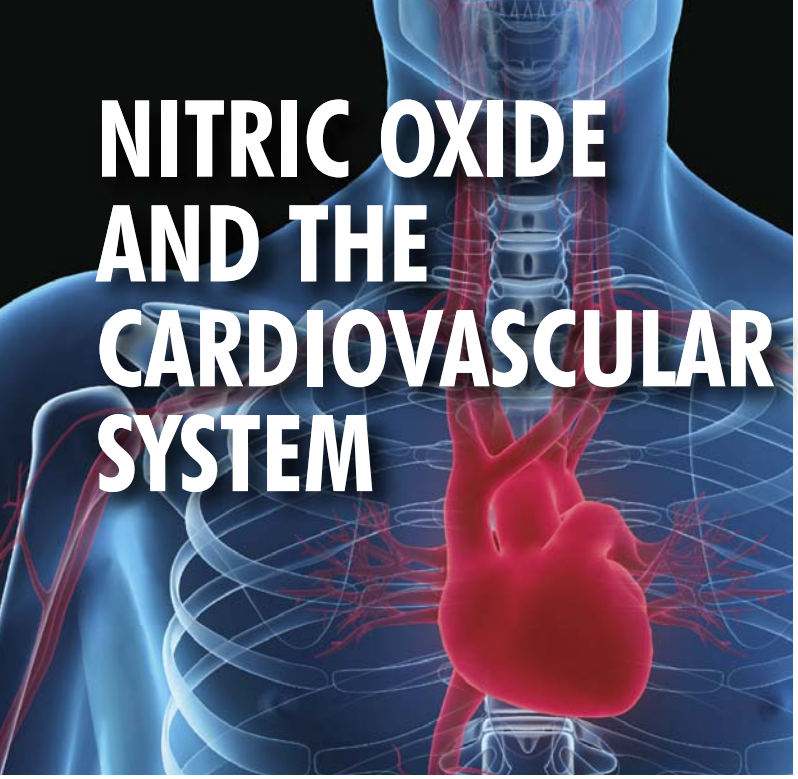

NITRIC OXIDE AND THE CARDIOVASCULAR SYSTEM

Cardiovascular Disease (CVD) which includes high blood pressure (hypertension), angina (ischaemia), arterial damage (atherosclerosis), stroke and heart attack is still the number one cause of morbidity and mortality world-wide. One in three Americans is affected by CVD, and over a billion people worldwide are affected by high blood pressure alone. In Canada, someone dies from heart attack or stroke every 7 minutes, with CVD accounting for 30% of all deaths in Canada annually. The costs associated with CVD are quite staggering with 2006 cost estimated in the EEC to be over 200 billion Euros. In Canada, the costs associated with CVD in 2009 were estimated at $22.2 billion in physician services, hospital costs, lost wages and decreased productivity.

In recognition of these alarming statistics there is a serious initiative on the part of the government to increase the daily intake of fruits, vegetables and fiber as well as to encourage people to exercise more. In North America, the previous recommendation of five servings of fruits and vegetables has been upgraded to a recommended nine servings per day.

Various epidemiological and cohort studies have repeatedly and unequivocally reported a lower incidence of CVD with higher intake of fruits and vegetables. These findings have culminated in the famed Dietary Approaches to Stop Hypertension (DASH) study. The DASH diet was specifically designed to lower blood pressure and recommends eight to ten servings of fruits and vegetables a day, along with consumption of low-fat dairy products. The results of the DASH diet in reducing blood pressure have been found to be as effective as a single prescription therapy for blood pressure!

Researchers from Johns Hopkins University reported an 18% reduction in the 10 year risk of heart disease with the DASH diet. The researchers stated that, "In addition to reducing blood pressure, the DASH diet should substantially reduce the risk of coronary heart disease".

Similarly, the lower incidence of CVD in patients consuming Mediterranean, vegetarian and Japanese diets has been attributed to the high fruit and vegetable intake associated with these diets. In fact some researchers have reported that the greatest protection against CVD is offered by diets with the highest content of leafy green vegetables (See Table 2). This has led scientists to ask some important questions; "What is the common denominator in such diets?" and therefore "What are potential CVD protective compounds associated with these diets?"

It appears that the riddle has been finally solved. A group of researchers principally from Sweden, the UK and the US have independently and collaboratively shown that the nitrate and nitrites in our fruits and vegetables may be the answer and have forwarded convincing evidence to support their hypothesis.

Are Inorganic Nitrates the Answer?

Nitrates can be classified into two categories: organic and inorganic. Organic nitrates are what most of us are familiar with; for example glyceryl trinitrate (GTN) is what is in the tiny tablets usually dispensed by pharmacists in glass vials and recommended to be placed under the tongue for anginal pains. Another common organic nitrate compound it isosorbide dinitrate, which is a more advanced and stable form of GTN. These organic nitrates have been in use since the mid 1800's. Then there are the inorganic nitrates,

which include potassium nitrate and sodium nitrate. These inorganic nitrates are commonly found as salt forms in soils, rocks and plants and have been used for thousands of years in gun powder, food preservation and for heart ailments by early Chinese physicians.

A recently discovered 1000 year old Chinese manuscript translated by scholars at Cambridge University shows the use of inorganic nitrates by Buddhist monks for heart ailments! (See Figure 5) It is quite remarkable that the Chinese physicians knew not only about the inorganic nitrates but more importantly they understood their proper use as evidenced by their advice to their patients to "Administer under tongue and have patient swallow the saliva" It is as if the physicians knew back then about the entero-salivary circulation of nitrate!

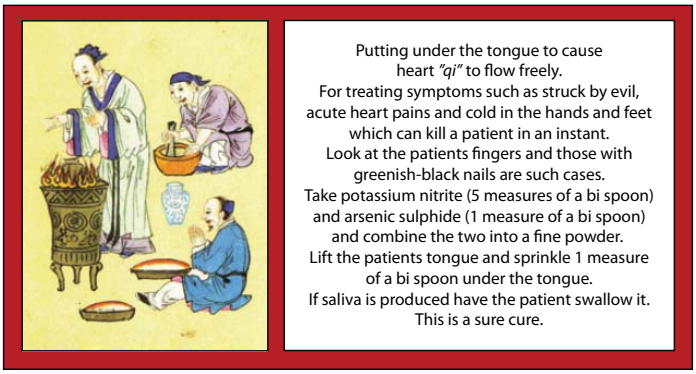

Figure 5. A medical recipe from Buddhist monks at Dunhuang from medieval times showing that the benefits of nitrates were recognized as early as 900 years ago. (From Lundberg et al., 2008)

Dr. Nathan Bryan and his colleagues have investigated the nitrate/nitrite content of various herbs and vegetables consumed in the West and by the Eastern cultures. Interestingly, they have found that herbs and plants that have been associated with a reduction in CVD are also those with the highest nitrate/nitrite content. For example the herb Radix miltiorrhizae, also known as Dan Shen, has a very high nitrate content and is a widely used Traditional Chinese Medicine (TCM) for conditions like angina, myocardial infarction or stroke. Its therapeutic efficacy has been associated with its ability to dramatically raise NO levels. Clinical studies have shown that Dan Shen's therapeutic benefit is nearly comparable to GTN. Furthermore, Dan Shen does not produce the tolerance that normally develops with most organic nitrates; inorganic nitrates seem to be free of this restriction.

Similarly, Bryan's research group has looked at various commonly consumed western vegetables and their nitrate/nitrite content. As can been seen from Table 2 the vegetables with the highest nitrate/nitrite content are spinach, cabbage and Bok Choy. Other vegetables such as beet root, collard greens and leeks also have very high nitrate content. Not only are these vegetables loaded with nitrate but many of them also have a high content of the enzyme nitrate reductace

which assists the conversion of nitrate into nitrite, thereby facilitating the conversion of nitrite into NO. This is particularly true for the Eastern herb Cinnamomum. This herb is rarely used by itself for

CVD because it has a fairly low nitrate/nitrite content. However, when it is combined with herbs like Dan Shen, which do have high nitrate content, the effect is pronounced! In this combination, Dan Shen provides the high nitrate content and Cinnamomum provides the necessary enzyme to reduce nitrate into nitrite and eventually to NO for therapeutic efficacy.

Table 2: The nitrate/nitrite content of various widley consumed western vegetables

VEGETABLE	NITRITE CONTENT (mg/100g)	NITRATE CONTENT (mg/100g)
Root Vegetables		
Carrot	0.002 - 0.023	92 - 195
Mustard Leaf	0.012 - 0.064	90 - 95
Green Vegetables		
Lettuce	0.008 - 0.215	12.3 - 267.8
Spinach	0 - 0.073	23.9 - 387.2
Cabbage		
Chinese Cabbage	0 - 0.065	42.9 - 161
Bok Choy	0.009 - 0.242	102.3 - 309.8
Cabbage	0 - 0.041	25.9 - 125.0
Cole	0.364 - 0.535	76.6 - 136.5
Melon		
Wax Gourd	0.001 - 0.006	35.8 - 68.0
Cucumber	0 - 0.011	1.2 - 14.3
Nightshade		
Eggplant	0.007 - 0.049	25 - 42.4

NO and Reduction of Blood Pressure

Hypertension or high blood pressure is defined as a Systolic Blood Pressure (SBP) of 140 mmHg or higher or a Diastolic Blood Pressure (DBP) of 90 mmHg or higher, with normal blood pressure being defined as 120/80 mmHg or lower. However, the risk of negative health effects and death is increased in individuals whose blood pressure is consistently 115/75 mmHg or higher, with the risk of cardiovascular disease doubling with each increment of 20/10 mmHg above 115/75 mmHg.

High blood pressure is associated with an increased risk of stroke, myocardial infarction (heart attack), heart failure, kidney failure and cognitive impairment. SBP above 115 is the most important determinant of the risk of death world-wide and is responsible for 7-8 million deaths annually and over 20,000 deaths each year in the United States. In Canada more than one in five people (22.7%) of Canadian adults aged 20 years and older were living with diagnosed hypertension. Since approximately 17% of individuals with hypertension are not aware of their condition, the true prevalence of hypertension is likely higher. The statistics in the US are similar, with approximately 22% of adults living with diagnosed hypertension. It has been suggested that in moderately hypertensive patients a 5 mmHg reduction in blood pressure might reduce the incidence of stroke by 22 % and coronary heart disease by 16 %!

The beneficial effects of fruits and vegetables on blood pressure are well known. For example, a comparison of the effect of a traditional Japanese diet (with a high nitrate content) to that of a diet with low nitrate content (as much as 20-times less nitrate) in the same 25 subjects (a cross-over study), demonstrated a significantly lower DBP by 5 mmHg.

Because vegetables are naturally rich in nitrates it seems reasonable to investigate whether the consumption of inorganic nitrate alone, corresponding to the amount present in a plate of salad for example, could affect blood pressure in healthy subjects. Dr. Jon Lundberg and Dr. Eddie Weitzberg's group in Sweden tested exactly this hypothesis in a small double-blind placebo controlled, cross-over designed study. Sodium nitrate in a dose of 7 millimoles of nitrate (see page 2: What is a mole?) per 70 kg adult male was administered for three days after which blood pressure was measured. It was found that DBP was indeed reduced by a significant 4 mmHg compared to the placebo (sodium chloride with equivalent amount of sodium). The researchers found that the rise in

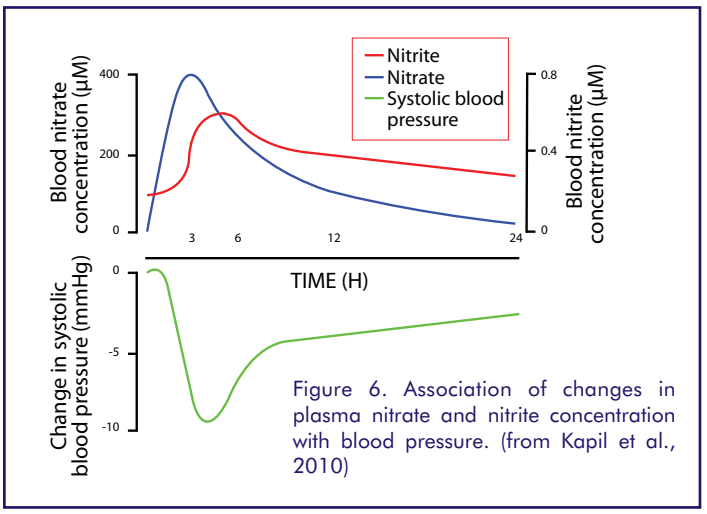

Figure 6. Association of changes in plasma nitrate and nitrite concentration with blood pressure. (from Kapil et al., 2010)

plasma nitrate levels resulting from consumption of sodium nitrate was accompanied by a reduction in blood pressure. This suggests that the blood pressure lowering effect was due to the nitrate being converted into nitrite and then into NO. Although such changes in blood pressure appear relatively small, it must be remembered that the dose of nitrate used was not large; equivalent to a single serving of salad (approximately a plate or 250 g). Even so, these small results could still have meaningful health effects when considered over the lifetime of the individuals. For instance, over the long term, even a small reduction in blood pressure will help reduce the stress put on blood vessel walls due to high blood pressure. Additionally, it is important to consider that the study was conducted in healthy patients with normal blood pressure levels. It is expected that a significantly greater fall in blood pressure would be observed in hypertensive patients.

Following up on the important findings of the Swedish researchers, Dr. Amrita Ahluwalia and her group in London conducted an elegant study to prove the point that the nitrate rich vegetables can reduce blood pressure via conversion into NO. Again in a double-blind randomized placebo controlled study, healthy adult volunteers were given 500 mL of beetroot juice to drink. The amount of nitrate present in the juice was three times the amount administered in the Lundberg study. Three hours after consuming the beetroot juice there were significant reductions in both SBP and DBP of 10 mmHg and 8 mmHg respectively. The beauty of this study was the clear demonstration of a cause and effect relationship between the nitrate/nitrite and the reduction in blood pressure. By monitoring plasma nitrate and nitrite levels as well as blood pressure levels, the researchers were able to show that increased plasma nitrate and nitrite levels corresponded directly to a decrease in blood pressure.

There are three noteworthy points that are raised by this study. First, the half to one hour delay that occurs between the intake of juice and the reduction in blood pressure represents the time taken for nitrite to be converted to nitrate by the bacteria in the mouth and then to be converted into NO in the stomach. Second, the study demonstrates a dose response relationship, meaning that the higher the dose the greater the effect. Finally, an exquisite relationship was established between raised plasma levels of nitrate/nitrite and blood pressure reduction. For scientists there is no better evidence than a physiological cause-and-effect relationship, and this study accomplishes this!

A second study by Dr. Ahluwalia's group provided further evidence, and also demonstrated that the blood pressure effect they observed could be achieved with a lower 250mL intake of beetroot juice. More importantly, they showed that the effects of dietary nitrate from beetroot juice can be sustained over a longer period, with the reduced blood pressure effects lasting for over 24 hours!

Lundberg and Weitzberg's group have also used animal hypertensive models to provide further evidence of the benefits of inorganic nitrates for reducing blood pressure. In one of these models they blocked the NOS enzyme (responsible for NO production via the L-Arginine pathway) with specific enzyme inhibitors of the enzyme. As a result of blocking this enzyme the animals developed hypertension. This effect was reduced by administering nitrites in the drinking water. In a more recent 2011 study, this research group has shown that administering nitrates to hypertensive animals (caused by removing the kidney) not only reduced blood pressure but also reduced scarring and other damage to the tissues in these animals. Furthermore, a much lower dose of nitrite that did not reduce blood pressure still protected against kidney damage, suggesting the use of much lower doses of nitrates as a preventive measure. More studies are needed in this exciting field.

Other CVD Protective Effects of Nitrates, Nitrites and NO

In addition to their blood pressure lowing effects, inorganic nitrates have been shown to have numerous other cardiovascular protective effects including improved endothelial function, improved platelet function, a reduction in the symptoms of metabolic syndrome and improved oxygen delivery to the tissues.

A Note on Sunlight: Is it good for your Heart?

In a recent publication of the European Heart Journal Dr. Martin Feelisch takes a deeper look at this question. Although there is a definite relationship between UV light as a predisposing factor for non-melanoma and melanoma skin cancers especially in light skinned people, there is fair amount of data suggesting that exposure to sunlight can reduce blood pressure as well as offering other health benefits. Previous researchers have attributed these benefits to the formation of vitamin D and melatonin by the skin, however, Dr. Feelisch presents a novel hypothesis to explain this phenomenon. He suggests that there is also a benefit due to the skin acting as a significant storage site for NO in the form of nitrate and nitrate from the diet. In fact the skin has a significantly higher nitrite concentration than the blood.

A recent human study has demonstrated that UVA radiation can increase plasma nitrite levels by over 40%. A similar increase in animals has been shown to offer significant cardiac and circulatory protection. In addition, all-cause mortality risk is tightly related to latitude, and seasonal variations in light intensity are correlated with seasonal variations in the incidence of CVD-related events and deaths. Rates of strokes, angina and heart disease are all higher in the winter months than summer. Of course other factors like diet and exercise need also to be taken into account. Dr. Feelisch argues that since nitrate and nitrite levels are under dietary control it could be possible to greatly enhance plasma levels and thereby allow the body to produce NO on demand, where and when the body desires to achieve optimal health benefits.

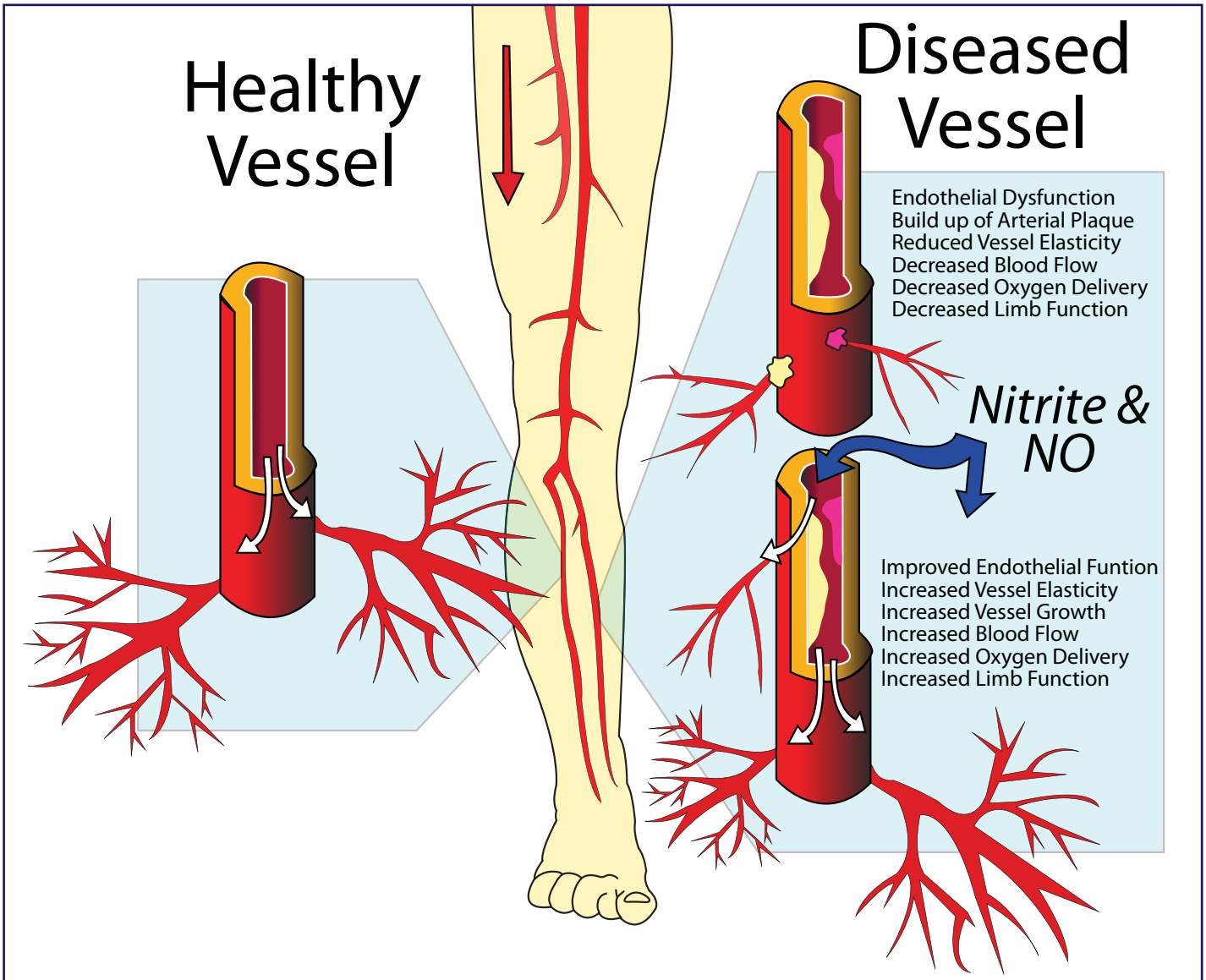

Figure 7. A comparison of a normal healthy blood vessel and a diseased showing damaged endothelial cells and a build-up of atherosclerotic plaque. The figure shows the beneficial effect of nitrite therapy on diseased blood vessels. (from Patillo et al., 2011).

The human circulatory system is the largest organ in the body. The health of this enormously important system depends on the proper functioning of a specific type of cell that lines the inside of the blood vessels. These cells are called endothelial cells, and they form the lining of every blood vessel in your body, from the large aorta to the tiniest capillaries. The endothelial layer is only one cell thick and plays a vital role in the health of the blood vessel. Any abnormality or damage leads to these cells leads to a condition called endothelial dysfunction, which is thought to be a key initiating factor in the pathology of CVD.

High blood pressure, inhaled or ingested toxins (e.g. tobacco smoke), excessive alcohol intake, poor diet (e.g. excessive cholesterol or sugar) and stress can all cause damage to the fragile endothelial layer. For example, high blood pressure results in an excess of mechanical sheer forces to be exerted on the layer, which can damage the endothelial cells. On the other hand, tobacco smoke may damage the cells by causing an excessive generation of free radicals or reactive oxygen species. As the name implies these are highly reactive molecules, and they can attack the endothelial cells, damaging them. Similarly, poor diet in the form of excessive consumption of sugar or cholesterol can initiate endothelial damage. In each case, the net result is the formation of plaques in the blood vessels. These plaques are highly dangerous as they can cause the rupture and release of blood clots, resulting in further damage to distant sites and can

also lead to blood vessel blockage - causing heart attacks or strokes. Once the damage to endothelial cells begins; it is like a chain reaction, with the damage spreading from endothelial cell to endothelial cell. Essentially, one plaque breeds more plaques, causing inflammation to spread like wildfire and contributing to atherosclerosis of the blood vessel. Atherosclerosis is a build-up of plaque inside the arteries, which can thicken and harden over time (see Figure 7, next pg). This results in a loss of blood vessel elasticity and flexibility which is critical to how the blood vessel responds to pressure changes. Any blood vessel that has reduced elasticity will have increased pressure inside it.

Endothelial cells are also important because they are one of the key sites in the body where NO is synthesized. NO acts as a local signaling molecule and hormone to the neighboring cells, allowing for monitoring of the environment in the vessel and ensuring that proper blood pressure and other factors are maintained. NO also has direct actions on the blood vessel itself. It can travel to the smooth muscle cells located directly beneath the endothelial cell lining where it caused vasodilatation or widening of the blood vessels. In a situation where excessive free radicals are present, NO is unable to do its job properly since the free radicals will act to "mop" up the NO, reducing the amount of NO that is able to act to cause vasodilatation, which is not a good thing!

Studies using genetic knockout mice (see page 2) that lack the endothelial NOS enzyme have shown that these animals are highly prone to cardiovascular complications. However, nitrite supplementation was able to reduce such endothelial disturbances and help to prevent resulting damage. The Ahluwalia study discussed above showed a similar beneficial effect in humans. They found that beetroot juice effectively reduced endothelial damage as assessed by various inflammatory markers like C-Reactive Proteins (CRP). Recently, a direct relationship between reduced endothelial damage and plasma nitrite levels has been established in humans (see Figure 8). Overall, the data points towards a very important role for nitrate/nitrite in the preservation and support of healthy endothelial function.

Platelet function

Platelets are cells present in the blood that are distinct from the other two major blood cell types, red and white blood cells. The primary function of the platelets is to aggregate at sites of damage causing clotting. Platelets are able to cause this clotting due to their innate tendency towards "togetherness', in other words they tend to aggregate or clump together. In normal circumstances this is a good thing, since the clotting caused by platelets is required to prevent excessive blood loss when an injury occurs. However, platelet adhesion, activation and aggregation are also known to be key factors in the progression of atherosclerosis and thus, modulation of platelet function is an important therapeutic strategy for the prevention of atherosclerosis. Both beetroot juice and potassium nitrate have been shown to reduce platelet aggregation. Reduced platelet aggregation or "stickiness" not only helps to prevent the inappropriate clumping of cells that can lead to colt formation and artery blockage, but also allows blood flow to take place more easily thereby reducing the burden on the heart.

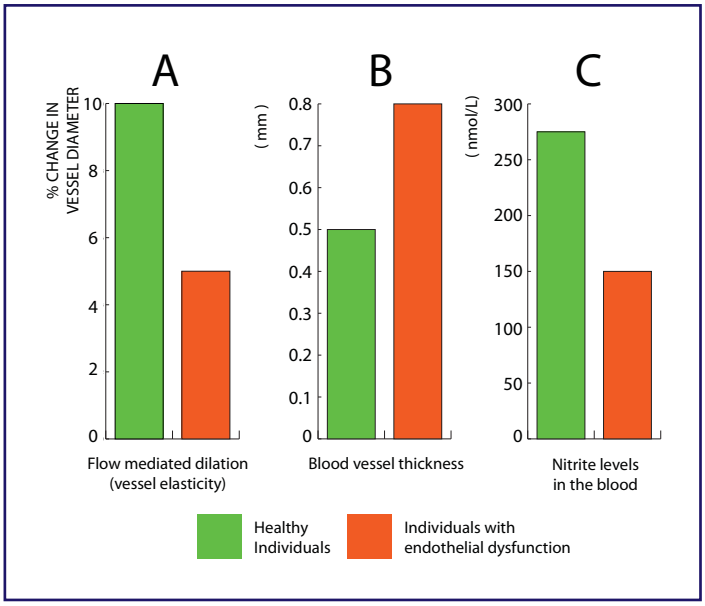

Figure 8. The association between blood nitrite levels and endothelial dysfunction. Healthy individuals show high nitrite levels which are associated with high levels of artery elasticity. Individuals with endothelial dysfunction show low nitrite levels, reduced artery elasticity and thicker artery walls.

Metabolic Syndrome

In 1985, Reaven coined the term Metabolic Syndrome to describe a cluster of symptoms that often appear together and may have a common initiating or causative event. This cluster of symptoms include: obesity, hypertension, high lipid/cholesterol levels and diabetes. These deadly conditions are the four horsemen of the apocalypse that have targeted one quarter of the world's adult population.

Collectively, these four conditions act to increase the risk of CVD in a synergistic fashion, meaning that the risk increases exponentially rather than additively as more and more factors become involved.

Animal studies using knockout mice lacking the NOS enzyme gene (thus preventing NO formation) result in full-blown metabolic syndrome. Various researchers have suggested that metabolic syndrome may be due to a deficiency of NO. Addition of nitrate and/or nitrite to the drinking water of these knock-out mice has been shown to protect the animals, significantly reduces their lipid and triglyceride levels and also reduces the incidence of diabetes and weight gain. These results present an intriguing possibility for the prevention and control of metabolic syndrome in humans, although human studies are required to verify these results.

Ischemia

Ischemia occurs when there is a lack of oxygen delivery to tissues, often due to partial or complete obstruction of blood flow. For example, endothelial dysfunction leading to atherosclerosis can result in ischemia. Angina (chest pains), heart attacks and strokes are examples of ischemic events. When ischemia occurs, the tissues that are deprived of blood and oxygen suffer irreversible damage which can result in death. Tissues like the heart, the kidneys and the brain are particularly sensitive to an interruption in blood supply. Besides helping to reduce the incidence of ischemic events in the first place through its ability to widen the blood vessels (thereby improving oxygen delivery to the tissues) and through its improvement of endothelial function, NO can also have direct effects in the prevention of ischemic injury. Animal studies have shown that during ischemic events, low oxygen acidic conditions result, which causes nitrite to be reduced to NO. This production of NO during ischemic events is the body's way of protecting the cells and tissues during periods of reduced oxygen supply. Studies in mice and rats have shown that an infusion of nitrite during an ischemic event can significantly improve recovery and reduce tissue damage and death in the heart, lungs, kidney and liver. Other animal studies have also found that pretreatment with inorganic nitrate helps to enhance blood flow to damaged areas following an ischemic event, which can help to minimize damage. Overall, the evidence points to a preventative and protective effect of nitrates and NO for ischemia. Further studies in humans will provide further support for the role of nitrates in these conditions.

Metabolic Syndrome:

Are you at risk?

Metabolic Syndrome is characterized by the following collection of symptoms:

For Men:

- Waist circumference > 40 inches
- Triglycerides > 150 mg/dL
- HDL Cholesterol < 40 mg/dL
- Blood Pressure > 130/85 mmHg
- Fasting Glucose > 100 mg/dL

For Women:

- Waist circumference > 35 inches
- Triglycerides > 150 mg/dL
- HDL Cholesterol < 50 mg/dL
- Blood Pressure > 130/85 mmHg
- Fasting Glucose > 100 mg/dL

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