importance of nitrates in reducing cardiovascular disease (CVD)

In the late 1960’s researchers discovered a naturally produced “factor” that would relax the blood vessels. They named it endothelium derived relaxing factor (EDRF). After considerable research, it was later discovered that EDRF was a simple molecule containing one atom of nitrogen and one atom of oxygen produced locally by the blood vessels, and was more correctly called nitric oxide (NO). This discovery eventually culminated in three researchers winning the Nobel Prize for medicine in 1998; sadly one of the key researchers, Salvador Moncada, was overlooked by the Nobel Committee. Who says there are no politics in science?

Cardiovascular Disease (CVD), which includes high blood pressure (hypertension), angina (ischemia), arterial damage (atherosclerosis), stroke and heart attack is still the number one cause of suffering and death world-wide. One in three Americans and Canadians are affected by CVD, and over a billion people are affected by high blood pressure alone. The costs associated with CVD are quite staggering, with a 2006 cost estimated in Western Europe to be over 200 billion Euros.

Government Initiative to Lower Cardiovascular Disease

In recognition of these alarming statistics, there is a serious initiative on the part of governments to promote the increased intake of fruits, vegetables and fiber along with increasing physical activity. The recommended consumption of fruits and vegetables has been upgraded from five daily servings to nine in North America.

Various studies of large populations have repeatedly and clearly reported a lower incidence of CVD in correlation with a higher intake of fruits and vegetables. These findings have culminated in the famed Dietary Approaches to Stop Hypertension (DASH) study. The DASH study 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 (see Figure 1) in reducing blood pressure have been found to be as effective as some prescription drugs for lowering blood pressure!

Researchers from John Hopkins University in Baltimore reported an 18% reduction in a 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 on the Mediterranean, vegetarian, and Japanese diets has been associated with a high fruit and vegetable intake. In fact, some researchers have reported that the greatest protection against CVD is offered by diets with the highest content of leafy green vegetables.

This has led scientists to ask an important question, “What is the common factor in such diets, and therefore the key CVD protective compound(s)? It appears that the riddle has been finally solved. A group of researchers from Sweden, the UK, and the US have independently and collaboratively shown that the nitrates and nitrites in fruits and vegetables may be the answer, and have forwarded convincing evidence to support their hypothesis.

Inorganic Nitrates the Answer?

Nitrates can be classified into two categories, organic and in-organic. Organic nitrates are what most of us are familiar with e.g. glyceryl trinitrate (GTN) – the tiny tablets usually dispensed by pharmacists in glass vials and recommended to be placed under the tongue for pains due to angina, or isosorbide di-nitrate a more upgraded and stable form of GTN. These organic nitrates have been in use in medicine since the mid-1800s.

Then there are the in-organic nitrates like potassium or sodium nitrate which are commonly found in soils, rocks and plants; they have been used for thousands of years, from the manufacture of gun powder to food preservation, and for heart ailments by early Chinese physicians.

Researchers have looked at various commonly consumed western vegetables and their nitrate/nitrite content. As can been seen from Table 1 on pg. 16, the
vegetables with the highest nitrate/nitrite content are spinach, cabbage, beet root, collard greens, leeks etc. Nathan Bryan at the University of Texas, and colleagues investigated the nitrate/nitrite content of various herbs and vegetables consumed in the West and by the Eastern cultures. Indeed, certain herbs with the highest nitrate/nitrite content are associated with a reduction in CVD. For example, the herb Radix miltiorrhizae has a high nitrate content and is widely used in Traditional Chinese Medicine (TCM); known as Dan Shen, for conditions such as angina, heart attack and stroke. The herb’s protective effect on the heart is recognized due to its ability to dramatically raise NO levels. Clinical studies have found Dan Shen almost comparable to nitroglycerin, and yet without the tolerance that normally develops with most organic nitrates. Inorganic nitrates seem to be free of this restriction.

Until the mid-1990’s, researchers believed that the only way to increase NO levels was by the conversion of the amino acid L-arginine into NO, with the help of various enzymes which require the presence of oxygen.

**Discovery of a new pathway!**

In the mid-1990’s, researchers from the UK and Sweden discovered a new pathway for NO generation from dietary nitrates. The Swedish researchers led by Jon Lundberg, Eddie Weitzberg, and Nigel Benjamin from London, were trying to find out why diets like the Mediterranean, vegetarian, Japanese or the DASH diet were particularly protective of the heart. Both groups independently reported that the composition of leafy green vegetables, 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 that did not require any enzymes or the presence of oxygen. (see Figure 2 on pg. 16)

Systolic blood pressure above 115 mmHg is the most important determinant of the risk of death, responsible for 7-8 million worldwide deaths annually. Indeed, it has been suggested that, in moderate hypertensive patients, just a 5mmHg reduction in blood pressure might reduce the incidence of stroke by 22% and coronary heart disease by 16%.

The beneficial effect of fruits and vegetables on blood pressure is well known. Comparison of the effect of traditional Japanese diet, which has a nitrate content and a diet with low nitrate content (20 fold less) in the same 25 subjects (a cross-over study), demonstrated a significantly lowered diastolic blood pressure (DBP) of 5mmHg in the nitrate rich diet.

Because vegetables are naturally rich in nitrates, it seems reasonable to investigate if inorganic nitrate alone, corresponding to the same amount present in a single serving of a plate of salad, could affect blood pressure in healthy subjects. Jon Lundberg and Eddie Weitzberg’s group in Sweden tested their hypothesis in a small double-blind placebo-controlled, cross-over designed study. A small dose of sodium nitrate was administered to adults for three days after which blood pressure was measured. Indeed, DBP was reduced by 4mmHg compared to placebo (sodium chloride providing an equivalent amount of sodium). The rise in blood nitrate levels was accompanied by a fall in blood pressure. This suggested that the blood pressure...
Advances

lowering effect was due to the nitrate being converted into nitrite and then into NO (see Figure 3).

Although such changes in blood pressure appear relatively small, it must be remembered that the dose used is equivalent to the nitrates found in only a single serving of salad (approximately a plate of 250 g). These results become more meaningful in the reduction of mechanical sheer and stress to the vascular endothelium over the entire lifetime of the individual. As well, the study was conducted in normotensive patients that were otherwise healthy. It is expected that a significantly greater fall in blood pressure would occur in hypertensive patients. Finally, the study was only three days long!

Following up on this important finding, Amrita Ahluwalia and her group at St. Bart’s Hospital, University of London, conducted an elegant study to prove the point that nitrate-rich vegetables can reduce blood pressure via conversion into NO via nitrite. Again in a double-blind randomized placebo-controlled study, healthy adult volunteers were given 500mL of beetroot juice to drink. The amount of nitrate present was three times the amount administered in the Lundberg study. Three hours later there was a significant reduction in SBP of 10 mmHg and DBP of 8 mmHg. The beauty of this study was that it demonstrated cause and effect. By monitoring plasma nitrate and nitrite levels and blood pressure, the researchers demonstrated that raised plasma nitrate and nitrite levels corresponded with a decrease in blood pressure.

Several points were raised in this study. First, there is a small delay of a half to one hour or so that occurs in blood pressure reduction after the intake of juice; this represents the time taken for conversion of nitrate into nitrite by the bacteria in the mouth and then the conversion of nitrite into NO in the stomach. Second, the study demonstrates a dose-response relationship, the higher the dose the greater the effect. Finally, a clear relationship was established between raised plasma levels and blood

### Table 1. Nitrate and nitrite contents of vegetables (adapted from Wang et al. 2000)

<table>
<thead>
<tr>
<th>Vegetable types and varieties</th>
<th>Nitrite mg/100 g fresh weight</th>
<th>Nitrate mg/100 g fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Root vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td>0.002–0.023</td>
<td>92–195</td>
</tr>
<tr>
<td>Mustard leaf</td>
<td>0.012–0.064</td>
<td>70–95</td>
</tr>
<tr>
<td><strong>Green vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>0.008–0.215</td>
<td>12.3–267.8</td>
</tr>
<tr>
<td>Spinach</td>
<td>0–0.073</td>
<td>23.9–387.2</td>
</tr>
<tr>
<td><strong>Cabbage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>0–0.065</td>
<td>42.9–161.0</td>
</tr>
<tr>
<td>Bok choy</td>
<td>0.009–0.242</td>
<td>102.3–309.8</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0–0.041</td>
<td>25.9–125.0</td>
</tr>
<tr>
<td>Kale</td>
<td>0.364–0.535</td>
<td>76.6–136.5</td>
</tr>
<tr>
<td><strong>Melon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wax gourd</td>
<td>0.001–0.006</td>
<td>35.8–68.0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0–0.011</td>
<td>1.2–14.3</td>
</tr>
<tr>
<td><strong>Nightshade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggplant</td>
<td>0.007–0.049</td>
<td>25.0–42.4</td>
</tr>
</tbody>
</table>

### Classification of vegetables in terms of their nitrate content (adapted from Santanaria P., 2006)

<table>
<thead>
<tr>
<th>Nitrate content (mg/100 g fresh weight)</th>
<th>Vegetable varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low, &lt;20</td>
<td>Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom,</td>
</tr>
<tr>
<td></td>
<td>pea, pepper, potato, summer squash, sweet potato, tomato, watermelon</td>
</tr>
<tr>
<td>Low, 20 to &lt;50</td>
<td>Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory</td>
</tr>
<tr>
<td>Middle, 50 to &lt;100</td>
<td>Cabbage, dill, turnip, savoy cabbage</td>
</tr>
<tr>
<td>High, 100 to &lt;250</td>
<td>Celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley</td>
</tr>
<tr>
<td>Very high, &lt;250</td>
<td>Celery, cress, chervil, lettuce, red beetroot, spinach, rocket (rucola)</td>
</tr>
</tbody>
</table>

![Figure 2. Nitrate-Nitrite-NO pathway](image-url)
pressure reduction. Scientists love to show a physiological cause and effect relationship - this study does this!

A second study by Ahluwalia’s group showed that the blood pressure effect could be achieved with a lower intake of 250mL beetroot juice. More importantly, the effects of dietary nitrate from beetroot juice can be sustained over a longer period. The blood pressure effect lasts for over 24 hours!

Lundberg and Weitzberg’s group, using elaborate animal hypertensive models, have shown that nitrates can reduce blood pressure. In one model, blocking the NOS enzyme (responsible for NO production) with specific inhibitors of the enzyme caused hypertension in the animals. This effect was reduced by administering nitrites in the drinking water.

In a more recent 2011 study, the Swedes have used another model of high blood pressure (caused by removing the kidney) to show that nitrates not only reduced the blood pressure, but also reduced the fibrosis (scarring) and other damage caused in the animals. Furthermore, a much lower dose of nitrite that did not reduce blood pressure still protected against kidney damage, suggesting the use of a much lower dose as a preventive measure. More studies are needed on this exciting topic.

Other CVD protective effects of nitrates/nitrites/NO

Inorganic nitrates have been shown to have numerous cardiovascular protective effects including: endothelial function, platelet function and metabolic syndrome.

1. Endothelial function

The human vasculature is the largest organ in the body. The endothelial cells line the largest blood vessel, the aorta, to the tiniest blood capillaries. The endothelium is one cell thick and plays an important role in the health of the vessel. Any abnormality or damage to these cells therefore leads to endothelial dysfunction which may be the initiating factor in the pathology of CVD.

Endothelial cells are one of the key sites in the body where NO is synthesized. NO acts as a local hormone to the neighbouring cells, thereby monitoring the local environment. NO diffuses to the smooth muscle cells located directly beneath the endothelial cell lining causing vasodilation or widening of blood vessels. Excessive free radicals will often “mop” up the NO resulting in the loss of the ability to execute vasodilatation which is not a good thing.

Animal studies with genetic knockout mice, lacking the enzyme that helps produce NO, are highly prone to cardiovascular complications. Nitrite supplementation reduces such endothelial disturbances and prevents damage. The Ahluwalia study showed that beetroot juice could effectively reduce endothelial damage as assessed by various inflammatory markers like C-Reactive Proteins (CRP). Recently, a direct relationship between reduced endothelial damage and higher plasma nitrite levels has been established. So the data seems to be very supportive of nitrates/nitrites preserving endothelial function.

2. Platelet function

The main function of platelets in the blood is to amass at sites of damage, causing clotting. Platelets have an innate ability of “togetherness”. The role of platelet activation, adhesion and aggregation in atherosclerosis is well known.

Reduced platelet aggregation or “stickiness” may not only prevent inappropriate clumping of cells causing thrombosis or clots, but it also allows blood to flow more easily, reducing the burden on the heart. Modulation of platelet function is an important therapeutic strategy in preventing atherosclerosis. Both beetroot juice and...
potassium nitrate have been shown to prevent platelet aggregation.14

3. Metabolic Syndrome
In 1985, Reaven coined the term Metabolic Syndrome to represent a cluster of symptoms that often appear together and may have a common initiating or causative event. Obesity, hypertension, high lipid levels and diabetes are the ‘four horseman of the apocalypse’ that have targeted one quarter of the world’s adult population. These four risk factors collectively increase the risk of CVD.

Animal studies in knockout mice lacking the NOS enzyme gene (thus preventing NO formation) results in full-blown metabolic syndrome. Various researchers have suggested that metabolic syndrome may be due to a deficiency of NO. The addition of nitrate and/or nitrite to the drinking water of the animals protects the animals and significantly reduces the lipid and triglyceride levels as well as the incidence of diabetes and weight gain.16 These results present an intriguing possibility for preventing and/or controlling metabolic syndrome. However, human studies are warranted.

4. Ischemia
Ischemia is the lack of oxygen delivery to tissues due in part to partial or complete obstruction of blood flow. Angina and heart attack are examples of an ischemic event. When ischemia occurs, the tissue with an obstructed blood supply (and thus oxygen) suffers irreversible damage. Damage to the blood vessels restricts blood flow leading to poor circulation.

Nitrates and nitrites have been shown to reduce the damage to the tissues and thus improve the circulation, leading to improvement in the symptoms.21

Conclusion
Nitric oxide is a versatile and an important molecule that plays a major role in heart and blood vessel health. Since its recent discovery there has been tremendous research of how it is generated, its mechanism of action and its therapeutic potential. In the last ten years there has been an explosion in the research with clinical studies confirming its safety and its effectiveness.

References
6. Butler A R and Feilisch M “Therapeutic uses of inorganic nitrite and nitrate: From the past to the future” Circulation 2008; 117:2151-2159
14. Webb A J et al. “Acute blood pressure lowering, vasoprotective and antiplatelet properties of dietary nitrate via bioconversion to nitrite” Hypertension 2008; 51:784-790
17. Carlstrom M et al. “Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension” Cardiovascular Research 2011; 89:574-585

Additional References