitrate diet (rich in green leafy vegetables) for 1-2 weeks had little/no effect on the blood pressure of study subjects [57; 175; 176]. The exact cause of this variation across studies remains unclear, yet could be due to methodological differences including the study population (e.g. healthy subjects vs. hypertensive subjects) or the conditions in which NO_x was consumed (e.g. food vs supplement, dosing or altered environmental conditions such as exercise stress). Nevertheless, this question remains unclear and will require further investigation, in order to better understand the usefulness of dietary/inorganic nitrate/nitrite within the general population.

While the acute effects of dietary inorganic nitrate on blood pressure has been extensively investigated, very few studies have investigated long-term effects. Sobko et al. investigated the effects of a traditional Japanese diet on blood pressure which provided approximately 1140 mg of nitrate per day for a 10 day period [23]. The Traditional Japanese diet lead to a lower diastolic blood pressure than seen in the non-Japanese diet group (71.3 ± 7.9 vs 75.8 ± 7.8, P=0.0066), indicating that dietary inorganic nitrate consumption for longer-periods of time may have some blood pressure lowering effects in healthy people, however a 10 day intervention can hardly be classified as a long-term intervention [23]. In another four week intervention Kapil et al. assigned hypertensive patients to receive a daily dose of either 250 mL of beetroot juice or placebo (nitrate depleted beetroot juice)[29]. Notably, Kapil et al. found daily dietary nitrate supplementation to significantly reduced mean clinic blood pressure (7.7/2.4 mm Hg (3.6-11.8/0.0-4.9), P<0.001, P=0.05), mean 24-hour ambulatory blood pressure (7.7/5.2 mm Hg (4.1-11.2/2.7-7.7), $P<0.001$ for both) and mean home blood pressure (8.1/3.8 mm Hg (3.8-12.4/0.7-6.9), $P<0.001$, $P<0.01$) [29].

Currently, the longest intervention study conducted in this area is a 10 week intervention trial from DeVan et al [125]. In this study, healthy 50-79 year old subjects were recruited to consume either 0 mg, 80 mg or 160 mg of sodium nitrite per day for a 10 week period [125]. Results indicated no significant changes in blood pressure at week 10 compared with baseline blood pressure values, however a significant time by treatment effect for carotid diameter in the nitrite groups was detected, as well as improved endothelial function of the brachial artery, suggesting improved vascular function with chronic inorganic nitrite supplementation despite a lack of an effect seen with blood pressure [125]. However, it is worth noting that the only perspective cohort study on this topic conducted by Golzarand et al. found that a higher dietary intakes of nitrate containing vegetables (~427.6 g/day) in normotensive individuals may have a protective effect against the development of hypertension (Highest tertile of nitrate containing vegetables, OR: 0.63 (0.41-0.98), $P=0.05$)[177].

Endothelial dysfunction is one of the key early events involved in the development of atherosclerosis [178]. Flow mediated dilatation is commonly used as a measure of endothelial function as reduced flow mediated dilatation is an indicator of endothelial dysfunction (caused by reduced NO bioavailability) and has been associated with increased severity and duration of blood pressure elevations [179]. More recently, dietary inorganic nitrate interventions have been shown to significantly improve flow mediated dilatation in healthy and hypertensive humans consuming spinach, beetroot juice or sodium nitrate capsules [29; 168; 180; 181]. Joris et al. tested the effects of beetroot juice (containing approximately 500 mg nitrate) with a dietary load of fat (56.6 g fat) in overweight and obese subjects (BMI: 30.1 ± 1.9 kg/m^2) [182]. While the control drink group saw impaired flow mediated dilatation with dietary fat intake, the consumption of beetroot juice appeared to attenuate this impairment (Beetroot juice: $-0.37 \pm 2.92\%$ vs Control: $-1.56 \pm 2.9\%$, $P=0.03$) [182]. Additionally, flow mediated dilatation has been shown to be reduced by approximately 40% after vascular ischemia, however Ingram et al. has demonstrated that sodium nitrite pre-conditioning (providing nitrite dose prior to ischemic event) will prevent ischemic reperfusion injury by preventing reductions in flow mediated dilatation and endothelial dysfunction [183]. Similar findings have been reported by Kapil et al. and Webb et al. with beetroot juice pre-conditioning, indicating that higher plasma NO_x concentrations achieved by inorganic NO_x consumption may have a role for improving cardiovascular outcomes post vascular ischemic events [29; 33].

In addition to flow mediated dilatation, CACs have been identified as an important indicator of vascular endothelial function, as they have a critical role in vascular repair [184]. The number of CACs have also been shown to predict the occurrence of cardiovascular disease and death [168]. Therefore it is of interest to note that Heiss et al. have indicated an important role for dietary nitrate for increasing CACs , showing that a single dose of sodium nitrate (12.7 mg/kg body weight) can double the number of CACs 1-2 hours post nitrate ingestion [168].

Pulse wave velocity and augmentation index are accepted measurements of arterial stiffness and atherosclerosis, to which higher readings are associated with increased cardiovascular disease risk [185; 186]. The role for dietary inorganic nitrate in preventing arterial stiffness has been established, as Kapil et al. found a 4 week beetroot juice intervention to reduce pulse wave velocity and augmentation index in hypertensive subjects [29]. Zamani et al. also saw a significantly reduced augmentation index with beetroot juice consumption in patients with symptomatic heart failure (Beetroot juice: 132.2 ± 16.7%; Placebo: 141.2 ± 21.9%; mean change -9.1 ± 15.4%; P=0.03) [187]. Rammos et al. investigated the effect of a 4 week sodium nitrate supplementation trial in elderly volunteers with mild hypertension, and found that vascular stiffness was significantly improved in the nitrate supplemented volunteers [188]. This is a very significant finding given that vascular stiffness tends to naturally increase with age [189].

In an RCT conducted by Jones et al. participants prone to MI and undergoing primary percutaneous coronary intervention (non-surgical intervention to treat stenosis) were administered with either a high-dose bolus injection of $NaNO_2^-$ (1.8 µmol) or NaCl placebo[190]. The nitrite group experienced a significantly (P=0.05) improved myocardial savage index (established indicator of cardio protective benefit) relative to placebo[190]. In addition, a sub-set of participants which exhibited a blocked blood vessel experienced a 19% reduction in infarct size with nitrite treatment compared to placebo[190]. A one-year follow-up of study participants also found the nitrite group experienced a significant reduction in major adverse cardiac events ($NaNO_2^-$: 2.6% vs NaCl: 15.8%, P=0.04)[190].

Conclusion

Cardiovascular disease remains the major killer from any disease across the developed world. Currently the available evidence indicates a role for dietary nitrate for improving cardiovascular disease risk factors, a highly valuable finding given that dietary nitrate from beetroot and green leafy vegetables could represent a relatively simple and cost effective treatment/preventative strategy for reducing CVD and its sequelae. However, at present it remains unclear whether incidence of cardiovascular disease morbidity or mortality can be reduced with long-term dietary intakes of inorganic nitrate, as such evidence investigating this question directly has not yet been published. At present, there is an overwhelming need for epidemiological research to be conducted to identify the potential long-term effects of sustained inorganic nitrate and nitrite consumption on the development of cardiovascular disease and its consequences.

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Conflict of Interest

None

Authorship

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References

1. World Health Organization (2011) Global status report on noncommunicable diseases 2010. http://www.who.int/nmh/publications/ncd_report_full_en.pdf (accessed December 2015).
2. Buttar HS, Li T & Ravi N (2005) Prevention of cardiovascular diseases: Role of exercise, dietary interventions, obesity and smoking cessation. *Exp Clin Cardiol* **10,** 229.
3. Cordain L, Eaton SB, Sebastian A *et al.* (2005) Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* **81**, 341-354.
4. Barengo NC, Hu G, Lakka TA *et al.* (2004) Low physical activity as a predictor for total and cardiovascular disease mortality in middle-aged men and women in Finland. *Eur Heart J* **25**, 2204-2211.
5. Clair C, Rigotti NA, Porneala B *et al.* (2013) Association of smoking cessation and weight change with cardiovascular disease among adults with and without diabetes. JAMA **309**, 1014-1021.
6. Isomaa B, Almgren P, Tuomi T *et al.* (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes care* **24**, 683-689.
7. Carpentier Y & Komsa-Penkova R (2011) Clinical Nutrition University. The place of nutrition in the prevention of cardiovascular diseases (CVDs). *E Spen Eur E J Clin Nutr Metab* **6**, e272-e282.
8. Harnack L, Block G & Lane S (1997) Influence of selected environmental and personal factors on dietary behavior for chronic disease prevention: a review of the literature. *J Nutr Educ* **29**, 306-312.
9. Sofi F, Cesari F, Abbate R *et al.* (2008) Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* **337**, a1344.
10. Dauchet L, Amouyel P, Hercberg S *et al*. (2006) Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr* **136**, 2588-2593.
11. Verlangieri A, Kapeghian J, El-Dean S *et al.* (1985) Fruit and vegetable consumption and cardiovascular mortality. *Med Hypotheses* **16**, 7-15.
12. He FJ, Nowson CA & MacGregor GA (2006) Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* **367**, 320-6.
13. Bazzano LA, He J, Ogden LG *et al.* (2002) Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Am J Clin Nutr* **76**, 93-9.
14. Wang X, Ouyang Y, Liu J *et al.* (2014) Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* **349**, g4490.
15. Ness AR, Powles JW & Khaw K-T (1996) Vitamin C and cardiovascular disease: a systematic review. *J Cardiovasc Risk* **3**, 513-21.
16. Chong MF-F, Macdonald R & Lovegrove JA (2010) Fruit polyphenols and CVD risk: a review of human intervention studies. *Br J Nutr* **104**, S28-S39.
17. Threapleton DE, Greenwood DC, Evans CE *et al.* (2013) Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* **347**, f6879.
18. Vivekananthan DP, Penn MS, Sapp SK *et al*. (2003) Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* **361**, 2017-2023.
19. Hord NG (2011) Dietary nitrates, nitrites, and cardiovascular disease. *Curr Atheroscler Rep* **13**, 484-492.
20. Bhupathiraju SN, Wedick NM, Pan A *et al.* (2012) Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. *Am J Clin Nutr*, ajcn-066381.
21. Joshipura KJ, Ascherio A, Manson JE *et al.* (1999) Fruit and vegetable intake in relation to risk of ischemic stroke. *JAMA* **282**, 1233-1239.
22. 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.
23. Sobko T, Marcus C, Govoni M *et al.* (2010) Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. *Nitric Oxide* **22**, 136-40.
24. L'hirondel J (2002) Nitrate and man: toxic, harmless or beneficial?: CABI
25. Lundberg J, Weitzberg E (2010) Nitric oxide formation from inorganic nitrate and nitrite. *Nitric Oxide* **2**.

26. McKnight G, Smith L, Drummond R *et al.* (1997) Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut* **40**, 211-214.

27. Kapil V, Weitzberg E, Lundberg J *et al.* (2014) Clinical evidence demonstrating the utility of inorganic nitrate in cardiovascular health. *Nitric Oxide* **38**, 45-57.

28. Hobbs DA, Kaffa N, George TW *et al*. (2012) Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread products in normotensive male subjects. *Br J Nutr* **108**, 2066-2074.

29. 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.

30. Bailey SJ, Winyard P, Vanhatalo A *et al.* (2009) Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* **107**, 1144-1155.

31. Kelm M (1999) Nitric oxide metabolism and breakdown. *Biochim Biophys Acta* **1411**, 273-289.

32. Hotchkiss J (1988) Nitrate, nitrite balance, and de novo synthesis of nitrate. *Am J Clin Nutr* **47**, 161-162.

33. Webb AJ, Patel N, Loukogeorgakis S *et al.* (2008) Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* **51**, 784-790.

34. Li H & Förstermann U (2000) Nitric oxide in the pathogenesis of vascular disease. *J Pathol* **190**, 244-254.

35. Green LC, De Luzuriaga KR, Wagner DA *et al.* (1981) Nitrate biosynthesis in man. *Proc Natl Acad Sci* **78**, 7764-7768.

36. Palmer R, Ashton D & Moncada S (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664-666.

37. Guzik TJ, Mussa S, Gastaldi D *et al.* (2002) Mechanisms of increased vascular superoxide production in human diabetes mellitus Role of NAD (P) H oxidase and endothelial nitric oxide synthase. *Circulation* **105**, 1656-1662.

38. Taddei S, Virdis A, Ghiadoni L *et al.* (2001) Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* **38**, 274-279.

39. Feron O, Dessy C, Moniotte S *et al*. (1999) Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. *J Clin Invest* **103**, 897-905.

40. Kharitonov SA, Robbins RA, Yates D *et al*. (1995) Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* **152**, 609-612.

41. Böger RH, Bode-Böger SM, Szuba A *et al.* (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction its role in hypercholesterolemia. *Circulation* **98**, 1842-1847.

42. Ichiki K, Ikeda H, Haramaki N *et al*. (1996) Long-term smoking impairs platelet-derived nitric oxide release. *Circulation* **94**, 3109-3114.

43. Bryan NS & Loscalzo J (2011) *Nitrite and nitrate in human health and diesease*. Springer Science & Business Media.

44. Sibal L, C Agarwal S, D Home P *et al*. (2010) The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. *Curr Cardiol Rev* **6**, 82-90.

45. Creager MA, Gallagher SJ, Girerd XJ *et al*. (1992) L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* **90,** 1248.

46. Bednarz B, Jaxa-Chamiec T, Maciejewski P *et al*. (2005) Efficacy and safety of oral l-arginine in acute myocardial infarction. Results of the multicenter, randomized, double-blind, placebo-controlled ARAMI pilot trial. *Kardiol Pol* **62**, 421-427.

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. The Natural Standard Research Collaboration (2013) Drug and Supplement Arginine Mayo Foundation for Medical Education and Research. http://www.mayoclinic.org/drugs-supplements/arginine/safety/hrb-20058733 (accessed June 2016).

49. Nakaki T & Hishikawa K (2002) The arginine paradox. *Nihon Yakurigaku Zasshi* **199**, 7-14.

50. Mirmiran P, Bahadoran Z, Ghasemi A *et al*. (2016) The Association of Dietary l-Arginine Intake and Serum Nitric Oxide Metabolites in Adults: A Population-Based Study. *Nutrients* **8**, 311.
51. Weitzberg E & Lundberg J (1998) Nonenzymatic nitric oxide production in humans. *Nitric Oxide* **2**, 1-7.
52. Zweier JL, Samouilov A & Kuppusamy P (1999) Non-enzymatic nitric oxide synthesis in biological systems. *Biochim Biophys Acta* **1411**, 250-262.
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**, 210-22.
54. van Velzen AG, Sips AJ, Schothorst RC *et al*. (2008) The oral bioavailability of nitrate from nitrate-rich vegetables in humans. *Toxicol Lett* **181**, 177-181.
55. Lundberg JO & Govoni M (2004) Inorganic nitrate is a possible source for systemic generation of nitric oxide. *Free Radic Biol Med* **37**, 395-400.
56. Pannala AS, Mani AR, Spencer JP *et al*. (2003) The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radic Biol Med* **34**, 576-584.
57. 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.
58. Tannenbaum S, Weisman M & Fett D (1976) The effect of nitrate intake on nitrite formation in human saliva. *Food Cosmet Toxoicol* **14**, 549-552.
59. Benjamin N, O'Driscoll F, Dougall H *et al*. (1994) Stomach NO synthesis. *Nature* **368**, 502.
60. Coggan AR, Leibowitz JL, Spearie CA *et al*. (2015) Acute Dietary Nitrate Intake Improves Muscle Contractile Function in Patients With Heart Failure A Double-Blind, Placebo-Controlled, Randomized Trial. *Circ Heart Fail* **8**, 914-920.
61. Lundberg JO, Weitzberg E & Gladwin MT (2008) The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* **7**, 156-167.
62. Sobko T, Reinders C, Norin E *et al*. (2004) Gastrointestinal nitric oxide generation in germ-free and conventional rats. *Am J Physiol Gastrointest Liver Physiol* **287**, G993-G997.
63. Bondonno CP, Liu AH, Croft KD *et al*. (2015) Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. *Am J Hypertens* **28**, 572-575.
64. Govoni M, Jansson EÅ, 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.
65. Duncan C, Dougall H, Johnston P *et al*. (1995) Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nat Med* **1**, 546-51.
66. Zhang Z, Naughton D, Winyard PG *et al*. (1998) Generation of nitric oxide by a nitrite reductase activity of xanthine oxidase: a potential pathway for nitric oxide formation in the absence of nitric oxide synthase activity. *Biochem Biophys Res Commun* **249**, 767-772.
67. Millar TM, Stevens CR, Benjamin N *et al*. (1998) Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. *FEBS letters* **427**, 225-228.
68. Webb A, Bond R, McLean P *et al*. (2004) Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia–reperfusion damage. *Proc Natl Acad Sci U S A* **101**, 13683-13688.
69. Duranski MR, Greer JJ, Dejam A *et al*. (2005) Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. *J Clin Invest* **115**, 1232-1240.
70. Gladwin MT, Raat NJ, Shiva S *et al*. (2006) Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signaling, cytoprotection, and vasodilation. *Am J Physiol Heart Circ Physiol* **60**, H2026.
71. Cosby K, Partovi KS, Crawford JH *et al*. (2003) Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* **9**, 1498-1505.
72. Brooks J (1937) The action of nitrite on haemoglobin in the absence of oxygen. *Proc R Soc Lond B Biol Sci* **123**, 368-382.
73. Doyle MP, Pickering RA, DeWeert TM *et al*. (1981) Kinetics and mechanism of the oxidation of human deoxyhemoglobin by nitrites. *J Biol Chem* **256**, 12393-12398.

74. 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.
75. Lundberg J, Weitzberg E, Lundberg J *et al*. (1994) Intragastric nitric oxide production in humans: measurements in expelled air. *Gut* **35**, 1543-1546.
76. Mowat C, Carswell A, Wirz A *et al*. (1999) Omeprazole and dietary nitrate independently affect levels of vitamin C and nitrite in gastric juice. *Gastroenterol* **116**, 813-822.
77. Gago B, Lundberg JO, Barbosa RM *et al*. (2007) Red wine-dependent reduction of nitrite to nitric oxide in the stomach. *Free Radic Biol Med* **43**, 1233-1242.
78. Crawford NM (1995) Nitrate: nutrient and signal for plant growth. *Plant cell* **7**, 859.
79. Ysart G, Clifford R & Harrison N (1999) Monitoring for nitrate in UK-grown lettuce and spinach. *Food Addit Contam* **16**, 301-306.
80. Kaiser WM & Brendle-Behnisch E (1991) Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis I. Modulation in vivo by CO2 availability. *Plant Physiol* **96**, 363-367.
81. Muramoto J (1999) Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Center for Agroecology and Sustainable Food Systems, University of California, Santa Cruz.
82. Ekart K, Gorenjak A, Madorran E *et al*. (2013) Study on the influence of food processing on nitrate levels in vegetables. In *European Food Safety Authority* pp. 1-150.
83. Omar SA, Artime E & Webb AJ (2012) A comparison of organic and inorganic nitrates/nitrites. *Nitric Oxide* **26**, 229-240.
84. Du S-T, Zhang Y-S & Lin X-Y (2007) Accumulation of nitrate in vegetables and its possible implications to human health. *Agricultural Sciences in China* **6**, 1246-1255.
85. Food Standards Australia New Zealand (2015) Survey of Nitrates and Nitrites in Food and Beverages in Australia. http://www.foodstandards.gov.au/consumer/additives/nitrate/Pages/default.aspx (accessed November 2015)
86. NHMRC N (2011) *Australian drinking water guidelines paper 6 national water quality management strategy*. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra.
87. US Food and Drug Administration (2006) *Food additive status list*. US Food and Drug Administration (FDA), Rockville, MD.
88. Ward MH, DeKok TM, Levallois P *et al*. (2005) Workgroup report: Drinking-water nitrate and health-recent findings and research needs. *Environ Health Perspect,* 1607-1614.
89. Shuval HI & Gruener N (1972) Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am J Public Health* **62**, 1045-1052.
90. Jukes D (2013) Food additives in the European Union. The Department of Food Science and Technology.
91. Food Standards Agency (2015) Food Additives Legislation Guidance to Compliance. http://www.food.gov.uk/sites/default/fi;es/multimedia/pdfs/guidance/food-additives-legislation-guidance-to-compliance.pdf (accessed December 2016).
92. United States Department of Health and Human Services (2014) Food additives permitted for direct addition to food for human consumption. www.fda.gov/Food/ingredientsPackagingLabeling/FoodAdditivesUngredients.ucm091048.htm (accessed June 2016).
93. Weitzberg E & Lundberg JO (2013) Novel aspects of dietary nitrate and human health. *Annu Rev Nutr* **33**, 129-159.
94. Machha A & Schechter AN (2011) Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits. *Eur J Nutr* **50**, 293-303.
95. Kelm M & Schrader J (1990) Control of coronary vascular tone by nitric oxide. *Circ Res* **66**, 1561-1575.
96. Cornwell TL, Arnold E, Boerth NJ *et al*. (1994) Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol Cell Physiol* **267**, C1405-C1413.

97. Radomski M, Palmer R & Moncada S (1987) Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* **330**, 1057-1058.
98. Kubes P, Suzuki M & Granger D (1991) Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci* **88**, 4651-4655.
99. De Caterina R, Libby P, Peng H-B *et al*. (1995) Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* **96**, 60.
100. Versari D, Daghini E, Virdis A *et al*. (2009) Endothelium-dependent contractions and endothelial dysfunction in human hypertension. *Br J Pharmacol* **157**, 527-36.
101. Vanhoutte P (1997) Endothelial dysfunction and atherosclerosis. *Eur Heart J* **18**, Suppl E, 19-29.
102. Kleinbongard P, Dejam A, Lauer T *et al*. (2006) Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. *Free Radic Biol Med* **40**, 295-302.
103. Landmesser U & Drexler H (2007) Endothelial function and hypertension. *Curr Opin Cardiol* **22**, 316-20.
104. Al Suwaidi J, Hamasaki S & Higano ST (2000) Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* **101**, 948-954.
105. Neunteufl T, Katzenschlager R, Hassan A *et al*. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* **129**, 111-118.
106. Drexler H, Hayoz D, Münzel T *et al.* (1992) Endothelial function in chronic congestive heart failure. *Am J Cardiol* **69**, 1596-1601.
107. Gokce N, Keaney JF, Hunter LM *et al*. (2003) Predictive value of noninvasivelydetermined endothelial dysfunction for long-term cardiovascular events inpatients with peripheral vascular disease. *J Am Coll Cardiol* **41**, 1769-1775.
108. Vita JA & Keaney JF (2002) Endothelial function a barometer for cardiovascular risk? *Circulation* **106**, 640-642.
109. Schächinger V, Britten MB & Zeiher AM (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **101**, 1899-1906.
110. Katz SD, Hryniewicz K, Hriljac I *et al*. (2005) Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. *Circulation* **111**, 310-314.
111. 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.
112. Lundberg JO, Gladwin MT & Weitzberg E (2015) Strategies to increase nitric oxide signalling in cardiovascular disease. *Nat Rev Drig Discov* **14**, 623-641.
113. Jeerooburkhan N, Jones LC, Bujac S *et al.* (2001) Genetic and environmental determinants of plasma nitrogen oxides and risk of ischemic heart disease. *Hypertension* **38**, 1054-1061.
114. Panza JA, Quyyumi AA, Brush Jr JE *et al*. (1990) Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* **323**, 22-27.
115. Casino PR, Kilcoyne CM, Quyyumi AA *et al*. (1993) The role of nitric oxide in endothelium-dependent vasodilation of hypercholesterolemic patients. *Circulation* **88**, 2541-2547.
116. Henry RM, Ferreira I, Kostense PJ *et al*. (2004) Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not: The Hoorn Study. *Atherosclerosis* **174**, 49-56.
117. Tsuchiya M, Asada A, Kasahara E *et al*. (2002) Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation* **105**, 1155-1157.
118. Green DJ, Maiorana A, O'driscoll G *et al*. (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* **561**, 1-25.
119. Lopez-Garcia E, Schulze MB, Fung TT *et al*. (2004) Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* **80**, 1029-1035.
120. Celermajer DS, Sorensen KE, Spiegelhalter DJ *et al*. (1994) Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* **24**, 471-476.

121. Celermajer D, Sorensen K, Georgakopoulos D *et al.* (1993) Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* **88**, 2149-2155.

122. Fuentes F, Lopez-Miranda J, Sanchez E *et al*. (2001) Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* **134**, 1115-1119.

123. O'driscoll G, Green D & Taylor RR (1997) Simvastatin, an HMG–coenzyme A reductase inhibitor, improves endothelial function within 1 month. *Circulation* **95**, 1126-1131.

124. Hornig B, Maier V & Drexler H (1996) Physical training improves endothelial function in patients with chronic heart failure. *Circulation* **93**, 210-214.

125. DeVan A, Brooks F, Evans T *et al*. (2014) Safety and efficacy of sodium nitrite supplementation for improving vascular endothelial dysfunction in middle-aged and older healthy adults. *FASEB Journal* **1**.

126. Wilson J & Hunt T (2014) *Molecular biology of the cell: the problems book*: Garland Science.

127. Channon KM, Qian H & George SE (2000) Nitric Oxide Synthase in Atherosclerosis and Vascular Injury Insights From Experimental Gene Therapy. *Arterioscler Thromb Vasc Biol* **20**, 1873-1881.

128. Hobbs DA, Goulding MG, Nguyen A *et al.* (2013) Acute ingestion of beetroot bread increases endothelium-independent vasodilation and lowers diastolic blood pressure in healthymen: A randomized controlled trial. *J Nutr* **143**, 1399-1405.

129. Simon DI, Stamler JS, Jaraki O *et al*. (1993) Antiplatelet properties of protein S-nitrosothiols derived from nitric oxide and endothelium-derived relaxing factor. *Arterioscler Thromb Vasc Biol* **13**, 791-799.

130. Clapp BR, Hingorani AD, Kharbanda RK *et al*. (2004) Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc Res* **64**, 172-178.

131. Vallance PJ & Webb DJ (2003) *Vascular endothelium in human physiology and pathophysiology*. vol. 7: CRC Press.

132. Gago B, Nyström T, Cavaleiro C *et al*. (2008) The potent vasodilator ethyl nitrite is formed upon reaction of nitrite and ethanol under gastric conditions. *Free Radic Biol Med* **45**, 404-412.

133. Klemenska E & Beresewicz A (2009) Bioactivation of organic nitrates and the mechanism of nitrate tolerance. *Cardiol J* **16**, 11-19.

134. Bassenge E, Fink N, Skatchkov M *et al*. Dietary supplement with vitamin C prevents nitrate tolerance. *J Clin Invest* **102**, 67.

135. Gori T, Burstein JM, Ahmed S *et al*. (2001) Folic acid prevents nitroglycerin-induced nitric oxide synthase dysfunction and nitrate tolerance. *Circulation* **104**, 1119-23.

136. Fontaine D, Otto A, Fontaine J *et al*. (2003) Prevention of nitrate tolerance by long-term treatment with statins. *Cardiovasc Drugs Ther* **17**, 123-128.

137. Greer FR & Shannon M (2005) Infant methemoglobinemia: the role of dietary nitrate in food and water. *Pediatrics* **116**, 784-786.

138. Martínez A, Sánchez-Valverde F, Gil F *et al*. (2013) Methemoglobinemia induced by vegetable intake in infants in northern Spain. *J Peadiatr Gastroenterol Nutr* **56**, 573-577.

139. Milkowski A, Garg HK, Coughlin JR *et al*. (2010) Nutritional epidemiology in the context of nitric oxide biology: A risk–benefit evaluation for dietary nitrite and nitrate. *Nitric Oxide* **22**, 110-119.

140. Avery AA (1999) Infantile methemoglobinemia: reexamining the role of drinking water nitrates. *Environ Health Perspect* **107**, 583.

141. Bouvard V, Loomis D, Guyton KZ *et al*. (2015) Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* **16**, 1599.

142. Bingham SA (1999) High-meat diets and cancer risk. *Proc Nutr Soc* **58**, 243-248.

143. Gilchrist M, Winyard PG & Benjamin N (2010) Dietary nitrate–good or bad? *Nitric Oxide* **22**, 104-109.

144. Magee PN & Barnes J (1956) The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *Br J Cancer* **10**, 114.

145. Assembly of Life Sciences (1981) The Health Effects of Nitrate, Nitrite, and N-nitroso Compounds: Part 1 of a 2-Part Study. *National Academy Press*.

146. Kapil V, Milsom AB, Okorie M *et al*. (2010) Inorganic nitrate supplementation lowers blood pressure in humans. *Hypertension* **56**, 274-281.

147. Asgary S, Afshani M, Sahebkar A *et al*. (2016) Improvement of hypertension, endothelial function and systemic inflammation following short-term supplementation with red beet (Beta vulgaris L.) juice: a randomized crossover pilot study. *J Hum Hypertens.*

148. Richardson G, Hicks S, O'Byrne S *et al*. (2002) The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. *Nitric Oxide* **7**, 24-29.

149. Baker JE, Su J, Fu X *et al*. (2007) Nitrite confers protection against myocardial infarction: Role of xanthine oxidoreductase, NADPH oxidase and K ATP channels. *J Mol Cell Cardiol* **43**, 437-444.

150. Lansley KE, Winyard PG, Bailey SJ *et al*. (2011) Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc* **43**, 1125-1131.

151. Dykhuizen R, Frazer R, Duncan C *et al*. (1996) Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defense. *Antimicrob Agents Chemother* **40**, 1422-1425.

152. Carlström M, Persson AEG, 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.

153. Petersson J, Carlström M, Schreiber O *et al*. (2009) Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic Biol Med* **46**, 1068-1075.

154. Kanematsu Y, Yamaguchi K, Ohnishi H *et al*. (2008) Dietary doses of nitrite restore circulating nitric oxide level and improve renal injury in L-NAME-induced hypertensive rats. *Am J Physiol Renal Physiol* **295**, F1457-F1462.

155. Ferguson SK, Hirai DM, Copp SW *et al*. (2013) Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *J Physiol* **591**, 547-557.

156. Bryan NS, Calvert JW, Elrod JW *et al*. (2007) Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. *Proc Natl Acad Sci* **104**, 19144-19149.

157. Shiva S, Sack MN, Greer JJ *et al*. (2007) Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. *J Exp Med* **204**, 2089-2102.

158. Minicucci MF, Azevedo PS, Polegato BF *et al*. (2011) Heart failure after myocardial infarction: clinical implications and treatment. *Clin Cardiol* **34**, 410-414.

159. Nieswandt B, Pleines I & Bender M (2011) Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. *J Thromb Haemost* **9**, 92-104.

160. Raskob GE, Angchaisuksiri P, Blanco AN *et al.* (2014) editors. Thrombosis: a major contributor to global disease burden. *Seminars in thrombosis and hemostasis* **40**, 724-735.

161. Park JW, Piknova B, Huang PL *et al*. (2013) Effect of blood nitrite and nitrate levels on murine platelet function. *PLoS ONE* **8**, e55699.

162. Apostoli G, Solomon A, Smallwood M *et al*. (2014) Role of inorganic nitrate and nitrite in driving nitric oxide–cGMP-mediated inhibition of platelet aggregation in vitro and in vivo.*J Thromb Haemost* **12**, 1880-1889.

163. Moraes DL, Colucci WS & Givertz MM (2000) Secondary pulmonary hypertension in chronic heart failure. *Circulation* **102**, 1718-1723.

164. Hunter CJ, Dejam A, Blood AB *et al*. (2004) Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nat Med* **10**, 1122-1127.

165. Zuckerbraun BS, Shiva S, Ifedigbo E *et al*. (2010) Nitrite potently inhibits hypoxic and inflammatory pulmonary arterial hypertension and smooth muscle proliferation via xanthine oxidoreductase–dependent nitric oxide generation. *Circulation* **121**, 98-109.

166. Casey DB, Badejo AM, Dhaliwal JS *et al*. (2009) Pulmonary vasodilator responses to sodium nitrite are mediated by an allopurinol-sensitive mechanism in the rat. *Am J Physiol Heart Circ Physiol* **296**, H524-H533.

167. Hendgen-Cotta UB, Luedike P, Totzeck M *et al*. (2012) Dietary nitrate supplementation improves revascularization in chronic ischemia. *Circulation* **126**, 1983-1992.

168. Heiss C, Meyer C, Totzeck M *et al*. (2012) Dietary inorganic nitrate mobilizes circulating angiogenic cells. *Free Radic Biol Med* **52**, 1767-1772.

169. Sindler AL, Fleenor BS, Calvert JW *et al*. (2011) Nitrite supplementation reverses vascular endothelial dysfunction and large elastic artery stiffness with aging. *Aging Cell* **10**, 429-437.

170. Cai H & Harrison DG (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* **87**, 840-844.

171. Stokes KY, Dugas TR, Tang Y *et al*. (2009) Dietary nitrite prevents hypercholesterolemic microvascular inflammation and reverses endothelial dysfunction. *Am J Physiol Heart Cir Physiol* **296**, H1281-H1288.

172. Carlström M, Larsen FJ, Nyström T *et al*. (2010) Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice. *Proc Natl Acad Sci* **107**, 17716-17720.

173. 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.

174. 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.

175. 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.

176. 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.

177. Golzarand M, Bahadoran Z, Mirmiran P *et al*. (2016) Consumption of nitrate-containing vegetables is inversely associated with hypertension in adults: A prospective investigation from the Tehran Lipid and Glucose Study. *J Nephrol* **29**, 377-384.

178. Raitakari OT & Celermajer DS (2000) Testing for endothelial dysfunction. *Ann Med* **32**, 293-304

179. Hadi HA, Carr CS & Suwaidi J (2005) Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Ris Manag* **1**, 183.

180. Bondonno CP, Yang X, Croft KD *et al*. (2012) Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: a randomized controlled trial. *Free Radic Biol Med* **52**, 95-102.

181. Rodriguez-Mateos A, Hezel M, Aydin H *et al*. (2015) Interactions between cocoa flavanols and inorganic nitrate: Additive effects on endothelial function at achievable dietary amounts. *Free Radic Biol Med* **80**, 121-128.

182. Joris PJ & Mensink RP (2013) Beetroot juice improves in overweight and slightly obese men postprandial endothelial function after consumption of a mixed meal. *Atherosclerosis* **231**, 78-83.

183. Ingram TE, Fraser AG, Bleasdale RA *et al*. (2013) Low-dose sodium nitrite attenuates myocardial ischemia and vascular ischemia-reperfusion injury in human models. *J Am Coll Cardiol* **61**, 2534-2541.

184. Heiss C, Jahn S, Taylor M, Real WM *et al*. (2010) Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. *J Am Coll Cardiol* **56**, 218-224.

185. The Reference Values for Arterial Stiffness' Collaboration (2010) Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors:'establishing normal and reference values'. *Eur Heart J* **31**, 2338-2350.

186. Chirinos JA, Zambrano JP, Chakko S *et al*. (2005) Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease. *Hypertension* **45**, 980-985.

187. Zamani P, Rawat D, Shiva-Kumar P *et al*. (2014) The effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation* **CIRCULATIONAHA**, 114.

188. 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.

189. 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**, 123-30.

190. Jones DA, Pellaton C, Velmurugan S *et al*. (2014) Randomized phase 2 trial of intra-coronary nitrite during acute myocardial infarction. *Circ Res* **CIRCRESAHA**, 114.

191. Alexander J, Benford D, Cockburn A *et al*. (2008) Nitrate in vegetables Scientific Opinion of the Panel on Contaminants in the Food chain. *EFSA J* **689**, 1-79.

192. Siciliano J, Krulick S, Heisler EG *et al*. (1975) Nitrate and nitrite content of some fresh and processed market vegetables. *J Agric Food Chem* **23**, 461-464.

193. Jackson W, Steel J, Boswell V (1967) Nitrates in edible vegetables and vegetable products. *Proc Amer Soc Hort Sci* **90**, 349-352.
194. Corré WJ (1979) *Nitrate and nitrite in vegetables*.
195. Richardson W (1907) THE OCCURRENCE OF NITRATES IN VEGETABLE FOODS, IN CURED MEATS AND ELSEWHERE. *J Am Chem Soc* **29**, 1757-1767.
196. Wilson J (1949) Nitrate in foods and its relation to health. *Agron J* **41**, 20-22.
197. Lee DH (1970) Nitrates, nitrites, and methemoglobinemia. *Environ Res* **3**, 484-511.
198. White JW (1975) Relative significance of dietary sources of nitrate and nitrite. *J Agric Food Chem* **23**, 886-891.
199. 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.
200. Öztekin N, Nutku MS, Erim FB (2002) Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis. *Food Chem* **2002**, 103-106.
201. Tamme T, Reinik M, Roasto M *et al*. (2006) Nitrates and nitrites in vegetables and vegetable-based products and their intakes by the Estonian population. *Food Addit Contam* **23**, 355-361.
202. Hsu J, Arcot J & Lee NA (2009) Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians. *Food Chem* **115**, 334-339.
203. Thomson B, Nokes C & Cressey P (2007) Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. *Food Addit Contam* **24**, 113-121.
204. 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.
205. Wang Z, Wei Y & Li S (2000) Nitrate accumulation and its regulation by nutrient management in vegetables. *Balanceable fertilization and high quality vegetables continual production Beijing: China Agricultural University*.
206. Petersen A, Stoltze S (1999) Nitrate and nitrite in vegetables on the Danish market: content and intake. *Food Addit Contam* **16**, 291-299.
207. Ysart G, Miller P, Barrett G *et al*. (1999) Dietary exposures to nitrate in the UK. *Food Addit Contam* **16**, 521-532.
208. Sušin J, Kmecl V & Gregorčič A (2006) A survey of nitrate and nitrite content of fruit and vegetables grown in Slovenia during 1996–2002. *Food Addit Contam* **23**, 385-390.
209. Pickston L, Smith J & Todd M. Nitrate and nitrite levels in fruit and vegetables in New Zealand and the effect of storage and pressure cooking on these levels. *Food Technology in New Zealand*.
210. Zhong W, Hu C & Wang M (2002) Nitrate and nitrite in vegetables from north China: content and intake. *Food Addit Contam* **19**, 1125-1129.
211. Panalaks T, Iyengar J & Sen N (1973) Nitrate, nitrite, and dimethylnitrosamine in cured meat products. *Ass Offic Anal Chem J*.
212. Sen NP & Baddoo PA (1997) Trends in the levels of residual nitrite in Canadian cured meat products over the past 25 years. *J Agric Food Chem* **45**, 4714-4718.
213. Sen NP, Baddoo PA & Seaman SW (1994) Rapid and sensitive determination of nitrite in foods and biological materials by flow injection or high-performance liquid chromatography with chemiluminescence detection. *J Chromatogr A* **673**, 77-84.
214. Panalaks T, Iyengar JR, Donaldson BA *et al*. (1974) Further survey of cured meat products for volatile N-nitrosamines. *J Assoc Off Anal Chem* **57**, 806.
215. Meah M, Harrison N & Davies A (1994) Nitrate and nitrite in foods and the diet. *Food Addit Contam* **11**, 519-532.
216. Buege D, Lee M & Cassens R (1978) Residual nitrite levels in meat products manufactured by Wisconsin meat processors [Meat curing, cancer-causing nitrosamines]. *Publication-Cooperative Extension Programs.*
217. Reinik M, Tamme T, Roasto M *et al*. (2005) Nitrites, nitrates and N-nitrosoamines in Estonian cured meat products: intake by Estonian children and adolescents. *Food Addit Contam* **22**, 1098-1105.
218. Cassens RG (1997) Composition and safety of cured meats in the USA. *Food Chem* **59**, 561-566.
219. Kerr R, Marsh C, Schroeder W *et al*. (1926) The Use of Sodium Nitrite in the Curing of Meat. *J Agric Res* **33**, 541-551.

220. Coppola ED, Wickroski AF & Hanna JG (1976) Nitrite in meat products determined by fluorescence quenching of p-aminobenzoate ion. *J Assoc Off Anal Chem* **59**, 783-786.
221. Siu DC & Henshall A (1998) Ion chromatographic determination of nitrate and nitrite in meat products. *J Chromatogr A* **804**, 157-160.
222. Greenberg R (1977) Nitrosopyrrolidine in United States cured meat products. *Proceedings of the International Symposium on Nitritein Meat Products*.
223. Sen N, Donaldson B & Charbonneau C (1975) Formation of nitrosodimethylamine from the interaction of certain pesticides and nitrite. *IARC Scientific Publications (IARC)*.
224. Sen N, Donaldson B, Seaman S *et al*. (1977) Recent nitrosamine analyses in cooked bacon. *J Can Inst Food Sci Technol.*

Tables

Table 1. Permissions for nitrate and nitrite in Food Products

Product	Additive	Maximum Permitted Level (mg/kg)
	Nitrite Salt	150 [90]
	Nitrate Salt	50 [85; 90]
Commercially Sterile Canned Dried Meat	Nitrite Salt	50 - 150 [85; 91]
	Nitrite Salt	125 [90]
	Nitrate Salt	150 [85]
	Nitrite Salt	125 - 200 [85; 90]
	Nitrate Salt	175- 500 [85; 92]
	Nitrite Salt	125 - 175 [85; 90]
	Nitrate Salt	150 – 300 [90; 91]
	Nitrite Salt	150 [90]
	Nitrate Salt	500 [85; 90]

Nitrate salt: Potassium Nitrate and Sodium Nitrate. Nitrite salt: Potassium Nitrite and Sodium Nitrite

Table 2. Vegetable sources of nitrate and nitrite with estimated nitrate and/or nitrite contents.

Vegetable Type	Nitrate Content (mg/kg) Mean (range)	Nitrite Content (mg/kg) Mean (range)
Rocket	3624 (1550-7316)[111; 191]	NA
Turnip Greens	3467[192; 193; 194]	NA
Spinach	2485 (2-6700)[22; 79; 85; 111; 191; 193; 194; 195; 196; 197; 198; 199; 200; 201; 202; 203; 204; 205]	15 (ND-162)[22; 85; 200; 202; 203; 205]
Swiss chard	2363[199]	NA
Turnip	2174 (10-4800)[111; 194; 195; 197; 201]	NA
Rhubarb	1999 (55-6500)[191; 193; 194; 196; 197; 201; 204]	NA
Celery	1964 (19-5300)[85; 191; 193; 194; 195; 196; 197; 198; 199; 201; 203]	2.5 (ND-6)[85; 191]
Beetroot	1992 (100-8100)[85; 111; 193; 194; 195; 196; 197; 198; 199; 201; 203; 204; 206; 207; 208]	1.7 (ND-110)[85; 199; 203; 209]
Chinese Cabbage	1855 (111-8050)[201; 202; 206; 208; 210]	0.9 (ND-14.3)[206; 208]
Radish	1773 (60-9000)[111; 191; 193; 194; 195; 196; 201]	NA
Lettuce	1689 (10-13000)[79; 85; 111; 191; 193; 194; 195; 196; 197; 198; 199; 201; 202; 203; 204; 205; 206; 208; 209]	0.8 (ND-5)[85; 203; 205; 206; 208]
Watercress	1640 (890-2790)[203]	2.5 (ND-5)[203]
Buk Choy	1620 (1023-3098)[202]	20 (0.09-30)[202]
Kale/ Mustard Greens	1318 (19-5500)[22; 191; 192; 193; 194; 197; 205]	(0.03-0.64)[22; 205]
Silver beet	1255 (190-1770)[203; 209]	2.5 (ND-5)[203; 209]
Endive	975 (10-3800)[194; 199]	NA
Broccoli	793 (ND-2300)[22; 85; 193; 194; 196; 197; 198; 199; 203; 204]	3 (ND-110)[22; 85; 203]
Cabbage	756 (1-3100)[85; 193; 194; 195; 196; 197; 198; 199; 201; 203; 204; 207; 208; 209; 210]	0.8 (ND-26)[85; 203; 208]
Cauliflower	547 (ND-4500)[191; 193; 194; 195; 196; 197; 198; 199; 201]	NA
Mixed Salad	540 (80-821)[22; 111; 191; 201]	1.3[22]
Eggplant	479 (31-1500)[191; 194; 195; 198; 199]	NA
Leek	399 (56-841)[111; 195]	NA
Pumpkin / Squash	389 (ND-2200)[85; 191; 194; 195; 196; 197; 198; 199; 201; 203]	6 (ND-194)[85; 203]
Green Onion	366 (4-1676)[111; 201]	NA
Fennel	363[199]	NA
Green Beans	315 (6-1100)[85; 111; 193; 195; 197; 199; 208]	7 (0.16-57)[85; 208]
Cucumber	184 (1-1236)[85; 111; 191; 194; 195; 198; 199; 208; 209; 210]	3 (ND-1164)[85; 208]
White Potato	184 (ND-5521)[22; 85; 111; 191; 193; 194; 195; 196; 197; 198; 201; 203; 207; 208; 209; 210]	1 (ND-10.3)[22; 85; 203; 208]
Carrot	182 (ND-2800)[22; 85; 111; 191; 193; 194; 195; 196; 197; 198; 199; 201; 203; 204; 205; 207; 208]	0.7 (ND-7.5)[22; 85; 203; 205; 208]
Garlic	163 (1-462)[111; 191; 199]	NA
Lima Beans	160 (54-310)[193; 195; 198]	NA
Brussels Sprouts	118 (ND-170)[194]	NA
Onion	100 (ND-2300)[85; 191; 194; 195; 196; 199; 201]	0.5 (ND-2.2)[85]
Mushroom	92 (ND-400)[85; 191; 194]	NA
Asparagus	84 (13-700)[194; 196; 198]	NA
Tomato	71 (ND-392)[22; 85; 111; 191; 193; 194; 195; 196; 198; 199; 201; 204; 207; 208; 209; 210]	0.6 (ND-13)[22; 85; 208]
Sweet Potato	55 (ND-66)[191; 193; 194; 195; 198]	NA
Peas	32 (ND-124)[85; 191; 193; 194; 195; 198; 199]	(ND-22)[85]
Dry Beans	30 (9-68)[195; 198]	NA
Corn	30 (ND-45)[85; 195; 198]	(ND-7.5)[85]
Artichoke	30[199]	NA
Preserved Olives	22 (21-23)[85]	NA
Avocado	18 (ND-51)[85]	(ND-7.5)[85]

Vegetable Type	Nitrate Content (mg/kg) Mean (range)	Nitrite Content (mg/kg) Mean (range)
Baked Beans	17 (ND-23)[85]	1.7 (ND-7.5)[85]

Data is combined nitrate and nitrite estimates from various published papers, government documents and reviews. ND: Not Detected. NA: Data not available.

Table 3. Meat based sources of nitrate and nitrite with estimated nitrate and/or nitrite contents.

Meat Type	Nitrate Content (mg/kg) Mean (range)	Nitrite Content (mg/kg) Mean (range)
Salami	94 (ND-450)[85; 202; 203; 211; 212; 213]	31 (ND-108)[85; 202; 203; 211; 212; 213]
Bolonga	65 (4-98)[211; 214; 215]	14 (ND-55)[211; 214; 215; 216]
Frankfurt/Hot dog	64 (8-81)[22; 85; 202; 203]	39 (0.5-95)[22; 85; 202; 203]
Shelf-Stable, Canned Cured Meat	63 (ND-840)[211; 212; 214]	31 (ND-19)[211; 212; 214]
Sausages	58 (15-240)[85; 202; 211; 214; 217; 218]	33 (ND-940)[91; 202; 211; 214; 216; 217; 218; 219; 220]
Ham	55 (ND-1400)[22; 85; 202; 203; 211; 215; 217; 221]	47 (ND-640)[22; 85; 202; 203; 211; 217; 219; 221; 222]
Bacon	42 (ND-310)[22; 85; 202; 203; 211; 214; 215]	29 (ND-430)[22; 85; 202; 203; 211; 212; 213; 214; 215; 218; 219; 222; 223; 224]
"Luncheon Meat"	32 (<10-70)[85; 203; 215]	31 (ND-130)[85; 203; 215]
Pork	21 (ND-19)[22; 215]	(ND-8)[22; 215]
Corned Beef	14 (4-36)[203; 215]	3 (ND-8)[203; 215]
Minced Beef	12 (ND-24)[202; 203]	NA

Data is combined nitrate and nitrite estimates from various published papers, government documents and reviews. ND: Not Detected. NA: Data not available.

Table 4. Fruit sources of nitrate and nitrite with estimated nitrate and/or nitrite contents.

Fruit Type	Nitrate Content (mg/kg) Mean (range)	Nitrite Content (mg/kg) Mean (range)
Melon	325 (38-600)[194; 195; 196; 199; 201]	NA
Strawberries	172 (96-233)[85]	18 (8-80)[85]
Banana	76 (45-200)[22; 85]	2 (ND-11)[22; 85]
Apple	20 (ND-56)[85]	(ND-7.5)[85]
Grapes	19 (ND-52)[85]	10 (ND-19.4)[85]
Sultanas	16 (9-22)[85]	0.8 (ND-5.5)[85]
Peach	10 (7-18)[85]	17 (ND-22)[85]
Orange	9 (ND-21)[22; 85]	0.2 (ND-7.5)[85]
Mango	9 (ND-12)[85]	6 (ND-15)[85]
Watermelon	8 (7-18)[85]	(ND-16.4)[85]
Pineapple	7 (ND-12)[85]	17 (10-22)[85]

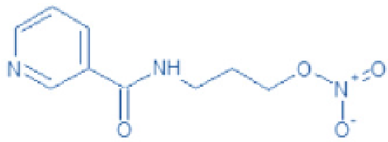
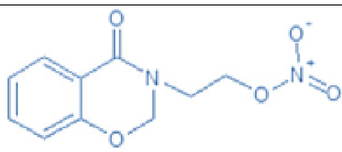
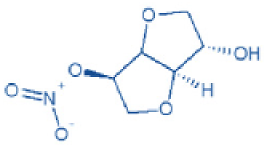
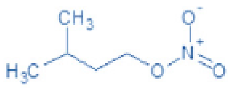
Data is combined nitrate and nitrite estimates from various published papers, government documents and reviews. ND: Not Detected. NA: Data not available.

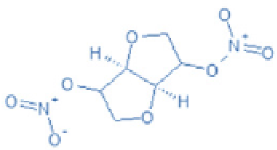
Table 5. Nitrate and nitrite containing herbs with estimated nitrate and/or nitrite contents.

Herb Type	Nitrite Content (mg/kg) Mean (range)	Nitrite Content (mg/kg) Mean (range)
Dill	2590 (2236-3267)[200; 201]	102[200]
Parsley	1304 (ND-4467)[85; 194; 195; 196; 200; 201]	(ND-94)[85; 200]
Tea	3 (2-3)[85]	(ND-0.3)[85]

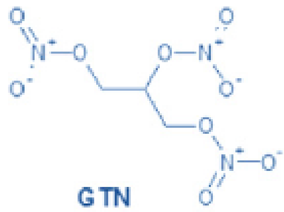
Data is combined nitrate and nitrite estimates from various published papers, government documents and reviews. ND: Not Detected. NA: Data not available.

Table 6. Chemical structure of inorganic nitrate/nitrite compared with organic mono-, di-, tri- and tetra nitrates/nitrites.

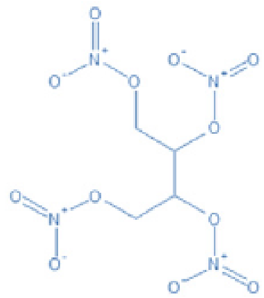
	Inorganic Nitrate/Nitrite	
		
	Organic Mono-Nitrates/Nitrites	
 nicorandil		 sinitrodil
 5-ISMN	 Ethyl nitrite	 Amyl nitrite
Organic Di	**Organic Tri**	**Organic Tetra**



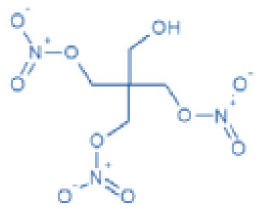
ISDN



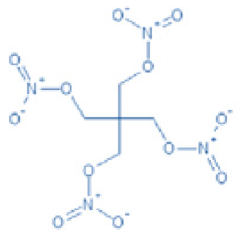
GTN



ETN



PETriN



PETN

Table from Omar, Artime and Webb, 2012 [83].