See corresponding editorial on page 11.

Food sources of nitrates and nitrites: the physiologic context for potential health benefits[1–3]

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. Nitrites 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 and nitrite consumption from plant foods; a comprehensive reevaluation of the health effects of food sources of nitrates and nitrites is appropriate. 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. *Am J Clin Nutr* 2009;90:1–10.

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.

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 content of inorganic nitrate (NO_3^-) in certain vegetables and fruit can provide a physiologic substrate for reduction to nitrite (NO_2^-), nitric oxide, and other metabolic products (NO_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 antianginal actions,

[1] From the Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI (NGH); the Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center, Houston, TX (YT and NSB); and The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX (NSB).

[2] Supported by the Michigan Agricultural Experiment Station, Michigan State University, and the American Heart Association National, 0735042N (to NSB).

[3] Address correspondence to NS Bryan, Center for Cell Signaling, Institute of Molecular Medicine, The University of Texas–Houston Health Science Center, 1825 Pressler Street, 530B, Houston, TX 77030. E-mail: nathan.bryan@uth.tmc.edu.

Received October 19, 2008. Accepted for publication March 9, 2009.

First published online May 13, 2009; doi: 10.3945/ajcn.2008.27131.

respectively (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 NO_x 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 vasodilation, blood pressure regulation, inhibition of endothelial inflammatory cell recruitment, and platelet aggregation (21). As a result, the normal production of nitric oxide and nitrite and the ability of the endothelium to respond to these species may prevent various types of cardiovascular disease, including hypertension, atherosclerosis, and stroke (24).

The biological effects of nitric oxide are caused by the initiation of cyclic GMP (cGMP)–mediated intracellular signals in the vascular wall. Two other members of the NOS family have neuronal functions (nNOS) and inflammatory immune functions (inducible NOS or iNOS) (25). In neuronal tissue, nNOS provides nitric oxide for normal neuron function. The function of iNOS is an essential signaling mechanism in the innate immune response (26). In tissues experiencing chronic inflammation, such as inflamed bowel tissue in ulcerative colitis, iNOS can generate high concentrations of nitric oxide that promote carcinogenesis by inhibiting apoptosis, enhance prostaglandin formation, and promote angiogenesis in the early stage of carcinogenesis (27–29). In atherosclerosis, hypoxic conditions combined with an oxidative environment can limit eNOS-derived nitric oxide production; nitrite can directly induce vasodilation in hypoxic endothelium (30). Indeed, the Bryan laboratory has shown that nitrite can restore vascular tone after ischemia/reperfusion and substitutes for loss of eNOS-derived nitric oxide in eNOS-deficient mice (31, 32).

Unlike the provision of eNOS-derived nitric oxide to the endothelium to maintain vasomotor tone, nitric oxide production from nitrite occurs primarily in tissues (33). There are 2 systems of reducing nitrate to nitrite in mammals. The first system identified to accomplish this was the action of commensal gram-negative bacteria on the tongue to reduce salivary nitrate (34). Concentrations of plasma nitrate 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 nitrite (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 mucin 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 nitrates, does not induce tolerance (45, 46). Nitrite has also been shown to play a role in mitochondrial respiration (47), cardiac function (48), activation the α 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 nitrate contributes to the pool of NO_x 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 NO_x species (nitrate, nitrite, and nitric oxide)[1]

NO_x species	Fasting plasma	Half-life	Exogenous or endogenous source
	nmol/L		
Nitrate	20–50,000	5–8 h	Diet or endogenous oxidation of nitrite
Nitrite	100–500	1–5 min	Endogenous nitrate, diet, oxidation of nitrite
Nitric oxide	<1	1–2 ms	Endogenous nitrite

[1] Data from references 44 and 51.

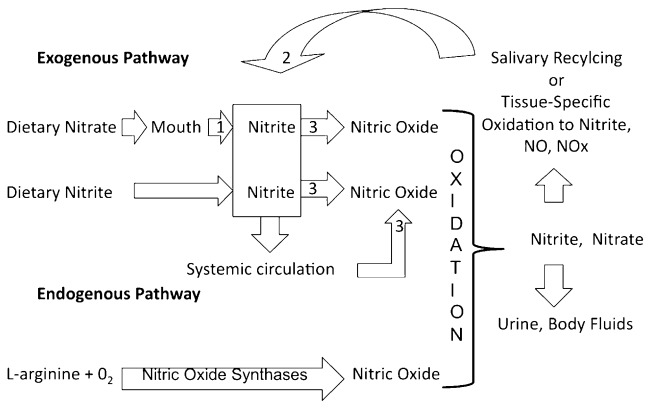

FIGURE 1. A schematic diagram of the physiologic disposition of nitrate, nitrite, and nitric oxide from exogenous (dietary) and endogenous sources. The action of bacterial nitrate reductases on the tongue and mammalian enzymes that have nitrate reductase activity in tissues are noted by the number 1. 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 NO_x 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 NO_x 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 (NO_x) (Figure 1), the effect of physiologic conditions such as atherosclerosis and inflammatory disease, dietary sources of NO_x, 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 nitrite 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 NO_x 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 NO_x (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 nitrite 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 > influorescence > 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 Dehli, 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.

DIETARY NITRATE AND NITRITE INTAKE ESTIMATES

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.

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

TABLE 2
Nitrate and nitrite contents of edible components of vegetables[1]

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

[1] Data from reference 67.

TABLE 3
Classification of vegetables according to nitrate content[1]

Nitrate content (mg/100 g fresh weight)	Vegetable varieties
Very low, <20	Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low, 20 to <50	Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Middle, 50 to <100	Cabbage, dill, turnip, savoy cabbage
High, 100 to <250	Celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley
Very high, >250	Celery, cress, chervil, lettuce, red beetroot, spinach, rocket (rucola)

[1] Data from reference 68.

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"

TABLE 4
Mean nitrate and nitrite contents of a convenience sample of juices

Juices	Nitrate	Nitrite
	mg/L; ppm	*mg/L; ppm*
Acai	0.56	0.013
Carrot	27.55	0.036
Cranberry	9.12	0.145
Green tea	0.23	0.007
Pomegranate	12.93	0.069
Vegetable juice[1]	26.17	0.092

[1] V8; Campbell Soup Co (Camden, NJ).

TABLE 5
Mean nitrate and nitrite contents of a convenience sample of fruit, vegetables, meats, and processed meats[1]

	Nitrates	Nitrites
	mg/100 g	*mg/100 g*
Fruit		
Apple sauce	0.3	0.008
Banana	4.5	0.009
Fruit mix	0.9	0.08
Orange	0.8	0.02
Vegetables		
Broccoli	39.5	0.07
Carrots	0.1	0.006
Cole slaw	55.9	0.07
French fries	2.0	0.17
Ketchup	0.10	0.13
Mustard greens	116.0	0.003
Salad mix	82.1	0.13
Spinach	741	0.02
Tomato	39.2	0.03
Vegetable soup	20.9	0.001
Desiccated vegetable dietary supplement[2]	27,890	10.5
Meats/processed meats		
Bacon	5.5	0.38
Bacon, nitrite-free	3.0	0.68
Ham	0.90	0.89
Hot dog	9.0	0.05
Pork tenderloin	3.3	0

[1] Nitrate and nitrite concentrations were quantified by ion chromatography (ENO 20 Analyzer; Eicom, Kyoto, Japan). Analysis of foods reflects the mean value from triplicate or quadruplicate analyses.

[2] Nature's Way Garden Veggies (1 capsule; 900 mg desiccated vegetables; Nature's Way Products Inc, Springville, UT).

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 nitrite. 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[1]

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

[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).

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 nitrites 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 $wt^{-1} \cdot d^{-1}$ (equivalent to ≈7.0 mg nitrate ion/kg body wt per day).

POTENTIAL 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 (Fe^{2+}) 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 nitrate 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$ 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 sacrosanct 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 nitrite 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) (21CFR 170.60, 172.170, and 172.175 for FDA and 9CFR 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 ≈500 g (≈18 oz) weekly of red meat can be consumed without cancer risk. However, review panelists 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 toxicological studies (91) and human epidemiologic investigations have not shown an unequivocal relation between nitrite exposure and the risk of cancer (71). It is reasonable to conclude that all food sources of nitrate and nitrite are not equal with regard to potential health benefits or risks. The association between nitrite consumption and gastrointestinal cancers was bolstered by findings that ingested nitrites may react with secondary amines or *N*-alkylamides to generate carcinogenic *N*-nitroso compounds (NOCs) (71). Although NOCs have been shown in animal models to be carcinogenic (92), proof in humans has been scant. The *N*-nitrosamides and *N*-nitrosoureas have been shown to be direct mutagens, whereas *N*-nitrosoamines do not act as direct mutagens but generally require activation by microsomal enzymes within the body, perhaps by cytochrome P450 enzymes (93). The use of nitrites in bacon must be accompanied by the use of either sodium erythorbate or sodium ascorbate (vitamin C), antioxidants that inhibit the nitrosation effect of nitrites on secondary amines (94). The use of these antioxidants, along with lower nitrate and nitrite levels in processed meats, has lowered residual nitrite levels in cured meat products in the US by ≈80% since the mid-1970s (95).

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*-nitrosopiperidine 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 nitrosodimethylamine, nitrosodiethylamine, and *N*-nitrosopiperidine 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.

ESTIMATING HUMAN NITRATE AND NITRITE EXPOSURE LEVELS

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 μmol/d, and an intake of 76 mg nitrate would equate to 894 μmol/d or roughly 1 mmol NO_x/d from diet. A 70-kg individual produces 1.68 mmol nitric oxide/d (based on 1 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ nitric oxide production) through the endogenous 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 from dietary sources. Assuming 50 μmol/L nitrite in saliva and a daily production of up to 1.5 L saliva/d, the total nitrite exposure from saliva alone is 75 μmol, or 5.18 mg. The enterosalivary concentration and circulation of nitrate and ultimately nitrite provides an essential pathway for health and host defense (98). If nitrite were, indeed, a carcinogen, we would be advised to avoid swallowing because saliva contains 50–100 μmol/L nitrite, which can increase to near millimolar levels (99) after a nitrate-rich meal. Even more convincing, studies of natives in the high altitude of Tibet have shown that increasing nitrite and nitrate concentrations within the body is a natural physiologic response that is not associated with harmful physiologic effects (100). These data show that normal physiologic exposure levels of nitrite and nitrate greatly exceed concentrations considered to produce health risks. These observations render as questionable the rationale supporting these regulatory limits.

DIETARY CONSUMPTION OF NITRATES AND NITRITES RELATIVE TO WHO ACCEPTABLE DAILY INTAKES

The WHO ADI for nitrate (0–3.7 mg/kg) translates into an equivalent of 222 mg nitrate for a 60-kg adult. Our calculations above indicate that an individual following a DASH dietary pattern with high-nitrate vegetable and fruit choices represented in our convenience sample would exceed this ADI by ≈550%. In

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 nitrates 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.

The authors' responsibilities were as follows—NGH: primary author; NSB: senior author who designed the experimental analysis; and YT: carried out the nitrate and nitrite analyses. No conflicts of interest were reported.

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