# Inorganic Nitrate Supplementation for Cardiovascular Health

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**ABSTRACT:** Nitric oxide (NO) is continually produced by the enzyme nitric oxide synthase (NOS) and is essential to the control and effectiveness of the cardiovascular system. However, there is a substantial reduction in NOS activity with aging that can lead to the development of hypertension and other cardiovascular complications. Fortunately, NO can also be produced by the sequential reduction of inorganic nitrate to nitrite and then to NO. Nitric oxide from inorganic nitrate supplementation has been found to have the same cardioprotective benefits of NO produced by NOS. Moreover, it can effectively compensate for declining NOS activity due to aging or NOS inhibition by oxidative stress, hypoxia, or other factors. This review covers some of the major cardiovascular regulatory actions of NO and presents evidence that NO from inorganic nitrate supplementation can provide (1) compensation when NOS activity is inadequate, and (2) cardioprotective benefits beyond that provided by a healthy NOS system. In addition, it discusses how to obtain a safe and efficacious range of inorganic nitrate.

## INTRODUCTION

Cardiovascular disease is the leading cause of death for both men and women and most ethnic groups in the United States. Research over the last 40 years has established that nitric oxide (NO) is a ubiquitous signaling molecule that is essential for a healthy cardiovascular system. This research was initially focused on the enzymatic production of NO by nitric oxide synthase (NOS) and its mechanisms of action. However, it has become apparent that the body can reduce inorganic nitrate and nitrite to NO and have the same biological effects as NO derived via the NOS system. Moreover, recent research suggests that diets consisting of foods high in nitrate, such as green leafy vegetables and dietary nitrate-based supplements, can provide added support to the NOS system and enhance the cardiovascular health benefits assigned to NO.

This review assesses the role of NO as it relates to cardiovascular health benefits and presents evidence documenting the importance of inorganic nitrate as a viable source of NO. It also discusses the potential cardioprotective effects of NO—including blood pressure regulation, control of the vascular endothelium, and improved myocardial contractility—in both healthy individuals and those with cardiovascular disease.

## NITRIC OXIDE PRODUCTION

Nitric oxide is a small gaseous molecule consisting of one oxygen atom and one nitrogen atom. It is a free radical with a half-life of less than a second. There are two basic mechanisms for the production of NO in the body (Figure 1). The first is the enzymatic conversion of L-arginine to NO and L-citrulline via NOS (the L-arginine-NO-synthase pathway), and the second is the sequential reduction of inorganic nitrate to nitrite to NO (the nitrate-nitrite-NO pathway). There are three isoforms of the NOS enzyme: nNOS or NOS-1, the isoform first found in neuronal tissue; iNOS or NOS-2, the isoform that is inducible in a wide range of cells and tissues; and eNOS or NOS-3, the isoform found in vascular endothelial cells.[1] Although there are some structural differences among these NOS enzymes, in their active form they are homodimeric and require the coenzymes NADPH, FAD, and FMN as well as heme iron, tetrahydrobiopterin (BH4), and oxygen.

Responsible for vascular conductance and homeostasis, eNOS can be activated by a number of clinically relevant agonists such as shear stress, acetylcholine, insulin, bradykinin, and aggregating platelets. However, uncoupling of the enzyme—which can result from oxidative stress, insufficient amounts of substrate or cofactors, hypoxia, or elevated levels of methylarginines such as asymmetric dimethylarginine—can significantly reduce its activity and NO bioavailability. Moreover, research indicates that the activity of eNOS declines by approximately 50% from ages 20 to 45.[2]

In the second mechanism of NO production (the nitrate-nitrite-NO pathway),[3] nitrate that is consumed from food or nitrate supplement is effectively taken up into the circulatory system in the upper gastrointestinal tract. The kidney clears approximately 75% of the nitrate while approximately 25% is circulated to the salivary glands, where it is concentrated up to 20-fold and secreted in saliva. Nitrate is then reduced to nitrite by commensal anaerobic bacteria found predominately under the tongue. Once the saliva is swallowed and enters the acidic stomach, some of the nitrite is spontaneously decomposed to

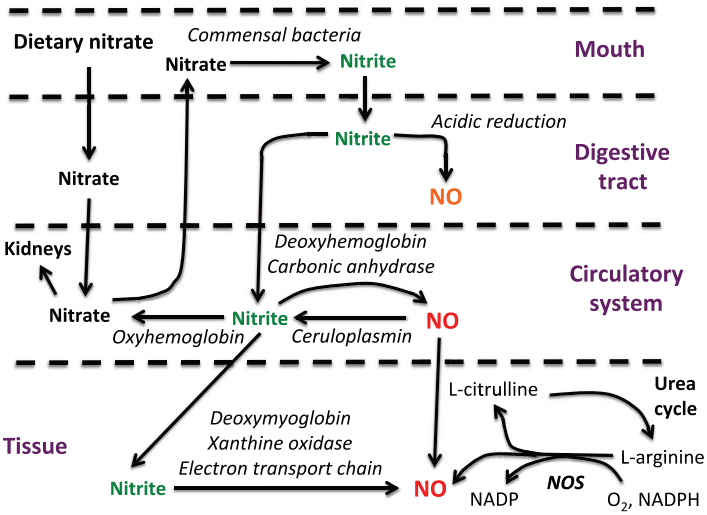


*Figure 1.*
Nitric oxide pathways via nitric oxide synthase (NOS) and nitrate reduction. This figure depicts the two pathways for the formation of nitric oxide (NO) and the areas within the body (designated in purple type) where the essential metabolic reactions occur. Arrows provide direction of substrate movement or chemical reactions. NO is produced continuously by NOS by catalyzing the five electron oxidation of the guanidino nitrogen of L-arginine. L-citrulline is a product of this reaction and can be converted back to L-arginine in the urea cycle. NO generated from the NOS system can be supported by the reduction of dietary nitrate to nitrite and then to NO. The majority of nitrate entering the body is initially cleared by the kidneys while the remainder is taken up from the circulation by the salivary glands and concentrated. This concentrated nitrate is then reduced in the mouth by commensal bacteria to nitrite and swallowed. A portion of the nitrite entering the digestive system is converted to NO by acidic reduction, which is enhanced by vitamin C and polyphenols. However, the majority of the nitrite is absorbed into the circulatory system, where it is reduced to NO by deoxyhemoglobin or reductase enzymes or enters tissues of the body, where it is reduced by deoxymyoglobin, xanthine oxidase, the electron transport chain, etc. NO can be converted back to nitrite and nitrate via oxidation by ceruloplasmin and oxyhemoglobin.

form NO and other bioactive nitrogen oxides.[3] This NO may be involved in the regulation of gastric activities such as intestinal peristalsis, gastric emptying, gastric mucus secretion, mucosal blood flow, and host defense.[3,4] However, the majority of the swallowed nitrite is rapidly taken up in the small intestines and enters the circulation and various tissues of the body. Here, the nitrite can be reduced to NO by several proteins and enzymes, including deoxyhemoglobin, deoxymyoglobin, xanthine oxidase, and cytochrome P-450 enzymes.[5] Nitrite reduction is greatly accelerated under hypoxic conditions, when the oxygen-dependent NOS pathway may be compromised. Thus, the nitrate–nitrite–NO pathway appears to function as a backup system to the NOS system when oxygen availability is limited. Although the half-life of NO is less than a second, it can be stabilized in the blood and body tissues by oxidation to nitrate and nitrite and converted back to NO under the appropriate physiological conditions.[6] Therefore, dietary nitrate can be a source of NO storage. In addition, a considerable amount of the nitrate initially cleared by the kidneys is reabsorbed. The fact that nitrate can be stored in the body, reabsorbed by the renal tubules, concentrated in the saliva, and continuously produced from the oxidation of nitric oxide strongly implies that it has an essential role in supporting normal human biology.

## HYPERTENSION

Cardiovascular disease (CVD) is the major cause of death in the United States and worldwide, and hypertension is a major risk factor. According to the American Heart Association, hypertension affects nearly 46% of all American adults, and it is estimated that another 30% are prehypertensive. Less than half of all hypertensive adults have their blood pressure under control.

NO has been found to play a significant role in regulating vascular tone, suggesting that limited NO production could be a primary contributor to the pathogenesis of essential hypertension. NO is continually produced by eNOS in the endothelium of the vasculature. In research, the infusing of the NOS inhibitor NG-monomethyl-L-arginine (L-NMMA) into animals results in a significant elevation in blood pressure.[7] Likewise, in a study by Vallance et al., local infusion of L-NMMA into the brachial artery of human volunteers resulted in a 50% fall in basal blood flow and attenuated the dilator response to infused acetylcholine.[8]

In humans and rats, the ingestion of nitrate is associated with acute and chronic blood pressure-lowering effects. The sources of nitrate, including inorganic salts such as potassium nitrate or dietary sources such as beetroot juice or green leafy vegetables, appear to be equally effective. For example, Webb et al. found that administering a single dose of beetroot juice containing 23 mmol nitrate to 14 healthy subjects reduced systolic blood pressure (SBP) by 10 mm Hg and diastolic blood pressure (DBP) by 8 mm Hg.[9] Blood pressure reached its nadir within 3 hours after consumption and corresponded with the peaking of plasma nitrite concentration. In a subsequent study from this group, the ingestion of only 5 mmol dietary nitrate resulted in a 5 mm Hg reduction in SBP. Multiple studies by Jones and associates demonstrating the beneficial effects of dietary nitrate on exercise performance and muscle-cell energetics also noted the blood pressure-lowering effects of nitrate supplementation.[10-12] In summary, their studies demonstrated an acute effect of dietary nitrate on SBP and DBP within 3 hours of ingestion and a chronic effect over 5

and 15 days with continued dietary nitrate supplementation. The average decline in blood pressure was approximately 4% for both SBP and DBP. This laboratory further demonstrated a dose response of blood pressure to dietary nitrate.[12] Acute ingestion of 4.2, 8.4, and 16.8 mmol dietary nitrate, administered in the form of beetroot juice, resulted in peak reductions of SBP of approximately 5, 10, and 9 mm Hg and peak reductions of mean arterial pressure of approximately 2, 5, and 5 mm Hg, respectively. Similarly, DBP dropped by approximately 3 and 4 mm Hg following ingestion of 8.4 and 16.8 mmol dietary nitrate but did not respond to a 4.2 mmol nitrate dose.

The blood pressure-lowering effects of inorganic nitrate have also been demonstrated in older subjects diagnosed with peripheral artery disease and primary hypertensive patients. Kenjale et al. found that a beetroot juice supplement containing 9 mmol nitrate lowered the DBP of patients with peripheral artery disease by 8 mm Hg and prolonged their walking time by reducing the onset of claudication pain.[13] In studying 15 stage 1 hypertensives who were not receiving medication, Ghosh et al. found that 3.5 mmol of dietary nitrate, an amount not shown to effectively lower blood pressure in normotensives, resulted in peak declines in SBP and DBP of approximately 12 and 9 mm Hg, respectively.[14] These peak declines occurred between 3 and 6 hours after nitrate consumption. SBP remained significantly lower than the control for 12 hours while DBP remained lower than the control for 6 hours.

## CONTROL OF THE ENDOTHELIUM

A healthy endothelium is paramount for proper functioning of the cardiovascular system. In this regard, several studies have shown endothelial dysfunction to be significantly related to atherosclerosis and development of CVD.[15] Injury to the endothelium can lead to endothelial dysfunction, which is characterized by reduced eNOS activity and imbalance between vascular relaxing and contracting factors.[15] Endothelial dysfunction can often be detected before any signs of atherosclerosis or CVD.

Oral nitrate salt supplements or foods high in nitrate content have been shown to mitigate the deleterious effects on endothelial function resulting from ischemia–reperfusion injury.[9] In a study by Webb et al., a mild ischemic insult in the forearm of healthy subjects significantly reduced flow-mediated dilation by approximately 60%. Supplementation with dietary nitrate imparted a significant protective effect that prevented endothelial dysfunction and restored flow-mediated dilation to near baseline levels.[9] A meta-analysis conducted by Lara et al. concluded that oral nitrate supplementation could improve endothelial dysfunction in a dose-dependent manner and provided evidence that oral nitrate supplementation can help support the eNOS system in regulating vascular tone.[16]

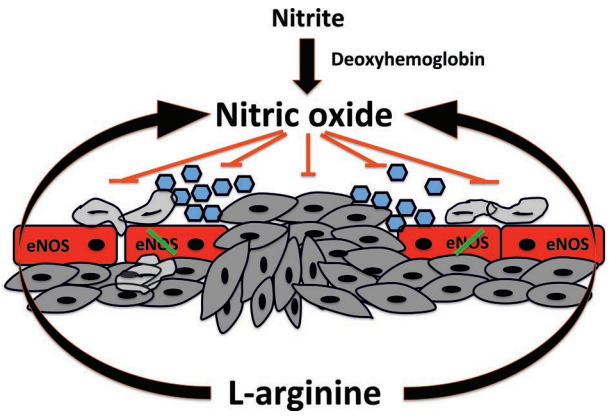


*Figure 2.*
Nitric oxide (NO) from nitrate reduction supports NO requirements when nitric oxide synthase (NOS) activity is low. Damage to the endothelium (red structure) can uncouple eNOS (green bar) and limit NO availability. This can result in inadequate regulation of platelet function (blue), leukocyte adhesion and migration (light gray), and vasculature smooth muscle cell proliferation (dark gray) and lead to the development of neointimal hyperplasia. However, the nitrate-nitrite-NO pathway can supplement the NO produced from eNOS and help to effectively regulate (inhibit, red lines) platelet aggregation and adhesion, leukocyte adhesion, and transendothelial migration and chemotaxis, control inflammation, and inhibit smooth muscle cell proliferation and migration. Without the nitrate-nitrite-NO pathway to supplement the NOS system, the probability of developing atherosclerosis and other cardiovascular diseases would likely be increased dramatically. This underscores the importance of consuming a diet sufficient in inorganic nitrate.

Endothelial dysfunction is also associated with insufficient control of anticoagulant substances and vascular inflammation. With a decline in eNOS activity comes a decline in regulation of platelet function, leukocyte adhesion and migration, and smooth muscle cell proliferation.[17] Therefore, increasing the bioavailability of NO through supplementation or dietary means is likely to have considerable cardioprotective effects (Figure 2).

The role of platelets is to survey the lining of our blood vessels, the endothelium. When there is acute damage, platelets are activated by contact with exposed collagen and aggregate together at the wound site to initiate a clotting process and stop bleeding. However, even mild injury to the endothelium (eg, due to high blood pressure) can cause platelets to adhere to the endothelium. Once these platelets are activated, an inflammatory response is initiated and predisposes the vasculature to complications. NO produced by eNOS is in part

responsible for controlling platelet aggregation and adhesion and proper development of the thrombus. Damage to the endothelium, however, can significantly reduce the activity of eNOS, thereby limiting NO production and diminishing its control of platelet activity. Uncontrolled platelet activity is associated with the development of atherosclerosis, thrombosis, and other cardiovascular complications.[17]

In the study by Webb et al. showing that dietary nitrate completely reduced endothelial dysfunction after an ischemic insult to the forearm,[9] the investigators also observed that it significantly attenuated ex vivo platelet aggregation in response to collagen and adenosine diphosphate. Similarly, Richardson et al. found that oral administration of 2 mmol potassium nitrate significantly impaired the ability of platelets to respond to a collagen stimulus.[18] In a separate experiment, these investigators also found that a dose of 0.5 mmol potassium nitrate achieved the same magnitude of effect as 2.0 mmol, but it took approximately 30 minutes longer. It should be noted that the amounts of potassium nitrate used in these experiments are quantities expected in a healthy diet. Accordingly, Velmurugan and colleagues reported no differences between nitrate salts and dietary nitrate supplements with regard to their inhibitory effect on ex vivo platelet aggregation.[19]

More recently, Apostoli et al. noted that plasma nitrite levels in wild-type mice were not significantly increased by the intraperitoneal administration of sodium nitrate (1 mmol $kg^{-1}$), nor was there an effect on in vivo platelet aggregation.[20] However, after administering sodium nitrate in eNOS knockout mice, there was a 5-fold increase in plasma nitrite concentration that significantly reduced in vivo platelet aggregation. The researchers surmised that inorganic nitrate exerts a specific effect on platelet function when there is endothelia dysfunction and eNOS deficiency. With a healthy endothelium, endogenous NO production is adequate for normal platelet function.

The arrival of platelets to an area of endothelial damage as well as the release of cytokines from the endothelium initiates an inflammatory response that attracts leukocytes to the site of damage. Adhesion of leukocytes to the endothelium is an early event in vascular inflammation and, with their subsequent transendothelial migration, a crucial step in the development of atherosclerosis.[21] Upon migration into the intima, leukocytes will secrete cytokines and growth factors that promote the proliferation and migration of vascular smooth muscle cell and plaque formation.[21,22] Research has emerged showing a clear regulatory effect of endothelium-derived NO on leukocyte adhesion regulation. Kubes et al. found that inhibition of eNOS increased leukocyte adherence by 15 fold.[23] Hypercholesterolemia has been found to inhibit eNOS, causing endothelial dysfunction and inflammation.[234] Using a high-cholesterol diet to induce hypercholesterolemia in C57B1/6J mice, Stokes et al. demonstrated that dietary nitrate could inhibit leukocyte adhesion to and emigration through the endothelium and reverse endothelial dysfunction.[25] Likewise, Jädert et al. found that 7 days of dietary nitrate supplementation significantly reduced leukocyte adhesion and transmigration in response to a proinflammatory insult.[26]

Platelet aggregation and leukocyte adherence and transendothelial migration at a site of vascular injury results in the migration of smooth muscle cells to the site; in turn, their subsequent proliferation results in neointima hyperplasia and ultimately stenosis. Due to the ability of NO to impede platelet aggregation and leukocyte activation, it is not surprising that NO has been found to influence smooth muscle proliferation. Early studies demonstrated that NO modulates vascular smooth muscle cell proliferation through a cGMP-mediated mechanism and impedes smooth muscle cell migration.[27,28] Moreover, there is evidence that a nitrite supplement can limit intimal hyperplasia following vascular injury. Alef et al. demonstrated that providing sodium nitrite in the drinking water of rats prior to balloon injury to their carotid arteries reduced intimal hyperplasia by approximately 50% and also reduced smooth muscle cell proliferation.[29] Furthermore, sodium nitrite supplementation 15 days after the vascular insult reversed the intimal hyperplasia. They also observed that the intimal hyperplasia was amplified in rats that were fed a diet low in nitrate and nitrite. The effectiveness of the sodium nitrite supplement was dependent on xanthine oxidoreductase, suggesting that reduction of nitrite to NO was essential.

In summary, endothelial dysfunction and the inability to provide an appropriate level of endogenous NO adversely affects vascular tone and limits control over anticoagulant processes and inflammation, directly influencing the development of atherosclerosis and CVD. However, research suggests that endothelial dysfunction can be overcome through nitrate supplementation or diets high in inorganic nitrate.

## SKELETAL AND MYOCARDIAL CONTRACTILITY

Dietary nitrate supplementation has been found to reduce oxygen consumption, SBP, and the heart rate-SBP product both at rest and at various submaximal exercise intensities. These findings suggest that, in healthy subjects, dietary nitrate decreases stress on the heart at rest and during aerobic exercise by decreasing cardiac afterload and myocardial oxygen demand. Furthermore, research suggests that nitrate supplementation may positively affect myocardial contractility.

Regular nitrate supplementation has been shown to increase force production in skeletal muscle in both mice and

humans.[30,31] Studies on humans have demonstrated that dietary nitrate can increase peak force and rate of force development during electrically stimulated isometric contractions and muscle speed and power in healthy volunteers during voluntary isokinetic contractions.[31,32] Even patients with systolic heart failure, who have markedly reduced skeletal muscle strength, velocity, and power, have been found to benefit from nitrate supplementation.[33] In fact, heart failure patients were found to benefit more from inorganic nitrate supplementation than healthy individuals.

To test the effects of dietary nitrate on myocardial contractility, Pironti et al. supplemented mice with sodium nitrate (~3.75 µmol per day) in their drinking water for approximately 2 weeks.[34] Hearts of sodium nitrate-treated and control mice were isolated and evaluated with the Langendorff heart procedure. Hearts from treated mice displayed 38% higher left ventricular developed pressure and 42% higher peak pressure rates than hearts from control mice. A faster ventricle relaxation rate was also observed in nitrate-treated mice hearts. The improved myocardial dynamics were attributed to enhanced $Ca^{2+}$ transients resulting from an increase in sarcoplasmic $Ca^{2+}$ storage and protein expression of the L-type $Ca^{2+}$ channel-dihydropyridine receptor. When the hearts were maximally stressed with isoproterenol, no differences in response were noted between the nitrate-treated and control hearts, which is analogous to the effect of nitrate supplementation on skeletal muscle.[34]

Myocardial dynamics have also been evaluated in heart failure patients with preserved ejection fraction after dietary nitrate or nitrite supplementation. Zamani et al. found that a single serving of nitrate-rich beetroot juice increased peak oxygen consumption and exercise capacity during a graded exercise test.[35] Maximal cardiac output and heart rate were significantly increased, with a trend for an increase in maximal stroke volume. Also, Borlaug et al. observed an increase in oxygen consumption at a standardized exercise workload coupled with a greater cardiac output in heart failure patients infused with 50 µg/kg/min of sodium nitrite per minute for 5 minutes.[36] The increase in cardiac output was due to an increase in stroke volume since exercise heart rate was unaffected by the nitrite treatment. Although a reduction in afterload could account, in part, for the increase in exercise stroke volume, the authors found that afterload-independent left ventricular stroke work also increased, suggesting that myocardial contractility was increased by the nitrite supplement.[36]

## EFFECTIVE NITRATE LEVELS

Acute cardioprotective effects such as reductions in blood pressure and platelet aggregation have been shown with nitrate salt supplements and dietary nitrate sources ranging between 4 to 20 mg/kg per supplement, with minimal adverse effects.[37] In general, the higher the nitrate concentration, the better and more sustained the benefit.[38] As previously discussed, nitrate can be stored in various forms in the body; therefore, high daily concentrations are probably not required to have significant sustained benefits. Based on the meta-analysis performed by Jackson et al., consuming between 4 and 12 mg/kg of nitrate (~300-800 mg/day) in supplement form—such as sodium nitrate, beetroot juice, or beetroot concentrates and powders—should provide a significant cardioprotective effect or improve conditions for those with CVD.[38]

Sufficient nitrate intake can also be achieved by dietary modification. Estimates of dietary nitrate intake in the United States range between 40 and 100 mg per day.[39] By increasing consumption of appropriate vegetables by three or more servings per day (100 g per serving), daily nitrate intake can be increased to levels shown to be cardioprotective. Vegetables high in nitrate include arugula, chervil, celery, spinach, collard greens, red beets, lettuce, leeks, and watercress.[40] Diets that have been found to benefit the cardiovascular system, such as the Dietary Approaches to Stop Hypertension (DASH), have been estimated to provide upwards of 1,200 mg of nitrate per day.[41] The nitrate content, however, is going to vary depending on soil composition. As such, the nitrate content of organic vegetables may be less than that of vegetables grown in the presence of nitrogen-containing fertilizers and may vary according to where it was grown.[41] For example, the nitrate concentration in 100 g of spinach can vary between 25 to 300 mg. Therefore, unless one is consuming a diet high in vegetables and fruits (eg, DASH or Mediterranean diet), daily nitrate supplements may be a more practical and sure means of achieving a healthy nitrate intake.

## CONCLUSION

Nitric oxide is essential for a healthy cardiovascular system and is continually produced via the L-arginine-NO-synthase pathway. When there is inadequate NOS activity, dietary supplementation with inorganic nitrate can meet the body's NO needs. Inorganic nitrate provided by either nitrate salts or food supplements has been found to support cardiovascular health by lowering blood pressure, protecting the endothelium when damaged, slowing metabolic reactions associated with atherogenesis and other CVDs, and strengthening the myocardium. The standard US diet is typically low in nitrate, and NOS-derived NO is significantly compromised with advancing age. Therefore, clinicians should consider recommending a nitrate supplement or diet high in nitrate to their patients, particularly when CVD is evident. An efficacious dose of nitrate can be safely met with commercially available nitrate supplements or a diet with multiple servings of nitrate-rich vegetables and fruits.

## KEY POINTS

- Nitric oxide (NO) is a signaling molecule essential to cardiovascular health.
- NO levels can be increased in the body by consuming inorganic nitrate supplements and foods high in nitrate, and the NO produced can be used to support the NO synthase.
- Evidence suggests that nitrate supplementation can help regulate blood pressure, limit progression of atherosclerosis, and improve myocardial contractility in both healthy individuals and those with cardiovascular disease.
- A prophylactic level of inorganic nitrate (300-800 mg/day) can be consumed through a diet encompassing vegetables and fruits with a high nitrate content or commercially available nutritional supplements.

*Conflict of Interest Disclosure:*
Dr. Ivy is a consultant for and shareholder in HumanN.

*Keywords:*
nitric oxide, nitric oxide synthase, nitrate, nitrite, cardiovascular disease, platelets, leukocytes

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