The Role of Nitric Oxide in Regenerative Medicine

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Structure of Presentation

• Brief Overview of Nitric Oxide (NO)
• NO effects on stem cells
• Safe and Effective Strategies to replete NO

Disclosure:
N.S. Bryan is the founder and Chief Science Officer for NeoGenis Labs, Inc.
Stock owner and advisor for SAJE Pharma
Consultant Bayer
“Research is to see what everybody else has seen, and to think what nobody else has thought”

Albert Szent-Gyorgyi
1937 Nobel Prize for Medicine
Stem Cell Theory of Aging

Aging is the result of the inability of various types of stem cells to continue to replenish the tissues of an organism with functional differentiated cells capable of maintaining that tissue’s original function.

The number of stem cells in young people is very much higher than older people and this cause a better and more efficient replacement mechanism in the young contrary to the old.

Aging is not a matter of the increase of damage, but a matter of failure to replace it due to decreased number of stem cells. They decrease in number and tend to loose the ability to differentiate.
“A man is as old as his arteries.”

~ Thomas Sydenham MD, English physician, 1624-1689
The Age of Your Arteries

In your 20s, your arteries are generally healthy and clear of obvious disease, though research shows the disease and aging process has already begun. Harm from tobacco smoke, cholesterol, and sedentary lifestyle can be accumulating.

In your 30s and 40s, accumulation of fatty deposits called plaque typically begins to accelerate in men; women are generally protected in their major arteries until after menopause though disease may begin in smaller vessels. Cholesterol, tobacco smoke, high blood pressure, and high blood sugar all cause persistent injury to the inner lining of the artery.

In your 50s, the assault on the inner lining of the arteries continues while the vessel wall becomes stiffer and more fibrous. High blood pressure creates damage apart from plaque build-up that makes arteries less elastic or compliant, increasing the workload on the heart. Injured tissue becomes scarred and calcified. For women, for whom disease tends to develop 10 to 15 years later than for men, the accumulation of plaque begins to accelerate.

In your 60s and beyond, the aging process, partly reflecting the arteries withstanding more than 100,000 heart beats a day, contributes to the attack on the lining of the arteries. Meantime, left, if not effectively checked, plaques can rupture or erode, leading to blood clots that can cause heart attacks, while an overworked or scarred heart increases the risk of heart failure.

Artery damage can be prevented or significantly mitigated by regular exercise, a healthy diet, refraining from smoking and adherence to heart medicines.
Inflammatory Biomarkers Diagnostic For Different Stages of Vascular Disease
Loss of NO is Associated with Atherosclerosis

As we age, we lose 85% of our ability to make Nitric Oxide.

In your 60's and beyond, the aging process partly reflecting the arteries withstanding more than 100,000 heart beats a day, contributes to the attack on the...
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 is required for red blood cell delivery of oxygen from the lungs to tissue.

Zhang et al Proc Natl Acad Sci U S A. 2015 May 19;112(20):6425-30

Prof. Stamler says "blood flow to tissues is actually more important in most circumstances than how much oxygen is carried by hemoglobin. The respiratory cycle is actually a three-gas system."
Nitric Oxide Plays a Key Role in the Regulation of Numerous Vital Biological Functions

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

**Cell Proliferation**
- Apoptosis
- Angiogenesis
- Tumor Cell Growth

**Peripheral Nervous System**
- Vasorelaxation
- Blood Cell Regulation
- Myocardial Contractility
- Microvascular Permeability

**Respiratory Tract**
- Bronchodilatation
- Asthma, ARDS

**Gastrointestinal/Urogenital Tract**
- Penile Erection
- Pre-term Labour

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

**Regeneration**
- Mobilization of resident stem cells
- Targeted differentiation

**Nitric Oxide**
- Contributes to numerous vital biological functions through various mechanisms.
Shear Stress

ACH

NOS

L-arginine

L-citrulline

NO

guanylyl cyclase (inactive)
guanylyl cyclase (active)
cGMP
GTP
Relaxation

PD5 inhibitors
Erectile Function in Men
Erectile Function in Women

- Glans clitoris
- Corpus cavernosum
- Crus clitoris
- Urethral opening
- Bulb of vestibule
- Vaginal opening
Nitric Oxide is the requisite signal for stem cell mobilization and differentiation into target cell types.

The bioavailability of NO in patients may predict stem cell therapy success or failure.

Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells

Nitric oxide-cyclic GMP signaling in stem cell differentiation.
Role of nitric oxide signaling components in differentiation of embryonic stem cells into myocardial cells.

Mujoo K, Sharin VG, Bryan NS, Krumenacker JS, Sloan C, Parveen S, Nikonoff LE, Kots AY, Murad F.

Proc Natl Acad Sci U S A. 2008 Dec 2;105(48):18924-9
Humans lose ability to produce NO with aging

Gerhard et al Hypertension 1996
Celermajer et al JACC 1994
Taddei et al Hypertension 2001
Egashira et al Circulation 1993
The L-Arginine-Nitric Oxide Pathway

**Health**
- L-Arg
- Diet
- Arginase
- ADMA

**Disease**
- ↓ L-Arg
- ↑ L-Arg
- ↑ Arginase
- ↑ ADMA
- ↓ Transport

Antioxidants
- NADH
- NAD
- GSSG
- GSNO
- GSNOR

NO
- Oxidation
- Bacterial Reduction
- NO₂⁻
- NO₃⁻

Mitochondria
- XO
- NADPH oxidase

O₂⁻

**Urea Cycle**
- BH4
- FAD+
- NADPH
- Heme iron

**L-Arg**
- Uncoupling
- Reduced Oxygen
- Reduced Cofactor + Substrate
- Oxidative Stress

**NOS**
- Ca/Cam
- FMN
- GSH
- O₂
Ways to Enhance NO Availability

Co-factor or Substrate Supplementation

L-Arginine
Ascorbic Acid
Folic Acid
Tetrahydrobiopterin (BH₄)

Require Functional NOS System

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

NOS-Independent Sources of NO
NOS Utilizes Intracellular L-Arginine from L-Citrulline for NO Production

Erez et al Nat Med 2011
Reduced NO availability is a hallmark of a number of cardiovascular disorders.

- **Endothelial dysfunction** is a physiological dysfunction of normal biochemical processes carried out by the endothelium, the cells that line the inner surface of all blood vessels including arteries and veins (as well as the innermost lining of the heart and lymphatics).

- Loss of endothelial NO function is associated with several cardiovascular disorders, including atherosclerosis, which is due either to decreased production or to increased degradation of NO (Davignon and Ganz 2004).

- Experimental and clinical studies provide evidence that defects of endothelial NO function, referred to as endothelial dysfunction, is not only associated with all major cardiovascular risk factors, such as hyperlipidemia, diabetes, hypertension, smoking and severity of atherosclerosis, but also has a profound predictive value for the future atherosclerotic disease progression (Schachinger, Britten et al. 2000; Halcox, Schenke et al. 2002; Bugiardini, Manfrini et al. 2004; Lerman and Zeiher 2005).

- The dysfunctional eNOS/NO pathway is considered as an early marker or a common mechanism for various cardiovascular disorders. Over the last two decades, it has become evident that decreased bioavailability of endothelial NO, produced from endothelial NO synthase (eNOS), plays a crucial role in the development and progression of atherosclerosis.
Cardiovascular disease (CVD) is the number one killer of both men and women in the U.S. Close to 1 million people die each year and more than 6 million are hospitalized due to CVD. The cost of CVD, in terms of health care and lost productivity, is over $270 billion and increasing as the baby boom population ages.

Ischemic heart disease, including myocardial infarction, remains the leading cause of morbidity and mortality in all industrialized nations.

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

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

Plasma NOx Levels

Nitrite [μM]

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>CS-eNOS-Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite</td>
<td>0.25</td>
<td>1.3</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.5</td>
<td>35.5</td>
</tr>
</tbody>
</table>

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
Atherogenesis

Monocyte → T Cells → Neutrophils

Vessel Lumen

LDL → Endothelium

Intima

LDL → Modified LDL

Modified LDL → Macrophage

Foam cells

Smooth Muscles

Proliferation
Atherogenic Diet
Atherogenic Diet + NO
Genetic Diversity

23,000 genes  3,000,000 genes
Bacteria

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2\text{OR} \rightarrow \text{N}_2 \rightarrow \text{NH}_3 \]

\[ 2e^- - 1e^- - 1e^- - 1e^- - 3e^- \]

**Ideal Community:**
- Higher Nitrate reduction efficacy
- No NiR enzyme; Nitrite can accumulate, enrich saliva to form NO when swallowed.
One week Chlorhexidine treatment caused 26 mmHg increase in systemic blood pressure. This was associated with change in bacterial communities that disrupted nitrate reduction and NO production.
Manipulating the NO System Through Diet and Nutrition

Oxidation

Beet, kale, etc

NO

Nitrate (NO₃⁻)

Facultative anaerobes 5-8%
Spiegelhalder 1976
Lundberg 2004

Oxyheme proteins

Nitrite (NO₂⁻)

Mammalian enzymes ~ 0.01%
Bryan Nat Chem Biol. 2005
Feelisch JBC 2008

Nitrate oxidases

Nitric oxide (NO)

L-arginine 50-90%

Reduction

Nitric oxide synthase

Nitric oxide synthase

Nitric oxide synthase
Development of Safe and Effective NO-based Technology

1. Provide an exogenous source of NO
2. Promote endogenous endothelial production of NO
3. Account for differences in non-responders to nitrate therapy and L-arginine
4. Plant-based natural product chemistry (clean and testable)
5. Clear product experience
6. Strong and sound basic science behind technology
7. Clinically proven in peer-reviewed, placebo-controlled trials
8. Intellectual Property for protection
Lowering blood pressure by 5 mmHg reduces risk of stroke by 34% and Ischemic heart disease by 21%


Lowering blood pressure to prevent myocardial infarction and stroke: a new preventive strategy.

Law M, Wald N, Morris J.
Nitric Oxide Clinical Trial Results

Strong & sustained Nitric Oxide activity

Zand et al Nutrition Research 2011
Nitric Oxide Clinical Trial Results

Significant reduction in patients with elevated triglycerides

Zand et al. Nutrition Research 2011
Hypertension Study Protocol

Active lozenge

Blood pressure | Ultrasound | BP | Pulse wave | BP | Endopat

0 | 10 min | 20 min | 30 min | 60 min | 4 hours

Placebo lozenge

Blood pressure | Ultrasound | BP | Pulse wave | BP | Endopat

0 | 10 min | 20 min | 30 min | 60 min | 4 hours

3 week washout
Hypertension Trial – Vanderbilt Univ

Houston, Hays JCH 2014
Representative Ultrasound Before and 10 minutes after NO

13% increase in vessel diameter causes a 34% increase in blood flow
NO Dilates Carotid Artery within 90 Seconds

Houston, Hays JCH 2014
Average Changes in 10 subjects After 30 minutes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AVG % Chg 7min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systole</td>
<td>-8.29%</td>
</tr>
<tr>
<td>Diastole</td>
<td>-8.15%</td>
</tr>
<tr>
<td>MAP</td>
<td>-7.91%</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>-1.09%</td>
</tr>
<tr>
<td>Central Systolic Pressure</td>
<td>-7.25%</td>
</tr>
<tr>
<td>Central Diastolic Pressure</td>
<td>-8.84%</td>
</tr>
<tr>
<td>Cardiac Output</td>
<td>5.31%</td>
</tr>
<tr>
<td>Total Vascular Resistance</td>
<td>-12.72%</td>
</tr>
<tr>
<td>Augmentation Pressure</td>
<td>-46.76%</td>
</tr>
<tr>
<td>Aug. Index@75 [90% C]</td>
<td>-60.37%</td>
</tr>
<tr>
<td>Pulse Wave Velocity [90% C]</td>
<td>-1.96%</td>
</tr>
</tbody>
</table>
Thermographic Images

Before NO

10 min After NO

49 yof chronic smoker with Raynauds
Pre-hypertension trial
Cedars Sinai Medical Center
PI: Ernst Schwarz MD, PhD
Pre-Hypertension Trial – Cedars Sinai School of Medicine

Figure 1

Blood Pressure

<table>
<thead>
<tr>
<th>mm Hg</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow Up</td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Systole

Diastole

Biswa et al JCPT 2015
## 30 Day Placebo controlled Trial

<table>
<thead>
<tr>
<th></th>
<th><strong>Group 1 (mean ± SD)</strong></th>
<th><strong>Group 2 (mean ± SD)</strong></th>
<th><strong>Baseline: NO vs placebo (p-value)</strong></th>
<th><strong>Follow-Up: NO vs placebo (p-value)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Follow-Up</strong></td>
<td><strong>Δ</strong></td>
<td><strong>Baseline</strong></td>
<td><strong>Follow-Up</strong></td>
</tr>
<tr>
<td><strong>BP (mmHg, systole; diastole)</strong></td>
<td>138±12; 84±5</td>
<td>126±12; 78±4</td>
<td>12; 6 reduction (p&lt;0.001)</td>
<td>138±21; 80±8</td>
</tr>
<tr>
<td><strong>Heart Rate (bpm)</strong></td>
<td>75±9</td>
<td>76±8</td>
<td>N.S.</td>
<td>80±10</td>
</tr>
<tr>
<td><strong>6-Minute Walk Test (meters)</strong></td>
<td>596±214</td>
<td>650±197</td>
<td>55 improvement (p&lt;0.005)</td>
<td>590±8</td>
</tr>
<tr>
<td><strong>SF-36v2 (PCS; MCS)</strong></td>
<td>48±10; 40±9</td>
<td>50±8; 45±7</td>
<td>p&lt;0.05</td>
<td>43±10; 37±9</td>
</tr>
</tbody>
</table>

Biswa et al JCPT 2015
Prehypertension Trial Conclusions

- In 30 days, NO lozenge reduces blood pressure in prehypertensive patients by 12 mmHg systolic and 6 mmHg diastolic.
  - By comparison, the DASH diet reduces BP by 5.5 mmHg systolic and 3 mmHg diastolic*

- NO also improves distance traveled in a 6-minute walk test and improves both mental and physical quality of life.

NO Leads to Plaque Regression

Edwin Lee MD – case report
NO Supplementation Rescues Inborn Error in Metabolism
The Urea Cycle converts ammonia to urea for excretion.
ASL deficiency is an Inborn error in metabolism

• Hyperammonemia
• In addition:
  – Progressive liver dysfunction and cirrhosis
  – Coagulopathy
  – Neurological dysfunction independent of recurrent hyperammonemia
  – Hypertension
  – Renal dysfunction
• More than hyperammonemia?
Echocardiogram measurements before and after initiation of NO supplementation.

<table>
<thead>
<tr>
<th>Left ventricle (LV) parameters</th>
<th>Before NO supplementation (z-score)</th>
<th>After NO supplementation (z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diastolic septal thickness</td>
<td>2.26</td>
<td>1.33</td>
</tr>
<tr>
<td>LV diastolic dimension</td>
<td>-2.10</td>
<td>-0.36</td>
</tr>
<tr>
<td>LV diastolic wall thickness</td>
<td>3.59</td>
<td>2.24</td>
</tr>
<tr>
<td>LV systolic septal thickness</td>
<td>4.08</td>
<td>1.94</td>
</tr>
<tr>
<td>LV systolic dimension</td>
<td>-2.08</td>
<td>-0.67</td>
</tr>
<tr>
<td>LV systolic wall thickness</td>
<td>3.01</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Echocardiogram measurements of LV dimensions taken before and 5 months after initiation of NO supplementation. All parameters demonstrate normalization. 

Also increased the number of circulating endothelial progenitor cells
How to recognize superior technology?

1. Provides an exogenous source of NO
2. Promotes endothelial production of NO
3. Provides the body the nutrients necessary for repairing endothelium
4. Plant based natural product chemistry (all-natural)
5. No tolerance development
6. Drug-like effects without side-effects
7. Clear product experience
8. Patented technology (5 issued patents, 6 pending worldwide)
Who Needs NO?

Anyone who is getting stem cell therapy
Anyone who is aging
Anyone over the age of 40
People with circulation issues
Diabetics
People with low energy
People with sexual dysfunction or who desire improved performance in bedroom
Anyone on antacids
Anyone interested in disease prevention
CONCLUSIONS

- Nitric oxide controls and regulates stem cell function

- There is an age-related decline in NO production that asserts its effect on all stem cell mobilization and differentiation

- Restoring NO production can lead to better stem cell function as well as success of stem cell injections and therapies

- Strategies to restore NO production/homeostasis will have a profound impact on public health and on the aging process

- Any anti-aging strategy should include NO as a first line of defense.
The Nitric Oxide Solution:

How to boost the body's miracle molecule to prevent and reverse chronic disease.

by Nathan S. Bryan, PhD and Janet Zand, OMD

with Eli Gottlieb

Book Highlights:
Restoring nitric oxide production in the body thereby combating:

- High blood pressure
- Heart attack
- Stroke
- Diabetes
- Arthritis
- Kidney disease
- Memory loss
- Osteoporosis
Nitrite and Nitrates in Human Health and Disease delivers a comprehensive review of nitrite and nitrates in biology, from basic biochemistry to the complex physiology and metabolism of these two naturally occurring molecules in the human body. Well-organized and well-referenced chapters cover the rich history of nitrite and nitrates, sources of exposure, and the physiological effects when consumed through foods containing nitrite and nitrates. The chapters are written by leading experts, all of whom share their research and perspectives in order to help define the context for benefits vs. any potential risks associated with nitrate and nitrates, whether through dietary ingestion or therapeutic dosing. This diverse collection of authors includes vascular biologists, physiologists, physicians, epidemiologists, cancer biologists, registered dieticians, chemists, and public health experts from five countries in both academia and government. Nitrite and Nitrates in Human Health and Disease provides a balanced view of nitric oxide biochemistry, and nitrates and nitrite biochemistry in physiology and in the food sciences.
All truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident.

—

Arthur Schopenhauer,
German philosopher (1788 – 1860)
At Neogenis, we want to help you create the most successful doctor-patient experience possible with regard to the use of our products for NO restoration.

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