NitroFX and NitroXtreme

The Science behind Kyäni's Nitric Oxide Precursors

Clair A. Francomano, M.D. Abbas Qutab, M.D. Thomas Burke, PhD

Nitric oxide (NO) is a short-lived gaseous molecule that plays a key role in numerous biological processes essential for human health. As of late October 2011, over 112,000 papers discussing NO were listed on PubMed, the National Library of Medicine' s web site. Kyani's NitroFX and NitroXtreme both contain concentrates of the Noni plant, which has high levels of nitrates and nitrites. These molecules serve as precursors of NO. This paper will discuss the biochemical processes whereby nitrates and nitrites are converted into NO, as well as the physiological functions served by NO throughout the body. In addition, we discuss the additional ingredients that make NitroXtreme even more powerful than NitroFX.

History:

The key role that NO plays in human biology was first recognized in 1980 (1-3) and was reviewed in the *New England Journal of Medicine* in 1993 and 2006 (4,5). The importance of this discovery was underscored in 1998 by the awarding of the Nobel Prize in Physiology or Medicine to three scientists involved in its discovery: Robert Furchgott, Louise Ignarro and Ferid Murad.

Nitrogen gas (N_2) is the most abundant element in the atmosphere and in this form represents the largest pool of nitrogen on our planet. The nitrogen cycle serves to convert N_2 to a form that can be used in biological processes. In the first step, atmospheric nitrogen is converted into the ammonium ion i(NH_{4+}) a process called nitrogen fixation. Ammonium is then modified to a number of different nitrogen oxides, including nitrite (NO_2^-) and nitrate (NO_3^-). The process of denitrification completes the cycle: Nitrate is reduced first to nitrite, nitric oxide, nitrous oxide and finally back to nitrogen gas (N_2), which can then diffuse back into the atmosphere. As a gas, NO has an extremely short half life but even during that short time it can activate a number of enzymes and these activated enzymes cause NO-mediated effects that can last for several hours. Any NO that is not used immediately in a biologic process is rapidly converted back to nitrite and nitrate. The metabolic steps in the nitrogen cycle depend heavily on bacteria, which have metabolic pathways not found in plants or animals to catalyze the different steps of the cycle.

Pathways for NO production:

The amino acid arginine was the first recognized precursor of nitric oxide synthesis. A metabolic pathway catalyzed by enzymes called nitric oxide synthases (NOSs) converts arginine to nitric oxide through a complex five-step process. However, in 1994 it was found, by two independent research groups, that nitric oxide could be made independent of the NOSs and arginine (6,7). These groups found

that ingestion of nitrate ***greatly*** enhanced production of nitric oxide. High concentrations of nitric oxide are produced from nitrates by the acidity in the stomach. This process depends, in part, on nitrites derived from saliva. In the gastrointestinal tract, circulation of nitrogen containing molecules occurs in the following manner: Any nitrate that escapes conversion to NO in the stomach enters the circulation or passes into the small intestine and is absorbed into the circulation; subsequently nitrate is delivered to the salivary glands and the saliva containing nitrate is actively secreted into the mouth. Bacteria in the mouth then convert ("reduce") nitrate to nitrite (8). The process of nitrate reduction also occurs in the heart (9) and multiple other organ systems throughout the body.

The generation of NO is thought to be involved in multiple biological processes. Among those physiologic functions known to be dependent on NO signaling are regulation of blood flow (10), cellular signaling, and response to hypoxia, or low oxygen levels (11,12). (Unlike the arginine-based pathway for generation of NO, the nitrate-nitrite-NO pathway is up-regulated in the hypoxic state.) Other functions known to be highly dependent on NO concentrations include the inhibition of platelet stickiness (13), lung function (14), immunity (15), metabolic (energy) regulation (16), nerve transmission (17), and pain perception (18). Low NO levels are thought to play a key role in a number of different diseases of the cardiovascular system as well as in the metabolic syndrome of obesity, hypertension and hyperlipidemia.

However NO is produced (either from L-arginine or nitrates), it is can be rapidly oxidized to produce nitrates and nitrites, with concentrations of nitrates being at least two orders of magnitude higher than those of nitrites. This is due, in part, to the differences in the half-lives of these two molecules; the half-life for nitrate is 5-6 hours, while the half-life for nitrite is only 20 minutes.

In humans, vegetables, especially green leafy vegetables, are a rich source of nitrate. Studies have found that systemic concentrations of nitrate increase markedly following ingestion of these vegetables. In fact, one study found that one serving of a green leafy vegetable contains more nitrate than the nitrate formed from L-arginine by all the NOS enzymes in the body combined during a day (12). The noni plant, from which NitroFX and NitroXtreme are made, is a particularly good source of nitrates and nitrites.

Up to 25% of circulating nitrate is taken up by the salivary glands, and nitrate is concentrated 10-20 fold in saliva (8). After nitrate is ingested and absorbed through the stomach and small intestines, salivary concentrations become very high (19,20). Bacteria in the mouth convert nitrate to nitrite by means of a particular set of enzymes that are not found in human cells. When saliva is swallowed and enters the acidic environment of the stomach, part of the nitrite is converted to nitrous acid (HNO_2) that decomposes to form nitric oxide (6,7). Low pH (the acidity of the stomach) and reducing compounds such as ascorbic acid and polyphenols enhance this reaction. Most of the nitrite in the saliva, however, is not converted to NO but is absorbed into the bloodstream. Interestingly, the circulating nitrite can be taken up by cells lining the blood vessels and converted to nitric oxide.

Nitric Oxide in the Stomach:

It is thought that the high concentrations of nitric oxide in the stomach may constitute a first line of defense against potential pathogens, as nitric oxide in high concentrations is known to kill bacteria (21). Gastric nitric oxide is also thought to play a role in the control of blood flow to the cells of the stomach, as well as the production of mucus, which is very important for protecting the lining of the stomach wall from damage by the acid environment (22,23).

Seriously ill patients in intensive care unit settings who are on ventilators do not produce much saliva, and they do not swallow much of what they produce. Moreover, they are often treated with potent antibiotics to prevent infection, as well as H2 blockers or proton-pump inhibitors which increase the pH in the stomach. These patients have extremely low levels of nitric oxide in the stomach (24, 25), and they are at high risk of developing stomach ulcers and bacterial infections in the stomach. One study found that nitric oxide levels in the stomach can be increased by putting nitrite directly into the stomach through a feeding tube; circulating levels of nitrite are also increased in these patients (24). The advantage of any maneuver which increases nitric oxide in extremely ill patients should be obvious.

Bioactivation of Nitrite:

Beyond the simple process that occurs in the stomach to add a proton to nitrite and create nitrous acid, there are multiple different enzymatic pathways in the body that serve to convert systemic nitrite to nitric oxide (11,12). Molecules and enzymes including hemoglobin, myoglobin, neuroglobin, xanthine oxidoreductase, aldehyde oxidase, carbonic anhydrase, endothelial nitric oxide synthase (eNOS) and the enzymes in the mitochondria all play a role in activation of nitrite in the body. Different pathways play different roles in different tissues at different times, depending on numerous factors including pH, oxygen status, concentration of oxygen free radicals.

While the important role of hemoglobin in transporting oxygen throughout the body has long been recognized, the interaction between hemoglobin and NO was recognized much more recently. The oxygen binding status of hemoglobin affects its ability to convert nitrite to NO (26-28). Gladwyn and his colleagues demonstrated that nitrite bioactivation (that is, conversion to NO) is most active during periods of rapid deoxygenation. This provides a mechanism whereby the body is able to produce more NO in times of low oxygen, resulting in increased dilation of the blood vessels, more blood flow and hence more oxygen delivery to hypoxic tissues. The authors suggest that this may be one of the mechanisms used by the body to increase vasodilation in times of hypoxia (26). Another route of NO production occurs in red blood cells that use the enzyme endothelial nitric oxide synthase (eNOS) to produce NO from L-arginine. Patients who are anemic are known to be at risk from the events associated with low NO availability including hypertension. Given the evidence that NO can be generated by leafy green vegetables and by NitroFX and NitroXtreme, this may provide a nutritional way to help support optimal health in patients who are anemic.

Myoglobin is also known to play a role in nitrite bioactivation. In the instance of myocardial ischemia, myoglobin converts nitrite to NO in the heart muscle in much the same way that hemoglobin does in the

intravascular space. Studies have demonstrated that nitrite has a cardio-protective effect mediated through myoglobin (29). A similar molecule in the nervous system, called neuroglobin, also has the ability to convert nitrite to NO through the process of reduction. Several other proteins, including xanthine oxidase (30), several different mitochondrial enzymes (31-35), and the mammalian cytochrome P450 enzymes (36) also play a role in reduction of nitrite to NO. As mentioned above, one of the enzymes involved in the generation of NO from arginine, eNOS, can also convert nitrite to NO under the right conditions, including low oxygen levels and low pH.

Summary:

There are multiple mechanisms whereby nitrite can be converted to nitric oxide and other nitrogen oxides. NOS-dependent production of NO is somewhat dependent on oxygen levels and pH. However the enzymatic pathways involved in nitrite reduction are highly dependent on low oxygen levels and low pH, situations in which the nitric oxide synthases may not be working optimally.

Nitrates, Nitrites and the Cardiovascular System:

The first reports of dilating the coronary arteries by pharmacologic doses of inorganic nitrite were published almost 100 years ago (37). Recent studies have found a vasodilatory effect of much lower levels of circulating nitrite (38-41). Organic nitrites, derived from plants, are much more potent than inorganic nitrite. The preferential conversion of nitrite to NO under conditions of hypoxia may well have important clinical applications in the care of patients with myocardial ischemia (39, 40, 42).

Multiple studies have demonstrated that increased consumption of fruits and vegetables provides a cardio-protective effect (43-46). It has also been shown that inorganic sodium nitrate, administered in the quantities corresponding to the amount present in a serving of nitrate-rich vegetable, reduced diastolic blood pressure by 4 mm Hg in one study (47) and had a comparable effect on systolic pressure in a later study with a larger number of participants (48). In a model of high blood pressure and renal disease induced by chronic blockage of NOS, nitrite supplementation ameliorated the blood pressure and a low dose of oral nitrite protected against kidney injury (49).

Administration of nitrate, either inorganic or through natural sources, has been shown to have demonstrable therapeutic effects in multiple organ systems in many different species, including humans. These include improved cardiovascular parameters in mouse and rat models of cardiac ischemia (50-52), decreased ulcer formation and improved mucus secretion in the stomach in a rat model of gastric ulcers (53, 54), decreased platelet aggregation, decreased blood pressure, improved endothelial function, decreased oxygen consumption with moderate or maximal exercise, and improved work efficiency in human studies (55-62). Administration of inorganic nitrite has been found to have a similarly wide-ranging list of benefits in rodent, canine and human studies (29,36,38, 49, 63-75).

In studies involving mouse models of sepsis, or disseminated bacterial infection, causing dangerously low blood pressure or septic shock, administration of nitrite improved survival and reduced mitochondrial damage, tissue damage from infarction, hypothermia and oxidative stress (75).

Because nitric oxide is a gas, it is possible to administer it through inhalation, and this approach has been used to treat babies with high blood pressure in the pulmonary circulation (76). In animal studies, inhaled nitrite has improved hypertension in the pulmonary circulation as well (42).

Transplantation studies:

In a rat model of cardiac transplantation, oral supplementation of nitrite prolonged graft survival from 50 days in animals on a control diet to over 120 days in animals receiving nitrite supplementation in their drinking water. Animals receiving a low-nitrite diet had reduced survival of the allograft, to an average of 31 days (64).

Antimicrobial effects:

The ability of NO to act against multiple bacterial pathogens has been well established (77-79). In an animal model of cystic fibrosis, nitrite was successfully used to clear *Pseudomonas aeruginosa*, a common bacterial infection in this disease (80).

The conversion of nitrite to nitric oxide in acidified urine may be why vitamin C and Cranberry juice are successful in preventing and treating urinary tract infections (81). In the laboratory setting, the combination of vitamin C with nitrite is comparable to antibiotics (82). Carlsson and colleagues used nitrite and ascorbic acid in a laboratory model of the urinary tract, successfully killing two different forms of *E. Coli* growing in the urine (83).

Dietary Considerations:

Because there are ample data that administration of nitrate and/or nitrite provides substantial benefit in multiple clinical situations, and because many epidemiological studies have demonstrated similar benefit from diets rich in fruits and vegetables, it has been speculated that nitrate may be the active ingredient in diets such as the Mediterranean diet (43) or the fruit- and vegetable-rich diet advocated by the Dietary Approaches to Stop Hypertension (DASH) program (44).

NitroXtreme:

Some may question why we have included 5 additional ingredients in NitroXtreme.

CoQ10:

Coenzyme Q10 is a natural, fat-soluble nutrient present in virtually every cell in the body but often it can be depleted by various drugs, including statins used to treat high cholesterol (84). Since it is vital to the production of adenosine triphosphate (ATP) (X), which is 90% of the energy in the body, one can see that anyone who exercises needs to produce as much ATP as possible (85). ATP is necessary for nerve and muscle function…with diminished ATP, you will "hit the wall" more quickly. CoQ10 has been shown to reduce atherosclerosis (hardening of the arteries), angina, and congestive heart failure. Therefore CoQ10 is beneficial in treating and preventing cardiovascular disorders (CVD) (86). Finally CoQ10 is beneficial for patients with diabetes, immune dysfunction, periodontal disease, breast and prostate

cancer, and those with different neurological conditions. There were 353 reviews on the medical effects of CoQ10 as of October 1, 2011. The reader who is interested in a particular problem is encouraged to do a search on PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). The noni, nitrate based, related improvement in circulation speeds the delivery of CoQ10 to all cells in the body so that it can carry out the important functions mentioned above.

Niacin:

Niacin plays an important role in the release of energy from carbohydrates. It also aids in the breakdown of protein and fats, in the synthesis of fats and certain hormones and in the formation of red blood cells. Red blood cells carry oxygen AND nitric oxide so adding niacin to NitroXtreme increases the likelihood that nitric oxide will be available for delivery to all peripheral tissues. Niacin also increases skin blood flow, called "flushing" through increases in arachidonic acid and prostaglandins . Therefore vasodilation is achieved by niacin in NitroXtreme as well as from the nitrates and nitrites. Several studies also show that niacin is effective against more than one type of carcinogen. One example notes that breast cancer patients have lower than normal levels of niacin. If all this wasn't enough, niacin decreases both cholesterol and triglycerides in the blood. There is evidence that niacin may prevent cardiovascular disease, specifically the risk of a heart attack; it also may be an adjunct in the treatment of coronary artery disease. Many physicians suggest that starting niacin supplementation early in life may even prevent, or markedly delay the onset, the development of heart disease. In fact the FDA has recently approved a drug based on niacin for this very indication. Finally, niacin is a building block in nucleosides, nucleotides, nucleic acids, RNA and DNA. To summarize, it may help in preventing several forms of cancer, it stabilizes the nervous system, ensures motility and absorption of nutrients in the intestine and helps regulate hormone production (87-91).

Magnesium:

A meta-analysis of 20 randomized trials suggests that magnesium helps to maintain systolic and diastolic blood pressure (92). In addition, magnesium promotes glucose metabolism thus delaying or preventing increases in blood glucose (hyperglycemia/diabetes). This is not a short-term phenomenon. In one 15-year study involving 4,637 young adults, higher intakes of magnesium were associated with healthy cardiovascular function and glucose utilization. Magnesium also plays an important role in the conversion of carbohydrates, protein and fats to energy, in the synthesis of proteins and the synthesis of genetic material within each cell. It also supports muscle relaxation and contraction and nerve transmission. It appears certain that people in the US are either not consuming enough magnesium or are losing it in the urine at too high a rate because plasma levels are uniformly low in most adults (93).

Zinc:

Zinc is a component of numerous enzymes and which play a role in protein synthesis, in controlling blood sugar, in stimulating wound healing, and maintaining brain function. Zinc is important in the maintenance of healthy skin, the immune system, nervous, digestive and reproductive systems, the genetic code and normal blood levels of vitamin A. Finally, zinc is necessary for a healthy, growing fetus and in men, a healthy prostate. The National Institutes of Health has beautifully summarized the benefits of diets containing zinc and the devastating consequences of zinc deficiency (94). Please look at

this easy to read summary; you can see why Kyani's founders and the Medical and Scientific Advisory Board have incorporated zinc into the NitroXtreme formula.

Chromium:

The second most serious problems facing people (after cardiovascular disease) is obesity/diabetes. Chromium helps in the uptake of blood sugar into muscle and other cells and therefore helps to maintain blood sugar levels. It also reduces the risk of insulin resistance -- therefore may reduce the risk of people in developing type 2 diabetes. Less diabetes means less cardiovascular disease, less vision problems (retinopathy), less kidney disease, less, gastrointestinal problems, less neuropathy (pain and loss of sensation), fewer falls, and a better chance of actually engaging in exercise that is recommended by the American Diabetes Association (95). Finally chromium also helps control weight and supports the maintenance of a lean body mass. The value of chromium to the obesity problem faced by the western world cannot be over emphasized (96)

Summary:

Thus, there are multiple studies in the medical literature clearly documenting numerous health benefits of ingested nitrates and nitrites. NitroFX and NitroXtreme provide a ready source of organic nitrates and nitrites in a convenient, easily accessible form. Both are produced in a GMP manufacturing facility with highly reproducible concentrations of nitrates and nitrites. NitroXtreme, which contains somewhat higher amounts of the noni plant, is designed to provide a stronger boost in nitric oxide production over a longer period of time and has additional ingredients to aid in the production and utilization of NO in the body. These include: Coenzyme Q10, magnesium, zinc, chromium and niacin, as well as a proprietary ingredient designed to enhance absorption.

Kyäni recommends that NitroFX be employed for regular daily use, while NitroXtreme should be reserved for more demanding days. NitroFX has a milder flavor and has the benefits of NO production. NitroXtreme has a quicker result with a longer duration of NO production. The taste is bolder. Also, there are additional ingredients that increase Nitric Oxide production and improve the body's ability to use the NO. Those who have demanding lives or circumstances, such as athletes, will more fully benefit from using NitroXtreme.

References:

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-6, 1980.
2. Ignarro IJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84:9265-9, 1987.
3. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-6, 1987.
4. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Eng J Med 329:2002-12, 1993.

5. Murad F. Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. N Eng J Med 355: 2003-11, 2006.
6. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intra-gastric nitric oxide production in humans: Measurements in expelled air. Gut 35:1543-6, 1994.
7. Benjamin N, O'Driscoll F, Dougall H, Duncan C, Smith L, Golden M, McKenzie H. Stomach NO synthesis. Nature 368:502, 1994.
8. Spiegelhalder B, Eisenbrand G, Preussman R. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to in vivo formation of N-Nitroso compounds. Food Cosmet Toxicol 14:545-8, 1976.
9. Zweier FL, Li H, Samouilov A, Liu X. Mechanisms of nitrate reduction to nitric oxide in the heart and vessel wall. Nitric Oxide 22:83-90, 2010.
10. Bian K, Doursout MF, Murad F. Vascular system: Role of nitric oxide in cardiovascular diseases. J Clin Hypertens (Greenwich) 10:302-10, 2008.
11. Van Faassen EE, Bahrami S, Feelisch M, et al. Nitrite as regulator of hypoxic signaling in mammalian physiology. Med Res Rev 29:683-741, 2009.
12. Lundberg JO, Gladwin MT, Ahluwalia A et al. Nitrate and nitrite in biology, nutrition and therapeutics. Nat Chem Biol l 5:865-9, 2009.
13. Alonso D, Radomski MW. Nitric oxide, platelet function, myocardial infarction and reperfusion therapies. Heart Fail Rev 8:47-54, 2003.
14. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. Physiol Rev 84:731-65, 2004.
15. Bogdan C. Nitric oxide and the immune response. Nat Immunol 2:907-16, 2007.
16. Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nat Rev Mol Cell Biol 3:214-20, 2002.
17. Calabrese V, Mancuso C, Calvani M et al. Nitric oxide in the central nervous system: Neuroprotection versus neurotoxicity. Nat Rev Neurosci 8:766-75, 2007.
18. Miclescu A, Gordh T. Nitric oxide and pain: "Something old, something new." Acta Anaesthesiol Scand 53:1107-20, 2009.
19. Lundberg JO, Govoni M. Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radic Biol Med 37:395-400, 2004.
20. Govoni M Jansson eA, weitzberg E, Lundberg JO. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide 19:333-7, 2008.
21. Fang FC. Antimicrobial reactive oxygen and nitrogen species: Concepts and controversies. Nat Rev Microbiol 2:820-32, 2004.
22. Bjorne HH, Petersson J, Phillipson M, et al. Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. J Clin Invest 113:106-14, 2004.
23. Petersson J, Phillipson M, Jansson EA, et al. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. Am J Physiol Gastrointest Liver Physiol 292:G718-24, 2007.
24. Bjorne H, Govoni M, Tornberg DC, Lundberg JO, Weitzberg E. Intragastric nitric oxide is abolished in intubated patients and restored by nitrite. Crit Care Med 33:1722-7, 2005.

25. Bolvin MA, Flack Ca, Kennedy JC, Iwamoto GK. Etiology of decreased gastric nitric oxide in the critically ill. J Investig Med 54: 484-9, 2006.
26. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med 9:19-505, 2003.
27. Nagababu E, Ramasamy S, Abernethy Dr, Rifkind JM. Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. J Biol Chem 278:46349-56, 2003.
28. Huang Z, Shiva S, Kim-Shapiro DB et al. Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. J Clin Invest 115:2099-107, 2005.
29. Hendgen-Cotta UB, Merx MW, Shiva S, et al. Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury. Proc Nat Acad Sci USA 105:10256-61, 2008.
30. Godber BL, Doel JJ, Spkota GP, Blake DR, et al. Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. J Biol Chem 275:7757-63, 2000.
31. Kozlov AV, Staniek K, Nohl H. Nitrite reductase activity is a novel function of mammalian mitochondria. FEBS Lett 454:127-30, 1999.
32. Castello PR, David PS, McCLure T, Crook Z, Poyton R. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: Implications for oxygen sensing and hypoxic signaling in eukaryotes. Cell Metab 3:277-87, 2006.
33. Nohl H, Staniek K, Sobhian B, et al. Mitochondria recycle nitrite back to the bioregulator nitric monoxide. Acta Biochim Pol 47:913-21, 2000.
34. Li H, Cul H, Kundu TK, Alzawahra w, Zweler JL. Nitric oxide production from nitrite occurs primarily in tissues not in the blood: Critical role of xanthine oxidase and aldehyde oxidase. J Biol Chem 283:17855-63, 2008.
35. Golwala NH, Hodenette C, Murthy SN, Nossaman BD, Kadowitz PH. Vascular responses to nitrite are mediated by xanthine oxidoreductase and mitochondrial aldehyde dehydrogenase in the rat. Can J Physiol Pharmacol 87:1095-101, 2009.
36. Kozlov AV, Dietrich B, Nohl H. Various intracellular compartments cooperate in the release of nitric oxide from glycerol trinitrate in liver. Br. J Pharmacol 139:989-97, 2003.
37. Butler AR, Reelisch M. Therapeutic uses of inorganic nitrite and nitrate: From the past to the future. Circulation 117:2151-9, 2008.
38. Mack AK, McGowan VR, Tremonti CK, et al. Sodium nitrite promotes regional blood flow in patients with sickle cell disease: A phase I/II study. Br J Haematol 142:971-8, 2008.
39. Ingram TI, Pinder AG, Bailey DM, et al. Low-Dose sodium nitrite vasodilates hypoxic human pulmonary vasculature by a means that is not dependent on a simultaneous elevation in plasma nitrite. Am J Physiol Heart Circ Physiol 298:H331-9, 2010.
40. Maher AR, Milsom AB, Gunaruwan P, et al. Hypoxic modulation of exogenous nitrite-induced vasodilation in humans. Circulation 117:670-7, 2008.
41. Dejam A, Hunter CJ, Tremonti C, et al. Nitrite infusion in humans and nonhuman primates: Endocrine effects, pharmacokinetics, and tolerance formation. Circulation 116:1821-31, 2007.

42. Hunter CJ, Dejam A, Blood AB, et al. Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. Nat Med 10:1122-7, 2004.
43. Willett WC. Diet and health: What should we eat? Science 264:532-7, 1994.
44. Liese Ad, Nichols M, Sun X, D'Agostino RB Jr, Haffner SM. Adherence to the DASH Diet is inversely associated with incidence of type 2 diabetes: The insulin resistance atherosclerosis study. Diabetes Care 32:1434-6, 2009.
45. Joshipura KJ, Hu FB, Manson JE, et al. The effect of fruit and vegetable intake on risk for coronary heart disease. Ann Intern Med 134:1106-14, 2001.
46. Joshipura KJ, Ascherio A, Manson JE, et al. Fruit and vegetable intake in relation to risk of ischemic stroke. JAMA 282:1233-9, 1999.
47. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. N Eng J Med 355:2792-3, 2006.
48. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. Free Radic Biol Med 48:342-7, 2010.
49. Kanematsu Y, Yamaguchi K, Ohnishi H, et al. Dietary doses of nitrate restore circulating nitric oxide level and improve renal injury in L-NAME-induced hypertensive rats. Am J Physiol Renal Physiol 295:F1457-62, 2008.
50. Bryan NS, Calvert JW, Elrod JW, et al. Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Proc Natl Acad Sci USA 104:19144-9, 2007.
51. Jansson EA, Huang L, Malkey R, et al. A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. Nat Chem Biol 4:411-7, 2008.
52. Petersson J, Carlstrom M, Schreiber O, et al. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. Free Radic Biol Med 46:1068-75, 2009.
53. Myoshi M, Kasahara E, Park AM, et al. Dietary nitrate inhibits stress-induced gastric mucosal injury in the rat. Free Radic Res 37:85-90, 2003.
54. Jansson EA, Petersson J, Reinders C, et al. Protection from nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers by dietary nitrate. Free Radic Biol Med 42:108, 2007.
55. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 51:784-90, 2008.
56. Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. N Engl J Med 355:2792-3, 2006.
57. Richardson G, Hicks Sl, O'Byrne S, et al. The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. Nitric Oxide 7:24-9, 2002.
58. Kapil V, Milsom AB, Okorie M, et al. Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. Hypertension 56:274-81, 2010.

59. Larsen JF, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. Acta Physiol (Oxf) 191:59-66, 2007.
60. Vanhatalo A, Baily SJ, Blackwell JR, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. Am J Physiol Reg Integr Comp Physiol 299:R1121-31, 2010.
61. Bailey Sj, Fulford J, Vanhatalo A, et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. J Appl Physiol 109-135-48, 2010.
62. Sobko T, Marcus C, Govoni M. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Nitric Oxide 22:136-40, 2010.
63. Jung KH, Chu K, Ko SY, et al. Early intravenous infusion of sodium nitrite protects brain against in vivo ischemia-reperfusion injury. Stroke 37:2744-50, 2006.
64. Zhan J, Nakao A, Sugimoto R, et al. Orally administered nitrite attenuates cardiac allograft rejection in rats. Surgery 146:155-65, 2009.
65. Webb A, Bond R, McLean P, et al. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. Proc Natl Acad Scie USA 101:13683-8, 2004.
66. Duranski MR, Greer JJ, Dejam A, et al. Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver. J Clin Invest 115:1232-40, 2005.
67. Shiva S, Sack MN Greer JJ, et al. Nitrite augments tolerance to ischemia/reperfusion injury via the modulation of mitochondrial electron transfer. J Exp Med 204:2089-102, 2007.
68. Ryan NS, Calvert JW, Elrod JW, et al. Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Proc Natl Acad Sci USA 104:19144-9, 2007.
69. Gonzalez FM, Shiva S, Vincent PS, et al. Nitrite anion provides potent cytoprotective and antiapoptotic effects as adjunctive therapy to reperfusion for acute myocardial infarction. Circulation 117:2986-94, 2008.
70. Dezfulian C, Shiva S, Alekseyenko A, et al. Nitrite therapy after cardiac arrest reduces reactive oxygen species generation, improves cardiac and neurological function, and enhances survival via reversible inhibition of mitochondrial complex I. Circulation 120:897-905, 2009.
71. Kumar D, Branch BG, Pattillo CB, et al. Chronic sodium nitrite therapy augments ischemia-induced angiogenesis and arteiogenesis. Proc Nat Acad Sci USA 105:7540-5, 2008.
72. Raat NJ, Noguchi Ac, Liu VB, et al. Dietary nitrate and nitrite modulate blood and organ nitrite and the cellular ischemic stress response. Free Radic Biol Med 47:510-7, 2009.

73. Tripatara P, Patel NS, Webb A, et al. Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury in vivo: Role for xanthine oxidoreductase. J Am Soc Nephrol 18:570-80, 2007.
74. Milsom Ab, Patel NS, Mazzon E, et al. Role for endothelial nitric oxide synthase in nitrite-induced protection against renal ischemia-reperfusion injury in mice. Nitric Oxide 22:141-8, 2010.
75. Cauwels A, Buys ES, Thoonen R, et al. Nitrite protects against morbidity and mortality associated with TNF- or LPS-induced shock in a soluble guanylate cyclase-dependent manner. J Exp Med 206:2915-24, 2009.
76. Kinsella JP, Neish SR, Shaffer E, Abman SH. Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340:819-20, 1992.
77. Lundberg JO, Weitzberg E, Cole JA, Benjamin N. Nitrate, bacteria and human health. Nat Rev Microbiol 2:593-602, 2004.
78. Dykhuizen RS, Fraser A, McKenzie H, et al. *Helicobacter pylori* is killed by nitrite under acidic conditions. Gut 42:234-7, 1998.
79. Dykhuizen RS, Frazer, Duncan C, et al. Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. Antimicrob Agents Chemother 40:1422-25, 1996.
80. Yoon SS, Coakley R, Lau GW, et al. Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrate derivatives under cystic fibrosis airway conditions. J Clin Invest 116:436-46, 2006.
81. Jepson RG, Craig JC. Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev CD001321, 2008.
82. Carlsson S, Govoni M, Wiklund NP, Weitzberg E, Lundberg JO. In vitro evaluation of a new treatment for urinary tract infections caused by nitrate-reducing bacteria. Antimicrob Agents Chemother 47:3713-8, 2003.
83. Carlsson S, Weitzberg E, Wiklund P, Lundberg JO. Intravesical nitric oxide delivery for prevention of catheter-associated urinary tract infections. Antimicrob Agents Chemother 49:2352-5, 2005.
84. Folkers K, Langsjoen P, Willis R, Richardson P, Xia LJ, Ye CQ, Tamagawa H. Lovastatin decreases coenzyme Q levels in humans. Proc Natl Acad Sci U S A. 1990 Nov; 87(22): 8931-4.
85. Crane FL. Biochemical functions of coenzyme Q10. J Am Coll Nutr. 2001; 20: 591-8.

86. Thomas SR, Witting PK, Stocker R. A role for reduced coenzyme Q in atherosclerosis? Biofactors. 1999; 9: 207-24

87. Kamanna VS, Ganji SH, Kashyap ML. The mechanism and mitigation of niacin-induced flushing Int J Clin Pract. 2009; 63: 1369–1377.

88. Jacobson EL. Niacin deficiency and cancer in women. J Am Coll Nutr August 1993; 12: 412-416.

89. Crouse JR 3rd. New developments in the use of niacin for treatment of hyperlipidemia: new considerations in the use of an old drug. Coron Artery Dis. 1996; 7: 321-6.

90. Taylor AJ, Villines TC, Stanek EJ, Devine PJ, Griffen L, Miller M, Weissman NJ, Turco M. Extended-release niacin or ezetimibe and carotid intima-media thickness. N Engl J Med. 2009; 361(22): 2113-22.

91. http://www.niaspan.com/?s_mcid=google-niaspan-branded (accessed 10/3/2011).

92. Jee SH, Miller ER, Guallar E, Singh VK, Appel LJ, Klag MJ. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. American Journal of Hypertension 2002; 15: 691-696.

93. http://www.magnesiumdirect.com/whymag.aspx (accessed10/01/2011)

94. http://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/ (accessed 10/3/2011)

95. http://www.diabetes.org/food-and-fitness/fitness/ (accessed 10/3/2011).

96. Cefalu WT, Hu FB. Role of Chromium in Human Health and in Diabetes. Diabetes Care 2004; 27: 2741-2751.