Mr Nathan Bryan

Biochemist

Baylor College of Medicine Houston

Texas

Saturday, June 10, 2017

11:00 – 11:30 Nitric Oxide - So What?

Nitric Oxide, So What?

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Baylor College of Medicine

BCM
Baylor College of Medicine

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

“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

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.

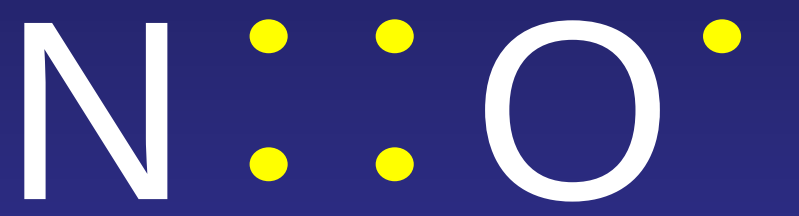
It is one of the most important signaling molecule in the body of mammals including humans, one of the few gaseous signaling molecules known.

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

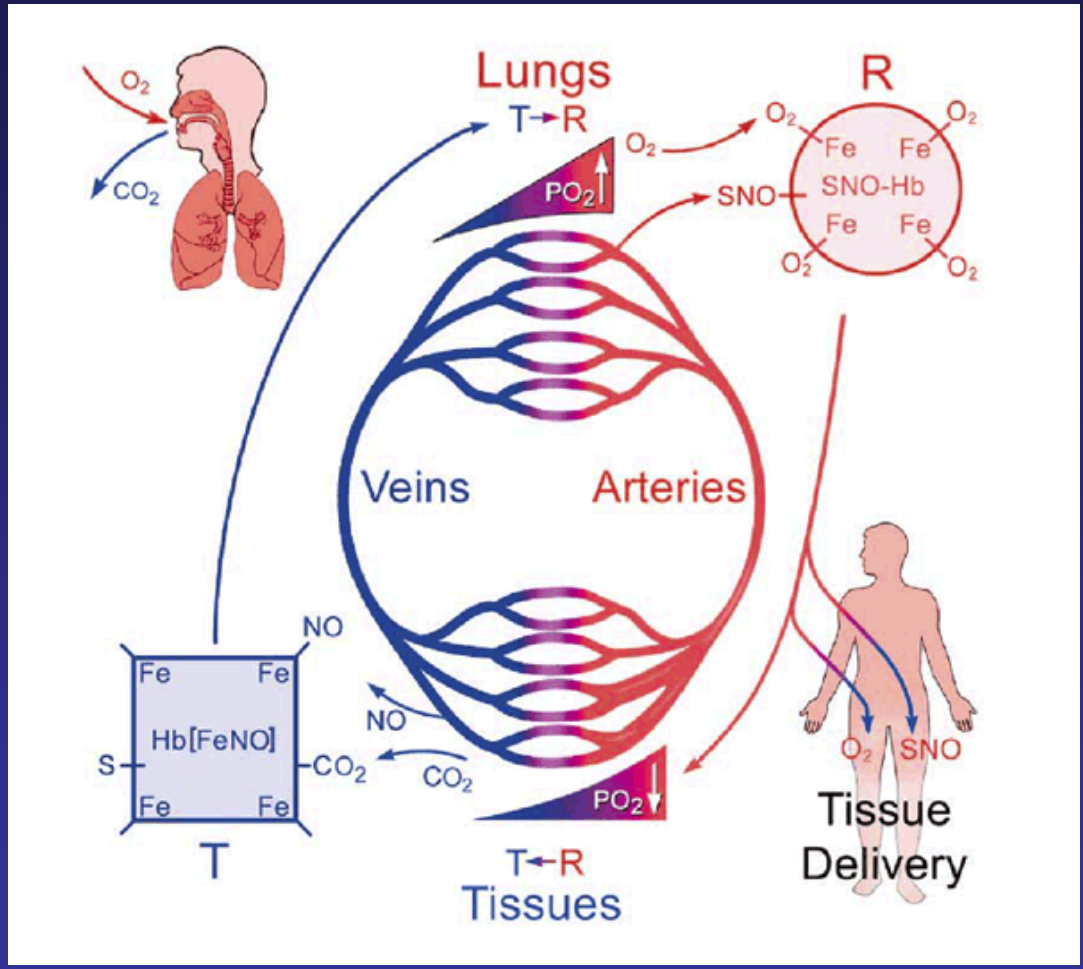
NO should not be confused with nitrous oxide (N_2O), a general anesthetic, or with nitrogen dioxide(NO_2) which is another poisonous air pollutant.

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

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Regulation
Contractility
lar Permeability

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rve-mediated
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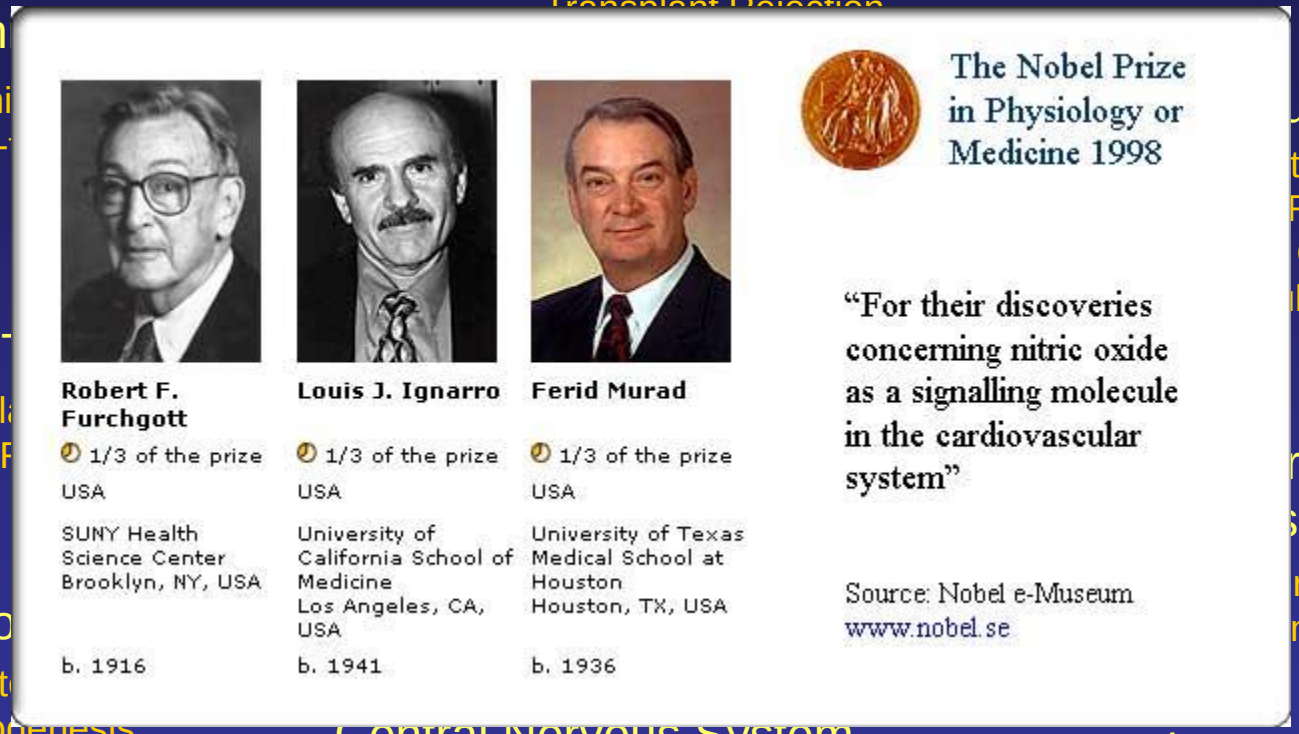
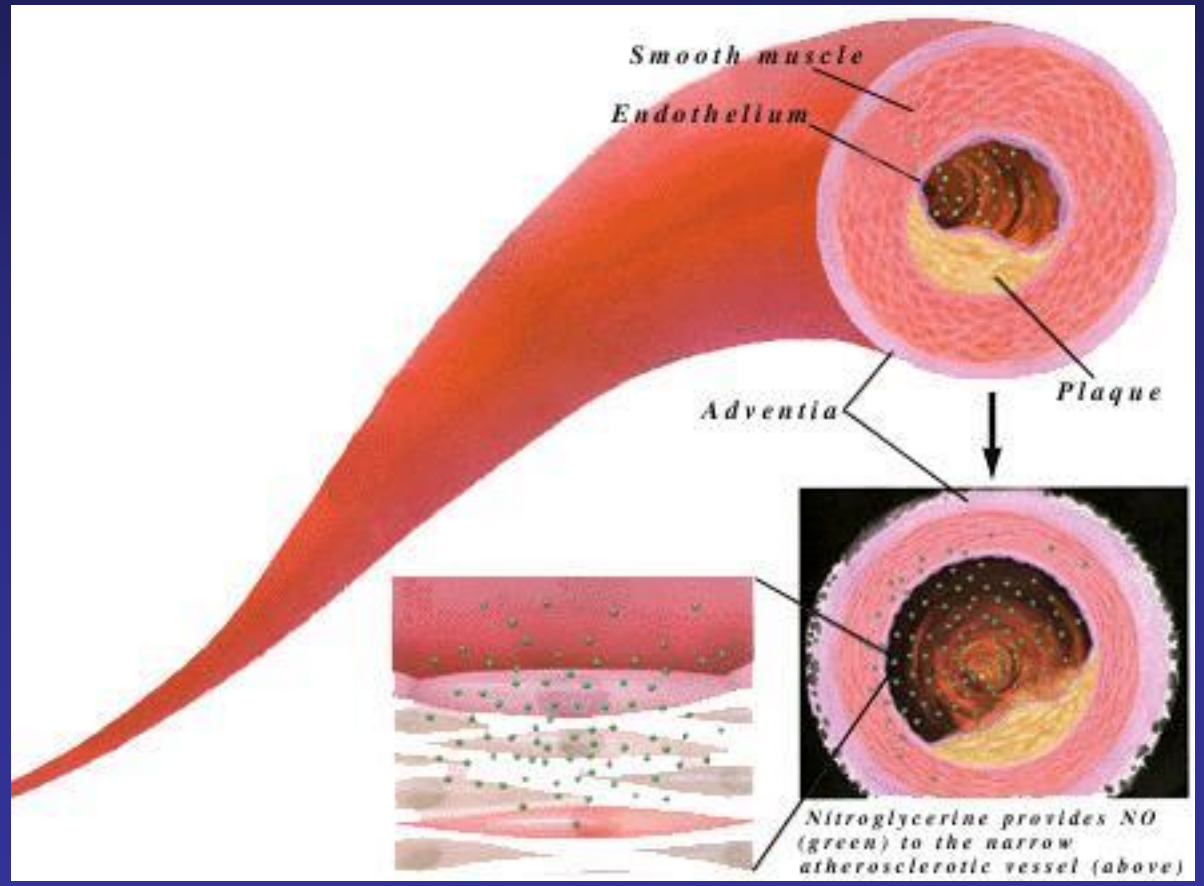


"The discovery of NO and its function is one of the most important in the history of cardiovascular medicine."

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.

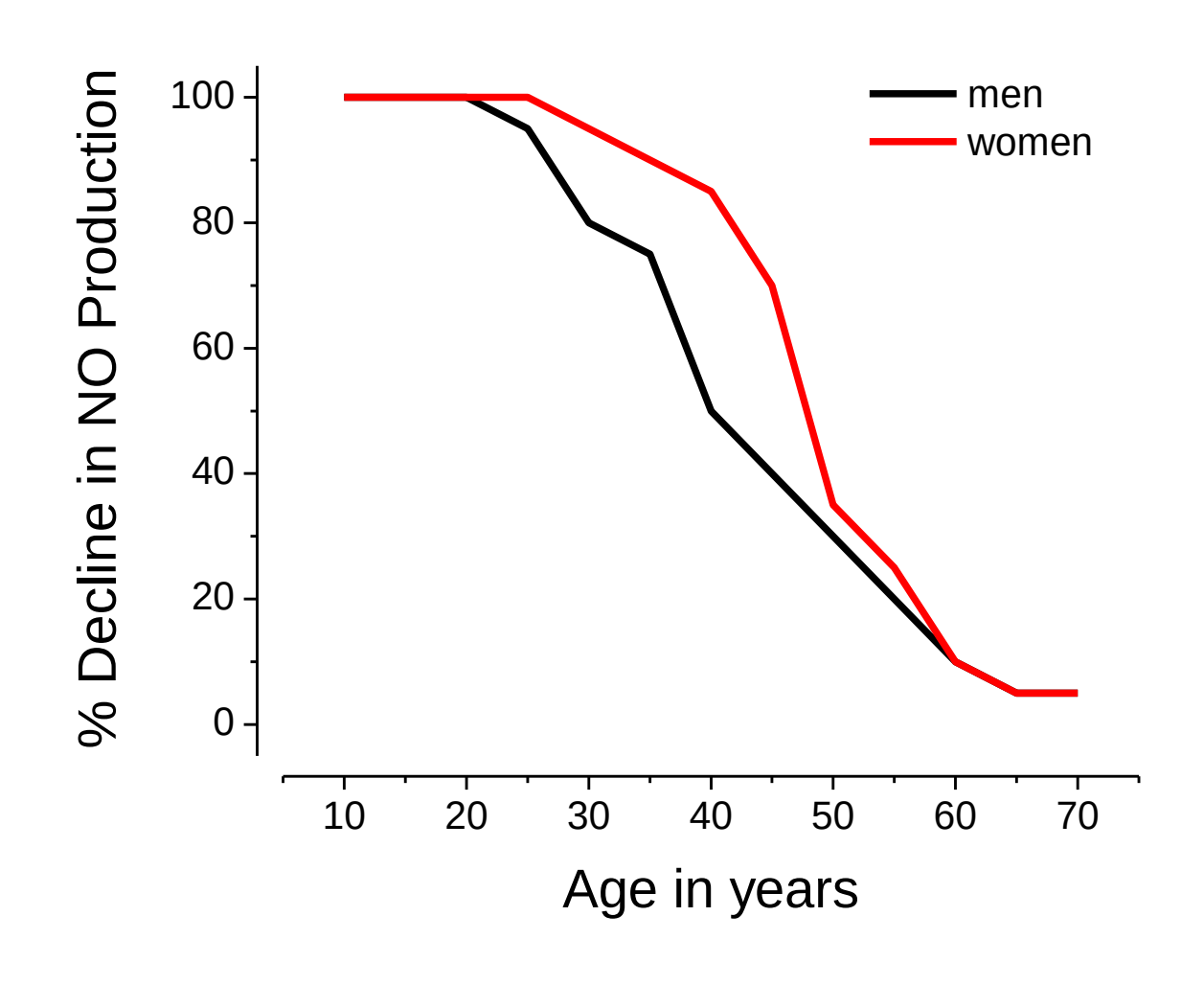
- **Endothelial dysfunction** is a physiological dysfunction of normal biochemical processes carried out by the endothelium, the cells that line the inner surface of all blood vessels including arteries and veins (as well as the innermost lining of the heart and lymphatics).

- 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

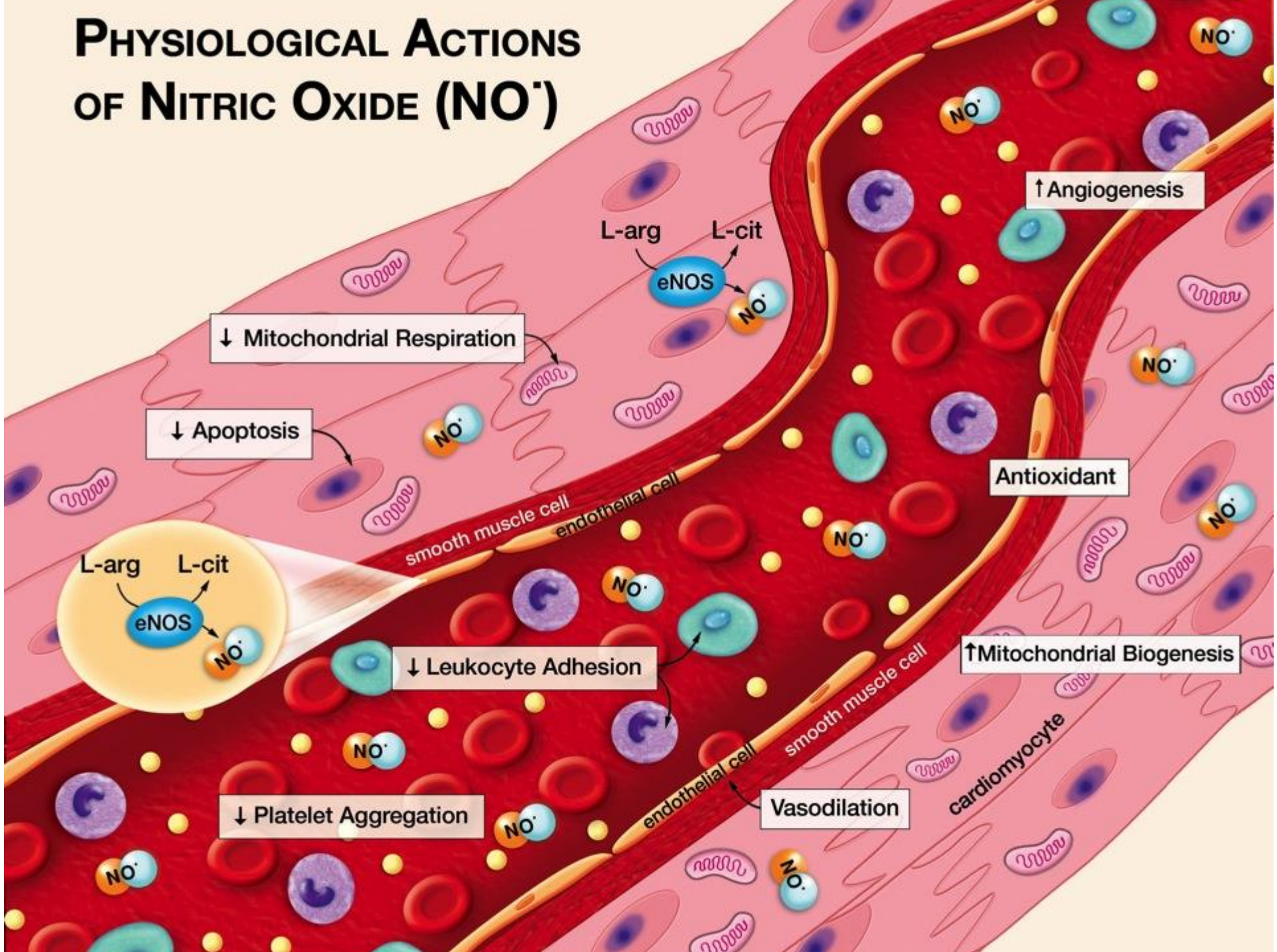
Current Options for Nitric Oxide Therapies

Drug Therapy

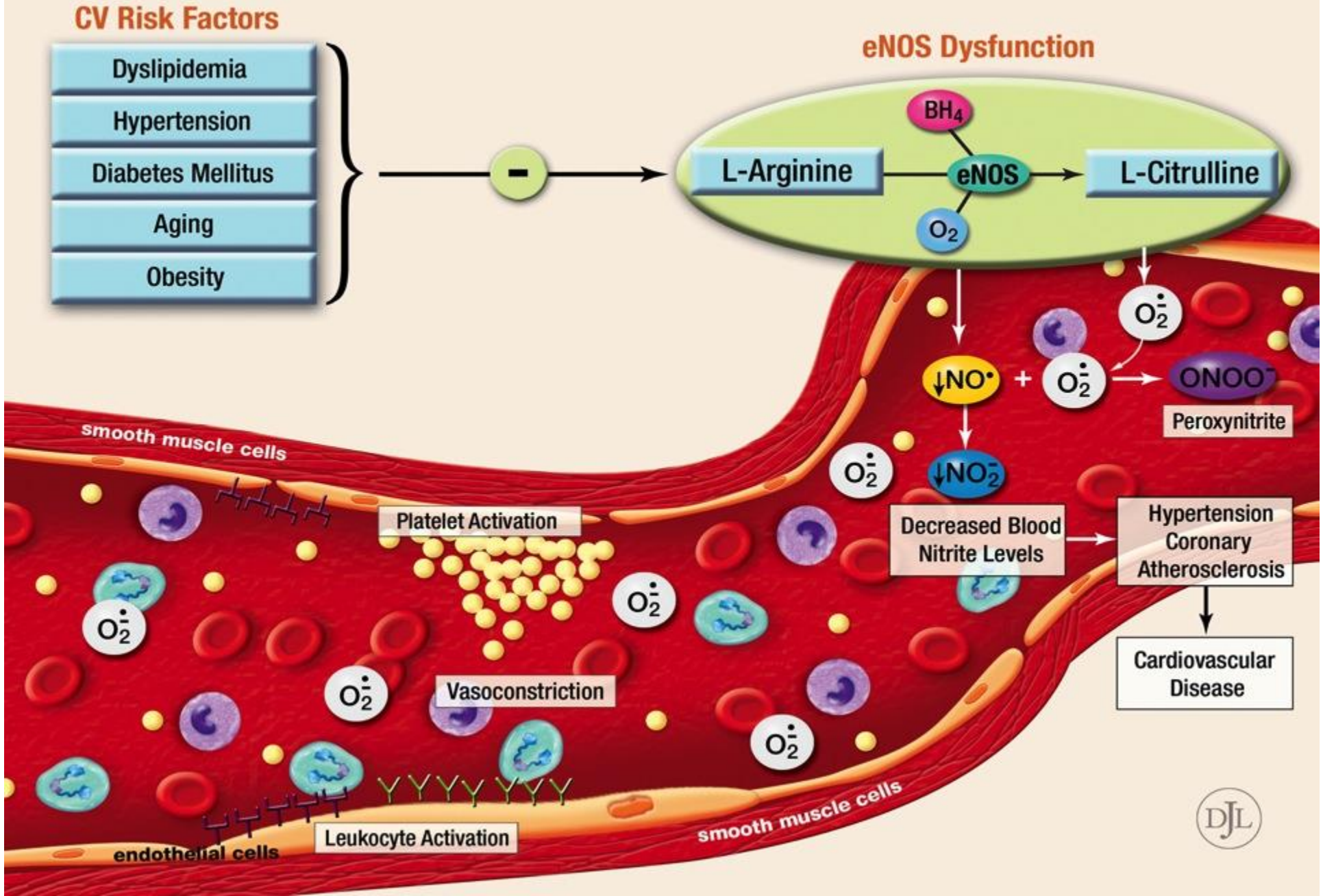
- organic nitrates (used for over 150 years for treatment of angina)
- inhalative NO therapy for neonates with pulmonary hypertension
- phosphodiesterase inhibitors such as sildenafil (Viagra) which act downstream from NO

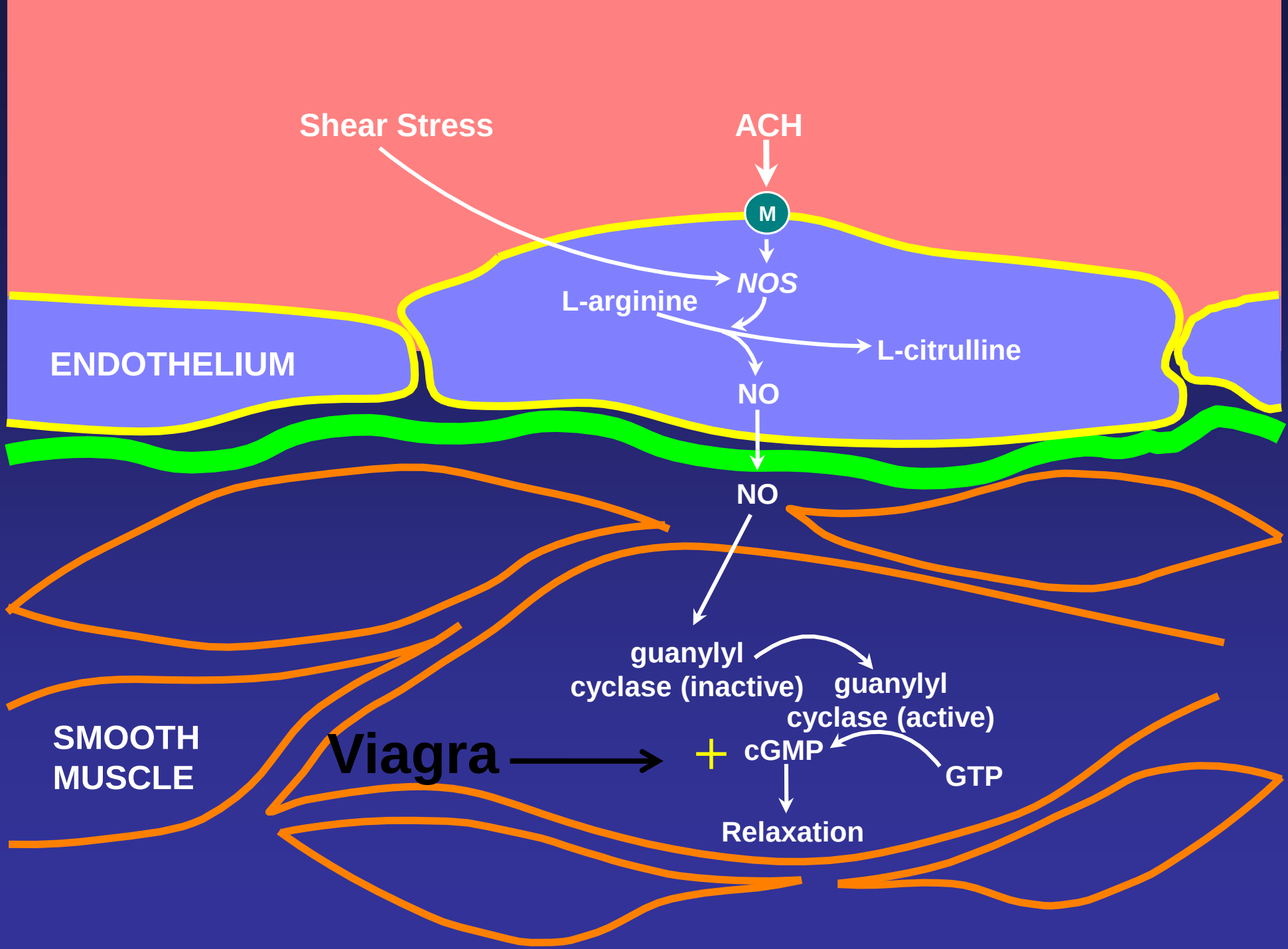
Lifestyle modification

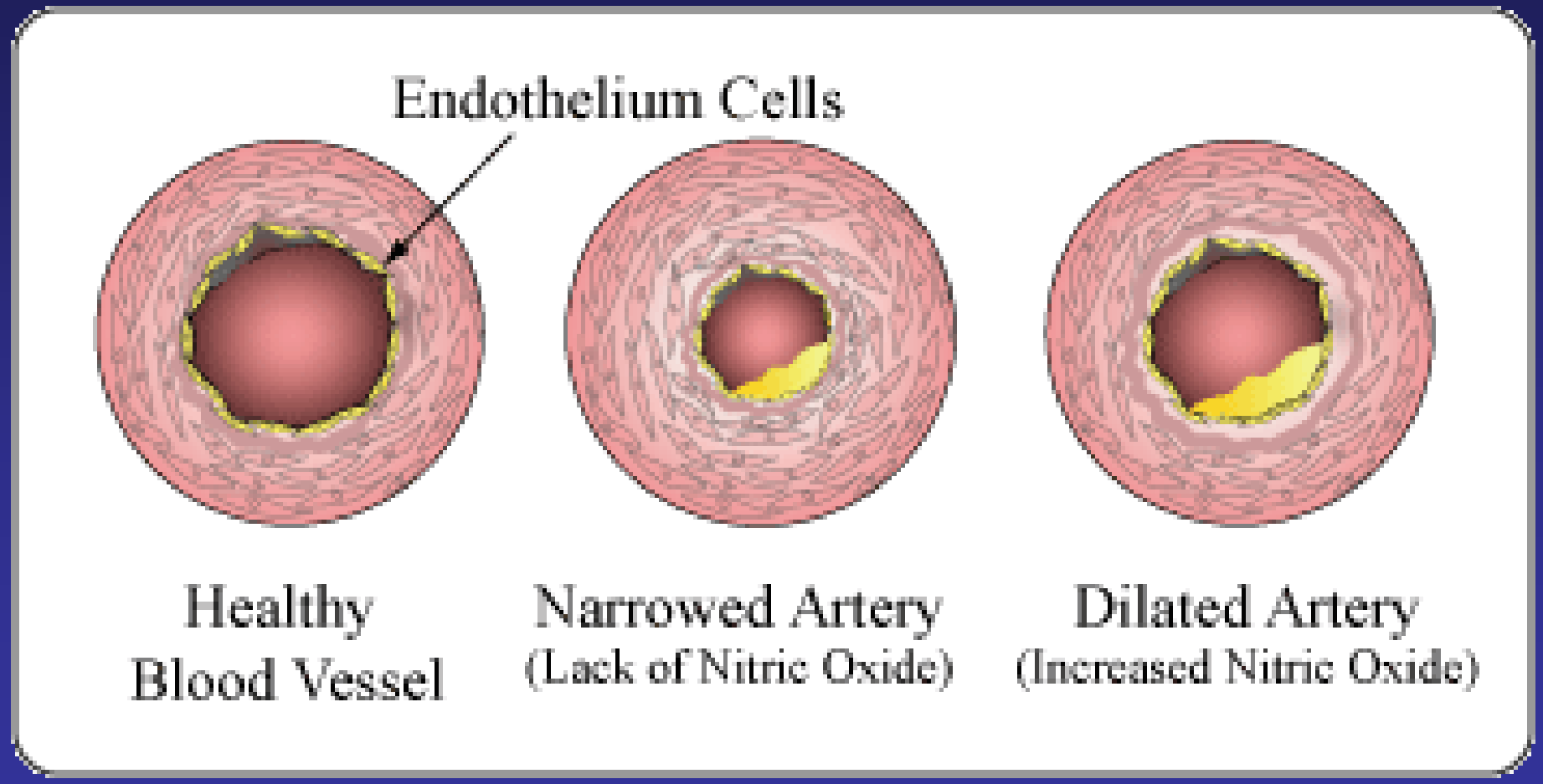
1. Vegetarian diet (high nitrate green leafy vegetables)
2. Moderate physical exercise (induces NO production – stimulation of eNOS)


PHYSIOLOGICAL ACTIONS OF NITRIC OXIDE (NO·)
L-arg
L-cit
eNOS
NO·
↓ Mitochondrial Respiration
↓ Apoptosis
smooth muscle cell
endothelial cell
↓ Leukocyte Adhesion
↓ Platelet Aggregation
↑Angiogenesis
Antioxidant
↑Mitochondrial Biogenesis
Vasodilation
cardiomyocyte

Vascular Endothelial Dysfunction

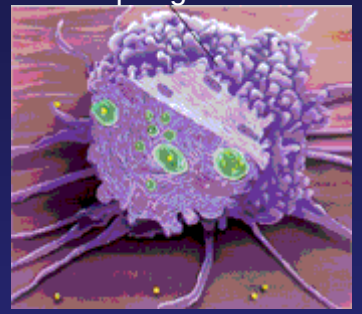



Shear Stress
ACH
M
NOS
L-arginine
L-citrulline
ENDOTHELIUM
NO
NO
guanylyl cyclase (inactive)
guanylyl cyclase (active)
SMOOTH MUSCLE
Viagra
+
cGMP
GTP
Relaxation


Endothelium Cells
Healthy
Blood Vessel
Narrowed Artery
(Lack of Nitric Oxide)
Dilated Artery
(Increased Nitric Oxide)

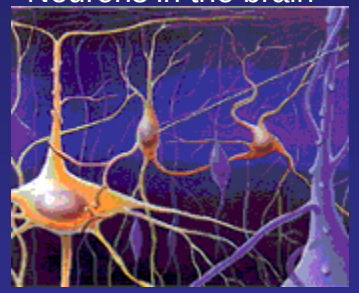
NO has Potential for Many Clinical Applications

Macrophage



NO is involved in the normal defense against bacterial and parasitic infections

Neurons in the brain

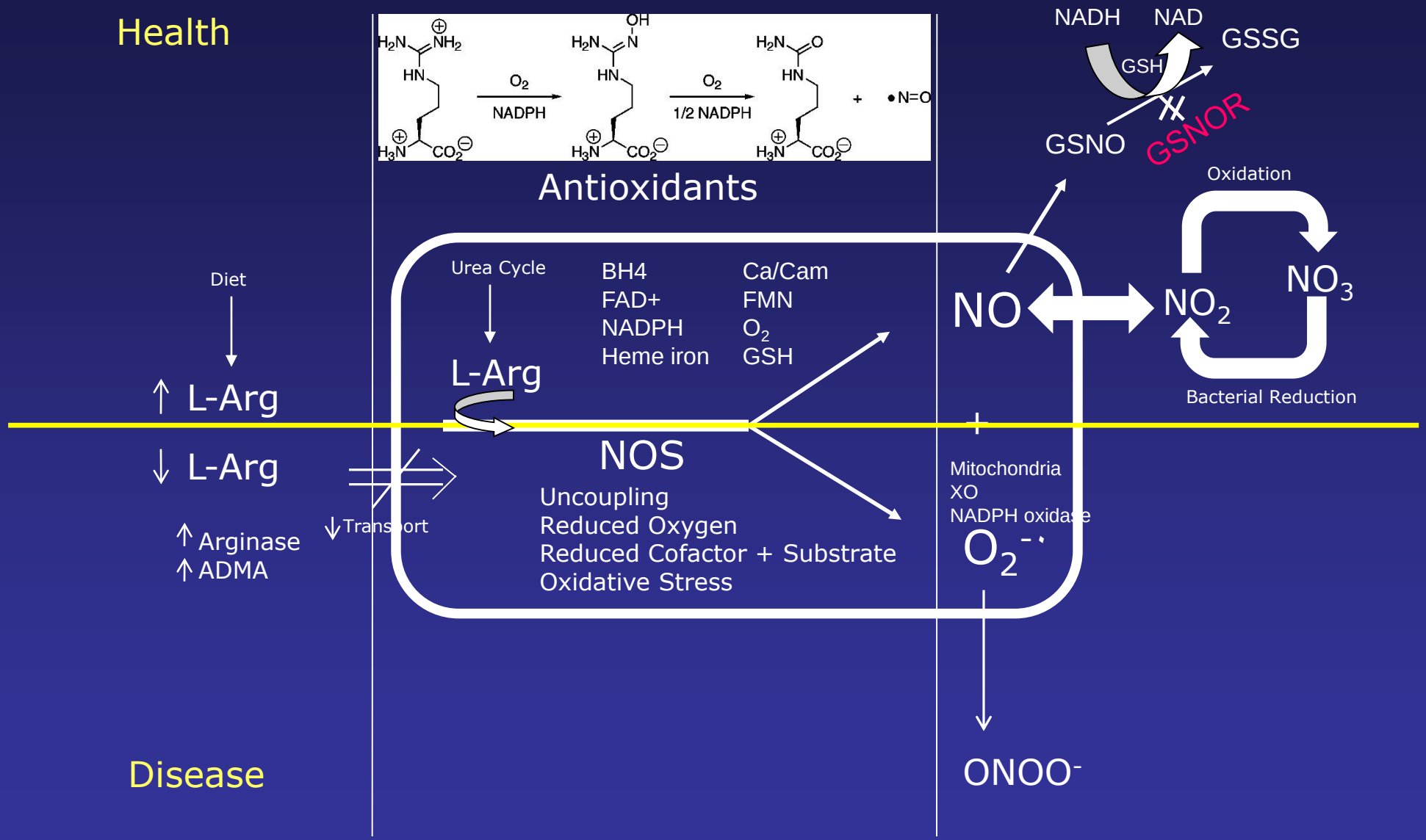


NO is important for signaling between nerve cells in the brain

NO can be inhaled to specifically treat high blood pressure in the lungs of newborns

How do we control and regulate NO production?

The L-Arginine-Nitric Oxide Pathway



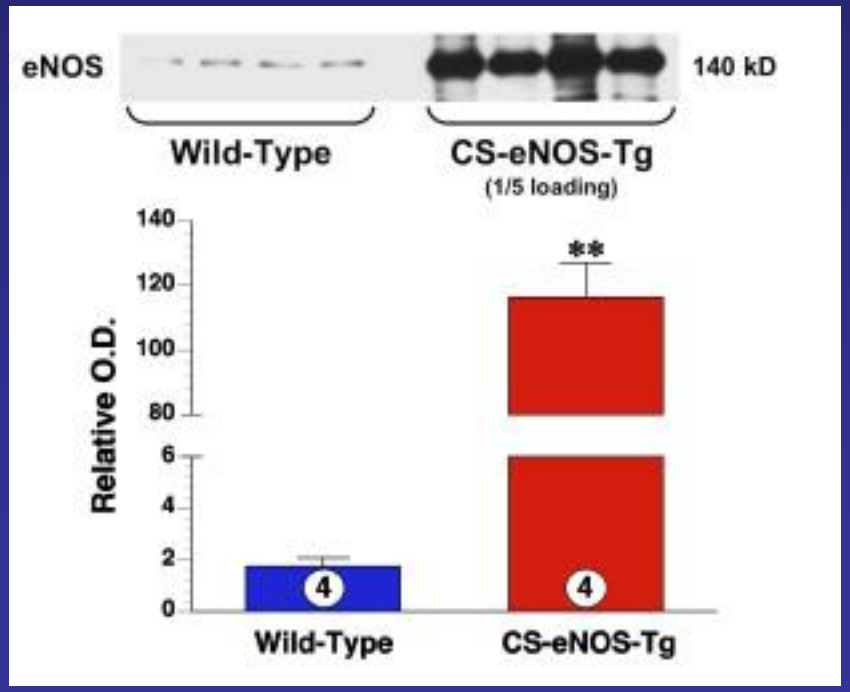
FACTS

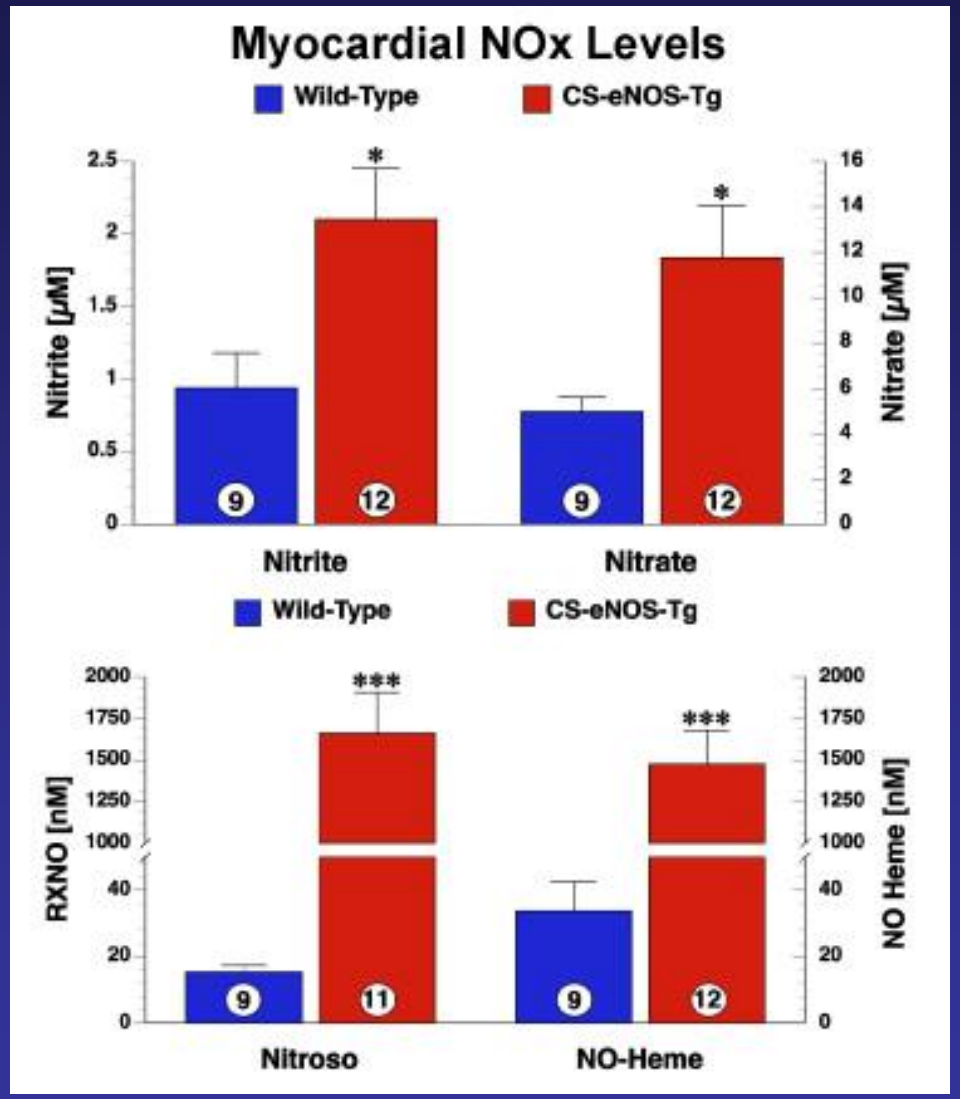
Cardiovascular disease (CVD) is the number one killer of both men and women in the U.S. Close to 1 million people die each year and more than 6 million are hospitalized due to CVD. The cost of CVD, in terms of health care and lost productivity, is over $270 billion and increasing as the baby boom population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

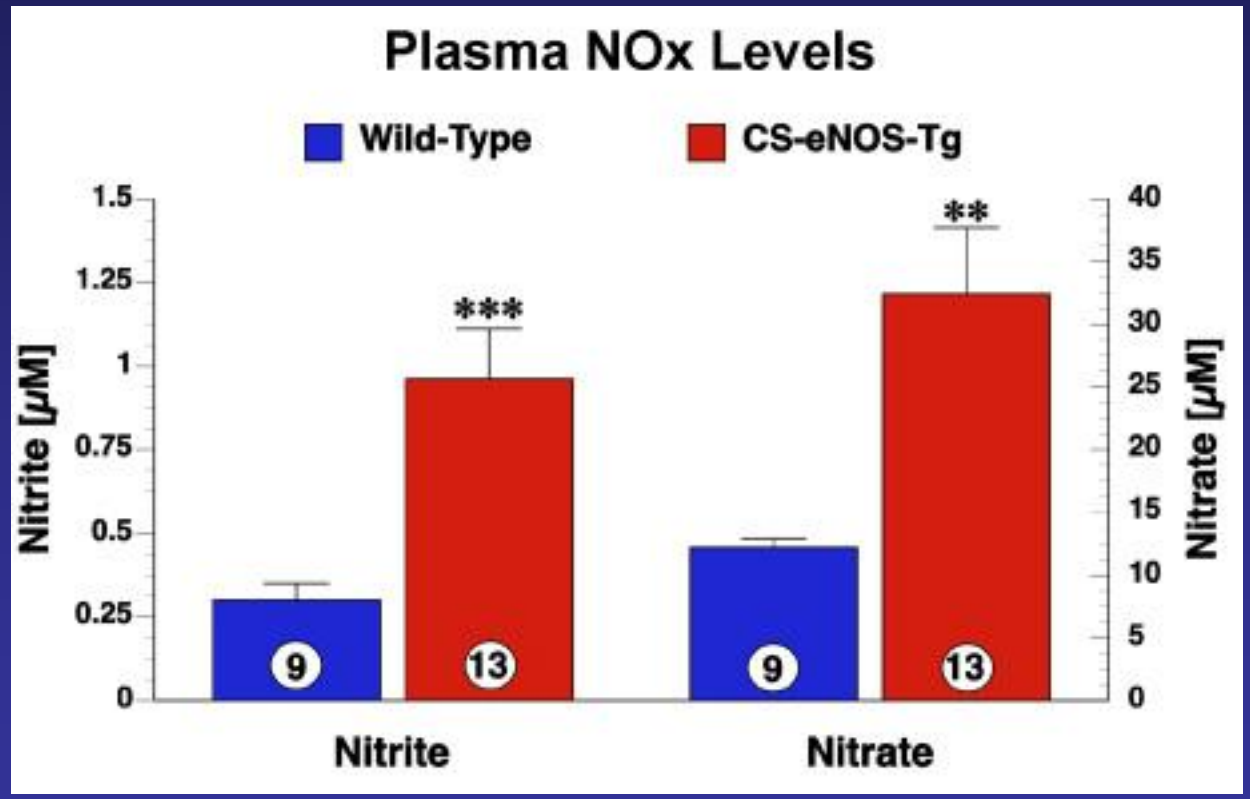
Cardiac Specific Overexpression of eNOS results in Increased Cardiac NO Production and Protects from I/R Injury





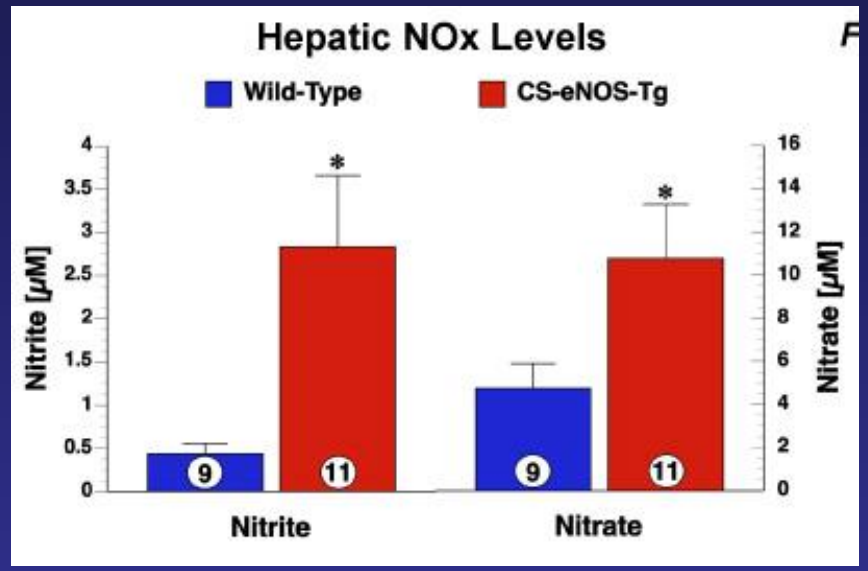
Elrod et al ATVB 2006

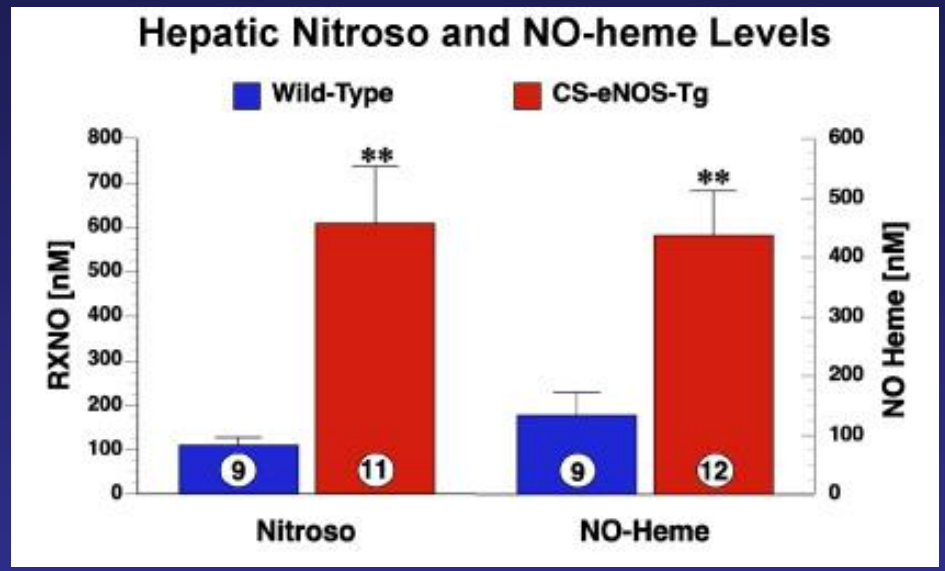
Increased Cardiac NO Production Results in Increased Circulating Nitrite and Nitrate

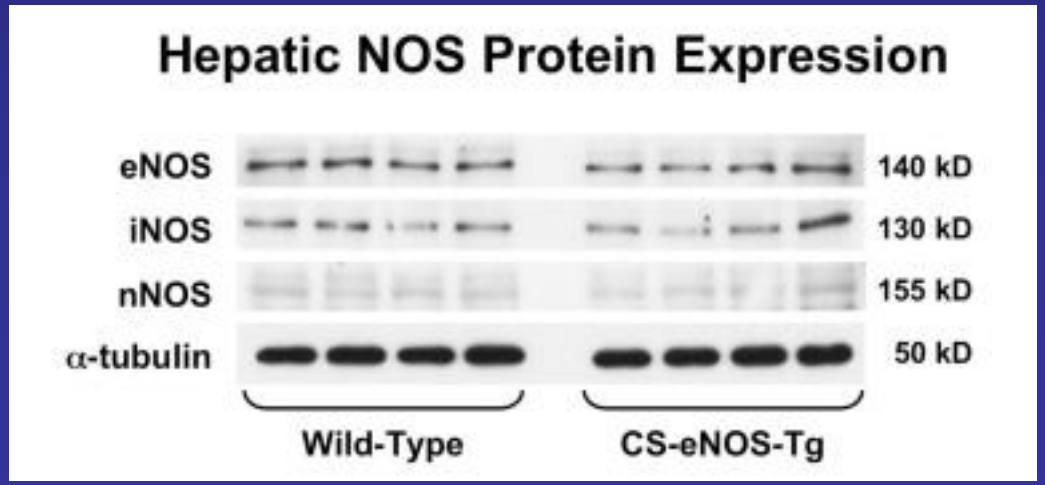


Elrod, PNAS 2008

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

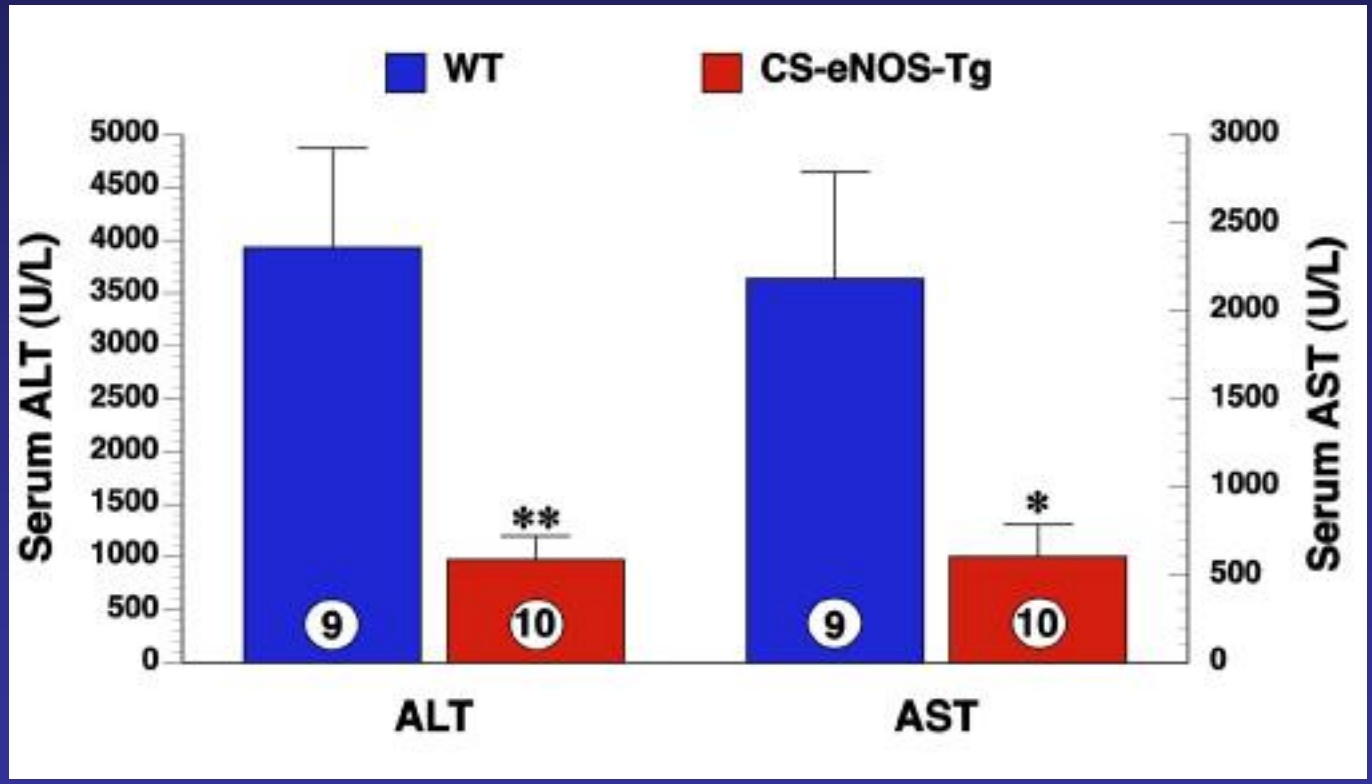






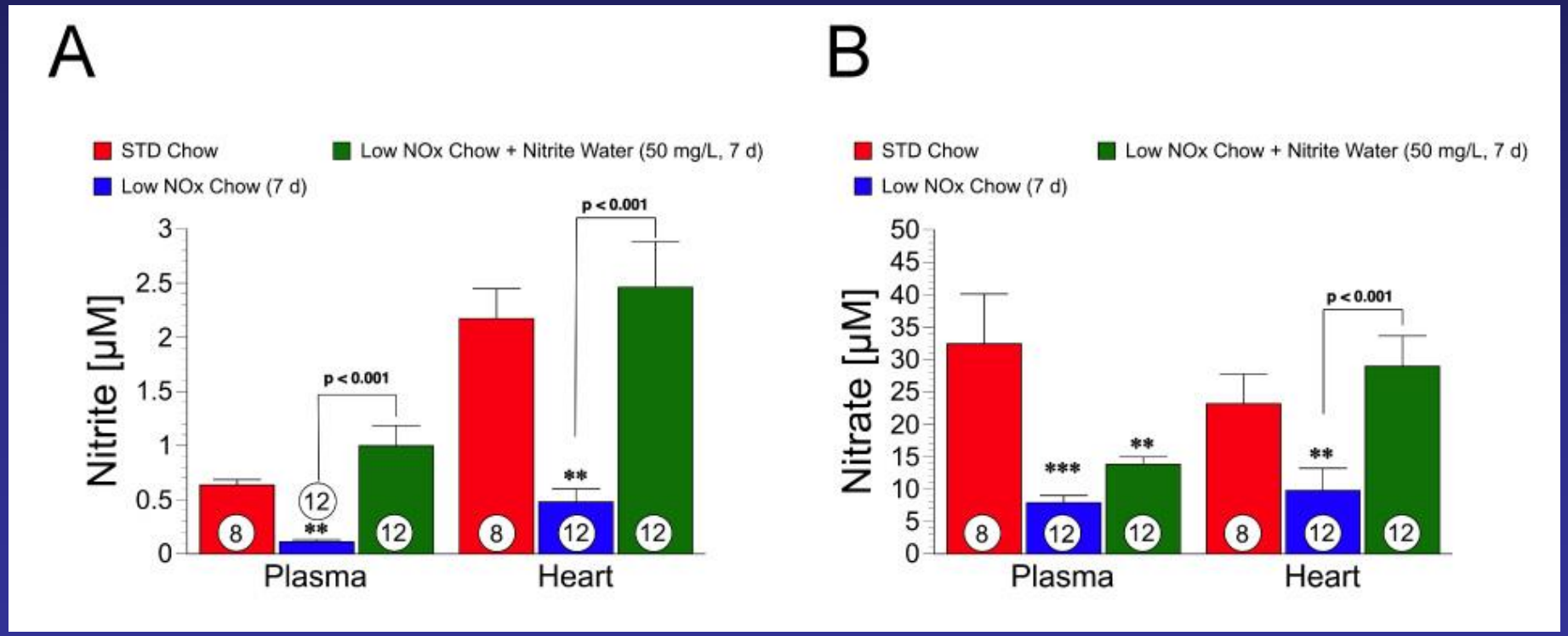
Elrod, PNAS 2008

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



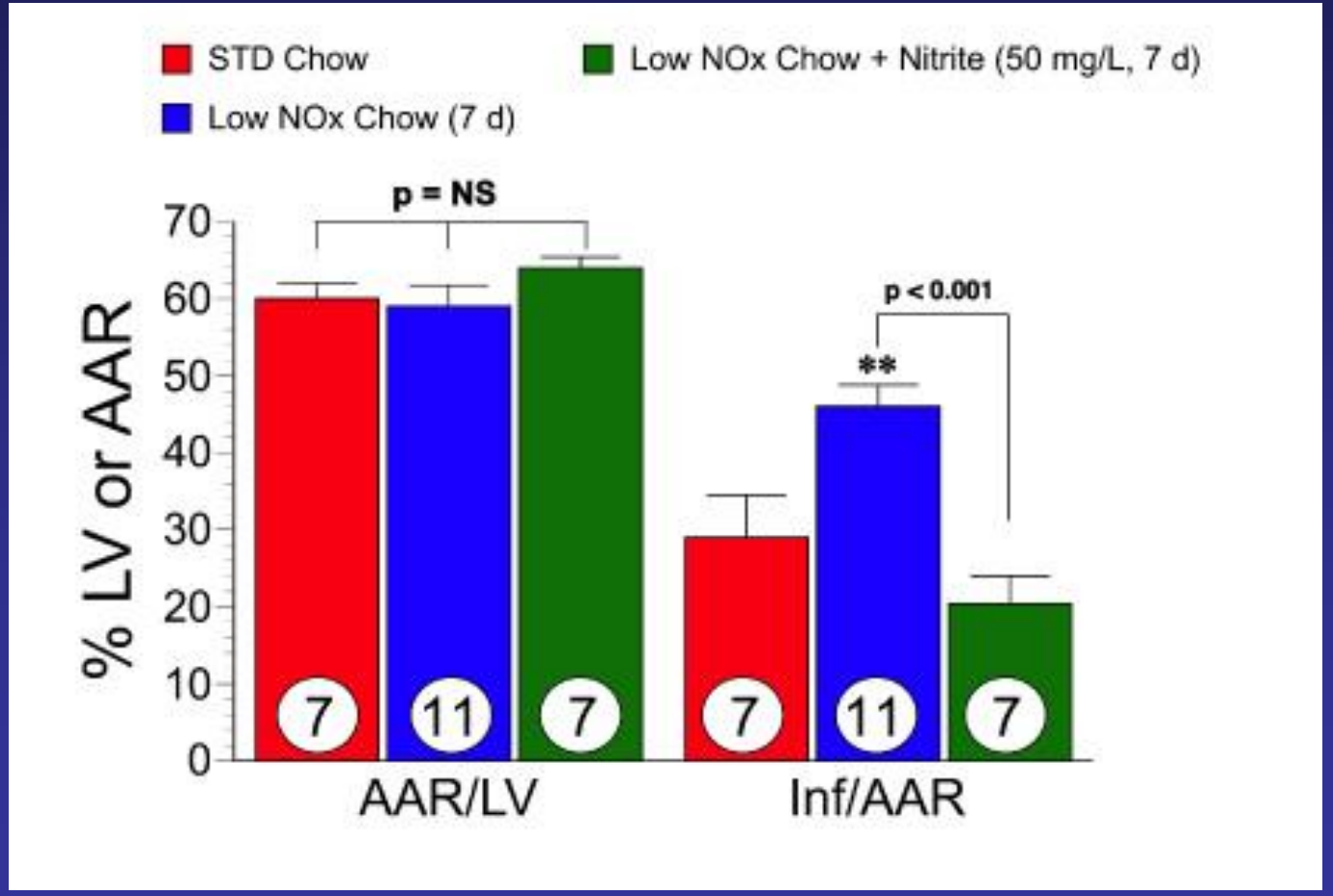
Elrod, PNAS 2008

Mice on Low NOx Diet for 1 Week Reveal Diminished Plasma and Cardiac Nitrite and Nitrate and is Restored with NO Supplementation



Bryan *et al* PNAS (2007)

Mice on Low NOx Diet for 1 Week Reveal Increase Injury from Heart Attack which is Reversed with NO Supplementation

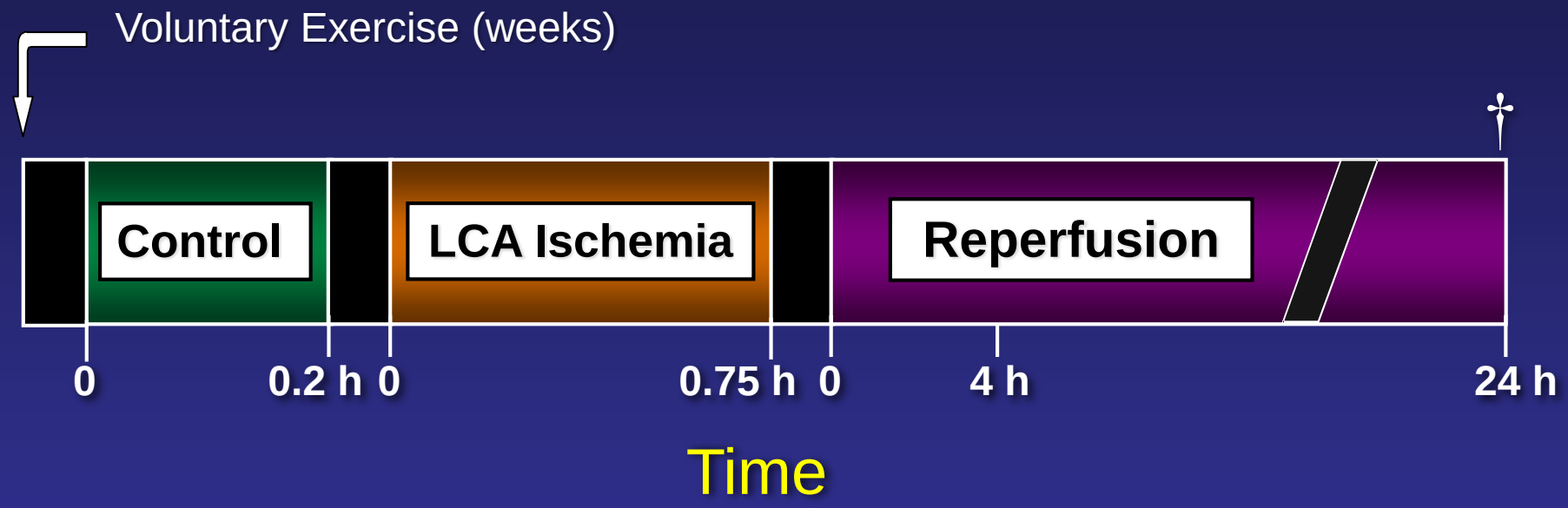


Bryan *et al* PNAS (2007)

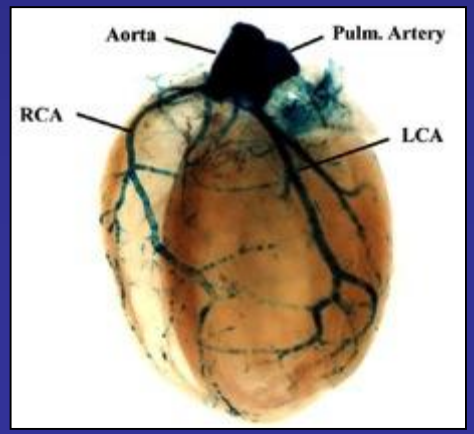
What about Exercise?

Can Exercise Protect from Injury from heart attack in healthy animals?

Murine *in vivo* Myocardial Ischemia-Reperfusion Protocol

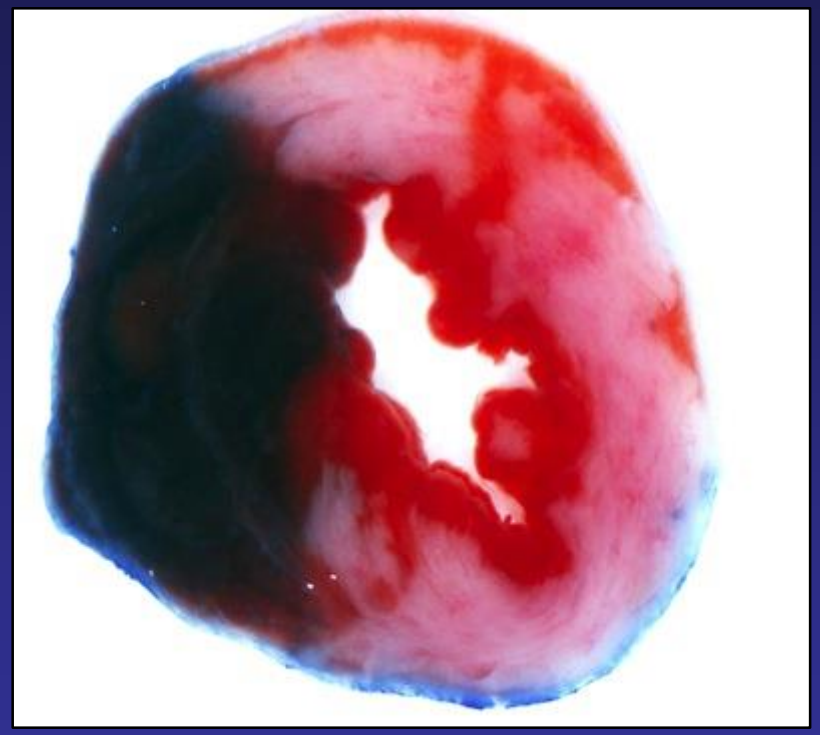


† Myocardial Infarct Size
- Area-at-risk (Evans Blue dye)
- Infarct size per Area-at-risk (TTC)

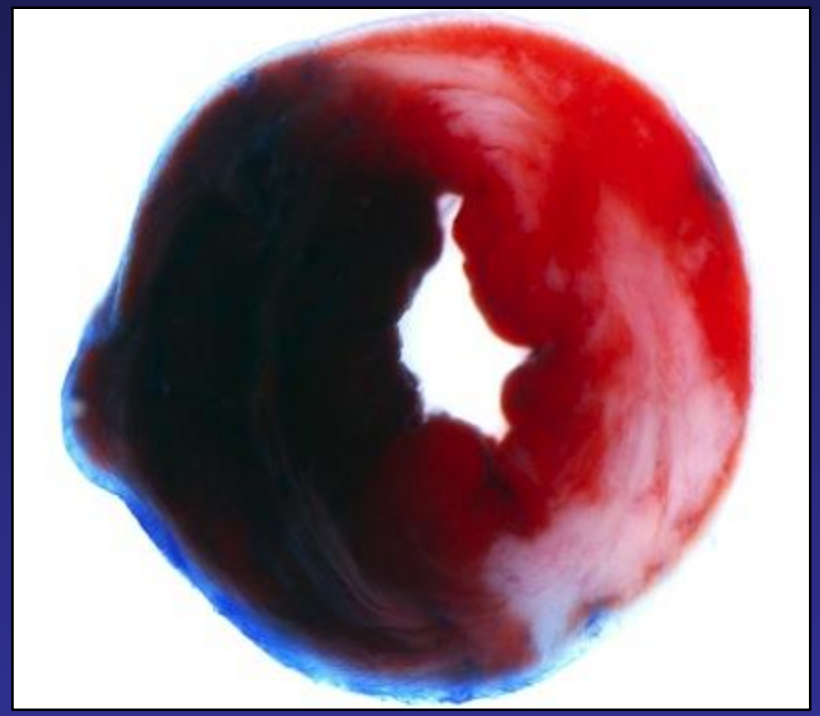


Myocardial Injury

45 min Ischemia and 24 hr Reperfusion



Sedentary



Voluntary Exercise (4 weeks)

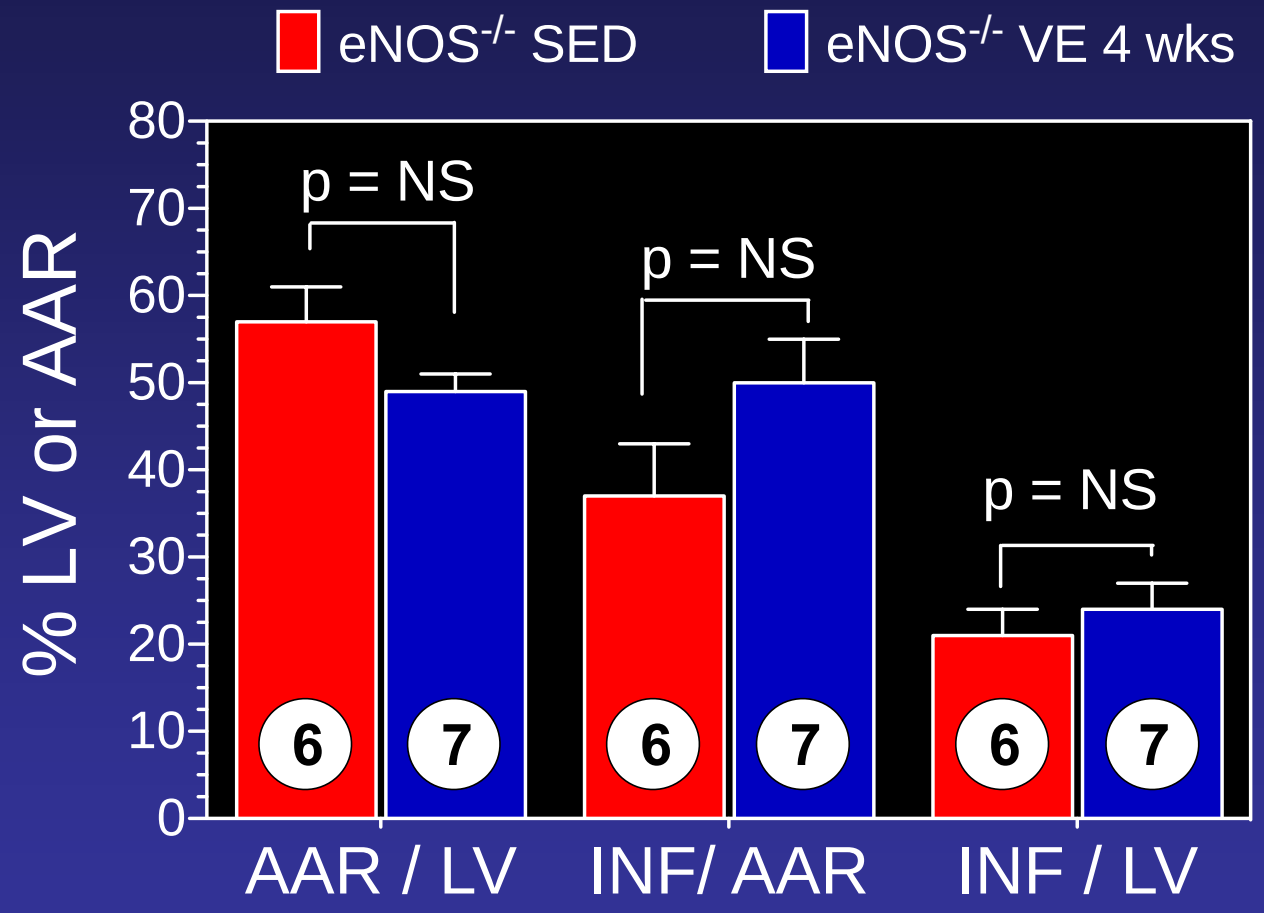
Calvert et al., *Circulation Research*, 2011

What about Exercise?

Can Exercise Protect from injury from heart attack in animals that cannot make NO?

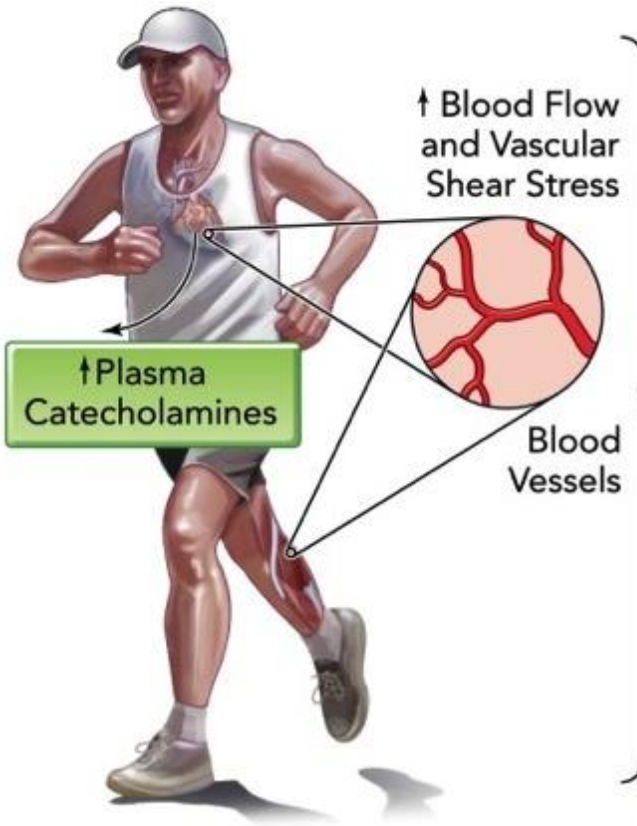
Myocardial Infarct Size

45 min of Ischemia and 24 hrs of Reperfusion



Calvert et al., *Circulation Research*, 2011

Exercise and Nitric Oxide Homeostasis



↑Plasma Catecholamines

↑ Blood Flow and Vascular Shear Stress

Blood Vessels

↑eNOS Phosphorylation

↑eNOS Activity

↑NO

Nitric Oxide

↑NO Metabolite Stores (Nitrite and Nitrosothiols)

Cardiac Ischemia

↑NO Bioavailability

↑Nitrosothiol Signaling

Cardioprotection

Patients Do Not Comply with Dietary Recommendations

J Community Health. 2014 Aug;39(4):732-6

Frequency and predictors of non-compliance to dietary recommendations among hypertensive patients.

Khan MS, Bawany FI, Mirza A, Hussain M, Khan A, Lashari MN.

Compliance to dietary recommendations among hypertensive people is a major health care issue. Non-compliance can nullify the effects of even the most scientific and optimum treatment plan. The main aim of this study was to determine the frequency and predictors of non-adherence in our region. We also investigated the possible factors based on patient opinions that could increase compliance. A sample of 400 adult patients, diagnosed with hypertension for at least 3 years, who visited Civil Hospital during the time period April-June 2013 were included in this cross sectional study. Patient data and opinions were collected by two trained interviewers using a pre-coded questionnaire. Compliance was assessed based on patients self report. More than three quarters (n = 310, 77.5%) of the hypertensive patients were non-compliant. More than one social gathering in a week, peer-influence, no friends to follow the recommended diet plan and lack of believe regarding diet as an effective measure to control blood pressure were found to be the significant predictors of non-compliance (P values <0.0001). There is an urgent need for doctors and nurses to counsel their patients effectively to prevent future morbidities and mortalities because of non-compliance.

Patients Do Not Comply with Exercise Programs

Approximately 50% of individuals who start an aerobic exercise programme will stop within the first 6 months, even though it is well known that to obtain the health benefits associated with physical activity, participation must be maintained.

Sports Med. 1994 Jan;17(1):39-52.
Adherence to exercise programmes. Recommendations.
Robison JI, Rogers MA.

Can Nitrite Overcome Variability Of Oral Microbiome and Inability to Reduce Nitrate and Affect Blood Pressure?

Safety Considerations

Acute toxicity:
1. methemoglobinemia
2. hypotension

The fatal dose of nitrite is 22-23 mg/kg body weight
(1.85g in 80kg human)
100 fold less than LD50 – FDA benchmark for safety

Long term toxicity: propensity to react with secondary amines to form carcinogenic low molecular weight N-nitrosamines

Prevent nitrosative chemistry by providing supra-stoichiometric Vitamin C or polyphenols relative to nitrite

Human body makes 20-40 mg nitrite per day
1. from NO oxidation (~10 mg)
2. from nitrate reduction (150mg X 0.05% = 7.5 mg)
3. saliva (15 mg per day)

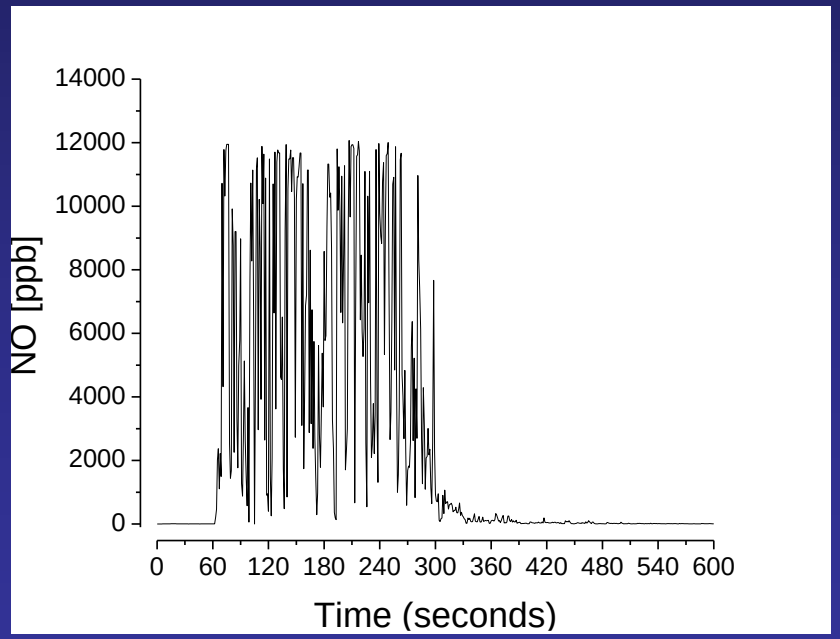
15 mg Nitrite
Oxygen independent nitrite reductase
Vitamin C (nitrosation inhibitor)

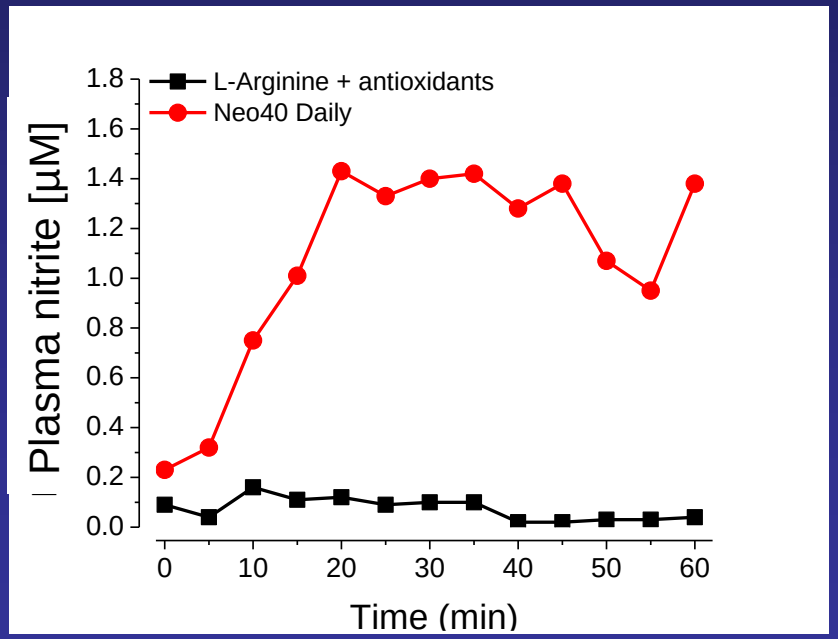
Orally disintegrating tablet in inert matrix that keeps active separated until dissolution

Generates 25-30 ppm NO gas upon dissolution for 5-6 minutes

NO lozenge Clinical Trial Results

Strong & sustained Nitric Oxide activity





Zand et al Nutrition Research 2011

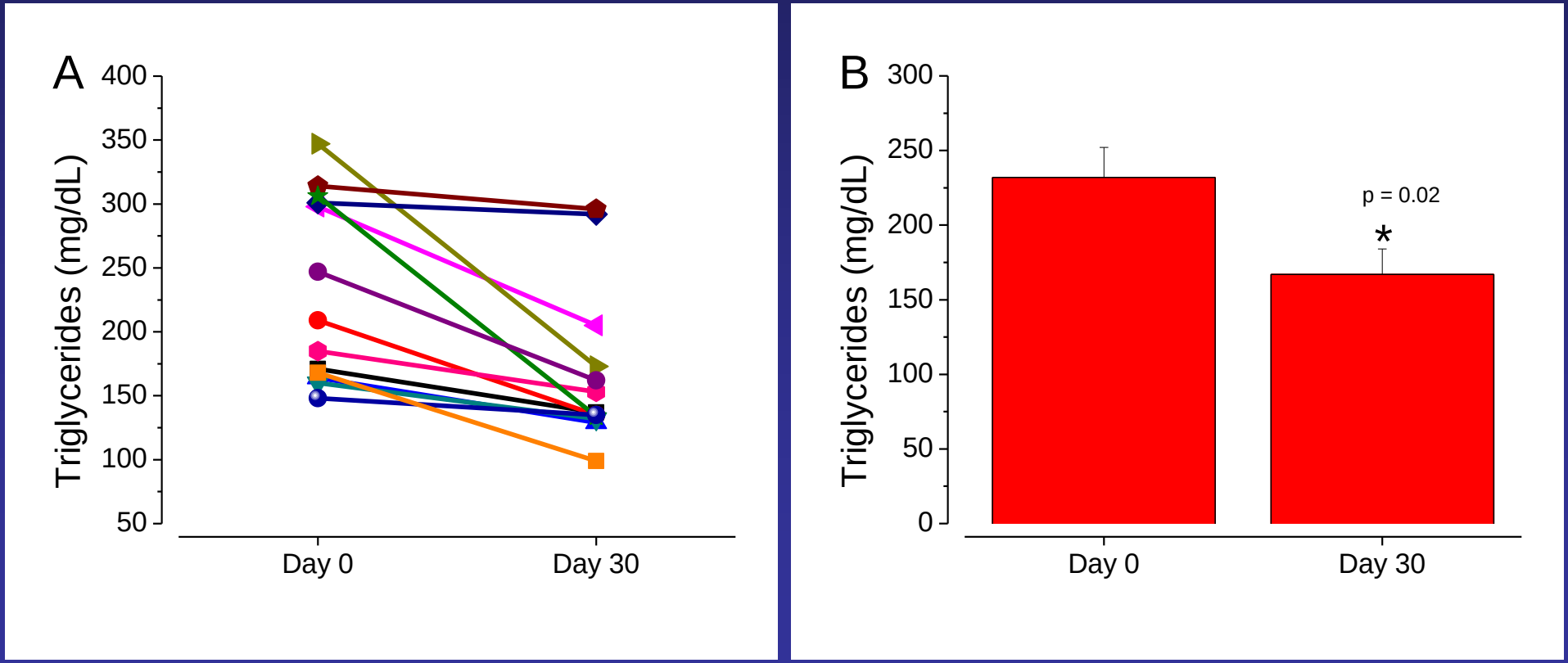
What Causes Arteries to Age?

1. Inflammation
2. Immune Dysfunction
3. Oxidative Stress

Increased oxidative stress is associated with elevated triglycerides

NO Clinical Trial Results

Significant reduction in patients
with elevated triglycerides

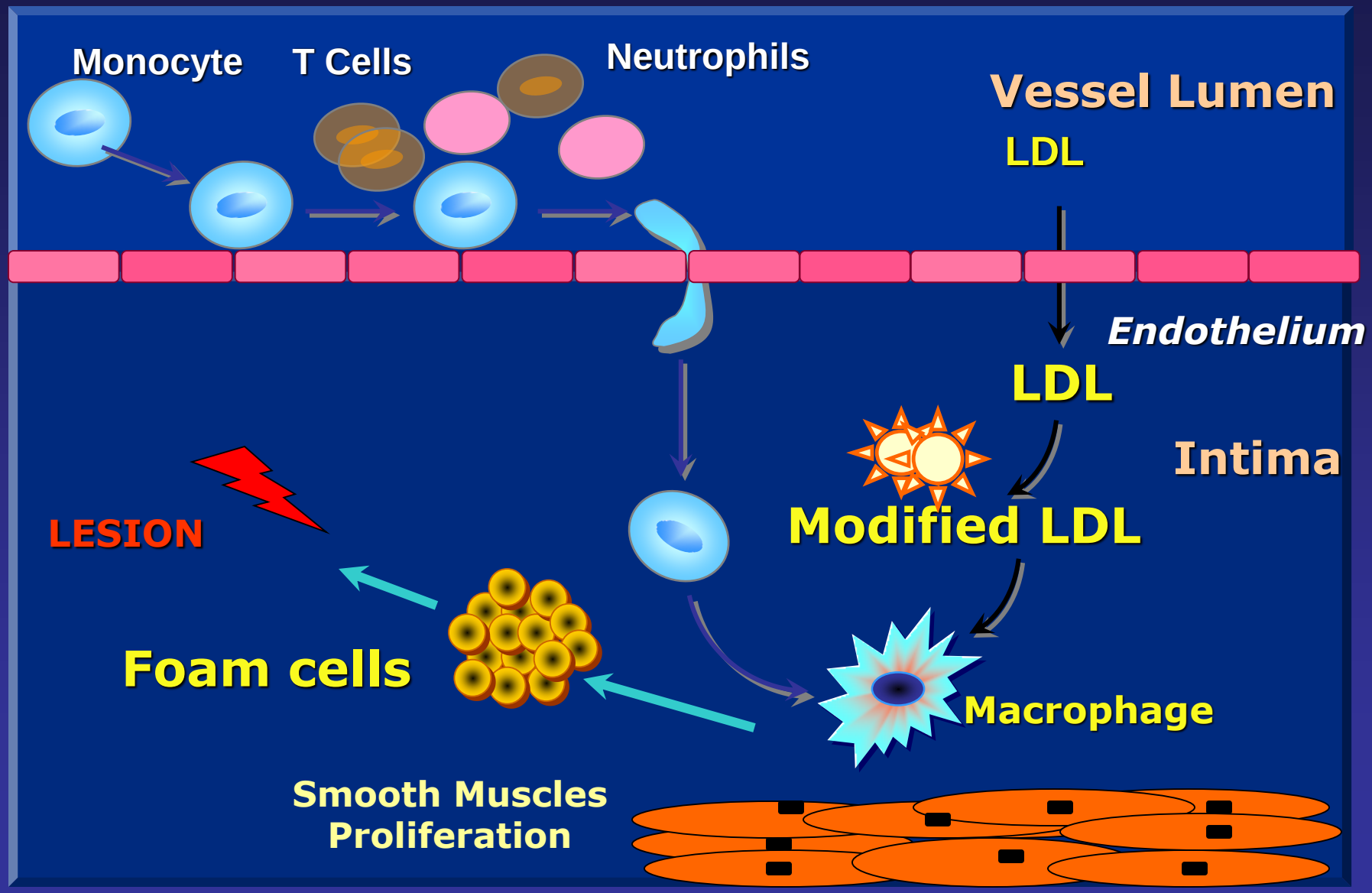


Zand et al Nutrition Research 2011

What Causes Arteries to Age?

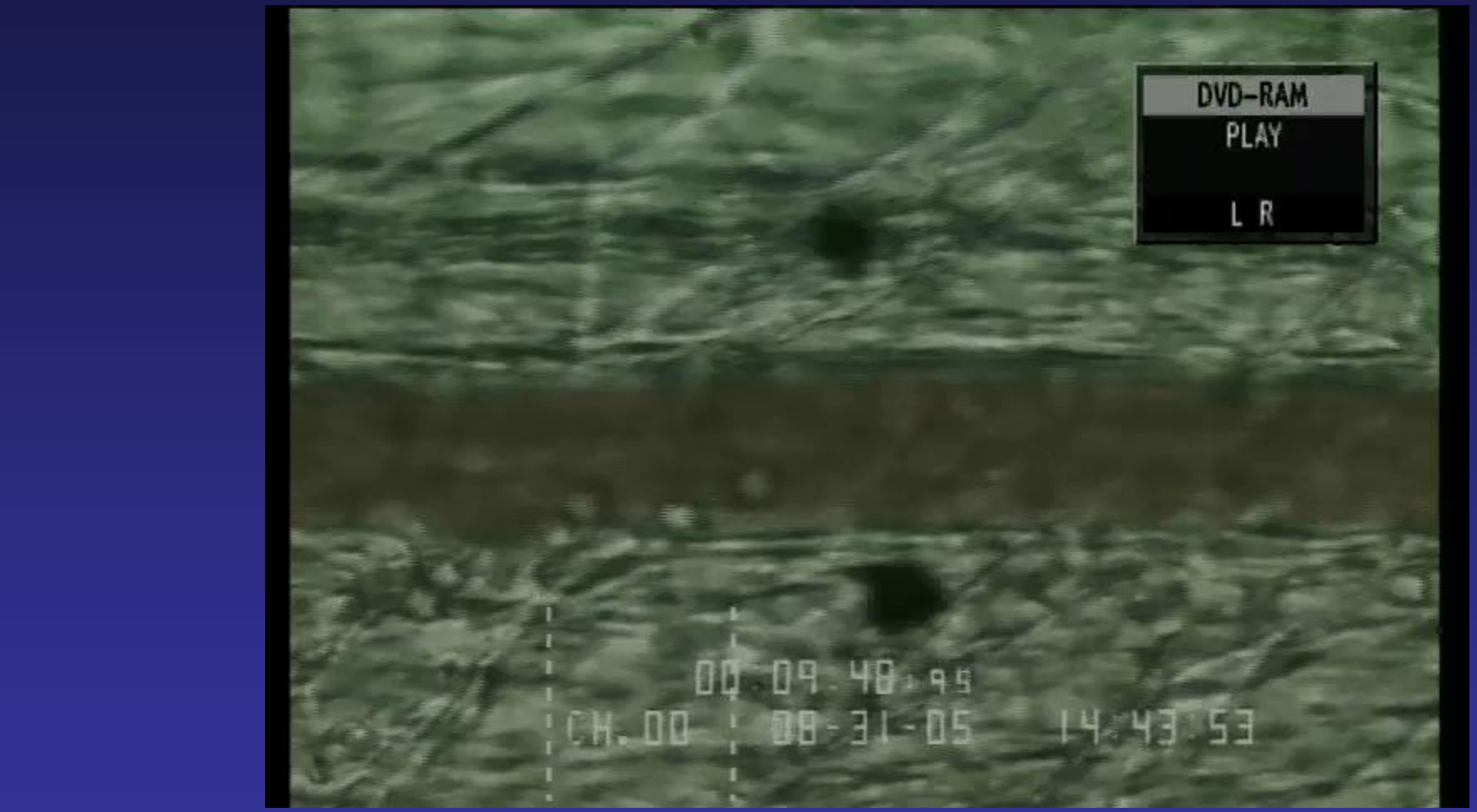
1. Inflammation
2. Immune Dysfunction
3. Oxidative Stress

Atherogenesis



Atherogenic Diet

Atherogenic Diet + NO



What Causes Arteries to Age?

1. Inflammation
2. Immune Dysfunction
3. Oxidative Stress

hsCRP is an acute marker of Inflammation

NO reduces hsCRP by 37% in 30 days

US Patent 9,119,823

What Causes Arteries to Age?

1. Inflammation
2. Immune Dysfunction
3. Oxidative Stress

Having high blood pressure puts you at risk for heart disease and stroke, which are leading causes of death in the United States

Achieving a target systolic pressure of 120 millimeters of mercury (mm Hg), reduced rates of cardiovascular events, such as heart attack and heart failure, as well as stroke, by almost a third and the risk of death by almost a quarter, as compared to the target systolic pressure of 140 mm Hg.

SPRINT trial

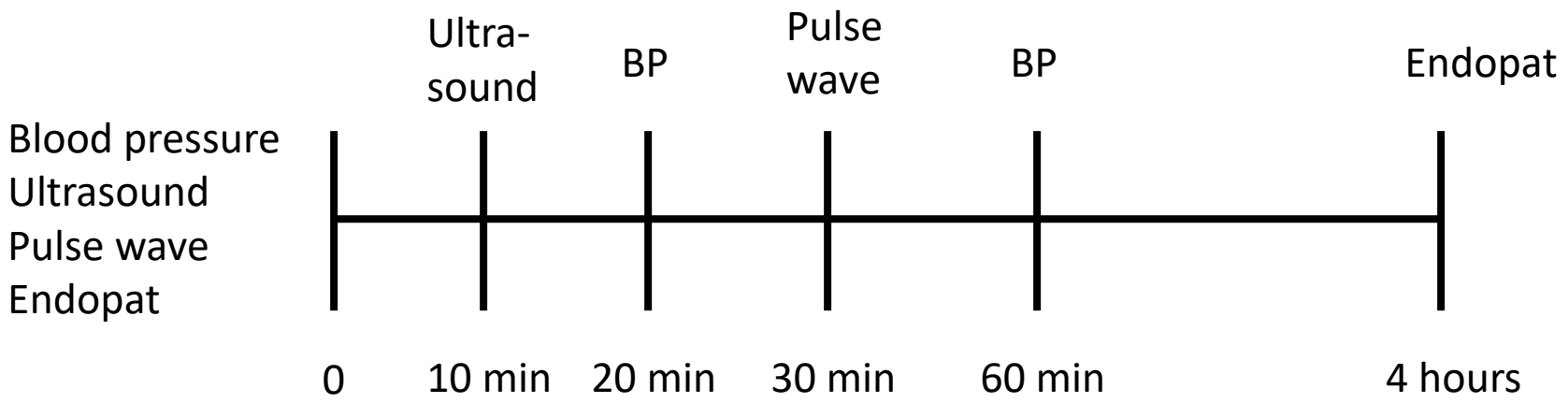
There are over 400 risk factors or markers for CVD

Most if not all can be corrected by restoring NO production

Hypertension is the #1 risk factor for development of cardiovascular disease

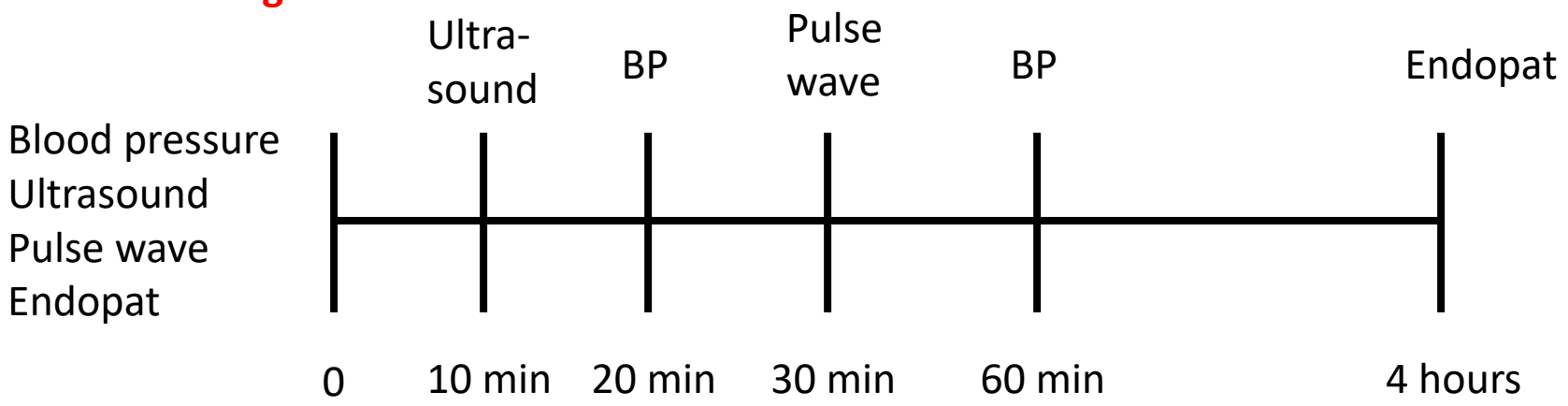
Hypertension Study Protocol

Active lozenge

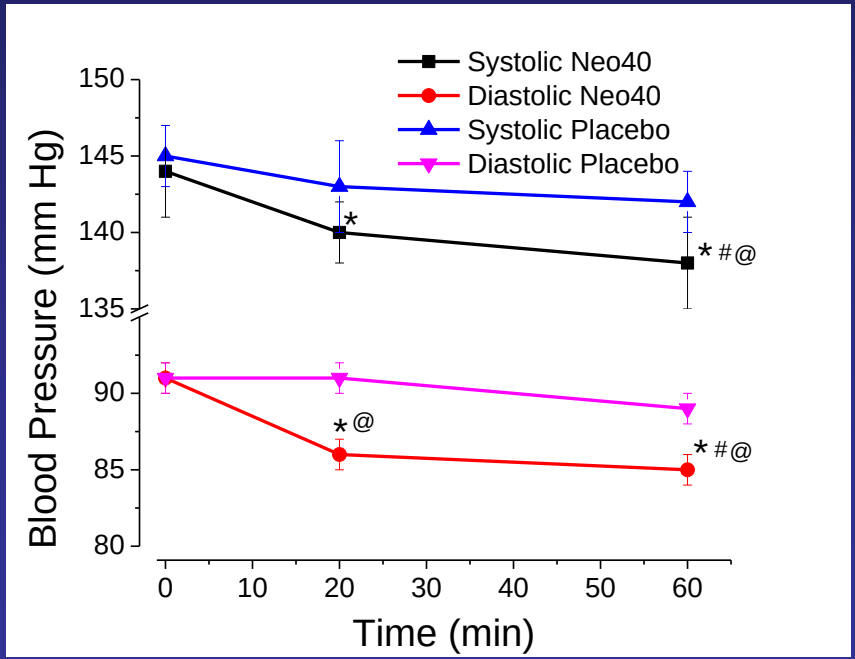


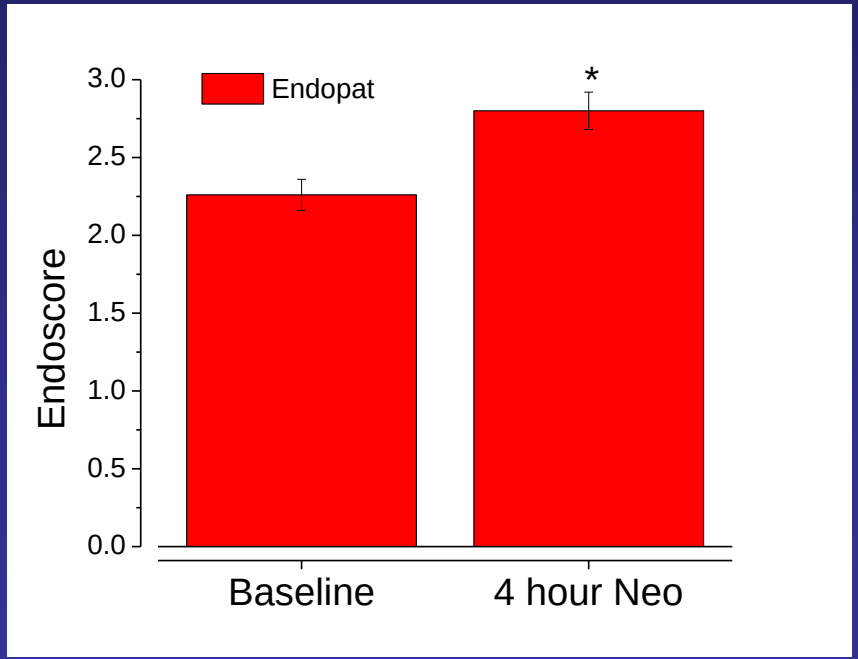
3 week washout

Placebo lozenge



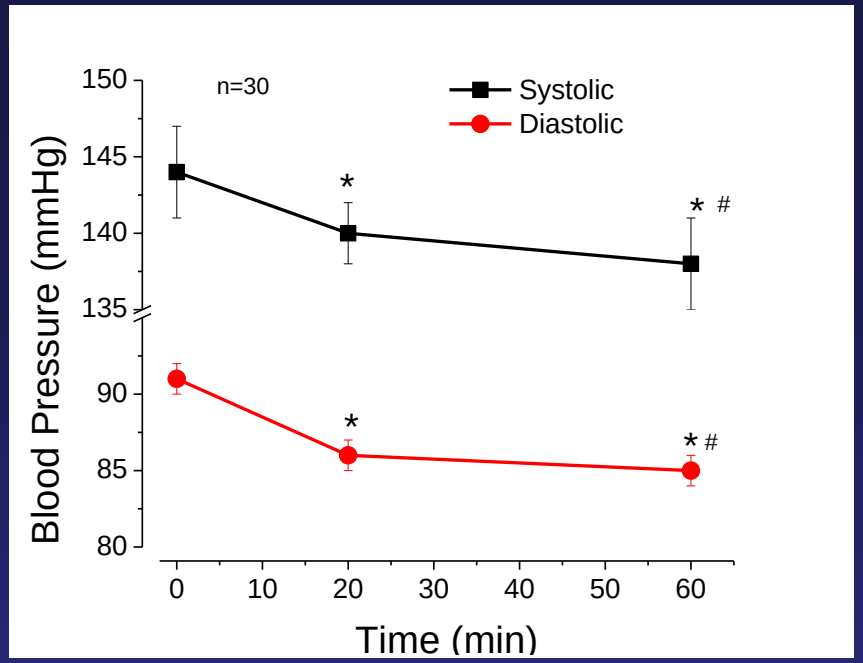
Hypertension Trial – Vanderbilt Univ

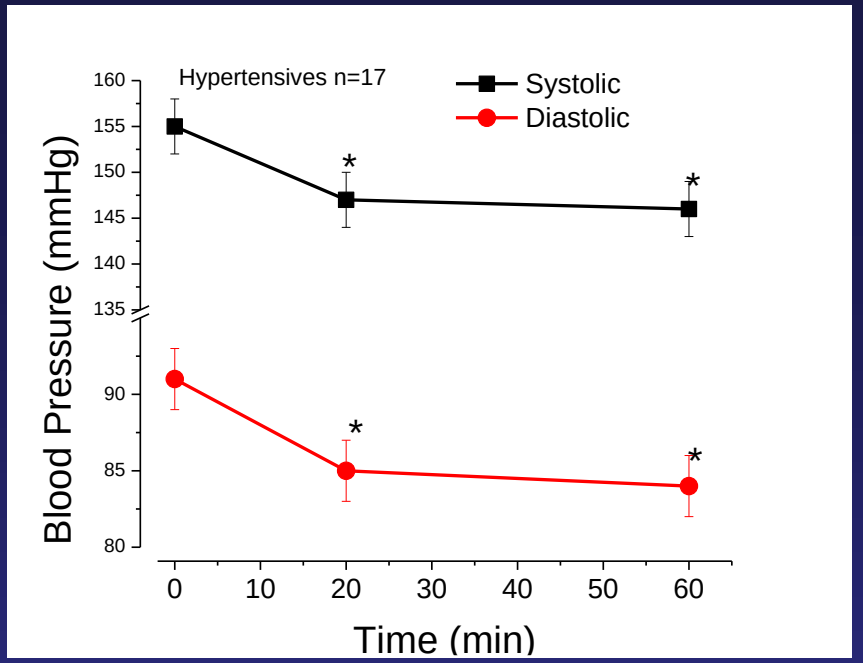


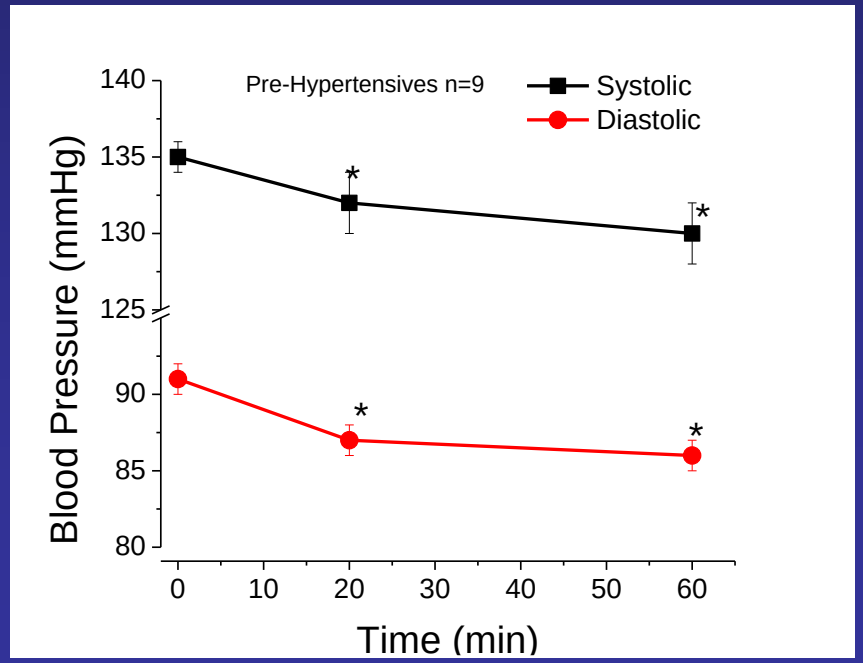


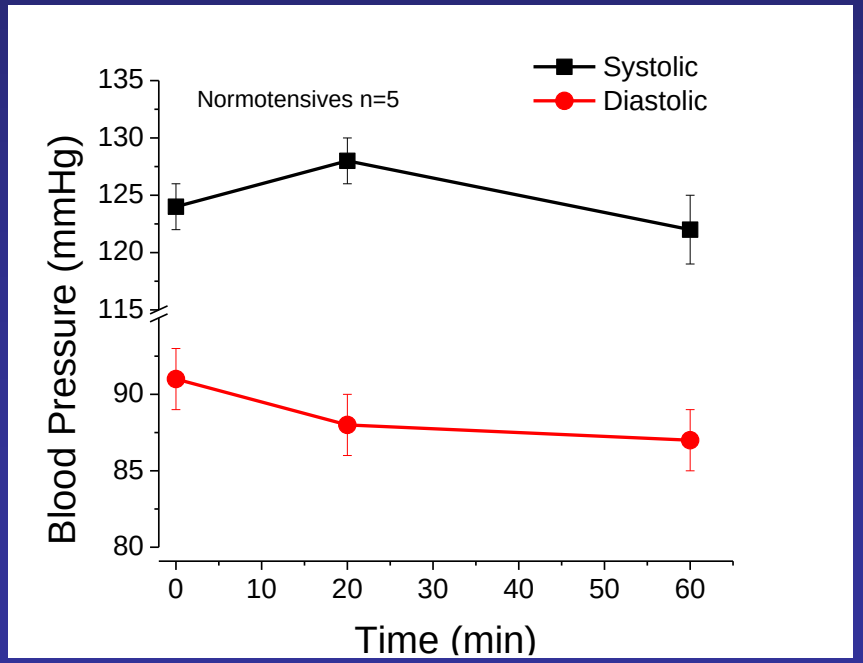
Flow mediated dilation

Houston, Hays JCH 2014

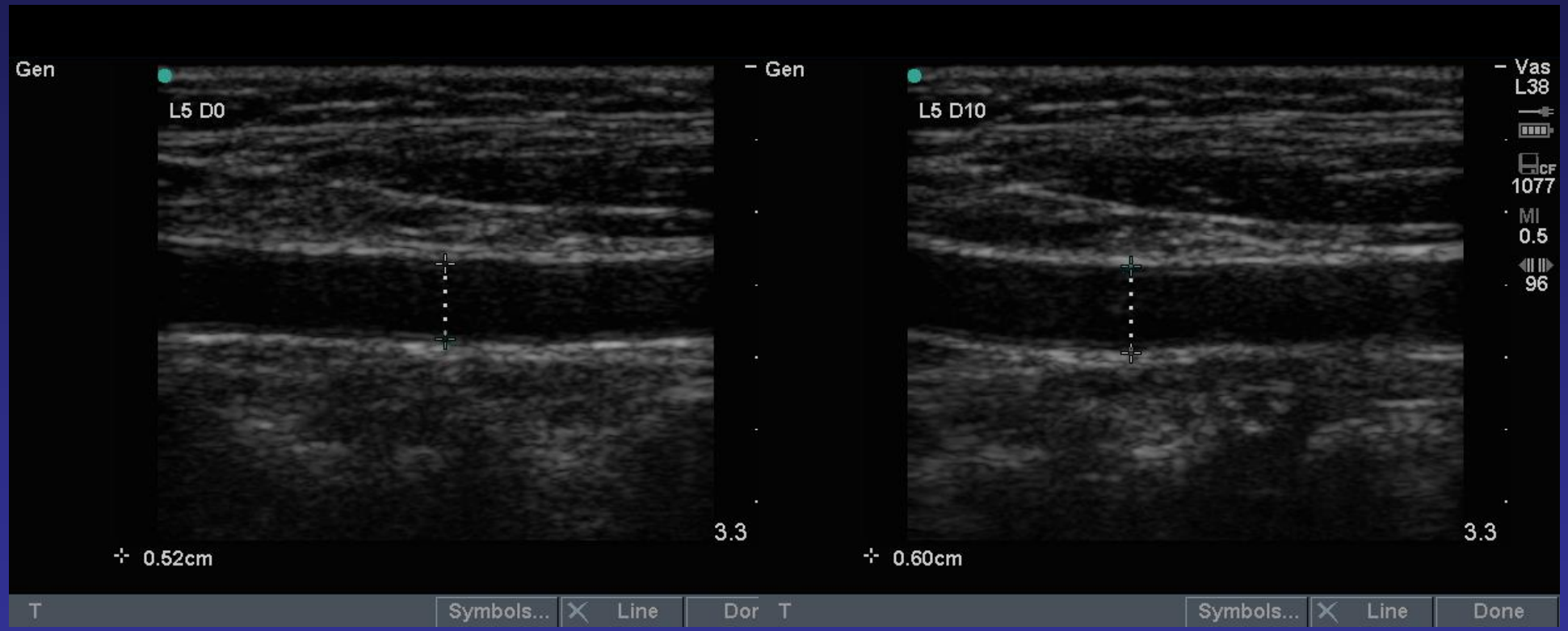

n=30
Systolic
Diastolic
Blood Pressure (mmHg)
Time (min)
150
145
140
135
90
85
80
0
10
20
30
40
50
60
*
*
* #
*
*
* #


Hypertensives n=17
Systolic
Diastolic
Blood Pressure (mmHg)
Time (min)
160
155
150
145
140
135
90
85
80
0
10
20
30
40
50
60
*
*
*
*


Pre-Hypertensives n=9
Systolic
Diastolic
Blood Pressure (mmHg)
Time (min)
140
135
130
125
90
85
80
0
10
20
30
40
50
60
*
*
*
*


Normotensives n=5
Systolic
Diastolic
Blood Pressure (mmHg)
Time (min)
135
130
125
120
115
90
85
80
0
10
20
30
40
50
60

Representative Ultrasound Before and 10 minutes after NO Lozenge



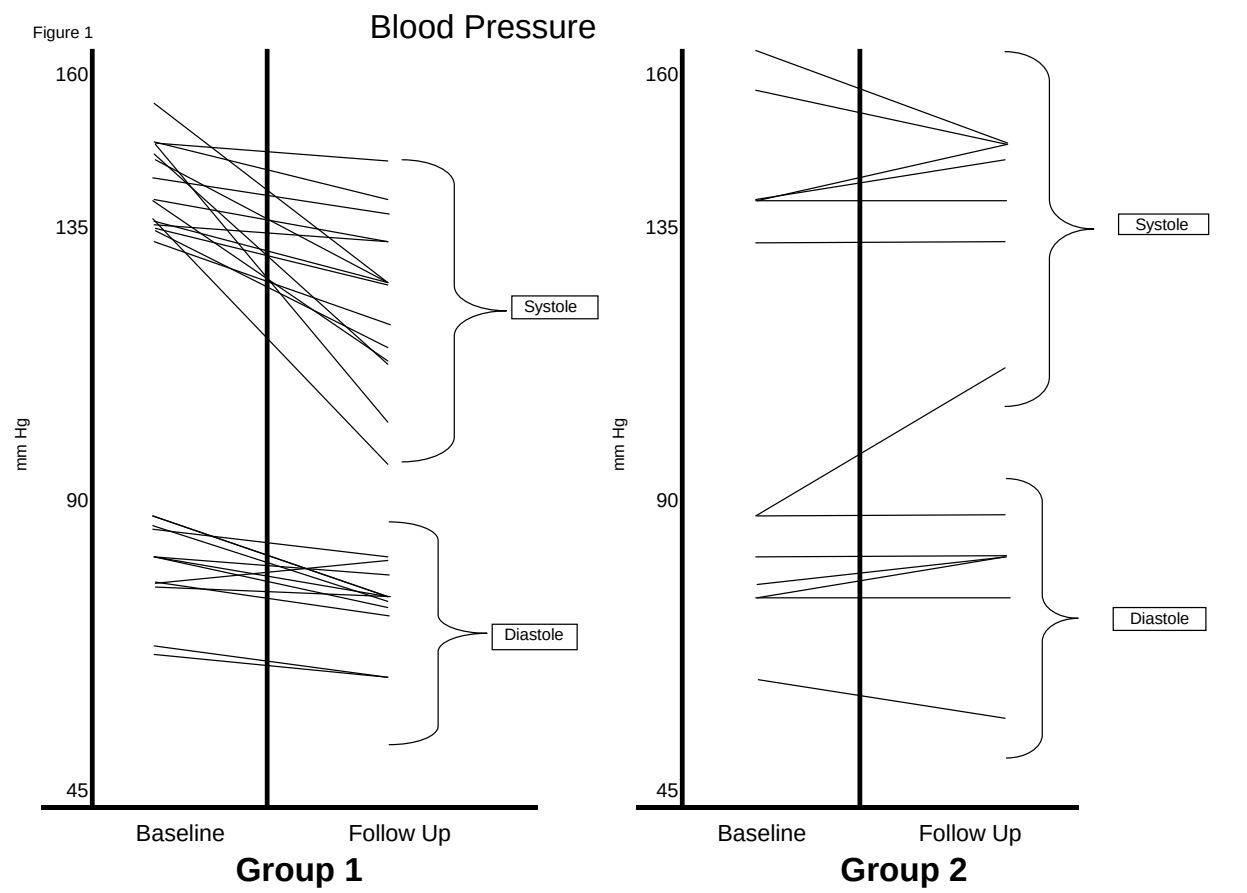
13% increase in vessel diameter causes a 34% increase in blood flow

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



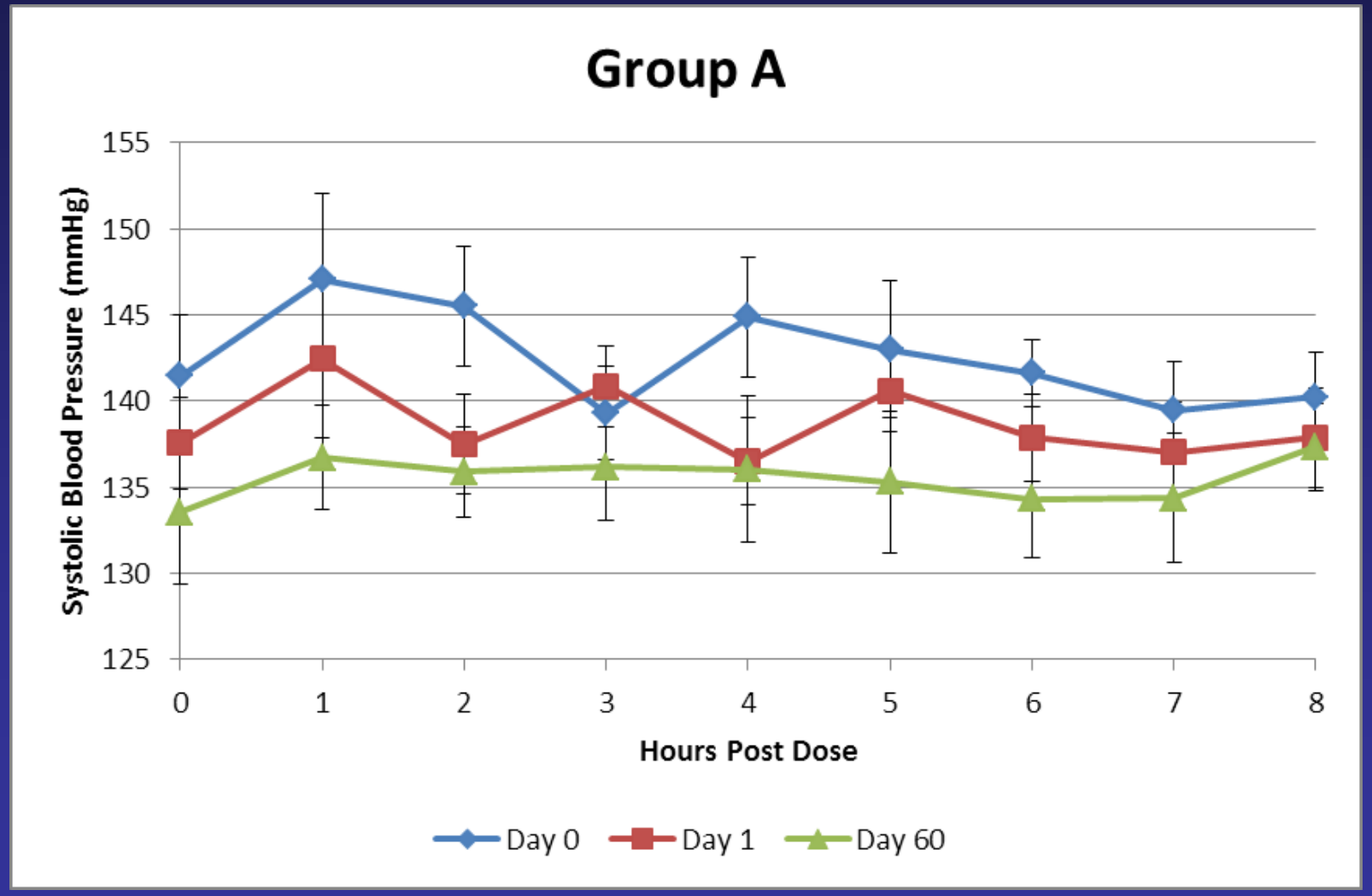
Biswas et al JCPT 2015

30 Day Placebo controlled Trial

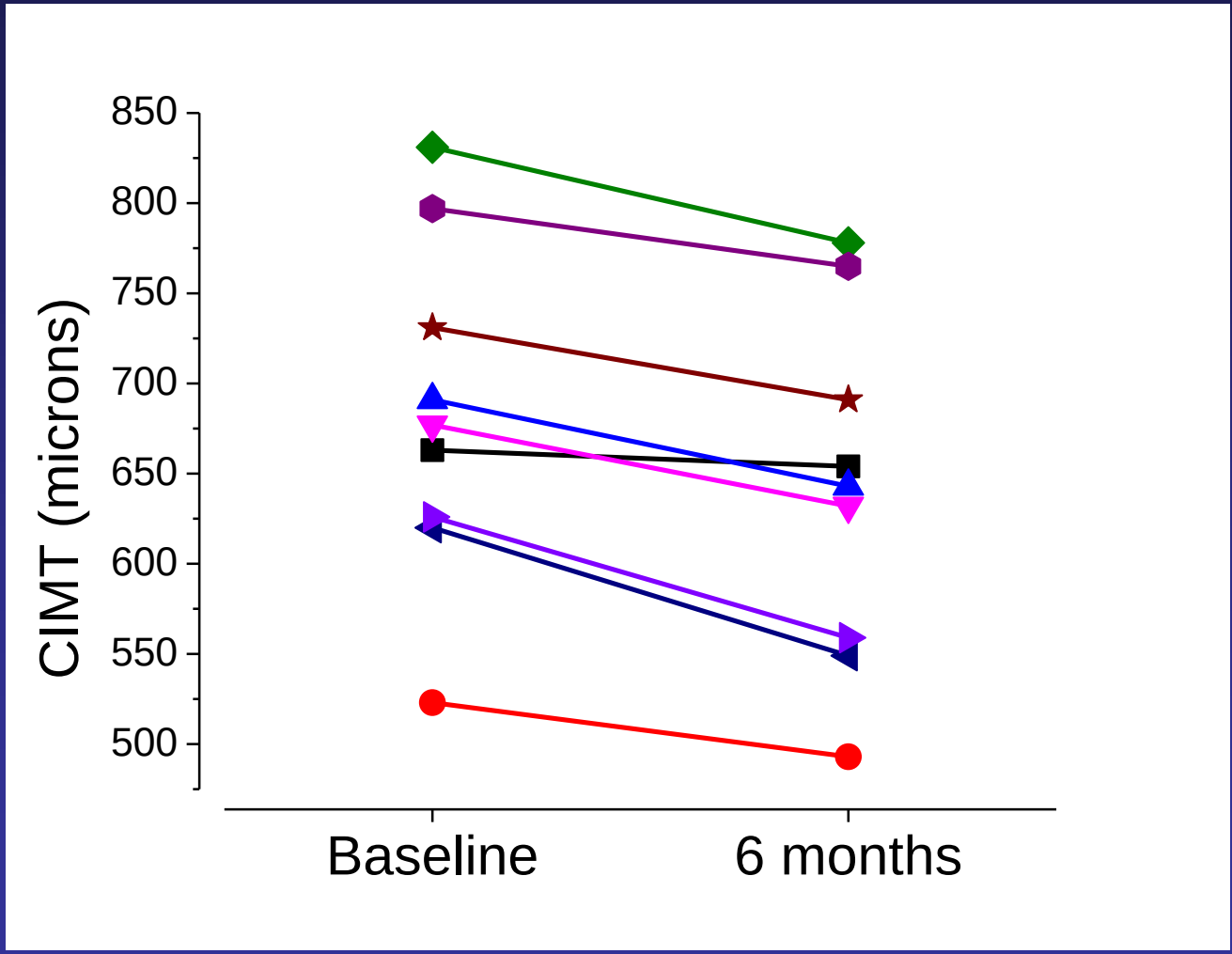
	Group 1 (mean ± SD)			Group 2 (mean ± SD)			Baseline: NO vs placebo (p-value)	Follow-Up: NO vs placebo (p-value)
	Baseline	Follow-Up	Δ	Baseline	Follow-Up	Δ		
BP (mmHg, systole; diastole)	138±12; 84±5	126±12; 78±4	12; 6 reduction (p<0.001)	138±21; 80±8	135±17; 82±8	N.S.	0.19; 0.012	0.26; 0.25
Heart Rate (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

Hypertension 60 day trial



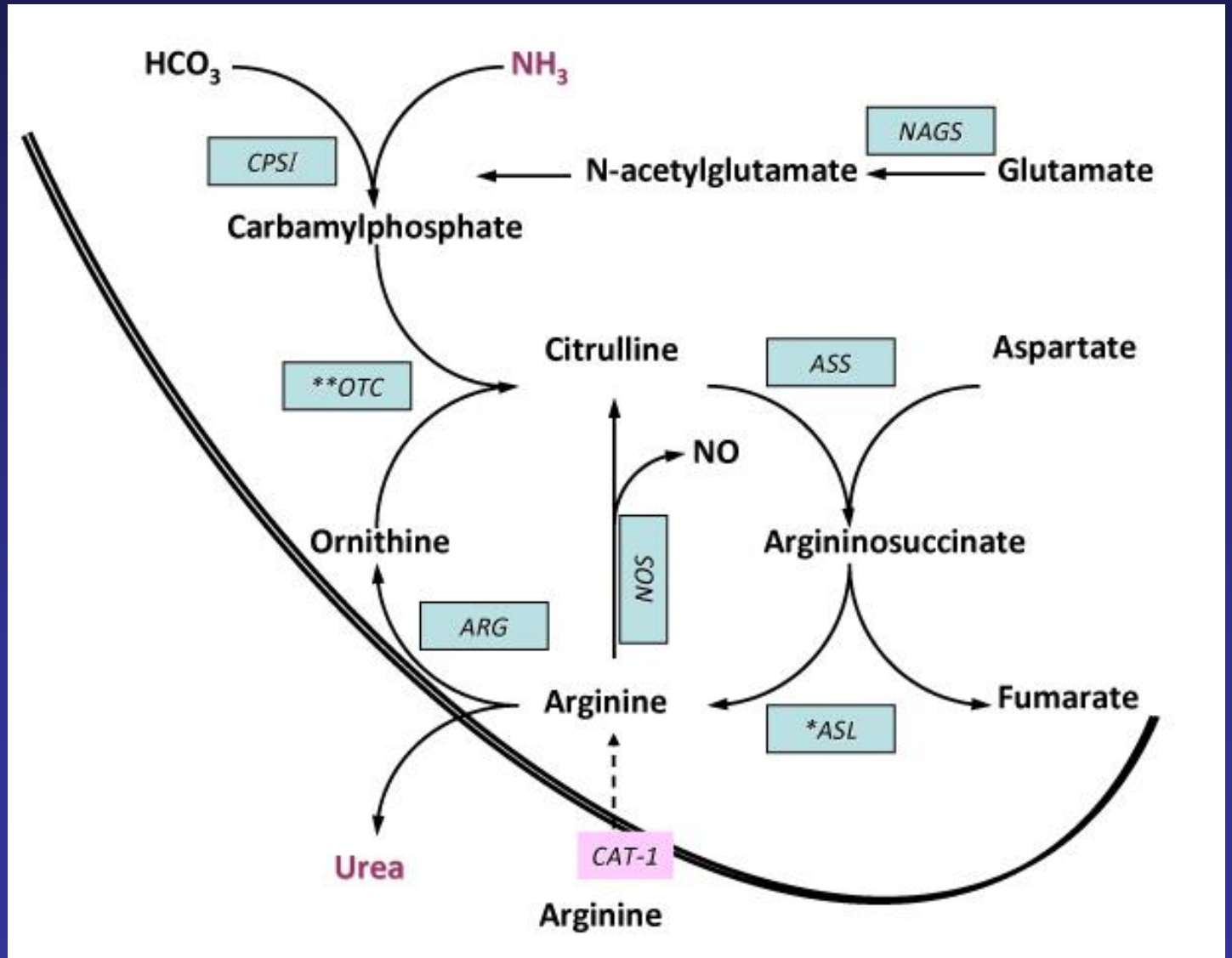
NO Supplement Leads to Plaque Regression



Edwin Lee MD – case report

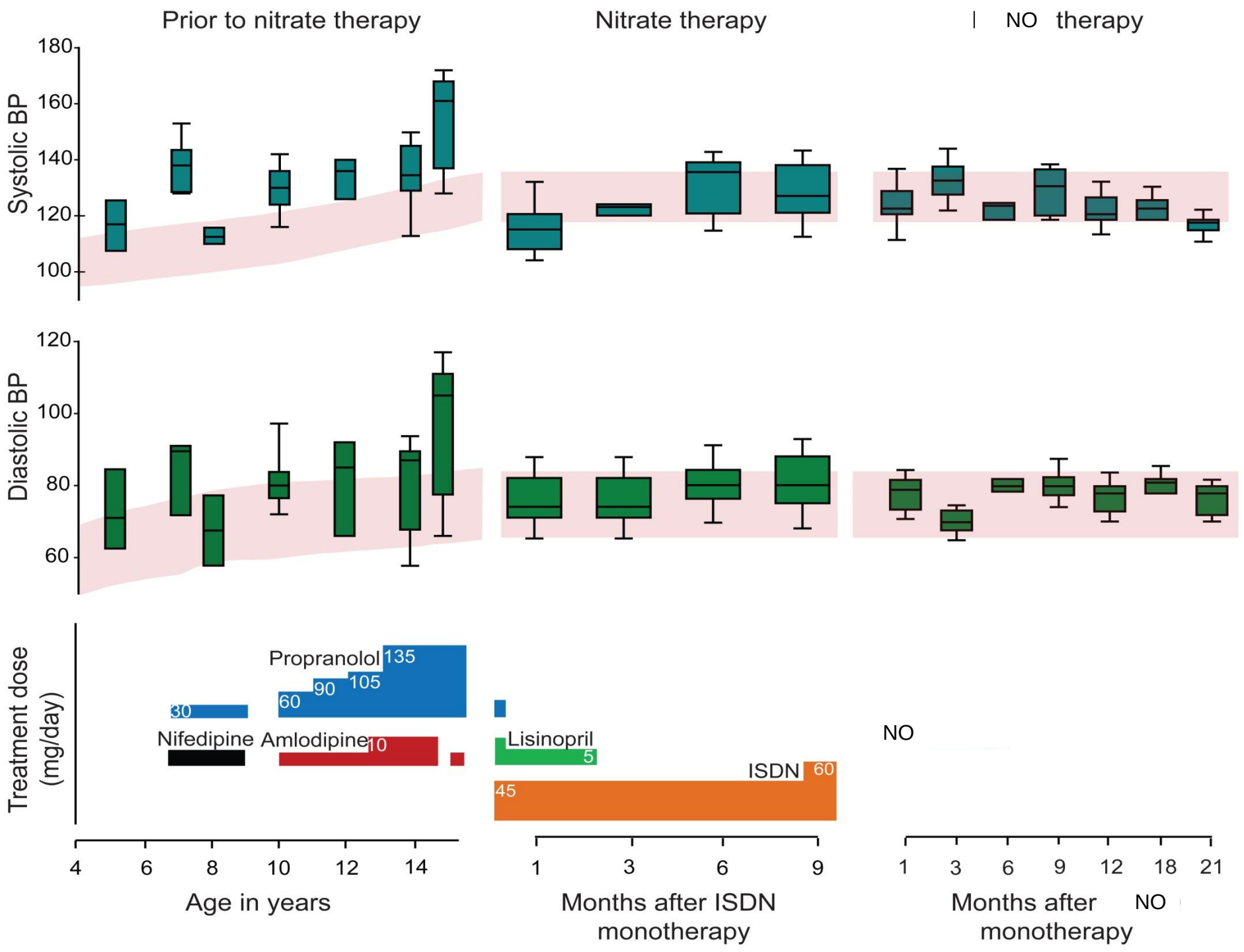
NO Lozenge Rescues Inborn Error in Metabolism

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?


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
105
90
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

NEW PARADIGM FOR NO REGULATION

- There exists specific bacterial communities that provide the human body with continuous sources of nitrite and NO from dietary nitrate.

- Contributes to optimal cardiovascular health (regulates BP)

- Absence of these oral bacterial communities affects NO homeostasis.

- Individuals deficient in these commensal bacteria would be NO deficient and perhaps at increased risk for cardiovascular disease

- Low dose nitrite can overcome oral microbiome diversity and normalize blood pressure and reduce inflammation

CONCLUSIONS

- There are profound effects of NO on health and disease
- Recognizing NO insufficiency is critical for prevention of disease
- Implementing lifestyle modification and strategies to restore NO production is fundamentally important is health and well-being
- Implementing the basics of physiology and cell biology is critical for optimal health (Identify cause of disease, remove toxicant and restore physiology)
- Patients will not get better without restoration of NO

You cannot practice medicine effectively without correcting NO status in patients

CONCLUSIONS

- NO deficiency leads to increased risk of heart disease and severity of Injury after MI
- Restoration of NO mitigates risk of heart disease and protects from injury from MI
- Taking steps to improve NO status in primary care patients may lead to better management of disease
- Compliance to diet and exercise programs are largely ineffective
- Providing patients a convenient daily NO product may improve compliance

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Booth 16 – Functional Regenerative Medicine

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

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Humana Press