NOS Uncoupling and the Impact of Nitrate Supplementation

NOS Uncoupling

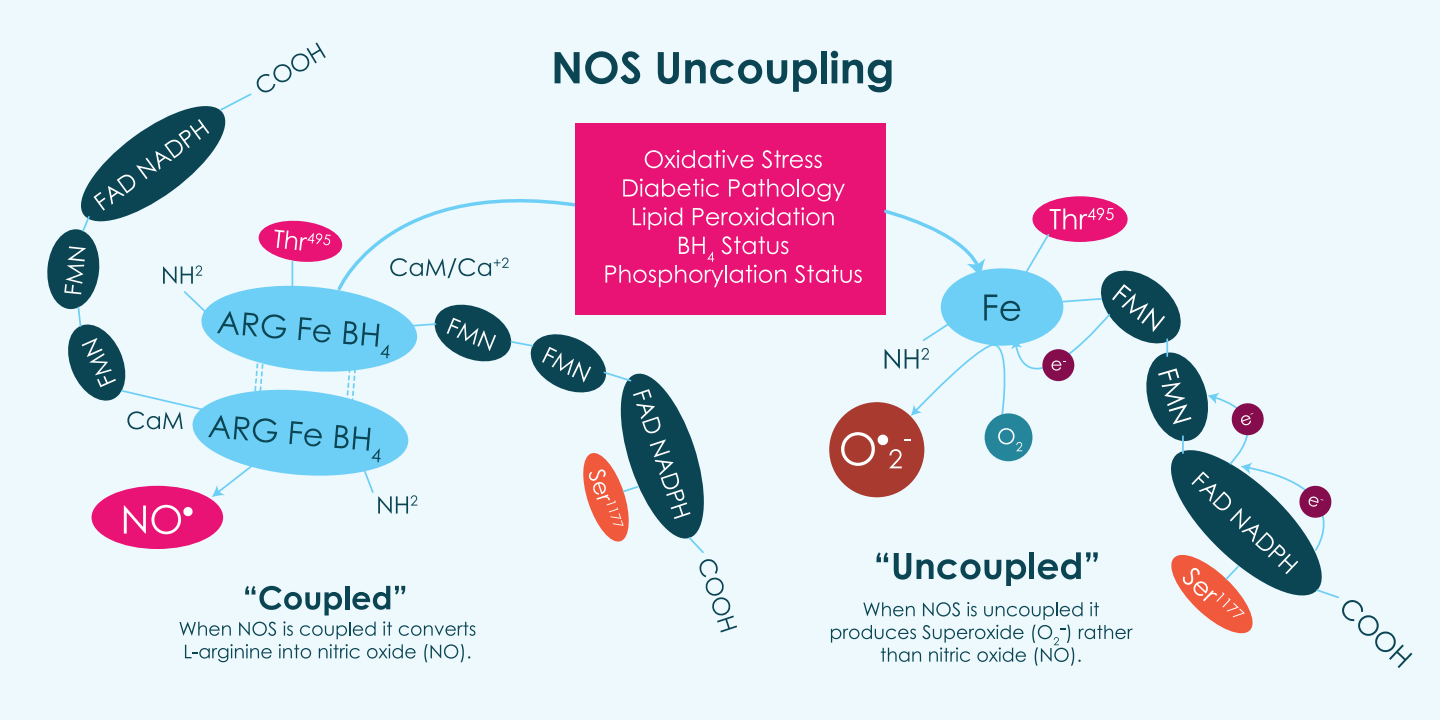
Under its standard, or 'coupled' state, nitric oxide synthase (NOS) converts L-arginine to nitric oxide (NO), with L-citrulline as a by-product. Nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen (O_2) are co-substrates in the reaction. The enzymatic action of NOS is also critically regulated by the cofactor tetrahydrobiopterin (BH4)[1].

However, an alternative synthesis process occurs in the L-arginine NOS pathway under certain biological conditions which cause BH4 levels to become depleted. These include genetic mutations, heavy metals like lead, mercury and aluminum, pollution, medications and oxidative stress. This causes NOS to generate superoxide (O_2^-) rather than NO due to changes in the NOS enzyme complex at the heme domain (Iron (Fe) as illustrated in Figure 1). The superoxide formed in these "uncoupled" NOS reactions can be converted to hydrogen peroxide or will scavenge NO to form peroxynitrite ($ONOO^-$).

This NO scavenging further decreases the available nitric oxide[2]. Single nucleotide polymorphism (SNP) in the MTHFR gene also contribute to BH4 deficiency, rendering individuals with these genetic variants NO deficient[3]. **These variants commonly affect at least 40% of individuals.**

NOS uncoupling plays a significant role in several diseases such as diabetes, hypercholesterolemia and hypertension[2]. NOS uncoupling can also occur as a concurrent adverse affect of medication such as birth control pills, NSAIDs and PPIs. In addition to this, antifungals such as imidazole and miconazole uncouple NOS by directly binding to the NOS enzyme complex heme group[4].

Figure 1



Lefer, David J. (2017). Nitric Oxide Biochemistry and Physiology. Research presented at the A4M Nitric Oxide: Applications for Clinical Practice meeting, Las Vegas, NV.

FAD: Flavin Adenine Dinucleotide
NADPH: Nicotinamide adenine dinucleotide phosphate
FMN: Flavin Mononucleotide
CaM: Calmodulin
CaM/Ca: Calmodulin/Calcium
ARG: L-arginine

FE: Iron
BH4: Tetrahydrobiopterin
Ser1177: Serine
Thr495: Threonine
NH2: Amine Group

NOS Uncoupling Exacerbates Oxidative Stress

The overproduction of reactive oxygen species (ROS), a category of free radicals, may lead to nucleic acid damage, inflammation and cell death. Accordingly, studies implicate ROS-mediated cellular damage in accelerated aging, cardiovascular disease, impaired cognitive function, diabetes and cancer.

NADPH oxidase (NOX) enzymes, present within cell membranes, are critical producers of ROS[5]. NOX is normally dormant but is activated during respiratory bursts forming superoxide. This is a normal a function of the immune system designed to kill bacteria and fungi. NOX can, however, be overstimulated by factors such as smoking, pollution, oxalates, histamine and mTOR.

These superoxides inactivate critical metabolic enzymes and can initiate lipid peroxidation, damage iron-sulphur clusters, liberate redox-active iron, and allows for generation of indiscriminate oxidants such as the hydroxyl (OH-) radical.

NADPH is also depleted by the over-stimulation of NOX. Amongst other things, NADPH is involved in protecting against the toxicity of ROS, allowing for the regeneration of gluthathione. When NADPH is depleted glutathione is unable to scavenge ROS[17].

Together, NOS uncoupling and increased NOX function result in a vicious cycle of ROS generation and oxidative damage. Several mechanisms link NOS uncoupling to oxidative damage, including:

- Decreased levels of NO, diminishing the body's antioxidant reserves
- Increased production of ROS such as superoxide and hydrogen peroxide, as well as reactive nitrogen species (RNS) like peroxynitrite
- Heightened superoxide-mediated metabolic enzyme inactivation and LDL oxidation
- Amplified oxidized LDL-mediated peroxynitrite formation and NOX activation, which further enhance ROS production[6]
- A reduction in the effect of the cellular NO
- Increased peroxynitrite-mediated BH4 oxidation, leading to BH4 deficiency and enhanced NOS uncoupling[7]

Aging Increases NOS Uncoupling

Production of NO significantly decreases during an individuals lifespan, with a 10-12% decline noted per decade. By age 40, NO production through the NOS pathway is reduced by a staggering 50%[9] and by the time we are 60 decreases to 15%. Importantly, NOS uncoupling is a significant driver of decreased NO production during aging. Other factors which affect NO production are poor diet, stress, sedentary lifestyle, pollution, genetic SNPs and medications.

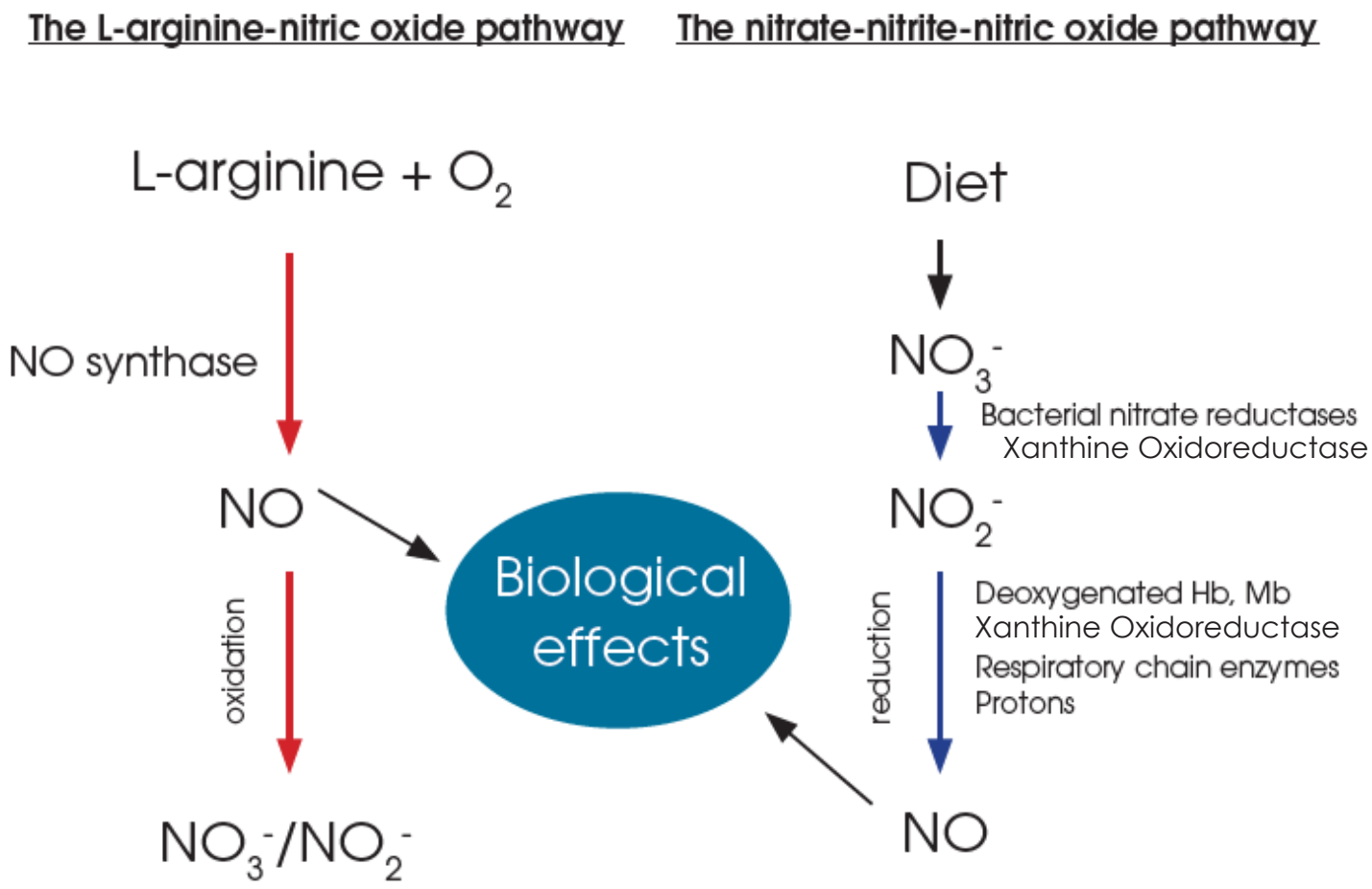
As the aging population experiences a heightened risk of hypertension, diabetes, cancer, neurodegeneration and arthritis, NOS uncoupling and impaired NO production may play an essential role in advancing these age related diseases. NOS uncoupling also limits the effectiveness of supplementation strategies utilizing the NOS pathway in the aging population, such as L-arginine based supplementation products.

NOS Uncoupling and NO Supplementation Strategies

Dietary L-arginine supplementation is a historically prevalent method to enhance NO production. However, this strategy relies exclusively on the L-arginine NOS pathway to work effectively, requiring NOS in its coupled state to efficiently generate NO. Recent research studies have highlighted the importance of an alternative route for NO generation, the nitrate-nitrite-NO pathway[1].

The nitrate-nitrite-NO pathway utilizes both dietary nitrate or endogenous nitrate/nitrite, which is produced as an end-product of the L-arginine NOS pathway. Dietary nitrates are converted to nitrites by the action of nitrate reductase enzymes produced by anaerobic oral bacteria. Once swallowed, nitrite is converted to NO in the acidic environment of the stomach, or absorbed into the bloodstream and converted by tissue nitrite reductases, like xanthine oxidoreductase, to NO.

Figure 2



NOS Uncoupling Limits the Efficacy of L-arginine Supplementation

Despite the popularity of L-arginine supplementation, **the L-arginine pathway is impaired during NOS uncoupling**. When NOS uncoupling intensifies during aging and inflammatory disease states, supplementing with L-arginine may stimulate superoxide rather than NO production in BH4-depleted NOS. The superoxide, together with peroxynitrite and hydrogen peroxide, subsequently exacerbate oxidative stress.

Importantly, cells do not use extracellular L-arginine to make NO. Instead, cells use intra-cellular L-citrulline to produce L-arginine, which is then funneled into the NOS enzyme pathway. Some studies also suggest that physiological concentrations of L-arginine are sufficient to saturate NOS, so L-arginine supplementation may not promote increased NOS activity and NO production[10].Therefore, L-arginine supplementation is not an ideal method to increase NO stores, particularly in the aging population and those with cardiovascular or other chronic diseases.

Nitrate Supplementation Effectively Bypasses NOS Uncoupling

By circumventing the L-arginine NOS pathway and utilizing the nitrate-nitrite-NO pathway, dietary nitrate promotes NOS-independent NO production. Via this mechanism, **nitrate supplementation can replenish NO stores, even under conditions of heightened NOS uncoupling.** Recent clinical and animal model studies have demonstrated that nitrate supplementation enhances vasodilation and reduces oxidative stress, arterial stiffness and organ injuries in models of kidney and cardiovascular disease[11,12,13].

In one such study, dose-dependent decreases in blood pressure and enhanced vasoprotection were observed after inorganic nitrate ingestion[14]. Dietary nitrates are also likely responsible for the health benefits of the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets, including their role in cardiovascular protection and increased longevity.

In addition to utilizing the nitrate-nitrite-NO pathway, dietary nitrate increases NO production and reduces ROS production through a variety of other mechanisms, including:

- Inhibiting NOX activation thereby increasing NADPH levels. This supports the cells' ability to decrease oxidative stress and prevents many chronic diseases.[15]
- Organic nitrates up-regulate GTP Cyclohydrolase I (GTPCH1) thus increasing BH4 biosynthesis from guanosine 5 triphosphate (GTP) [17]
- Preventing NOS uncoupling by inducing the activity of superoxide dismutase (SOD), an antioxidant protein that scavenges superoxide
- Increasing functional NO bioavailability by inducing heme oxygenase and decreasing serum xanthine oxidase activity[16]
- Suppressing free radical formation by scavenging both ROS and RNS, therefore decreasing oxidative stress

Key Points: NOS Uncoupling and Nitrate Supplementation

- Uncoupled NOS produces superoxide rather than NO, exacerbating oxidative stress
- NOS uncoupling is amplified during aging and chronic disease states
- L-arginine supplementation is not an effective method to increase NO stores in the aging population and those with chronic diseases
- **By circumventing the enzyme dependent NOS pathway, dietary nitrates are a safe and effective way to restore NO bioavailability, decrease oxidative damage and recouple NOS**

For more information on NOS uncoupling and the role of nitrate supplementation,
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Beth Shirley RPh CCN has developed expertise as a pharmacist and certified clinical nutritionist during a 40+ year career. Her specialties include stress-induced hormonal imbalance, intestinal dysfunction, autoimmune and chronic inflammatory issues, detoxification, and super-normal oxidative stress. Over the last nine years Beth has spent time working with some of the leading thought leaders in the world of nitric oxide research and through this has developed an in-depth knowledge on the topic and it's potential applications in Patient care. She currently is the Executive Director of the Berkeley Life Scientific Advisory Board.

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