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Nitric oxide and geriatrics: Implications in diagnostics and treatment of the elderly

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Abstract

The nation's aging population is growing rapidly. By 2030, the number of adults age 65 and older will nearly double to 70 million. Americans are living longer and older adults can now live for many years with multiple chronic illnesses but with a substantial cost to health care. Twenty percent of the Medicare population has at least five chronic conditions i.e., hypertension, diabetes, arthritis, etc. Studies in experimental models and even humans reveal that constitutive production of nitric oxide (NO) is reduced with aging and this circumstance may be relevant to a number of diseases that plague the aging population. NO is a multifunctional signaling molecule, intricately involved with maintaining a host of physiological processes including, but not limited to, host defense, neuronal communication and the regulation of vascular tone. NO is one of the most important signaling molecules in our body, and loss of NO function is one of the earliest indicators or markers of disease. Clinical studies provide evidence that insufficient NO production is associated with all major cardiovascular risk factors, such as hyperlipidemia, diabetes, hypertension, smoking and severity of atherosclerosis, and also has a profound predictive value for disease progression including cardiovascular and Alzheimers disease. Thirty plus years after its discovery and over 13 years since a Nobel Prize was awarded for its discovery, there have been no hallmark therapeutic breakthroughs or even NO based diagnostics. We will review the current state of the science surrounding NO in the etiology of a number of different diseases in the geriatric patient. From these observations, it can be concluded that enzymatic production of NO declines steadily with increasing age in healthy human subjects. Implementing strategies to diagnose and treat NO insufficiency may provide enormous benefit to the geriatric patient.

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1 Introduction

The mammalian biosynthesis of nitric oxide (NO) discovered in the 1980's for its roles in the immune,[1,2] cardiovascular[3–5] and nervous[6] systems established a startling new paradigm in the history of cellular signaling mechanisms. Prior to this discovery, NO was widely recognized as a toxic molecule: a common air pollutant, a constituent of cigarette smoke, and a toxic gas, which appears in the exhaust of motor cars and jet airplanes, causes acid rain, and destroys the ozone layer. Thus, it was essentially inconceivable that cells would intentionally produce a toxic gas as part of normal physiology. NO is now recognized as one of the most important signaling molecules in the body, and is involved in virtually every organ system where it is responsible for modulating an astonishing variety of effects. The primary targets for NO are metals and thiols. NO can bind to soluble gualylyl cyclase (sGC) and cause an increase in second messenger cGMP,[3] and mediate a number of physiological functions. This pathway was considered the basis of NO based signaling until it was recognized that NO elicited a number of physiological and biological effects that were not dependent upon cGMP. It is now recognized that NO can react directly with thiyl radicals to form nitrosothiols or other reaction products of NO *i.e.*, nitrite, N_2O_3, N_2O_4, which can post-translationally modify thiols to affect protein structure and function.[7] NO has been shown to be involved in and affect practically every organ system in the body.[8] One can then imagine a host of diseases or conditions and multi-systemic symptoms may be caused or affected by the body's dysregulation of NO production/signaling (Figure 1).

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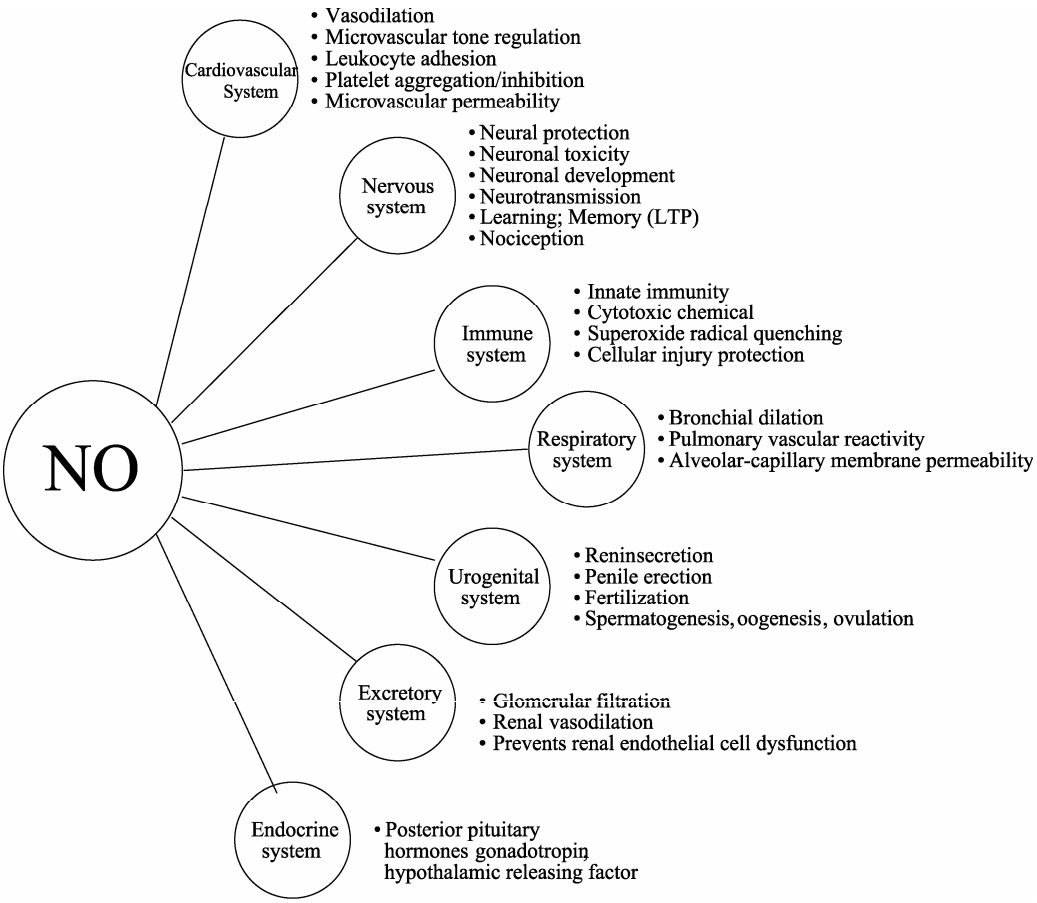

Figure 1. An overview of nitric oxide (NO) in various physiological, biochemical and pathological systems.

Maintaining NO homeostasis is critical for optimal health and disease prevention. Developing novel NO based diagnostics and therapies is central to better patient care, especially in the geriatric patient.

2 Nitric oxide production pathways

The first pathway to be discovered for the endogenous production of NO was involving *L*-arginine,[2] from a group of enzymes call nitric oxide synthase (NOS). NOS enzymes produce NO by catalyzing a five electron oxidation of the guanidino nitrogen of *L*-arginine. Oxidation of *L*-arginine to *L*-citrulline occurs via two successive mono-oxygenation reactions producing N^G-hydroxy-*L*-arginine as an intermediate. Two moles of O_2 and 1.5 moles of nicotinamide adenine dinucleotide phosphate (NADPH) are consumed per mole of NO formed.[9] NOS enzymes are the only enzymes known to simultaneously require multiple bound cofactors/ prosthetic groups: flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, glutathione, NADPH, tetrahydrobiopterin (BH_4) and Ca^{2+}-calmodulin. There are three isoforms of NOS, the genetic sequence of each residing on three distinct chromosomes. One type is constitutive, Ca^{2+}/calmodulin dependent and releases NO for short time periods in response to receptor or physical stimulation. NO released by this enzyme acts as a transduction mechanism underlying several physiological responses. The other enzyme type is induced after activation of macrophages, endothelial cells and a number of other cells by cytokines and once expressed, synthesizes NO for long periods of time. Furthermore, this enzyme is Ca^{2+} independent since calmodulin is already bound to the enzyme, and its induction is inhibited by glucocorticoids.[8] Endothelial NOS (eNOS), neuronal NOS (nNOS) which are both constitutively expressed in mammalian cells have now been well characterized in the cardiovascular system and nervous system respectively, and an inducible NOS (iNOS) which was first believed to be expressed only when activated by an immune response. Now it is appreciated that eNOS is found in other cells and tissues besides the endothelium, iNOS is found constitutively in some tissues, and there are inducible forms of both eNOS and nNOS,

adding confusion to the nomenclature as it was first described. In an attempt to clarify the nomenclature, the three different isoforms are now commonly referred to as NOSI, NOSII, and NOSIII for neuronal, inducible and endothelial isoforms, respectively, based on the order in which they were first purified and cloned.

For years, scientists and physicians have investigated *L*-arginine supplementation as a means to enhance NO production. This strategy has been shown to work effectively in young healthy individuals with functional endothelium or in older patients with high levels of asymmetric dimethyl *L*-arginine (ADMA) where the supplemental *L*-arginine could outcompete this natural inhibitor of NO production. Patients with endothelial dysfunction, however, by definition, are unable to convert *L*-arginine to NO and, therefore, this strategy has failed in clinical trials. Schulman *et al*.[10] found that *L*-arginine, when added to standard postinfarction therapies, did not improve vascular stiffness measurements or ejection fraction and was associated with higher postinfarction mortality. *L*-arginine should not be recommended following acute myocardial infarction (MI). However, there are also a number of studies showing benefit to patients taking *L*-arginine just as many showing no benefit, no harm.[11]

Understanding the complex and complicated reaction pathway for NOS mediated production of NO from *L*-arginine helps us define the context for rational interventions. Using *L*-arginine supplementation therapy alone may not be effective due to oxidative stress in geriatric patients resulting in constitutive NOS uncoupling by redox-based post translational modifications. Supplementing *L*-arginine with anti-oxidants to prevent oxidation of reduced co-factors such as BH_4, might prevent NOS uncoupling and lead to better results. In a study by Taddei *et al*.[12], the role of oxidative stress on NO availability and endothelial dysfunction was examined in both younger and older aged populations. They found that NO availability was profoundly restored in older patients when oxidative stress is removed by antioxidants such as vitamin C. In older individuals (age > 60 years) characterized by a profound alteration in NO availability, vitamin C not only enhanced the response to the endothelial agonist but also restored the inhibitory effect of *L*-NMMA on vasodilation to acetylcholine. Although, it is demonstrated that anti-oxidant supplementation can be very beneficial for those experiencing oxidative stress and endothelial dysfunction, it showed no benefit in younger (age < 60) or healthy individuals with no endothelial dysfunction. Collectively, the literature suggests that strategies to enhance NO production through *L*-arginine supplementation are equivocal at best.

Although the *L*-arginine-NO pathway was the first to be discovered, it does not necessarily mean it is the primary pathway for the endogenous production of NO. In fact nitrogen cycling in bacteria and production of NO as an intermediate in denitrification may be one of the most primitive pathways known, dating back to the Archaean era.[13] The now recognized human nitrate-nitrite-nitric oxide pathway that still relies on bacteria may be a redundant system for overcoming the body's inability to make NO from *L*-arginine.[14] It appears that we have at least two systems for affecting NO production/homeostasis. The first is through the classical *L*-arginine-NO pathway. This is a complex and complicated five-electron oxidation of *L*-arginine and if any of the co-factors become limiting, then NO production from NOS shuts down, and in many cases, NOS produces superoxide instead.[15] The enzymatic production of NO normally proceeds very efficiently. However, in disease characterized by oxidative stress where essential NOS cofactors become oxidized, NOS uncoupling, or conditions of hypoxia where oxygen is limiting, this process can no longer maintain NO production.[16] This process is illustrated in Figure 2. Therefore, one can argue saliently that there has to be an alternate route for NO production. It is highly unlikely that nature devised such a sophisticated mechanism of NO production as a sole source of a critical molecule. This alternate route involves the provision of nitrate and nitrite reductively recycled to NO (Figure 3). The two-electron reduction of nitrate to nitrite occurs through symbiosis with facultative anaerobic bacteria that reside in the crypts of our tongue.[17] Nitrite reduction to NO can occur in a much simpler mechanism than nitrate. The 1-electron reduction of nitrite can occur by ferrous heme proteins (or any redox active metal) through the following reaction: $NO_2^- + Fe^{(II)} + H^+ \leftrightarrow NO + Fe^{(III)} + OH^-$.

This is the same biologically active NO as that produced by NOS, with nitrite rather than *L*-arginine as the precursor and is a relatively inefficient process.[18] Much of the recent focus on nitrite physiology is due to its ability to be reduced to NO during ischemic or hypoxic events.[19–21] Nitrite reductase activity in mammalian tissues has been linked to the mitochondrial electron transport system,[22,23] protonation,[20] deoxyhemoglobin,[24] and xanthine oxidase.[25,26] Therefore, for this reaction to occur, the tissues or biological compartment must have a sufficient pool of nitrite stored. Since plasma nitrite is a direct measure of NOS activity,[27] a compromised NOS system can also affect downstream nitrite production and metabolism, which can perhaps exacerbate any condition associated with decreased NO bioavailability. Considerable published data support the notion that exogenous nitrite contributes to whole body NO

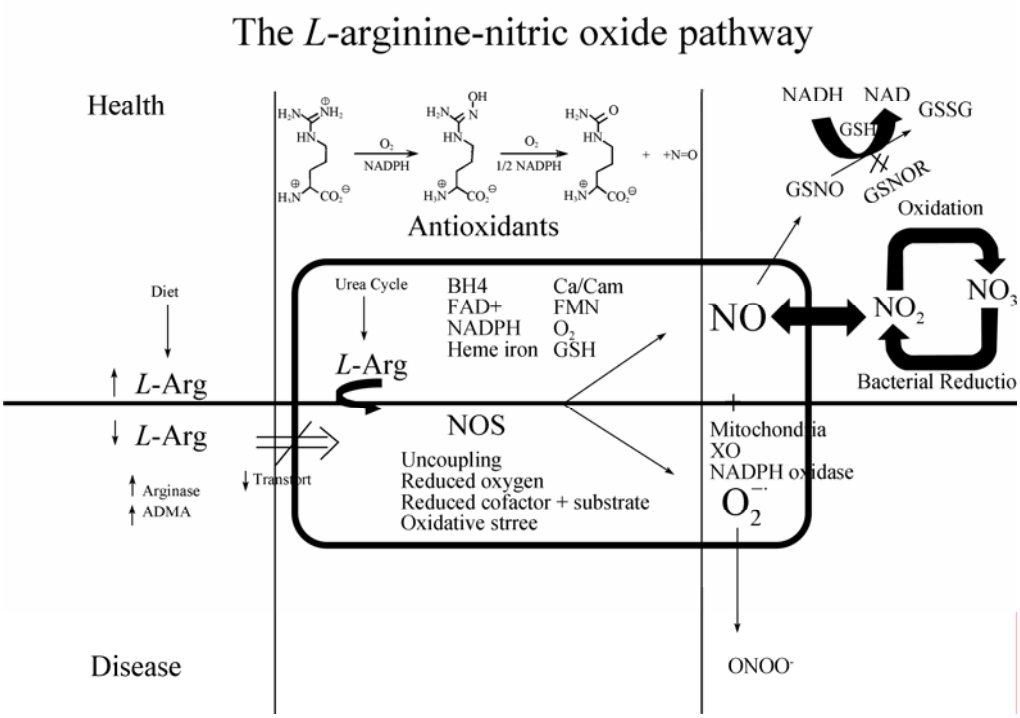

Figure 2. NO production and biochemistry. There are a number of critical steps for the NOS production of NO from *L*-arginine. Under healthy conditions (top), enzymatic function proceeds normally. Under disease conditions (bottom), there can be a number of problems with *L*-arginine availability, transport and conversion to NO due to enzyme uncoupling or insufficient co-factor availability. Once produced, NO can form nitrosothiols or become oxidized to nitrite and nitrate which now recognized can be recycled to regenerate NO. NO: nitric oxide; NOS: nitric oxide synthase; FAD: flavin adenine dinucleotide; FMN: flavin mononucleotide; GSH: glutathione; GSSG: oxidized glutathione; GSNO: nitrosoglutathione; NADPH: nicotinamide adenine dinucleotide phosphate; ADMA: asymmetric dimethyl *L*-arginine.

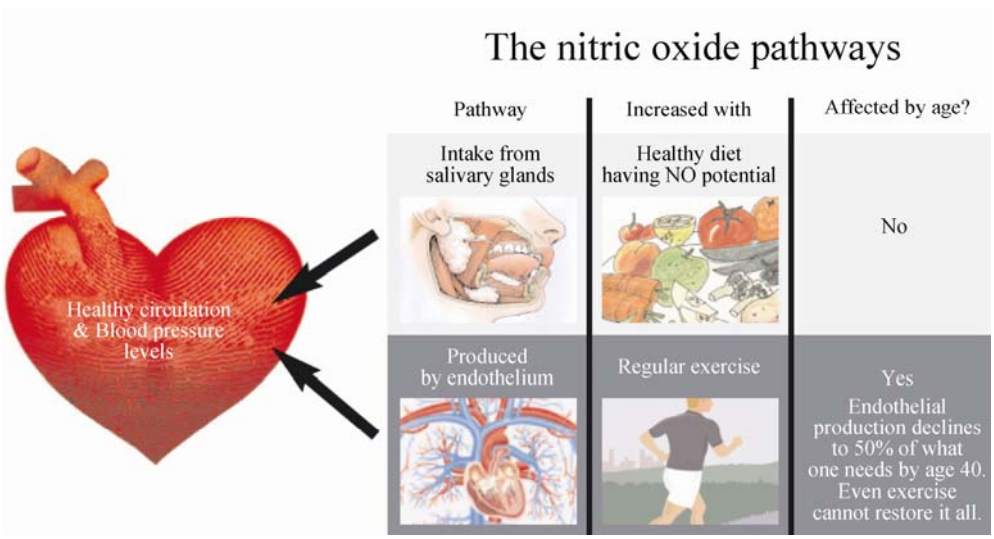

Figure 3. Two pathways for endogenous nitric oxide (NO) production. The *L*-arginine NO pathway can be enhanced through regular exercise, which becomes dysfunctional with age. The dietary pathway through reduction of nitrate and nitrite is not affected by age but is dependent on specific foods and diets. Both systems work in concert to maintain NO homeostasis.

production: NO produced from nitrite in the upper intestine is up to 10,000 times the concentrations that occur in tissues from enzymatic synthesis,[28] nitrite can act as a circulating NO donor,[29] and nitrite can itself perform many actions

previously attributable to NO[30] without the intermediacy of NO.[31] Experiments in primates revealed a beneficial effect of long-term application of nitrite on cerebral vasospasm.[32] Moreover, inhalation of nitrite selectively dilates the pulmonary circulation under hypoxic conditions *in vivo* in sheep.[33] Topical application of nitrite improves skin infections and ulcerations.[34]

Replenishing nitrate and nitrite through dietary means may then act as a protective measure to compensate for insufficient NOS activity under conditions of hypoxia or in a number of conditions characterized by NO insufficiency. Since a substantial portion of steady state nitrite concentrations in blood and tissue are derived from dietary sources,[31] modulation of nitrite and/or nitrate intake may provide a first line of defense for conditions associated with NO insufficiency.[21] The recognition of this mammalian nitrogen cycle has led researchers to explore the role of dietary nitrate and nitrite in physiological processes that are known to be regulated by NO.[35] Nitrite can transiently form nitrosothiols (RSNOs) under both normoxic and hypoxic conditions[19,31] and a recent study by Bryan *et al*.[31] demonstrates that steady state concentrations of tissue nitrite and nitroso are affected by changes in dietary nitrite and nitrate (collectively, NOx) intake. Furthermore, enriching dietary intake of nitrite and nitrate translates into significantly less injury from heart attack.[36] Previous studies demonstrated that nitrite therapy given intravenously prior to reperfusion protects against hepatic and myocardial ischemia/reperfusion (I/R) injury.[37] Additionally, oral nitrite has also been shown to reverse *L*-NAME induced hypertension and serve as an alternate source of NO *in vivo*.[38] These results have since been corroborated in humans. In fact, it has been reported that dietary nitrate reduces blood pressure in healthy volunteers.[39,40] Commercial development of nitrite and nitrate enriched dietary supplements has been shown to impact important cardiovascular risk factors in the aging population leading to a reduction in triglycerides and restoration of NO homeostasis.[41] Furthermore, in the stomach, nitrite-derived NO seems to play an important role in host defense[42] and in regulation of gastric mucosal integrity.[43] However, this is pH dependent. Since stomach acid production declines with age and many patients are prescribed proton pump inhibitors, this pathway may be disrupted in the geriatric patient causing additional problems with maintaining NO homeostasis.

Nitrite and nitrate therapy may then offer an all natural, over the counter and cost effective regimen for conditions associated with NO insufficiency. This has the potential to provide the basis for new preventive or therapeutic strategies and new dietary guidelines for optimal health. From a public health perspective, we may be able to make better recommendations on diet and dramatically affect the incidence and severity of cardiovascular disease and the subsequent clinical events.

3 NO diagnostics

The major pathway for NO metabolism is the stepwise oxidation to nitrite and nitrate. For years, both nitrite (NO_2^-) and nitrate (NO_3^-) have been used as surrogate markers of NO production in biological tissues, but there have not been any new developments in the use of NO biomarkers in the clinical setting for diagnostic or prognostic utility. In fact, NO status is still not part of the standard blood chemistry routinely used for diagnostic purposes. This is simply unacceptable given the critical nature of NO in many disease processes and new technologies should be developed.[44] The only true measure of endothelial NO production (endothelial function) is through flow mediated dilatation (FMD). FMD is a non-invasive ultrasound-based method where arterial diameter is measured in response to an increase in shear stress, which causes release of NO from the endothelium and consequent endothelium dependent dilatation. FMD has been shown to correlate with invasive measures of endothelial function, as well as with the presence and severity of the major traditional vascular risk factors.[45] Nitrite and nitrate have recently been shown to be biomarkers for cardiovascular and other diseases from both diagnostic and therapeutic aspects.[46] However, it is not known if levels of NOx correlate with FMD. In addition to blood, urinary levels of NOx provide a means to assess systemic NO production *in vivo*, or renal handling of these anions which may be compromised in the geriatric patient.[47] A report by Kleinbongard *et al*.[48] demonstrated that plasma nitrite levels in humans progressively decrease with increasing cardiovascular risk load. Risk factors included age, hypertension, smoking, and hypercholesterolemia, conditions all known to reduce the bioavailability of NO. Although a correlation exists in the plasma, it is not known whether the situation is mirrored in the heart or other tissue of interest in specific disease. The recent recognition of a human nitrogen cycle whereby nitrate and nitrite are reduced to NO by an enterosalivary circulation of nitrate[14] now opens up the potential for using saliva as a potential biomarker for NO status in certain diseases.

4 Aging and NO production

Aging and hypertension are well-documented cardio-

vascular risk factors.[49,50] Most of the functional and structural vascular alterations that lead to cardiovascular complications are similar in aging and hypertension.[51] Moreover, these vascular changes associated with essential hypertension are generally considered to be an accelerated form of the changes seen with aging.[52] When we are young and healthy, the endothelial production of NO through *L*-arginine is efficient and sufficient; however, as we age we lose our ability to synthesize endothelial derived NO. Most of the works on the activity of NO in cells and tissues agree that the bioavailability or the generation of NOS derived NO decreases with aging. It has been proposed that superoxide can scavenge NO to form peroxynitrite and thereby reduce its effective concentrations in cells.[53] It has also been reported that there is decreased NOS expression with aging both in constitutive and inducible isoforms.[54,55] Berkowitz *et al.*[56] observed the upregulation of arginase (an enzyme that degrades the natural substrate for NOS, *L*-arginine) in aged blood vessels and the corresponding modulation of NOS activity. Taddei *et al.*[12] have shown that there is a gradual decline in endothelial function due to aging with greater than 50% loss in endothelial function in the oldest age group tested as measured by forearm blood flow assays. Egashira *et al.*[57] reported more dramatic findings in the coronary circulation of aging adults whereby there was a loss of 75% of endothelium-derived nitric oxide in 70-80 year old patients compared to young, healthy 20 year olds. Vita *et al*.[58] demonstrated that increasing age was one predictor of abnormal endothelium-dependent vasodilation in atherosclerotic human epicardial coronary arteries. Gerhard *et al*.[59] concluded from their 1996 study that age was the most significant predictor of endothelium-dependent vasodilator responses by multiple stepwise regression analysis. Collectively, these important findings illustrate that endothelium-dependent vasodilation in resistance vessels declines progressively with increasing age. This is illustrated in Figure 4. This abnormality is present in healthy adults who have no other cardiovascular risk factors, such as diabetes, hypertension, or hypercholesterolemia. Most of these studies found that impairment of endothelium-dependent vasodilation was clearly evident by the fourth decade. In contrast, endothelium-independent vasodilation does not change significantly with aging, demonstrating that the responsiveness to NO does not change only the ability to generate it. These observations enable us to conclude that reduced availability of endothelium-derived NO occurs as we age, and to speculate that this abnormality may create an environment that is conducive to atherogenesis and other vascular disorders, including Alzheimers disease. It appears

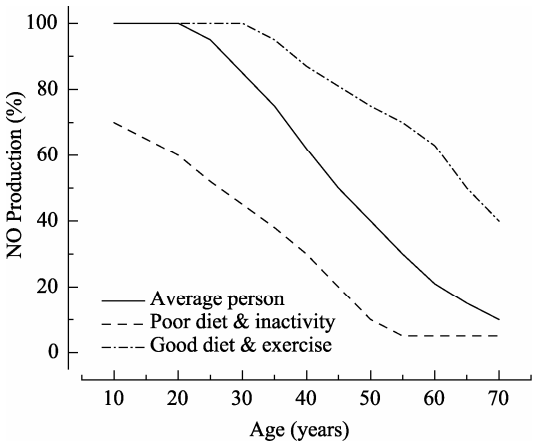

Figure 4. Hypothetical representation of nitric oxide (NO) production based on diet and lifestyle.

that aging interrupts NO signaling at every conceivable level, from production to inactivation. Given that NO is a necessary molecule for maintenance of health and prevention of disease, restoration of NO homeostasis may provide a new treatment modality for age and age related disease.

5 Consequences of NO insufficiency in the aging population

Aging is considered the single largest risk factor related to cardiovascular related diseases and deaths. Cardioprotection decreases with increasing age and is attributed to a decline in NO. The lack of NO production can lead to hypertension, atherosclerosis, peripheral artery disease, heart failure, and thrombosis leading to heart attack and stroke, the leading cause of death for all Americans, especially in geriatrics. Remarkably, all of these conditions have been shown to be positively affected by dietary nitrite and nitrate interventions.[14,35,60]

5.1 Hypertension and NO

Hypertension, along with aging is a well-known cardiovascular risk factor that leads to functional and structural alterations in the heart and vasculature.[12] Vascular changes associated with hypertension, such as endothelial dysfunction, are an accelerated form of the type of changes seen with aging.[12] Additionally, the presence of acetylcholine alongside a NO synthase inhibitor (*L*-NMMA) was tested for NO availability in the vasculature. A noteworthy finding is that after the age of 60 years old, the inhibiting effect of L-NMMA on response to acetylcholine was extremely weak, suggesting that NO availability is completely compromised in older populations. These results indicate that essential hypertension is characterized by an age-related

reduction of endothelial function by mechanisms that appear to be similar to those observed in older normotensive individuals. NO based therapies can reduce blood pressure. Transdermal nitroglycerin has been shown to reduce blood pressure in patients with recent stroke.[61] Dietary intervention with nitrite and nitrate has been shown to modestly reduce blood pressure in humans[39–41,62] showing remarkable efficacy using this approach.

5.2 Atherosclerosis and NO

Atherosclerosis is the major source of morbidity and mortality in the developed world. The magnitude of this problem is profound, as atherosclerosis claims more lives than all types of cancer combined and the economic costs are considerable.[63] Reduced NO availability is a hallmark of atherosclerosis. The endothelium-derived NO plays a crucial role in regulating a wide spectrum of functions in the cardiovascular system, including vasorelaxation, inhibition of leukocyte-endothelial adhesion, vascular smooth muscle cell (SMC) migration and proliferation, as well as platelet aggregation.[8] The concept of endothelial dysfunction arises from variations in blood flow observed in patients with atherosclerosis compared with healthy subjects. In healthy subjects, activation of eNOS causes vasodilation in both muscular conduit vessels and resistance arterioles. In contrast, in subjects with atherosclerosis, similar stimulation yields attenuated vasodilation in peripheral vessels and causes paradoxical vasoconstriction in coronary arteries, thus indicating a decrease in the production and/or bioavailability of NO.[64,65] Interestingly, endothelial dysfunction can be demonstrated in patients with risk factors for atherosclerosis in the absence of atherosclerosis itself.[66,67] These observations lend credence to the concept that endothelial dysfunction is integral to the development and progression of disease. Impaired endothelium may abnormally reduce vascular perfusion, produce factors that decrease plaque stability, and augment the thrombotic response to plaque rupture.[68] There are a number of studies showing that insufficient NO production from the endothelium is associated with all major cardiovascular risk factors, such as hyperlipidemia, diabetes, hypertension, smoking and severity of atherosclerosis, and importantly also has a profound predictive value for the future atherosclerotic disease progression.[69–72] Augmentation of NO or restoration of NOS function seems a logical means to inhibit atherosclerosis. Absence of eNOS in apoE-knockout mice accelerates atherosclerosis that is not caused by hypertension.[73,74] Paradoxically, however, overexpression of endothelial NOS accelerates lesion formation in apoE-deficient mice[75] demonstrating that enhanced NOS derived NO may not always be beneficial. Supplementation with tetrahydrobiopterin (BH4) reduced the lesion size to those seen in Apo E knockout mice revealing the requirement of enzyme cofactors. Even with BH4 supplementation there was still no effect on lesion development. These studies demonstrate the complexity of endothelium derived NO in the setting of atherosclerosis but clearly illustrate the dysfunctional eNOS/NO pathway as an early marker or a common mechanism for various cardiovascular disorders and therefore provides an ideal target for therapeutic or preventive intervention including alternative NOS independent sources of NO. Stokes *et al*.[76] have demonstrated that supplementing nitrite in the drinking water inhibits the adhesion and emigration of leukocytes to the vascular endothelium, one of the earliest events of atherogenesis suggesting this nitrate-nitrite-NO pathway may be useful in preventing chronic vascular disease.[77]

5.3 Thrombosis and NO

Thrombosis affects nearly 1 million patients in the United States annually. Out of those million, nearly 300,000 are reported as thrombosis related deaths. A majority of current treatment options are centered on preventative anticoagulants, such as warfarin but with risk of bleeding. NO inhibits platelet activation, adhesion, and aggregation by influencing several signaling pathways, including activation of sGC and increasing intracellular cGMP;[78] inhibition of phosphatidylinositol-3 kinase;[79] and inhibition of capacitative cation influx and agonist-dependent increases in intracellular calcium.[80] These molecular events lead to an impairment of platelet activation, adhesion, secretion, fibrinogen-binding to glycoprotein lib/IIIa,[81] and, ultimately, aggregation. In addition, NO promotes platelet disaggregation. In conjunction with its vasorelaxing actions, these anti-platelet effects of NO maintain blood fluidity and tissue perfusion. A constitutive NO synthase has been found in both human platelets and megakaryocytic cells[82] and this isoform is active[83]. Using an NO-selective microelectrode adapted to a platelet aggregometer, Freedman *et al*.[84] recently showed that this platelet-derived NO not only modestly modulates platelet activation to strong and weak agonists but, more importantly, markedly inhibits platelet recruitment to the growing platelet thrombus. NO, derived both from the endothelial cell and the platelet, modulates platelet activation, adhesion, and aggregate formation, thereby serving as an important deterrent to platelet-mediated arterial thrombosis.[85] NO insufficiency, either through reduced production or oxidative inactivation leads to thrombotic events.[85] Efforts to restore the normal vascular redox balance and/or to restore normal NO availability may

provide one therapeutic avenue for reducing platelet-dependent arterial thrombosis in older patients. In fact, recent studies have shown that dietary nitrate can inhibit platelet aggregation[40] again demonstrating this dietary approach to replete NO may provide first line of defense for NO insufficiency.

5.4 Alzheimers disease and NO

The most feared disease of the geriatric population is Alzheimer's disease (AD). In the United States, around 5.4 million people live with AD, a type of dementia.[86] Patients with AD lose brain function, resulting in problems with language, perception and memory. AD can start before age 60 (early onset) or after age 60 (late onset). The risk for AD increases as a person ages—and that rising risk is being seen as the baby boomers start turning 65 years old. Out of every eight baby boomers, one will get AD after she turns 65 years old; at age 85, that risk grows to one in two. With the 65 and over population in the United States expected to double by 2030, there may be up to 16 million people with AD by 2050; there may be almost one million new AD cases diagnosed each year. Each year in the United States, more than 800,000 people die from this neurological disease. It is the sixth leading cause of death, with the number of deaths rising 66 percent from 2000 to 2008.

There is becoming a clear and convincing association with AD and NO. Decreased levels of NOx has been detected in patients with different forms of dementia especially AD.[87] The exact etiology of sporadic AD is unclear, but it is interesting that cardiovascular risk factors including hypertension, hypercholesterolemia, diabetes mellitus, aging, and sedentary lifestyle are associated with higher incidence of AD.[88] The link between cardiovascular risk factors and AD has yet to be identified; however, a common feature is endothelial dysfunction, specifically, decreased bioavailability of NO.[89] The pathogenesis of Alzheimer's disease is closely associated with the accumulation of amyloid-β (Aβ) peptides, which eventually form neuronal deposits known as senile plaques on the outside surface of the neurons[90] and lead to neuron death. AD is characterized by progressive loss of neurons, cognitive decline, and two defining histopathologies: extracellular amyloid plaques and intracellular tangles composed primarily of Aβ peptide and hyperphosphorylated tau, respectively.[91] Furthermore, AD is often accompanied by cerebrovascular dysfunction, as well as amyloid deposition within the cerebral vessels, termed cerebral amyloid angiopathy.[92] NO in the brain can be produced either by iNOS in microglia and astrocytes, or by constitutive NOS in neurons and endothelial cells (nNOS and eNOS). A large body of evidence suggests that the NO produced by neuronal and endothelial constitutive NOS is responsible for neuroprotection during Aβ-induced cell death, while NO production in the case of iNOS activation plays a neurotoxic role due to the inflammatory response caused by the over generation of other reactive nitrogen species from NO.[93] A decrease in nNOS and an increase in hippocampal iNOS have been demonstrated in aged rats,[94] suggesting the dual roles and complexity of NO signaling in the brain and during AD. In mice, higher levels of constitutive NO produced by NOS protects beta-amyloid transgenic mice from developing most typical human symptoms of AD.[95] The protective role of NO in AD pathogenesis has been linked to NO/sGC/cGMP/Protein Kinase G (PKG) signaling cascades. Treatment with NO donors and cGMP analogues suppresses cell death,[96] and increasing intracellular cGMP levels prevents inflammatory responses in brain cells.[97] Moreover, the use of the NO donors, sGC stimulators, and cGMP-analogs reverses learning and memory impairment through PKG activation, in part by reestablishing the enhancement of the transcription factor cAMP-responsive element binding protein (CREB), which is phosphorylated during long term potentiation.[98] It has also been shown that NO modulates expression and processing of amyloid beta precursor protein.[99]

However, an accumulation of Aβ inhibits the NO signaling pathway and therefore may suppress the protective effects of endogenous NO in the brain. Chronic administration of fibrillar Aβ decreases the expression of sGC in cultured rat astrocytes, desensitizing them to treatment with sodium nitroprusside.[100] Acute Aβ administration blocks NO-induced vasoactivity in rats[101] and inhibits NO-stimulated phosphorylation of CREB.[98] In postmortem temporal cortex from a series of AD patients there was reduced NO responsive sGC providing the first evidence for a loss of NO responsive sGC activity in AD brain.[102]

Alternatively, current research suggests S-nitroslylation, may be responsible for cGMP independent mechanisms of NO and can contribute to neurotoxicity in neurogenerative diseases such as AD. Consequently, NO does not always protect against disease and may help facilitate neurodegenerative disorders through nitrosative stress and dysregulation of production. A common theme in many neurodegenerative disorders is the finding of abnormal aggregates of misfolded proteins. Recent findings have implied that NO-related species may significantly participate in the process of protein misfolding through protein S-nitrosylation under degenerative conditions. Qu *et al*.[103] demonstrate that Cdk5 activity, a cyclin dependent kinase responsible for neuronal

functions, is regulated by S-nitroslylation. They found significantly increased S-nitrosylated Cdk5 (SNO-Cdk5) levels in postmortem human brain tissues of patients with AD compared to control brain tissue. Significantly, SNO-Cdk5 was not detectable in control human brains, thus indicating that measurable levels of SNO-Cdk5 are representative of a diseased state. Additionally, researchers reported that formation of SNO-Cdk5 contributes to NMDA-induced spine loss, neuronal damage, and to Aβ-induced loss of synaptic spines. In conclusion, the SNO-Cdk5 mediated pathway may contribute to the pathogenesis of AD and serve as a unique therapeutic for restoring spine damage in AD and other neurodegenerative diseases.

Collectively, the literature demonstrates a critical role for NO in the development of AD. It appears that normal and sufficient NO production/availability can modulate and inhibit the expression and formation of Aβ but once Aβ becomes present it further compromises NO activity. This creates a perpetual system of NO insufficiency and a feed forward mechanism that may accelerate AD progression. The NO pathway (both cGMP and through S-nitrosylation) may be an important therapeutic target in preventing and treating mild cognitive impairment, as well as AD. In fact, a high nitrate diet has been shown to increase regional cerebral blood flow to the frontal lobe in older patients.[104]

6 Conclusions

It appears that the inability to produce sufficient NO under the right preclinical conditions enhances the risk for a number of diseases that plague the older population. If true, then there exist an opportunity to intervene early during this process, implement strategies to restore NO homeostasis, and, perhaps, delay or prevent the onset and progression of certain diseases. This gradual loss of NO activity with age can be sped up or slowed down based on individual lifestyle and diet. This idea is illustrated in the hypothetical graphical representation in Figure 4. Adopting healthy habits such as a good diet and exercise can prolong the precipitous drop in NO production with age. To the contrary a poor diet along with physical inactivity can accelerate the process and lead to a faster decline in NO production at a younger age. Therapeutic strategies directed at improving endothelial function or providing an alternative source of NO should be the primary focus because they may reduce the incidence of atherosclerosis or other diseases that occur with aging, even perhaps AD.

The role of diet in the prevention and control of morbidity and premature mortality due to non-communicable diseases has been well established by vast population-based epidemiological studies carried out during the last decade.[105] NO is essential for maintaining normal blood pressure, preventing adhesion of blood cells to the endothelium, and preventing platelet aggregation; it may, therefore, be argued that this single abnormality, the inability to generate NO, puts us at risk for diseases that plague us later in life, such as atherosclerosis, myocardial infarction, stroke, and peripheral vascular disease. Are dietary and nutritional strategies utilizing nitrite and/or nitrate to restore NO homeostasis the best approach? More clinical trials are needed to define the context for risks *vs.* benefit. Although modestly increased associations between consumption of foods containing nitrite and nitrate and certain cancers have been reported in some prospective epidemiologic studies,[106–108] findings across studies have been largely inconsistent and equivocal[109–112] and thus the overall burden of proof remains inconclusive.[113–119] As with any therapy or treatment regimen, a risk benefit evaluation should be considered and understood for certain persons or patient populations. However, given the emerging data on the growing number of benefits from diets and foods enriched in nitrite and nitrate, we predict the benefits will far outweigh any risks. What remains clear is developing strategies and new technologies designed to restore NO availability is essential for inhibiting the progression of certain common chronic diseases.

Disclosure

Bryan NS has a financial interest in Neogenis, Inc., as a paid consultant and stockowner. Bryan NS also has stock options in SAJE Pharma and has received honoraria for consulting services to Bristol-Myers Squibb. His financial and research conflicts of interest are managed by UTHSCH. Conflicts of Interest Management Plans, developed from and reviewed by the Research Conflicts of Interest Committee and approved by the Executive Vice President for Research at the University of Texas Health Science Center at Houston.

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