The Power of Nitric Oxide: Say Yes to NO!

What is Nitric Oxide?

Nitric Oxide (NO) is a simple molecule consisting of one atom of nitrogen and one of oxygen, making it even simpler than water itself! Research into the effects of this small but important molecule began in the 1970's when scientists started to examine why blood vessels relaxed when certain compounds were added. This led to the discovery of NO and it's amazing effects in the body, with NO being named the molecule of the year in 1992! In 1998, twenty-some years after this research first began, a Nobel Prize was awarded to these researchers for their breakthrough discoveries regarding NO. Unfortunately, one of the pioneering researchers in the field of NO-research, Salvador Moncada, was overlooked by the Nobel Prize committee, showing that there is indeed a political side to science! Over the last two decades NO research has continued to grow, and there has been an exponential increase in the number of publications on this fascinating molecule.

NO is now known to be a signaling molecule; meaning that is acts as a messenger to facilitate communication between cells, the relaying of messages and other cellular chatter. The body relies on a wide variety of signaling molecules including hormones, nerve impulses and chemicals like cytokines, hydrogen and carbon dioxide. These signaling molecules help the body perform vital functions including the mobilization of the body's immune defense systems against invading foreigners like viruses, bacteria, toxins and carcinogens, as well as the modulation of other body functions like temperature and blood pressure.

Besides being a simple molecule consisting of two of the most abundant and well know elements, NO is also a radical. This means that NO has an unpaired electron, making it a very reactive molecule! NO's unpaired electron means that it has extensive reactivity with virtually anything and everything from body cells and tissues to other chemicals and molecules circulating in the system. This reactivity is essential in enabling NO to carry out its many biological functions.

How is NO generated?

The conventional method of NO synthesis is from the amino acid L-Arginine (see Figure 1). L-Arginine is oxidized via a series of steps involving a family of enzymes called nitric oxide synthases (NOSs). There are essentially three common NOSs termed isozymes; these are iNOS, eNOS and nNOS. Each of these enzymes plays a different role in the generation of NO in different tissues like the nerves, endothelium or blood vessels or on demand. In each case however, normal oxygen conditions (also called "normoxia") as well as a neutral to high (alkaline) pH level are

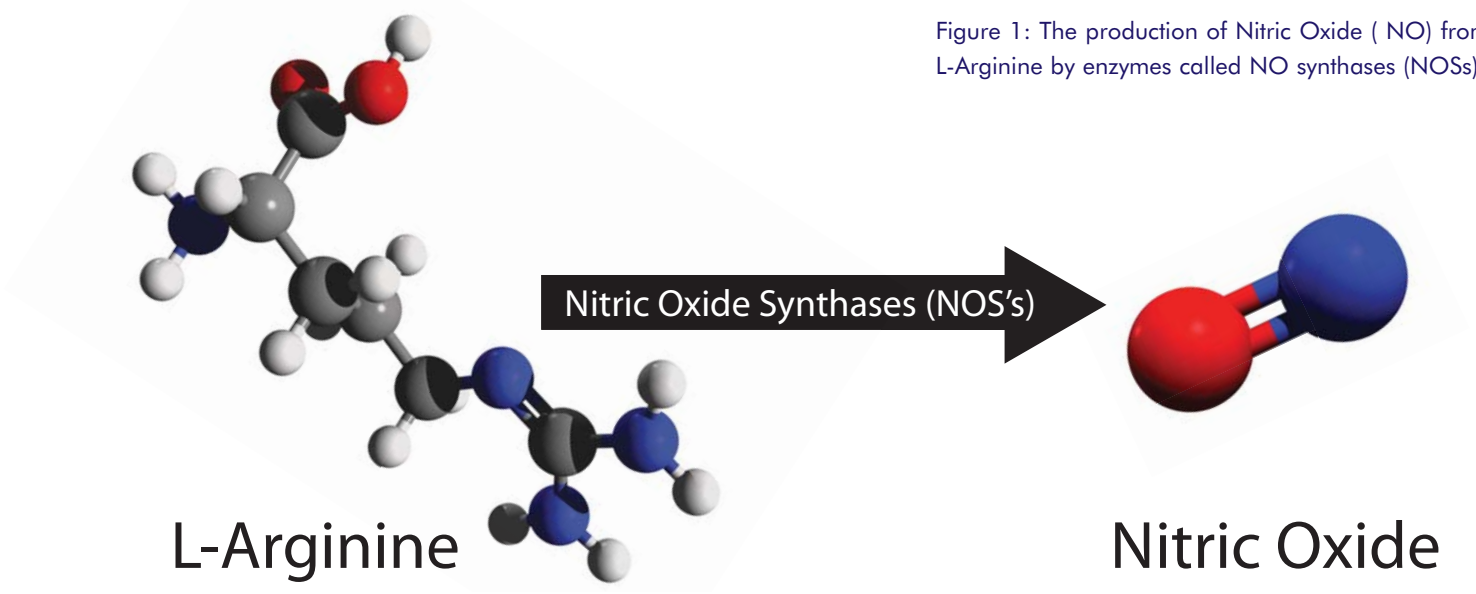


Figure 1: The production of Nitric Oxide (NO) from L-Arginine by enzymes called NO synthases (NOSs)

KEY TERMS AND DEFINITIONS

Mediterranean Diet

Consumption of fruits and vegetables has been known for some time to protect against Cardiovascular Disease (CVD) and Cancer. In the mid 1960's an ambitious study called the Seven Countries Epidemiological Study was conducted. The study included the following countries: United States, Italy, Greece, Japan, Holland, the former Yugoslavia and Finland. A five year follow up of their diet, including intake of fruits, vegetables, saturated fats and meat was recorded as well as the incidence of cardiovascular disease and cancer. The study was unique in that it standardized for a number of factors, including risk factors of heart disease, accurate measurement of diet and blinding (meaning that no one would know who was on which diet). A careful and extensive analysis of the data revealed that the incidence of CVD was significantly lower in Italy, Japan and Greece than in the other four countries included in the study. Dr. Ancel Keys, the lead author, coined the term "The Mediterranean Diet" to describe the diet followed by the Japanese, Italians and the Greeks. In each of these countries the intake of fruits and vegetables was significantly greater and corresponded to a reduced rate of cardiovascular disease. The Mediterranean diet is now universally recognized as a healthy diet.

DASH Diet

(Dietary Attempts to Stop Hypertension) is modeled after the Mediterranean diet. In the DASH diet an intake of fruits and vegetables of upwards of 8-10 servings per day is recommended.

Oxidation

Oxidation is a term that means an increased number of oxygen atoms (strictly speaking it refers to the loss of electrons) for example when NO undergoes oxidation the number of oxygen atoms increases: NO→NO2→NO3.

Hypoxia

Hypoxia is a condition when oxygen availability is greatly reduced. For example, such a condition is likely to occur during ischemia, which is when there is an interruption of blood flow to the tissues (e.g. the heart during a heart attack).

Reduction

Reduction is a term that is the opposite of oxidation and refers to the loss of oxygen atoms (again strictly speaking it is the gain of electrons) for example when NO undergoes reduction the number of oxygen atoms decreases: NO3→NO2→NO.

NOS

NOSs are Nitric Oxide Synthases, which are a diverse range of enzymes that facilitate the formation of NO from various starting molecules (substrates) like L-Arginine.

Free Radicals

Free Radicals are molecules that are very reactive due to an unpaired electron. Free radicals are generally considered to have negative health implications but that isn't the universal case. For example, NO itself is a free radical!

Genetic Knock-Out

Genetic knock-out mice/rats are widely used to test what happens when a certain gene is deleted. When a gene is deleted the mouse can no longer produce a certain protein or enzyme. For example, using rodents lacking a specific gene that codes for NOS enzymes, one can test the effect of a lack of NO and its health implications.

Bioavailability

Bioavailability is a term that refers to the amount of an active molecule that reaches the target site of action. Poor bioavailability is a huge problem for large number of natural and pharmaceutical compounds. There is active research into various ways of delivering more of the molecule at lower doses.

Mole

Mole is a measurement widely used in chemistry that equates how much of the active molecule is present. The mole and/or milli-mole (mM - a thousandth of a mole) is widely used to express the concentration of an active compound in the blood.

Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) is another term for free radicals; species that are very reactive.

Half Life

Half-life is the time taken for the concentration of a compound to be reduced by half. Various metabolic processes in the body act to ensure that molecules do not "linger" about for longer than is necessary by degrading them via enzymatic function, binding with various proteins, elimination via increased excretion by the kidneys, and other mechanisms.

required. When these conditions are met the NOS-dependent conversion of L-Arginine occurs efficiently. Production of NO is the primary reason for the dietary intake of L-Arginine. However, under low oxygen conditions (also called "hypoxia") the conversion of L-Arginine to NO is severely limited. Low oxygen conditions can occur for a variety of reasons. For example, partial or complete blockage of a blood vessel (ischemia), conditions of extreme physical exercise or high altitude situations like mountaineering can all result in reduced blood flow and thus reduced oxygen delivery to the body's tissues and cells. Moreover, such low oxygen conditions are also accompanied by an increased production of lactic acid which reduces pH making the tissue condition both hypoxic and acidic. As such, a body builder pumping iron or an energetic aerobic class or a middle to long distance runner derive little if any benefit from L-Arginine in terms of NO generation.

A New Discovery! A Second Pathway to NO Generation: The NOx 3,2,1 pathway

However, there is good news for these athletes! Recently a novel pathway to NO generation from dietary nitrates has been discovered by researchers at the Karolinska Institute in Stockholm, Sweden and by researchers from the University of London, England. The Swedish researchers, led by Jon Lundberg and Eddie Weitzberg, along with Nigel Benjamin from London, were trying to discover why certain diets, like the Mediterranean diet, vegetarian diets, Japanese diets and the famed DASH diet (Dietary Advice for Stopping Hypertension) were particularly protective of the heart. Both groups independently reported that the key to the success of these diets was the consumption of leafy green vegetables, and that a key component of all these diets was the high nitrate content. The researchers proposed that the nitrate was converted into NO via a reductive process as follows:

Essentially, the highly nitrate-rich vegetables are reduced (see key terms pg 2) in the mouth by bacteria that are normally present on the back of the tongue. These specialized bacteria use the nitrate to help them make energy in the form of ATP. In return, the bacteria utilize their own nitrate reducing enzyme called Nitrate Reductase to generate nitrite, which as we shall see is very useful to us! This special relationship is an interesting example of human-bacteria symbiosis: a mutually beneficial relationship.

The nitrite is a much more active molecule than nitrate and is present in high concentrations in the saliva which is swallowed into the stomach where conditions of low oxygen (relative to the mouth) and high acid are present. These conditions are ideal for further reduction of nitrite into NO. The entire reduction process of nitrate into nitrite and then into NO occurs without the intervention of NOS enzymes. These enzymes wouldn't be active in these low oxygen and low pH conditions anyway. It should be noted that low oxygen and low pH conditions don't just occur in the stomach, they can also occur throughout the body in certain situations including extreme physical exercise, heart disease and psychological and physical stress! Both pathways of NO generation are depicted in Figure 3, below.

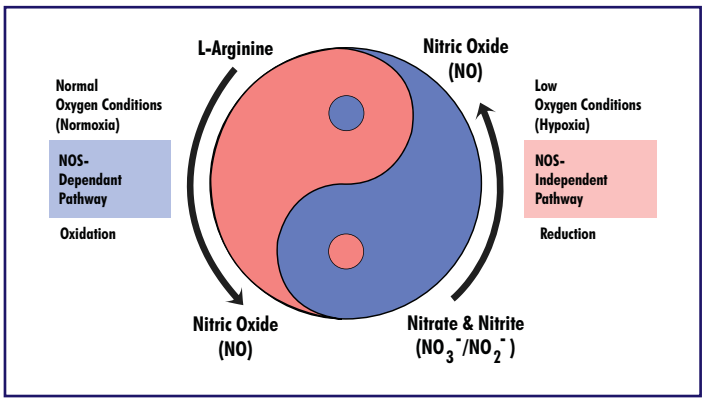


Figure 3. There are two ways that the body generates NO depicted as Ying Yang. Each pathway has special requirements. NO can be produced from L-Arginine and oxygen by a family of enzymes, the NO synthases (NOSs). In a more recently discovered pathway, inorganic nitrate and nitrite are reduced to form NO in blood and tissues when oxygen levels are low. Although NO generation by the L-Arginine pathway is ineffective as oxygen levels fall, the nitrate–nitrite–NO pathway is enhanced! (From Lundberg et al., 2008)

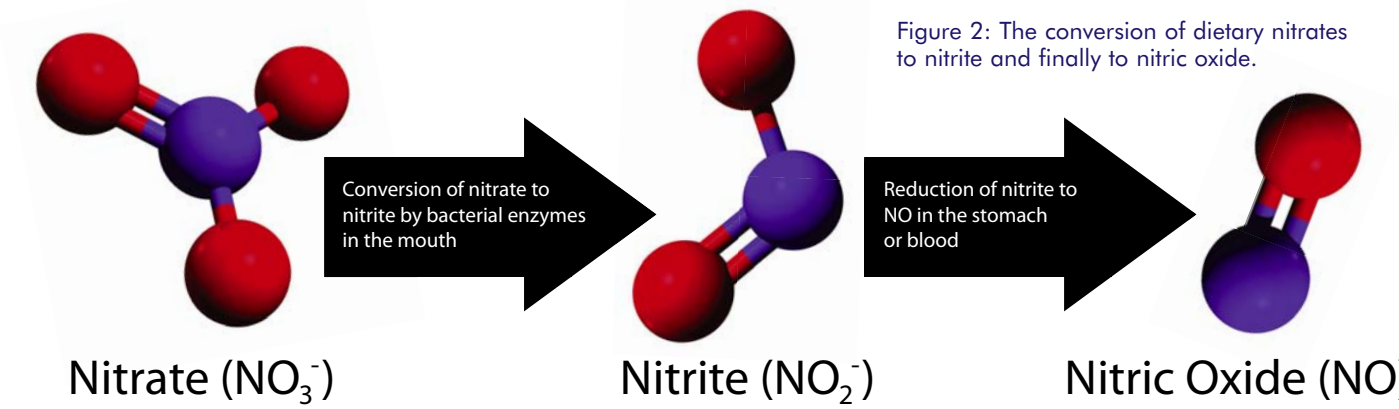


Figure 2: The conversion of dietary nitrates to nitrite and finally to nitric oxide.

Oxidation is a step-wise increase in the number of oxygen and reduction is the opposite with oxygen being reduced from 3 to 2 to 1; hence the term NOx3,2,1 pathway. Besides specific conditions like oxygen status and pH, other factors like the presence of certain reducing compounds will also help to enhance the conversion of nitrates into NO. These helpful reducing compounds include vitamin C, alpha lipoic acid and many polyphenols like green tea catechins (EGCG) and resveratrol.

Once NO is generated in the stomach it is rapidly absorbed across the cell membrane into the blood vessels and from there it is carried all over the body. However, as mentioned before, NO is a free radical and is therefore a highly reactive species that lasts only a few milliseconds (a few thousandths of a second) before it is oxidized back into both nitrite and nitrates.

See Table 1 for a detailed comparison between nitrate, nitrite and NO.

All of this begs the question: "How is it that a molecule with such a short half-life can exert biological effects at

Table 1: Comparing Nitrate, Nitrite and NO (from Hord et al., 2009). See Box 1 for an explanation of terms used in this table.

NOx Species	Concentration in Blood (nmol/L)	Half Life	NOx Species
Nitrate	20,000 - 50,000	5-8 Hours	From diet or from the oxidation of nitrite in the body
Nitrite	100 - 500	1-5 Minutes	From diet, nitrate, or from the oxidation of NO
Nitric Oxide	Less than 1	1-2 Miliseconds	From nitrite in the body

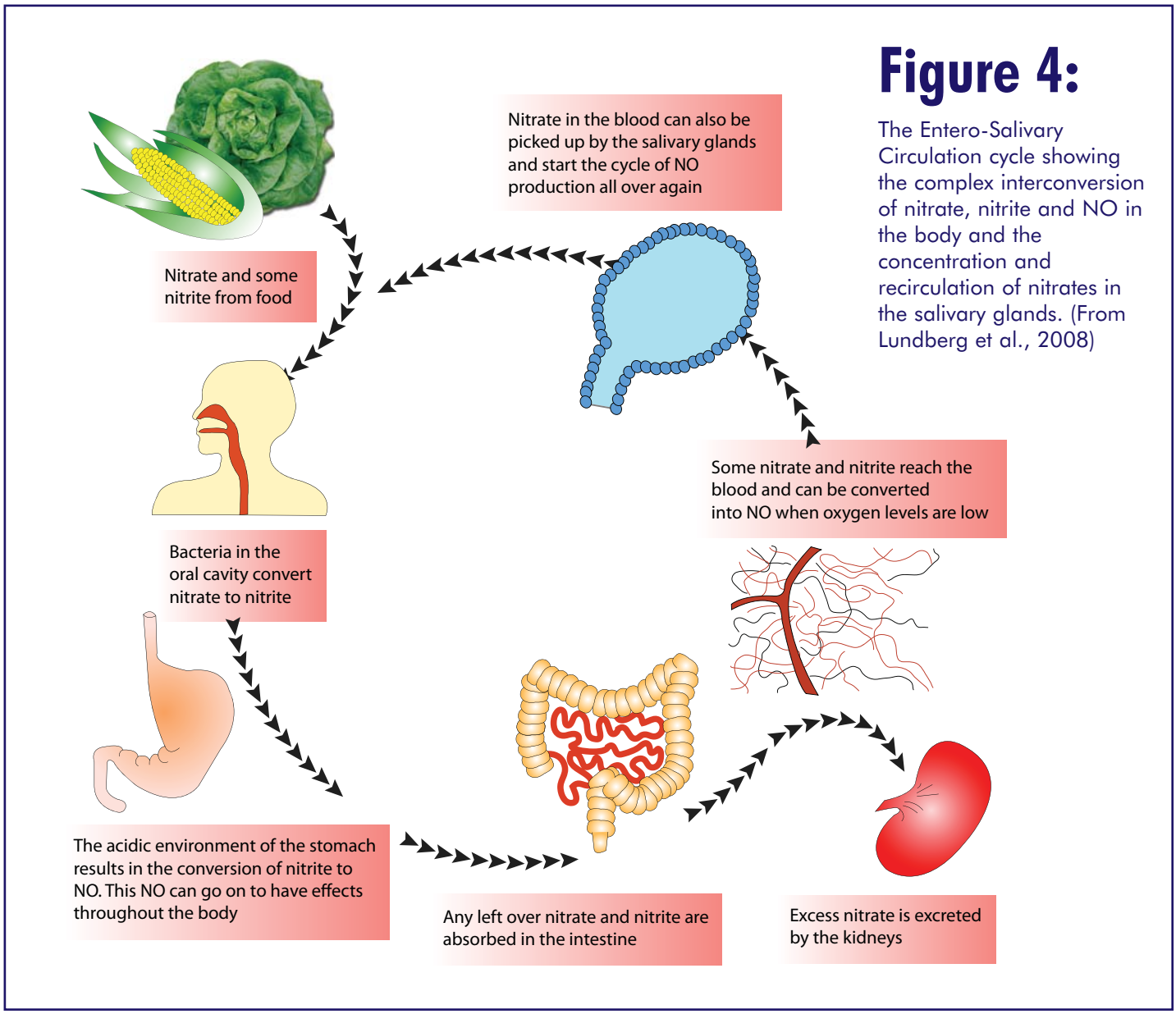


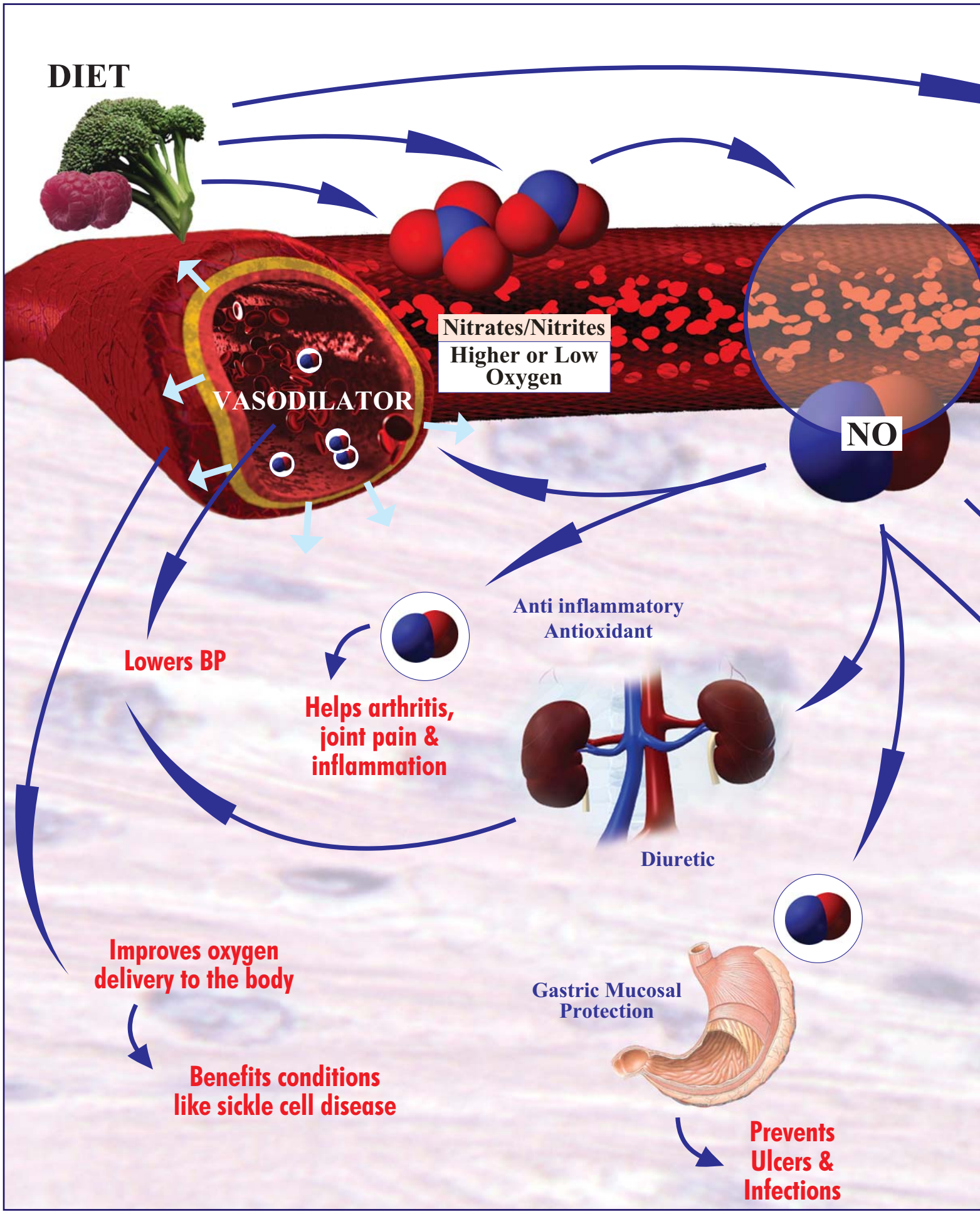
Figure 4:

The Entero-Salivary Circulation cycle showing the complex interconversion of nitrate, nitrite and NO in the body and the concentration and recirculation of nitrates in the salivary glands. (From Lundberg et al., 2008)

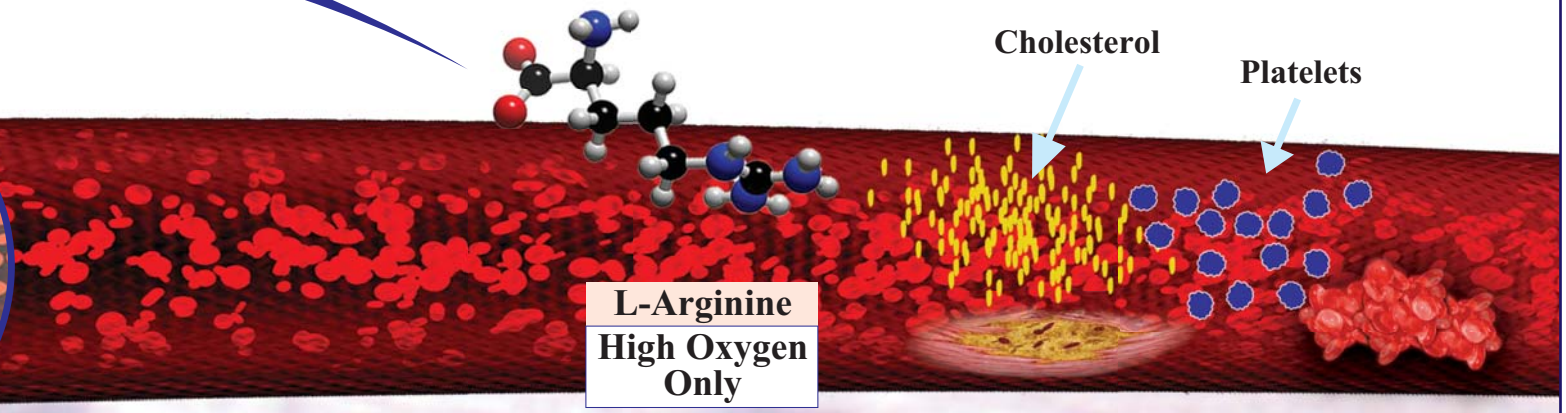
distant sites in the body?" The answer, it seems, lies in the fact that NO is converted back into nitrite and nitrate. Although NO lasts only a short time and may only act directly and locally before it is oxidized into nitrite and then nitrate, this rapid oxidation to both nitrite and especially nitrate, allows these much more stable molecules to then travel to distant sites for re-conversion back into NO as and when the specific tissue requires. In effect, both the nitrite and nitrate act as pro-drug forms of NO; in other words, nitrate and nitrite act as a stable reservoir and a depot for future NO requirements by all cellular tissues throughout the body. In this manner NO can act both locally and at distant sites in a similar manner to various hormones secreted by the thyroid or adrenal glands.

An interesting feature of nitrate in the blood is that approximately 25% of the nitrate is concentrated back in the salivary glands via the entero-salivary circulation cycle (See Figure 4). The concentration of nitrate in the salivary glands is 100-fold greater than in the blood and that of nitrite it is 1000-fold greater! The rest of the nitrate is eventually excreted in the urine. So both urine and saliva are particularly rich in nitrate and nitrite. It is interesting then why animals lick their wounds or that urinating on an injury is considered beneficial – the potential benefits of these behaviours will be discussed in detail in later articles in this magazine.

In summary the opposite biochemical processes of oxidation and reduction of L-arginine and nitrate bioconversion into NO respectively may be viewed as "ying-yang" with the more conventional L-Arginine pathway occurring in conditions when there is plentiful of oxygen and the nitrate pathway as a "back-up" system occurring in conditions when there is lack of oxygen. The key point is that without this vital back-up system our bodies would not be able to carry out a number of biological functions.


DIET
Nitrates/Nitrites
Higher or Low Oxygen
VASODILATOR
NO
Anti inflammatory
Antioxidant
Lowers BP
Helps arthritis, joint pain & inflammation
Diuretic
Improves oxygen delivery to the body
Gastric Mucosal Protection
Benefits conditions like sickle cell disease
Prevents Ulcers & Infections

THE ROLE OF NITRATES IN THE BODY



Anti-platelet
Prevents
LDL Oxidation

Prevents Clots
Prevents
Arterial Plaque

Prevents
Heart
Atttacks

Anti-bacterial

Prevents
Ulcers &
Infections

Prevents
Strokes