The Role of Nitric Oxide in Regenerative Medicine

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Dec 14-17, 2017 Las Vegas, NV

Learning Objectives

- Identify the nitric oxide production pathways in humans
- Describe evidence on how NO affects stem cell function
- Define evidenced based nutritional strategies to restore and replete NO production in humans

“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

Well vascularized tissues are more resistant to infections and capable of localizing/containing offending agents. By contrast, poorly vascularized tissues are relatively inefficient in responding to inflammatory stimuli.

Robbins Pathology book page 58

What's the Problem?

Heart Disease – 1 in every 4 death
610,000 die of heart disease every year
Every 42 seconds someone has a heart attack
Each minute someone dies from a heart disease related event

The amount of people dying from cardiovascular disease is equivalent to 4 jumbo jets crashing and killing everyone on board every single hour of every single day each year

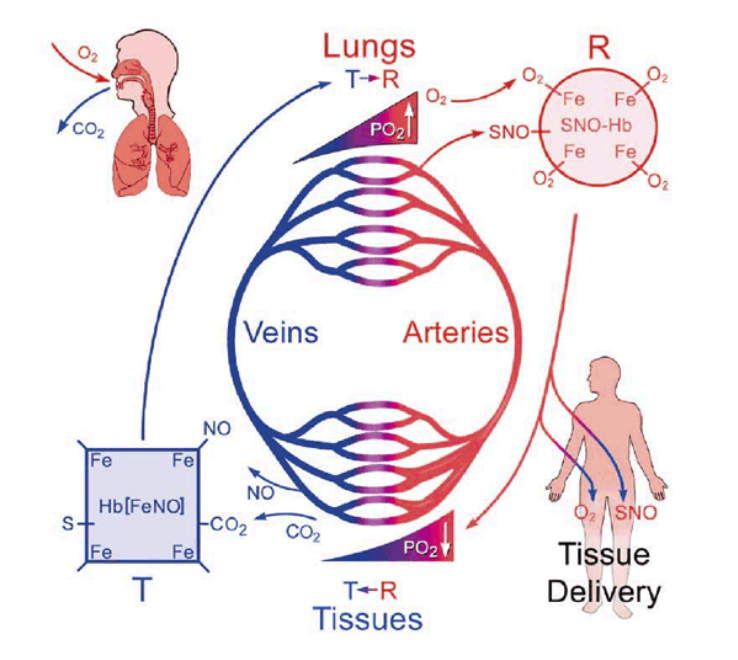
What Causes Heart Disease?

Cholesterol? No chance
Smoking? Yes
Poor diet? Yes
Poor oral hygiene/oral infections? Yes
Sedentary lifestyle? Yes
Inflammation? Yes
Drug therapy? Yes

What is the common denominator?

Nitric Oxide

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

Gastrointestinal/ Urogen

Peni
Pre-

Respiratory

Bronchodil
Asthma, AF

Cell Pro

Apopt
Angiogenesis
Tumor Cell Growth

ular System

tion
Regulation
Contractility
lar Permeability

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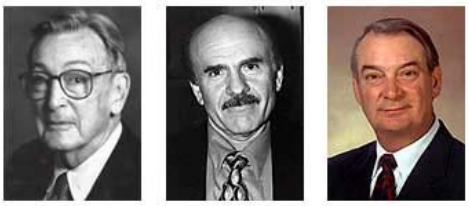
Central Nervous System

Learning and Memory
Pain Sensitization
Epilepsy
Neurodegeneration
Central BP Control

Regeneration

Mobilization of resident stem cells
Targeted differentiation



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 at Houston Houston, TX, USA
b. 1936

The Nobel Prize in Physiology or Medicine 1998

"For their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system"

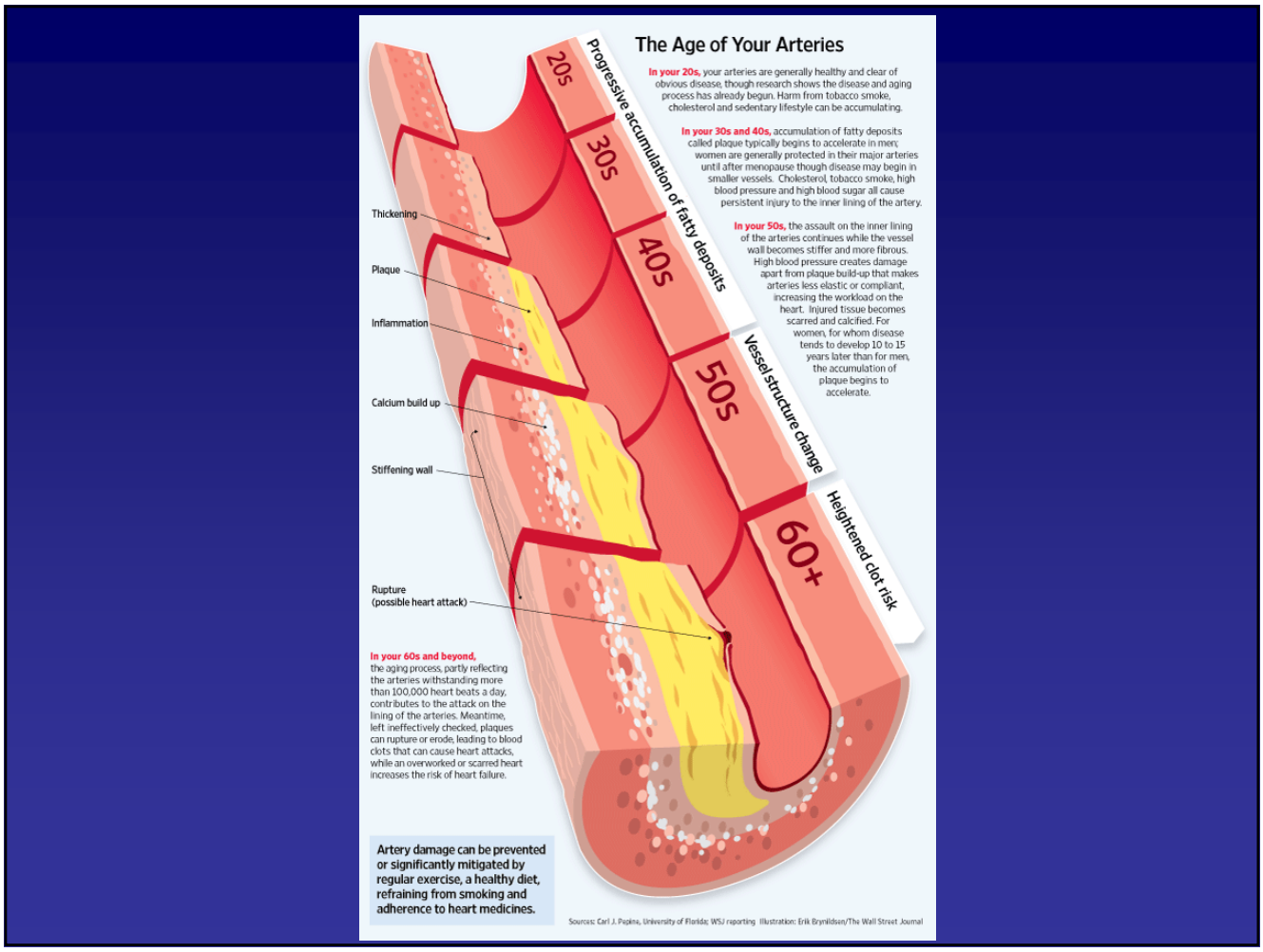
Source: Nobel e-Museum
www.nobel.se

Who Needs Nitric Oxide?

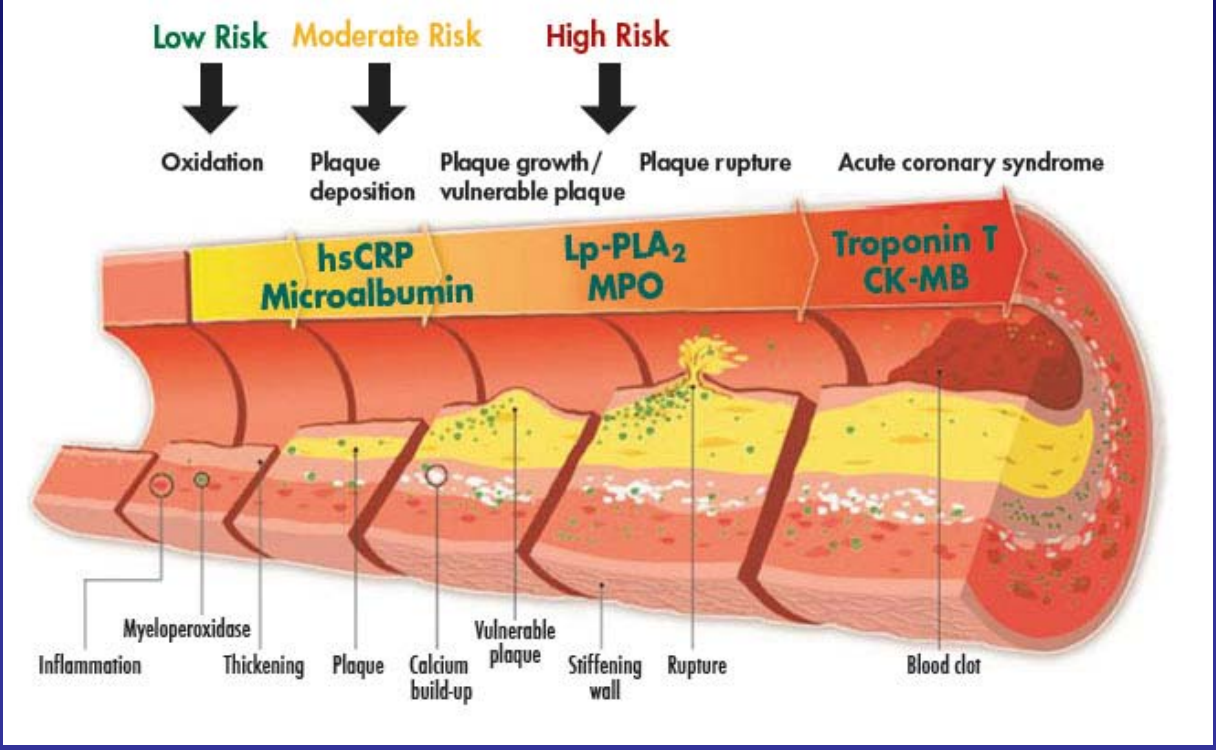
Anyone who is aging
Anyone over the age of 40
People with circulation issues
Diabetics
People with low energy
People with sexual dysfunction or who desire improved performance in bedroom
Anyone on antacids
Anyone interested in disease prevention
Anyone getting stem cell therapy

"A man is as old as his arteries."

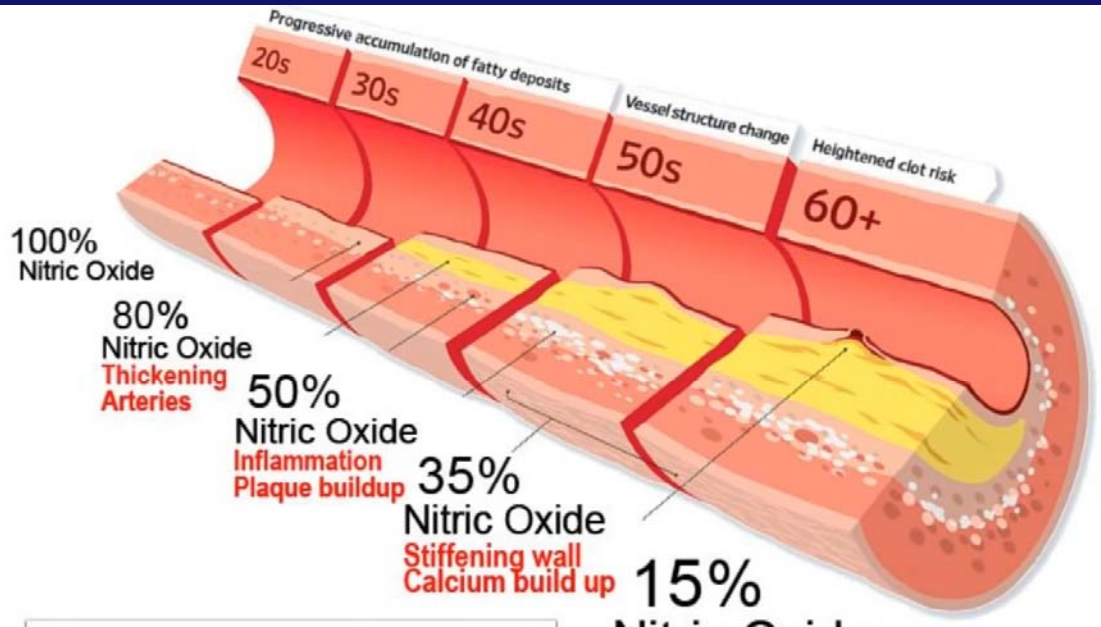
~ Thomas Sydenham, English physician, 1624-1689


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.
20s
30s
40s
50s
60+
Progressive accumulation of fatty deposits
Vessel structure change
Heightened clot risk
Thickening
Plaque
Inflammation
Calcium build up
Stiffening wall
Rupture (possible heart attack)
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 ineffectively 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.
Sources: Carl J. Pepine, University of Florida; WSJ reporting Illustration: Erik Brynildsen/The Wall Street Journal

Inflammatory Biomarkers Diagnostic For Different Stages of Vascular Disease



Loss of NO is Associated with Atherosclerosis



What causes aging and is involved In regenerative medicine?

Three main hypotheses:

1. Telomere shortening
2. Mitochondrial dysfunction
3. Loss of stem cell function and repair

Unified Theory of Aging

Nitric Oxide controls and regulates

1. Telomerase activity
2. Mitochondrial biogenesis and function
3. Mobilization of resident stem cells

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
Aicher et al Nature Medicine 9, 1370 - 1376 (2003)

Nitric oxide-cyclic GMP signaling in stem cell differentiation.
Free Radic Biol Med. 2011 Dec 15;51(12):2150-7

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.

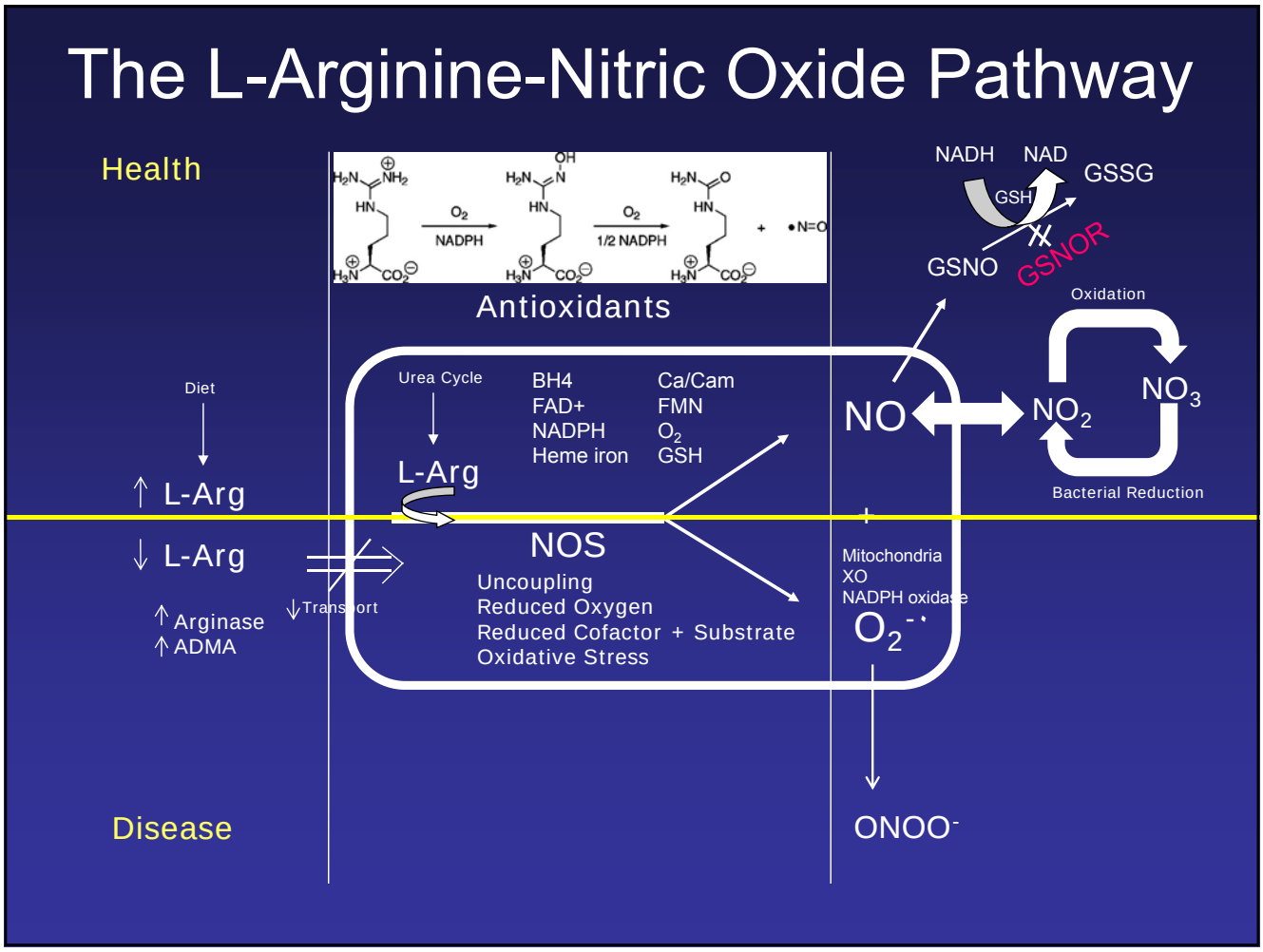
Proc Natl Acad Sci U S A. 2008 Dec 2;105(48):18924-9

How do we control and regulate NO production?

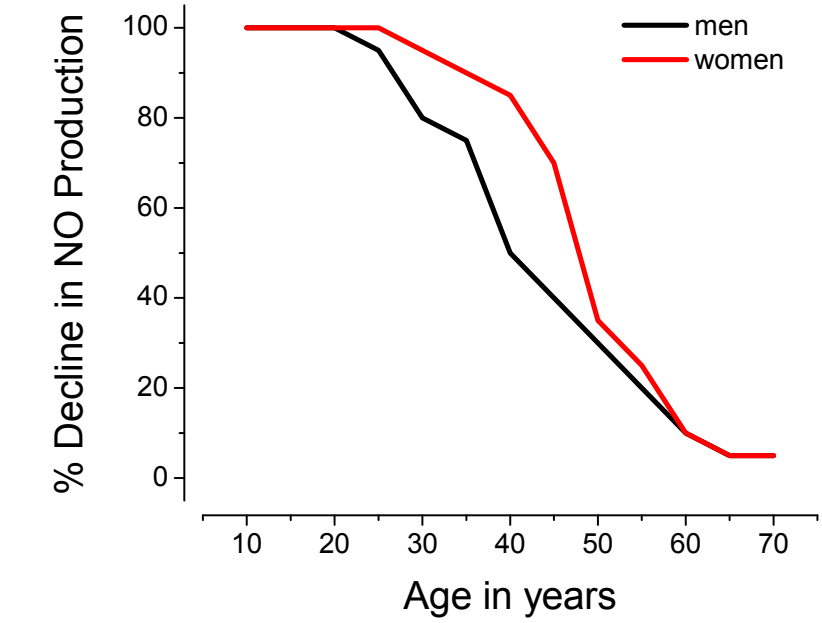
Two NO Production Pathways

1. Oxidation of L-arginine (NOS)
2. Nitrate-Nitrite-Nitric Oxide

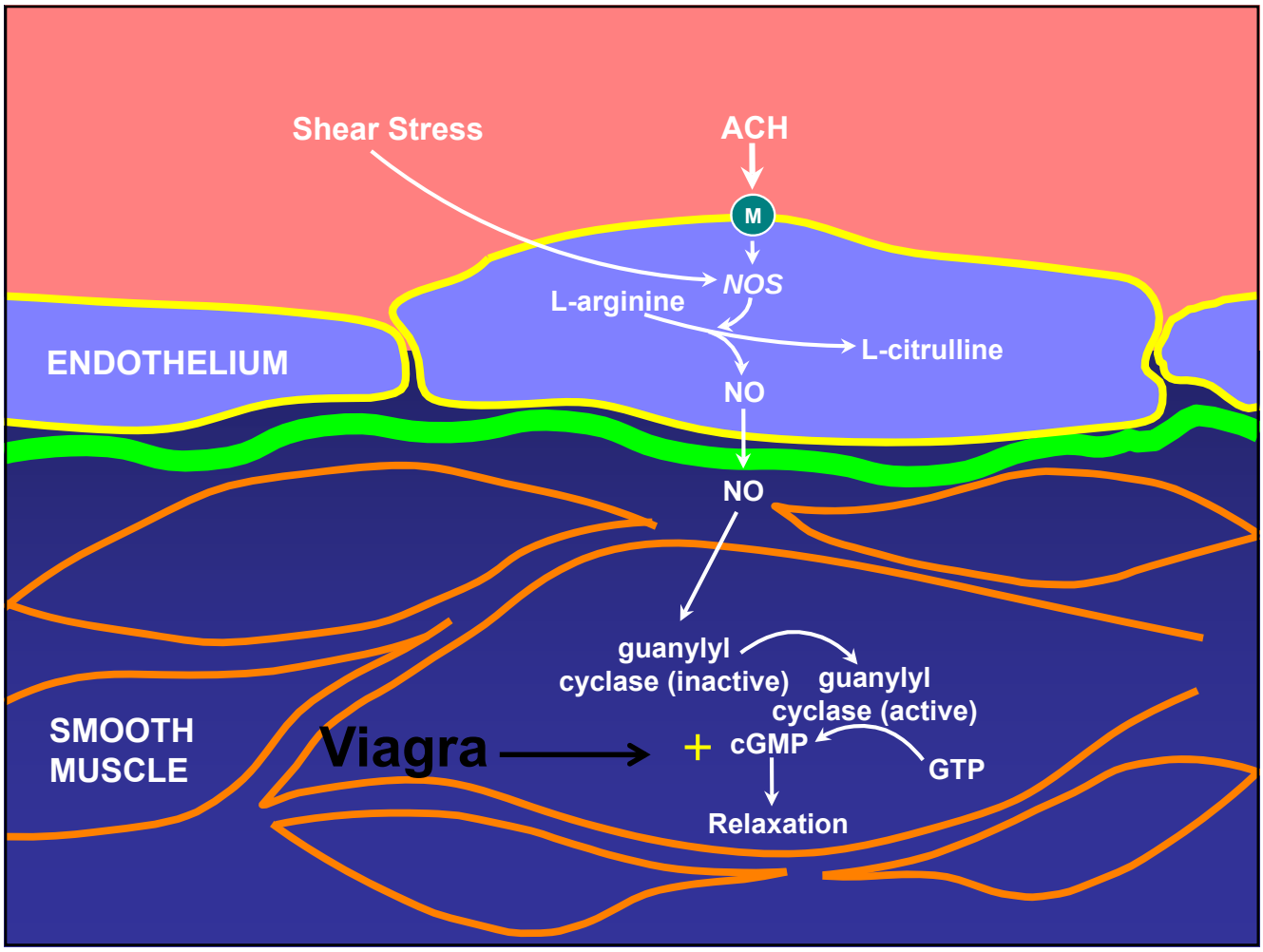
Each pathway provides about 50% of total body NO production


The L-Arginine-Nitric Oxide Pathway
Health
Antioxidants
NADH
NAD
GSSG
GSH
GSNO
GSNOR
Oxidation
Diet
↑ L-Arg
↓ L-Arg
↑ Arginase
↑ ADMA
↓ Transport
Urea Cycle
L-Arg
BH4
FAD+
NADPH
Heme iron
Ca/Cam
FMN
O_2
GSH
NOS
Uncoupling
Reduced Oxygen
Reduced Cofactor + Substrate
Oxidative Stress
NO
NO_2
NO_3
Bacterial Reduction
Mitochondria
XO
NADPH oxidase
$O_2^{-\cdot}$
Disease
$ONOO^-$

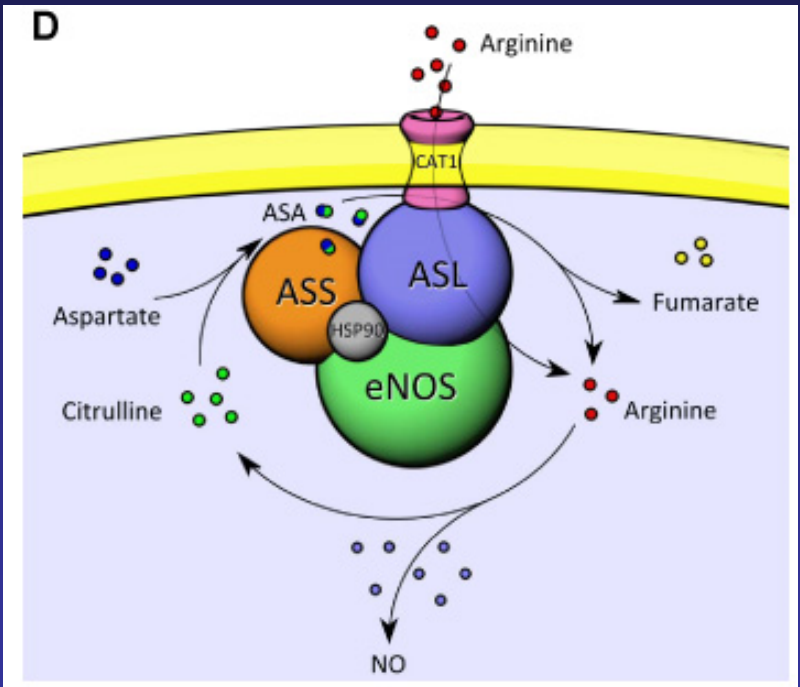
Endothelial Production of NO Declines with Age



Gerhard et al Hypertension 1996
Celermajer et al JACC 1994
Taddei et al Hypertension 2001
Egashira et al Circulation 1993


Shear Stress
ACH
M
NOS
L-arginine
L-citrulline
ENDOTHELIUM
NO
NO
guanylyl cyclase (inactive)
guanylyl cyclase (active)
SMOOTH MUSCLE
Viagra
+ cGMP
GTP
Relaxation

NOS Utilizes Intracellular L-Arginine from L-citrulline for NO Production



Erez et al Nat Med 2011

Nitric oxide synthase-derived plasma nitrite predicts exercise capacity

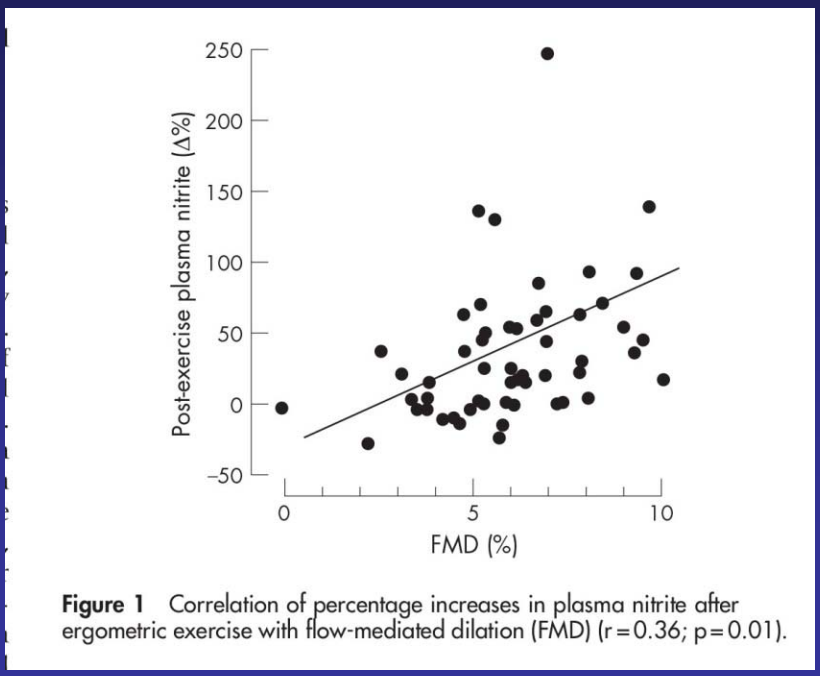


Figure 1 Correlation of percentage increases in plasma nitrite after ergometric exercise with flow-mediated dilation (FMD) (r = 0.36; p = 0.01).

Rassaf T, et al
Br J Sports Med 2007;41:669–673

Age-dependent endothelial dysfunction is associated with failure to increase plasma nitrite in response to exercise

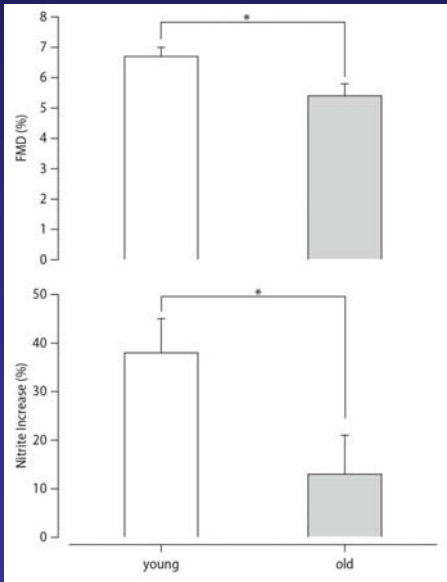


Fig. 1 Brachial artery flow mediated dilation (FMD) at baseline (*upper panel*) and changes in plasma nitrite after exercise (*lower panel*) in young and old subjects. *Asterisks* indicate significantly different at the $P < 0.05$ level

Lauer et al
Basic Res Cardiol 103:291–297 (2008)

L-arginine utilization is controlled by the enzymatic function of nitric oxide synthase

Dietary nitrate utilization is controlled by amount of nitrate consumed and the oral nitrate reducing bacteria

Important Considerations for L-Arginine/L-Citrulline-Based Technologies

1. Must have a functional NOS
   - most NO-deficient patients have dysfunctional NOS
   - must also have supporting co-factors to recouple NOS
2. L-arginine can activate Herpes virus
3. High dose L-arginine can do more harm than good
   - VINTAGE trial (Schulman JAMA 2006)
   - PAD trial (Wilson Circulation 2007)
4. There is not a single human disease caused by L-arginine deficiency

Important Considerations for Nitrate-Based Technologies

1. All beets/vegetables are not created equal
   - growth conditions
   - time of harvest
   - processing, heat, packaging, etc.
2. Liquid products can de-stabilize NO
   - pH < 3 (acid labile components)
3. More is not better
   - safety first
4. Patients/consumers must have oral nitrate-reducing bacteria
5. Patients/consumers using PPI and antiseptic mouthwash will not respond

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.

FACTS

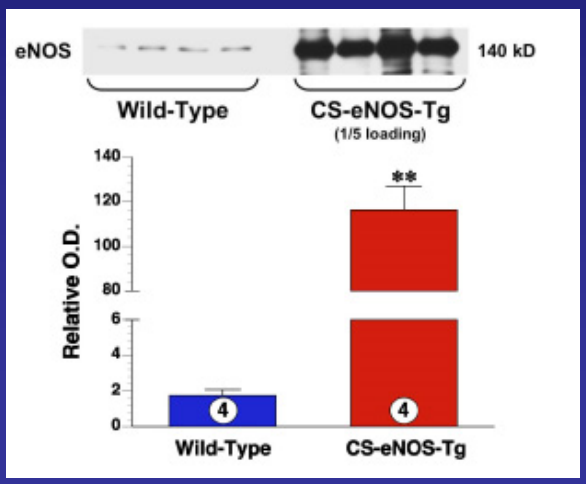
Cardiovascular disease (CVD) is the number one killer of both men and women in the U.S. Close to 1 million people die each year and more than 6 million are hospitalized due to CVD. The cost of CVD, in terms of health care and lost productivity, is over $270 billion and increasing as the baby boom population ages.

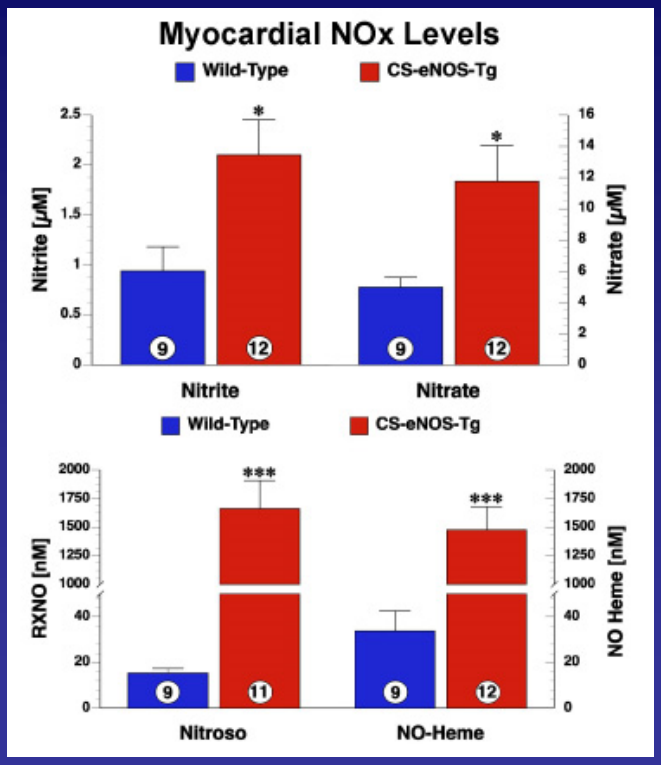
Ischemic heart disease, including myocardial infarction, remains the leading cause of morbidity and mortality in all industrialized nations

What is the physiological consequence of enhanced NO production in Ischemia-reperfusion injury?

Can we trace the phenotype biochemically?

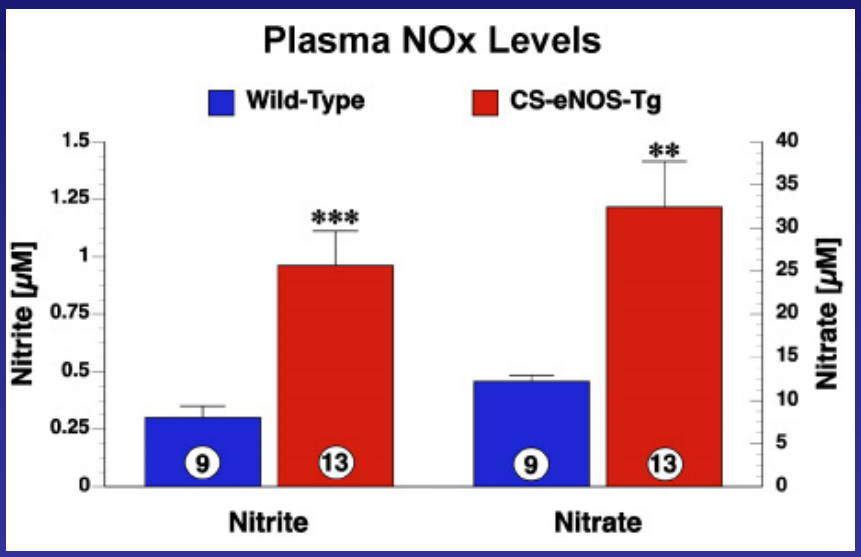
Cardiac Specific Overexpression of eNOS results in Increased Cardiac NO Production and Protects from I/R Injury





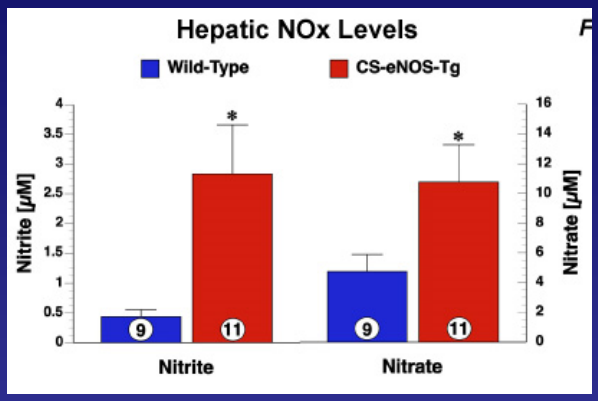
Elrod et al ATVB 2006

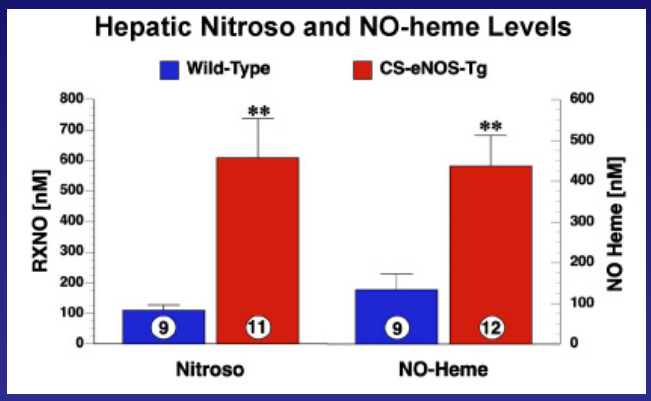
Increased Cardiac NO Production Results in Increased Circulating Nitrite and Nitrate

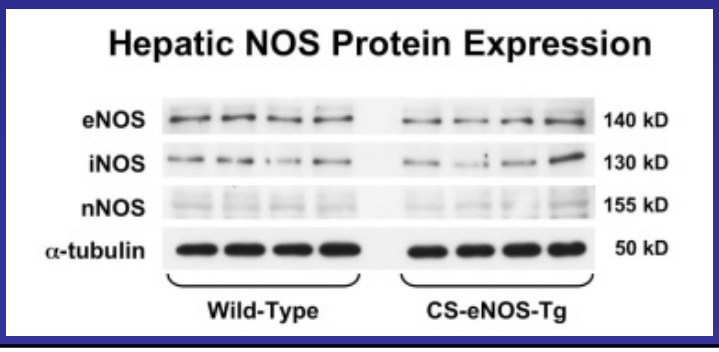


Elrod, PNAS 2008

Local NO Production in the Heart Results in Accumulation of NO Products in the Liver

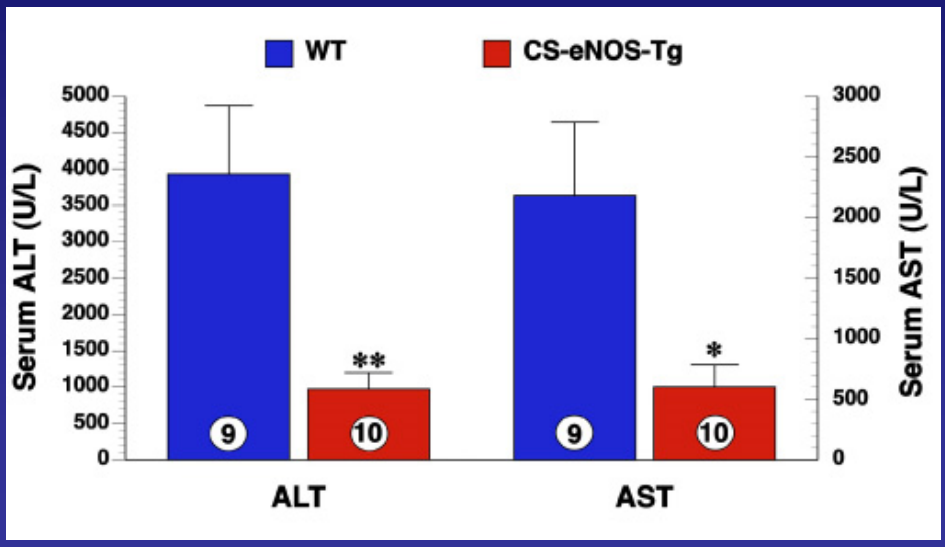






Elrod, PNAS 2008

Cardiac Derived NO Promotes Distant Organ Protection: Evidence for an Endocrine Role of Nitrite and GSNO

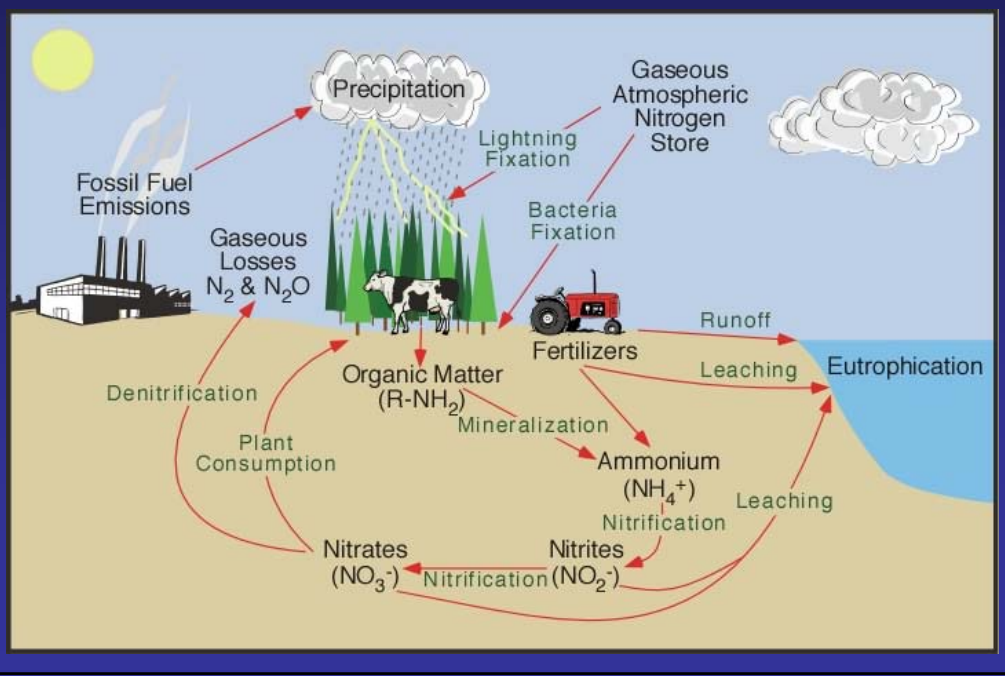


Elrod, PNAS 2008

Can proper nutrition, food or Supplements provide NO support?

Atmospheric Nitrogen Cycle

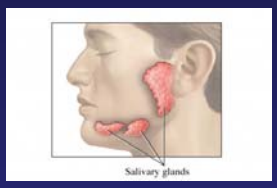
The store of nitrogen found in the atmosphere, where it exists as a gas (mainly N2), plays an important role for life. Most plants can only take up nitrogen in two solid forms: ammonium ion (NH4+) and the nitrate ion (NO3-). Most plants obtain the nitrogen they need as nitrate from the soil. When released, most of the ammonium is often chemically altered by a specific type of bacteria (genus Nitrosomonas) into nitrite (NO2-). Further modification by another type of bacteria (genus Nitrobacter) converts the nitrite to nitrate. All nitrogen obtained by animals can be traced back to the eating of plants at some stage of the food chain.



New Paradigm - Human Nitrogen Cycle

Dietary nitrate and nitrite intake

Commensal bacteria reduce 20% of nitrate to nitrite



25% circulating Nitrate concentrated in salivary glands

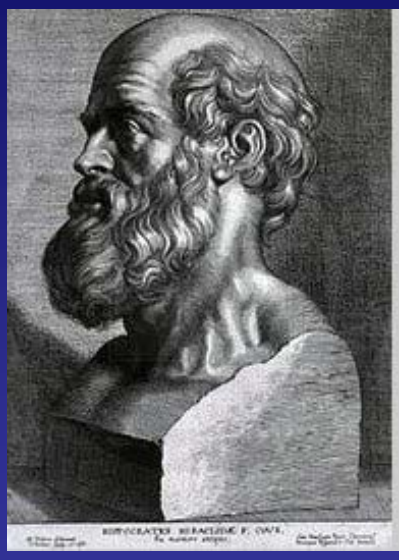
Majority of Nitrate Filtered and reabsorbed

Systemic circulation – nitrite and nitrate delivered throughout the body

Low gastric pH reduces nitrite to NO

Nitrate and nitrite absorbed by intestines

50% or more of NO bioactivity is determined and dictated by foods and diets containing nitrite and nitrate and oral bacteria



"Let food be thy medicine and medicine be thy food"
– Hippocrates

PALEOSPIRIT COM

How much nitrate do we need?

300-400 mg nitrate necessary to see changes in blood pressure or improvement in exercise capacity

Estimated that US population consumes ~150 mg nitrate per day (over 2-3 meals)

We are a Nitrate Deficient Population

Nitrate Comparison Between Beet Root Powders

We have analyzed over 30 different beet root powders, both from ingredient companies and from beet root powder purchased at retail

There is much as a 500 fold difference in nitrate content of beet root powder from one supplier to the next (we use several of these as a placebo in our clinical trials)

Many companies use beet root powder as window dressing

A Survey of Nitrate and Nitrite Concentrations in Conventional and Organic-Labeled Raw Vegetables at Retail

Regional and Category Differences In Vegetable Nitrate Values

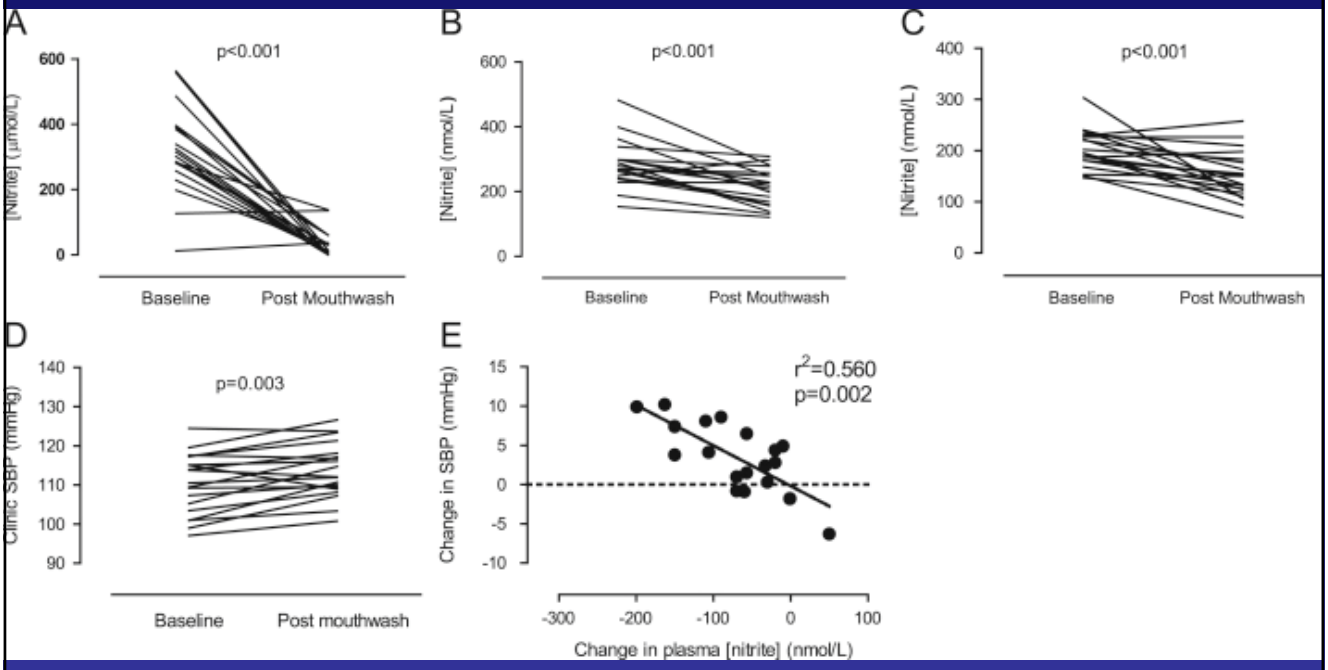
Table 2. Mean nitrate (NO_3?) concentrations[a] (ppm)[b] of raw vegetables classified as conventional from each city

Product category	Chicago	Dallas	Los Angeles	New York	Raleigh
Broccoli	271 ± 89 (61-822)	357 ± 50 (165-664)	512 ± 85 (164-1140)	279 ± 80 (29-1009)	553 ± 28 (374-680)
Cabbage	475 ± 46 (256-670)	256 ± 33 (63-434)	800 ± 142 (275-1831)	193 ± 28 (37-283)	364 ± 79 (72-882)
Celery	230 ± 19 (147-359)	2052 ± 156 (918-2973)	2651 ± 339 (608-4269)	88 ± 17 (20-157)	2201 ± 112 (1397-2727)
Lettuce	207 ± 32 (79-425)	1370 ± 93 (870-1909)	1051 ± 122 (422-1495)	568 ± 93 (321-970)	986 ± 185 (450-2171)
Spinach	647 ± 69 (162-875)	4923 ± 327 (2377-6473)	4138 ± 451 (2141-8000)	564 ± 174 (65-1545)	3155 ± 145 (2478-4168)

[a]Mean value with standard error; minimum and maximum nitrate values in parentheses.
[b]mg/ kg of fresh weight.

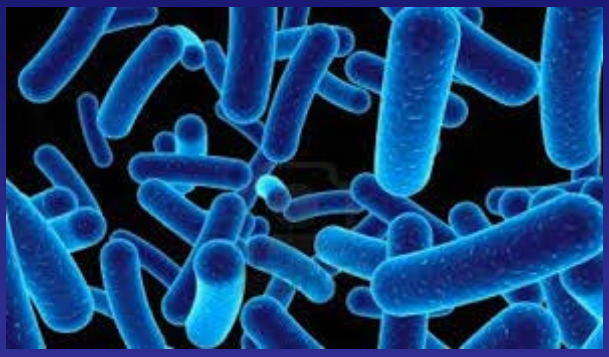
Nitrate is inert in Humans.
Nitrate must be reduced to nitrite by commensal bacteria

Physiological Role for Nitrate-Reducing Oral Bacteria in Blood Pressure Control



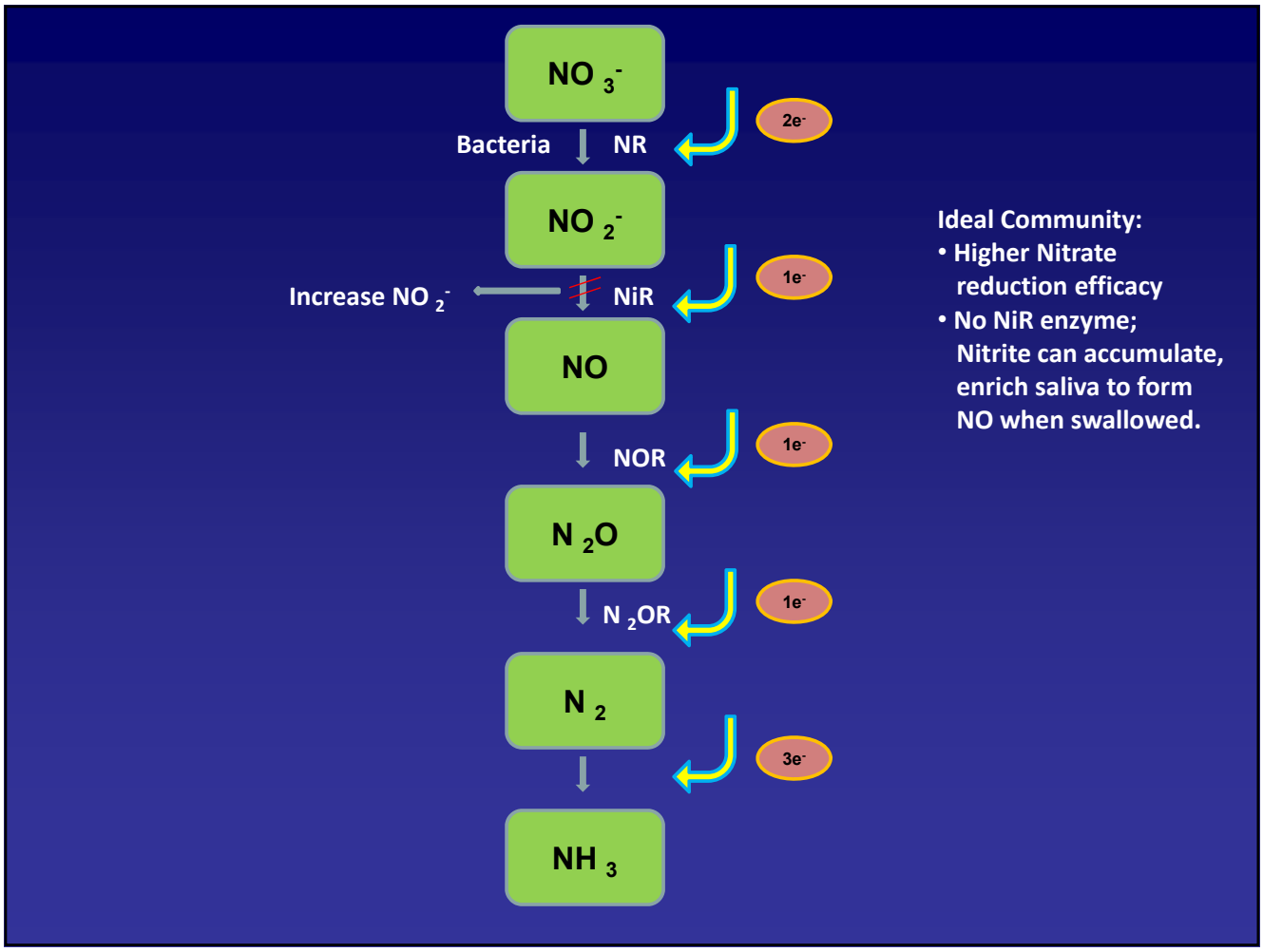
Kapil et al Free Radic Biol Med. 2013 Feb;55:93-100

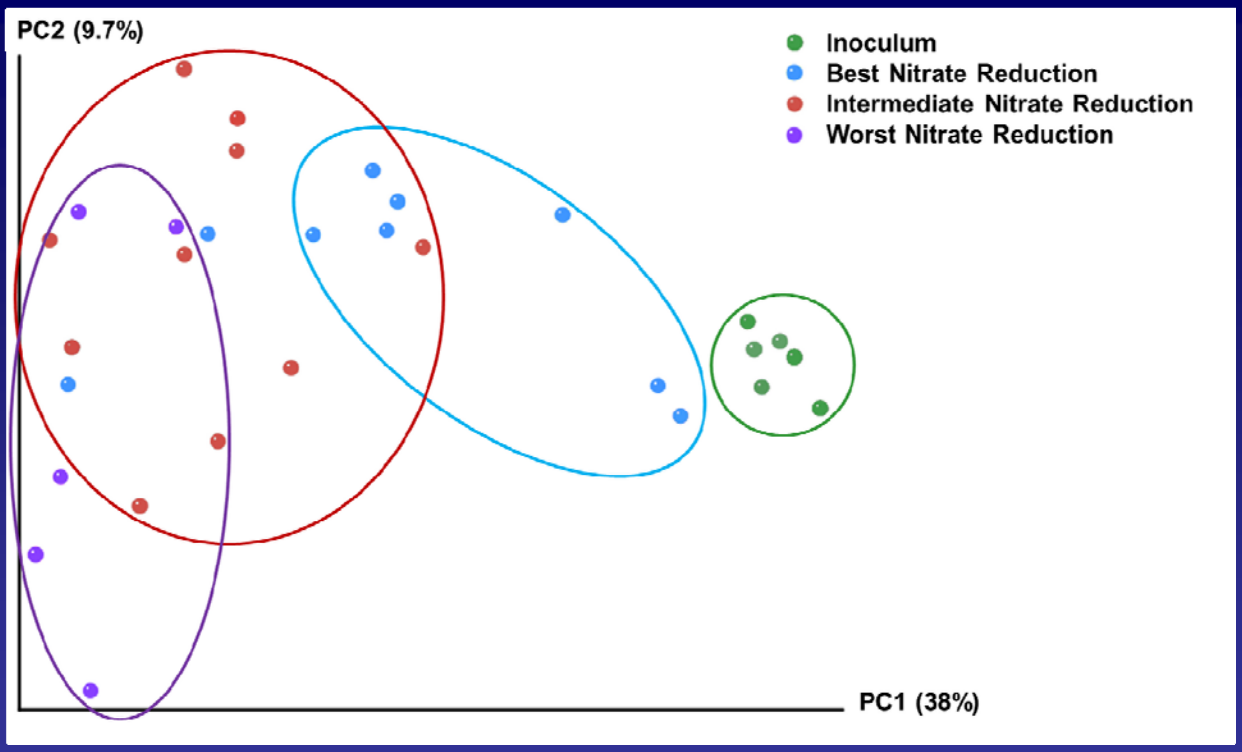
Genetic Diversity



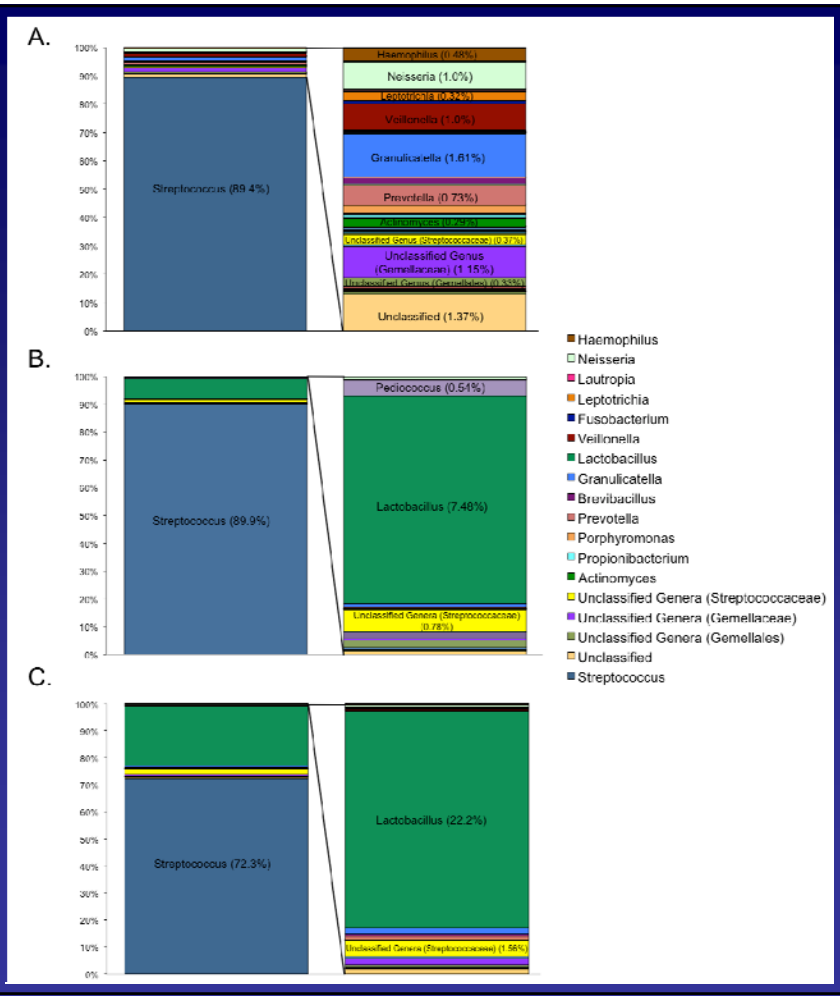
23,000 genes

3,000,000 genes


NO 3-
Bacteria
NR
2e-
NO 2-
Increase NO 2-
NiR
1e-
NO
NOR
1e-
N 2O
N 2OR
1e-
N 2
3e-
NH 3
Ideal Community:
• Higher Nitrate reduction efficacy
• No NiR enzyme; Nitrite can accumulate, enrich saliva to form NO when swallowed.



The microbial community structure changes as nitrate reduction decreases. Unweighted UniFrac-based Principal Coordinate Analysis (PCoA) illustrates the first two principal coordinates (PCs) for inocula, best reduction, intermediate reduction, and worst reduction groups. Unweighted UniFrac is a phylogenetic-tree based method that determines the similarity of two microbial communities based on the amount of shared branch length; thus, similar communities cluster closely on PCoA. Each dot represents a single sample and the amount of variance explained by each PC is indicated in parenthesis next to each axis.



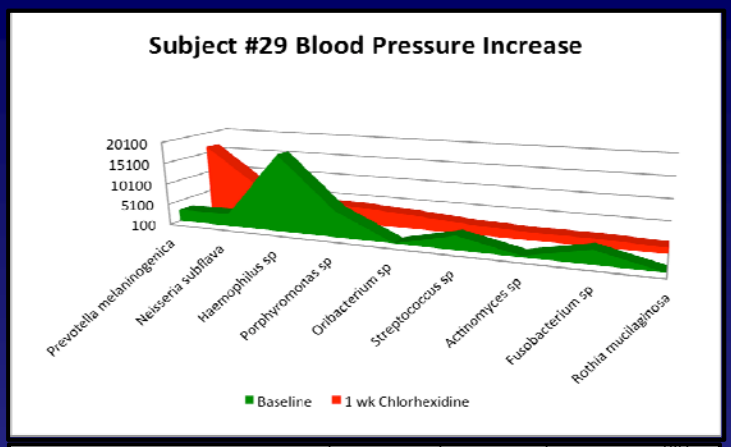
Best

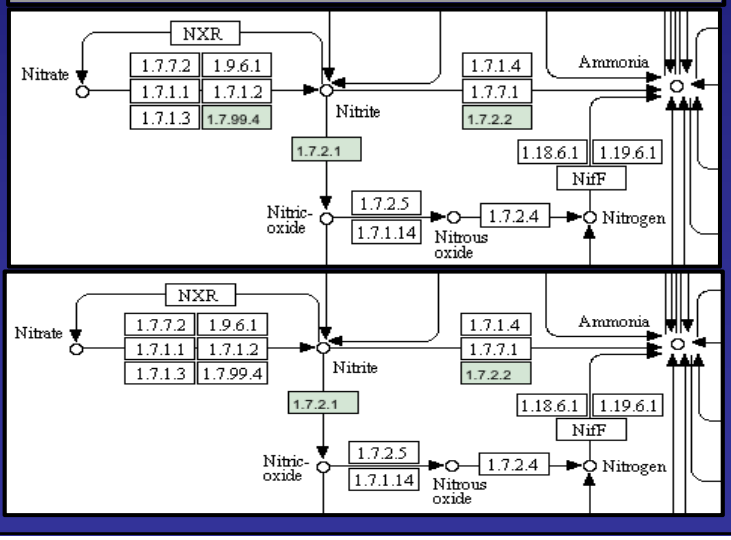
Intermediate

Worst

Hyde et al PLoS One (2014)

How Does Chlorhexidine Treatment Affect Diversity of Oral Microbiome And Nitrate Reduction in Healthy Subjects and what Effect Does this have on Systemic Blood Pressure?





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

Oral Hygeine Survey

Brush teeth	Count	Percent
twice a day	22	81%
three times a day	5	19%

Floss	Count	Percent
several times per month	1	4%
several times per week	8	30%
once a day	16	67%
twice a day	2	7%

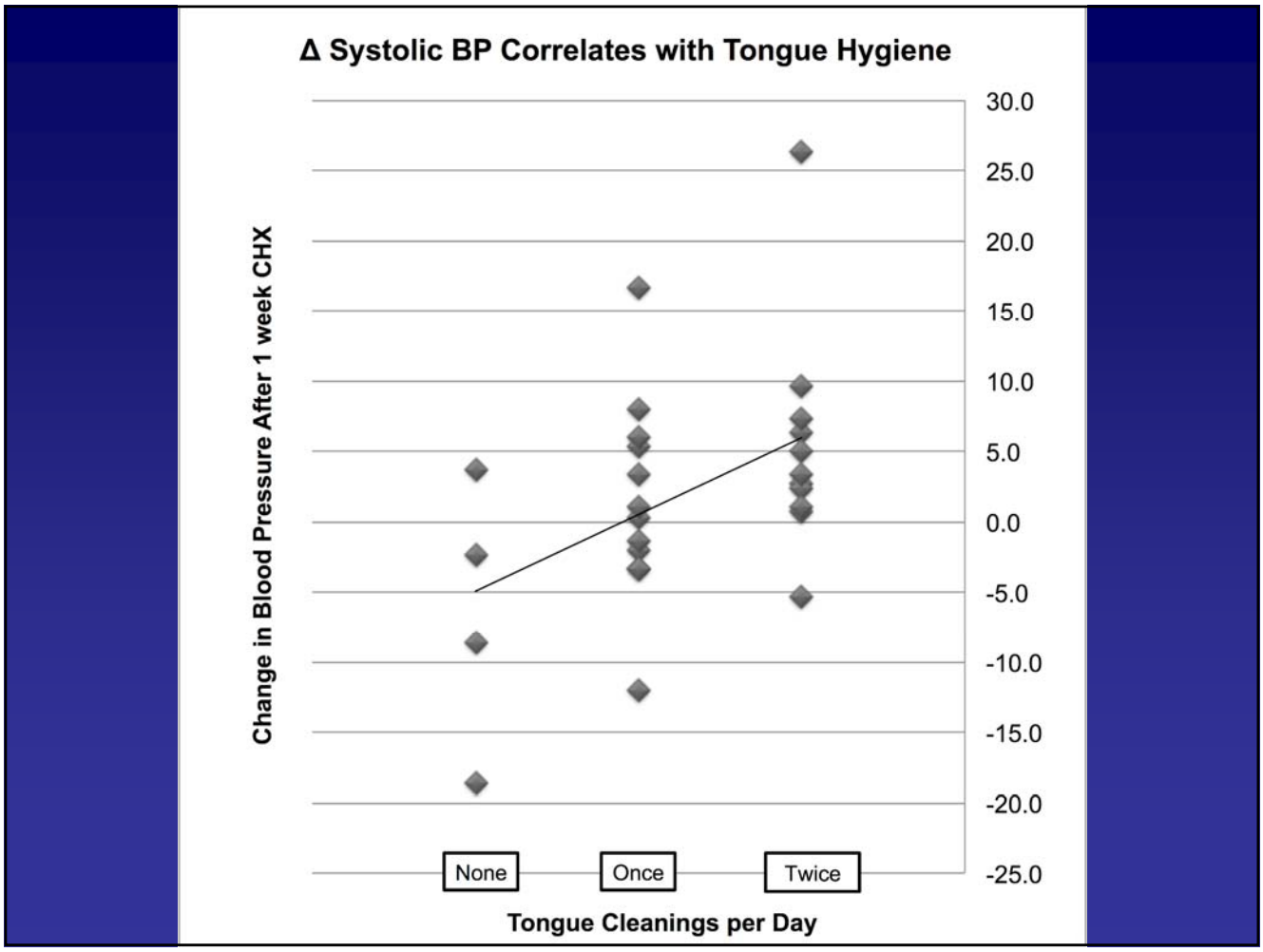
Clean tongue	Count	Percent
Less than once a week	4	15%
Once a day	13	48%
Twice a day or more	10	37%

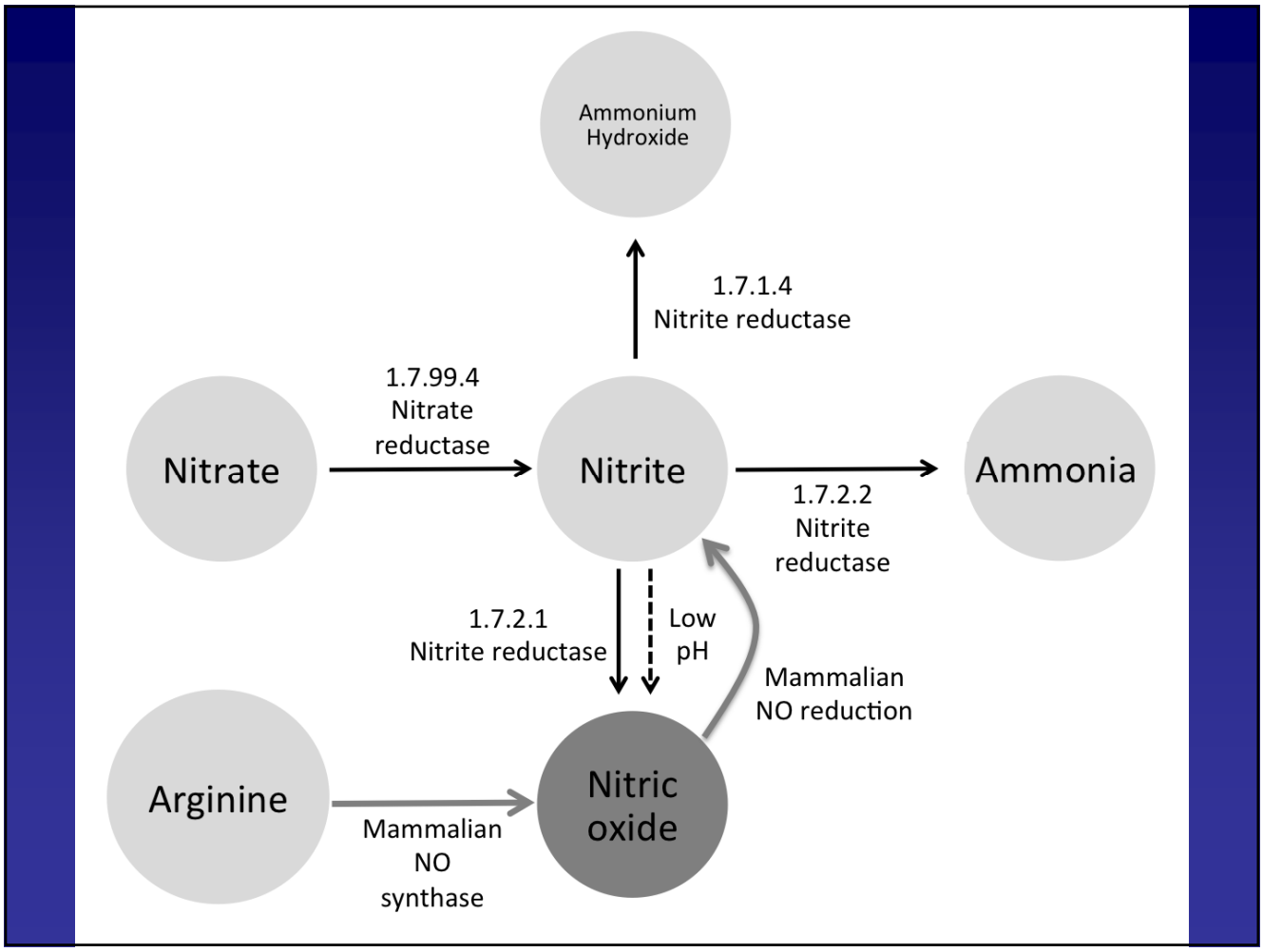
Mouthwash use	Count	Percent
As needed	5	19%
Once a day	16	59%
Twice a day	6	22%

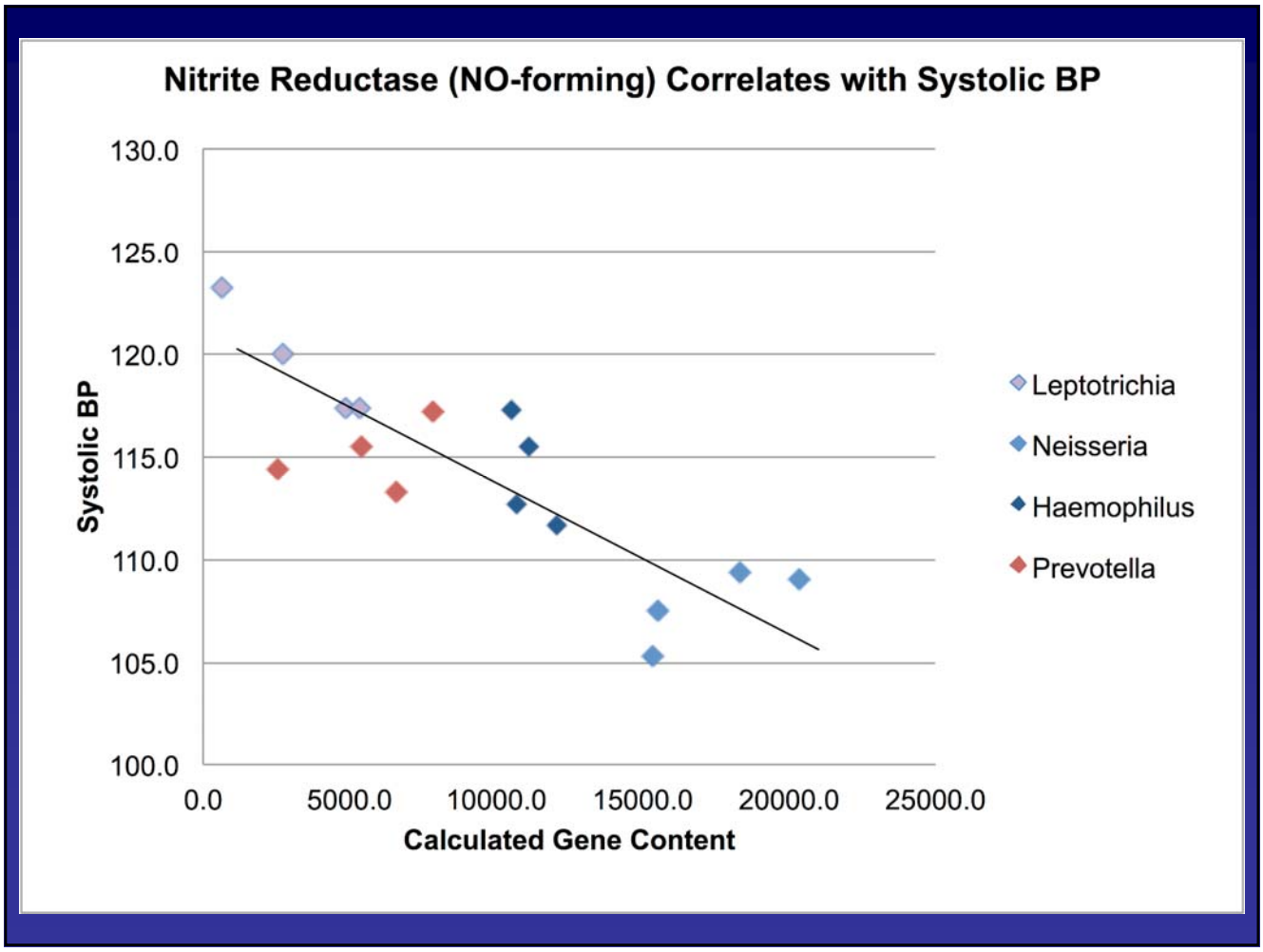
Mouthwash ingredient	Count	Percent
Essential oils	11	41%
Cetylpyridinium chloride	10	37%
No response	6	22%

Type of Toothbrush	Count	Percent
Manual	10	37%
Electric	12	44%
Both manual and electric	5	19%

Visits to dentist per year	Count	Percent
None	1	4%
Once	12	44%
Twice or more	14	52%


Δ Systolic BP Correlates with Tongue Hygiene
Change in Blood Pressure After 1 week CHX
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
None
Once
Twice
Tongue Cleanings per Day


Ammonium Hydroxide
1.7.1.4 Nitrite reductase
1.7.99.4 Nitrate reductase
Nitrate
Nitrite
Ammonia
1.7.2.2 Nitrite reductase
1.7.2.1 Nitrite reductase
Low pH
Mammalian NO reduction
Arginine
Nitric oxide
Mammalian NO synthase


Nitrite Reductase (NO-forming) Correlates with Systolic BP
130.0
125.0
120.0
115.0
110.0
105.0
100.0
Systolic BP
0.0
5000.0
10000.0
15000.0
20000.0
25000.0
Calculated Gene Content
Leptotrichia
Neisseria
Haemophilus
Prevotella

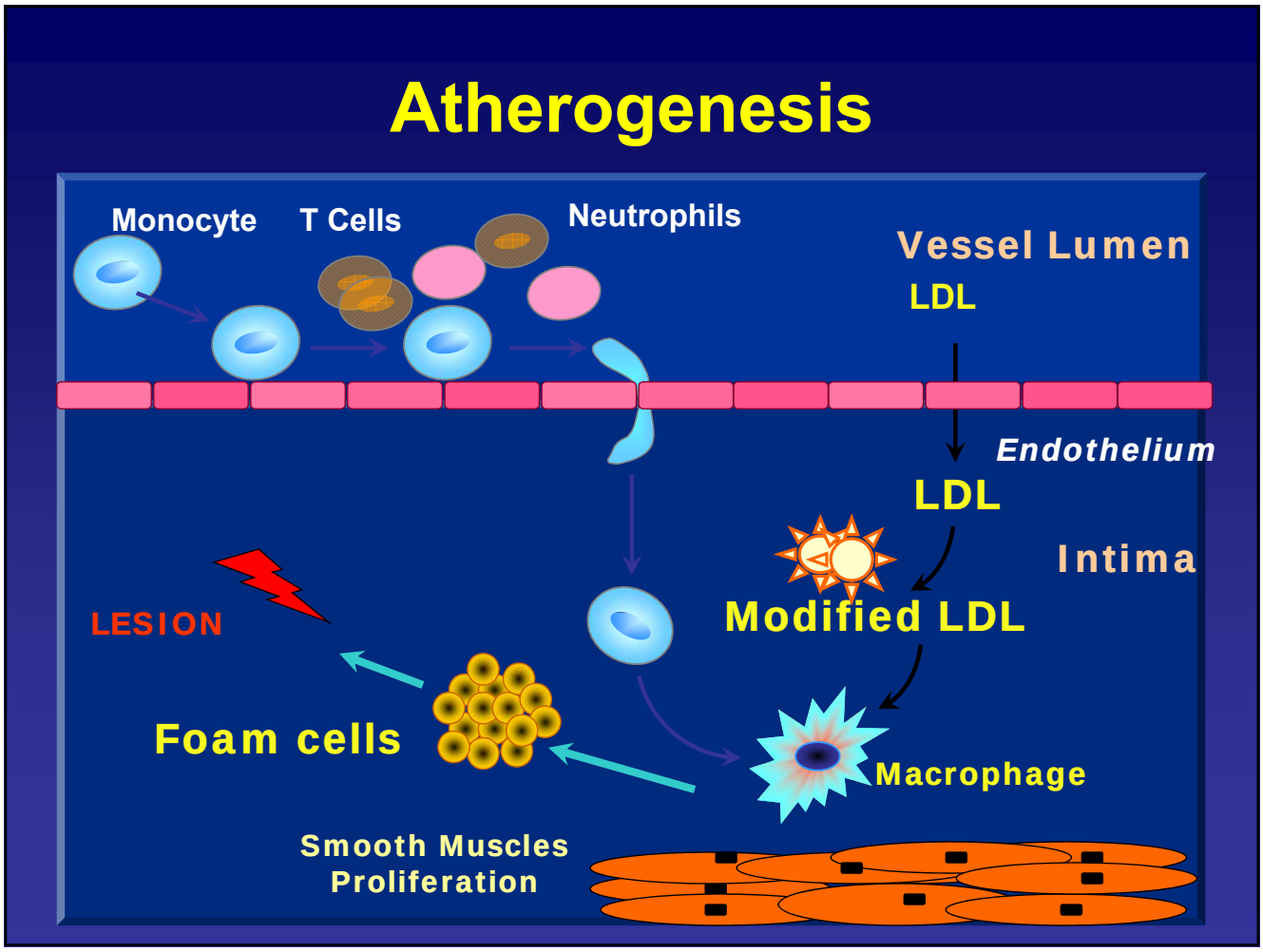
Disruption of Nitrate-Nitrite-NO Pathway

1. Insufficient dietary intake of nitrate/nitrite rich foods
   (green leafy vegetables, beets, etc)
2. Problems with nitrate uptake in duodenum
   (sialin (SLC17A5) transporter mutations – Salla Disease)
3. Insufficient saliva production
   (Sjogrens syndrome)
4. Lack of oral commensal bacteria to reduce nitrate to nitrite
   (use of antibiotics/antiseptic mouthwash, poor oral hygeine)
5. Insufficient stomach acid production – Achlorhydria
   (use of PPI's, H. Pylori infection, iron overload)
6. Increased oxidative stress that scavenges NO

What might this mean?

- Absence of these select bacteria - a new risk factor for cardiovascular disease.
- Patients with periodontal disease , affecting the NO producing communities - possibly linking oral health to cardiovascular disease risk by disruption of NO production
- Use of antiseptic mouthwash or overuse antibiotics can disrupt nitrate reducing communities
- Patients taking proton pump inhibitors to suppress stomach acid production
- Develop this pathway as a primary therapeutic target to affect NO production

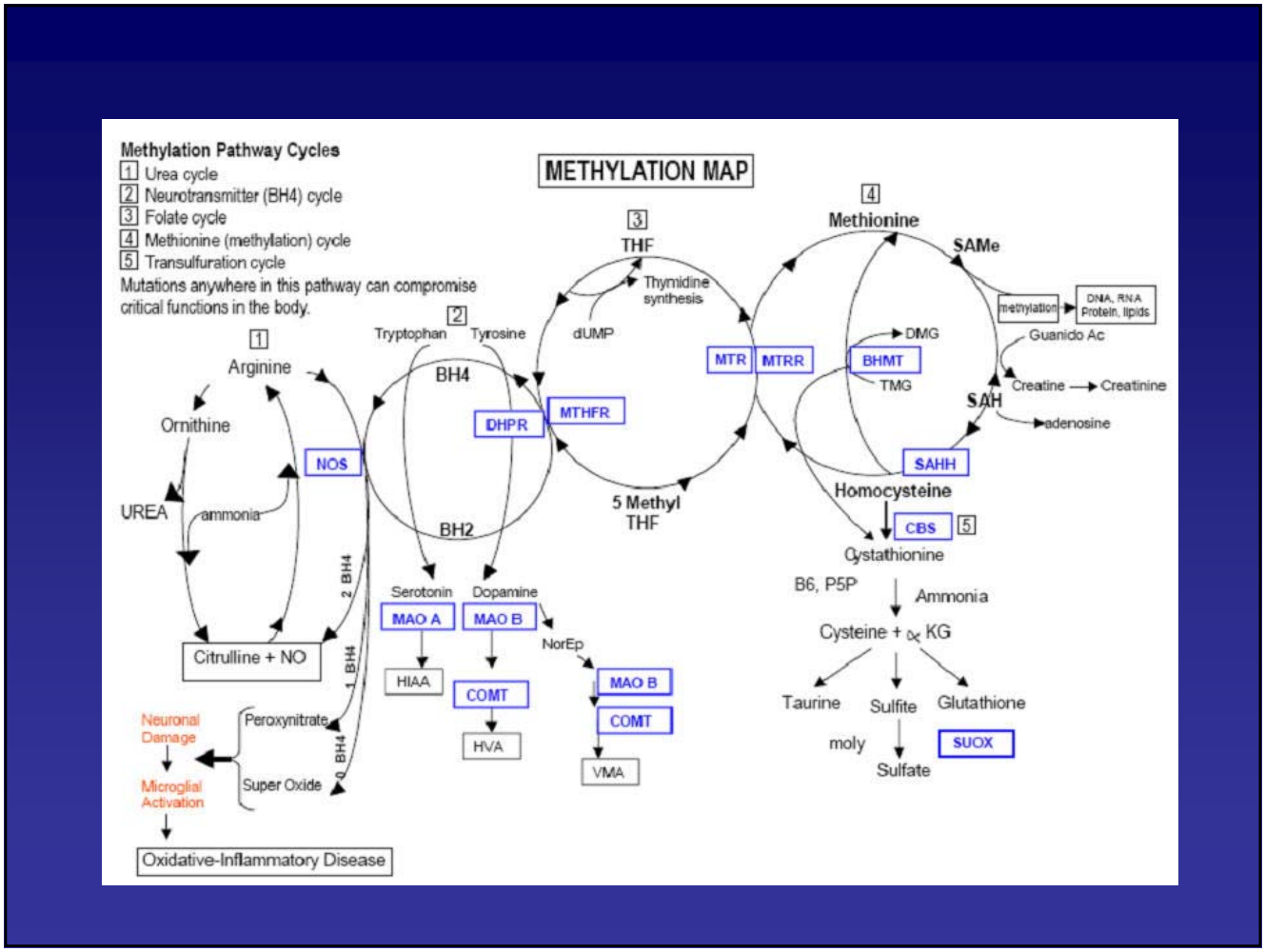
Can we overcome variability in nitrate reduction based on differences in oral bacteria?


Atherogenesis
Monocyte
T Cells
Neutrophils
Vessel Lumen
LDL
Endothelium
LDL
Intima
Modified LDL
LESION
Foam cells
Macrophage
Smooth Muscles
Proliferation

Atherogenic Diet

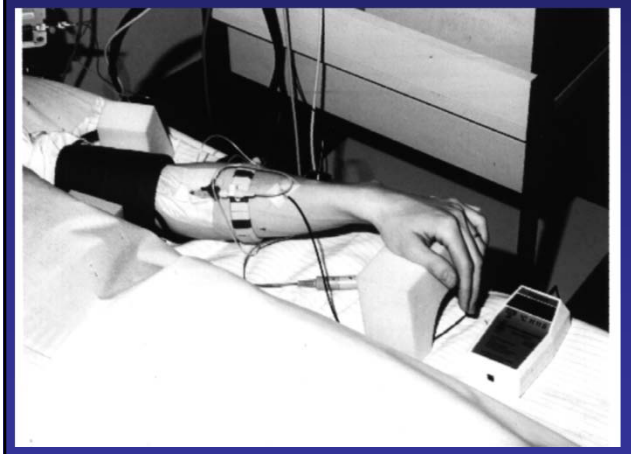
Atherogenic Diet + Nitrite

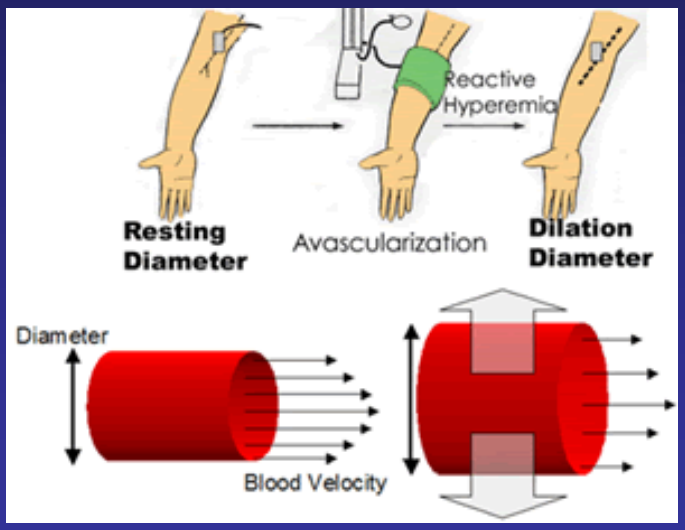
What about Genetic Testing?
Do Specific SNPs Affect NO Production


Methylation Pathway Cycles
1 Urea cycle
2 Neurotransmitter (BH4) cycle
3 Folate cycle
4 Methionine (methylation) cycle
5 Transulfuration cycle
Mutations anywhere in this pathway can compromise critical functions in the body.
METHYLATION MAP
1
Arginine
Ornithine
UREA
ammonia
NOS
Citrulline + NO
2
Tryptophan
Tyrosine
BH4
BH2
DHPR
MTHFR
2 BH4
1 BH4
0 BH4
Neuronal Damage
Peroxynitrate
Super Oxide
Microglial Activation
Oxidative-Inflammatory Disease
3
THF
Thymidine synthesis
dUMP
5 Methyl THF
MTR
MTRR
Serotonin
Dopamine
MAO A
MAO B
HIAA
COMT
HVA
NorEp
MAO B
COMT
VMA
4
Methionine
SAMe
methylation
DNA, RNA Protein, lipids
Guanido Ac
Creatine
Creatinine
adenosine
DMG
TMG
BHMT
SAH
SAHH
Homocysteine
5
CBS
Cystathionine
B6, P5P
Ammonia
Cysteine + ∝ KG
Taurine
Sulfite
Glutathione
moly
Sulfate
SUOX

NO Diagnostics

Flow Mediated Dilatation for Endothelial Function



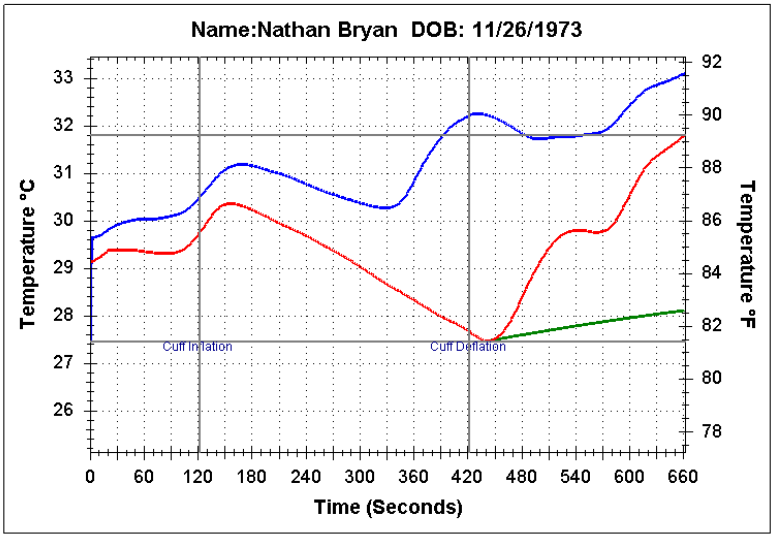


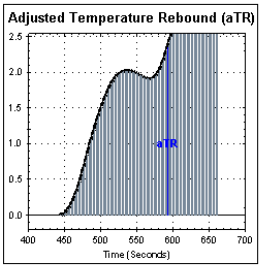
VENDYS®

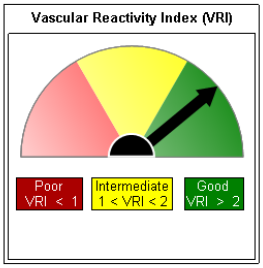
Vascular Function Test

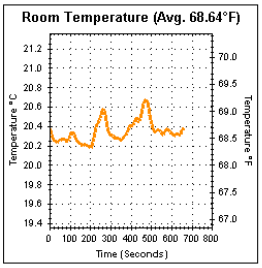
Digital Thermal Monitoring of Endothelial Function and Vascular Reactivity

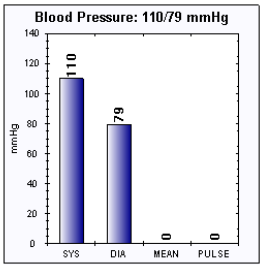
Right Finger Temperature — Left Finger Temperature — Zero Reactivity Curve











Quality Check:

- Cold Finger
- Sympathetic Response
- Stabilization
- Finger Room Delta
- Cold Room
- Fluctuating Room Temp
- Right vs Left

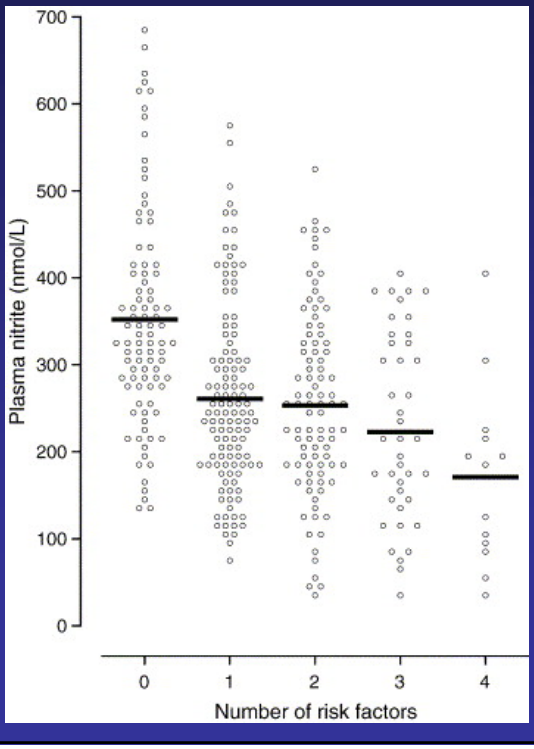
*Note: Cold Finger Flag check was disabled.

Vascular Reactivity Index
VRI = 2.34

ENDOTHELIX

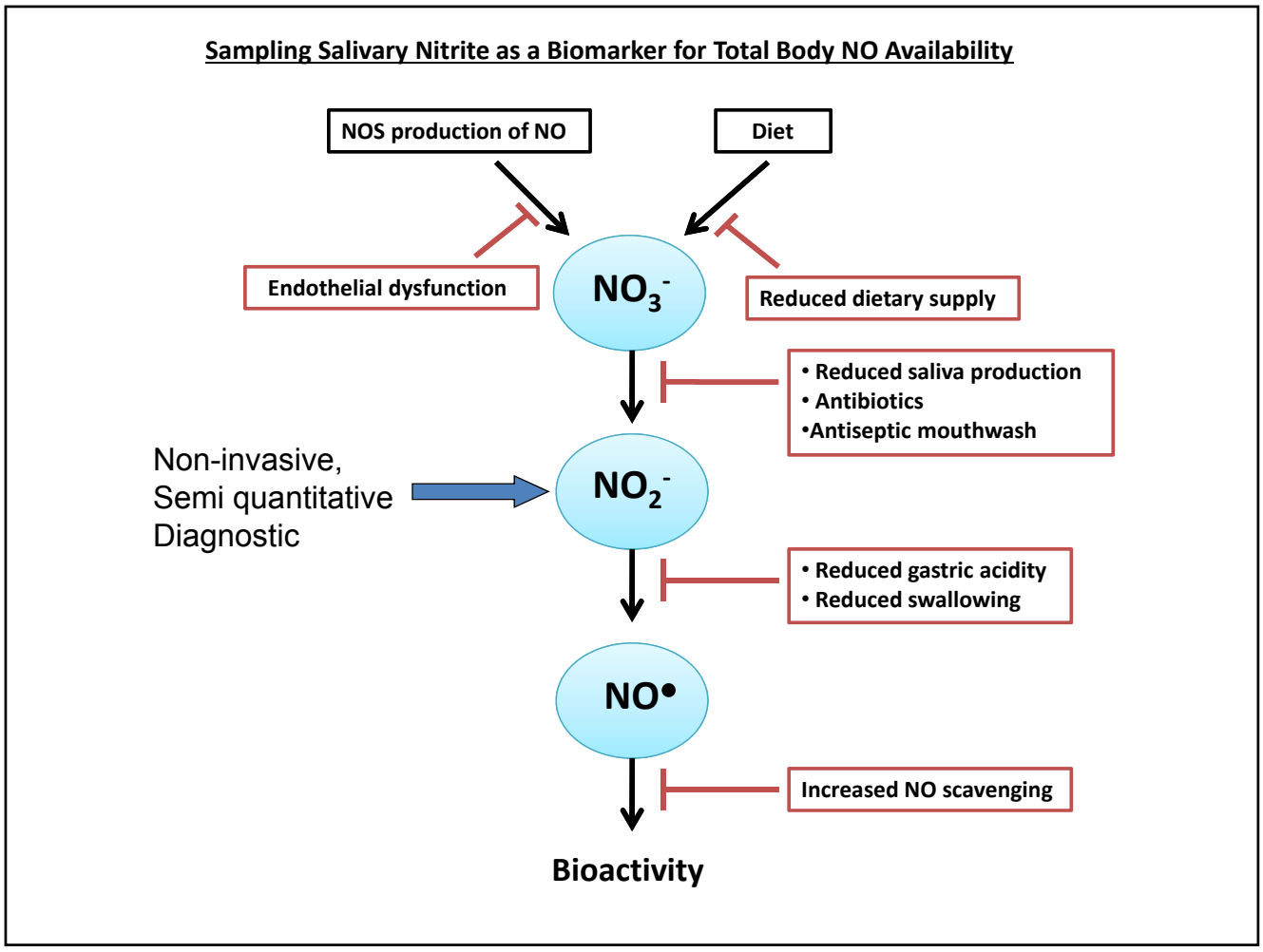
Test Date:
4/26/2014 1:04:56 PM

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


Sampling Salivary Nitrite as a Biomarker for Total Body NO Availability
NOS production of NO
Diet
Endothelial dysfunction
NO_3^-
Reduced dietary supply
• Reduced saliva production
• Antibiotics
•Antiseptic mouthwash
Non-invasive,
Semi quantitative
Diagnostic
NO_2^-
• Reduced gastric acidity
• Reduced swallowing
NO•
Increased NO scavenging
Bioactivity

The First and Only Non-invasive NO Diagnostic

NO Controls Stem Cell Mobilization And Differentiation

Sick patients have insufficient NO and dysfunctional cells

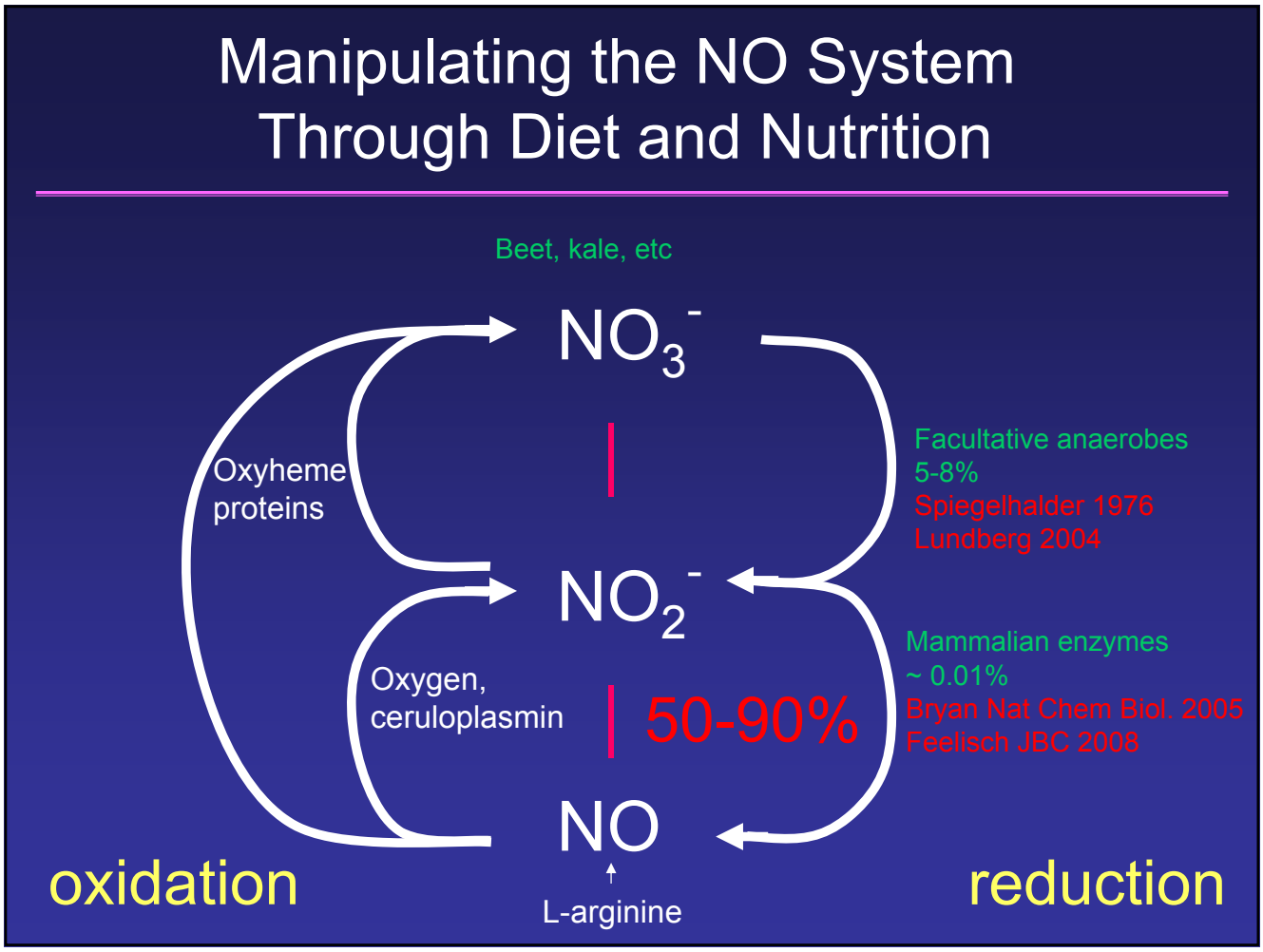
How do we make these cells more functional prior to isolation and deployment?

Restore body's own ability to make NO

Development of Safe and Effective NO-based Technology

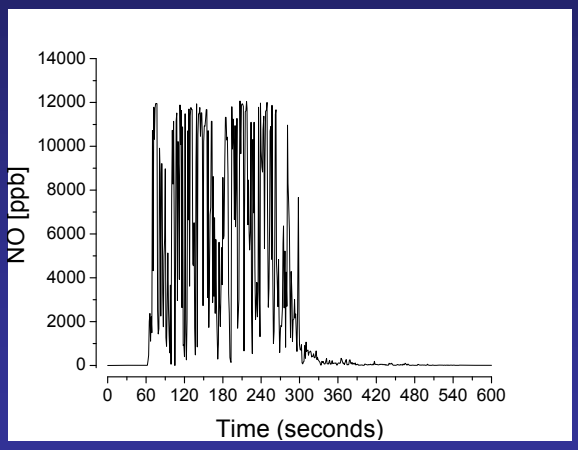
1. Provide an exogenous source of NO
2. Promote endogenous endothelial production of NO
3. Account for differences in non-responders to nitrate therapy and L-arginine
4. Plant-based natural product chemistry (clean and testable)
5. Clear product experience
6. Strong and sound basic science behind technology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

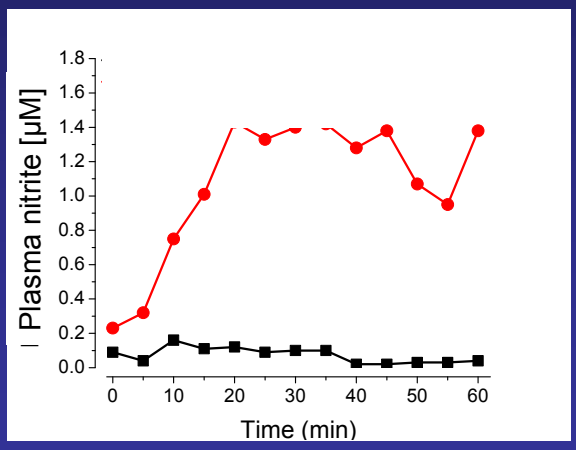
Manipulating the NO System Through Diet and Nutrition



NO Clinical Trial Results

Strong & sustained Nitric Oxide activity





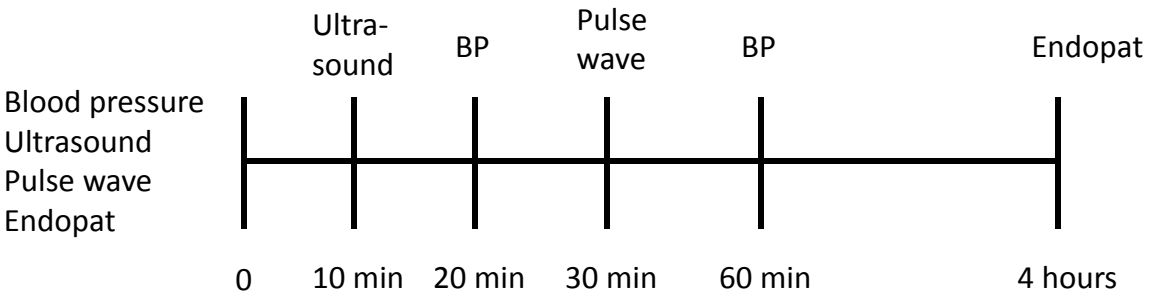
Zand et al Nutrition Research 2011

There are over 400 risk factors or markers for CVD

Most if not all can be corrected by restoring NO production

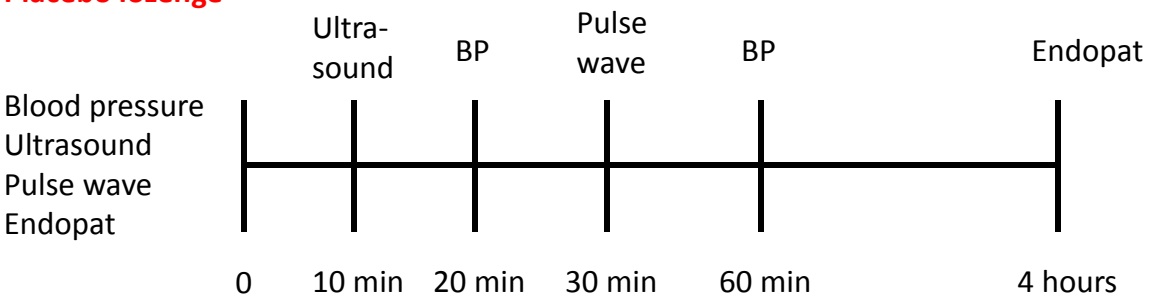
Blood Pressure Study Protocol

Active lozenge

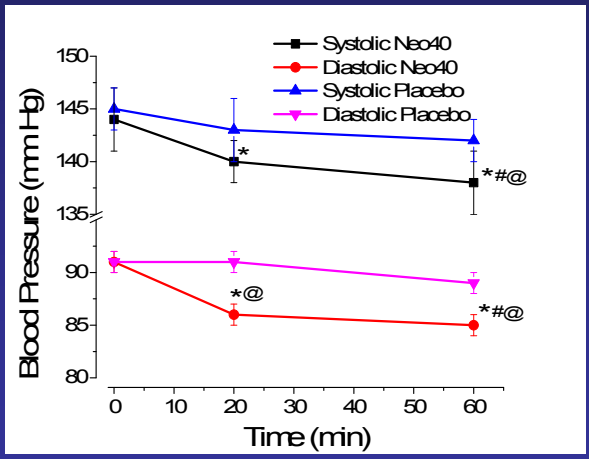


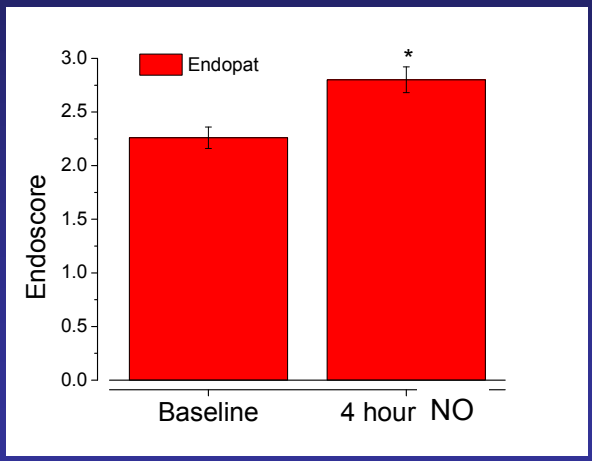
3 week washout

Placebo lozenge



NO Supports Healthy Blood Pressure And Improves Endothelial Function

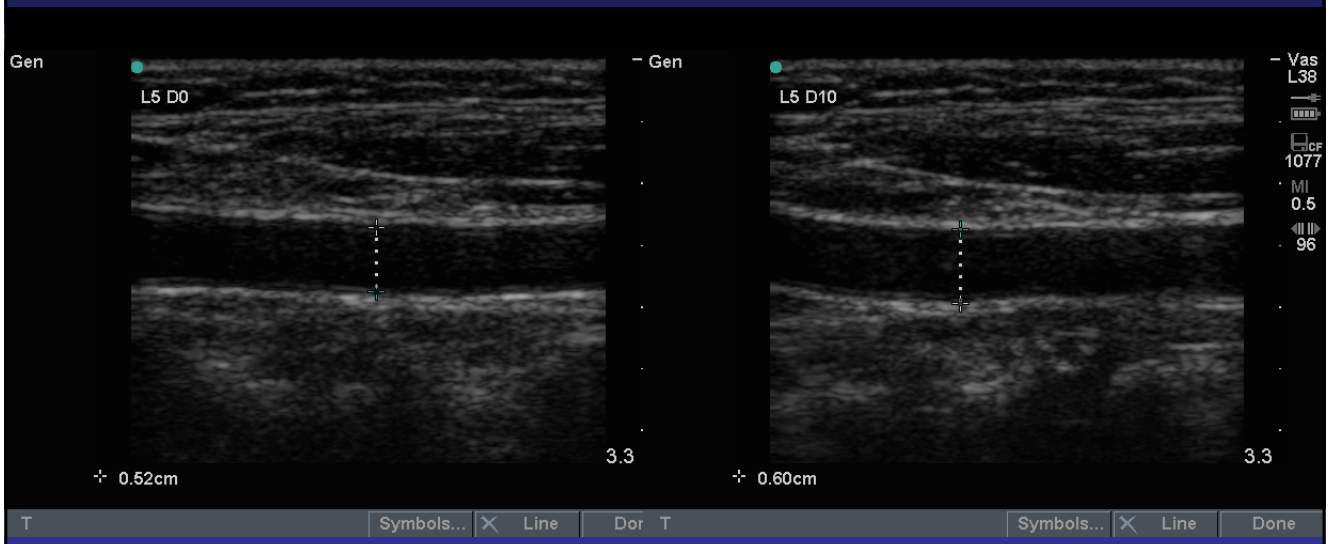




Flow mediated dilation

Houston, Hays JCH 2014

Representative Ultrasound Before and 10 minutes after NO Lozenge



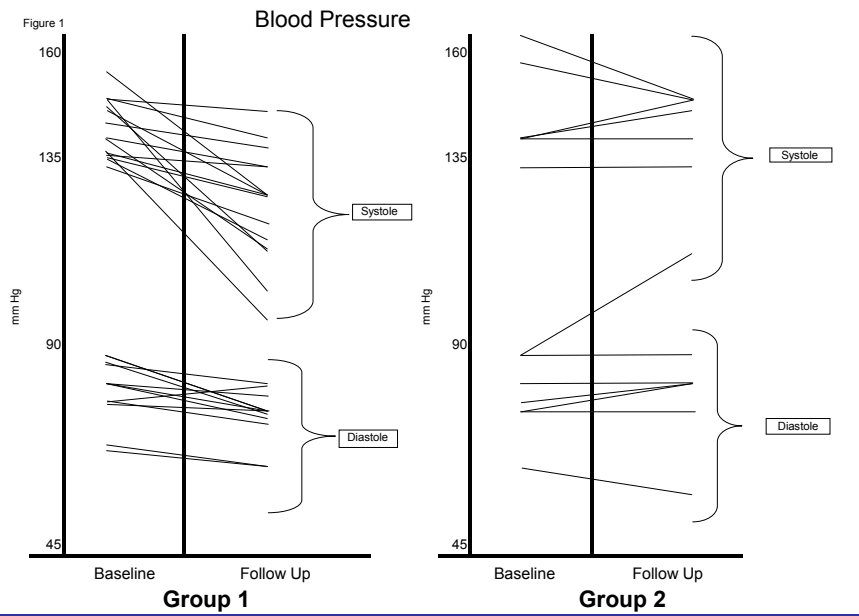
13% increase in vessel diameter causes a 34% increase in blood flow

Average Changes in 10 subjects After 30 minutes

	AVG % Chg 7min
Systole	-8.29%
Diastole	-8.15%
MAP	-7.91%
Heart Rate	-1.09%
Central Systolic Pressure	-7.25%
Central Diastolic Pressure	-8.84%
Cardiac Output	5.31%
Total Vascular Resistance	-12.72%
Augmentation Pressure	-46.76%
Aug. Index@75 [90% C]	-60.37%
Pulse Wave Velocity [90% C]	-1.96%

Pre-hypertension trial
Cedars Sinai Medical Center
PI: Ernst Schwarz MD, PhD

Pre-Hypertension Trial – Cedars Sinai School of Medicine



Biswas et al JCPT 2015

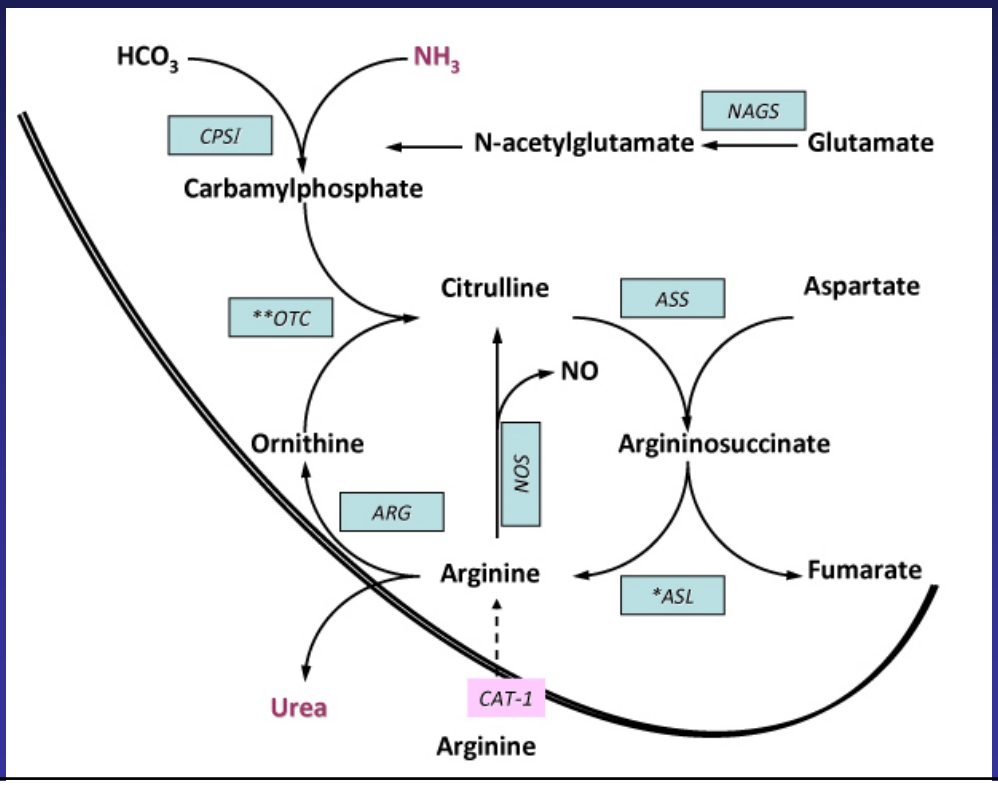
30 Day Placebo controlled Trial

	Group 1 (mean ± SD)			Group 2 (mean ± SD)			Baseline: NO vs placebo (p-value)	Follow-Up: NO vs placebo (p-value)
	Baseline	Follow-Up	Δ	Baseline	Follow-Up	Δ		
BP (mmHg, systole; diastole)	138±12; 84±5	126±12; 78±4	12; 6 reduction (p<0.001)	138±21; 80±8	135±17; 82±8	N.S.	0.19; 0.012	0.26; 0.25
Heart Rate (bpm)	75±9	76±8	N.S.	80±10	79±8	N.S.	0.14	0.33
6-Minute Walk Test (meters)	596±214	650±197	55 improvement (p<0.005)	590±8	606±225	N.S.	0.25	0.35
SF-36v2 (PCS; MCS)	48±10; 40±9	50±8; 45±7	p<0.05	43±10; 37±9	37±11; 37±7	significant worsening (p<0.05)	0.08; 0.06	0.08; 0.03

Biswas et al JCPT 2015

NO Lozenge Rescues Inborn Error in Metabolism

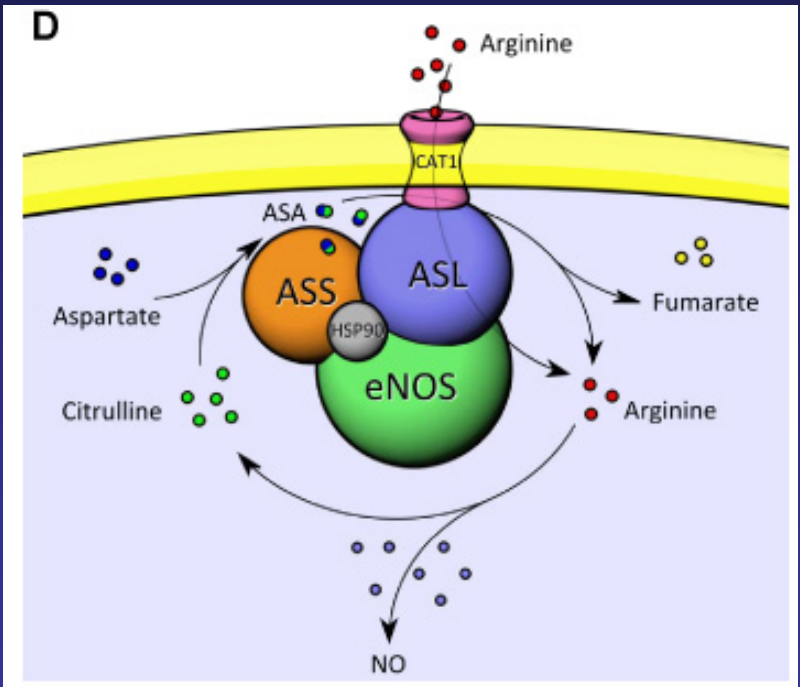
The Urea Cycle converts ammonia to urea for excretion



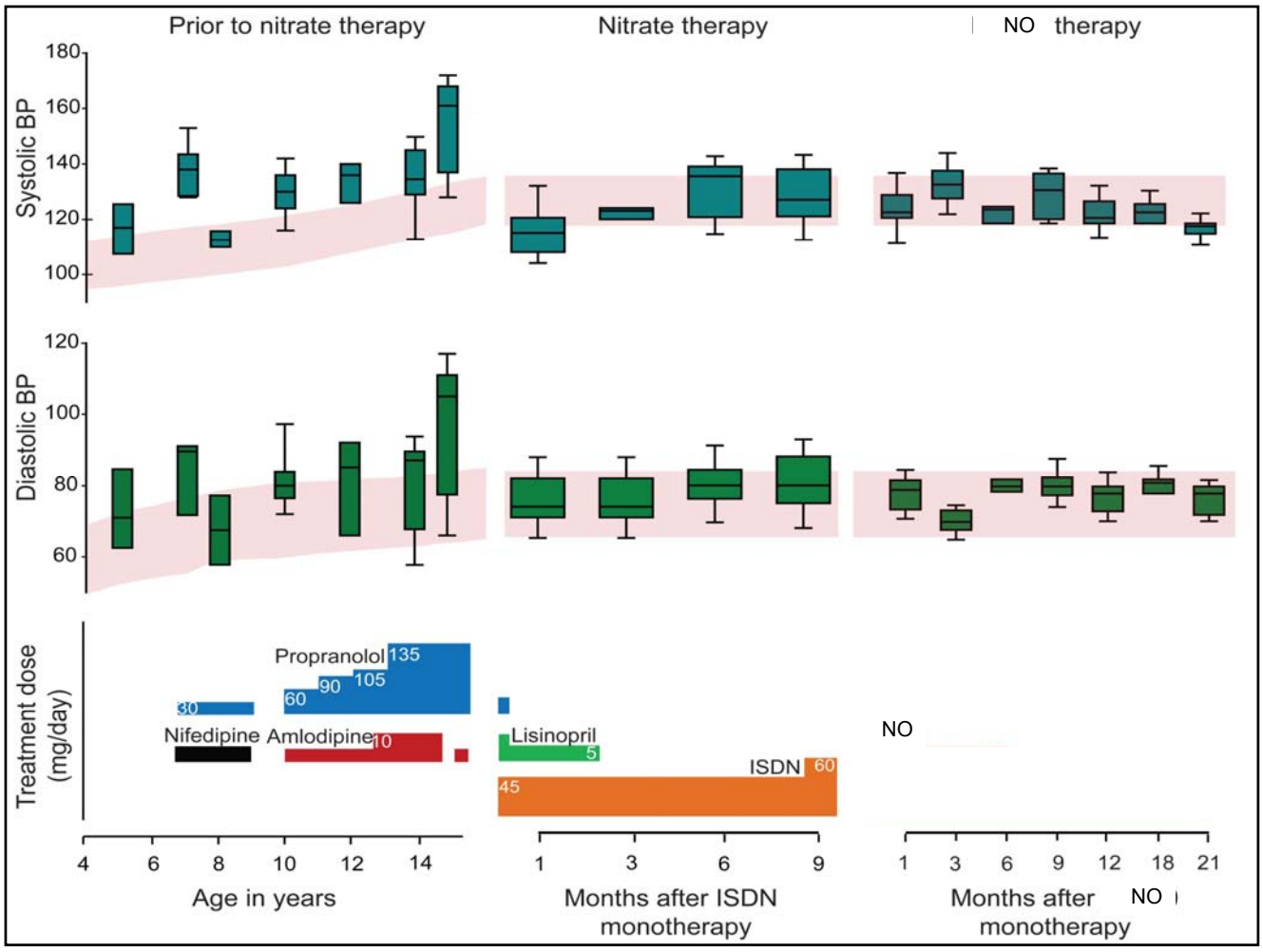
ASL deficiency is an Inborn error in metabolism

- Hyperammonemia
- In addition:
  - Progressive liver dysfunction and cirrhosis
  - Coagulopathy
  - Neurological dysfunction independent of recurrent hyperammonemia
  - Hypertension
  - Renal dysfunction
- More than hyperammonemia?

NOS Utilizes Intracellular L-Arginine from L-citrulline for NO Production



Erez et al Nat Med 2011

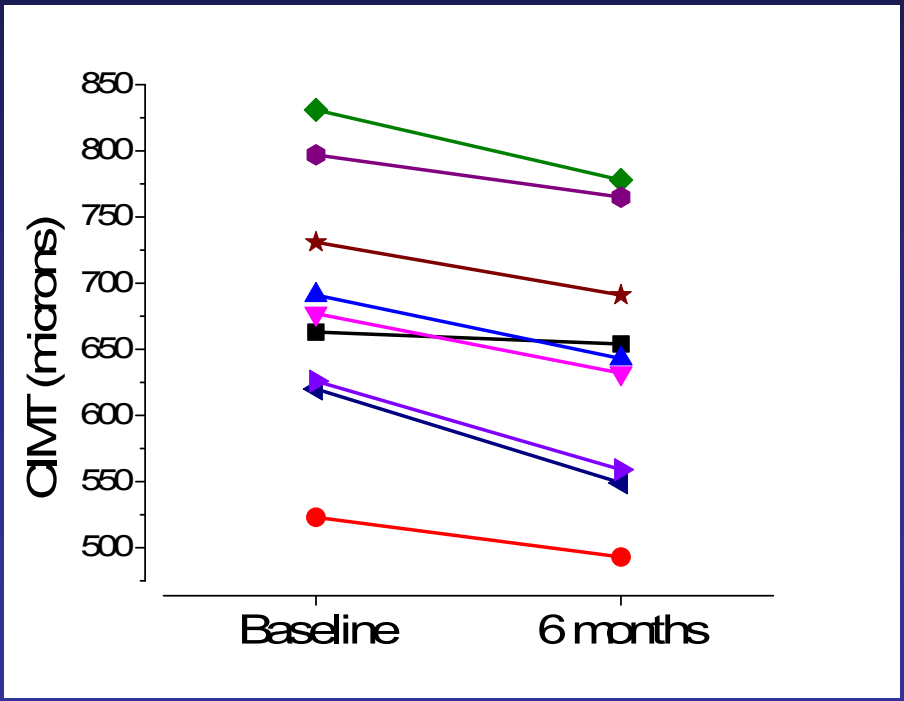

Prior to nitrate therapy
Nitrate therapy
NO therapy
Systolic BP
180
160
140
120
100
Diastolic BP
120
100
80
60
Treatment dose (mg/day)
Propranolol
135
90
105
60
30
Nifedipine
Amlodipine
10
Lisinopril
5
ISDN
60
45
NO
4
6
8
10
12
14
Age in years
1
3
6
9
Months after ISDN monotherapy
1
3
6
9
12
18
21
Months after NO monotherapy

Echocardiogram measurements before and after initiation of NO supplementation.

Left ventricle (LV) parameters	Before NO supplementation (z-score)	After NO supplementation (z-score)
LV diastolic septal thickness	2.26	1.33
LV diastolic dimension	-2.10	-0.36
LV diastolic wall thickness	3.59	2.24
LV systolic septal thickness	4.08	1.94
LV systolic dimension	-2.08	-0.67
LV systolic wall thickness	3.01	1.53

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**

NO Supplement Leads to Plaque Regression



Edwin Lee MD – case report

What are the Experts Saying?

Recently, the oxidative 'waste' products of nitric oxide, nitrite and nitrate, have been evaluated in a new context, due to their own ability form NO independent of nitric oxide synthase enzymes, through reductive electron exchanges. …….

Furthermore we may finally have an explanation for the many known and undisputed benefits of the Mediterranean diet. Perhaps now we should consider nitrite and nitrate as the bioactive food components that account for the protective phenotype of certain foods and diets…

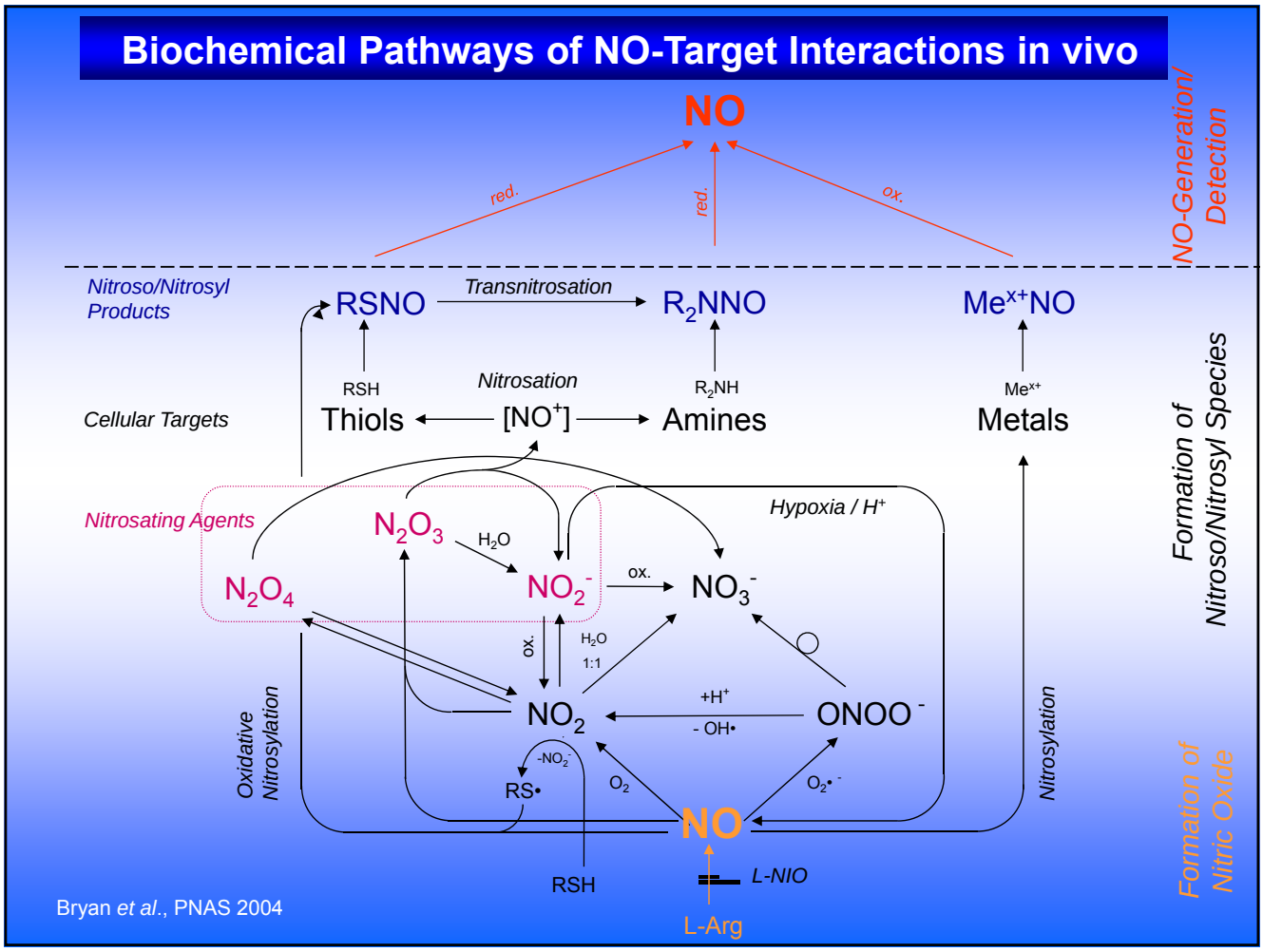
The future use of nitrite/nitrate in dietary considerations will likely have a significant impact on current public health policy.

Louis J. Ignarro, Ph.D.
1998 Nobel Laureate in Medicine
Foreword – Nitrite and Nitrate in Human Health and Disease; Springer Humana Press 2011

Beware of Pretenders!!!

1. Ask for clinical evidence that NO products work
2. Make them show you it works
3. Demand published research on the product

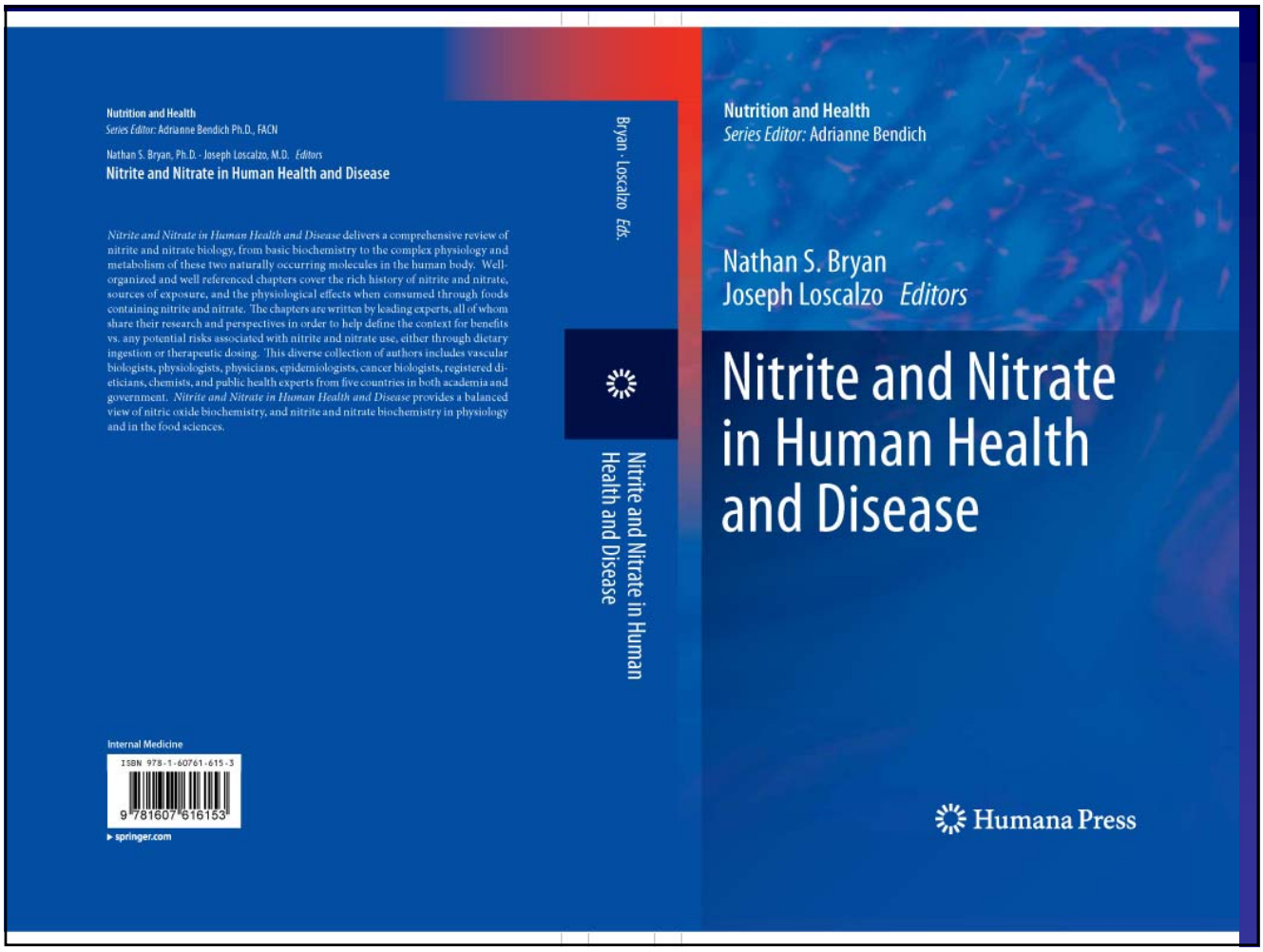
If they cannot provide you these 3 simple requests, then RUN


Biochemical Pathways of NO-Target Interactions in vivo
NO
red.
red.
ox.
NO-Generation/ Detection
Nitroso/Nitrosyl Products
RSNO
Transnitrosation
R2NNO
Mex+NO
RSH
Nitrosation
R2NH
Mex+
Cellular Targets
Thiols
[NO+]
Amines
Metals
Formation of Nitroso/Nitrosyl Species
Nitrosating Agents
N2O3
H2O
Hypoxia / H+
N2O4
NO2-
ox.
NO3-
ox.
H2O
1:1
Oxidative Nitrosylation
NO2
+H+
- OH•
ONOO-
-NO2-
RS•
O2
O2•-
Nitrosylation
NO
RSH
L-NIO
L-Arg
Formation of Nitric Oxide
Bryan et al., PNAS 2004

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.

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)


Nutrition and Health
Series Editor: Adrianne Bendich Ph.D., FACN
Nathan S. Bryan, Ph.D. · Joseph Loscalzo, M.D. Editors
Nitrite and Nitrate in Human Health and Disease
Nitrite and Nitrate in Human Health and Disease delivers a comprehensive review of nitrite and nitrate 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 nitrate, sources of exposure, and the physiological effects when consumed through foods containing nitrite and nitrate. 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 nitrite and nitrate use, either 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 Nitrate in Human Health and Disease provides a balanced view of nitric oxide biochemistry, and nitrite and nitrate biochemistry in physiology and in the food sciences.
Internal Medicine
ISBN 978-1-60761-615-3
9 781607 616153
springer.com
Bryan · Loscalzo Eds.
Nitrite and Nitrate in Human Health and Disease
Nutrition and Health
Series Editor: Adrianne Bendich
Nathan S. Bryan
Joseph Loscalzo Editors
Nitrite and Nitrate in Human Health and Disease
Humana Press

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