Nitric Oxide, a Powerful Clinical Therapy

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Nitric oxide (NO) is a gas that has significant effects on many physiologic processes in the body. This gas plays a role in inflammation, the immune response, and neurotransmission in the brain, as well as in the functioning of the cardiovascular system. Dysfunction in the NO pathway is associated with many diseases. Conditions such as atherosclerosis, coronary artery disease (CAD), diabetes, hypertension, erectile dysfunction, and stroke are correlated with NO pathology.

Current research suggests that manipulation of NO activity may have profound effects on overall health. Many pharmaceuticals as well as nutrients, supplements, and diet are being investigated and prescribed to modulate NO activity.

The Physiology of NO

NO is most notably produced in endothelial cells, macrophages, and neurons. It is a toxic free-radical gas with a half-life of 6–10 seconds, thus providing only localized effects. NO is synthesized by combining L-arginine with oxygen to form L-citrulline and nitric oxide. The enzyme responsible for this conversion is nitric oxide synthase (NOS), which is found in three forms.[1]

One form is found in endothelial cells and platelets and is a calcium-calmodulin–dependent enzyme that is made at a constant rate regardless of physiologic demand. The second form is a calcium-independent inducible form of the enzyme, and exists in macrophages, neutrophils, cardiac cells, and hepatocytes.[2,3] The third form is found in neural cells.

To do its job properly, NOS requires several cofactors, such as tetrahydrobiopterin, heme, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD).[4] FAD and FMN are both active forms of riboflavin. Many factors have been shown to modulate NOS activity.

NO has several physiologic functions. One particularly significant role concerns the relaxation of vascular smooth muscle. Endothelium-derived relaxing factor, which is either identical or closely related to NO, is produced by the endothelial cells. Rapid blood flow through the arteries causes induction of the enzyme, increasing NO availability.

The presence of NO activates the enzyme guanylate cyclase, causing an increase in cyclic guanosine monophosphate (cGMP). This leads to relaxation of the vascular smooth muscle and, thus, vasodilation.

Bradykinin and acetylcholine also can stimulate NO release from the endothelial cells. The ability of NO to decrease platelet aggregation and adhesion is also significant for cardiovascular health.[5] New studies have suggested that NO also plays a role in lipid metabolism regulation.[6]

White blood cells—including macrophages and neutrophils—produce NO. In the immune system, NO functions as a localized bacteriocidal and tumoricidal agent. NO combines with superoxide anions to form highly toxic, bacteriocidal free radicals. Macrophage NOS synthesis is increased in response to lipopolysaccharides from bacteria and cytokines such as interferon-γ.[7] Further research is likely to demonstrate a link between plant-based polysaccharides and NOS production.

NO is also formed in the mitochondria. NOS found in the mitochondria is similar to the isoform found in neural cells.[8] NO in the mitochondria regulates the mitochondrial membrane's proton gradient, its membrane potential, and cellular respiration.[9] In the mitochondrial respiratory chain, NO competes with oxygen to inhibit, reversibly, cytochrome c oxidase, which is the terminal electron acceptor.[8] Studies have shown that mitochondrial NOS activity in the heart increases with increased altitude.[10]

In addition, NO exhibits activity in the nervous system. NO has been shown to act as a signaling molecule in the brain. Studies have also found that NO plays a role in neurogenesis in both the embryonic and adult brain.[11] NO also operates in the parasympathetic nerve endings in the penis, causing vasodilation and penile erection.

NO is important in bone metabolism, and is produced in bone cells in response to the presence of proinflammatory cytokines, estrogen, and mechanical loading. Endothelial NOS produces NO that affects normal anabolic osteoblast function. The inducible NOS pathway produces NO that regulates the effects of the proinflammatory cytokines interleukin-1 and tumor necrosis factor, both of which stimulate bone resorption.[12]

Natural Therapies for Increasing Nitric Oxide Synthase Levels

Supplement	Dose
L-Arginine	3 g 1–3 times per day
Folic acid	25–100 mg per day
Vitamin B_6	800–3000 mcg per day
Vitamin B_{12}	500–2000 mcg per day
Vitamin E	400–800 IU per day
N-acetyl-l-cysteine	1500–2000 mg per day
Pycnogenol®[a] (French pine bark; *Pinus maritima*)	100 mg 3 times per day
Garlic (*Allium sativum*)	600–1200 mg per day
Grape (*Vitis vinifera*)	75–300 mg per day
American ginseng (*Panax quinquefolius*)	400 mg per day
Artichoke (*Cynara scolymus*)	320–640 mg 3 times per day
Dehydroepiandrosterone (DHEA)[b]	25–100 mg per day

[a]Horphag Research, Ltd., Geneva, Switzerland.

[b]DHEA levels should be measured prior to and monitored after beginning this therapy.

IU = international units.

Artichoke (*Cynara scolymus;* also called *Cynara cardunculus*).

When There Is Not Enough NO

NO deficiencies in the body may present as a variety of different health conditions. Most commonly, individuals with low levels of NO will have signs and symptoms of cardiovascular disease such as atherosclerosis, hypertension, CAD, and stroke. In addition, NO deficiency can present as inflammatory conditions or erectile dysfunction. Because NO produced by white blood cells is bacteriocidal and tumoricidal, individuals with deficiencies may present with infections and possibly malignancies.

Because of their physiologic effects on the body, manipulation of NO levels can provide avenues for health modification. Cardiovascular diseases such as atherosclerosis, stroke, hypertension, and CAD can be attenuated by increases in NO synthesis and activity. Anemia, cancer, diabetes, and erectile dysfunction can also be ameliorated by NO manipulation. Both natural therapies and pharmaceuticals can augment NO bioavailability.

NO and Circulation

Atherosclerosis, hypertension, and CAD have all been shown to have a connection to endothelial dysfunction, which often is the result of lowered NO levels. Platelet aggregation and adhesion as well as arterial stiffness are also affected by NO activity. These cardiovascular conditions have been shown to be the result of abnormal NOS expression, decreased NO availability, or resistance to endothelium-dependent vasodilators.[13,14]

Studies have indicated that NO, when combined with low oxygen levels as a result of circulatory insufficiency, sensitizes neurons to hypoxia-induced death. Thus, neurons in the brain may be more likely to be damaged from ischemic or hypoxic damage from NO produced as a result of excitotoxicity or inflammation.[15] However, NO combined with normal oxygen levels does not increase neuronal cell death.[16]

In addition, NO increases cerebral blood flow, which may actually mean that NO can be protective during an ischemic attack.[17] In short, NO's modulatory effects appear to have a built-in protective effect regardless of oxygen status. With increased NO levels, oxygen delivery is enhanced, thus providing protection from otherwise-ischemic conditions.

NO and Anemia

Individuals with various forms of anemia have been shown to experience endothelial-cell dysfunction. Pulmonary hypertension—the result of such dysfunction—is the leading cause of death in both sickle-cell anemia and thalassemia, both of which have chronic anemia and intravascular hemolysis as their hallmarks. Chronic intravascular hemolysis is associated with endothelial dysfunction and results in decreased NO availability with a lack of vasodilation.[18]

NO and Diabetes

Individuals with diabetes are more vulnerable to oxidative stress, leading to an increase in superoxide anions and a subsequent decrease in NO bioavailability.[19] Endothelium-dependent vasodilation is impaired in individuals with either type 1 or type 2 diabetes. Many explanations have been suggested for this impairment, including abnormalities in substrate availability or signal transduction, release of endothelium-derived relaxing factors, destruction of endothelium-derived relaxing factors, decreased sensitivity of the vascular smooth muscle to endothelium-derived relaxing factors, and increased release of endothelium-constricting factors.[20]

Studies have shown that NO increases basal and insulin-stimulated glucose uptake in skeletal muscle in rats with type 2 diabetes. However, high concentrations of NO inhibit this uptake.[21]

NO and Erectile Dysfunction

Erectile dysfunction is significantly increased in individuals with cardiovascular disease, diabetes, and hypercholesterolemia. Erectile dysfunction is believed to be a condition of vascular origin that may be caused by endothelial damage or dysfunction. Such damage has been correlated with oxidative stress and the resulting decrease in NO.[22] Research has demonstrated that men with erectile dysfunction and without overt cardiovascular disease or diabetes do, in fact, have endothelial dysfunction.[23] Although there are many noncirculatory forms of erectile dysfunction—such as psychogenic, drug-induced, and other types—NO modulation helps patients who experience the condition as a result of circulatory problems.

NO and Neoplasia

The role of NO in neoplastic disease is controversial; research indicates that inducible NOS may have either negative or positive influences on metastasis, malignant transformation, and angiogenesis. NO derived from macrophages has a potentially cytotoxic action on malignant cells.[24] Studies indicate that tumor cells that produce high levels of NO undergo apoptosis. Induction of inducible NOS in sarcoma cells increases NO such that the tumors studied showed complete regression.[25]

NO and Various Other Conditions

Many other diseases have been shown to have a connection to either deficiencies or excesses in NO production. Ocular diseases—from cataracts to glaucoma to diabetic retinopathy—are among such NO-related conditions.[26,27] Other conditions include psoriasis, systemic sclerosis, Parkinson's disease, Alzheimer's disease, and diabetic nephropathy.[28–32] In addition, NO abnormalities may be correlated with diseases such as Huntington's disease, amyotrophic lateral sclerosis, migraine headache, hypertrophic pyloric stenosis, and muscular dystrophy.[33]

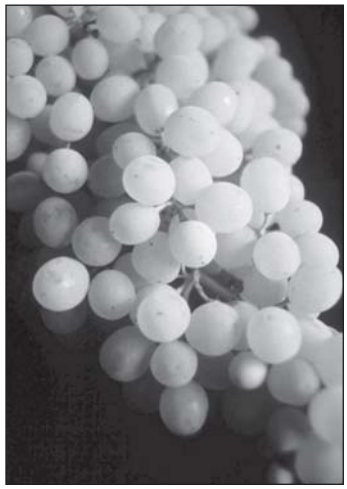
NO-Friendly Nutrients and Herbs

L-Arginine

L-Arginine is commonly found in red meat, dairy products, fish, and poultry. As previously mentioned, it is the substrate in the NOS pathway. Many studies have shown that supplementation with L-arginine improves small-vessel coronary endothelial function and promotes vasodilation in individuals with CAD or hypercholesteremia.[34,35] In addition, researchers have found that arginine supplementation improved blood pressure in patients with type 2 diabetes who also had mild hypertension.[36] L-Arginine is commonly taken at 3 g 3 times per day. Clinically, there remains a concern because of anecdotal evidence that arginine exacerbates herpes simplex and thus L-arginine is still commonly avoided as a treatment in the presence of herpes.

Folic Acid

Several studies have demonstrated that folic acid supplementation can improve endothelium-dependent vasodilation in individuals with CAD.[37,38] Studies have also suggested that folic acid may improve nitrate tolerance in individuals on continuous nitroglycerin as well as improving NOS function, possibly via regenerating tetrahydrobiopterin.[39] Folate inhibits intracellular superoxide production, which increases the half-life of NO, allowing for greater vasodilation. In addition, folic acid decreases homocysteine levels, which are an independent risk factor for cardiovascular disease.[40]



Grape (*Vinus vitifera*) cluster, left, and garlic (*Allium sativium*), right.

Vitamins B_6 and B_{12}

Vitamins B_6 and B_{12}, in addition to folic acid, will decrease homocysteine levels. High levels of homocysteine are suspected of causing endothelial damage, resulting in decreased NO release rates.[41,42]

Vitamin E

Vitamin E is a fat-soluble vitamin, found in grains, fruits, vegetables, and animal products, which has antioxidant and anti-inflammatory actions. Studies performed with mixed tocopherols have demonstrated that this form of vitamin E supplementation activates endothelial NOS, increases NO release, and decreases platelet aggregation in vivo. While alpha-tocopherol has also been shown to increase NOS activity and NO production, it has a less significant effect.[43]

N-Acetyl-L-Cysteine

N-acetyl-L-cysteine (NAC) is a thiol derived from the amino acid cysteine. It is the precursor to the potent antioxidant glutathione. NAC has been shown to directly decrease platelet aggregation by increasing the bioavailability of platelet NO.[44]

Pycnogenol

Pycnogenol® (Horphag Research, Ltd., Geneva, Switzerland) is an extract from French maritime pine bark (*Pinus maritima*). This product is commonly used to treat venous insufficiency, asthma, and hypertension. Pycnogenol has anti-inflammatory and antioxidant properties; it also decreases platelet aggregation and prevents oxidation of low-density

Dandelion (*Taraxicum officinale*).

lipoproteins.[45,46] Pycnogenol has been shown to increase NOS activity in endothelial cells in vitro, resulting in an increase in NO.[47]

Garlic

Garlic (*Allium sativum*) is frequently used for preventing atherosclerosis, hypertension, and hyperlipidemia, and as an antifungal agent and a cancer preventative.[48–50] Studies have suggested that garlic increases NO production. One study on rats showed that arterial hypertension caused by N omega-nitro-L-arginine-methyl-ester, which inhibits NOS, is prevented by garlic supplementation. Additionally, NO metabolites were measurably higher in the group treated with garlic, suggesting increased NO production.[51]

Grapes

Vitis vinifera is commonly known as the classic wine grape. Grapes and their juices are high in flavonoids, which are believed to give red wine its cardiovascular protective qualities. Research has shown that grape products such as grape juice and red wine increase NO release from platelets, and decrease platelet aggregation and superoxide production.[52]

More specifically, research on a constituent of grape skins and seeds known as resveratrol has produced effects on NO. Resveratrol has been demonstrated to have estrogen-like activity and antioxidant, antiplatelet, anti-inflammatory, and anticarcinogenic properties. Studies indicate that resveratrol upregulates the gene expression of endothelial NOS with a resulting increase in NO levels.[53]

American Ginseng

The root of American ginseng (*Panax quinquefolius*) is often used as a supplement because of its adaptogenic properties. It is often used for immune modulation and stress resistance, to treat diabetes and hormone imbalances, and as a stimulant. Studies have indicated that ginseng stimulates NO release in vitro.[54]

Artichoke

Cynara scolymus (or *Cynara cardunculus*) is commonly known as the artichoke. This plant lowers lipids, has antioxidant properties, and calms the digestive system. Luteolin and cynaroside are two flavonoids found in artichoke, which affect NO. These flavonoids increase NOS expression in endothelial cells.[55]

Quercetin

Quercetin is a citrus bioflavonoid used for treating conditions such as atherosclerosis, coronary heart disease, hypercholesterolemia, vascular insufficiency, diabetes, and allergies. Many studies on rats with diabetes have demonstrated that quercetin increases NO availability and induces vasorelaxation via the endothelial NOS pathway.[56,57]

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is an androgen made in the adrenal glands, liver, and testes. This androgen is converted to androstenedione, which is metabolized to other androgens and estrogen. Studies have suggested that DHEA decreases atherosclerosis via an NO-dependant system; researchers have measured increases in NO with DHEA supplementation. This may be partially explained by DHEA's conversion to estrogen.[58]

Melatonin

Melatonin has been shown to decrease mitochondrial NOS induction from bacteria as a result of lipopolysaccharides. It has been suggested that age-related decreases in melatonin may be correlated with the mitochondrial damage that increases with aging. However, this may protect the body from oxidative damage because large amounts of NO are produced in sepsis and shock.[59]

Herbs That Reduce NO Levels

A number of botanicals and nutrients have been shown to decrease—rather than increase—NO levels. Many of these are herbs known for their anti-inflammatory and antioxidant properties. They include:

- Green tea (*Camellia sinensis*), which is composed of multiple catechins such as epigallocatechin gallate, which is believed to provide much of the physiologic activity of this herb. This particular flavanol has been shown to inhibit NOS activity.[60]
- Devil's claw (*Harpagophytum procumbens*), which is often used to produce anti-inflammatory and analgesic effects. Studies have indicated that this botanical inhibits NOS expression.[61]
- Dandelion (*Taraxacum officinale*), which offers anti-inflammatory, diuretic, and digestive support among its many actions. Research has show that dandelion inhibits expression of the NOS enzyme as well as cyclo-oxygenase-2.[62]
- Beefsteak plant (*Perilla frutescens*), which is high in rosmarinic acid as is basil (*Ocimum basilicum*), mint (*Mentha* spp.), and sage (*Salvia* spp.). Rosmarinic acid is an antioxidant that has inhibited NO activity and NOS synthesis in macrophages.[63]

Pharmaceuticals That Affect NO Levels

While there are clearly a large number of natural therapies that can address NO dysfunctions, there are also pharmaceuticals that are aimed at the problem. Nitroglycerin, sodium nitroprusside, and other nitrates and nitrites increase NO in the endothelium and cause vasodilation of the arteries. These drugs are used to treat hypertensive crisis, angina pectoris, acute myocardial infarction, and heart failure.

Dysfunction in the nitric acid pathways can cause various disease processes.

Tadalafil (Cialis), vardenafil (Levitra), and sildenafil (Viagra) are pharmaceuticals prescribed for erectile dysfunction. They are phosphodiesterase inhibitors that cause vasodilation and hypotension. They are contraindicated for patients who are taking nitrates because NO increases cGMP by activating the enzyme guanyl cyclase and phosphodiesterase metabolizes cGMP. In other words, the inhibition of NO by pharmaceuticals may potentiate the effects of the nitrates.[64] It is prudent to use these pharmaceuticals cautiously, especially with patients who have leukemia, sickle-cell anemia, and other underlying health conditions.

Some studies have shown that NO may play a significant role in increasing the chemosensitivity of cancer cells. Via the cGMP pathway, NO may be used therapeutically to improve the efficacy of chemotherapeutic agents such as doxorubicin.[65]

Statin drugs—or 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors—are prescribed to lower cholesterol and decrease the risk of cardiovascular disease and stroke. Studies performed on the statin drug Mevastatin have shown that some of its cardioprotective benefits are the result of this drug's ability to increase endothelial NOS, which has been shown to improve cerebral blood flow in addition to relaxing vascular smooth muscle and inducing vasodilation.[66]

Steroid hormones, such as estrogen and DHEA, have also been shown to be cardioprotective and affect NO activity. Studies using 17 β-estradiol showed an increase in activity of endothelial NOS, which may at least partially explain the antiatherosclerotic effects of estrogen.[67]

Making NO-Positive Changes in Diet and Lifestyle

NO levels can be manipulated by dietary intake of specific nutrients such as folic acid, L-arginine, fish oil, and soy foods. The Mediterranean diet—which is high in vegetables, olive oil, red wine, and fish—has been shown to improve endothelial function as well.[68] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 fatty acids found in the oils of fish such as salmon, cod, and mackerel. These fatty acids compete with arachidonic acid in the lipo-oxygenase and cyclo-oxygenase pathways, leading to a decrease in inflammatory eicosanoids.[69–71] Fish oils high in DHA and EPA reduce the production of proinflammatory cytokines including interleukin-1, interleukin-2, and tumor necrosis factor.[72–74] Folic acid increases these fatty acids, which, in turn, increases endothelial NO.[8]

Cofactors for NOS such as vitamins B_2 and B_3 and folic acid will improve NO production. In addition, antioxidants such as vitamin C, vitamin E, glutathione, and α-lipoic acid improve NO levels by decreasing reactive oxygen species.[75] Vitamins B_6 and B_{12} can decrease cardiovascular risk factors further by decreasing homocysteine.

Physical activity has been shown to increase NO activity.[76] Studies have indicated that low-frequency exercise improves endothelial function as well as decreasing blood pressure (at least in patients with mild hypertension) and raising high-density lipoprotein levels.[77]

Conclusions

Dysfunction in the NO pathways can cause various disease processes. Natural therapeutics provide many ways to augment NO synthesis and bioavailability and improve overall health. Nutrients, diet, and botanical supplementation are proven methods for modulating NO and addressing health conditions that result from its deficiency. As with all health interventions, of course, treatment should be individualized to each patient. □

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