Current Atherosclerosis Reports (2018) 20: 21
https://doi.org/10.1007/s11883-018-0723-0

NUTRITION (P. KRIS-ETHERTON, SECTION EDITOR)

Functional Nitric Oxide Nutrition to Combat Cardiovascular Disease

Nathan S. Bryan[1]

Published online: 17 March 2018

Abstract

Purpose of review To reveal the mechanisms of nitric oxide (NO) production in humans and how lifestyle, drug therapy, and hygienic practices can decrease NO production. Furthermore, to show how functional nitric oxide nutrition can overcome these limitations to restore endogenous NO production and combat cardiovascular disease.

Recent findings Research over the past decade has revealed that inorganic nitrate and nitrite found naturally in green leafy vegetables and other vegetables such as beets can provide the human body with a source of bioactive nitric oxide. NO is one of the most important molecules produced within the cardiovascular system that maintains normal blood pressure and prevents inflammation, immune dysfunction, and oxidative stress, hallmarks of cardiovascular disease. This pathway is dependent upon the amount of inorganic nitrate and nitrite in the foods we eat, the presence of oral nitrate-reducing bacteria, and sufficient stomach acid production.

Summary The concept of food being medicine and medicine being food has lost its place in the practice and implementation of modern medicine over the past century. Certain dietary patterns and specific foods are known to confer very significant protective effects for many human diseases, including cardiovascular disease, the number one killer of men and women in the developed world. However, identification of single or multiple bioactive molecules that are responsible for these effects has escaped scientists and nutritionists for many years. This review will highlight the biochemical, physiological, and epidemiological basis for functional nitric oxide nutrition that can be safely and effectively utilized in patients.

Keywords Dietary nitrate · Nitrite · Microbiome · Stomach acid · Beets · Vegetarian

Introduction

Cardiovascular Disease: Are we Doing Enough?

Despite advances in diagnostic and imaging modalities to detect early stages of cardiovascular disease (CVD) and many new drugs approved for treating symptoms of CVD such as hypertension, hyperlipidemia, diabetes, etc., heart attacks and strokes still remain the number 1 and number 3 killer of men and women worldwide, respectively. Over 600,000 people die of heart disease every year. Each minute of every day, someone dies from an event due to heart disease. The amount of people dying from cardiovascular disease is equivalent to four jumbo jets crashing and killing everyone on board every single hour of every single day each year. These statistics are unacceptable, especially since science has revealed the cause of cardiovascular disease and that it can be mitigated by diet and lifestyle. Moderate physical exercise and a diet rich in vegetables is proven to prevent or even reverse heart disease [1–3]. The mechanism of action of both physical exercise and a vegetarian diet is activation and promotion of nitric oxide [4, 5, 6••]. Hypertension, the number one modifiable risk factor for development of cardiovascular disease, is poorly managed. In the USA, about 78 million (1 out of every 3) people have high blood pressure or hypertension. Another one out of three have pre-hypertension (CDC fact sheet). That puts over 150 million Americans at risk for heart disease. Despite major advances in understanding the pathophysiology of hypertension and availability of antihypertensive drugs, suboptimal blood pressure control is still the most important risk factor for cardiovascular mortality. According to the AHA 2015

This article is part of the Topical Collection on *Nutrition*

✉ Nathan S. Bryan
Nathan.bryan@bcm.edu

[1] Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building for Biomedical Research R-850, Houston, TX 77030, USA

Statistics Fact Sheet, 75% of people that know they have hypertension and take medication for their high blood pressure, only about half of those are adequately managed. Since blood pressure remains elevated in approximately half of all treated hypertensive patients [7, 8], new safe and cost-effective solutions are desperately needed. We now know from the SPRINT trial that better management of blood pressure reduces all-cause mortality [9]. Adding additional drug therapy is not the solution. Loss of nitric oxide production and signaling is the cause of hypertension [10, 11]. Providing evidence-based nitric oxide functional nutrition may provide a simple, cost-effective, and most importantly safe strategy for preventing, treating, or reversing cardiovascular disease. This approach is supported by biochemical, physiological, and epidemiological evidence. The objective of this review is to summarize these findings and illustrate how diet and lifestyle disrupt NO production and, most importantly, illustrate how we can overcome these roadblocks to normalize NO-based cell signaling to restore normal cardiovascular function.

Nitric Oxide

The discovery of nitric oxide production in the body of mammals and humans over 30 years ago opened a new field of cardiovascular research. Now, with over 150,000 scientific papers published on this molecule, a Nobel Prize awarded to the three US scientists responsible for its discovery, and a growing awareness around NO, it can no longer be ignored by medical healthcare practitioners. Surprisingly, there have been no hallmark therapeutic breakthroughs in terms of drug therapy around NO. Perhaps this is due to the fact that NO itself may not be "drugable." NO, once produced, has a half-life of less than 1 s [12]. NO binds to redox-active metals and initiates its cell signaling actions, primarily through activation of soluble guanylyl cyclase (sGC) and a subsequent increase in cyclic guanosine monophosphate (cGMP) [10]. Redox-active metabolites of NO can also bind to cellular thiols to initiate cGMP-independent signaling cascades likely due to changes in protein conformation and functionality [13]. Most drugs today are inhibitors of specific enzymatic reactions. We know that it is low NO and loss of production of bioactive NO that is responsible for cardiovascular disease, so an enzyme inhibitor for NO is not prudent. Although phosphodiesterase inhibitors to inhibit the breakdown of cGMP have been successful treatments for erectile dysfunction and pulmonary hypertension [14, 15], 30–35% of patients fail to respond to this therapy [16]. This is due to insufficient nitric oxide production to activate sGC to lead to any accumulation of cGMP. Therefore, erectile dysfunction is really a symptom of insufficient NO production or endothelial dysfunction. This type of vascular dysfunction occurs throughout the entire cardiovascular system and leads to loss of regulation of blood flow and circulation upon demand. As a result, focus should be on restoring NO production and not downstream targets.

Loss of functional endothelial NO production, termed endothelial dysfunction, precedes the structural changes in the vasculature by many years, sometimes decades, and correlates with cardiovascular risks [17]. Aging and hypertension are established and validated cardiovascular risk factors [18, 19]. The functional and structural vascular changes that lead to the complication of cardiovascular disease share similar characteristics in older subjects that have aged normally to those younger adults that have aged with high blood pressure [20]. Furthermore, the vascular changes seen in essential hypertension are considered to be an accelerated progressive form of vascular structure and function seen with normal aging [21]. Young healthy individuals have normal and sufficient endothelial production of NO through L-arginine. However, as we age, we lose our ability to synthesize NO from L-arginine; this is termed endothelial dysfunction. Most of the work on the production of NO in cells, tissues, and humans agree that the bioavailability or the generation of NOS-derived NO decreases with aging. Increased oxidative stress through production of superoxide can scavenge NO thereby reducing its effective concentrations and signaling actions in cells [22]. Aging also causes a decrease in NOS enzyme expression [23, 24]. There is also an upregulation of arginase (an enzyme that degrades the natural substrate for NOS, L-arginine) in the blood vessels as we age that causes a reduction in NO production [25] due to a shuttling of L-arginine away from the NOS enzyme. Aging causes a gradual decline in NO production with a greater than 50% loss in endothelial function in some aged populations [26]. Some studies show a more than 75% loss of NO in the coronary circulation in patients in their 70s and 80s compared to young, healthy 20-year olds [27]. Others have shown [28] that age was the most significant predictor of endothelium-derived NO production. These data clearly demonstrate that NO production from L-arginine declines as we get older. This is due to uncoupling of the NOS enzyme which is then unable to convert L-arginine into NO. This process can be accelerated or decelerated depending on diet and lifestyle. The majority of studies reveal that loss of NO production was clearly evident by 40 years of age. However, the vasodilation to exogenous NO (endothelium-independent vasodilation) does not change over time with aging, illustrating that the body does not lose its ability to respond to NO; it only loses its ability to generate it with age. These observations allow scientists and physicians to conclude that reduced production of NO occurs as we age, and this creates the environment that is conducive to the onset and progression of cardiovascular disease. This is illustrated in Fig. 1.

Over the past several decades, the development of novel biomarkers allows physicians to gain a perspective on the degree of vascular disease based on the presence of specific biomarkers in peripheral blood. Functional measurements in endothelial NO production also allow us to determine endothelial function (endothelial NO production) throughout the

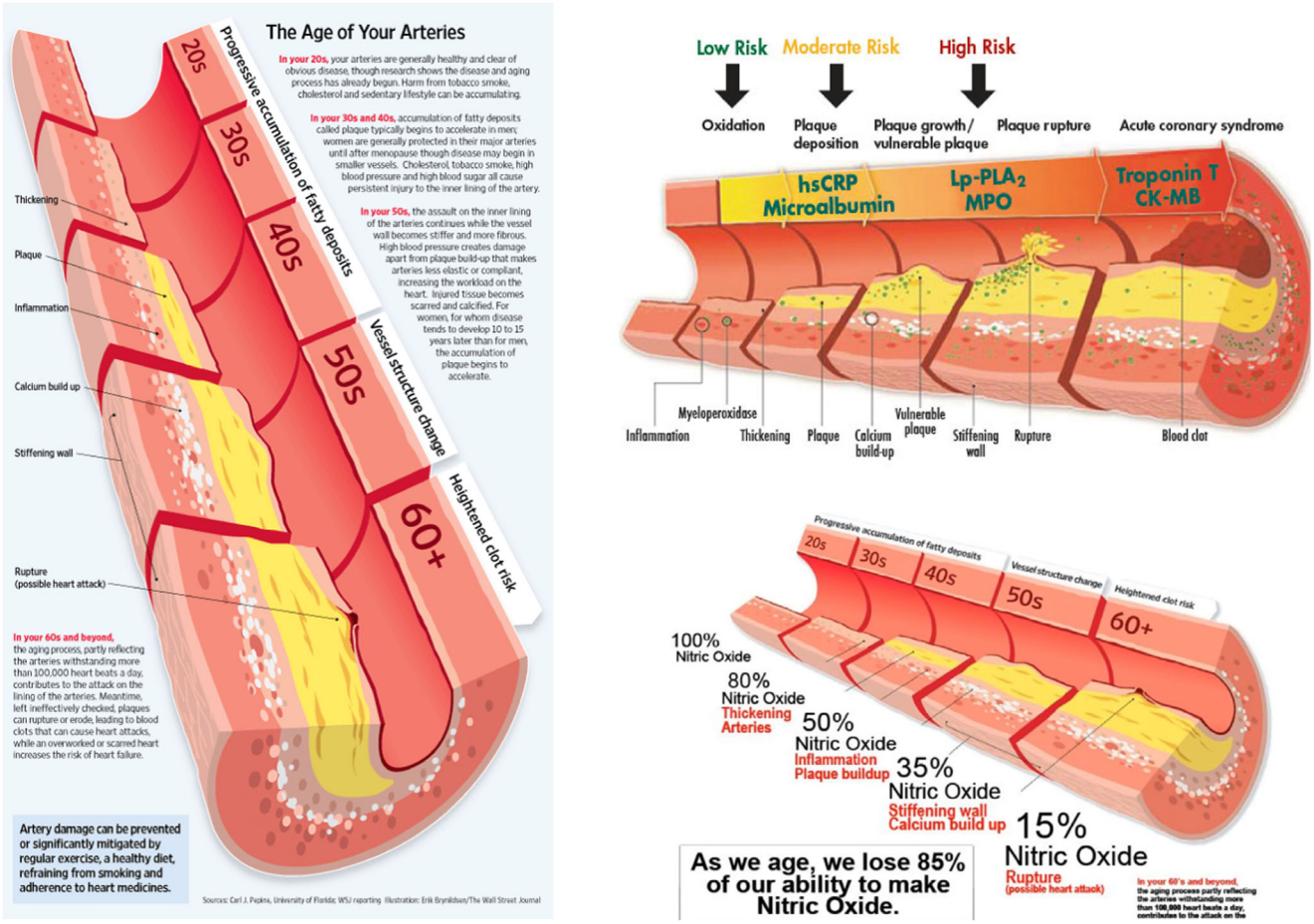

Fig. 1 (Left) Fat deposition and increased thickness of the media along with plaque formation occurs as we age. (Upper right) Specific blood biomarkers correlate with different stages of vascular disease and/or plaque vulnerability. (Lower right) Loss of endothelial nitric oxide production precedes presence of biomarker and structure changes that occur during progression of CVD

progression of cardiovascular disease. It is this functional loss of NO that precedes the appearance of the biomarkers and the structural changes seen during atherosclerosis. Since loss of NO precedes all biochemical biomarkers and the structural changes along the vascular tree, restoration of NO production/signaling will very likely allow for new treatment strategies for age and age-related disease.

NO Production Pathways

The discovery of NO originated from the observation that L-arginine was required for nitrite production involving a group of enzymes called nitric oxide synthase (NOS) [29]. NOS enzymes are homodimers that catalyze the five electron oxidation of the guanidino nitrogen of L-arginine. These enzymes are the only enzymes known to simultaneously require multiple bound cofactors/prosthetic groups: flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, glutathione, nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (BH_4), and Ca^{2+}-calmodulin. This is the pathway that becomes dysfunctional with age due to NOS uncoupling.

Understanding how the body makes NO and what goes wrong with these production pathways allows us to define the context for safe and effective interventions. In most cases, loss of NO production occurs due to oxidative stress and oxidation of tetrahydrobiopterin (BH4) which causes NOS uncoupling [30]. Therefore, using L-arginine supplementation alone will not recouple the NOS enzyme. The concentration of available L-arginine (100 to 800 μmol/L) is orders of magnitude greater than the Michaelis constant for binding to NOS (Km; 3 μmol/L) for NOS and is even higher in the circulation [31, 32]. In addition, cells can make their own L-arginine from L-citrulline through the partial urea cycle [33, 34]. As a result, L-arginine is never the rate-limiting step in NO production. Supplementation with antioxidants to help prevent oxidation of BH4 may be a better approach. Studies have demonstrated the role of oxidative stress on NO production and availability. With antioxidants such as vitamin C, NO production can be improved in older subjects [26]. In the oldest of subjects (age > 60 years) that had clear and evident endothelial

dysfunction, supplementing with vitamin C enhanced NO production in response to an endothelial agonist and also restored the inhibitory effect of L-NMMA on vasodilation to acetylcholine [26]. Although the antioxidant supplementation was effective for older subjects with oxidative stress, it showed no benefit in a younger healthy population. Collectively, the literature suggests that strategies to enhance NO production through L-arginine supplementation are equivocal at best. In some patient populations, administering L-arginine can do more harm than good. L-arginine, when added to standard post-infarction therapies, did not improve vascular stiffness measurements or ejection fraction and was associated with higher post-infarction mortality. The authors concluded L-arginine should not be recommended following acute myocardial infarction [35]. Similarly, in patients with PAD, long-term administration of L-arginine does not increase nitric oxide synthesis or improve vascular reactivity. Furthermore, the expected placebo effect observed in studies of functional capacity was attenuated in the L-arginine-treated group. As opposed to its short-term administration, long-term administration of L-arginine is not useful in patients with intermittent claudication and PAD [36].

Although the production of NO from L-arginine was discovered first, today we know that it may not always be the primary pathway for endogenous NO production. In fact, nitrogen fixation and denitrification in bacteria with production of NO as an intermediate may be one of the most primitive pathways, dating back to the Archaean era [37]. The now recognized human nitrate-nitrite-nitric oxide pathway that still relies on commensal bacteria may be a redundant system for overcoming the body's inability to make NO from L-arginine [38]. This alternate route involves the nitrate and nitrite as substrates that are reduced to NO. This concept is presented in Fig. 2. The two-electron reduction of nitrate to nitrite occurs through symbiosis with facultative anaerobic bacteria that reside in the crypts of our tongue [39]. Nitrite reduction to NO is a much simpler process than the reduction of nitrate to nitrite. The one-electron reduction of nitrite can occur by ferrous heme proteins [40] (or any redox active metal) through the following reaction:

$$NO_2^- + Fe^{2+} + H^+ \leftrightarrow NO + Fe^{3+} + OH^-$$

This is biologically active NO just as that produced by NOS. This pathway depends on nitrite availability and not on L-arginine. This is a reduction of nitrite rather than an oxidation of L-arginine but is still a relatively inefficient process [41]. Therefore, for biologically active amounts of NO to be produced from nitrite, the tissues or biological compartment must have a sufficient pool of nitrite stored. Since nitrite concentrations in plasma and tissue is a direct measure of NOS activity [42], a dysfunctional NOS system can lead to nitrite deficiency, which will lead to even less NO produced from nitrite. Since nitrite can also be derived from dietary nitrate from certain foods, we have the ability to compensate for loss of NO production from NOS, provided all systems are in place for nitrate metabolism. Therefore, it is possible that consuming nitrate-enriched vegetables has the ability to restore nitrite levels and replete NO-based signaling.

How Much Nitrate Is Required in Our Diet?

There are now a growing number of studies showing health benefits from inorganic nitrate found in vegetables [6••]. These studies establish that 300–400 mg of nitrate is required to see any changes in blood pressure or improvements in exercise performance [6••]. This can be achieved through the consumption of specific high nitrate foods, such as spinach, beets, kale, and other green leafy vegetables. In order to determine just how much of any given food one would have to consume to reach 300–400 mg of dietary nitrate, we analyzed five different foods from five different cities across the USA for nitrate content. We found a more than tenfold difference in nitrate content of the same vegetable across five US cities and also more than tenfold difference in nitrate between different vegetables [43•]. Interestingly, organically grown vegetables had much less nitrate than conventionally grown, in some cases up to ten times less. What this means in terms of dietary consumption patterns, for example, is that if you live in Dallas, Texas, you could eat roughly 60 g of spinach (small salad plate) and consume 300 mg nitrate. However, if you live in Chicago, Illinois, you would have to eat over 450 g of spinach to consume over 300 mg nitrate. Just because you are eating vegetables does not mean that you are consuming sufficient nitrate to affect NO production. Nitrate assimilation is dependent upon soil conditions, time of harvest, amount of fertilizer added, and water availability (drought) [44]. Based on existing databases, the mean estimated intake for nitrate and nitrite in the USA and Europe varies but is consistent and somewhat comparable. International estimates of nitrate intakes from food are 31–185 mg/day in Europe and in the USA about 40–100 mg/day [45, 46]. We know from above that 300–400 mg as a bolus is required for NO production and improvements in blood pressure and exercise performance. Most people are consuming only half of this amount over two to three meals and not as a bolus. Therefore, the US diet is depleted in nitrate and as a result, Americans are a nitrate-deficient society. The research suggests that this deficiency may be partly responsible for the increased incidence of all cardiovascular-related diseases in the US population [47]. Consistent with this notion, certain diets provide much more nitrate. For example, the Dietary Approaches to Stop Hypertension diet can provide as much as 1200 mg nitrate per day from selection of certain foods [48]. The Japanese diet also provides sufficient nitrate that has been shown to

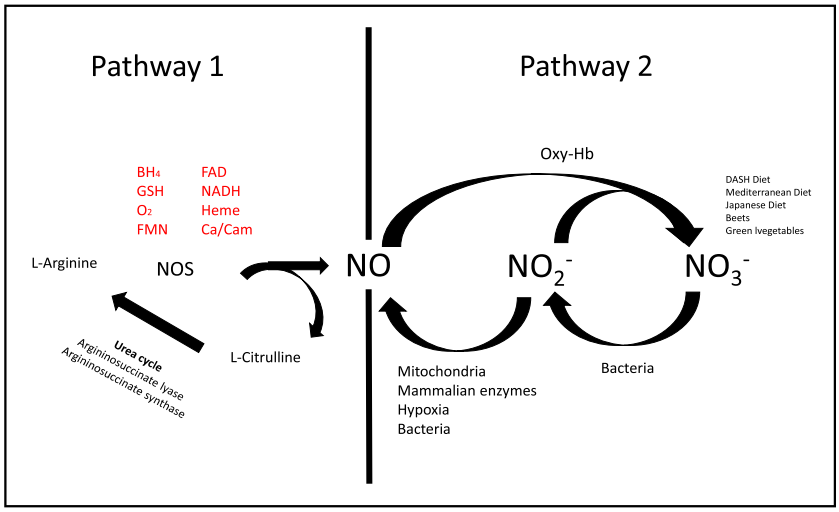

Fig. 2 There are two production pathways the human body generates NO. Pathway 1 involves oxidation of L-arginine to NO through a complex series of reactions involving nitric oxide synthase (NOS) enzymes. This pathway becomes dysfunctional with age. Pathway 2 involves the provision of nitrate and nitrite found in our food supply and generated from oral nitrate-reducing bacteria. This pathway becomes disrupted with insufficient nitrate ingestion, antiseptic mouthwash/antibiotic use, and antacid therapy. One can compensate for the other but when both systems fail, NO-based signaling is completely inhibited and disease ensues

reduce blood pressure and improve performance [49]. Similarly, the Mediterranean diet provides sufficient nitrate along with antioxidants to support reduction of nitrate to nitrite and nitric oxide [50]. There is strong evidence that it is the inorganic nitrate in these foods and diets that confer the protective and health-promoting activities [47]. Although many clinical trials have been performed to try to identify the mechanism of action of these diets, looking primarily at antioxidants, vitamins, and minerals, most if not all have failed to recapitulate the effects of whole-food diets. Evidence strongly suggests that it is the nitrate-nitrite content along with the antioxidants that account for the effects.

Nitrate Is Inert in Humans

Even if we consume sufficient nitrate from our diet, if it is not first metabolized and reduced to nitrite, the human body cannot utilize it to make nitric oxide. Humans do not have a functional nitrate reductase gene. These are mostly bacteria gene products from commensal bacteria that live in and on our body [51, 52]. We and others have identified a number of facultative anaerobic bacteria that are efficient at the two-electron reduction of nitrate to nitrite in the oral cavity [51, 53]. Nitrate ingested from the diet is rapidly absorbed in the small intestine, taken up by the sialin transporter [54], mixes with the endogenous nitrate from oxidation of nitric oxide and is readily distributed throughout the body [55] and mostly concentrated in our salivary glands. About 25% of oral nitrate from diet is concentrated and excreted by salivary glands [56], so that salivary nitrate concentration is approximately 10 times higher in the saliva than in plasma. Approximately 20% of salivary nitrate can be reduced to nitrite in the mouth by facultative anaerobic bacteria which are found on the dorsal surface of the tongue [39, 51], if nitrate-reducing bacteria are present, resulting in about a 5% reduction of total ingested nitrate to nitrite. If we are consuming 300–400 mg nitrate from our diet, which is the dose known to be effective at reducing blood pressure and enhancing performance, then this reduction efficacy results in 15–20 mg nitrite produced from dietary nitrate through the enterosalivary circuit. Nitrate when consumed through the diet reaches a peak plasma concentration in about 1 h [57]. The plasma half-life of nitrate is roughly 5 h [57]. Nitrate is a small anion and is not protein-bound, and its pharmacokinetics and half-life suggest that it is reabsorbed in the renal tubules. Nitrate can be converted to urea then excreted in the urine [58]. Nitrate clearance from blood to urine is approximately 20 ml/min in adults [59], indicating considerable renal tubular re-absorption of the ion. It is estimated that 96% of the filtered nitrite and nitrate is reabsorbed in the renal tubules [60]. Other studies in dogs suggest that approximately 80% of filtered nitrate is reabsorbed [61]. The high concentration of nitrate and nitrite in saliva and other tissue, continuous production from nitric oxide, and the re-absorption from renal tubules strongly suggest that nitrate and nitrite have a definite role in normal human physiology and is not just an unwanted toxin. It serves as an important substrate for NO production provided the body can utilize it.

Cardiovascular Health Benefits of Nitrate

Over the past 10 years, there have been many published studies showing health benefits in humans of dietary nitrate including blood pressure regulation and sports performance [6••]. For the majority of studies in humans, investigators have

used beetroot juice that is standardized to a known amount of nitrate. These studies reveal that 450–550 mg nitrate must be administered at least 2.5 h before the measurable endpoints in order for the enterosalivary circulation and reduction of nitrate to nitrite and NO to show any improvements in performance. The data reveal that increasing plasma nitrite levels can increase NO production and improve oxygen efficiency and athletic performance [62–64]. In studies in older adults, nitrate supplementation with beetroot juice (400 mg nitrate per dose twice per day (816 mg total)) increased plasma nitrite concentration, reduced blood pressure, and positively influenced physiological responses to exercise [65]. Nitrate supplementation at a dose of 6.62 mg/kg can also enhance exercise performance in patients with peripheral artery disease [66] whereas L-arginine was ineffective in these patients.

Dietary nitrate can also lower blood pressure. Consuming 0.5 L of beetroot juice containing 15 mM (638 mg) nitrate lowers blood pressure 6 h later in healthy adults [67]. To demonstrate it was indeed the nitrate responsible for these effects, Kapil et al. gave potassium nitrate capsules containing 700 or 1488 mg of nitrate or had patients drink beetroot juice that contained 468 mg of nitrate and found that all forms of nitrate led to a dose-dependent decrease in blood pressure beginning after 90 min and lasting for several hours [68]. Other studies show that giving 500–700 mg nitrate for 3 days led to a statistically significant reduction in diastolic pressure [69]. Similarly, drinking 442 mg nitrate from beetroot juice acutely reduces blood pressure and the oxygen cost of submaximal exercise. These effects are maintained for at least 15 days if supplementation is continued [64].

Antiseptic Mouthwash Eradicates the Benefits of Nitrate

All of the above studies confirm nitrate as the active agent since a nitrate-depleted beetroot juice was used as the placebo. Furthermore, the effects of dietary nitrate are completely abolished if subjects are not allowed to swallow or they use an antiseptic mouthwash to eradicate the oral microbiome [70–73]. Kapil demonstrated that use of antiseptic mouthwash for 7 days caused a decrease in salivary and plasma nitrite with a concomitant increase in systolic and diastolic blood pressure [71]. Increases in salivary and plasma levels of nitrite are completely abolished if subjects use an antiseptic mouthwash [70] demonstrating that the removal of these bacteria with an antibacterial mouthwash will very likely attenuate the NO-dependent biological effects of dietary nitrate. Over 180 million Americans use mouthwash on a daily basis, and in 2015 alone, approximately 269 million antibiotic prescriptions were dispensed from outpatient pharmacies in the USA, enough for five out of every six people to receive one antibiotic prescription each year. Interestingly, at least 30% of these antibiotic prescriptions were unnecessary [74]. Use of both antiseptic mouthwash and antibiotics disrupts the oral microbiome and leads to a complete lack of nitrate reduction or at least a decreased efficiency of nitrate reduction. Also, given the diversity and variability of the oral microbiome between certain individuals and cultures, it is uncertain how many people have the correct nitrate-reducing bacteria [53]. With prevalence of antibiotic and antiseptic mouthwash use in the USA along with periodontal disease and poor oral hygiene, it would not be surprising if over half of the population is unable to reduce dietary nitrate. This means that although they may be consuming what is considered a healthy diet even with sufficient nitrate, they are unable to get a nitric oxide benefit due to lack of nitrate reduction by bacteria. This should be a new consideration in patient assessment.

Stomach Acid Is Required for Optimal NO Production

Even if you have the right oral nitrate-reducing bacteria, this does not always mean you will get a benefit from the nitrate consumed in your diet that is then reduced to nitrite. Stomach acid is required for optimal effects of salivary nitrite that results from consuming dietary nitrate. Nitrite concentration in the saliva from reduction of dietary nitrite when swallowed becomes protonated (nitrite pKa ~3.4) to form nitrous acid. Nitrous acid can spontaneously release NO [75]. Proton pump inhibitors (PPIs) by inhibiting stomach acid production and increasing gastric pH may prevent formation of nitrous acid from inorganic nitrite, and, accordingly, NO release. Indeed, giving PPIs to rodents blocks the blood pressure-lowering effects of orally administered sodium nitrite [76]. Furthermore, PPIs blunt the favorable effects of antioxidants on nitrite-to-NO conversion in the stomach [77] and disrupts the formation of S-nitrosothiols. S-nitrosothiols are an important circulating reservoir of NO that also contributes to the blood pressure-lowering effects of orally administrated nitrates/nitrites [78]. PPIs also specifically lead to the accumulation of asymmetric dimethylarginine [79]. Asymmetric dimethylarginine (ADMA) is generated during metabolism of cellular proteins containing methyl-arginine residues. ADMA is broken down by the enzyme dimethylarginine dimethylaminohydrolase [80], an intracellular enzyme ubiquitously expressed in many cells. The inhibition of the dimethylaminohydrolase (DDAH) enzyme is known to be the major contributor to increases in ADMA in animal models and patients with cardiovascular risk factors [81, 82]. Evidence now reveals that PPI drugs directly inhibit DDAH activity [83]. In addition to inhibiting DDAH, PPIs affect NOS expression. Both phosphorylated (active) and unphosphorylated endothelial NOS proteins were downregulated by omeprazole [83]. Altogether, these findings provide proof-of-concept that PPIs are able to impair nitric oxide pathway in the endothelium as well as NO production from the nitrate-nitrite-NO pathway. There are approximately 64.6

million prescriptions written for gastroesophageal reflux disease (GERD) medications in the USA on an annual basis [68] accounting for over $11 billion in total healthcare expenditures in the USA, and this does not even include the OTC market. Any therapy that increases stomach pH will interrupt NO generation from salivary nitrite. Clear evidence is emerging that PPIs have adverse cardiovascular effects. These effects may be mediated primarily or at least in part through a disruption in NO production/signaling and should be considered when PPIs are prescribed, especially in patients at increased cardiovascular risk.

How Do You Overcome These Limitations?

Collectively, the evidence suggests that the US diet is deficient in nitrate. Furthermore, common drug therapy and lifestyle decisions disrupt metabolism of nitrate into nitrite and nitric oxide. So, how does one overcome deficiencies in dietary nitrate, variability between individual microbiome, mouthwash use, PPI use, or achlorhydria? We and others have focused on nitrite [84, 85]. Nitrite is the two-electron reduction product of nitrate that can be utilized and metabolized directly into nitric oxide by mammalian enzymes and is not dependent upon oral bacteria [86]. Nitrite is derived directly from exogenous dietary nitrate but also from the oxidation of endogenously produced nitric oxide [87]. Nitrite is found naturally in colostrum and breast milk [88], small amounts found naturally in green leafy vegetables [43], and small amounts added to cured meats [89]. Nitrite intake from food varies from 0 to 20 mg/day [90]. NO production rate measured with stable isotopes reveal that daily NO production varies between 0.15 and 2.2 μmol/kg/h in healthy subjects [91, 92]. This NO is quickly oxidized to nitrite and nitrate through the reaction with oxygen and oxyhemoglobin respectively [12]. For a 70–80-kg person, this would equate to approximately 20–200 mg nitrite and nitrate daily. Due to approximately 5% reduction of nitrate (average of 150 mg per day in US diet plus 200 mg from oxidation of NO), this would equate to 17.5 mg endogenous nitrite production. Therefore, total daily nitrite exposure in a normal healthy individual on a Western diet is roughly 20–40 mg. In the same healthy individual consuming more of a vegetarian diet or DASH diet that included 400–1200 mg nitrate per day [48], endogenous nitrite production could exceed 70 mg per day. These nitrite levels are dramatically reduced in people with endothelial dysfunction, insufficient vegetable consumption, or consuming vegetables without sufficient nitrate along with use of antibiotics/antiseptic mouthwash and/or PPIs. These data beg the question that if most people are nitrite deficient, can we safely and adequately supplement back what is missing? This approach is no different than vitamin D for example. If labs demonstrate we are low in vitamin D, then you supplement what is missing in order to normalize your levels. This has been our approach with nitrite and nitrate.

There are a number of published studies in humans showing the safety and efficacy of nitrite within a large range of doses. Sodium nitrite capsules at doses of 160 and 320 mg were used to determine toxicity and pharmacokinetics. Nitrite even at a dose of 320 mg did not show any clinically toxic levels of methemoglobinemia (< 15%) [93]. However, some subjects reported mild headache and nausea that resolved after half hour. This study also revealed that nitrite is 98% orally available. In another study using sodium nitrite capsule in diabetics demonstrated that a single administration of 80 mg sodium nitrite was well tolerated with no significant changes in methemoglobin, sulfhemoglobin, pulse rate, laboratory tests, or other safety parameters with the possible exception of headache and a hot flush feeling in 2 of the 12 subjects (17%) [94]. The 80-mg nitrite dose led to a significant drop in systolic blood pressure with no effect on diastolic pressures. Plasma nitrite levels increased to 3–4 μM or approximately 10 times higher than normal steady state. More chronic studies using 80–160 mg nitrite capsules for 10 weeks in a randomized, placebo control, double-blind study increased plasma nitrite acutely and chronically and was well tolerated without symptomatic hypotension or clinically relevant levels of methemoglobin. Endothelial function, measured by brachial artery flow-mediated dilation, was significantly improved without changes in body mass or blood lipids. Carotid artery elasticity (as measured by ultrasound and applanation tonometry) improved. These functional changes were related to 11 plasma metabolites identified by untargeted analysis with glycerophospholipids and fatty acyls, predicting these vascular changes with nitrite [95]. Similarly, in another study, using 80 and 160 mg nitrite capsules for 10 weeks showed improvement in performance on measures of motor and cognitive outcomes in healthy middle-aged and older adults (62 ± 7 years) [96]. These studies provide evidence that sodium nitrite supplementation is well tolerated, increases plasma nitrite concentrations, improves endothelial function, lessens carotid artery stiffening, and improves motor and cognitive function in middle-aged and older adults, perhaps by altering multiple metabolic pathways. Other studies have investigated sodium nitrite directly infused in critically ill patients with subarachnoid hemorrhage. Infusion of sodium nitrite over 14 days at a maximum dose of 64 nmol/kg/min (622 mg of nitrite per day for 14 days) showed no toxicity or systemic hypotension, and blood methemoglobin levels remained at 3.3% or less in all patients [97]. The authors state that the results of their study suggest that safe and potentially therapeutic levels of nitrite can be achieved and sustained in critically ill patients after a ruptured cerebral aneurysm [97]. The effects of nitrite are not dependent upon oral nitrate-reducing bacteria and appear to be safe even at doses that far exceed daily human production.

The doses of nitrite used in these studies are typically more than one would normally consume in an ordinary diet. This is in part due to the fact that nitrite is inefficiently reduced to NO along the physiological oxygen gradient [41, 86] and therefore more is needed to get any appreciable amount of NO produced, especially in people that are NO deficient. Through the discovery of natural product chemistry of an oxygen-independent nitrite reductase, lower supplemental doses of nitrite can more effectively reduce nitrite to NO and therefore provide an exogenous source of NO in the oral cavity [98]. The premise of this technology is that if your body cannot make NO due to endothelial dysfunction, oral dysbiosis, antiseptic mouthwash, or PPI use, then this will provide an exogenous source of NO. Studies using 15–20 mg of supplemental sodium nitrite (Neo40™, HumanN, Inc™) to account for differences in endogenous production along with the natural product chemistry in the form of an orally disintegrating tablet found that it could modify cardiovascular risk factors in patients over the age of 40, significantly reduce triglycerides, and reduce blood pressure [99]. Single administration of this lozenge leads to peak plasma levels of nitrite around 1.5 μM. In patients with argininosuccinic aciduria (ASA), the nitrite lozenge led to a significant reduction in blood pressure when prescription medications were ineffective, improved renal function and cognition, and reversed cardiac hypertrophy [100]. Another randomized controlled study using the nitrite lozenge reveals that a single lozenge can significantly reduce blood pressure, dilate blood vessels, and improve endothelial function and arterial compliance in hypertensive patients [101]. Furthermore, in a study of pre-hypertensive patients (BP > 120/80 < 139/89), administration of one lozenge twice daily leads to a significant reduction in blood pressure (12 mmHg systolic and 6 mmHg diastolic) after 30 days [102] along with improvements in functional capacity as measured by a 6-min walk test. In an exercise study, the nitrite lozenge significantly improved exercise performance [103]. Most recently, in subjects with stable carotid plaque, the NO lozenge led to a 11% reduction in carotid plaque after 6 months [104]. To put this in perspective, meta-analysis of trials using treatment with statins (mean treatment duration of 25.6 months) reveal that a total of seven trials showed regression and four trials showed slowing of progression of CIMT of approximately 2.7% (− 0.04) after over 2 years [105]. Using the nitric oxide lozenge, the data show an average of 0.073 mm or 10.9% after 6 months [104]. Similarly, this same technology in the form of a concentrated beet root powder (Superbeets™, HumanN, Inc.™) attenuates peripheral chemoreflex sensitivity without concomitant change in spontaneous cardiovagal baroreflex sensitivity while also reducing systemic blood pressure and mean arterial blood pressure in older adults [106]. These studies clearly demonstrate the safety and efficacy of low supplemental doses of nitrite in humans that can correct for any insufficiencies from dietary exposure, pharmacological inhibition by antiseptics, or PPIs.

Is This What Nature Intended?

If supplementing deficient nitrite and nitrate in populations is a viable strategy for combatting cardiovascular disease or any condition associated with insufficient nitric oxide availability, are there examples in epidemiology or in specific populations that can provide justification for such? The answer may come in the form of nature's most perfect food, breast milk. We and others have published that early breast milk and colostrum contain high concentrations of nitrite until the bacteria begin to colonize in the oral and digestive tract of growing infants [88, 107, 108]. Once commensal bacteria have colonized, then breast milk changes from nitrite to nitrate so that the infant's body can utilize the nitrate-reducing bacteria to provide a more extended exposure to nitrite. Commercial infant formulas lack any nitrite and have very little nitrate [88]. The health disparities between breast-fed and formula-fed babies are well known. Supplementing nitrite that is missing in formula can protect from necrotizing enterocolitis [109]. These data clearly demonstrate that nitrite missing in formula that is present in breast milk when supplemented back into formula affords protection. Perhaps it is the missing nitrite in formula that accounts for some of the differences from that in breast milk.

Additionally, anthropological studies on native Tibetans reveal that their acclimatization to living at high altitude and reduced oxygen is through increased nitric oxide production with 20–50 times higher circulating nitrite and nitrate than those that live at sea level [110]. People who live at or near sea level increase their NO production and plasma levels of nitrite and nitrate as they ascend to altitude [111]. Increasing nitrite and nitrate availability is a physiological response to low oxygen and the adaptive response to allow us to acclimate to different environments. In other words, increasing steady-state concentrations of nitrite and nitrate appears to be a natural physiological response that allows the body to adapt to changing oxygen environments, whether environmental or physiological.

Conclusions

The most reasonable conclusion that can be made from the data reviewed is that humans are adapted to receive dietary nitrite and nitrate from birth and throughout life and therefore may not pose significant risks at levels naturally found in certain foods. In fact, we believe that the absence of an essential nutrient, namely nitrite and/or nitrate in our foods and diets, may be involved in many of the chronic health problems facing the entire developing world. Advancements in science and research over the past 30 years have illuminated the essential nature of nitrite and nitrate in our food supply as well as how our body makes these natural molecules. Eating a well-balanced, nutritious diet and performing moderate exercise

comprise the ideal model of routinely good health and disease prevention. The role of a proper diet in the prevention of disease is well established by many population-based epidemiological studies [43•]. Nothing affects our health more than what we choose to eat. Nitric oxide is essential for maintaining normal blood pressure, preventing adhesion of blood cells to the endothelium, and preventing platelet aggregation; it may, therefore, be argued that this single abnormality, the inability to generate NO, puts us at risk for diseases that plague us later in life, such as atherosclerosis, myocardial infarction, stroke, Alzheimer's disease, and peripheral vascular disease. Therefore, developing strategies and new technologies designed to restore NO availability is essential for inhibiting the progression of certain common chronic diseases. The provision of dietary nitrate and nitrite may allow for such a strategy. This review reveals the beneficial effects of nitrite and nitrate and how each step in their metabolism may be affected by lifestyle, hygiene habits, and/or drug therapy. Nutrition can play a key and cost-effective role in decreasing the risks of different chronic diseases. Hippocrates himself said, "let food be thy medicine and medicine be thy food". Understanding how the body utilizes key dietary nutrients, specifically nitrate, will help scientists and physicians develop more effective treatment strategies for overcoming key limitations in our diet or metabolism of dietary constituents. Implementing functional nitric oxide nutrition has the potential to profoundly change the face of health and disease since it can overcome any inherent problems in nitrate metabolism.

Compliance with Ethical Standards

Conflict of Interest NSBryan is the Founder and Shareholder of HumanN

NSBryan is a Shareholder and Advisor for SAJE Pharma

NSBryan receives royalties from patents from the University of Texas Health Science Center at Houston

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Tuso P, Stoll SR, Li WW. A plant-based diet, atherogenesis, and coronary artery disease prevention. Perm J. 2015;19(1):62–7.
2. Cooper KH, Pollock ML, Martin RP, White SR, Linnerud AC, Jackson A. Physical fitness levels vs selected coronary risk factors. A cross-sectional study. JAMA. 1976;236(2):166–9.
3. Blair SN, Kohl HW 3rd, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. JAMA. 1989;262(17):2395–401.
4. Rassaf T, Lauer T, Heiss C, Balzer J, Mangold S, Leyendecker T, et al. Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. Br J Sports Med. 2007;41(10):669–73. discussion 673
5. Green DJ, O Driscoll G, Blanksby BA, Taylor RR. Control of skeletal muscle blood flow during dynamic exercise: contribution of endothelium-derived nitric oxide. Sports Med. 1996;21(2):119–46.
6. •• Bryan NS, Ivy JL. Inorganic nitrite and nitrate: evidence to support consideration as dietary nutrients. Nutr Res. 2015;35(8):643–54.
7. Wang YR, Alexander GC, Stafford RS. Outpatient hypertension treatment, treatment intensification, and control in Western Europe and the United States. Arch Intern Med. 2007;167(2):141–7.
8. Cutler JA, Sorlie PD, Wolz M, Thom T, Fields LE, Roccella EJ. Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988-1994 and 1999-2004. Hypertension. 2008;52(5):818–27.
9. Wright JT Jr, et al. A randomized trial of intensive versus standard blood-pressure control. N Engl J Med. 2015;373(22):2103–16.
10. Arnold WP, Mittal CK, Katsuki S, Murad F. Nitric oxide activates guanylate cyclase and increases guanosine 3′:5′-cyclic monophosphate levels in various tissue preparations. Proc Natl Acad Sci U S A. 1977;74(8):3203–7.
11. Bryan NS, Bian K, Murad F. Discovery of the nitric oxide signaling pathway and targets for drug development. Front Biosci. 2009;14:1–18.
12. Kelm M. Nitric oxide metabolism and breakdown. Biochim Biophys Acta. 1999;1411:273–89.
13. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, et al. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci U S A. 1992;89:444–8.
14. Boolell M, Allen MJ, Ballard SA, Gepi-Attee S, Muirhead GJ, Naylor AM, et al. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int J Impot Res. 1996;8(2):47–52.
15. Weimann J, Ullrich R, Hromi J, Fujino Y, Clark MWH, Bloch KD, et al. Sildenafil is a pulmonary vasodilator in awake lambs with acute pulmonary hypertension. Anesthesiology. 2000;92(6):1702–12.
16. McMahon CN, Smith CJ, Shabsigh R. Treating erectile dysfunction when PDE5 inhibitors fail. BMJ. 2006;332(7541):589–92.
17. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation. 1990;81(2):491–7.
18. Lakatta EG, Yin FC. Myocardial aging: functional alterations and related cellular mechanisms. Am J Phys. 1982;242(6):H927–41.
19. Kannel WB, Gordon T, Schwartz MJ. Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study. Am J Cardiol. 1971;27(4):335–46.
20. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340(2):115–26.
21. Soltis EE. Effect of age on blood pressure and membrane-dependent vascular responses in the rat. Circ Res. 1987;61(6):889–97.
22. van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, et al. Enhanced peroxynitrite formation is associated with vascular aging. J Exp Med. 2000;192(12):1731–44.
23. Pie JE, Baek SY, Kim HP, Ryu SD, Chung WG, Cha YN, et al. Age-related decline of inducible nitric oxide synthase gene expression in primary cultured rat hepatocytes. Mol Cells. 2002;13(3):399–406.
24. Zhou XJ, Vaziri ND, Zhang J, Wang HW, Wang XQ. Association of renal injury with nitric oxide deficiency in aged SHR:

prevention by hypertension control with AT1 blockade. Kidney Int. 2002;62(3):914–21.
25. Berkowitz DE, White R, Li D, Minhas KM, Cernetich A, Kim S, et al. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. Circulation. 2003;108(16):2000–6.
26. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, et al. Age-related reduction of NO availability and oxidative stress in humans. Hypertension. 2001;38(2):274–9.
27. Egashira K, Inou T, Hirooka Y, Kai H, Sugimachi M, Suzuki S, et al. Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. Circulation. 1993;88(1):77–81.
28. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension. 1996;27(4):849–53.
29. Hibbs JB Jr, Taintor RR, Vavrin Z. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science. 1987;235(4787):473–6.
30. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest. 2003;111(8):1201–9.
31. Lortie MJ, Ishizuka S, Schwartz D, Blantz RC. Bioactive products of arginine in sepsis: tissue and plasma composition after LPS and iNOS blockade. Am J Phys Cell Physiol. 2000;278(6):C1191–9.
32. Aisaka K, Gross SS, Griffith OW, Levi R. L-arginine availability determines the duration of acetylcholine-induced systemic vasodilation in vivo. Biochem Biophys Res Commun. 1989;163(2):710–7.
33. Erez A, Nagamani SCS, Shchelochkov OA, Premkumar MH, Campeau PM, Chen Y, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med. 2011;17(12):1619–26.
34. Hecker M, Mitchell JA, Harris HJ, Katsura M, Thiemermann C, Vane JR. Endothelial cells metabolize NG-monomethyl-L-arginine to L-citrulline and subsequently to L-arginine. Biochem Biophys Res Commun. 1990;167(3):1037–43.
35. Schulman SP, Becker LC, Kass DA, Champion HC, Terrin ML, Forman S, et al. L-arginine therapy in acute myocardial infarction: the vascular interaction with age in myocardial infarction (VINTAGE MI) randomized clinical trial. JAMA. 2006;295(1):58–64.
36. Wilson AM, Harada R, Nair N, Balasubramanian N, Cooke JP. L-arginine supplementation in peripheral arterial disease: no benefit and possible harm. Circulation. 2007;116(2):188–95.
37. Moir JWB, editor. Nitrogen cycling in bacteria: molecular analysis. Norfolk: Caister Acedemic Press; 2011.
38. Bryan NS, Loscalzo J. Nitrite and nitrate in human health and disease. In: Bendich A, editor. Nutrition and health. New York: Humana Press; 2011.
39. Lundberg JO, Weitzberg E, Cole JA, Benjamin N. Nitrate, bacteria and human health. Nat Rev Microbiol. 2004;2(7):593–602.
40. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med. 2003;9:1498–505.
41. Feelisch M, Fernandez BO, Bryan NS, Garcia-Saura MF, Bauer S, Whitlock DR, et al. Tissue processing of nitrite in hypoxia: an intricate interplay of nitric oxide-generating and -scavenging systems. J Biol Chem. 2008;283(49):33927–34.
42. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, et al. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. Free Radic Biol Med. 2003;35(7):790–6.
43.• Nunez de Gonzalez MT, et al. A survey of nitrate and nitrite concentrations in conventional and organic-labeled raw vegetables at retail. J Food Sci. 2015;80(5):C942–9.
44. Bryan NS, Van Grinsven H. The role of nitrate in human health. In: Sparks DL, editor. Advances in agronomy. New York: Elsevier; 2013. p. 153–76.
45. Mensinga TT, Speijers GJ, Meulenbelt J. Health implications of exposure to environmental nitrogenous compounds. Toxicol Rev. 2003;22(1):41–51.
46. Gangolli SD, van den Brandt P, Feron VJ, Janzowsky C, Koeman JH, Speijers GJ, et al. Nitrate, nitrite and N-nitroso compounds. Eur J Pharmacol. 1994;292(1):1–38.
47. Lundberg JO, Feelisch M, Björne H, Jansson EÅ, Weitzberg E. Cardioprotective effects of vegetables: is nitrate the answer? Nitric Oxide. 2006;15(4):359–62.
48. Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr. 2009;90(1):1–10.
49. Sobko T, Marcus C, Govoni M, Kamiya S. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Nitric Oxide. 2010;22(2):136–40.
50. Nadtochiy SM, Redman EK. Mediterranean diet and cardioprotection: the role of nitrite, polyunsaturated fatty acids, and polyphenols. Nutrition. 2011;27(7–8):733–44.
51. Doel JJ, Benjamin N, Hector MP, Rogers M, Allaker RP. Evaluation of bacterial nitrate reduction in the human oral cavity. Eur J Oral Sci. 2005;113(1):14–9.
52. Li H, Duncan C, Townend J, Killham K, Smith LM, Johnston P, et al. Nitrate-reducing bacteria on rat tongues. Appl Environ Microbiol. 1997;63(3):924–30.
53. Hyde ER, Andrade F, Vaksman Z, Parthasarathy K, Jiang H, Parthasarathy DK, et al. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. PLoS One. 2014;9(3):e88645.
54. Qin L, Liu X, Sun Q, Fan Z, Xia D, Ding G, et al. Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane. Proc Natl Acad Sci U S A. 2012;109(33):13434–9.
55. Walker R. The metabolism of dietary nitrites and nitrates. Biochem Soc Trans. 1996;24(3):780–5.
56. Spiegelhalder B, Eisenbrand G, Preussmann R. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds. Food Cosmet Toxicol. 1976;14(6):545–8.
57. McKnight GM, Smith LM, Drummond RS, Duncan CW, Golden M, Benjamin N. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut. 1997;40(2):211–4.
58. Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, et al. Nitrate biosynthesis in man. Proc Natl Acad Sci U S A. 1981;78(12):7764–8.
59. Wennmalm A, Benthin G, Edlund A, Jungersten L, Kieler-Jensen N, Lundin S, et al. Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. Circ Res. 1993;73(6):1121–7.
60. Rahma M, et al. Effects of furosemide on the tubular reabsorption of nitrates in anesthetized dogs. Eur J Pharmacol. 2001;428(1):113–9.
61. Godfrey M, Majid DS. Renal handling of circulating nitrates in anesthetized dogs. Am J Phys. 1998;275(1 Pt 2):F68–73.
62. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. J Appl Physiol. 2010;109(1):135–48.
63. Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, et al. Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. J Appl Physiol. 2011;110(3):591–600.
64. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological

responses to moderate-intensity and incremental exercise. Am J Phys Regul Integr Comp Phys. 2010;299(4):R1121–31.
65. Kelly J, Fulford J, Vanhatalo A, Blackwell JR, French O, Bailey SJ, et al. Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults. Am J Phys Regul Integr Comp Phys. 2013;304(2):R73–83.
66. Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, VanBruggen M, et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. J Appl Physiol (1985). 2011;110(6):1582–91.
67. Coles LT, Clifton PM. Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: a randomized, placebo-controlled trial. Nutr J. 2012;11:106.
68. Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, et al. Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. Hypertension. 2010;56(2):274–81.
69. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med. 2006;355(26):2792–3.
70. Govoni M, Jansson EÅ, Weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide. 2008;19(4):333–7.
71. Kapil V, Haydar SMA, Pearl V, Lundberg JO, Weitzberg E, Ahluwalia A. Physiological role for nitrate-reducing oral bacteria in blood pressure control. Free Radic Biol Med. 2013;55:93–100.
72. Petersson J, Carlström M, Schreiber O, Phillipson M, Christoffersson G, Jägare A, et al. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. Free Radic Biol Med. 2009;46(8):1068–75.
73. Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension. 2008;51(3):784–90.
74. Fleming-Dutra KE, Hersh AL, Shapiro DJ, Bartoces M, Enns EA, File TM Jr, et al. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. JAMA. 2016;315(17):1864–73.
75. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intragastric nitric oxide production in humans: measurements in expelled air. Gut. 1994;35(11):1543–6.
76. Pinheiro LC, Montenegro MF, Amaral JH, Ferreira GC, Oliveira AM, Tanus-Santos JE. Increase in gastric pH reduces hypotensive effect of oral sodium nitrite in rats. Free Radic Biol Med. 2012;53(4):701–9.
77. Amaral JH, Montenegro MF, Pinheiro LC, Ferreira GC, Barroso RP, Costa-Filho AJ, et al. TEMPOL enhances the antihypertensive effects of sodium nitrite by mechanisms facilitating nitrite-derived gastric nitric oxide formation. Free Radic Biol Med. 2013;65:446–55.
78. Pinheiro LC, Amaral JH, Ferreira GC, Portella RL, Ceron CS, Montenegro MF, et al. Gastric S-nitrosothiol formation drives the antihypertensive effects of oral sodium nitrite and nitrate in a rat model of renovascular hypertension. Free Radic Biol Med. 2015;87:252–62.
79. Porst H, Padma-Nathan H, Giuliano F, Anglin G, Varanese L, Rosen R. Efficacy of tadalafil for the treatment of erectile dysfunction at 24 and 36 hours after dosing: a randomized controlled trial. Urology. 2003;62(1):121–5; discussion 125-6.
80. Perros F, Ranchoux B, Izikki M, Bentebbal S, Happé C, Antigny F, et al. Nebivolol for improving endothelial dysfunction, pulmonary vascular remodeling, and right heart function in pulmonary hypertension. J Am Coll Cardiol. 2015;65(7):668–80.
81. Cooke JP, Ghebremariam YT. DDAH says NO to ADMA. Arterioscler Thromb Vasc Biol. 2011;31(7):1462–4.
82. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuehlinger MC, Wang BY, et al. Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. Circulation. 2003;108(24):3042–7.
83. Ghebremariam YT, LePendu P, Lee JC, Erlanson DA, Slaviero A, Shah NH, et al. Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. Circulation. 2013;128(8):845–53.
84. Bryan NS. Nitrite in nitric oxide biology: cause or consequence? A systems-based review. Free Radic Biol Med. 2006;41(5):691–701.
85. Kevil CG, Kolluru GK, Pattillo CB, Giordano T. Inorganic nitrite therapy: historical perspective and future directions. Free Radic Biol Med. 2011;51(3):576–93.
86. Bryan NS, Fernandez BO, Bauer SM, Garcia-Saura MF, Milsom AB, Rassaf T, et al. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nat Chem Biol. 2005;1(5):290–7.
87. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008;7(8):156–67.
88. Hord NG, Ghannam JS, Garg HK, Berens PD, Bryan NS. Nitrate and nitrite content of human, formula, bovine, and soy milks: implications for dietary nitrite and nitrate recommendations. Breastfeed Med. 2011;6(6):393–9.
89. Nunez De Gonzalez MT, et al. Survey of residual nitrite and nitrate in conventional and organic/natural/uncured/indirectly cured meats available at retail in the United States. J Agric Food Chem. 2012;60(15):3981–90.
90. Pennington J. Dietary exposure models for nitrates and nitrites. Food Control. 1998;9(6):385–95.
91. Luiking YC, Deutz NE. Isotopic investigation of nitric oxide metabolism in disease. Curr Opin Clin Nutr Metab Care. 2003;6(1):103–8.
92. van Eijk HM, Luiking YC, Deutz NE. Methods using stable isotopes to measure nitric oxide (NO) synthesis in the L-arginine/NO pathway in health and disease. J Chromatogr B Anal Technol Biomed Life Sci. 2007;851(1–2):172–85.
93. Hunault CC, van Velzen AG, Sips AJAM, Schothorst RC, Meulenbelt J. Bioavailability of sodium nitrite from an aqueous solution in healthy adults. Toxicol Lett. 2009;190(1):48–53.
94. Greenway FL, Predmore BL, Flanagan DR, Giordano T, Qiu Y, Brandon A, et al. Single-dose pharmacokinetics of different oral sodium nitrite formulations in diabetes patients. Diabetes Technol Ther. 2012;14(7):552–60.
95. DeVan AE, et al. Effects of sodium nitrite supplementation on vascular function and related small metabolite signatures in middle-aged and older adults. J Appl Physiol (1985). 2015;p jap 00879 2015.
96. Justice JN, Johnson LC, DeVan AE, Cruickshank-Quinn C, Reisdorph N, Bassett CJ, et al. Improved motor and cognitive performance with sodium nitrite supplementation is related to small metabolite signatures: a pilot trial in middle-aged and older adults. Aging (Albany NY). 2015;7(11):1004–21.
97. Oldfield EH, Loomba JJ, Monteith SJ, Crowley RW, Medel R, Gress DR, et al. Safety and pharmacokinetics of sodium nitrite in patients with subarachnoid hemorrhage: a phase IIA study. J Neurosurg. 2013;119(3):634–41.
98. Tang Y, Garg H, Geng YJ, Bryan NS. Nitric oxide bioactivity of traditional Chinese medicines used for cardiovascular indications. Free Radic Biol Med. 2009;47(6):835–40.
99. Zand J, Lanza F, Garg HK, Bryan NS. All-natural nitrite and nitrate containing dietary supplement promotes nitric oxide production and reduces triglycerides in humans. Nutr Res. 2011;31(4):262–9.
100. Nagamani SC, et al. Nitric-oxide supplementation for treatment of long-term complications in argininosuccinic aciduria. Am J Hum Genet. 2012;90(5):836–46.

101. Houston M, Hays L. Acute effects of an oral nitric oxide supplement on blood pressure, endothelial function, and vascular compliance in hypertensive patients. J Clin Hypertens (Greenwich). 2014;16(7):524–9.
102. Biswas OS, Gonzalez VR, Schwarz ER. Effects of an oral nitric oxide supplement on functional capacity and blood pressure in adults with prehypertension. J Cardiovasc Pharmacol Ther. 2014;
103. Lee J, Kim HT, Solares GJ, Kim K, Ding Z, Ivy JL. Caffeinated nitric oxide-releasing lozenge improves cycling time trial performance. Int J Sports Med. 2015;36(2):107–12.
104. Lee E. Effects of nitric oxide on carotid intima media thickness: a pilot study. Altern Ther Health Med. 2016;22(S2):32–4.
105. Bedi US, Singh M, Singh PP, Bhuriya R, Bahekar A, Molnar J, et al. Effects of statins on progression of carotid atherosclerosis as measured by carotid intimal—medial thickness: a meta-analysis of randomized controlled trials. J Cardiovasc Pharmacol Ther. 2010;15(3):268–73.
106. Bock JM, et al. Inorganic nitrate supplementation attenuates peripheral chemoreflex sensitivity but does not improve cardiovagal baroreflex sensitivity in older adults. Am J Physiol Heart Circ Physiol. 2017;p ajpheart 00389.
107. Ohta N, Tsukahara H, Ohshima Y, Nishii M, Ogawa Y, Sekine K, et al. Nitric oxide metabolites and adrenomedullin in human breast milk. Early Hum Dev. 2004;78(1):61–5.
108. Cekmen MB, Balat A, Balat O, Aksoy F, Yurekli M, Erbagci AB, et al. Decreased adrenomedullin and total nitrite levels in breast milk of preeclamptic women. Clin Biochem. 2004;37(2):146–8.
109. Yazji I, Sodhi CP, Lee EK, Good M, Egan CE, Afrazi A, et al. Endothelial TLR4 activation impairs intestinal microcirculatory perfusion in necrotizing enterocolitis via eNOS-NO-nitrite signaling. Proc Natl Acad Sci U S A. 2013;110(23):9451–6.
110. Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, et al. Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. Proc Natl Acad Sci U S A. 2007;104(45):17593–8.
111. Levett DZ, et al. The role of nitrogen oxides in human adaptation to hypoxia. Sci Rep. 2011;1:109.