Nitric Oxide: The Overlooked Molecule In Patient Care

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Structure of Presentation

- Review of Nitric Oxide (NO) and its discovery
- Biological effects of deficiency and therapies surrounding NO
- Current Environment for Drug Discovery
- Strategies to Replete NO
- A New Paradigm – Nature’s NO

Disclosure: N.S. Bryan is a paid consultant and Chief Science Officer of NeoGenis Labs, Inc.
"The discovery of NO and its function is one of the most important in the history of cardiovascular medicine."

Dr. Valentin Fuster
1998 President of American Heart Association
What is Nitric Oxide?

The chemical compound nitric oxide is a gas with chemical formula NO⁻.

It is an important signaling molecule in the body of mammals including humans, one of the few gaseous signaling molecules known.

It is also a toxic air pollutant produced by automobile engines and power plants.

NO should not be confused with nitrous oxide (N₂O), a general anesthetic, or with nitrogen dioxide(NO₂) which is another poisonous air pollutant.

The nitric oxide molecule is a free radical, which is relevant to understanding its high reactivity. It reacts with the oxygen in air to form nitrogen dioxide, signaled by the appearance of the reddish-brown color.
Nitric Oxide Plays a Key Role in the Regulation of Numerous Vital Biological Functions

**Immunology**
- Unspecific Immunity
- Inhibition of Viral Replication
- Transplant Rejection

**Central Nervous System**
- Learning and Memory
- Pain Sensitization
- Epilepsy
- Neurodegeneration
- Central BP Control

**Gastrointestinal/Urogenital Tract**
- Apoptosis
- Angiogenesis
- Tumor Cell Growth

**Respiratory Tract**
- Bronchodilatation
- Asthma, ARDS
- NANC nerve-mediated Relaxation

**Cell Proliferation**
- Myocardial Contractility
- Microvascular Permeability

**Vasorelaxation**
- Penile Erection
- Pre-term Labour

**Nitric Oxide**

Robert F. Furchgott
- 1/3 of the prize USA
- SUNY Health Science Center Brooklyn, NY, USA
- b. 1916

Louis J. Ignarro
- 1/3 of the prize USA
- University of California School of Medicine Los Angeles, CA, USA
- b. 1941

Ferid Murad
- 1/3 of the prize USA
- University of Texas Medical School Houston, TX, USA
- b. 1936

The Nobel Prize in Physiology or Medicine 1998

“For their discovery concerning nitric oxide as a signalling molecule in the cardiovascular system”
Ferid Murad Discovers NO activates sGC which is responsible for vessel relaxation

Ferid Murad knew that nitroglycerine caused relaxation of smooth muscle cells. The enzyme, guanylyl cyclase, was activated and increased cyclic GMP, causing relaxation of the muscle. Did nitroglycerin act via release of nitric oxide, NO? He bubbled NO-gas through tissue containing the enzyme; cyclic GMP increased! A new mode of drug action had been discovered!

(Arnold WP, PNAS 1977)

Acetylcholine stimulates the endothelial cells to produce a factor, NO, which penetrates into and activates the muscle cells causing relaxation.
...In investigating this apparent discrepancy, we discovered that the loss of relaxation of ACh in the case of the strip was the result of unintentional rubbing of its intimal surface against foreign surfaces during its preparation. If care was taken to avoid rubbing of the intimal surface during preparation, the tissue, whether ring, transverse strip or helical strip, always exhibited relaxation to ACh, and the possibility was considered that rubbing of the intimal surface had removed endothelial cells.

Furchgott Nature 1980
Robert F Furchgott showed that acetylcholine-induced relaxation of blood vessels was dependent on the endothelium. His "sandwich" experiment set the stage for future scientific development. He used two different pieces of the aorta; one had the endothelial layer intact, in the other it had been removed. (Furchgott, Zawadzki Nature 1980)
Louis Ignarro Discovers EDRF is Identical to NO

Hemoglobin (yellow) exposed to endothelial cells that were stimulated to produce EDRF (green)

Hemoglobin (yellow) directly exposed to NO (green)

The shift of absorption curves is identical, hence EDRF is NO

Ignarro, LJ et al. PNAS 1987
L-arginine \( \rightarrow \) \( \text{Ca/CaM BH}_4 \) \( \text{NADPH} \) \( \underset{O_2}{\longrightarrow} \) \( \text{H}_2\text{O} \) \( N\)-hydroxy-L-arginine \( \rightarrow \) \( \text{Ca/CaM BH}_4 \) \( 1/2 \text{NADPH} \) \( \underset{O_2}{\longrightarrow} \) \( \text{H}_2\text{O} \) \( \rightarrow \) L-citrulline

L-arginine \( \rightarrow \) \( N\)-hydroxy-L-arginine \( \rightarrow \) L-citrulline + NO
Half-Life of NO

- Water (10-1 μM, 37°C, pO₂ 150mmHg, pH 7.4): 1-3 min
- Blood (5*10⁹ erythrocytes): 0.5 - 2 ms
- In presence of HbO₂ (15g/dL): 2 µs
Cardiovascular Effects of NO

- Regulation of vascular tone and blood pressure
- Inhibition of migration and proliferation of smooth muscle cells
- Inhibition of aggregation of thrombocytes and of adhesion of thrombocytes, monocytes and granulocytes at the endothelium
- > anti-arteriosclerotic properties
Nitroglycerine, a 100 year old explosive and heart medicine

In atherosclerosis, plaques reduce blood flow in the arteries. This decreases oxygen supply to the heart muscle causing chest pain (angina pectoris) and sometimes even myocardial infarction. Treatment with nitroglycerine provides NO, dilates the vessels, and increases blood flow. Thanks to the 1998 Nobel Laureates we now understand how nitroglycerine, an important heart medicine, works. It acts as a NO donor, causes dilation of the blood vessels, increases oxygen supply and protects the heart from damage and cell death.
Detection of Nitric Oxide-Related Metabolites

**Ca^{2+}**

NO-Synthases

\[ \text{NOS-mRNA} \sim <10 \text{ kb} \]

\[ \begin{array}{c}
\text{NH}_2 \\
\text{R-N-C-NH}_2 \\
\text{H} \\
\text{L-Arg}
\end{array} \]

\[ \rightarrow \]

\[ \begin{array}{c}
\text{NOH} \\
\text{R-N-C-NH}_2 \\
\text{H} \\
\text{NOH-Arg}
\end{array} \]

\[ \rightarrow \]

\[ \begin{array}{c}
\text{O} \\
\text{R-N-C-NH}_2 \\
\text{H} \\
\text{L-Cit}
\end{array} \]

\[ + \text{NO}^\cdot \rightarrow \text{cGMP} \uparrow \]

**Products of oxidative decomposition**

\[ \begin{array}{c}
\text{NO}_2^- \\
\text{NO}_3^- \\
\text{HPLC}
\end{array} \]

Potential Targets and Nitros(yl)ation Sites

**Heme**

\[ \text{R-SH} \]

\[ \text{Chemiluminescence} \]

\[ \text{R}_2^-\text{NH} \]
Why Study Nitric Oxide?

- Many diseases are associated with either an impaired or enhanced production/availability of nitric oxide.

- *In vivo* characterization of the NO status in health and disease is crucial for selection of the optimal therapeutic intervention.

- This mandates accurate and interference-free determination of NO-related species.

- It also requires intense efforts to understand why, how and when NO is metabolized to other species and which of the latter elicit what biological response *in vivo*. 
The L-Arginine-Nitric Oxide Pathway

**Health**

- **Diet**
  - ↑ **L-Arg**
  - ↓ **L-Arg**
    - ↑ Arginase
    - ↑ ADMA

**Antioxidants**

- Urea Cycle
  - BH4
  - FAD+
  - NADPH
  - Heme iron
  - Ca/Cam
  - FMN
  - O2
  - GSH

**L-Arg**

**NOS**

- Uncoupling
- Reduced Oxygen
- Reduced Cofactor + Substrate
- Oxidative Stress

**Oxidation**

- NO → NO2 → NO3

**Bacterial Reduction**

- NO → NO2 → NO3

**Disease**

- Oxidative Stress
- Mitochondria
- XO
- NADPH oxidase

- ONOO-

- O2·−
Reduced NO availability is a hallmark of a number of cardiovascular disorders.

- Endothelial dysfunction is a defect in processes carried out by cells lining arteries, veins & vessels

- Endothelial NO function loss associated with atherosclerosis (Davignon & Ganz 2004)

- Defects in endothelial NO function associated with all major CV risk factors & has predictive value for disease progression (Schachinger, Britten et al. 2000; Halcox, Schenke et al. 2002; Bugiardini, Manfrini et al. 2004; Lerman and Zeiher 2005).
Age Associated Decline in NO Production: Modifiable by Diet and Lifestyle

![Graph showing the decline in NO production with age for different lifestyles.]

- **average person**
- **poor diet & inactivity**
- **good diet & exercise**

Gerhard et al Hypertension 1996
Celermajer et al JACC 1994
Taddei et al Hypertension 2001
Egashira et al Circulation 1993
How the road from promising scientific breakthrough to real-world remedy has become all but a dead end.

From 1996 to 1999, the U.S. food and Drug Administration approved 157 new drugs. In the comparable period a decade later—that is, from 2006 to 2009—the agency approved 74. Not among them were any cures, or even meaningfully effective treatments, for Alzheimer’s disease, lung or pancreatic cancer, Parkinson’s disease, Huntington’s disease, or a host of other afflictions that destroy lives.

Newsweek, May 15, 2010
Time and Money

Discovery to FDA Approved Drug – 10 years and $800,000,000

“It takes physicians an average of 17 years to adopt widely the findings from new basic research.”

U.S. Senator and thoracic surgeon, Dr. William Frist
2005 Shattuck Lecture published in the New England Journal of Medicine:
Despite almost 30 years of research after its discovery, very few clinical drugs on the market for NO.
- organic nitrates (used for over 150 years for treatment of angina)
- inhalative NO therapy for neonates
- phosphodiesterase inhibitors such as sildenafil (Viagra) which act downstream from NO

L-Arginine supplementation is ineffective and in fact detrimental in some populations

L-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. (JAMA. 2006 Jan 4;295(1):58-64)
CONCLUSIONS: L-arginine, when added to standard postinfarction therapies, does not improve vascular stiffness measurements or ejection fraction and may be associated with higher postinfarction mortality. L-arginine should not be recommended following acute myocardial infarction.

L-Arginine Paradox
Km for NOS is 5µM. Plasma levels of L-arginine ~100µM
How can you get modest improvement in NO output when enzyme theoretically saturated with substrate?

Physiological systems are fraught with redundancy. Where is the redundant NO pathway? Why does such a critical molecule only have a singular complex and complicated pathway to production????
Ways to Enhance NO Availability

Co-factor or Substrate Supplementation

L-Arginine
Ascorbic Acid
Folic Acid
Tetrahydrobiopterin (BH$_4$)

Nitrosothiols
Nitrite/Nitrate
Nitro-fatty acids
Nitroglycerin/organic nitrates
NO hybrid drugs (NO-NSAIDS)

Require Functional NOS System

NOS-Independent Sources of NO
FACTS

#1 killer in America, led by ischemic heart disease

- Over 1 million/year die
- Over 6 million/year hospitalized
- $270 billion in lost productivity & healthcare

Two critical questions:

- What is the physiological consequence of enhanced NO production in ischemia-reperfusion injury?
- Can we trace the phenotype biochemically?
Cardiac Specific Overexpression of eNOS results in Increased Cardiac NO Production and Protects from I/R Injury

Myocardial NOx Levels

- Nitrite
  - Wild-Type
  - CS-eNOS-Tg

- Nitrate
  - Wild-Type
  - CS-eNOS-Tg

Relative O.D.

- Wild-Type
- CS-eNOS-Tg

RXNO [nM]

- Nitroso
- NO-Heme

NO Heme [nM]

- 9
- 11
- 9
- 12

Elrod et al ATVB 2006
Increased Cardiac NO Production Results in Increased Circulating Nitrite and Nitrate

Elrod, PNAS 2008
Local NO Production in the Heart Results in Accumulation of NO Products in the Liver

Elrod, PNAS 2008
Cardiac Derived NO Promotes Distant Organ Protection: Evidence for an Endocrine Role of Nitrite

Elrod, PNAS 2008
Can we intervene naturally through diet to enhance NO bioavailability?
Dietary nitrate is rapidly absorbed into the bloodstream, where it mixes with endogenous nitrate from the NOS/NO pathway. A large portion of nitrate is taken up by the salivary glands, secreted with saliva and reduced to nitrite by symbiotic bacteria in the oral cavity. Salivary-derived nitrite is further reduced to NO and other biologically active nitrogen oxides in the acidic stomach. Remaining nitrite is rapidly absorbed and accumulates in tissues, where it serves to regulate cellular functions via reduction to NO or possibly by direct reactions with protein and lipids. NO and nitrite are ultimately oxidized to nitrate, which again enters the enterosalivary circulation or is excreted in urine.
Mice on Low NOx Diet for 1 Week Reveal Diminished Plasma and Cardiac Nitrite and Nitrate and is Restored with Supplemental Nitrite

Bryan et al PNAS (2007)
Mice on Low NOx Diet for 1 Week Reveal Increase Injury from Heart Attack which is Reversed with Supplemental Nitrite

Bryan et al PNAS (2007)
Circulating and Tissue Nitrite/Nitrate are Affected By Both NOS and Diet and Restored by Supplemenatal Nitrite

Bryan et al, FRBM 2008
Supplemental Nitrite Reverses MIR Injury in eNOS -/- mice

Myocardial Infarct Size

eNOS -/- mice

- Standard Chow
- STD Chow + Nitrite Water 50 mg/L, 7 days

![Graph showing Myocardial Infarct Size](image)

Bryan et al, FRBM 2008
Atherogenesis

Endothelium

Vessel Lumen

Monocyte

T Cells

Neutrophils

LDL

VLDL

LDL

Intima

Modified LDL

Macrophage

Foam cells

Smooth Muscles

Proliferation

Lesion
Hypothesis: Dietary nitrite acts as an alternate source of NO and provides anti-inflammatory properties to inhibit leukocyte adhesion to endothelium.

Nitrite in food controls and stabilizes the oxidative state of lipids in meat products (Shahidi and Hong 1991), thus preventing lipid oxidation. However nitrite is known as a strong oxidant in human physiology by its propensity to form methemoglobinemia. Emerging physiological evidence reveal that nitrite may have anti-inflammatory properties as well akin to NO. The goal is to determine if supplemental nitrite can inhibit leukocyte rolling and adhesion in a mouse model of vascular inflammation.

Group I - Controls: C57 mice fed normal diet for 3 weeks with nitrite free MQ water ad libitum

Group II – HC diet: C57 mice fed high cholesterol diet for 3 week with MQ water ad libitum

Group III – low nitrite: C57 mice fed high fat diet for 3 weeks with 50mg/L nitrite added to drinking water ad libitum

Group IV – high nitrite: C57 mice fed high fat diet for 3 weeks with 150mg/L nitrite

The 50mg/L dose of nitrite has been shown to be cardioprotective in I/R injury and inhibit plaque formation in atherosclerotic mice without producing any measurable changes in methemoglobin.
Nitrite Supplementation Reduces Triglycerides in Hypercholesterolemic Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>MAP (mmHg)</th>
<th>WSR (s⁻¹)</th>
<th>Leukocyte Count (#/mL blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND+water</td>
<td>71±2.8</td>
<td>57.0±27</td>
<td>64±3.3</td>
<td>733±66.8</td>
<td>4830±990.9</td>
</tr>
<tr>
<td>HC+water</td>
<td>116±4.2*</td>
<td>75.3±14.05</td>
<td>67±0.7</td>
<td>594±27.0</td>
<td>6767±1238.2</td>
</tr>
<tr>
<td>HC+50mg/L nitrite</td>
<td>117±8.9*</td>
<td>47.3±5.68**</td>
<td>69±1.2</td>
<td>708±65.8</td>
<td>6590±785.7</td>
</tr>
<tr>
<td>HC+150 mg/L nitrite</td>
<td>123±7.7*</td>
<td>ND</td>
<td>69±2.7</td>
<td>756±44.8</td>
<td>6080±761.3</td>
</tr>
</tbody>
</table>

* P<0.005 vs. ND+H2O
** P<0.01 vs. ND+H2O
ND – not determined

Stokes, et al. AJP 2009
High Fat Diet
High Fat Diet + Nitrite
High Fat Diet Induces Microvascular Inflammation that Is inhibited by nitrite supplementation

Stokes, et al. AJP 2009
High Fat Diet Causes Endothelial Dysfunction that Is reversed by Nitrite Supplementation

Stokes, et al. AJP 2009
High Fat Diet Causes Increase in C-reactive Protein. Nitrite Lowers CRP Induced by High Fat Diet

Stokes, et al. AJP 2009
Nitric Oxide Activity in Breast Milk

Nature’s Most Perfect Food
Nitrite in Breast Milk is Absent in Formula

Hord et al
Breastfeeding Medicine
2010

0.25mg/kg
Ratio of Anions Change With Progression of Milk

Hord et al
Breastfeeding Medicine
2010
Nitrite is Enriched in Colostrum and Declines As Gut Bacteria Colonize

Hord et al
Breastfeeding Medicine 2010
Study on Sub-Population of High Altitude Natives

Is increasing nitrite and nitrate plausible in humans?
Is this a natural phenomenon?
Tibetan Highlanders Have Increased Blood Flow to Compensate for Decreased Ambient Oxygen

Erzurum et al. PNAS (2007)
Increased Blood flow is due to increased NO production and accumulation of nitrite and nitrate

Erzurum et al. PNAS (2007)
Inherent Inefficiencies in Nitrite Reduction

Feelisch et al JBC 2008
What about plants and botanicals as a source of NO?

Rich source of nitrate (beet root, artichokes, etc)

Contain the active nitrate and nitrite reductase, antioxidants and polyphenols that can generate nitrite and NO
Traditional Chinese Medicine (TCM) has been used as a main stream of medical care throughout Asia for centuries. But it is still considered an alternative medical system in the western world. Mostly because of a lack of understanding of their mechanisms of action and/or the active compounds.

Many cardiovascular diseases are characterized by a NO insufficiency. There are a number of published reports on the association of TCM and NO-related effects in cardiovascular field. However, their mechanism of action is far from clear.
NO is now well established as a key signaling molecule in cardiovascular system. There are two ways to form NO: oxidation of L-arginine and reduction of nitrite. All nitrogen obtained by animals can be traced back to the eating of plants at some stage of the food chain.
Traditional Chinese Medicines
<table>
<thead>
<tr>
<th>Name of Chinese herb</th>
<th>Chinese</th>
<th>Latin</th>
<th>English</th>
<th>Primary indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>DanShen</td>
<td><em>Radix salviae</em> miltorrhizae</td>
<td>Danshen root</td>
<td>CAD, acute MI, hyperlipidemia</td>
</tr>
<tr>
<td>GL</td>
<td>GuaLou</td>
<td><em>Fructus</em> trichosanthis</td>
<td>Snakegourd fruit</td>
<td>CAD, acute MI, hyperlipidemia</td>
</tr>
<tr>
<td>XB</td>
<td>XieBai</td>
<td><em>Bulbus allii</em> macrostemi</td>
<td>Longstamen onion bulb</td>
<td>CAD, acute MI, hyperlipidemia</td>
</tr>
<tr>
<td>SC</td>
<td>SanChi</td>
<td><em>Radix notoginseng</em></td>
<td>Sanchi</td>
<td>CAD</td>
</tr>
<tr>
<td>RX</td>
<td>RuXiang</td>
<td><em>Resina olibani</em></td>
<td>Frankincense</td>
<td>Hypertension</td>
</tr>
<tr>
<td>CS</td>
<td>ChiShao</td>
<td><em>Radix paeonia rubra</em></td>
<td>Red Peony Root</td>
<td>CAD</td>
</tr>
<tr>
<td>HS</td>
<td>HongSheng</td>
<td><em>Radix ginseng</em></td>
<td>Ginseng</td>
<td>Heart failure, CAD, Increase other herb's function for CAD or brain disease</td>
</tr>
<tr>
<td>SBP</td>
<td>BingPiang</td>
<td><em>Borneolum syntheticum</em></td>
<td>Borneol</td>
<td>Increase other herb's function for CAD or brain disease</td>
</tr>
<tr>
<td>NBP</td>
<td>TianRanBingPiang</td>
<td><em>Cinnamomum</em></td>
<td>Borneol</td>
<td>Increase other herb's function for CAD or brain disease</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease; MI, myocardial infarction.

Tang et al FRBM 2009
Kinetics of NO Formation after Rational Combination of Herbs
Herbs Generate NO and Relax Blood Vessels and Reverse Endothelial Dysfunction

![Graphs showing relaxation and force changes with various treatments.](image-url)
Should *we* be concerned about our nitrite status and dietary habits?
Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans.

RISK FACTORS
Hyperlipidemia
Arterial hypertension
Smoking
Age (45 males: 55 females)

Kleinbongard et al FRBM 2006
Daily NOx Intake Varies Depending on Diet

<table>
<thead>
<tr>
<th></th>
<th>Western Menu</th>
<th>Mediterranean Menu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Bagel w/ Cream Cheese</td>
<td>Toast w/ Jam and Butter</td>
</tr>
<tr>
<td></td>
<td>Black Coffee (12 oz)</td>
<td>Cappuccino (Espresso+Milk)</td>
</tr>
<tr>
<td>AM Snack</td>
<td>Carrot Nut Muffin</td>
<td>Yogurt (Strawberry)</td>
</tr>
<tr>
<td></td>
<td>Diet Coke (12 oz)</td>
<td>Carrot Juice (12 oz)</td>
</tr>
<tr>
<td>Lunch</td>
<td>Big Mac</td>
<td>Mediterranean Wrap</td>
</tr>
<tr>
<td></td>
<td>Large French Fries</td>
<td>Garden Vegetable Soup</td>
</tr>
<tr>
<td></td>
<td>Diet Coke (12 oz)</td>
<td>Mineral Water (12 oz)</td>
</tr>
<tr>
<td>PM Snack</td>
<td>Snickers</td>
<td>Trail Mix</td>
</tr>
<tr>
<td></td>
<td>Black Coffee (12 oz)</td>
<td>Orange Juice (12 oz)</td>
</tr>
<tr>
<td>Dinner</td>
<td>Cheese Pizza (4 slices)</td>
<td>Salmon (Smoked)</td>
</tr>
<tr>
<td></td>
<td>Diet Coke (12 oz)</td>
<td>Red Wine (12 oz)</td>
</tr>
</tbody>
</table>

H. Garg, Master's Thesis
NOx and Antioxidant Capacity of Common Beverages

![Chart showing NOx and Polyphenol GAE for various beverages including grape juice, apple juice, pomegranate, V8 vegetable juice, carrot juice, reisling, shiraz, merlot, green tea, red bull, rx stress, and perrier.](image-url)
New Paradigm: Two Pathways For NO Production

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Increased with</th>
<th>Affected by age?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake from salivary glands</td>
<td>Healthy diet having NO potential</td>
<td>No</td>
</tr>
<tr>
<td>Produced by endothelium</td>
<td>Regular exercise</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Healthy Circulation &amp; Blood Pressure Levels</strong></td>
<td>Endothelial production declines to 50% of what one needs by age 40. Even exercise cannot restore it all.</td>
<td></td>
</tr>
</tbody>
</table>
Manipulating the System Through Natural Product Chemistry

Beet, artichoke, etc

Oxidation: NO₃⁻ → NO₂⁻ → NO
Reduction: NO → NO₂⁻ → NO₃⁻

Oxyheme proteins
Oxygen, ceruloplasmin

Probiotics
Herbs with reductive capacity

L-arginine
Rich source of nitrate (beet root, artichokes, etc)

Contain the active nitrate and nitrite reductase, antioxidants and polyphenols that can generate nitrite and NO
# The Ultimate Nitric Oxide Index

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Nitric Oxide Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale</td>
<td>6825</td>
</tr>
<tr>
<td>Swiss Chard</td>
<td>2055</td>
</tr>
<tr>
<td>Arugula</td>
<td>1452</td>
</tr>
<tr>
<td>Spinach</td>
<td>1123</td>
</tr>
<tr>
<td>Chicory</td>
<td>938</td>
</tr>
<tr>
<td>Wild Radish</td>
<td>814</td>
</tr>
<tr>
<td>Bok Choy</td>
<td>775</td>
</tr>
<tr>
<td>Collard Greens</td>
<td>697</td>
</tr>
<tr>
<td>Beets</td>
<td>632</td>
</tr>
<tr>
<td>Chinese Cabbage</td>
<td>499</td>
</tr>
<tr>
<td>Lettuce</td>
<td>388</td>
</tr>
<tr>
<td>Cabbage</td>
<td>312</td>
</tr>
<tr>
<td>Mustard greens</td>
<td>226</td>
</tr>
<tr>
<td>Cauliflower, Raw</td>
<td>167</td>
</tr>
<tr>
<td>Parsley</td>
<td>150</td>
</tr>
<tr>
<td>Kohlrabi</td>
<td>136</td>
</tr>
<tr>
<td>Carrot</td>
<td>127</td>
</tr>
<tr>
<td>Broccoli</td>
<td>122</td>
</tr>
</tbody>
</table>
What is your Nitric Oxide level?

Introducing the first and only saliva test to measure your body’s Nitric Oxide level. Nitric Oxide is a critical molecule naturally produced in your body that helps maintain optimal circulation. Neo40™ Daily increases your Nitric Oxide levels which affects important cardiovascular risk factors. Learn more at www.neogenis.com.

**Neogenis™ Test Strips**  1 Sample Nitric Oxide Test

(μmol/L) <20 Depleted  25-100 Low  100-300 Normal  >300 Neo Optimal
The First and Only Saliva Test for NO

Simple to use
Instant, easy-to-read results
CONCLUSIONS

- Current paradigm for NO production through L-arginine is complex and inefficient, especially in the aging population.
- Strategies to restore NO production/homeostasis will have a profound impact on public health.
- Utilizing activity from natural products can overcome our inherent inefficiencies and provide an essential source of bioactive NO.
- Rational design of NO rich herbs may provide a natural, cost effective and over the counter product for conditions related to NO insufficiency to improve health, performance and prevent disease.
- Recognizing foods rich in NO potential may provide information into the health benefits of certain foods/diets (Nitric Oxide Index).
Acknowledgements

The University of Texas Health Sciences Center at Houston
Harsha Garg
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Book Highlights:
Restoring nitric oxide production in the body thereby combating:
• High blood pressure
• Heart attack
• Stroke
• Diabetes
• Arthritis
• Kidney disease
• Memory loss
• Osteoporosis
Food, Nutrition and the Nitric Oxide Pathway: Biochemistry and Bioactivity

Edited by: Nathan S. Bryan, Ph.D.
University of Texas, Houston

This book provides a scientific analysis of the effects of foods and nutrients on the NO pathway in humans. Contributors to the book clarify novel chemical and biochemical connections between dietary intake and nitric oxide, particularly in cases of NO deficiency. In this context, the book addresses how specific foods can restore nitric oxide production and bioactivity—without medical interventions. A variety of evidential data is presented showing how NO-rich dietary elements are implicated in disease prevention and modulation. The book offers new knowledge for food technologists, food manufacturers, nutrition researchers, and healthcare practitioners.

From the Foreword by Louis J. Ignarro, Nobel Laureate in Physiology/Medicine

"The body of work contained in this volume, linking NO to food and nutrition, may have revolutionary implications in terms of developing strategies to combat heart disease and many other contemporary diseases associated with NO deficiency. Proving that a natural and inexpensive regimen of foods rich in nitric oxide activity does restore NO homeostasis can have profound effects on human health...The research presented in this text provides an important expansion of NO work...and Dr. Nathan Bryan, the editor...is to be congratulated for...communicating new knowledge and assembling the world's experts in their fields."

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