Food sources of nitrates and nitrites: the physiologic context for potential health benefits

Norman G Hord, Yaoping Tang, and Nathan S Bryan

ABSTRACT
The presence of nitrates and nitrites in food is associated with an increased risk of gastrointestinal cancer and, in infants, methemoglobinemia. Despite the physiologic roles for nitrate and nitrite in vascular and immune function, consideration of food sources of nitrates and nitrites as healthful dietary components has received little attention. Approximately 80% of dietary nitrates are derived from vegetable consumption; sources of nitrites include vegetables, fruit, and processed meats. Nitrates are produced endogenously through the oxidation of nitric oxide and through a reduction of nitrate by commensal bacteria in the mouth and gastrointestinal tract. As such, the dietary provision of nitrates and nitrites from vegetables and fruit may contribute to the blood pressure–lowering effects of the Dietary Approaches to Stop Hypertension (DASH) diet. We quantified nitrate and nitrite concentrations by HPLC in a convenience sample of foods. Incorporating these values into 2 hypothetical dietary patterns that emphasize high-nitrate or low-nitrate vegetable and fruit choices based on the DASH diet, we found that nitrate concentrations in these 2 patterns vary from 174 to 1222 mg. The hypothetical high-nitrate DASH diet pattern exceeds the World Health Organization’s Acceptable Daily Intake for nitrate by 550% for a 60-kg adult. These data call into question the rationale for recommendations to limit nitrate in the DASH diet pattern to prevent hypertension (11, 12). The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in vegetables (ie, 8–10 servings) and low-fat dairy products can lower blood pressure to an extent similar to that achieved with single hypotensive medications (13, 14). The blood pressure–lowering effect of this diet was hypothesized to be attributable to the high calcium, potassium, polyphenols, and fiber contents and low sodium and animal protein contents (15). These and other findings point to a less widely acknowledged but biologically plausible hypothesis: the intake of inorganic nitrate (NO\textsubscript{3}\textsuperscript{−}) in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite (NO\textsubscript{2}\textsuperscript{−}), nitric oxide, and other metabolic products (NO\textsubscript{x}) that produce vasodilation, decrease blood pressure, and support cardiovascular function (16–18). Interestingly, both potassium nitrite, in 1880, and potassium nitrate, in 8th century China, were known to mediate hypotensive and antiangiial actions, consistently show a lack of efficacy and an increased risk of mortality (7). Clearly, more research is needed to identify the nutrients and food components of vegetables and fruit associated with a decreased risk of cardiovascular disease and cancer.

 Whereas the health benefits of vegetables and fruit may derive from the contribution of their constituents to food patterns such as the Mediterranean-type pattern (8–10), recent research has found specific foods to be associated with a decreased risk of cardiovascular disease. Recent prospective epidemiologic studies have shown that green leafy vegetables are among the foods most protective against coronary heart disease and ischemic stroke risk (11, 12). The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in vegetables (ie, 8–10 servings) and low-fat dairy products can lower blood pressure to an extent similar to that achieved with single hypotensive medications (13, 14). The blood pressure–lowering effect of this diet was hypothesized to be attributable to the high calcium, potassium, polyphenols, and fiber contents and low sodium and animal protein contents (15). These and other findings point to a less widely acknowledged but biologically plausible hypothesis: the intake of inorganic nitrate (NO\textsubscript{3}\textsuperscript{−}) in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite (NO\textsubscript{2}\textsuperscript{−}), nitric oxide, and other metabolic products (NO\textsubscript{x}) that produce vasodilation, decrease blood pressure, and support cardiovascular function (16–18). Interestingly, both potassium nitrite, in 1880, and potassium nitrate, in 8th century China, were known to mediate hypotensive and antiangiial actions, consistently show a lack of efficacy and an increased risk of mortality (7). Clearly, more research is needed to identify the nutrients and food components of vegetables and fruit associated with a decreased risk of cardiovascular disease and cancer.

 INTRODUCTION
The health effects of the dietary consumption of vegetables and fruit have been attributed to their constituents, including vitamins, minerals, fiber, and so-called nonnutritive substances such as flavonoids and glucosinolates to name a few (1–3). Dietary supplements containing food components such as β-carotene and antioxidant vitamins such as vitamin A and E have been used in secondary prevention trials for the prevention of lung cancer (4, 5). These trials found that β-carotene, alone or in combination with vitamin E or retinyl palmitate, increased the incidence of lung cancers and cardiovascular disease mortality rates (6). Indeed, meta-analyses of primary and secondary cancer prevention trials of dietary antioxidant supplements consistently show a lack of efficacy and an increased risk of mortality (7). Clearly, more research is needed to identify the nutrients and food components of vegetables and fruit associated with a decreased risk of cardiovascular disease and cancer.

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respective (19, 20). The goals of this review are to 1) provide a physiologic context for the potential health benefits of dietary nitrite and nitrate from plant foods, and 2) support a growing consensus for a comprehensive reevaluation of the health benefits and risks associated with dietary sources of nitrates and nitrites.

NITRITE AND NITRIC OXIDE PRODUCTION IN THE VASCULATURE AND IN TISSUES: 2 SYSTEMS, REDUNDANT FUNCTIONS

New discoveries in the field of nitrate and nitrite biology have provided mechanistic insights into the potential new physiologic roles of dietary nitrate and nitrite and their potential health benefits. A brief introduction to the biology of nitric oxide production in the vasculature and nitric oxide–requiring tissues will provide the appropriate context for understanding the importance of dietary nitrate and nitrite. There is a consensus that dietary nitrates are essentially inert and acquire biological activity only after reduction to nitrite. As such, nitrate serves as a source, via successive reduction, for the production of nitrite and nitric oxide as well as other metabolic products. The late Speaker of the US House of Representatives, Representative Thomas “Tip” O’Neil, famously stated “All politics is local.” There is no more apropos analogy in biology than the regulation of the availability of nitrogen oxides—by localization, oxygen tension, pH, inflammatory microenvironment, and organ and tissue specificity—that determines how much nitrate, nitrite, nitric oxide, and other NO₃ species to which tissues will be exposed.

VASCULAR NITRIC OXIDE PRODUCTION

Normal functioning of human vasculature requires both the presence of nitrite and nitric oxide along with the necessity to respond to these important signaling molecules (21, 22). The generation of up to 70% of systemic nitric oxide is accomplished by endothelial nitric oxide synthase (eNOS), one of 3 members of the NOS family of enzymes, in the vascular endothelium (23). These enzymes synthesize nitric oxide from the amino acid L-arginine and molecular oxygen to accomplish this was the action of commensal gram-negative bacteria on the tongue to reduce salivary nitrate (34). Concentrations of plasma nitrite in the saliva occur as part of enterosalivary circulation of dietary nitrate (35). Approximately 25% of ingested nitrate is secreted in saliva, where some 20% (or 5–8% of the nitrate intake) is converted to nitrite by commensal bacteria on the tongue (36). These anaerobic bacteria on the dorsal surface of the tongue use nitrate as an alternative electron acceptor to produce energy. Indeed, use of an antibacterial mouthwash after consumption of dietary nitrate (10 mg/kg in water) attenuates the expected postprandial rise in plasma nitrate (37). In the proximal small intestine, nitrate is rapidly absorbed with high bioavailability (100%) (38). The nitrite supplied to the gastrointestinal tract serves to enhance gastric mucus production (39) and can serve as a substrate for generation of nitrogen oxides for antimicrobial actions and support of gastric homeostasis (40).

NITRIC OXIDE PRODUCTION IN TISSUES

Recently, nitric oxide synthesis in healthy tissues has been shown to occur independently of the L-arginine–NOS pathway (41); dietary provision of nitrates and nitrites may account for approximately half of steady state nitric oxide concentrations. Because inorganic nitrate is considered a biologically inert compound, the reduction of nitrate to nitrite is necessary for nitrite to serve as a substrate for nitric oxide production. The Lundberg group at the Karolinska Institute has shown, for the first time, that mammalian enzymes have nitrate reductase activity—a function previously thought to be carried out only by bacterial nitrate reductases (41). As such, several different mammalian enzymes and metalloproteins have been shown to possess nitrate reductase activity, including xanthine oxidoreductase (XOR), aldehyde oxidase (AO), heme proteins, and mitochondria (41, 42). Nitric oxide synthesis in tissues, therefore, can occur through a reduction of nitrate to nitrite and nitrite can be subsequently reduced to nitric oxide. Nitrite reduction to nitric oxide can be carried out by numerous metalloproteins, enzymes, and compounds with redox potential, including hemoglobin (43), deoxyhemoglobin, deoxymyoglobin, XOR, vitamin C, and polyphenols (41). As noted above, nitrite reduction to nitric oxide is greatly enhanced during the stress of hypoxemia and ischemia (44). These redundant physiologic systems for the provision of nitric oxide under normoxic or hypoxic conditions indicate that nitrite may serve as systemic reservoir for nitric oxide production.

Emerging evidence from animal models and human clinical studies indicates that nitrite exerts unique intracellular signaling properties that mediate physiologic functions independent of its role as a source of nitric oxide in tissues by reduction (24). Nitrite
infusion in humans induces rapid local vasodilation, reduces blood pressure acutely, serves as an endocrine reservoir of nitric oxide, and, unlike organic nitrites, does not induce tolerance (45, 46). Nitrite has also been shown to play a role in mitochondrial respiration (47), cardiac function (48), activation of the 2 form of the estrogen receptor (49), and exertion of antiapoptotic effects (50). Because nitrite is a biologically active compound resulting from nitrate reduction in tissues, significant physiologic benefits may be associated with the provision of nitrite from dietary sources.

REGULATION OF THE NITRITE ECONOMY: ROLE OF DIET, TISSUE NITRATE REDUCTASES, AND DISEASE STATES

The stepwise reduction of nitrate to nitrite to nitric oxide is, by necessity, an inefficient process by which each step yields a 3-log lower concentration of product than substrate (41). Therefore, a 10 mg/kg infusion of nitrate given over 5 min yielded a plasma concentration of nitrite of ≈1 μmol/L and resulted in ostensibly nitric oxide–mediated vasodilation after experimentally induced ischemia (41). Typical plasma concentrations, half-lives, and sources of nitrate, nitrite, and nitric oxide are shown in Table 1 (45, 51). The 1- to 5-min half-life of nitrite is intermediate between that of nitrate (5–8 h) and nitric oxide (milliseconds) (44). Notably, the short half-life of nitric oxide results from efficient oxidation of nitric oxide to nitrite and other nitrogen oxides, such as N-nitroso compounds by enzymes (so-called nitric oxide oxidases) that use transition metals in their active sites, such as copper-containing ceruloplasmin (52), myeloperoxidase (which uses heme iron as a cofactor), and even endothelial NOS (53). Oxidation of nitric oxide to nitrite and nitrite to nitric oxide contributes to the pool of NOx compounds that serve as signaling molecules systemically or as a local substrate for nitric oxide production. In situations such as iNOS-mediated inflammatory processes in ulcerative colitis, the large concentrations of nitric oxide produced can lead to high concentrations of more stable nitric oxide oxidation products such as nitrite and nitrate. The elegant physiologically redundant mechanisms by which nitrite and nitrate are produced and reformed by oxidation of nitric oxide to ensure an abundant supply for the myriad processes that require them for adequate functioning are illustrated in Figure 1. Dietary sources of nitrate and nitrite may bolster the reserve of these compounds for optimal functioning through periods of physiologic stress and diseases characterized by endothelial dysfunction (31, 32).

**TABLE 1**

Plasma concentrations, half-lives, and sources of NOx species (nitrate, nitrite, and nitric oxide)

<table>
<thead>
<tr>
<th>NOx species</th>
<th>Fasting plasma</th>
<th>Half-life</th>
<th>Exogenous or endogenous source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>20–50,000 nmol/L</td>
<td>5–8 h</td>
<td>Diet or endogenous oxidation of nitrate</td>
</tr>
<tr>
<td>Nitrite</td>
<td>100–500 nmol/L</td>
<td>1–5 min</td>
<td>Endogenous nitrate, diet, oxidation of nitrite</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>&lt;1 nmol/L</td>
<td>1–2 ms</td>
<td>Endogenous nitrite</td>
</tr>
</tbody>
</table>

* Data from references 44 and 51.

**FIGURE 1.** A schematic diagram of the physiologic disposition of nitrate, nitrite, and nitric oxide from exogenous (dietary) and endogenous sources. The action of bacterial nitrate reductases on the tongue and mammalian enzymes that have nitrate reductase activity in tissues are noted by the number 1. Bacterial nitrate reductases are noted by the number 2. Mammalian enzymes with nitrite reductase activity are noted by the number 3.

**SOURCES OF ENDOGENOUS AND DIET- DERIVED NITRIC OXIDE GENERATION**

In addition to the provision of nitrate and nitrite by diet or via the oxidation of nitric oxide to nitrite, vascular and gastrointestinal nitric oxide production can be enhanced through various means based on lifestyle and food choices. Physical activity, commensal bacteria, and dietary factors can influence nitric oxide production. Exercise enhances nitric oxide production in vascular endothelium (54) and postexercise plasma nitrite concentrations have been proposed as an index of exercise capacity (55). In fact, aging is associated with an impaired capacity of the vasculature to increase plasma nitrite during exercise (56). Strikingly, it has been found that dietary nitrate supplementation, at concentrations achievable by vegetable consumption, results in more efficient energy production without increasing lactate concentrations during submaximal exercise (57).

Foods can increase the generation of nitric oxide in the gastrointestinal tract via the polyphenolic content of, for example, apples or red wine (58, 59). Pomegranate juice has been shown to protect nitric oxide from oxidation while enhancing its biological activity (60). The metabolic activity of commensal bacteria in the gastrointestinal tract and probiotic bacteria also provide nitric oxide from nitrite, and to a lesser extent, from nitrate (61, 62). Whereas data estimating the contribution of the microbiota, including probiotic bacteria, to the generation of nitric oxide are speculative, they raise the possibility that the gastrointestinal production of nitric oxide and NOx is biologically plausible. These data add layers of complexity to the estimation of nitrate/nitrite exposure levels in vivo and the determination of whether specific foods or lifestyle choices can significantly affect the production and metabolic disposition of dietary and endogenous NOx species.

**QUANTIFYING THE NITRITE ECONOMY**

Given the complex interactions between nitrite and nitrate of dietary origin, the endogenous production of nitrate and nitrite from nitric oxide and other nitrogen oxides (NOx) (Figure 1), the effect of physiologic conditions such as atherosclerosis and inflammatory disease, dietary sources of NOx, and physical
activity, nitrate, and nitrite balance studies are not, at present, feasible. Therefore, a simple characterization of an optimal concentration of dietary nitrate and nitrite based on an overall picture of the nitrate and nitrite economy is not possible. However, we can make 2 generalizations that summarize our current knowledge. First, most nitrate utilization and nitric oxide production occur in healthy individuals in 2 compartments: vascular and somatic tissues. Normal vascular function requires nitric oxide production from the L-arginine-NOS pathway; in ischemic conditions, nitrite can substitute for L-arginine-NOS-derived nitric oxide (31, 32). Most healthy somatic tissues possess mammalian enzymes that exert nitrate reductase activity (the tongue utilizes nitrate reductases of commensal bacteria) to generate biologically active nitrite to maintain gastrointestinal and cardiovascular health. In inflammatory conditions, iNOS in epithelial and immune cells can produce nitric oxide as part of the innate immune response. The second generalization is that in cardiovascular disease states characterized by hypoxia and/or ischemia/reperfusion injury, eNOS-supplied nitric oxide may be limiting and nitrite may be used to support vascular function under these conditions. However, concern has been expressed that nitrite may be reduced to nitric oxide under normoxic conditions (63) and that, under these conditions, nitrate and nitrite may inhibit steroidogenesis in vitro and in vivo (64).

The data supporting the in vivo conversion of nitrates and nitrites to nitric oxide has implications for dietary consumption of foods high in nitrate and nitrite. As such, nitrate- and nitrite-containing foods may supply nitrite in situations in which substrates for endogenous NOx production are limiting, as in cardiovascular conditions, to support cardiovascular and gastrointestinal function. As such, when the dietary intake of nitrate and nitrite is low and there is no additional endogenous sources of NOx (eg, gastrointestinal infections involving iNOS activation), the endogenous production of nitrate, via oxidation of nitric oxide and nitrite, provides more substrate for nitric oxide production than dietary sources. Long-term consumption of diets containing high levels of nitrate and nitrite may have important implications for providing health benefits by ensuring high concentrations of nitrogen oxides as a “reserve” for tissue defense and homeostasis in stress and disease.

DIETARY SOURCES OF NITRATE AND NITRITE

Dietary nitrate intake is determined by the type of vegetable consumed, the levels of nitrate in the vegetables (including the nitrate content of fertilizer), the amount of vegetables consumed, and the level of nitrate in the water supply (65). As such, the nitrate content of organic vegetables may be less than that of vegetables grown in the presence of nitrogen-containing fertilizers. The primary determinants of nitrate consumption are the levels of nitrites in cured, processed meats and the consumption level of these products. A recent survey of vegetable nitrate concentrations in the European Union states and Norway based on ~42,000 submitted analytic results showed a variation ranging from a low of 0.1 mg/100 g (peas and Brussels sprouts) to a high of 480 mg/100 g (rucola or rocket) (66). The nitrate and nitrite contents of edible vegetable components are listed in Table 2 (from reference 67). A list of vegetable varieties grouped in ascending order of nitrate content are shown in Table 3 (from reference 68). In terms of plant anatomy, the nitrate content of vegetable organs can be listed in descending order (most to least) as petiole > leaf > stem > root > fluorescence > tuber > bulb > fruit > seed (69). The accumulation of nitrate is subject to factors such as genotype, soil conditions, growth conditions (ie, nitrate uptake, nitrate reductase activity, and growth rate), and storage and transport conditions (65, 70). 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 (FW) (70). These data dictate that caution be observed in linking the biological effects of leafy vegetables (and other nitrate-containing vegetables and fruit) to specific health effects, particularly in observational epidemiologic studies.

A CASE STUDY IN NITRATE AND NITRITE INTAKE ESTIMATES BASED ON A CONVENIENCE SAMPLE

Due to the variability in nitrate and nitrite concentrations of foods reported in Tables 2 and 3, we conducted nitrate and nitrite intake estimates based on a convenience sample (42,000 submitted analytic results showed a variation ranging from a low of 0.1 mg/100 g to a high of 480 mg/100 g for nitrate and nitrite). The mean intake estimates for nitrate and nitrite in the United States and Europe vary by investigator but are consistent and comparable. International estimates of nitrate intakes from food are 31–185 mg/d in Europe and ~40–100 mg/d in the United States (71, 72). The bioavailability of dietary nitrate is 100% (38). Nitrite intakes vary from 0 to 20 mg/d (65). Nitrate intakes from sources other than vegetables, including drinking water and cured meats, has been estimated to average 35–44 mg/person per day for a 60-kg human (66). On the basis of a conservative recommendation to consume 400 g of different fruits and vegetables per day at median nitrate concentrations, the dietary concentration of nitrate would be ~157 mg/d (66). In the European Union, where fruit consumption (average nitrate concentration: ~10 mg/kg FW) constitutes more than half of the recommended intake of 400 g, actual nitrate intakes would be ~81–106 mg/d before additional nitrate losses from washing, peeling, and/or cooking are taken into consideration.

<table>
<thead>
<tr>
<th>Vegetable types and varieties</th>
<th>Nitrate mg/100 g fresh weight</th>
<th>Nitrite mg/100 g fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>0.002–0.023</td>
<td>92–195</td>
</tr>
<tr>
<td>Mustard leaf</td>
<td>0.012–0.064</td>
<td>70–95</td>
</tr>
<tr>
<td>Green vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>0.008–0.215</td>
<td>12.3–267.8</td>
</tr>
<tr>
<td>Spinach</td>
<td>0–0.073</td>
<td>23.9–387.2</td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>0–0.065</td>
<td>42.9–161.0</td>
</tr>
<tr>
<td>Bok choy</td>
<td>0.009–0.242</td>
<td>102.3–309.8</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0–0.041</td>
<td>25.9–125.0</td>
</tr>
<tr>
<td>Cole</td>
<td>0.364–0.535</td>
<td>76.6–136.5</td>
</tr>
<tr>
<td>Melon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wax gourd</td>
<td>0.001–0.006</td>
<td>35.8–68.0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0–0.011</td>
<td>1.2–14.3</td>
</tr>
<tr>
<td>Nightshade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggplant</td>
<td>0.007–0.049</td>
<td>25.0–42.4</td>
</tr>
</tbody>
</table>

† Data from reference 67.

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analyses on a convenience sample of vegetables, a commercial vegetable juice beverage (V8; Campbell Soup Co, Camden, NJ), fruit, fruit juices, as well as fresh and processed meats (Tables 4 and 5). Vegetables with the highest nitrate concentrations in our sample included spinach (740 mg/100 g FW), collard greens (320 mg/100 g FW), mustard greens (120 mg/100 g), broccoli (40 mg/100 g FW), and tomato (39 mg/100 g FW). Banana, apple sauce, and oranges had nitrate and nitrite concentrations (mg/100 g FW) of 5 and 0.009, 0.3 and 0.008, and 0.8 and 0.015, respectively. Vegetable and fruit juices had nitrate and nitrite concentrations (mg/L FW) of 27.6 and 0.04 (carrot juice), 26.1 and 0.09 (V8 juice), 12.9 and 0.07 (pomegranate juice), 9.1 and 0.14 (cranberry juice), and 0.6 and 0.01 (acai juice). Note that a desiccated vegetable dietary supplement (Nature’s Way Garden Veggies; Nature’s Way Products Inc, Springville, UT) had the highest nitrate and nitrite concentrations of any food tested, ie, 27,890 and 10.5 mg/100 g FW, respectively. Each capsule of this supplement contains 900 mg desiccated vegetable product, and label recommendations suggest a daily intake of 2 capsules daily, which equates to >500 mg nitrate and 0.2 mg nitrite per day. Hot dogs, ham, pork tenderloin, bacon, and nitrate- or nitrite-free bacon had nitrate and nitrite concentrations (mg/100 g FW) of 9 and 0.05, 0.9 and 0.89, 3 and 0.6, and 0.38, and 3 and 0.68, respectively.

MODELING NITRATE AND NITRITE INTAKES BASED ON THE VEGETABLE AND FRUIT CONTENT IN THE DASH DIET PATTERN

Our data, considered together with data in Tables 2 and 3, make it plain that, because of the wide variation in nitrate and nitrite contents of vegetables, fruit, and their juices, practicing the oft-quoted dietary recommendation “Eat your fruits and vegetables” may not translate into high nitrate and nitrite concentrations in the diet. We set out to model this variation by using the vegetable and fruit components of the DASH dietary pattern (73) that involved choosing particular foods with a high or low nitrate content. Two hypothetical vegetable and fruit consumption patterns based on the DASH diet (1 cup raw leafy vegetables, 1/2 cup cut-up raw or cooked vegetables, 1/2 cup vegetable juice, 1 medium fruit, 1/4 cup dried fruit, 1/2 cup fruit juice, or 1/2 cup fresh, frozen, or canned fruit), which contains foods that are low or high in nitrate, are shown in Table 6. The high-nitrate DASH diet would result in the consumption of 1222 mg nitrate and 0.351 mg nitrite compared with the low-nitrate DASH diet that yields 174 mg nitrate and 0.41 mg nitrate. These analyses make evident that consuming a dietary pattern such as the DASH diet can yield differences in nitrate intake that vary by ~700%.

POTENTIAL HEALTH RISKS OF EXCESSIVE NITRATE AND NITRITE EXPOSURE

Analogous to all essential or indispensable nutrients, intake of excess nitrate and nitrite exposure is, in specific contexts, associated with an increased risk of negative health outcomes. A set of Dietary Reference Intake (DRI) categories are set by the Food and Nutrition Board of the National Academy of Sciences for essential nutrients to clearly define, where possible, the contexts in which intakes are deficient, safe, or potentially excessive. These DRI categories include the Recommended Dietary Allowance (RDA), Adequate Intake (AI), Tolerable Upper Level Intake (TUL), and Estimated Average Intake (EAI) (74). The
TABLE 6
Hypothetical dietary nitrate and nitrite intakes based on food and juice serving recommendations for vegetables and fruit based on the Dietary Approaches to Stop Hypertension (DASH) dietary pattern

<table>
<thead>
<tr>
<th>Food pattern and serving size</th>
<th>Nitrate content</th>
<th>Nitrite content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/serving</td>
<td>mg/serving</td>
</tr>
<tr>
<td>DASH food pattern with high-nitrate or high-nitrate food choices (4–5 servings each of vegetables and fruit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cup raw spinach</td>
<td>926</td>
<td>0.027</td>
</tr>
<tr>
<td>1/2 cup cooked collard greens</td>
<td>198</td>
<td>0.06</td>
</tr>
<tr>
<td>1/2 cup vegetable juice</td>
<td>42.5</td>
<td>0.02</td>
</tr>
<tr>
<td>1 medium banana</td>
<td>6.75</td>
<td>0.014</td>
</tr>
<tr>
<td>1/4 cup raisins</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1 medium orange</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>1/2 cup pomegranate juice</td>
<td>47</td>
<td>0.21</td>
</tr>
<tr>
<td>Total</td>
<td>1222</td>
<td>0.351</td>
</tr>
<tr>
<td>DASH food pattern with low-nitrate or low-nitrate food choices (4–5 servings each of vegetables and fruit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cup raw leaf lettuce</td>
<td>103</td>
<td>0.17</td>
</tr>
<tr>
<td>1/2 cup broccoli</td>
<td>25</td>
<td>0.09</td>
</tr>
<tr>
<td>1/2 cup vegetable juice</td>
<td>42.5</td>
<td>0.02</td>
</tr>
<tr>
<td>1 medium apple</td>
<td>0.40</td>
<td>0.01</td>
</tr>
<tr>
<td>1/4 cup raisins</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1/2 cup canned fruit cocktail</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>1/2 cup orange juice</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Notes:
1. Analysis of foods reflects the mean value from triplicate analyses. Nitrate and nitrite concentrations were quantified by ion chromatography (ENO20 Analyzer; Eicom, Kyoto, Japan).

The process of setting DRIs for nutrients considers a broad range of physiologic factors, including nutritional status and potential toxicities. Rational methodologies such as these, including the consideration of normal dietary consumption patterns of nitrate- and nitrite-containing foods, have not been applied in setting exposure limits or in considering the potential health benefits of dietary nitrates and nitrites.

Whereas accidental toxic exposures of nitrates and nitrites have occurred (75), the health risks due to excessive nitrate and nitrite consumption appear only in specific subgroups of the population. The permissible concentration of nitrate in drinking water is 50 mg nitrate/L in the European Union and 44 mg/L in the United States in accordance with World Health Organization recommendations first established in 1970 and reaffirmed in 2004 (76). The US Environmental Protection Agency limits human exposure to inorganic nitrates to >10 mg/L (or 10 ppm nitrate nitrogen) and nitrates to 1 ppm nitrite nitrogen (77). The Joint Food and Agricultural Organization/World Health Organization has set the Acceptable Daily Intake (ADI) for the nitrate ion at 3.7 mg/kg body wt and for the nitrite ion at 0.06 mg/kg body wt (66). Likewise, Environmental Protection Agency has set a Reference Dose for nitrate of 1.6 mg nitrate nitrogen · kg body wt⁻¹ · d⁻¹ (equivalent to ≈7.0 mg nitrate ion/kg body wt per day).

POSSIBLE CONTEXTS FOR NITRATE- AND NITRITE-ASSOCIATED TOXICITIES

Two types of exposure place susceptible individuals at high risk to the adverse effects of excess nitrite exposure. First, infants younger than 6 mo of age may be exposed to excess nitrates in bacterially contaminated well water, which reduces nitrate to nitrite (78). Infants consuming excess nitrite experience methemoglobinemia or “blue baby syndrome” because of the nitrite-mediated oxidation of ferric (Fe³⁺) iron in oxyhemoglobin that leads to hypoxia and cyanosis (16, 79). As such, an American Academy of Pediatrics consensus panel concluded that all prenatal and well-infant visits should include questions about the home water supply; if the water source is a private well, the water should be tested for nitrates (80). The panel concluded that infants fed commercially prepared infant foods are generally not at risk of nitrate poisoning, but that home-prepared infant foods from vegetables (eg, spinach, beets, green beans, squash, and carrots) should be avoided until infants are 3 mo of age or older. Breastfed infants are not at risk of excessive nitrate exposure from mothers who ingest water with a high nitrate content (up to 100 ppm nitrite nitrogen) because the nitrate concentration does not increase significantly in breast milk (80).

Note that the few human nitrate and nitrite exposure studies, including children and adults, have not produced methemoglobinemia. Infants exposed to 175–700 mg nitrate/d did not have methemoglobin concentrations >7.5%, which suggests that nitrate alone does not cause methemoglobinemia (81). A more recent randomized 3-way crossover study exposed healthy adults to single doses of sodium nitrite that ranged from 150 to 190 mg per volunteer to 290–380 mg per volunteer (82). The observed methemoglobin concentrations were 12.2% for volunteers receiving the higher dose of nitrite ion and 4.5% for those receiving the lower dose. Recent nitrite infusion studies of up to 110 µg · kg⁻¹ · min⁻¹ for 5 min induced methemoglobin concentrations of only 3.2% (45). These data have led scientists to propose alternative explanations for the observed methemoglobinemia in infants, including gastroenteritis and associated iNOS-mediated production of nitric oxide induced by bacteria-contaminated water (83, 84). These studies call into question the mechanistic basis for exposure regulations for nitrate and nitrite. At best, these findings highlight a serious, but context-specific, risk associated with nitrite overexposure in infants.

Experts have questioned the veracity of the evidence supporting the hypothesis that nitrates and nitrites are toxic for healthy adolescent and adult populations (16, 17, 66). It appears that the biologically plausible hypothesis of nitrite toxicity (eg, methemoglobinemia) has essentially transformed a plausible hypothesis into saccrosanct dogma (16), despite the lack of proof (83, 84).

The second context in which nitrate and nitrite exposure has been associated with negative health effects is through the consumption of cured and processed meats (85). Nitrates added to meats serve as antioxidants, develop flavor, and stabilize the red color in meats but must be converted to nitrite to exert these actions. Sodium nitrate is used as a colorant, flavor enhancer, and antimicrobial agent in cured and processed meats. Nitrate and nitrite use in meat products, including bacon, bologna, corned beef, hot dogs, luncheon meats, sausages, and canned and cured meat and hams is subject to limits put forth in Food and Drug Administration (FDA) and US Department of Agriculture (USDA) regulations. These regulations can be found in the Code of Federal Regulations (CFR) (21 CFR 170.60, 172.170, and 172.175 for FDA and 9 CFR 318.7 for USDA regulations, respectively).
Consumption of red and processed meats is associated with an increased risk of certain types of cancer and chronic obstructive pulmonary disease (85–89). On the basis of the association with cancer risk, the American Institute for Cancer Research’s Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective contains the following recommendation “Limit consumption of red meats (such as beef, pork and lamb) and avoid processed meats” (90). A systematic review indicated that up to \( \sim 500 \) g (\( \sim 18 \) oz) weekly of red meat can be consumed without cancer risk. However, review panels could not determine a safe consumption level for processed meat; cancer risk was shown to increase with any consumption of processed meats based on a meta-analysis of cohort studies showing an increased risk of colorectal cancer with increased intakes of processed meats (summary estimate of relative risk per 50 g/d: 1.21; 95% CI: 1.04, 1.42) (92). It is worth noting that nitrite or nitrate is not added to fresh meats. A discussion of this association in the context of nitrate and nitrite consumption and gastric physiology is warranted to illuminate the processes relevant to this association.

Direct evidence of the participation of nitrate and nitrite in human carcinogenesis is lacking, despite extensive epidemiologic and animal studies (84). Rodent toxicologic studies (91) and human epidemiologic investigations have not shown an unequivocal relation between nitrite consumption and gastrointestinal cancers was bolstered by findings that ingested nitrate and nitrite are not equal with regard to potential health benefits or risks. The association between nitrite consumption and gastric physiology is warranted to illuminate the processes relevant to this association.

The recent demonstration of the vasoprotective, blood pressure-lowering, and antiplatelet aggregation properties of nitrite alone, or of nitrite originating from dietary nitrate, suggests that a reexamination of the health effects of dietary sources of nitrate and nitrite would be beneficial (31, 32, 46). An illustrative example of human exposure to nitrate, nitrite, and nitric oxide will serve to support the apparent safety of these exposure levels. Based on an estimated daily intake of 0.77 mg nitrite, nitric oxide production would equate to 11.1 \( \mu \text{mol}/\text{d} \), and an intake of 76 mg nitrate would equate to 894 \( \mu \text{mol}/\text{d} \) or roughly 1 mmol NO\( _{x}/\text{d} \) from diet. A 70-kg individual produces 1.68 mmol nitric oxide/d (based on 1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) nitric oxide production) through the endogenous \( \text{L-arginine} \) pathway. Notably, the amount of nitrite and nitrate consumed as dietary nitrate and nitrite results in nitric oxide production approximately equal to endogenous sources if, as discussed above, we assume most of the endogenous nitric oxide goes to stepwise oxidation to nitrite and nitrate. Therefore, up to 50% of human steady state concentrations of nitrite and nitrate, which are routinely used as clinical biomarkers of nitric oxide activity, are derived from foods consumed by the average person in the US. A recent study has yielded new insights into the ability of vitamin C to modulate the formation of carcinogenic NOCs under conditions simulating the proximal stomach during the digestion of foods such as processed meats (96). Nitrite in processed meats may be converted to nitrosating species and NOCs by acidification in the presence of thiocyanate at low gastric pH. The formation of NOCs was examined under these conditions in the presence and absence of vitamin C and lipid. In the absence of lipid, vitamin C prevented the formation of \( N\)-nitrosodiethylamine and \( N\)-nitroso-piperidine and decreased the formation of \( N\)-nitrosodimethylamine and \( N\)-nitrosomorpholine 5-fold and 1000-fold, respectively. In the presence of 10% lipid (a food matrix component for processed meats), the presence of vitamin C increased the formation of nitrosodiethylamine, nitrosodiethylamine, and \( N\)-nitroso-piperidine 8-, 60-, and 140-fold, respectively. Thus, the presence of lipid converts vitamin C from inhibiting to promoting acid nitrosation. This effect is attributable to the ability of vitamin C to assist in the generation of nitric oxide in the aqueous phase, which enables the regeneration of nitrosating species by reacting with oxygen in the lipid phase (96). Whereas these data require confirmation in animal models and in humans, it provides a biologically plausible mechanism for the observed association between processed meat consumption and gastrointestinal cancer risk. Others have postulated that gastric formation of NOCs may be inhibited by nutrients and other components of vegetables and fruit (97). Clearly, more research is needed to address the potential mechanisms by which certain NOCs are related to cancer risk.
fact, as has been observed previously and confirmed here, a portion of spinach commonly consumed in one serving of salad can exceed the ADI for nitrate (51). The fact that typical consumption patterns of vegetables and fruit exceed regulatory limits for dietary nitrites calls into question the rationale behind current nitrate and nitrite regulations. The physiologic basis for regulating human consumption of plant foods containing nitrates and nitrites should be reevaluated to include potential health benefits.

CONCLUSIONS

The DASH diet forms the basis for public dietary health recommendations in the United States (eg, MyPyramid.gov) and is widely recommended by private health agencies, such as the American Heart Association (101). Taken together, the data considered here support the conclusions of the European Food Safety Authority (66) that benefits of vegetable and fruit consumption outweigh any perceived risk of developing cancer from the consumption of nitrate and nitrite in these foods. Note that the nitrate and nitrite concentrations measured in our convenience sample may differ from samples taken from more disparate geographic locations. We conclude that the data on nitrate and nitrite contents of vegetables and fruit bolster the strength of existing evidence to recommend their consumption for health benefits.

Despite the demonstration of physiologic roles for nitrate and nitrite in vascular and immune function, food sources of nitrates and nitrites as healthful dietary components have received little attention (18). The questionable practice of causal inference with regard to the etiologic roles of dietary nitrates and nitrites in methemoglobinemia and cancer has exerted a detrimental effect on research supporting the health benefits of nitrate- and nitrite-containing foods. This has occurred despite the observed benefits of nitrate and nitrite in medical therapeutics (102). Indeed, data from observational epidemiologic and human clinical studies support the hypothesis that nitrates and nitrites of plant origin play essential physiologic roles in supporting cardiovascular health and gastrointestinal immune function. We support the recent call for a multidisciplinary and systematic review of the biological consequences of dietary nitrate and nitrite consumption (84). The strength of the evidence linking the consumption of nitrate- and nitrite-containing plant foods to beneficial health effects supports the consideration of these compounds as nutrients.

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