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Texas

Saturday, June 10, 2017

8:15 - 8:30 NO in Heart Disease

Nitric Oxide in Heart Disease

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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)

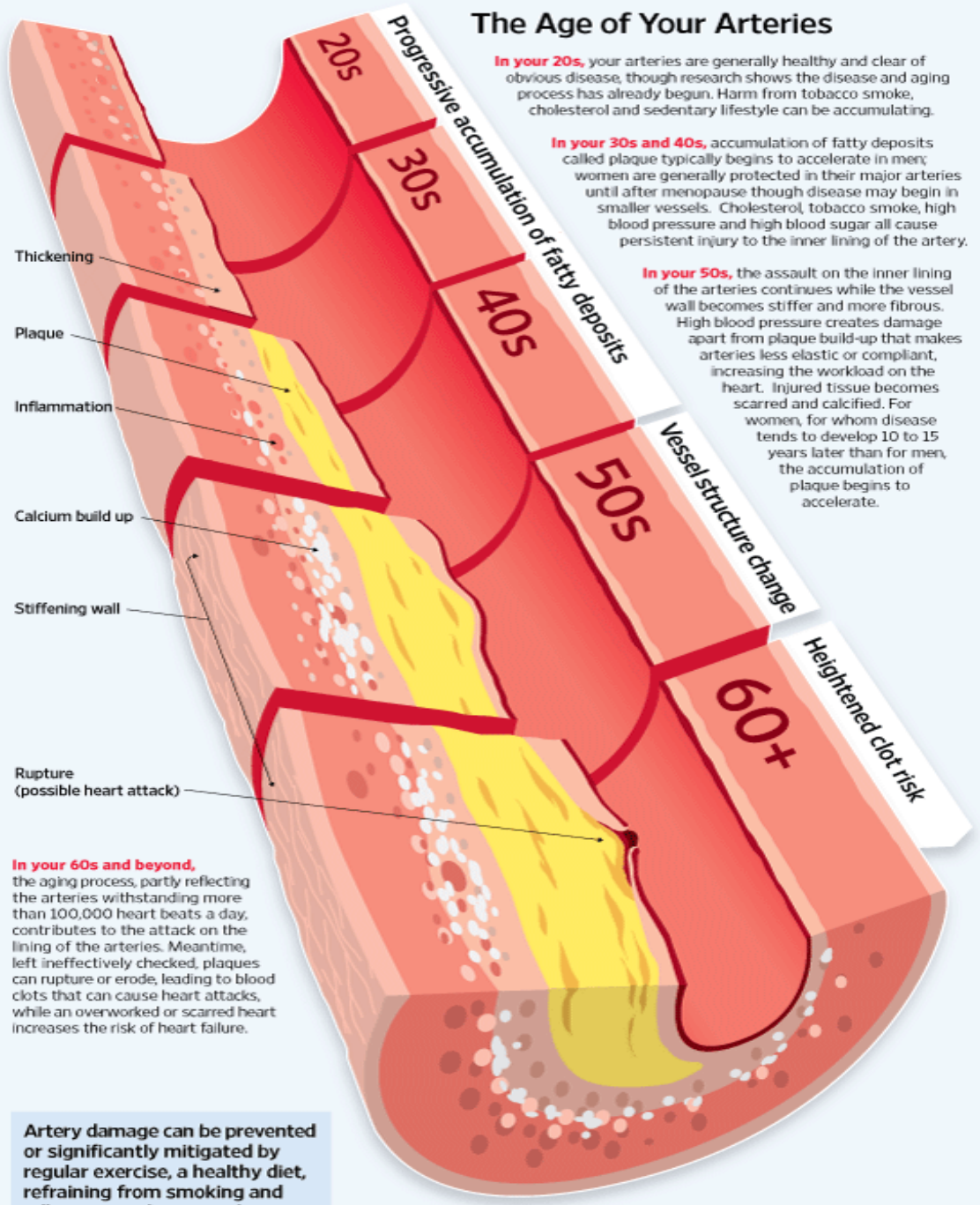
Objectives

- Provide an education on Nitric Oxide (NO) Biology
- Understand the role of NO in heart disease
- How do diet and exercise affect heart disease

“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.

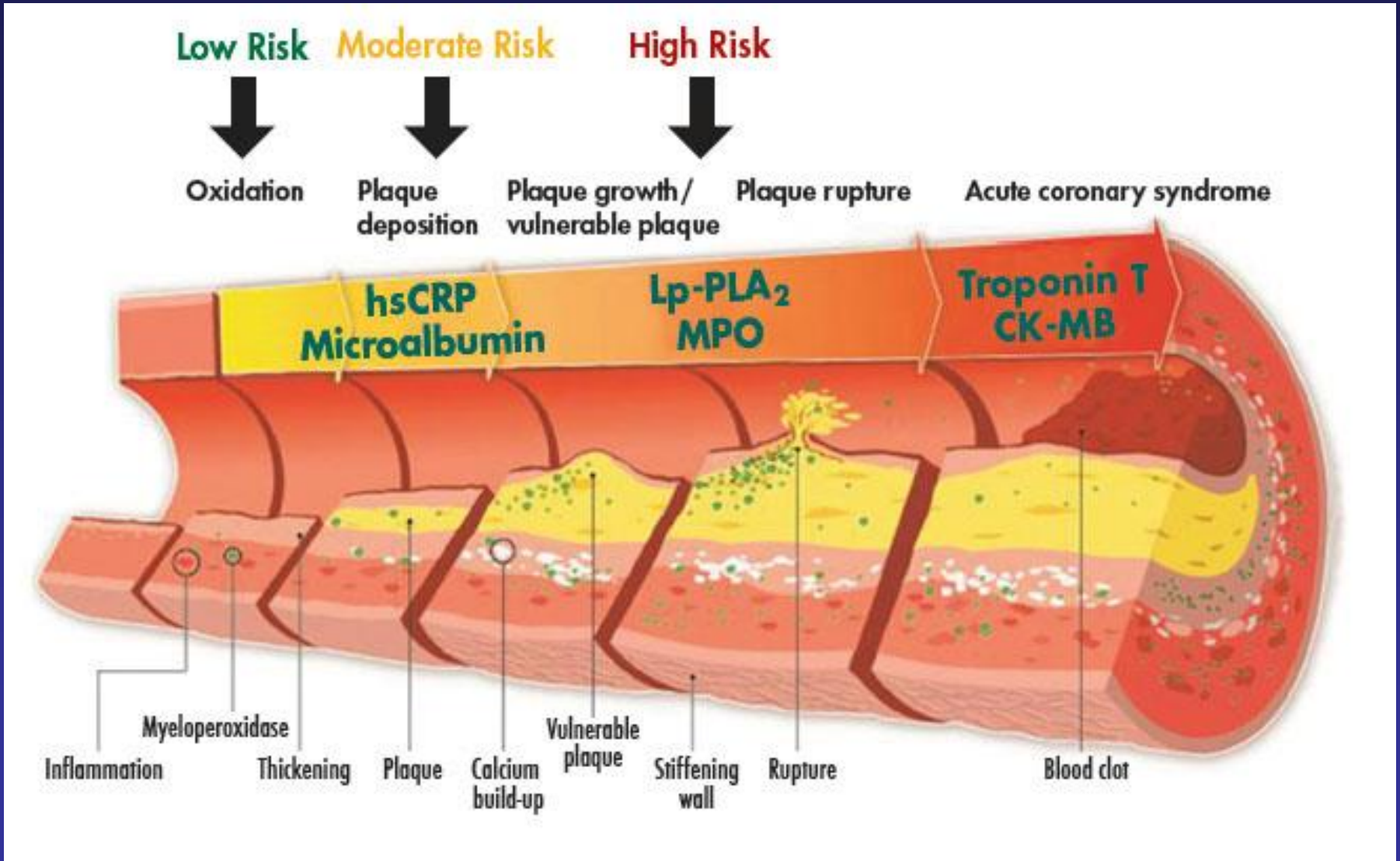
In your 50s, the assault on the inner lining of the arteries continues while the vessel wall becomes stiffer and more fibrous. High blood pressure creates damage apart from plaque build-up that makes arteries less elastic or compliant, increasing the workload on the heart. Injured tissue becomes scarred 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.

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.

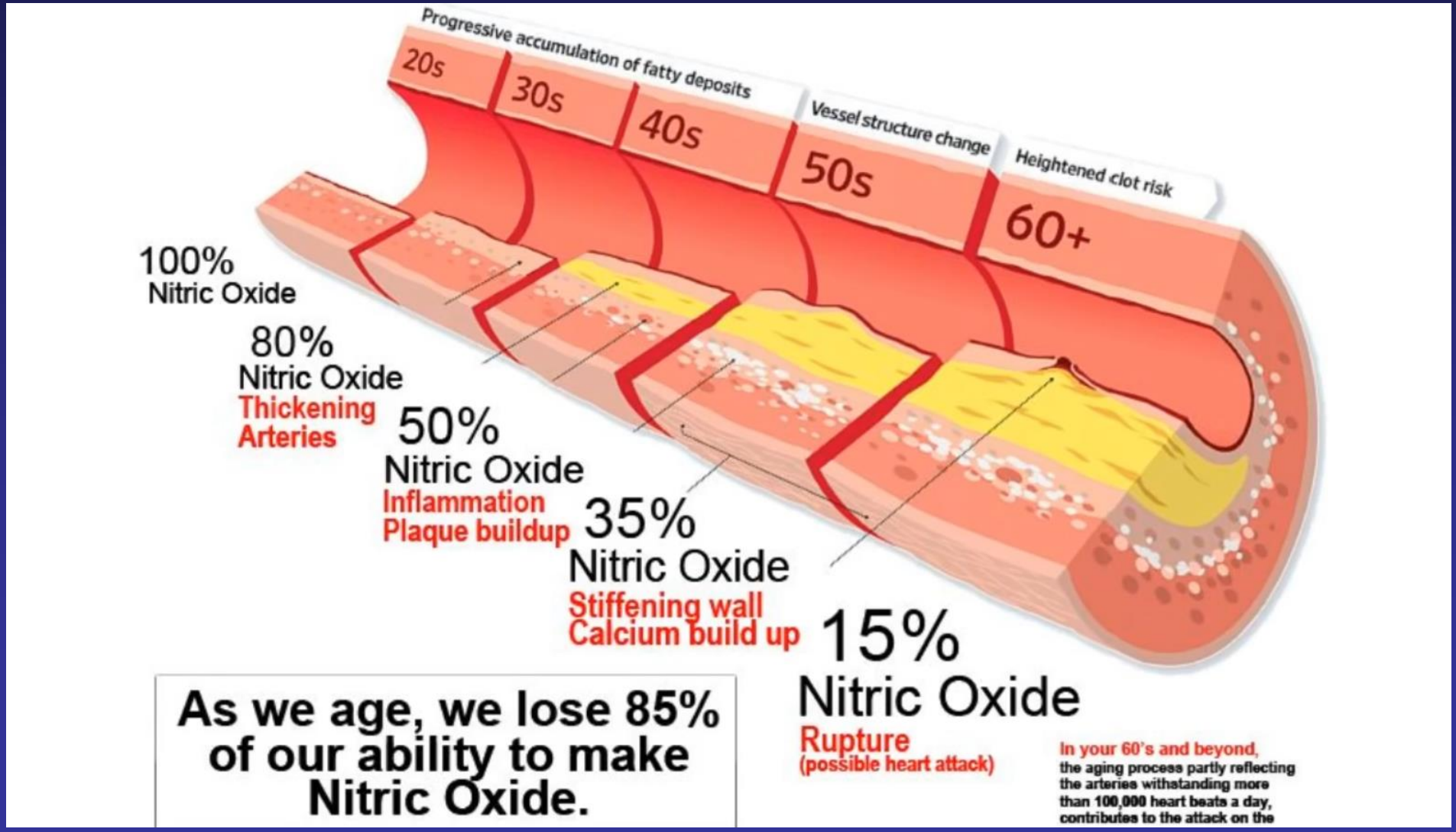
Artery damage can be prevented or significantly mitigated by regular exercise, a healthy diet, refraining from smoking and adherence to heart medicines.

Sources: Carl J. Pepine, University of Florida; WSJ reporting Illustration: Erik Brynildsen/The Wall Street Journal

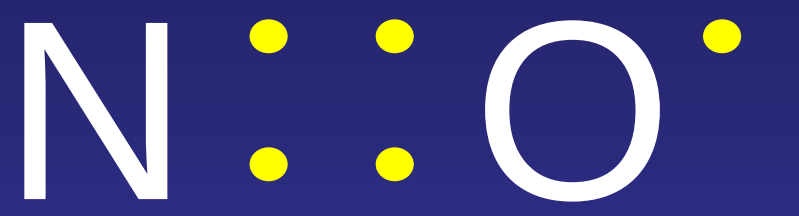
Inflammatory Biomarkers Diagnostic For Different Stages of Atherosclerosis

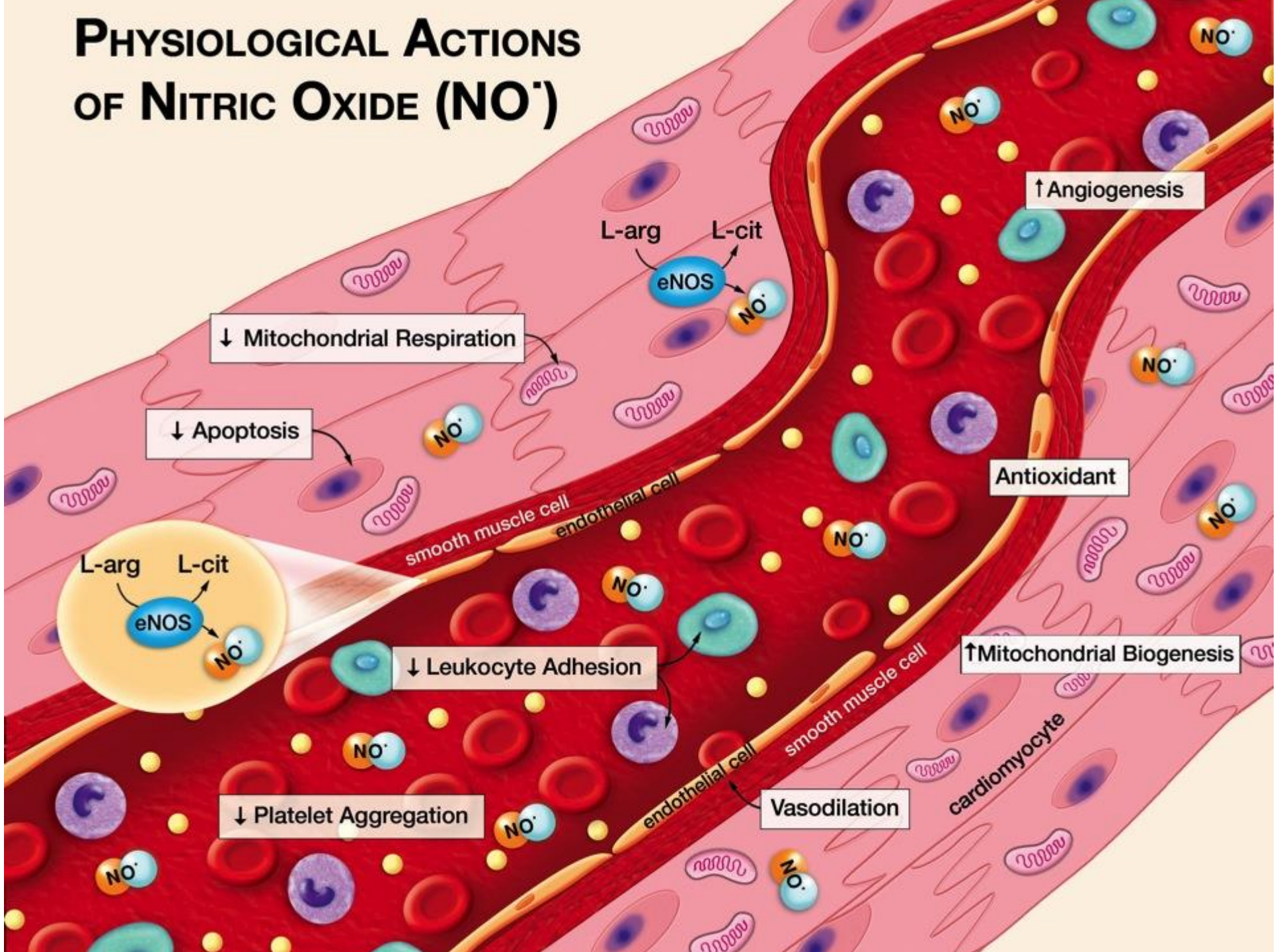


Loss of NO is Associated with Atherosclerosis

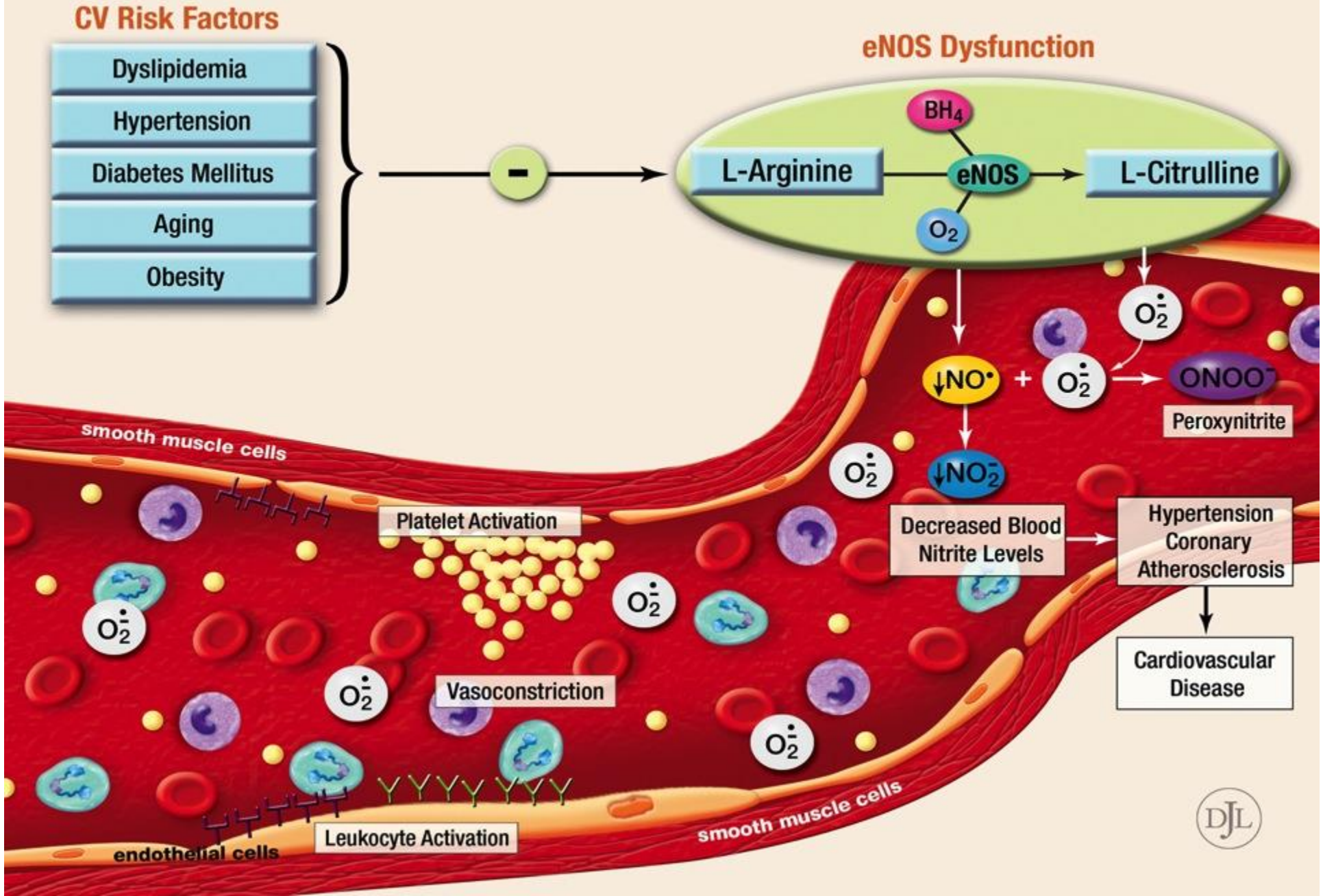


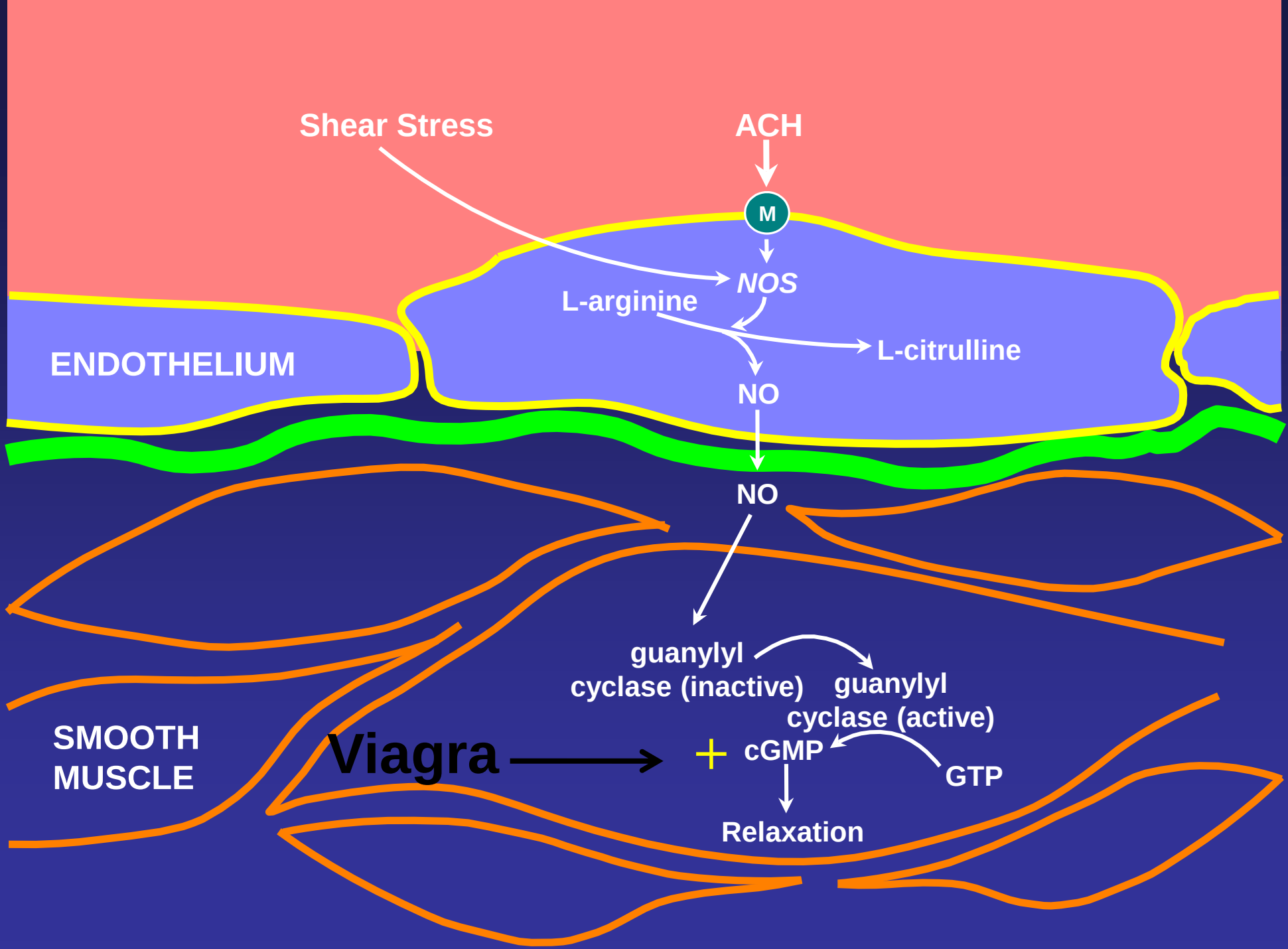
Nitric Oxide

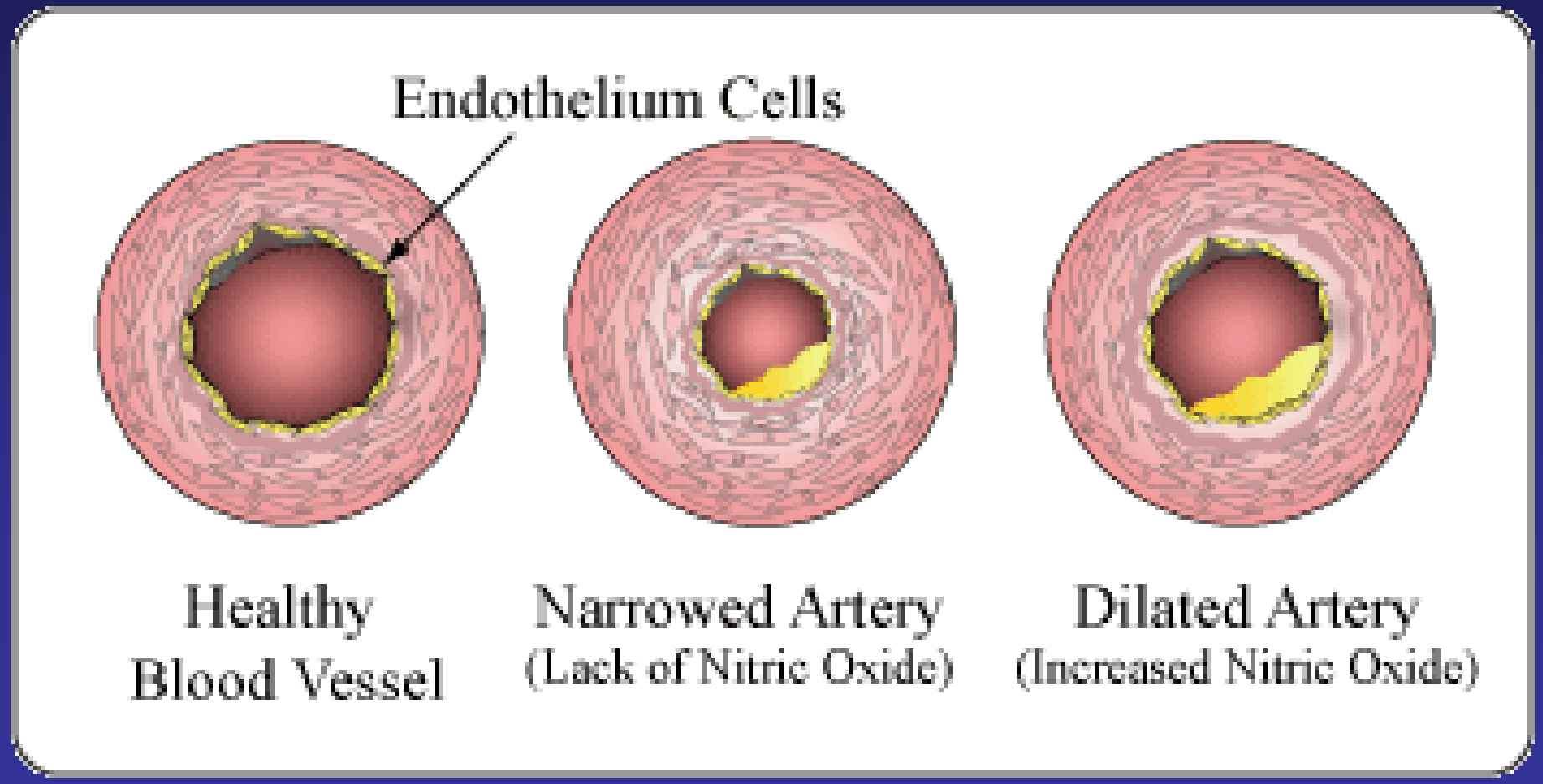



PHYSIOLOGICAL ACTIONS OF NITRIC OXIDE (NO·)
L-arg
L-cit
eNOS
NO·
↓ Mitochondrial Respiration
↓ Apoptosis
smooth muscle cell
endothelial cell
↓ Leukocyte Adhesion
↓ Platelet Aggregation
↑Angiogenesis
Antioxidant
↑Mitochondrial Biogenesis
Vasodilation
cardiomyocyte

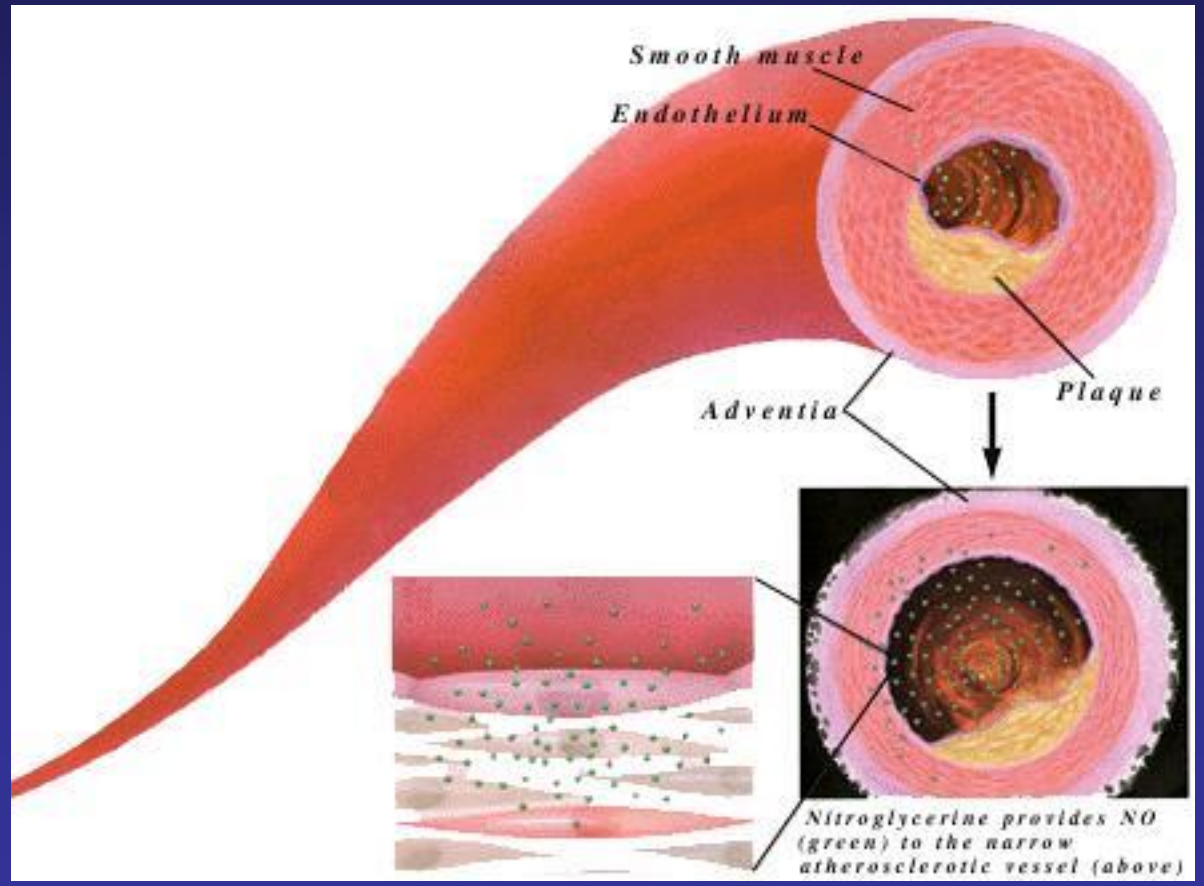
Vascular Endothelial Dysfunction




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)

Nitroglycerine, a 100 year old explosive and heart medicine

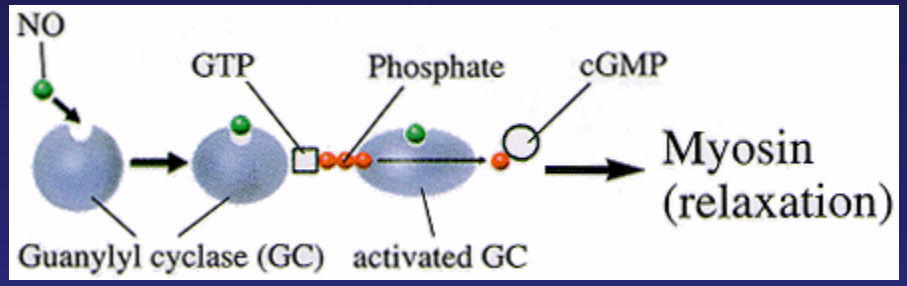


In atherosclerosis, plaques reduce blood flow in the arteries. This decreases oxygen supply to the heart muscle causing chest pain (angina pectoris) and sometimes even myocardial infarction.
Treatment with nitroglycerine provides NO, dilates the vessels, and increases blood flow.
Thanks to the 1998 Nobel 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.

Half-Life of NO

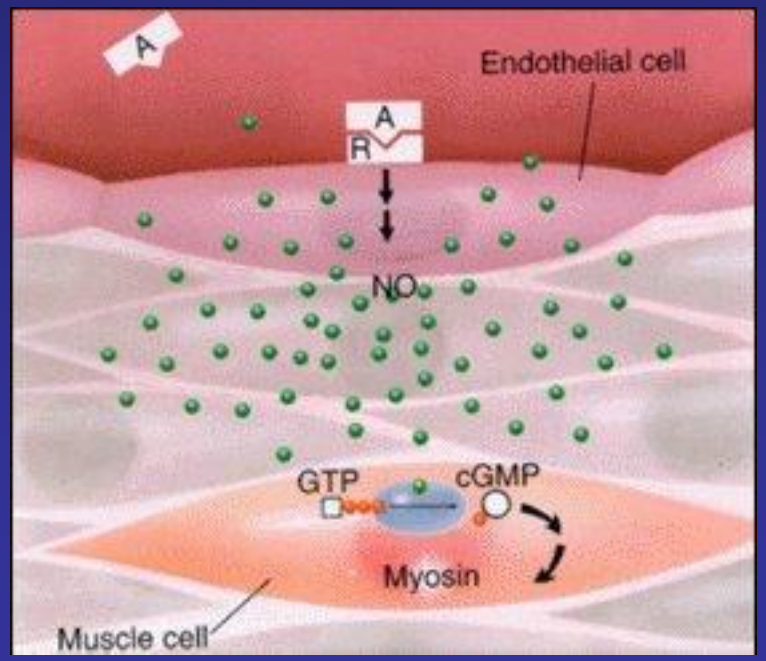
water (10-1 µM, 37°C, pO_2 150mmHg, pH 7.4):	**1-3**	**min**
blood ($5*10^9$ erythrocytes):	**0.5 - 2**	**ms**
in presence of HbO_2 (15g/dL):	**2**	**µs**

Ferid Murad Discovers NO activates sGC which is responsible for vessel relaxation



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

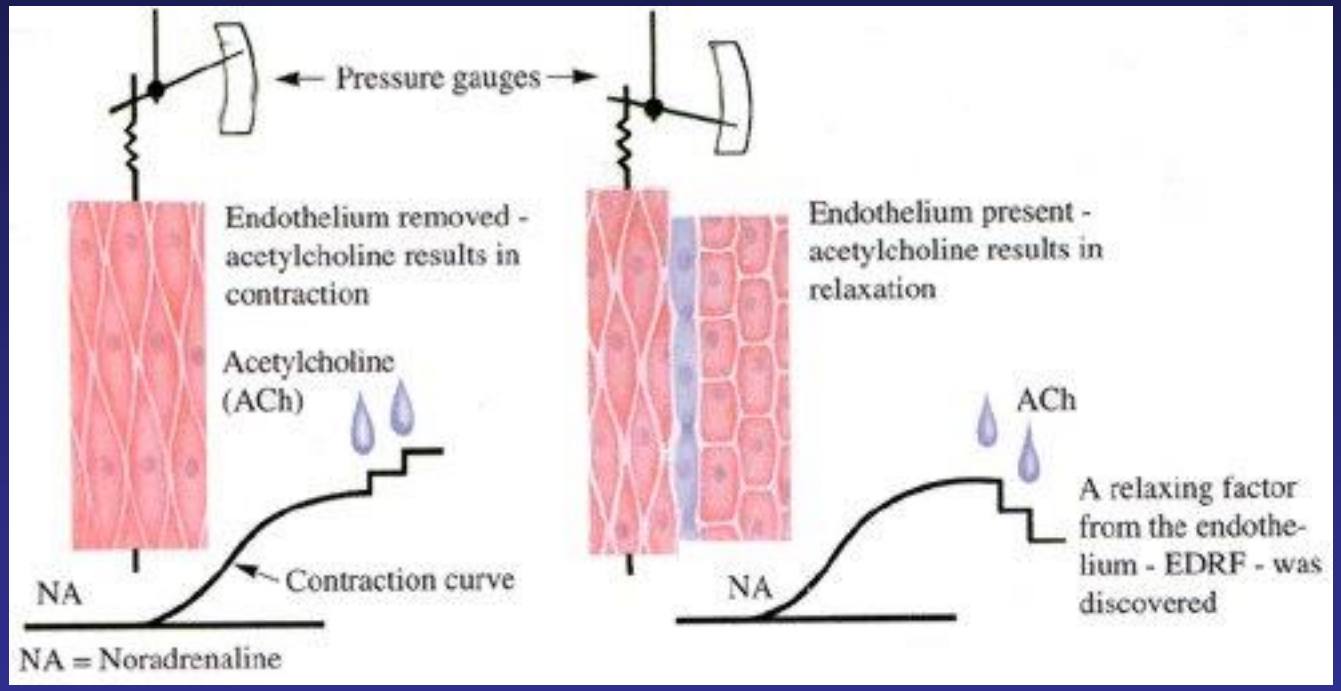
(Arnold WP, PNAS 1977)



Acetylcholine stimulates the endothelial cells to produce a factor, NO, which penetrates into and activates the muscle cells causing relaxation.

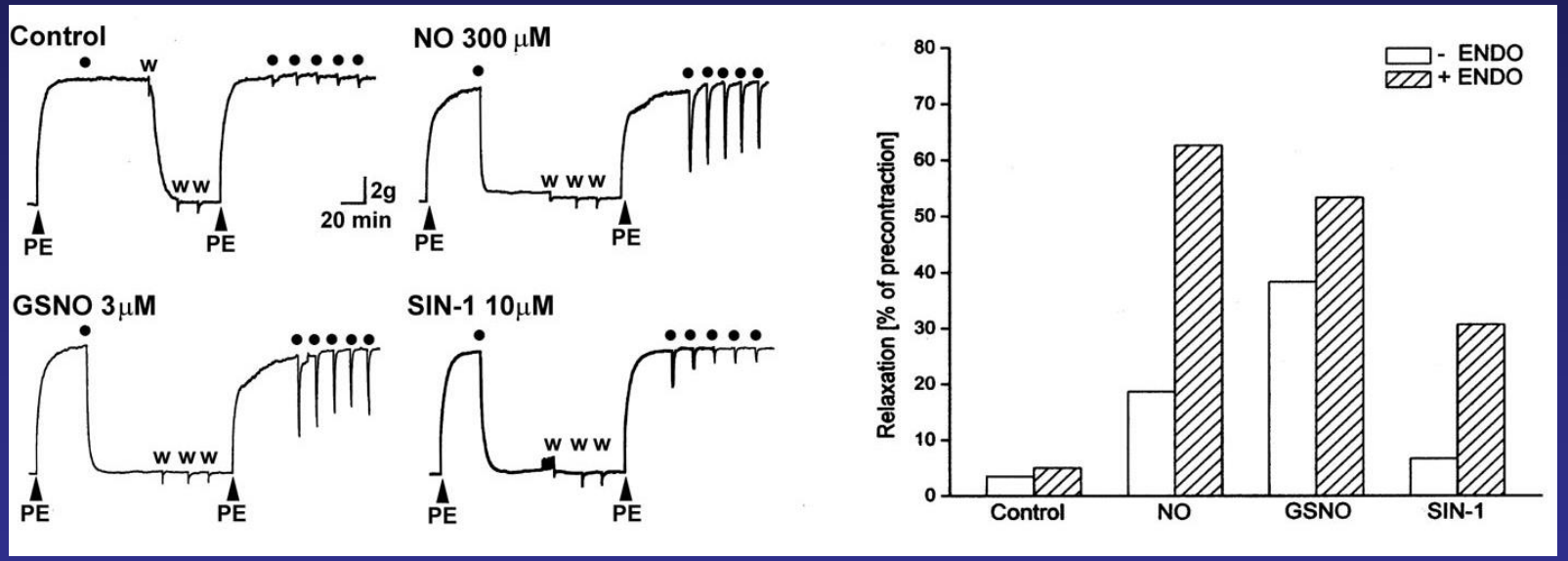
…In investigating this apparent discrepancy, we discovered that the loss of relaxation of ACh in the case of the strip was the result of unintentional rubbing of its intimal surface against foreign surfaces during its preparation. If care was taken to avoid rubbing of the intimal surface during preparation, the tissue, whether ring, transverse strip or helical strip, always exhibited relaxation to ACh, and the possibility was considered that rubbing of the intimal surface had removed endothelial cells. Furchgott Nature 1980

Robert Furchgott Discovers Endothelium-Dependent Relaxing Factor (EDRF)



Robert F Furchgott showed that acetylcholine-induced relaxation of blood vessels was dependent on the endothelium. His "sandwich" experiment set the stage for future scientific development. He used two different pieces of the aorta; one had the endothelial layer intact, in the other it had been removed. (Furchgott, Zawadzki Nature 1980)

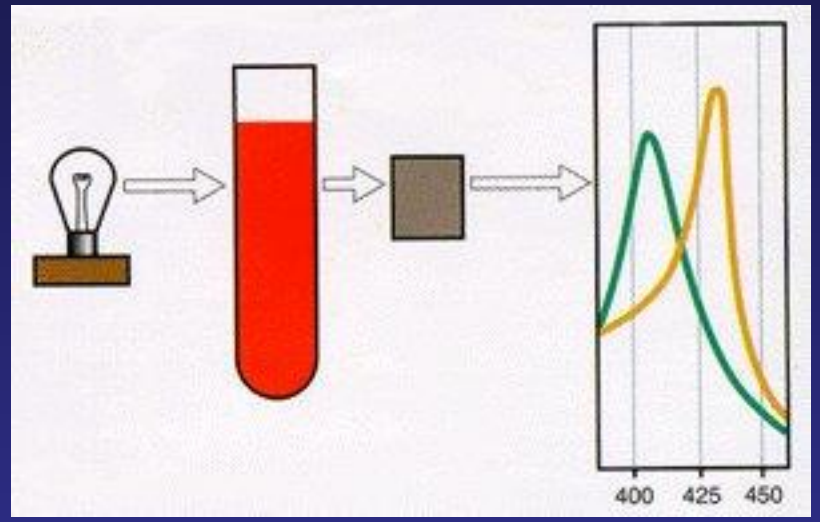
Photorelaxation



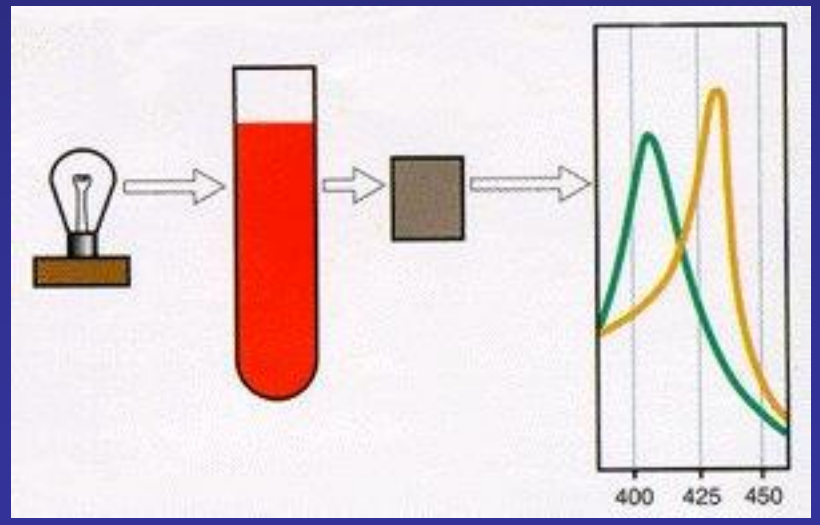
Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri, G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987 Dec;84(24):9265-9

Palmer RM, Ferrige AG, Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987 Jun 11-17;327(6122):524-6.

Louis Ignarro Discovers EDRF is Identical to NO



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



Hemoglobin (yellow) directly exposed to NO (green)

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

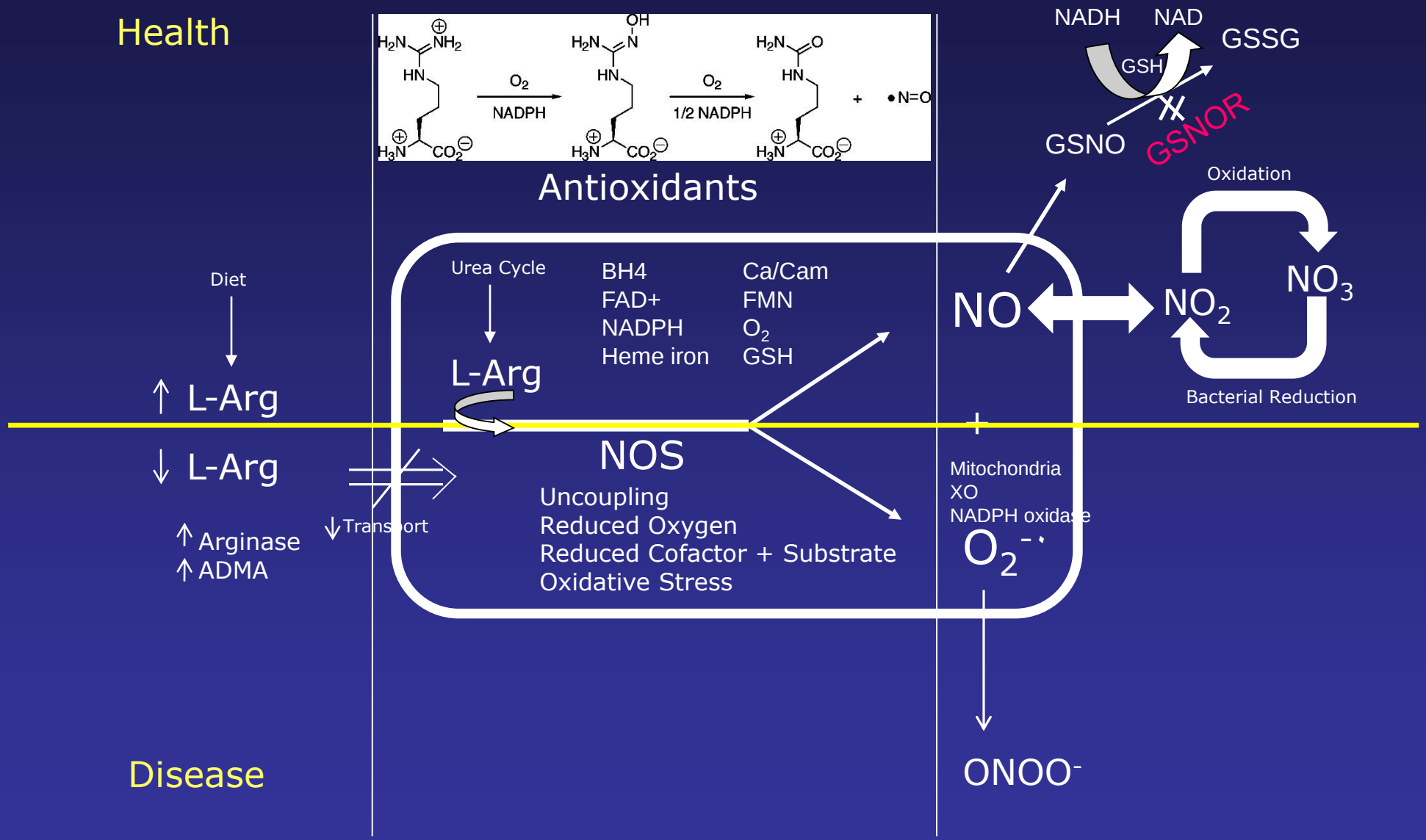
Ignarro, LJ et al. PNAS 1987

What Warranted Nobel Prize for Discovery of NO?

Nitric Oxide, NO, is a short-lived, endogenously produced gas that acts as a signaling molecule in the body. Signal transmission by a gas, produced by one cell, which penetrates membranes and regulates the function of other cells is an entirely new principle for signaling in the human organism.

How do we control and regulate NO production?

The L-Arginine-Nitric Oxide Pathway



Mitochondrial Theory of Aging

1. Free radicals play a major role in aging and most are mitochondrially produced.

2. Mitochondrial DNA lacks the protective DNA-binding protein (called histones), has less efficient DNA repair, and is close to the free radical-producing ETC, resulting in high levels of mitochondrial DNA damage compared to nuclear DNA.

3. Caloric restriction, the only known treatment to increase the mammalian lifespan, reduces mitochondrial free radical production and mitochondrial DNA oxidative damage.

4. Fibroblasts injected with mitochondria from old rats degenerate more rapidly than fibroblasts injected with mitochondria from young animals.

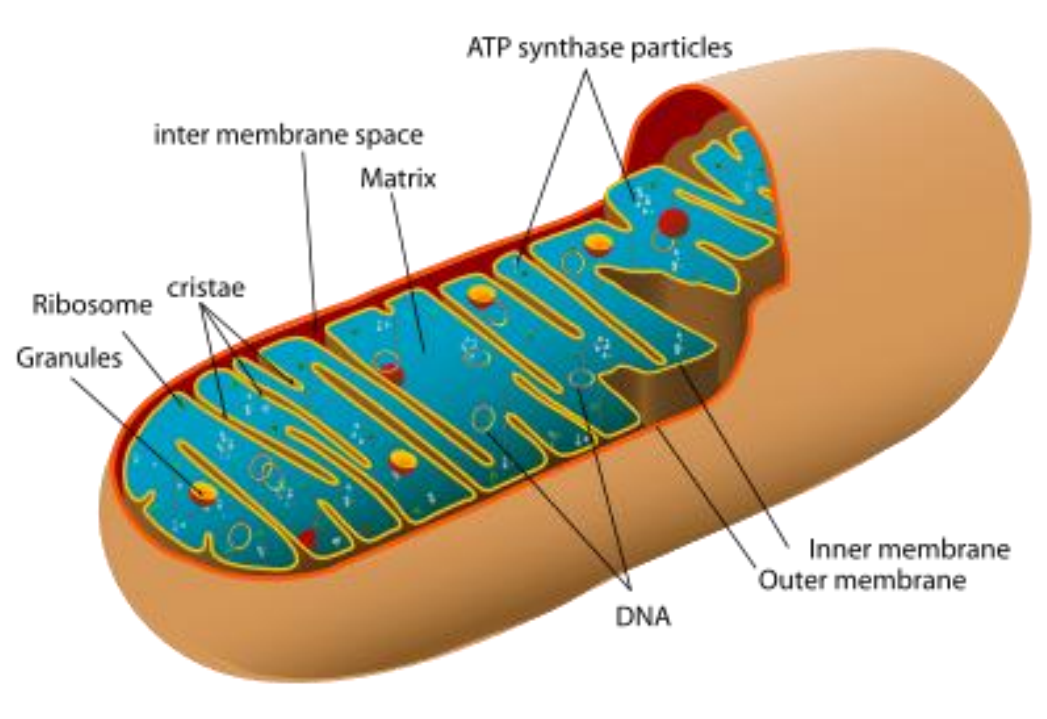
5. The mitochondria of older mammals are often larger and less efficient than those from younger mammals.

6. Targeted increased mitochondrial catalase (an enzymatic anti-oxidant) expression increases the mouse lifespan by around 20%.

7. Studies between different species have demonstrated that longer-lived species typically have lower mitochondrial DNA oxidative damage and lower free radical production.

8. Targeted mutation of the mitochondrial DNA polymerase-g, which causes an increased mitochondrial mutation rate with aging, results in a premature aging phenotype.

Mitochondria – Cellular Power Plants



Mitochondria are found in nearly all eukaryotes. They vary in number and location according to cell type. A single mitochondrion is often found in unicellular organisms. Conversely, numerous mitochondria are found in human liver cells, with about 1000–2000 mitochondria per cell, making up 1/5 of the cell volume.

Generate ATP, used as a source of chemical energy

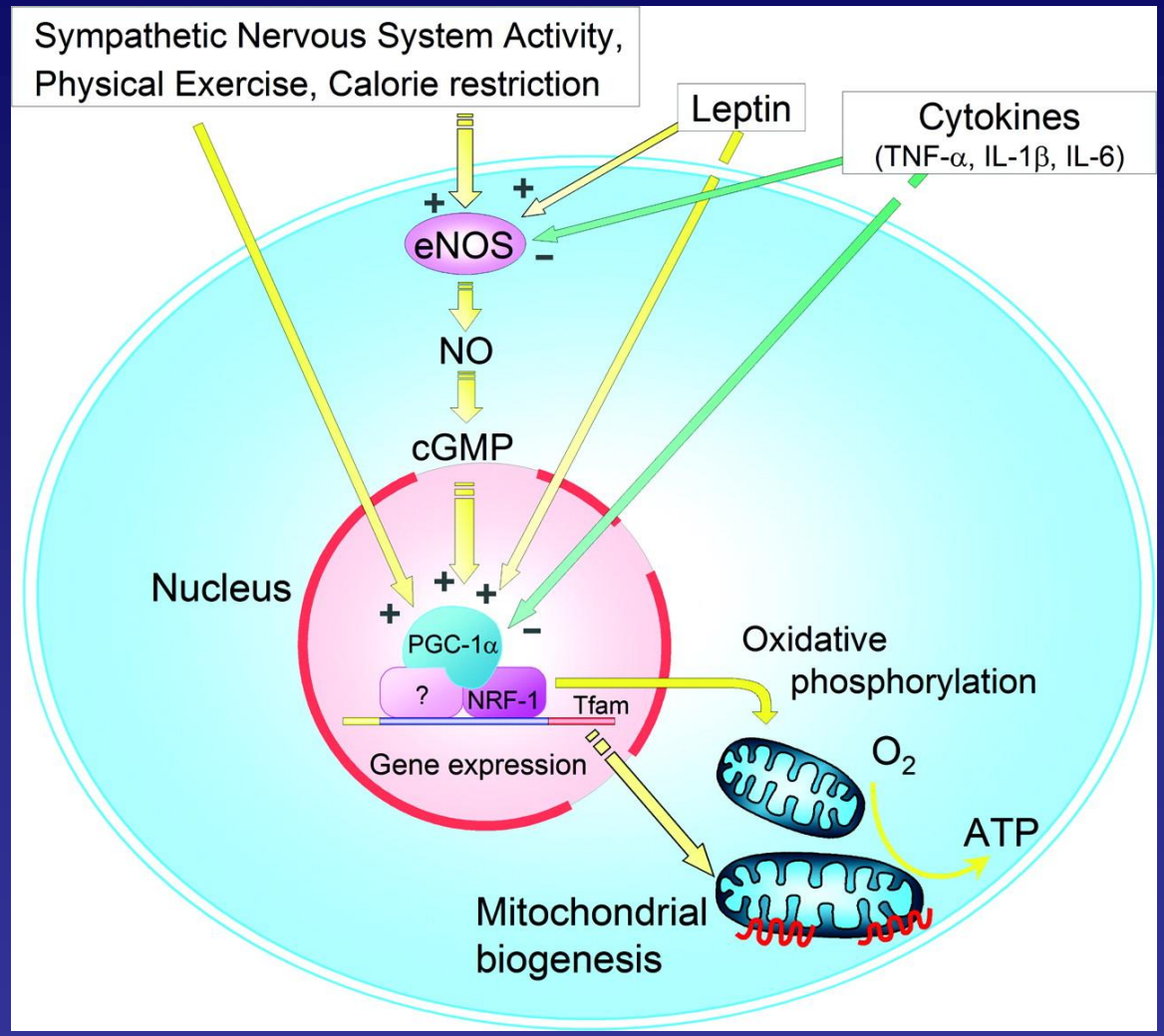
Involved in cell signaling
Heat Production
Cellular metabolism
Cellular differentiation
Cell death
Control of the cell cycle (cancer)
Cell growth
Steroid synthesis

Mitochondria have been implicated in many human diseases and are critical in the aging process.

Nitric Oxide Controls and Regulates Mitochondrial:

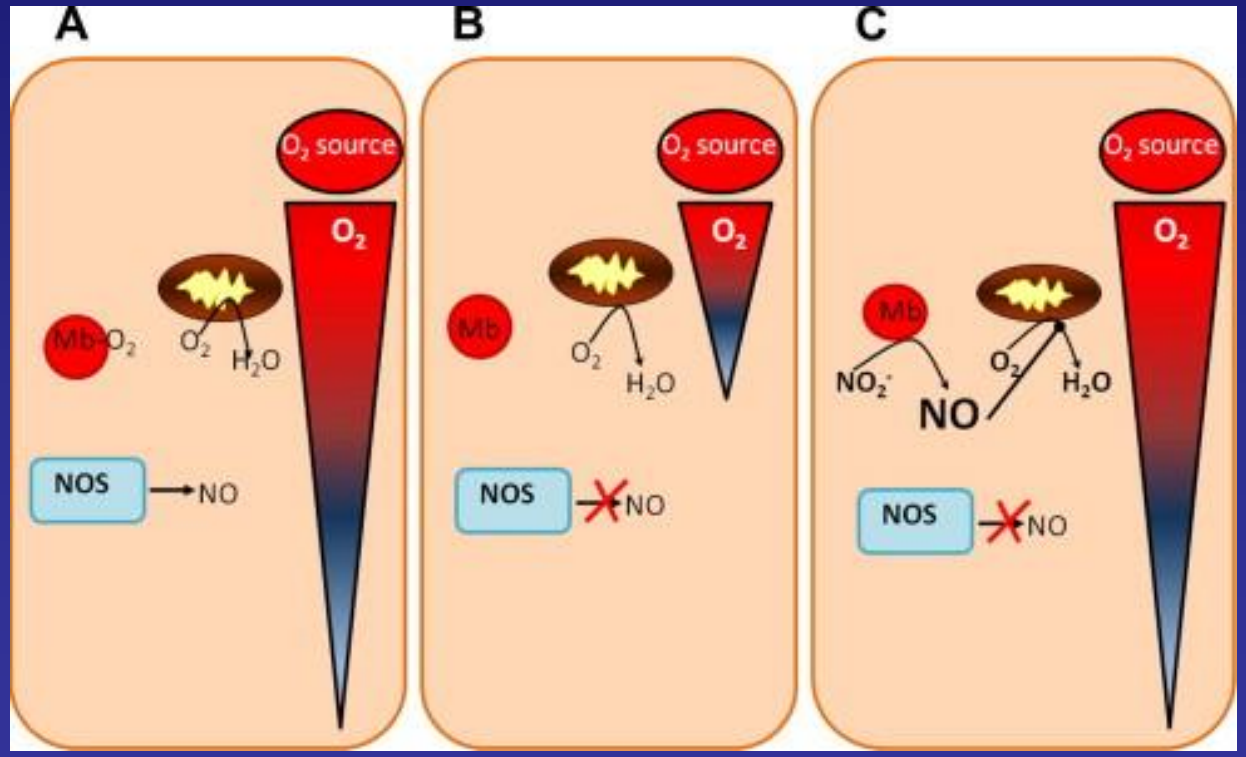
ATP synthesis
Reactive Oxygen Species
Cell Signaling
Apoptosis (Cell Cycle)
Biogenesis
Metabolism/Bioenergetics

Nitric Oxide and Mitochondrial Biogenesis



Nisoli E et al. Circulation Research 2007;100:795-806

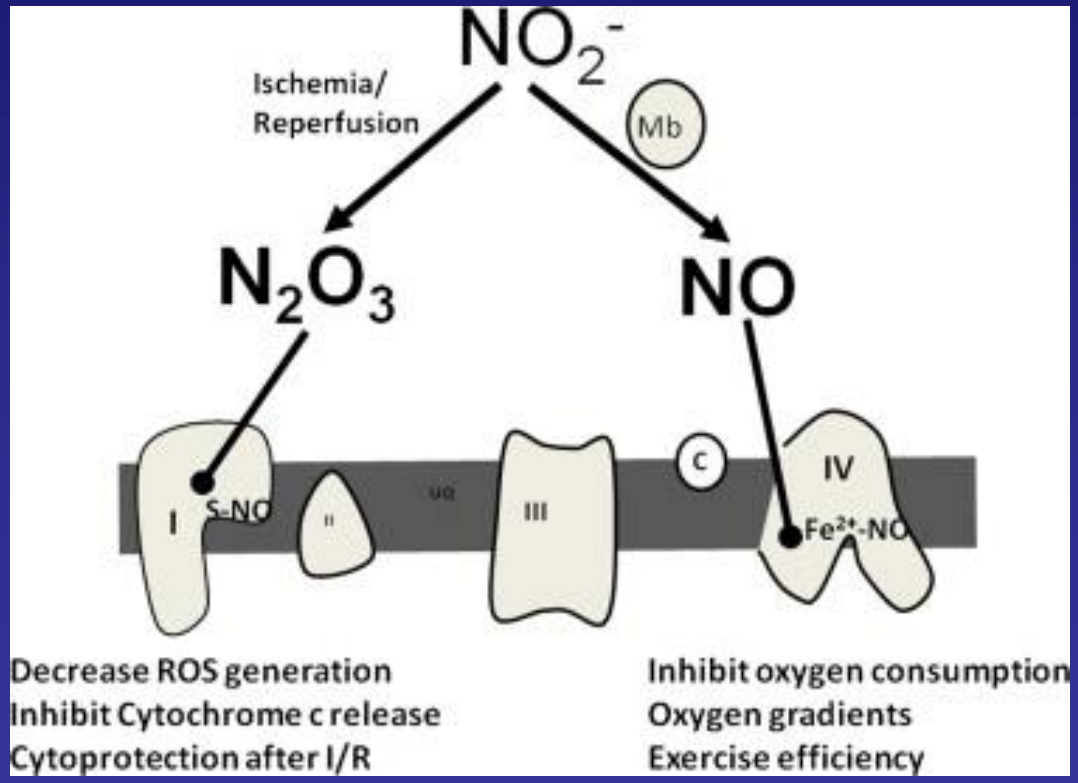
Extension of Tissue Oxygen Gradients and Modulation of Exercise Capacity by Nitrite Reduction to NO



Nitrite-dependent extension of oxygen gradients. (A) During normoxia, NOS is functional, myoglobin is oxygenated, and sufficient oxygen is available to diffuse from the source of oxygen through the tissue. (B) In hypoxic conditions, NOS is substrate limited and cannot make NO and myoglobin becomes deoxygenated. The majority of oxygen present is consumed by mitochondria close to the oxygen source, leading to a shortened oxygen gradient. (C) If nitrite is present during hypoxic conditions, it can be reduced by deoxygenated myoglobin. The NO generated can then partially inhibit mitochondrial oxygen consumption, allowing more oxygen to diffuse past these mitochondria and further into the tissue (elongation of oxygen gradient).

Shiva S. Nitric Oxide 2010 Feb 15;22(2):64-74

Nitrite Regulates Mitochondrial Function At Different Stages of ETC



Nitrite regulates mitochondrial function. During hypoxia, nitrite is reduced to NO by deoxygenated myoglobin and nitrosylates the binuclear center of complex IV. This results in the inhibition of oxygen consumption which may contribute to the regulation of oxygen gradients and the modulation of exercise efficiency. During ischemia/reperfusion, nitrite is converted to a nitrosating species (possibly N2O3 through its reductive anhydrase reaction with heme) and S-nitrosates complex I at reperfusion. This leads to decreased ROS generation and inhibition of cytochrome c release, which contribute to cytoprotection after I/R.

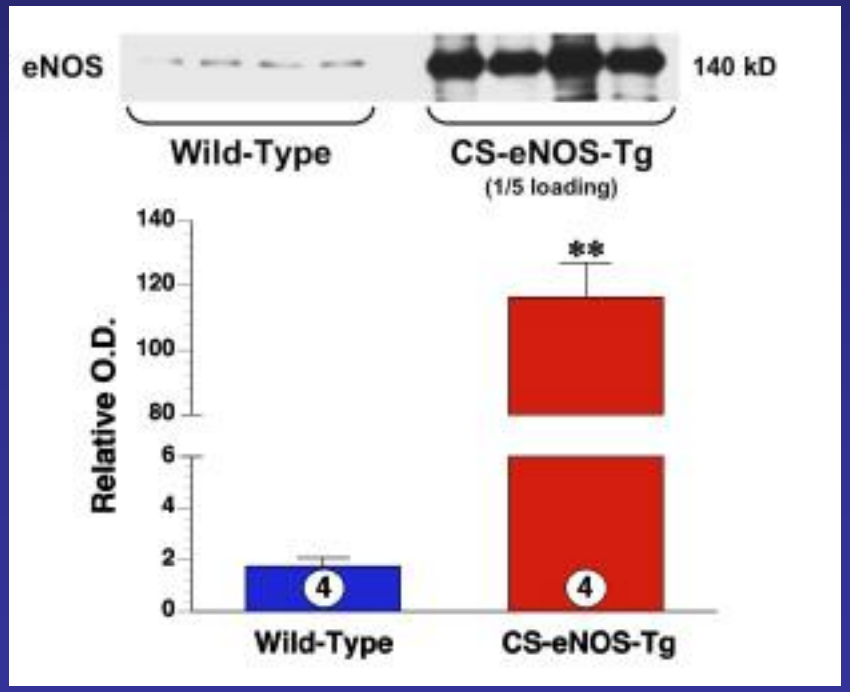
FACTS

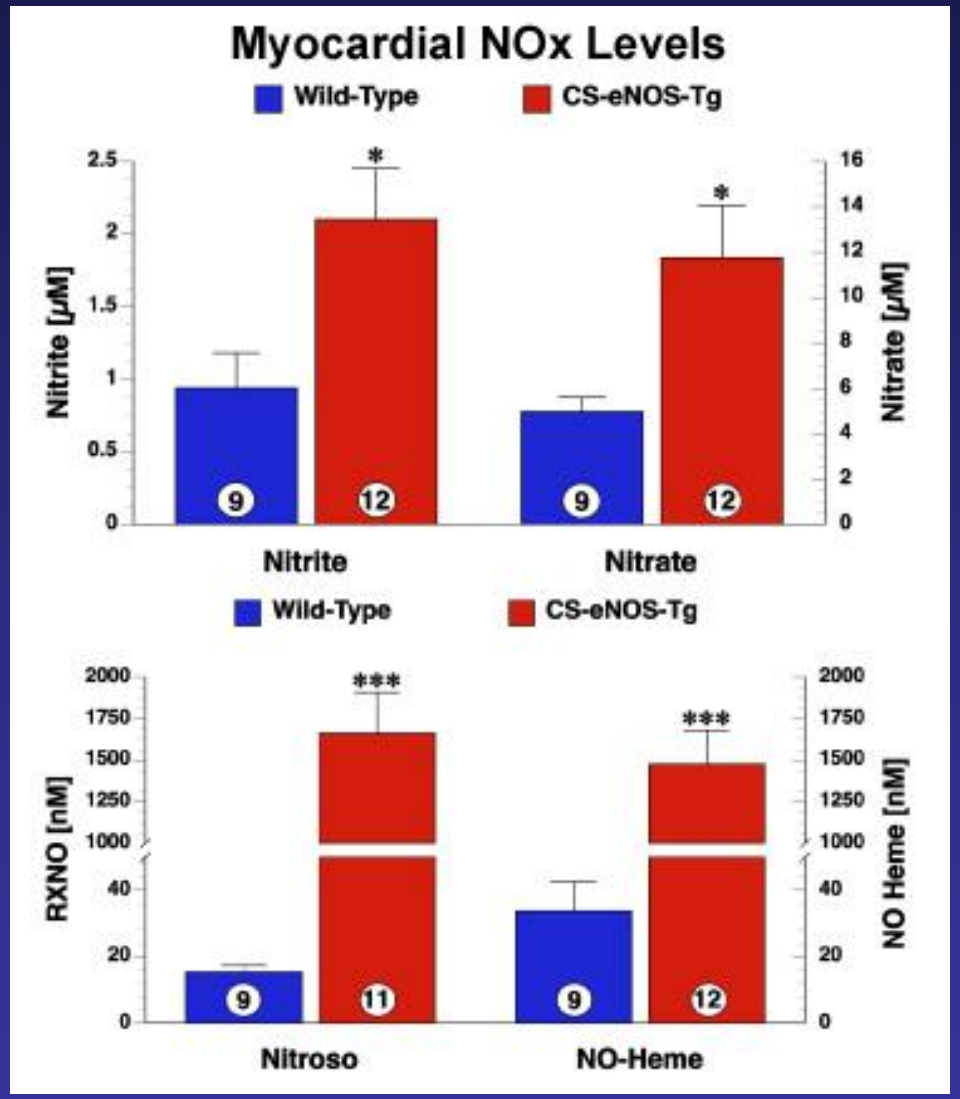
Cardiovascular disease (CVD) is the number one killer of both men and women in the U.S. Close to 1 million people die each year and more than 6 million are hospitalized due to CVD. The cost of CVD, in terms of health care and lost productivity, is over $270 billion and increasing as the baby boom population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

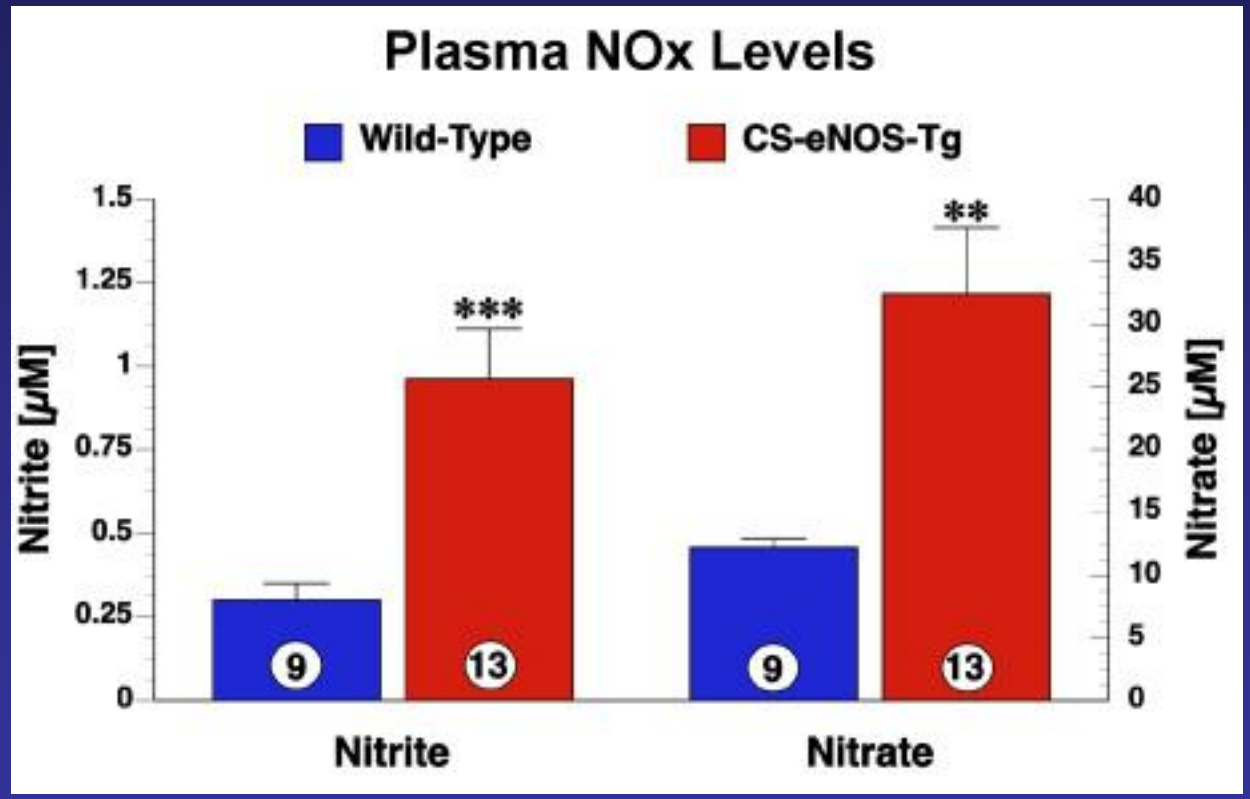
Cardiac Specific Overexpression of eNOS results in Increased Cardiac NO Production and Protects from I/R Injury





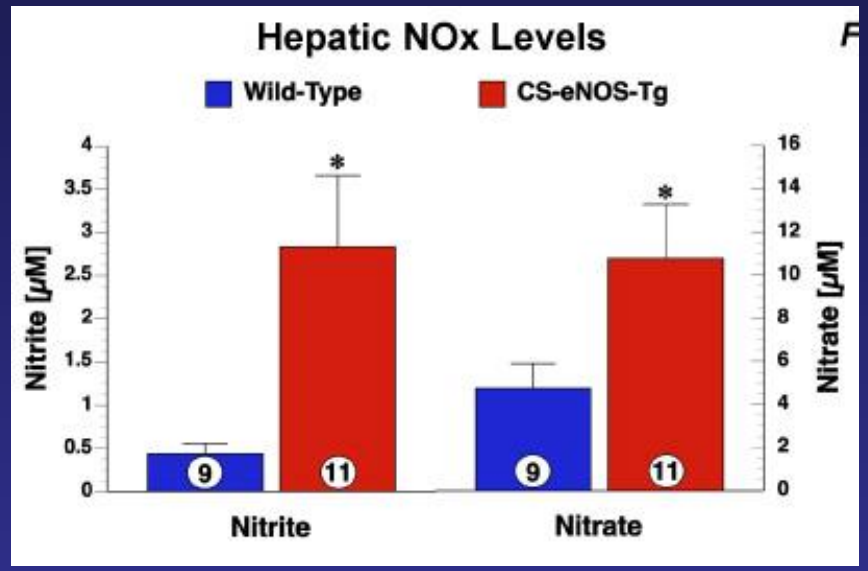
Elrod et al ATVB 2006

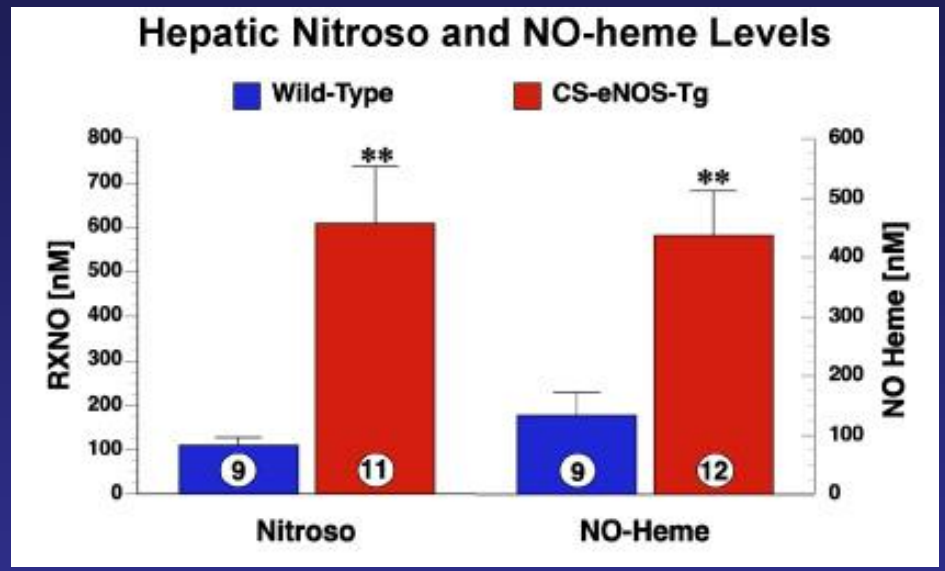
Increased Cardiac NO Production Results in Increased Circulating Nitrite and Nitrate

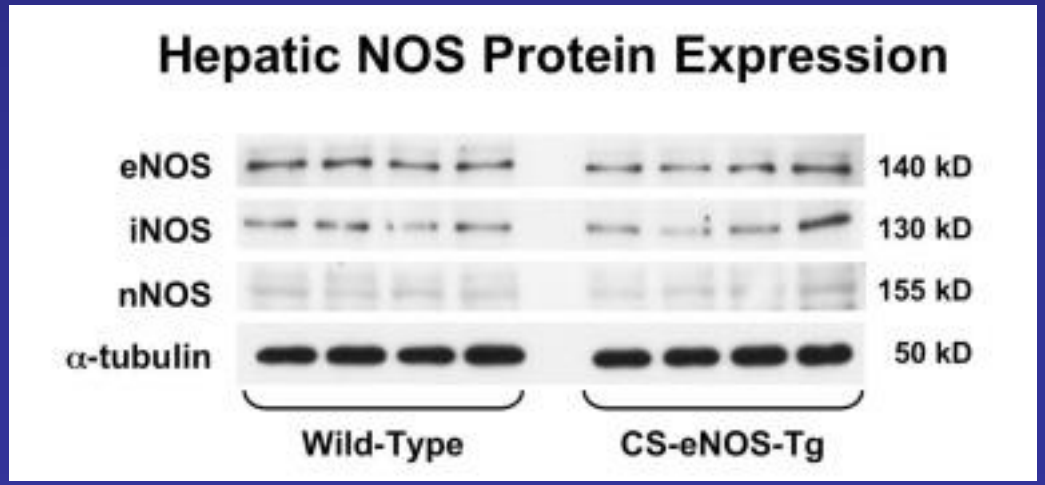


Elrod, PNAS 2008

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

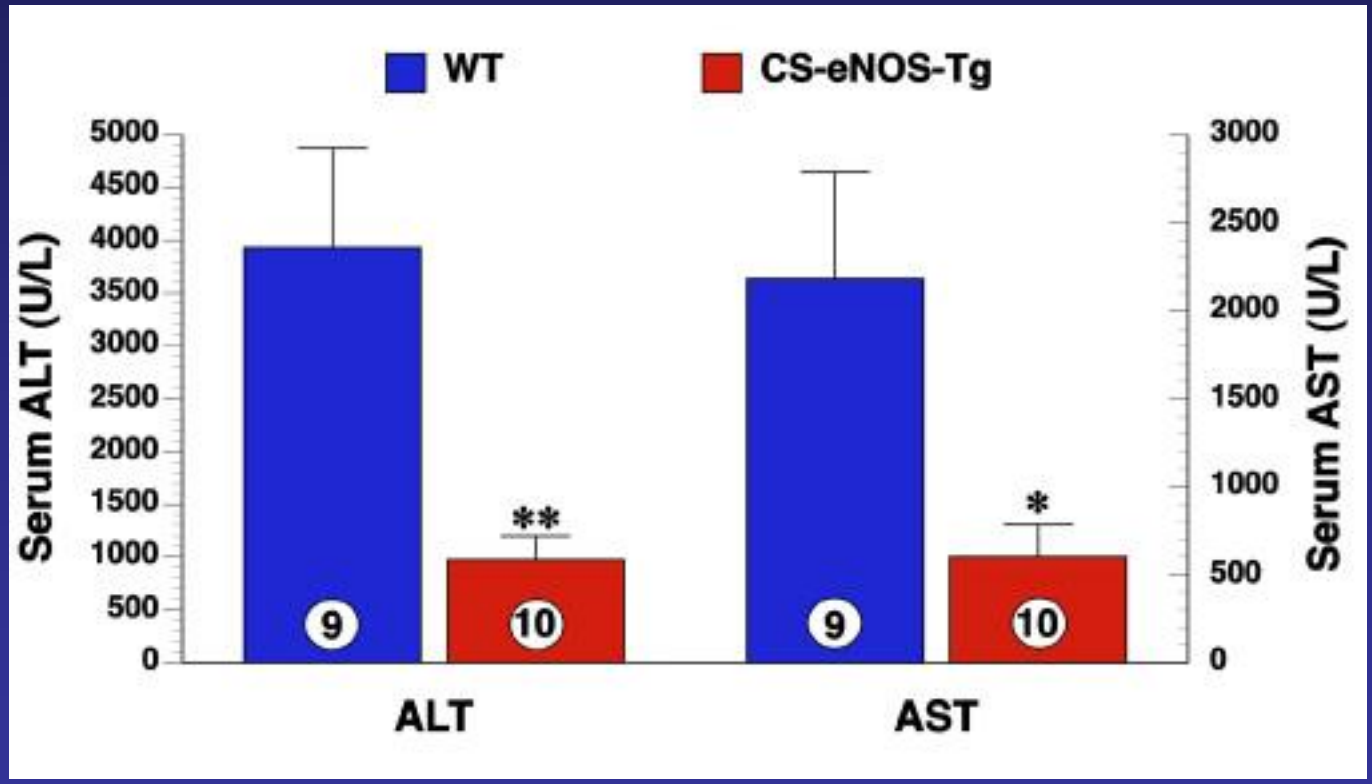






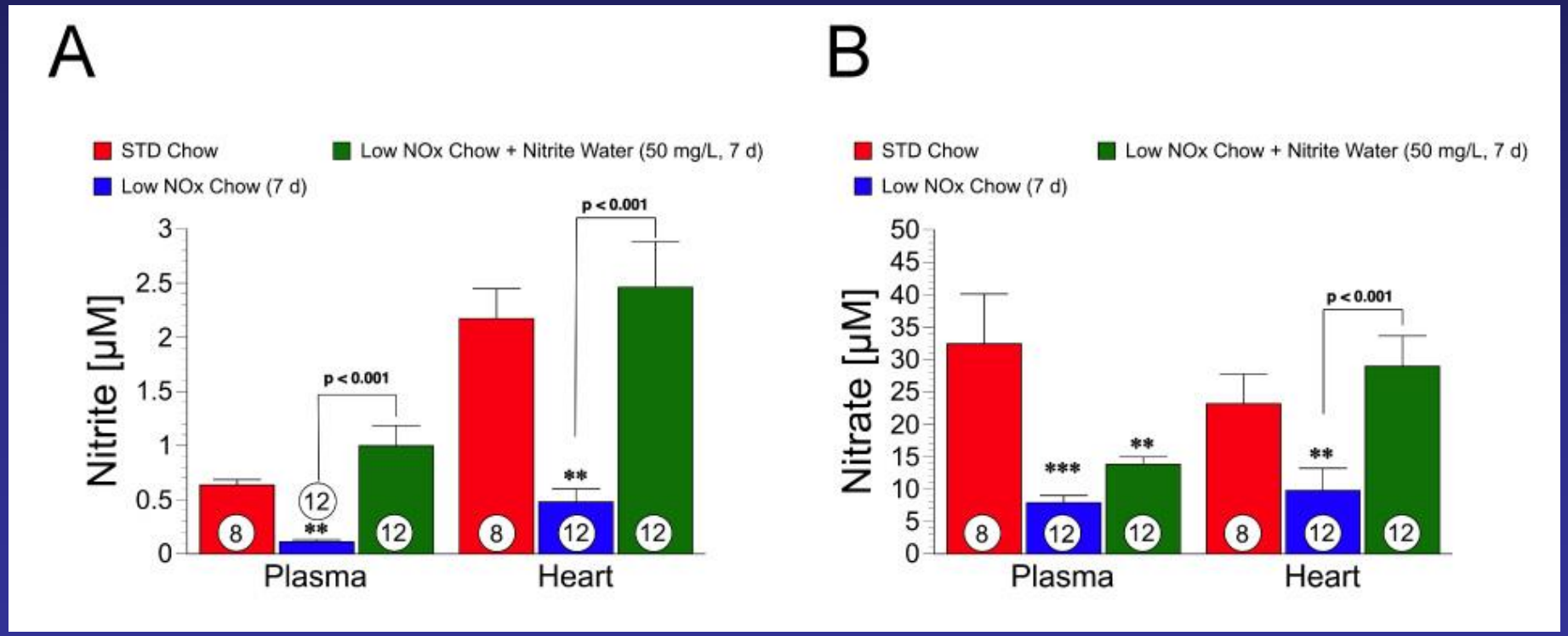
Elrod, PNAS 2008

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



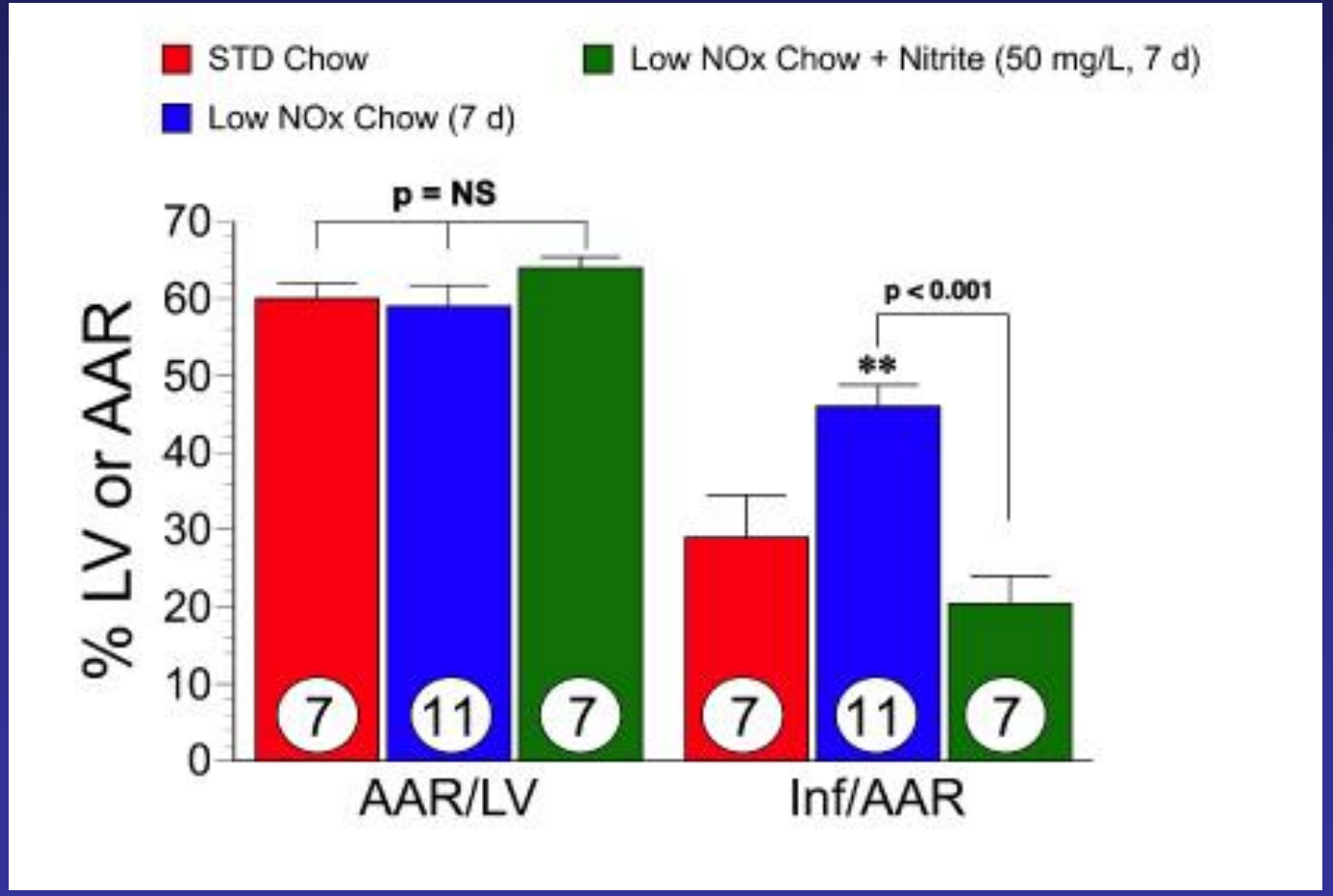
Elrod, PNAS 2008

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

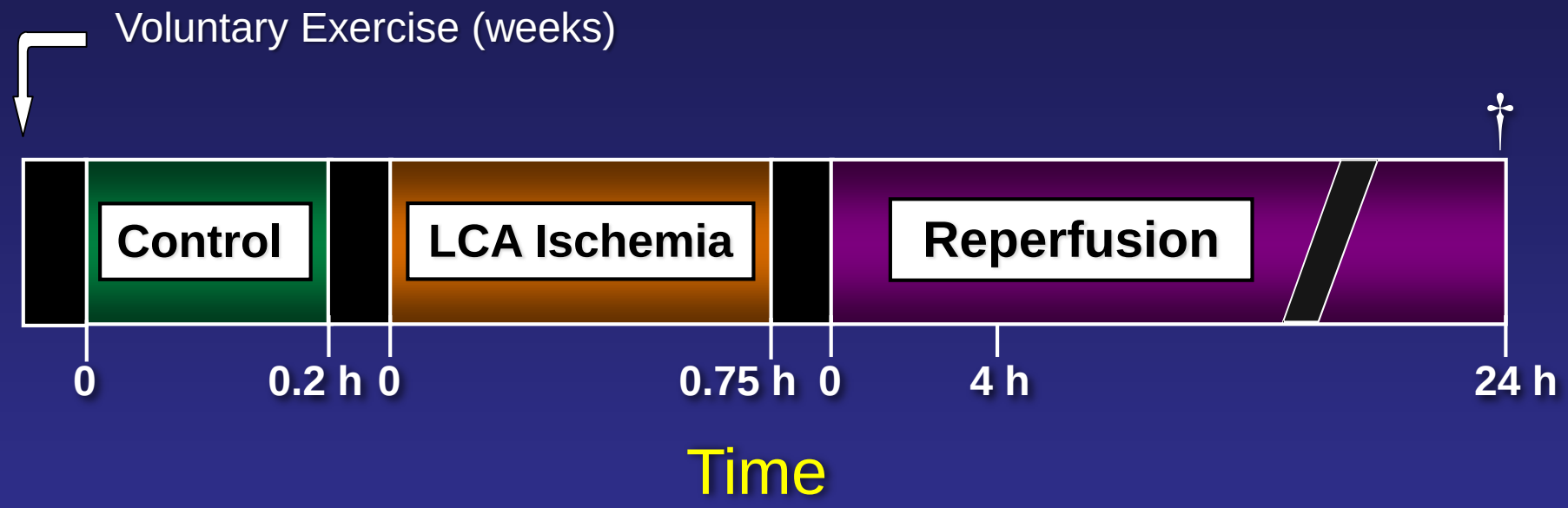


Bryan *et al* PNAS (2007)

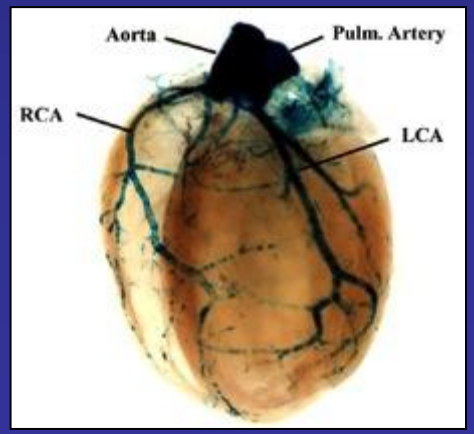
What about Exercise?

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

Murine *in vivo* Myocardial Ischemia-Reperfusion Protocol

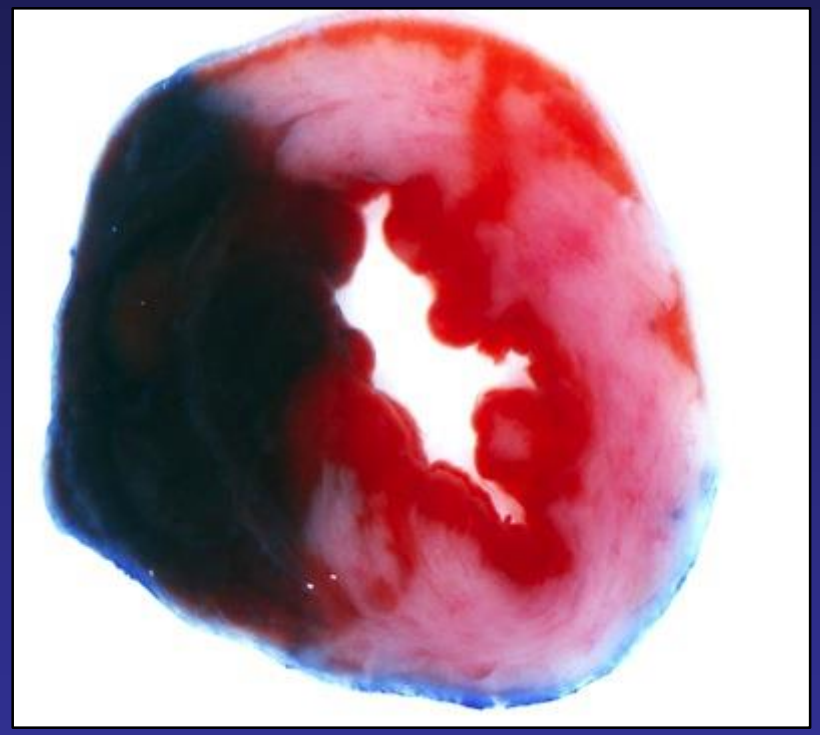


† Myocardial Infarct Size
- Area-at-risk (Evans Blue dye)
- Infarct size per Area-at-risk (TTC)

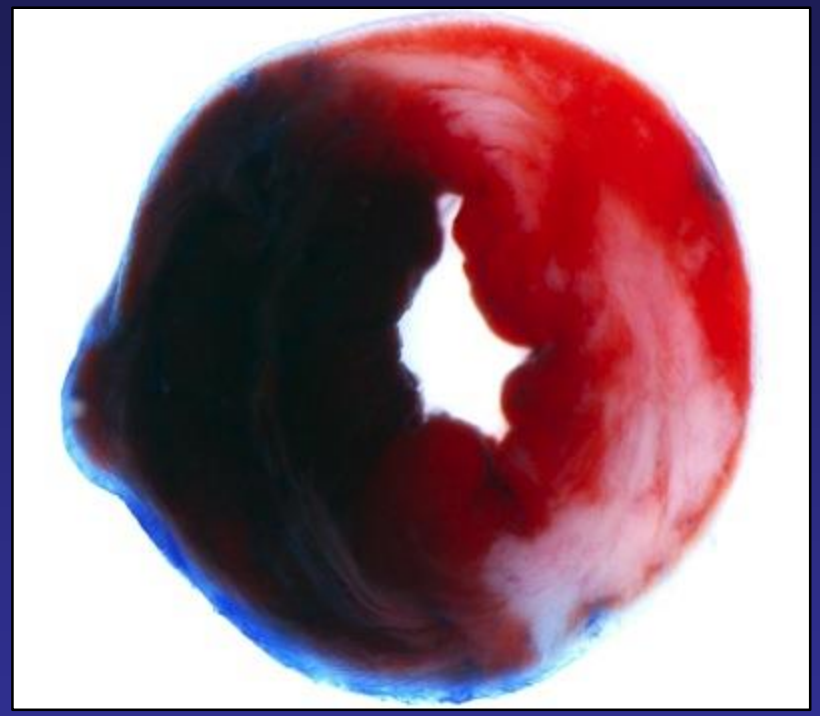


Myocardial Injury

45 min Ischemia and 24 hr Reperfusion



Sedentary



Voluntary Exercise (4 weeks)

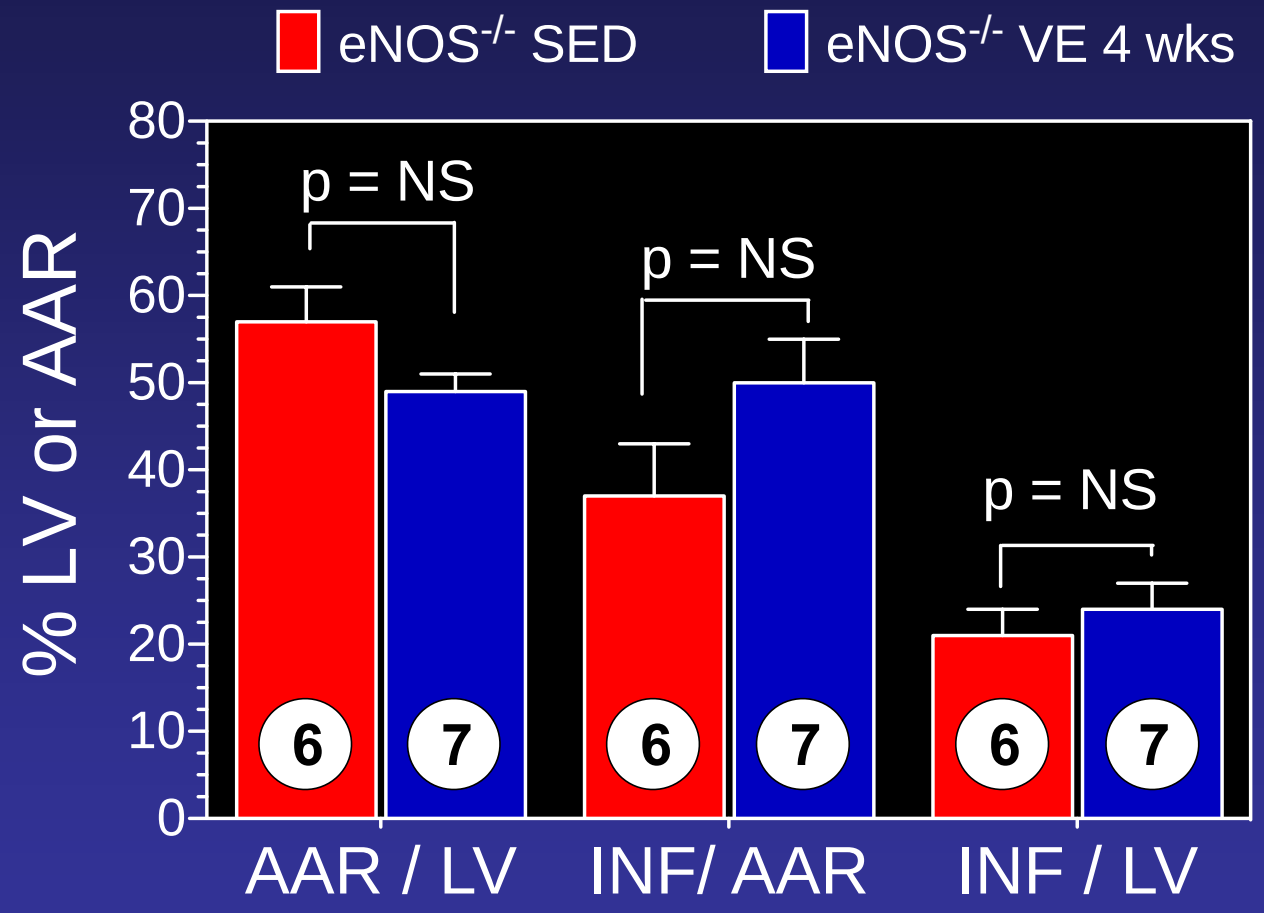
Calvert et al., *Circulation Research*, 2011

What about Exercise?

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

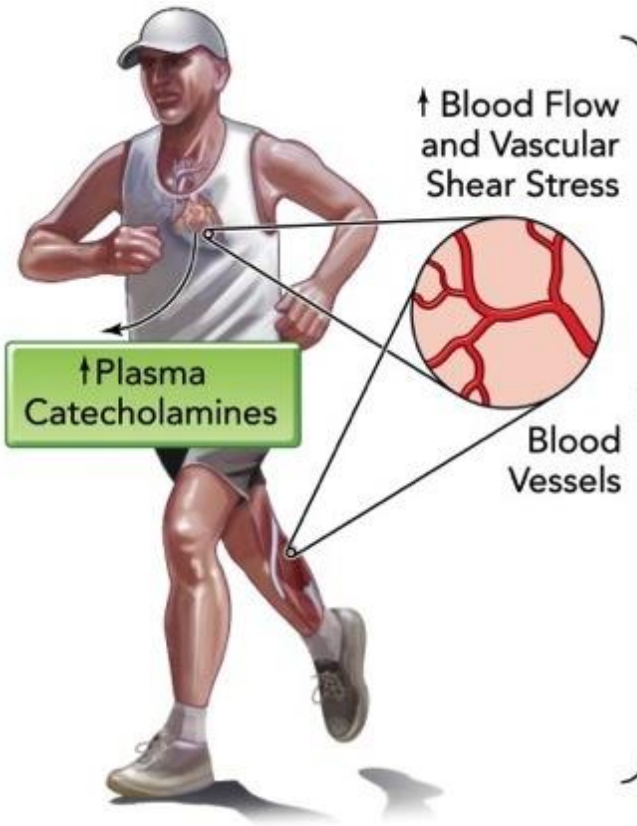
Myocardial Infarct Size

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.

NITRITE (NO_2^-) AND CARDIOVASCULAR DISEASE

Dietary and insufficency

NO_2^- NO_3^-

↓NO_2^- Uptake in PLASMA

↓NO_2^- Uptake in PLASMA

eNOS Dysfunction

↓$NO^\bullet$ + O_2

↓ Systemic Bioavailability in PLASMA NO_2^-

↓ Nitrite Transport to TISSUES

↓ TISSUE NO_2^- STORAGE

Nitrite Metabolism Impairment

↓ $NO^\bullet$ BIOAVAILABILITY

↑ CV DISEASE RISK

+

↑ CV DISEASE SEVERITY

ATHEROSCLEROSIS

Coronary Artery

Heart

ACUTE MYOCARDIAL INFARTION

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

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Nathan S. Bryan, Ph.D. · Joseph Loscalzo, M.D. *Editors*

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