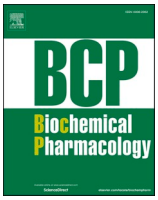
Biochemical Pharmacology 206 (2022) 115325

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm



Review

Nitric oxide deficiency is a primary driver of hypertension

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ARTICLE INFO

Keywords:
Therapeutics
High blood pressure
Nitrite
Nitrate
Endothelial function
Hypertension

ABSTRACT

Hypertension remains a global health crisis. High blood pressure is the number one modifiable risk factor in the onset and progression of cardiovascular disease. Despite many different classes of drug therapies approved for hypertension, the use of polypharmacy and recommendations on lifestyle modification, many patients still suffer from uncontrolled or unmanaged hypertension. Nitric oxide is a naturally produced vasodilator that controls and regulates vascular tone and therefore controls and regulates blood pressure. Research over the past 40 years reveals that loss of nitric oxide production, termed endothelial dysfunction, is the earliest event in the development of hypertension. Strategies aimed at preventing the loss of nitric oxide production and/or therapeutic strategies designed to restore nitric oxide production will likely have a positive effect on patients' health and lead to better management of blood pressure. This review article will focus on the loss of nitric oxide production as the primary contributor to hypertension and also discuss safe and clinically proven strategies to restore nitric oxide production and recapitulate nitric oxide based signaling in humans.

1. Hypertension

Hypertension or commonly known as high blood pressure is the leading cause and risk factor for cardiovascular disease (CVD), including heart attack and stroke [1], the number one cause of death worldwide. Based on the latest guidelines, hypertension is defined by consistently having blood pressure greater than 130 mmHg systolic and greater than 80 mmHg diastolic [2]. Recent reports reveal that approximately 116 million Americans or nearly-one in two people suffer from hypertension [3]. Globally, an estimated 1.28 billion adults aged 30–79 years have hypertension [4]. The incidence of hypertension increases with age. More than half of all people between the ages of 60–69 have hypertension and more than 75 % of all people over the age of 70 have hypertension [5]. Strikingly, those that do not currently have hypertension at age 55–65 years, 90 % will develop hypertension by the age of 80–85 years [6]. Men have a higher prevalence of hypertension (24 %) than women (20 %). After the age of 60, women tend to have higher blood pressures than aged matched men even though men develop high blood pressure starting at a younger age [6,7]. The number of people with hypertension continues to increase [8]. From 1990 to 2015, the number of people with a systolic blood pressure of greater than 140 mmHg has doubled from 442 million to 874 million [8].

Interestingly and alarmingly, approximately 46 % of adults with hypertension are unaware that they have an elevation in blood pressure [9]. Hypertension is known as the "silent killer". Less than half of adults (42 %) with hypertension are even diagnosed and therefore not treated. Only about 21 % with hypertension have it under control [9]. Some symptoms may occur from an acute increase in blood pressure. These include nosebleeds, blurred vision, headaches, irregular heart-beat, and the occasional buzzing in the ears. Severe hypertension if left untreated can cause nausea, fatigue, confusion, anxiety, chest pain and sometimes muscle tremors.

Nearly 18 million people died from CVDs in 2019. This represented 32 % of all deaths globally. Heart attack and stroke made up 85 % of those deaths [8]. Results of the SPRINT (Systolic Blood Pressure Intervention Trial) study clearly showed that lowering systolic blood pressure to 120 mm Hg resulted in significantly lower rates of all cardiovascular events and death from any cause [10]. Despite improvement in detection and treatment, there were more people that did not achieve effective blood pressure control in 2019 than in 1990 because of the large increase in the number of people with hypertension [4]. Historically high blood pressure has not been well treated or cured because, in most patients, the cause has not been identified. If left uncontrolled, many vital organs such as the heart, brain, kidney, and retina can be affected. The primary treatment of hypertension is through drug therapy. In 2021, global revenue from antihypertensive drugs reached US$ 30.2 billion and the global market is predicted to grow at a compounded annual growth rate (CAGR) of 3 % over the next decade [11]. Antihypertensive medications have been shown to improve blood pressure [12], mitigate the resulting cardiovascular diseases caused by hypertension [13] and

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https://doi.org/10.1016/j.bcp.2022.115325
Received 15 August 2022; Received in revised form 18 October 2022; Accepted 19 October 2022
Available online 5 November 2022

reduce the rate of death and morbidity associated with CVD [14]. The primary treatment medications for hypertension are calcium channel blockers (CCBs), angiontensin converting enzyme (ACE) inhibitors, angiontensin II receptor blockers (ARBs), diuretics, beta blockers, alpha blockers and vasodilators. Studies show that beta blocker, CCBs and ARBs are somewhat effective at normalizing blood pressure in pre-hypertensive and hypertensive patients but not enough to get to goal [15]. After monotherapy of each for 8 months of treatment, patients still had average blood pressure of 138/82 mmHg, still an unsafe elevation in blood pressure [15]. These drugs also caused side effects in most of the patients. Patients taking beta blockers experienced cough (3.70 %), skin rashes (44.44 %), dry mouth (33.33 %), stomach discomfort (7.40 %), fatigue (7.40 %), nausea (3.70 %). CCB complaints included sedation (66.66 %), vomiting (13.33 %), swelling (13.33 %) and stomach pain (6.66 %). Those patients taking the ARBs experienced nausea (31.57 %, skin rashes (31.57 %), headache (21.05 %) and cough (15.78 %). Other side effects of beta blockers such as bronchospasm, heart failure, depression, bradycardia, nightmares, heart block and sexual dysfunction also occur [16].

Since different therapeutic classes have various pharmacodynamics and kinetics, they may also be combined. The prevalence of polypharmacy varies between 10 % and 90 % depending on several factors such as age, comorbidities, geographical area, tolerance and management of blood pressure [17]. Combination drug therapy can lead to better management of blood pressure but not without severe side effects, adherence and an increase in costs to the patients [18]. Today, many patients with high blood pressure do not reach a target blood pressure despite the widespread use of antihypertensive drugs and treatment strategies. Many patients with hypertension fail to achieve target blood pressure control, a condition called resistant hypertension. As mentioned above roughly 50 % of those patients treated with medications still have an unsafe elevation blood pressure. Resistant hypertension is defined as have blood pressure greater than 140/80 mmHg even after treatment. Resistant hypertension remains a major clinical problem. Resistant hypertension can cause organ damage including chronic kidney disease (CKD), and premature cardiovascular diseases [19]. These data reveal that hypertension may be poorly understood, at least regarding the targets of therapy. Collectively, scientists and physicians must find a safer and more effective manner to manage blood pressure. Scientific discoveries over the past few decades reveal that nitric oxide (NO) may be the solution.

Historically the use of nitrovasodilators have been effective in treating hypertensive crises. In the U.S. two main drugs are used clinically, organic nitrates and sodium nitroprusside. Due to tolerance development (tachyphylaxis) with organic nitrates, their clinical use for chronic management is very limited [20]. Sodium nitroprusside (SNP) can spontaneously generate NO although several cellular proteins have been shown to facilitate this process.[21] SNP diffuses into erythrocytes where it is metabolized to release NO via interaction with oxyhemoglobin, which is oxidized to methemoglobin. In this process, cyanide radicals are generated creating cyanomethemoglobin, which exists in equilibrium with free cyanide.[22] In the liver, additional cyanide is detoxified via thiosulfate sulfotransferase with thiosulfate as a substrate, converting it to thiocyanate, which is subsequently cleared by the kidneys [23]. To prevent cyanide toxicity, thiosulfate may be co-administered. The onset of action of SNP is rapid, but is very short acting at around 2 min. SNP is not orally absorbed, so continuous intravenous infusion is needed to maintain its activity [22]. Therefore, its clinical utility is reserved for hypertensive crisis in an in-patient setting. With hypertension being a global problem, we must look for new solutions. Risk factors that contribute to hypertension that can be modified include unhealthy diets, physical inactivity, being overweight or obese, low intake of green leafy vegetables and consumption of tobacco and alcohol.

2. Nitric oxide – Vasodilator and anti-Inflammatory

Nitric oxide or NO is a relatively new discovery in science and medicine. It was first realized only about 40 years ago and has since opened up a new area in cardiovascular research. Over the past 4 decades, there are now more than 180,000 scientific articles published on NO. In 1998, the Nobel Prize in Physiology or Medicine was awarded to three U.S. Scientists responsible for its discovery. The science is clear on what NO is and how it affects every major chronic disease, including hypertension. It can no longer be ignored by medical health care practitioners. Surprisingly, despite years of research and billions of dollars in development costs, safe and effective NO therapeutics have been slow and largely unsuccessful. Perhaps this is due to the fact that NO is a gas with an extremely short half-life once it is produced [24]. However, there is clear evidence that NO can act as a hormone affecting distal tissue from original site of production [25]. NO exerts is biological effects as a cell signaling molecule that binds to redox active metals. Its primary target is soluble guanylyl cyclase (sGC) where once activated converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) [26]. NO metabolites can also bind to sulfur groups on protein thiols to elicit a change in protein structure and function. These signaling actions are independent of cGMP production [27]. Most drugs, historically and even today are inhibitors of specific enzymatic reactions. Since hypertension is a lack of nitric oxide production, development of inhibitors of NO production are not prudent. Inhibitors of downstream enzymes have proven successful. Phosphodiesterase inhibitors (PDE5i) to inhibit the breakdown of cGMP are successful drug for the treatments for erectile dysfunction and pulmonary hypertension [28,29]. However, 30–35 % of patients do not respond to PDE5i therapy [30]. This is because NO is required for activation of sGC and without sufficient NO, there is insufficient cGMP produced to elicit a biological effect. Therefore, there is limited therapeutic utility to PDE5i drugs.

The functional loss of NO production through the enzyme nitric oxide synthase (NOS) in the endothelium is termed endothelial dysfunction. The functional loss of NO production precedes the structural changes (arterial stiffness, plague deposition, etc) in the blood vessel by many years sometimes decades and correlates with cardiovascular risks [31–34]. This type of vascular dysfunction occurs throughout the entire cardiovascular system. Without sufficient NO production, this leads to the loss of regulation of blood flow and circulation in every organ. As a result, blood pressure, inflammation, oxidative stress and immune dysfunction increase. Aging and hypertension are well-documented cardiovascular risk factors [35,36].

The functional and structural vascular changes that lead to cardiovascular disease in normal aging is accelerated in younger patients with hypertension [37,38]. Young and healthy persons can produce NO through the oxidation of L-arginine by the NOS enzyme. However, as we age, we lose our ability to synthesize NO from L-arginine (endothelial dysfunction). Early work in cells and tissues reveal that the generation of NOS derived NO decreases with aging and is responsible for the age-related prevalence of hypertension [33]. Increased oxidative stress and superoxide production scavenges NO before it has a chance to reach its cellular target thereby reducing its biological effects [39]. The genetic expression of the NOS enzyme is also decreased with aging [40,41]. There is also an upregulation of arginase (an enzyme that degrades the natural substrate for NOS, L-arginine) as we age that leads to a decrease in NO production [42] due to a shuttling of L-arginine away from the NOS enzyme. The older we get, the less NO produced in the endothelium causing a greater than 50 % loss in endothelial function in older patients [33]. Other studies reveal as much as a 75 % loss of endothelium produced NO in the coronary arteries of people 70–80 years old compared to young, healthy 20 year old controls [32]. In fact, age is the most accurate predictor of endothelium-dependent vasodilator responses [43]. Collectively, these data reveal that vascular NO production in resistance vessels decreases with increasing age. Once the NOS enzyme becomes uncoupled, it is no longer able to convert L-arginine into NO

[44]. This process can be accelerated or decelerated depending on diet and lifestyle and standard risk factors for CVD. On average, impairment of endothelium-dependent vasodilation is clearly evident by 40 years of age [33]. 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 the onset and progression of hypertension and cardiovascular disease. This is illustrated in Fig. 1. Similar to eNOS, neuronal NOS or NOS1 is constitutively expressed and has same degree of regulation as eNOS. However, the inducible (iNOS) NOS2, expression becomes induced from cytokines in response to inflammation due to infection or injury. Chronic over-activity of iNOS can lead to over production of NO and cause hypotension. Increased activity of iNOS leads to a feedback inhibition of the constitutive isoforms and can lead to decreased systemic NO production [45].

The production of NO by the NOS enzymes is a very complex and complicated reaction whereby the guanidino nitrogen of L-arginine undergoes a five electron oxidation with L-citrulline as a bi-product [46]. Once NO is generated, as a gas it diffuses into the smooth muscle and binds to sGC to activate smooth muscle relaxation. The resulting vasodilation causes a reduction in systemic blood pressure allowing for improvements in blood flow and oxygen delivery. In persons with a healthy endothelium, activation of eNOS causes vasodilation in both muscular conduit vessels and resistance arterioles. In contrast, in subjects with endothelial dysfunction or atherosclerotic vascular disease, similar activation or stimulation causes reduced vasodilation in peripheral vessels and causes paradoxical vasoconstriction in coronary arteries [31]. These data reveal a disruption in NOS derived NO likely due to BH4 oxidation and NOS uncoupling [47,48]. Endothelial dysfunction and the inability to produce NO when activated or stimulated is associated with the development of hypertension and the onset and progression of cardiovascular disease [49]. Hypertension accelerates atherosclerosis. Endothelial dysfunction is apparent in patients with risk factors for atherosclerosis but absent of atherosclerosis itself [50,51]. Loss of NO production is associated with all major cardiovascular risk factors, such as diabetes, hypertension, hyperlipidemia, smoking and the severity of atherosclerotic disease [52]. Furthermore, loss of NO is also predictive for future atherosclerotic disease progression [53]. Abnormal endothelium that cannot produce NO causes inflammation and thickening of the intima of the blood vessels. Loss of NO also promotes thrombosis and vasoconstriction, leading to hypertension, inflammation, oxidative stress and immune dysfunction which ultimately leads to the rapid growth of atherosclerotic plaques and further plaque instability [54]. The science is clear, essential hypertension is characterized by a loss of NO production. Hypertension can only be solved and treated by restoring NO production.

3. The oral microbiome regulates systemic blood pressure

The human microbiome constitutes many different bacterial species. In fact, there are ten times more bacteria that live in and on the human body than our own human cells. They are there to provide essential functions that are essential for survival of the human host, a true symbiotic relationship. Although much of the research has been conducted on the gut microbiome, the oral microbiome is gaining attention. A human nitrogen cycle has been identified that requires specific oral bacteria. This pathway is commonly referred to as the entero-salivary nitrate-nitrite-nitric oxide pathway and contributes bioactive nitrite and nitric oxide to the host that is independent of the NOS derived NO [55,56]. Similar to the environmental nitrogen cycle where soil bacteria convert atmospheric nitrogen or ammonia into usable forms for plant growth, primarily nitrate (nitrification), the human nitrogen cycle

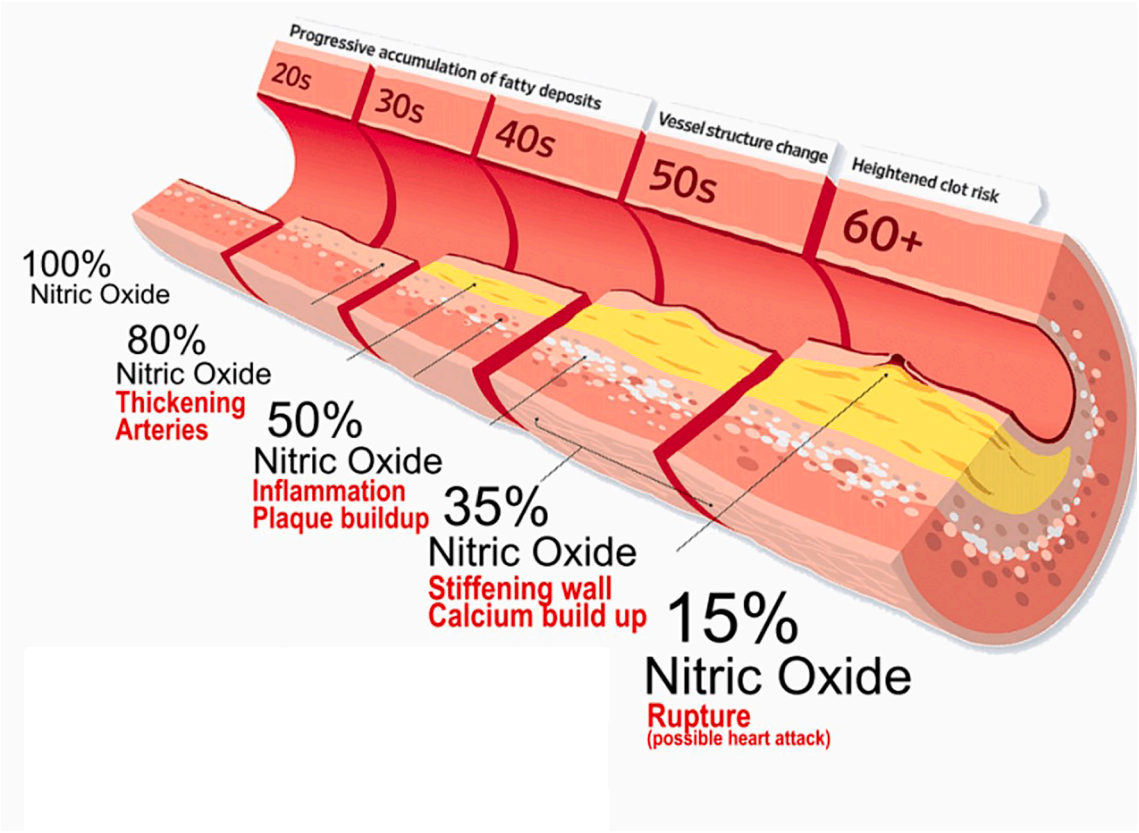

Fig. 1. The functional loss of endothelial nitric oxide production precedes the structural changes that occur in cardiovascular disease by many years. There is an age-related decline in nitric oxide production that leads to the onset and progression of CVD.

reduces nitrate from the diet back into nitrite and nitric oxide (denitrification). Human nitrate reduction is dependent upon bacteria since humans do not express a nitrate reductase gene and thus cannot metabolize nitrate.

Inorganic nitrite (NO_2^-) and nitrate (NO_3^-) were once considered harmful food additives to cure meats. However, they are naturally found in green leafy vegetables [57,58] and are produced endogenously in humans from the oxidation of NO [24]. Nitrate and nitrite are now considered essential nutrients [59,60] necessary to form NO and other bioactive nitrogen oxides [61–64]. The reduction of nitrate and nitrite to NO is complementary and compensatory to any disruptions in the NOS oxidation of L-arginine, such as during anaerobic conditions or uncoupling of NOS. Since mammals lack a nitrate reductase enzyme, the reduction of nitrate from dietary (mainly green leafy vegetables) or endogenous sources (oxidation of NO) requires the presence of oral bacteria to form nitrite [65]. The nitrate that comes from our diet, is absorbed through the duodenum into the blood. Once absorbed in the blood, it is indistinguishable from nitrate formed from the oxidation of endogenous NO. After consuming a meal rich in nitrate, plasma nitrate increases after about half an hour and remain elevated for several hours (the plasma half-life of nitrate is 5–6 h). Nitrate in blood is filtered through the kidney and mostly excreted in the urine. However, approximately 25 % of the nitrate found in blood is actively taken up by the salivary glands and concentration can increase up to 20-fold in saliva [66,67]. Now for the next 8–10 h, each time we salivate, commensal facultative anaerobic bacteria found primarily in the crypts of the dorsal tongue reduce salivary nitrate to nitrite [56,65]. This leads to an increase in plasma nitrite approximately 90 min after consumption of a nitrate rich meal [67]. This only occurs if nitrate reducing bacteria are present. In this case, salivary nitrate and nitrite levels can approach 10 mM and 1–2 mM, respectively [67]. Now for the next 8–10 h every time saliva is swallowed and enters the acidic stomach (1–1.5 L per day), nitrite becomes protonated and forms nitrous acid (HNO_2; pKa ~ 3.3), which then becomes NO [63,64]. A simplified human nitrogen cycle is illustrated in Fig. 2. Nitrite does not have to be protonated and can be absorbed directly. Nitrite is about 98 % bioavailable when swallowed [68].

Salivary nitrate is metabolically reduced (2 electrons) to form nitrite during anaerobic respiration by nitrate reductase enzymes from facultative and obligate anaerobic commensal oral bacteria [65,69]. Human cells do not have this capability. There are specific bacteria in the oral cavity can reduce nitrate to form nitrite in the saliva. See Fig. 3 Although a few nitrate-reducing bacteria have been identified in the oral cavity [70], my lab and others have analyzed nitrate reduction by bacterial communities present in tongue-scrapings from healthy human volunteers. Using 16S rRNA gene pyrosequencing and whole genome shotgun (WGS) sequencing, we have identified specific taxa that are responsible for nitrate reduction. The oral microbiome is a complex community that requires interaction amongst different bacteria [71]. The most effective nitrate reducers found from tongue scrapings were *Granulicatella adiacens, Haemophilus parainfluenzae, Actinomyces odontolyticus, Actinomyces viscosus, Actinomyces oris, Neisseria flavescens, Neisseria mucosa, Neisseria sicca, Neisseria subflava, Prevotella melaninogenica, Prevotella salivae, Veillonella dispar, Veillonella parvula, and Veillonella atypica*. Additionally, *Fusobacterium nucleatum* and *Brevibacillus brevis* were also recognized as effective nitrate reducers even though they were not as abundant [71]. Prior data from 2005, revealed that five genera of bacteria could effectively reduce nitrate on the tongues of healthy individuals: *Veillonella, Actinomyces, Rothia, Staphylococcus,* and *Propionibacterium* [70]. Our data corroborated their findings. *Veillonella* species were the most abundant group of nitrate reducers isolated from the tongue, followed by *Actinomyces spp*. *Veillonella* appears to be the most abundant nitrate-reducing genus detected in tongue scrapings. *Prevotella, Neisseria,* and *Haemophilus* were all found at high abundance. These studies also revealed that not all healthy donors had nitrate-reducing bacteria in their oral cavity could be cultured. This suggests that are a number of people who do not possess the nitrate reducing bacteria and may be NO deficient. Are these people at increased risk for hypertension or cardiovascular disease? New data suggest this may in fact be the case [72,73]. The presence or absence of oral nitrate reducing bacteria may be a new determinant NO bioavailability and hypertension in humans and, thus, a new risk factor for cardiovascular disease and

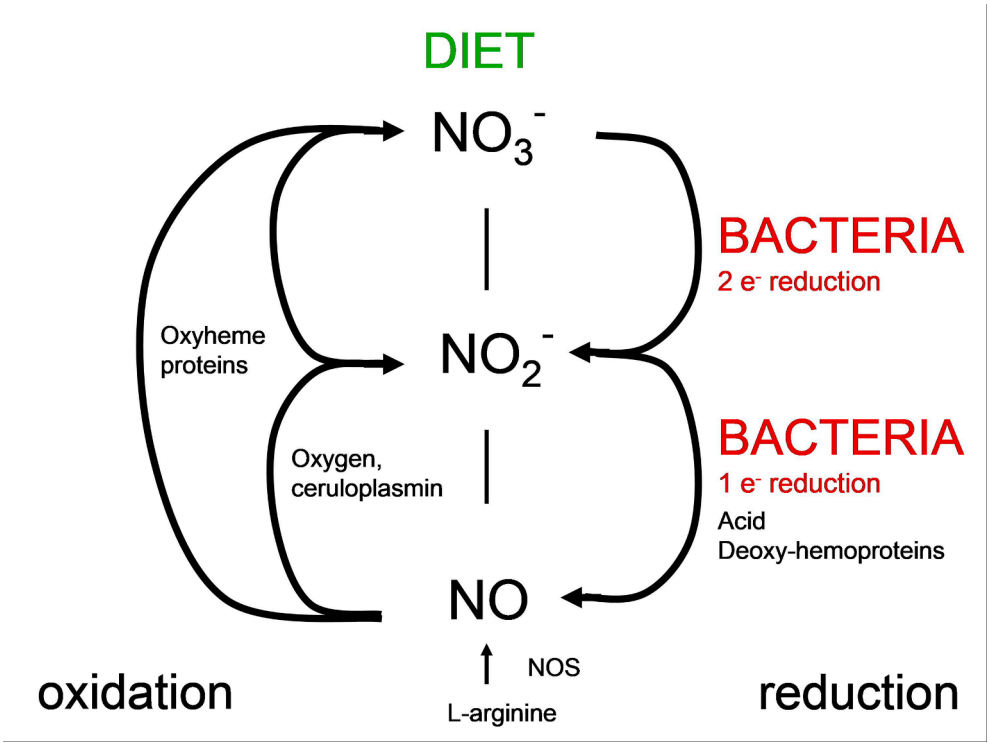

Fig. 2. The human nitrogen cycle whereby nitrate is serially reduced to nitrite and NO providing the host with a source of bioactive NO.

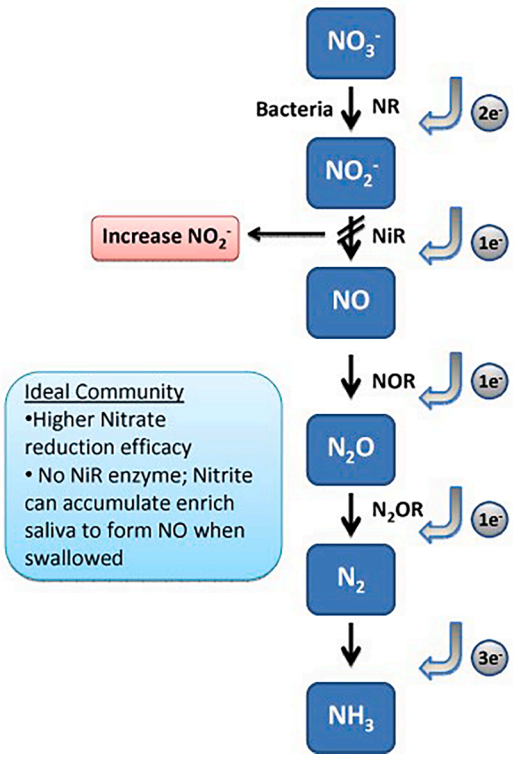

Fig. 3. The biological nitrogen cycle. Nitrate is reduced all the way down to elemental nitrogen by a series of enzymatic steps from nitrate reductase (NR), nitrite reductase, NO reductase (NOR), nitrous oxide reductase (N2OR). Identifying bacteria or communities that only reduce nitrate and/or nitrite will allow for nitrite accumulation and more efficient NO production.

potential target for hypertensive drugs [74].

To demonstrate the essential and important role of oral nitrate reducing bacteria, many studies have been conducted to investigate what happens when these bacteria are eliminated through antiseptic mouthwash. Supplementing with nitrate from the diet has been shown to reduce blood pressure, protect against ischemia–reperfusion injury, restore NO homeostasis which leads to cardioprotection, increased vascular regeneration after chronic ischemia, and a reversal of endothelial dysfunction in the elderly [59,60,75,76]. The benefits of nitrate are abrogated or completely abolished with antiseptic mouthwash that disrupts the oral microbiome [75,77]. The increase in nitrite in plasma and saliva after nitrate supplementation are abolished in healthy subjects taking an antiseptic mouthwash [78]. Nitrate reduces blood pressure in healthy volunteers [79,80], but the blood pressure lowering effects are not realized if subjects use an oral antiseptic mouthwash [81]. It appears that any antiseptic mouthwash disrupts this pathway but stronger antiseptics can influence the blood pressure response during low-intensity exercise [82]. Even without any dietary modifications, use of antiseptic mouthwash for seven-days caused an increase in systolic and diastolic blood pressure that correlated with reduced salivary and plasma nitrite levels in healthy human volunteers [81]. Continuous antiseptic mouthwash has been shown to increase systolic blood pressure in some patients by more than 20 mmHg [83]. The oral microbiome appears to be highly dynamic. Four days after cessation of mouthwash, the oral microbiome re-establishes and blood pressure normalized in those patients that experienced an increase in blood pressure from mouthwash [83]. We were able to identify the bacteria that disappeared when the blood pressure increased and identify the bacteria that reappeared when blood pressure normalized. Administration of nitrite can normalize blood pressure that was increased after use of antiseptic mouthwash [84]. Collectively, these studies provide evidence that the oral microbiome contribute to systemic NO production through the reduction of nitrate to nitrite and play an important role in the maintenance of systemic blood pressure. It appears that providing nitrite can overcome the absence of microbial nitrate reduction. These observations begin to provide an explanation for resistant hypertension. Patients that do not respond to ACE inhibitors, ARBs or CCBs with better blood pressure may be due to oral dysbiosis and the disruption in NO production and therefore cannot and will not respond to pharmacotherapy. The only rational therapy is to discontinue mouthwash and allow the oral microbiome to populate. Over 200 million Americans use mouthwash daily. Not coincidently, over 200 million Americans have an unsafe elevation in blood pressure. It is time to consider the unintended consequences of mouthwash, fluoride and other antiseptic practices on human health.

4. Sodium nitrite as a drug therapy

Now that we have a better understanding of what contributes to hypertension, we can begin to rationalize safe and effective therapies. As we began to solve this problem, we had certain requirements for any therapeutic innovation.

1. If the patient could not produce nitric oxide, either due to endothelial dysfunction or from inadequate dietary nitrate consumption or use of antiseptics, then the technology must provide an exogenous source of NO.
2. The therapy should recapitulate NO based signaling throughout the body, not just in terms of vasodilation.
3. The therapy must not cause tolerance and must restore the body's own ability to produce NO.

Sodium nitrite is an ideal candidate for drug therapy and for treating a number of human diseases caused by deficiency in NO production [85]. Nitrite can form NO through a one-electron reduction [62], nitrite can activate sGC and lead to accumulation of cGMP [61], nitrite can post-translationally modify proteins through nitrosylation [86,87], nitrite can form nitro-fatty acids and nitrite is naturally occurring, safe at effective doses and can recapitulate all NO based signaling. We've previously shown that nitrite prevents the oxidation of tetrahydrobiopterin (BH4) and recouples the NOS enzyme [88]. Nitrite does not cause tolerance [89].

4.1. Nitrite safety Studies

First do no harm. In consideration of new drug development, safety must be established at efficacious doses. There are a number of clinical studies demonstrating safety of sodium nitrite over a wide range of doses. In a safety study in diabetic patients, 80 mg sodium nitrite was well tolerated with no significant changes in methemoglobin, sulfhemoglobin, pulse rate, laboratory tests, or other safety parameters. A select few subjects, 2 out of 12 subjects (17 %) experienced a headache and a hot flush feeling [90]. In this study 80 mg sodium nitrite capsule caused a significant drop in systolic blood pressure with no change on diastolic pressures but not to unsafe levels. Plasma nitrite levels increased to 3–4 μM which is about 10 times higher than normal steady state concentrations in healthy subjects. In a sub-chronic study using 80–160 mg sodium nitrite capsules for ten weeks revealed that there was an acute and chronic increase in plasma nitrite, was well tolerated without symptomatic hypotension or clinically relevant increases in methemoglobin. Sodium nitrite at 80 mg dosing improves endothelial function and carotid artery elasticity without changes in body mass or blood lipids [91]. The changes in endothelial function and vascular elasticity were related to 11 plasma metabolites that could predict the vascular changes with nitrite [91]. Other studies investigating 80 and 160 mg nitrite capsules for 10 weeks revealed healthy middle aged and older adults improved their performance on measures of motor and cognitive outcomes [92]. These studies clearly show that sodium nitrite

is safe at doses from 80 to 320 mg, well tolerated, can increase plasma nitrite concentrations, improve motor and cognitive function, lessen carotid artery stiffening and improve endothelial function. Other studies using much higher doses of sodium administered intravenously (622 mg sodium nitrite per day over 14 days) in critically ill patients with subarachnoid hemorrhage revealed no systemic hypotension and blood methemoglobin levels remained at 3.3 % or less in all patients [93]. These data suggest that nitrite is safe and potentially therapeutic without any signs of toxicity even in critically ill patients after a ruptured cerebral aneurysm [93]. We have monitored methemoglobin levels in a 15 year old pediatric patient taking five doses of a 20 mg sodium nitrite tablet (100 mg daily) and found no increase in methemoglobin levels [94].

The effects of nitrite are not dependent upon oral nitrate reducing bacteria and appear to be safe even as doses that far exceed daily human production. The relatively high doses of sodium nitrite used in the above studies are super-physiological in dosing but yet reveal good safety profile with remarkable clinical effects. There are many physiological effects of nitrite that appear to be independent of NO production [61]. As discussed earlier, nitrite reduction to NO is inefficient along the physiological oxygen gradient [61,95]. Therefore, to get a NO response, more nitrite is needed, especially in people that are NO deficient. Alternatively, a mechanism to more efficiently and effectively reduce nitrite to NO is needed. We have discovered natural product chemistry that provides oxygen independent nitrite reductase that can be combined with nitrite to more effectively reduce nitrite to NO and therefore be able to provide less nitrite while providing an exogenous source of NO in the oral cavity [96]. The purpose and objective of this technology is to provide an exogenous source of NO in patients that have endothelial dysfunction, hypertension, oral dysbiosis, use antiseptic mouthwash or proton pump inhibitors (PPIs).

4.2. Sodium nitrite tablet for treatment of hypertension in a human subject with ASA

Hypertension has been anecdotally reported as a complication in human argininosuccinic aciduria (ASA) subjects [97,98]. It is known that ASA patients have decreased NO production but their vasodilatory response to NO donors is normal [99]. This is similar to patients with essential hypertension [100]. It appears that in patients with ASA that lack the enzyme argininosuccinate lyase (ASL), the cellular signaling pathway downstream from NO are intact and that their primary vascular pathology and hypertension is due to a lack of NO production. In one patient, he was diagnosed with ASA at 3 years of age, and developed idiopathic hypertension at 5 years of age. For a period of ten years, he suffered from severe hypertension even with L-arginine supplementation that was initiated for the prevention of hyperammonemia. Evaluations for secondary causes of hypertension including serum electrolytes, renal function, plasma renin, aldosterone, and renal arterial duplex ultrasonography were unremarkable. Strategies to normalize his blood pressure were introduced including salt restriction, diuretics, and ACE inhibitors were introduced but did not lower his blood pressure. The patient was then started on a regimen of beta blockers and CCBs. In spite of adequate dosing and compliance (evidenced by significant bradycardia), his blood pressure did not decrease. At the age of 15, the patient presented with hypertensive urgency with systolic BP between 160 and 170 mm of Hg. The physicians rationalized that his hypertension could be due to insufficient NO production. Over a four-day period, the patient was started isosorbide dinitrate (ISDN), an organic nitrate (starting at 0.2 mg kg^{-1} $day^{-1}$and titrated to 0.6 mg kg^{-1} day^{-1}). At that time, amlodipine was discontinued and propranolol was weaned to 0.6 mg kg^{-1} day^{-1}. The subject's systolic, diastolic, and mean arterial pressures began to normalize. An echocardiogram revealed mild concentric left ventricular hypertrophy (LVH) with a normal left ventricular systolic function. Over the course of the next three months, the mean blood pressure was 120/74 mm Hg, allowing for the discontinuation of the remaining antihypertensive medications. On isosorbide dinitrate (ISDN) alone, blood pressure remained normal for nine months. An echocardiogram performed after five months of ISDN treatment showed an improvement in left ventricular parameters and mild residual hypertrophy. In addition, urinanalysis showed resolution of kidney disease by a decrease in proteinuria. After nine months of treatment with ISDN, the subject's blood pressure started to increase, typical with tolerance development to organic nitrate therapy [101]. The patient was then started on a 20 mg sodium nitrite tablet together with natural products that effectively reduce nitrite to NO without tolerance. Administration of sodium nitrite normalized blood pressure for the next 10 months without the need for any additional therapy. Even in a patient with hypertension due to an inborn error metabolism, sodium nitrite normalized his blood pressure when no other known therapy was effective.

4.3. Nitrite tablet lowers blood pressure in hypertensive patients

In another study using a 20 mg sodium nitrite lozenge at the Hypertension Institute in Nashville, the investigators assessed the effects in hypertensive patients. Forty subjects 18–80 years of age with blood pressure reading of greater than 140/90 mm Hg on two occasions and who are able to be evaluated by a non-invasive Endopat measurement were included in the study. Subjects enrolled in the study were given either a placebo or single nitrite lozenge and blood pressure was monitored after 20 and 60 min. Measures of endothelial function by Endopat were also collected at baseline and after 4 h. The average blood pressure at baseline was 144 ± 3 / 91 ± 1 mm Hg. Twenty minutes after a single lozenge administration, both systolic and diastolic pressure significantly decreased to 140 ± 2 / 86 ± 1 mm Hg ($p = 0.003$ for systolic and $p = 0.002$ for diastolic pressures). Blood pressure measurements after one hour revealed a further statistically significant decrease in both systolic and diastolic pressure to 138 ± 3 / 85 ± 1 mm Hg ($p = 0.0001$ for systolic and $p = 0.00001$ for diastolic vs baseline and $p = 0.04$ for systolic and $p = 0.04$ for diastolic when compared to data at 20 min. After 4 h, the group receiving nitrite tablet had a significant improvement in endothelial function ($p = 0.003$) demonstrating the nitrite tablet restores endothelial function.

4.4. Nitrite lozenge dilates the carotid arteries

The use of ultrasound allows clinicians to measure any changes in carotid intima media thickness and any change in vasodilation. In an attempt to determine if the nitrite tablet could dilate blood vessels, an ultrasound of the left common carotid was taken before and ten minutes after the nitrite lozenge. The sodium nitrite lozenge resulted in an average 8.5 % increase in blood vessel diameter after 10 min. This demonstrates that the nitrite lozenge generates NO that is bioactive. Using simple laws of hemodynamics, (Poiseuille's Law), the radius of the blood vessel is inversely proportional to the rate of blood flow through the vessel. This degree of vasodilation causes a 34 % increase in blood flow through these arteries [102].

4.5. Nitrite lozenge improves vascular compliance

Arterial stiffness can be measured by a device that determines augmentation index, which is a sensitive marker of arterial status and has been shown to be a predictor of adverse cardiovascular events in a variety of patient populations [103]. Using the Mobilograph instrument (Cardiograde, Inc.), measurements were taken at baseline and then again 30 min after administration of a single nitrite lozenge. The 20 mg nitrite lozenge was designed to have an oral resident time of about 4–6 min. After thirty minutes from taking the lozenge, there was a decrease in the central aortic pressure, mean arterial pressure and pulse pressure. There was also a significant improvement in augmentation pressure, augmentation index and pulse wave velocity [102]. These data reveal

that nitrite improves arterial compliance, an important predictor of adverse cardiovascular events.

4.6. Nitrite lozenge improves endothelial function

Flow-mediated dilation (FMD) is a valuable clinical tool to evaluate early changes in endothelial function in arteries, using a high-resolution ultrasound [34]. The data from this device are expressed as a percent change of the arterial diameter after a period of five minutes of no flow ischemia. FMD has been extensively validated in clinical research and it is considered a standard for a noninvasive assessment of endothelial function [104]. Using such a device, Endopat, patients were evaluated at baseline and then repeated four hours after a single 20 mg nitrite lozenge. Four hours after the nitrite was administered patients experienced an average of 15 % improvement in endothelial function. These data reveal that a single lozenge not only has acute NO bioactive effects in terms of dilating the carotid artery and lowering blood pressure but also improve endogenous endothelial NO production [102].

4.7. Nitrite lozenge lowers blood pressure in pre-hypertensive patients

To determine the effects of a nitrite lozenge on blood pressure over 30 days in pre-hypertensive patients, thirty randomly selected patients with blood pressure readings in the range associated with pre-hypertension but without overt chronic cardiovascular disease were enrolled. Study participants were then randomized to receive either the 20 mg nitrite lozenge or a placebo twice a day for thirty days. Blood pressure, heart rate, quality of life and a walking test were conducted at baseline and at the conclusion of the thirty days. The average baseline blood pressure in the active nitrite group ($n = 17$) was 138 ± 11.66 mmHg in systole and 84 ± 5.09 mmHg in diastole; the baseline heart rate was 74.7 ± 9.24 beats per minute (bpm). Baseline blood pressure in placebo group ($n = 12$) was 138 ± 21.37 mmHg in systole and 80 ± 7.68 mmHg in diastole; the baseline heart rate was 80.4 ± 10.44 bpm. At follow-up, blood pressure in group 1 at rest was 126 ± 11.86 mmHg in systole and 78 ± 4.26 mmHg in diastole ($p < 0.001$ versus baseline), indicating a 12 mmHg reduction in systolic and 6 mmHg reduction in diastolic pressures. Heart rate was 76.2 ± 7.63 bpm (non-significant versus baseline). In the placebo group, blood pressure at rest was 135 ± 17.28 mmHg in systole and 82 ± 7.68 mmHg in diastole (non-significant versus baseline), heart rate was 79.3 ± 8.01 bpm (non-significant versus baseline) [105].

4.8. Nitrite lozenge improves functional capacity of the heart and quality of life

In order to determine if there was any improvement in the functional capacity of the heart, patients were put on a treadmill for a 6-minute walk test. The average distance of the six minute walk test in the nitrite lozenge group at baseline was 595.63 ± 213.66 m versus 650.31 ± 196.64 m at follow-up ($p < 0.005$ versus baseline) revealing a 55 m improvement in the treatment group [105]. Quality of life was assessed using a standardized SF-36 questionnaire that computed eight scaled scores. The present study utilized the Physical Composite Score (PCS) and the Mental Composite Score (MCS) that were created based on the questionnaire responses [106]. The average PCS and MCS scores for the nitrite lozenge group at baseline were 48.1 ± 10.00 and 40.1 ± 9.20, respectively. At follow-up, PCS was 50.4 ± 8.46 and MCS was 44.6 ± 6.55, respectively ($p < 0.05$ versus baseline for both comparisons). PCS and MCS scores for the placebo group at baseline were 43.1 ± 9.54 and 36.7 ± 8.84, respectively, versus 37.2 ± 10.64 and 36.6 ± 6.55 at follow-up.

5. The future of nitric oxide-based drug therapies

It is evident that loss of endogenous nitric oxide production and homeostasis is at least partly responsible for hypertension and is associated with most cardiovascular risk factors [107–112]. Diet and lifestyle are critically important to controlling and combatting hypertension. Current drug therapy is not sufficient to manage hypertension. Moderate physical exercise promotes endothelial NO production [113]. The Dietary Approaches to Stop Hypertension (DASH) diet is proven to lower blood pressure moderately. The DASH diet lowered systolic blood pressure significantly in untreated subjects with systolic blood pressure < 160 mmHg and diastolic blood pressure 80–95 mmHg by 5.5/3.0 mmHg [114]. This diet as well as the Mediterranean diet is thought to be effective at lowering blood pressure due to the inorganic nitrate content of green leafy vegetables that can be utilized to regenerate nitrite and NO [55,115,116]. Therefore, everything we know about preventing, treating and curing cardiovascular disease, including hypertension is dependent upon sufficient nitric oxide production. Now with this important consideration, physicians and other health care practitioners can have a new weapon in their armament to treat hypertension. We start with making basic lifestyle recommendations. In order to help promote nitric oxide production, we must advise patients to stop the activities that disrupt or inhibit NO production and begin implementing the lifestyle strategies that are clinically proven to promote NO production. Daily activities that disrupt or inhibit nitric oxide production include daily use of mouthwash, fluoride-based toothpastes and rinses and antacids. Mouthwash and fluoride are antiseptic and kill the nitrate reducing bacteria causing a disruption in nitrite and NO production resulting in an increase in blood pressure. Antacids, specifically proton pump inhibitors disrupt NO production [117]. Physicians should immediately begin recommending patients discontinue mouthwash and eliminate fluoride from toothpaste and drinking water. Physicians should begin to wean patients off of PPIs and encourage more green leafy vegetables. These strategies are proven scientifically and clinically to help with hypertension. The association of hypertension with cardiovascular (CV) morbidity and mortality is well established. Abundant epidemiological data have shown that the risk of CV disease rises with increasing blood pressure (BP) levels, starting at $\geq$ 115/75 mm Hg, in a strong, independent, graded and continuous manner [46]. Lowering blood pressure by 5 mmHg diastolic reduces the risk of stroke by an estimated 34 % and ischemic heart disease by 21 % from any pre-treatment level; there is no threshold [118].

Given the very good safety profile of sodium nitrite and its efficacy at restoring and recapitulating NO based signaling, it is a primary focus for drug development. Nitrite's ability to reduce blood pressure, provide an exogenous source of NO when administered and lack of any tolerance development makes it an ideal drug candidate. With the growing epidemic of hypertension in a growing population, new, safe and effective therapies for hypertension will be required. The science is clear. Lack of nitric oxide production causes and contributes to hypertension. Hypertension is the primary cause of cardiovascular disease which remains the number one killer of men and women worldwide. Nitric oxide-based therapies can and should have a profound impact on hypertension and public health around the globe.

CRediT authorship contribution statement

Nathan S. Bryan: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, product administration, resources, software - origin, excel, supervision, validation, visualization, writing, reveiw and editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [N.S. Bryan is the inventor on dozens of issued U.S. and International patents. He receives royalties on his patents from the University of Texas Health sciences Center at Houston. Dr. Bryan is company Founder of

HumanN, Nitric Oxide Innovations, Pneuma Nitric Oxide,Bryan Nitriceuticals and Nitric Oxide Research Institute].

Data availability

No data was used for the research described in the article.

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