Role of Vascular Biology, Nutrition and Nutraceutical Supplements in the Prevention and Treatment of Vascular Aging and Cardiovascular Disease

Module II Cardiology 2019

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Disclosure

MARK HOUSTON MD MS MSc has indicated that he has no conflicts of interest related to this lecture

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MODULE 2 CVD

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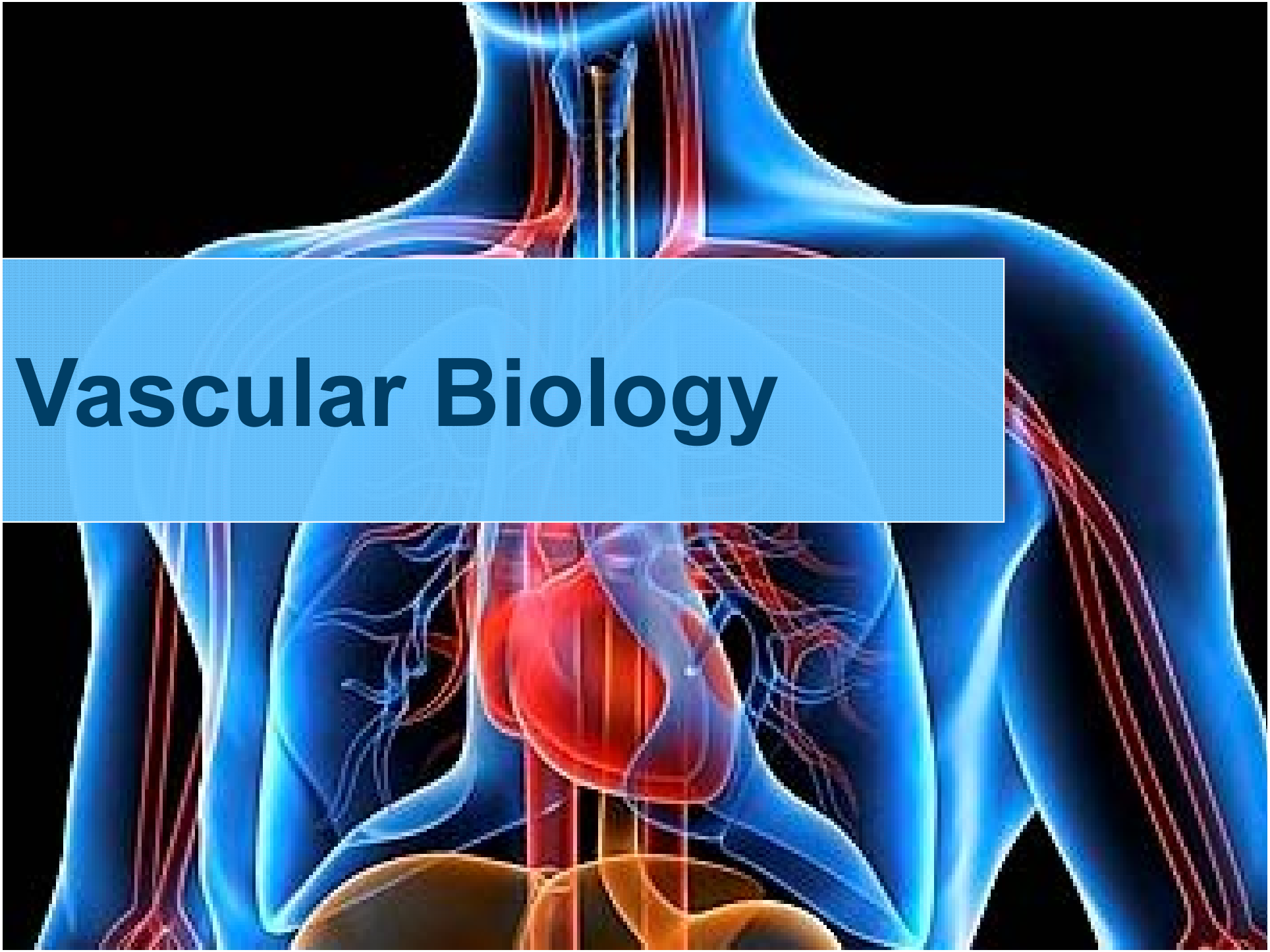
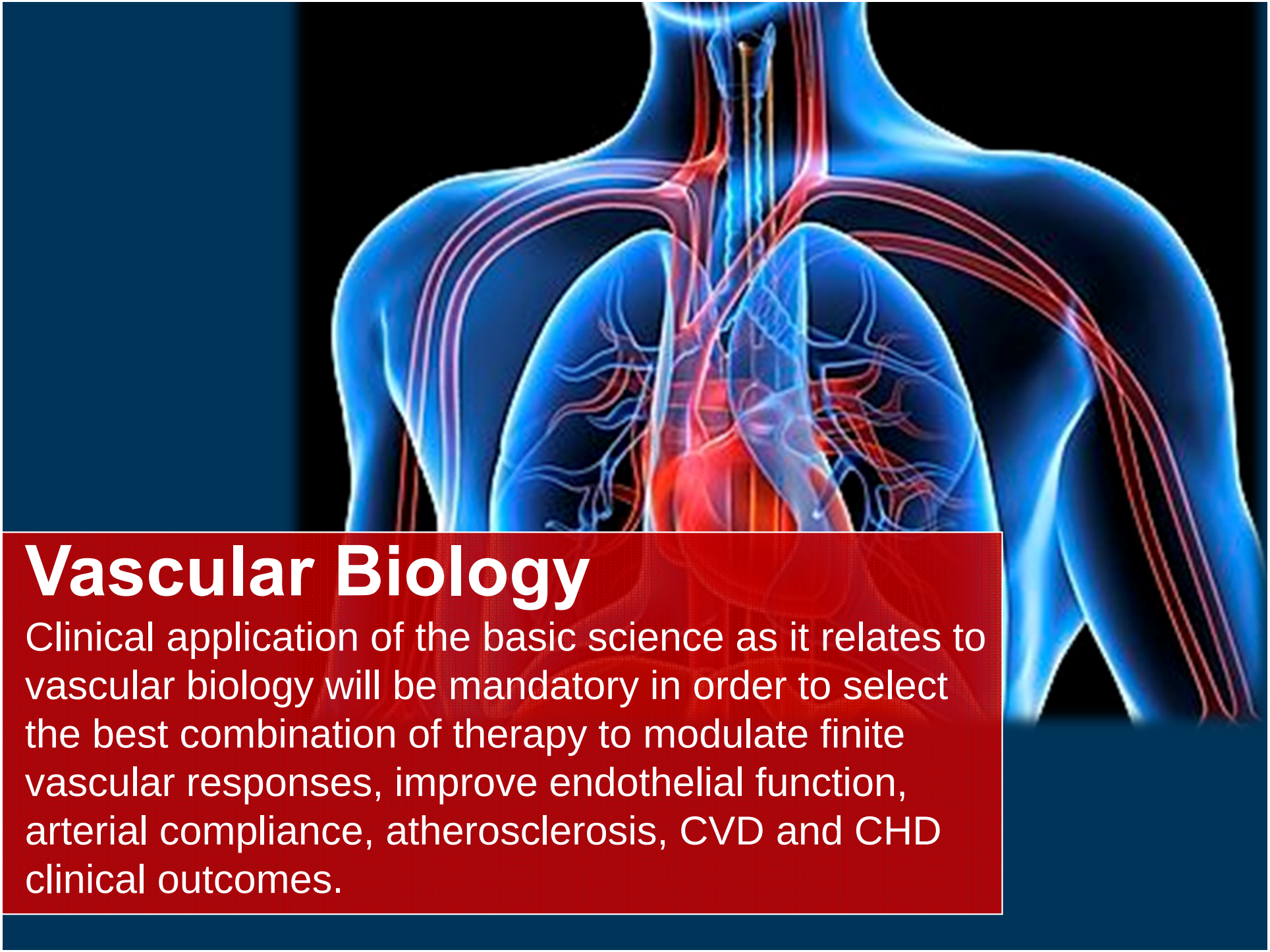


Vascular Biology

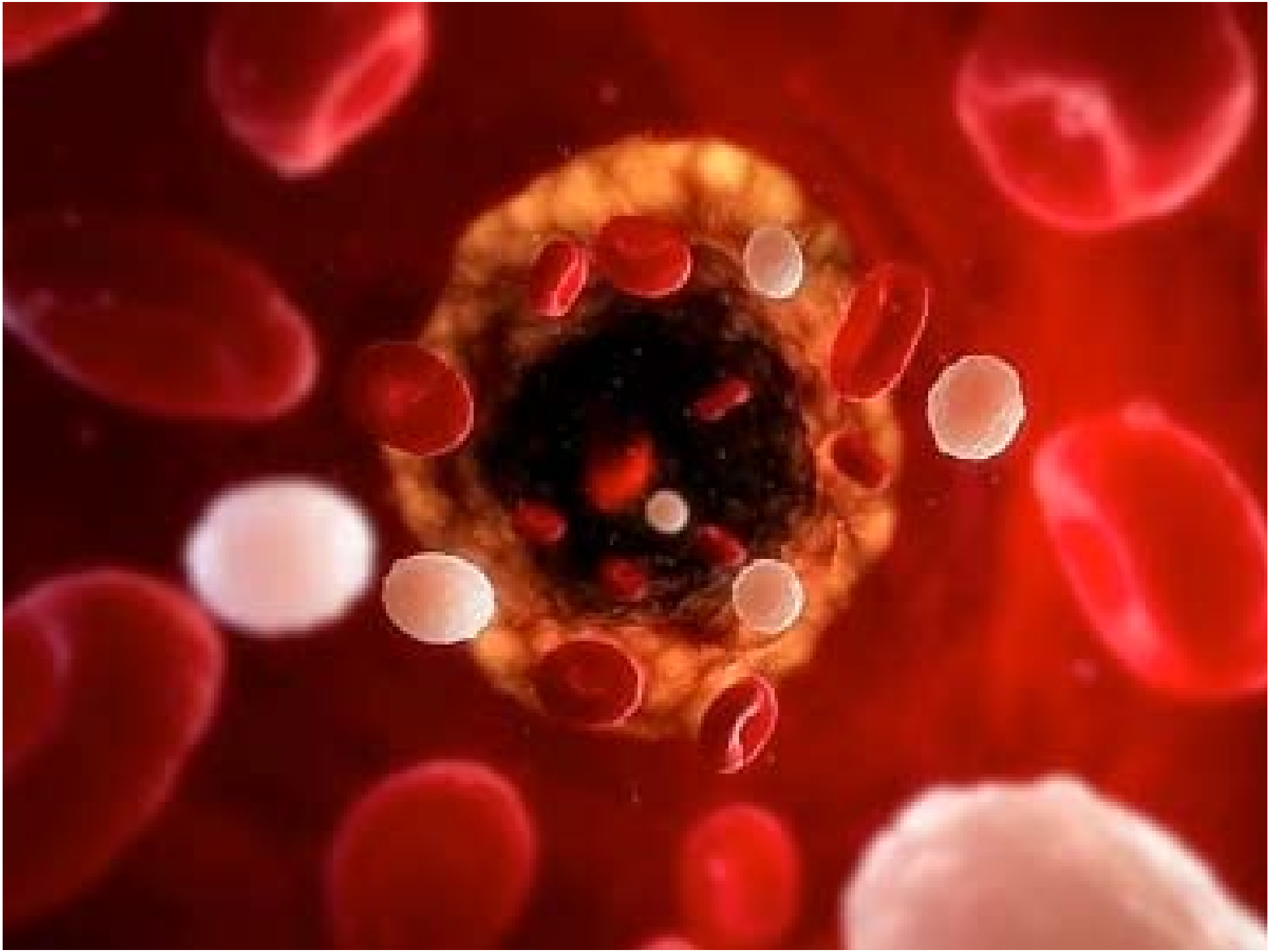


Vascular Biology

Clinical application of the basic science as it relates to vascular biology will be mandatory in order to select the best combination of therapy to modulate finite vascular responses, improve endothelial function, arterial compliance, atherosclerosis, CVD and CHD clinical outcomes.

Vascular Biology/Vascular Aging
Learning Objectives

1. Understand the role of vascular biology in atherosclerosis vascular disease, CHD and CVD.
2. Understand the role of endothelial dysfunction, nitric oxide, angiotensin II and aldosterone in atherosclerosis, CHD and CVD.
3. Understand how inflammation, oxidative stress and vascular immune dysfunction promote CVD and CHD.
4. Define the relationships of the finite vascular responses to the “infinite” range of vascular insults.
5. Be familiar with the pathways of vascular aging, pathogenesis, and how they relate to the prevention and treatment of atherosclerosis, CHD and CVD.



Vascular Disease is a Balance

MC Houston. Vascular Biology in Clinical Practice. Hanley and Belfus 2000
MC Houston. Handbook of Hypertension Wiley Blackwell Oxford UK 2009

Vascular Injury

Nitric oxide vs angiotensin II endothelin and aldosterone

VS

Vascular Repair

Endothelial Progenitor Cells (EPC's)

The blood vessel has a finite number of responses: inflammation, oxidative stress and immune vascular dysfunction to an infinite number of insults. (Houston 2002)

There are over **400 CHD** risk factors and mediators, but there are 25 TOP modifiable risk factors for CHD.

J of Nutritional Biochemistry 2012;23:39-50

Top 25 Modifiable CHD Risk Factors

Houston MC. What Your Doctor May Not Tell You About Heart Disease 2012

- Hypertension (24 hour ABM)
- Dyslipidemia (advanced lipid analysis)
- Hyperglycemia, metabolic syndrome, insulin resistance and diabetes mellitus
- Obesity
- Smoking
- Hyperuricemia
- Renal disease
- Elevated fibrinogen
- Elevated serum iron
- Trans fatty acids and refined carbohydrates
- Low dietary omega 3 fatty acids
- Low dietary potassium and magnesium with high sodium intake
- Inflammation: increased HSCRP, MPO, interleukins
- Increased oxidative stress and decreased defense
- Increased immune dysfunction
- Lack of sleep
- Lack of exercise
- Stress, anxiety and depression
- Homocysteinemia
- Subclinical hypothyroidism
- Hormonal imbalances in both genders
- Chronic clinical or subclinical infections
- Micronutrient deficiencies: numerous ones such as low vitamin D and K etc.
- Heavy metals
- Environmental pollutants

Vascular Disease is the "correct" but chronic dysregulated response with an exaggerated outcome. The blood vessel is an innocent bystander.

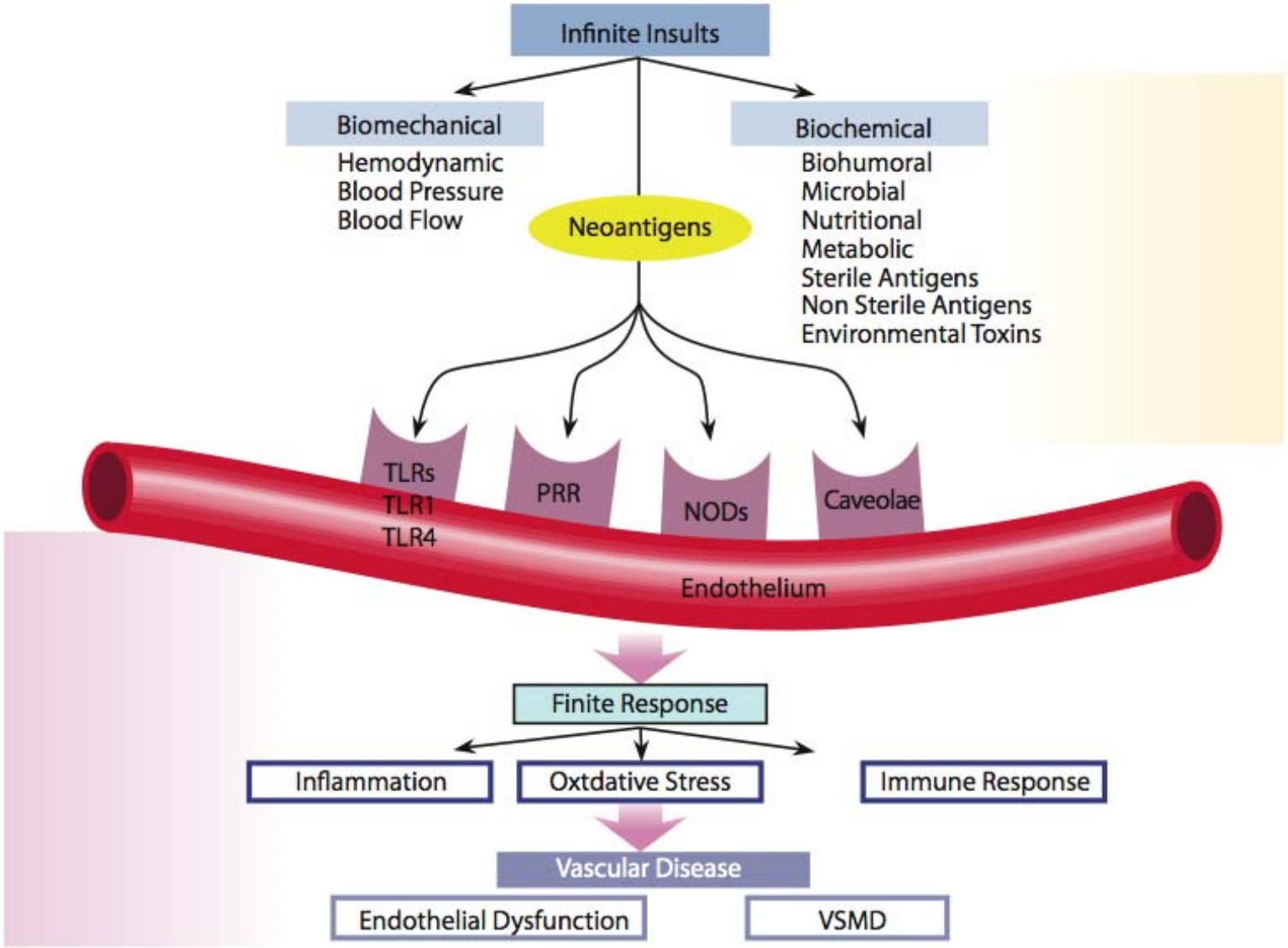
MC Houston. Vascular Biology in Clinical Practice. Hanley and Belfus 2000 MC Houston. Handbook of Hypertension Wiley Blackwell Oxford UK 2009

- **Vascular disease is the "correct" but chronic dysregulated and exaggerated response to infinite vascular insults with finite responses and environmental-gene expression patterns.**

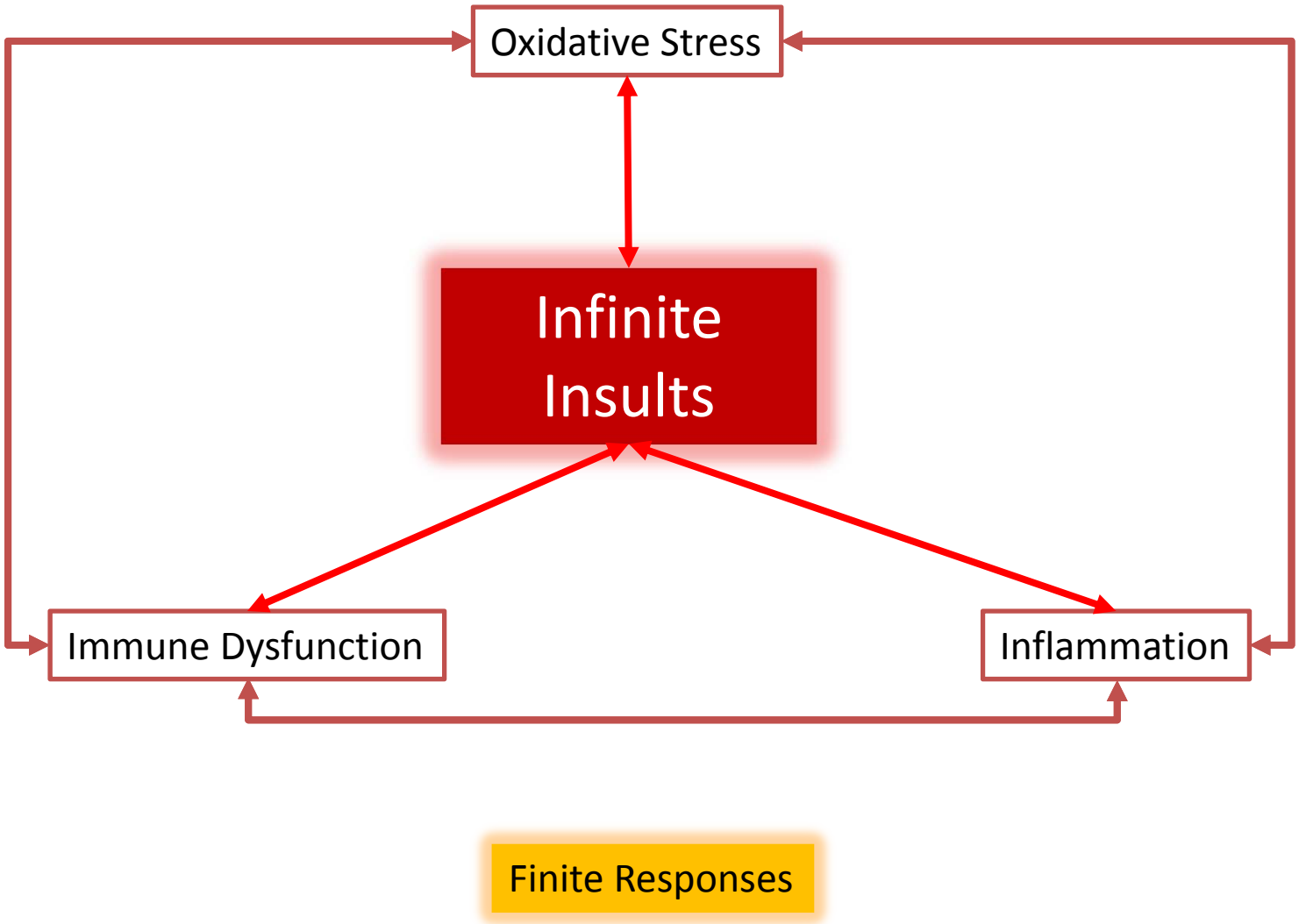
- **The vascular system as an innocent bystander leading to endothelial dysfunction (ED), cardiac dysfunction and VSMH (vascular smooth muscle hypertrophy).**

Houston 2011

Infinite Insults



Mechanism Of Model



Pathophysiology of Vascular Disease

J of the American Society of Hypertension 2010;4:272
Circulation 2007;115:1020
Expert Rev in CV Therapy 2010;8:821
Nephrol Dial Transplant 2006:21:850

1. **Oxidative Stress (ROS-radical oxygen species- and RNS- radical nitrogen species)** : increased in arteries and kidneys with decreased oxidative defense.
2. **Inflammation**: increased in the vasculature and kidneys: increased hsCRP, leukocytosis with increased neutrophils and decreased lymphocytes. Increased RAAS(renin angiotensin aldosterone system) in the kidney.
3. **Autoimmune dysfunction** of the arteries and kidneys: leukocytosis, involvement of CD4+(T-helper cells) and CD 8+(cytotoxic T –cells), IL-17(interleukin) and TNF-alpha.(tumor necrosis factor alpha)
4. **Abnormal vascular biology** with endothelial dysfunction (ED), vascular smooth muscle dysfunction (VSMD) and cardiac dysfunction.
5. **Epigenetics, genetics, genomics, and gene expression patterns.**

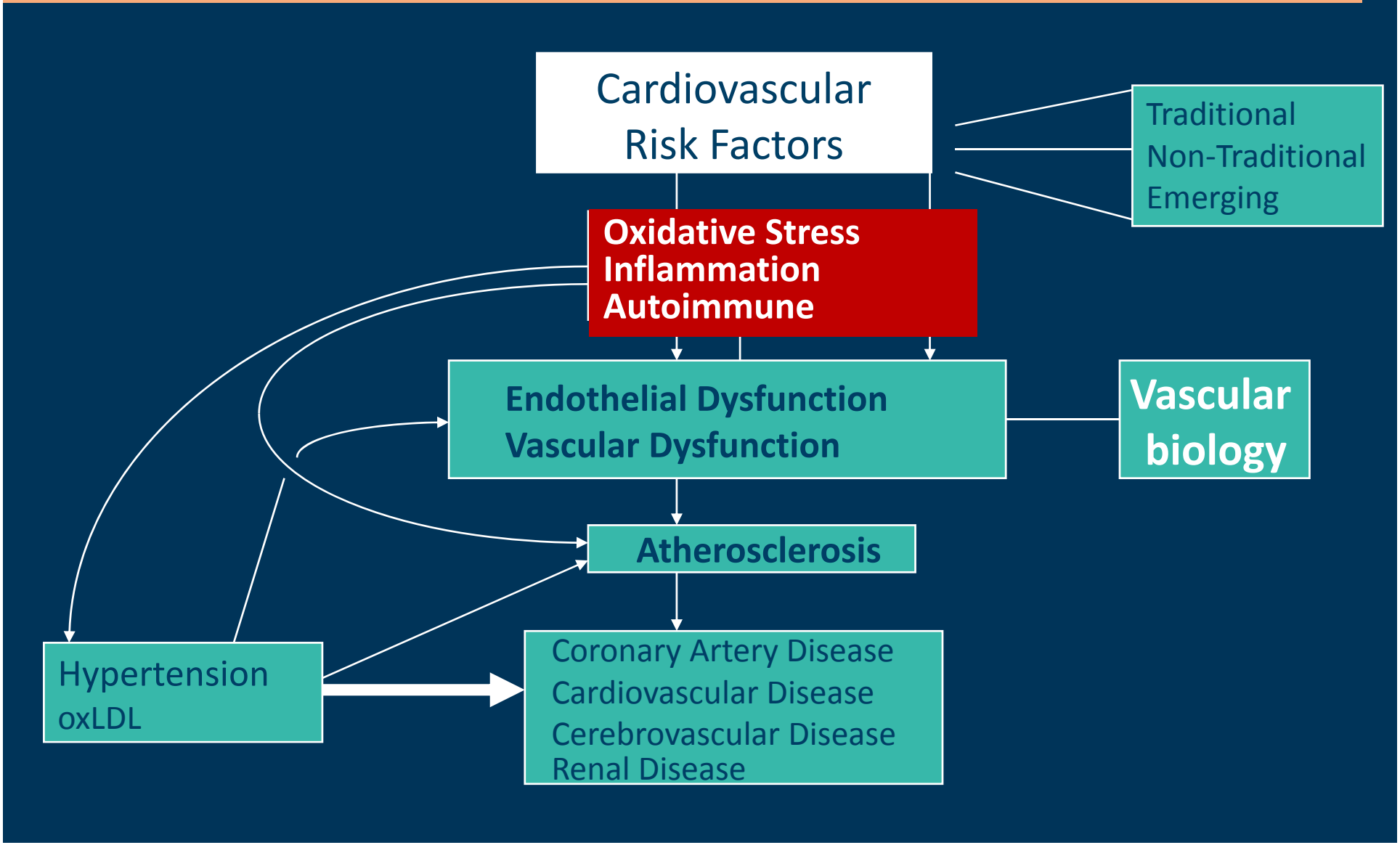
Atherosclerosis,
Vascular Disease and Meals

J of Nutritional Biochemistry 2011; 22:1105

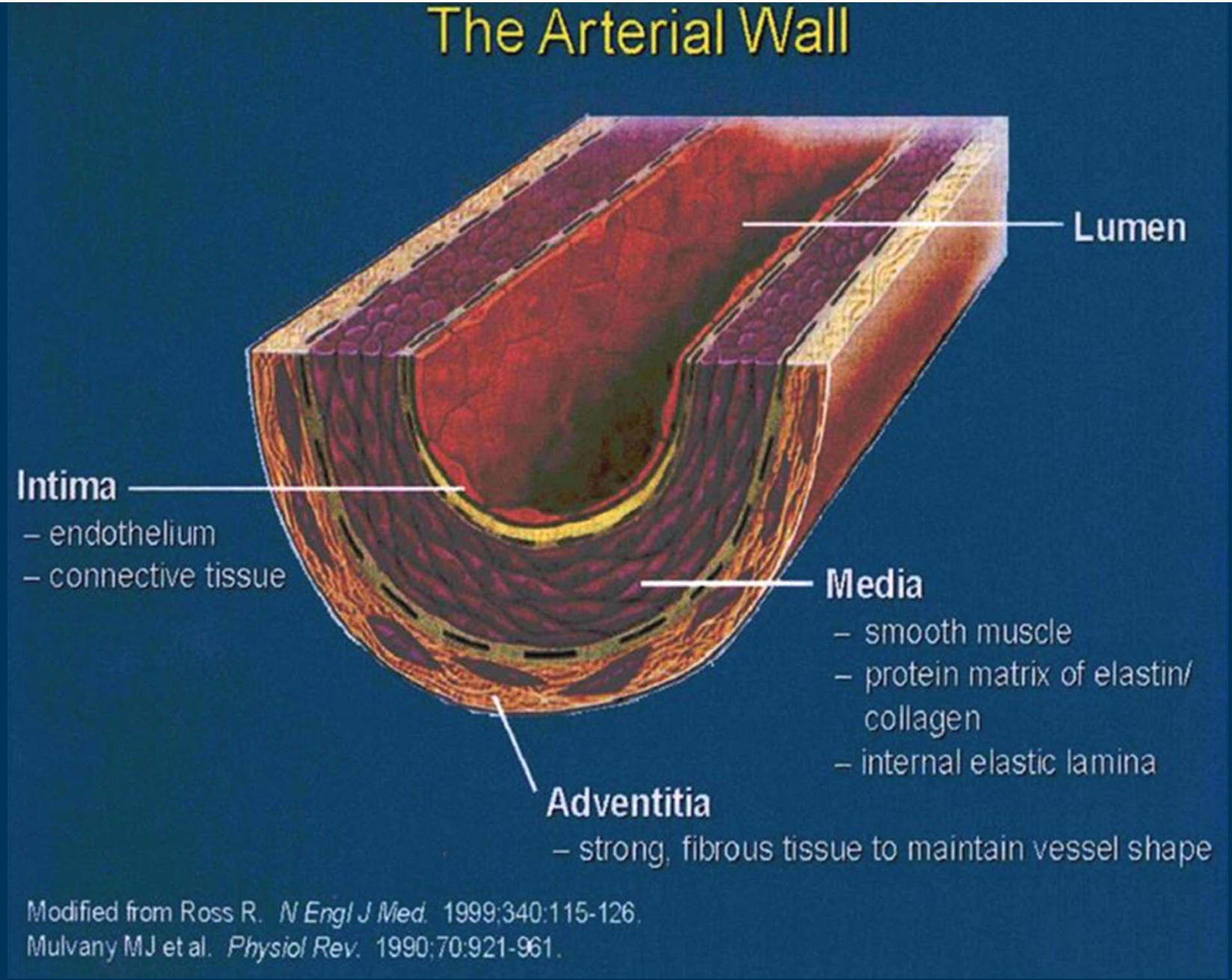
- Atherosclerosis and vascular disease are post-prandial phenomena.
- Inflammatory foods, coupled with hyperglycemia and hypertriglyceridemia, induce inflammation, oxidative stress, autoimmune vascular dysfunction with metabolic endotoxemia and metabolic memory.
- These same responses occur with other vascular and endothelial insults such as microbial infections, metabolic, toxic, biochemical and biomechanical mediators and insults.

Key Concept in Endothelial Dysfunction, Atherosclerosis, Cardiovascular Disease, and CHD

Houston MC. Handbook of Hypertension Wiley Blackwell Oxford UK 2009



The Arterial Wall



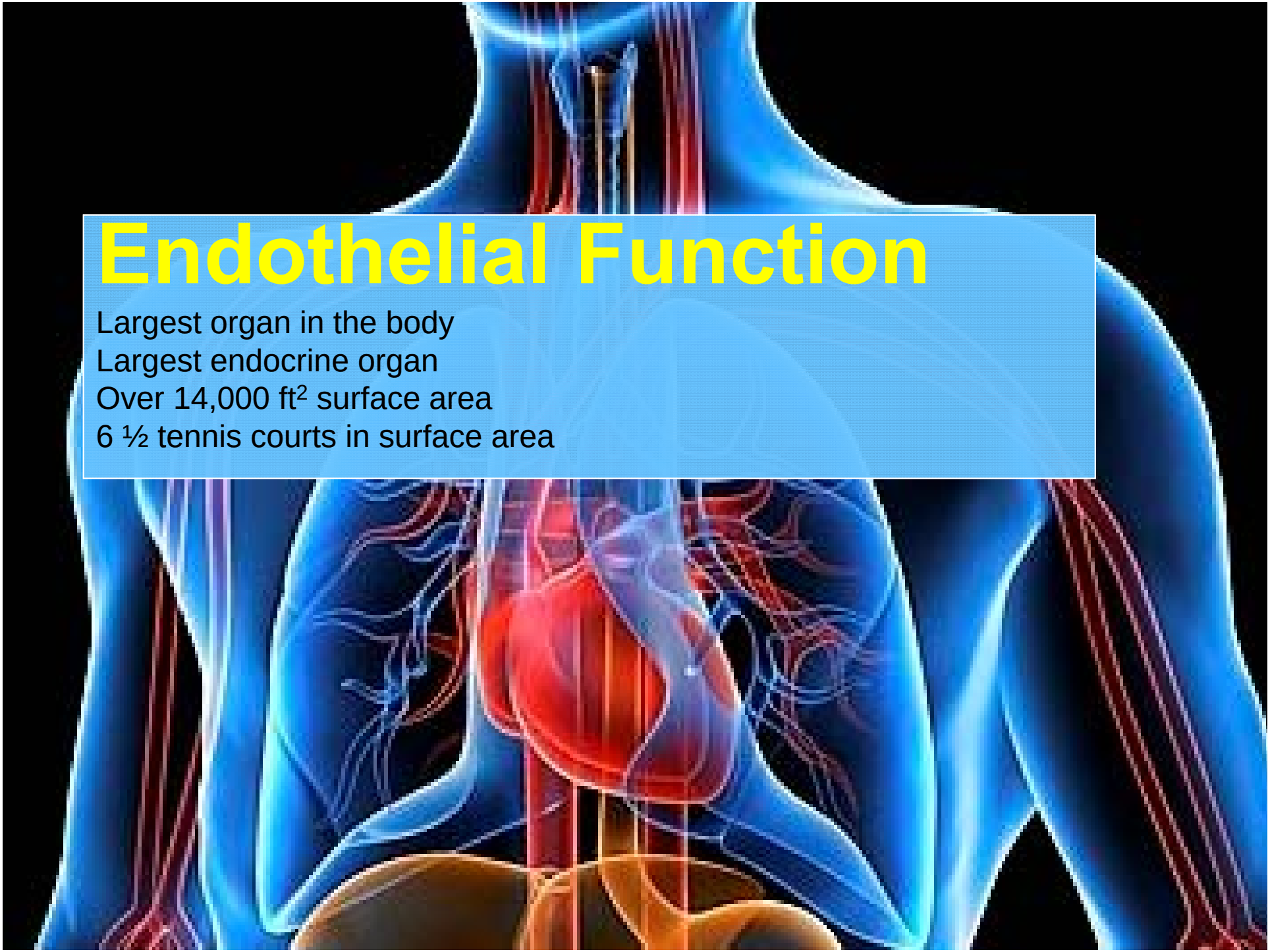
Modified from Ross R. *N Engl J Med.* 1999;340:115-126.
Mulvany MJ et al. *Physiol Rev.* 1990;70:921-961.

MC Houston. Vascular Biology in Clinical Practice. Hanley and Belfus 2000
MC Houston. Handbook of Hypertension Wiley Blackwell Oxford UK 2009

Clinical Pearls I

Develop treatments to:

1. Increase bioavailability of nitric oxide (NO), increase EPCs and decrease effects of A-II, aldosterone and endothelin.
2. Reduce infinite insults and the 400 CHD risk factors. Treat the TOP 25 modifiable risk factors.
3. Control the 3 finite vascular responses, ED and VSMH, metabolic memory, "neo-antigens", epitopes, adverse nutrient-gene interactions and innocent bystander vascular damage.
4. Consume smaller, frequent meals with anti-oxidants, non-inflammatory foods combined with nutraceutical supplements to avoid postprandial metabolic endotoxemia, ED and atherosclerosis.

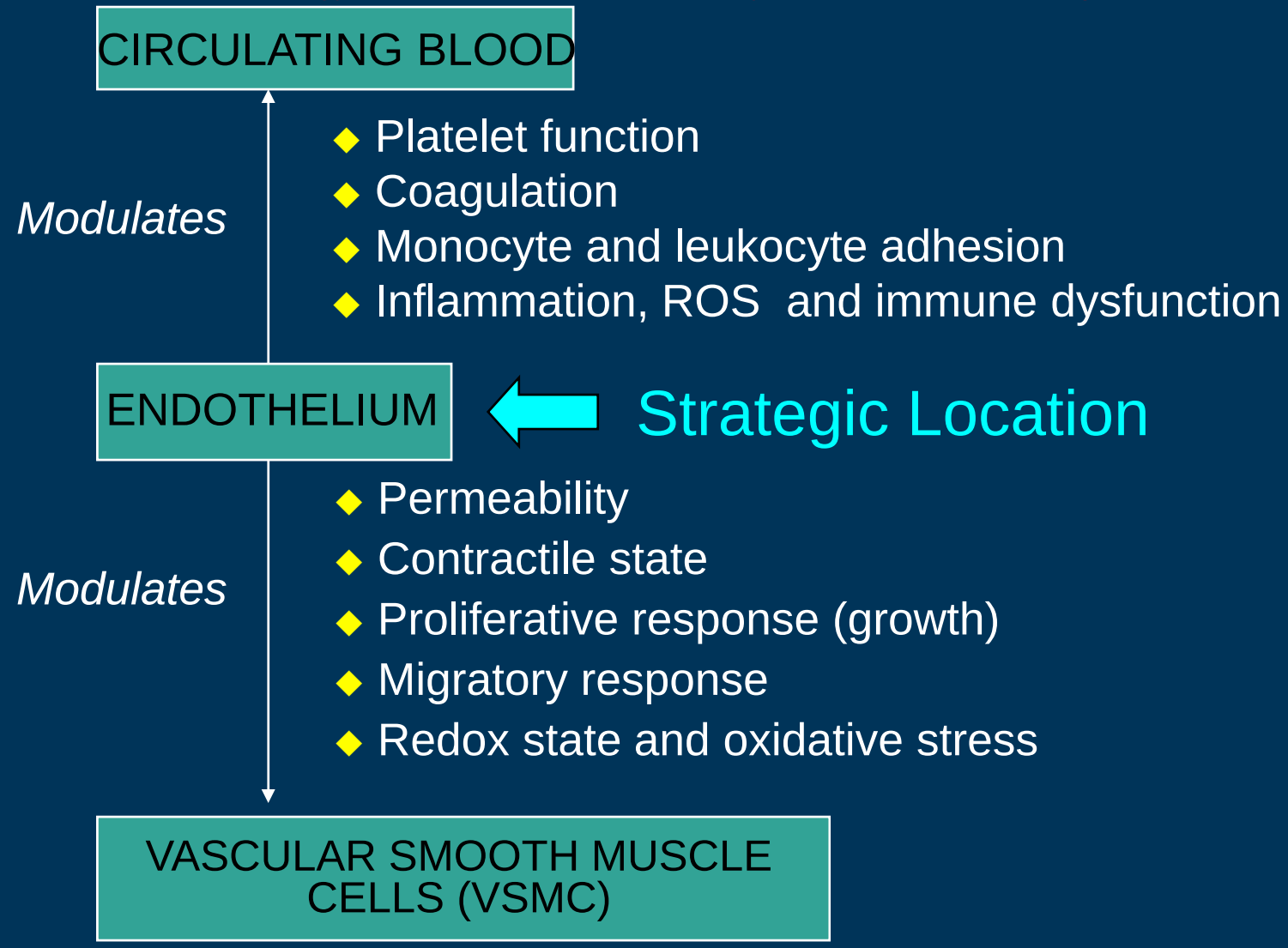


Endothelial Function

Largest organ in the body
Largest endocrine organ
Over 14,000 ft^2 surface area
6 ½ tennis courts in surface area

Vascular endothelium : Strategic anatomical position to maintain vascular homeostasis

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

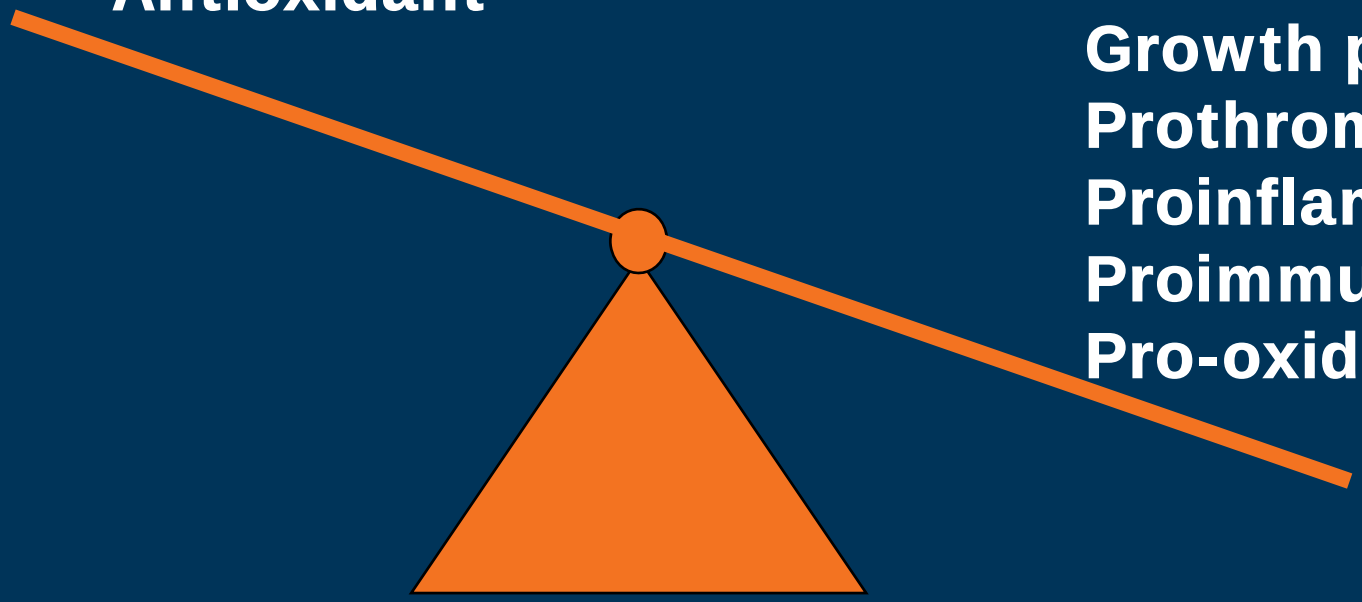


The Endothelium Maintains Vascular Health

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

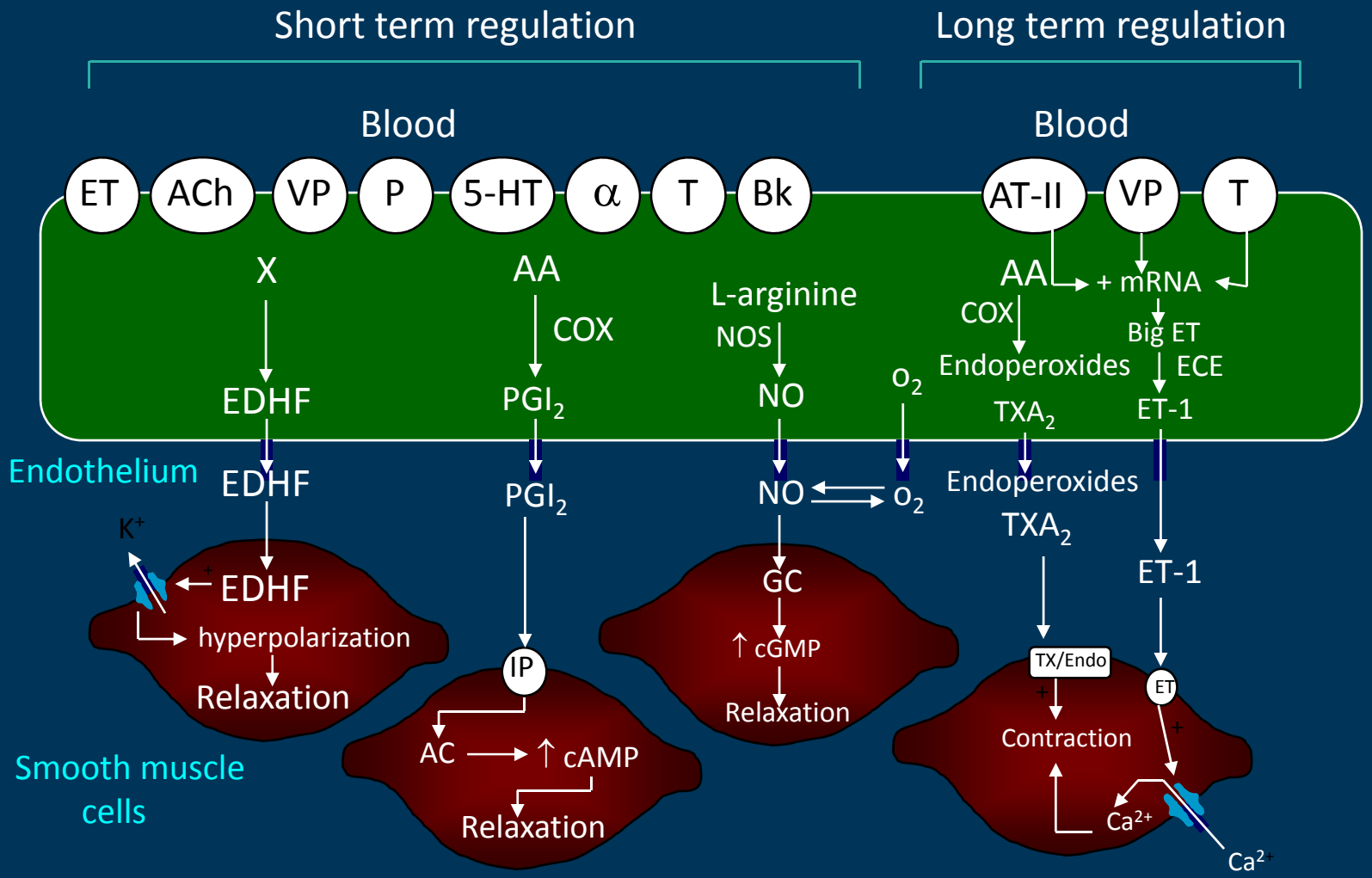
Journal of Hypertension 2016;34:1464-1472

Dilatation
Growth inhibition
Antithrombotic
Anti-inflammatory/immune
Antioxidant



Constriction
Growth promotion
Prothrombotic
Proinflammatory
Proimmune
Pro-oxidant

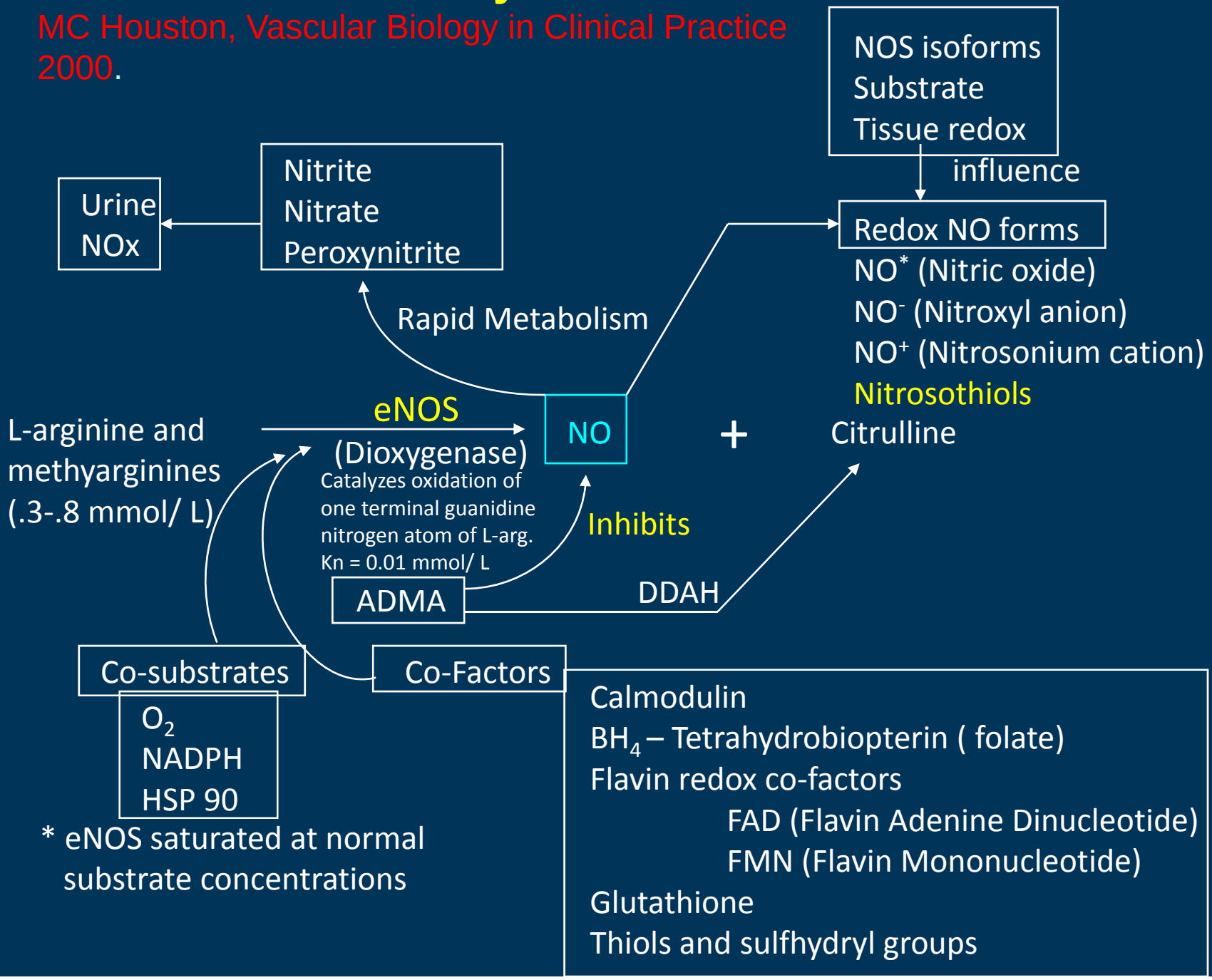
Endothelium-Dependent Responses (not present in all blood vessels)



MC Houston. Vascular Biology in Clinical Practice. 2000.
MC Houston. Handbook of Hypertension. 2009

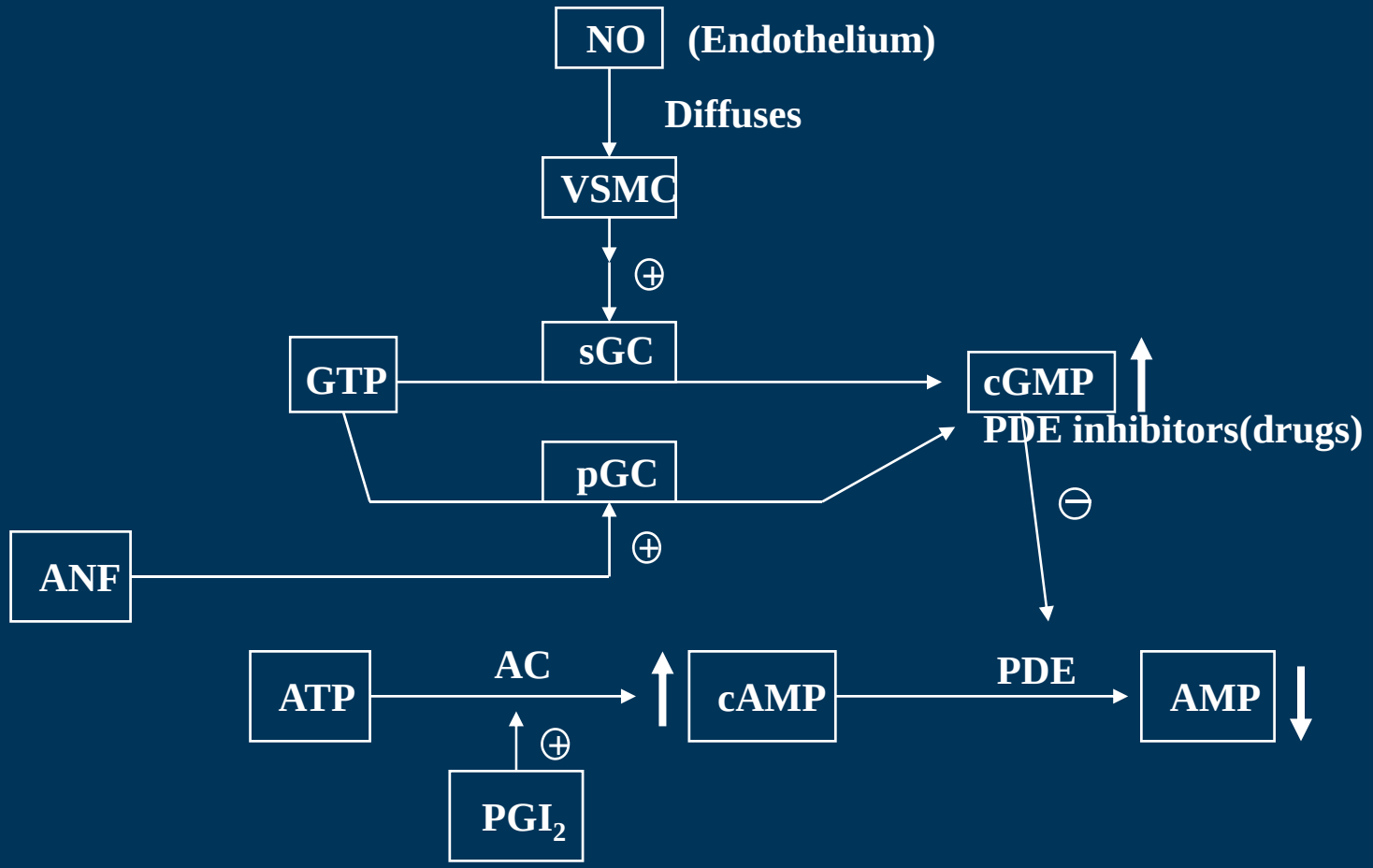
Nitric Oxide : Pathways & Metabolic Products

MC Houston, Vascular Biology in Clinical Practice 2000.



* eNOS saturated at normal substrate concentrations

Nitric oxide, soluble/ particulate guanylyl cyclase (sGC) (pGC), adenyl cyclase (AC), guanosine 3` 5` monophosphate (cGMP) guanosine triphosphate (GTP), ATP, cAMP, ANF, PGI_2



NO increases cGMP which increases cAMP
cAMP increased by via AC by PGI_2 .
ANF increases cGMP via pGC which increases cAMP

Vascular Biology in Clinical Practice, Oct. 2000; Mark C. Houston, MD

Summary:

Relationship of NO, cGMP, and cAMP

MC Houston. Vascular Biology in Clinical Practice. Hanley and Belfus 2000
MC Houston. Handbook of Hypertension Wiley Blackwell Oxford UK 2009

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- cAMP mediates the effects of nitric oxide and results in:
  - Vasodilation
  - Reduced endothelial and vascular permeability
  - Reduced monocyte and leukocyte adhesion
  - Decrease VSMH, proliferation, growth and migratory response
  - Decrease thrombosis and inhibits platelet activation
  - Reduced oxidative stress, inflammation and immune vascular dysfunction
  - Improved insulin resistance and glucose metabolism
  - Reduced atherosclerosis and CHD

Clinical Pearls 2 A

1. Balance the vascular endothelial and vascular biology "scale" with treatments to increase vasodilation, decrease endothelial cell permeability, growth, thrombosis, inflammation, oxidative stress and immune dysfunction in arteries.
2. Increase eNOS and NO: oral nitrates/nitrites(beet root juice/extracts, dark green leafy vegetables, cruciferous vegetables, polyphenols, vitamin C, arginine, methylarginines, citrullene, grapefruit juice with PDE inhibitors, PDE inhibitors (viagra, cialis, levitra), BH4, folate, B vitamins, NADH, NADPH, glutathione(GSH), whey protein, selenium, sulfhydryls, thiols (R-lipoic acid, NAC, MSM), curcumin, quercetin. STOP mouthwash.
3. Lower ADMA and increase DDAH activity
    1. Lower glucose, AIC, AGE's, homocysteine, LDL, oxLDL, TG, BP, hsCRP, VCAM, ICAM, proteinuria, Cr, weight. STOP tobacco.
    2. Increase tocopherols, antioxidants, vitamin A, omega 3 FA.
    3. Exercise(increase EPCs).
    4. Medications: ACEI, ARB, DRI, nebivolol, metformin, statins, estrogen, ASA, PDE inhibitors, block aldosterone with SARAs (serum aldosterone receptor antagonists) like spironolactone and eplerenone. STOP PPIs.

Clinical Pearls 2 B

1. Increase cAMP by increasing NO and cGMP which increases cAMP which generates all vascular effects. (arginine, citrullene, dark green leafy vegetables, beets and beet extract)
2. Increase PGI 2 and PGE2 levels: Stop COX2 inhibitors and NSAIDs.
3. Increase ATP: D-Ribose, CoQ10, carnitine, lipoic acid, Mg++, ALCAR, cordyceps, ginseng, B-vitamins, FAD, NADH, nicotinamide riboside exercise, optimal iron and copper.
4. Increase adenylyl cyclase (AC): beta adrenergic stimulation, Forskolin, caffeine, theophylline.
5. Increase ANF (atrial naturiuretic factor): caloric restriction, exercise, beta adrenergic stimulation.
6. Reduce superoxide anion and oxidative stress via AT1R blockade and reduce NADPH oxidase (NAC-n acetyl cysteine-, resveratrol, ARB, ACEI) which neutralizes NO via superoxide anion which decreases bioavailability of NO.
7. Increase NO and lower calcium influx into arteries with CCB (calcium channel blockers)
8. Increase NO, BK and angiotensin 1-7 with ACEI and ARB.

Endothelial Vasodilators

Nitric Oxide

Circulation Research 2016;119:375-396

Nitric Oxide (NO) in Cardiovascular Disease
The Big Picture: Nitrosothiols are the storage form for Nitric Oxide

Expert Opin Drug Discov 2011;6(11): 1139-54

- Nitric oxide is short-lived gas produced in the endothelium which diffuses across the endothelial cell into the vascular smooth muscle, stimulates soluble guanylyl cyclase (sGC), increases cGMP and cAMP.
- Modifies protein nitrosothiols via nitrosylation reactions to form storage form of NO call nitrosothiols which promote vasodilation and other CV effects.
- Regulates cell function (autocrine and paracrine)
- Perturbations in nitric oxide production, signaling and bioavailability induce many diseases especially CHD.
- Maintain **nitric oxide homeostasis** and physiological levels.
- Balance is key with avoidance of too little or too much nitric oxide.

S –Nitrosothiols (RSNO) Storage form of NO. Glutathione (GSH) role

Expert Opin Drug Discov 2011;6(11): 1139-54

- S-Nitrosothiols (RNSO) are thiol esters of nitrite (sulfur esters)
- Storage site for nitric oxide which are released on demand.
- RSNO are intermediates in NO-dependent but sGC – independent signaling processes that mediate vasodilation, platelet, anti-atherosclerotic and other CV effects.
- Longer circulating half life compared to nitric oxide.
- Decomposition of S-nitrosothiols occurs with metal ions like copper, mercury, iron, lead as well as enzymatic, photochemical and UV reactions.
- GSH (GSNO)(glutathione) + NADH --------GSNOR----→ GSSG + NAD.
- Reduced blood levels of GSNO decrease NO
  - S-Nitrosocysteine
  - S-Nitrosoglutathione (GSNO) / GSNOR S- nitrosoglutathione reductase
  - S-Nitrosoalbumin

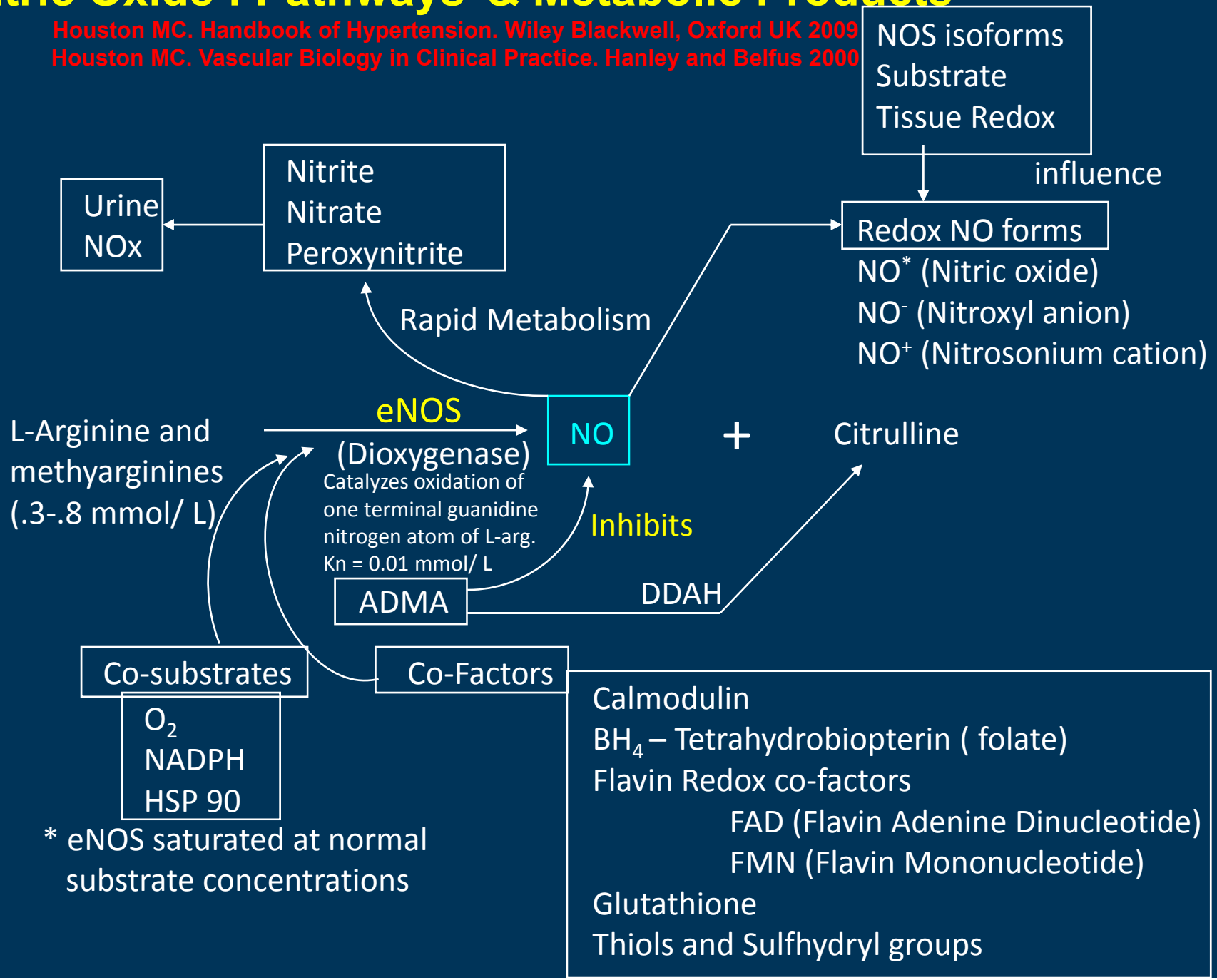
Nitric Oxide: Most Potent Endogenous Vasodilator Ca

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

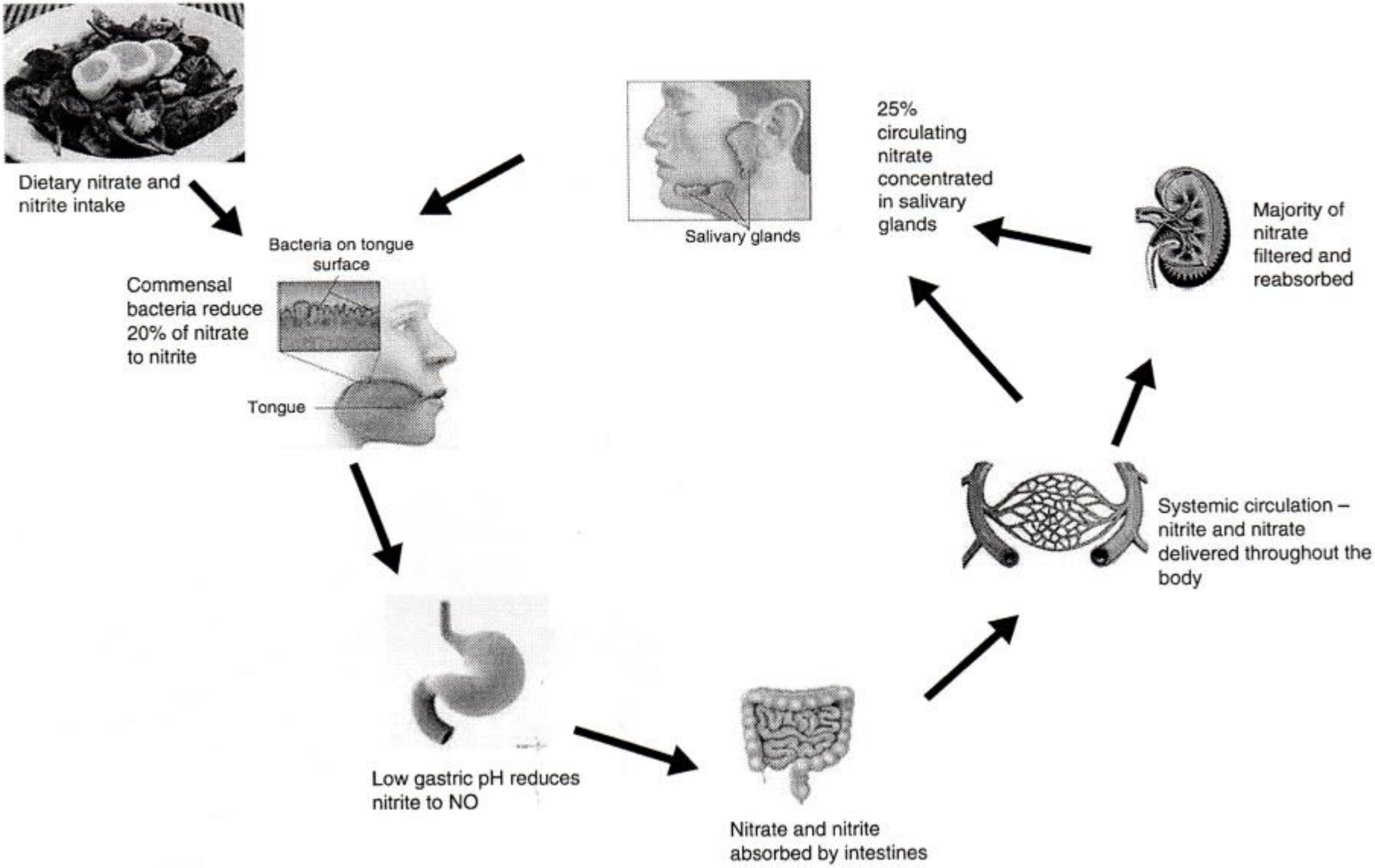
- Vasodilation (VSMC): ↑ cGMP, ↑ cAMP (secondary), ↓ ET-1(endothelin)
- Anti-atherosclerotic anti-inflammatory and anti-immune: modulates leukocyte-vessel wall interaction: ↑ cGMP, ↓ CAMs (cell adhesion molecules), ↓ chemokines and cytokines
- Anti-platelet: ↑ cGMP, ↑ cAMP, ↑ PGI (prostaglandin I), ↑ tPA (tissue plasminogen activator)
- Anti-growth: VSM hypertrophy, proliferation, migration
- Anti-oxidant: ↓ O_2^- (superoxide anion), ↓ oxLDL
- Synthesized by eNOS (endothelial nitric oxide synthase)

Nitric Oxide : Pathways & Metabolic Products

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus 2000

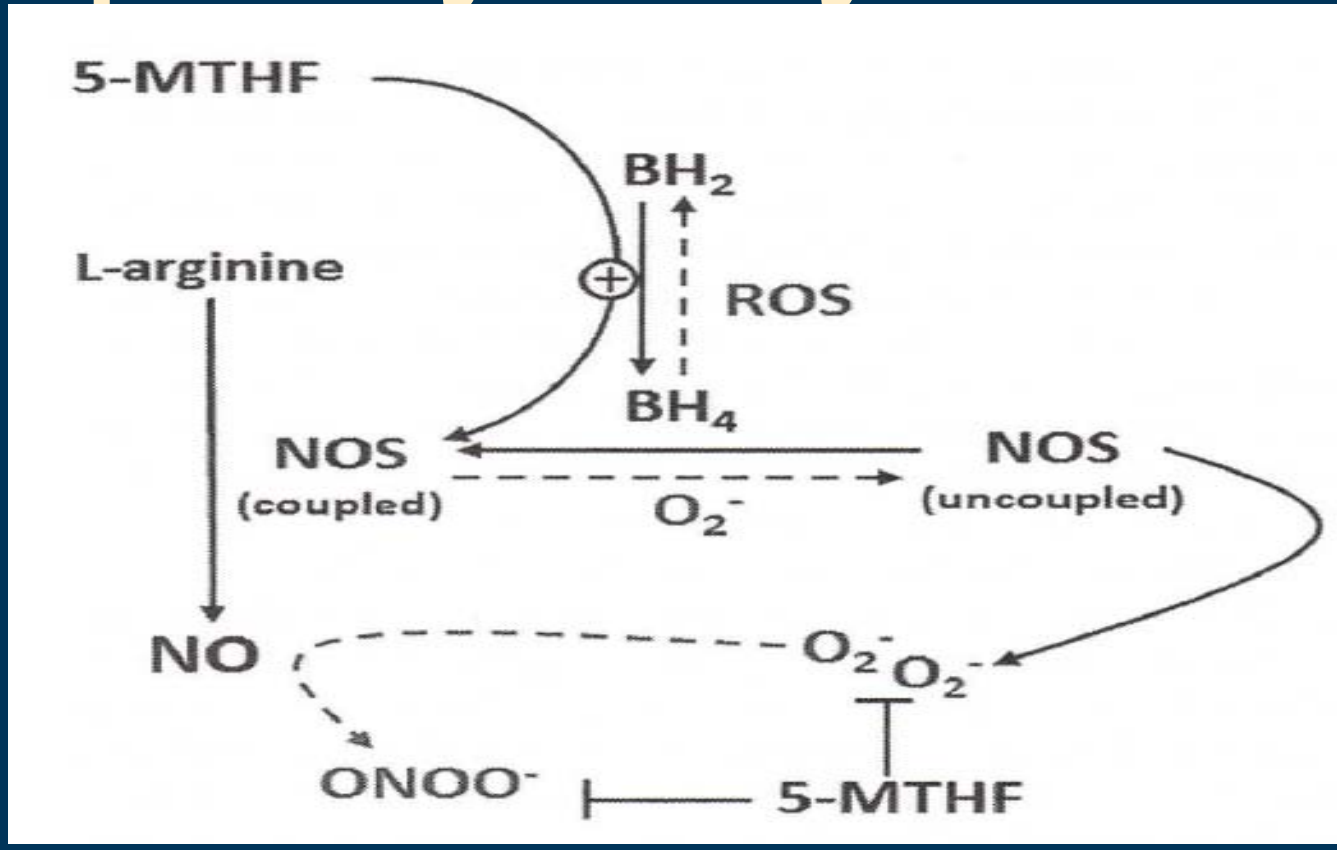


Application of nitric oxide in drug discovery and development



Expert Opin Drug Discov. 2011 Nov;6(11):1139-54

5-MTHF and NO Synthesis and Bioavailability : 5 mg per day methyl folate



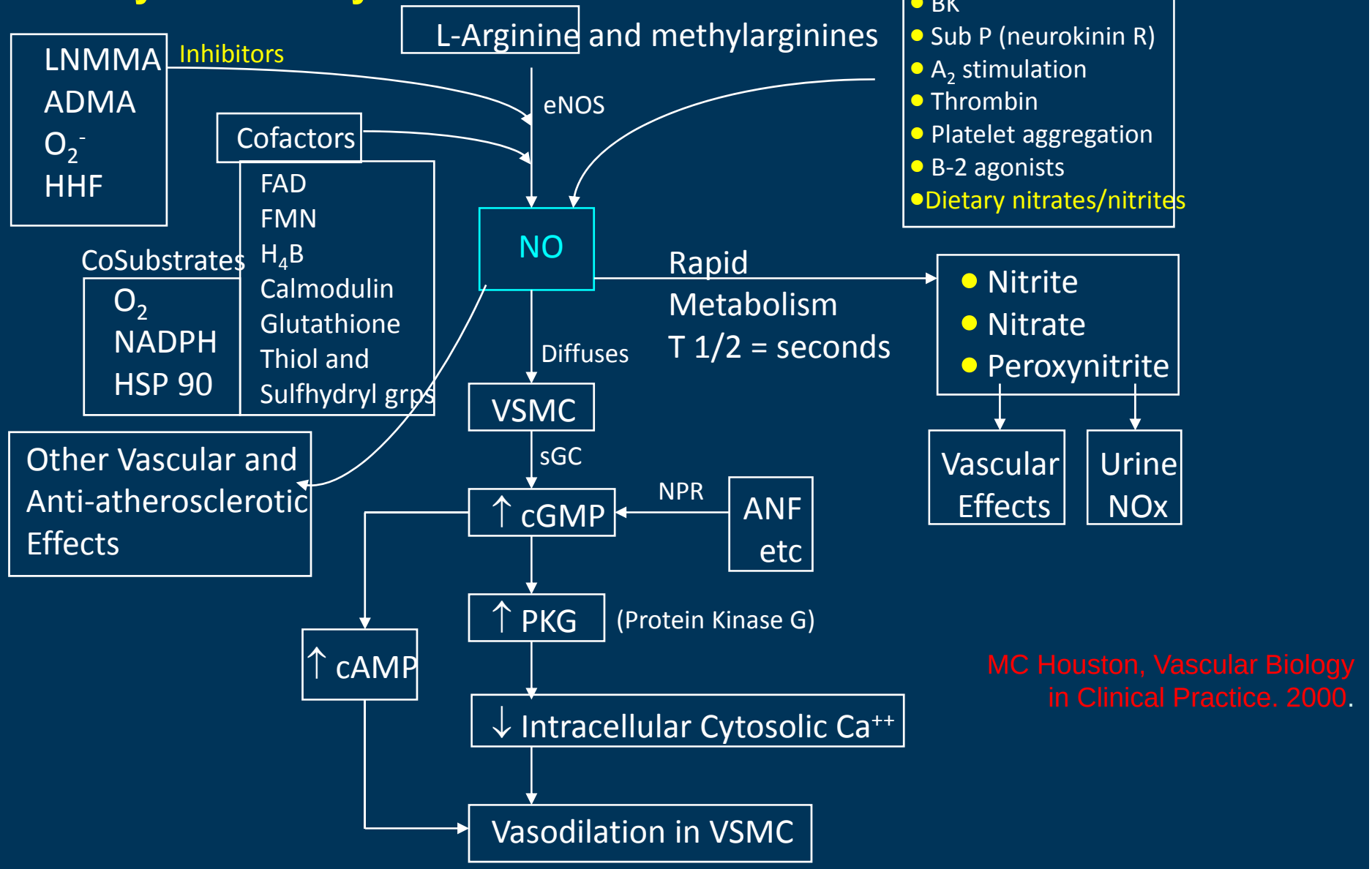
Nutrition Reviews® Vol. 75(1):51-70.

PPI increase CHD Risk

MPR July 16,2013, Circulation Research, May 10, 2016
AHA May 10, 2016
PLOS One June 10,2015 1-16

- PPIs elevate ADMA and decrease NO and induce ED. ADMA elevated 20-30%.
- Inhibit DDAH (dimethylarginine dimethylaminohydrolase enzyme) that degrades ADMA. DDAH clears 80% of ADMA
- Impairs acid production in endothelial cell lysosomes that prevent waste removal and accelerate endothelial cell aging
- Increases risk for CHD
- Also increase risk for CKD

Endothelium Vasodilators : Nitric Oxide : Synthesis
Summary of Pathways Stimulants



MC Houston, Vascular Biology in Clinical Practice. 2000.

ADMA

(Asymmetric Di-methyl Arginine)

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

- Inhibits endothelial nitric oxide synthase (eNOS) and reduces NO
- Elevated in PPI use, diabetes, chronic renal insufficiency, smokers, hypertension, dyslipidemia, homocysteinemia, elderly, atherosclerosis, vascular cell adhesion molecule (VCAM), inflammation and ox LDL
- Levels of ADMA : Normal: 1.0 $\pm$ 0.1 μmol/L
- eNOS + ADMA $\rightarrow$ O_2^- $\rightarrow$ NFkB activation $\rightarrow$ $\uparrow$ MCP-1(monocyte chemotactic protein)

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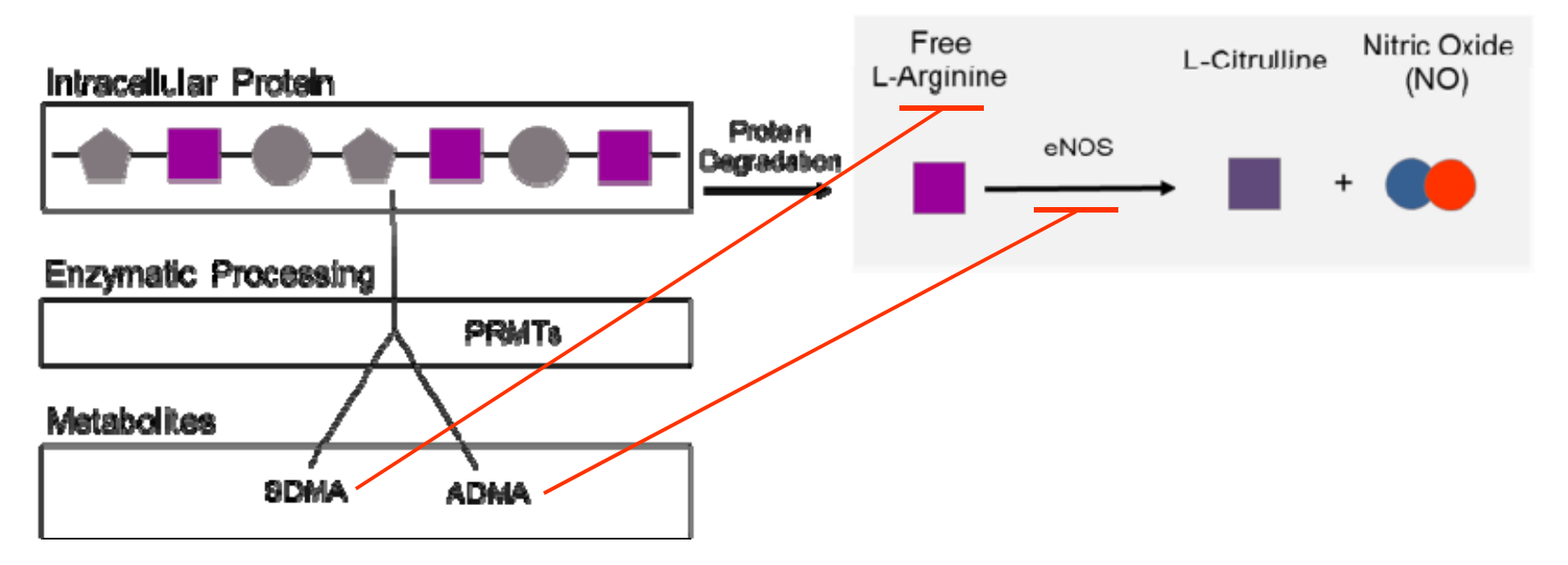
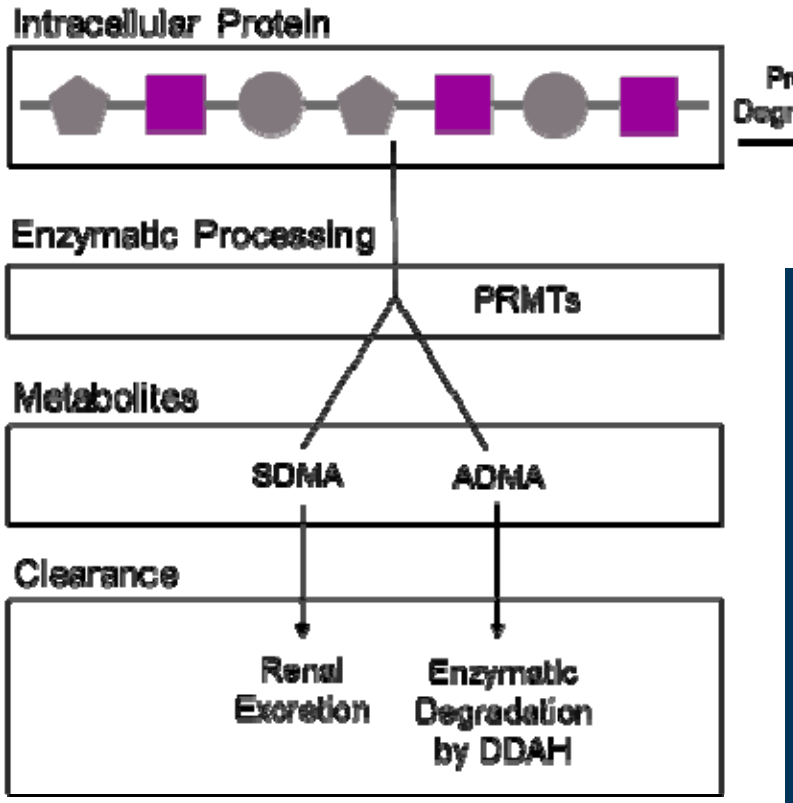
Regulation of NO production by ADMA and SDMA



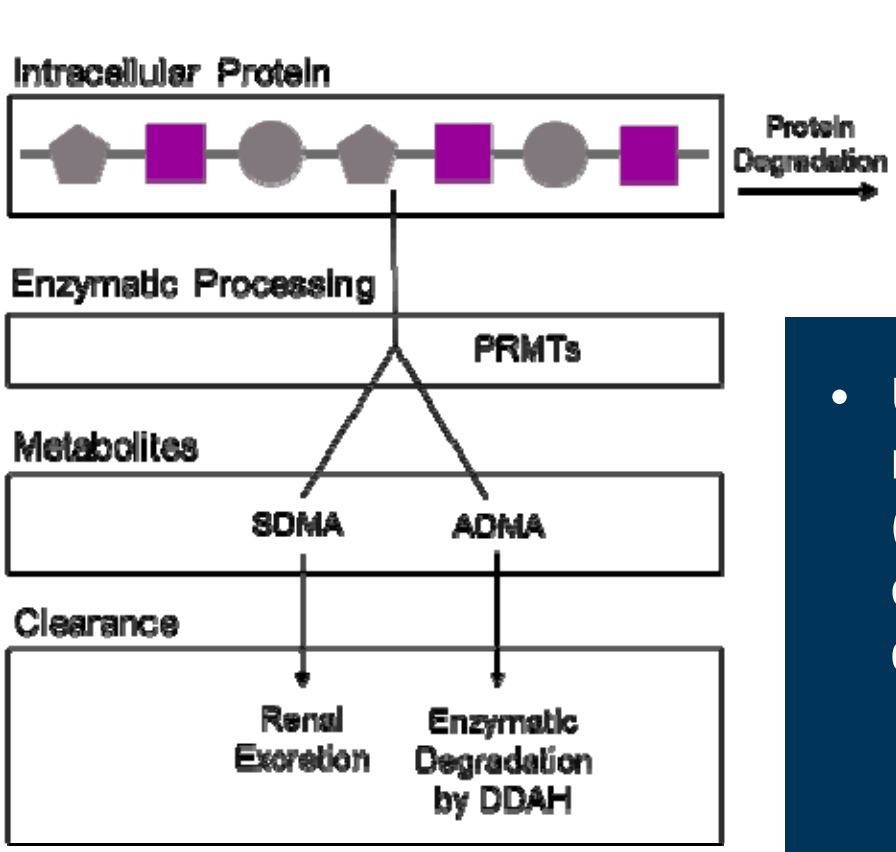
- ADMA directly blocks eNOS to inhibit NO production
- SDMA indirectly blocks NO production by inhibiting the availability of free L-Arginine.

ADMA and SDMA are excreted/degraded by distinct mechanisms, and therefore manifest differently



- ADMA is cleared gradually by degradation while SDMA is cleared rapidly through the urine
- Therefore, **ADMA identifies endothelial dysfunction and CV risk whereas SDMA identifies renal insufficiency and subsequent renal failure**

Uncontrolled risk factors modulate the production of ADMA and SDMA



- Uncontrolled risk factors[1-4] modulate ADMA and SDMA (through regulation of key enzymes) resulting in endothelial damage
  - Promote formation through PRMTs
  - Inhibit degradation of ADMA by DDAH

[1]Lin KY et al. *Circulation*. 2002; 106: 987-92.
[2]Böger RH et al. *Circ Res*. 2000; 87: 99-105.
[3]Osanai, T et al. *Hypertension*. 2003; 42: 985-90.
[4]Mah E and Bruno RS. *Nutrition Research*. 2012; 32: 727-740.

Overview

Clinical Study	Cohort	ADMA is associated with:
Framingham Offspring Study	'Asymptomatic'	Individuals who have an abnormal CIMT and disease burden
		Individuals who are at risk of all-cause mortality
Athero*Gene*	With known CAD	Individuals with known disease at risk of events
LURIC	With and without known CAD	Individuals with known disease at risk of CV-related mortality

Clinical Study	Cohort	SDMA is associated with:
LURIC	With and without known CAD	Reduced renal function and CV-related mortality

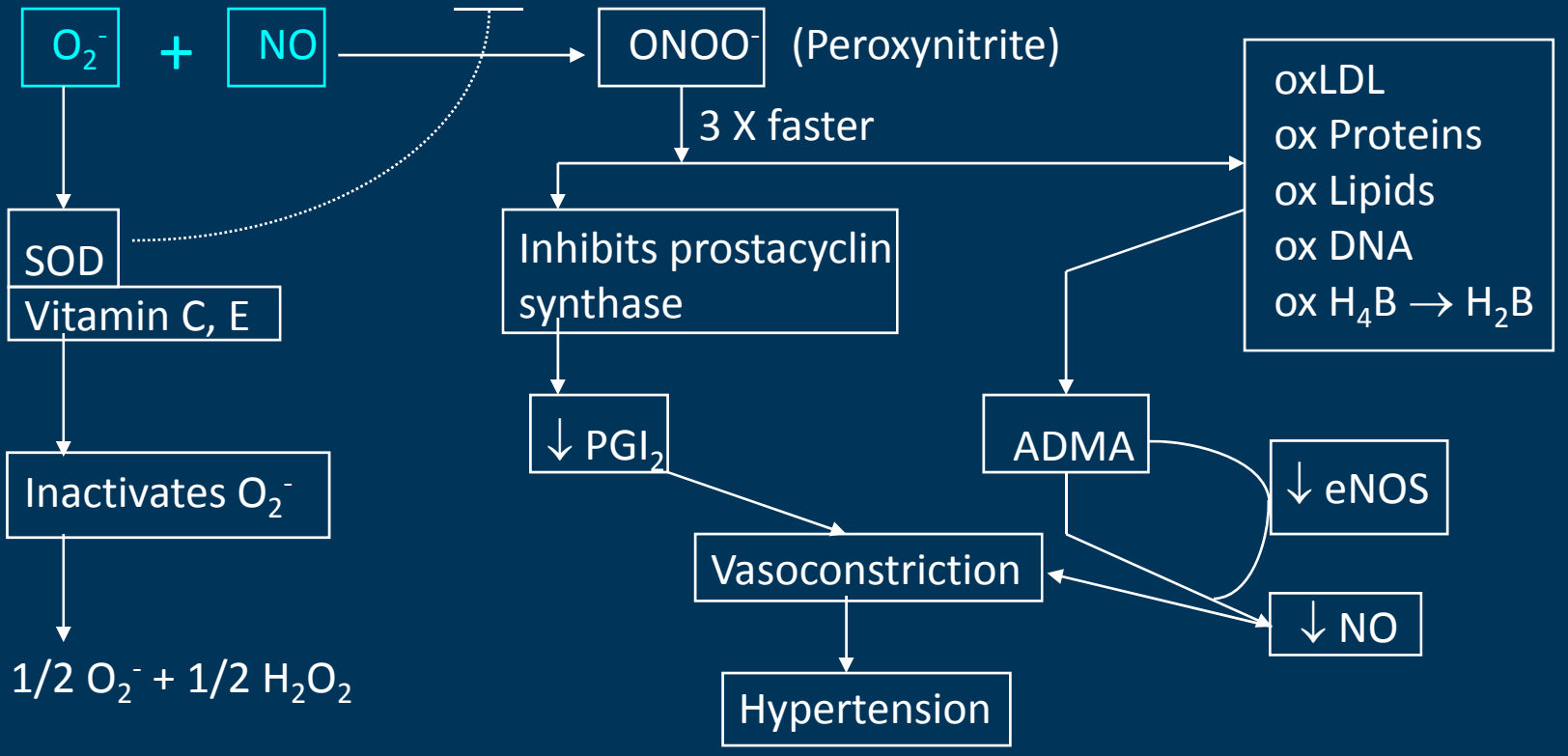
Clinical Interpretation

Test			Interpretation
ADMA		SDMA	
Low		Low	• Normal endothelial function
Med / High		Low	• Endothelial dysfunction and possible presence of pre-diabetes/diabetes or CVD
Low		High	• Reduced renal function
Med / High		High	• Endothelial dysfunction and possible presence of pre-diabetes/diabetes or CVD • Possible renal failure

Nitric Oxide and Hypertension-II (NO and O_2^- and Peroxynitrite ADMA)

Superoxide Anion (O_2^-) sources

- NADH oxidase
- eNOS + ADMA
- Cyclooxygenase
- Cytochrome P-450
- Lysophosphatidylcholine (LPC) via PKC



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Nitric Oxide and Hypertension Therapeutic Relationship

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

- L-arginine, methylarginine and oral nitrates and nitrites, aged garlic, lycopene, vitamin D, coenzyme Q 10 improve EDV (endothelial vasodilation) via eNOS and NO
- Increase NO levels with antihypertensive drugs
  - ACEI (angiotensin converting enzyme inhibitors) ($\uparrow$ BK $\rightarrow$ $\uparrow$ NO + $\downarrow$ O_2^-) and increase Angiotensin 1-7
  - CCB (calcium channel blockers) ($\uparrow$ NO, $\downarrow$ ROS)
  - ARB(angiotensin receptor blockers) ($\uparrow$ BK, $\uparrow$ NO, $\downarrow$ ROS) and increase Angiotensin 1-7
  - DRI (direct renin inhibitors) (decrease plasma renin activity (PRA), angiotensin-I and II, increase NO, angiotensin (1-7)
- NO increased also by:
  - Statins
  - Estrogens ($\uparrow$ eNOS)
  - Exercise ($\uparrow$ Shear stress $\rightarrow$ $\uparrow$ eNOS)
  - BH_4 ($\uparrow$ eNOS, $\downarrow$ BH_4 in HLP)
  - Antioxidants ($\downarrow$ O_2^-, $\downarrow$ ROS)
  - FAD, FMN, NADPH, GSH, thiols. sulfhydryl groups, folate
  - ASA ($\uparrow$ iNOS in VSMC, $\downarrow$ platelet activity

.

Summary Functions of
Nitric Oxide

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

1. Most powerful endogenous vasodilator (↓ VSMC contraction)
2. Maintains basal vascular tone
3. Inhibition of migration of VSMC
4. Inhibition of leukocyte adhesion to endothelium
5. Inhibition of platelet aggregation and granule secretion
6. Major role in architecture and remodeling of blood vessels
7. Inhibits VSMC proliferation
8. Inhibits oxidation LDL
9. Inhibits ET-1(endothelin) production and NO inhibits expression of mRNA for ET-1 production
10. NO inhibits action of ET-1 at ET-A receptor
11. Promotes apoptosis

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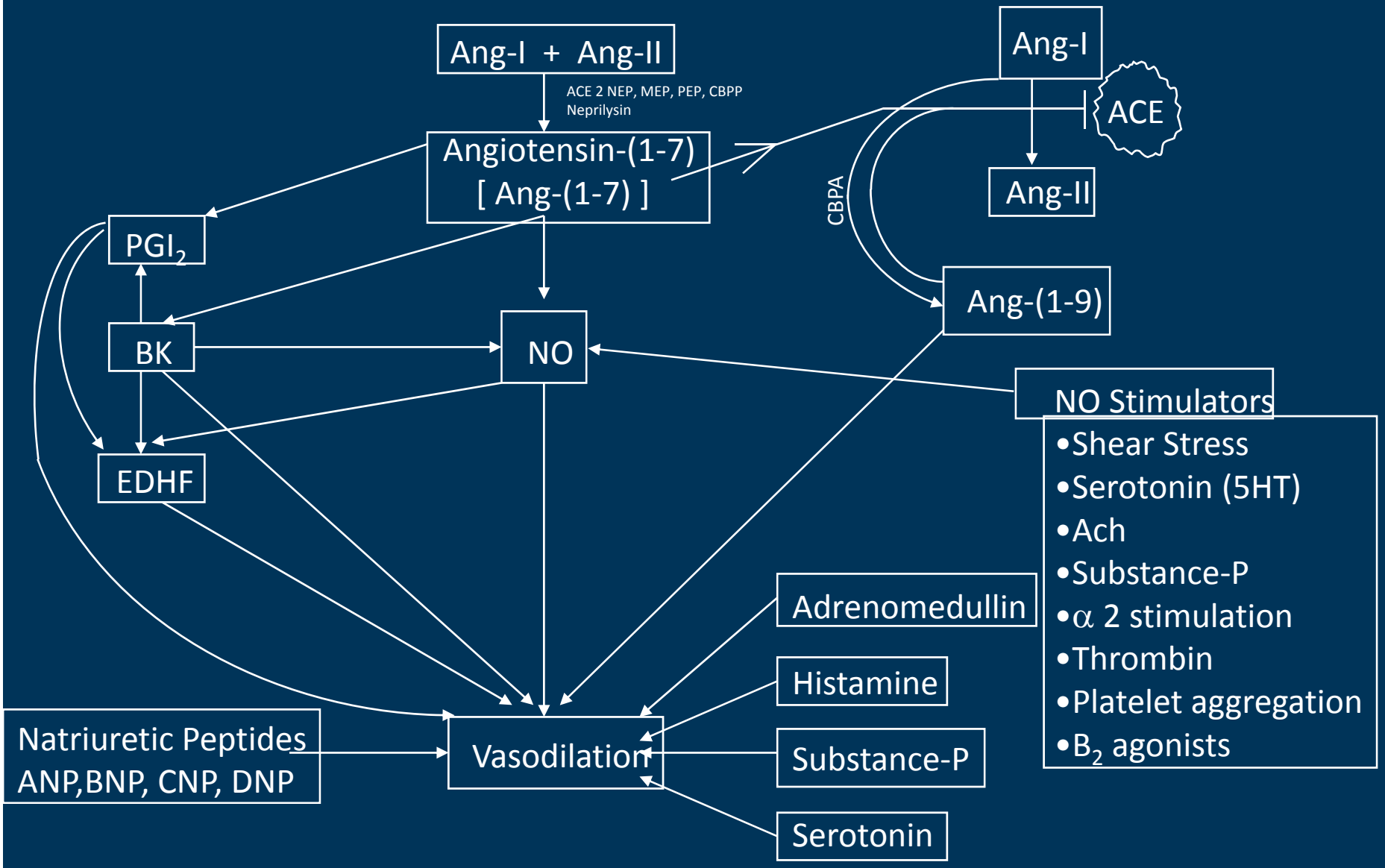
Summary Functions of
Nitric Oxide

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

12. Protects against target organ damage. (LVH, renal, cardiac, cerebral)
13. Decreases endothelial permeability
14. Inhibits expression of adhesion molecules (CAM's)
15. Suppresses TNFα induced NF-kB activation in endothelium
16. Renal vasodilation with diuresis and natriuresis
17. Inhibits aldosterone secretion in ZG (zona glomerulosa) of adrenal gland
18. Inhibits platelet - endothelium denuded vessel wall interaction
19. Inhibits platelet adhesion to endothelial cell monolayers
20. Inhibits pro-inflammatory cytokines
21. Modulates baroreceptor reflexes

Endothelium Vasodilators : Summary



Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

Endothelium Vasoconstrictors

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

1. Endothelin (ET-1)
2. Angiotensins
    1. Angiotensin-I (Ang-I)
    2. Angiotensin-II (Ang-II)
    3. Angiotensin-III (Ang-III)
    4. Angiotensin-IV (Ang-IV)
    5. Angiotensin 1-7
    6. Angiotensin-"X" (Ang-X)
3. Aldosterone
4. Cyclooxygenase products
    1. Prostaglandin 2 (PG_2)
    2. Prostaglandin H_2 (PGH_2)
    3. Thromboxane A_2 (TxA_2)
    4. Endoperoxides (Vasoconstrictor prostanoids)(F_2 isoprostane)
    5. Arachidonic Acid derivatives - others (AA)
5. Thrombin (T)
6. Nicotine (N)
7. Serotonin (ST) - (Variable VC or VD)

.

Endothelin

Clinical Treatment Considerations

Houston MC. Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Houston MC. Vascular Biology in Clinical Practice. Hanley and Belfus. Philadelphia 2000

- Increased endothelin (ET-1) in areas of intimal hyperplasia in atherosclerotic human coronary arteries and aorta
  1. ET-1 → mediates collagen type-I synthesis (CT-1)
  2. Ang-II → mediates collagen type-II synthesis (CT-II)
  3. CT-I and CT-II account for most of the hyperplasia and restenosis after PCTA(angioplasty)
  4. ET-1 is an active mitogen in atheroma plaque
- Anti-hypertensive therapy effects
  - CCB : Most effective available ET-1 inhibitors
    Reduce vasoconstriction, vascular remodeling, plaque rupture and restenosis after PCTA
  - ACEI : ↓ Ang-II, ↓ ET-1 ↓ CT-I, ↓ CT-II
  - ARB : ↓ Ang-II effects ↓ CT-I, ↓ CT-II

.

Clinical Pearls 3

1. Increase nitric oxide and nitrosothiols (storage form of NO). Check heavy metals
2. Control inflammation, oxidative stress and immune vascular dysfunction
3. Increase citrulline and arginine if deficient or if high ADMA.
4. Increase eNOS (BH4, folate, FAD, FMN, GSH, thiols etc.)
5. Decrease ADMA and increase DDAH activity
6. Decrease superoxide anion with SOD (superoxide dismutase) and antioxidants, block NADPH oxidase with NAC, resveratrol, statins, ARB and ACEI.
7. Decrease peri-oxynitrate (NO/O2- reaction)
8. Increase ANG 1-7 vasodilator (ACEI, ARB)
9. Decrease ET-1(endothelin) vasoconstrictor and CT-I and CT-II (CCB,ACEI,ARB)
   - ACEI : ↓ Ang-II, ↓ ET-1 and ↓ CT-I, ↓ CT-II
   - ARB : ↓ Ang-II effects and ↓ CT-I, ↓ CT-II
   - CCB: reduce ET-1 best.
10. Decrease angiotensin II and increase angiotensin 1-7:
    ACEI, ARB and DRI.

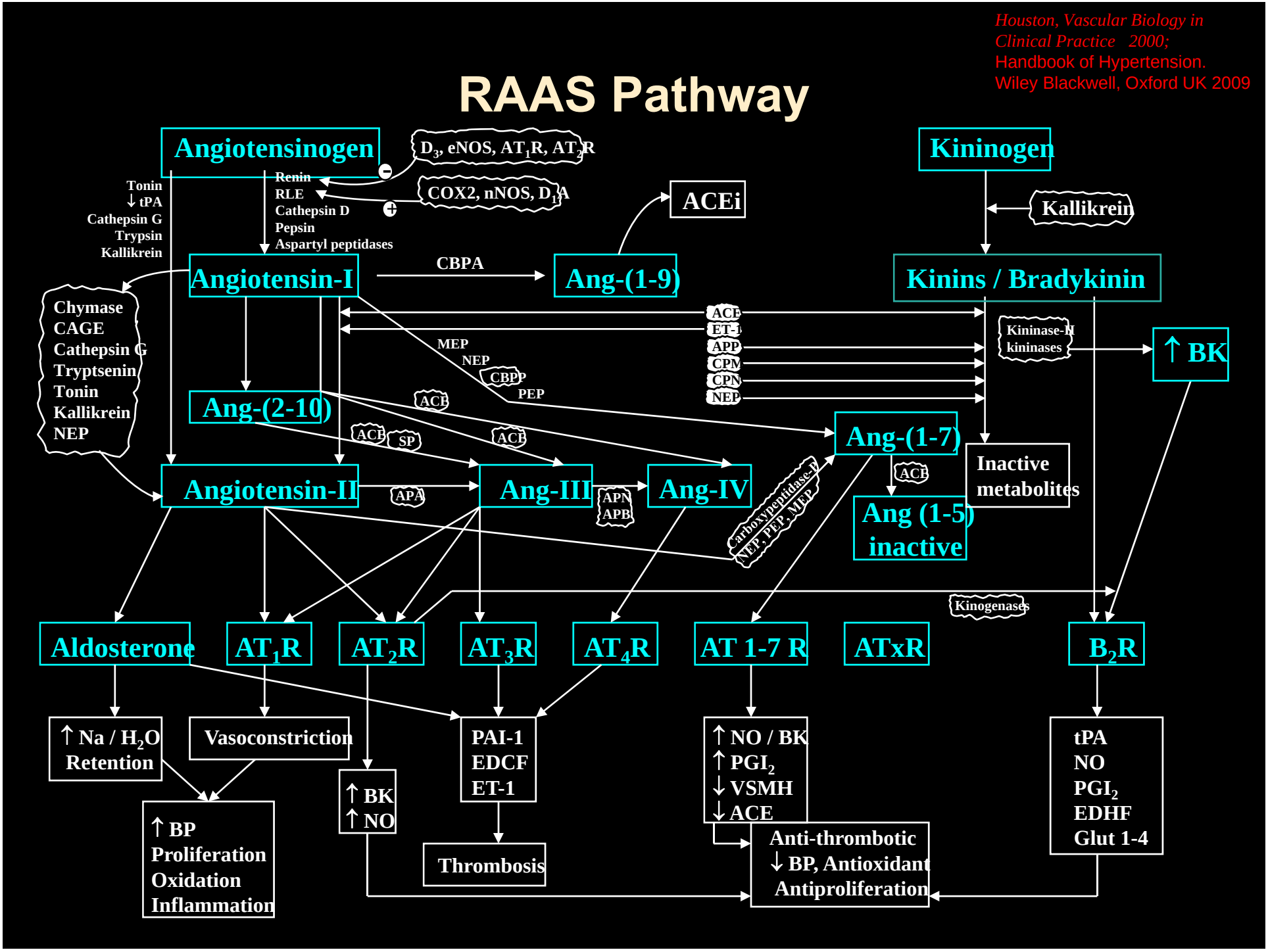
The **Renin Angiotensin Aldosterone System** (RAAS)

Endothelium Vasomediators

MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Angiotensin I
Angiotensin II
Angiotensin III
Angiotensin IV
Angiotensin (2-10)
Angiotensin (4-8)
Angiotensin (1-7)

Angiotensin (1-5)
Angiotensin (1-9)
Angiotensin (2-7)
Angiotensin (2-9)
Angiotensin 10
Angiotensin (1-12)
Aldosterone


RAAS Pathway
Houston, Vascular Biology in Clinical Practice 2000;
Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
Angiotensinogen
D3, eNOS, AT1R, AT2R
COX2, nNOS, D1A
Renin
RLE
Cathepsin D
Pepsin
Aspartyl peptidases
Tonin
↓ tPA
Cathepsin G
Trypsin
Kallikrein
ACEi
Kininogen
Kallikrein
Angiotensin-I
CBPA
Ang-(1-9)
Kinins / Bradykinin
Chymase
CAGE
Cathepsin G
Tryptsenin
Tonin
Kallikrein
NEP
ACE
ET-1
APP
CPM
CPN
NEP
MEP
NEP
CBPP
PEP
Kininase-II
kininases
↑ BK
Ang-(2-10)
ACE
ACE
SP
ACE
Ang-(1-7)
Angiotensin-II
APA
Ang-III
APN
APB
Ang-IV
ACE
Inactive
metabolites
Carboxypeptidase-P
NEP, PEP, MEP
Ang (1-5)
inactive
Kinogenases
Aldosterone
AT1R
AT2R
AT3R
AT4R
AT 1-7 R
ATxR
B2R
↑ Na / H2O
Retention
Vasoconstriction
PAI-1
EDCF
ET-1
↑ NO / BK
↑ PGI2
↓ VSMH
↓ ACE
tPA
NO
PGI2
EDHF
Glut 1-4
↑ BK
↑ NO
↑ BP
Proliferation
Oxidation
Inflammation
Thrombosis
Anti-thrombotic
↓ BP, Antioxidant
Antiproliferation

Renin Angiotensin Aldosterone System
General and Major Points

MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

- Two RAAS: Renin angiotensin aldosterone systems
  1. Classic RAAS: BP regulation. Circulating ACE 10%
  2. Tissue RAAS: Regulates *vascular* and *cardiac structure* and *function* (90%).
- ACE is an zinc dependent ecto-enzyme located on:
  1. Vascular endothelial cells: lumen and vasa vasorum
  2. Media of VSMC
- Angiotensin-II is potent vasoconstrictor, hypertensive, growth promoter, thrombogenic, pro-oxidant, pro-inflammatory, pro-immune and atherogenic hormone.

Postulated Role of Angiotensin II and ACE

90 % of ACE is tissue located RAAS: ACE is ectodermal enzyme on endothelium and VSMC

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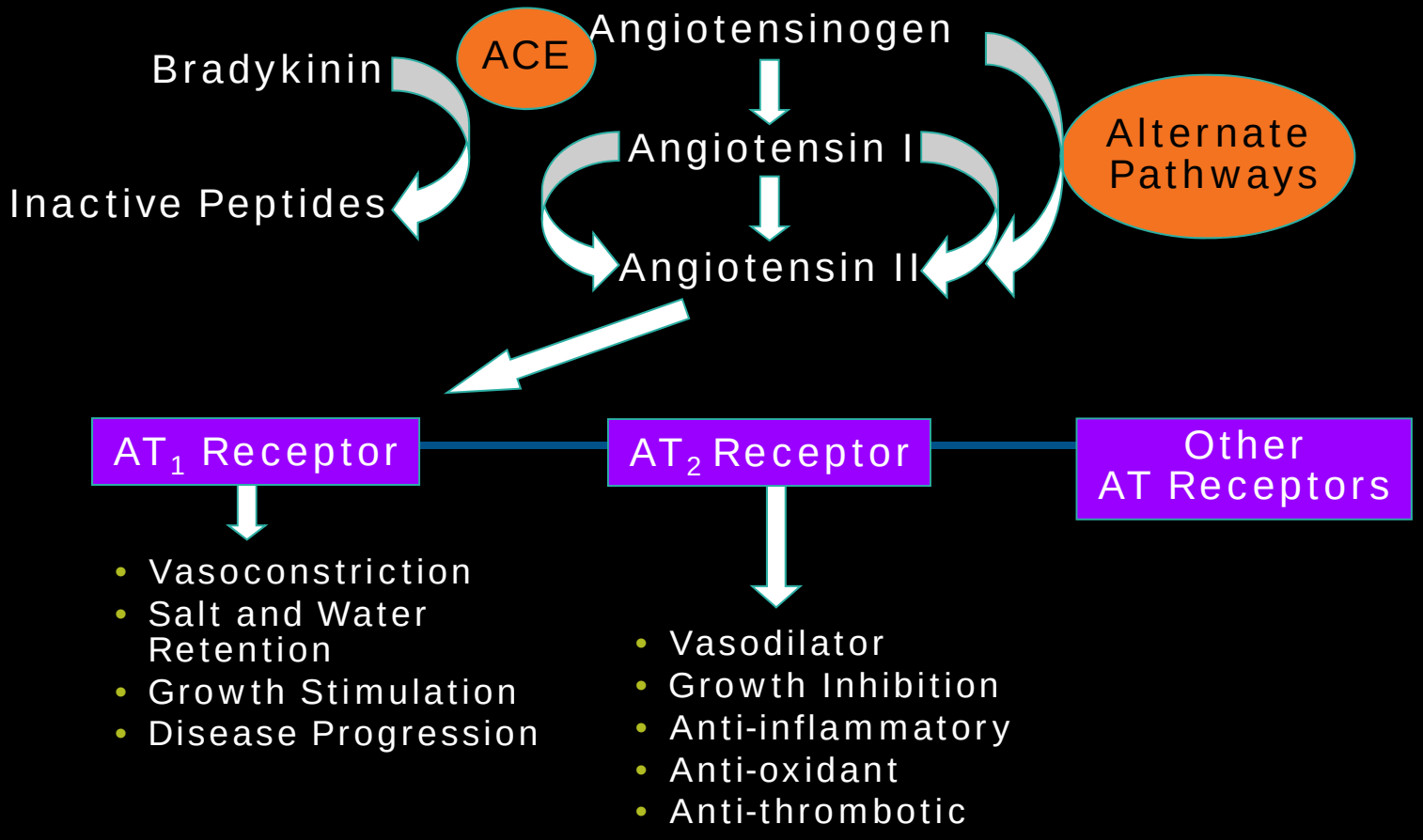

Angiotensin II (Ang-II)

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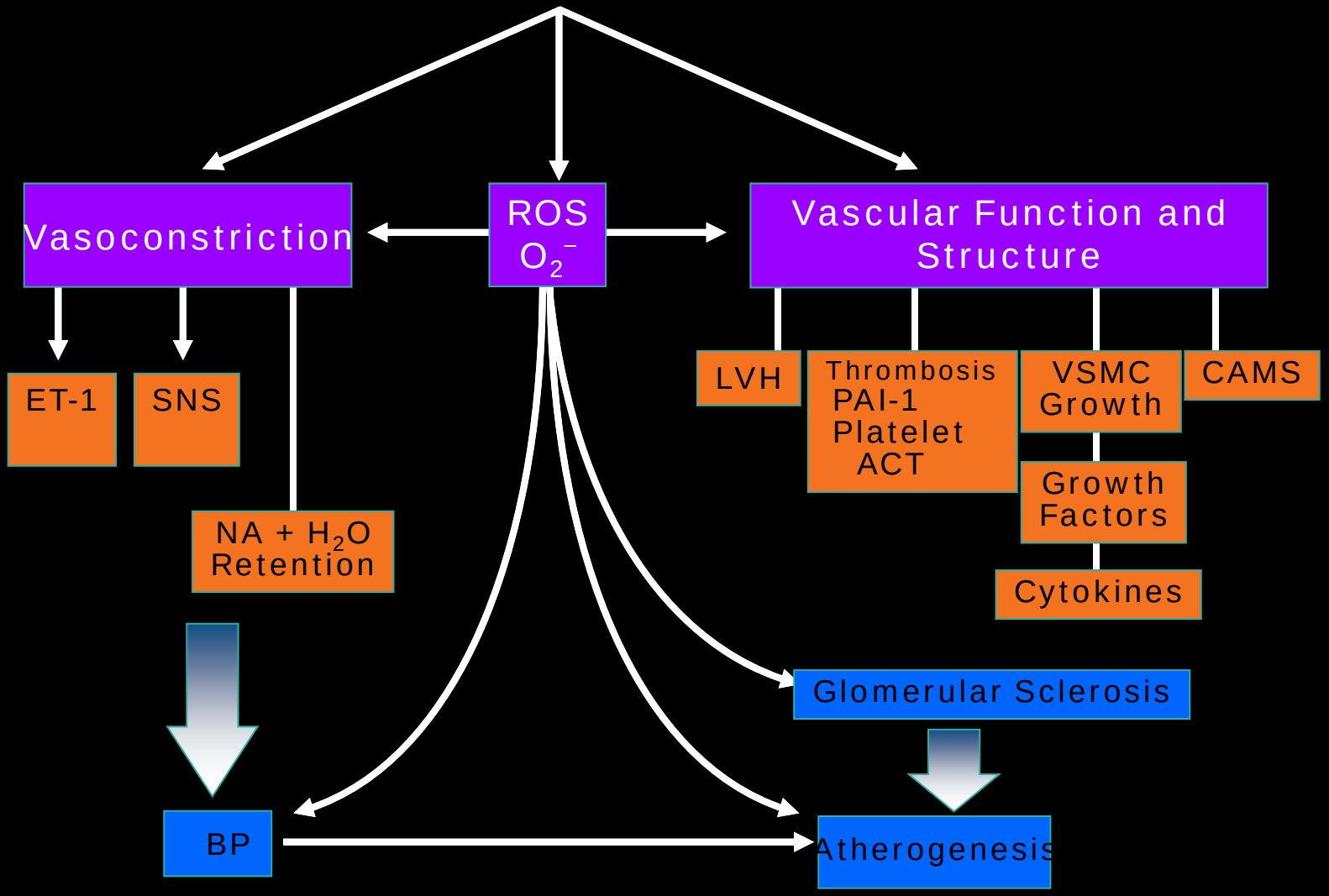


Caveolae Membrane Lipid Rafts that contain Nitric Oxide and eNOS

Therapeutic Interventions

J of Nutritional Biochemistry 2011;22:807

Omega 3 Fatty Acids (specially DHA)

- Alter lipid environment of raft microdomains and down stream signaling events and lipid raft disruption.
- Decreased lipid raft cholesterol, reduce ICAM, VCAM, TNF-alpha.
- Displaces caveolin-1 and eNOS with increase in NO
- Modulate TLR 4 (toll like receptor) activation response to LPS and lauric acid.
- Inhibits NADPH oxidase and superoxide production which increases NO.
- Attenuates atherosclerosis

Caveolae

Therapeutic Interventions

J of Nutritional Biochemistry 2011;22:807

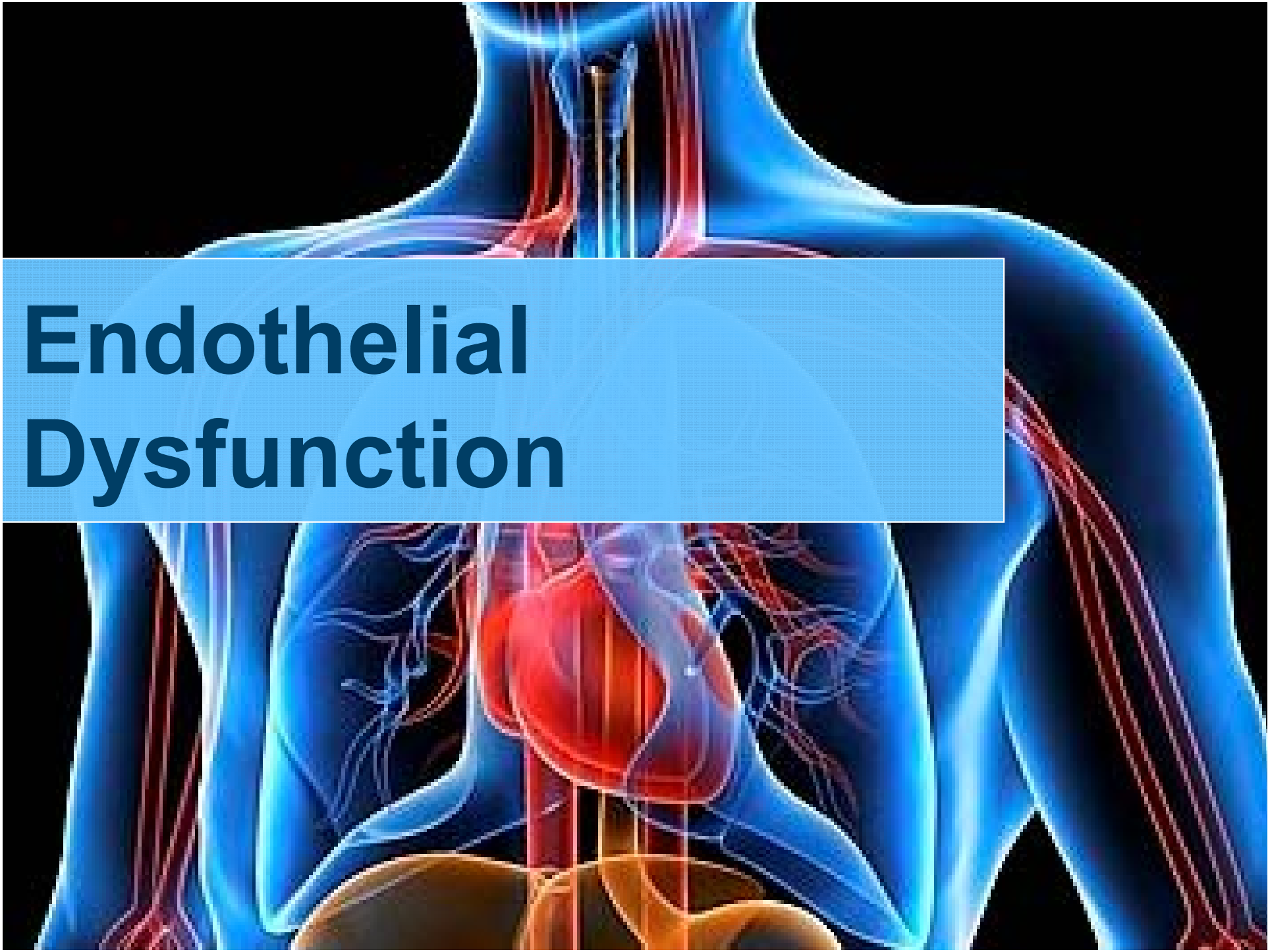
Plant-derived polyphenols
Fruits and vegetables
Resveratrol
Quercetin
Red wine

Tea: EGCG
Dark chocolate
Various flavonoids
Daidzein and genistein
Curcumin

- Decrease inflammatory stimulation for endothelial activation
- Selective and avid uptake into caveolae
- Increase NO
- Increase mitochondrial uptake of compounds

Clinical Pearls 4

1. Balance AT1R with AT2R (ACEI, ARB).
2. Improve caveoli function (caveolin-1) and membrane function for intracellular signaling and increase eNOS and nitric oxide: lower ox LDL, add dietary omega 3 FA, phytonutrients, polyphenols, flavonoids and drugs such as ARB, ACEI, DRI and CCB)
3. Increase angiotensin- 1-7: ACEI and ARB.
4. Decrease inflammation (interleukins, hsCRP, TNF alpha) oxidative stress and immune dysfunction by balance of Th1 and Th2 response with sterolins and phytosterols.
5. A-II has effects similar to aldosterone. Block both with ARB, ACEI, DRI and SARA or natural compounds.



Endothelial Dysfunction

Two Paradigms of Endothelial Activation:

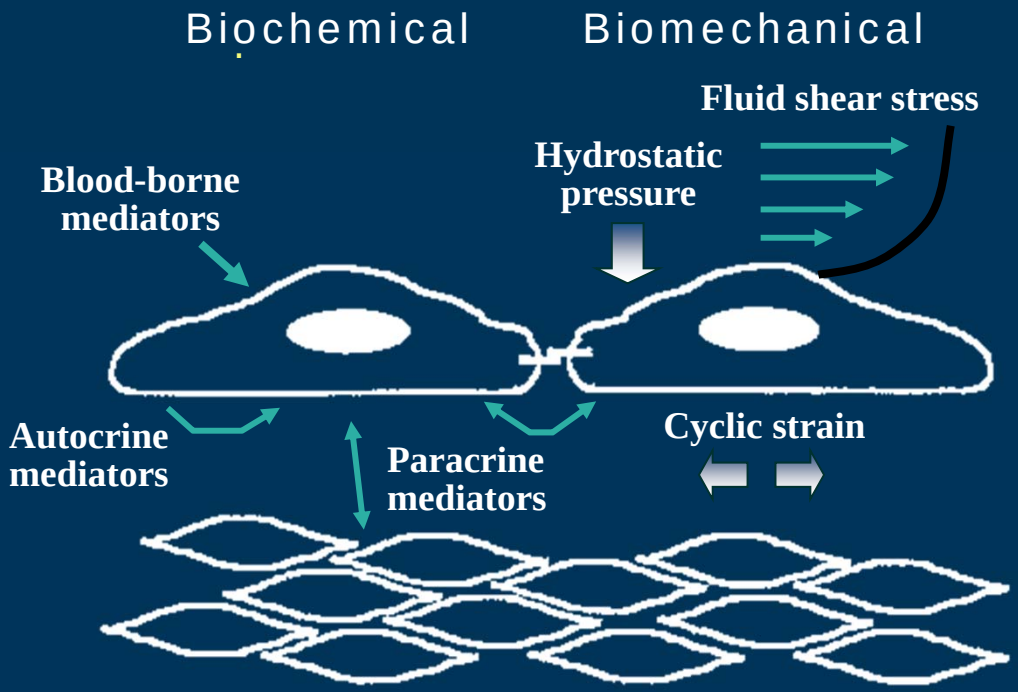
Biochemical and Biomechanical

Set off three finite responses

MC Houston. Vascular Biology in Clinical Practice. 2000.

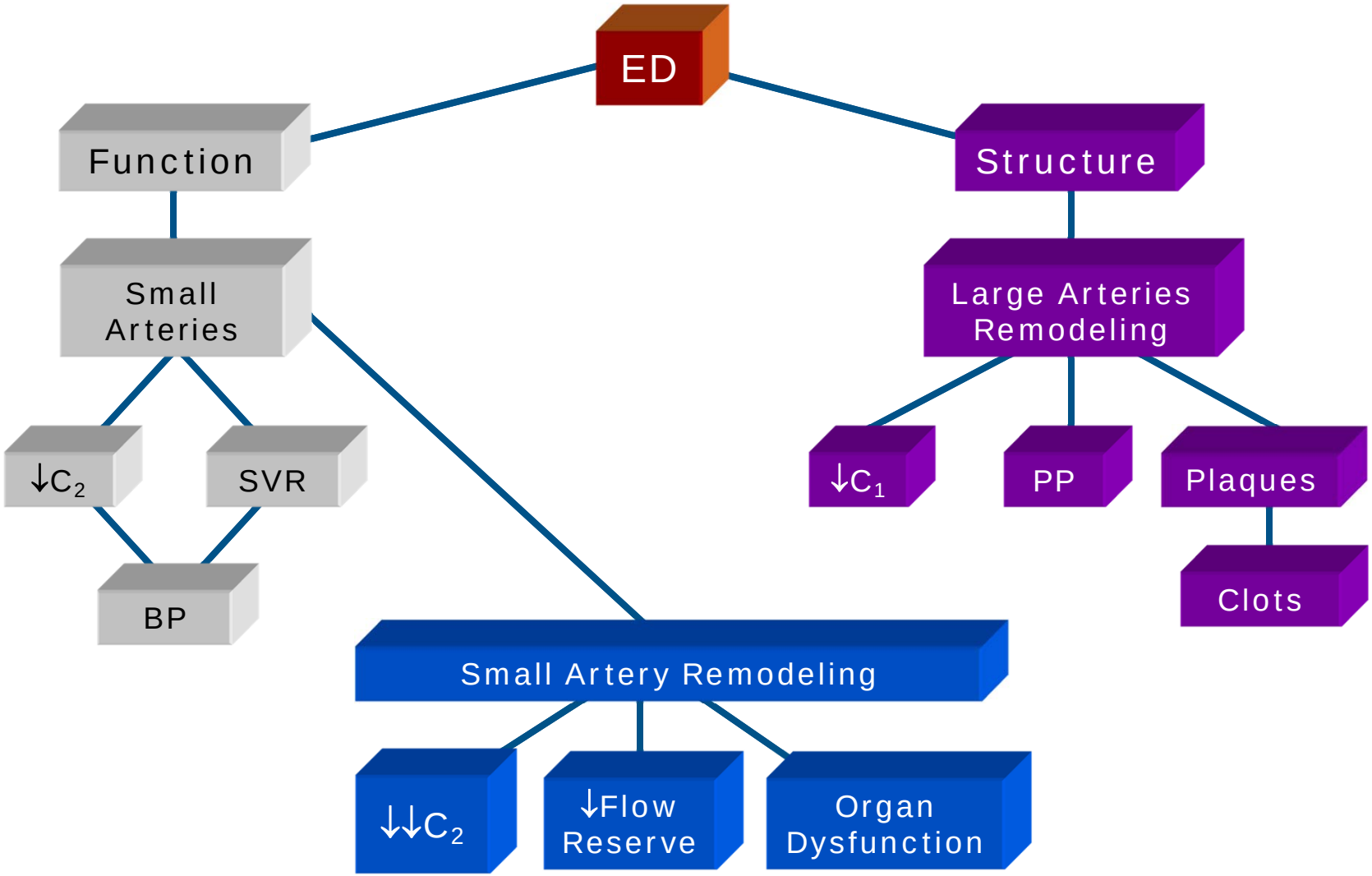
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Activation or dysfunction = Phenotypic Modulation of Structure and Function of Resting Endothelial Cell



The term *endothelial activation* is used to connote the modulation of endothelial functional phenotype, in response to physiologic and pathophysiologic stimuli, which can have both adaptive and nonadaptive consequences. By virtue of its position at the interface between flowing blood and tissues, endothelium is exposed to a vast array of both biochemical and biomechanical stimuli that can induce endothelial activation. The biochemical stimuli (hormones, growth factors, cytokines and bacterial products) can be delivered via the blood and also in an autocrine (acting on the cell of origin) or paracrine (acting on adjacent cells) manner. The biomechanical stimuli consist of wall shear stresses (tractive forces generated at the luminal endothelial interface by blood flow), pressures (hydrostatic forces that act perpendicular to the endothelial interface), and cyclic strains (circumferential stretching of endothelium and other cells within the vessel wall, as a consequence of pulsatile blood flow).

Endothelial Dysfunction



Cohn, J. ISH, August 2000

Atherosclerosis

Atherosclerosis and Inflammation Endothelial Dysfunction (ED)

MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

- Atherosclerosis and inflammation share similar mechanism during the early phases which involve increased interactions between vascular endothelium and circulating leukocytes.
- Membrane phospholipids are major modulators of cell responsiveness to cytokines.
- Primary atherosclerotic site is at arterial bifurcations, branch points, convex side with low or oscillatory shear stress favors passive transport of blood components into the vessel wall.
- Fatty streak is earliest stage of plaque development and is reversible. Present in 50% children age 10-14 years on autopsy.

The Inflammatory Response Atherosclerosis

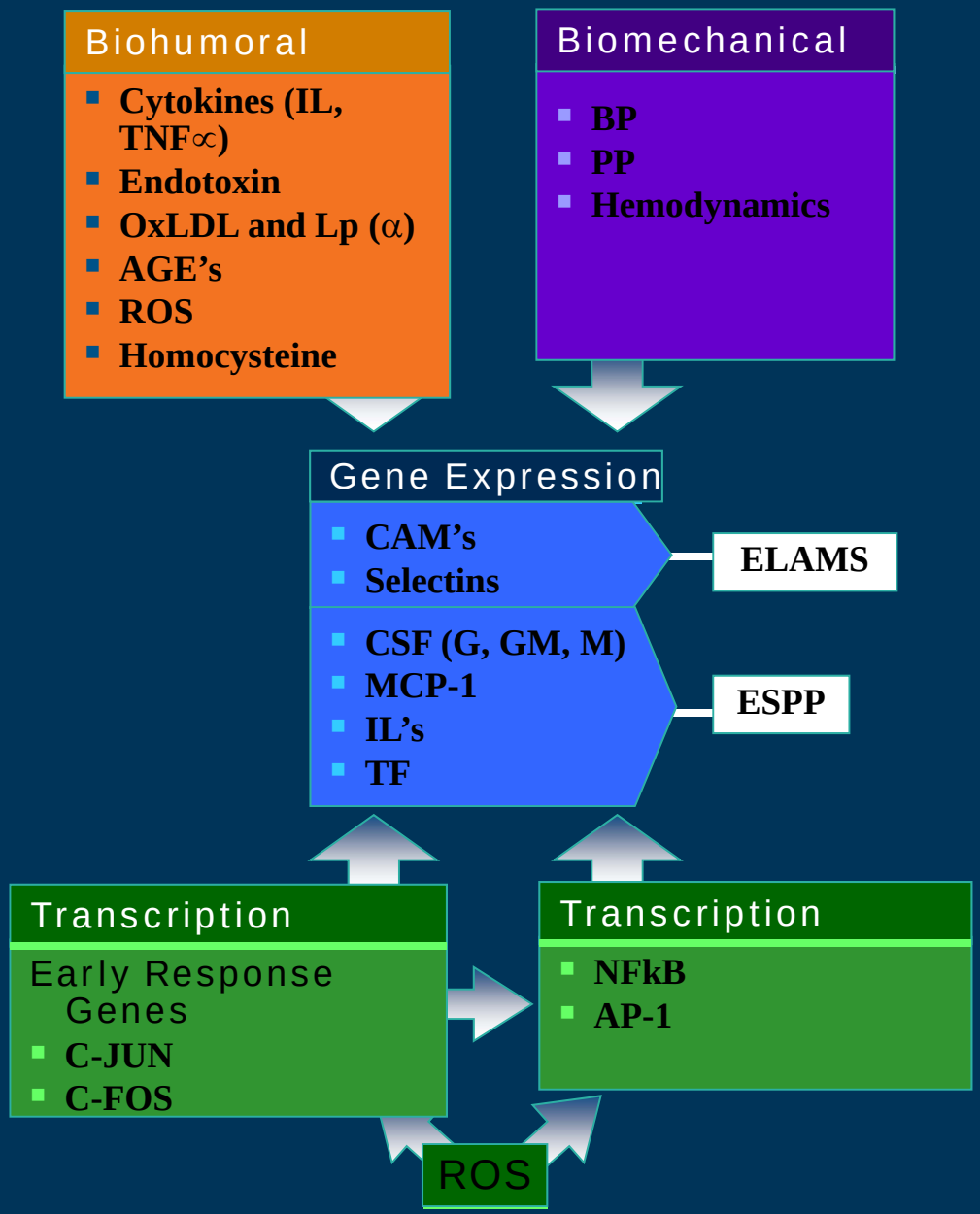
MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

- Leukocyte recruitment to the endothelium is mediated by the interaction of adhesion molecule receptors expressed on the surface of endothelial cells with counterreceptors expressed on immune cells.
- Leukocyte classes involved in atherogenesis include:

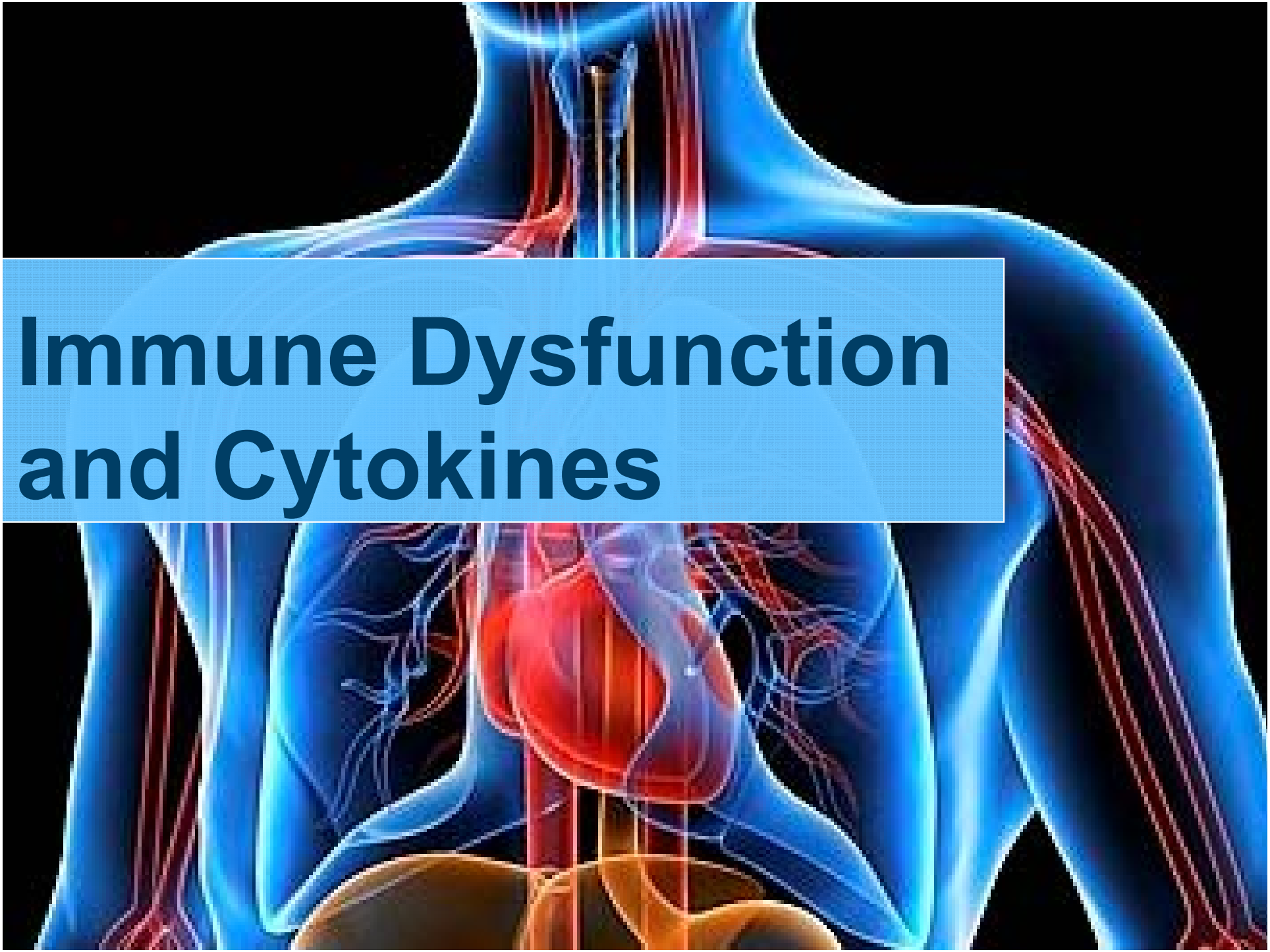
1. **Mononuclear cells**
   - **Mononuclear phagocytes**
   - **Monocytes**
   - **Macrophages**
2. **Lymphocytes**
   - **T cells ($CD4^+$, $CD8^+$)**
   - **B cells**
   - **Plasma cells**
3. **PMN's**
   - **Granulocytes**
   - **Eosinophils**
4. **Mast cells**

Endothelial Activation:

Endothelial-Leukocyte Interaction

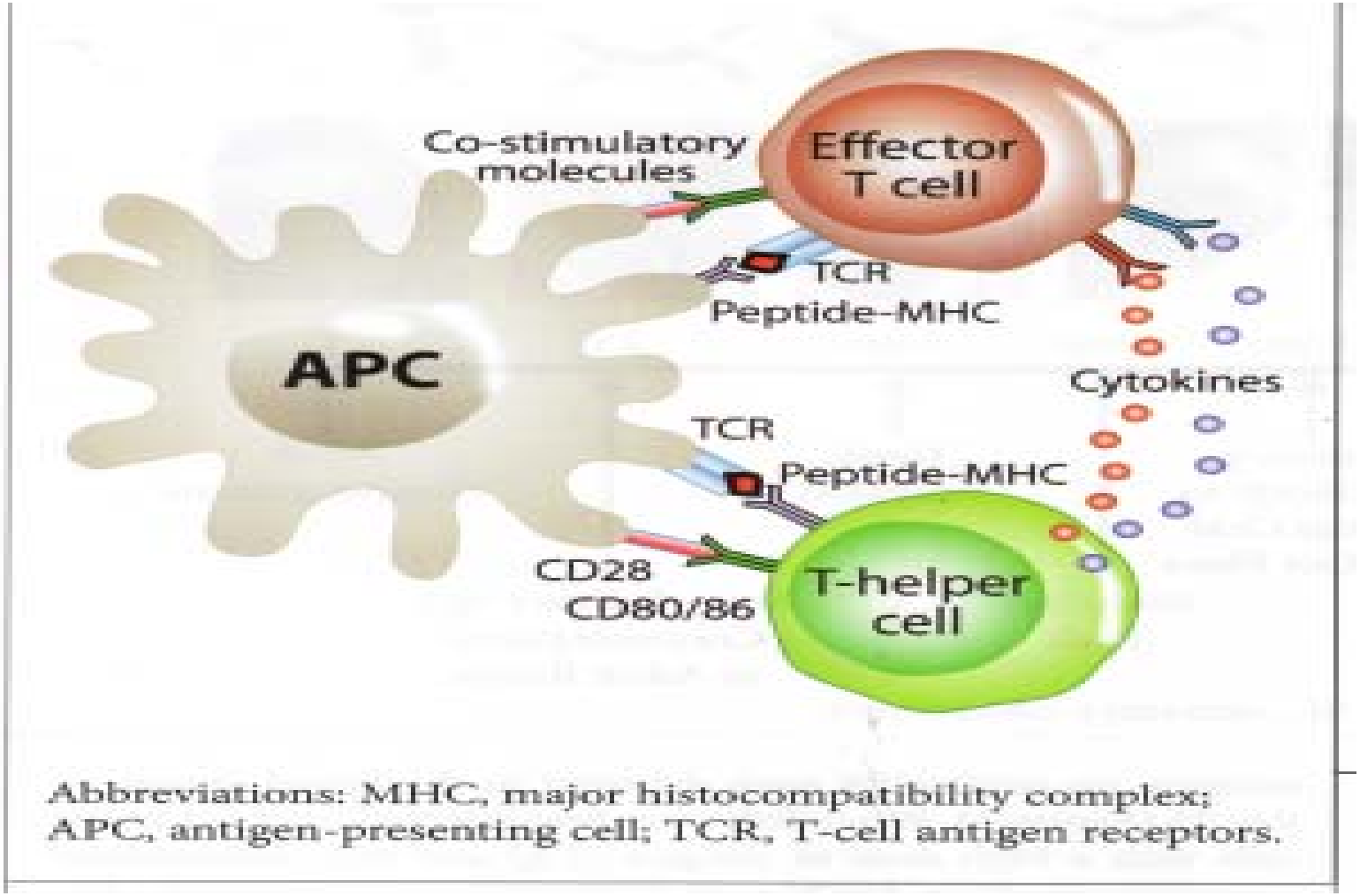


MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009



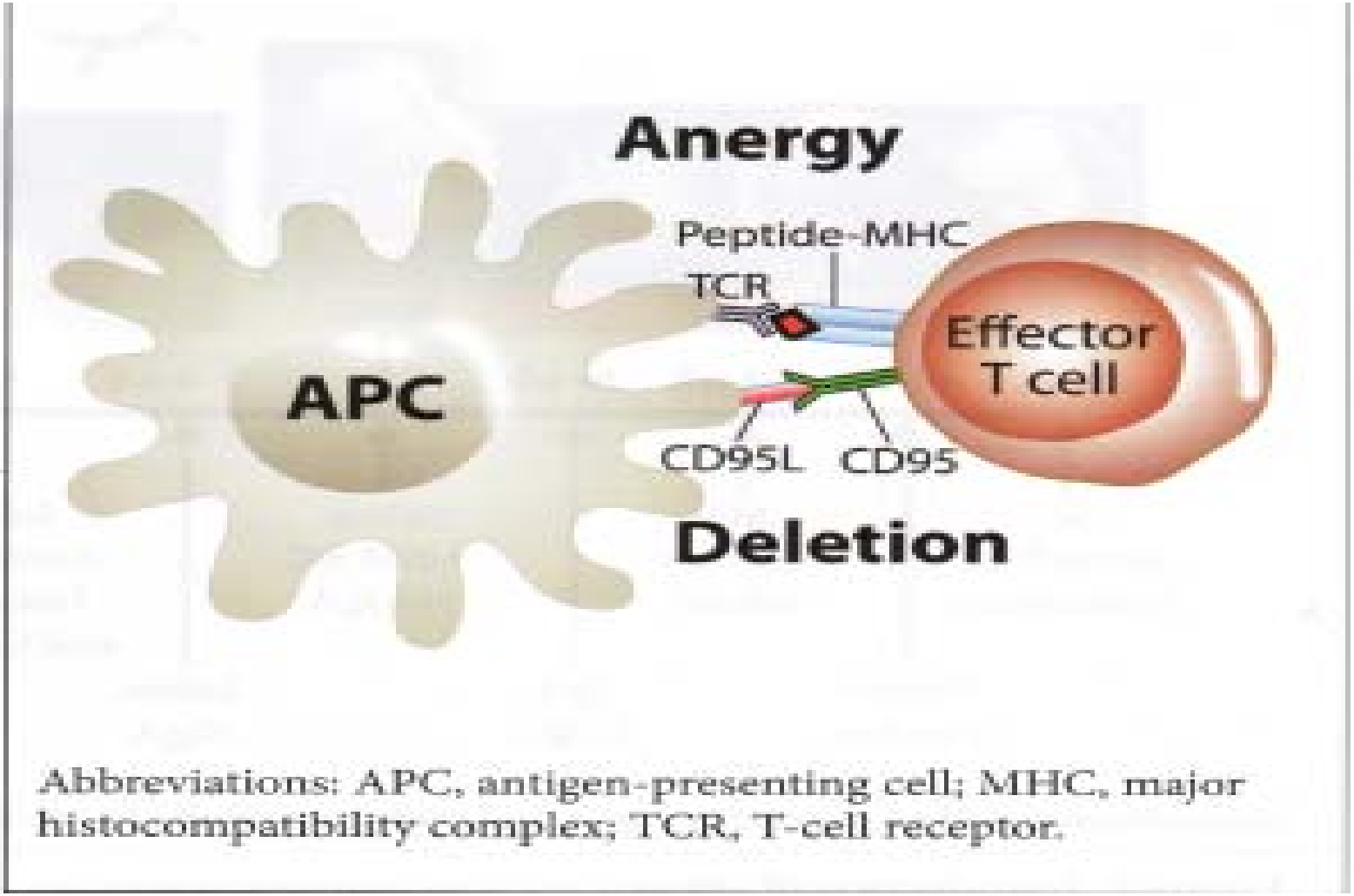
Immune Dysfunction and Cytokines

Generation of an Immune Response



Alternative Therapies, Vol 21, Suppl 1; 26

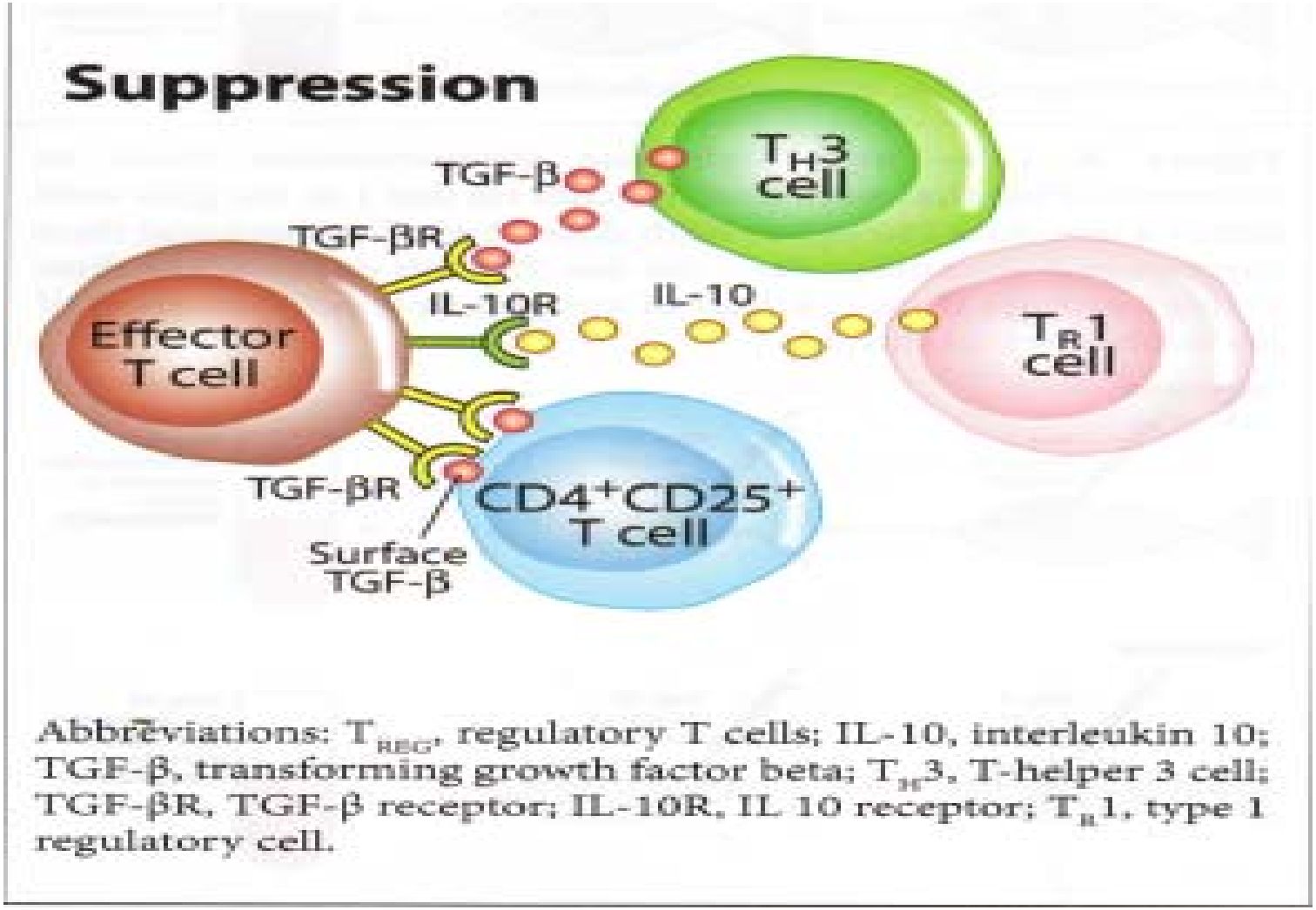
High-Dose Mechanism



Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

Alternative Therapies, Vol 21, Suppl 1; 26

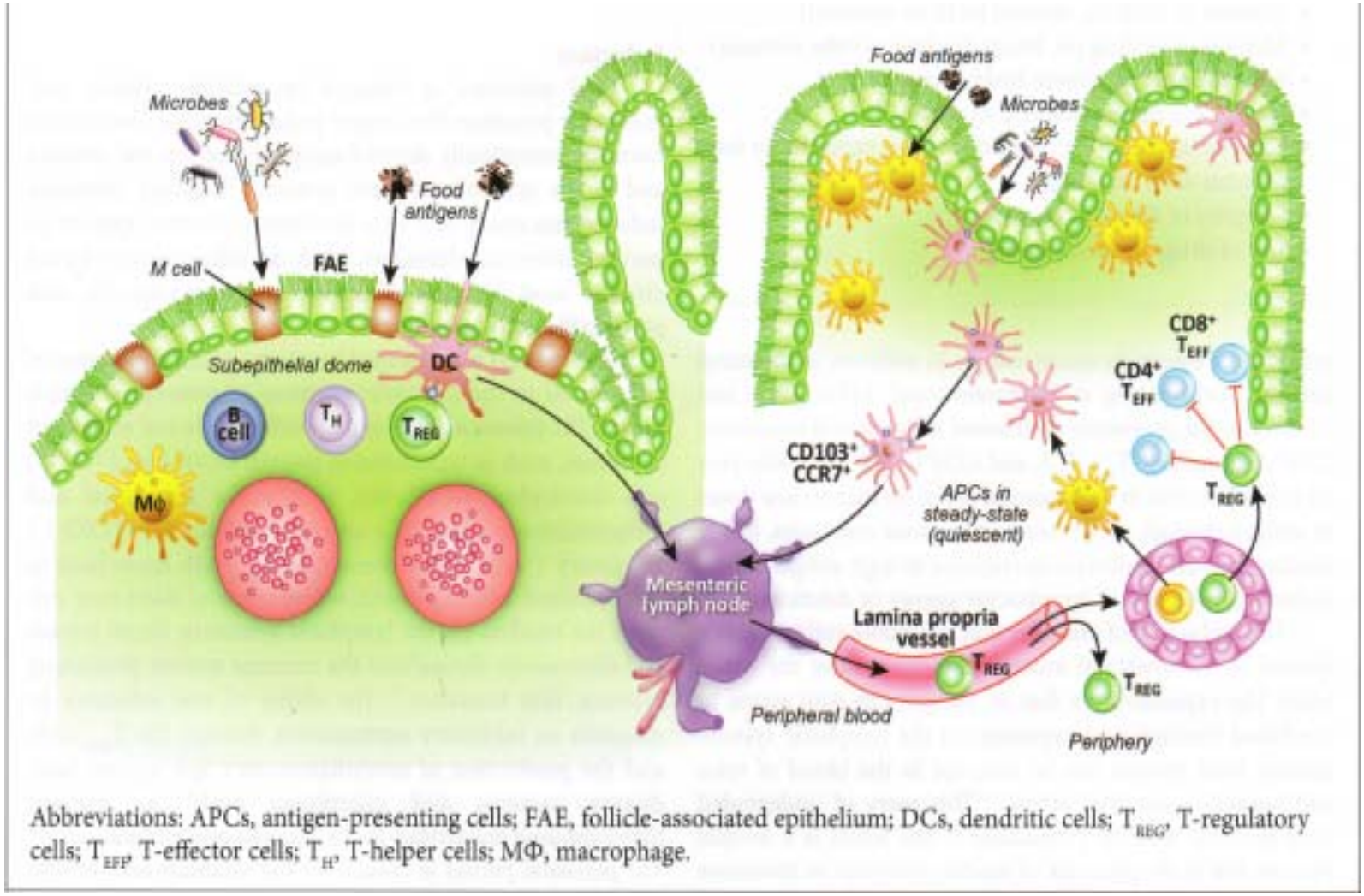
Low-Dose Mechanism



Abbreviations: T_{REG}, regulatory T cells; IL-10, interleukin 10; TGF-β, transforming growth factor beta; T_H3, T-helper 3 cell; TGF-βR, TGF-β receptor; IL-10R, IL 10 receptor; T_R1, type 1 regulatory cell.

Alternative Therapies, Vol 21, Suppl 1; 26

The Immunoregulatory Network



Abbreviations: APCs, antigen-presenting cells; FAE, follicle-associated epithelium; DCs, dendritic cells; T_{REG}, T-regulatory cells; T_{EFF}, T-effector cells; T_H, T-helper cells; MΦ, macrophage.

Alternative Therapies, Vol 21, Suppl 1; 28

Cytokines Mediators of inflammation & immunity—Definitions:

MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

- Small proteins with multiple biologic activities produced by immunologically active cells in response to external stimuli that contribute to: immune responses, shock inflammation, endothelial cell activation, atherosclerosis, CHD, MI, CHF, and endothelial dysfunction
- Arrive at endothelial cells from circulating blood, endothelium abluminal sites, leukocytes, pericytes, VSMC, macrophages fibroblasts
- Act in autocrine or paracrine manner
- Cytokines mediate **inflammation, oxidative stress** and **immune function/dysfunction.**

Cytokine Classification

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Pro-Inflammatory Cytokines

1. Interleukin 1 (IL-1)
2. Interleukin 6 (IL-6)
3. Interleukin 8 (IL-8)
4. Tumor necrosis factor (TNF-α)

Colony-Stimulating Factors

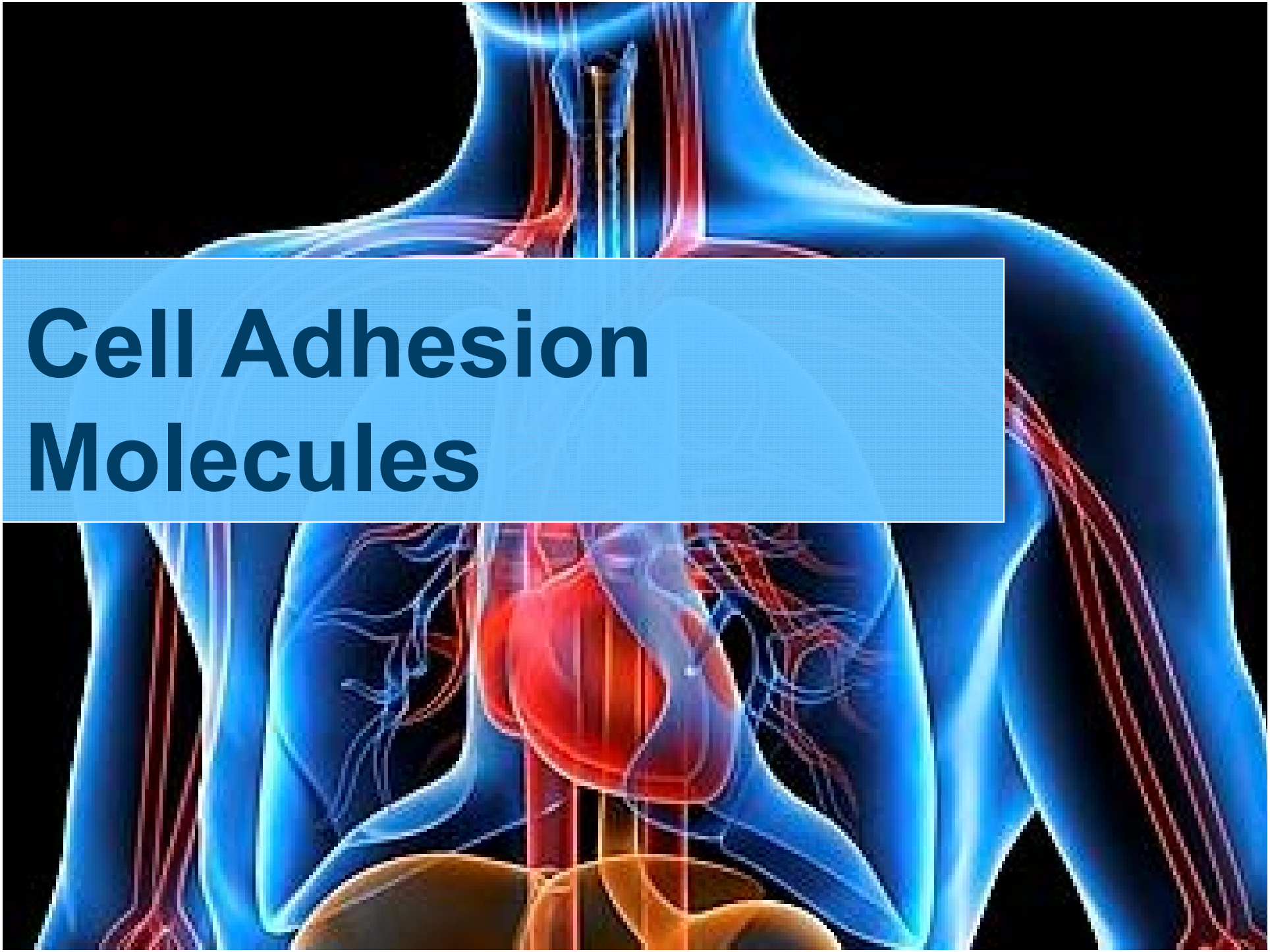
1. Granulocyte colony stimulating factor (G-CSF)
2. Monocyte-colony stimulating factor (M-CSF)
3. Granulocyte-monocyte colony stimulating factor (GM-CSF)

Cytokine Classification

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Chemotactic Factors (Chemo-attractants)

1. Monocyte chemoattractant protein – 1 (MCP-1)
2. Macrophage inhibitory protein – 1B (MIP-1B)
3. Platelet activating factor (PAF)
4. Leukotriene B4 (L-B4)
5. Complement components
6. N-formyl peptides
7. GRO - α



Cell Adhesion Molecules

Cell Adhesion Molecules

(CAMs)

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

The molecular interactions responsible for cellular adhesion either cell to cell or cell to extracellular matrix is well orchestrated and under sophisticated control. They serve a broad range of biologic processes including platelet aggregation, hemostasis, leukocyte adhesion and extravasation, immune response, inflammation and maintenance of endothelial and vascular integrity. Disease states may result from loss of adhesion interaction or stimulation of excessive adhesion. High levels correlate with vascular disease, DM, HLP, HBP, CHD, PCTA restenosis.

Cell – Cell Adhesion

Cell – P-ECM Adhesion

Homotypic
vs.
Heterotypic Interactions

Cell Adhesion Molecule

Classification

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Selectins

(Slowing and Rolling of Leukocyte on Endothelium)

1. P-Selectin (Platelet/endothelium selectin)
2. E-Selectin (Endothelial selectin)
3. L-Selectin (Leukocyte selectin)
4. CD-34 (Cluster of differentiation 34)

Immunoglobulin Superfamily

(Adhesion, Immune Response, Inflammation, Atherosclerosis)

1. ICAM-1, ICAM-2, ICAM-3, ICAM-4, ICAM-5 (Intracellular adhesion molecule)
2. VCAM (Vascular cell adhesion molecule)
3. MADCAM-1 (Mucosal-adhesion cell adhesion molecule)
4. PECAM-1 (Platelet-endothelial cell adhesion molecule)

Cell Adhesion Molecule

Classification

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Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Cadherins

(Epithelial Integrity and Correct Architecture)

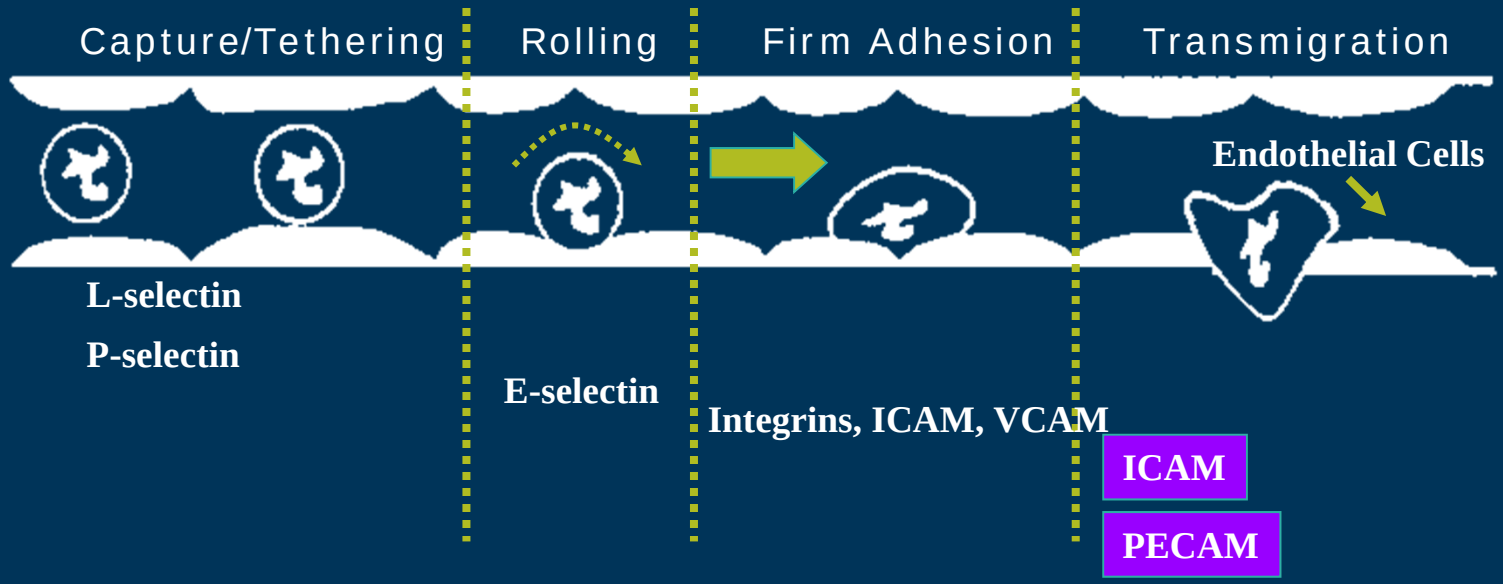
1. N, P, R, B, E Cadherins
2. Desmogleins 1 and 3
3. Desmocollins

Integrins

(External Cell Membrane to Internal Signal Proteins)

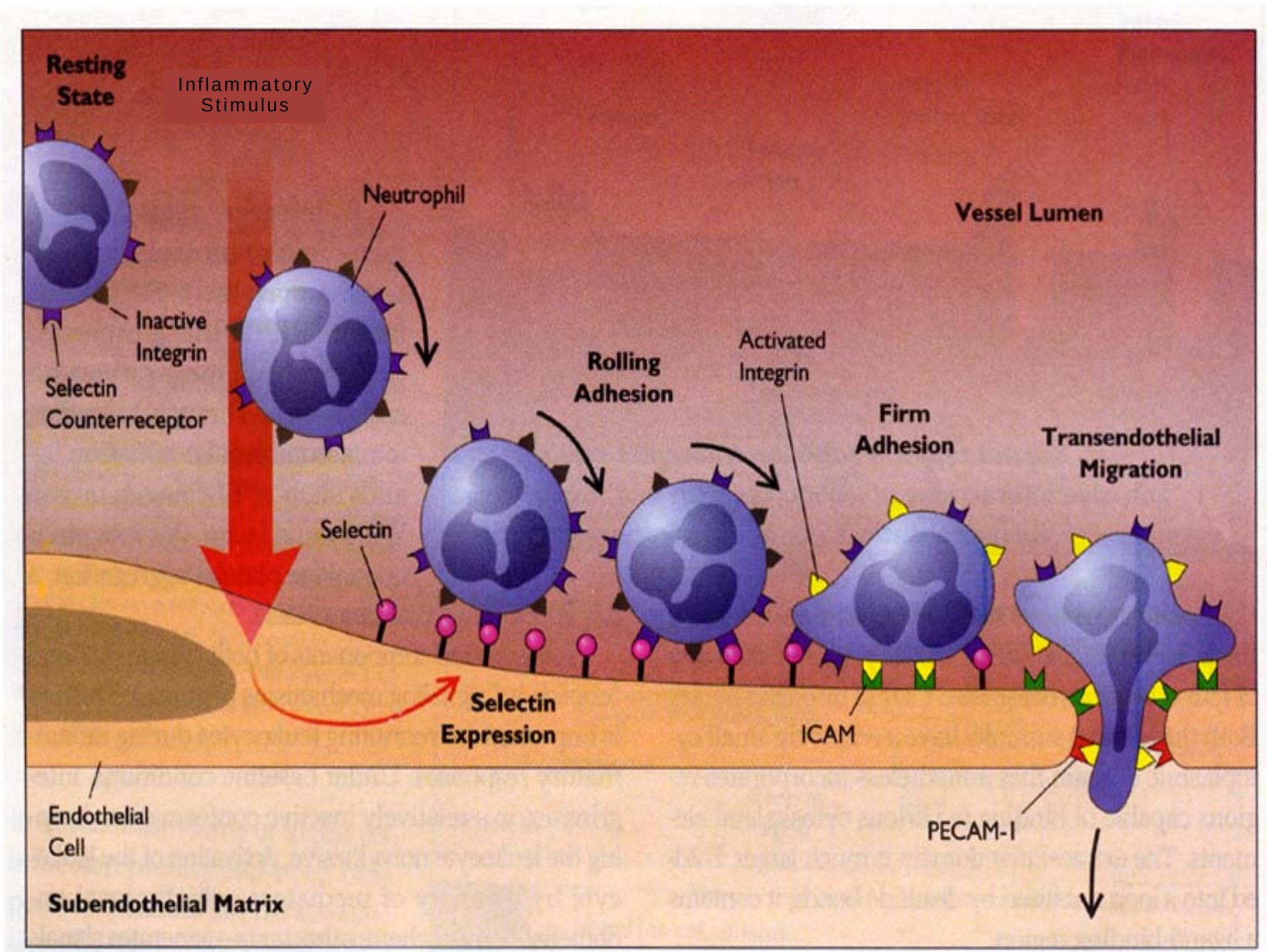
1. B-1 (Leukocyte - ECM) VLA
2. B-2 (Leukocyte - ICAM)
3. B-3 (Platelets)
4. B-4 - B-8
5. Subunits attached to all B subunits (> 20 Types)

Sequential Steps of Leukocyte Adhesion



The four step model of leukocyte adhesion and transmigration across an endothelial monolayer under dynamic flow conditions at sites of inflammation. *Leukocyte tethering and rolling* (steps 1 and 2) are mediated primarily by selectin - carbohydrate interactions, although knock-out murine models suggest that immunoglobulin family members may participate in this step. Also, $\alpha_4\beta_1$ integrins, not expressed by resting neutrophils, are also capable of initiating primary lymphocyte adhesion to endothelial cells through binding to VCAM-1. *Firm adhesion* (step 3) follows if leukocytes encounter activating signals while rolling along the endothelium. Activation-dependent attachment of β_2 integrins (Mac-1, LFA-1) on neutrophils to endothelial ICAM-1 supports this firm or secondary cell adhesion to the vessel wall. In addition, monocytes and lymphocytes may use the $\alpha_4\beta_1$/VCAM-1 pathway in this step. *Transmigration* (step 4) may occur if a favorable chemotactic gradient exists across the monolayer. Platelet/endothelial cell adhesion molecule 1 (PECAM-1) expressed at endothelial cell junctions appears to be required for transmigration by binding homophilically to PECAM-1 expressed on leukocytes. Also, antibodies against β_2/ICAM-1 pathway that block firm adhesion exert a similar effect on neutrophil transendothelial migration.

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009


Resting State
Inflammatory Stimulus
Neutrophil
Vessel Lumen
Inactive Integrin
Selectin Counterreceptor
Rolling Adhesion
Activated Integrin
Firm Adhesion
Transendothelial Migration
Selectin
Selectin Expression
ICAM
Endothelial Cell
PECAM-1
Subendothelial Matrix

Endothelial Injury and Inflammatory Responses in Atherogenesis

MC Houston. Vascular Biology in Clinical Practice. 2000

Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

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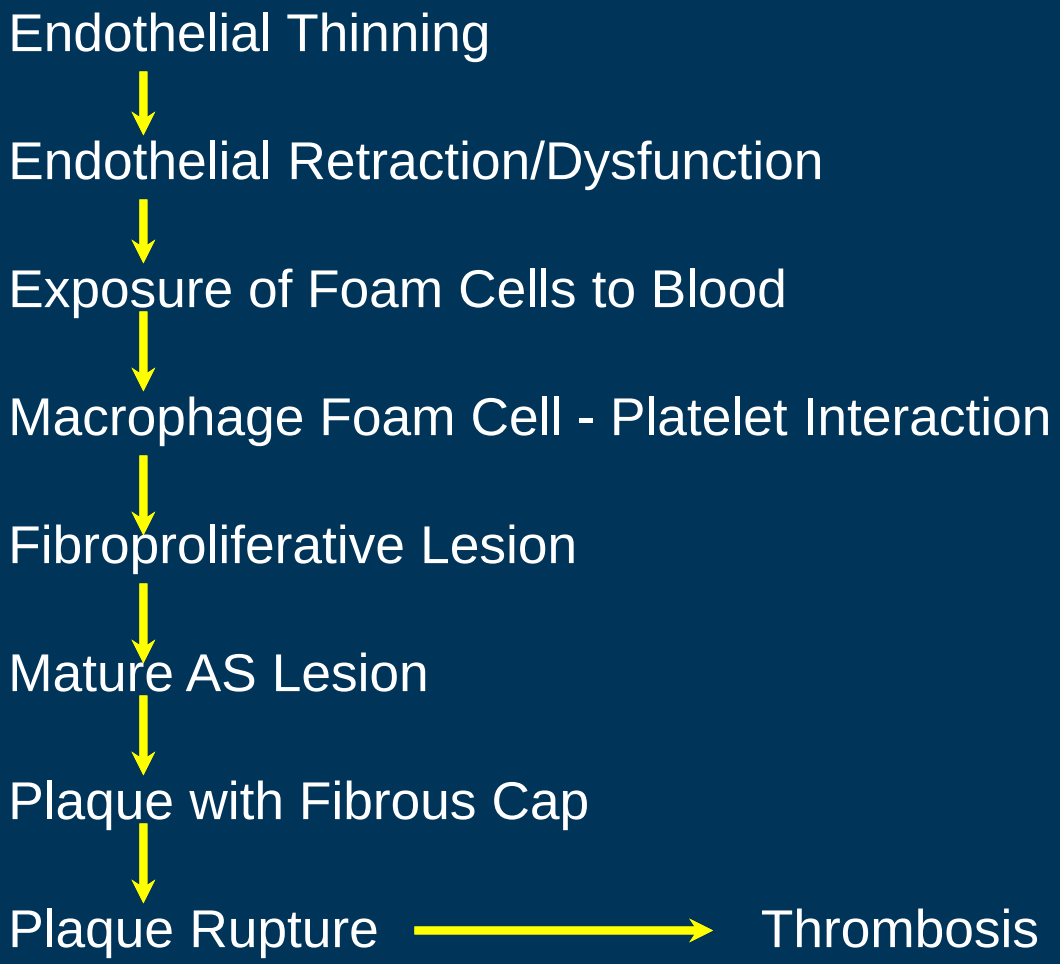


Atherosclerosis

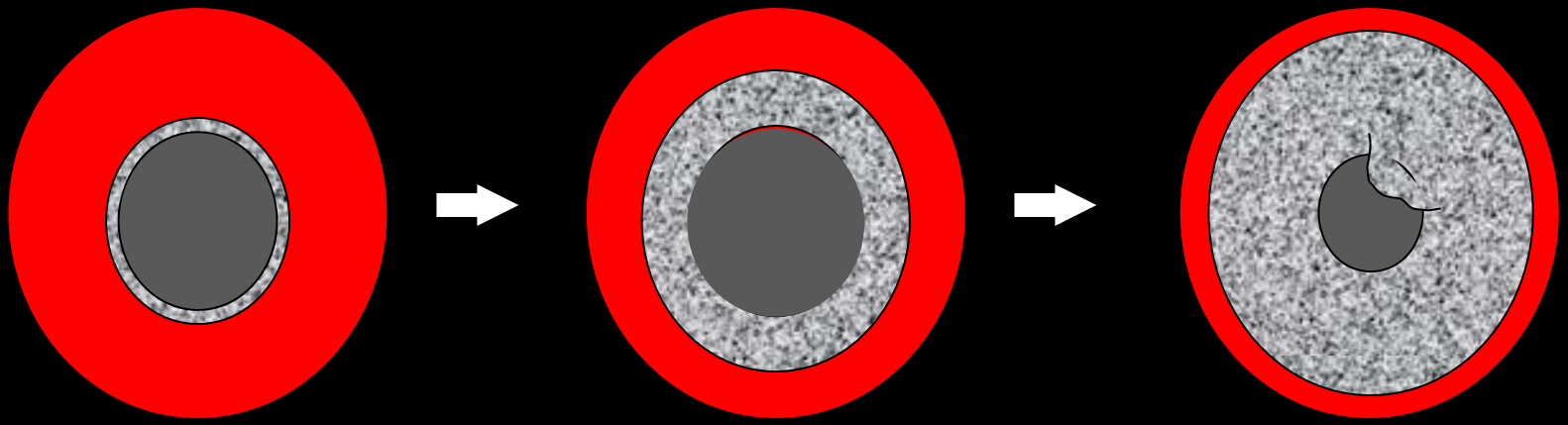
is a initially a disease of the

vessel wall

It is NOT initially a disease of the vascular lumen

CHD is an extra-luminal disease
Until the later phases

CHD: Extraluminal Disease:
Glagov Principal



Minimal to mild CHD
Lumen Normal
Mild extraluminal atheroma

Moderate CHD
Lumen normal size
Mild extraluminal atheroma

Severe CHD
Lumen Stenosis
Severe extraluminal and intraluminal atheroma

95 - 99%

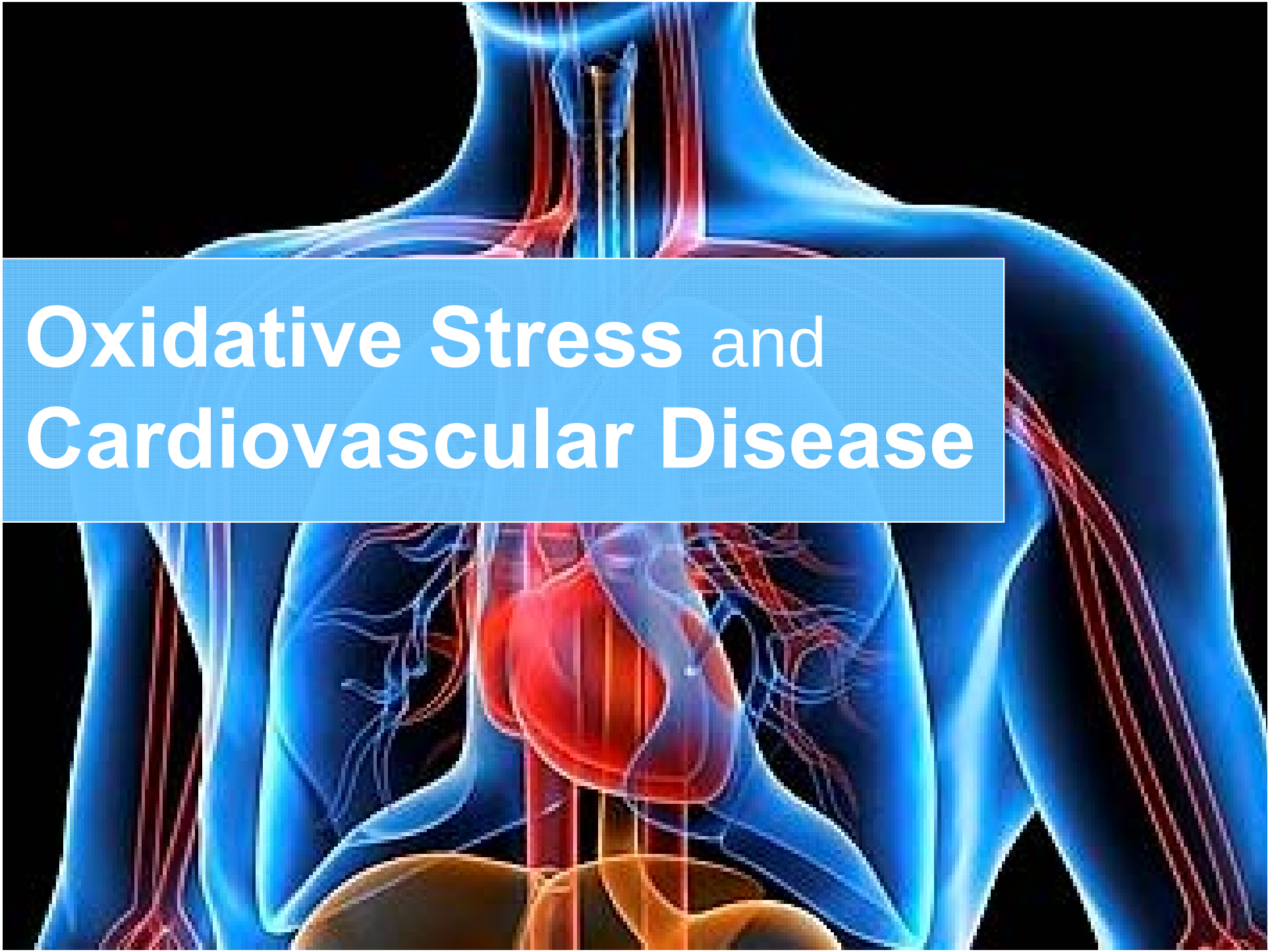
1 -5%

- 68% of MI: < 50% Stenosis
- 14% of MI: Significant Stenosis
- 62% men 1st **symptom** of CHD is MI
- 46% women 1st symptom of CHD is MI

Nissen, S.
ISH, August 2000

Clinical Pearls 6

1. Reduce infinite biochemical and biomechanical insults to reduce 3 finite responses in the arteries of inflammation, oxidative stress and immune vascular dysfunction.
2. Improve both functional and structural abnormalities of the blood vessel.
3. Improve large and small arterial compliance and elasticity (C1 and C2) (omega 3, resveratrol, increase NO, ACEI, ARB, statin).
4. Alter nutrient gene interaction and early and late gene transcription of inflammatory and immune mediators.
5. Reduce endothelial activation by reducing leukocyte recruitment, inflammatory cytokines, CAMs (NAC, GSH, thiols, sulfhydryls, omega 3, phytonutrients, NO).
6. Early detection of endothelial dysfunction (ENDOPAT) and aggressive prevention strategies to slow progression to atherosclerosis and CHD.
7. CHD is initially an extra-luminal disease.



Oxidative Stress and Cardiovascular Disease

Oxidative Stress and Cardiovascular Disease

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Cardiovascular diseases related to **increased oxidative stress and decreased oxidative defense**

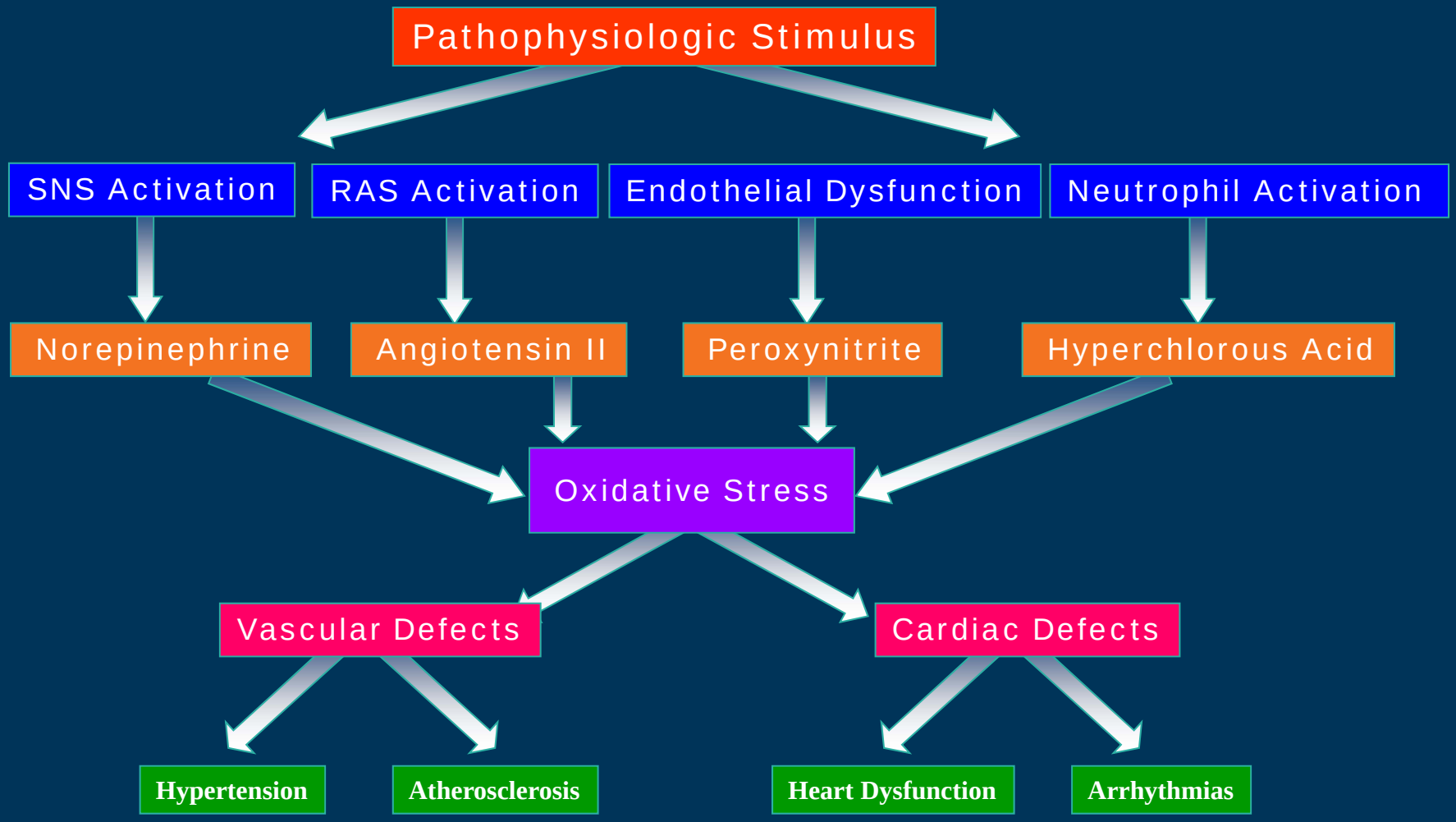
Atherosclerosis, hypertension, NIDDM, acute ischemic syndromes/CHD, ischemic reperfusion injury, hyperlipidemia and CHF.

Oxidative Stress and CVD Causes/Mechanisms

MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

- Increased oxidative stress (ROS)
  1. Increased generation (ROS): ED, AA, catecholamines, etc.
  2. Decreased antioxidant reserve
     A. Intracellular (SOD, CAT, GTP)
     B. Extracellular (albumin, TIBC, vitamin C, ceruloplasmin)
     C. Enzymatic and nonenzymatic: vitamin E, C, A, beta carotene, sulfhydryl compounds, Co Q 10, thioesters, uric acid and flavonoids.

- Primary and ultimate mechanism of ROS damage is intracellular calcium overload via damage of subcellular organelles



Role of different extra-cardiac and extra-vascular systems in the genesis of oxidative stress and development of cardiovascular abnormalities. SNS, sympathetic nervous system; RAS, renin-angiotensin system.

Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

The Cytotoxic Reactive Oxygen Species and the Natural Defense Mechanisms

MC Houston. Vascular Biology in Clinical Practice. 2000.

Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Reactive Oxygen Species

Free Radicals

$O_2 \bullet^-$	Superoxide anion radical
OH •	Hydroxyl radical
ROO •	Lipid peroxide (peroxyl)
RO •	Alkoxyl
RS •	Thiyl
NO •	Nitric oxide
NO_2 •	Nitrogen dioxide
$2H_2O$	
$ONOO^-$	Peroxynitrite
CCl_3 •	Trichloromethyl

Non-radicals

H_2O_2	Hydrogen peroxide
HOCl	Hypochlorous acid
$ONOO^-$	Peroxynitrite
$^1O_2$	Singlet oxygen

Antioxidant Defense Mechanisms

Enzymatic Scavengers

SOD	Superoxide dismutase
	$2O_2 \bullet^- + 2H^+ \rightarrow H_2O_2 + O_2$
CAT	Catalase (peroxisomal-bound)
	$2H_2O_2 \rightarrow O_2 + H_2O$
GTP	Glutathione peroxidase
	$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$
	$2GSH + ROOH \rightarrow GSSG + ROH +$

Nonenzymatic scavengers

Vitamin A
Vitamin C (ascorbic acid)
Vitamin E (α-tocopherol)
β-carotene
Cysteine
Coenzyme Q
Uric Acid
Flavonoids
Sulfhydryl group
Thioether compounds

The superscripted bold dot indicates an unpaired electron and the negative charge indicates a gained electron. GSH, reduced glutathione; GSSG, oxidized glutathione; R, lipid chain. Singlet oxygen is an unstable molecule due to the two electrons present in its outer orbit spinning in opposite directions.

Oxidative Stress & CVD

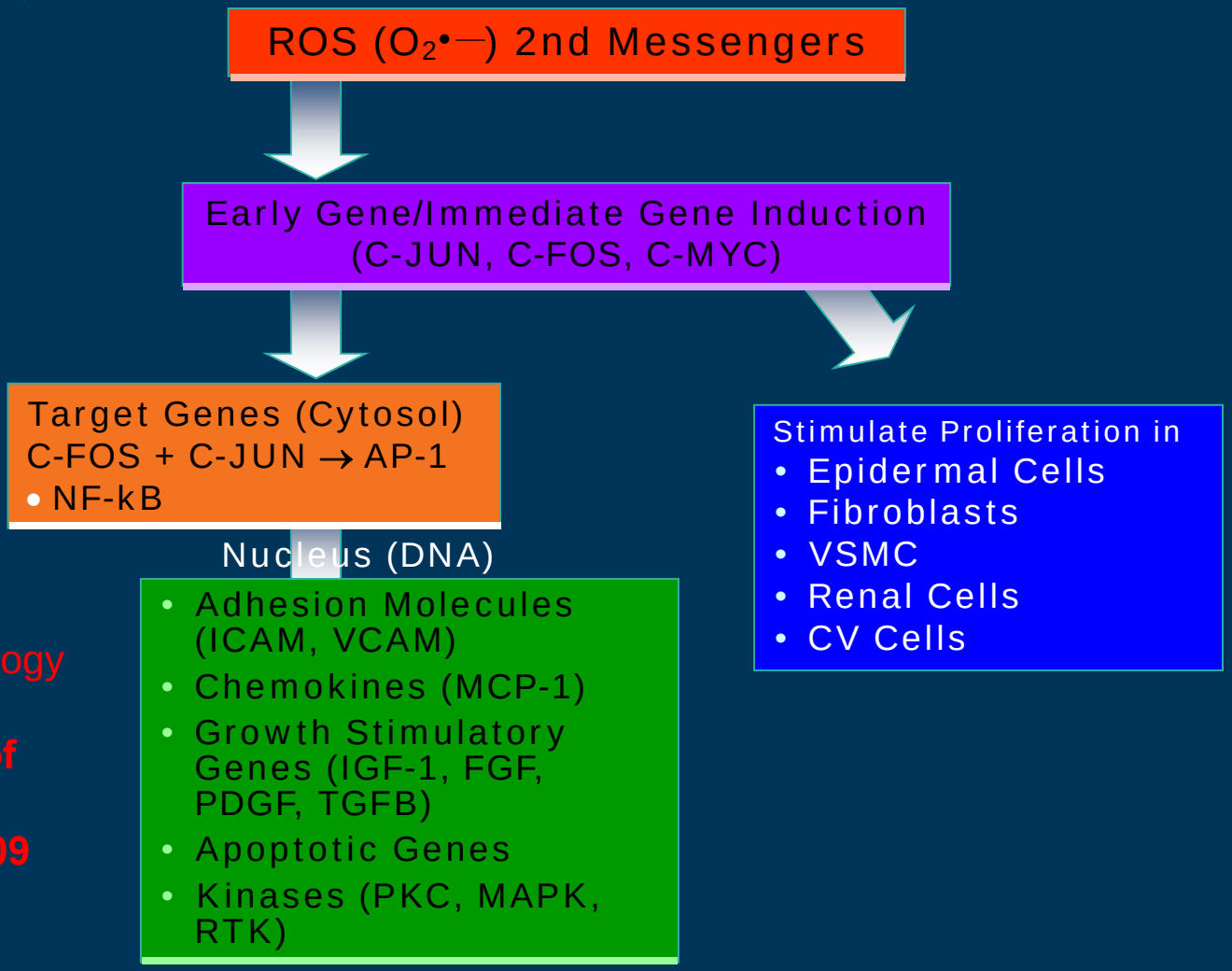
MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

Cardiovascular diseases related to **increased oxidative stress**: atherosclerosis, hypertension, NIDDM, acute ischemic syndromes/CHD, ischemic reperfusion injury, hyperlipidemia, CHF.

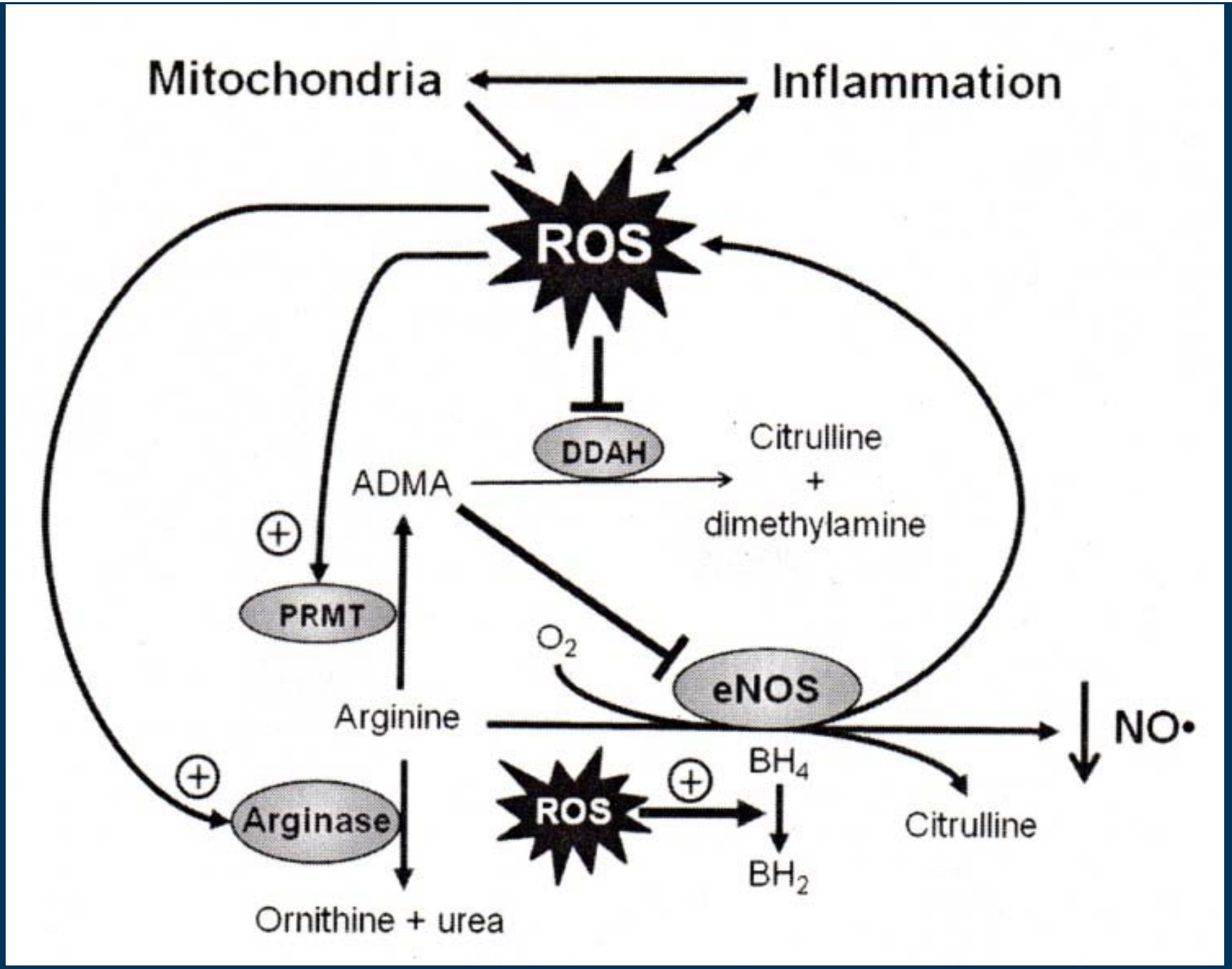
ROS EFFECTS

- Lipid Peroxidation: (PUFA in membrane lipid bi-layer)
- Protein oxidation: induces lipid and CHO auto-oxidation proteolysis
- Carbohydrate oxidation
- DNA oxidation and damage
- Organic molecule oxidation
- Genetic machinery and gene expression
- Transcription factors and DNA synthesis

ROS are Intracellular Signal Transduction Systems and Modulators of Transcriptional Pathways



MC Houston. Vascular Biology in Clinical Practice. 2000.
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

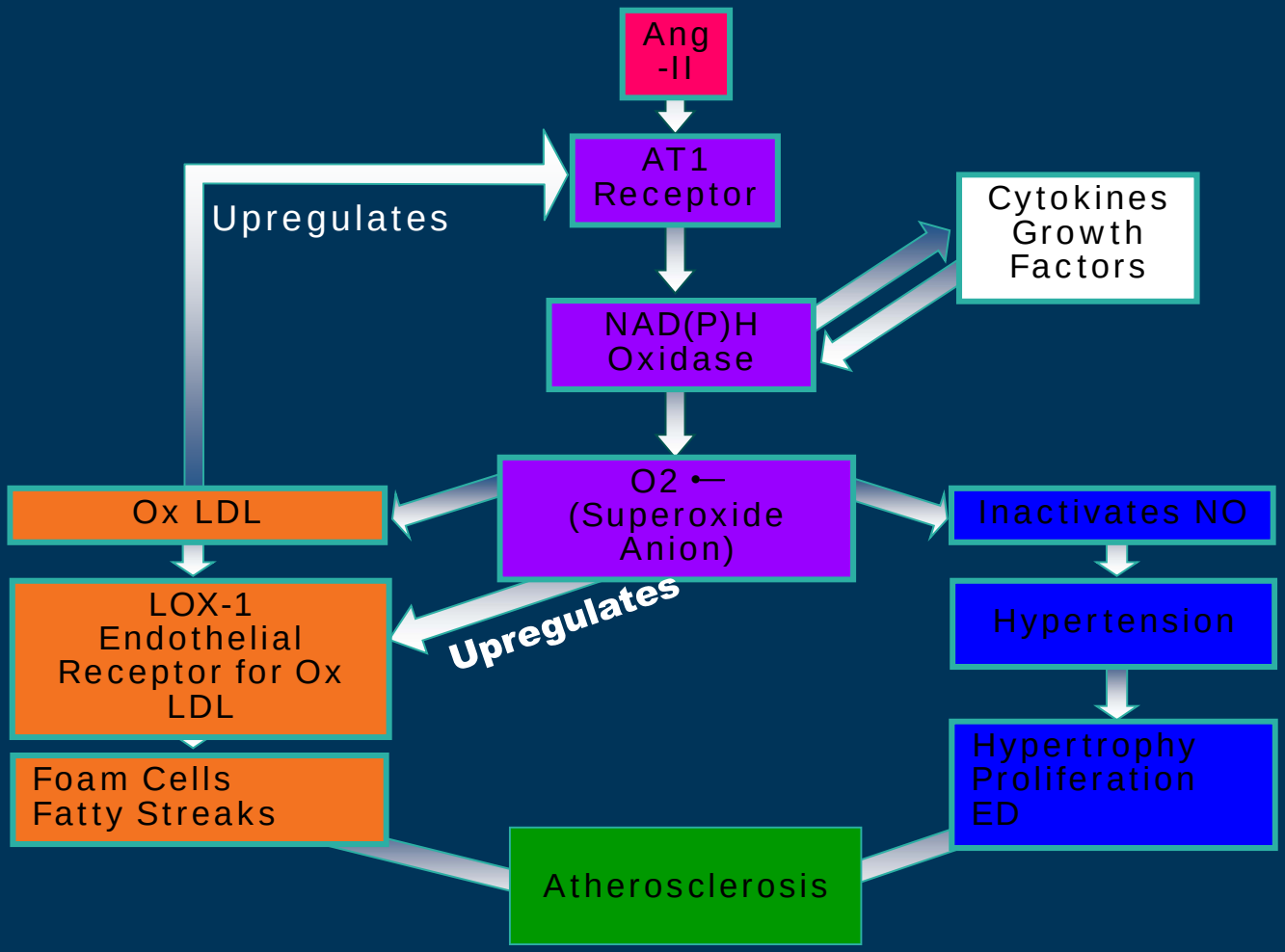


Nutrition Research 32 (2012) 727-740

Receptor Regulation: Role of ROS on Hypertension and Hyperlipidemia and Atherosclerosis

MC Houston Vascular Biology in Clinical Practice. 2000

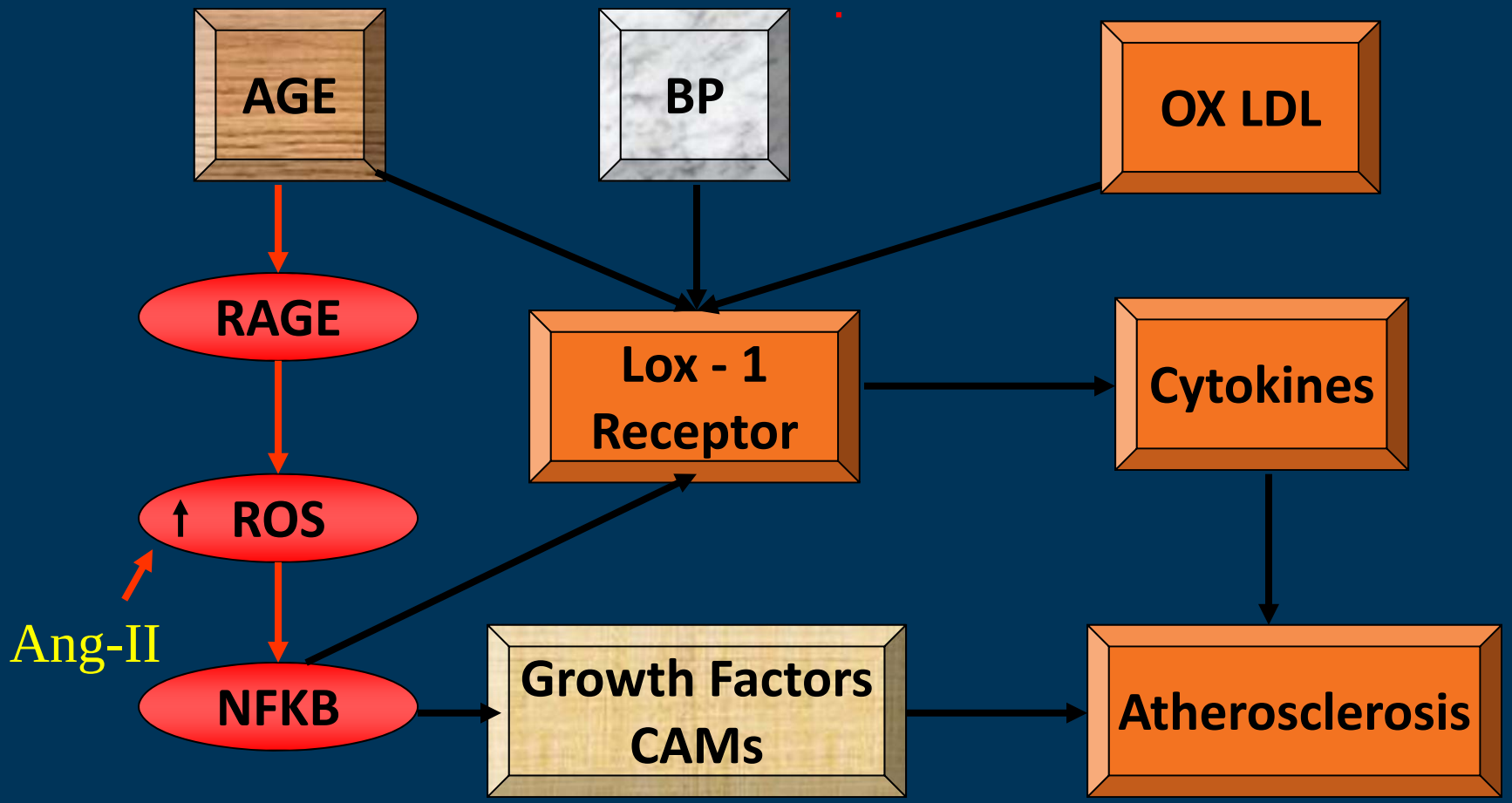
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009

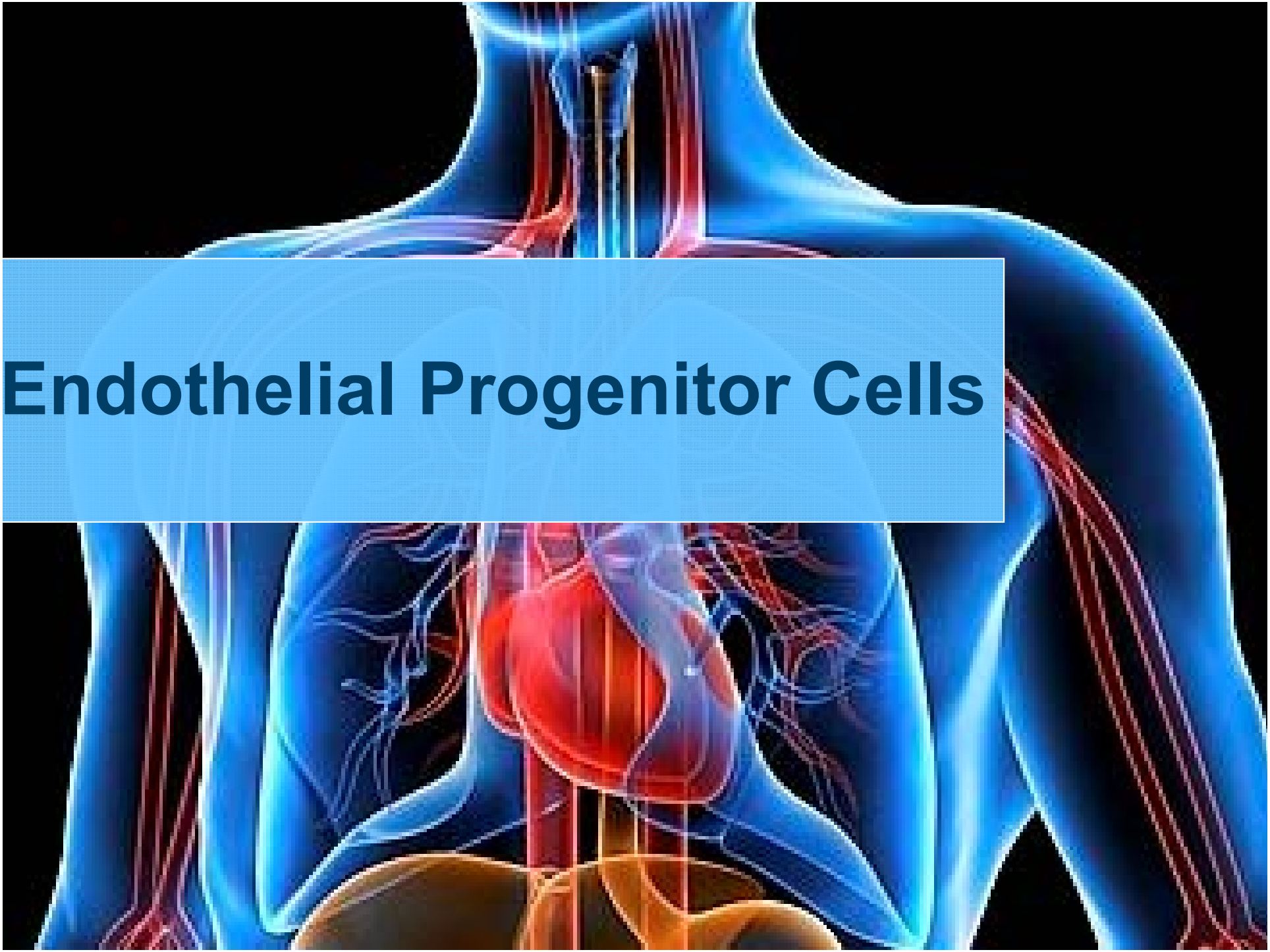


Receptor CrossTalk: Lox1 gene and receptor: common link BP, Lipids and DM

Vascular Biology in Clinical Practice. 2000

Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009





Endothelial Progenitor Cells

Endothelial Progenitor Cells (EPC)

NEJM 2003: 348:593
Therapeutic Advances in Cardiovascular Disease 2010;4:55-69
JCI 2001;108:399
Am J Clin Nutr 2010;92:161

- Most predictive of all known cardiovascular risk factors
- Correlates with hyperlipidemia, hypertension, diabetes, Framingham risk score, FMD-BA(flow mediated vasodilator of brachial artery), CVD, CHD, age, glucose and smoking.
- High risk patient has a lower number and early senescence of EPC's, higher risk CHD, CVA, and CVD
- EPC's are a marker of neovascularization and vascular repair
- Bone marrow, monocytes and cord blood
- Steps of EPC production are:
  - Mobilization or release,
  - Migration or homing and
  - Incorporation and promotion of angiogenesis/vasculogenesis
- EPC have higher content of MnSOD and GPx-1

Endothelial Progenitor Cells (EPC) Increased Production

NEJM 2003;348:593 JCI 2001;108:399
Am J Clin Nutr 2010;92:161
Am J Cardiol 2010;106:1606

- eNOS, nitric oxide
- Omega 3 fatty acids
- ACEI
- ARB
- Statins
- Resveratrol
- Red Wine
- Exercise
- Estrogen E2
- GH
- IGF-1
- Prostacyclin

Endothelial Progenitor Cells (EPC) Decreased Production

NEJM 2003;348:593,
JCI 2001;108:399
Am J Clin Nutr 2010;92:161

- Age
- Angiotensin-II
- Oxidative stress
- Inflammation
- Immune dysfunction
- Obesity
- Lack of exercise
- Menopause and andropause

Clinical Pearls 7

1. Balance SNS, PNS and RAAS
2. Balance oxidative stress and defense
3. Reduce adverse ROS transcription reactions
4. Reduce receptor interaction and upregulation of LOX, AT1R and RAGE
5. Increase EPCs (nitric oxide, omega 3 FA, exercise, weight loss, ACEI, ARB, statins, resveratrol, red wine, estradiol, lower oxidative stress and inflammation)

Arterial Compliance Structure/Function - Treatment

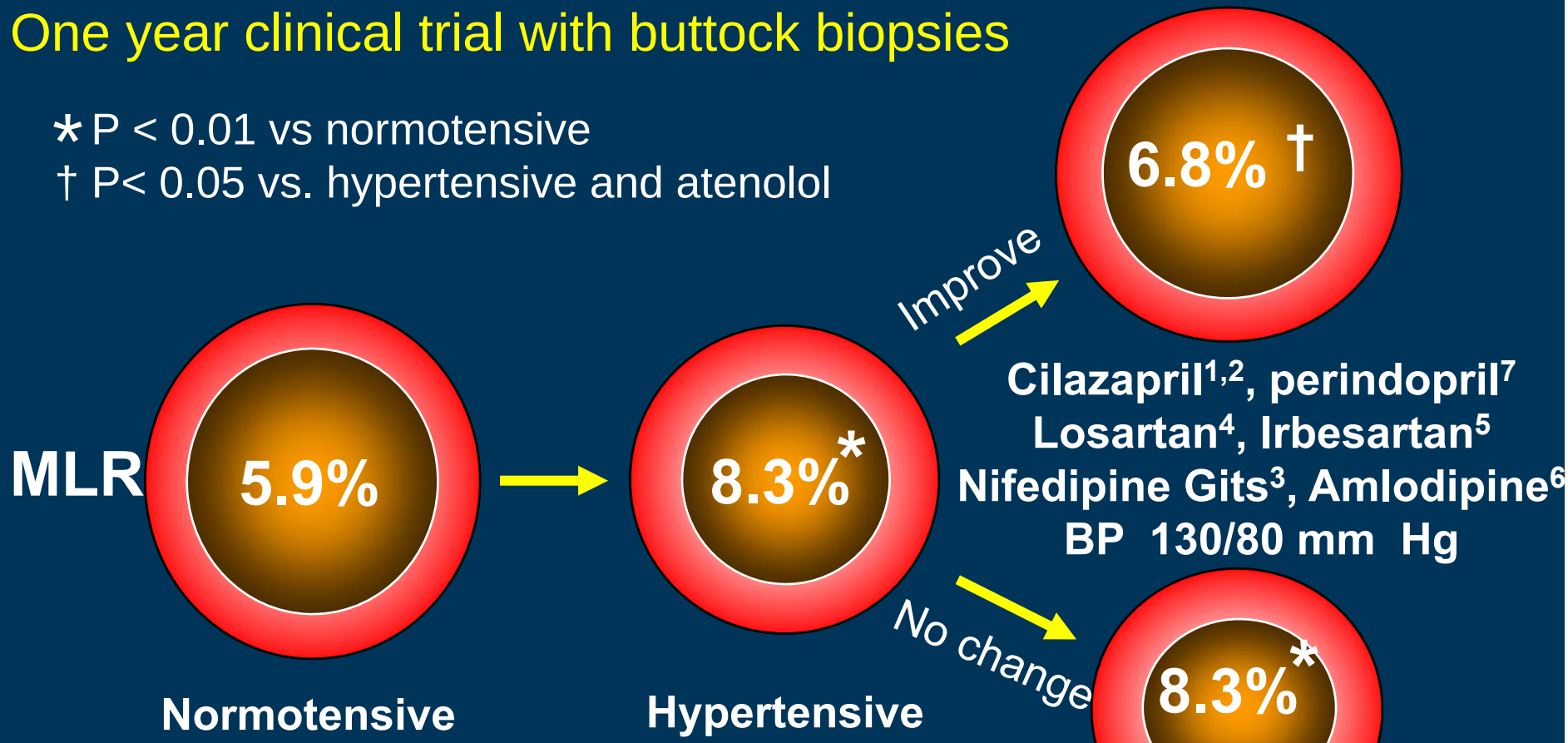
MC Houston. Vascular Biology in Clinical Practice. 2000
Houston MC Handbook of Hypertension. Wiley Blackwell, Oxford UK 2009
1) Schiffrin et al. Hypertension 1994, 2) Am J Hypertens 1995,
3) J Hypertens 1996, 4) Circulation 2000, 5) J. Hypertension 2002
6) Am. J. Hypertens 2002 7) J Hypertension 1996;14:1237
8) Thybo et al. Hypertension 1995 9) J Hypertens 2009;27:1107
10) Am J Hypertens 2006;19:477 . 11) Am J Hypertens 2010;23:1136

- Human trials with gluteal artery biopsies to assess vascular wall structure and function
- Goal is to correct both structure and function
- Treatment for 12 months to same blood pressure
- Increase small artery diameter, increase arterial compliance (AC), improve endothelial function (ED), decrease media/lumen ratio (MLR), decrease SVR(systemic vascular resistance) and blood pressure with remodeling of arterioles and blood pressure reduction:
  - ACEI
  - CCB
  - ARB
  - DRI
- Diuretics(HCTZ, chlorthalidone) and Beta blockers of first and second generation (not carvediol or nebivolol) did not change on ED, AC, MLR, arterial diameter

Effect of antihypertensive drugs on small artery remodeling

Effect on media-to-lumen ratio (MLR)
One year clinical trial with buttock biopsies

* P < 0.01 vs normotensive
† P< 0.05 vs. hypertensive and atenolol



1) Schiffrin et al. Hypertension 1994, 2) Am J Hypertens 1995,
3) J Hypertens 1996, 4) Circulation 2000, 5) J. Hypertension 2002
6) Am. J. Hypertens 2002 7) J Hypertension 1996;14:1237
8) Thybo et al. Hypertension 1995 9) J Hypertens 2009;27:1107
10) Am J Hypertens 2006;19:477 . 11) Am J Hypertens 2010;23:1136

Prevention and Treatment of ED and CVD 1

1. **Diet and dietary nitrate : 0.1 mmol/kg of body weight /day. 10 servings of fruits (4)(berries) and vegetables (6) with dark green leafy vegetables. DASH 2 and Mediterranean diets. Caloric restriction (12.5/12.5 EE with overnight fast. 30 % protein, 30 % MUFA and omega 3 FA with limited SFA and no trans fat, minimal refined CHO(50 grams), more complex CHO (40%). Consume smaller meals more frequently with antioxidants/meal Minimal caffeine depending on CYP 1A2 status.**
2. **Vitamin C sustained release : 250-500 mg bid.**
3. **Vitamin K 2 MK 7 200 mcg per day**
4. **Polyphenols: 20 grams dark chocolate (>70%), EGCG 500 mg bid or green tea 32 oz/day (decaffeinated), 6 ounces red wine.**
5. **Quercetin 500-1000 mg/day.**
6. **Curcumin 500 mg-1000 mg bid.**
7. **2 gram sodium, 10 gram potassium, 1000 mg magnesium /day**
8. **500 mg beetroot juice: 45 mmol/L or 2.79 g/L inorganic nitrate/day.**
9. **Pomegranate seeds: one cup per day or juice 6 ounces/day.**

Prevention and Treatment of ED and CVD 2

9. **BH4** 2mg/kg/day with 5 methyl folate 1000 -5000ug per day with B complex vitamins.
10. **R-lipoic acid (RLA)** 100 mg per day with biotin 5000 ug/day for GSH (glutathione) and acetyl –L-carnitine 1000 mg/d (mitochondrial function with RLA)
11. **NAC(n-acetyl cysteine)** 500 mg bid for GSH (glutathione) etc.
12. **Whey protein** 30-40 grams per day for GSH (glutathione)
13. **Niacinamide** 500-1000 mg bid for GSH (glutathione) etc.
14. **MSM** 500 mg bid
15. **Branched chain amino acids** (leucine , valine, isoleucine 4:1:1 ratio) 5000 mg/d
16. **D-Ribose** 5 grams tid and nicotinomide riboside 125 mg/d for NADH and ATP
17. **Trans-resveratrol** 250 mg per day with grape seed extract 500 mg bid
18. **Balanced omega 3 FA** (DHA, EPA, GLA with gamma delta tocopherols: 2- 5 grams per day
19. **Exercise** 60 min /day 6 days per wk. (aerobic/ resistance)(ABCT)
20. **8 hours of sleep.** Circadian rhythm. Early to bed and early to rise.
21. **Stress Reduction and IBW and composition (M 16%, F 22%)**

Prevention and Treatment of ED and CVD 3

1. **Plant sterols** 2.5 grams per day and sterolins.
2. **Reishi and Shiitake mushrooms**: one serving per day.
3. **Vitamin D3** to level of 60 ng/ml.
4. **AGED garlic (Kyolic)** CV formulation: 600 mg bid.
5. **Co enzyme Q 10** :100 mg per day to level of 3 ug/dl and **PPQ** 20 mg/d
6. **Lycopene**: 20 mg/d(supplement, tomato, pink grapefruit, watermelon)
7. **Carnosine** 500 mg bid
8. **Berberine** 500-1000 mg per day
9. **High quality varied multivitamin and fruit and vegetable extracts**
10. **Probiotics:** 50 billion CFU per day.

Pharmacologic treatments

ARB (telmisartan 80mg qd) or ACEI (perindopril 16 mg qd).

Amlodipine 5mg qd

Rosuvastatin 5mg qd or intermittent therapy

Metformin 500 mg qd

Colchicine 0.5 mg qd

Bezafibrate 200 mg per day

ASA and BIHRT ?

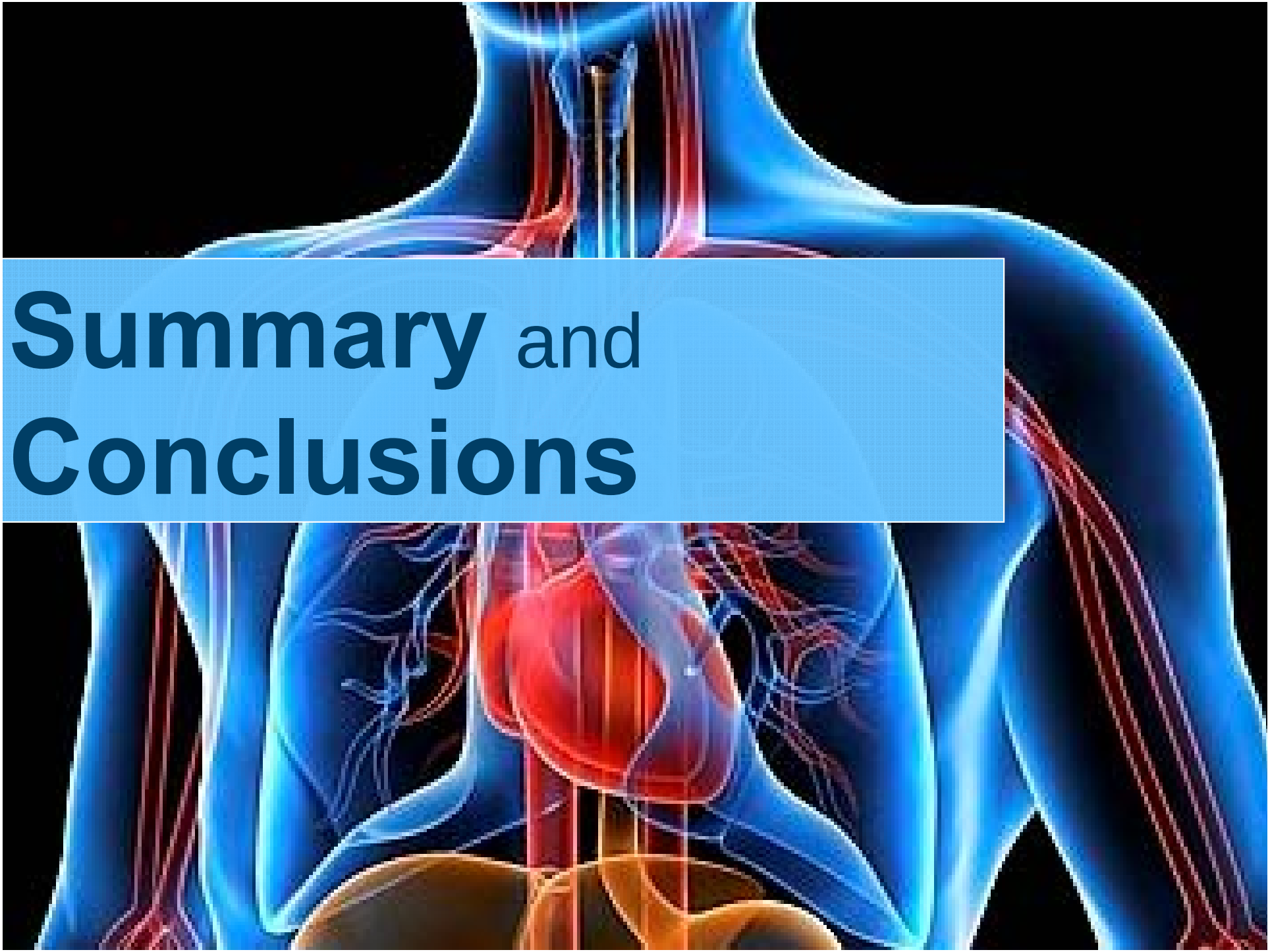
Reduce inflammation, oxidative stress and immune dysfunction and control all CV risk factors and insults.

Therapeutic Implications Phytochemicals and PRR, TLR (Toll Like Receptors and NLRs (NOD like Receptors)

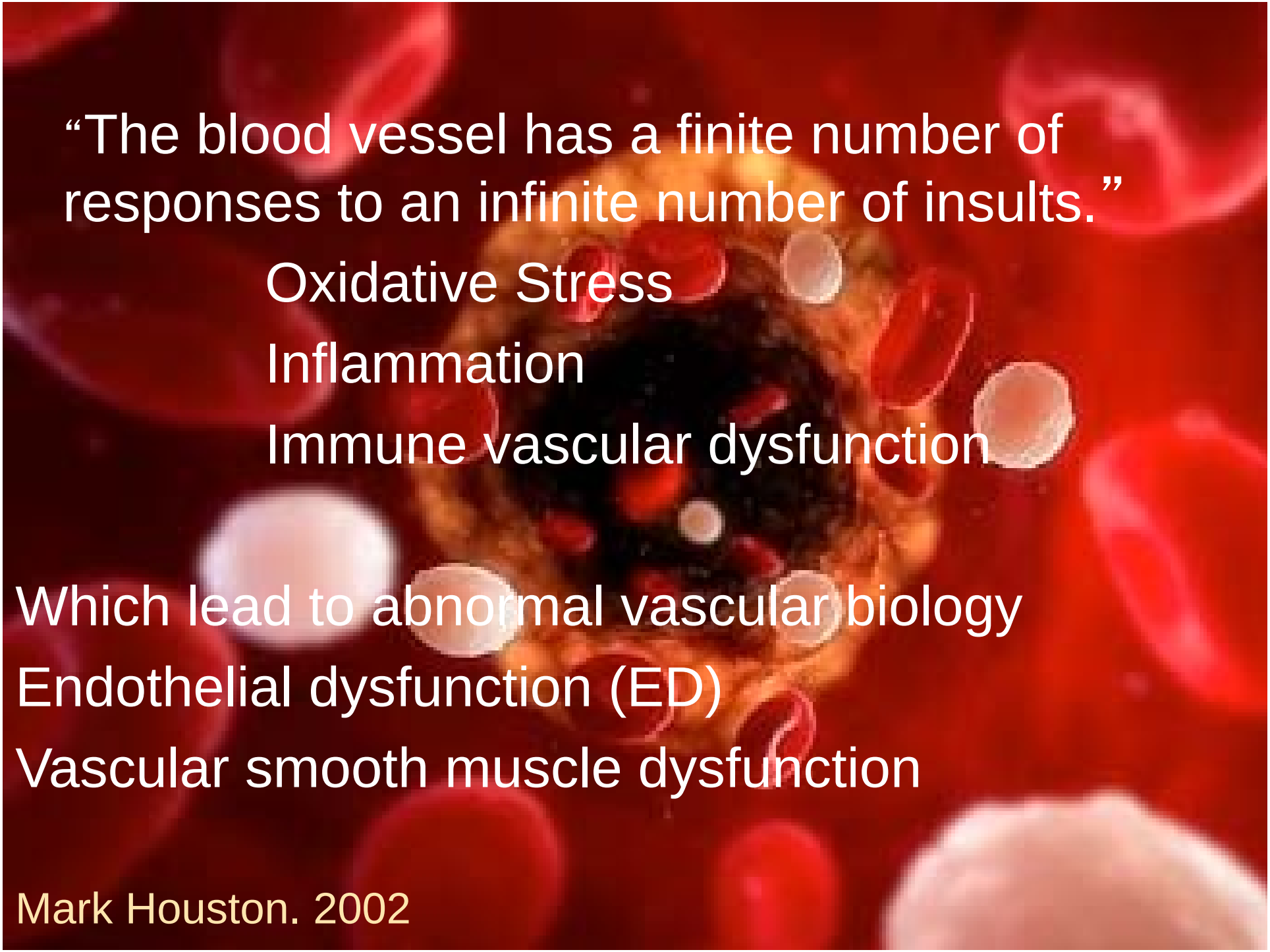
J of Nutritional Biochemistry 2012;23:39-50

Inhibit PRR vascular receptors

- **Curcurmin** (Tumeric): TLR 4, NOD 1 and NOD 2
- **Cinnamaldehyde**: Cinnamin : TLR 4
- **Sulforaphane**: Broccoli : TLR 4
- **Resveratrol**: nutritional supplement, red wine, grapes and grape seed extract: TLR 1
- **EGCG (green tea)** :TLR 1
- **Luteolin** :celery, green pepper, rosemary, carrots, oregano, oranges, olives : TLR 1
- **Quercetin**: Tea, apples, onion, tomatoes, capers :TLR 1.
- **Chrysin**: TLR 1



Summary and Conclusions


“The blood vessel has a finite number of responses to an infinite number of insults.”
Oxidative Stress
Inflammation
Immune vascular dysfunction
Which lead to abnormal vascular biology
Endothelial dysfunction (ED)
Vascular smooth muscle dysfunction
Mark Houston. 2002

Blood Vessel

The **blood vessel** should be the initial and primary target of both non pharmacologic and pharmacologic therapy to prevent and treat **atherosclerosis and CVD**.

Therapy should be aimed at the **endothelium** and the **arterial wall.**

Atherosclerosis, Vascular Disease and **Meals**

- Atherosclerosis and vascular disease are post-prandial phenomena.
- Inflammatory foods, coupled with hyperglycemia and hypertriglyceridemia, induce oxidative stress, autoimmune vascular dysfunction, metabolic endotoxemia and inflammation.

Endothelial Dysfunction

- ED is the initial and earliest event in vascular disease that eventually leads to functional and structural changes in the blood vessel.
- ED can occur in the absence atherosclerosis when only risk factors are present.
- ED precedes atherosclerosis and CV events by decades.
- ED occurs with loss of the homeostasis underlying vascular tone, growth thrombotic potential, inflammation, oxidation, immune dysfunction and vessel wall permeability.

Endothelial Dysfunction (ED) Arterial Compliance (AC)

Abnormalities or dysfunctions of the endothelium (ED) and the arterial wall (AC) are associated with increased future cardiovascular events. Correction and treatment of ED and abnormal AC **reduces** cardiovascular events.

Endothelium Functions

- Vasomotor tone
- Growth
- Thrombosis
- Inflammation
- Redox Modulation and oxidative stress/defense
- Permeability
- Immune function

Nutrition and Supplements to improve endothelial function, nitric oxide and vascular elasticity

Curr Opin Lipidol 2012;23:147-156 Nutrition 2013;29:71-75
Nutrition 2015;31:28; Am J Clin Nut 2016;103:25

- DASH diet
- Mediterranean diet with EVOO
- Nut consumption: 20 % reduction death with 7 servings per wk
- Vitamin D 4000IU per day
- Vitamin C 500 mg per day
- Beet Root extract with arginine, citrullene and hawthorne in the form of NEO 40 from NeoGenis labs
- Dietary nitrate at 0.1 mmol/kg of body weight per day (high intake of F and V) reduces DBP 3.5 mm Hg.
- Effect is potentiated by Vitamin C and polyphenols.
- 500 mg beetroot juice with 45 mmol/L of 2.79 g/L of inorganic nitrate lowers BP 10.4/8.1 mm Hg, inhibits platelet aggregation by 20% and increased FMD 30%.
- Lycopene 20 mg per day
- Omega 3 fatty acids 5 grams per day EFA Sirt Supreme from BIotics Labs
- Polyphenols, Flavonoids and Flavonoid-rich foods. Best data with flavones and flavonols. Pomegranate seeds and juice. ½ cup per day of seeds or 6 oz of juice per day.

Nutrition and Supplements to improve endothelial function, nitric oxide and vascular elasticity

Curr Opin Lipidol 2012;23:147-156 Nutrition 2013;29:71-75
Nutrition 2015;31:28; Am J Clin Nut 2016;103:25

- Resveratrol 250mg trans form per day (ResveraSirt from BIotics)
- Grape seed extract 500 mg twice per day
- EGCG 500 to 1000mg twice per day
- Co Enzyme Q 10 100 mg twice per day
- Cacao and dark chocolate 30 grams per day
- Tea and catechins: EGCG 500 mg bid
- Curcumin 1000 to 2000 mg twice per day
- Quercetin 500 mg bid
- Berry anthocyanins and pomegranate seeds
- Orange juice and hesperidin
- Wine polyphenols: Pinot Noir is the best 4- 6 ounces per day
- Rhodiola extract: 200 to 500 mg /day
- Kyolic Aged Garlic extract- KYOLIC GARLIC (AGE): 600 mg twice per day
- Arginine 2 grams bid
- Citrullene 2 grams bid
- Methyl folate 400 micrograms bid
- Superoxide dismutase (Biotics) one bid
- VasculoSIRT from Biotics

SUMMARY Concepts

- Interrelations of nitric oxide, cAMP and cGMP are key
- Vascular health is balance of injury and repair: Nitric oxide and Angiotensin-II and EPC's(endothelial progenitor cells)
- RAAS system is lifesaving acutely, but if chronically over-stimulated promotes vascular disease. The blood vessel becomes and innocent bystander.
- Membranes, membrane fluidity, receptors and ion channels determine external to internal cell communication and cell signaling
- Role of caveolae with eNOS and nitric oxide in membranes
- Cytokines: pro-inflammatory, colony stimulating and chemotactic
- CAMS: cell adhesion molecules: first step in atherogenesis
- Atherosclerosis and CHD start as extra-luminal diseases

SUMMARY Concepts

- Concept of oxidative stress and anti-oxidants as direct and indirect actions with redox modulation and hormesis with cell stress adaptation and HSP.

- Pattern recognition receptors with PAMPs, DAMPs, TLRs(toll like receptors) and NODs (NLR’s) that recognize various mediators, antigens, bacteria that are internal and external, but have same downstream vascular responses.

Detection and **Prevention of Vascular Disease**

Noninvasive measurements of endothelial and vascular function (ED and arterial compliance) are necessary and important; they will become standard clinical practice to detect vascular disease and institute appropriate lifestyle, non-pharmacologic, pharmacologic, and integrative therapies earlier, before the onset of clinical disease. In addition, prognostic information is obtained using these measurements.

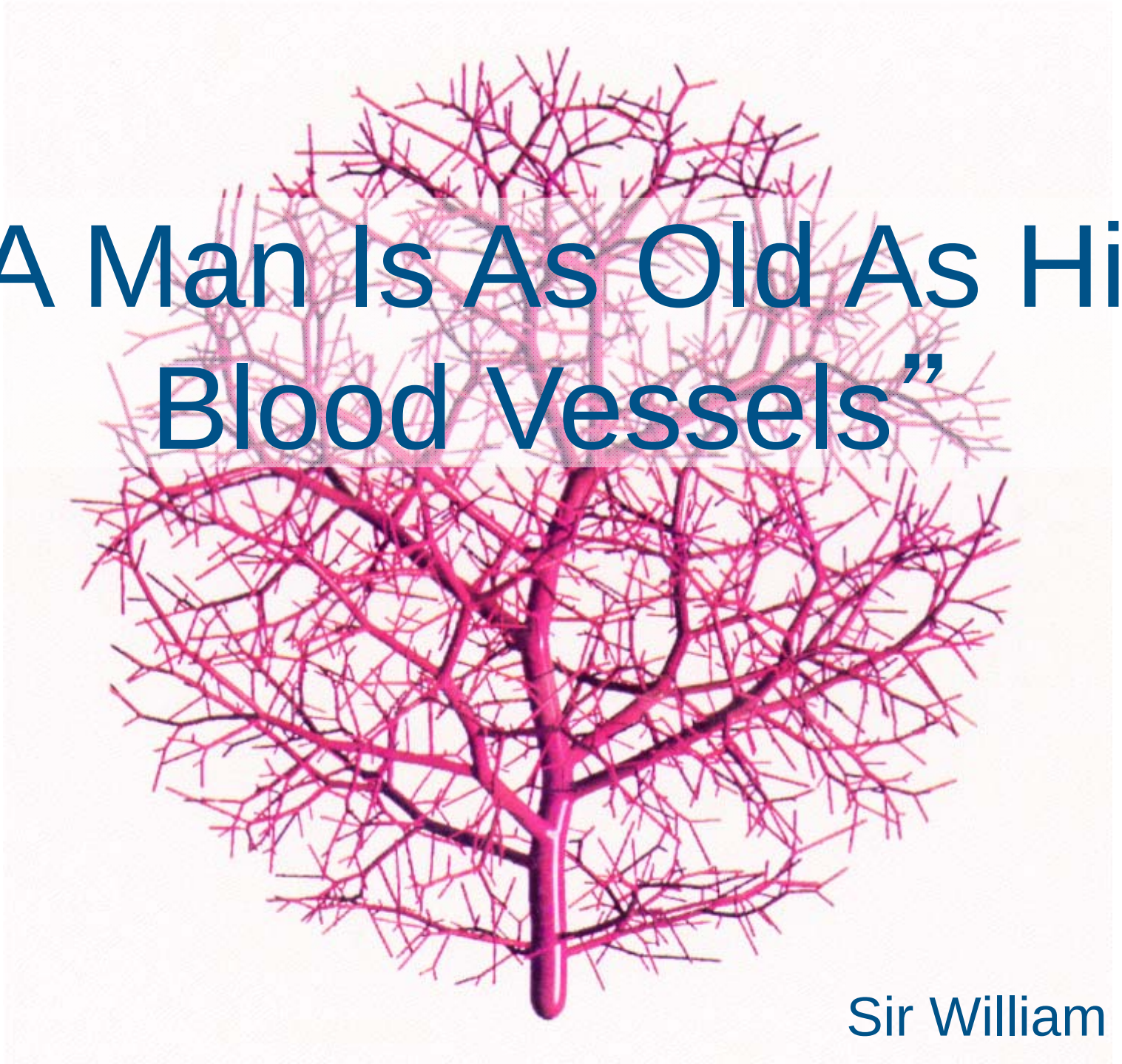
Holistic Approach to Vascular Health and Vascular Disease

- Understand and treat the **mechanism, the processes and the WHY,** not the ***manifestation***.
- Emphasis should be on pathophysiology of vascular disease. What are the metabolic and functional medicine causes?
- Relate the epidemiologic/genetic, epigenetic and environmental-genetic interactions, proteomics, nutrigenomics and metabolomics with systems biology and a dynamic approach to cardiovascular disease.

Vascular Aging

Mark Houston MD, MS, MSc

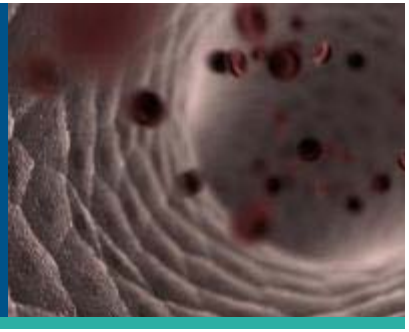
“A Man Is As Old As His Blood Vessels”



Sir William Osler

Vascular Aging

Nat Rev Cardiol 2010;96:1-8. J Gerontology: Biol Sci Med Sci 2010;65:1028-41 Nutrition Reviews 2010;69:65-75 Am J Pathol 2007; 170:388 Circ Res 2007;100:15 J Cardiovasc Pharmacol 2006; 48:88 Semin Nucl Med 2007; 37:120

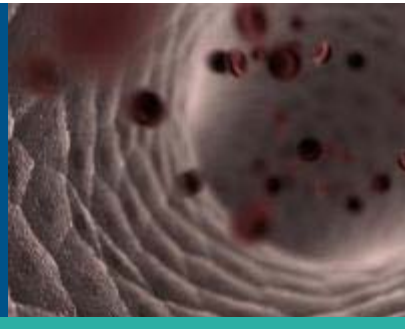


Vascular aging is characterized by progressive arterial stiffness, loss of arterial elasticity and arterial compliance, increase in PWV and pulse pressure, and mechano-sensitive gene expression from a myriad of structural and functional changes in the endothelium and vascular media and adventitia with altered gene expression:

Increased extracellular matrix
Endothelial dysfunction loss of NO, increase cytokines & chemokines
Altered vascular smooth muscle (VSMC) and MLR
Altered adventitia
Inflammation
Loss of elasticity and increase elastase and collagen
Calcium deposition

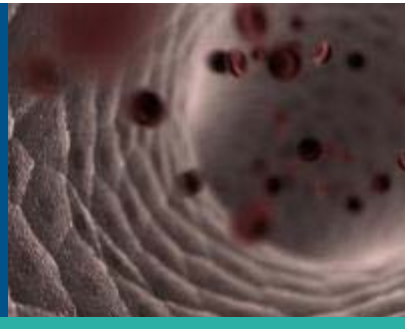
Vascular Aging Summary

Nat Rev Cardiol 2010;96:1-8. J Gerontology: Biol Sci Med Sci 2010;65:1028-41 Nutrition Reviews 2010;69:65-75 Am J Pathol 2007; 170:388 Circ Res 2007; 100:15 J Cardiovasc Pharmacol 2006; 48:88



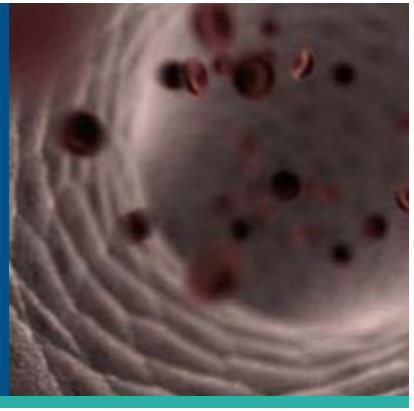
1. Arterial stiffness with reduced elasticity and compliance with increased pulse wave velocity (PWV) and augmentation index (AI)
2. Inflammation
3. Oxidative stress (NADPH-oxidase, xanthine oxidase)
4. Immune vascular dysfunction
5. Thrombosis
6. Growth and hypertrophy
7. Permeability (microalbuminuria)

Vascular Aging Summary Continued



8. Reduced angiogenesis
9. Impaired “circadian clock” genes
10. Increased sympathetic and decreased parasympathetic nervous system activity (SNS > PNS)
11. Vasoconstriction
12. Vascular calcification

Vascular Cell Senescence Contributors to Atherosclerotic Vascular Disease: Eight mechanisms



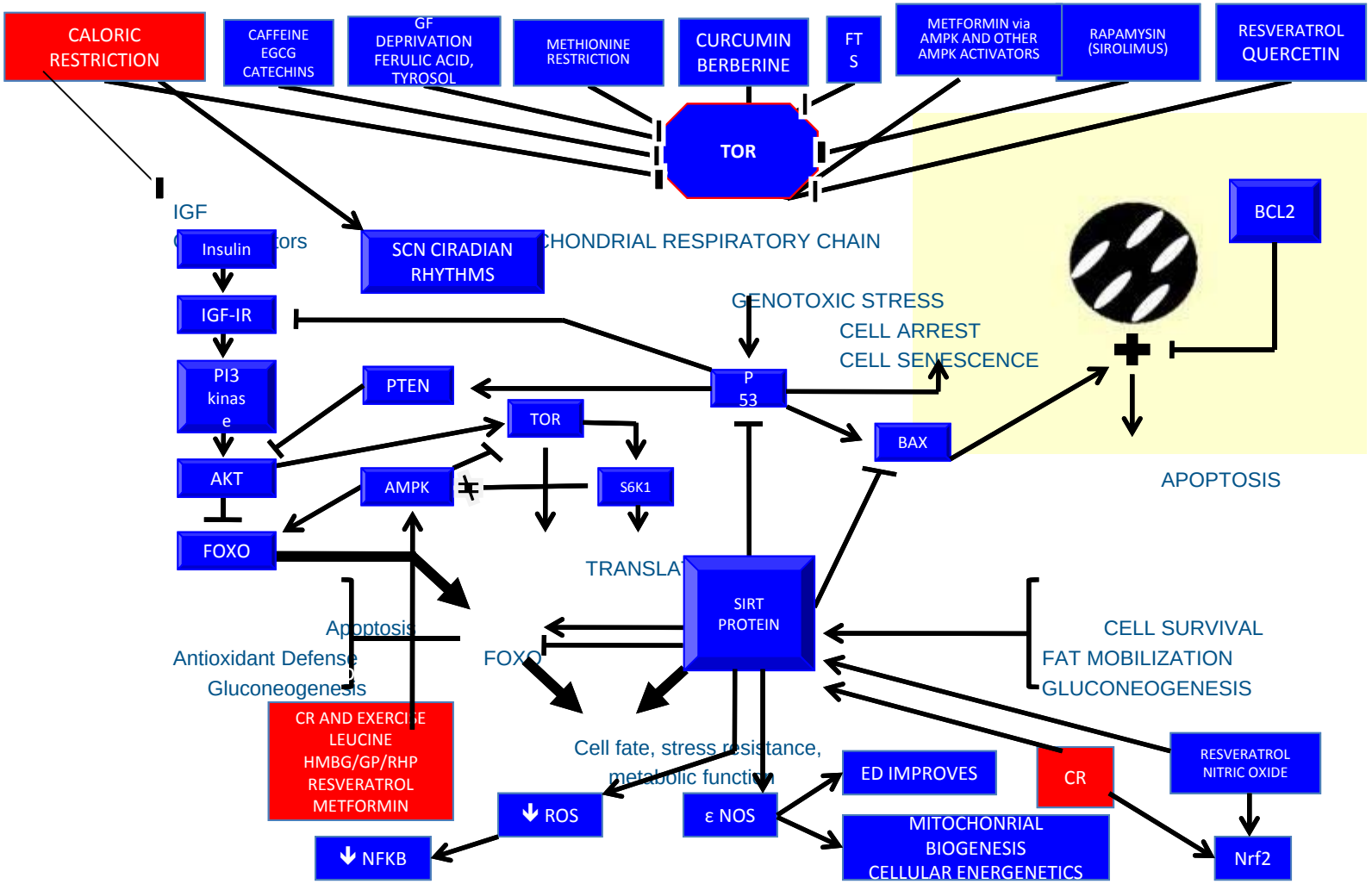
Circ Res 2007; 100:15-26

Circ Res 2007; 100:460
Nutrition Reviews 2010;68:38

1. DNA repair system
2. Telomere maintenance system
3. Tumor suppression pathway
4. Insulin / AKt pathway
5. Angiotensin II and RAAS pathway
6. Mitochondrial energy / metabolism
7. Endothelial progenitor cells (EPC)
8. Nutrient-gene interaction, nutrigenomics and epigenetics

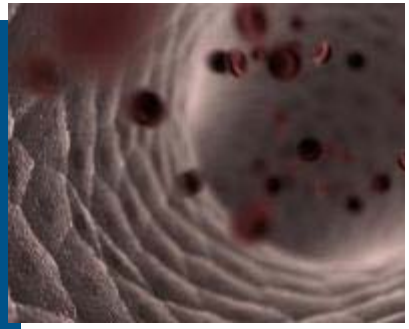
Cellular Senescence alters gene expressions to down-regulate repair mechanisms. Inadequate response to oxidative stress, inflammation and immune vascular dysfunction. Inborn replicative limit at which division potential is lost (Hayflick Limit) which is about 50 -60 replications.

AGING PATHWAYS



Key Vascular Aging Enzymes and Genes

Drugs Aging 2012;28:779, Circ Research 2012;110:1238
Nat Rev Cardiol 2010;96:1-8, Circ Research 2012;110:1109
Lipidology 2012;23:226



Alterations in enzyme activity and gene transcription determine vascular aging and generalized aging

- TOR: Target of Rapamycin: serine/theonine protein kinase
- SIRT: Silent information regulator of transcription: HDAC III sirtuins: NAD+dependent class III histone protein deacetylases.
- AMPK: adenosine monophosphate activated kinase
- S6K1: ribosomal protein kinase 6. Also 4EBP1 and P66SHC
- Akt or PKB: protein kinase B. Serine/theonine protein kinase
- FOXO: Forkhead box subgroup O: group of transcription factors
- P53: Protein 53 tumor suppression protein

To slow Aging: Block TOR, S6K1 and Akt,
Activate SIRT, AMPK, FOXO and P53

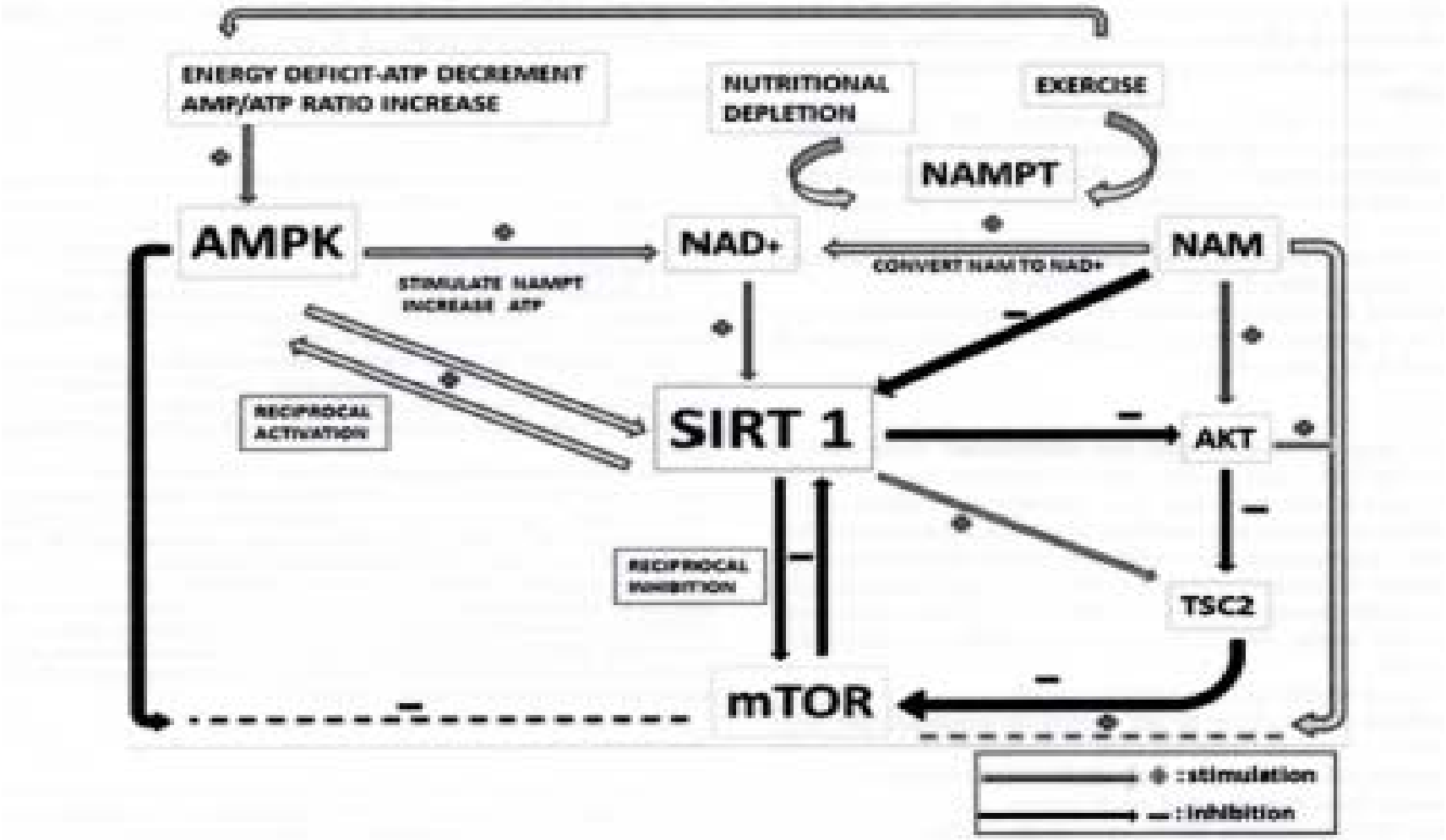
How to Increase Life Expectancy
Enzyme and Metabolic Pathways

NEJM 2009;361:2669; Aging 2010;2:514; Science 2009;326:140

- Inhibition of TOR
- Stimulation of SIRT 1 and 2 increase life expectancy
- Increase AMPK (energy sensor) blocks TOR
- Block IGF-1 and insulin (IIS pathway) and aKT (PKB: activate TOR
- Block PKA
- Decrease S6K1 (ribosomal) enhances mRNA
- Increase FOXO
- Block P53 and BAX

- TOR stimulates S6K1 which activates mRNA and blocks AMPK activation (sensor of energy status)

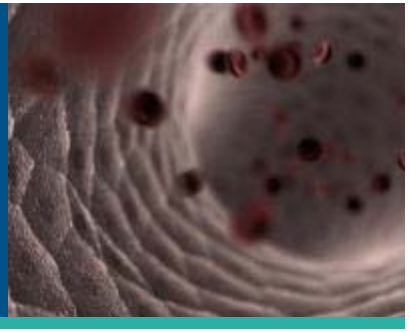
Regulation of SIRT1



Nutrition 34 (2017) 82-96

SIRT Activators

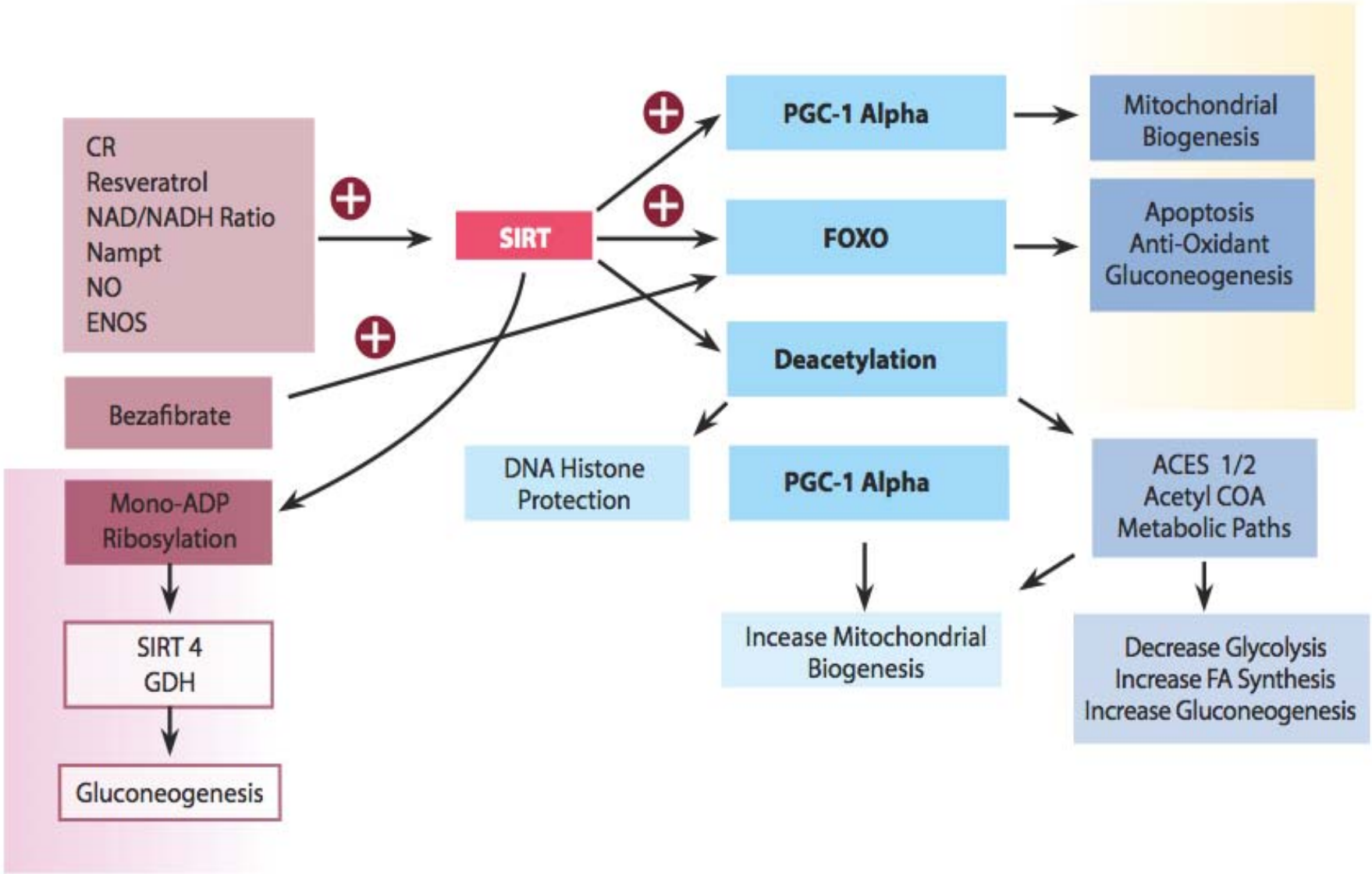
Trends in Biochem Sci;2010; 32 (1); 1-4
Nutrition Reviews 2012;32:648



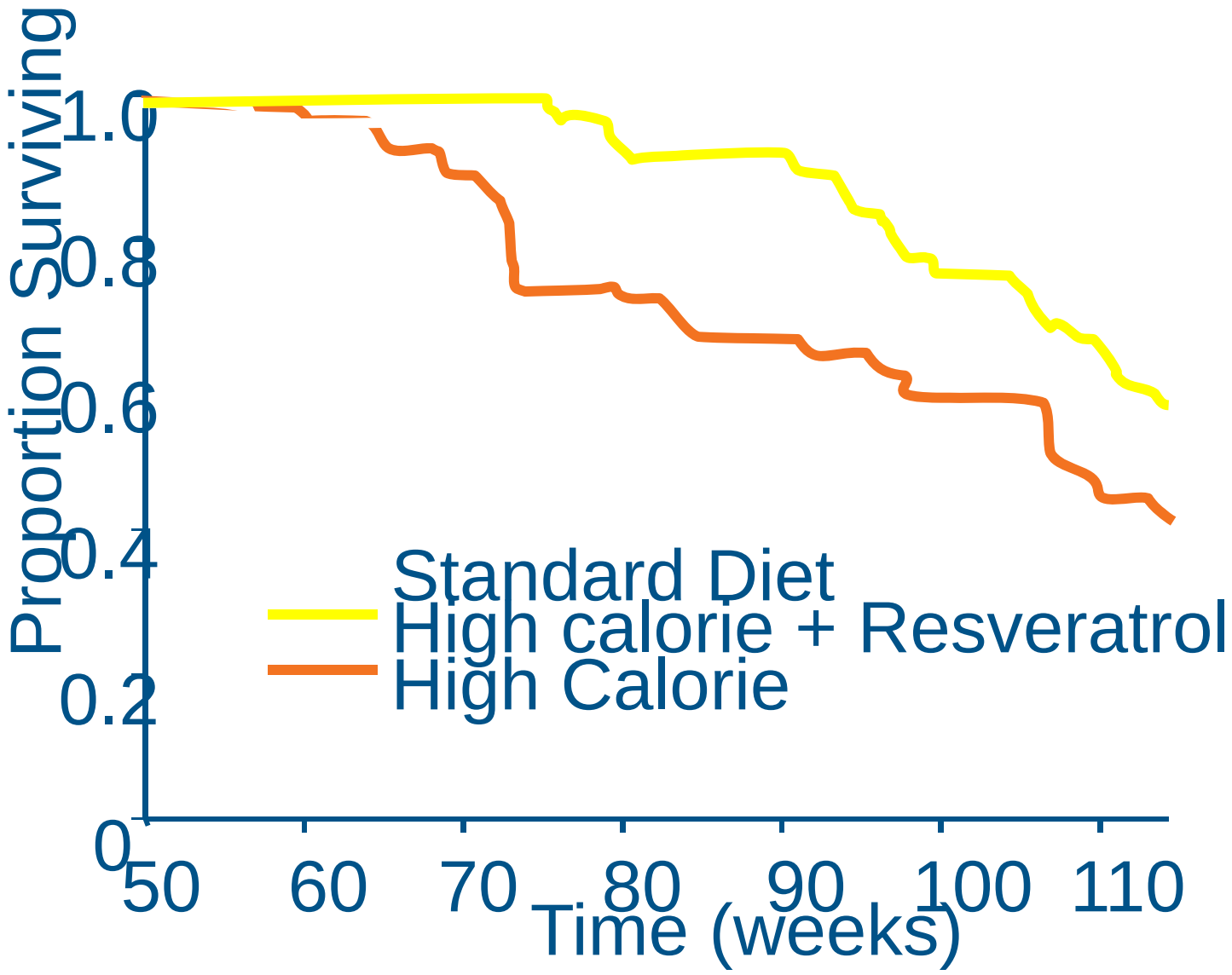
- Caloric restriction
- Resveratrol
- Increased NAD/NADH ratio
- Nampt (nicotinamide phosphoribosyltransferase)
- Nitric oxide/eNOS

SIRT Activation

HDAC III SIRITUINS



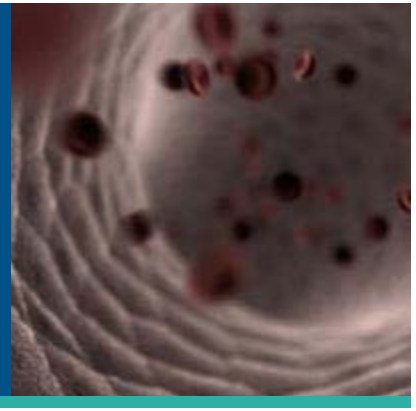
Resveratrol Improves Survival



Nature; Nov 16, 2006; 444: 337-42

Curcuminoids and CHD, MI and CABG

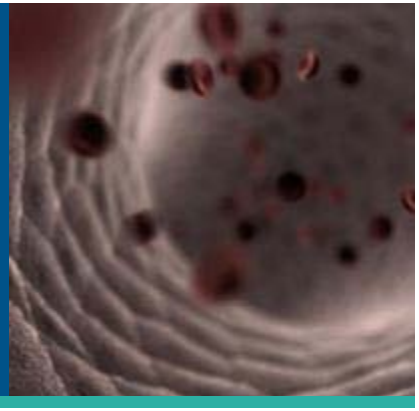
Am J Cardiology 2012;110;40
J Pharmacol Exp Ther 2009;329(3):959-66
J Cardiovasc Med 2010;11(1): 1-6
J of Nutritional Biochemistry 2012;23:1514



- Decrease proinflammatory cytokines during CABG procedure
- Decrease cardiomyocyte apoptosis after cardiac ischemic-reperfusion injury
- Reduce MI post CABG from 30 to 13 % ($p<0.038$) at 4 grams per day given 3 days before and 5 days after CABG.
- Decrease HS CRP, MDA and NtBNP
- Block TLR 2, reduce inflammation, oxidative stress MI.
- Lower NFkB, COX 2, LOX, MMP 2, MMP 9 and iNOS.
- Membrane stabilizing effect on cardiac myocytes
- Inhibit platelet activation.
- Inhibits VSMC proliferation and arterial stenosis

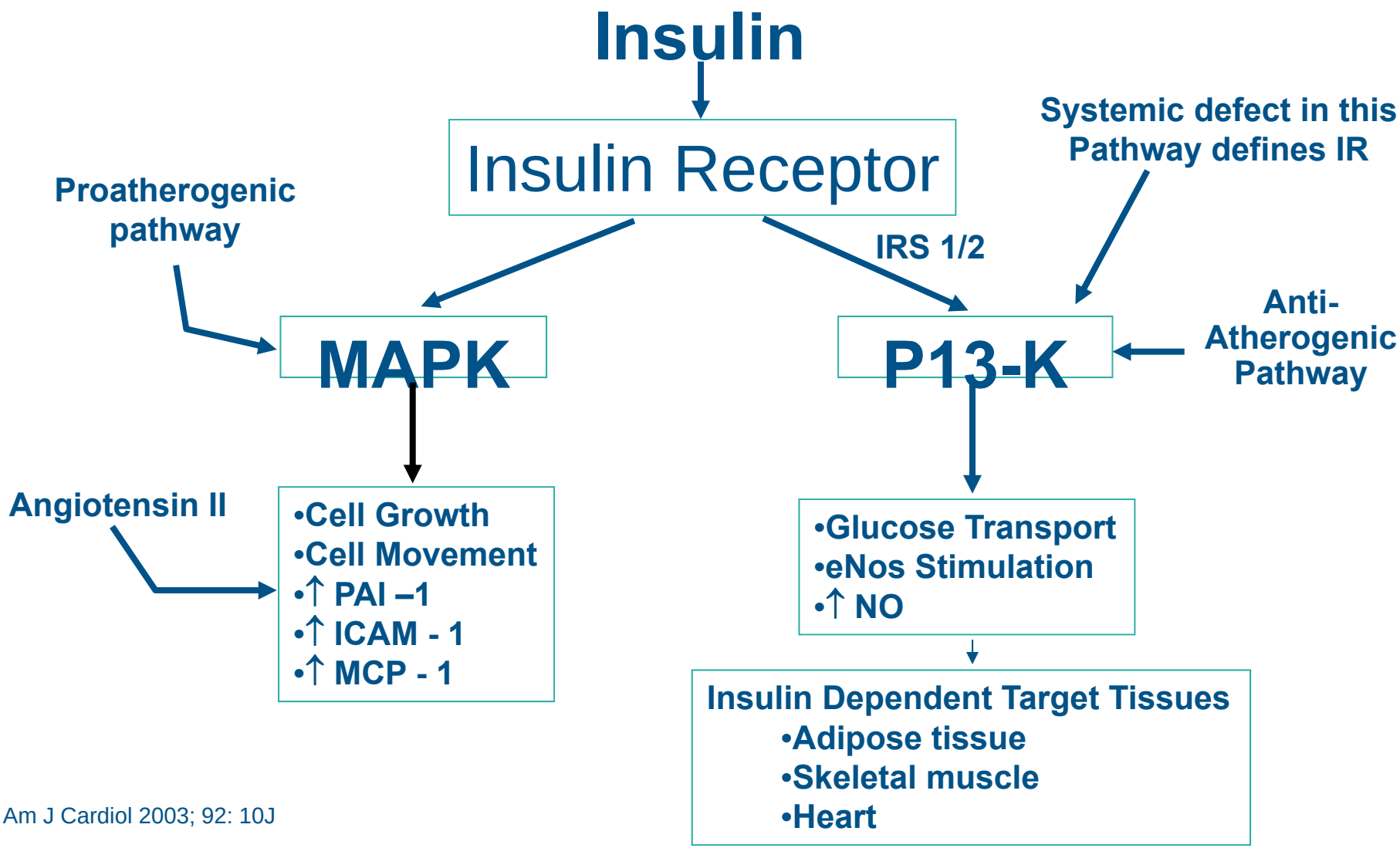
Metformin

Current Opinion in Lipidology 2011;22:445



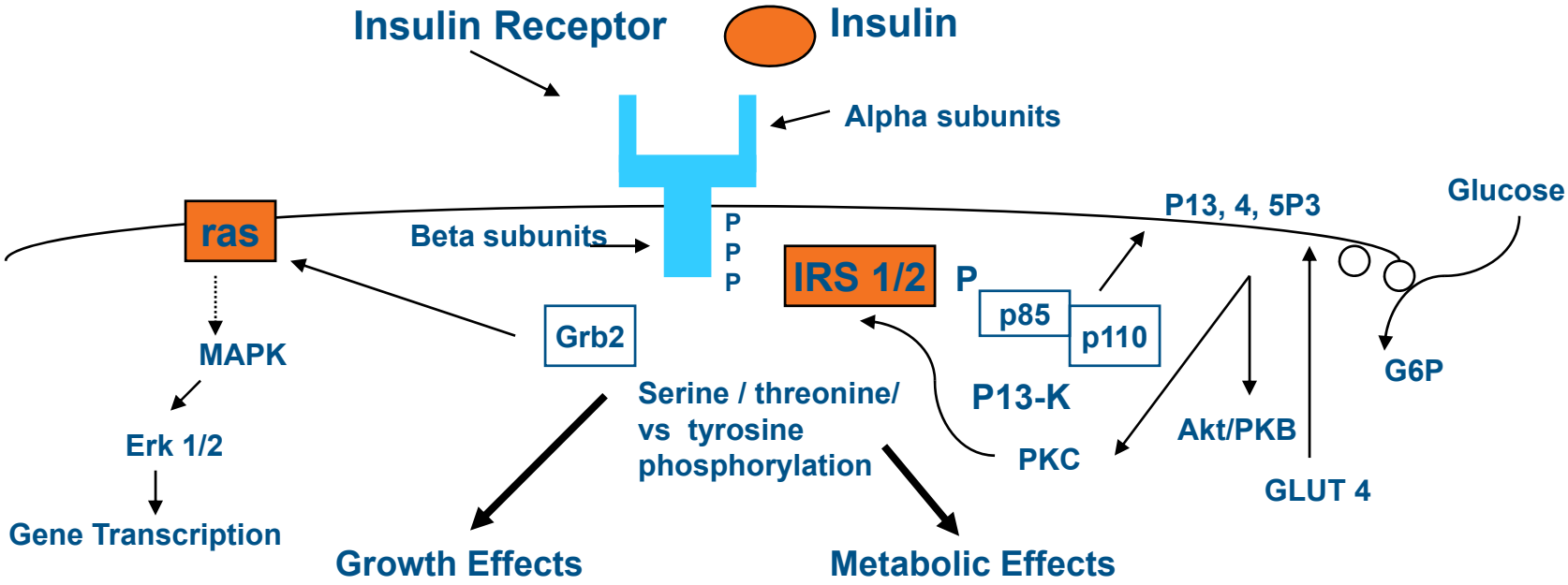
- Cardioprotective and anti-atherosclerotic: 39% reduction in MI and all cause mortality in UKPDS
- Reduces infarct size and reduces CHF
- Limits myocardial ischemic reperfusion injury
- Inhibits gluconeogenesis 36%
- Increases skeletal muscle/ adipocyte insulin sensitivity and glucose uptake, lowers insulin 25%
- Changes mitochondrial complex I AMP/ATP ratio to stimulate AMPK, increase PCG-1 alpha
- Reduces FFA, lowers TG, LDL and increases HDL
- Improves ED and increases NO
- Reduces coagulation
- Reduces carotid IMT

Stimulation of the MAPK Pathway in Endothelial and VSM Cells: Tyrosine vs Serine Phosphorylation



Am J Cardiol 2003; 92: 10J

Insulin Signaling



Autophosphorylation of IR (Tyrosine Kinase)
Tyrosine Residues and serine vs tyrosine phosphorylation
IR Kinase Activity
Phosphorylation of Insulin Receptor Substrates
GLUT 4 Translocation

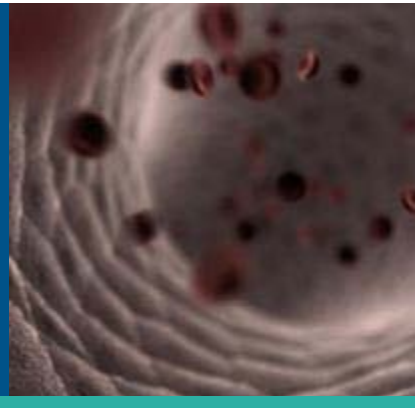
IRS = Variable deficiencies in intracellular signaling pathways

IRS = Insulin receptor substrate
P13-K = phosphoinositide-3 kinase
PK = Protein kinase
Erk = Extracellular signal-regulated kinase
GLUT 4 = Glucose transporter 4

J Nutr 2001; 131:2782S-86S

Mitochondria & Aging

Nutrition Reviews 2010;59:65



SIRT 1 activated by

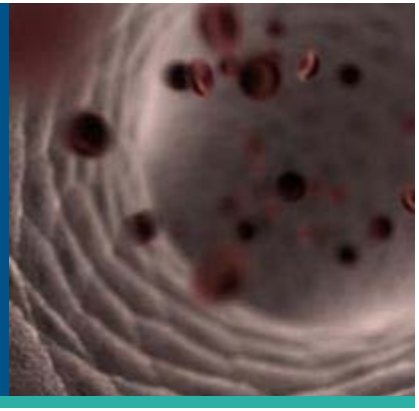
- NOS and NO
- High NAD/NADH ratio
- Nampt (nicotinamidephosphoribosyltransferase) pathway
- Caloric restriction (CR)

CR activation of SIRT I and 2

- Increase mitochondrial respiration,
- Beta oxidation in skeletal muscle and WAT
- Mitochondrial autophagy and biogenesis.

SIRT and bezafibrate activate PGC -1 alpha (peroxisome proliferator activated gamma co-activator 1) which increases the expression of nuclear genes involved in mitochondrial biogenesis, mass, OXPHOS and regulates PPAR.

PPAR/PGC-1 alpha - Bezafibrate



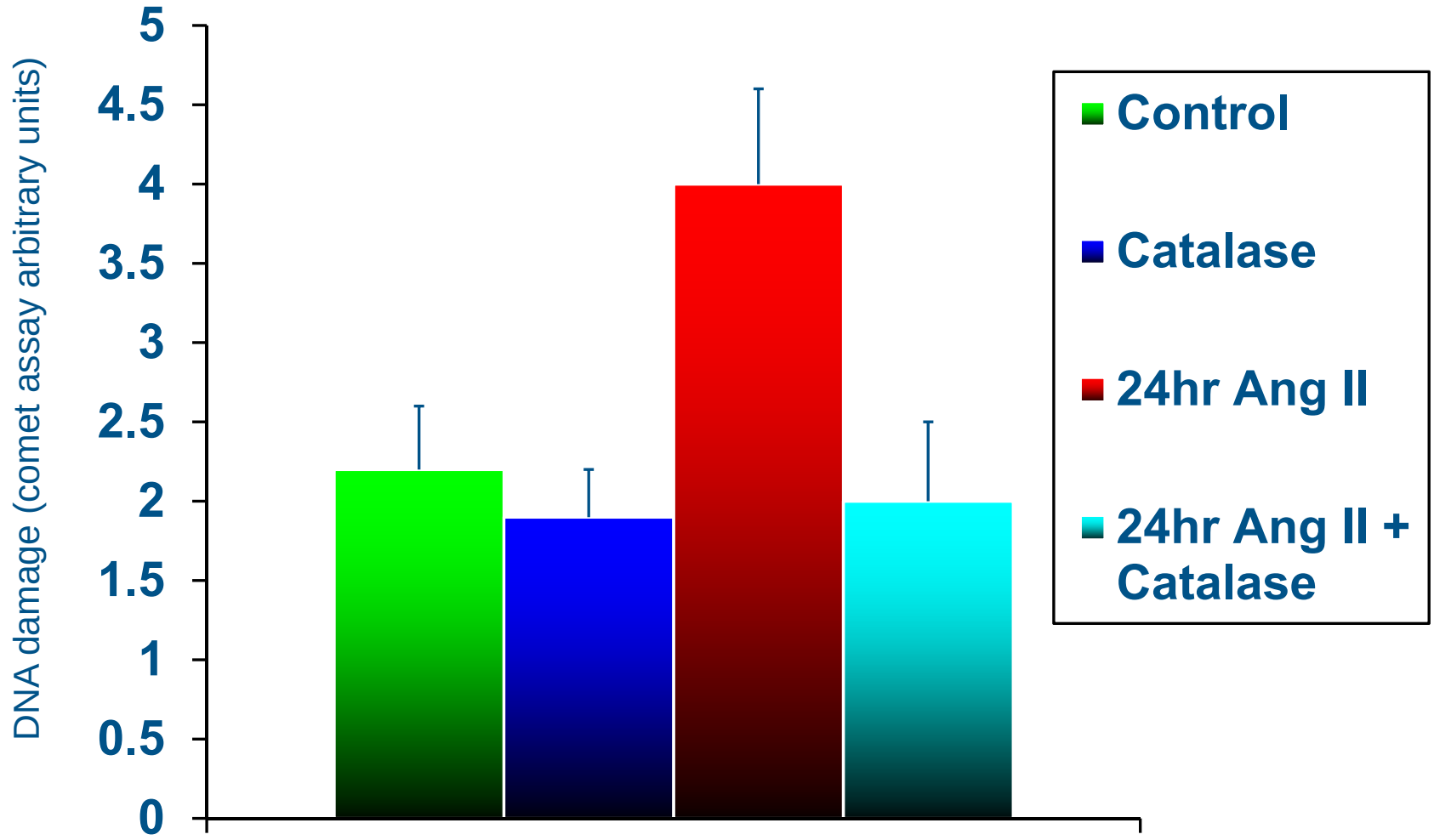
Cell Metab 2008;8(3):249-56

- PGC-1 alpha is a transcriptional coactivator of nuclear receptors and other transcription factors, including NRF-1 and 2, ERR alpha and mtDNA transcription factor A.
- PGC-1alpha regulates PPAR gamma.
- PPAR gamma increases mitochondrial biogenesis.
- Activation of PPAR gamma increases mitochondrial mass, enhances mitochondrial function and biogenesis. OXPHOS capacity.

- **Bezafibrate** is a pan PPAR agonist that increases mitochondrial biogenesis. It increased life expectancy, ATP and delayed onset of myopathy in mouse model with cytochrome C deficiency.

ANG II Induced DNA Damage

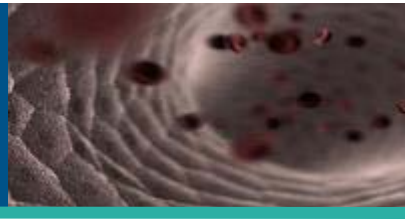
Effects of Catalase

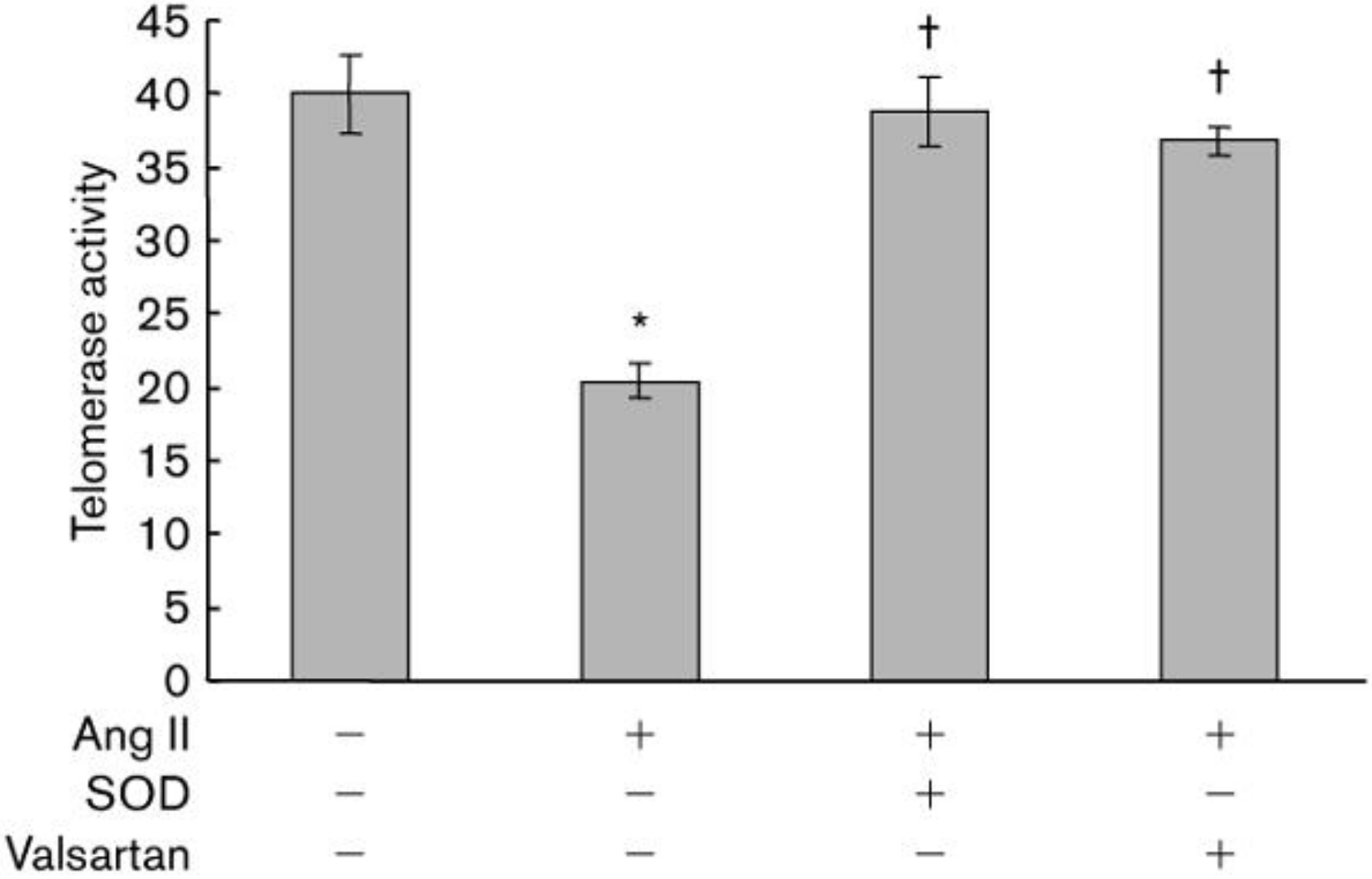


Williams, B. AJH 2002, 15(4):13A

ANG II Cell Senescence and Telomere

Williams, B. AJH 2002, 15(4):13A

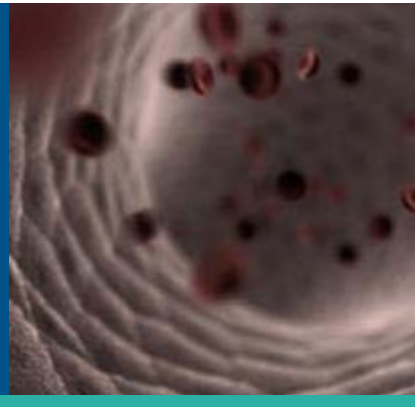




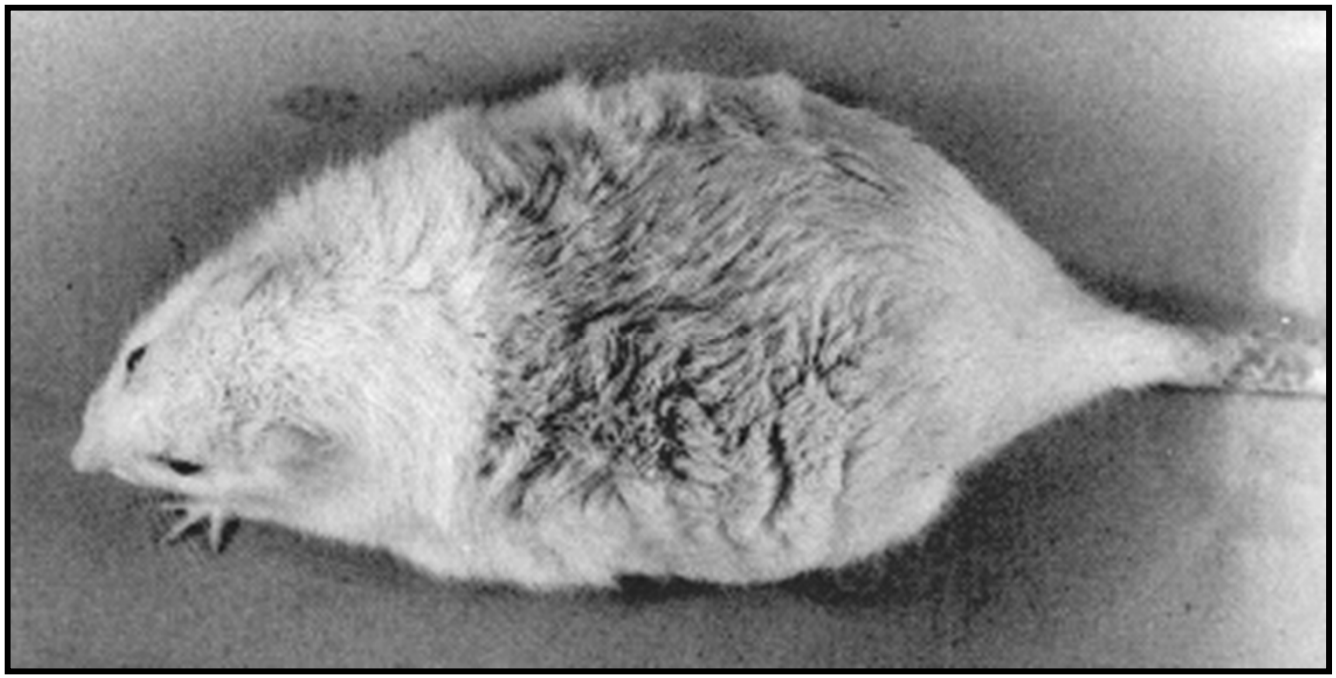
AT1R and Aging

Curr Opin in Nephrology and Hypertension 2011;20:84

J Clin Invest 2009;119:524

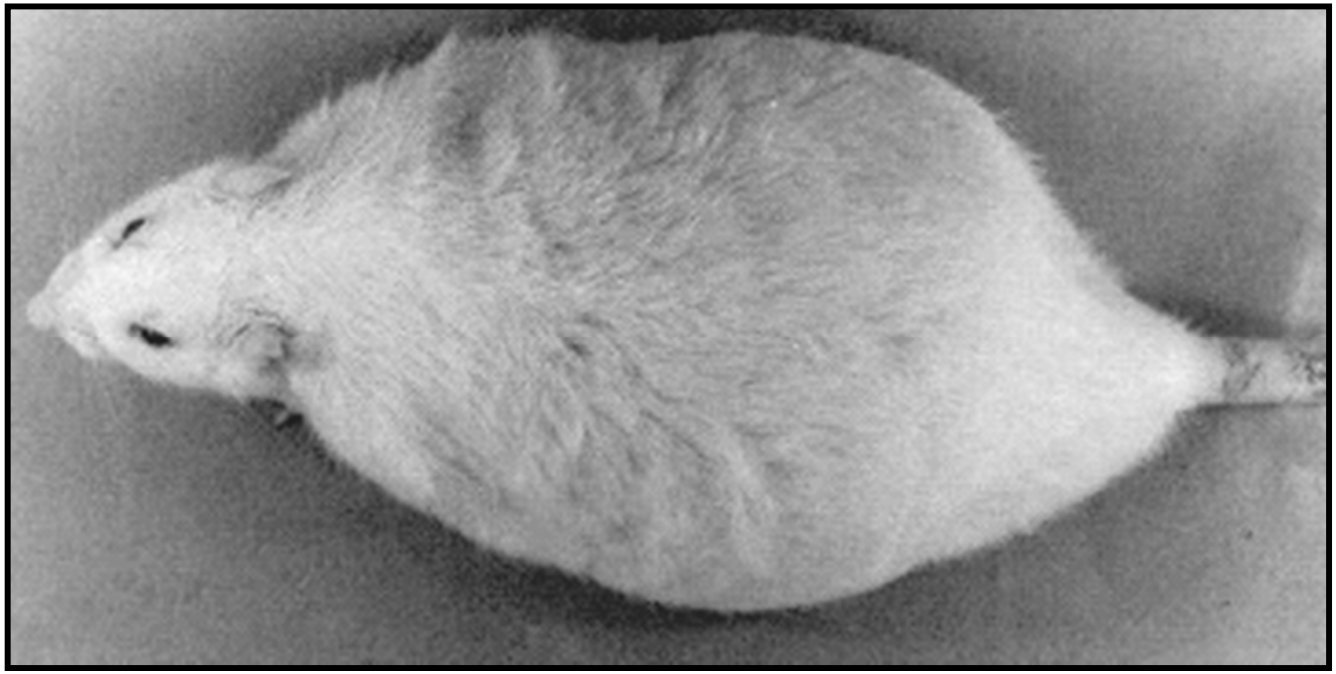


- Mice lacking the AT1R have 26 % increase in life span ($p<0.0001$). 31.2 months vs 24.81 months.
- Increased expression of survival genes like Nampt (nicotinamide phosphoribosyltransferase) and (Sirtuins) SIRT 3 and mRNA for Sirt -3
- Decreased ROS, decreased perioxynitrite and nitrotyrosine.
- Improved mitochondrial function, number and survival with increased mitochondrial NAD+.
- Reduced CV and vascular aging and damage. Decreased atherosclerotic lesions, cardiac damage, interstitial collagen
- Decrease autoimmune dysfunction and T cells
- Decreased TGF-b and fibrosis and inflammation



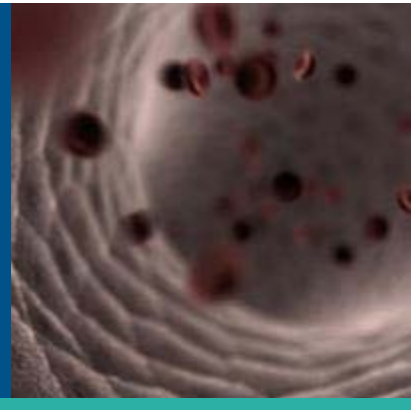
Wistar Rats
24 Months

Control



Enalapril

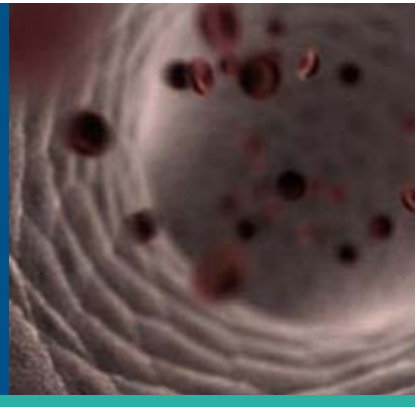
Caloric Restriction Actions



European J of Clinical Nutrition 2007;61:160-65
Science 2003;299:572
Proc Natl Acad Sci USA 2003;100:6216
Physiol Rev 2002;82:637

- Decrease oxidative stress, inflammation and autoimmunity
- Increase mitochondrial biogenesis and cellular energetics
- Increase SIRT
- Decrease TOR, S6K1, 4EBP1 and P66SHC
- Increase AMPK
- Increase FOXO
- Increase Nrf2
- Increase NO and eNOS
- Decrease NFKb
- Decrease TGF-1
- Decrease P53 and BAX and cell apoptosis

CALORIE RESTRICTION (CR) in Rhesus Monkey

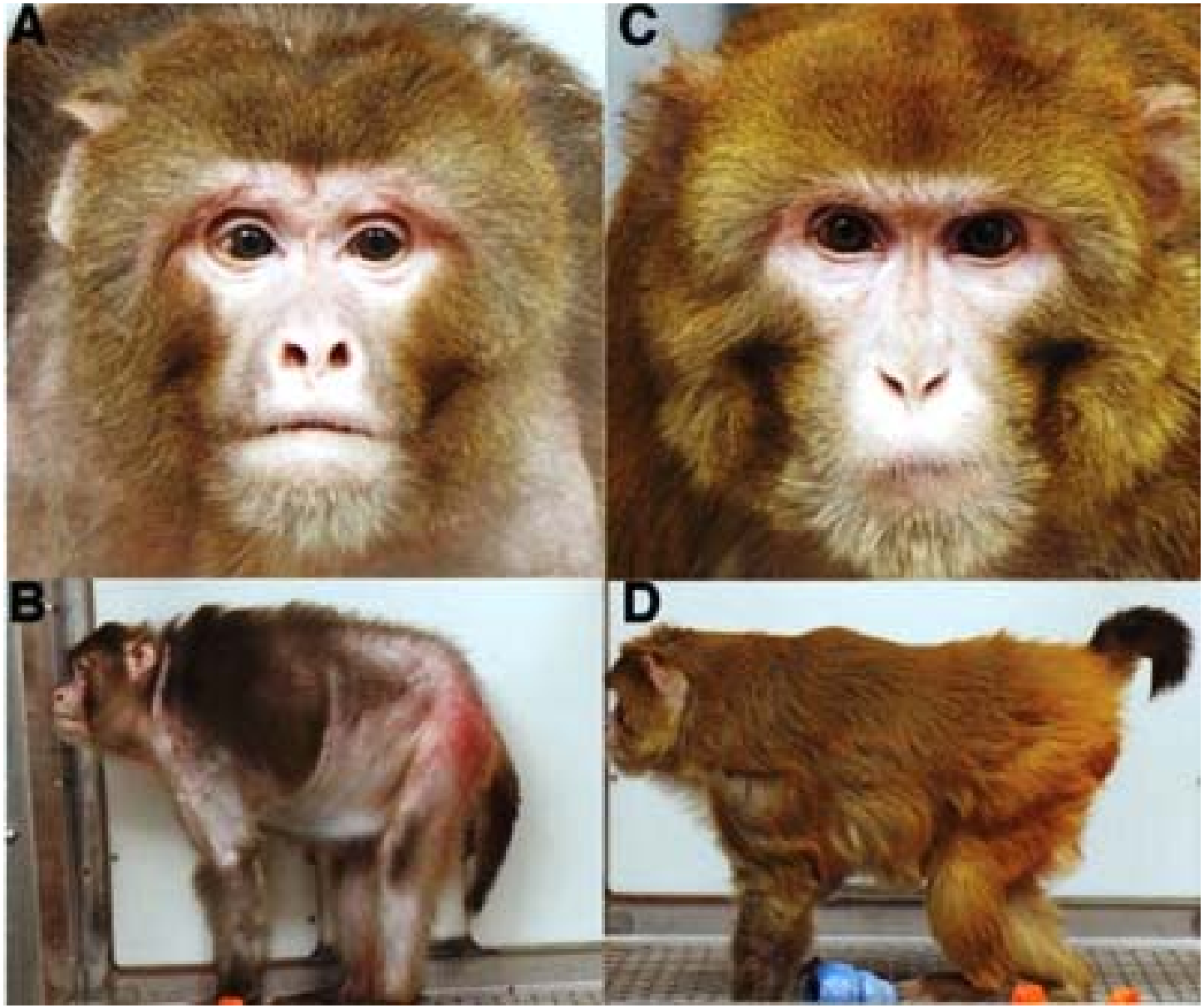


Exp Gerontol 2003;38:35 and 2011;46:23 and 2010 45:208
Biogerontology 2006;7:169 and J Neurolsci 2010;30:7940 **Science 2009;325(5937):201-204 and Neuroimage 2010;51:987 Toxicol Pathol 2009;37:47 and Neurobiol Aging 2010;8/4 epub Exp Gerontol 2009;44:356 and Clin Geriatr Med 2009;25:733

20 year longitudinal study in Rhesus Monkey with 30% CR

50% of control fed animals survived vs 80% survival of the CR animals
50% reduction in all age related diseases:
Less body fat and weight and less sarcopenia (via PPAR)
Improved insulin sensitivity, lower glucose and decrease DM
Improved serum lipids with increased HDL 2b, lower LDL particles
Lower BP.
Lower body temp.
Higher DHEA and melatonin.
Reduced cancer
Reduced CVD
Reduced brain atrophy, brain excision repair, reduced neural iron and reduced effects of homocysteine on brain. Better hearing
Shifts gene expression with increases in energy metabolism genes and reduces over 50 inflammatory genes

Caloric Restriction in Primates



Calorie Restriction in Humans: CALERIE *Civitarese AE, et al. CR Increases Muscle Mitochondrial Biogenesis in Healthy Humans. PLOS Medicine. 2007 March 4(3) e 76. Pennington Biomedical Research Center: Baton Rouge, LA*

- Study design in humans
  - 36 overweight subjects @ 6 months BMI 28
  - 3 groups
    - CR 25%
    - CR+EX: CR 12.5% + ↑ EE 12.5%
    - Control: 100% calories

STUDY RESULTS

Civitarese AE, et al.

	Control	CR-25%	CR-12.5%+EX-12.5%
• 24 hour EE	No changes	↓ 135 Kcal/d	↓ 117 Kcal/d (p = 0.008)
• Gene Coding Proteins for Mitochondrial Function	No changes	↑ PPAR-G-CIA ↑ TFAM ↑ ENOS ↑ SIRT-I ↑ PARL (All p < 0.05)	↑ PPAR-G-CIA ↑ TFAM ↑ ENOS ↑ SIRT-I ↑ PARL (All p < 0.05)
• Mitochondrial DNA	No changes	↑ 35% (p = 0.005)	↑ 21% (p = 0.004)

STUDY RESULTS

Civitarese AE, et al.

	Control	CR25%	CR12.5%+EX12.5%
• Mitochondrial Enzymes TCA + ETC	No change	No change	No change
• DNA Damage	No change	↓ 0.56 AU (p = 0.003)	↓ 0.45 AU (p = 0.011)
• Myotubules	No change	↑ NO donor ↑ mito biogenesis	↑ NO donor ↑ mito biogenesis

Caloric Restriction in Humans

Sci Transl Med. 2017 Feb 15;9(377). pii: eaai8700. doi: 10.1126/scitranslmed.aai8700ion

Randomized 100 generally healthy participants into two study arms and tested the effects of a fasting-mimicking diet (FMD)-low in calories, sugars, and protein but high in unsaturated fats-on markers/risk factors associated with aging and age-related diseases.

Subjects followed 3 months of an unrestricted diet vs subjects who consumed the FMD for 5 consecutive days per month for 3 months.

Three FMD cycles reduced body weight, trunk, and total body fat; lowered blood pressure; and decreased insulin-like growth factor 1 (IGF-1). No serious adverse effects were reported.

After 3 months, control diet subjects were crossed over to the FMD program, resulting in a total of 71 subjects completing three FMD cycles.

A post hoc analysis of subjects from both FMD arms showed that body mass index, blood pressure, fasting glucose, IGF-1, triglycerides, total and low-density lipoprotein cholesterol, and C-reactive protein were more beneficially affected in participants at risk for disease than in subjects who were not at risk.

Thus, cycles of a 5-day FMD are safe, feasible, and effective in reducing markers/risk factors for aging and age-related diseases.

Vitamin K and D and Arterial Stiffness

J of Nutritional Biochemistry 2017:46:83

- Low Vitamin K with a low vitamin D is synergistic or additive in increasing arterial stiffness and increasing the pulse wave velocity.

Metformin

Current Opinion in Lipidology 2011;22:445
Current Opinion in Lipidology 2014;25:446

- Cardioprotective and anti-atherosclerotic: 39% reduction in MI and all cause mortality in UKPDS. Reduces infarct size and reduces CHF.
- Inhibits gluconeogenesis 36%
- Increases skeletal muscle/ adipocyte insulin sensitivity and glucose uptake, lowers insulin 25%.
- Changes mitochondrial complex I AMP/ATP ratio to stimulate AMPK, increase PCG-1 alpha
- Reduces FFA, lowers TG, LDL and increases HDL
- Improves ED and increases NO
- Reduces coagulation
- Reduces carotid IMT
- Limits myocardial ischemic reperfusion injury, ischemic preconditioning by closing of MPTP (mitochondrial permeability transition pore) via RISK and PI3K/Akt pathways, increased adenosine receptor stimulation and AMPK. Statins and adenosine also close MPTP.

Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls

Diabetes Obes Metab.2014 Nov;16(11):1165-73.

- With reference to observed survival in diabetic patients initiated with metformin monotherapy [survival time ratio (STR) = 1.0], adjusted median survival time was 15% lower (STR = 0.85, 95% CI 0.81-0.90) in matched individuals without diabetes and 38% lower (0.62, 0.58-0.66) in diabetic patients treated with sulphonylurea monotherapy.
- CONCLUSIONS:
- Patients with type 2 diabetes initiated with metformin monotherapy had longer survival than did matched, non-diabetic controls. Those treated with sulphonylurea had markedly reduced survival compared with both matched controls and those receiving metformin monotherapy. This supports the position of metformin as first-line therapy and implies that metformin may confer benefit in non-diabetes. Sulphonylurea remains a concern

Treatment Summary Specifics

- **Caloric Restriction**: 12 hour overnight fast 4 to 7 days per week with CR of 12.5 % and EE of 12.5%
- **Nutrition**: 12.5% CR with 10 F/V per day, 30 % protein, 30 % MUFA and Omega 3 with limited SFA and no trans fat, minimal refined CHO
- **Ideal body weight (IBW) and composition**: < 22% body fat for women and <16% for men.
- **Exercise**: ABCT exercise regimen for 60 minutes daily with 40 min resistance and 20 minute interval aerobics
- **Reduction in inflammation and oxidative stress**: IBW, no tobacco, foods and supplements that reduce these.
- **Sleep and stress reduction**: 8 hours sleep per night, early to bed and early to rise re circadian rhythm.

Treatment Summary

- **Sirtuins**: Trans Resveratrol : ResveraSirt HP 250 mg per day (Biotics)
- **Vitamin K2- MK7**: 100-500 micrograms/day . Tri K(DFH) or Vasculosirt 5 capsules twice/day (Biotics)
- **Vitamin D** to blood level of 60 ng/ml
- **Omega 3 Fatty Acids**: balanced DHA,EPA,GLA and gamma/delta tocopherol. 3-5 grams/day with EFA SIRT SUPREME 6 capsules twice/day (Biotics)
- **Curcumin**: 800 mg twice/day with Curcumin C3 Complex (400mg) 2 capsules twice/day (DFH)
- **Glutathione support program**: Whey protein 40 grams a day, R lipoic acid 100 twice a day, NAC 500 mg twice a day and niacinamide 1000 mg twice a day.
- **BCAA** 5000 mg per day after exercise

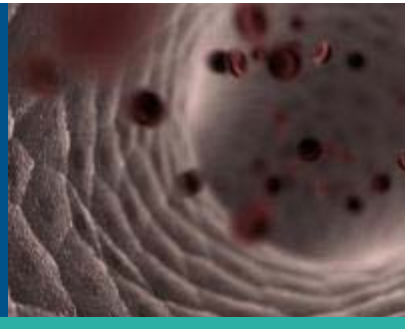
Treatment Summary

- Acetic acid (vinegar)
- Mushroom extracts
  1. Reishi F-3 polysaccharide (Gandoderma lucidum)
  2. Mycelium fractions of Chan-Chih (A. camphorata)
  3. Lions Mane (H erinaceus)

Treatment Summary: Specifics

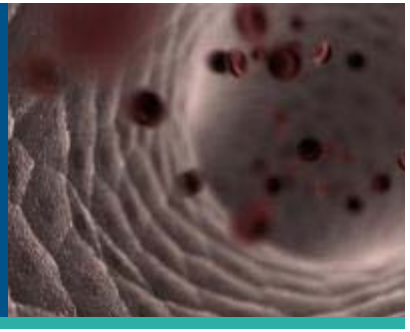
- CHD Risk Factor Control: per new aggressive guidelines noted in previous slides.
- Micronutrients and Nutraceuticals : See next 3 slides
- ACEI: Perindopril 16 mg /day or
- ARB: Telmisatan 80 mg/day
- Statin: Rosuvastatin: 5 mg every other day
- Metformin ER : 500 – 1000 mg per night
- Colchicine 0.5 mg qd to bid
- Bezafibrate
- ASA: 81 mg/day
- BIHRT: as indicated

Vascular Aging Summary



- Vascular aging parallels aging (ED, increased SVR, loss of elasticity, stiffness, inflammation, ROS, immune function).
- Pathways involve TOR, AKT, PI3K,SK6, P66SHC,4EBP-1, IGF(IIS), Sirtuins, FOXO and mitochondrial biogenesis.
- Vascular aging is slowed by blocking TOR and S6K1
- Vascular aging is slowed by stimulation of SIRT-1 and SIRT-2 and AMPK (stimulated by caloric restriction (CR), resveratrol, Curcumin and metformin).
- Vascular cell senescence due to many pathways: DNA damage, telomeres, tumor suppression pathway, insulin and AKt, RAAS, mitochondria, EPCs and nutrient-gene interactions.
- Diagnostic Testing for vascular structure and function and vascular risk factors, and treat all CHD risk factors to optimal levels. Early detection is key.
- Aggressive prevention and treatment will slow aging by nutrition, exercise, weight loss, CR, various nutrients, and drugs.

Major Points & Summary

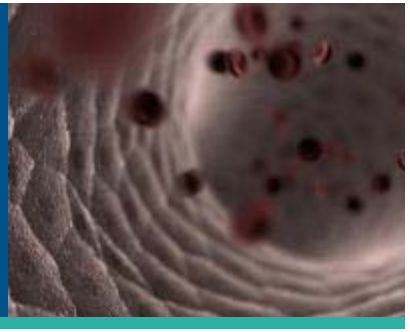


Translational risk factor-vascular disease concept:

- Correlate and validate all risk factors and mediators to the actual presence of endothelial dysfunction (functional) and vascular structural changes with presence of vascular disease with sensitive non-invasive tests such as ENDOPAT and CAPWA.

- Early detection (and aggressive and early prevention) and treatment are required. Use global incremental risk reduction and integrative therapies.

Vascular Aging



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