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Review Article

HOLISTIC SUPPLEMENT OF NO SAYS NO TO CARDIOVASCULAR AILMENTS

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Abstract:

NO is a gaseous signalling molecule. It is a key vertebrate biological messenger, playing a role in a variety of biological processes. It is a known bioproduct in almost all types of organisms, ranging from bacteria to plants, fungi, and animal cells. Nitric oxide, known as an endothelium–derived relaxing factor (EDRF), is biosynthesized endogenously from L–arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. One of the main enzymatic targets of nitric oxide is guanylyl cyclase. The binding of nitric oxide to the haem region of the enzyme leads to activation, in the presence of iron. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signalling molecule. Once nitric oxide is converted to nitrates and nitrites by oxygen and water, cell signalling is deactivated. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Sildenafil (Viagra) is a common example of a drug that uses the nitric oxide pathway. Sildenafil does not produce nitric oxide, but enhances the signals that are the downstream of the nitric oxide pathway by protecting cyclic guanosine monophosphate (cGMP) from degradation by cGMP–specific phosphodiesterase type 5 (PDE5) in the corpus cavernosum, allowing for the signal to be enhanced, and thus vasodilation. Another endogenous gaseous transmitter, hydrogen sulfide (H_2S) works with NO to induce vasodilatation and angiogenesis in a cooperative manner.

Keywords: EDRF, NOS, NADPH, PDE5, cGMP, Q10, CoQ10, Nitric oxide, Nitrates, Nitrites, L–Arginine, Citrulline

Introduction

Preamble: Nitric oxide is a vital molecule produced in the body that impacts many aspects of health. It helps blood vessels dilate to promote proper blood flow and may provide various health benefits, including improved exercise performance, lower blood pressure and better brain function. Switching up your diet is one of the best and most effective ways to naturally boost levels of this important molecule.[1]

Here are the 10 best foods to boost your nitric oxide levels:

1. Beets: Beets are rich in dietary nitrates, which your body can convert to nitric oxide. According to one study in 38 adults, consuming a beetroot juice supplement increased nitric oxide levels by 21% after just 45 minutes. Similarly, another study showed that drinking 3.4 ounces (100 ml) of beetroot juice significantly increased nitric oxide levels in both men and women. Thanks to their rich content of dietary nitrates, beets have been linked to a number of health benefits, including improved cognitive function, enhanced athletic performance and lower blood pressure levels. [***Beets are high in nitrates, which can significantly increase levels of nitric oxide in your body***.]

2. Garlic: Garlic can boost nitric oxide levels by activating nitric oxide synthase, the enzyme that aids in the conversion of nitric oxide from the amino acid L–arginine. One animal study showed that aged garlic extract temporarily increased blood nitric oxide levels

by up to 40% within an hour of consumption. Another test–tube study found that aged garlic extract also helped maximize the amount of nitric oxide that can be absorbed by the body. Both human and animal studies indicate that garlic's ability to increase nitric oxide levels may have a beneficial effect on health and can help lower blood pressure and improve exercise tolerance. [***Garlic can enhance the bioavailability of nitric oxide and may increase levels of nitric oxide synthase, the enzyme that converts L–arginine to nitric oxide.***]

3. Meat: Meat, poultry and seafood are all excellent sources of coenzyme Q10, or CoQ10 — an important compound believed to help preserve nitric oxide in the body. In fact, it's estimated that the average diet contains between 3–6 mg of CoQ10, with meat and poultry supplying about 64% of the total intake. Organ meats, fatty fish and muscle meats like beef, chicken and pork contain the highest concentration of CoQ10. Studies show that getting enough CoQ10 in your diet not only preserves nitric oxide but can also help improve athletic performance, prevent migraines and promote heart health. [***Meat, poultry and seafood are high in CoQ10, a key compound that helps preserve nitric oxide in your body.***]

4. Dark Chocolate: Dark chocolate is loaded with flavanols — naturally occurring compounds that boast an extensive list of powerful health benefits. In particular, research shows that the flavanols found in cocoa can help establish optimal levels of nitric oxide in your body to promote heart health and protect cells against oxidative damage. One 15–day study in 16 people showed that consuming 30 grams of dark chocolate daily led to significant increases in nitric oxide levels in the blood. What's more, participants experienced decreases in both systolic and diastolic blood pressure levels — the top and bottom number of the blood pressure reading. Because of its rich content of nitric–oxide–boosting flavanols, dark chocolate has been associated with improved blood flow, enhanced brain function and a lower risk of heart disease, too. [***Dark chocolate is high in cocoa flavanols, which increase levels of nitric oxide to promote heart health and prevent cell damage.***]

5. Leafy Greens: Leafy green vegetables like spinach, arugula, kale and cabbage are packed with nitrates, which are converted to nitric oxide in your body. According to one review, regular consumption of nitrate–rich foods like green leafy vegetables can help maintain sufficient levels of nitric oxide in the blood and tissues. One study even showed that eating a nitrate–rich meal containing spinach increased salivary nitrate levels eightfold and significantly decreased systolic blood pressure (the top number). Other research has found that consuming high–nitrate leafy greens may also be associated with a reduced risk of heart disease and cognitive decline. Leafy green vegetables are high in dietary nitrates, which can be converted to nitric oxide and may help maintain proper levels in your blood and tissues.

6. Citrus Fruits: Citrus fruits like oranges, lemons, limes and grapefruit are all excellent sources of vitamin C, an important water–soluble vitamin that plays a central role in health. Vitamin C can enhance levels of nitric oxide by increasing its bioavailability and maximizing its absorption in the body. Research shows that it may also bump up levels of nitric oxide synthase, the enzyme needed for the production of nitric oxide. Studies indicate that citrus fruit consumption may be linked to decreased blood pressure, improved brain function and a lower risk of heart disease — all of which may be due in part to their ability to boost nitric oxide levels. [***Citrus fruits are high in vitamin C, which can enhance the bioavailability of nitric oxide and increase levels of nitric oxide synthase***.]

7. Pomegranate: Pomegranate is loaded with potent antioxidants that can protect your cells against damage and preserve nitric oxide. One test–tube study showed that pomegranate juice was effective in protecting nitric oxide from oxidative damage while also increasing its activity. Another animal study found that both pomegranate juice and pomegranate fruit extract were able to increase levels of nitric oxide synthase and boost the concentration of nitrates in the blood. Human and animal studies have found that antioxidant–rich pomegranate can improve blood flow, which may be especially beneficial in treating conditions like high blood pressure and erectile dysfunction. [***Pomegranate can help protect nitric oxide against damage, enhance the activity of nitric oxide and increase levels of nitric oxide synthesis***.]

8. Nuts and Seeds: Nuts and seeds are high in arginine, a type of amino acid that is involved in the production of nitric oxide. Some research suggests that including arginine from foods like nuts and seeds in your diet can help increase nitric oxide levels in your body. For

example, one study in 2,771 people showed that a higher intake of arginine–rich foods was associated with higher levels of nitric oxide in the blood. Another small study found that supplementing with arginine increased levels of nitric oxide after just two weeks. Thanks to their arginine content and stellar nutrient profile, regularly eating nuts and seeds has been associated with lower blood pressure, improved cognition and increased endurance. [***Nuts and seeds are high in arginine, an amino acid needed for the production of nitric oxide***.]

9. Watermelon: Watermelon is one of the best sources of citrulline, an amino acid that's converted to arginine and, ultimately, nitric oxide in your body. One small study found that citrulline supplements helped stimulate nitric oxide synthesis after just a few hours but noted that it may take longer to see positive effects on health. Meanwhile, another study in eight men showed that drinking 10 ounces (300 ml) of watermelon juice for two weeks led to significant improvements in nitric oxide bioavailability. Recent research suggests that upping your intake of watermelon not only enhances nitric oxide levels but can also improve exercise performance, decrease blood pressure and boost blood flow. [***Watermelon is high in citrulline, which is converted to arginine and then later used in the production of nitric oxide.***]

10. Red Wine: Red wine contains many powerful antioxidants and has been tied to a multitude of health benefits. Interestingly, some studies have found that drinking red wine could also increase levels of nitric oxide. One test–tube study showed that treating cells with red wine increased levels of nitric oxide synthase, an enzyme involved in the production of nitric oxide. Another test–tube study had similar findings, reporting that certain compounds found in red wine enhanced nitric oxide synthase and increased the release of nitric oxide from the cells that line the blood vessels. For this reason, it's not surprising that moderate consumption of red wine has been shown to reduce blood pressure and improve heart health. [***Red wine can increase levels of nitric oxide synthase, which can help enhance nitric oxide levels.***]

N_2 NO $-NO_2$ $-NO_3$ → → →

Overview: Nitric oxide is produced by nearly every type of cell in the human body and one of the most important molecules for blood vessel health. It's a vasodilator, meaning it relaxes the inner muscles of your blood vessels, causing the vessels to widen. In this way, nitric oxide increases blood flow and lowers blood pressure. Supplements that increase nitric oxide in the body make up one of the most popular supplement categories today. These supplements don't contain nitric oxide itself. However, they contain compounds that your body can use to make nitric oxide and have been shown to provide many benefits for health and performance.[2]

Here are 5 health and performance benefits of taking nitric oxide supplements:

1. Help Treat Erectile Dysfunction: Erectile dysfunction (ED) is the inability to achieve or maintain an erection firm enough for sex. L–citrulline is an amino acid that may help treat erectile dysfunction by increasing the production of nitric oxide. Nitric oxide is needed for the muscles in the penis to relax. This relaxation allows chambers inside the penis to fill with blood so the penis becomes erect. In one study, L–citrulline was found to improve erection hardness in 12 men with mild erectile dysfunction. Researchers concluded that L–citrulline was less effective than prescription drugs used to treat ED, such as Viagra. Nevertheless, L–citrulline proved to be safe and well tolerated. Two other nitric–oxide–boosting supplements have been shown to treat erectile dysfunction — the amino acid L–arginine and Pycnogenol, a plant extract from the pine tree. In several studies, a combination of L–arginine and Pycnogenol significantly improved sexual function in men with ED. When taken together, L–arginine and Pycnogenol also appear safe.[3]

Summary: Nitric oxide plays an important role in erectile function. Several supplements, including L–citrulline, L–arginine and Pycnogenol, have been shown to increase levels of nitric oxide in men with erectile dysfunction (ED).

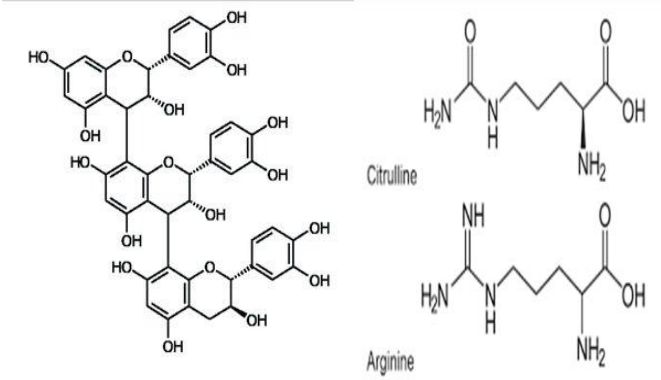

Figure 1: Pycnogenol and Citrulline & Arginine

2. May Decrease Muscle Soreness: A form of L–citrulline called citrulline malate not only increases nitric oxide production, but also decreases muscle soreness. Muscle soreness is an uncomfortable experience that tends to occur after strenuous or unaccustomed exercise. This soreness is referred to as delayed–onset muscle soreness and usually feels the strongest 24–72 hours after exercise. In one study, 41 people were randomized to receive either 8 grams of citrulline malate or a placebo one hour before performing as many repetitions as possible on a flat barbell bench press. Those given citrulline malate reported 40% less muscle soreness at 24 and 48 hours after the exercise, compared to those who took the placebo. Citrulline malate increases nitric oxide production, which increases blood flow to active muscles. In turn, citrulline malate is thought to increase nutrient delivery and clear waste products that are related to muscle fatigue, such as lactate and ammonia. However, a later study on the effects of citrulline after leg exercises did not find citrulline malate helpful for the treatment of muscle soreness. One explanation for this difference in findings is that the people in the leg exercise study were given 6 grams of citrulline malate, which is 2 grams less than the previous study. Therefore, the ability of citrulline malate to decrease muscle soreness may depend on the dose and exercise. However, more research on this is needed.

Summary: Citrulline malate is a form of L–citrulline that may help alleviate muscle soreness by increasing nitric oxide. The dose and type of exercise may affect the ability of citrulline malate to decrease muscle soreness.

3. Lower Blood Pressure: People with high blood pressure are thought to have an impaired ability to use nitric oxide in their bodies. High blood pressure occurs when the force of your blood pushing against the walls of your arteries is consistently too high. Over time, high blood pressure can lead to health issues such as heart disease and kidney disease. It has been shown that a diet high in fruits and vegetables decreases blood pressure and therefore lowers the risk of disease. This has led researchers to test the beneficial effects of certain compounds found in fruits and vegetables on blood pressure levels.

Nitrate: Nitrate is a compound found in beetroot and dark leafy greens like spinach and arugula. When you consume nitrate, your body converts it to nitric oxide, which in turn causes blood vessels to relax and dilate, lowering blood pressure. Many studies have shown nitrate may help lower blood pressure by increasing the production of nitric oxide. One review analysed the effects of taking nitrate supplements on blood pressure in adults. Of the 13 studies analysed, six found significant reductions in systolic blood pressure and diastolic blood pressure when participants took nitrate supplements. What's more, another review of 43 studies found participants' systolic and diastolic blood pressures decreased by an average of 3.55– and 1.32–mm Hg, respectively, after they took nitrate supplements.[4]

Flavonoids: Like nitrates, flavonoid extracts have been shown to improve blood pressure. Flavonoids have powerful antioxidant effects and are found in almost all fruits and vegetables. Scientists believe flavonoids not only increase production of nitric oxide but also decrease its breakdown, promoting higher levels overall. However, nitrates have more research supporting their blood–pressure–lowering effects than flavonoids do.

Summary: Vegetables and fruits contain several compounds, such as nitrate and flavonoids, that may help keep blood pressure under control by increasing nitric oxide levels.

4. Boost Exercise Performance: Nitric oxide is involved in many cell processes, including the widening of the blood vessels, or vasodilation. Wider blood vessels help increase the delivery of nutrients and oxygen to working muscles during exercise, thus enhancing exercise performance. This has made nitric oxide supplements popular among athletes and recreational gym–goers. These supplements often contain several ingredients that are said to increase nitric oxide, such as nitrate or the amino acids L–arginine and L–citrulline. In many analyses, nitrate has been shown to improve exercise performance in cyclists, runners, swimmers and even kayakers. On the other hand, L–arginine has not proven to be effective for improving exercise performance in many studies. This is likely because most of the L–arginine that is ingested is metabolized or broken down before it gets a chance to reach the bloodstream, whereas L–citrulline is not. For this reason, L–citrulline is more effective than L–arginine at increasing nitric oxide and therefore exercise performance.

Summary: Supplements intended to increase nitric oxide are commonly promoted as performance

enhancers. While the performance–enhancing benefits of L–arginine are minimal, nitrate and L–citrulline may be worthwhile.

5. May Help Manage Type 2 Diabetes: Nitric oxide production is impaired in people with type 2 diabetes. This leads to poor blood vessel health, which can lead to conditions like high blood pressure, kidney disease and heart disease over time. Therefore, supplements that increase nitric oxide may have important implications for diabetes treatment and disease prevention. One study found that when people with type 2 diabetes took L–arginine, their nitric oxide production increased. This increase in nitric oxide also led to increased insulin sensitivity, allowing for better blood sugar control. Another study in 144 people looked at the effects of L–arginine on preventing or delaying the progression of type 2 diabetes. While L–arginine did not prevent people from developing diabetes, it did increase insulin sensitivity and improve blood sugar control. But until more research is available, it is premature to recommend taking L–arginine supplements to treat diabetes.[5]

Summary: People with diabetes have impaired nitric oxide production, which can lead to harmful health effects. L–arginine has been shown to improve blood sugar control in people with diabetes, but more research is needed before it can be recommended.

Side Effects: Nitric oxide supplements are generally safe when taken in appropriate amounts. However, there are some side effects to be aware of. L–arginine taken in doses above 10 grams can cause stomach discomfort and diarrhoea. Beetroot juice supplements can also turn your urine and stool a dark red color. This is a common but harmless side effect. Before taking any supplements to increase nitric oxide, consult your doctor or dietitian.

Summary: Nitric oxide supplements are generally considered safe. However, there are some side effects to be aware of, including potential stomach discomfort and diarrhoea, as well as dark red stool and urine.

The Bottom Line: Nitric oxide is a molecule that plays many important roles in human health. Many supplements are claimed to increase nitric oxide in the body and provide impressive benefits for health and performance. They usually contain ingredients like nitrate or the amino acids L–citrulline and L–arginine. However, other supplements, such as Pycnogenol, have also been shown to increase or maintain nitric oxide levels.

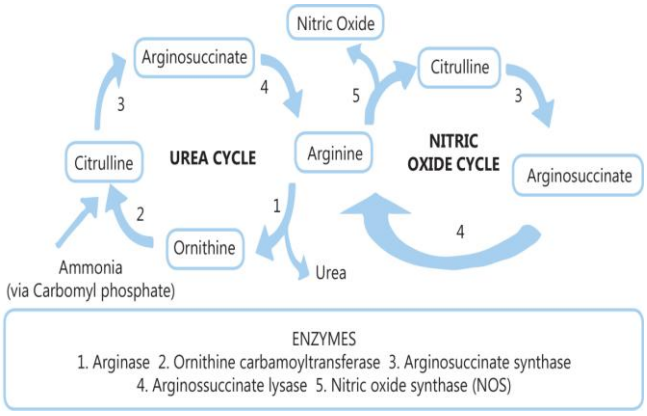

Figure 2: Biochemical pathways of Urea Cycle & Nitric Oxide Cycle

Nitric oxide (nitrogen monoxide) is a molecule and chemical compound with chemical formula of NO. In mammals including humans, nitric oxide is a signalling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a half–life of a few seconds in the blood. Standard pharmaceuticals such as nitroglycerine and amyl nitrite are precursors to nitric oxide. Low levels of nitric oxide production are typically due to ischemic damage in the liver. As a consequence of its importance in neuroscience, physiology, and immunology, nitric oxide was proclaimed "Molecule of the Year" in 1992. Research into its function led to the 1998 Nobel Prize for elucidating the role of nitric oxide as a cardiovascular signalling molecule.[6]

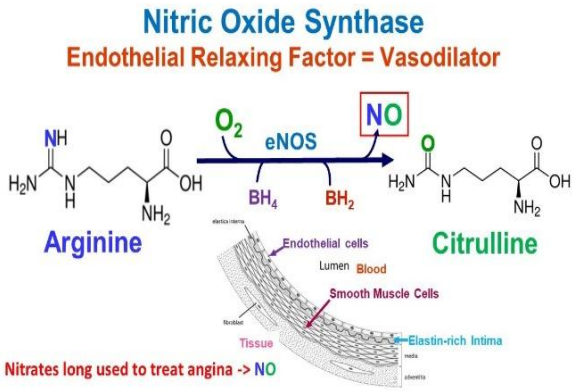

Figure 3: Role of NOS (Nitric Oxide Synthase) in Nitric Oxide Biosynthesis

Nitric oxide biosynthesis: Platelet–derived factors, shear stress, acetylcholine, and cytokines stimulate the

production of NO by endothelial nitric oxide synthase (eNOS). eNOS synthesizes NO from the terminal guanidine–nitrogen of L–arginine and oxygen and yields citrulline as a byproduct. NO production by eNOS is dependent on calcium–calmodulin and other cofactors. Nitric oxide synthases (NOSs) synthesize the metastable free radical nitric oxide (NO). Three isoforms are known for the NOS enzyme: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) – each with separate functions. The neuronal enzyme (NOS–1) and the endothelial isoform (NOS–3) are calcium–dependent and produce low levels of this gas as a cell signalling molecule. The inducible isoform (NOS–2) is calcium–independent and produces large amounts of gas that can be cytotoxic.

NOS oxidizes the guanidine group of L–arginine in a process that consumes five electrons and results in the formation of NO with stoichiometric formation of L–citrulline. The process involves the oxidation of NADPH and the reduction of molecular oxygen. The transformation occurs at a catalytic site adjacent to a specific binding site of L–arginine. NO is an important regulator and mediator of numerous processes in the nervous, immune, and cardiovascular systems. These include vascular smooth muscle relaxation, resulting in arterial vasodilation and increasing blood flow. NO is also a neurotransmitter and has been associated with neuronal activity and various functions such as avoidance learning. NO also partially mediates macrophage cytotoxicity against microbes and tumour cells. Besides mediating normal functions, NO is implicated in pathophysiologic states as diverse as septic shock, hypertension, stroke, and neurodegenerative diseases. Pathway for nitrosylation of heme–thiolate, steps in cell signalling (porphyrin is depicted as the square).[7]

Figure 4: Nitric Oxide rich diets

Exogenous NO (NO–delivery drugs): Exogenous NO sources constitute a powerful way to supplement NO when the body cannot generate enough for normal biological functions. Certain endogenous compounds can act as NO–donors or elicit NO–like reactions *in–vivo*. Nitroglycerin and amyl nitrite serve as vasodilators because they are converted to nitric oxide in the body. The vasodilating antihypertensive drug minoxidil contains an ·NO moiety and may act as a NO agonist. Likewise, Sildenafil citrate, popularly known by the trade name Viagra, stimulates erections primarily by enhancing signalling through the nitric oxide pathway. Prominent examples are S–nitrosothiols, certain organic nitrates, nitrosylated metal complexes, dinitrosyl iron complexes (DNIC), and even nitrite anions (NO_2^-) under hypoxic conditions. A high salt intake attenuates NO production in patients with essential hypertension, although bioavailability remains unregulated.[8]

Other, including dietary: Dietary nitrate is also an important source of nitric oxide in mammals. Green, leafy vegetables and some root vegetables (such as beetroot) have high concentrations of nitrate. When eaten and absorbed into the bloodstream, nitrate is concentrated in saliva (about 10–fold) and is reduced to nitrite on the surface of the tongue by a biofilm of commensal facultative anaerobic bacteria. This nitrite is swallowed and reacts with acid and reducing substances in the stomach (such as ascorbate) to produce high concentrations of nitric oxide. The purpose of this mechanism to create NO is thought to be both sterilization of swallowed food (to prevent food poisoning) and to maintain gastric mucosal blood flow.

The **nitrate–nitrite–nitric oxide** pathway elevates nitric oxide through the sequential reduction of dietary nitrate derived from plant–based foods. Nitrate–rich vegetables, in particular leafy greens, such as spinach and arugula, and beetroot, have been shown to increase cardioprotective levels of nitric oxide with a corresponding reduction in blood pressure in pre–hypertensive persons. For the body to generate nitric oxide through the nitrate–nitrite–nitric oxide pathway, the reduction of nitrate to nitrite (by nitrate reductase, a bacterial enzyme) occurs in the mouth, by commensal bacteria, an obligatory and necessary step. Monitoring nitric oxide status by saliva testing detects the bioconversion of plant–derived nitrate into nitric oxide. A rise in salivary levels is indicative of diets rich in leafy vegetables which are often abundant in anti–hypertensive diets such as the DASH diet.[9]

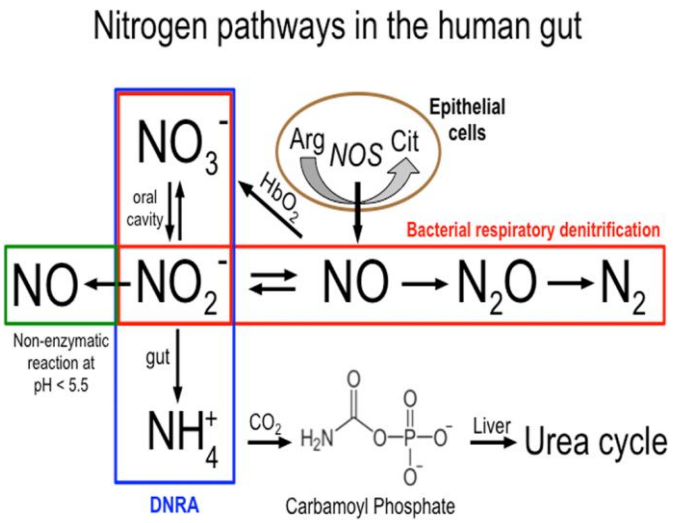

Figure 5: Nitrogen pathway

A related mechanism is thought to protect the skin from fungal infections, where nitrate [–NO_3] in sweat is reduced to nitrite [–NO_2] by skin commensal organisms and then to NO on the slightly acidic skin surface. In alternative fashion, nitrite anions on sun–exposed skin may be photolyzed to free nitric oxide radicals by UVA in sunlight. This mechanism may elicit significant changes to the systemic blood circulation in humans and be exploited for therapeutic purposes. Nasal breathing also produces nitric oxide within the body.[10]

Immune response: A dinitrosyl iron complex (DNIC), the product from the immune responsive attack of NO on Fe–S proteins. Nitric oxide is generated by phagocytes (monocytes, macrophages, and neutrophils) as part of the human immune response. Phagocytes are armed with inducible nitric oxide synthase (iNOS), which is activated by interferon–gamma (IFN–γ) as a single signal or by tumour necrosis factor (TNF) along with a second signal. On the other hand, transforming growth factor–beta (TGF–β) provides a strong inhibitory signal to iNOS, whereas interleukin–4 (IL–4) and IL–10 provide weak inhibitory signals. In this way, the immune system may regulate the armamentarium of phagocytes that play a role in inflammation and immune responses. Nitric oxide is secreted as free radicals in an immune response and is toxic to bacteria and intracellular parasites, including Leishmania and malaria; the mechanism for this includes DNA damage and degradation of iron sulfur centers into iron ions and iron–nitrosyl compounds. The inducible pathway (iNOS) of nitrogen oxide synthesis in phagocytes can generate large amounts of NO that trigger apoptosis and kill other cells. *In–vitro* studies indicate that phagocyte–dependent generation of NO at concentrations greater than 400–500 nM triggers apoptosis in nearby cells and that this effect may act in a manner similar to Specialized pro–resolving mediators to dampen and reverse inflammatory responses by neutralizing and then speeding the clearance of pro–inflammatory cells from inflamed tissues. However, the role of ·NO *free radical* in inflammation is complex with model studies involving viral infection suggesting that this gaseous mediator can also promote inflammation. In response, many bacterial pathogens have evolved mechanisms for nitric oxide resistance. Because nitric oxide might serve as an inflammometer (meter of inflammation) in conditions like asthma, interest has increased in the use of exhaled nitric oxide as a breath test in diseases with airway inflammation. Reduced levels of exhaled NO have been associated with exposure to air pollution in cyclists and smokers, but, in general, increased levels of exhaled nitric oxide are associated with exposure to air pollution. Molecular effects of NO on biological systems: In cells, two broad classes of reactions of nitric oxide involve the S–nitrosation of thiols [–SH] and the nitrosylation of some metalloenzymes.[11]

S–nitrosation of thiols: S–nitrosation involves the (reversible) conversion of thiol groups, including cysteine residues in proteins, to form S–nitrosothiols (RSNOs). S–Nitrosation is a mechanism for dynamic, post–translational regulation of most or all major classes of protein.

Nitrosylation of metal centers, especially iron: Nitric oxide to a transition metal ion like iron or copper, forming metal nitrosyl complexes. Typical cases involve

the nitrosylation of heme proteins like cytochromes, thereby disabling the normal enzymatic activity of the enzyme. Nitrosylated ferrous iron is particularly stable. Hemoglobin is a prominent example of a heme protein that may be modified by NO by both direct attack by NO and, independently, via attack by S–nitrosothiols, involving NO transfer from S to Fe. The iron–containing proteins ribonucleotide reductase and aconitase are deactivated by NO. NO has been demonstrated to activate NF–κB in peripheral blood mononuclear cells, a transcription factor in iNOS gene expression in response to inflammation.

Guanylate cyclase: Although NO affects many metalloproteins, it does so by deactivating them. Guanylate cyclase is a key component of the famous smooth–muscle relaxing properties of NO. It is a heme–containing enzyme that is acted on by NO, which binds to the heme. Cyclic–GMP activates protein kinase G, which causes reuptake of Ca^{2+} and the opening of calcium–activated potassium channels. The fall in concentration of Ca^{2+} ensures that the myosin light–chain kinase (MLCK) can no longer phosphorylate the myosin molecule, thereby stopping the crossbridge cycle and leading to relaxation of the smooth muscle cell.[12]

Smooth muscles:

Vasodilation: Nitric oxide dilates blood vessels, raising blood supply and lowering blood pressure. Conversely, it helps protect tissues from damage due to low blood supply. Also, a neurotransmitter, nitric oxide acts in the nitrergic neurons active on smooth muscle, abundant in the gastrointestinal tract and erectile tissue. Sildenafil (Viagra) works to inhibit the enzyme phosphodiesterase PDE5, which increases the cGMP concentration by inhibiting the conversion to GMP. Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways. Nitric oxide (NO) is a mediator of vasodilation in blood vessels. It is induced by several factors, and once synthesized by eNOS it results in phosphorylation of several proteins that cause smooth muscle relaxation. The vasodilatory actions of nitric oxide play a key role in renal control of extracellular fluid homeostasis and is essential for the regulation of blood flow and blood pressure. NO also plays a role in erection of the penis and clitoris.[13]

Cardiac effects: Nitric oxide also acts on cardiac muscle to decrease contractility and heart rate. NO contributes to the regulation of cardiac contractility. Emerging evidence suggests that coronary artery disease (CAD) is related to defects in generation or action of NO. Reduced levels of exhaled NO have been associated with exposure to traffic related air pollution.

Effects on plants: In plants, nitric oxide can be produced by any of four routes: (i) L–arginine–dependent nitric oxide synthase, (although the existence of animal NOS homologs in plants is debated), (ii) plasma membrane–bound nitrate reductase, (iii) mitochondrial electron transport chain, or (iv) non–enzymatic reactions. It is a signalling molecule, acts mainly against oxidative stress and also plays a role in plant pathogen interactions. Treating cut flowers and other plants with nitric oxide has been shown to lengthen the time before wilting. In plants, NO also regulates some plant–pathogen interaction, promotion of the plant hypersensitive response, symbiosis (for example, with organisms in nitrogen–fixing root nodules), development of lateral and adventitious roots and root hairs, and control of stomatal opening. Nitric oxide is known to be produced by cellular organelles, including mitochondria, peroxisomes, and chloroplasts. It plays a role in antioxidant and reactive oxygen species responses. Nitric oxide sensing in plants is mediated by the N–end rule of proteolysis and controls abiotic stress responses such as flooding–induced hypoxia, salt and drought stress. Nitric oxide interactions have been found within signalling pathways of plant hormones such as auxin, ethylene, Abscisic acid and cytokinin. Atmospheric nitric oxide can enter the stomates of most vascular species, and can have effects ranging from leaf blemishing, to stunting of growth, to necrosis.[14]

Effects in insects: Blood–sucking insects exploit vasodilation induced by NO to ensure their blood meal. These insects include *Cimex lectularius* (bed bug) and *Rhodnius proxlixus* (kissing bug). These insects deliver NO from its carrier nitrophorin carrier, which is in their saliva.

Effects in bacteria: While nitric oxide is typically known to halt bacterial growth as part of an immune response, in one case NO protects a bacterium. The bacterium *Deinococcus radiodurans* can withstand extreme levels

of radioactivity and other stresses. In 2009 it was reported that nitric oxide plays an important role in this bacteria's recovery from radiation exposure: The gas is required for division and proliferation after DNA damage has been repaired. A gene that increases nitric oxide production after UV radiation was described, and in the absence of this gene the bacteria were still able to repair DNA damage, but would not grow.[15]

Medical uses

Nitric oxide:

Trade names: Inomax, Noxivent, Genosyl. Routes of administration: Inhalation

IUPAC name: Nitrogen monoxide, Half–life: 2–6 seconds, CAS Number: 10102–43–9, Molar mass: 30.006 $g \cdot mol^{-1}$

Contraindications: Inhaled nitric oxide is contraindicated in the treatment of neonates known to be dependent on right–to–left shunting of blood. This is as the nitric oxide decreases the pulmonary circulation's resistance by dilating pulmonary blood vessels. The increased pulmonary return increases pressure in the left atrium, causing closure of the foramen ovale and reducing the blood flow through the ductus arteriosus. Closing these shunts can kill neonates with heart malformations that rely on the right–to–left shunting of blood.[16]

Dosage and strength: In the United States, nitric oxide is a gas available in concentrations of only 100 ppm and 800 ppm. Overdosage with inhaled nitric oxide will be seen by elevations in methemoglobin and pulmonary toxicities associated with inspired ·NO. Elevated NO may cause acute lung injury.

Fatty liver disease: Nitric oxide production is associated with non–alcoholic fatty liver disease (NAFLD) and is essential for hepatic lipid metabolism under starvation.

Lung infection: Nitric oxide is a potential therapeutic intervention in acute and chronic lung infections.

Mechanism of action: Nitric oxide is a compound produced by many cells of the body. It relaxes vascular smooth muscle by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic–guanosine 3',5'–monophosphate, which then leads to vasodilation. When inhaled, nitric oxide dilates the pulmonary vasculature and, because of efficient scavenging by hemoglobin, has minimal effect on the vasculature of the entire body. Inhaled nitric oxide appears to increase the partial pressure of arterial oxygen (PaO_2) by dilating pulmonary vessels in better–ventilated areas of the lung, moving pulmonary blood flow away from lung segments with low ventilation/perfusion (V/Q) ratios toward segments with normal or better ratios.[17]

Neonatal use: Nitric oxide/oxygen blends are used in critical care to promote capillary and pulmonary dilation to treat primary pulmonary hypertension in neonatal patients and post–meconium aspiration related to birth defects. These are often a last–resort gas mixture before the use of extracorporeal membrane oxygenation (ECMO). Nitric oxide therapy has the potential to significantly increase the quality of life and, in some cases, save the lives of infants at risk for pulmonary vascular disease.

Pathology: People with diabetes usually have lower levels of nitric oxide than patients without diabetes. Diminished supply of nitric oxide can lead to vascular damage, such as endothelial dysfunction and vascular inflammation. Vascular damage can lead to decreased blood flow to the extremities, causing the diabetic patient to be more likely to develop neuropathy and non–healing ulcers, and to be at a greater risk for lower limb amputation.

Paediatric and adult use: The primary use is in the form of nitroglycerin, either pill or liquid spray forms, which, as a prodrug, is denitrated and releases the active metabolite nitric oxide (NO). As with all supplements of nitric oxide, the response is short–lived because, as a normally produced internal physiologic control mechanism, increased concentrations lead to increased rates of clearance, which is the reason that the effectiveness of sustained use of nitroglycerin for vasodilation fades to none after hours to days. In the United States, ongoing direct use of nitric oxide use is only approved for neonates. In the adult ICU setting, inhaled ·NO can improve hypoxemia in acute lung injury, acute respiratory distress syndrome, and severe pulmonary hypertension, although the effects are short–lived and there are no studies demonstrating improved clinical outcomes. It is used on an individualized basis in ICUs as an adjunct to other definitive therapies for reversible causes of hypoxemic respiratory distress.

Pharmacokinetics: Nitric oxide is absorbed systemically after inhalation. Most of it moves across the pulmonary capillary bed where it combines with haemoglobin that

is 60% to 100% oxygen–saturated. Nitrate has been identified as the predominant nitric oxide metabolite excreted in the urine, accounting for >70% of the nitric oxide dose inhaled. Nitrate is cleared from the plasma by the kidney at rates approaching the rate of glomerular filtration.[18]

Pharmacology: Nitric oxide is considered an antianginal drug. It causes vasodilation, which can help with ischemic pain, known as angina, by decreasing the cardiac workload. By dilating (expanding) the arteries, nitric oxide drugs lower arterial pressure and left ventricular filling pressure. Nitric oxide can contribute to reperfusion injury when an excessive amount produced during reperfusion (following a period of ischemia) reacts with superoxide to produce the damaging oxidant peroxynitrite. In contrast, inhaled nitric oxide has been shown to help survival and recovery from paraquat poisoning, which produces lung tissue–damaging superoxide and hinders NOS metabolism. This vasodilation does not decrease the volume of blood the heart pumps, but rather it decreases the force the heart muscle must exert to pump the same volume of blood. Nitroglycerin pills, taken sublingually (under the tongue), are used to prevent or treat acute chest pain. The nitroglycerin reacts with a sulfhydryl group (–SH) to produce nitric oxide, which eases the pain by causing vasodilation. There is a potential role for the use of nitric oxide in alleviating bladder contractile dysfunctions, and recent evidence suggests that nitrates may be beneficial for treatment of angina due to reduced myocardial oxygen consumption both by decreasing preload and afterload and by some direct vasodilation of coronary vessels.

Pulmonary embolism: Nitric oxide is also administered as salvage therapy in patients with acute right ventricular failure secondary to pulmonary embolism.[19]

Research: COVID-19

As of April 2020, studies and trials are underway that examine the possible benefits of nitric oxide in the treatment of COVID-19. This research is based on the fact that nitric oxide was investigated as an experimental therapy for SARS. Brian Strickland, MD, a fellow in Wilderness Medicine at Massachusetts General Hospital who studies "acute respiratory distress" in high altitudes, is applying this research towards COVID-19. He is involved in clinical trials which apply the use of inhaled nitric oxide as a treatment for COVID-19. This approach was inspired by the work of Associate Professor of Emergency Medicine at the Harvard Medical School N. Stuart Harris, who has been studying the effects of altitude sickness on mountain climbers, such as those who climb Mount Everest. Harris noticed that the consequences of high–level altitude sickness on the human body mirrored COVID-19's dysfunctional impact on the lungs. His focus on nitric oxide comes from its role in being able to breathe in high altitudes. According to WCVB–TV, similar trials are being conducted at Tufts Medical Center. Other studies speculate that replacing mouth breathing (which decimates NO) with nasal breathing (which increases NO) is a "lifestyle change" that "may also help to reduce SARS–CoV–2 viral load and symptoms of COVID-19 pneumonia by promoting more efficient antiviral defence mechanisms in the respiratory tract."[20]

Conclusion: Until a few decades ago, researchers paid little attention to nitric oxide's role in the body. It has long been known that we exhale tiny amounts of this chemical compound, and that it's an air pollutant from cars and factories. But its molecules are so small and have such a short life span (just a few seconds) that scientists didn't think that nitric oxide did much in the body. That changed in the 1980s and 1990s, when the significance of nitric oxide as a chemical messenger in all mammals was established. In 1992, the American Association for the Advancement of Science proclaimed it the "molecule of the year." Nitric oxide's physiological importance was officially recognized in 1998 when a Nobel Prize was awarded to three scientists who discovered that it's a key molecule in the cardiovascular system and helps keep blood vessels healthy and regulate blood pressure. Since then, nitric oxide has also been found to be essential in many other bodily systems (such as the immune system and the nervous system, including the brain) and in many chronic conditions and diseases (such as chronic inflammation, erectile dysfunction, and cancer). This has led researchers to focus on nitric oxideas a potential target for medical therapies. Like many key compounds in the body, nitric oxide can be a double–edged sword. Produced by the body in small amounts where it is needed, it is essential and helps maintain health. But in larger amounts, it can be harmful and damage cells.

How does nitric oxide affect the heart and blood vessels?

Released by the inner layer of cells (endothelium) of blood vessels, nitric oxide relaxes the vessels and keeps them flexible, allowing them to dilate, boosting blood flow, and helping to control blood pressure. Nitric oxide also has anti–inflammatory effects and helps prevent platelets and white blood cells from adhering to the lining of blood vessels, thus reducing the risk of plaque development. As we age, our cells produce less nitric oxide, so blood vessels become less flexible, which contributes to hypertension, inflammation in blood vessels, and atherosclerosis (plaque buildup)—that is, cardiovascular disease. And there's a vicious cycle: increasing blood pressure, inflammation, and atherosclerosis, in turn, impair the production and action of nitric oxide. Recent lab research also suggests that nitric oxide is involved in the respiratory cycle by helping red blood cells to release the oxygen they carry to the body's tissues. By the way, the drug nitroglycerin helps relieve chest pain by becoming nitric oxide and thus improving flow of blood and oxygen to the heart. Cardioprotective drugs such as statins and ACE inhibitors also increase nitric oxide availability.

What's the connection between nitric oxide and erectile dysfunction?

Nitric oxide is well known these days because it is the target of drugs like sildenafil (Viagra), which treat erectile dysfunction (ED). Nitric oxide helps relax blood vessels and smooth muscle in the penis, allowing for the increased blood flow needed to initiate and maintain an erection. Interestingly, sildenafil was originally developed to treat angina, though it turned out that it works better for ED. It's now well known that ED is connected to cardiovascular disease, and reduced nitric oxide may help explain the link. In fact, some research has found that men who have ED as a result of reduced nitric oxide are at increased risk for cardiovascular disease. Currently researchers are experimenting with ways of delivering nitric oxide directly to the penis via application to its skin. Keep in mind, ED can be caused by other problems than low nitric oxide, so drugs like sildenafil don't work for all men with ED. Many medical and emotional factors can contribute to ED.

What does nitric oxide do in the brain?

It plays key roles in a variety of neurological processes, including as a neurotransmitter. Both its underproduction and overproduction of nitric oxide may impact memory and cognitive function and be involved in conditions such as depression, Parkinson's disease, and Alzheimer's disease. A reduction in nitric oxide production can lessen blood flow to the brain, as well as possibly increase inflammation and oxidative stress, and thus may contribute to cognitive problems. In addition, nitric oxide in the endothelium can modulate a protein that's a precursor to amyloid (found in plaques that are a hallmark of Alzheimer's).

How does nitric oxide affect the immune system?

Certain immune cells generate nitric oxide, which is important as a defence molecule against bacteria and other pathogens. It also regulates the activity, growth, and death of many immune and inflammatory cell types.

What is nitric oxide's role in cancer?

The double–edged nature of nitric oxide is noteworthy when it comes to cancer. For instance, as a signalling molecule, nitric oxide can cause cancer cells to destroy themselves (apoptosis). But it can also promote growth of blood vessels (angiogenesis), which cancer needs in order to grow. Some chemotherapy drugs cause cells to release nitric oxide in order to trigger apoptosis in tumours. Whether nitric oxide has pro– or anti–cancer effects depends on many factors, including its concentration and the type of tumour.

What foods boost nitric oxide?

Vegetables—notably leafy green vegetables and beets—contain nitrate, which can be converted in the body to nitrites and then to nitric oxide. Studies have shown, for instance, that beet juice can help lower elevated blood pressure, which has been attributed to the ability of its nitrates to boost nitric oxide. A recent review in the American Journal of Clinical Nutrition summarized research showing the beneficial effects of nitrate consumption on blood pressure, arterial stiffness, platelet function, and cerebral blood flow. Many plant foods, including fruits, chocolate, and red wine, also provide polyphenols and other compounds that can increase nitric oxide production in the body via a variety of pathways. Cells use arginine, an amino acid (one of the building blocks of protein), to make nitric oxide. High–protein foods such as nuts, beans, seeds, turkey, seafood, and dairy products supply arginine. A 2016 study in the journal Nutrients found that higher dietary intake of arginine was strongly linked with higher blood levels of nitrites and nitrates, which are a measure of nitric oxide production.

So, then, are arginine supplements a good idea?

Studies have shown that arginine supplements can boost the body's nitric oxide production. A few have also suggested that the supplements can improve the function of blood vessels, enhance coronary blood flow, lower blood pressure, and even reduce angina or other symptoms in people with cardiovascular disease. Ads for arginine supplements often boast that they are based on Nobel Prize–winning research. Though they have to avoid explicit medical claims, they can make vaguer claims such as "supports nitric oxide production and blood flow for the healthy function of the heart, brain and other organs," "supports healthy blood pressure levels and blood vessel elasticity," and "helps keep blood vessels toned and flexible." But several clinical trials have found that supplemental arginine did not help in treating people with cardiovascular disease and may actually have worsened their condition. For instance, two well–designed studies raised red flags about arginine supplements and the heart. One, a study in the Journal of the American Medical Association in 2006, found that arginine given to heart attack patients dramatically increased deaths. The study had to be halted; researchers warned strongly against using arginine for heart attack patients. And a study in Circulation in 2007 found that arginine supplements did not help people with peripheral artery disease and may even have made matters worse. Some dietary supplements that claim to improve sexual performance and sex drive also contain arginine because of its effect on nitric oxide—along with other ingredients (most of them dubious and some possibly dangerous). It's unknown whether supplemental arginine gets to blood vessels in the penis. Indeed, some studies have found little or no improvement compared to a placebo. In sum, the benefits of arginine supplements are un-certain and their long–term safety unknown. Increased amounts of one isolated amino acid may create imbalances with other amino acids and thus have adverse effects. And increased nitric oxide might be dangerous for people with certain medical conditions.

Should you have your nitric oxide level measured by your doctor or an OTC test kit?

No. Nitric oxide is difficult to assess except in laboratories doing experimental research. Blood or saliva levels of nitrite may reflect nitric oxide bioavailability in the body. But currently there is no valid, reliable test available to the public, according to a paper in Nitric Oxide: Biology and Chemistry in 2016. Saliva test strips on the market that claim to measure nitric oxide are "not likely to accurately assess nitric oxide bioavailability," according to the authors.

Bottom line: The best way to maintain a healthy nitric oxide level and keep blood vessels healthy is to eat a varied plant–based diet. Exercising regularly also helps enhance nitric oxide generation. This is one way a heart–healthy diet and exercise help improve cardiovascular health and health in general. And here's another reason not to smoke: Long–term smoking impairs nitric oxide function in the body.

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