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Positive biofeedback of nitric oxide in human body

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Abstract
Nitric oxide is a molecule that our body produces to help its 50 trillion cells communicate with each other by transmitting signals throughout the entire body. Following its benchmark discovery, nitric oxide (NO) is now known to play important functional roles in a variety of physiological systems. Within the vasculature, NO induces vasodilation, inhibits platelet aggregation, prevents neutrophil/platelet adhesion to endothelial cells, inhibits smooth muscle cell proliferation and migration, regulates programmed cell death (apoptosis) and maintains endothelial cell barrier function. NO generated by neurons acts as a neurotransmitter, whereas NO generated by macrophages in response to invading microbes acts as an antimicrobial agent. Because neurons, blood vessels and cells of the immune system are integral parts of the reproductive organs, and in view of the important functional role that NO plays in those systems, it is likely that NO is an important regulator of the biology and physiology of the reproductive system. Indeed, in the past 10 years, NO has established itself as a polyvalent molecule which plays a decisive role in regulating multiple functions within the female as well as the male reproductive system. This review provides an overview of the role of NO in various reproductive organs under physiological and pathological conditions.

Keywords: radical chemistry; nitrosative stress; bioenergetics; nitrergic transmission; neurodegeneration; mitochondria; haem proteins pathophysiology; warburg effect; melatonin

Introduction

Nitric oxide (NO) is a free radical, actively produced in human body. NO exerts crucial roles in vascular and neuronal signal transduction, smooth muscle contractility, bioenergetics, platelet adhesion and aggregation, immunity, and cell death regulation. The evidence accumulated over the last 25 years suggests that a defective control of the NO levels causes pathologies, such as hypertension, cardiovascular dysfunctions, neurodegeneration, arthritis, asthma and septic shock. Despite dealing with NO, the boundary between health and disease is still blurry, although the feeling is that pulses of NO in the low concentration range (Pico Nanomolar) are by and large physiological, whereas cell persistence in the high concentration range (micromolar) may turn to pathological. Evidence is growing that the dark side of NO resides on its concentration levels and on the production of peroxynitrite and other reactive oxygen and nitrogen species; last but not least, the type of biomolecule reacting with NO and, when present, the cell bioenergetic changes induced strongly contribute to physiological or pathological outcomes. However, in the late 1970s, researchers who were investigating the dilation mechanisms of the anti-angina drug nitroglycerin made an important discovery: the drug was being metabolised into nitric oxide, and it was this NO that was producing the potent vasodilation effects. This finding initiated a whole new raft of research into NO, but the real breakthrough came in the late 1980s, when it was discovered that not only does the human body produce its own NO, but that this NO also acts as an important *neurotransmitter* – a chemical signaller that tells cells in the body how to behave [1]. And when NO was then discovered to be the neurotransmitter to erectile tissue, it spawned the development of Viagra – and the rest, as they say, is history!

We now know that nitric oxide is not just an important neurotransmitter, but is in fact the most widespread signalling molecule in the body, helping to *control* a range of processes in the body, including nerve signalling, immune function, tissue turnover and the dilation of blood vessels. In particular, nitric oxide acts as a messenger molecule that acts on a variety of endothelial tissues in the circulatory system (eg. blood vessels and capillaries), causing them to 'relax'. As they relax or deconstrict, they open up, allowing more blood to flow.

Synthesis of Nitric Oxide

Nitric oxide is produced by a group of enzymes called nitric oxide synthases. These enzymes convert arginine into citrulline, producing NO in the process. Oxygen and NADPH are necessary co-factors. There are three isoforms of nitric oxide synthase (NOS) named according to their activity or the tissue type in which they were first described. The isoforms of NOS are neuronal NOS (or nNOS), endothelial NOS (or eNOS) and inducible NOS (or iNOS). These enzymes are also

sometimes referred to by number, so that nNOS is known as NOS1, iNOS is known as NOS2 and eNOS is NOS3. Despite the names of these enzymes, all three isoforms can be found in a variety of tissues and cell types. The general mechanism of NO production from NOS is illustrated below.

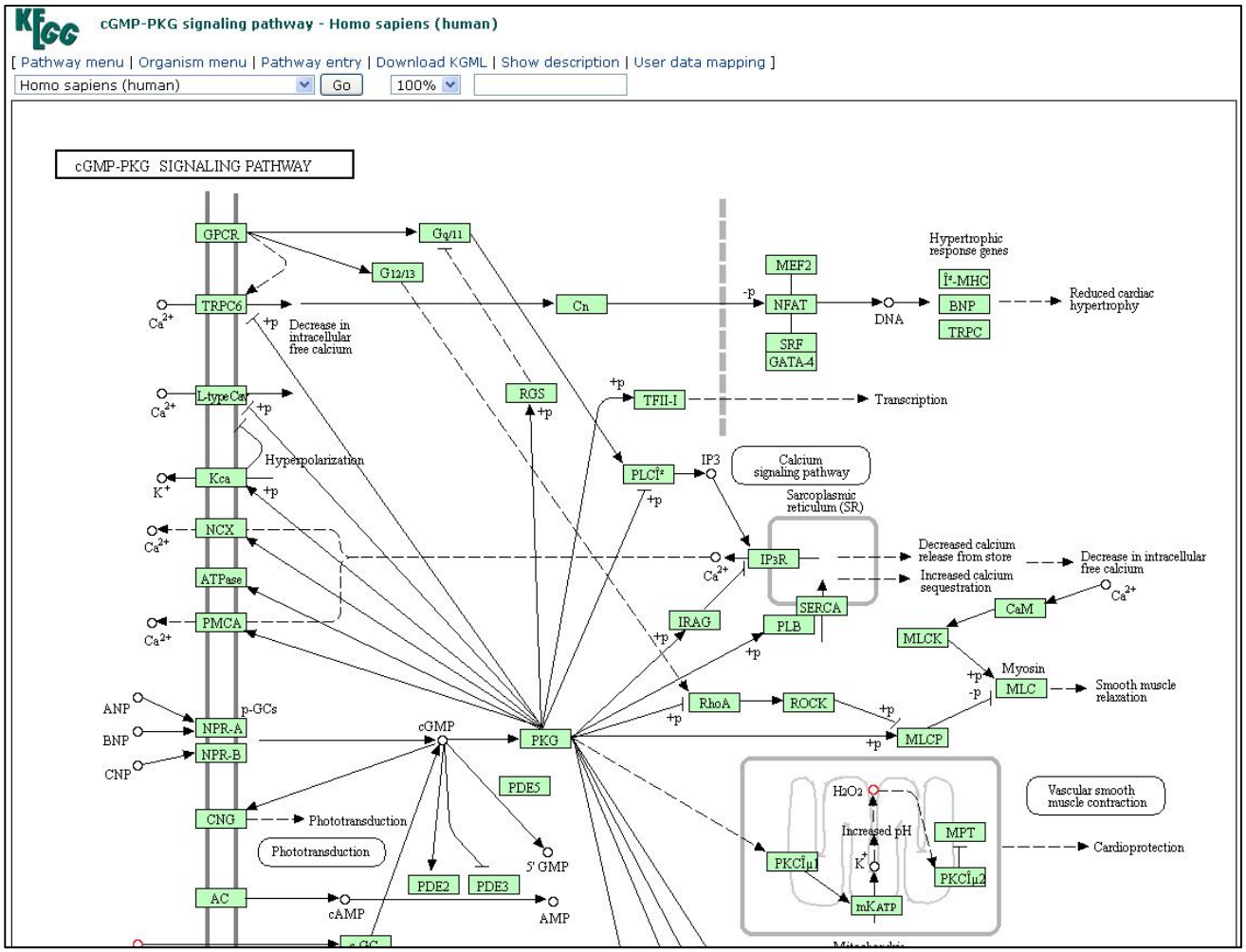

Fig: cGMP-PKG Signalling Pathway

Two of the enzymes (nNOS and eNOS) are constitutively expressed in mammalian cells and synthesise NO in response to increases in intracellular calcium levels. In some cases, however, they are able to increase NO production independently of calcium levels in response to stimuli such as shear stress.

iNOS activity is independent of the level of calcium in the cell, however its activity - like all of the NOS isoforms - is dependent on the binding of calmodulin. Increases in cellular calcium lead to increases in levels of calmodulin and the increased binding of calmodulin to eNOS and nNOS leads to a transient increase in NO production by these enzymes. By contrast iNOS is able to bind tightly to calmodulin even at very low cellular concentration of calcium. Consequently iNOS activity doesn't respond to changes in calcium levels in the cell. As a result the production of NO by iNOS lasts much longer than from the other isoforms of NOS, and tends to produce much higher concentrations of NO in the cell. The production of NO by iNOS can, however, be controlled through transcription. In most cell types iNOS protein levels are either very low or undetectable. However, stimulation of these cells with, for example, cytokines or growth factors, can lead to increased transcription of the iNOS gene, with subsequent production of NO.

In theory, any nutritional approach that can enhance tissue turnover and vasodilation is a good thing for an athlete; all the nutrients required for muscle-tissue growth and repair are transported via the circulatory system and delivered by tiny capillaries, and waste products such as *lactate* are also removed via this route. Encouraging more vasodilation enhances circulation to the working muscles and, as well as producing a better pump during workouts, should theoretically improve nutrient delivery. These vasodilation benefits go a long way to explaining the current popularity of NO-enhancing supplements.

If we accept that boosting NO production in the body is a good thing, the obvious question is whether there are any nutritional strategies that can increase NO generation in the body. The answer to this question is yes; it just so happens that we can synthesise NO in our bodies from the amino acid L-arginine. We obtain L-arginine from dietary protein we eat because L-arginine is one of the basic amino acid building blocks of protein foods.

Because NO has such a short lifetime in the body, it's synthesised 'in situ' from the amino acid L-arginine, which (in the presence of oxygen) is broken down by an *enzyme* known as 'nitric oxide synthase', into nitric oxide and another amino acid called L-citrulline. In other words, L-arginine is a major precursor of NO in the body and numerous studies have shown that increasing arginine intake increases the production of NO [2], which explains why arginine supplementation has found favour among some sportsmen and women as an 'NO booster'.

There are two main claims made for L-arginine-based NO boosters:

The NO-mediated increased vasodilation enhances blood flow, ensuring a better delivery of blood and nutrients to working muscles and more efficient removal of by-products such as lactate, all of which lead to better performance; L-arginine supplementation also stimulates the body's natural production of *growth hormone*, which in turns helps *recovery* and particularly muscle/strength gains.

Arginine and growth hormone

Let's start with the second claim, which is based on evidence derived mainly from animal studies and from sedentary human subjects. In recent years, trials with arginine supplementation or infusions in rats have shown that it can increase the activity of genes known to be responsible for the synthesis of growth hormone [3, 4]. Moreover, a study carried out by Danish scientists showed that healthy young men who had an infusion of arginine demonstrated an increase in release rates of growth hormone, compared to those who didn't [5]. This seemed to confirm the work of British scientists three years earlier, who also found that, compared to a *placebo*, an arginine infusion boosted growth hormone release in 18 young healthy males. Another study demonstrated that arginine was able to boost growth hormone production in children aged 5-14[6], a finding mirrored in a study of healthy elderly people who took either oral arginine supplements or who had an infusion [7].

The problem with the arginine-growth hormone claim, however, comes when you look at studies carried out on physically active subjects, where the evidence is not just weak but actually suggests that arginine supplementation may be counter-productive! For example, Californian scientists looked at the combined effects of both arginine and *resistance training* in both young and old adults [8]. Exercise, particularly resistance training, is known to produce a natural release of growth hormone, and the researchers wanted to see whether giving extra arginine augmented the growth hormone boost provided by training.

The subjects (20 with average age 22 and eight with average age 68) had growth hormone levels measured under three conditions:

1. At rest after 5g of arginine.
2. Following three sets or 8-10 reps at 85% of 1 rep max on 12 separate resistance exercises.
3. After 5g of arginine plus the same exercise protocol above.

The results showed that not only did the arginine infusion at rest fail to produce a significant increase in growth hormone release, it also failed to augment growth hormone release when exercise was performed. Even worse, in the young subjects, arginine seemed to blunt the release of growth hormone compared to exercise only.

A very recent scientific review paper published earlier this year by US scientists supports the findings above [9]. In the paper, researchers looked carefully at the evidence for the effects or otherwise of L-arginine supplementation on growth hormone response when combined with exercise. The key finding was that most studies using oral arginine have shown that 5-9g of arginine alone increases the resting growth hormone levels at least 100%, while exercise can increase growth hormone levels by 300-500% over resting levels. Worryingly, however, the combination of oral arginine plus exercise attenuates the exercise-only growth hormone response, and increases growth hormone levels by only around 200% compared to resting levels.

The reasons why extra arginine may diminish exercise-induced growth hormone release are unclear, but what is clear from the available evidence is that the effects of exercise on growth hormone release dramatically outweigh any produced by arginine, so those seeking to maximise the muscle-building effects of a workout have little to gain by taking arginine and possibly something to lose!

Activation of NOS activity

One of the main activators of NOS enzyme activity is changes in cellular calcium levels. The constitutive isoforms of NOS, eNOS and nNOS, show increased activity following increases in calcium, and therefore calmodulin, in the cell. In addition both eNOS and nNOS are known to show further increases in activity following phosphorylation. There are at least 5 regulating phosphorylation sites on eNOS and these can be regulated by a number of different kinases and phosphatases, with the protein kinase Akt being one of the best understood regulators of eNOS activity. It is also known that nNOS has both activating and inhibiting phosphorylation sites, but much less is known about the post-translational regulation of iNOS. There is some suggestion that iNOS activity can be regulated by Src mediated tyrosine phosphorylation, but it appears that iNOS activity is mostly regulated at the transcriptional level and through its intracelular distribution.

Endogenous inhibitors of NOS

His synthesis of NO can also be inhibited by two methylated analogues of arginine, asymmetric dimethylarginine (ADMA) and mono methylarginine (L-NMMA). These inhibitors are endogenously produced competitive inhibitors of NOS formed by the post-translational methylation of arginine residues in proteins and liberated upon their hydrolysis.

Free ADMA is found in plasma and urine, while the intracellular concentration of ADMA is about 5 times higher than the extracellular concentration. We have shown that circulating ADMA falls during normal pregnancy but is elevated early in pregnancies complicated by preeclampsia. There is also evidence that elevated levels of ADMA are important in a number of other conditions including diabetes, atherosclerosis and renal failure. Interestingly, the administration of NOS inhibitors to pregnant rats results in features similar to preeclampsia, including a reduction in placental size and utero-placental blood flow, both of which can be reversed by the administration of L-arginine, suggesting a specific NO-mediated effect. Within cells the concentration of ADMA and L-NMMA, but not symmetric

dimethylarginine (SDMA), is regulated by the activity of the cytoplasmic enzyme dimethylarginine dimethylaminohydrolase (DDAH). Inhibition of DDAH activity leads to elevated ADMA in culture and inhibits endothelium-dependent relaxation. These experiments demonstrate that DDAH is basally active and that inhibition of DDAH leads to local accumulation of ADMA which reaches concentration sufficient to inhibit NOS.

Arginine as an NO booster

Given that the evidence for arginine as an effective growth hormone booster is patchy to say the least, what about its other claim – as an NO enhancer? There are two issues here: firstly, does taking extra arginine significantly boost NO production in the body and if so, does this produce any real benefits for sportsmen and women?

As far as extra arginine goes, studies have shown that both diabetic rats [10,11] and human patients [12,13] have markedly decreased concentrations of arginine in the blood, and clinical and experimental studies have shown beneficial effects of arginine administration in improving vascular function (via vasodilation) in diabetic subjects [14,15]. Moreover, studies in rats have shown that extra dietary arginine boosts NO synthesis in diabetic rats [16]. But what's the evidence that the same is true in exercising humans?

Results from studies are far from conclusive. For example, a double-blind, placebo-controlled US study showed that 8.4g of arginine a day for two weeks significantly reduced platelet aggregation (blood cell stickiness) in patients suffering from high blood cholesterol [17]. The researchers surmised that this effect occurred as a result of increased NO production because their previous studies had shown that reduced vascular activity of nitric oxide in rabbits suffering from this condition was restored by arginine supplementation.

However, another US study carried out just last year produced very different results. In the study, scientists set out to determine the effects of long-term administration of 3g of arginine for six months on vascular reactivity and functional capacity in 133 patients with peripheral arterial disease [18]. Importantly, the researchers not only looked at the effects of arginine on symptoms of this condition (such as calf pain during exercise), they also directly measured NO availability to the tissues using a number of different techniques. They found that arginine supplementation did not increase nitric oxide synthesis or improve measures of vascular health; indeed, when they looked at the symptoms of this condition, those who had taken a placebo (ie. no arginine) actually fared better than those who took the arginine!

Arginine as a performance enhancer

The evidence in favour of using arginine as an NO booster is far from convincing – but surely, if it helps you deliver the goods performance-wise, that's what matters, isn't it? Unfortunately for devotees of NO enhancers, the scientific evidence for this is even less convincing!

A glimmer of hope for NO-enhancers' fans came from a Polish study that looked at the exercise capacity of 21 patients with congestive heart failure who took 9g of arginine a day for a week [19]. The researchers found that, compared to a placebo, arginine supplementation did enhance exercise duration time. However, the researchers were not able to ascertain the cause of this increase in performance because there were no signs of changes in NO production in the patients.

By contrast, studies of arginine supplementation and subsequent performance using trained athletes are very thin on the ground, and those that have been conducted have drawn a blank. In one such example, Swiss scientists investigated whether daily intake of two different dosages of arginine aspartate (AA, a type of arginine supplement purported to be readily absorbed and utilized) over a four-week period would affect selected parameters of overtraining syndrome, such as performance and metabolic parameters, in 30 male endurance-trained athletes [20].

The athletes were split into three groups and ingested either a high dose of AA (containing 5.7g arginine and 8.7g aspartate), a low dose of AA (2.8g arginine and 4.2g aspartate) or a placebo. Maximal oxygen uptake and time to exhaustion were determined on a cycling ergometer in an incremental exercise test before and after supplementation. The researchers found that regardless of dose, the arginine aspartate had absolutely no influence on performance or any of the metabolic parameters measured. Indeed, they went on to comment: 'There seems to be no apparent reason why the supplementation of arginine aspartate should be an effective ergogenic aid and the practice of using arginine aspartate as potential ergogenic aid should be critically re-evaluated.'

Taiwanese scientists drew another blank in a study using well-trained male athletes, the report of which was published just a few months ago. It investigated the effect of short-term arginine supplementation on performance in intermittent anaerobic exercise [21]. Ten elite male college judo athletes participated and consumed 6g per day of arginine or a placebo for three days and then performed an intermittent anaerobic exercise test on a cycle ergometer. The researchers found that there was no significant differences between the two trials in blood nitrate and nitrite concentrations, suggesting an absence of increased NO production. Moreover, there was no difference in peak and average power during exercise.

Protein Nitrosylation

There are a number of mechanisms through which the effects of NO are mediated, but the reaction of NO with cysteine residues in proteins, a process known as nitrosylation, is emerging as one of the most important mechanisms. Nitrosylation is a physiologically important post-translational modification that affects a wide variety of proteins involved in a number of cellular processes. The role of nitrosylation in regulating signal transduction has been largely overlooked until relatively recently. This is because the production of the small, highly reactive NO molecule had been thought to lack the specificity and control observed in other post-translational modifications such as phosphorylation. However, evidence has now emerged that suggests that in fact nitrosylation shares many properties with phosphorylation. Both modifications exhibit substrate specificity, strict spatial and temporal regulation and are reversible.

The functional consequences of protein nitrosylation depends on the protein that is affected. In many cases, such as the caspases, nitrosylation has been shown to inhibit enzyme activity. In other proteins, such as matrix metalloproteinases, it is thought that nitrosylation increases the activity of the enzymes. Despite its importance in many aspects of cell

biology our understanding of protein nitrosylation and its regulation remains poorly understood.

Recently a technique was developed that has greatly increased our ability to study nitrosylation. This technique is known as the biotin-switch technique, and exploits differences in chemistry between NO-reacted cysteines (nitrosocysteines) and normal, unmodified cysteines to specifically label the nitrosocysteines with biotin. Following biotinylation the proteins can be easily isolated from the non-nitrosylated proteins using streptavidin beads.

Isolation of proteins using this method allows us to determine which proteins are nitrosylated in cells either by using specific antibodies in Western blots or by using a proteomic approach to identify them. If nitrosylated proteins are isolated from trophoblast cells using this approach and then run on a 2D gel, the results can be seen in the figure below.

Approximately 60 spots, corresponding to around 60 different nitrosylated proteins can be seen in this gel. These proteins were isolated from untreated cells, but if NO activity is increased several hundred spots can be seen, suggesting that many more proteins are capable of being nitrosylated.

It has recently been demonstrated that there is specificity of nitrosylation within a protein so that although a target protein may possess many available cysteines only one of them, and always the same one, is nitrosylated. It is not entirely clear how this specificity is achieved, but it has been suggested that there may be a consensus motif around the cysteine to be nitrosylated that facilitates the reaction. Recently it has been shown that such consensus motifs may not always be found in the primary amino sequence, but in many cases may be a product of the tertiary structure of the protein.

Fruit and vegetables as NO boosters

Swedish scientists have carried out a fascinating study indicating that rather than using NO-boosters, simply eating more fruit and vegetables may be a route to enhance NO activity and performance [22]. Fruit and vegetables are naturally rich in nitrate and nitrite, both of which can be metabolized into NO in the body. The researchers investigated the effects of a simulated high fruit and vegetable intake by giving nine healthy, young, well-trained men a nitrate supplement and compared its effects on submaximal and maximal work tests on a cycle ergometer, to a placebo. They discovered that the nitrate supplementation resulted in a lower oxygen demand during sub-maximal work and that this effect occurred without an accompanying increase in *blood lactate* concentration, indicating that the energy production had become more efficient. If this effect is subsequently confirmed, it could be yet another reason to eat those greens!

Role of Nitric Oxide in apoptosis

Apoptosis is a form of programmed cell death in which cells die in a controlled and regulated manner. There are a number of ways in which apoptosis can be triggered, including activation of death receptors such as Fas or TRAIL, cellular stresses leading to release of cytochrome C from the mitochondria, DNA damage and viral infection.

NO regulates apoptosis in wide range of cell types, although its precise effects are dependent on the amount of NO used and the type of cell. It has been shown to both induce and inhibit apoptosis. Nitric oxide has been demonstrated to inhibit apoptosis in a number of cell types including leukocytes, hepatocytes, trophoblasts and endothelial cells.

Nitric oxide is able to affect apoptotic signalling at multiple points in the pathway:

1. NO can regulate the expression of the death receptors in a cGMP dependent manner
2. NO can alter the expression of protein such as acid sphingomyelinase that help regulate the early signalling events in death receptor signalling such as ceramide production. This reduces DISC (Death Inducing Signalling Complex) formation and is also mediated through the production of cGMP.
3. The activity of the caspases can be directly affected by NO through nitrosylation of the active site, leading to inhibition of protein function.
4. The effect of NO on caspase activity and DISC formation leads to reduced cleavage of Bid and therefore a lack of amplification of apoptotic signalling through the mitochondria.
5. NO can also affect the expression of many members of the Bcl-2 protein family, including both pro- and anti-apoptotic proteins. This can affect the release of cytochrome C and other factors from the mitochondria and is mediated through the production of cGMP.
6. DISC formation can also be affected through the recruitment of the anti-apoptotic protein cFLIP. One of the proteins that can affect cFLIP recruitment to the DISC is PKC and the activity of PKC can be regulated by NO through nitrosylation.

Generally the anti-apoptotic effects of NO can be mediated through a number of mechanisms such as the nitrosylation and inactivation of many of the caspases including caspase 3, caspase 1 and caspase 8. Other mechanisms include activating p53, upregulating heat shock protein 70 (and consequently blocking recruitment of procaspase 9 to the Apaf-1 apoptosome), upregulating Bcl-2 and Bcl-XL (with subsequent inhibition of cytochrome C release from the mitochondria) and activating cGMP signaling leading to activation of cGMP-dependent protein kinases and suppression of caspase activity.

Nitric Oxide as a Part of the Immune System

The role of NO in the immune system is much different from its role in the cardiovascular or neuronal system. In humans, most every type of cell in the body can express NOS.

The signal to translate the DNA sequence for the NO-producing enzyme comes from certain cytokines, which are produced by the infected cells. Any kind of infection (including bacteria, viruses, or cancer) will lead to the production of cytokines. Cytokines carry the message of the infectious state to the surrounding cells of humans, which will start to produce NOS enzyme. Immediately after translation is complete and the prosthetic groups are in place, the enzyme will continuously produce large amounts of NO for an extended period of time (several hours). The total NO production will be therefore much higher than that produced by endothelial cells (few minutes) or neurons (few seconds). The enzyme produces a sufficient concentration of NO to locally inhibit DNA synthesis; hence, the profound cytostatic effect of NO on the proliferation of rapidly dividing tumor cells or pathogens. In addition, DNA synthesis is a

fundamental step in normal cell proliferation. Even normally high NO concentrations from constitutive NOS can inhibit the ribonucleotide reductase, thus halting the proliferation of smooth muscles around major arteries and cardiac myocytes. NO is not toxic even at higher biological con-centration. Therefore, it is unlikely that NO can actually kill tumor cells, it will just limit their proliferation.

Pathology of Nitric Oxide Release

NO is one of the 10 smallest molecules found in biological systems. There is now little question that NO is essential to the everyday activities of many cells and tissues in the body. There-fore, any pathology of NO production in the body can lead to many diseases (Table II). Because of this, NO is also called a vital-poison, the right amount of NO production is essential for life, but too much or too little can be deadly poisonous. In most life-threatening diseases like hypertension, atherosclerosis, and diabetes, the net concentration of NO is lower than in a healthy system. This does not necessarily mean that the expression of the enzyme is lower. In some of these diseases, enzyme expression is even higher (hypertension).

Why? Endothelial L-arginine concentration is deficient in hypertensive mammals usually due to the formation atherosclerotic plaques on the membrane. Huk & Malinski *et al*. (1997), have demonstrated in L-arginine- starved *in vivo* environments that the NO-producing enzyme can also donate an electron to its other substrate oxygen (O_2) to form superoxide (O_2^-). When NO and O_2^- are produced simultaneously, in close proximity, they chemically react very quickly.

The product of this reaction is called peroxynitrite ($OONO^-$). In the presence of certain reactive centers, HOONO may undergo homolytic cleavage to a hydroxyl free radical ($^{\bullet}OH$) and nitrogen dioxide free radical ($^{\bullet}NO_2$), or heterolytic cleavage to a nitronium cation (NO_2^+) and hydroxide anion (OH^-). Three of these cleavage products ($^{\bullet}OH$, $^{\bullet}NO_2$ radicals and NO_2^+) are among the most reactive and most damaging species in biological systems and may be major contributors to the severe damage of the heart and brain. The low concentration of NO produced in the heart in atherosclerotic cardiovascular system is a prime cause of heart attack. Also, a continuously produced high concentration of NO in the heart under emotional stress can lead to heart attack. Heart failure is a major cause of death (in USA 185 deaths per 100,000 population per year).

Table 1: Pathology of Nitric Oxide Release

Nitric Oxide Concentration	
too low	**too high**
hypertension	septic shock
atherosclerosis	hypotension
diabetes	excessive bleeding
ischemia (stroke,	meningitis
heart Alzheimer's disease)	
Parkinson's disease	rheumatoid arthritis
fibrosis	
cancer	

Another serious disease associated with NO is septic shock, which threatens the life of 50–70 million people per year worldwide. The affliction is usually initially caused by a bacterial toxin, which enters the blood circulation through incidental infections, wounds, and surgical procedures. Up to 60% of the people with septic shock do not survive this condition. Septic shock is due to the extensive release of NO. The immune system, attempting to fight infection, releases so much NO that the system goes out of control. Too high a concentration of NO dramatically decreases blood pressure, which is followed by failure of vital organ system, especially the liver, kidney and heart.

During a heart attack, brain stroke, and any kind of condition which limits the supply of blood and O_2 (ischemia) to an organ, a massive release of NO can be observed. There-fore, if blood flow cannot be restored within several minutes, the NOS starts to produce superoxide in addition to NO. The simultaneous, proximal release of both NO and O_2 may lead to serious, sometime irreversible damage especially in the brain or heart.

Knowing the activity and targets of NO in the body, it is possible to develop new drugs which can enhance or inhibit the chemistry of NO production. New drugs, based on inhibition of NO production have been already developed as well as drugs to increase NO concentration (nitric oxide donors) in the body when needed. NO donors which are more effective and controllable will replace nitroglycerin. Also, the proper NO concentration in the body can be maintained by scavenging O_2^- or inhibiting the major sources of O_2^-. Many natural vitamins and other compounds can be used as effective scavengers of O_2^- production. Even though there are many sources of O_2^- in humans, a proper diet with foods containing a low amount of fat will help to prevent deposition of cholesterol on the endothelial membrane and subsequent damage by NOS, a major source of O_2^-.

The variety of vital roles played by NO is a direct consequence of its unique chemical properties in the biological cells. NO is small and chargeless, therefore, it can freely diffuse through the cell membrane as well as through cytoplasm just like O_2 and N_2. NO has modest chemical reactivity (making it a somewhat selective reducing or oxidizing agent), al-lowing its survival at least for several seconds in the biological matrix. NO has an unpaired electron and binds to quickly to O_2^- and more slowly to several metals in the biological matrix including iron in hemoglobin. This reaction leads to the eventual disposal of NO in the body. The story of the role of NO is fascinating and far from being complete. It is also an excellent example of utilization of chemistry in solving important medical problems.

Conclusion

Increasing nitric oxide has become the new secret weapon for athletes and bodybuilders. Athletes are now taking supplements with L-arginine and L-citrulline to support the flow of blood and oxygen to the skeletal muscle. They also use them to facilitate the removal of exercise-induced lactic acid build-up which reduces fatigue and recovery time. Since arginine levels become depleted during exercise, the entire arginine-nitric oxidecitrulline loop can lose efficiency, causing less-than-ideal nitric oxide levels and higher lactate levels. Supplements can help restore this loop allowing for better workouts and faster recovery from workouts.

With nitric oxide deficiencies due to aging, inactivity, smoking, high cholesterol, fatty diets, and lack of healthy foods, increasing your nitric oxide levels can help increase

your energy, vitality and overall wellness. The basic adage of eating well and staying active all makes sense now.

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