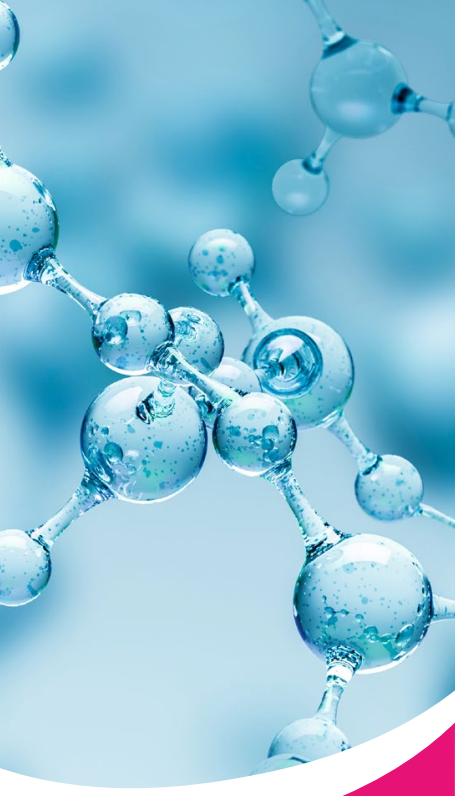

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UPDATED

# Nitric Oxide and Immune Health


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One aspect of immune health that often does not receive the attention it deserves is nitric oxide's role in the immune competence. The vulnerable populations in the current pandemic may have lower levels of endogenously produced nitric oxide (NO) generated through the nitric oxide synthase (NOS) enzyme system. Aging is the largest risk factor for severity and mortality in adult COVID-19 (C19). NO synthesis drops with age. Production of NO through NOS decreases to around 50% by the time we are 40 and to around 15% by the time we are 60. Furthermore, patients with chronic vascular inflammation, such as in type 2 diabetes, metabolic syndrome, chronic obstructive pulmonary disease, obesity, autoimmune disorders, and hemoglobinopathies may produce less NO.[1] These types of diseases are also the same underlying conditions tied to worse outcomes in C19. Further suggesting a connection between NO levels and C19 is the finding that increased risk of C19 death is significantly associated with disease cases that have lower vascular NO concentrations.[2]

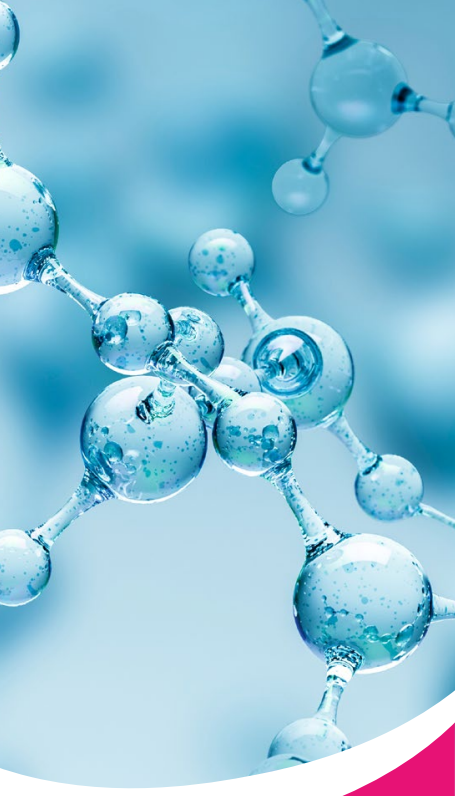
Other factors that impair the production of NO are the SAD (Standard American Diet) devoid of essential nutrients, cofactors, and lack of nitrate-rich vegetables, lack of exercise, medications such as antibiotics, antidepressants, birth control pills, NSAIDS, and proton pump inhibitors (PPIs), pollution, any process that increases oxidative stress, the herbicide glyphosate, genetic single nucleotide polymorphisms (SNPs) in NOS, impaired activity of superoxide dismutase (SOD) and catalase, methylenetetrahydrofolate reductase (MTHFR) polymorphisms, or glutathione levels, as well as stress, a particularly important factor involved in depletion of NO.[3] Each of these factors is highly common in today's world and may predispose to susceptibility to immune threats.

## Nitric Oxide's Role in the Immune Response

NO is a gasotransmitter, a gaseous neurotransmitter. It plays an important role in development of the innate immune response to many bacterial and viral threats. At the same time, it regulates vascular physiology.[4] NO plays an essential role in the function of the immune response as defense against infectious organisms, an inducer or suppressor of apoptosis, or an immunoregulator.[5] Inducible NOS (iNOS) or NOS2 production of NO is part of the immune response. NO is toxic to viruses, bacteria, fungi and other pathogens. NO regulates the functional activity, growth, and death of many immune and inflammatory cell types including macrophages, T lymphocytes, antigen presenting cells, mast cells, neutrophils, and natural killer (NK) cells.[5] NO may inhibit an early stage in viral replication and prevent viral spread and promote viral clearance and recovery. Likewise, NO donors have been shown to inhibit viral replication.[6]

## Clinical Data:

- **2004** – use of nitric oxide (NO) in patients suffering from SARS-CoV, reversed pulmonary hypertension, improved severe hypoxia and decreased duration of ventilation support.
- **2005** – endogenous production of NO stopped the SARS-CoV viral replication process. In fact, an 82% decrease in viral replication was observed[2].
- **2020** – NO gas inhalation for SARS-CoV study undertaken[3].
- UK & Israel using intranasal spray of nitric oxide to kill coronavirus



"Well-vascularized tissues are more resistant to infections and capable of localizing/containing offending agents. By contrast, poorly vascularized tissues are relatively inefficient in responding to inflammatory stimuli. This means that if you have good circulation and blood flow to every tissue in the body, then this allows your immune system to mobilize a strong defense against any invading pathogen. If you don't have good blood flow and circulation, the infection takes hold and makes you sick and can sometimes kill you. This is basic physiology. The regulation of blood flow and circulation is based on your ability to produce nitric oxide[9]."

*– Nathan S. Bryan, Ph.D*

## Promotion of Immunity and Vascular Integrity

In C19, there is impaired endothelial NOS (eNOS) or NOS3 production of NO, which governs the circulation and microcirculation. As noted earlier, increased risk of C19 death is significantly associated with disease cases that have lower vascular NO levels.[2] Emerging evidence suggests that the virus that causes C19 can affect the endothelial lining of the blood vessels. Severe cases of C19 are related to vascular damage with evidence of direct viral infection in the endothelial cells.[2] Furthermore, enhanced clotting and sluggish blood flow result in systemic hypoxia in oxygen-sensitive organs such as the kidneys. Oxygen is required for a functional NOS enzyme. Therefore, hypoxia decreases NO production through NOS.[7]

The lung is the beneficiary of the most blood flow of any organ of the body, so the potential for trouble to develop in the lung due to vascular insult is very high. Vasculature depleted of NO suffers from persistent inflammation and blunted delivery of oxygen and removal of toxic byproducts through stagnant blood flow into and out of hypoxic tissue.[1]

According to Nathan S. Bryan, Ph.D., "*Pathology is taught during the first and sometimes second year of medical school. The primary textbook used in many medical schools is Robbins Pathology. On page 58, it clearly states, 'Well vascularized tissues are more resistant to infections and capable of localizing/containing offending agents. By contrast, poorly vascularized tissues are relatively inefficient in responding to inflammatory stimuli.' This means that if you have good circulation and blood flow to every tissue in the body, then this allows your immune system to mobilize a strong defense against any invading pathogen. If you don't have good blood flow and circulation, the infection takes hold and makes you sick and can sometimes kill you. This is basic physiology. The regulation of blood flow and circulation is based on your ability to produce nitric oxide.*"[8]

## The Relationship Between Iron Dysregulation and Nitric Oxide

Iron dysregulation can negatively affect outcome in C19 with increased ferritin levels corelated with disease severity.[9] SARS-CoV-2 attacks the hemoglobin and oxidizes Fe2+, which then displaces the oxidized Fe3+ to become "cell-free heme." This cell-free heme is unable to carry oxygen. Hypoxia pathways are stimulated and this activates more virus as well as stimulating pro-inflammatory cytokines. Cell-free heme causes endothelial cell injury including in the lungs.[10] The resultant cell-free heme is scavenged by NO, essentially removing NO from the circulation and making NO non-bioavailable.[11] The rapid loss of NO leads to hypertension, coagulation, and systemic inflammation.[11] Additionally, loss of endogenous NO reduces the ability to suppress viral replication.[9] It is the main reason for the failure of the immune system.

## How Nitric Oxide Modulates Immunity

The lymphatic system is an essential part of the immune system for protecting the body from pathogens while keeping bodily fluids in balance. eNOS in lymphatic endothelial cells is necessary for strong lymphatic contractions under normal conditions but is not able to sustain these contractile motions during inflammation.[12] Suppression of lymphatic contraction is

linked to a decline in autoreactive responses to antigens.[12]

Nitrosation of reactive thiols on the surface of red blood cells (RBCs) and on the beta chain of the hemoglobin tetramer protects against hemolysis and oxidative damage, supporting the potential of NO to control the RBC-associated harmful processes occurring in SARS-CoV-2.[1]

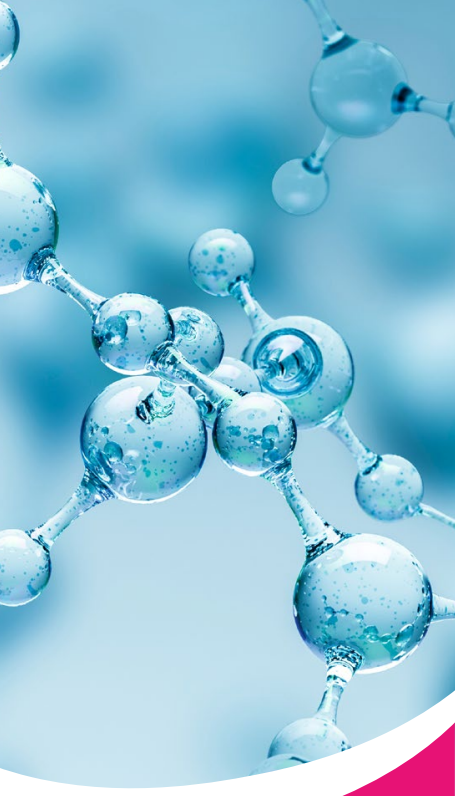
The stress response, no matter if it's physical, psychological, or emotional, increases cortisol production. Cortisol inhibits both iNOS (affecting the immune response) and eNOS (affecting the health of the cardiovascular system). Stress increases ROS in the mitochondria, from NADPH oxidase (NOX) and xanthine oxidase. This increased oxidative stress uncouples NOS leading to increased production of superoxide. Cortisol decreases synthesis of BH4 which increases NOS uncoupling even more. Additionally, cortisol decreases the membrane transport of l-arginine. All of these cortisol-mediated effects decrease the production of and/or bioavailability of NO.[13]

## Optimizing Nitric Oxide

During inflammatory states, NO supplementation blocks cytokine storm, restores the functional capillary density essential for oxygen delivery and removal of waste, stops hypoxia/reperfusion injury, and is protective of organs that are oxygen sensitive such as the kidneys.[1] Restoring NO/ROS balance may reduce cellular damage by reoxygenation and reperfusion. NO down-regulates inflammatory cytokines (NLRP3, IL1B, IL6, IL18) and decreases mast cell degranulation and histamine release. These are upregulated during exposure to SARS-CoV-2 and have everything to do with the severity of the disease.[14]

A good portion of our immune system is located in our intestinal tract. Therefore, supporting the health of our intestinal tract increases the health of our immune response. Addressing the gut microbiome using nitrate therapy and probiotic therapies might help decrease inflammatory response of viral pathogenesis and respiratory symptoms by strengthening the host immune system, ameliorating gut dysbiosis, and improving gut barrier function.[15] Nitrate helps support the health, richness, and diversity of the microbiomes. Nitrate, nitrite, and NO support the health of the intestinal mucus lining as well as mucous membranes throughout the body, our first defense against pathogens. Nitrate, nitrite, and NO also enhance mucosal blood flow and mucus thickness and suppress microbial infections.[16] Nitrate prevents the loss of tight junction proteins that control intestinal permeability and regulates events leading to inflammation.[16]

Another way in which NO is involved in immunity is through oxygen availability. NO synthesis by the NOS enzyme requires oxygen and is inhibited in hypoxic conditions. In lung damage that involves respiratory and/or metabolic acidosis—for example, in Acute Respiratory Response Syndrome (ARDS)—a reduced local pH in the injured



capillary may allow for the reduction of nitrite to NO because that pathway is stimulated under hypoxic and acidic conditions.[17] Therefore, nitrate and nitrite that regulate NO bioavailability are worth considering both for clinical treatment and prevention of C19.[17]

Restoring NO through the nitrate/nitrite/NO pathway, independent of eNOS, may prevent endotheliitis and play a role in pulmonary vasodilation as well as suppress thrombosis and viral activity.[18] NO and NO donors show promise in treating viral conditions due to their combined role as antimicrobials and antithrombotics.[4] NO-based therapeutic strategies are highly beneficial in direct combat of viruses and mitigation of secondary infections that occur in conjunction with viral exposure.[4]

Furthermore, oral supplementation of nitrate is protective of endothelial function and resulted in significant protection against ischemia/reperfusion (I/R) damage in the myocardial, hepatic, renal, pulmonary, and cerebral vascular system.[19] In multiple studies, dietary inorganic nitrate was effective at re-establishing endothelial function, ameliorating pulmonary and arterial hypertension, and encouraging antimicrobial activity.[18]

Adusumilli and colleagues proposed that delivering NO "at the early stages of C19 infection might limit the progression toward ARDS and fulminant systemic failure, particularly in vulnerable patients who may have decreased levels of endogenous NO due to increased age or comorbid conditions. This approach should decrease viral replication, downregulate ACE, prevent the onset of any hypoxia-reoxygenation/ischemia reperfusion-based inflammation, control the cytokine cascade, allow for removal of cell debris, limit lipid peroxidation and concomitant cell damage, reduce detrimental vascular permeability, and maintain proper blood flow."[1]

## Benefits of Nitric Oxide Plus Ascorbic Acid

Ascorbic acid can work synergistically with NO.[20] Ascorbic acid can reverse or inhibit the damage from cell-free heme in lung tissue.[21] Ascorbic acid also is the only molecule that can reduce BH3 back to BH4, a rate-limiting cofactor in the production of NO through the NOS pathway. Without sufficient ascorbic acid, BH3 oxidizes to BH2 to uncouple NOS, increasing superoxide production. Nitrate upregulates GTP Cyclohydrolase, increasing synthesis of BH4 to recouple NOS.[3] Ascorbic acid also supports the production of NO through the nitrate/nitrite/NO pathway.

## Conclusion

Optimal NO is essential for prevention as well as for recovery from C19. Numerous factors, including aging, inhibit the NOS enzyme production of NO, leading to an impaired immune response and cardiovascular complications. Nitrate and nitrite positively modulate NO bioavailability. Therefore, supporting the nitrate to nitrite to NO pathway through a nitrate-rich diet or nitrate supplement which not only increases NO production outright, but also helps recouple the NOS enzyme, decreasing oxidative stress and increasing NO.[3] This can lead to a robust antiviral response as well as supporting lung and cardiovascular health.

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