14 Nitrate and Human Health
An Overview

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14.1 GENERAL OVERVIEW OF NITRATE AND HUMAN HEALTH

The organic nitrite, amyl nitrite ($C_5H_{11}ONO$), was introduced in 1867 as a therapeutic agent in the treatment of angina pectoris, which is characterized by inadequate oxygen supply to cardiac muscle (hypoxia) due to stenosis or blockage of arteries characteristic of coronary artery disease (1). In 1879, glyceryl trinitrate, formerly nitroglycerin, was introduced by William Murrell as an organic treatment for angina pectoris (2). Serendipitous observation had shown that a drop on the tongue could immediately initiate headaches characteristic of marked cranial vasodilation and neural impingement of the trigeminal nerve (2). Subsequently, organic nitrate replaced amyl nitrite because of facilitated delivery and extended duration of action as a vasodilator, although marked side effects resulted, including nitrate tolerance (loss of efficacy) and headache (1). Since these initial discoveries, a considerable amount of information has been gleaned and confirmed regarding the myriad health benefits of nitrate largely on the vasculature, but also in numerous other tissues. Moreover, the bioactive agents responsible and likely mechanism and pathways have been elucidated.

14.1.1 Definition of Nitrate

Nitrate has been used as an anti-ischemic pharmacological agent and exogenous donor of nitric oxide (NO) for more than 130 years without the realization, previously, of the mechanism of action or the specific bioactive entity responsible for its effects. Traditionally, the inorganic anions nitrite (NO_2^-) and nitrate (NO_3^-) have been considered inert end products of NO metabolism and largely undesired residues in the food chain (3, 4). Recent studies, however, show that naturally occurring nitrate and nitrite are physiologically recycled in blood and tissue to form NO as well as myriad other bioactive nitrogen oxides (5, 6). As a result, nitrate seems to function as storage depots for NO-like bioactivity as an adjunct to the endogenous enzymatic pathways (4). The two major sources of NO are the endogenous, enzymatic NO synthase (NOS) pathway requiring L-arginine and oxygen as substrates and the exogenous consumption of nitrate, primarily in dietary vegetables. For each, the formation of NO requires bioactivation of nitrate via chemical reduction to nitrite, which is largely due to commensal bacteria in the oral cavity (7). Estimates indicate there are >300 species of bacteria with nitrite reductase activity in the oral cavity, which has stimulated considerable research involving the oral microbiome (8, 9). Although these tend to be the primary means of conversion, there are numerous other endogenous molecules (both enzymatic and non-enzymatic) that can produce NO, including hemoglobin, myoglobin, xanthine oxidoreductase, ascorbate, and polyphenols (10).

14.1.2 Natural Environmental Sources

As previously mentioned, nitrogen represents the largest component of atmospheric air and is the fourth most prevalent element in cellular biomass (11). The cycle between inert atmospheric nitrogen and bioactive nitrogen-encompassing reactions

in cellular metabolism and increased biomass is largely controlled by microbial activities and, in fact, is critical for the insertion of nitrogen into genetic material, that is, RNA and DNA, and ultimately protein (11). A specific mandatory process is the ability of bacteria to "fix" or capture N_2 converting it to ammonium (NH_4^+). Subsequently, this can be further converted via oxidation to form numerous nitrogen oxide entities, including nitrite and nitrate (12). The environmental nitrogen cycle (Figure 14.1) continues with the serial reduction of nitrate to NO, nitrous oxide, and ultimately atmospheric nitrogen.

The environmental nitrogen cycle captures nitrogen and distributes to plants where it may be accumulated and consumed by mammals with ultimate increased human plasma levels rendering nitrate bioavailable. Dietary nitrate intake ultimately depends on the type and amount of vegetables consumed, the concentrations of nitrate in the vegetables (including the nitrate content of fertilizer), and the level of nitrate in the water supply (13). Concentrations can vary considerably and occur in high levels in the petiole (leaf, stem stalk) followed by the leaf, stem, root, influorescence (group or cluster of flowers), tuber, bulb, fruit, and seed (14, 15). For example, the average nitrate content of spinach collected from 3 different markets in Delhi, India, varied from 71 to 429.3 mg/100 g fresh weight (16). The relative accumulation of nitrate also depends on factors such as plant genotype, soil quality, growth environment, and storage and transport conditions (16).

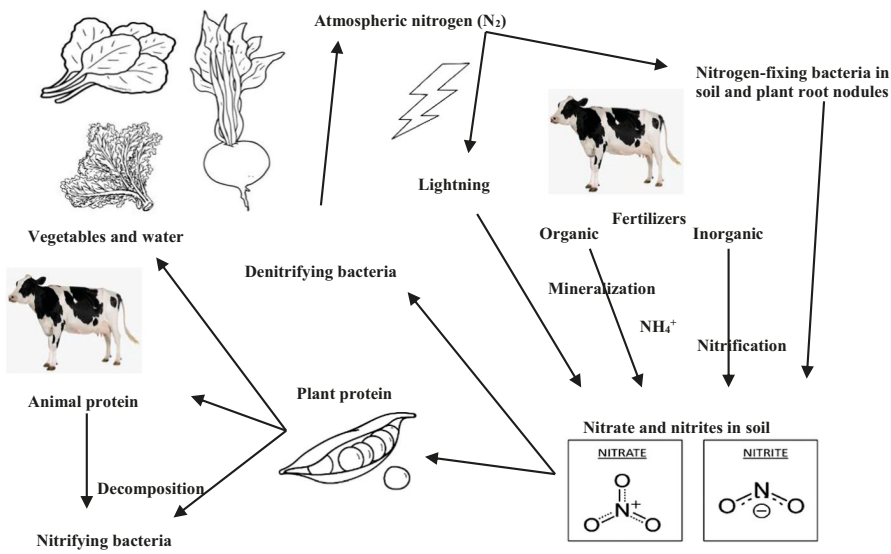

FIGURE 14.1 Environmental nitrogen cycle. Atmospheric nitrogen is assimilated, or fixed, by symbiotic bacteria associated with plants where it can be transported within the structures of plants, for example, leaves and accumulated. Plants may then be consumed by humans, which increases plasma levels of nitrate. Nitrate can then be reduced to other bioactive nitrogen oxides including the potent vasodilator, nitric oxide.

14.1.3 Use in Soils

Atmospheric nitrogen, after capture, is essential for successful plant growth, but artificial fertilization (inorganic and organic) as part of agricultural practices can be more efficient for increasing plant biomass and nitrogen composition (17). Both nitrogenous fertilizers can be used in cropping systems. For the former, many agricultural practices include the application of bovine manure (organic matter) containing many nutrients, for example, phosphorous, calcium, and so forth, as well as nitrogenous compounds. However, after the application of organic fertilizers, inorganic nitrogen is released via mineralization and absorbed by plants as inorganic nitrate, the preferential chemical speciation (18). Interestingly, inorganic nitrogen compounds such as nitrate, nitrite, and ammonium represent <5 percent of the total nitrogen in soil but are the prevalent forms used by plants (19). Other factors influence nitrogen accumulation from fertilizers, including agronomic practices, presence of microorganisms, soil properties, ambient temperature, and water content, which may influence translocation to leaves and ultimately accumulation (20). As such, the amounts of nitrate in farmed vegetables, based on the continuing public concern for dietary nitrate and cancer, have justified governmental limitations on allowable nitrate concentrations in farmed vegetables.

14.1.4 Potential Harmful Effects of Nitrate

There are two primary areas of concern regarding toxicities and/or adverse effects from dietary nitrate, including methemoglobinemia and gastric cancer. Methemoglobinemia is a potentially fatal condition in which hemoglobin is oxidized to methemoglobin (>1% of total hemoglobin) with significant reduction, due to oxidation of ferric iron in oxyhemoglobin, in the ability of iron to bind and transport oxygen, leading to hypoxia and cyanosis, or "Blue Baby" syndrome (21–23). Infants <6 months of age may be especially vulnerable when exposed to nitrate from sources such as well water contaminated with bacteria, which reduces and bioactivates nitrate to nitrite (24). As a result, it is vital that potable well water that may be provided to infants directly or indirectly be tested for nitrate/nitrite concentrations (25). Although the bulk of dietary nitrate is derived from vegetables, and infants fed commercially prepared foods with vegetables are not considered to be at risk for excessive nitrate, with the caveat that home-prepared foods from nitrate-rich vegetables such as red spinach, beetroot, squash, and so forth should be avoided until infants are >3 months of age.

Dietary nitrate consumption presents a conundrum during pregnancy. Recommendations to NO-deficient pregnant women for increased consumption of nitrate, for example, beetroot has increasingly been suggested to mitigate hypertension and pre-eclampsia, improve placental blood flow and markedly improve maternal and neonatal health (26). However, caution has also been recommended since potentially lethal outcomes may result in methemoglobinemia, alteration in embryonic cells and malignant transformation, and thyroid disorders. Epidemiologic evidence suggests an association between nitrate-rich water consumption and spontaneous abortions, intrauterine growth restriction, and various birth defects, although the data are limited (27).

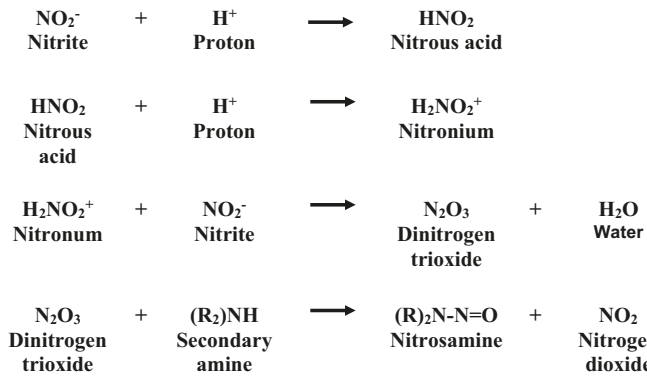

FIGURE 14.2 Formation of nitrosoamines from nitrite. Under proton-rich environments with low pH, nitrite can be reduced to nitrous acid and the nitronium ion. Subsequent reaction with nitrite produces dinitrogen trioxide, which can react with secondary amines to form nitrosoamine.

Although a concern, few nitrate and nitrite exposure studies in humans, including either children or adults as subjects, have resulted in the elaboration of methemoglobinemia causing many to proffer alternative explanations for the etiology (21). For example, in one study infants exposed to 175–700 mg nitrate/day did not display methemoglobin concentrations >7.5 percent, which suggests that nitrate alone did not cause methemoglobinemia (28). In a recent study, healthy adults were provided a bolus dose of sodium nitrite (low, 150 to 190 mg or high, 290–380 mg) (29). Methemoglobin concentrations were 12.2 percent and 4.5 percent for the high and low dose, respectively. The data suggest other factors in the etiology of methemoglobinemia such as gastroenteritis or bacteria-induced NO production as an immune response to infection (30, 31).

The second concern with dietary nitrate involves the capacity of nitrate (Figure 14.2) to form carcinogenic nitrosamines (R_2N-NO) at low pH (pH<3) and low pO_2 in the gastric lumen (32, 33). Indeed, in a population-based cohort study, there were increased risks for all-cause mortality due to 9 different dietary sources (causes). All were associated with both processed and unprocessed meats via, in part, heme iron, nitrate and nitrites (34).

Although there are ongoing concerns with dietary nitrate-derived cancer, direct evidence from extensive epidemiologic and animal studies has been equivocal and inadequate (31). That is, neither rodent nor human epidemiologic investigations have clearly shown a direct correlation between dietary nitrite exposure and the risk of cancer (35). The basis in large part for the linkage of nitrate/nitrites and gastrointestinal cancer relies on observations that nitrites can empirically and chemically react with secondary amines, for example, proteins, or *N*-alkylamides (bioactive compounds in plants) to generate carcinogenic *N*-nitroso compounds, that is, nitrosoamines as shown in Figure 14.2 (35). Oddly, however, demonstration that nitrosoamines cause cancer in humans has also been inadequate. It is known that enzymatic activation is needed to produce a direct-acting carcinogen and interruption of this process may

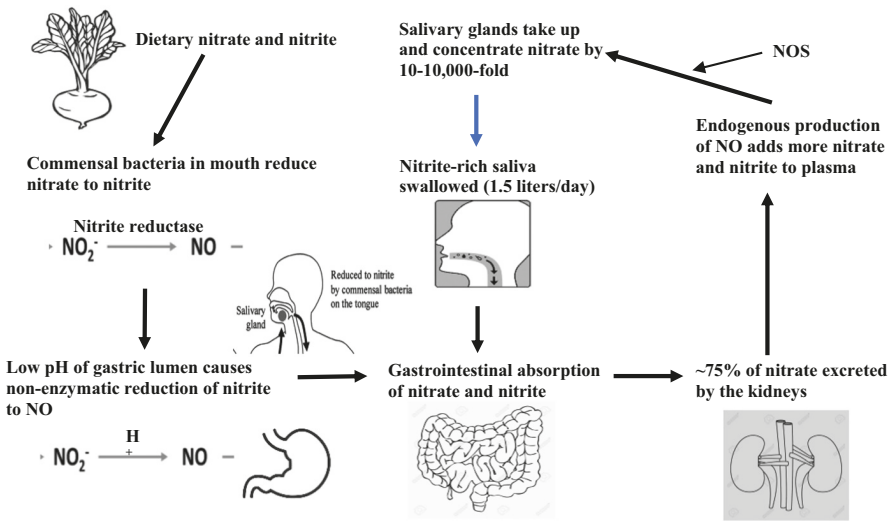

FIGURE 14.3 Endogenous salivary cycle illustrating disposition of ingested nitrate. After absorption in the gastrointestinal tract, nitrate enter the bloodstream for systemic circulation. Approximately 75 percent is excreted by the kidneys and 25 percent remains in circulation and enters the enterosalivary cycle where it is reduced to nitrite in the oral cavity by bacteria with nitrite reductase activity. Nitrite-rich saliva is swallowed (~1.5 liters per day) and continues in the cycle.

be a reason for the discrepancies. This has prompted the food industry to include as additives along with nitrate/nitrites, antioxidants that can interrupt this bioactivation process, reducing the potential for nitrosamine formation. Other "green" approaches have been developed substituting other naturally occurring nitrate-rich molecules for the synthetic nitrate.

The occurrence of the enterosalivary cycle known as the nitrate-nitrite-NO pathway, in humans with retention, marked concentration, and conversion of nitrate to nitrite, upon swallowing, can serve as a source for NOS-independent gastric generation of NO and nitrosamines as shown in Figure 14.3 (6, 36, 37).

This occurs after secretion and ionization of hydrochloric acid (HCl), which protonates nitrite in this microenvironment (38, 39). This process can be abolished by proton pump inhibitors such as omeprazole (Prilosec), esomeprasol (Nexium), and so forth, which reduce gastric pH (40). Interestingly, NO levels increase with dietary consumption particularly in the presence of reducing agents such as the antioxidants ascorbic acid and polyphenols, suggesting a detrimental effect (41). However, nitrite in combination with gastric acid is considerably more potent in killing gastric pathogens such as *Helicobacter pylori* than acid alone (42). This was attributed to increased gastric mucosa blood flow and mucus formation protecting the stomach and supports a role for gastric NO as a defense against pathogens (43, 44).

Most salivary nitrite is absorbed systemically, obviating the potential for gastric NO formation. Plasma levels peak around 30 minutes and remain elevated for several

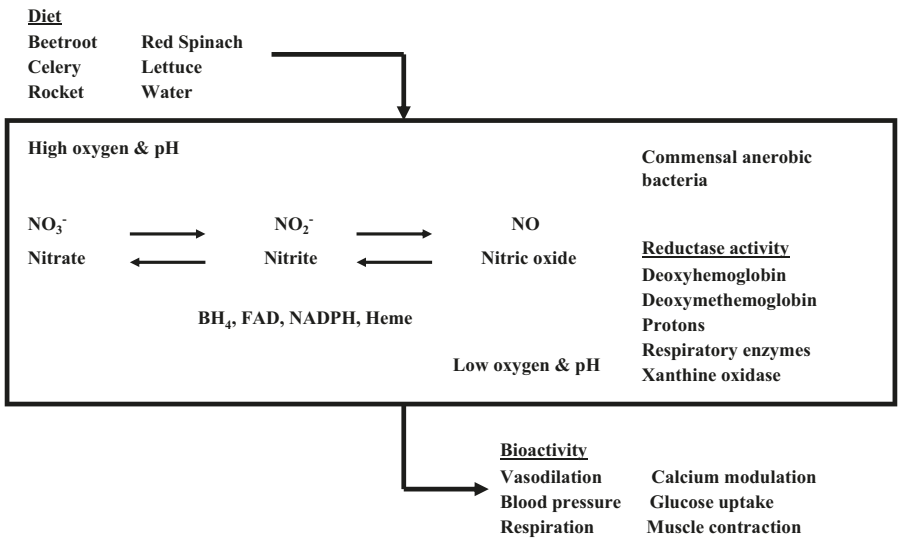

FIGURE 14.4 Endogenous nitrate-nitite-nitric oxide cycle. Dietary consumption of nitrate-rich vegetables is absorbed and increases plasma nitrate. In the salivary glands, nitrate is reduced to nitrite by commensal bacteria, with further reduction to bioactive nitrogenous compounds, that is, nitric oxide, via other endogenous mechanisms particularly under hypoxic conditions. With oxygen, endogenous enzymatic systems, viz., NOS, nitric oxide may be produced via the oxidation of arginine. (Adapted from reference 139.)

hours, although the half-life is approximately 20–30 minutes, likely due to recirculation of nitrate. In the systemic circulation, myriad proteins, including enzymes, can catalyze one electron reduction of nitrite to NO (Figure 14.4).

Several examples are xanthine oxidoreductase, deoxyhemoglobin, cytochrome P450, mitochondrial proteins, carbonic anhydrase, aldehyde oxidase, and eNOS as well as protons, polyphenols, vitamins E and C (45–47). The mechanism of nitrite reduction ranges from simple protonation to enzymatic activity, and a common observation is enhanced nitrite reduction by the former during hypoxia in the presence of low pH (48). Classical endogenous NOS-dependent NO production requires molecular oxygen and arginine as substrates. Thus, it appears that the nitrate-nitrite-NO pathway functions under homeostasis, whereas nitrite reduction to NO is a backup, or auxiliary, function during periods of ischemia/hypoxia. This redundancy permits NO generation from nitrate as a mechanism of hypoxic vasodilation when oxygen tension falls.

14.1.5 Potential Health Benefits of Nitrate

The concentration of inorganic nitrate in vegetables can serve as a substrate for reduction to nitrite, NO, and other metabolic products (NO_x) that produce vasodilation, decrease blood pressure, improve and maintain endothelial function, as well as modulate glucose homeostasis, improve muscle contractility, enhance mitochondrial function, and facilitate respiration. Chronic and acute beetroot juice supplementation

has been used as a therapeutic approach for diabetes and insulin hemostasis, renal health, and modulation of the microbiome (49, 50). Thus, collectively these observations have obvious implications for cardiovascular health and exercising muscles, and subsequently athletic performance (51–53). As a result, many experts have suggested increasing vegetable intake, particularly nitrate-rich sources as a heart-healthy strategy and potent ergogenic aid despite the ongoing stigma (54–57).

14.2 STRUCTURE AND FUNCTION OF NITRATE AND NITRITES

Nitrate and nitrite are naturally occurring chemical compounds produced from the reaction of diatomic nitrogen and oxygen to form polyatomic anions (NO_3^- and NO_2^-, respectively) that can form salts with cations, for example, Na+, K+, to form classes of compounds called nitrates or nitrites, respectively. Both molecules are rich sources of inorganic nitrate (without carbon) in human diets deriving largely from leafy vegetables with differential enrichment depending on the anatomical plant structure. Since almost all nitrates are soluble in water, drinking water is also a significant dietary source. As a result, the WHO has established an upper limit of 10 mg nitrate/L for municipal water supplies (17).

Uptake of nitrate in the salivary glands and subsequent excretion in the saliva is a pivotal, necessary step for conversion of nitrate or nitrite in the oral cavity. Several species of facultative anaerobic bacteria in the crypts of the tongue efficiently convert nitrate to nitrite (58, 59). Due to various intrinsic biological mechanisms, salivary concentrations of both may approach 10–10,000 times that of plasma levels reaching millimolar levels, which may contribute to nitrosamine formation in the low pH environment of the stomach as shown in Figure 14.3 (33, 60–62). As such, there continue to be restrictions on acceptable nitrate levels in farmed vegetables as recommended by the WHO (63).

Several proteins, such as hemoglobin, cytochrome P450 reductase, and cytochrome P450, can catalyze the reduction of nitrite or nitrate to generate NO (10). Interestingly, hemoglobin has enzymatic activity as a nitrite reductase under hypoxic conditions (when hemoglobin is 40–60% saturated) and, moreover, is a sensor and effector of hypoxic vasodilatation (64). Cytochrome P450 reductase also causes NO release by reducing nitrate, which facilitates the production of S-nitrosothiols (65). NO can also be formed via the decomposition of biological molecules to produce reactive products. For example, S-nitrosothiols (R-S-N=O), such as S-nitrosoglutathione and S-nitrosohemoglobin, are also sources of NO when exposed to transition metals, for example, iron, or light (66, 67). S-nitrosothiols can also undergo trans-S-nitrosation with other thiol groups (R-S-H), which can alter cell or protein function of NO via mechanisms analogous to phosphorylation and ubiquitinylation (68, 69).

Endogenous enzymatic production of NO occurs via a family of enzymes called NO synthases (NOS) with endothelial NOS (eNOS) producing ~70 percent in the vascular endothelium (70, 71). This causes vasodilation, blood-pressure regulation, anti-inflammation, and reduced platelet aggregation, which may be pivotal in preventing various types of cardiovascular disease, hypertension, atherosclerosis, and stroke. The two other NO-producing isoforms are neuronal NOS (nNOS) and

cytokine-inducible NOS (iNOS). Although eNOS is the major NOS isoform, both nNOS and iNOS exhibit important functional roles in certain tissues and environments (72). For example, vascular injury induces expression of nNOS in smooth muscle cells, and activation of iNOS by proinflammatory cytokines within vascular smooth muscle causes vasodilation in sepsis as part of the innate immune response.

Numerous mechanisms of action have been identified for dietary nitrate. For example, NO induces formation of cyclic guanosine monophosphate (cGMP) by activating soluble guanylyl cyclases in vascular SMC. After this initial step, numerous downstream effectors can be activated such as cGMP-dependent protein kinase (protein kinase G [PKG]), cGMP-gated ion channels, and cGMP sensitive phosphodiesterases (69, 73). PKG induces reuptake of calcium into the sarcoplasmic reticulum (membrane-bound structure in muscle cells that store calcium), extracellular movement of calcium within a cell, and opening of calcium-activated potassium channels. Collectively, these signaling processes cause smooth muscle cell relaxation, vasorelaxation, and improved blood flow.

Although it is becoming clear that NO is critical for homeostasis, another seeming paradox exists. Many argue that free radicals (with an unpaired electron), capable of generating oxidative stress, are causative in the etiology of many, if not most, chronic diseases (74). However, NO is itself a free radical and interacts readily with other free radicals and/or minerals – for example, iron – and can induce nitrosative stress via reactive nitrogen species (75). Moreover, NO can generate noxious, toxic molecules in states of disequilibrium. For example, eNOS may become "uncoupled" producing less NO and more superoxide (O_2^-), a reactive oxygen species, which causes endothelial dysfunction found in atherosclerosis, diabetes, hypertension, cigarette smoking, hyperhomocysteinemia, and ischemia/reperfusion injury (65, 76). Major mechanisms of eNOS uncoupling include depletion of its cofactor tetrahydrobiopterin, deficiency of l-arginine, the eNOS substrate, competition with endogenous arginase activity, and/or eNOS S-glutathionylation (77). Uncoupling can lead to the diffusion-controlled reaction of NO with superoxide radical to form highly reactive peroxynitrite ($ONOO^-$), which readily kills and damages any molecule within proximity (78). Conversely, cytokine activation of iNOS in the innate immune system significantly increases ONOO- to eliminate infections by bacteria, viruses, or fungi and iNOS-derived NO modulates pathways of glucose and lipid metabolism during inflammation (79).

Nitrite can be bioactivated via numerous non-enzymatic routes (80, 81). NO binds with ferrous iron (a source of electrons) as part of heme – although potentially problematic – and is the basis for many of the signaling activities of NO and a rapid means of modulating NO levels (82). The reduces the half-life of NO to a few seconds with the ultimate formation of nitrate, which can reenter the endogenous nitrogen cycle. NO can also react with Complex I and cytochrome c oxidase (Complex IV), inducing either problematic or protective outcomes, depending on NO, cytochrome, and O_2 concentrations (83). NO may also bind to myriad amino acids of diverse proteins to form adducts (two molecules form one) via nitrosation and nitration, which has been shown as a mechanism of altering protein function. Lastly, sulfur-linked nitrosylated proteins can exert NO-like endocrine activity (84, 85). S-nitrosylation involves covalent post-translational modifications of cysteine moieties, which then can propagate NO-triggered signals (86).

14.3 SOURCES OF NITRATE

14.3.1 Foods Containing Nitrate

Vegetables accumulate significant amounts of nitrate (~80% of total dietary) from nitrogen-based fertilizers, which are used for rapid and enhanced growth (16). Leafy vegetables, such as lettuce or spinach, and beetroot contain the highest concentrations of nitrate (Table 14.1) (87).

Other examples include radishes, turnips, watercress, bok choy, Chinese cabbage, kohlrabi, chicory leaf, celery, onion, and garlic (7, 88–90). Fruits also contain nitrate,

TABLE 14.1
Nitrate and Nitrite Content of Vegetables

Vegetable	Nitrate content (mg/kg)	Nitrite content (mmol/100 g)
High		
Arugula (Rocket)	2597	4.19
Spinach	2137	3.45
Lettuce	1893	3.05
Radish	1868	3.01
Beetroot	1459	2.35
Cabbage	1388	2.24
Average	1890	3.05
Medium		
Turnip	624	1.00
Cabbage	513	0.83
Green beans	496	0.80
Leeks	398	0.64
Spring onion	353	0.58
Cucumber	240	0.39
Carrot	222	0.36
Potato	220	0.35
Garlic	183	0.30
Sweet pepper	117	0.19
Green pepper	111	0.18
Average	316	0.51
Low		
Onion	87	0.14
Tomato	69	0.11
Average	78	0.13
Water (250 mL)		
Tap	26	0.13
Mineral	3	0.01

Data were collated from numerous sources. (52, 88, 138).

but at low levels. Examples include watermelon, apples, bananas, grapes, kiwi, pears oranges, and strawberries (91). Interestingly, diets recommended by expert panels that are associated with reduced chronic disease risk tend to be considerably higher in the vegetable intake and include, for example, the Dietary Approaches to Stop Hypertension (DASH) diet and the Mediterranean Diet, with the former estimated to provide >1,200 mg nitrate/d. As a note, current estimates of dietary nitrate consumption in the US ranges from 40–100 mg/d (35).

14.3.2 Nitrate as a Food Additive

Nitrates are routinely added to processed, cured meats as antioxidants, flavor enhancers, color stabilizers (red or pink color in meats) and antimicrobial agents. In fact, they are critical for minimizing or preventing the growth of noxious, disease-causing bacteria such as *Clostridium botulinum*, the causative agent of botulism (92). Examples of processed foods include bacon, bologna, corned beef, hot dogs, ham, luncheon meats, sausages, canned meat, cured meat and hams, all of which are regulated by the FDA and USDA (93). According to the Code of Federal Regulations, the level of sodium nitrite in cured meat products must be ≤200 ppm and sodium nitrate must be ≤500 ppm. Although generally a public-health concern, food additives are not major contributors to the total estimated intake of nitrate (Table 14.2). Vegetables remain the major contributor, followed by water and other relatively minor sources, including cereals and non-water beverages (94–96).

Nitrate, which can form nitrites, reacts with naturally occurring components of protein, viz., amines, and lead to the formation of nitrosamines, known cancer-causing compounds. Concern over these reactions nearly caused a ban of nitrites and nitrate from food use in the 1970s (97). Paradoxically, vegetables may also naturally contain nitrosamines without artificial addition, although the level is low. An intriguing observation is the perpetuating stigma of food additives (nitrates) as cancer-causing agents, yet individuals frequently consume identical molecules from dietary vegetables. Although cancer risk has been discussed for years, others have recently suggested that nitrate and nitrite be considered essential nutrients because they promote

TABLE 14.2
Nitrate and Nitrite Levels in Processed Foods

Product	Nitrate (mg/kg)	Nitrite (mg/kg)
Sausage (cooked)	**48**	**32**
Sausage (smoked)	**56**	**20**
Ham	**42**	**24**
Salami	**85**	**10**
Bacon	**55**	**4**
Bacon (nitrite-free)	**30**	**7**
Hot dog	**90**	**1**
Pork tenderloin	**33**	**0**
Cheese	**34**	**10**

NO production and consequently contribute to cardiovascular health. Nonetheless, this has driven the food industry to seek natural nitrate compounds due to high consumer demand (98). Indeed, alternatives to nitrate and nitrites are the object of many studies, with heightened focus on the addition of vitamins, fruits, chemicals, and natural products containing nitrate (99).

14.3.3 Nitrate as a Dietary Supplement

Inorganic nitrate and nitrite are plant nutrients and legally permitted food additives primarily for processed meats and components of foods. They also may be included in dietary supplements and nutraceuticals since they are associated with blood-pressure–lowering and performance-enhancing effects. It is not surprising that they are immensely popular among consumers with cardiovascular disorders, as well as athletes. Although dietary supplement blends and specific bioactive compounds such as betaine and arginine are generally thought to indirectly produce NO, oddly there is inadequate research to support this assertion (100–103). The most commonly used dietary constituent is nitrate-rich beetroot because of its high nitrate concentration (>250 mg nitrate/100 g [3.5 oz]) coupled with compelling evidence that it does, in fact, increase blood nitrate and/or nitrite concentrations following acute and chronic ingestion as shown in Table 14.3 (104–106).

Others have shown that dietary nitrate supplementation with ~0.1 mmol/kg body mass significantly reduces blood pressure, reduces oxygen cost associated with exercise, improves muscle efficiency, and exerts ergogenic properties (107–110. Valenzuela and colleagues compared dietary supplements, that is, nitrates, and found sufficient evidence for nitrate supporting its acute beneficial effects on muscle strength (111). The mechanism of action is thought to result from enhanced NO bioavailability and subsequent vasodilation leading to improved cardiovascular health and enhanced exercise performance (112).

The results of some clinical trials report that the intake of other nutraceuticals (hawthorn, coenzyme Q10, l-carnitine, d-ribose, carnosine, vitamin D, probiotics, n-3 PUFA, and beet nitrates) might improve self-perceived quality of life and/or functional parameters such as blood flow (left ventricular ejection fraction, stroke volume and cardiac output) in heart failure (HF) patients, with minimal or no side effects (113, 114). This supports the usefulness of supplementation of some nutraceuticals, that is, beet nitrates to improve HF management as an adjunct to evidence-based pharmacological therapy, although corroboration of its efficacy is still needed (115).

There are many other nitrate-rich supplements, either in development or already on the market, that have been shown to be efficacious. For example, the combination of the plant-based ingredients (beetroot, red spinach (*Amaranthus tricolor*), and aronia berry extracts) yields a significant increase in NO metabolites following acute ingestion (100). In other studies based on a meta-analysis, consuming 4–12 mg/kg of nitrate (300–00 mg/d) as a dietary supplement – for example, beetroot juice, beetroot concentrates powders, and/or sodium nitrate – are needed to confer significant cardioprotection (90). Reductions in blood pressure and platelet aggregation have

TABLE 14.3
Nitrate and Nitrite Content of Nitrate-Rich Dietary Supplements

Dietary supplement product	Serving size	Nitrate mmol	Nitrite mmol
Redibeets	**100 g**	**10.75**	**0.75**
Superbeets	**100 g**	**20.60**	**2.80**
Endurance beets	**100 g**	**9.80**	**0.00**
BeetBoost	**100 g**	**16.20**	**0.00**
BeetElite	**100 g**	**21.60**	**2.20**
PureClean powder	**100 g**	**25.60**	**0.00**
Beet power	**1 L**	**1.02**	**0.00**
Beet performer	**1 L**	**3.01**	**0.00**
Unbeetable Fizz	**1 L**	**3.52**	**0.00**
Beet performer with passion fruit	**1 L**	**3.97**	**0.02**
Beet blast	**1 L**	**3.96**	**0.06**
Ginger beet juice	**1 L**	**6.67**	**0.06**
Red rush	**1 L**	**2.39**	**0.01**
Beet performance supplement	**1 L**	**2.76**	**0.00**
BeetActive	**1 L**	**2.76**	**0.00**
Beet it organic beetroot shot	**1 L**	**5.93**	**0.00**
Beet it: Sport elite shot	**1 L**	**6.41**	**0.00**
Biotta beet juice	**1 L**	**4.81**	**0.00**
Beet it beet juice	**1 L**	**7.55**	**0.00**
Beet juice-Knudsen	**1 L**	**12.54**	**0.00**
Beet juice-Lakewood	**1 L**	**18.77**	**0.02**

Table adapted from (121).

also been observed after supplementation with 4–20 mg/kg (116). There are many other nitrate-rich dietary supplements and nutraceuticals based mostly on beetroot on the market (Table 14.3).

An evolving conundrum regarding nitrate supplementation exists as to whether benefit outweighs detriment with a reasonable intake of dietary nitrate, which may readily exceed recommendations for daily intake. For example, consumption of a single serving of a nitrate-rich food or dietary supplement can exceed the acceptable daily intake (ADI) for nitrate (222 mg/d for a 60-kg adult) per the WHO (35). Moreover, recommendations for dietary supplement intake in exercise are 300–600 mg of nitrate (<10 mg/kg or 0.1 mmol/kg) or 500 mL beetroot juice (3–6 whole beets) within 1.5 h of exercise commencement (117). An additional recommendation is multi-day dosing for around 6 days prior to exercise or an athletic event. Given the potential health benefits and risks for dietary nitrate and nitrite intakes, there is a need for rational dietary guidance regarding nitrate- and nitrite-containing foods and dietary supplements to achieve optimal cardiovascular health and athletic performance, while taking into account the potential negative health risks.

14.3.3.1 Nitrate and Nitrite Salts

As one might surmise, the notion of supplementing directly with inorganic sodium or potassium nitrate has been considered by many individuals, including endurance athletes. Indeed, both are available either from supermarkets or the internet. However, the indiscriminate use of salts and the assumption of safety have generated considerable concern amongst researchers and experts specifically for nitrite, which has an LD50 (lethal dose) similar to cyanide and can cause death (119). Inorganic nitrate is nontoxic at high doses, but inorganic nitrite can be injurious at much lower doses. The LD50 for nitrite is 100–200 mg/kg body weight, which would be 7–14 g (0.25–0.5 ounces) for a 70 kg (154 pound) human (119). The conversion rate (%) of nitrate to nitrites from dietary sources – that is, vegetables – is relatively low and, thus, even with high dietary nitrate intake, nitrite generation is low, thus safe.

In 2017, the European Food Safety Authority (EFSA) initiated a reevaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. EFSA calculated nitrate exposure in humans based on salivary secretion rates of nitrate (20–25%), oral conversion rates to nitrite (5–36%), and conversion percentage of 1–9 percent. The ADI range was 1.05–9.4 mg nitrate/kg/d. EFSA compared this to the ADI for nitrite (0.07 mg nitrite/kg body/d) and concluded that intake was within the current, designated ADI of 3.7 mg/nitrate/kg/d (118).

14.3.3.2 Beets and Beetroot Juice

Currently, the most commonly used and most prevalently researched dietary constituent thought to enhance plasma NO levels is beetroot or its juice because of its high nitrate concentration and observations that consumption increases plasma nitrate and/or nitrite subsequent to both acute and chronic ingestion (104, 105, 120). The results have been conflicting, however, and may be due to the wide range of concentrations of nitrate in vegetables and their juices due largely to agronomic practices: for example, nitrate-rich fertilizer use (121, 122). The consensus, however, is that nitrate-rich beetroot juice is efficacious in several aspects of cardiovascular health, as well as physical performance (68). Alternatives, such as the addition of vitamins, fruits, chemicals products, natural products containing nitrite or spices, which have similar properties of nitrites, are in evaluation (99).

Beetroot juice is increasingly used more in athletics due to emerging and cumulative evidence for its role in vasodilation and subsequent reductions in blood pressure and increases in oxygenation of various tissues, particularly exercising muscle that may experience hypoxia. Indeed, others have shown that dietary nitrate can increase muscle efficiency, exercise tolerance, and markedly improve endurance (123). Thus, for athletes the use of dietary nitrate may optimize training leading to significant improvements in athletic performance. Other modulated functions include increased blood flow, improved gas exchange, enhanced mitochondrial biogenesis and efficiency, and strengthening of muscle contraction indicating potential benefit in ergogenic effects and cardiovascular endurance (124). In a meta-analysis of 22 studies, the results showed that beetroot supplementation significantly improved cardiorespiratory endurance, exercise efficiency, athletic

performance over a range of distances, time to exhaustion at submaximal intensities, and cardiorespiratory performance at intensities approaching anaerobic threshold and VO_{2max} (124).

Supplementation with dietary nitrate, that is, beetroot juice, is popular amongst not only elite athletes but also recreational exercisers because of its effects on training physiology and exercise performance in both healthy and diseased populations. As a caveat, support for evidence-based sports performance supplements (caffeine, creatine, nitrate/beetroot juice, β-alanine, and bicarbonate) depends on the type of event, the conditions of the event, and the individual responsiveness to the supplement. Indeed, Rothschild and colleagues corroborate these observations, asserting that effects are dependent on dose and duration of the exercise (125). Although conflicting results exist, time to exhaustion seems to increase, and ergogenic benefits may depend on individual aerobic fitness levels. That is, individuals with a lower fitness level may derive greater benefits regarding athletic or exercise performance after nitrate consumption, than well-trained athletes. The purported mechanisms for these effects seem to be improved oxygen cost and consumption during exercise via greater adenosine triphosphate (ATP) production and lesser ATP consumption. In a meta-analysis of beetroot juice studies, nitrate consumption and its relative concentration exerted a substantial effect on athletic or exercise performance when averaged across athletes, non-athletes, and modes of exercise (126). Dietary nitrate supplementation appears to have some effect on training performance in patients with peripheral artery disease, heart failure, and chronic pulmonary obstructive disease. However, larger randomized controlled trials are necessary to determine the overall utility of beetroot as a dietary supplement (127).

14.3.3.3 Red Spinach

Red spinach extract (RSE) is another popular nitrate-rich dietary supplement. In one study, RSE significantly increased plasma nitrate 30 min post-ingestion, with significant acute microvascular (that is, resistance vasculature) reactivity increases in the lower limb of apparently healthy humans (128). Moreover, the acute effect of RSE (1000 mg dose; ~90 mg nitrate) was determined using performance markers during graded exercise testing (GXT). Plasma concentrations of nitrate increased pre and post GXT with RSE, but not with placebo. During GXT, VO_2 at the ventilatory threshold was significantly higher with RSE compared to placebo, though time-to-exhaustion and maximal aerobic power (i.e., VO_2 peak) were non-significantly lower with RSE. Overall, this suggests that RSE supplementation may exert ergogenic properties by delaying the ventilatory threshold (129). In a study of 17 recreationally active men (n = 9, 22.2 ± 3.8 years) and women (n = 8, 22.8 ± 3.5 years) undergoing 2 randomized testing sessions, subjects supplemented daily with 1 g of RSE or placebo for 7 days prior and 1 hour before completing a 4-km cycling time trial test (130). Compared to placebo, RSE supplementation significantly lowered post-exercise diastolic blood pressure and significantly improved 4-km completion time, average power, relative power, and average speed. Only females displayed significant improvement during RSE trials. Interestingly, it has been proposed that sex differences do, in fact, exist in response

to dietary nitrate supplementation, but this proposal lacks firm support because females are underrepresented in research (131). In a different study, RSE supplementation significantly reduced time-to-completion, increased measures of power and speed, and lowered post-exercise diastolic blood pressure during a 4-km cycling time trial without altering subjects' perceived exertion or subjective measures of muscle fatigue.

14.4 POTENTIAL HARM OF NITRATE

14.4.1 Historical View and Rationale

Nitrites and nitrates are used as preservatives in cured meats such as bacon, salami, sausages, and hot dogs. The historical basis for concern with dietary nitrate began in the 1950s and 1960s when it was realized that nitrite, which can form from nitrate, react with naturally occurring amines in protein at low pH, such as the gastric lumen, and potentially form carcinogenic nitrosamines. This has prompted many to insist on a ban of synthetic nitrate and nitrites from foods. The potential for methemoglobinemia in infants from nitrate and nitrite exposure from drinking water and some foods has also prompted concern. This stigma surrounding nitrate has continued into recent years, even with the lack of compelling, definitive evidence of harm – coupled with emerging, cumulative evidence of the critical importance of dietary nitrate in health.

14.4.2 Current View and Rationale

Emerging evidence regarding the relationship between dietary nitrate/nitrites and health presents a conundrum. Although negatively viewed by many, the high natural nitrate content of beetroot juice has been credited with significantly lowering blood pressure and enhancing exercise performance. Nitrate is also the active ingredient in some medications for angina and have been used for >130 years (amyl nitrite, nitroglycerin). The addition of nitrate and nitrites to processed foods is legal and an effective preservative. The indisputable health benefits of nitrate from dietary vegetable consumption has supported the notion that they are, in fact, dietary nutrients and the benefit surpasses the risk (89, 132).

The rationale for the current view is based on the observation that gastric nitrosation largely occurs via an attack on secondary amines (of proteins), thiols, and phenol groups and the nitric dioxide radical is also formed from nitrite in the stomach. While both NO and nitrite are produced in equimolar amounts, the redox conditions of the microenvironment can shift the reaction in either direction. For example, the presence of reducing agents such as the antioxidants ascorbic acid or polyphenols will synthesize as the principle product, NO. Interestingly, other nitration products can be generated that exert protective effects. For example, nitrate may control the activity of pepsin via nitration or form electrophilic compounds, which react with fatty acids producing anti-inflammatory adducts (133). Moreover, ethanol from alcoholic beverages can be nitrosated by dietary nitrite to form the potent vasodilator ethyl nitrite. Ethyl nitrite, once absorbed, may also release NO at physiological pH (pH=7.4) (134).

In summary, many diverse and potentially reactive nitrogen products are formed in the acidic environment of the stomach. The end products may exert beneficial or detrimental effects depending on the microenvironment and the molecule formed. This argues that the nitrite-producing bacteria of the oral cavity and the acid-producing stomach have pivotal roles in modulating nitrogen oxide signaling throughout the body.

14.4.3 Toxicity from Ingestion

Dietary nitrate and nitrites may be associated with cancer risk, but the data are inconsistent. It is, however, estimated that nitrate intake >600 mg/d, nitrite intake >0.2 mg and nitrosamine intake >0.2 μg increase the relative risk. EFSA has recommended an ADI of nitrate for a human adult of 3.7 mg/kg body weight/day, that is for a person with body weight of 60 kg, it is 222.0 mg/day (135). An interesting calculation has been presented by Hord and colleagues, who posit that if nitrite were a carcinogen, the public would be advised to avoid swallowing because saliva contains 50–100 μmol/L nitrite, which can increase to near millimolar levels after a nitrate-rich meal (136). Overall, the data support that normal physiological levels of nitrite and nitrate clearly exceed concentrations considered at-risk. Collectively, this contributes to the conundrum of safety versus toxicity based on regulatory limits. Moreover, in a descriptive cross-sectional study, a total of 90 vegetable samples were collected from nine farms and analyzed for nitrate content. The authors concluded that the amount of nitrate in raw vegetables did not reach the standard limit level for toxicity and, thus, did not cause health problems for consumers (88).

14.5 POTENTIAL HEALTH BENEFITS OF NITRATE

Nitrate may exert numerous health benefits. They can produce NO, which readily reduces blood pressure via its vasodilatory effects. Subsequently, the risk for cardiovascular disease, coronary heart disease, myocardial infarction, and stroke are markedly mitigated (137). In a meta-analysis of 34 studies, inorganic nitrate consumption significantly reduced blood pressure, improved endothelial function, reduced arterial stiffness, and reduced platelet aggregation (90, 138). Dietary nitrate consumption also significantly improves and/or reduces the risk for gastric ulcers, renal failure, and metabolic syndrome (135). NO has been implicated in many other physiological functions including neural function and immunity (139, 140).

14.5.1 Increased NO

Numerous studies have detected plasma nitrate after ingestion. Ingestion of high-dose nitrate, either as synthetic sodium nitrate or natural beetroot juice, in eight young, healthy individuals rapidly increased plasma nitrate concentration up to threefold, a study maintained for two weeks (105). Mayra and colleagues showed in a crossover trial that those consuming high-nitrate leafy salad twice daily for ten days significantly increased fasting plasma nitrate/nitrite concentrations and significantly improved

flow-mediated dilation (FMD) by 17 percent (141). Ormesher and colleagues tested in hypertensive pregnant females in a double-blind, placebo-controlled study the effects on blood pressure of daily nitrate consumption (70 mL for 8 days). Dietary nitrate significantly increased plasma and salivary nitrate/nitrite concentrations compared to placebo and was significantly correlated with corresponding reductions of diastolic BP (142). Others have shown, in different experimental models, significant increases in plasma nitrate after consumption of nitrate-rich vegetables and dietary supplements with subsequent beneficial effects. For studies demonstrating protective effects after nitrate consumption, but without measuring plasma nitrate/nitrite levels, there is an assumption that levels did increase to elicit the effect, provided the study was placebo-controlled and well-designed with fairly stringent inclusion/exclusion criteria.

An important source of nitrite is via the enterosalivary NO cycle and the commensal bacteria of the oral cavity. Several studies have shown that elimination of bacteria via the use of antiseptic mouthwash can lead to reduced plasma levels of nitrate and nitrite, increased blood pressure, and lack of gastroprotection as well as contributing to the risk of cardiovascular disease and chronic kidney disease (143–145). It has also been noted that the frequency of tongue cleaning significantly impacts the composition of the human tongue microbiome and enterosalivary circulation of nitrate (146). These observations may be responsible, in part, for reported effects after acute dietary nitrate supplementation, where alterations of the microbiome occur but without subsequent effects on vascular responses (147).

14.5.2 Improved Blood Flow: Response to Hypoxia

Due to clear efficacy, nitrate such as nitroglycerin has been valuable in the treatment of cardiovascular disease for decades and continue as an important therapeutic agent in clinical settings. Nitrate causes potent vasodilatation of the capacitance veins and markedly enhance ventricular filling pressure in the heart, and dilate the epicardial (inner layer of the pericardium) coronary arteries, improving coronary blood flow, particularly in ischemic (hypoxic) tissue (148). Bioactivation of organic nitrate generates NO, which induces vasorelaxation via its effect on vascular smooth muscle cells as well as impairs platelet activation and potential aggregation, reducing the risk for subsequent blood clots – for example, thrombi, emboli.

Exercise during hypoxia reduces muscle oxidative function and impairs exercise tolerance (capacity to sustain aerobic exercise). Vanhatalo and colleagues provided beetroot juice (750 ml) to subjects and noted the limit of tolerance was reduced during hypoxia in the placebo group but was restored in those consuming beetroot juice (120). There was also notable attenuation of muscle metabolic perturbation during hypoxic exercise. Moreover, beetroot consumption improved muscle energetics and functional capacity in hypoxic environments. Engan and colleagues provided beetroot juice concentrate to healthy subjects and observed small, yet significant, changes in arterial oxygen saturation between placebo and nitrate after 2 min of static apnea (holding breath while submerged in water) (149). Additionally, maximal apneic duration was prolonged by 11 percent. In contrast, Schiffer and colleagues found no difference after 2 min of apnea, with a lower arterial oxygen saturation after 4 min of

static apnea after nitrate intake compared to placebo, but the maximal apneic duration was 5 percent shorter (149).

Kapil and colleagues showed in healthy adults (n=35) that consumption of 250 mL beetroot juice compared to water prevented endothelial dysfunction caused by ischemia-reperfusion (150). This group further showed reduced arterial stiffness after beetroot juice consumption. Asgary and colleagues further showed in hypertensive untreated adults that 500 mL beetroot juice for 15 days significantly reduced endothelial intracellular and vascular cell adhesion molecule expression as well as E-selectin expression, which reduces the risk for atherogenesis (151). Velmurugan and colleagues showed in patients with hypercholesterolemia that consumption of 250 mL beetroot juice per day significantly improved FMD as an indicator of improved vascular response (152). In several studies, acute ingestion of beetroot juice between 2–3 h with doses of 341–1488 mg demonstrated benefits on ischemic reperfusion injury as assessed by FMD as an indicator of endothelial impairment via noxious ischemic reperfusion (150, 153).

14.5.3 Reduction in Blood Pressure

Many clinical studies have addressed the capacity for nitrate-rich beetroot juice or some other NO donor to reduce blood pressure (154–157). In 2006 Larsen and colleagues showed that sodium nitrate (8.5 mg/kg/d) consumed for three days increased plasma levels of nitrate and significantly reduced DBP by 4 mmHg in young, healthy volunteers. The dose corresponded to 2–3 beetroots or 200–300 g spinach. A bolus dose of beetroot (500 ml; 1,400 mg) reduced both systolic and diastolic blood pressures by 10 and 8 mmHg, respectively. Subsequent use of a lower dose of beetroot juice (250 ml) caused a 5 mmHg reduction but only in systolic blood pressure. Plasma cGMP was also increased, indicating conversion of soluble guanylyl cyclase involved in NO production and ultimate vasodilation. Several studies have now confirmed the blood-pressure–lowering activity of dietary nitrate mostly in healthy young adults. Similar effects, however, have been shown in older adults, although some report a lack of effect (104, 158). Provision of a single bolus dose of beetroot juice to older adults with peripheral artery disease significantly reduced blood pressure, increased time of claudification pain (leg cramping with exercise caused by arterial obstructions), and prolonged peak walking time. Husmann and colleagues showed that dietary nitrate supplementation mitigated muscle fatigue by alleviating exercise-induced damage to contractile muscle function. Moreover, dietary nitrate reduced the perception of effort and leg muscle pain during exercise (159). Hobbs and colleagues showed that 3 doses of beetroot juice (100, 250, 500 mL) given over 24 h reduced ambulatory blood pressure in healthy adults and was dose-dependent with reduced systolic, but not diastolic blood pressure (160, 161). In the same study, red and white beetroot effects were similar, suggesting that betalains (red and yellow tyrosine-derived pigments) were not responsible for the antihypertensive effect.

Numerous studies have been conducted that demonstrate the hypotensive action of nitrate and nitrate-rich vegetables (141, 162, 163). Keen and colleagues demonstrate in healthy non-smokers in a randomized, double-blind, placebo-controlled study that daily consumption of 70 mL of beetroot juice significantly reduced both mean arterial

and diastolic blood pressures (164). Hobbs and colleagues showed in two separate clinical trials, ranging from 100–583 g in healthy adults, significant reductions in systolic and diastolic blood pressure in a dose-dependent fashion as well as subsequent increased endothelium-independent vasodilation (160, 161). In a systematic, meta-analysis, of 22 studies, the data showed that dietary consumption of beetroot juice significantly reduced Systolic Blood Pressure (SBP) and a positive correlation was observed between consumption and resultant mean difference in blood pressure via NO-independent effects (165). In other studies, oral inorganic nitrate and nitrite infusion significantly dilated peripheral arteries leading to increases in forearm blood flow (153, 166). Phase 2 studies in hypertensive patients revealed that dietary inorganic nitrate (beetroot juice) caused sustained blood pressure drops in hypertensive patients (167, 168). The results showed consistent blood pressure drops with the four weeks of the treatment period and therefore no development of tolerance, a frequent side effect of chronic nitrate use. Compelling evidence demonstrates that dietary consumption of synthetic dietary nitrate or nitrate-rich vegetables significantly decreases blood pressure within hours of ingestion and is correlated with dose (165, 169–171). In a study comparing the effects of dietary nitrate of vegetarians and omnivores, the authors noted that vegetarian diets did not alter nitrate or nitrite homeostasis, or the oral microbiome compared to the omnivore diet (170).

From a therapeutic perspective, nitrate-rich beetroot has been routinely used as an adjunct treatment by many for hypertension. In a meta-analysis of 22 studies (2009–2017), the results indicated a significant reduction in both SBP (-3.55 mm Hg) and DBP (-1.32 mm Hg) (165). Moreover, the mean difference of SBP was greater for longer, rather than shorter, time periods (≥14 versus <14 d) and higher, rather than lower (500 versus 70–140 mL/d), doses of beetroot juice (-4.78 compared with -2.37 mm Hg) (165). The beetroot dose was positively correlated with the subsequent blood pressure differences. In a different meta-analysis, studies (2006–2012) with collectively more than 254 subjects (7–30 per study) with durations of 2–15 d were evaluated. The results indicated greater changes in SBP (-4.4 mm Hg] than DBP [-1.1 mm Hg), and there was a clear association between daily nitrate dose and effects on SBP. In a meta-analysis by Bonilla and colleagues, the authors also concluded that beetroot juice supplementation reduced blood pressure in different populations as well as mitigated risk for cardiovascular events and subsequent mortality (169).

14.5.4 General Cardioprotection

Cardioprotection includes all mechanisms that collectively contribute to the protection of the heart with mitigation of myocardial injury. Many studies have shown that nitrate consumption reduces infarct size and improves clinical outcomes, but the mechanisms underlying the effect of nitrate against reperfusion injury (RI) are currently unknown. It is, however, well known that nitrate is a NO donor, and NO has a cytoprotective effect via activation of its downstream pathways (172, 173). Since ischemia-reperfusion is characterized as a NO deficit, replacement or restoration of physiological levels of NO should improve vasodilation and mitigate pathologies, although studies are inconsistently demonstrating the need for additional studies (174–176).

Nitrate can favorably influence myocardial infarction, an ischemic, hypoxic event, through several mechanisms (177, 178). Nitrate can reduce infarct size through hemodynamic (blood flow) effects and increased collateral flow of microvessels (arterioles, metartioles, capillaries, venules) typically not open under normal conditions. Moreover, they can accelerate and stabilize reperfusion or prevent adverse remodeling changes, for example, post-injury changes in size, mass, function, in refractory patients. Nitrate also redistributes coronary flow to ischemic regions of the heart that has low oxygen tension. In a study of coronary heart disease (associated with inadequate perfusion of the heart), the highest quintile of nitrate consumption compared to the lowest, there was a protective association for CHD (relative risk 0.77) (179). However, this effect was reduced after adjusting for largely modifiable lifestyle factors, that is, smoking, physical activity, and so forth.

NO is essential also in maintaining the function of the vascular endothelium largely via its vasodilatory, antiaggregatory, and antiadhesive effects. Divakaran and colleagues showed that FMD in healthy individuals was acutely improved after oral sodium nitrate, but the response to intravenous nitroglycerin did not alter the vascular smooth muscle response (1). It did improve endothelial function. Webb and colleagues demonstrated that a 20-minute ischemic insult to the forearm followed by a 20-minute perfusion reduced the FMD response by 60 percent in control subjects. Consumption of beetroot juice (500 ml) completely abrogated the effect indicating preservation of endothelial integrity and function. Moreover, the result aligns with the observation that, in humans, plasma levels of inorganic nitrate closely correlate with normal endothelial function and, as a result, vascular homeostasis (160, 180). This was further corroborated by the observations that daily dietary intake of inorganic nitrate improved endothelial dysfunction (155). In contrast, other studies have shown equivocal results with no apparent improvement to endothelial function, although plasma levels of nitrate significantly increased. The disparities in research results require further research to fully understand the reasons.

In patients with congestive heart failure (CHF), peripheral abnormalities are apparent, including a high degree of vasoconstriction relative to a maximally dilated state and ultrastructural changes to the cellular architecture of skeletal muscle. Recent studies in patients with HF with preserved ejection fraction (% blood ejected divided by maximally filled left ventricle) show that beetroot juice and potassium nitrate improved exercise capacity, presumably via reduced vascular resistance, increased muscle power, and markedly improved vascular compliance (the relationship between blood volume of vessel and BP that is generated). Others have shown that synthetic sodium nitrite provided either by inhalation or intravenous infusion exerts significant effects on biventricular central filling pressures, cardiac output, and improved exercise capacity. NO-generating nitroglycerin was the first and most frequently used organic nitrate for clinical treatment of angina pectoris because it causes vasodilatation of the capacitance veins and improves ventricular filling pressure as well as dilating the epicardial coronary arteries, improving coronary blood flow, particularly in ischemic zones, reducing infarct size and improving clinical outcomes.

Nitrate via synthetic or dietary means – that is, green leafy vegetables – is pivotal in cardioprotection. Cardiac anomalies leading to hypoxia disrupt energetics and

mitochondrial function of the heart (181, 182). Thus, the provision of dietary nitrate may reverse hypoxia-induced effects on respiration, mitochondrial complex I levels and activity, and oxidative stress that occurs concomitant with hypoxia (183). Since hypoxia and arginase deficiency (leading to reduced NO) are key features of heart failure, dietary nitrate may confer protection, particularly since the non-enzymatic pathway of NO production is preferred at hypoxic sites (184).

14.5.5 Improved Cognition

Cerebral blood flow regulation exerts an important role in cognitive function and ischemia and/or energy depletion (185). NO is critical in regulating cerebral blood flow and the coupling of neural activity to perfusion in the brain (186). In a study by Presley and colleagues, older adults were given high-dose and low-dose nitrate. The former significantly increased regional cerebral perfusion in the frontal lobe of the brain in regions involved in executive functioning (working memory, flexible thinking, self-control) (187). Oral nitrate supplementation differentially altered cerebral arterial blood velocity and subsequent prefrontal oxygenation under normoxic conditions but not hypoxic conditions (188, 189). In a study by Kelly and colleagues, older subjects were given beetroot juice for 2.5 days. However, no significant effects were noted using a panel of cognitive tests or in concentrations of brain metabolites (190).

The increased blood flow and regional perfusion in the brain due to beetroot consumption suggests a means of improving mental function and reducing the progression of age-related cognitive decline as well as dementia. In a study by Wightman and colleagues, healthy adults were recruited to assess the effect of dietary nitrate consumption on cognitive performance and cerebral blood-flow to the prefrontal cortex (191). Forty healthy adults were randomized to groups for either placebo or 450 ml beetroot juice (~5.5 mmol nitrate). After the 90-minute consumption period, participants completed an array of cognitive tasks known to activate the frontal cortex. The bioconversion of nitrate to nitrite was confirmed in plasma. Moreover, dietary nitrate modulated the hemodynamic response associated with task performance, with an initial increase in cerebral blood flow tapering off for the last of the three tests. Cognitive performance was also improved. Collectively, the results demonstrate that a bolus dose of nitrate can modulate cerebral blood flow during task performance and potentially improve cognition. Other studies have shown that dietary nitrate improves oxygenation and cerebral flow during hypoxia (186, 192).

Previous studies have shown that both acute and prolonged supplementation of dietary nitrate in older adults can significantly improve oxygen uptake and agility in exercise, and increase time to fatigue (delayed tiredness), thus promoting improved exercise performance (171). Furthermore, nitrate supplementation can significantly improve cognitive performance, as shown by enhanced reaction times in older adults, although the potential benefits of dietary nitrate supplementation are limited. Studies have also shown that, in older adults, consuming nitrate reduced blood pressure and improved blood flow to the brain and muscle, suggesting cardiovascular and cerebrovascular benefits. Older adults frequently display reduced vasodilation (reduced blood flow), cardiovascular function, cognitive function, and mood. As a result,

dietary nitrate supplementation or increased nitrate-rich vegetable intake may be particularly efficacious for older adults.

14.5.6 Erectile Dysfunction

Erectile dysfunction is a common, multifactorial disorder associated with aging and a range of organic, hormonal, and psychogenic conditions and is considered a marker for cardiovascular disease (193). Given the vascular involvement in ED, NO deficiency is involved in the etiology, since it is a key vasoactive neurotransmitter of penile tissue (194). NO is secreted by neural and endothelial cells of the corpora cavernosa, where it activates, as described earlier, soluble guanylyl cyclase, which increases cGMP levels releasing calcium from intracellular stores in smooth muscle cells (57). This can also interact with vasorelaxation-inducing contractile proteins. As one might surmise, the absence or impairment of NO bioactivity and its vasorelaxing properties are major contributors to erectile dysfunction. The efficacy of such drugs as sildenafil, viz., Viagra, illustrates the importance of the NO-cGMP pathway. Sildenafil is a PDE-5 inhibitor that prevents the degradation of NO-generated cGMP (195). With this knowledge, other drugs are being investigated that function as activators of guanylyl cyclase, donors of NO, and so forth. Recent evidence suggests that neuronal and endothelial NOS (nNOS and eNOS, respectively) play major roles also in causing NO bioactivity necessary for erectile function. Moreover, S-nitrosylation/denitrosylation has been shown to regulate eNOS activity via S-nitrosoglutathione reductase, contributing directly and indirectly to erectile function/dysfunction (196).

Demonstration that blood-flow-dependent generation of NO involves phosphorylation of penile eNOS questions the current paradigm of NO-dependent erectile mechanisms. Regulation of erectile function may not be mediated exclusively by neurally derived NO, but by fluid shear stress in the penile vasculature. This stress stimulates phosphatidyl-inositol 3-kinase to phosphorylate PKB which, in turn, phosphorylates eNOS to generate NO as discussed earlier (197). Thus, working in tandem, nNOS may initiate cavernosal tissue relaxation, while eNOS initiates and sustains full erection.

14.5.7 Improvement in Aerobic Exercise Performance

Increasingly, there has been considerable interest in the beneficial effects of dietary nitrate supplementation on athletic performance and exercise in general. In the last 5 years, >180 publications have been published on dietary nitrate and exercise, more than double that published in the 10 years before that (117). Indeed, dietary nitrate has been touted as an ergogenic aid and potential exercise therapeutic (139). Others have reviewed supplements and their use and the myriad beneficial effects to endurance athletes, physical performance, and exercise performance (108–110).

Dietary nitrate supplementation may exert enhanced effects on aerobic exercise performance and improved exercise tolerance, as reported in numerous studies (198–202). Many studies have also shown that dietary nitrate exerts ergogenic effects associated with lower oxygen cost during submaximal exercise (203–205). Mechanisms have been posited as greater production of mitochondrial ATP and thriftiness in ATP

use during the work of skeletal muscles. Many effects are also based on the capacity to significantly enhance vascular function, but also modulation of both metabolism and muscle function (206). Acute dietary nitrate supplementation (five days) has also been shown to reduce muscle fatigue primarily caused by lower exercise-induced dysfunction in contractile capacity (207).

Dietary nitrate can improve performance during high-intensity exercise. Dominguez reports in a meta-analysis of nine studies on the effects of beetroot juice supplementation and high-intensity exercise. Beetroot juice given as a single dose or over a few days improved muscle fatigue and exercise performance at intermittent, high-intensity efforts with short rest periods in between (124). A potential mechanism proffered is enhanced phosphocreatine (an energy store in muscle) resynthesis mitigating the rate of depletion. Wylie and colleagues have shown that nitrate concentration in skeletal muscle is indeed considerably higher than blood concentrations, and is further increased by dietary nitrate ingestion (208). Further, the authors show that high-intensity exercise reduces the skeletal nitrate store following dietary supplementation, presumably via conversion of nitrite to NO. Nitrate-rich beetroot juice also improved muscle power output, presumably due to more rapid muscle shortening velocity (124). Kelly and colleagues report the effects of dietary nitrate in nine recreationally active male subjects in a double-blind, randomized, crossover study by measuring and calculating critical power and the curvature constant of power duration, which describe the relationship of tolerance to severe-intensity exercise. Dietary nitrate significantly enhanced endurance in recreationally active subjects (209). The data, however, are inconsistent, and some studies do not report beneficial effects. For example, Bescos and colleagues report that sodium nitrate supplementation did not enhance performance in endurance athletes (188).

In runners, Thompson and colleagues have shown that dietary nitrate improves sprint and high-intensity running performance, as well as cognitive function (210, 211). Margaret and colleagues also showed that nitrate-rich whole beetroot consumption acutely improved running performance (212). As a result, many nitrate-containing supplements and regimens have been proposed for optimal function and performance enhancement of track and field athletes (127).

In rowers, high-dose (8.4 mmol) dietary nitrate provided 2 hours prior to rowing has been shown to improve performance in well-trained rowers in simulated 2,000-meter rowing ergometer tests (108). Bond and colleagues also showed that nitrate supplementation, as beetroot juice, improved maximal rowing-ergometer repetitions (213). Muggeridge and colleagues showed that a single dose of beetroot concentrate can improve performance in trained flatwater kayakers (214).

In a different study, 6 d of nitrate supplementation (beetroot concentrate, 140 ml/d; 8 mmol) reduced VO_2 during submaximal exercise and improved time-trial performance in trained cyclists (215). Regarding endurance, dietary sodium nitrate (10 mg/kg) supplementation to 11 cyclists reduced the VO_2 peak without compromising maximal exercise performance (216). RSE supplementation (1 g/d; 7 d and 1 hour prior) was provided to 17 males and females prior to a 4-km cycling time-trial. RSE significantly reduced time-to-completion, increased power and speed, and lowered diastolic BP (130). Conversely, in an acute study, a single dose of beetroot juice (500

mL beetroot juice) was provided prior to a 50-mile time-trial performance in 8 well-trained male cyclists. The data indicated no significant improvement in performance (217). Garnacho-Castano and colleagues gave 70 mL beetroot juice (6.5 mmol) to 12 well-trained male triathletes and conducted endurance tests on cycle ergometers and noted no differences between groups on cardiovascular efficiency/economy, VO_2 time-trial, energy expenditure, carbohydrate oxidation, or fat oxidation (218). Both authors suggest that the training status of subjects may have blunted the physiological and performance responses.

Wylie reports that dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance (219). Vanhatale and colleagues further elaborates on both the acute and chronic effects in 8 healthy subjects of dietary nitrate (beetroot juice, 500 mL/d 4–6 days). The results indicated that dietary nitrate acutely reduced BP and the oxygen cost of submaximal exercise, which was maintained for 15 days with continuous consumption (106). Kramer and colleagues supplemented athletes with potassium nitrate (8 mmol/d for 6 d), followed by subject participation in a heavily power-dependent CrossFit sport regimen. The authors concluded that peak power improved during one test, viz., Wingate, but not the others testing strength or endurance (220). The results showed that plasma nitrate and the O_2 cost of moderate-intensity exercise were altered dose-dependently with beetroot juice, but there was no additional improvement in exercise tolerance after doubling the dose (16.8 mmol) (221). Moreover, positive effects occur even with chronic disease, such as peripheral artery disease, and treated, but uncontrolled, hypertension, and thus is important in maintaining cardiovascular health (222–224).

Regarding tolerance, beetroot juice amplifies oxygen uptake kinetics and significantly improves exercise tolerance during severe-intensity exercise with elevated metabolic rates (225). Nitrate also reduces skeletal muscle metabolism perturbation under conditions of hypoxia (199). In a meta-analysis by Van de Walle and colleagues the effect of nitrate on exercise tolerance and performance was compared. A total of 29 studies using the time to exhaustion (TTE) as the outcome variable revealed a significant effect of nitrate supplementation on exercise compared with placebo, although it revealed no significant effect of nitrate supplementation on exercise performance compared with placebo. Thus, it appears that nitrate supplementation improves exercise tolerance and capacity, which may subsequently improve exercise performance (226).

In a meta-analysis by McMahon and colleagues, data from 76 trials were reviewed and analyzed for time-trial performance, TTE, and graded-exercise test (GXT) protocols. The results indicated small but non-significant effects for the time and GXT trials and TTE data displayed small to moderate statistically significant effects. The data suggest nitrate supplementation elicits a positive outcome when testing endurance exercise capacity, but is less effective for time-trial performance (227). In a meta-analysis by Campos and colleagues, 54 studies were analyzed for the effects of dietary nitrate supplementation on human performance. Nitrate supplementation was ergogenic in non-athletes using long-duration, open-ended tests, but, overall, did not enhance the performance of athletes. Thus, the present study suggests that dietary nitrate supplementation improves physical performance in non-athletes but others were non-refractory (228).

Regarding oxygen use, dietary nitrate supplementation has been shown to significantly reduce the oxygen cost of exercise of walking and running (200, 229). It also reduced maximal oxygen consumption while maintaining work performance in maximal exercise (204). Bailey and colleagues have shown that dietary nitrate supplementation reduces oxygen cost of low-intensity exercise and enhances tolerance to high-intensity exercise (203). Nitrate from red spinach consumption has increased the ventilatory threshold during graded exercises (129). Masschelein and colleagues show as well that dietary nitrate improves skeletal muscle oxygenation during exercise in hypoxia (192). Cermak and colleagues, however, report that nitrate-rich beetroot did not improve endurance performance after a single dose (215). Bescos and colleagues have also reported that sodium nitrate supplementation does not enhance the performance of endurance athletes (188).

14.5.8 Diabetes, Glycemia, and Insulin Resistance

There is compelling evidence that NO modulates carbohydrate metabolism, and lack of NO contributes to the development of type 2 diabetes (230). A review of five human studies was analyzed and reported as significantly reducing blood glucose levels and beneficially affecting both glycemic and insulin responses (231). In 16 healthy adults, participants were given 225 mL beetroot juice. Postprandial insulin response was reduced in the early phase (0–60 min) and the glucose response was reduced in the 0–30 min phase (232). Subjects were provided three beverages, including (1) beetroot with lemon, (2) beetroot with glucose, fructose, and sucrose and (3) beetroot juice with added glucose. A positive correlation was observed with beetroot juice plus lemon, but not the two other beverages, and glycemic response was lower with both the first two glycemic responses significantly lower than the third. In a second study, there was a trend for 35 percent reduction in plasma glucose in 30 subjects consuming the beverage over longer periods and 10 percent for 4 weeks, suggesting that chronic consumption must be maintained (233, 234). In a study of 57 individuals, co-ingestion of beetroot juice and glucose caused greater elevation of glucose in obese versus non-obese up to 90 min. The authors concluded that obesity with an intrinsic higher risk for developing insulin resistance may receive greater benefit than non-obese (235) in a meta-analysis of 173 (11) studies (2004–2019). Interestingly, preliminary experimental findings strongly support the hypothesis that NO_3 can be considered as a natural anti-obesity agent (231).

14.6 DIRECTIONS FOR FUTURE RESEARCH ON NITRATE RELATED TO HEALTH

Given the relatively recent appreciation of the capacity of dietary nitrate to generate NO, and the clear beneficial effects of NO, there are many areas of research that require attention. First, elucidation of the lowest effective, efficacious dose and the duration needed to significantly lower blood pressure and improve cardioprotection are needed from dietary intervention studies. Moreover, the demonstration of the potential for these effects in target “at-risk” populations or those with overt chronic disease (hypertensive) is needed. More specific biomarkers with greater sensitivity

and validity are needed, since plasma NOx are currently measured colorimetrically as nitrite and nitrate, which can be metabolized and/or interconverted. The clear capacity for other dietary components to interact both positively and negatively requires further investigation to elicit dichotomous effects and the microenvironments that may contribute to their action. The ongoing conundrum of whether dietary nitrate cause gastric cancer requires resolution, thus a reliable risk-benefit analysis would be helpful. Future cancer research should focus on at-risk populations, such as tobacco smokers, supplement users (particularly sodium and potassium nitrate and nitrite), and specific dietary components that might yield pro-carcinogenic and/or mutagenic molecules. By extension, a comparison of health or disease status to overall nitrate-rich vegetable intake (amounts and types) and water would be helpful in establishing risk versus benefit. Other recommendations have been made, including the development and/or expansion of dietary databases derived from epidemiological studies to assist with more accurate estimates of dietary consumption patterns and amounts. As such, the cardiovascular benefits may be correlated to intakes. Furthermore, studies of total intake coupled with excretion or at least urinary and plasma concentrations should be incorporated into studies to clarify the disposition of nitrate.

Given the effects of nitrate based largely on the assumption that NO is specifically produced from nitrate and nitrites, and that the vasculature is a specific target, then most pathologies and/or conditions that are based in part on vasoconstriction and/or altered hemodynamics should merit additional research. Regarding exercise and sports performance, there is a need for elaborating the impact of dietary nitrate on anaerobic exercise, identifying interactions with other dietary components, determining efficacious, non-toxic doses, and optimal time of supplementation. Collectively, this may help unravel the many noted discrepancies in research results.

14.7 CONCLUSION

Dietary vegetables represent a nitrate-rich source for consumption by humans. Both dietary nitrate and nitrites can be bioactivated endogenously to form NO, as well as other nitrated bioactive molecules, with subsequent reabsorption of 25 percent, which enters the nitrate-nitrite-NO pathway or enterosalivary cycle and is concentrated 10–10,000-fold. The relative critical importance of NO and its vasodilatory function, among many others, has garnered considerable research interest despite the ongoing stigma that nitrate can form carcinogenic nitrosamines at low pH. Indeed, nitrate can be problematic regarding methemoglobinemia, and perhaps some cancers, but data are inconsistent. Nonetheless, there is considerable interest in exogenous dietary nitrate for endogenous protective functions such as reducing the risk for cerebrovascular incident, myocardial infarction, cardioprotection, angina pectoris, hypertension, erectile dysfunction, athletic performance, gastric ulcers, and so forth. Dietary consumption of nitrate and nitrite, although low for the latter, do not cause harm in humans. Dietary supplementation with purified salts, in particular, and certain nutraceutical formulations may be problematic regarding safety. Given the considerable myriad protective effects, dietary nitrate and nitrite are pivotal in physiological health and homeostasis largely via the formation of NO either enzymatically via NOS or

non-enzymatically. With the clear observations of benefit – tempered, however, with cautious concerns for safety – further investigation is needed regarding biological functions, efficacy, dosing, and duration, as well as therapeutic applications of dietary nitrate.

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