

Dietary Nitrates, Nitrites, and Food Safety: Risks Versus Benefits

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Abstract

Atmospheric nitrogen, via the environmental nitrogen cycle, is captured, or fixed, by symbiotic bacteria interacting with plants. Nitrates, as a result, are intermediates in the movement of atmospheric nitrogen into the food chain with rich dietary sources including red spinach, beetroot, etc. Nitrate-rich fertilizers may further increase nitrogen content of plants. Other sources include potable water, dietary supplements and food additives. Although prevalent in the diet, nitrates have been viewed negatively because they chemically form carcinogenic nitrosamines in acidic environments, e.g. stomach, purportedly leading to gastric cancer as well as neoplasia of the intestine, brain, pancreas, and contributing to Non-Hodgkin's lymphoma. Other reports indicate associations with hyperthyroidism and diabetes mellitus. A second major concern with dietary nitrate consumption is the development of methemoglobinemia particularly in infants caused by increases in methemoglobin where heme iron is oxidized to ferric iron and unable to bind and transport oxygen. In contrast, the recent discoveries of the vital gasotransmitter function of nitric oxide derived from dietary nitrates and nitrites, endogenous production, and endogenous recycling and considerable concentration in saliva have revealed the importance of nitrates to health including improved cardiovascular function (reductions in blood pressure), improved cognition, reduced platelet aggregation, and enhanced blood flow to ischemic (hypoxic) and/or exercising tissues. As a result, dietary supplementation may represent an effective, inexpensive option for individuals with vascular disorders and a safe, efficacious means of enhancing performance in both recreational and competitive athletes. Given the myriad beneficial effects tempered by the purported negative effects, it is timely to revisit the conundrum of risk versus benefit from dietary nitrates.

Keywords: Dietary Nitrates; Food Safety; Soil Quality

Natural environmental sources of nitrates

The environmental nitrogen cycle captures atmospheric nitrogen and distributes it throughout plants where it may be accumulated and ultimately consumed by mammals. Dietary nitrate intake is largely via vegetable intake, which ultimately depends on the type and amount of vegetable consumed, the concentrations of nitrate in the vegetables (including the nitrate content of fertilizer), and the level of nitrate in the water supply [1]. Concentrations can also vary considerably by up to 50-fold in different anatomical structures within the same plant occurring in high levels

in the petiole (leaf, stem stalk) followed by the leaf, stem, root, inflorescence (flower head: stems, stalks, bracts, and flowers), tuber, bulb, fruit, and seed [2]. The relative accumulation of nitrate also depends on factors such as plant genotype, soil quality (nitrate content), growth environment, and storage and transport conditions. 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 [3].

Atmospheric nitrogen is essential for successful plant growth, and artificial fertilization (inorganic and organic) as part of agri-

cultural practices can be more efficient for increasing plant biomass and nitrogen composition. For the former, many agricultural practices include application of organic matter containing myriad nutrients, e.g., phosphorous, potassium, etc. as well as nitrogenous compounds. After application of organic fertilizers, inorganic nitrogen is released via mineralization (decomposition of chemical compounds) and absorbed by plants as inorganic (without carbon) nitrates. Although inorganic nitrogen compounds such as nitrate, nitrite and ammonium represent <5% of the total nitrogen in soil, they are the prevalent forms used by plants [4]. Other environmental factors affect 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 [5]. As such, the amounts of nitrates in farmed vegetables has led to governmental limitations on allowable nitrate concentrations due to safety concerns.

Foods containing nitrate

Vegetables accumulate significant amounts of nitrate from the atmosphere and more so from nitrogen-based fertilizers, which are used for rapid and enhanced plant growth. The amount of available nitrate in soil (depending on the content of artificial fertilizer) appears to be major factor determining the nitrate content in vegetables. An estimated daily dose of nitrates consumed by humans is 75-100 mg, of which 80-90% come from vegetables and 5-10% come from water [5]. Leafy vegetables, such as lettuce or spinach, and beetroot contain the highest concentrations of nitrate [4]. Other examples of rich sources include radishes, turnips, watercress, bok choy, Chinese cabbage, kohlrabi, chicory leaf, celery, onion and garlic. Fruits including watermelon, apples, bananas, grapes, kiwi, pears oranges, and strawberries also contain nitrates but at low levels [3]. Interestingly, diets recommended by expert advisory panels that are associated with reduced chronic disease risk tend to be considerably higher in fruit and 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 nitrates/d.

Potential adverse effects

Methemoglobinemia

Methemoglobinemia is a potentially fatal condition in which hemoglobin is oxidized to methemoglobin (> 1% of total hemo-

globin) with significant reduction, due to oxidation of ferrous iron in oxyhemoglobin to ferric iron, in the ability of iron to bind and transport oxygen leading to hypoxia and cyanosis, or "Blue Baby" syndrome [6]. Infants < 6 months of age may be especially vulnerable when exposed to nitrate from sources such as well water contaminated with bacteria, which can reduce and bioactivate nitrate to nitrite [1]. As a result, it has been strongly advised that potable well water that may be provided to infants directly or indirectly be tested for nitrate/nitrite concentrations to minimize health risk [7]. Although the bulk of dietary nitrates are derived from vegetables, infants fed commercially prepared foods with vegetables are not considered to be at risk for excessive nitrates as long as home-prepared nitrate-rich foods are avoided until infants are >3 months of age.

Although a concern, few nitrate and nitrite exposure studies in humans, including either children or adults as subjects, have resulted in elaboration of methemoglobinemia causing many to proffer alternative explanations for the observed etiology. For example, in one study infants exposed to 175 - 700 mg nitrate/day did not display methemoglobin concentrations > 7.5%, which suggests that nitrate alone was not the causative factor [6]. In a recent study, healthy adults were provided a bolus dose of supplemental sodium nitrite (low, 150 to 190 mg or high, 290 - 380 mg) [1]. Methemoglobin concentrations were 12.2% and 4.5% for the high and low dose, respectively. These data suggest other factors as well in the etiology of methemoglobinemia such as gastroenteritis or bacteria-induced NO production as an immune response to infection.

Although thought due to nitrate-rich water, methemoglobinemia is now more commonly thought to be due, not by nitrate per se, but by fecal bacteria with nitrate reductase capacity that infect the infants and produce nitrite and ultimately nitric oxide, in their gut (nitric oxide can convert hemoglobin to methemoglobin). Indeed, some studies have shown that infants exposed to high levels of nitrate (~700 mg/day) did not develop methemoglobinemia [7]. Recent studies on both nitrate and nitrite in healthy adult and adolescent populations also have not demonstrated any negative health effects. As a result, many experts remain skeptical as to whether nitrates (and nitrites) in food and water really do pose a risk for this condition.

Dietary nitrate consumption presents a conundrum during pregnancy. Recommendations to NO-deficient pregnant women for increased consumption of nitrates, e.g., beetroot has been advised to suggested to mitigate hypertension and pre-eclampsia, improve placental blood flow and markedly improve maternal and neonatal health [8]. In another study, the authors demonstrate that breast-fed babies did not display methemoglobinemia when mothers ingested high amounts of nitrate [7]. However, caution has also been advised since methemoglobinemia, alteration in embryonic cells and malignant transformation (neoplasia), and thyroid disorders may occur. Epidemiologic evidence also suggests an association between nitrate-rich water consumption and spontaneous abortions, intrauterine growth restriction, and various birth defects although the data are limited [8].

Methemoglobinemia may occur after consuming water containing > 10 mg/L nitrate. Concentrations over > 45 mg/L have caused methemoglobinemia in infants and particularly children younger than 6 months old. In 214 clinical cases, 2% of methemoglobinemia cases occurred at concentrations of 49 - 88 mg/L and 80% at > 220 mg/L. In a different study, children aged 1-8 years consumption of nitrate-rich water (97 - 491 mg/L) did not display increased risk [1]. In contrast, in infants consuming nitrate-rich water (44.3 mg/L) the prevalence significantly increased. In a study by the WHO, there was also no correlation between aquatic nitrates and methemoglobinemia. Collectively, results of several studies have been conflicting and, as a result, has been generated speculation that exposure to other environmental chemicals such as sulfate, chlorite, chloramines, chlorate and pharmacological agents such as sulfonamides and nitroglycerin [9].

Gastrointestinal tract neoplasia

Nitrates can be reduced to nitrites and react with naturally occurring components of protein, viz., amines, which can lead to formation of well-recognized carcinogenic nitrosamines. This has generated considerable concern regarding the safety of ingested nitrates. Paradoxically, vegetables may also naturally contain nitrosamines without artificial addition although the level is low [10]. Individuals are exposed to many other sources of nitrosamines such as through smoke, beer, water, work environments, and tobacco use especially cigarettes which contain 100-1000 times more nitrosamines than the typical diet [10].

The second primary concern with dietary nitrates involves the capacity to form carcinogenic nitrosamines at low pH and low pO₂ in the gastric lumen. Indeed, in a population-based cohort study, there were increased risks for all-cause mortality due to 9 different dietary sources [11]. All were associated with both processed and unprocessed meats via, in part, heme iron, nitrates and nitrites. Interestingly, food additives, viz., nitrates are regarded as cancer-causing agents, yet individuals frequently consume identical chemicals of nitrates from dietary vegetables. Others have recently suggested that nitrate and nitrite should be considered essential nutrients because they promote NO production and consequently contribute to cardiovascular health and exercise performance [12]. Indeed, alternatives to nitrates and nitrites are the object of many studies with heightened focus on the addition of vitamins, fruits, chemicals, and natural products containing nitrate.

The association of dietary nitrite with gastric cancer is unresolved and often contradictory amongst results of epidemiological studies. In a large cohort study, nitrate and nitrite were not associated with esophageal or gastric cancer but there positive associations between red meat intake and esophageal squamous cell carcinoma [13]. Other studies have also shown no correlation between high intake of nitrate (52 - 423 mg/day) and cancer in several countries [14]. Dietary nitrates and nitrites may be associated with cancer risk but the data continue to be inconsistent. It has however been estimated that nitrate intake >600 mg/day, nitrite intake > 0.2 mg/day and nitrosamine intake > 0.2 µg/day increase the relative cancer risk [4].

The notion that all gastric nitrosation occurs primarily via attack on secondary amines of proteins may be misleading when considering gastric cancer risk. Thiols, phenol groups, and the nitric dioxide radicals are also formed from nitrite in the stomach. Moreover, gastric NO and nitrite are equimolar and the redox conditions of the gastric microenvironment can shift this reversible reaction between the two in either direction. For example, the presence of reducing agents mentioned previously, i.e., ascorbic acid and polyphenols, shift the reaction largely towards NO production. Other nitration products may also be generated that exert protective effects such as modulating pepsin enzymatic activity via nitration or forming electrophilic compounds, i.e., fatty acid adducts, that are

anti-inflammatory [15]. Moreover, ethanol from alcoholic beverages can be nitrosated by dietary nitrite to form the potent vasodilator ethyl nitrite, which can release NO at physiological pH (pH = 7.4) [16]. Patients with achlorhydria (absence of hydrochloric acid [HCl]) and bacterial overgrowth of the stomach are at high risk of developing gastric cancer purportedly due to nitrosamine formation. However, this process can be mitigated by the co-presence of polyphenols and other antioxidants such as vitamin C, which can inhibit nitrosylation of secondary amines. As such, dietary nitrate could decrease the occurrence of gastric cancer because the presence of myriad antioxidants and reductants [11].

In a study of colon cancer, there was no significant correlation between the concentration of nitrate in drinking water and risk of colon cancer although cancers appearing in the placebo group were non-colonic and the treatment group displaying largely colon cancer. In a cross-sectional study, the odds ratio for colon cancer ranged from 0.84 to 1.14 with 98% confidence [17]. In two cohort studies, there were no associations between systemic nitrate concentrations and the occurrence of gastrointestinal cancers after 6 - 24 years of follow up. In addition, there was no correlation between dietary reductants and cancer [18]. In contrast, a case-control study showed that individuals consuming low amounts of dietary antioxidants but high amounts of nitrates displayed a substantially increased risk of gastric cancer. In a study comparing different water concentrations of nitrates and cancer incidence, the incidence of gastric cancer was slightly greater in those consuming nitrate-rich water (30 mg/ml) In similar studies comparing this relationship, mortality from gastric cancer increased with increasing exposure to nitrate (50 mg/L) as did the relative risk for prostate cancer (RR = 1.86) and gastric cancer (RR = 1.91) for males 55 - 75 years of age [19].

In an ecologic study, the relationship between stratified dietary nitrate consumption and cancer occurrence was determined with no associations noted for kidney or bladder cancer. Using a nitrate data base from public water supplies in Central Europe, nitrate concentrations were stratified into low (0 - 10 mg/L), medium (10.1 - 20 mg/L), or high (20.1 - 50 mg/L) [19]. There was a positive association between nitrate-rich water consumption and Non-Hodgkin lymphoma (NHL) and colorectal cancer but no associations for kidney or bladder cancer. In contrast, no association

between nitrate in drinking water and NHL incidence was noted using a population-based incidence register in Northern England. These results contrast with previous studies where NHL was associated with increased levels of nitrate although negative results were also reported rendering the overall outcome equivocal. NHL incidence was compared to nitrate monitoring data of community water supplies in several locations in Italy and revealed limited evidence among men but not women. Collectively, the association of NHL and cancer risk have generated inconsistent results.

Non-Hodgkin's lymphoma

In a cohort study, females displayed a weak inverse relationship between nitrate concentrations (> 2.46 mg/L) in drinking water and risk of NHL and after eliminating confounding variables was strengthened [20]. Others have shown a dose-response relationship and doubling of the risk of NHL at high nitrate concentrations in drinking water even after eliminating confounding variables prompting the conclusion that long-term exposure led to increased risk of NHL [21]. Long-term consumption of municipal water with > 4 mg/L nitrogen was positively associated with risk [OR = 2.0]. In contrast, most studies have reported that nitrate concentrations in drinking water is not associated with Hodgkin's Lymphoma, or alternatively have mitigated the risk. Moreover, potential confounding agents, e.g. vitamin C, have weakened the relationship between dietary nitrate and lymphoma incidence

Urinary tract neoplasia

The effect of nitrate concentrations in drinking water on the prevalence of urinary tract cancer in 2 Central European cohorts exposed to different concentrations of nitrate (10 - 60 mg/L) was investigated. With greater exposure, a positive correlation was observed between urinary tract cancer in both males and females, but negative correlations with testicular and kidney cancer. No association was observed between urinary tract neoplasia in either males or females consuming nitrite-rich food sources suggesting no increased risk of bladder cancer. Positive associations between bladder cancer and nitrate in drinking water (2.5 mg/L) have been reported in both a case-control and cohort study [22]. However, no association was noted in subsequent similar studies. It has been posited that bladder cancer risk is substantially increased after long-term consumption of nitrate-rich water at levels below regulatory limits highlighting the importance of both drinking water and dietary nitrate as risk factors for bladder cancer [22].

Effect on the thyroid gland

The thyroid gland is purportedly another potential target for dietary nitrates with ensuing enlargement and potential neoplasia. Studies have been conducted in two population groups, viz., pregnant females and children (3-6 years of age) who consumed high and low concentrations of nitrate in drinking water to determine the impact of nitrate on iodine (required for thyroid hormone synthesis). In pregnant women, but not children, the frequency of goiter formation was significantly increased representing perhaps a specific at-risk population for detrimental effects from nitrate exposure [23]. In a different study, two groups exposed to contaminated nitrate-rich water were evaluated for size and function of the thyroid gland. With high nitrate exposure (> 50 mg/L), the thyroid gland volume increased purportedly due to the interaction and competition between iodine and nitrate for uptake [20,23]. Nitrate competes with uptake of iodide by the thyroid with adverse effects on function and subsequent hypertrophy (enlargement of the thyroid gland). At lower nitrate exposures, there were no differences in thyroid volume.

Potential health benefits of nitrate

Food safety: Nitrate and nitrite as food additives

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 [12]. 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 [12]. 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 nitrates.

Nitrates and nitrites are routinely used as food additives in the meat curing process. Approximately 10% - 20% of residual nitrites remain in food products marketed to the consumer although levels decrease upon storage [14]. The average levels of residual nitrites in cured processed meats is estimated at 4 - 14 mg//kg for many industrialized countries although levels of 50 mg/kg have been reported for France [14].

The myriad benefits and aesthetic qualities imparted by nitrates and nitrites to foods seemingly renders them vital to the food supply. This is due to nitrate/nitrite-induced effects on color enhancement, flavor profile, antimicrobial effects and antioxidative activities.

Cardiovascular benefits

A considerable body of literature reports the cardiovascular benefits of dietary nitrate and nitrite consumption. These beneficial effects include blood pressure reductions, mitigation of ischemia/reperfusion injury, reduction of platelet aggregation, and enhanced endothelial function [24,25]. Moreover, many studies use flow mediated dilation (FMD) as a meaningful outcome of artery diameter since the dilatory effects on the brachial artery are correlated with plasma nitrate and nitrite levels.

Dietary nitrates whether in vegetables, water, or supplemental nitrate salt dose-dependently and acutely reduce blood pressure in numerous experimental models. For example, individuals consuming nitrate-rich foods (18.8 mg/day/kg body weight) displayed a significant reduction in diastolic blood pressure (DBP) of almost 5 mmHg [26]. In other studies, it has been demonstrated that nitrate-rich beetroot juice (500 mL; 341 mg nitrate) or inorganic potassium nitrate (4, 12, or 24 mmol nitrate) significantly induced dose-dependent increases in plasma nitrate and nitrite which led to reductions of both systolic (SBP) and DBP at the highest dose [24]. Studies have shown that females displayed significantly higher baseline plasma nitrite levels compared to males suggesting a sex-specific effect. Moreover, those with higher salivary nitrite displayed lower resting and ambulatory SBP. Interestingly, female subjects displayed two-fold higher oral nitrite production compared to males after a bolus supplement of potassium nitrate [27]. These results suggest that females produce more nitrite derived from enterosalivary circulation than males contributing to the reduced BP and perhaps the observation of reduced risk of cardiovascular disease in females.

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, in-

creased blood pressure, and lack of gastroprotection as well as contributing to the risk of cardiovascular disease and chronic kidney disease [27]. It has also noted that the frequency of tongue cleaning significantly impacts the composition of the human tongue microbiome and enterosalivary circulation of nitrate. 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.

NO exerts a critical role in maintaining cardiovascular health since it regulates vascular tone, smooth muscle cell proliferation and growth, platelet activity and aggregation, leucocyte trafficking, expression of adhesion molecules and inflammation [27]. However, when the bioavailability of NO is compromised, the beneficial effects of NO are impaired or negated and endothelial dysfunction predominates due to the imbalance created between the release of vasoconstrictors and vasodilators (such as NO). NO can also prevent the development of atherosclerosis via its antiplatelet effects, anti-proliferative effects, anti-inflammatory, and antioxidant effects [27]. It is well-accepted that NO can bind or react with a variety of chemical biomolecules within the cellular environment, including metal-containing proteins, membrane receptors, ion channels, enzymes, transcription factors and oxygen species although the specific mechanisms are unclear.

Mitigation of diabetes and insulin resistance

The potential toxic effects of nitrates on pancreatic β cell have remained controversial over the past two decades. In one study, epidemiological studies were reviewed and the associations between nitrates exposure was investigated, from both diet and drinking water to determine whether these compounds contribute to development of type 1 diabetes. Limited data were available regarding the potential diabetogenic effect of nitrite from drinking water, although there was evidence indicating high dietary nitrite increase risk for development of type 1 diabetes.

Prior studies reported that nitrites and nitrates prevalent in red meat could increase insulin resistance, dysregulate blood glucose levels, and elevate oxidative stress, which all lead to chronic diseases [28]. Indeed, a significant relationship between the prevalence of diabetes mellitus type 1 and concentration of nitrate in drinking water has been reported in children (1 - 18 years old) [29]. However, other studies report no increased risk in children and no relationship between different concentrations of nitrate and nitrite

in drinking water and insulin-dependent type 1 diabetes mellitus [29]. Data are limited with many contradictions, thus more research is needed to fully elaborate an effect if present.

In contrast to purported negative effects in diabetes, there is also compelling evidence that NO beneficially modulates carbohydrate metabolism and the lack of NO, in fact, contributes to the development of type 2 diabetes [30]. A review of 5 human studies were analyzed and reported as significantly reducing blood glucose levels and beneficially affecting both glycemic and insulin responses [31]. In 16 healthy adults consuming 225 mL beetroot juice, postprandial insulin response was reduced over 0-60 min and the glucose response was reduced in the 0-30 min phase [31]. Subjects were provided 3 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 response significantly lower than the third. In a second study, there was a trend for 35% reduction in plasma glucose in 30 subjects consuming the beverage over longer periods and 10% for 4 weeks suggesting that chronic consumption must be maintained. 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 suggesting that obese individuals with intrinsic higher risk for developing insulin resistance may receive greater benefit than non-obese [31]. Interestingly, this observation has fostered support for the hypothesis that nitrates are natural anti-obesity agents.

Improved cognition

Cerebral blood flow regulation exerts an important role in cognitive function and ischemia and/or energy depletion [32]. NO regulates cerebral blood flow and couples neural activity to perfusion in the brain [33]. Older adults were given high-dose and low-dose nitrate where 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) [34]. Oral nitrate supplementation differentially altered cerebral arterial blood velocity and subsequent prefrontal oxygenation under normoxic conditions but not hypoxic conditions [35]. In a study by Kelly, *et al.* 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 [36].

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, *et al.* healthy adults were recruited to assess the effect of dietary nitrate consumption on cognitive performance and cerebral blood-flow to the prefrontal cortex [33]. Forty healthy adults were randomized to either groups for 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. There are other studies that have shown that dietary nitrate improves oxygenation and cerebral flow during hypoxia [34].

Previous studies have shown that both acute and prolonged supplementation of dietary nitrate in older adults can significantly improve oxygen uptake, agility in exercise, and increase time to fatigue (delayed tiredness), thus promoting improved exercise performance [37]. 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 in older adults consuming nitrates 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.

Attenuated 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 [38]. Given the vascular involvement in ED, NO deficiency is involved in the etiology since it is a key vasoactive neurotransmitter of penile tissue. 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. This can also interact with vasorelaxation-inducing contractile proteins [38]. As one might surmise, the absence or impairment of NO bioactivity and its vasorelaxing properties is a major contributor 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, which prevents the degradation of NO-generated cGMP. With this knowledge, other drugs are being investigated that function as activators of guanylyl cyclase, donors of NO, etc. 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 [39].

Improvement in aerobic exercise performance

There has been increasing considerable interest in the beneficial effects of dietary nitrate supplementation on athletic performance and exercise in general. Indeed dietary nitrates have been touted as an ergogenic aid and potential exercise therapeutic [40]. Others have reviewed supplements and their use and the myriad beneficial effects to endurance athletes, physical performance, and exercise performance [41].

Dietary nitrate supplementation may exert enhanced effects on aerobic exercise performance and improved exercise tolerance as reported in numerous studies [42]. Many studies have also shown that dietary nitrate exerts ergogenic effects associated with lower oxygen cost during submaximal exercise [43]. Mechanisms have been posited as greater production of mitochondrial ATP and thriftiness in ATP use during 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. Acute dietary nitrate supplementation (5 days) has also been shown to reduce muscle fatigue primarily caused by lower exercise-induced dysfunction in contractile capacity [42].

Exercise reduces the risk of cardiovascular disease. A preliminary sign is suppressed endothelial reactivity after ischemic reperfusion, which generates a period of hypoxia followed by re-

oxygenation Age-dependent endothelial dysfunction is correlated with inadequate increases in plasma nitrite levels in response to exercise. This occurs, in part because exercise increases endothelial NOS-dependent NO production via shear stress on the inner vasculature wall. Accumulating evidence shows that dietary nitrate can markedly reduce the oxygen cost of exercise and enhance endurance around 15%, purportedly via modulation of mitochondrial efficiency mediated by improvement in the efficiency of oxidative phosphorylation [41,44]. This leads to reductions in oxygen cost for energy production during exercise.

Defense against pathogens

Helicobacter pylori is globally the most commonly observed infection and a major risk factor for gastric cancer development. In fact, gastric adenocarcinoma is one of the leading causes of cancer-related mortality worldwide and *Helicobacter pylori* infection is the strongest known risk factor for this disease. Many bacterial species survive and thrive in the stomach leading to an intricate interplay between *H. pylori* and other residents of the gastric microbiota.

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 can serve as a source for NOS-independent gastric generation of both NO and nitrosamines. This occurs after secretion and ionization of HCl, which protonates nitrite in the gastric lumen. This process can be abolished by proton pump inhibitors such as Prilosec, Nexium, etc., which reduce gastric pH [45]. 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. However, nitrite in combination with gastric acid is considerably more potent in killing gastric pathogens such as *Helicobacter pylori* than acid alone. This is 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.

Production of gastric NO from dietary nitrite, and presumably via dietary nitrate, kills many different enteropathogens including *Salmonella*, *Shigella*, *Helicobacter pylori*, *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium difficile*, and *Candida albicans* [46].

Aside from protective bactericidal activity, NO is key in host defense in the GI tract largely via stimulation of mucus and fluid secretion, regulation of the epithelial barrier, mediation of vascular smooth muscle tone, mitigation of immune cell binding to the endothelium, mucosal repair, and release of inflammatory mediators [47].

Gastric ulcers and cancer

Dietary nitrates secreted from saliva protect against gastric ulcers by promoting gastric NO production and stimulating concomitant mucus formation. In a rodent study, stress-induced gastric damage was induced demonstrating that stress promotes salivary nitrate secretion by up to 50-fold and nitrite formation. Exogenous nitrate administration (5 mmol/L NaNO₃) recovered gastric mucosal blood flow and gastric NO levels, thereby mitigating the gastric damage [48]. Others have shown that NO production from dietary induced stomach mucosa injury can improve the thickness of the protective mucus layer in the stomach. Collectively, these observations have stimulated research and new opportunities into drug development.

Current ADI conundrum

The acceptable daily intake (ADI) is defined as the maximum amount of a dietary component, viz., chemical that can be ingested daily over a lifetime with no appreciable health risk. That is, it is based on the highest dietary intake or level that exhibits no observable adverse effects (NOAEL). An evolving conundrum regarding nitrate supplementation exists as to whether benefits outweigh potential detriment with a reasonable intake of dietary nitrates, which may readily exceed recommendations. For example, consumption of a single serving of a nitrate-rich food or dietary supplement can readily exceed the ADI for nitrate (222 mg/d for a 60-kg adult) per the World Health Organization (WHO) [49]. 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 starting exercise also providing a substantial amount of dietary nitrate [44]. An additional recommendation that provides high dietary nitrate levels is multi-day dosing for around 6 days prior to exercise for an athletic event. Given both risks and benefits, there is a need for expert evidence-based dietary guidance to achieve optimal cardiovascular health and athletic performance.

The EPA has designated maximum levels of 10 mg/L (or ppm) for nitrate (~44 mg nitrate/L) and 1 mg/L (or ppm) for nitrite (~3.3 mg nitrite/L). The FDA advocates 10 mg/L nitrate (44 mg nitrate/L), 1 mg/L nitrite (~3.3 mg nitrite/L), and 10 mg/L total nitrate and nitrite (as nitrogen) as allowable levels in bottled water. Neither OSHA nor NIOSH have not established a recommended limit for nitrate or nitrite in air [49]. The recommended intakes are helpful particularly because there is a wide range of lethal concentrations of nitrite and nitrate to humans (due likely to sensitivity) have been reported. For nitrate, the lethal dose is 4-50 grams and from 67-833 mg/kg body weight and for nitrite the lethal dose is 1.6-9.5 grams and from 33-250 mg/kg body weight [49].

The European Food Safety Authority (EFSA) has recommended an ADI of nitrate for a human adult of 3.7 mg/kg body weight/day (222.0 mg/day for a 60 kg person) An interesting calculation has been presented by Hord., *et al.* who posits that if nitrite were a carcinogen, the public would be advised to avoid swallowing because saliva contains 50-100 $\mu\text{mol/L}$ nitrite, which can increase to near millimolar levels after a nitrate-rich meal [50]. 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 was collected from nine farms and analyzed for nitrate content. The authors concluded that the amount of nitrates in raw vegetables did not reach the standard limit level for toxicity and, thus, did not cause health problems for consumers.

Conclusion

Dietary nitrates and nitrites have presented an ongoing conundrum due to reported benefits but also risks at amounts obtainable from the human diet cumulatively through nitrate-rich vegetables, water, food additives in processed meats, dietary supplements and nutraceuticals. The majority of dietary nitrates are consumed through vegetables and the concentrations can vary considerably due to myriad reasons, e.g., agronomy, fertilizer use, anatomical part of plant, etc. Although dietary nitrate is generally considered safe, there are endogenous mechanisms such as the enterosalivary pathway that can reduce nitrate to nitrite via bacterial reductases forming ultimately beneficial, vasodilatory nitric oxide. Alternatively, nitrite can be significantly concentrated by up to 10,000-fold and converted to carcinogenic nitrosamines at low pH as occurs in

the gastric lumen and purportedly increase the risk of gastrointestinal cancer. Other problems that have been reported include other neoplasia, thyroid enlargement, and methemoglobinemia. In contrast, numerous reports demonstrate benefits including reduction of blood pressure, cardiovascular benefits including reduction of triglycerides and mitigation of stroke and reduced atherosclerosis risk. Other benefits are improved exercise performance, tolerance and efficiency, improved function during hypoxia, better glycemic control, and function as an anti-pathogenic agent. Collectively, there are reports of both benefit and risk from dietary nitrate consumption at levels obtainable (and easily exceeded beyond the DRI) through the food supply and/or supplementation. Currently, recommendations for acceptable intakes are in place but whether these should be reevaluated and modified is unclear.

The interplay between dietary nitrate consumption and endogenous production and recycling of nitrates and nitrites can substantially increase benefits or alternatively contribute to adverse events in those susceptible. As such, future research is needed in numerous areas. For example, nitrate and nitrite levels in plants can be manipulated by agronomical practices, thus a better understanding of nitrate uptake and assimilation into plants, i.e., vegetables and the resultant anatomical distribution and concentration is needed. To that end, better elucidation is needed for the molecular mechanisms modulating nitrate uptake, distribution, and assimilation under differing environmental conditions. This may permit more efficient use of nitrate-rich fertilizers requiring much less use and ultimately reduced concentrations of nitrates in vegetables. Other areas of research center on more effective growth strategies including "light recipes" with optimal spectra, e.g., percentage red light, along with optimal blends of micronutrients, temperature, and air composition. Collectively, these may reduce the need for potentially excessive application of nitrate-rich fertilizers.

Many adverse outcomes are the result of the interplay of several factors. First, some individuals may genetically and/or physiologically be more susceptible to endogenous nitrosation and nitrosamine formation particularly with high dietary nitrate consumption. Using the approach of personalized nutrition or personalized medicine via nutrigenetics (relationship between gene, diet, and health outcome) would be helpful in determining those most likely to experience detriment from dietary nitrate consumption. Indeed,

some recent studies of cancer and some birth defects have identified subgroups of the population with greater potential for potentially harmful endogenous nitrosation. Furthering this goal would also require the development and validation of methods of assessment.

The recent realization of the importance of the microbiome to health, and the existence of many biomes, i.e., salivary microbiome, suggest an optimal population and diversity exist. As a result, new methods for identifying and quantifying the nitrate-reducing bacteria in the oral microbiome and its inherent genetic variability and effect on health or disease may permit identification of high-risk subpopulations in epidemiologic studies. With more well-controlled studies, recommendations and potential interventions could be proffered. Additional studies aimed at illuminating relationships between environmental exposures, e.g., nitrate-rich well water, and incidences of colorectal cancer, thyroid disease, and birth defects, which show the most consistent associations with water nitrate ingestion, will be particularly useful for estimating relative cancer risks. Collectively, as data are collected from the studies listed above, in part, perhaps a clarification of the ADI and the seeming paradox between actual dietary exposure (exogenous and endogenous) and the recommended upper limit for intake, which may purportedly and routinely be exceeded without apparent detriment for most individuals. This information would permit development of a more robust knowledge base for recommendations regarding the safety of dietary nitrates and nitrites.

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