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Review Article

Dietary, Lifestyle, and Medicinal Factors that Influence Nitric Oxide Production-A Review

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Abstract

Nitric oxide (NO) functions as a vasodilator, playing key roles in cardiovascular, gastrointestinal, and endothelial systems. Elevated NO has multiple health and exercise benefits that include decreased blood pressure, reduced oxygen cost of exercise, increased fatigue resistance, enhanced gastric mucosal defenses, and improved glucose tolerance via diminishing insulin resistance and dyslipidemia observed in obesity and diabetes. It is of high interest, therefore, to better understand how consumption of specific foods and lifestyle factors may influence NO production. It is rare to observe high blood pressure in children because of the robust activity of the L-arginine to NO pathway during growth and development. However, as the L-arginine pathway is diminished in adulthood, elevated blood pressure becomes increasingly prevalent. Elevated high blood pressure can be mediated, however, via the enterosalivary nitrate-to-NO pathway that is initiated in the oral cavity. There are multiple dietary and lifestyle factors that can interact with the enterosalivary pathway, resulting in enhanced or diminished NO production. For instance, regular consumption of inorganic nitrate from dietary sources or nitrate salts enhances NO production, while chronic consumption of organic nitrate, as obtained from drugs such as nitroglycerin, is likely to diminish NO production. Additionally, nitrate-reducing oral bacteria are necessary for the conversion of nitrate to nitrite, but regular use of antibiotic mouthwash and/or antibiotic therapy have been found to decrease the activity of these bacteria, resulting in the reduced production of NO. Dietary factors, including high intake of high-fructose-corn-syrup and co-ingestion of glucosinolate-rich vegetables (i.e., cabbage family) may also impair NO production. Certain lifestyle factors, including smoking and chronic use of specific medications, such as proton-pump inhibitors, have also been found to negatively influence NO production. While it is important to consume a diet that is naturally high in inorganic nitrate to enhance NO production, it is also important to be aware of these other factors that may either positively or negatively influence the nitrate-to-NO pathway and, ultimately, impact health.

Keywords: Nitrate, Nitrite, Nitric Oxide

Abbreviations

NO – Nitric Oxide; BP – Blood Pressure; CLA – Conjugated Linoleic Acid; CHX – chlorhexidine; PPIs – Proton Pump Inhibitors; XOR – Xanthine oxidoreductase

INTRODUCTION

Nitric Oxide (NO), formerly referred to as *endothelium-derived relaxing factor*, has a vascular-smooth-muscle-relaxing effect in the human body (Das & Kumar, 1995). The vasodilatory effect of NO is now established as playing key roles in cardiovascular, gastrointestinal, and endothelial systems, resulting in lower blood pressure (BP) and enhanced oxygen and nutrient delivery to cells (Blot, 2021). NO is a small gaseous and lipophilic molecule synthesized endogenously from 2 metabolic pathways:

1) Via the endogenous L-arginine pathway, where NO synthase converts endogenous L-arginine to NO (Oliveira-Paula et al., 2019).

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2) Via the exogenous enterosalivary pathway, where nitrate-reducing bacteria reduce nitrate to nitrite, which is then further reduced to NO (Blot, 2021).

Nitrate reductase catalyzes the reduction in the enterosalivary pathway, and it is only expressed by nitrate-reducing oral bacteria and not through human body tissues. Studies have found that the abundance of nitrate-reducing bacteria in the oral cavity is associated with lower systolic BP in healthy individuals, likely the result of an enhanced nitrate-nitrite-NO conversion (Goh et al., 2019; Tribble et al., 2019).The L-arginine pathway, which directly creates NO in tissues, is robust in children but is diminished in adulthood due to decreased L-arginine availability and decreased NO synthase activity (Reckelhoff et al., 1994; Berenyiova et al., 2018). It is important, therefore, for adults to understand the important shift toward a greater reliance on food borne inorganic nitrate and the enterosalivary pathway for NO production. Failure to adequately produce NO has been associated with higher morbidity and mortality in hospitalized patients (Blot, 2021).

The source of nitrate used in the enterosalivary pathway is available in either organic or inorganic forms. Organic nitrates, such as glyceryl trinitrate, have a more complex chemical structure, as compared to inorganic nitrates, and are mostly medically obtained (Omar et al., 2012). These organic nitrate pharmaceutical agents are commonly used by individuals with heart conditions, such as angina (Tibballs, 1993). Although organic nitrates have potent vasodilatory effects, their chronic use results in elevated tolerance and endothelial dysfunction, which are associated with elevated oxidative stress and increased

risk for ischemia-reperfusion injury (Münzel & Daiber, 2018; Omar et al., 2012).

By comparison, inorganic nitrates naturally occur in certain foods, and can be found in relatively large quantities in beets and green leafy vegetables (See Table 1). It is important to note that consuming these vegetables as a juice enhances surface contact with oral bacteria and enhances nitrate reduction as compared to their whole food counterparts. Inorganic nitrates can also be synthesized from nitrate salts, such as sodium nitrate, which are commonly used as food preservatives in processed meats. In contrast to the organic form, chronic consumption of inorganic nitrates do not result in endothelial dysfunction because they participate in the enterosalivary circulation that prolongs the vasodilatory effect and prevents excess NO production, which would lead to oxidative stress (Omar et al., 2012). Inorganic nitrates also have a cytoprotective effect against ischemia-reperfusion injury (Omar et al., 2012).

In contrast, nitrosamines, which are produced by nitrosation between nitrates or nitrites and certain amines, increase cancer risk in various organs, including the stomach. Nitrosamines can be formed endogenously in the stomach from nitrous acid, which is reduced from nitrates, and amines via nitrosation (Dubrow et al., 2010; Song et al., 2015). Studies have found that fruits and vegetables are the primary source of dietary nitrate intake, and large quantities of amines are found in fermented and over-cooked foods (Said Abasse et al., 2022; Dubrow et al., 2010). Nitrosamines can also be formed exogenously in processed meats as a result of heating at high temperatures and fermentation. In addition, organic nitrate salts can react with secondary amines in meat during thermal processing to form nitrosamine. Therefore, while antioxidants, such as vitamins C, vitamin E, and polyphenols in fruits and vegetables inhibit nitrosation, heme in red meats stimulates the endogenous formation of nitrosamines (Dubrow et al., 2010). As a result, naturally occurring inorganic nitrate from fruits and vegetables contributes to NO production while decreasing cancer risk, but red and processed meat consumption may elevate cancer risk by inducing malignant tumors via gene mutation and DNA adductions (Song et al., 2015).

Inorganic nitrates, derived either as dietary nitrates from food or nitrate salts, were commonly used in research studies to investigate the effect of nitrate supplementation on BP and endothelial function. Recent studies have found that both dietary nitrate and nitrate salt supplementation can significantly enhance plasma nitrate and nitrite concentration and NO production, with a resultant reduction in BP. In healthy men and women, single administrations of dietary nitrate through beetroot juice, spinach juice, salad juice, or nitrate salts decreased BP (Kapil et al., 2010; Jonvik et al., 2016; O'Gallagher et al., 2021; Hughan et al., 2017). Similar BP-lowering effect of dietary nitrates provided in a single administration or over 14 consecutive days were observed in healthy young men and postmenopausal women (Sweazea et al., 2018; Kim et al., 2019). The BP-lowering effect of beetroot juice and nitrate salts remained true in individuals with metabolic disorders and Raynaud syndrome with acute or short-term (up to 2 weeks) supplementation (Siervo et al., 2013; Shepherd et al., 2019). Shepherd at al. (2019) also identified anti-inflammatory and endothelial benefits of beetroot juice in Raynaud syndrome. It was also found that inorganic nitrate supplementation through dietary nitrate or nitrate salts had a greater BP lowering effect on systolic BP than diastolic BP regardless of health comorbidities (Siervo et al., 2013).

There is increasing evidence suggesting that enhanced NO production as a result of dietary nitrate supplementation improves athletic performance through increasing time to exhaustion and decreasing the oxygen cost of exercise (Husmann et al., 2019; Lansley et al., 2011). Nitrate reduction to nitrite by oral bacteria lowers BP and increases skeletal muscle oxygenation, which results in a proportionately higher fat metabolism in healthy individuals (Cutler et al., 2019). The higher fat metabolism diminishes reliance on carbohydrate, for which humans have limited storage, and is likely the primary reason associated with greater fatigue resistance. Additionally, beetroot juice supplementation over 5 days reduced exercise-associated leg muscle pain, which consequently increased time to fatigue (Husmann et al., 2019). Acute or short-term (1-2 weeks) beetroot juice supplementation also reduced oxygen cost of exercise, which improved exercise economy and enhanced tolerance to high-intensity exercise (Bailey et al., 2009; Vanhatalo et al., 2010; Lansley et al., 2011).

Inorganic nitrate consumption has also been found to have a gastroprotective effect. In animal studies, one week of sodium nitrate supplemented in water attenuated mucosal damage

Table 1: Inorganic Nitrate Content of Commonly Consumed Foods

Nitrate Content (mg/100g fresh weight)	Foods
>250	Beetroot, spinach, lettuce, rocket, celery, garden cress, chervil
100-250	Celeriac, fennel, leek, endive, parsley
50-100	Cabbage, savoy cabbage, turnip, dill
20-50	Broccoli, carrot, cauliflower, cucumber, pumpkin
<20	Artichoke, asparagus, broad bean, Brussels sprout, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, sweet potato, tomato

Sources: Machha A, and Schechter AN. Inorganic nitrate: A major player in cardiovascular health benefits of vegetables? Nutrition Reviews. 2012; 70(6): 367-372; Hord NG, Tang Y, Bryan NS. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr* 2009; 90:1-10; Santamaria P. Nitrate in vegetables: Toxicity, content, intake and EC regulation *J Sci Food Agric*. 2006; 86:10-17.

through increasing gastric mucosal blood flow and mucus thickness (Petersson et al., 2007; Petersson et al., 2009; Pang et al., 2020). In male Mongolian gerbils with stress-induced gastric mucosal injury, sodium nitrate supplementation over 1 week effectively inhibited gastric ulceration through restoration of gastric mucosal blood flow (Pang et al., 2020). A meta-analysis investigated the relationship between dietary nitrate intake and risk of gastric cancer using a random-effects model found a significant association between higher nitrate intake and lower gastric cancer risk (Zhang et al., 2019).

In addition, inorganic nitrates have metabolic benefits on insulin resistance, glucose tolerance, and dyslipidemia, which are associated with obesity and diabetes (Khalifi et al., 2015; Li et al., 2016; Brunetta et al., 2020). In mice and rats with induced obesity or hyperglycemia, long-term (1-2 months) supplementation with dietary nitrate through spinach juice or nitrate salts improved their lipid profile (Khalifi et al., 2015; Li et al., 2016; Fischer et al., 2020). While sodium nitrate improved glucose tolerance in hyperglycemic rats, calcium nitrate reduced triglyceride content and liver fatty acid composition in obese female mice (Khalifi et al., 2015; Fischer et al., 2020). Spinach supplementation also relieved inflammation and enhanced endothelial function in hyperglycemic mice (Li et al., 2016). In addition, a higher abundance of nitrate-reducing bacteria in the oral cavity was found to be associated with lower insulin resistance and plasma glucose concentration in normotensive individuals (Goh et al., 2019).

The following review provides information on factors that enhance, inhibit, or alter the dose-related effect of nitrate on health. The goal is to provide information that results in the best strategy for optimizing the potential health benefits derived from consumption of naturally occurring high nitrate-containing foods.

INHIBITORS OF NITRIC OXIDE FORMATION

Dietary Factors

A diet rich in inorganic nitrate, as found in commonly consumed vegetables (See Table 1), is desirable because of the enhanced Nitric Oxide (NO) production it imparts. However, other dietary factors, such as high fructose intake and co-ingestion of high nitrate vegetables with glucosinolate-rich vegetables, may interfere with the expected NO production.

Fructose

Fructose is a monosaccharide also commonly referred to as “fruit sugar” because it is naturally occurring in ripe fruit. Although both fructose and glucose are monosaccharides, they are metabolized differently in the human body. While body cells metabolize glucose throughout the body, fructose is almost exclusively metabolized in the liver, where it has 3 primary metabolic pathways: Pathway 1: conversion to liver glycogen; Pathway 2: creation of triglyceride; and Pathway 3: creation of uric acid (Coronati et al., 2022; Meneses et al., 2022; Rodriguez-Iturbe et al., 2022). Hence, a high and chronic intake of fructose is associated with increased health risks as it can increase the risks of obesity, and also may result in insulin resistance and fatty liver disease (Lelis et al., 2020). High-fructose corn syrup is commonly used as a sweetener in sodas, fruit-flavored drinks, and certain condiments, such as ketchup. Recent studies have shown that high fructose intake increases the risk of developing cardiometabolic disorders and endothelial dysfunction, both of which impair NO production. Rats fed a high fructose diet for 8 or 12 weeks had decreased plasma nitrate/nitrite concentrations due to reduced production of endothelial NO synthase (Chou et al., 2013; Malakul et al., 2018). Similarly, acute fructose ingestion reduced plasma NO concentrations in healthy young individuals (Cai et al., 2018). The fructose ingestion also corresponded to an elevated systolic BP 1 to 3 hours post-ingestion (Cai et al., 2018). Due to the adverse cardiometabolic disorders associated with high fructose intake, it is important to consider the fructose content of the commonly consumed or used drinks and condiments.

Cruciferous Vegetables

Cruciferous vegetables of the family Brassicaceae, which include cauliflower, cabbage, kale, bok choy, and broccoli, are rich in thiocyanate. These vegetables are well-known for their anti-inflammatory and anti-cancerous effects and are considered ‘super-veggies’ (Soundararajan & Kim, 2018). However, cruciferous vegetables are also rich in glucosinolate, which impairs nitrate reduction in the enterosalivary pathway when co-ingested with nitrate-rich vegetables because it competes with nitrate for transport into the salivary glands (Oliveira-Paula et al., 2019). It was found that acute consumption of nitrate-rich vegetables alone increased plasma and salivary nitrate and nitrite concentration that resulted in lowered systolic BP in healthy individuals. However, when consumed in combination with thiocyanate-rich vegetables, the BP-lowering effect was not observed (Dewhurst-Trigg et al., 2018). This finding suggests that the potential benefits associated with different components and types of vegetables can be impaired if consumed at the same time. Hence, there is a need for an appropriate health-promoting strategy to consume nitrate-rich vegetables and cruciferous vegetables in separate meals.

Lifestyle Factors

Smoking: Research has found that cigarette smokers have higher plasma and salivary thiocyanate concentrations than non-smokers (Flieger et al., 2019; Madiyal et al., 2018). High circulating thiocyanate competes with nitrate for transport into the salivary glands, which compromises nitrate-to-NO reduction (Bailey et al., 2016). In healthy individuals, the BP-lowering effect associated with short-term (6 days) inorganic nitrate supplementation from beetroot juice was attenuated in cigarette smokers but not in non-smokers, as a result of the mechanism described above (Bailey et al., 2016). It is not surprising that the acute response to cigarette smoking is profound. In smokers, smoking a single cigarette rapidly reduces plasma nitrate, nitrite, and concentrations of antioxidants, resulting in uncontrolled reactive oxygen species (ROS), elevated BP, and increased cancer risk. (Tsuchiya et al., 2002). The effect of smoking on nitrate reduction appears to be, at least in part, associated with the enterosalivary pathway in the oral cavity. In an analysis of the

human tongue and oral nitrate reductase activity, smokers were found to have lower nitrate-reducing activity resulting from the inhaled toxicants of cigarette smoke (Ahmed et al., 2017).

Conjugated Lenoleic Acid Supplementation: Conjugated Linoleic Acid (CLA) is the isomer of linoleic acid, which is an essential fatty acid and is found mostly in meats and dairy products. CLA supplements are often marketed for their anti-cancer and muscle developing benefits (den Hartigh, 2019). Although dietary intake of adequate essential nutrients, such as CLA, from natural foods is beneficial to health, supplemental intake of CLA may negatively impact NO production. In healthy adults, acute supplementation of 3g CLA suppressed the increase in plasma nitrate and nitrite concentrations from nitrate salts administration, which attenuated the BP-lowering effect of nitrate (Hughan et al., 2017). The underlying mechanism of the inhibitory effect involves chemical reaction between CLA and nitrate that forms CLA-nitration products. Consequently, free nitrate and nitrite becomes less available for NO production, which inhibits the BP-lowering benefit of nitrate, resulting in higher health risks (Hughan et al., 2017).

Antiseptic Mouthwash: Nitrate-reducing bacteria in the oral cavity play a crucial role in the enterosalivary pathway. However, antiseptic mouthwash is designed to eliminate bacteria in the oral cavity to prevent infection, making it an obvious inhibitor of nitrate reduction. Hence, the use of antiseptic mouthwash is one of the most researched inhibitory factors in NO production. Chlorhexidine (CHX) is commonly used in antiseptic mouthwash for its powerful antibacterial properties. Hence, mouthwash containing CHX is often used in studies to better understand the effect of antiseptic mouthwash on nitrate reduction and NO production. In healthy individuals, the use of CHX mouthwash twice a day for 7 days reduced oral and plasma nitrite concentrations and was correlated with increased systolic BP (Tribble et al., 2019; Kapil et al., 2010; Bescos et al., 2020). It has also been demonstrated in healthy mice that the administration of CHX mouthwash twice a day for 7 days inhibited the growth and activity of enterosalivary nitrate-reducing bacteria and attenuated the gastroprotective and BP-lowering effects associated with nitrate supplementation (Petersson et al., 2009; Ahmed et al., 2017). Bescos et al. (2020) found that mouthwash associated changes in salivary bacterial communities resulted in a more acidic environment and reduced nitrite production. Tribble et al. (2019) found that lower levels of nitrite-reducing bacteria is associated with higher systolic BP. Hence, the use of antiseptic/CHX containing mouthwash is a potential health concern.

The inhibitory effect of antiseptic mouthwash is an even greater concern in obese and hypertensive individuals because of pre-existing endothelial dysfunction. A recent observational study investigated the relationship between use of antiseptic mouthwash and risk of hypertension in middle-aged obese individuals. Researchers found that a use frequency greater than or equal to twice daily was associated with an 85% higher risk of hypertension that is independent of major hypertension risk factors and other potential confounders (Joshipura et al., 2020). This finding is supported by other human and animal studies. In treated hypertensive men and women, 3-day use of CHX mouthwash blunted oral nitrate-reducing activity, decreased plasma nitrite concentrations, and increased systolic BP (Bondonno et al., 2015). In hypertensive rats, four weeks of CHX mouthwash administration disrupted the enterosalivary circulation of nitrate and attenuated the BP-lowering effects associated with sodium nitrite or L-arginine supplementation (Pinheiro et al., 2016; Batista et al., 2021). The use of CHX-containing mouthwash for a shorter period has demonstrated a similar result. Additionally, the disrupted nitrate reduction due to the use of antiseptic mouthwash was thought to be an important factor in the increased mortality risk in hospitalized patients due to worsening pathologies that lead to life-threatening complications, such as ischemic heart disease and sepsis (Blot, 2021). These studies have concluded that both acute and long-term use of antiseptic mouthwash may disrupt the nitrate-NO reduction pathway, attenuate health benefits associated with dietary inorganic nitrate, and increase the risk of hypertension and other diseases in individuals with or without currently diagnosed metabolic disorders.

Medicinal Factors: Similar to the antiseptic mouthwash, some medications may disrupt the nitrate reduction through inhibition of nitrate-reducing enzymes and/or bacteria. Proton pump inhibitors (PPIs), commonly prescribed to reduce stomach acid, have been found to impair the BP-lowering effect of nitrate or nitrite supplementation. In healthy individuals and normotensive or hypertensive rats, the PPI omeprazole lowered gastric acidity and blunted the BP-lowering effect associated with oral sodium nitrate or nitrite supplementation (Pinheiro et al., 2012; Pinheiro et al., 2015; Montenegro et al., 2017). It is important to note that PPIs are commonly used to reduce the acid reflux caused by increased abdominal pressure, which is often observed in obese patients, weightlifters, and pregnant women. Hence, these populations may be at higher risk for hypertension with chronic PPIs use. This is of especially high concern for obese individuals as they are already at high risk of hypertension due to excess fat mass and endothelial dysfunction that result in reduced NO availability.

Antibiotic therapy may also eliminate nitrate-reducing bacteria, which attenuates health benefits associated with nitrate. However, there are limited studies investigating this relationship. In healthy individuals, 2-days of broad-spectrum antibiotic amoxycillin intake destroyed nitrate-reducing bacteria and attenuated the increase in salivary nitrite concentrations that is associated with potassium nitrate supplementation (Dougall et al., 1995). In rat pulmonary alveolar macrophages studied *in vitro*, macrolide antibiotic use reduced NO production through inhibiting the production of type II NO synthase (Kohri et al., 2000). It is important to note that NO production may have a protective effect against *Candida albicans* infection (Elahi et al., 2001). Hence, elimination of nitrate-reducing bacteria via antibiotics may result in an elevated risk of Candida infections. This is a greater concern in populations that are already at risk of yeast infection, including individuals with diabetes mellitus, immunocompromised conditions, are under chemotherapy, are pregnant, and/or use hormonal birth control (Grigoriou et al., 2006).

Xanthine oxidoreductase (XOR) is an enzyme produced by body tissues that is involved in purine metabolism and uric acid production. Some medications are designed to inhibit XOR production to lower uric acid. These XOR inhibitors, such as oxypurinol and febuxostat, are commonly used to treat gout-associated hyperuricemia and several other related medical conditions. Because XOR enhances NO production, medications that inhibit XOR result in lower NO production. In hypertensive rats and mice, administration of oxypurinol or febuxostat blunted the BP-lowering effect associated with long-term or acute sodium nitrate or nitrate supplementation (Montenegro et al., 2014; Oliveira-Paula et al., 2016; Peleli et al., 2016). These findings suggest that nitrate or nitrite supplementation may not be effective in patients being treated for gout with XOR inhibitors, and that hypertension may be aggravated due to the inhibited NO production. It is important to also note that serum levels of uric acid are directly related to fructose intake, as one of the three primary fructose metabolic pathways stimulates uric acid production. This fructose-derived uric acid production may contribute to cardiometabolic disorders through reducing NO bioavailability (Caliceti et al., 2017).

ENHANCERS OF NITRIC OXIDE FORMATION

Dietary Factors

It is well established that consuming a diet rich in inorganic nitrate increases NO production. This is in contrast to fructose and cruciferous vegetables which may diminish NO production. Other dietary factors, including consumption of citrus fruit, berries, cocoa, tea, coffee, wine, olive oil, and nuts, may independently enhance NO production or bioavailability when co-ingested with nitrate.

Dietary factors that may enhance NO production include reducing agents, which include but are not limited to polyphenols and flavonoids. Polyphenols are naturally occurring bioactive compounds that are widely distributed in commonly consumed foods, and are known to be protective against cancers and cardiovascular diseases (Scalbert et al., 2002). Common polyphenol food sources include fruits, tea, coffee, red wine, and cocoa. Some types of polyphenols are widely present in food tannins that give the bitter taste to fruits, wine, and tea. In addition, they are present in the red color to fruits such as strawberry, blackcurrant, and grape. Specific polyphenols can only be obtained from single foods, such as isoflavone phytoestrogen, which can only be obtained from soy products. As for their chemical properties, all polyphenols are potent reducing agents that have antioxidant properties due to their phenolic structure and, therefore, have the ability to scavenge free radicals. Based on their structures, polyphenols can be further grouped into four subclasses (flavonoids, phenolic acids, lignans, and stilbenes) of which flavonoids are mostly commonly found in foods (Scalbert et al., 2002). Flavonoids are also naturally occurring reducing agents and antioxidants, and are found primarily in fruits and vegetables (Panche et al., 2016). They can be further grouped into six subclasses based their chemical structure, which are flavones, isoflavones, flavanones, flavanols, and anthocyanins (Panche et al., 2016). Due to the reducing potential of polyphenols and flavonoids, they may increase NO production through promoting nitrate-to-NO reduction.

Citrus Fruit & Citrus Flavonoids (hesperidin, naringin, and quercetin)

Grapefruit juice has been found to enhance the health benefits associated with dietary nitrate intake. A recent cross-over study in healthy individuals found that acute ingestion of grapefruit juice enhanced the beetroot juice's effect on lowering systolic BP and pulse rate (O'Gallagher et al., 2021). The co-ingestion of grapefruit and beetroot juices also slightly increased diastolic BP, which has a potential clinical application in patients with isolated systolic hypertension due to the adverse effect of low diastolic BP (O'Gallagher et al., 2021).

Several mechanisms have been proposed to explain the enhancing effect of grapefruit juice on NO production. One possible explanation is that the formation of other NO species outside the enterosalivary pathways contribute to the BP-lowering effect. Although grapefruit juice by itself lowered the pH in the oral cavity and stomach (i.e., made them more acidic), which is a favorable factor in nitrate reduction, co-ingestion of grapefruit juice and nitrate increased the pH level (O'Gallagher et al., 2021). As a result, the nitrate-to-nitrite conversion was inhibited, with an associated decrease in nitrite concentrations (O'Gallagher et al., 2021). Hence, the BP-lowering effect of grapefruit juice may be attributed to the vasodilatory effect of other NO species that were not directly measured in the study. Another possible explanation is that the reducing agents in grapefruit juice, including vitamin C and polyphenols, increased NO formation through facilitating nitrite-to-NO reduction in the stomach, which is discussed in detail in the following paragraphs. This mechanism can also explain the decreased plasma nitrite concentrations because, if more nitrite is reduced in the stomach, less nitrite will be absorbed into the plasma. In addition, some compounds in grapefruit might inhibit the uptake of organic anions and increased the uptake of inorganic ions, such as nitrate and nitrite, which enhance NO production.

As speculated by O'Gallagher et al. (2021), reducing agents that include naringin and hesperidin, which are found in grapefruit juice, may facilitate NO production through increasing NO bioavailability, which contributes to a BP-lowering effect. Flavonoids are the main type of polyphenols present in citrus fruit, of which hesperidin and naringin in the subclass of flavanones are the most abundant (Pontifex et al., 2021). Other flavonoids, including anthocyanins, flavones, and flavanols, are also present but in lower concentrations (Pontifex et al., 2021). Malakul et al. (2018) found that 4 weeks of oral naringin administration (100 mg/kg/d) in fructose-fed rats reduced fructose-induced metabolic disorders and endothelial dysfunction caused by 8 weeks of a high fructose diet. These vascular effects of naringin were associated with significantly increased NO bioavailability and enhanced endothelial NO synthase activity (Malakul et al., 2018). In stroke-prone hypertensive rats, 4 weeks of diet mixed with hesperidin, G-hesperidin (a water-soluble derivative of hesperidin), and naringin significantly enhanced NO production, suppressed the age-related increase in BP, and decreased risks for

cerebral thrombosis (Ikemura et al., 2012). Similar effects were observed in humans. In individuals with metabolic syndromes, 3 weeks of oral hesperidin administration (500 mg/d) stimulated NO production from endothelial NO synthase, increased vasodilation, and reduced inflammatory markers associated with hypertension (Rizza et al., 2011).

In addition to flavanones, quercetin is another important vasoactive flavonol found in citrus fruit that may play a role in NO production. Quercetin is well-known for its antioxidant property and there is increasing evidence suggesting its cardiovascular protective effect (Loke et al., 2008). In healthy men, acute oral administration of 200 mg quercetin dissolved in water increased the concentration of plasma nitrite and NO species, which improved endothelial function (Loke et al., 2008). Quercetin administration also significantly reduced the concentration of endothelin-1, a potent vasoconstrictor, resulting in a promotion of vasodilation (Loke et al., 2008). Similar effects were observed in animal models of hypertension and diabetes. In hypertensive rats, 30 days of oral quercetin administration (25 or 50 mg/kg/day) reduced the activity of NO inhibiting enzymes (arginase) and increased plasma NO levels (Olabiyi et al., 2022). In male diabetic or euglycemic rats, long-term (120 days) quercetin administrated in drinking water (40 mg/day) enhanced NO bioavailability in the jejunum and prevented NO-related morphological changes in diabetes (Martins-Perles et al., 2020). However, in allergic rhinitis (studied in human nasal epithelial cells stimulated with interleukin-4) and inflammatory periodontal disease (studied in murine macrophages stimulated with lipopolysaccharide from Prevotella intermedia), quercetin administration inhibited NO production from inducible NO synthase (Ebihara Cho)(Cho & Kim, 2013; Ebihara et al., 2018). These findings suggest that quercetin has beneficial effect on cardiovascular health that is mediated through NO production, which is retained in individuals with hypertension or diabetes. However, the effect of quercetic may be undesirable in certain medical conditions, such as allergic rhinitis and inflammatory periodontal disease.

In summary, findings highlight the importance of having a varied and balanced diet that is rich in inorganic nitrate and reducing agents that contribute to greater NO production. Simply focusing on high-nitrate foods may inhibit the magnitude of the desired effect. Studies also suggest the BP-lowering and NO-enhancing effect of citrus flavonoids appear to have, at least partially, the same beneficial effect observed with grapefruit consumption. Although grapefruit and related hybrids are the most significant source of naringin and hesperidin among commonly consumed foods, other citrus fruit, such as orange and lemon and their corresponding juices, have similar contents of reducing agents. Hence, while they are likely to also enhance the health benefits associated nitrate-rich foods, there is a lack of research on this topic. It is important to consider that individuals with diagnosed medical conditions, other than hypertension or diabetes, may experience different effects from the consumption of these foods.

Cocoa & Cocoa Flavanols

Research has shown a cardiovascular protective potential of cocoa flavanols that may be the result of NO production (Rodriguez-Mateos et al., 2015). Cocoa flavanols are bioactive compounds found in raw cocoa that can be consumed from food sources including cocoa drinks and dark chocolate. In healthy males, acute consumption of inorganic nitrate (0.1–10 mg/kg) and cocoa flavanols (1.4–10.9 mg/kg), consumed separately or simultaneously, improved endothelial function through increasing flow-mediated dilation (Rodriguez-Mateos et al., 2015). It is important to note that the separate effects from cocoa flavanols and nitrate were additive at low intake levels, which was speculated to be related to the enhancing effect of cocoa flavanols on nitrate-related gastric NO formation (Rodriguez-Mateos et al., 2015). This theory is supported by findings of other studies. In healthy individuals, short-term (5 days) ingestion of flavanol-rich cocoa (821 ml/day) induced vasodilation in peripheral arteries, while the effect was not observed with flavanol-poor cocoa (Fisher et al., 2003). Similarly, acute administration of polyphenol-rich cocoa powder (total polyphenol 17 mg/g of powder; flavan-3-ols 46 mg/g of powder) decreased systolic BP in hypertensive male rats (Quiñones et al., 2011). In both studies, the vasodilatory effect of flavanol-rich cocoa was reversed when co-administrated with N omega-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, which suggests that the observed vasodilatory effect was directly dependent on NO production (Fisher et al., 2003; Quiñones et al., 2011).

The effect of cocoa flavanols on NO production was also investigated using arginase *in Vitro* and *in Vivo*. Arginase is a protein that participates in the L-arginine pathway, in which it inhibits NO production (Schnorr et al., 2008). *In Vitro*, cocoa flavonoids reduced the expression of arginase-2 mRNA in human umbilical endothelial cells, and this finding is supported by *in Vivo* studies (Schnorr et al., 2008). In healthy individuals, acute consumption of a high-flavanol drink (985 mg cocoa flavanols per serving) reduced arginase activity in erythrocytes while the effect was not observed with a low-flavanol drink (<90 mg). Similarly, long-term (28 days) supplementation of flavanol-rich cocoa in diet reduced arginase activity in the kidney in male Sprague–Dawley rats, where arginase expression is highly abundant (Schnorr et al., 2008). These findings suggest that cocoa flavanols may decrease arginase activity and enhance NO production, resulting in improved endothelial function.

In addition to cocoa drinks, dark chocolate is also a significant source of cocoa flavanol because of its high cocoa content and may also have cardiovascular benefits in individuals with prehypertension. In this population, short-term (15 days) consumption of dark chocolate (30 g/day) containing 70% cocoa increased plasma NO concentration and reduced systolic blood pressure to a greater extent than white chocolate (Sudarma et al., 2011). A similar effect was observed with long-term (18 weeks) consumption, where dark chocolate (6.3 g, 30 kcal /day) that contains 30 mg of polyphenols reduced systolic BP and increased plasma NO concentration while polyphenol-free white chocolate did not (Taubert et al., 2007). These findings suggest that short- to long-term consumption of high-flavanol dark chocolate may reduce BP and prevent the progression to hypertension

in individuals with prehypertension, which is at least partially mediated through NO production.

In summary, acute, or long-term ingestion of cocoa flavanols through dark chocolate containing a high cocoa content and high-flavanol cocoa drinks may exert BP-lowering and vasodilatory effect and improve endothelial function by enhancing the production and/or bioavailability of NO.

The Mediterranean Diet & Polyphenol Rich Supplement

Dietary patterns that are high in polyphenols, such as the Mediterranean diet that has a relatively high intake of vegetables, fruits, olive oil and nuts, may also improve NO production. In individuals with hypertension or who are at high risk for cardiovascular disease, long-term (2 to 12 months) intake of a high-polyphenol diet interventions had independent effects in decreasing BP and improving endothelial function, which is associated with the nitrate-nitrite-NO pathway (Moreno-Luna et al., 2012; Medina-Remón et al., 2015). Specifically, the Mediterranean diet that commonly includes extra virgin olive oil and nuts increased total polyphenol intake and was associated with elevated plasma NO concentration in individuals at high risk for cardiovascular diseases (Medina-Remón et al., 2015). In women with hypertensive disorder, the consumption of a diet containing polyphenol-rich olive oil elicited an increase in plasma nitrites and nitrates concentrations, while the effect was not observed for a diet containing polyphenol-free olive oil (Moreno-Luna et al., 2012). Although their specific effect on nitrate reduction when co-ingested with nitrate-rich food was not investigated, polyphenol content of these foods is likely to enhance the health benefits of nitrate through facilitating NO synthesis, decreasing BP, and improving endothelial function. Red wines contain a variety of polyphenols and are also a common component of the Mediterranean diet. Wines are discussed later in this paper due to its ethanol content, which may influence the impact of polyphenols on NO production.

A cross-over study investigated the effect of a polyphenol-rich supplement containing the reducing agents that included kaempferia parviflora methoxy flavones, pomegranate peel polyphenols, and moringa oleifera leaf saponins, which are common components of the Mediterranean diet. In healthy male athletes, acute oral ingestion of this supplement significantly increased plasma nitrate and nitrite concentrations for at least 12 hours post-ingestion, which was associated with improved performance and physical endurance (Jacob et al., 2018).

Summary

Foods containing reducing agents, including but not limited to flavonoids (naringin, hesperidin, quercetin, and cocoa flavanols) and other polyphenols, are likely to enhance the health benefits associated with dietary inorganic nitrate. While the precise mechanism for this enhancing effect is not well-established, the BP-lowering effect may be associated with enhanced endothelial NO synthase activity (nitrate-to-NO reduction). However, it is important to note that not all flavonoids have an enhancing effect on NO production and the effect may be reversed in certain medical conditions (Duarte et al., 2014). Although only citrus fruits, cocoa, and the Mediterranean diet are discussed in this review, other foods including berries, tea, and coffee, are also significant sources of polyphenols that may enhance NO production.

Lifestyle Factors

Tongue Brushing: Tongue brushing, a common strategy for maintaining oral hygiene, may initially disrupt the oral microbiome involved in nitrate reduction, but ultimately increases the abundance and activity of oral nitrite reducing bacteria and enhances nitrite production if performed regularly (Tribble et al., 2019). These alterations were found to be associated with lower BP in healthy normotensive individuals (Tribble et al., 2019). Tribble et al. (2019) proposed that the mechanism responsible for this effect involves the stimulation/activation of bacterial metabolism, which results in a favorable bacterial community for nitrate-reducing activity. It is important to note that the American Dental Association recommends regular tongue cleaning for its benefit in reducing halitosis severity (Brignardello-Petersen, 2017). The study by Tribble et al. (2019) brings new significance to tongue brushing and its BP-lowering benefit, but there is a lack of epidemiological data on the frequency and practices of tongue brushing to fully understand the potential health benefits at a population-level.

Medicinal Factors: Although medications such as PPIs, antibiotic therapy, and XOR inhibitors may disrupt the enterosalivary pathway, TEMPOL has a beneficial effect on nitrate reduction. TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) is a water-soluble, superoxide dismutase mimetic drug widely used as an antioxidant in *in vivo* and *in vitro* studies (Inagi, 2011). Similar to flavonoids and polyphenols, it is also a potent reducing agent that may enhance NO production. (Inagi, 2011). In hypertensive rats, acute TEMPOL administration enhanced the BP-lowering effects associated with sodium nitrite supplementation through promoting NO formation in the stomach (Amaral et al., 2013). This finding further supports the idea that reducing agents may facilitate NO synthesis.

Vitamin C

Similar to flavonoids and polyphenols mentioned earlier in this paper, vitamin C is also a reducing agent and thus may enhance NO production from nitrate and nitrite. However, it is discussed separately from other reducing agents because the effects of vitamin C on NO production may vary depending on dose. In both healthy and hypertensive individuals, administration of around 1000mg vitamin C enhanced NO production and the BP-lowering effect associated with nitrate (Ashor et al., 2020; Basaqr et al., 2021). In healthy individuals, acute vitamin C administration (20 mg/kg body weight) with or without potassium nitrate (7 mg/kg body weight) improved pulse wave velocity, which is a measure of arterial stiffness (Ashor et al., 2020). Importantly, while vitamin C did not increase plasma nitrate or nitrite concentrations, the BP-lowering effect associated with potassium nitrate was only observed when co-administrated with vitamin C (Ashor et al., 2020). A similar enhancing effect was found in

individuals with untreated hypercholesterolemia. Four weeks supplementation of inorganic nitrate from beetroot juice resulted in significantly higher plasma nitrate and nitrite concentrations when co-ingested with 1000mg vitamin C (Basaqr et al., 2021). The co-ingestion of nitrate and vitamin C were also associated with other health benefits including decreased plasma low-density lipoprotein and triglyceride concentrations and reduced oxidative stress (Basaqr et al., 2021). These findings suggest that supplemental intake of vitamin C of approximately 1000mg, but under 2000 mg, may enhance the health benefits associated with nitrate with or without increasing plasma nitrate and nitrite concentrations.

It is important to consider that excessively large doses of vitamin C at a level that exceeds 2000 mg may impair the BP-lowering of nitrate. In hypertensive rats, acute co-administration of sodium nitrite and vitamin C had contrasting effects in BP response and NO production depending on vitamin C dosages: they were unmodified by a 0.02 mmol/l dose, enhanced by a 0.2 mmol/kg dose, and attenuated by a 2 mmol/kg dose (Pinheiro et al., 2018). The bell-shaped profile in response to vitamin C suggests that vitamin C magnifies the BP-lowering effect from nitrite when it is administrated at doses below or equimolar to that of nitrite, which is partially mediated by increased gastric formation of NO species (Pinheiro et al., 2018). The study also explains the mechanism behind the contrasting effect in BP response: low doses of vitamin C that are below or equimolar to that of nitrite facilitate the formation of NO species that have vasodilatory functions; however, high doses that are greater than that of nitrite destroy NO species, which attenuates the BP-lowering effect (Pinheiro et al., 2018). These findings are supported by another human study, which found that, in healthy males three-days vitamin C infusion at a rate of 2.4 g/24 hours suppressed platelets release of O_2^- and NO (McVeigh et al., 2002). Although more human research is required to support this relationship, findings from these studies suggest that consuming nitrate-rich foods with low doses of vitamin C from dietary sources may enhance the BP-lowering effects associated with nitrate, while consuming nitrate-rich food with excessively high doses (above the level of 2000mg) of vitamin C from supplements or infusion may impair the BP-lowering effect and NO production. Together with the inhibitory effect of conjugated linoleic acid, discussed previously in this review, these findings further support that large doses of chemicals tend to interfere with other biochemical reactions and physiologic functions that are beneficial to health.

It is important to consider that the large dose-dependent inhibitory effect of vitamin C administration may be beneficial in preventing nitrate tolerance associated with continuous organic nitrate therapy, which is commonly used in clinical settings (McVeigh et al., 2002; Watanabe et al., 1998). The issue of nitrate tolerance is important because it limits the efficacy of nitrate therapy in ischemic heart disease and congestive heart failure (Watanabe et al., 1998). In patients with congestive heart failure, vitamin C infusion at a rate of 55 μg/kg per minute (around 2376mg/day) prevented the attenuation of vasodilatory and hemodynamic effect and cGMP production associated with continuous nitroglycerin infusion at a rate of 0.5 μg/kg per minute (around 21.6mg/day) at 18 hours of infusion (Watanabe et al., 1998). These studies suggest that the elevated production and activity of oxygen-derived free radicals contribute to nitrate tolerance in continuous nitrate therapy, which can be suppressed with large dose (above the toxicity level of 2000mg) vitamin C administration.

In summary, vitamin C supplementation in small doses (approx. 1000mg) may enhance the health benefits associated with inorganic nitrate supplementation while vitamin C administration in large doses (above the toxicity level of 2000mg) may inhibit NO production and BP-lowering effect. However, the resultant inhibitory effect can be beneficial in continuous organic nitrate therapy commonly used in patients with ischemic heart disease and congestive heart failure.

Alcohol

Alcohol-containing beverages are commonly consumed by humans, and its consumption represents a lifestyle factor that may influence the potential health benefits associated with nitrate. A universal ingredient of alcoholic beverages is the alcohol ethanol, which is produced by the fermentation of yeast, sugars, and starches. Similar to vitamin C, ethanol has a differential effect on nitrate metabolism based on its source and the amount consumed.

Not all alcoholic beverages have the same effect on the health benefits associated with nitrate. Wines, for instance, may have a more potent BP-lowering effect due to the reducing potential of its polyphenol content. However, hard alcohols, such as vodka and whisky, do not appear to have a similar beneficial effect. In healthy males, the high polyphenol content in red wine induced faster-acting BP-lowering effect than vodka if consumed at relatively low levels. (McDonagh et al., 2018). This finding is supported by another human study, where the consumption of nitrate-rich lettuce only elevated NO concentration when co-ingested with red wine, but not with whiskey (Rocha et al., 2015). These findings suggest that different alcohols differentially impact the nitrate-to-NO reduction, which signifies the importance of polyphenol in nitrite reduction.

Anthocyanin and caffeic acid are polyphenols found in wine.

Caffeic acid is a polyphenol present in both red and white wines that belongs to the subgroup of hydroxycinnamic acids. The effect of these polyphenols on NO metabolism were studied by Gago et al. (2007) *in vitro* and in healthy individuals. It was found that the mixture of sodium nitrate with red wine, wine anthocyanin fraction, or wine caffeic acid was found to be dose- and pH dependent in promoting NO production. NO levels increased with higher concentrations of wine phenolic compound and lower pH levels.

Caffeic acid, which is present in both white and red wines, was also studied independently to investigate its effect on oxidative stress-induced endothelial injury. In an experimental model of kidney ischemia-reperfusion injury in mice, acute administration

of low doses (1μM/ml) of caffeic acid, which was similar to those observed after moderate wine consumption, induced NO release independently from the endothelial NO synthase (Migliori et al., 2015). This modulation may have a protective effect on endothelial cell function and increase NO production when co-ingested with nitrate. These findings suggest that the polyphenol content of wine may enhance NO production from a nitrate-rich meal. However, the ethanol content of alcohol may influence the effect.

The ethanol content of alcohol interacts with nitrate metabolism to increase or decrease NO production, and one of the mechanisms involves the formation of ethyl nitrite. Ethyl nitrite is a potent vasodilator produced in the human stomach when ethanol and nitrate or nitrite are co-ingested (Rocha et al., 2015). It has an independent BP-lowering effect but can also release NO that has BP-lowering effect when diffused into smooth muscle (Rocha et al., 2015). In healthy male Sprague–Dawley rats, acute administration of sodium nitrite-ethanol blend had a dilatory effect on smooth muscles in the gastric and vascular system, which can be attributed to NO release from ethyl nitrite (Gago et al., 2008). However, when administrated separately in similar doses, nitrite had a minor vasodilatory effect and ethanol had a vasoconstricting effect (Gago et al., 2008). These findings suggest that low ethanol tissue exposure may have an enhancing effect on the BP-lowering effect of nitrite that involves ethyl nitrite production.

Similar to the dose-dependent lowering or increasing effect of vitamin C on BP, alcohol consumption at low and high doses may exert different effects on BP. In healthy male Sprague-Dawley rats, 8 weeks of 7.5% alcohol supplementation in water, which mimics chronic moderate alcohol consumption in humans, improved postischemic myocardial functions and enhanced maximum vasodilation (Abou-agag et al., 2005). The cardioprotective and BP-lowering benefits were attributed to increased NO production in vascular endothelial cells, which corresponds to the increased amount of endothelial NO synthase protein (Abou-agag et al., 2005). It is important to note that while long-term treatment of low doses of ethanol (1g/kg) decreased systolic and mean BP in rats, higher doses of ethanol (2, 4, and 6g/kg or 18% of total calories) elevated systolic and mean BP (Abou-agag et al., 2005; Husain et al., 2005). The dose-dependent effects are associated with NO metabolism: Low doses of ethanol increased NO production, which reduces BP, while higher doses of ethanol depleted NO and antioxidants, which induces hypertension (Husain et al., 2005). These findings suggest that chronic moderate alcohol consumption may improve cardiovascular functions, while chronic high alcohol consumption may impair cardiovascular functions.

Considering both the phytochemical and ethanol content of alcohol, the type and amount of alcohol matter in regard to the enhancing effect on nitrate reduction. The polyphenol content of wines may improve endothelial function and exert a stronger enhancing effect when co-ingested with nitrate through facilitating NO production. Hence, moderate consumption of wine should be preferred over other types of alcohol, such as vodka and whiskey, as it helps to maximize the BP-lowering effect of nitrate and minimize risks for other health problems. The maximum alcohol consumption recommended by the 2020-2025 Dietary Guidelines for Americans is 2 drinks or less in a day for men or 1 drink or less in a day for women (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020).

SUMMARY

Nitric Oxide (NO) can be synthesized from the L-arginine pathway or the alternative enterosalivary pathway. It is a vasodilator that plays key roles in cardiovascular, gastrointestinal, and endothelial systems. Nitrate derived from natural food can be reduced to form NO by nitrate-reducing bacteria in the enterosalivary pathway. However, some dietary, lifestyle, and medicinal factors may diminish the production of NO via the enterosalivary pathway through inhibition of nitrate reduction to NO or blunting of nitrate-reducing activity. High dietary intake of fructose and/or high-fructose-corn-syrup should be avoided as it may increase the risk for cardiometabolic problems and endothelial dysfunction. The wide use of high-fructose-corn-syrup in commonly used/consumed condiments and foods is a public health issue. Although cruciferous vegetables are nutritious and have important health benefits, they may impair NO production and should be consumed at different times than nitrate-rich vegetables to achieve their maximal positive health effects. Lifestyle factors, including large supplemental intake of conjugated linoleic acid and large doses vitamin C, smoking, and use of antiseptic mouthwash should also be avoided since they were found to reduce nitrate and nitrite concentrations and attenuate nitrate's BP-lowering effect. Note that studies have found that large doses of chemicals, as commonly found in nutrient supplements, tend to interfere with biochemical reactions and physiologic functions that are beneficial to health and should, therefore, be avoided.

Medicinal factors, including Proton Pump Inhibitors, antibiotic therapy, and Xanthine Oxidoreductase (XOR) inhibitors were found to blunt nitrate's BP-lowering effect. Common use of PPIs, as often seen in obese individuals, weight-lifters, and pregnant women, is of concern since this may place them at high risk of hypertension. Antibiotic therapy may lower oral bacterial, which has an inhibitory effect on the nitrate-to-nitrite-to NO pathway, resulting in elevated blood pressure. The use of XOR inhibitors to treat fructose-derived uric acid production should be avoided when possible as these may aggravate cardiovascular and cardiometabolic conditions because of their inhibitory effect on NO production.

Some dietary, lifestyle, and medicinal factors may enhance health benefits associated with nitrate. A diet rich in polyphenols from food sources, including citrus fruit, cocoa, olive oil and nuts, and regular tongue brushing should be encouraged since they may improve endothelial function, increase nitrate-reducing potential, and enhance the BP-lowering effect. It is important to having a varied and balanced diet, rather than a diet focusing on a few "perfect foods", to maximize clinical benefits of dietary nutrients and phytochemicals.

Some factors, including vitamin C and alcohol, appear to have both enhancing and inhibiting effect on nitrate's BP-lowering potential, depending on dose. While relatively low doses of vitamin C and alcohol may be beneficial, large doses of vitamin C, typically from supplemental intake, or high alcohol intake should be avoided. It is recommended that the current Dietary Guidelines for Americans (2020-2025) be used as a guide for both nutrient intake and alcohol consumption. Since vitamin C can be commonly found in fruits and vegetables in relatively high doses and the current average dietary intake meets the daily nutritional goal, large-dose vitamin C supplementation should be avoided.

In conclusion, enhanced NO formation is an important factor in good health by enhancing oxygen delivery to metabolically active cells and also reducing cancer risk. This lower cancer risk is primarily observed in people with higher intakes of fruits and vegetables, which are the primary sources of naturally occurring inorganic nitrate. To achieve optimal NO formation, individuals should have frequent consumption of a wide variety of nitrate-rich foods, while avoiding factors that are inhibitors of NO formation. Poor NO production is now clearly associated with multiple cardiometabolic factors that can be mediated through a varied diet that focuses on frequent consumption of fruits and vegetables and limits exposure to products/lifestyle factors that may inhibit NO production, including antibacterial mouthwash and high alcohol consumption.

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