Mr Nathan Bryan

Biochemist

Baylor College of Medicine Houston

Texas

Friday, June 9, 2017

8:30 - 8:55 The Role of Nitric Oxide in Primary Care

The Role of Nitric Oxide in Primary Care

Nathan S. Bryan, Ph.D.
Dept. of Molecular and Human Genetics
Baylor College of Medicine
Houston, TX USA

BCM
Baylor College of Medicine

General Practice Conference and Medical Education
Rotorua, NZ – June 8-11, 2017

Financial Disclosures

Founder and Shareholder of HumanN (www.humann.com)

Shareholder and advisor for SAJE Pharma

Receive royalties on patents from University of Texas Health Science Center at Houston

FxMed is exclusive distributor for UT patented NO products in New Zealand and Australia (Booth 16)

Objectives

- Provide an education on Nitric Oxide (NO) Biology
- Understand how to recognize NO deficient patients
- Provide Evidenced Based Therapeutic Solutions to Safely and Effectively Restore NO Production

Who Needs Nitric Oxide Therapy?

Patients with poor diet
Sedentary patients
Anyone over the age of 40
People with circulation issues
Diabetics
People with low energy
People with sexual dysfunction
Athletes wanting to improve performance
Anyone on antacids
Anyone interested in disease prevention

Consequences of NO insufficiency

Hypertension
Insulin resistant diabetes
Atherosclerosis
Thrombosis
Alzheimers (vascular dementia)
Erectile Dysfunction
Peripheral Artery Disease
Immune Dysfunction
Uncontrolled Cell Proliferation – Cancer
Chronic Inflammation
Poor wound healing

What is Nitric Oxide?

The chemical compound **nitric oxide** is a gas with chemical formula NO, with a half life of <1 second.

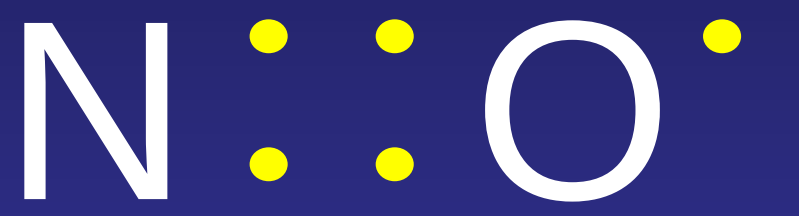
It is one of the most important signaling molecule in the body of mammals including humans, one of the few gaseous signaling molecules known.

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

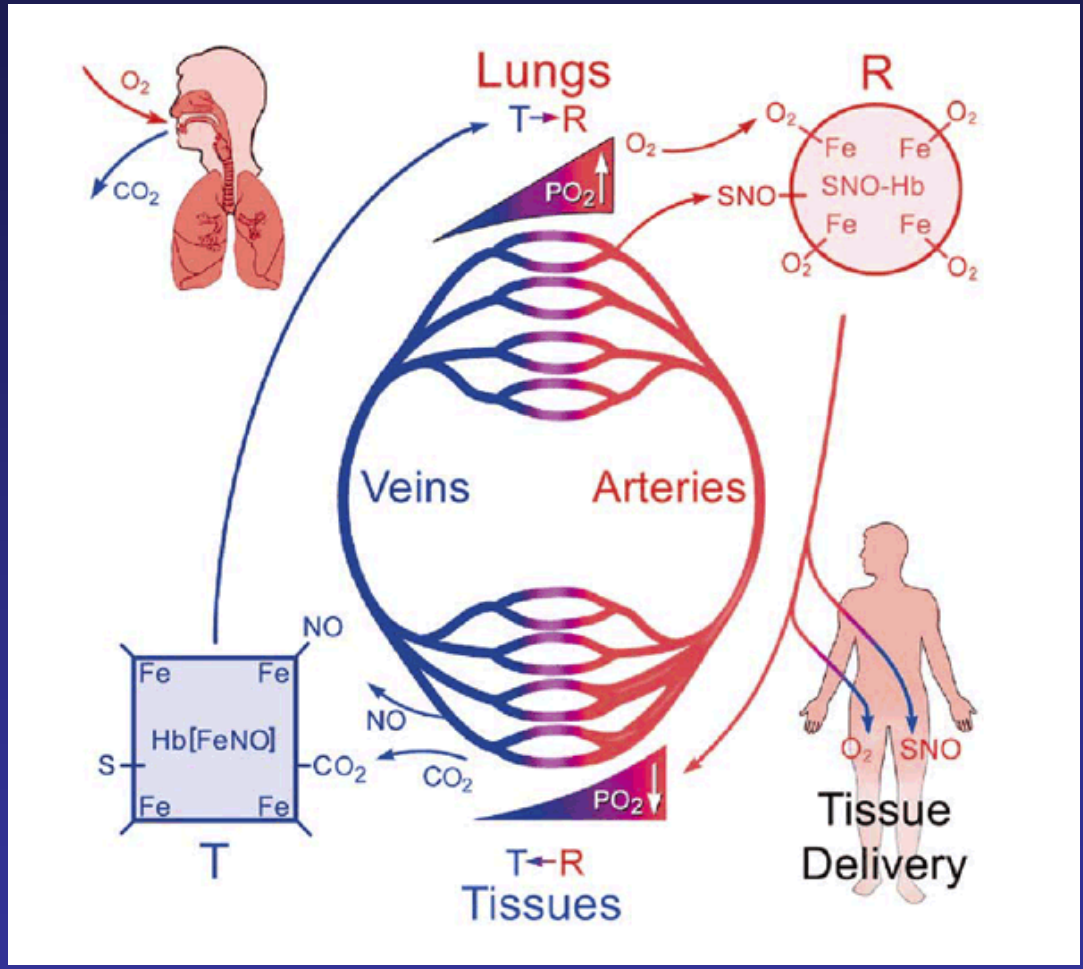
NO should not be confused with nitrous oxide (N_2O), a general anesthetic, or with nitrogen dioxide(NO_2) which is another poisonous air pollutant.

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



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."

When you cannot deliver oxygen to tissues and cells of the body, you are slowly dying

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

Nitric Oxide Plays a Key Role in the Regulation of Numerous Vital Biological Functions

Immunology

Unspecific Immunity
Inhibition of Viral Replication
Transplant Rejection

Gastrointestinal/ Urogen

Peni
Pre-

Respiratory T

Bronchodila
Asthma, AF

Cell Pro

Apopt
Angiogenesis
Tumor Cell Growth

Central Nervous System

Learning and Memory
Pain Sensitization
Epilepsy
Neurodegeneration
Central BP Control

Regeneration

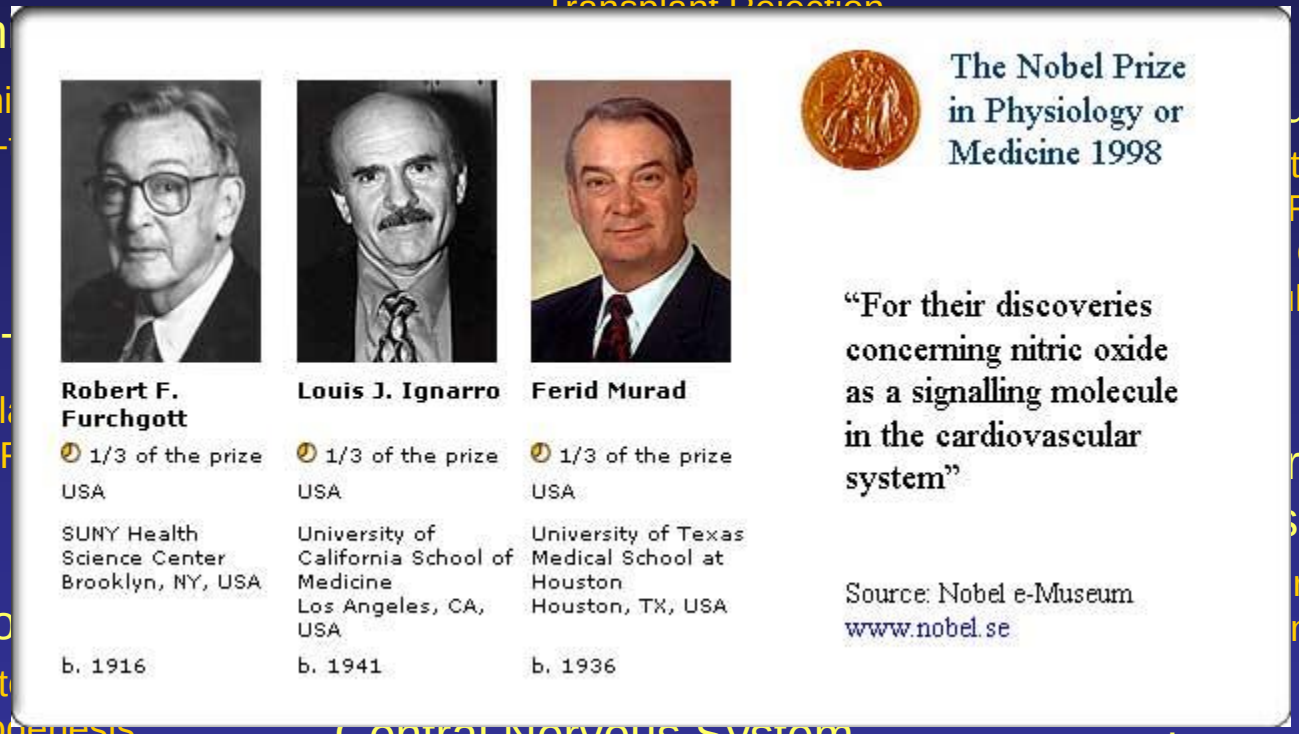
Mobilization of resident stem cells
Targeted differentiation

ular System

tion
Regulation
Contractility
lar Permeability

ral s System

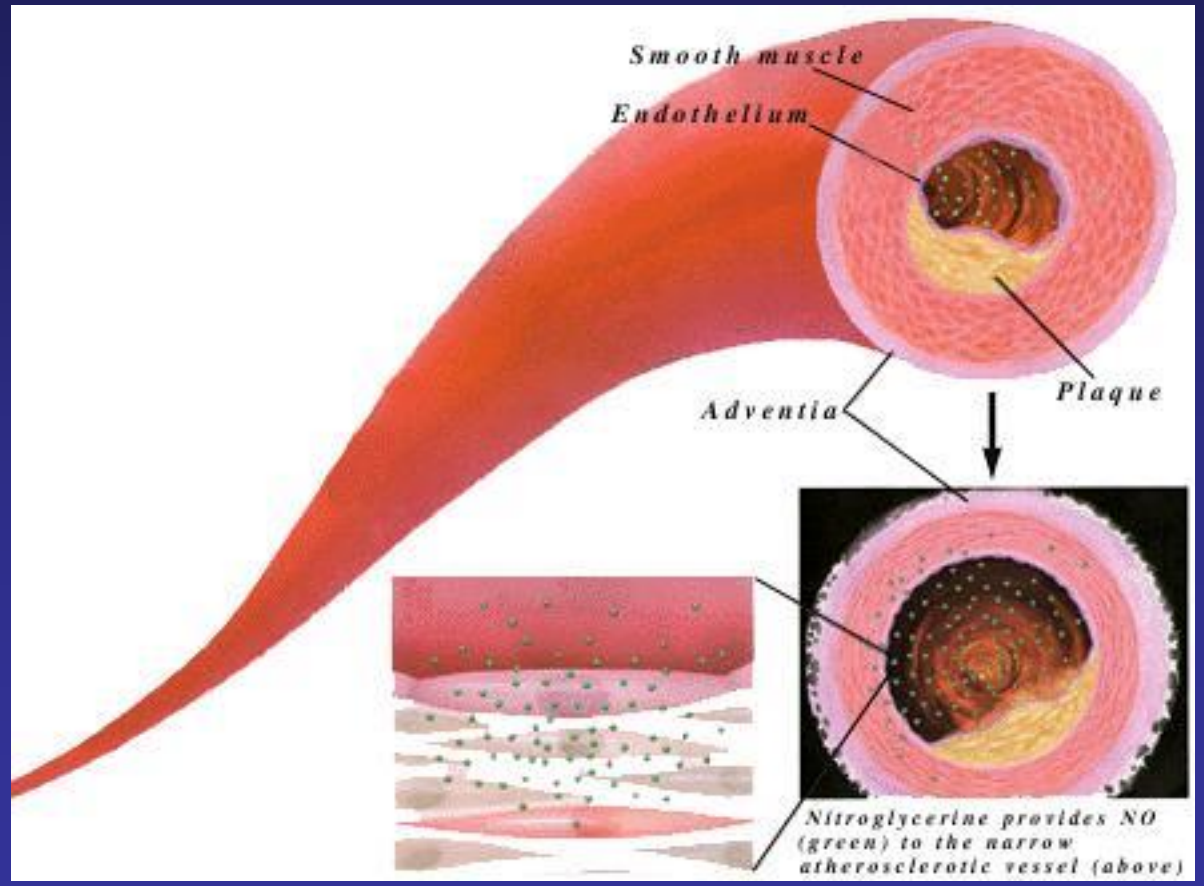
rve-mediated
n



"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

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.

Reduced NO availability is a hallmark of a number of cardiovascular disorders.

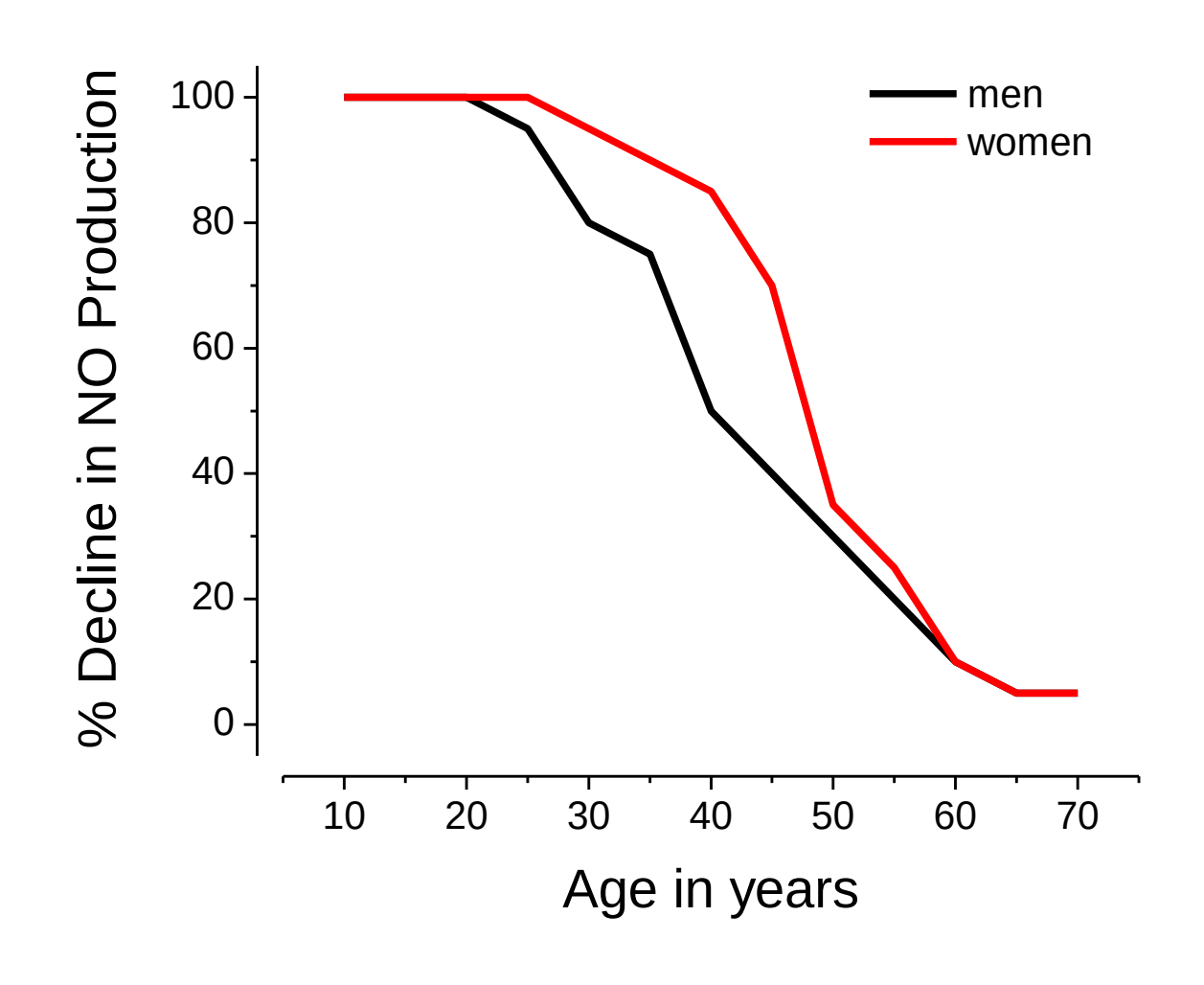
- **Endothelial dysfunction** is a physiological dysfunction of normal biochemical processes carried out by the endothelium, the cells that line the inner surface of all blood vessels including arteries and veins (as well as the innermost lining of the heart and lymphatics).

- 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.

NOS Derived NO Declines With Age



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

How do we control and regulate NO production?

1. Nitric oxide synthase production
2. Dietary nitrate and nitrite ingestion

Current Options for Nitric Oxide Therapies

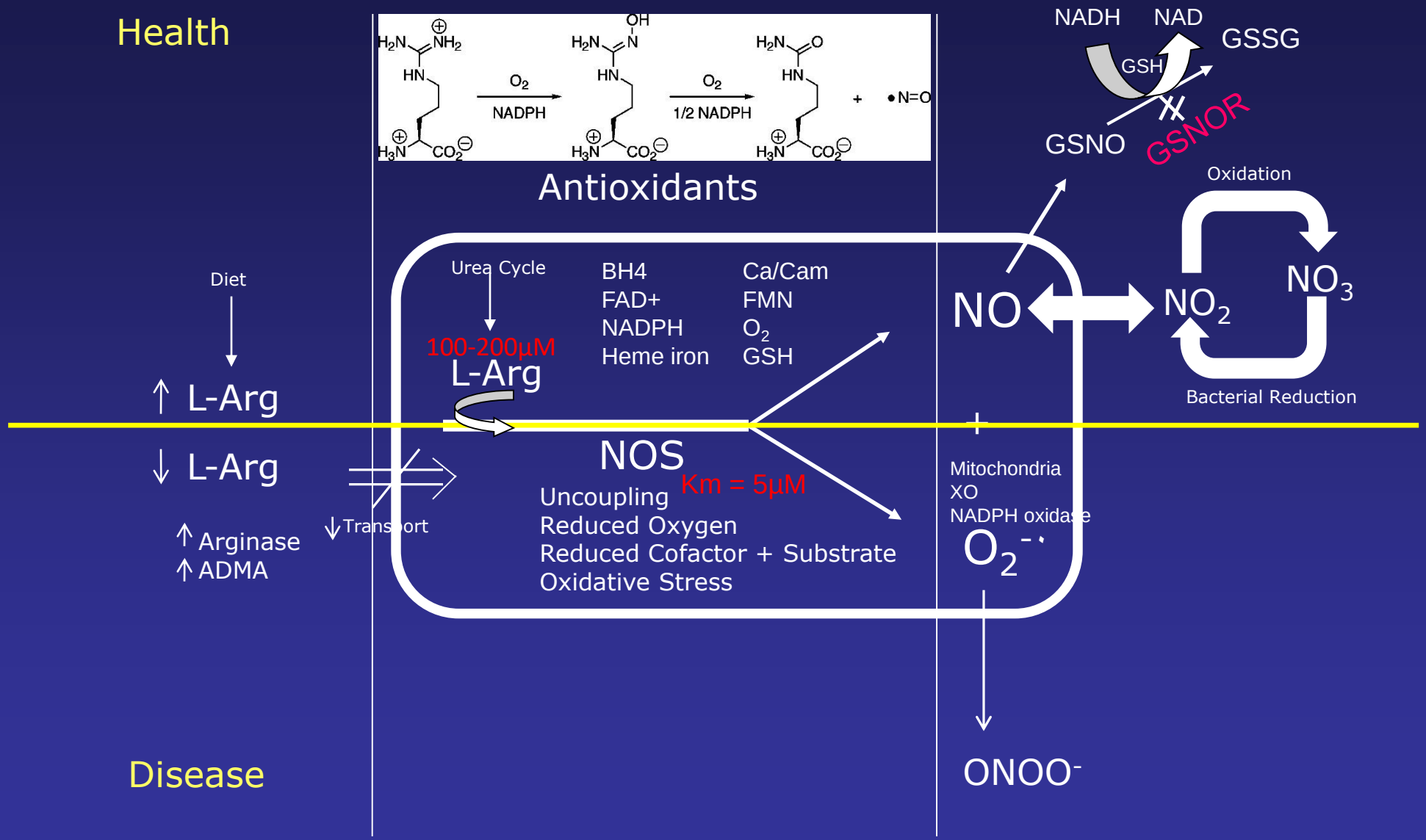
Drug Therapy

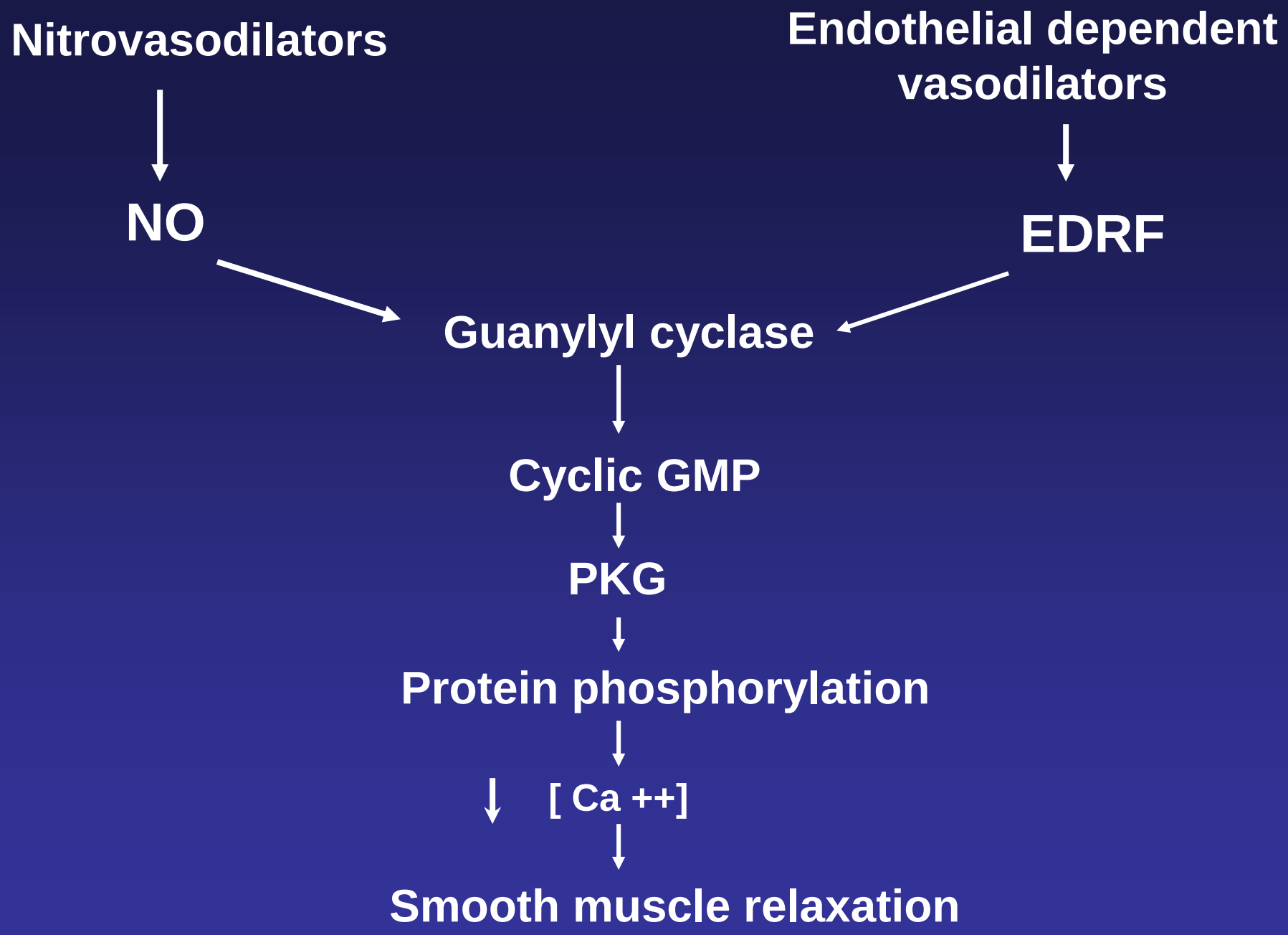
- organic nitrates (used for over 150 years for treatment of angina)
- inhalative NO therapy for neonates with pulmonary hypertension
- phosphodiesterase inhibitors such as sildenafil (Viagra) which act downstream from NO

Lifestyle modification

1. Vegetarian diet (high nitrate green leafy vegetables)
2. Moderate physical exercise (induces NO production – stimulation of eNOS)

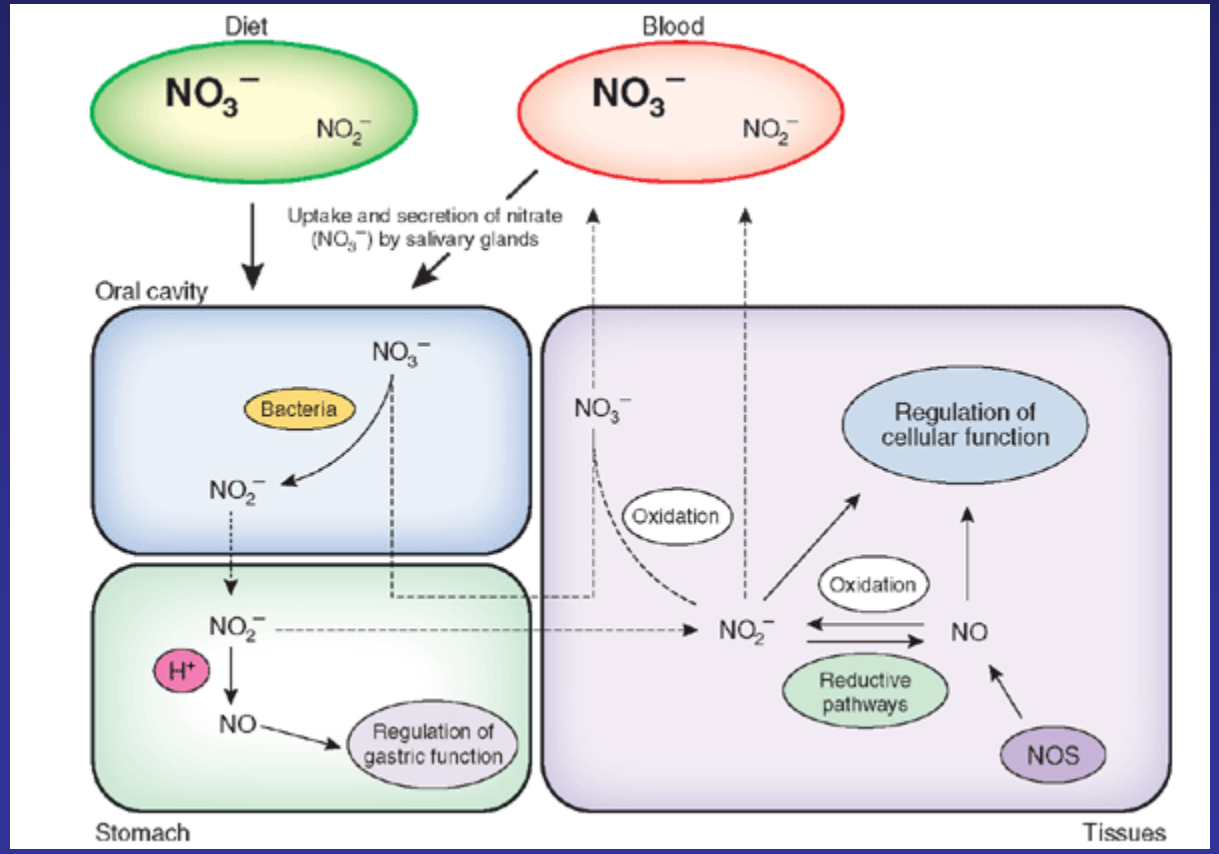
The L-Arginine-Nitric Oxide Pathway




Nitrovasodilators
Endothelial dependent vasodilators
NO
EDRF
Guanylyl cyclase
Cyclic GMP
PKG
Protein phosphorylation
[Ca ++]
Smooth muscle relaxation

New Paradigm - Human Nitrogen Cycle

One-electron reduction is favorable to five-electron oxidation



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.

Dietary Nitrate Can Be Metabolized to Nitrite and NO

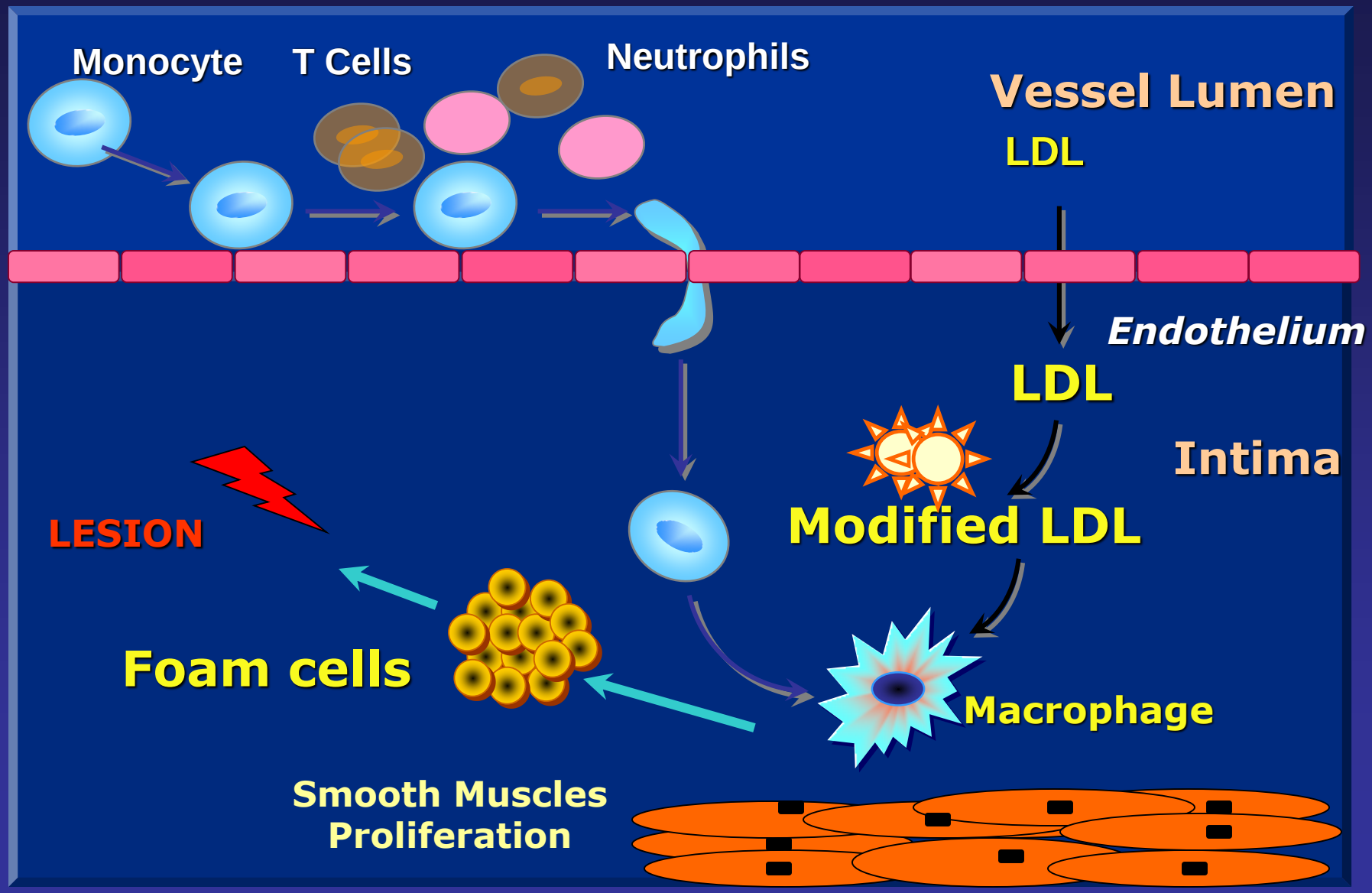
Nitrate, bacteria and human health
Lundberg JO, Weitzberg E, Cole JA, Benjamin N.
Nat Rev Microbiol. 2004 Jul;2(7):593-602

Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite.
Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, **Ahluwalia A**.
Hypertension. 2008 Mar;51(3):784-90.

Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans.
Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, **Jones AM**.
J Appl Physiol. 2009 Oct;107(4):1144-55.

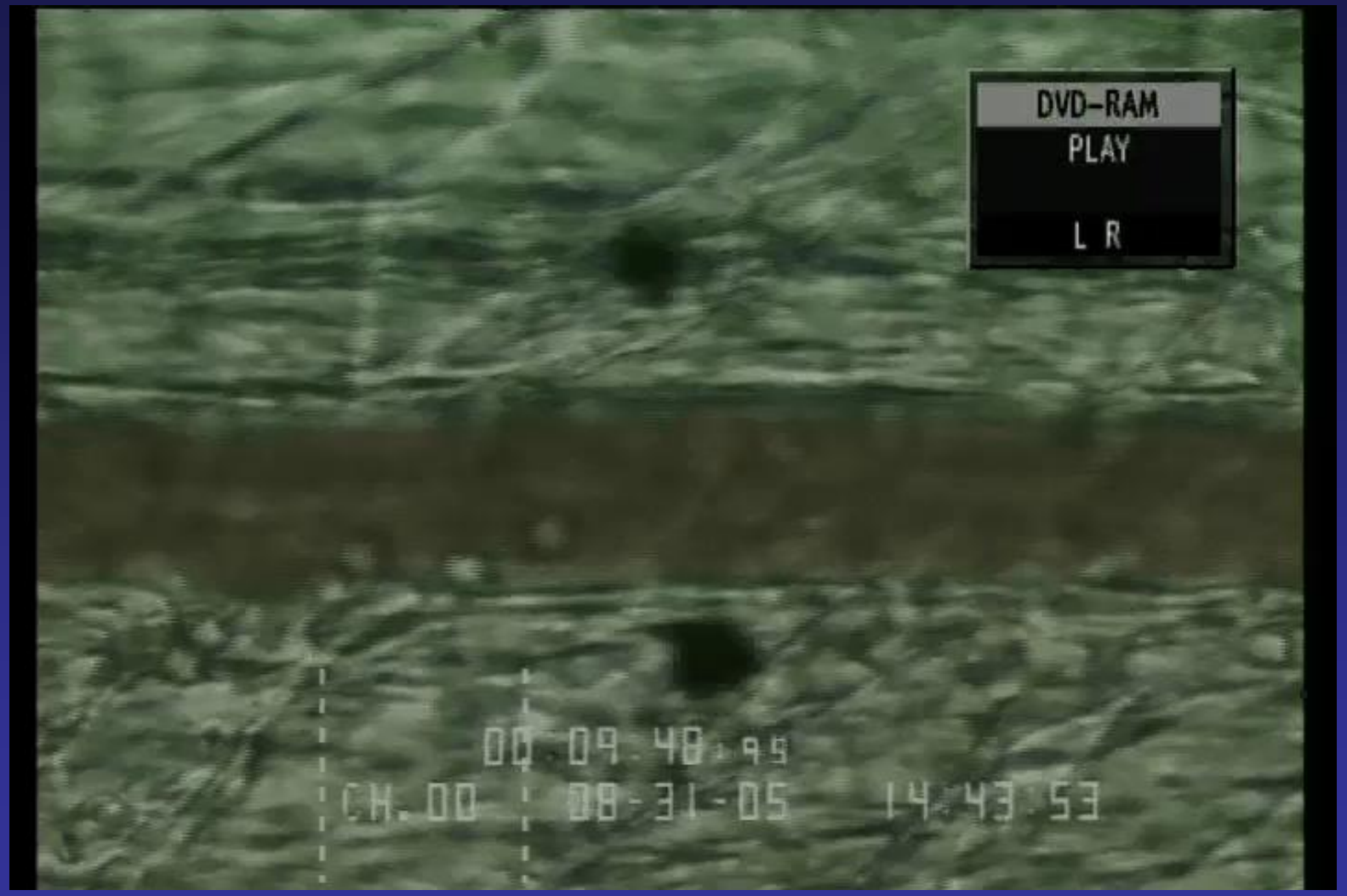
Physiological role for nitrate-reducing oral bacteria in blood pressure control
Kapil V, Haydar SM, Pearl V, Lundberg JO, Weitzberg E, Ahluwalia A.
Free Radic Biol Med. 2013 Feb;55:93-100.

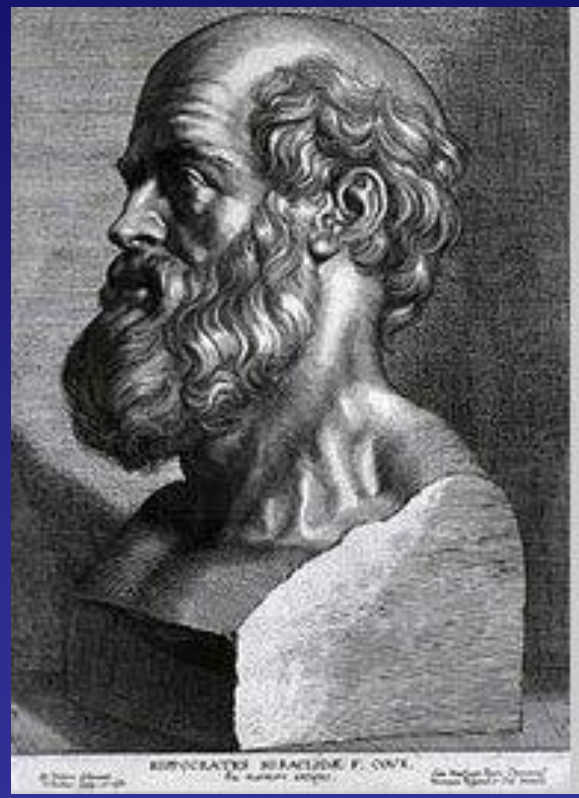
Atherogenesis



Atherogenic Diet

Atherogenic Diet + Nitric Oxide





"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

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

Regional and Category Differences In Vegetable Nitrate Values

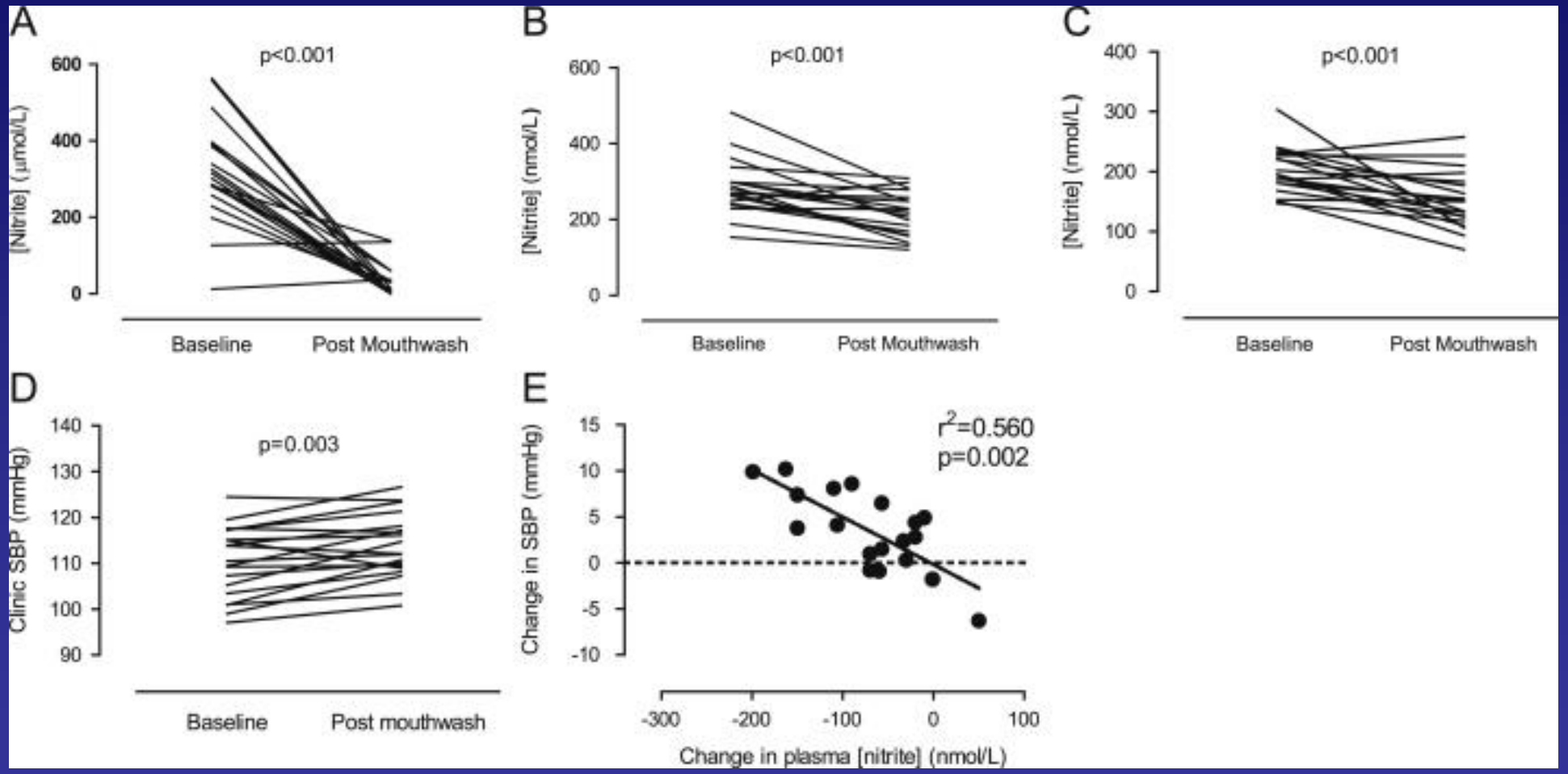
Table 2. Mean nitrate (NO_3?) concentrations[a] (ppm)[b] of raw vegetables classified as conventional from each city

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.

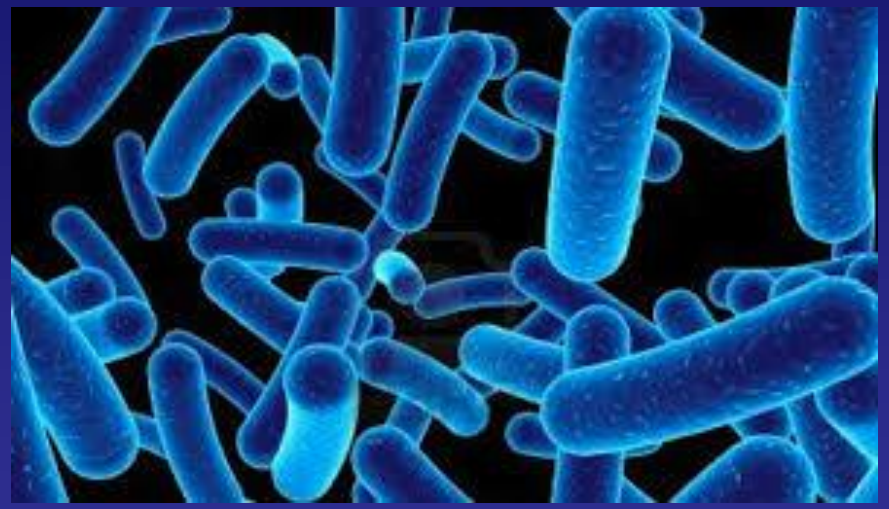
Nitrate is inert in Humans.
Nitrate must be reduced to nitrite
by commensal bacteria

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



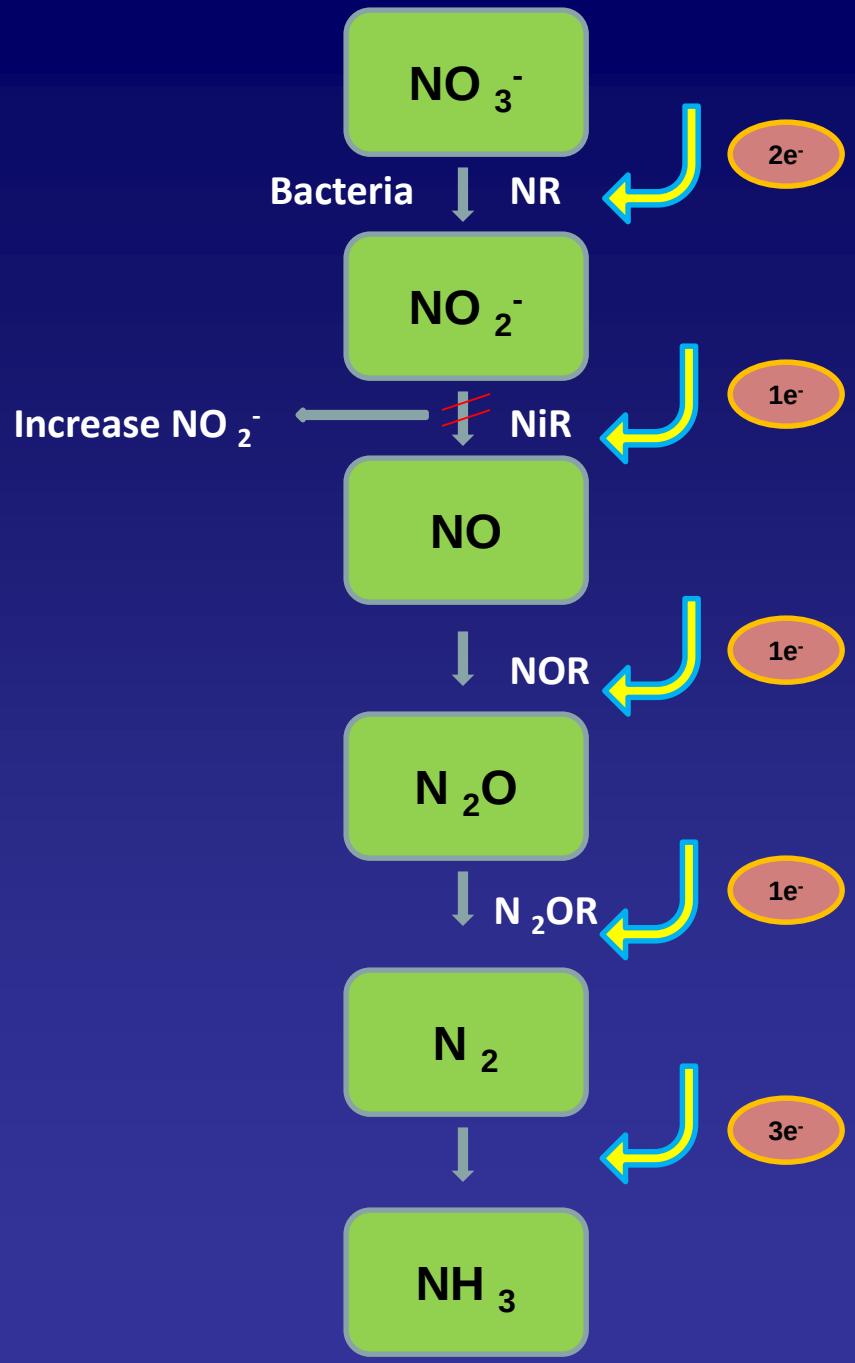
Kapil et al Free Radic Biol Med. 2013 Feb;55:93-100

Genetic Diversity



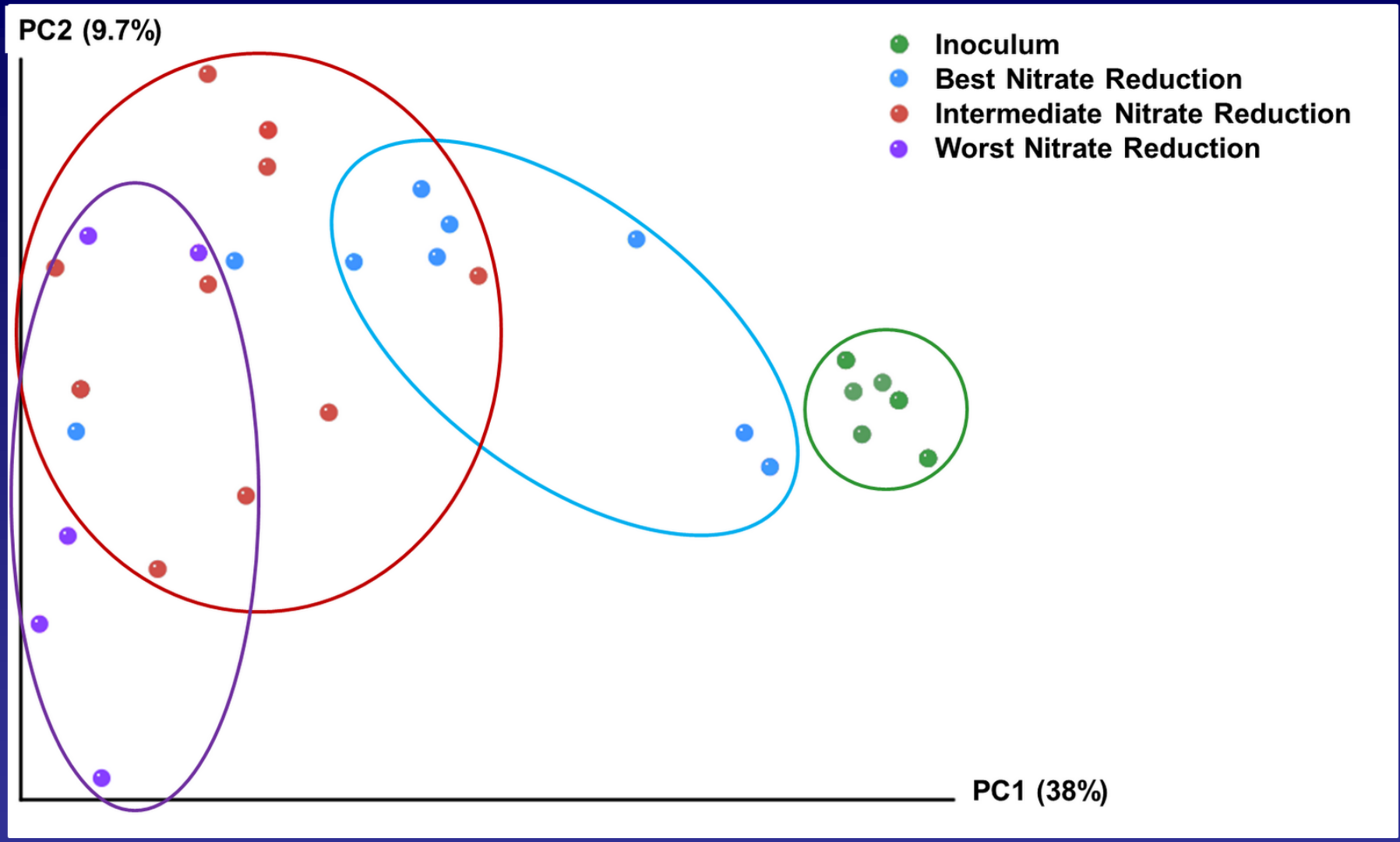
23,000 genes

3,000,000 genes

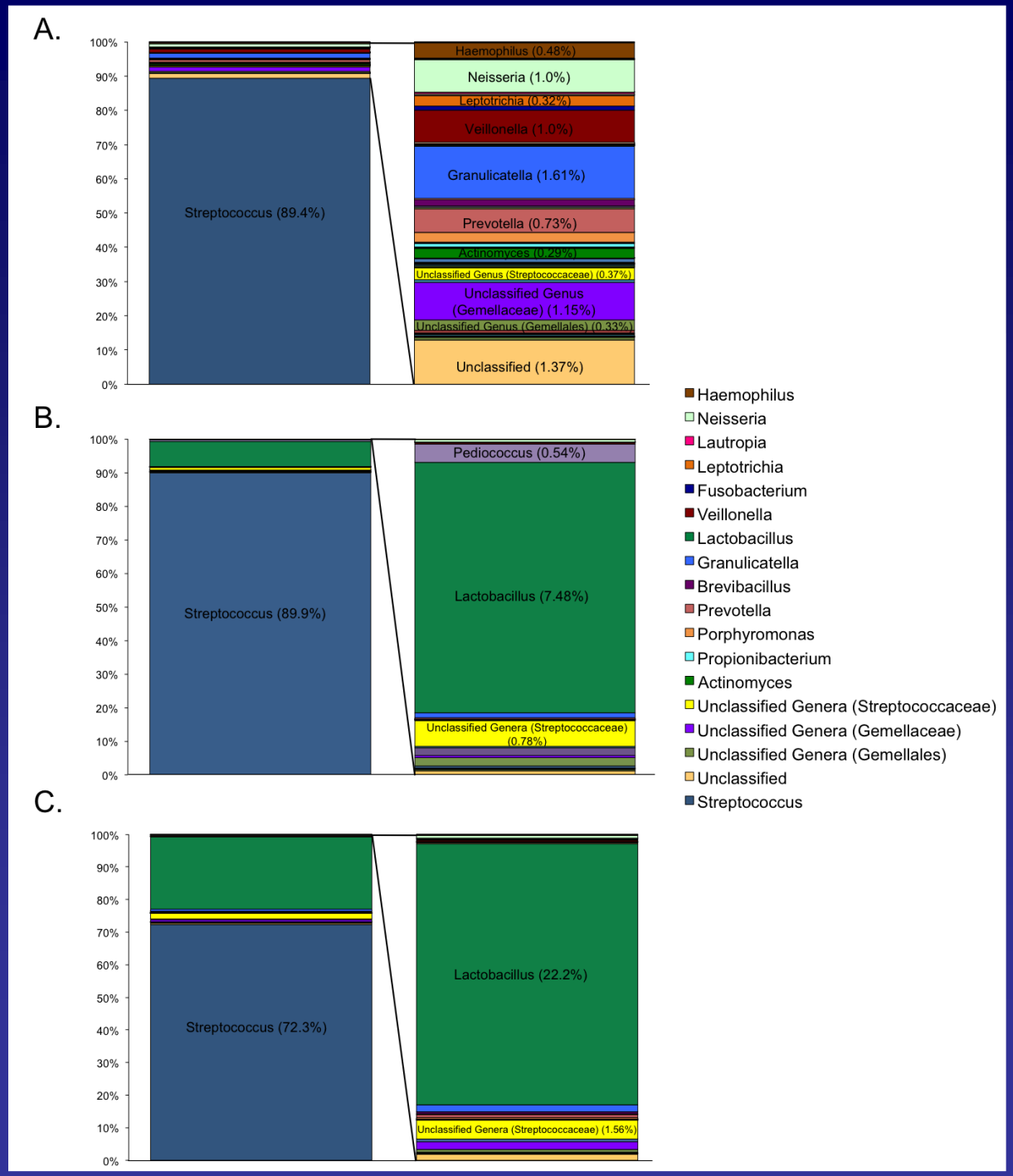


Ideal Community:

- 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)

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, parotidectomy)
4. Lack of oral commensal bacteria to reduce nitrate to nitrite
   (use of antibiotics/antiseptic mouthwash, poor oral hygiene)
5. Insufficient stomach acid production – Achlorhydria
   (use of PPI's, H. Pylori infection, iron overload)
6. Increased oxidative stress that scavenges NO
   (inflammation, mitochondrial dysfunction)

Acid Reflux Drug May Cause Heart Disease

The Wall Street Journal
July 10, 2013

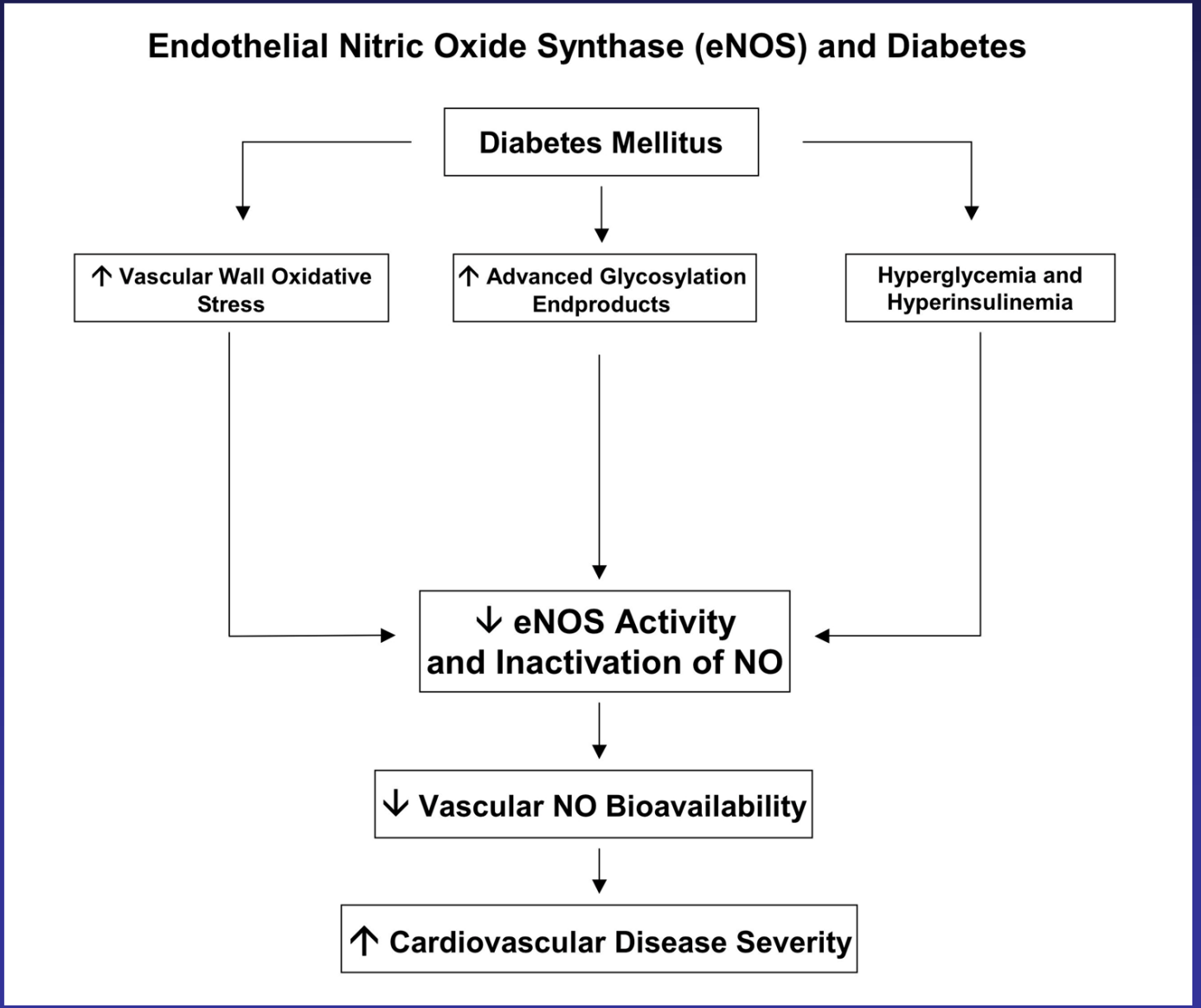
PLoS One. 2015 Jun 10;10(6).

Proton Pump Inhibitor Usage and the Risk of Myocardial Infarction in the General Population.

Shah NH, LePendu P, Bauer-Mehren A, Ghebremariam YT, Iyer SV, Marcus J, Nead KT, Cooke JP, Leeper NJ

CONCLUSIONS:
Consistent with our pre-clinical findings that PPIs may adversely impact vascular function, our data-mining study supports the association of PPI exposure with risk for MI in the general population. These data provide an example of how a combination of experimental studies and data-mining approaches can be applied to prioritize drug safety signals for further investigation

Diabetes Leads to Insufficient NO Production



Insufficient NO Production Leads to Diabetes

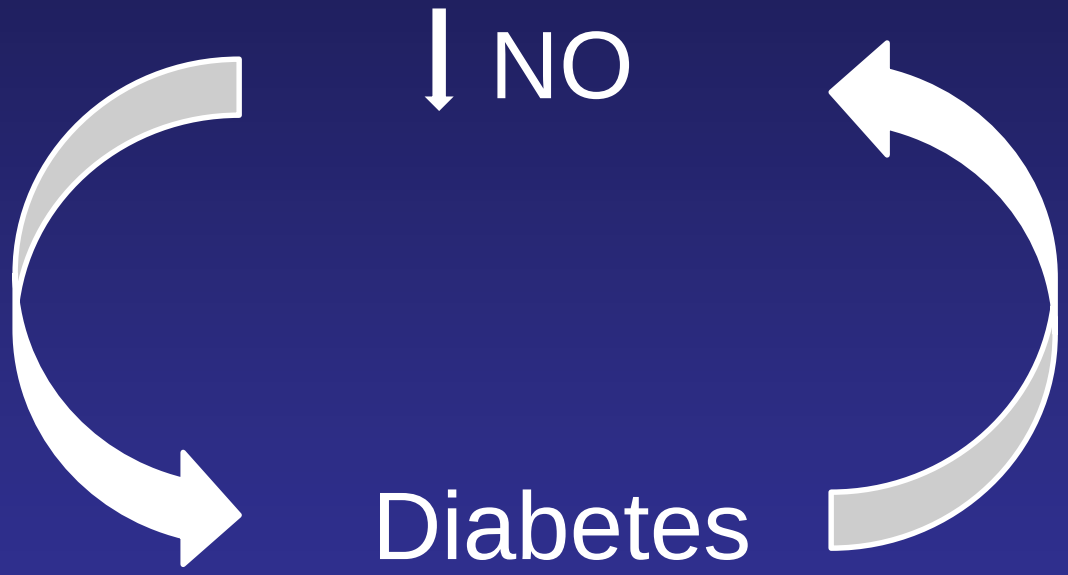
eNOS$^{-/-}$ mice display a disturbed blood-glucose concentration curve

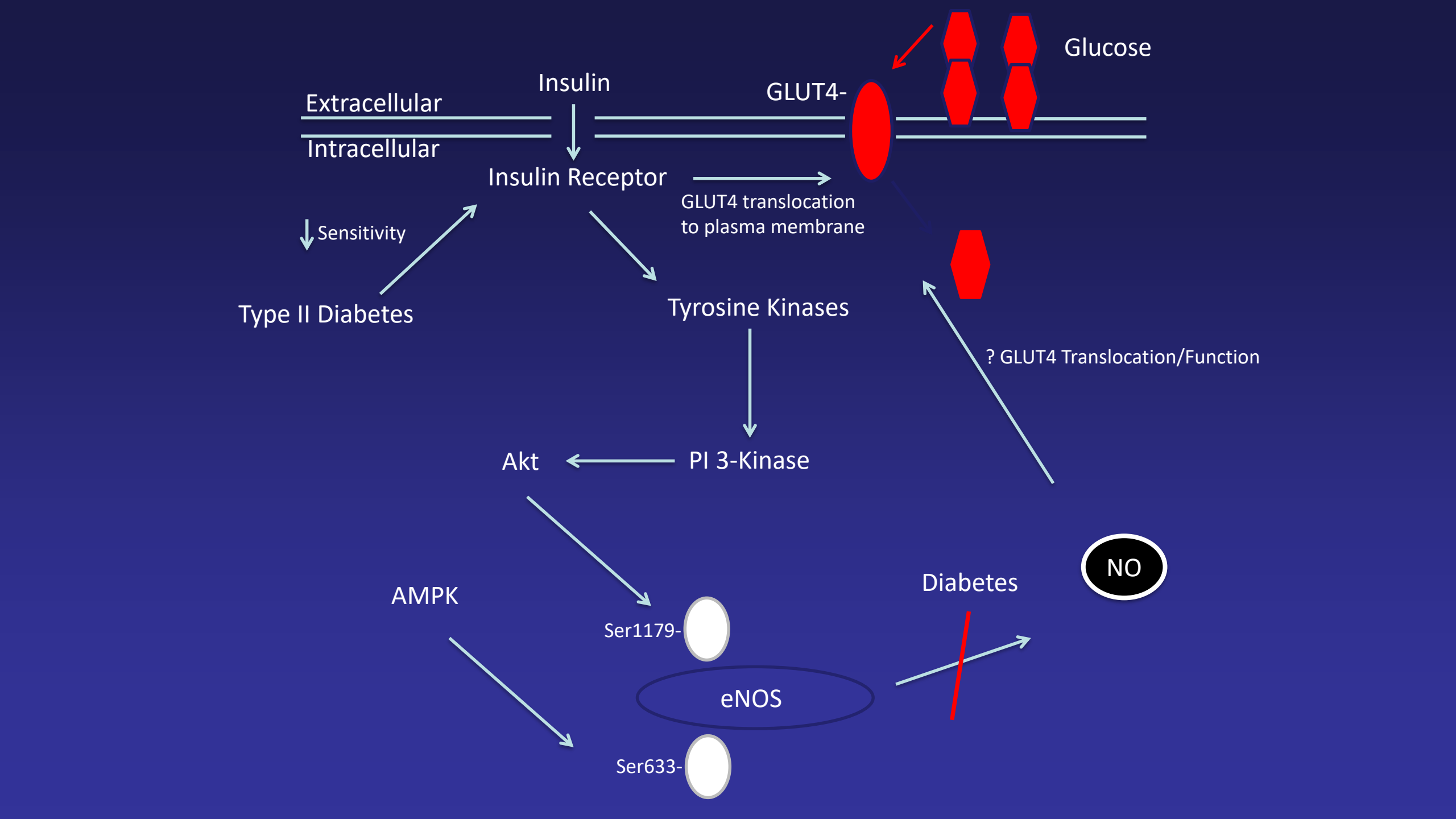
eNOS-/- mice have high proinsulin/insulin ratios

eNOS-/- mice have higher visceral fat

Diabetic patients suffer from higher incidence of hypertension, heart disease and stroke, high blood pressure, blindness, kidney disease, nervous system disease, amputation, and complications of pregnancy and surgery – all conditions associated with insufficient NO production.

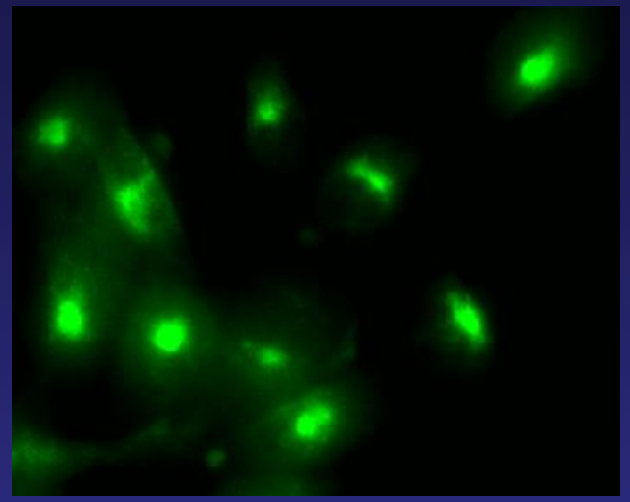
Perpetual Cycle of NO and Diabetes



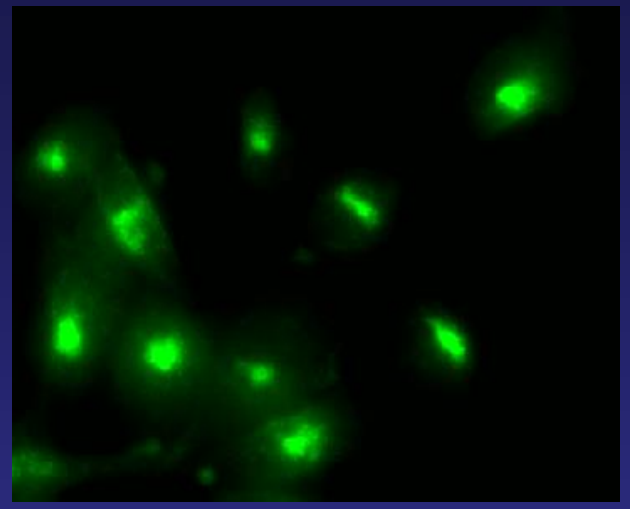

Glucose
Insulin
GLUT4-
Extracellular
Intracellular
Insulin Receptor
GLUT4 translocation
to plasma membrane
Sensitivity
Type II Diabetes
Tyrosine Kinases
? GLUT4 Translocation/Function
Akt
PI 3-Kinase
NO
Diabetes
AMPK
Ser1179-
eNOS
Ser633-

Control

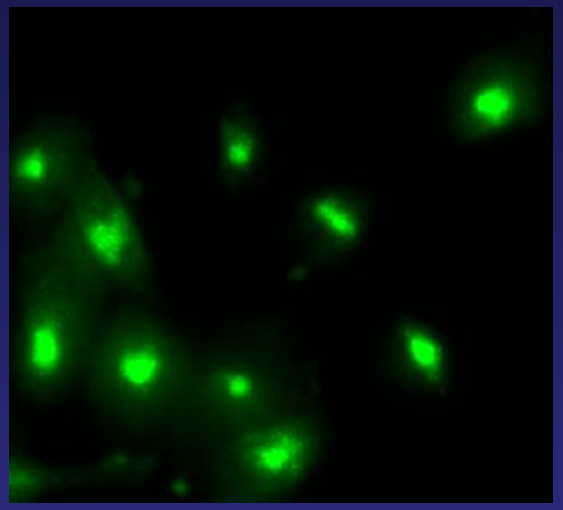
0 min



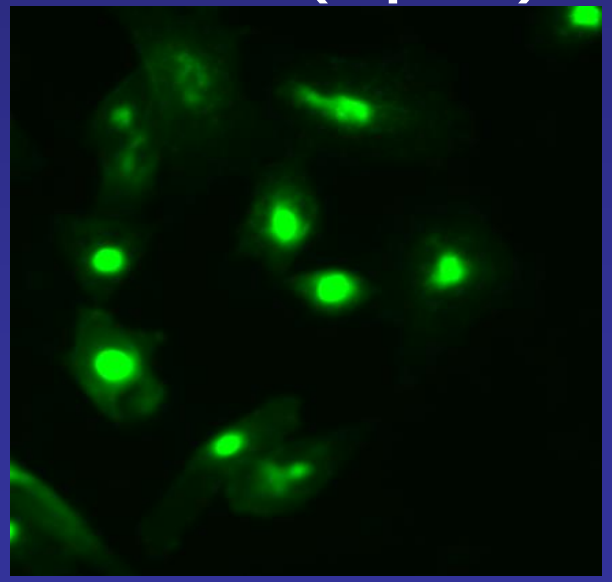
10 min

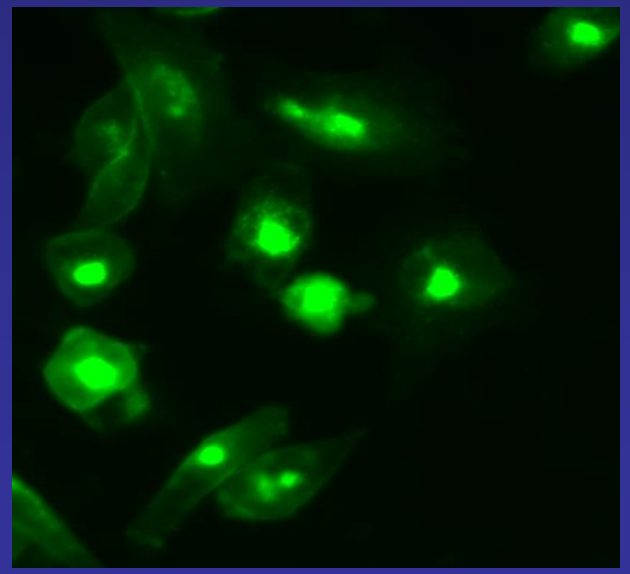


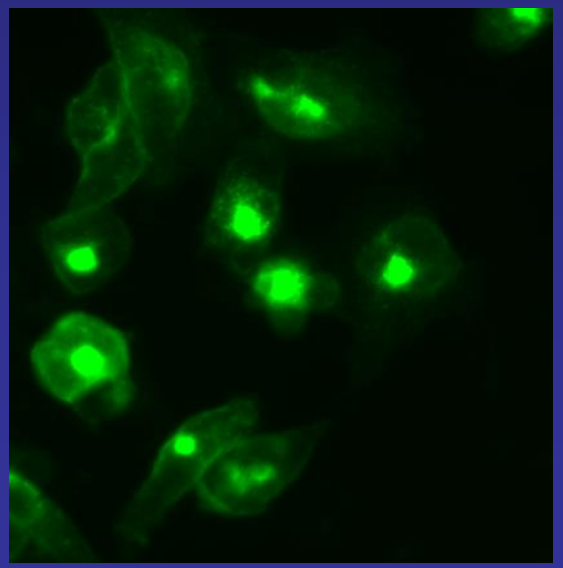
30 min

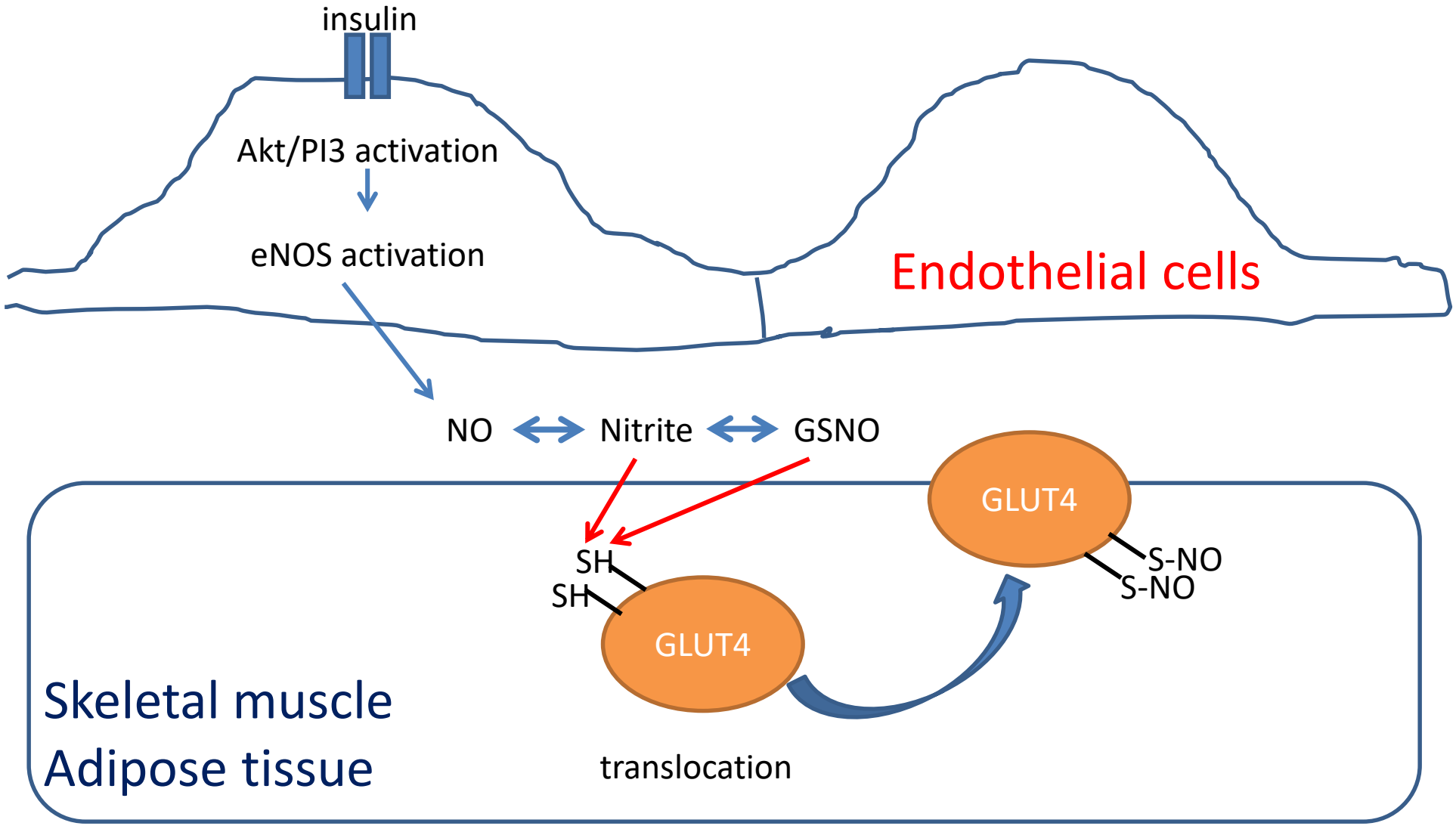


GSNO (1µM)



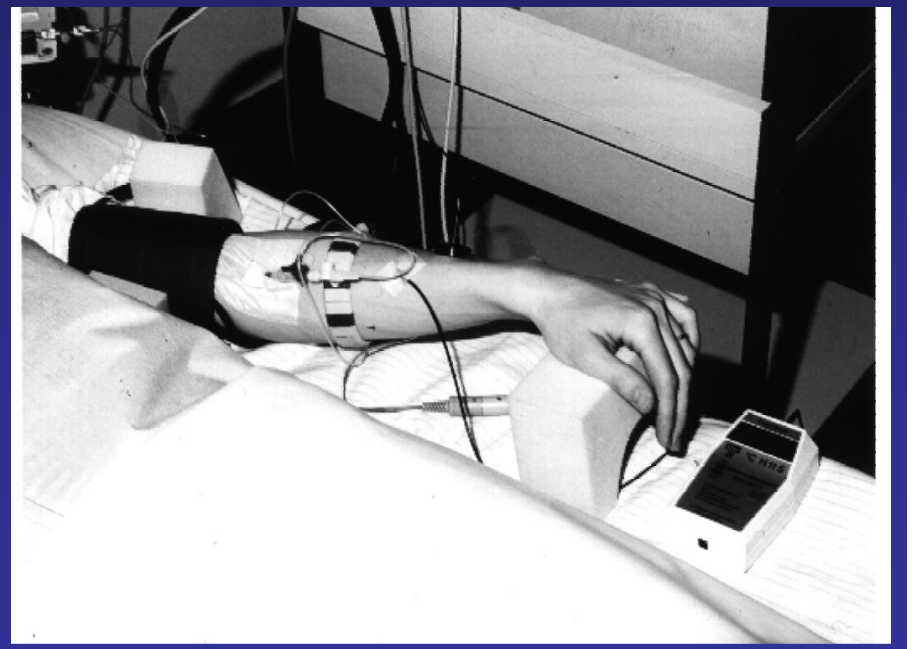


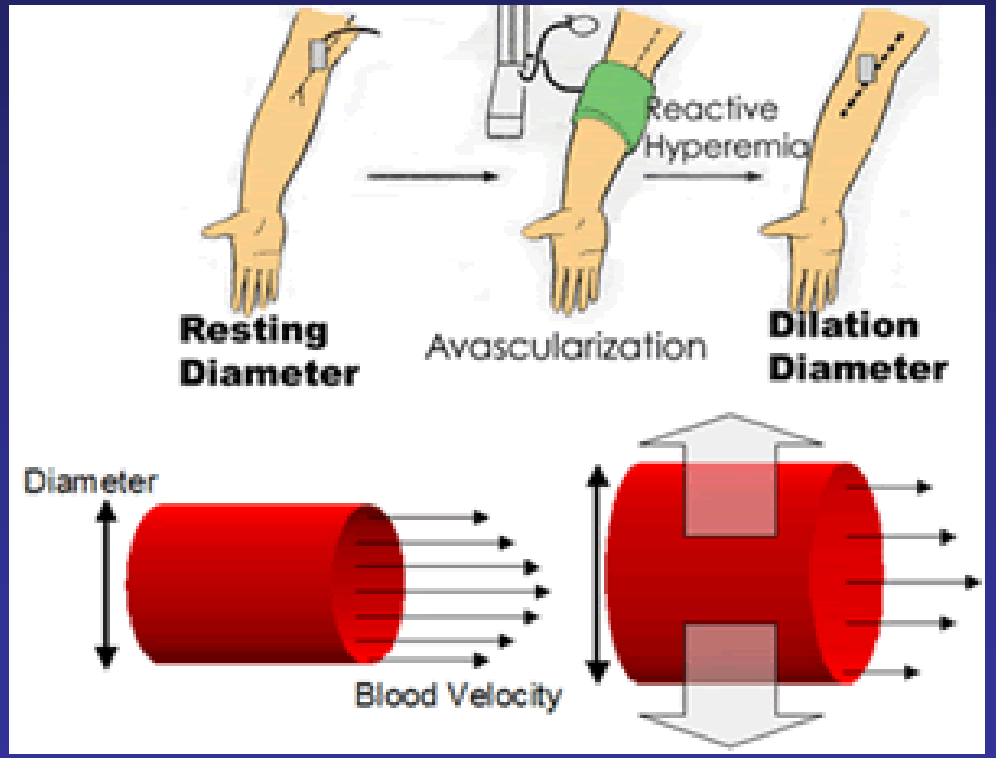



insulin
Akt/PI3 activation
eNOS activation
Endothelial cells
NO
Nitrite
GSNO
GLUT4
SH
SH
S-NO
S-NO
GLUT4
Skeletal muscle
Adipose tissue
translocation

NO Diagnostics

Flow Mediated Dilatation for Endothelial Function



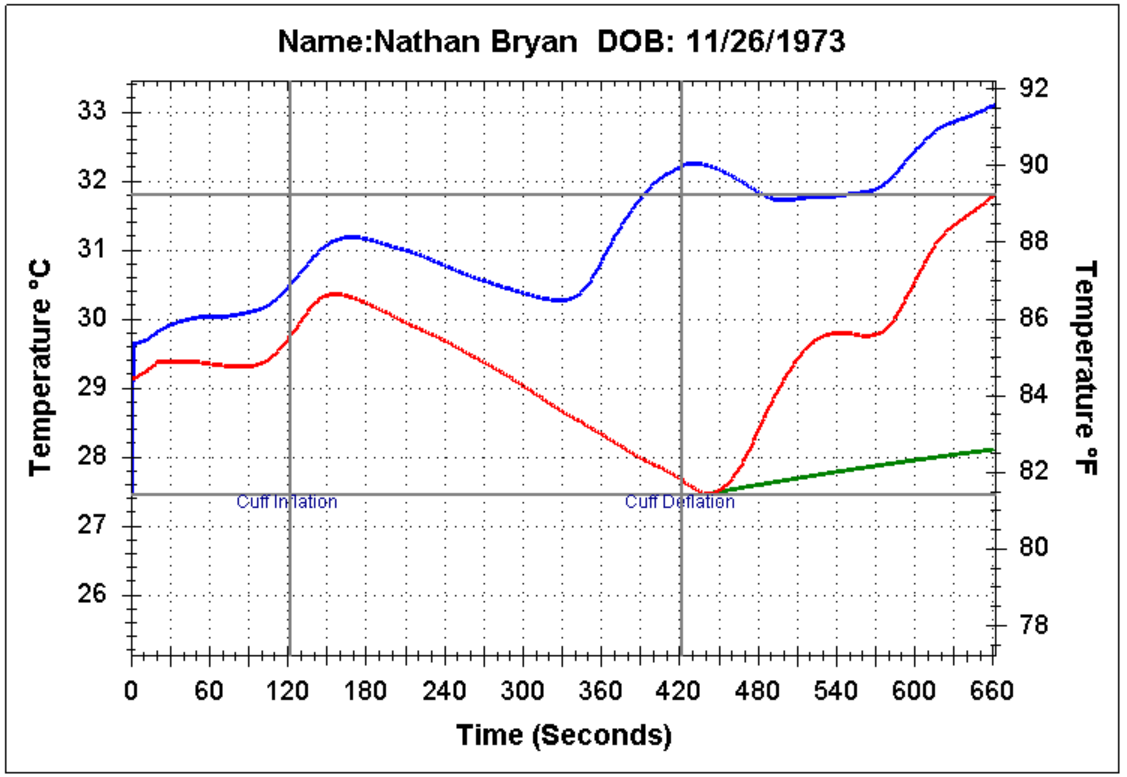


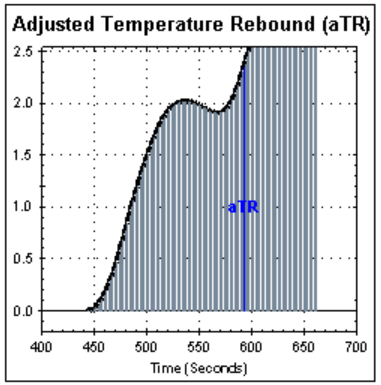
VENDYS®

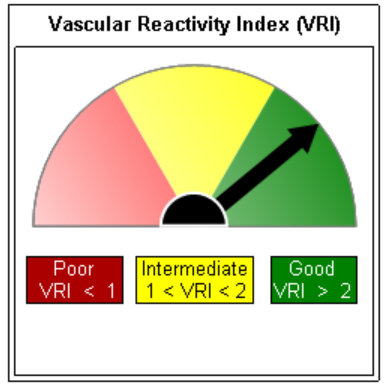
Vascular Function Test

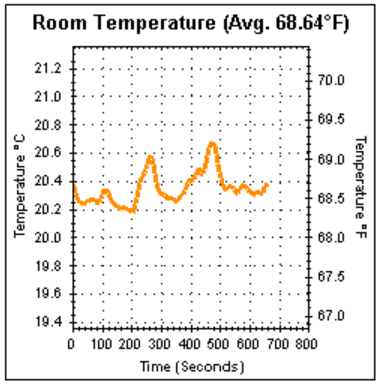
Digital Thermal Monitoring of Endothelial Function and Vascular Reactivity

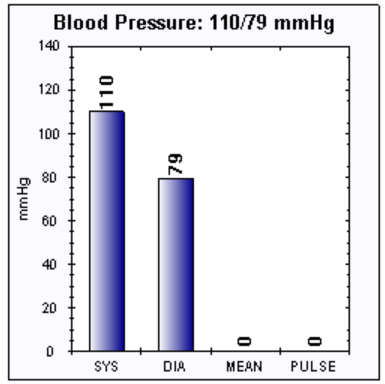
Right Finger Temperature — Left Finger Temperature — Zero Reactivity Curve











Quality Check:

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

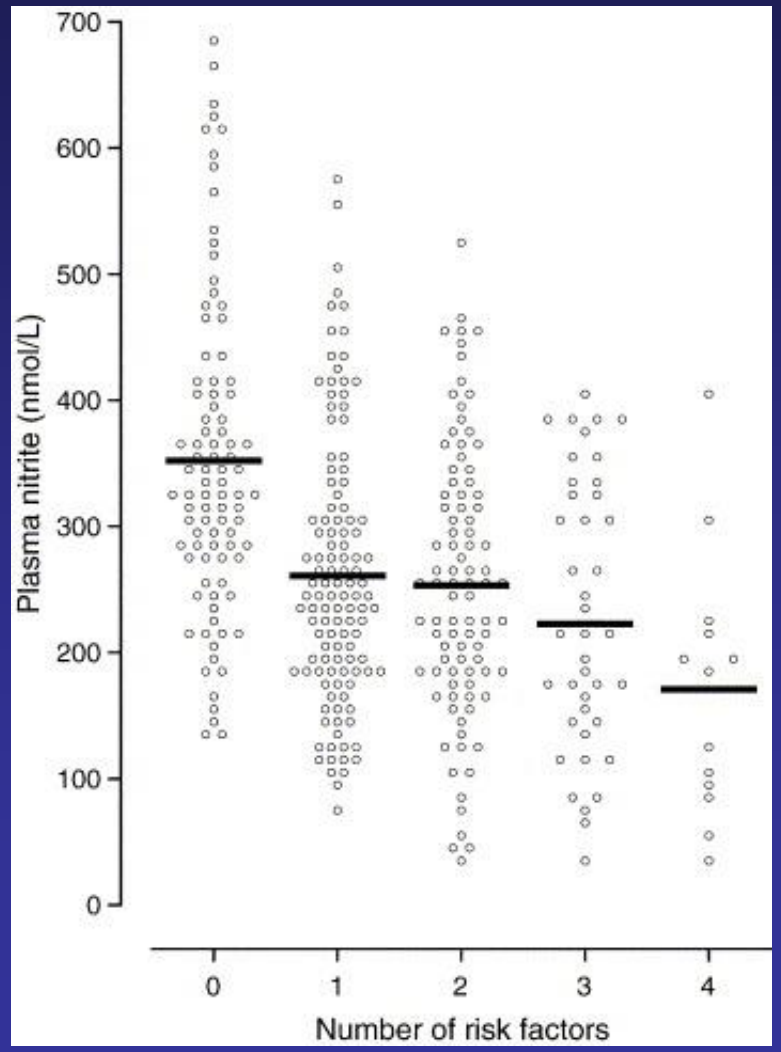
*Note: Cold Finger Flag check was disabled.

Vascular Reactivity Index
VRI = 2.34

ENDOTHELIX

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

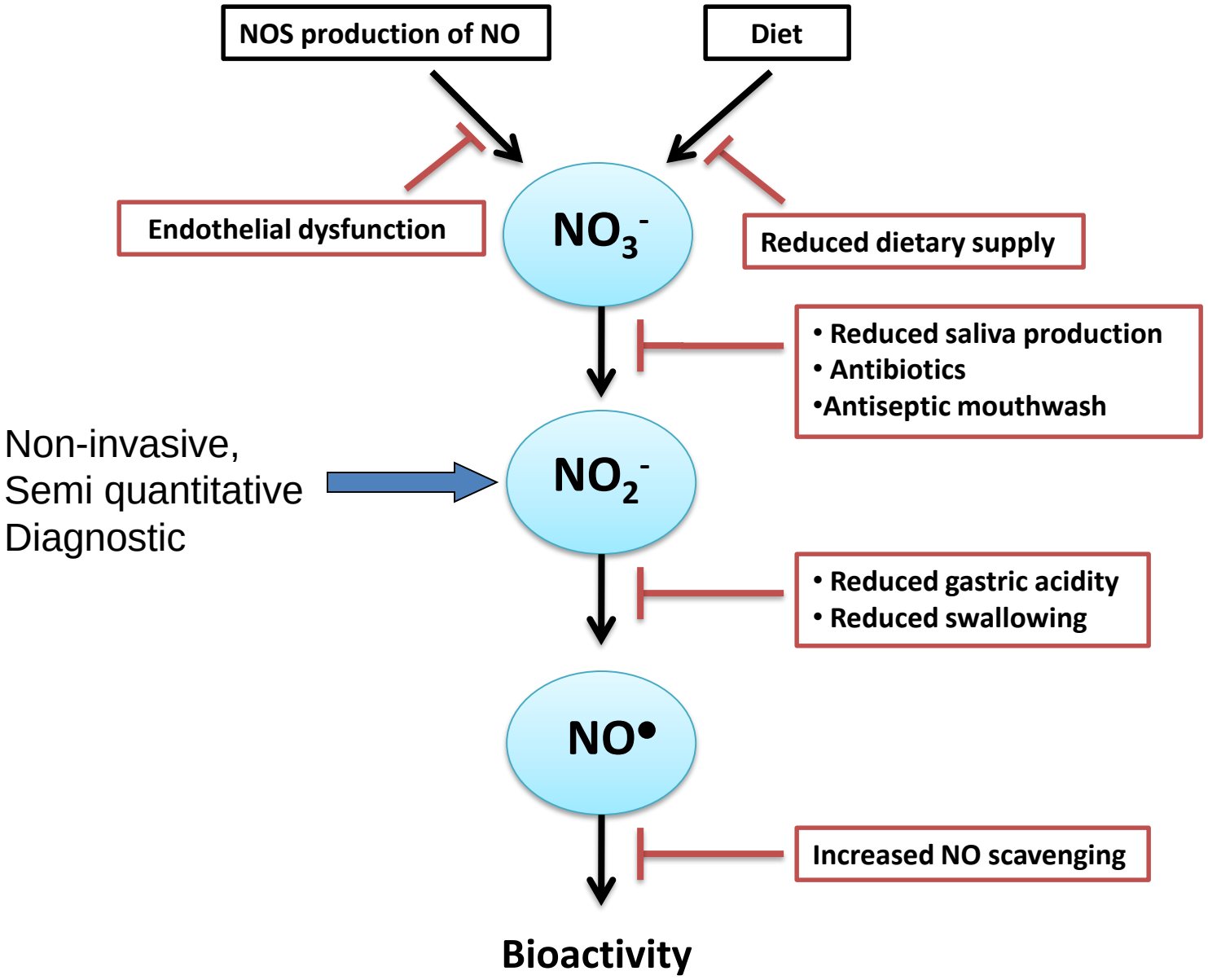
Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans.



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



Nitric oxide synthase-derived plasma nitrite predicts exercise capacity

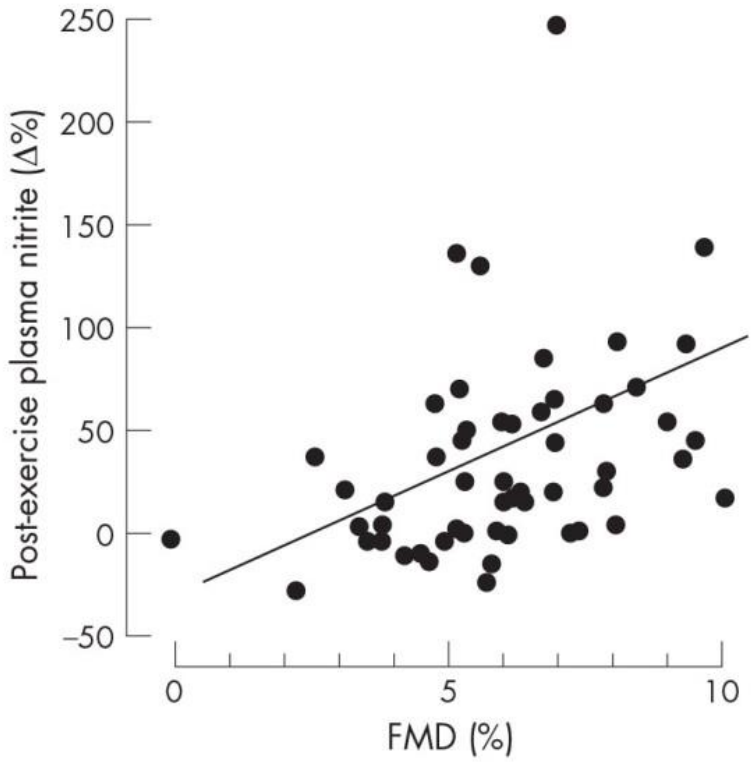


Figure 1 Correlation of percentage increases in plasma nitrite after ergometric exercise with flow-mediated dilation (FMD) ($r = 0.36$; $p = 0.01$).

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

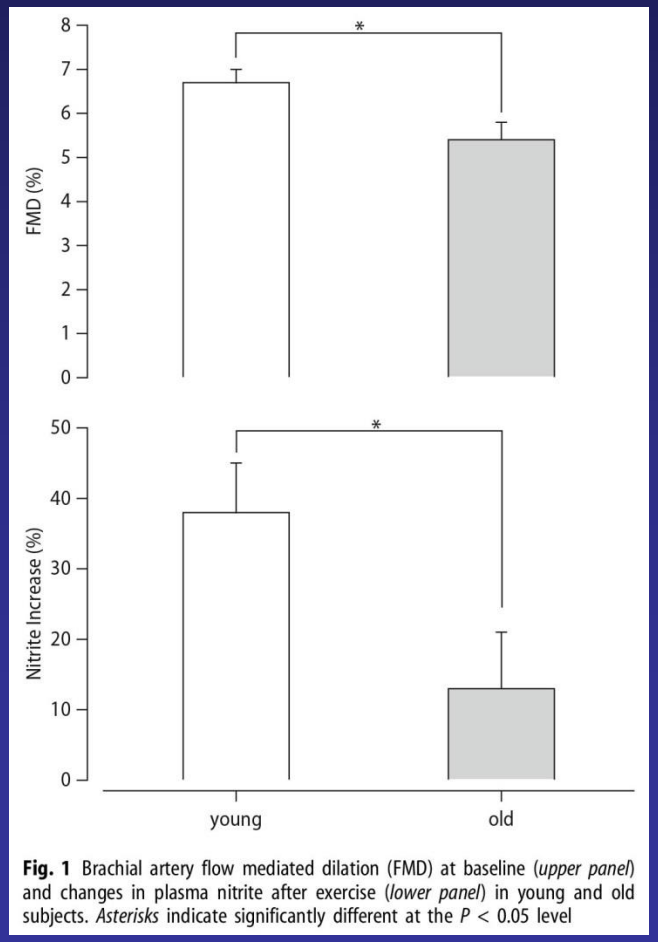
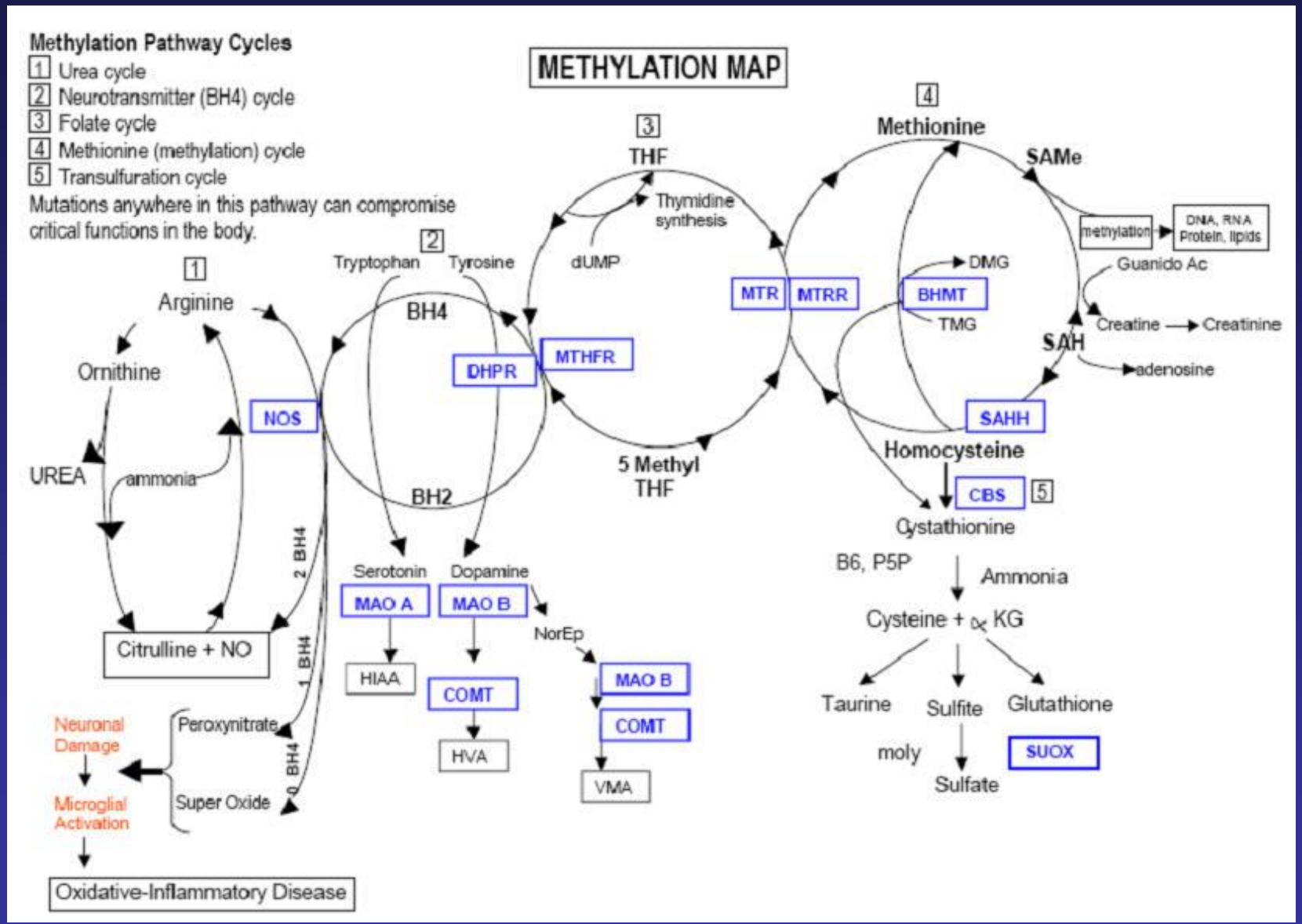


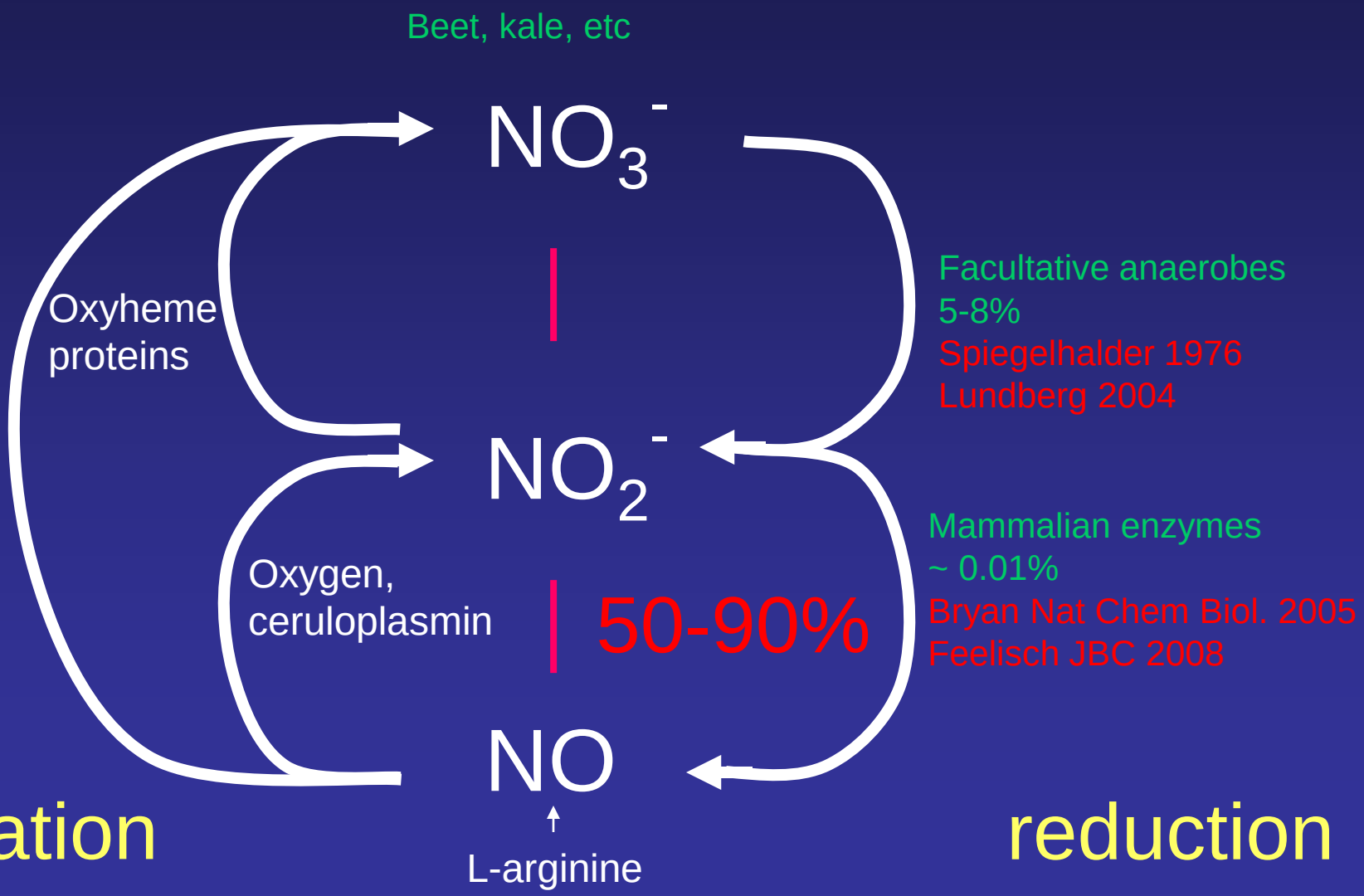
Fig. 1 Brachial artery flow mediated dilation (FMD) at baseline (*upper panel*) and changes in plasma nitrite after exercise (*lower panel*) in young and old subjects. *Asterisks* indicate significantly different at the $P < 0.05$ level

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

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
Serotonin
Dopamine
MAO A
MAO B
HIAA
COMT
HVA
NorEp
MAO B
COMT
VMA
3
THF
Thymidine synthesis
dUMP
5 Methyl THF
MTR
MTRR
4
Methionine
SAMe
methylation
DNA, RNA Protein, lipids
Guanido Ac
Creatine
Creatinine
DMG
BHMT
TMG
SAH
adenosine
SAHH
Homocysteine
CBS
5
Cystathionine
B6, P5P
Ammonia
Cysteine + α KG
Taurine
Sulfite
Glutathione
moly
Sulfate
SUOX
Neuronal Damage
Microglial Activation
Peroxynitrate
Super Oxide
Oxidative-Inflammatory Disease

Manipulating the NO System Through Diet and Nutrition



CONCLUSIONS

- There are profound effects of NO on health and disease
- Recognizing NO insufficiency is critical for prevention and/or progression of disease
- Taking steps to improve NO status in primary care patients may lead to better management of disease

Contact information:
Nathan.bryan@bcm.edu
Booth 16 - FxMed