Kyäni Nitro FX™ and Kyäni Nitro Xtreme™:

The Science Behind Kyäni's Nitric Oxide Precursors

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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. Kyäni Nitro FX™ and Kyäni Nitro Xtreme™ 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, this paper will discuss the additional ingredients that make Kyäni Nitro Xtreme™ even more powerful than Kyäni Nitro FX™. Please note this document discusses the formulation of Kyäni Nitro FX™ and Kyäni Nitro Xtreme™ for the United States. Some foreign countries may have formula variations.

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 nitrites (NO_2^-) and nitrates (NO_3^-). The process of denitrification completes the cycle: Nitrates are reduced first to nitrites, 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. 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 nitrites and nitrates. 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 nitrates ***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 nitrates that escapes conversion to NO in the stomach enters the circulation or passes into the small intestine and is absorbed into the circulation; subsequently nitrates are delivered to the salivary glands and the saliva containing nitrates are actively secreted into the mouth. Bacteria in the mouth then convert ("reduce") nitrates to nitrites (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 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 a nitrate is 5-6 hours, while the half-life for a nitrite is only 20 minutes.

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

Up to 25% of circulating nitrates are taken up by the salivary glands, and nitrates are concentrated 10-20 fold in saliva (8). After nitrates are ingested and absorbed through the stomach and small intestines, salivary concentrations become very high (19,20). Bacteria in the mouth convert nitrates to nitrites 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 nitrites are converted to nitrous acid (HNO2) 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 nitrites in the saliva, however, are not converted to NO but are absorbed into the bloodstream. Interestingly, the circulating nitrites 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 may be increased by putting nitrites directly into the stomach through a feeding tube; circulating levels of nitrites 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 Nitrites:

Beyond the simple process that occurs in the stomach to add a proton to nitrites and create nitrous acid, there are multiple different enzymatic pathways in the body that serve to convert systemic nitrites 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 nitrites in the body. Different pathways play different roles in different tissues at different times, depending on numerous factors including pH, oxygen status, and the 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 nitrites to NO (26-28). Gladwyn and his colleagues demonstrated that nitrites 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 Kyäni Nitro FX™ and Kyäni Nitro Xtreme™, 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 nitrites bioactivation. In the instance of myocardial ischemia, myoglobin converts nitrites to NO in the heart muscle in much the same way that hemoglobin does in the intravascular space. Studies have demonstrated that nitrites have a cardio-protective effect mediated through myoglobin (29). A similar molecule in the nervous system, called neuroglobin, also has the ability to convert nitrites 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 nitrites to NO. As mentioned above, one of the enzymes involved in the generation of NO from arginine, eNOS, can also convert nitrites to NO under the right conditions, including low oxygen levels and low pH.

Summary:

There are multiple mechanisms whereby nitrites 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 nitrites were published almost 100 years ago (37). Recent studies have found a vasodilatory effect of much lower levels of circulating nitrites (38-41). Organic nitrites, derived from plants, are much more potent than inorganic nitrites. The preferential conversion of nitrites 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 vegetables, 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 nitrites protected against kidney injury (49).

Administration of nitrates, 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 nitrites 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 nitrites 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 nitrites have improved hypertension in the pulmonary circulation as well (42).

Transplantation studies:

In a rat model of cardiac transplantation, oral supplementation of nitrites 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, nitrites were successfully used to clear *Pseudomonas aeruginosa*, a common bacterial infection in this disease (80).

The conversion of nitrites 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 nitrites is comparable to antibiotics (82). Carlsson and colleagues used nitrites 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 nitrates and/or nitrites provide 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 nitrates 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).

Kyäni Nitro Xtreme™:

Kyäni Nitro Xtreme™ contains a greater concentration of nitrates than Kyäni Nitro FX™. In fact, Kyäni Nitro Xtreme™ contains at least three and one-half times more nitrates than Kyäni Nitro FX™. The higher nitrates content helps produce a more profound effect in the body. In addition, Kyäni has included five additional ingredients in Kyäni Nitro Xtreme™. These ingredients are CoQ10, Niacin, Magnesium, Zinc and Chromium. Each ingredient was included to further enhance the production of nitric oxide in the body.

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 Kyäni Nitro Xtreme™ 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 Kyäni Nitro Xtreme™ 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 and 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 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 Kyäni has incorporated zinc into the Kyäni Nitro Xtreme™ 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 it 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 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. Kyäni Nitro FX™ and Kyäni Nitro Xtreme™ 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. Kyäni Nitro Xtreme™, 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 CoQ10, magnesium, zinc, chromium and niacin, as well as a proprietary ingredient designed to enhance absorption.

Kyäni recommends that Kyäni Nitro FX™ be employed for regular daily use, while Kyäni Nitro Xtreme™ be reserved for more demanding days. Kyäni Nitro FX™ has a milder flavor and has the benefits of NO production. Kyäni Nitro Xtreme™ 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, business professionals, even busy parents will more fully benefit from using Kyäni Nitro Xtreme™.

The Ability of Samples to Modulate Angiogenesis

The extract of Kyäni's proprietary noni blend was tested at two concentrations in the presence and the absence of Vascular Endothelial Growth Factor (VEGF) for its ability to inhibit angiogenesis using the aortic ring model.
The rings treated with VEGF alone exhibited growth of the microvessels over the 7 days of the investigation.
At no point of time was any microvessel growth observed when the aortic rings were cultured in the presence of two different concentrations of Kyäni's proprietary noni blend extract.
Furthermore the two concentrations of noni blend also completely prevented any vessel growth in rings treated with VEGF.

The full report from Trinity Bioactives.

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