

Nitric Oxide in Health and Physical Performance: Considering the Molecule 2 Nitrooxy Ethyl 2 Amino 3 Methylbutanoate

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Abstract

Nitric oxide (NO[·]) is an important signaling molecule that has received considerable attention in recent years for its beneficial impact on a variety of health related outcomes. The effects range from improving various aspects of circulatory health to aiding exercise performance. Although NO[·] is produced naturally within the body, methods have been proposed to increase circulating NO[·] within humans. Most notably, the performance of regular exercise, the use of pharmaceutical agents, and the use of nutritional ingredients has been studied. A newly developed molecule known as 2 nitrooxy ethyl 2 amino 3 methylbutanoate (VEEN™) is now being studied as a next generation NO[·] agent. This paper discusses the importance of NO[·] in human health, with a particular focus on methods to increase NO[·]. Attention is given to VEEN™, highlighting the studies performed to date using this molecule.

Keywords: Nitric oxide, Blood pressure, Exercise, Health, Nutritional supplements

1. Nitric Oxide Defined

Nitric oxide (NO[·]) is an important signaling molecule initially referred to as endothelium-derived relaxing factor (Furchgott and Zawadzki, 1980) and known to induce vasodilation (Collier and Vallance, 1989). Nitric oxide has multiple beneficial effects within the human body—in particular when present at low (nanomolar) concentrations. These include, but are not limited to decreased smooth muscle cell proliferation and platelet and leukocyte adhesion (Bian et al., 2008), regulation of neurotransmission (Thomas et al., 2008), regulation of immune defense (Tripathi, 2007), influence on excitation-contraction coupling and myofibrillar function (Sheffield-Moore et al., 2013), regulation of muscle hypertrophy

(Leiter et al., 2012), and stimulation of satellite cells (Anderson, 2000).

The effect that has garnered the greatest interest over the past several years relates to the ability of NO[·] to induce vasorelaxation of smooth muscle (Loscalzo, 2013). Theoretically, such vasorelaxation would enhance blood flow to specific body tissues, potentially resulting in a variety of cardiovascular benefits—including a reduction in blood pressure, a decrease in myocardial work, and an enhancement in sexual function. Increased blood flow may also provide an ergogenic benefit related to the performance of acute exercise bouts—as has been suggested in recent research focused on the ingestion of nitrates prior to exercise (Hoon et al., 2013; Ormsbee et al., 2013).

Although multiple methods have been proposed to increase circulating NO[·], this molecule is adequately synthesized within most humans by a family of enzymes known as nitric oxide synthases (Robinson et al., 2011), using a combination of the amino acid L-arginine, oxygen, and a variety of other cofactors. As stated, while relatively low concentrations of NO[·] can impart multiple favorable effects on human health, very high concentrations of this molecule favor cell cycle arrest and programmed cell death (Napoli et al., 2013); the effect which may be dependent on the flux and concentration of NO[·], the cell type, and the cellular redox status (Wang et al., 2010). This may be partly related to the potential formation of peroxynitrite, a harmful reactive nitrogen species which can be generated when NO[·] interacts directly with the superoxide radical (Beckman and Koppenol, 1996).

Nitric oxide appears to function via both a cyclic guanosine monophosphate (cGMP) dependent and independent signaling cascade (Gewaltig and Kojda, 2002). It does so while functioning as a gaseous chemical compound—making oral ingestion near impossible; however, inhaled NO[·] is widely used clinically (Bloch et al., 2007). In most circumstances, agents that are thought to stimulate the production of NO[·] must be provided, rather than the actual NO[·] itself. This has been an area of interest almost since the work of Furchgott and Zawadzki (1980) over 30 years ago. In fact, multiple lines of research related to NO[·] have been ongoing, as evidenced by the naming of NO[·] as “molecule of the year” by *Science* magazine in 1992, and the awarding of the Nobel Prize in Physiology or Medicine in 1998 for work related to NO[·] signaling within the cardiovascular system. Clearly, this molecule is of importance within the scientific and clinical community.

2. Nitric Oxide, Health and Physical Performance

The majority of NO[·] research over the past few decades has been focused on how this molecule can improve certain aspects of human health. While many areas of study have been of interest to scientists, the topic of NO[·]-stimulated blood flow appears at the forefront. In fact, a PubMed search performed in June 2015 using the term “nitric oxide and blood flow” returned more than 11,000 articles. Indeed, this is a topic of intense interest.

Enhanced blood flow may be mechanistically responsible for improving certain aspects of cardiovascular health such as reducing blood pressure. In turn, a reduction in blood pressure may have a direct effect on improving heart health (e.g., reduction in myocardial work due to the decreased afterload), as well as reducing the potential for stroke. While not a “life or

death” concern, improving blood flow to improve overall sexual function is also of great interest to individuals. Specifically, drugs that directly or indirectly impact NO[•] bioavailability have been used widely to aid erectile function in men. Certain nutritional ingredients with a focus on the NO[•] pathway have also been studied for purposes of improving sexual function (Estrada-Reyes et al., 2013).

2.1 Physical Performance

In relation to physical performance and sport supplementation, NO[•] is of great interest for its potential effects on increasing blood flow—with a secondary effect in regulating muscle atrophy and hypertrophy. Bodybuilding and fitness magazines are inundated with pages of advertisements for NO[•]-stimulating nutritional supplements. Unfortunately, there are few studies (aside from those focused on nitrate ingestion) demonstrating benefits of these supplements on measures of exercise performance and related outcomes (e.g., muscle “pumps”).

Acute exercise itself can induce an increase in NO[•] (Bode-Böger et al., 1994a; Gilligan et al., 1994; Hickner et al., 1997), likely owing to the shearing forces placed on the vasculature (Ramírez-Vélez et al., 2013) which provide the signal for endothelial cells to produce NO[•]. Likewise, the performance of regular exercise leads to an increase in circulating NO[•] metabolites nitrate (NO₃⁻) and nitrite (NO₂⁻), suggesting an increase in NO[•] production (Edwards et al., 2004; Poveda et al., 1997). Hence, individuals with the interest in increasing NO[•] for purposes of improving overall health and physical performance would be best served by performing regular and strenuous exercise—in addition to paying close attention to other lifestyle factors known to influence NO[•] production and availability (e.g., cessation of cigarette smoking, maintenance of an ideal body weight, reducing intake of saturated fat and sugar, maintaining low levels of oxidative stress) (Meldrum et al., 2012).

Although it remains to be directly demonstrated (Bloomer, 2010), the theoretical model suggests that increased NO[•] will result in increased blood flow to working skeletal muscle during and following exercise. This, in turn, will result in increased oxygen and nutrient delivery (e.g., amino acids, fatty acids, glucose) which should aid both exercise performance and exercise recovery—ultimately leading to muscle hypertrophy.

Aside from NO[•] regulation of exercise blood flow, several other mechanisms are involved with the redistribution of blood flow during and following exercise (Joyner and Wilkins, 2007; Tschakovsky and Joyner, 2008). These include muscle contraction-induced distortion of resistance vessels, flow mediated dilation, alterations in chemicals known to alter vessel diameter such as adenosine, endothelin, and prostacyclin, in addition to changes in muscle temperature, pCO₂, pO₂, and pH. Hence, NO[•] may play a smaller role in regulating exercise blood flow than many have been led to believe (Tschakovsky and Joyner, 2008).

3. Proposed Methods to Increase Nitric Oxide

As mentioned above, regular exercise has been demonstrated to increase NO[•] production. In addition, alteration of whole food intake in favor of nitrate-rich vegetables and low saturated fat/low sugar foods may prove helpful (Bloomer et al., 2011; Trepanowski et al., 2012). In

addition, a variety of pharmaceutical agents have been used in an attempt to alter NO[•] production and/or availability. Lastly, certain nutritional ingredients have yielded positive outcomes (e.g., beetroot).

In relation to pharmaceuticals, several agents have been used with success to either increase NO[•] biosynthesis or bioavailability, with the end result being enhanced vasodilatation (Burgaud et al., 2002). These agents include the sublingual and transdermal nitrates, intravenous propionyl-L-carnitine, intravenous L-arginine, and other medications used for the treatment of erectile dysfunction (e.g., phosphodiesterase-5 inhibitors). Related to the latter, although specifically prescribed for purposes of improving erectile function, evidence indicates the potential role of a phosphodiesterase-5 inhibitor (Sildenafil) to impact outcomes related to physical performance and adaptations to regular exercise (Hsu et al., 2006; Rinaldi et al., 2013; Sheffield-Moore et al., 2013). This fact suggests the likelihood that phosphodiesterase-5 inhibitors are now being used within the athletic doping world.

3.1 Nutritional Ingredients

If individuals are not interested in using pharmaceutical agents in an attempt to influence NO[•], they may opt for isolated nutritional ingredients that may have an effect. Although various nutritional ingredients have been studied for purposes of influencing NO[•] (e.g., citrulline, resveratrol, grape seed extract), only a few have a relatively large number of studies dedicated to their use in human subjects. These are discussed below.

Historically, the amino acid L-arginine has been suggested for use, as arginine is directly involved in the biosynthesis of NO[•]. In addition, a specific form of carnitine known as propionyl L-carnitine or glycine propionyl L-carnitine (GPLC) has been used with success. Finally and most recently, beetroot juice and its active component, nitrate has been used to increase NO[•] and improve associated parameters (e.g., blood pressure, exercise performance). The below text briefly discusses these ingredients.

3.2 L-Arginine

A review of dietary supplements marketed over the past several years which are designed to increase NO[•] indicates that many products contain L-arginine as the chief ingredient—typically at a dosage of approximately 3 grams per serving. While the overall basis for arginine inclusion may seem justified, as arginine is the precursor to NO[•] biosynthesis and has been associated with enhanced vasodilatation (Bode-Böger et al., 1994b; Giugliano et al., 1997), a careful review indicates that a relatively small oral dosage of L-arginine may provide little overall benefit.

Consider that the rationale for L-arginine inclusion is based largely on research using intravenous L-arginine, often at a dosage as high as 20 or 30 grams. Early studies involving direct comparisons between intravenous and oral L-arginine indicate no vasodilatory effect of oral L-arginine, which may be attributed to variability in oral L-arginine bioavailability (Bode-Böger et al., 1998)—possibly due to the fact that oral L-arginine intake is negatively influenced by extensive elimination due to intestinal arginase activity (Schwedhelm et al., 2008). In support of these findings, studies involving oral intake of L-arginine at dosages

ranging from 10 to 20 grams indicate no benefit with regards to increasing circulating NO[•] or, more importantly, impacting the more important end result of enhanced blood flow (Adams et al., 1995; Chin-Dusting et al., 1996). Finally, the possibility that L-arginine itself may not be the rate limiting component to NO[•] biosynthesis must be considered (Kurz and Harrison, 1997). Rather, nitric oxide synthase enzymes may be most important. Hence, simply adding L-arginine at high dosages may provide little to no benefit if the enzymes involved in NO[•] synthesis are not available at optimal activities to facilitate the formation of the molecule.

3.3 Propionyl-L-Carnitine (and Glycine Propionyl-L-Carnitine)

Propionyl-L-carnitine (PLC) has been used as a prescription drug in Europe, primarily for the treatment of intermittent claudication. At an intravenous dosage of 6 grams per day, PLC has been demonstrated to increase blood NO[•] metabolites (Loffredo et al., 2007). Glycine Propionyl-L-carnitine is a molecular bonded form of PLC and the amino acid glycine, sold as a dietary ingredient. Oral intake of GPLC at a dosage of 4.5 grams per day results in increased plasma NO₃⁻ + NO₂⁻. This has been reported in previously sedentary men and women following an eight week intervention (Bloomer et al., 2009) and in resistance trained men following a four week intervention (Bloomer et al., 2007). The mechanism of action for this apparent increase in NO[•] with PLC and GPLC appears mediated by a decrease in NADPH oxidase activation (Pignatelli et al., 2003). It is known that NADPH oxidase can lead to superoxide radical generation (Zalba et al., 2001), which can then interact with NO[•] to form peroxynitrite (Beckman et al., 1996) and decrease NO[•] availability. Propionyl-L-carnitine can also increase endothelial nitric oxide synthase (eNOS) (de Sotomayor et al., 2007), leading to increased NO[•] production.

3.4 Beetroot Juice and Nitrate

An emerging area of investigation is the study of beetroot juice (and the active ingredient contained within the beetroot juice—nitrate) to aid exercise performance and associated parameters (Hoon et al., 2013; Ormsbee et al., 2013). Recent reviews of literature indicate that approximately 60 studies have now been performed focused on beetroot juice, beetroot, or sodium nitrate to improve certain aspects of aerobic or anaerobic exercise performance, as well as mechanisms responsible for the ergogenic effect. One such mechanism is enhanced NO[•] production and/or bioavailability. Based on the volume of research performed to date using nitrate (either as a component of beetroot or as sodium nitrate), this may be the most promising nutritional ingredient available at the present time for purposes of enhancing NO[•] and yielding the desired effects of NO[•] elevation.

4. 2 Nitrooxy Ethyl 2 Amino 3 Methylbutanoate (Veen™)

While not technically a pharmaceutical agent or a nutritional ingredient, a new molecule known as “2 nitrooxy ethyl 2 amino 3 methylbutanoate” has been developed with the objective of delivering NO[•] to the circulation and enhancing health and physical performance. This molecule, trademarked as VEEN™ (Smartek International), has a mechanism of action that is proposed to differ from other NO[•] stimulators. Specifically, VEEN™ does not provide precursor amino acids or other agents targeting increased NO[•] biosynthesis (such as other

nutritional ingredients). Nor does VEEN™ seek to inhibit the degradation of NO in order to prolong the effects (such as medications used for erectile dysfunction). Rather, VEEN™ is claimed to deliver actual NO to the circulation.

Within cells, the physiological effects of VEEN™ appear related to a dual phase metabolism of the molecule. The first phase comprises the carboxylesterase cleavage of the valine from the nitrooxy ethyl alcohol, while the second phase involves the glutathione transferase regulated metabolism of nitrooxy ethyl alcohol in the cytosol of endothelial cells to NO. Variance in subject response to VEEN™ treatment may be partly explained through a genotypic difference in carboxylesterase 2 activity (Wu et al., 2004), which would limit the first cleavage reaction to produce the active intermediate. In addition, a laminar flow feedback mechanism (Chen et al., 2003) of glutathione transferase in the endothelial cells may contribute in the second phase reaction. Additional research is needed to confirm these hypotheses.

Presently, in human subjects the VEEN™ can be delivered either as a sublingual fast-dissolve tablet or as a transdermal rub-on gel. Both delivery systems have been studied recently, as detailed in the text below. In animal research, the VEEN™ molecule has been delivered via inhalation as well. This is also discussed in the relevant section below. In terms of safety, the VEEN™ molecule has recently been investigated extensively by AIBMR Life Sciences, Inc. and has been granted self-determination Generally Recognized as Safe (GRAS) status. A schematic of the potential mechanisms and impact of VEEN™ in human physiology is presented in Figure 1.

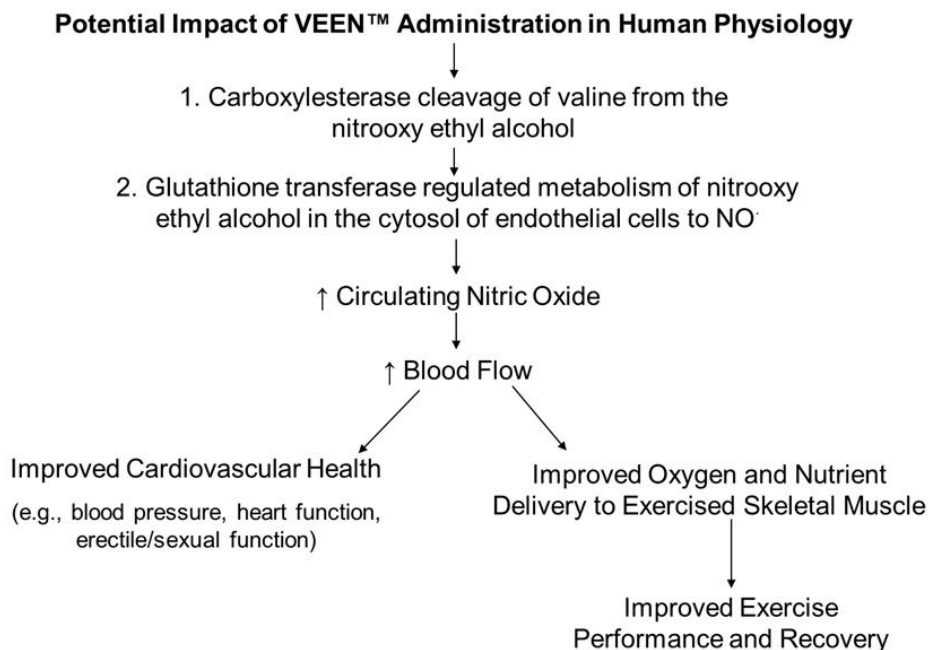


Figure 1. The Potential Impact of VEEN™ Administration in Human Physiology

4.1 Human Studies of VEEN™

In the first human study of VEEN™, 10 resistance-trained men received either a placebo or the VEEN™ fast-dissolved sublingual tablets on two separate occasions using a randomized, cross-over design (Bloomer et al., 2010). Blood samples were taken before and at 5, 15, 30, and 60 minutes after complete breakdown of the supplement or placebo. An approximate 7% increase in circulating nitrate/nitrite was noted at the 15 minute post-ingestion period for subjects when ingesting the VEEN™ tablets. Heart rate and blood pressure were measured at the times indicated above and noted to be nearly identical between the VEEN™ and placebo conditions, and relatively unchanged across time. No measures of physical performance were obtained in that study.

With consideration that oral delivery leads to systemic uptake of the molecule and potential for compartmentalization into specific tissues, the VEEN™ has also been imbedded within a topical, gel-based delivery system. The gel is applied to the skin and rubbed on; in much the same way as lotion would be applied. Using this gel-based delivery technology, a sample of 14 resistance-trained men received either a placebo gel or the VEEN™ gel to use for a period of seven days (with 7-10 days between each condition) using a randomized, cross-over design (Bloomer et al., 2012). Subjects applied the gel to their upper and lower arms each day and then reported to the lab to perform assessments of arm isometric force and muscular endurance (3 sets to fatigue using 80, 65, and 50% of 1 repetition maximum [1RM]: total of 9 sets). Subjects' heart rate and perceived exertion related to the exercise were measured after each set and were nearly identical between conditions; with the lack of change in heart rate highlighting the non-stimulatory nature of the molecule. While no performance measure was noted to be statistically significant, in 8 subjects who were noted as being “responders” to the VEEN™ treatment, 19.9% more repetitions were performed when using a load of 50% of 1RM. In 9 of the 14 subjects tested, higher blood NO⁻ metabolites were noted with greater total repetition number, suggesting the potential involvement of NO⁻ in exercise performance. Future experiments are needed to confirm this hypothesis.

Most recently, VEEN™ or placebo was provided to 15 healthy men in a fast-dissolved sublingual tablet on two separate occasions using a randomized, cross-over design (Bloomer et al., 2015). Subjects received their assigned condition before performing two, two-minute sets of sit-up exercise (exercise sets performed 20 minutes apart). Heart rate, as well as systolic (SBP) and diastolic (DBP) blood pressure were measured before and for 20 minutes following each set of exercise. In addition, blood was collected and analyzed for nitrate/nitrite. Results indicated that heart rate was unaffected by treatment. However, SBP and DBP was lower with VEEN™ as compared to placebo, in particular during the 20 minute recovery period following set two of exercise. Nitrate/nitrite was increased approximately 10% above baseline with VEEN™, suggesting a possible impact of NO⁻ on blood pressure regulation. Interestingly, the impact of VEEN™ on blood pressure appears to be in response to the acute elevation in blood pressure. That is, with relatively stable blood pressure, VEEN™ does not appear to induce any further reduction. However, when blood pressure is acutely elevated, the molecule appears to be activated and leads to a compensatory reduction in blood pressure. Further research is needed to extend these initial findings.

4.2 Animal Studies of VEEN™

A total of four animal studies using VEEN™ have been conducted to date. Two studies involving rats and two studies involving a ferret model have been completed (unpublished findings). In one rat study, the VEEN™ was delivered orally for a period of 90 days. Findings after 90 days of treatment indicated slight changes in the mean percentage of reticulocytes and organ weight (spleen, kidney, liver) in both male and female animals, with slight changes in reticulocytes and spleen weight relative to body weight in male animals only. Based on these findings, the study investigators concluded that the No Observed Adverse Effect Level (NOAEL) was 1000mg/kg body weight/day for both male and female rats.

When using a transdermal cream for a period of 10 days and compared against other antibiotic creams in the treatment of leishmaniasis in rats, the VEEN™ cream was noted to be more effective than all treatments except for one (a double antibiotic cream). Moreover, no adverse effects were observed.

The studies in the ferret model involved an investigation of the aerosolized VEEN™ molecule to impact H1N1 infected animals. In both studies, animals were treated with either the VEEN™ or vehicle (nebulized saline) twice daily for 5 days. Results indicated that the molecule was safe, with no reported adverse effects. In addition, blood nitrite levels were measured and noted to be elevated in animals treated with the VEEN™ molecule.

4.3 Future Studies with VEEN™

As with all new developments and discoveries, well-designed research studies are needed to explore their potential applications. In three human studies on normotensive individuals, VEEN™ has been shown to be a novel NO[•] donor and vasodilator; however these studies have been relatively small in size and scope. Additional human studies are needed to determine the influence of VEEN™ on aspects of human health, including blood pressure reduction, sexual function (and associated parameters), and physical performance in relevant populations. Select subject populations should be chosen for such work (e.g., those with hypertension, men with erectile dysfunction, recreationally active exercise enthusiasts).

5. Conclusion

Nitric oxide is an important signaling molecule that has great potential as a therapeutic aid. While lifestyle factors such as performance of regular exercise, maintenance of ideal body weight, ingestion of a healthy diet, and cessation of cigarette smoking are paramount if attempting to maximize circulating NO[•], adjunctive therapy is available in the form of pharmaceutical and nutritional agents. While additional research is certainly needed, the VEEN™ molecule may have potential as a next generation NO[•] agent. Ongoing and future research should provide more evidence regarding the potential application of this molecule and nutritional agents such as nitrate to aid human health and performance.

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