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All-natural nitrite and nitrate containing dietary supplement promotes nitric oxide production and reduces triglycerides in humans☆

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Abstract

There is an emerging paradigm that certain foods promote nitric oxide (NO) production from the stepwise reduction of nitrate to nitrite to NO, providing an endothelium independent source of bioactive NO. We hypothesize that a unique formulation containing nitrate-rich beetroot along with Hawthorn berry shown to have a robust nitrite reductase activity would improve NO status in humans and modify cardiovascular risk factors. The trial was conducted at the Houston Institute for Clinical Research in Houston, Texas. Inclusion criteria for this double-blinded, placebo-controlled study were patients older than 40 years with 3 or more of the following cardiovascular risk factors: hypertension, obesity, hyperlipidemia, smoking, sedentary, family history of cardiovascular disease, and diabetes. Subjects were instructed to take either the NO dietary supplement called Neo40 Daily® or placebo twice daily on an empty stomach for 30 days. Patients taking the NO dietary supplement twice a day for 30 days led to a significant increase in both plasma nitrite ($P < .01$) and nitrate ($P < .0001$), indicating an increase in systemic NO availability. There was a statistically significant reduction in 72% of patients with elevated triglycerides (>150 mg/dL) after 30 days compared with their starting levels before taking the NO dietary supplement (168 ± 17 mg/dL vs 232 ± 19 mg/dL, $P = .02$). The strategy of formulating a combination of natural products and botanicals chosen specifically for their NO activity shows promise in restoring NO homeostasis in human subjects at risk for cardiovascular disease for use as a dietary supplement.

Keywords: Nitric oxide; Dietary supplement; Triglycerides; Cardiovascular disease; Humans
Abbreviations: CVD, cardiovascular disease; CRP, C-reactive protein; FDA, Food and Drug Administration; NO, nitric oxide; NOS, nitric oxide synthase; TG, triglyceride.

1. Introduction

Nitric oxide (NO) is one of the most important signaling molecules in our body [1]. Although NO is involved in virtually every organ system within our body, it is known primarily for maintaining normal blood pressure and blood flow to tissues and protecting the cardiovascular system from insult and injury. A deficiency in NO production or availability is a hallmark of several disease conditions. Experimental and clinical studies provide evidence that defects of endothelial NO function, referred to as *endothelial*

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dysfunction, is not only associated with all major cardiovascular risk factors such as hyperlipidemia, diabetes, hypertension, smoking, and severity of atherosclerosis but also has a profound predictive value for future atherosclerotic disease progression [2-5].

It has been previously demonstrated that plasma nitrite reflects short-term changes in endothelial NO synthase (NOS) activity in various mammals [6] and, thus, may provide an accurate measurement of patients at risk for cardiovascular events. A report by Kleinbongard et al [7] demonstrated that plasma nitrite levels progressively decrease with increasing cardiovascular risk load indicative of a reduction in the production of NO. Risk factors considered included age, hypertension, smoking, and hypercholesterolemia.

The original discovery for the production of NO is through the oxidation of the semiessential amino acid L-arginine by NOS. Nitric oxide synthase enzymes produce NO by catalyzing a 5-electron oxidation of the guanidino nitrogen of L-arginine. Remarkably though, strategies to restore NO homeostasis by supplementing L-arginine and antioxidants have consistently failed in clinical trials. This is because L-arginine–NO pathway is dysfunctional in patients with endothelial dysfunction. In fact, it is now known that feeding the NO pathway through L-arginine supplementation may not be effective and can actually be detrimental in at-risk patients [8]. Interventions to enhance NO production with organic nitrates such as nitroglycerin have been equivocal in human trials. Early entry therapy with nitrates do not significantly improve survival in myocardial infarction but increases the beneficial effects of the angiotensin-converting enzyme–inhibitor enalapril by 50% [9]. Short-term experimental and clinical investigations suggest that nitrate tolerance induced by nitroglycerin is associated with toxic effects in the vasculature [10]. Long-term organic nitrate therapy has been associated with reduced survival when used in patients with coronary artery disease [11,12]. The observed endothelial dysfunction induced by a continuous treatment with nitroglycerin may be an additional risk of patients who receive continuous nitroglycerin to treat conditions such as unstable angina and acute heart failure [9]. As such, all current strategies have failed; so there is a large unmet need for safe and effective NO based interventions or strategies.

Despite NO's known and accepted importance in human physiology, there have been no hallmark therapeutic breakthroughs or effective strategies developed to enhance or restore NO homeostasis in humans at risk for cardiovascular disease (CVD). Developing such strategies or technologies to restore and replete NO availability, both through restoration of endothelial NO production and in an endothelium-independent manner, is of paramount importance and could potentially save millions of lives worldwide and lessen the burden on the health care system.

There is an emerging paradigm whereby functionalities of certain foods and diets may confer NO activity [13,14]. Nitrate in the diet (primarily from green leafy vegetables or beetroot) is reabsorbed in the proximal intestines and concentrated in the salivary glands. It was known from the literature that the salivary glands extract nitrate from plasma, but the reason for this active process was not explained [15]. This active process leads to levels of salivary nitrate that are 10- to 20-fold higher than in plasma. Oral facultative anaerobic bacteria residing mainly in the crypts of the tongue then reduce nitrate to nitrite by the action of nitrate reductase enzymes [15,16]. These bacteria use nitrate as an alternative electron acceptor to gain adenosine triphosphate in the absence of oxygen. Approximately 25% of ingested nitrate is secreted in saliva, where some 20% (or ~5%-8% of the nitrate intake) is converted to nitrite by commensal bacteria on the tongue [17]. This bacterial nitrate reduction results in salivary levels of nitrite that are 1000-fold higher than those found in plasma [18]. When nitrite-rich saliva meets the acidic gastric juice, nitrite is protonated to form nitrous acid (HNO_2) that then decomposes to NO and a variety of other nitrogen oxides [17,19] that are then transported throughout the body. This nitrate-nitrite-NO pathway has been shown in both animals and humans to reduce blood pressure [20,21], restore endothelial function [22,23], protect from myocardial ischemia-reperfusion injury [24], prevent microvascular inflammation, and reduce triglycerides (TGs) and C-reactive protein [23]. Therefore, there exists a pathway for increasing NO bioavailability within the body through supplementing NO-rich or NO-active food components that contain adequate amounts of nitrate and/or nitrite and antioxidants to facilitate reduction to NO and to inhibit any unwanted nitrosation reactions. Using a unique formulation from intellectual property whereby this pathway is optimized with natural products developed out of the University of Texas Health Science Center in Houston, Neogenis Laboratories, Inc, has the exclusive license to commercialize the technology. The all-natural formulation, called Neo40 Daily®, provides an innovative delivery system for generating NO in an endothelium-dependent and endothelium-independent manner. The formula is based on a proprietary blend of NO active herbs that act to replete and restore NO production in the human body by exploiting the nitrate-nitrite-NO pathway [25].

Our hypothesis, based on several preclinical and clinical studies, is that using a nitrate source such as beetroot along with an effective nitrite reductase, as found in Hawthorn, would enhance NO production through the efficient reduction of nitrate and nitrite to NO and thereby modify cardiovascular biomarkers of risk. Nitrate-enriched beetroot has been shown in several studies to promote NO production and reduce blood pressure and endothelial function in humans [20-22]. Screening of more than 100 herbs and botanicals for nitrite reductase activity, we found Hawthorn berry to have the highest activity. This formulation has been recently tested by an uninterested third party in a double-blinded, placebo-controlled clinical trial to modify biomarkers of cardiovascular risk and increase steady state levels of NO biomarkers in human subjects older than 40 years. The

information gained from this study will advance our understanding of human nutrition by providing a mechanistic explanation for the health benefits of certain foods, primarily nitrate-rich green leafy vegetables or beets, by their ability to generate NO in the proper context. Nearly 50% of Americans use dietary supplements [26] often without clinical evidence to support its safety and efficacy. Such a study is important for physicians and consumers.

2. Methods and materials

2.1. Patient enrollment

The trial was conducted at an institutional review board–approved site at the Houston Institute for Clinical Research in Houston, Texas. Our inclusion criteria for this double-blinded, placebo-controlled study were patients older than 40 years with 3 or more of the following cardiovascular risk factors: hypertension, obesity, hyperlipidemia (TGs >150 mg/dL), smoking, sedentary lifestyle (no formal exercise routine), family history of CVD, and diabetes. Patients were screened to ensure that they met the inclusion criteria, informed of the study protocol, and signed a consent form. Subjects' age ranged from 42 to 79 years, with a median age of 56 years, with 63% men and 37% women. Patients were excluded if they were currently taking organic nitrates for angina or nebivolol as a β blocker. Our objective was to investigate if increasing NO through the nitrate-nitrite-NO pathway could modify known risk factors for CVD such as TGs and C-reactive protein. Our preclinical studies in mice revealed that restoring NO homeostasis through this pathway could reduce TGs and C-reactive protein [23]. We began by enrolling only patients with elevated TGs (>150 mg/dL) and at least 2 of the other risk factors from above. Subjects were instructed to take a single lozenge of either the NO dietary supplement or placebo twice daily on an empty stomach for 30 days. Placebo was made of identical physical appearance and flavor as the active NO supplement. It contained the same excipients but of higher amounts to accommodate the identical tablet dimensions of the active comparator. In addition, patients were asked to maintain their normal dietary pattern and physical activity levels. After 30 days, the patients returned to the clinic for a blood draw and were asked to complete a survey. Compliance was confirmed by pill count, and subjects returned all the remaining lozenges upon completion of the study. Patients were randomized using a randomization table to receive the NO dietary supplement or placebo and asked to fast for 12 hours before all blood draws. Blood samples were sent to LabCorp (Houston, Tex, USA) for the following analysis: complete blood count with differential/platelet; complete metabolic panel 14; lipid panel; bilirubin, total/direct, serum; and phosphorus and serum as well as C-reactive protein. Separate blood samples were collected and analyzed for nitrite and nitrate at Dr Bryan's laboratory with a well-validated and sensitive high performance liquid chromatography (HPLC) system (ENO-20, Eicom Corp, San Diego, Calif, USA) [27]. A total of 30 patients were enrolled that include 23 on Neo40 and 7 receiving the placebo. The randomization schedule was intentionally and preferentially weighted to the Neo40 group in the first 30 patients. For the sample size calculations, we assumed an α of .05, power of 0.80, and a 2-tailed t test. The software package used was Stata Statistical Software, Release 10 (StataCorp LP, College Station, Tex, USA). Sample size was calculated based on the steady state plasma nitrite levels and fasted serum TGs. Power analysis based on preliminary data revealed that 25 subjects would be sufficient for significant changes in plasma nitrite levels and the ability to determine a 20% reduction in TGs. Both the clinical coordinator and data analyst were blinded to the study. All investigators completed and passed an institutional course on Health Insurance Portability and Accountability Act.

2.2. Product formulation

By screening more than 100 herbs and botanicals as well as dessicated food products, we identified beetroot as containing the highest nitrate content of any tested. This finding is consistent with others, as beetroot is routinely used as a source of nitrate in experimental and human studies [20,22,28]. Commensal bacteria are responsible for the first reductive step of nitrate to nitrite. The reduction of nitrite to NO is a very inefficient process and occurs primarily under low oxygen conditions [29]. Our most recent data on traditional Chinese medicines revealed that some herbs have a robust capacity to reduce nitrite to NO in an oxygen-independent manner [30]. We reported that GuaLou and Borneal contained the highest nitrite reductase of the traditional Chinese herbs with 133 pmol NO/mg per minute and 875 pmol NO/mg per minute, respectively. We have since tested more than 100 herbs and botanicals for their ability to reduce nitrite to NO. We found Hawthorn berry to have the highest nitrite reductase activity of any botanical tested, and its activity is unaffected by oxygen (activity, 434 369 pmol NO/mg per minute or 500 times higher than the Chinese herbs). The Hawthorn also contains 5% polyphenols that also help facilitate nitrite reduction while inhibiting nitrosation reactions [31]. The formulation also contains generally recognized as safe amounts of sodium nitrite for use as a preservative as well as a substrate for NO production. We have discovered a specific product ratio with the highest NO activity. This proprietary formulation with precise ratio of ingredients has been submitted as an unpublished patent application through the University of Texas Health Science Center at Houston. We had these ingredients sourced and formulated into a unique quick-dissolving lozenge by a good manufacturing practice–certified facility, Solara Inc, Miami, Florida. All ingredients were tested for microbiology and heavy metals. The Neo40 Daily® product ingredients list and packaging was submitted to FDA Office of Compliance by Neogenis Labs, Inc, for use

as a dietary supplement (registration no. 3008524085). All ingredients are listed on the FDA Generally Recognized As Safe list (therefore, no investigation new drug application is required).

2.3. Nitrite reductase activity

Nitrite reductase activity was quantified by adding 100 mg of the Neo40 product into a reaction vessel containing 5 mL of phosphate-buffered saline, purged with nitrogen or medical-grade air (21% oxygen) at 37°C. The area under the curve was integrated to quantify the total amount of NO generated.

2.4. Statistical analyses

Comparisons were made using a 2-tailed t test. Significance was considered $P < .05$. The software package used was Origin version 7.5 (OriginLab, Inc., Northampton, Mass, USA). Some of these data are presented as means + SEM.

3. Results

To test the NO activity of our rationally designed combination in its final product formulation, we injected 100 mg of the Neo40 product into a buffered solution (pH 7.4) connected to an ozone-based chemiluminescent analyzer (EcoPhysics, Ann Arbor, Mich, USA) and monitored the NO release profile. Immediately upon dissolving, the NO dietary supplement produced NO with a sustained release. We stopped the analysis after 100 minutes. The NO release kinetics is shown in Fig. 1A. In this in vitro system, the NO dietary supplement has a half-life of roughly 1 hour with respect to NO release. Based on this information, we then tested the pharmacokinetics of this formulation when administered to human subjects. Study participants were asked to place the lozenge in their mouth and let it dissolve. Baseline venous blood was drawn and then sampled every 5 minutes for 1 hour. The formulation for the NO dietary supplement is designed to act as a quick-dissolve lozenge that melts in your mouth and enriches your saliva. When allowed to dissolve in the mouth, this leads to a slow and steady rise in plasma nitrite of humans, as shown in Fig. 1B. These data indicate that the NO activity of the lozenge is absorbed and transported through the blood as nitrite.

To demonstrate proof of concept that an NO dietary supplement could restore systemic NO levels for a period of 30 days, we enrolled patients with known cardiovascular risk factors, older than age 40 years, and has hyperlipidemia. Patient's blood was analyzed at baseline for screening and inclusion. If subjects met inclusion criteria, they were administered placebo or the NO dietary supplement. After 30 days, subjects returned for follow-up laboratories for plasma levels of nitrite and nitrate, which are markers of NO production. It has been reported that normal plasma nitrite levels in healthy human volunteers is 250 to 500 nM [6,7]. Taking a single NO dietary supplement twice a day for 30

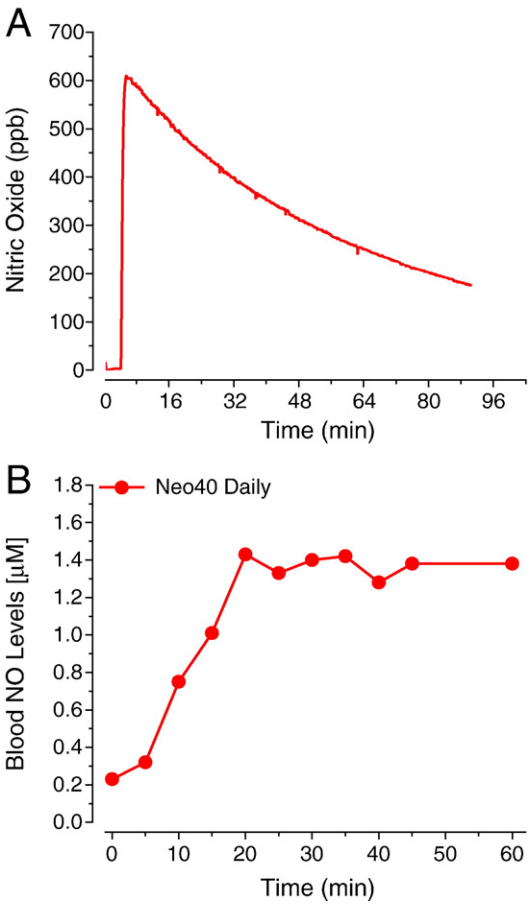

Fig. 1. A, Neo40 Daily® releases NO with a half-life of 1 hour (single representative tracing from n = 3). B, Plasma concentrations of nitrite after taking Neo40 (average of 2 patients).

days led to a modest but significant increase in both plasma nitrite ($P < .01$) and nitrate ($P < .0001$), indicating an increase in systemic NO availability. Results are shown in Fig. 2 below. Patients taking a placebo actually experienced a nonstatistically significant reduction in steady state nitrite and nitrate after 30 days.

Smoking, diabetes, high blood pressure, high cholesterol and TGs, sedentary lifestyle, obesity, and family history of CVD are all established risk factors that put patients at risk for developing CVD. Blood values can reveal levels of cholesterol and TGs as well as systemic inflammation based on C-reactive protein. We compared levels of cholesterol, TGs, and C-reactive protein of the patients taking the Neo40 Daily® product at baseline and after 30 days on the product. There was no significant change in total cholesterol or low-density lipoprotein, very–low-density lipoprotein, or high-density lipoprotein after 30 days on the NO dietary supplement or placebo (data not shown). Our preclinical data in mice indicated that restoring NO through the nitrate-nitrite-NO pathway could reduce TGs [23]. Therefore, we only enrolled patients with TGs greater than 150 mg/dL. Of these patients with baseline TGs greater than 150 mg/dL, 72% of the subjects on the NO dietary supplement

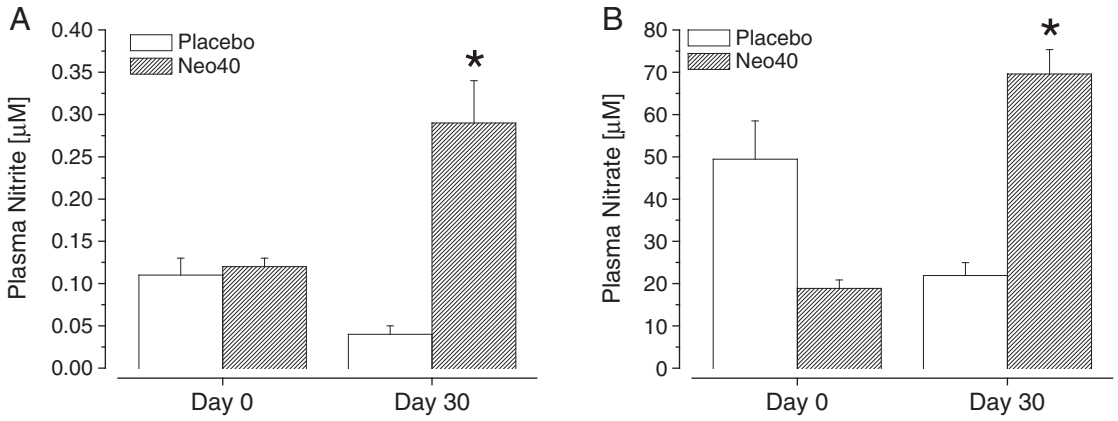

Fig. 2. Thirty-day twice per day regimen of Neo40 Daily® significantly increased plasma levels of nitrite (A) and nitrate (B) to normal healthy levels. There was a nonstatistically significant reduction in plasma nitrite and nitrate in patients taking the placebo. Data are means ± SEM of n = 23 patients for active and n = 7 for placebo.

experienced a reduction in TGs after 30 days. The individual changes in TGs are shown in Fig. 3A. There was a statistically significant 27% reduction in all patients' TGs after 30 days compared with their starting levels before taking NO dietary supplement (168 ± 17 mg/dL vs 232 ± 19 mg/dL, $P = .02$). These data are illustrated in Fig. 3B.

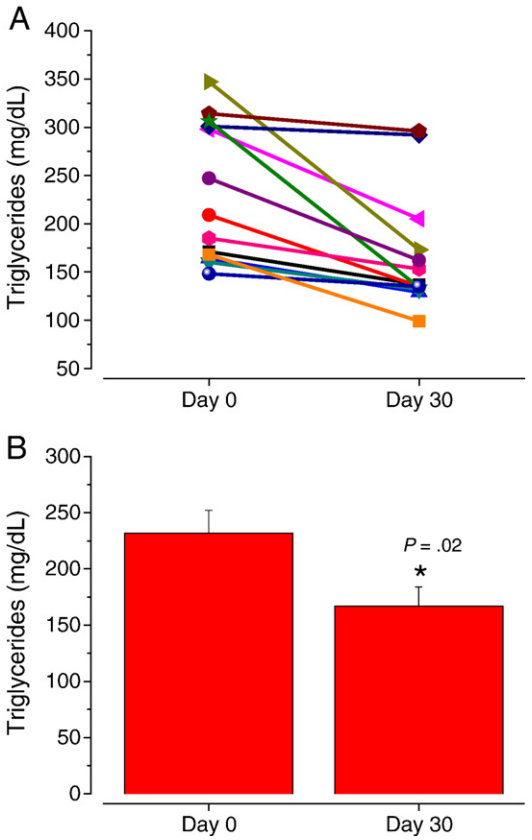

Fig. 3. A, Patients taking Neo40 Daily® for 30 days saw a 10% to 55% decrease in fasting TGs. Collectively, all patients taking Neo40 Daily® experienced a statistically significant reduction in fasting TGs (data are means ± SEM of n = 13 patients).

Only 4 of our 23 patients taking Neo40 Daily® had elevated C-reactive protein (>4.9 mg/dL) at baseline. Normal CRP levels are between 0.0 and 4.9 mg/L. However, all 4 patients who started with elevated CRP experienced a reduction after 30 days on NO dietary supplement. There was, anywhere, from a 6% to 37% reduction in CRP in these 4 subjects. We will need to enroll more patients with elevated CRP to reach any conclusions on CRP.

Nitric oxide is the main molecule responsible for controlling blood pressure. Of the 23 patients, 9 had blood pressure greater than 130/80 mm Hg (systolic range, 135-160 mm Hg). Of the 9, 5 were on prescription antihypertensive medications. The subjects taking the active NO dietary supplement experienced a nonstatistically significant reduction of 7 mm Hg systolic and 2.7 mm Hg reduction in diastolic blood pressure. More hypertensive patients are needed for specific effects on blood pressure. Such trials are currently underway.

Upon completion of the study, participants were asked to complete a subjective questionnaire based on how they felt. The results from the questionnaire are shown in Table 1, including patients taking placebo. Greater than 50% of the respondents taking the active product indicated that they had more energy and felt less anxious. Most strikingly, more than 75% of the subjects indicated that they would continue taking the product if available. There were no adverse health effects reported.

Table 1
Patient Questionnaire

Question	% yes Neo40	% yes Placebo
More relaxed	45.5	0
Sleep better	45.5	0
More energy	54.5	0
Less anxious	54.5	35
Continue taking product if available	78.6	35

4. Discussion

Nitric oxide is one of the most important signaling molecules produced within our body. The loss of NO generation because of a dysfunctional vascular endothelium is a very likely cause of heart disease [32]. Continuous generation of NO is essential for the integrity of the cardiovascular system because a decreased production and/or bioavailability of NO is central to the development of cardiovascular disorders [33,34]. Neogenis Labs Inc, has developed a unique and novel formulation of natural products designed to exploit the nitrate-nitrite-NO pathway to restore NO homeostasis in adults older than 40 years based on patent pending technology developed out of the University of Texas Health Science Center at Houston. As we age, we lose our ability to produce NO [35,36], which is the earliest event that puts us at risk for a host of conditions such as atherosclerosis, myocardial infarction, stroke, and even Alzheimer disease later in life. By the age of 40, most adults produce only about 50% of the NO produced when they were 20 [35-37]. The strategy of formulating a combination of natural products and botanicals chosen specifically for their NO activity shows enormous promise in restoring NO homeostasis in human subjects at risk for CVD for use as a daily dietary supplement.

Despite NO being recognized by the scientific and medical community as one of the most important molecules produced within our body and being named "Molecule of the Year" by *Science* in 1992 and a Nobel Prize in Physiology or Medicine awarded in 1998, there are currently only 3 products on the market directly related to NO: (1) organic nitrates such as nitroglycerin for the treatment of acute angina, (2) inhalative NO therapy for neonates for treatment of pulmonary hypertension because of underdeveloped lungs, (3) phosphodiesterase inhibitors such as sildenafil (Viagra®). These classes of drugs do not directly affect NO production but act through affecting the downstream second messengers of NO known as cyclic guanosine monophosphate. Therefore, there is an unmet need for safe and effective strategies to restore and enhance NO production, particularly in patients with endothelial dysfunction.

The first pathway to be discovered for the endogenous production of NO was through the 5 electron oxidation of the guanidino nitrogen group of L-arginine (a semiessential amino acid) by a group of enzymes called NOS localized to the vascular endothelium. For years, scientists and physicians have investigated L-arginine supplementation as a means to enhance NO production. However, patients with endothelial dysfunction, by definition, are unable to convert L-arginine to NO; and therefore, this strategy has failed in clinical trials. In fact, the study of Schulman et al, published in the *Journal of American Medical Association* in 2006, concluded that L-arginine, when added to standard post-infarction therapies, did not improve vascular stiffness measurements or ejection fraction and was associated with higher postinfarction mortality [8]. The Neo40 Daily® technology uses a natural source of NO through the reduction of nitrate and nitrite to NO and overcomes the endothelium's inability to generate NO. This is the first-of-its-kind technology using natural products for NO activity in patients insufficient in NO production. Neo40 Daily® provides a functional system to produce NO that is derived from natural products. There are numerous preclinical and some clinical trials underway that support this notion [25,38].

A daily regimen that can safely and effectively restore NO levels as well as reduce blood biomarkers routinely used to assess patient risks for developing CVD is novel with profound public health implications. Our preclinical data in mice fed a high cholesterol diet revealed that nitrite-based interventions could reduce TGs after 9 weeks on a high-fat diet [23]. We were surprised to see a significant reduction in TGs in our human subjects after only 30 days. Recently, the TG-lowering effects seen here were corroborated in eNOS-deficient mice and in rats, whereby 7 weeks of inorganic nitrate administration caused a significant reduction in TGs [39]. Furthermore, Hawthorn extract has previously been shown to reduce TGs in animal models [40]. There are several medications that reduce TGs, including fibrates, such as fenofibrate (Lofibra®, TriCor®) and gemfibrozil (Lopid®), niacin (Niaspan®), and ethyl esters of omega-3 fatty acids known as Lovaza®. Lovaza® is a combination of long chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), indicated as an adjunct to diet to reduce TG levels in adult patients with severe (≥500 mg/dL) hypertriglyceridemia. Lovaza® is FDA approved for patients with very high TGs (>500 mg/dL) and have been shown to reduce TGs by up to 43% [41]. However, most Americans who are hypertriglyceridemic have serum concentrations above 150 but below 500. Other than changing their diet, there is no effective regimen for this population. The results from this study suggest that an NO dietary supplement can indirectly restore NO homeostasis and reduce TGs, thereby reducing the overall burden of risk of the development of CVD.

Dietary supplementation of nitrite and nitrate in mice has shown to reverse endothelial dysfunction, suppress microvascular inflammation, reduce levels of C-reactive protein in mice subjected to a high-cholesterol diet [23], and protect from ischemia reperfusion injury [24,42,43]. Most recently, feeding the nitrate-nitrite-NO pathway has been shown to reverse symptoms of metabolic syndrome [39]. This proof of concept has now been extended to humans supplemented with dietary sources of nitrate. Dietary nitrate has also been shown to reduce blood pressure [20-22], inhibit platelet aggregation [22], and restore endothelial function [22]. What is clearly emerging is that there are 2 pathways for NO production: 1 through endothelial production through the L-arginine pathway and 1 through dietary sources of nitrite, nitrate, and antioxidants. The L-arginine pathway becomes dysfunctional with age, and we therefore need a backup system to compensate. Eating a diet rich in NO activity, that

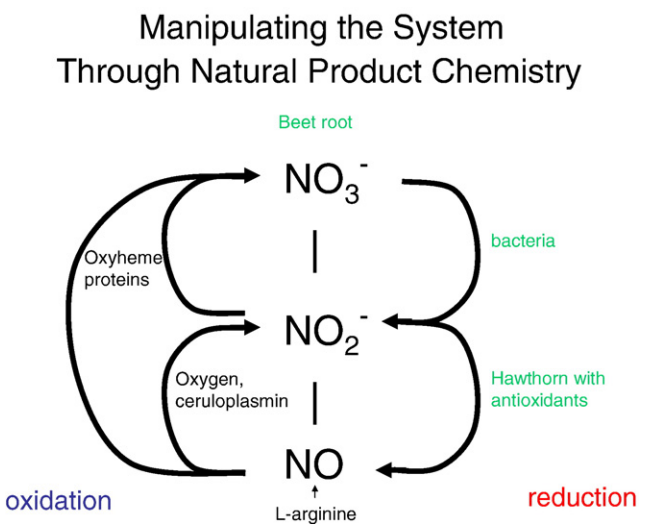

Fig. 4. The nitrate-nitrite-NO pathway. Providing a source of nitrate through beetroot is the first step in feeding this pathway. Bacteria that reside in and on our body reduce nitrate to nitrite. Using herbs with robust nitrite reductase activity can facilitate formation of NO from physiological concentrations of nitrite. This pathway is recycled in the body, providing a system for generating NO independent of endothelium derived NO.

is, sufficient nitrite and nitrate along with antioxidants and botanicals to facilitate reduction to NO, can appear to overcome an insufficiency in endothelial-derived NO. This dietary pathway does not appear to be affected by age. However, overuse of antibiotics or antiseptic mouthwashes can affect this pathway by killing off the commensal bacteria that are essential for the first step of nitrate reduction to nitrite. Furthermore, use of proton pump inhibitors can decrease the acid secretions in the stomach, thereby affecting the acidic disproportionation of nitrite to NO. This dietary pathway is reliant upon recognizing foods that are rich in NO activity.

We believe that the inherent NO bioactivity of certain cardioprotective foods (such as green leafy vegetables) or diets (Mediterranean or Dietary Approaches to Stop Hypertension diet) is a delicate balance between nitrite and nitrate content as well as antioxidant capacity to facilitate reduction to NO and to inhibit any unwanted nitrosation reactions. The nitrate-nitrite-NO pathway is illustrated above in Fig. 4. The 2 ways to promote NO production are shown. Providing a rich source of nitrate either through direct supplementation with nitrate salts or through nitrate-rich foods such as beetroot will increase circulating levels of nitrite [18,22,44]. However, because of the inherent inefficiencies that exist for reducing nitrite to NO along the physiological oxygen gradient [29], to effectively use nitrite to make NO, this step in the pathway must be enhanced. One could accomplish this by supplying more nitrate to generate supraphysiological concentrations of nitrite, or one could introduce a robust nitrite reductase to more effectively reduce nitrite to NO along the physiological oxygen gradient in vivo. We have found that the Hawthorn berry can serve such a role. This system uses physiological concentrations of nitrate and nitrite, supplied by the diet, to effectively generate NO through the stepwise reduction of nitrate and nitrite by supplying the necessary substrates and machinery to perform these steps. The ingredients in the NO dietary supplement tested here were selectively and intentionally included to affect each step of the nitrate-nitrite-NO pathway, providing a system for restoring NO homeostasis safely, effectively, and naturally.

The role of diet in the prevention and control of morbidity and premature mortality because of noncommunicable diseases has been well established by the vast population-based epidemiological studies carried out during the last decade [45]. Nitric oxide is essential for maintaining normal blood pressure, preventing adhesion of blood cells to the endothelium, and preventing platelet aggregation. It may be argued that this single event, the inability to generate NO, puts us at risk for diseases that plague us later in life, such as atherosclerosis, myocardial infarction, stroke, peripheral vascular disease, and even Alzheimer disease. Therefore, developing strategies and new technologies designed to restore NO availability in the body is essential for inhibiting the progression of certain diseases. We believe we have identified key components in the dietary pathway to promote NO production that can make this pathway more effective and overcome a deficiency in endothelial-derived NO from L-arginine. Although these studies were simply designed to show proof of concept for restoring NO homeostasis through a dietary supplement, therefore modifying risk factors for CVD, more studies are needed to more clearly define the role of NO dietary supplement for optimal health and nutrition. Future studies will determine if such strategies using NO active dietary supplements can help reduce the progression of CVD and the incidence of myocardial infarction and stroke.

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