Endothelial Function

Clinical Assessment

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Endothelium

Key Regulator of Vascular Homeostasis

Vita JA et al. Circulation. 2002;106:640–642

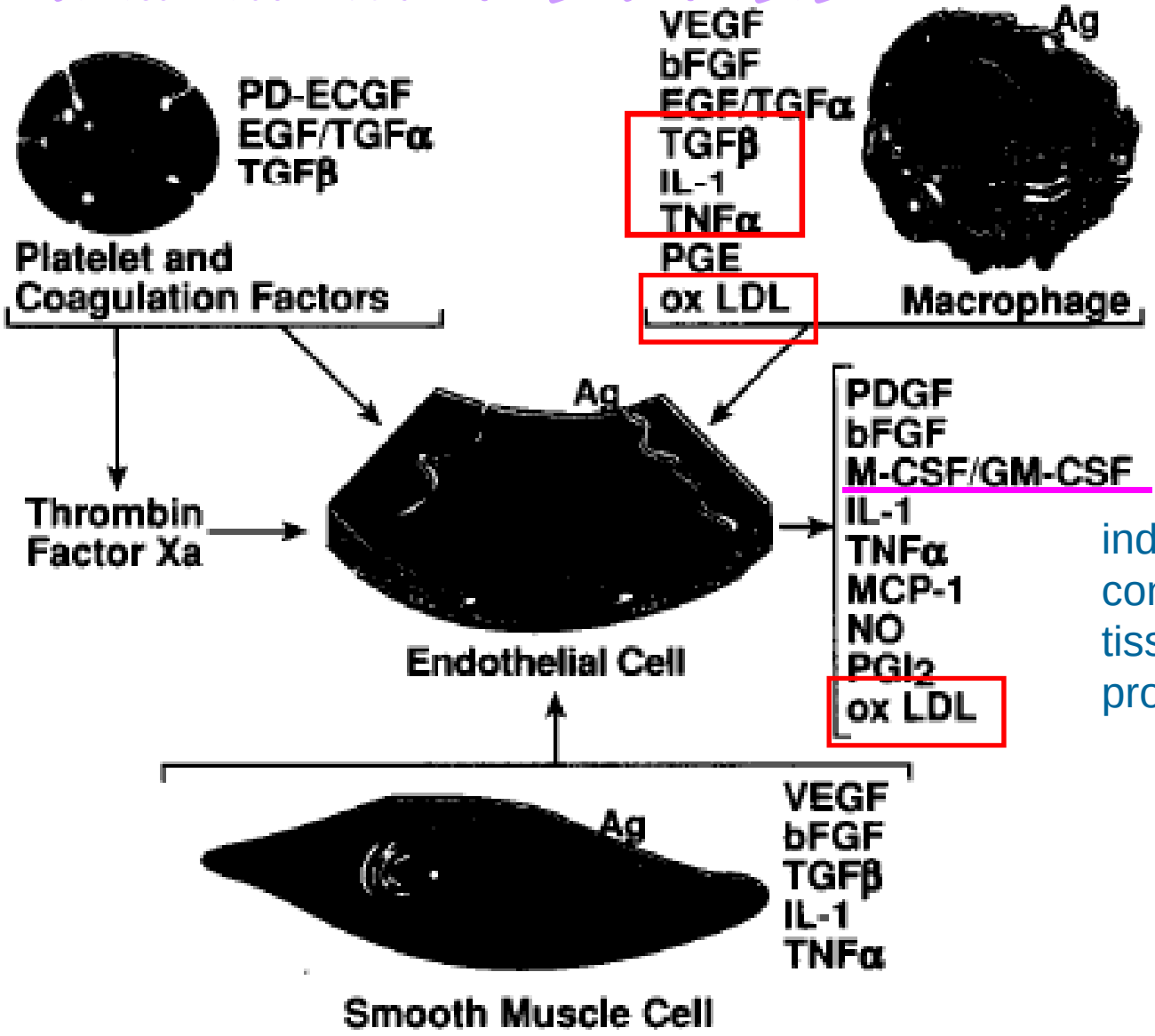
Endothelium in Normal Vessel

- Simple monolayer
- Respond to physical and chemical signals
- Wide range of factors

Regulate

- Vascular tone
- Cellular adhesion
- Thrombosis
- Smooth muscle cell proliferation
- Inflammation

Endothelium in atherosclerosis



induce connective tissue proliferation

Ross R. Nature. 1993;362:801–809

NO

Endothelium-Derived Hyperpolarizing Factor

Prostacyclin

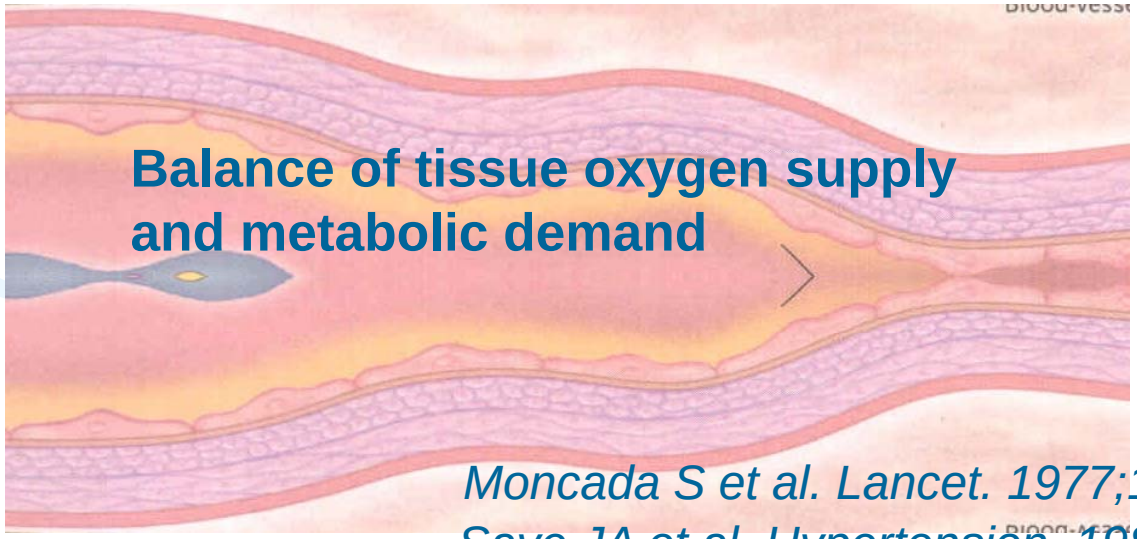
Endothelin

Vasoconstrictor prostanoids

Angiotensin II

Vasodilator tone

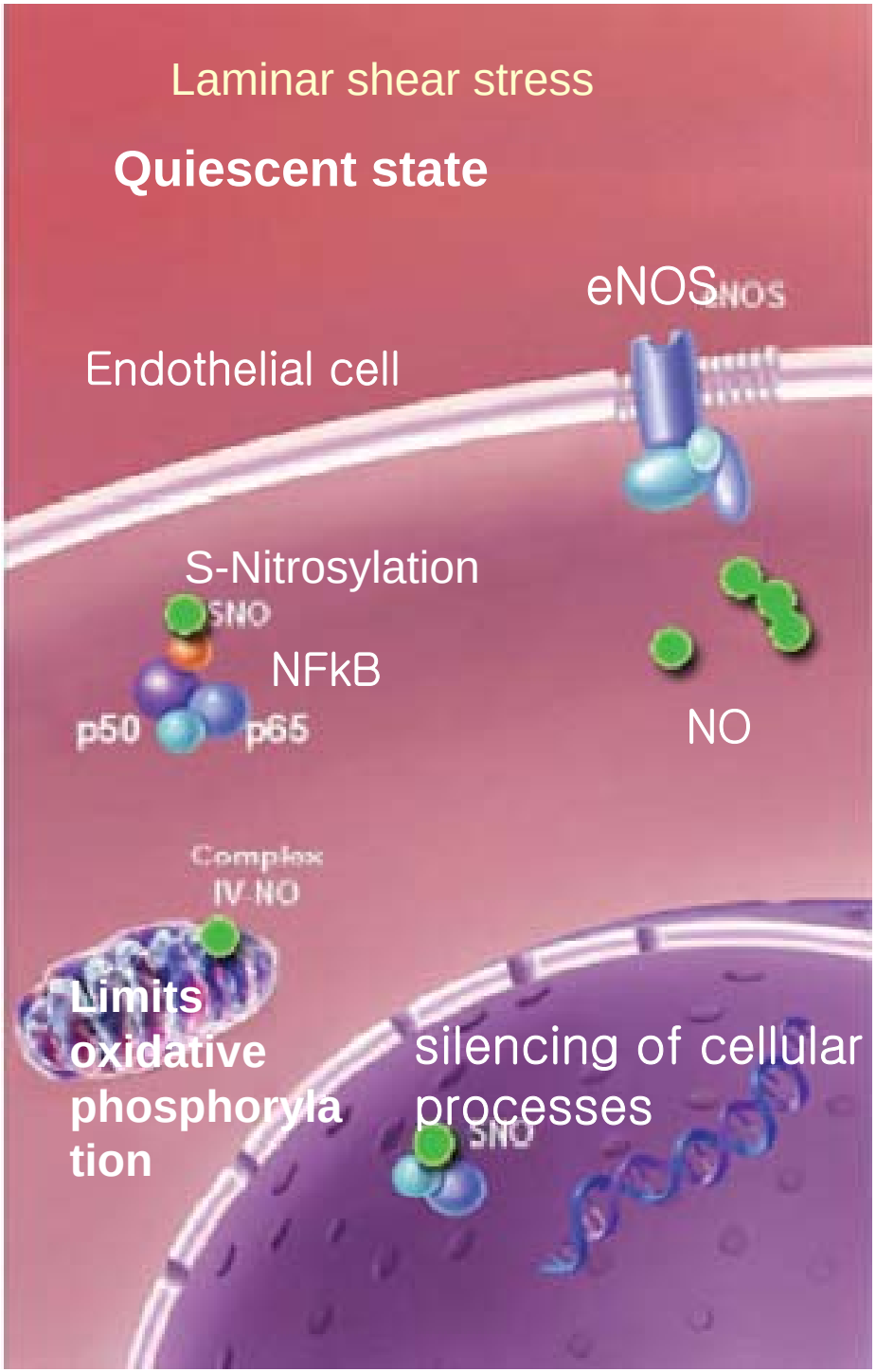
Constrictor tone



Moncada S et al. Lancet. 1977;1:18–20

Saye JA et al. Hypertension. 1984;6:216–221

Kinlay S et al. Circulation. 2001;104:1114–1118



In normal vascular physiology

"Quiescent state"

NO → Inhibit → Cell proliferation, Inflammation, Thrombosis

S-Nitrosylation of cystein residue of

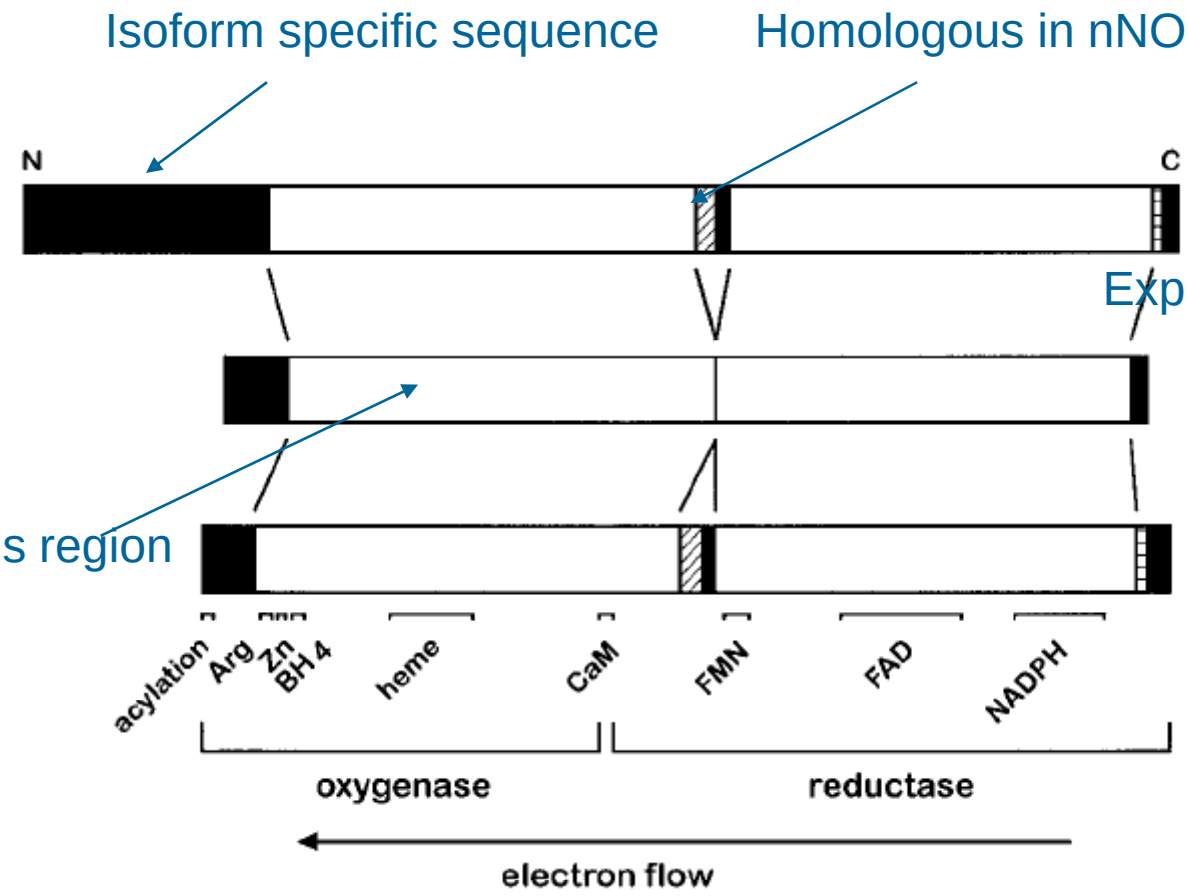
NF κ B,

cell cycle controlling proteins,

proteins involved in generation of tissue factor

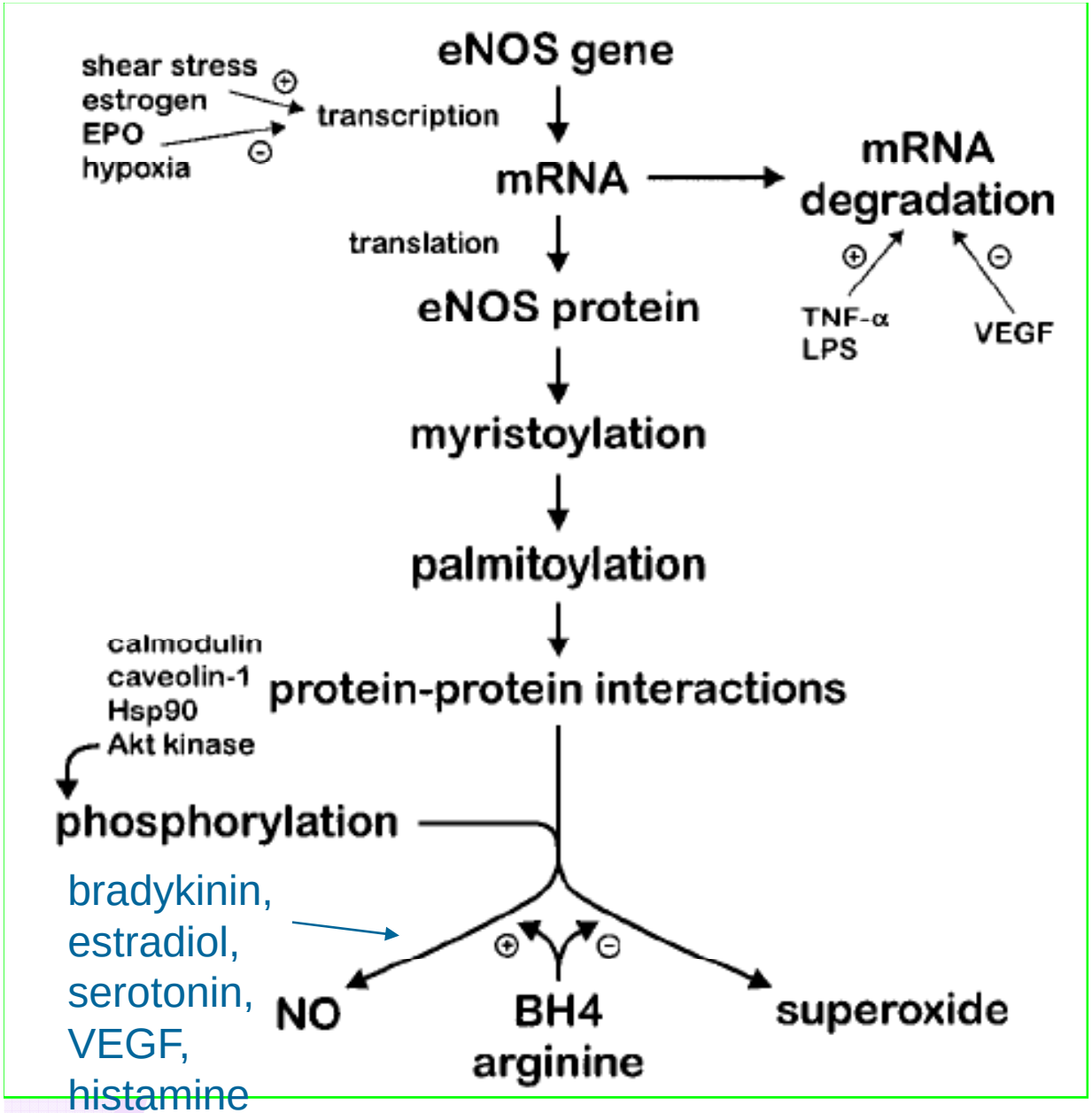
Deanfield JE et al. Circulation 2007;115:1285–95

Nitric Oxide Synthase (NOS) isoforms



Govers R et al. Am J Physiol Renal Physiol. 2001;280:F193–F206

Endothelial NOS



Arginine and cofactor BH4

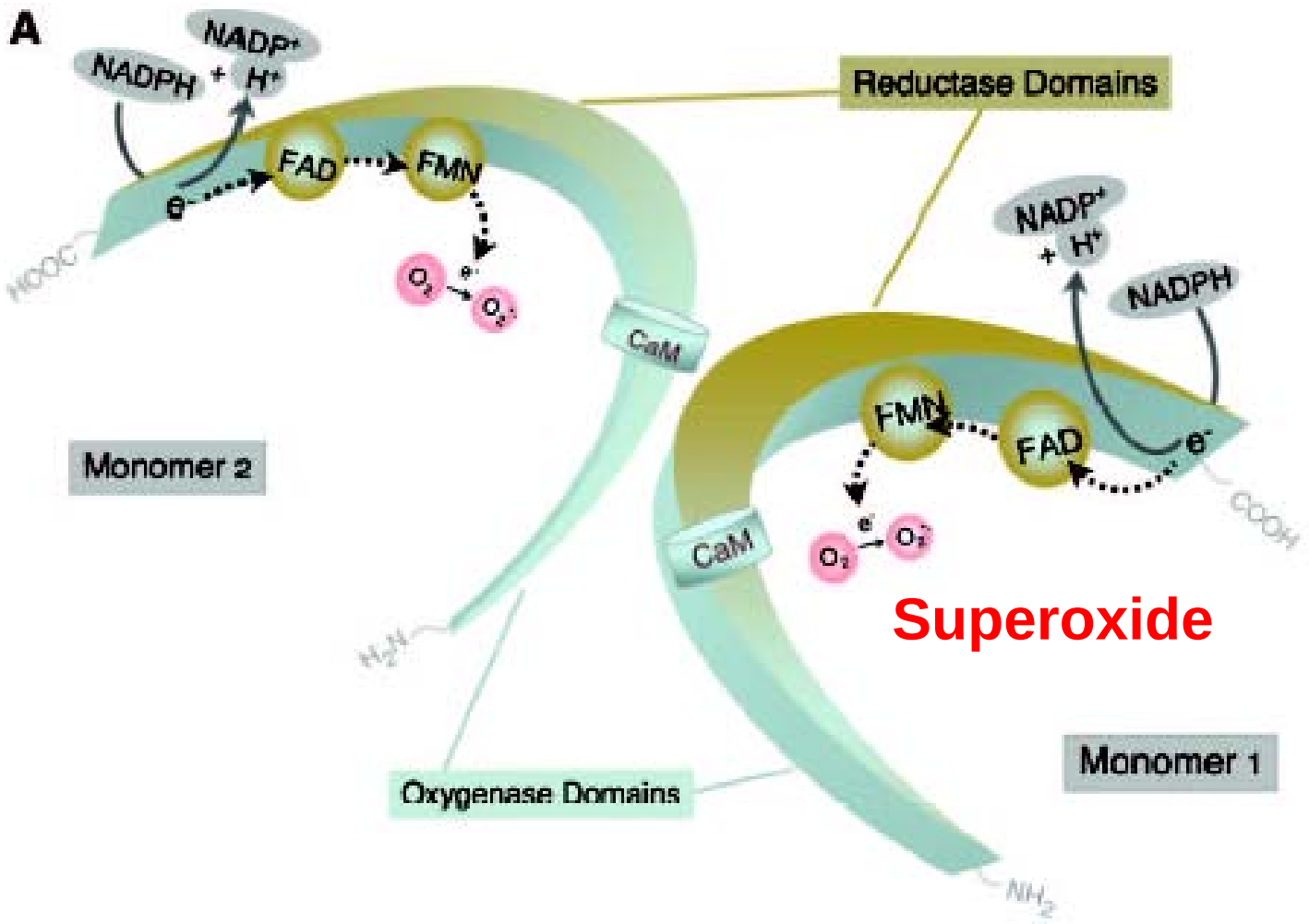
↓

eNOS

producing NO or Superoxide

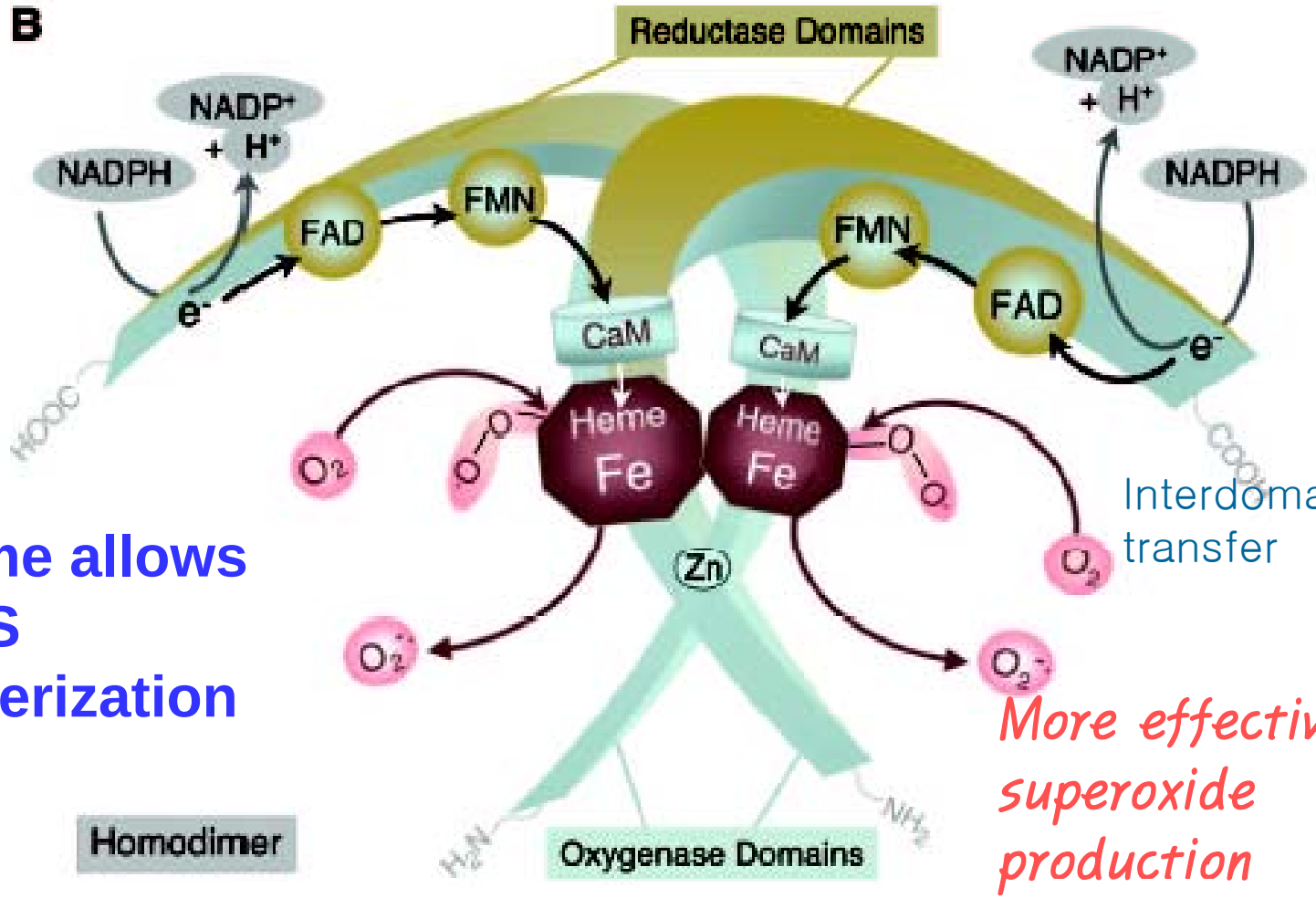
Govers R et al. Am J Physiol Renal Physiol. 2001;280:F193–F206

Monomers are unable to bind tetrahydrobiopterin (BH4) or l-arginine and cannot catalyze NO production.



Forstermann U et al. Circulation. 2006;113:1708–1714

Heme containing substrate free NOS dimers.



Heme allows NOS dimerization

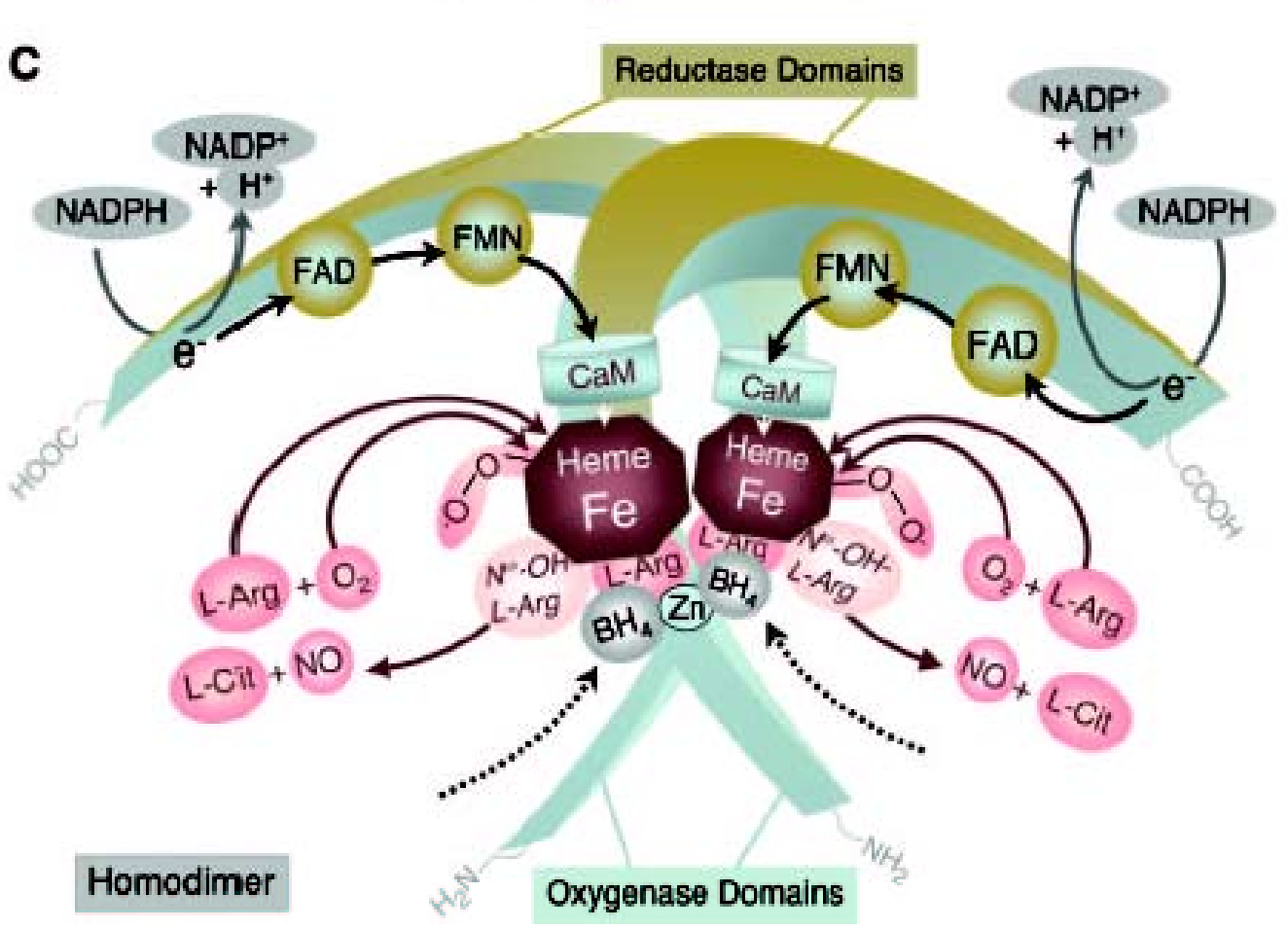
Interdomain electron transfer

More effective superoxide production

Forstermann U et al. Circulation. 2006;113:1708–1714

Sufficient L-arginine and BH4

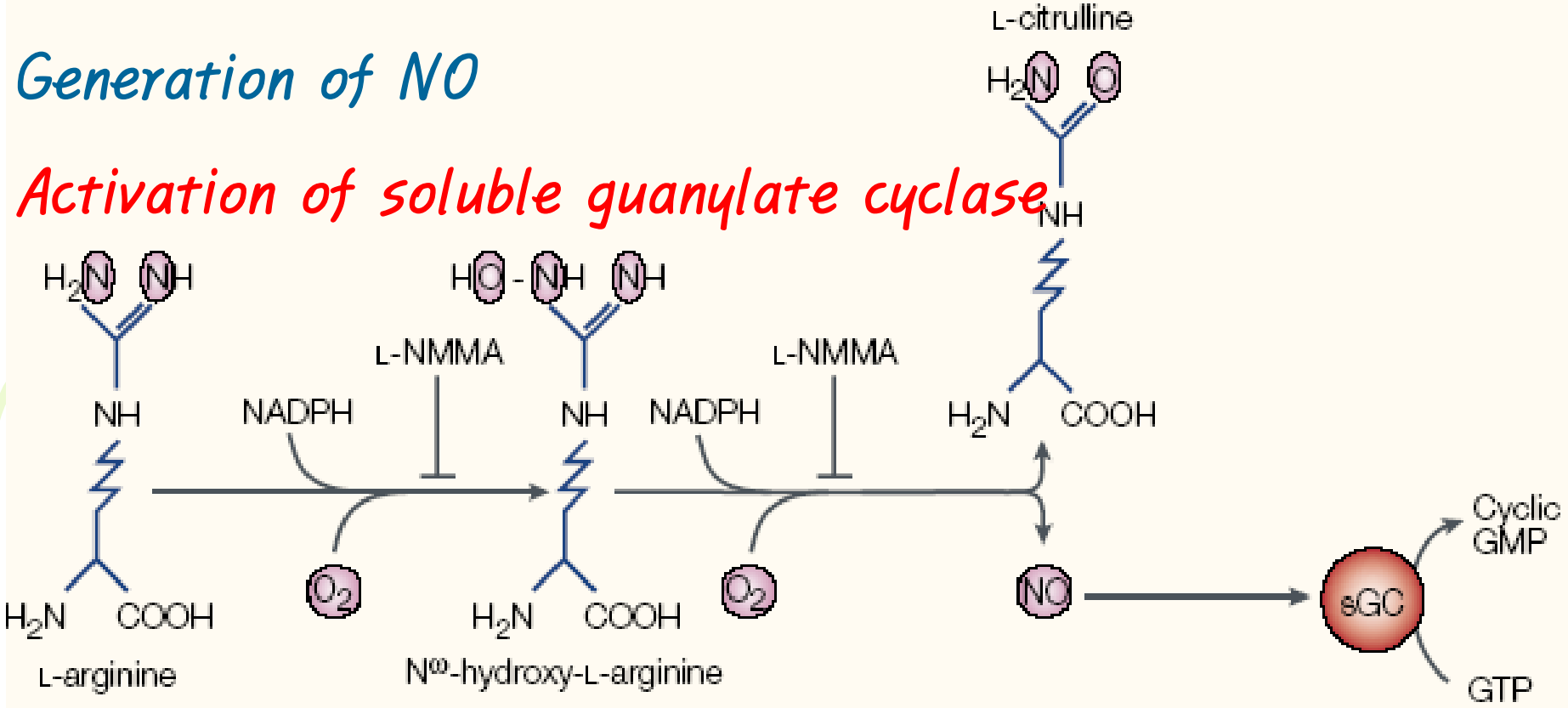
Intact NOS dimers couple heme and O2 reduction to the synthesis of NO.



Forstermann U et al. Circulation. 2006;113:1708–1714

Generation of NO

Activation of soluble guanylate cyclase



cGMP dependent protein kinase or cGMP gated ion channels

Vasodilation,

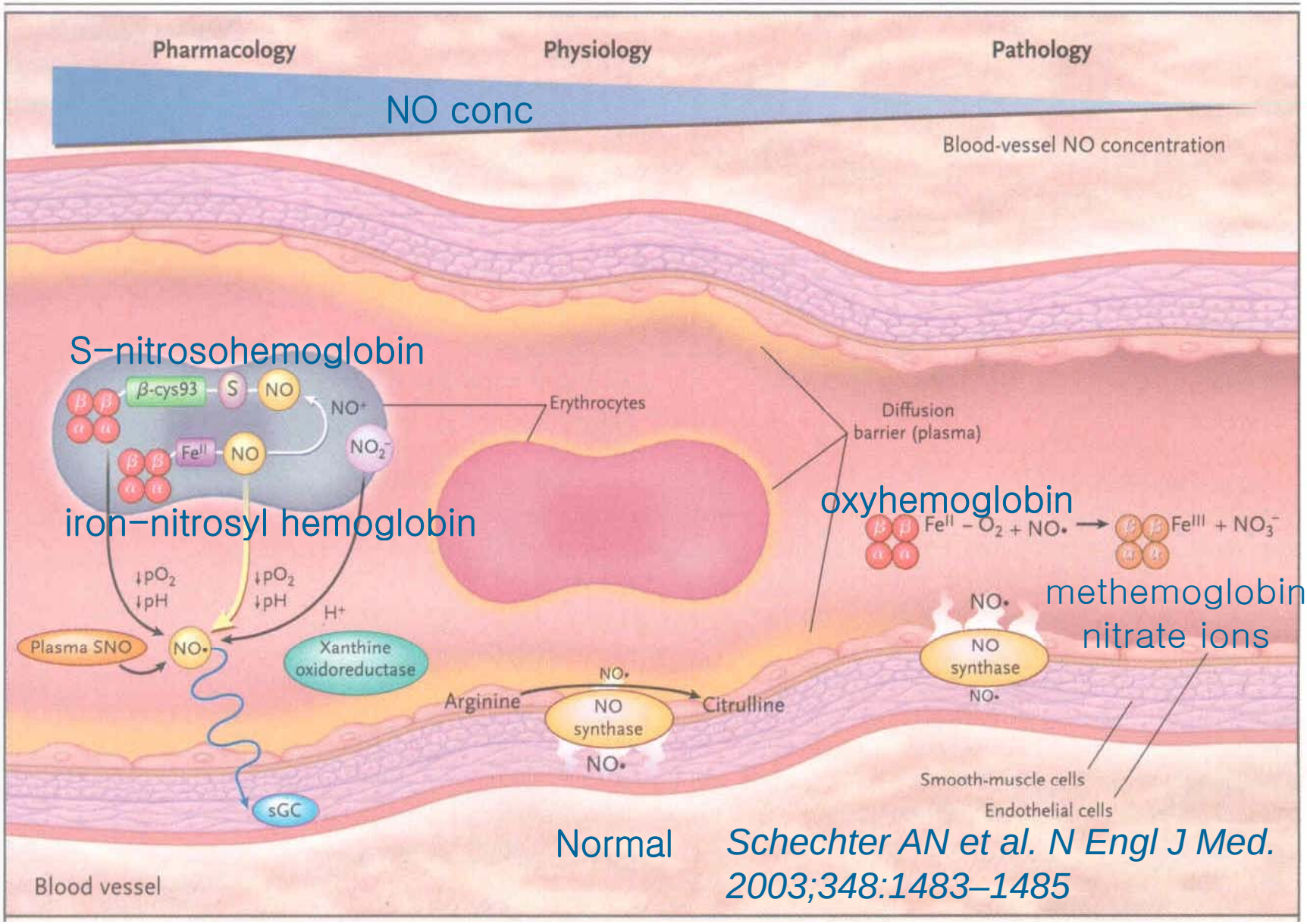
Inhibition of platelet and white cell activation

Peripheral and central neurotransmission,

Immunological modulation

Moncada S et al. Nat Rev Mol Cell Biol. 2002;3:214–220

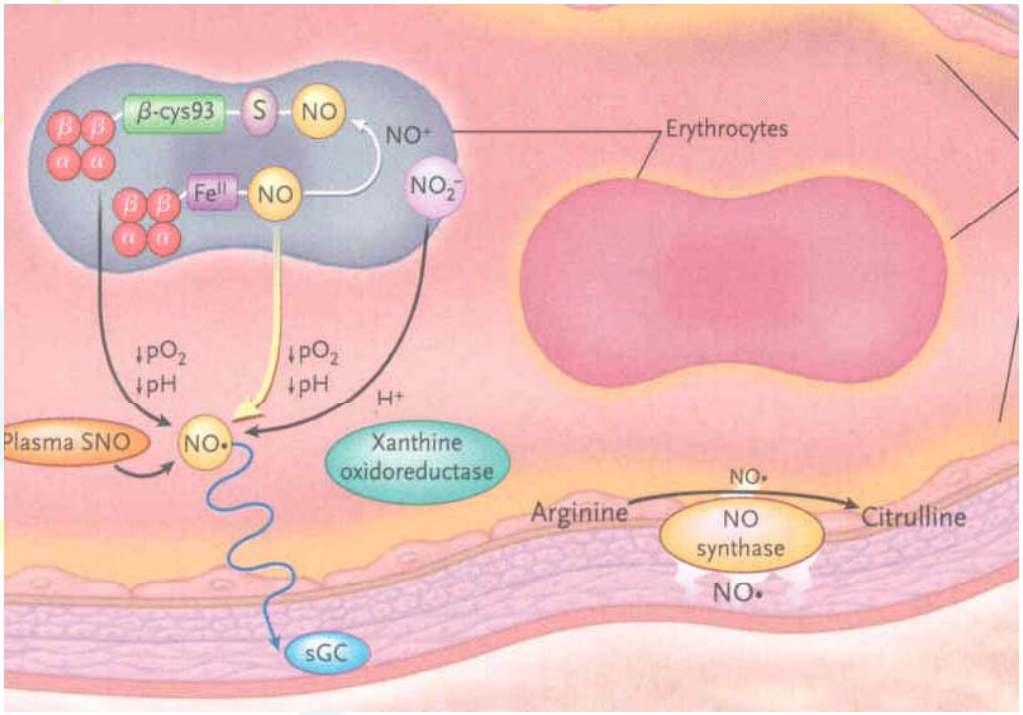
Hemoglobin as a Destroyer or Carrier of NO



Schechter AN et al. N Engl J Med. 2003;348:1483–1485

Shear stress, Key activator of eNOS

Adapts organ perfusion to changes in cardiac output.



Shear stress

↓

Phosphorylation of eNOS

↓

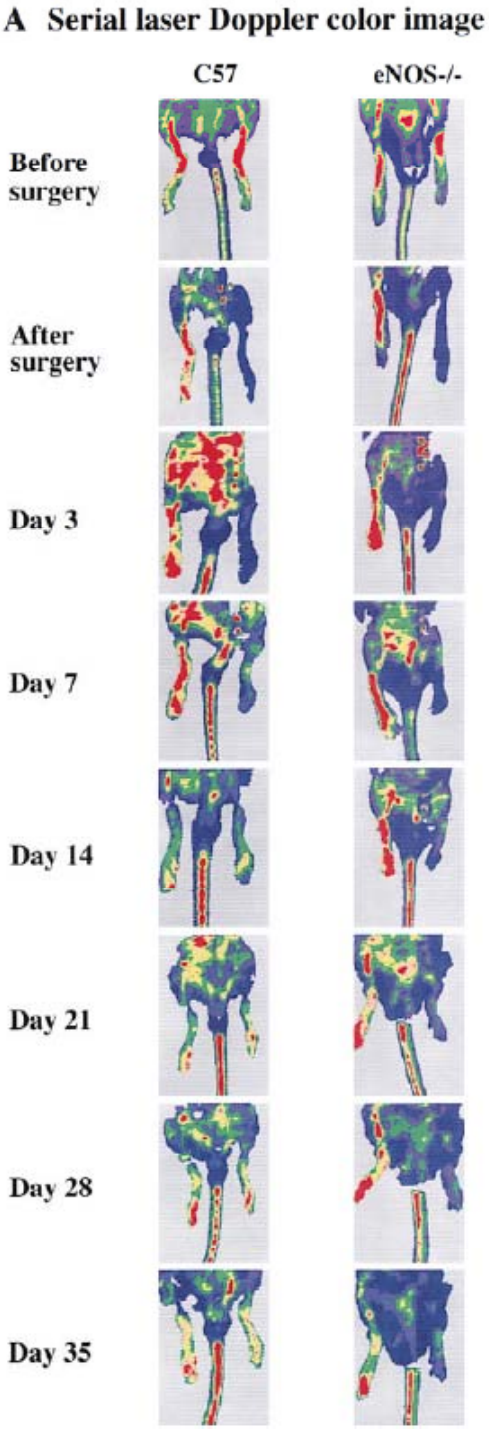
Increase NO production

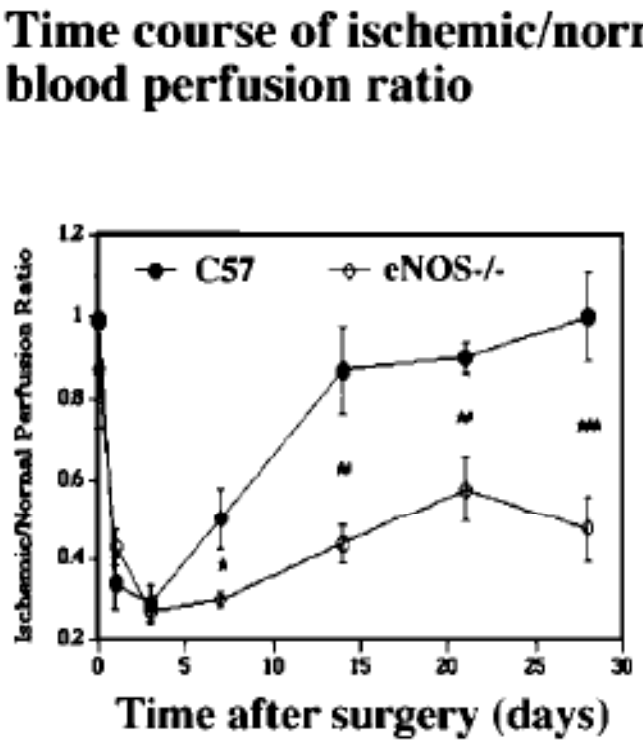
cGMP mediated vasodilatation

Corson MA et al. Circ Res. 1996;79:984–991

NOS modulates Angiogenesis

eNOS -/- mice

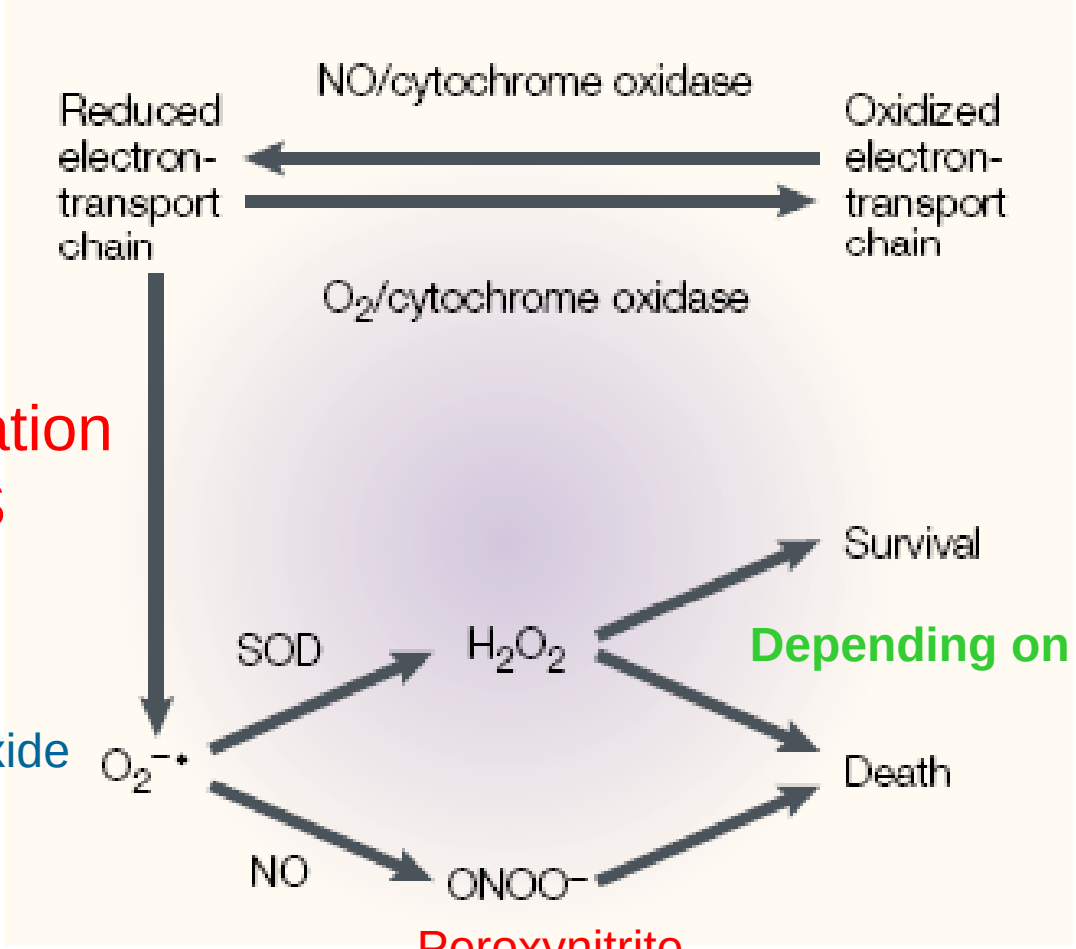




Murohara T et al. J Clin Invest. 1998;101:2567–2578

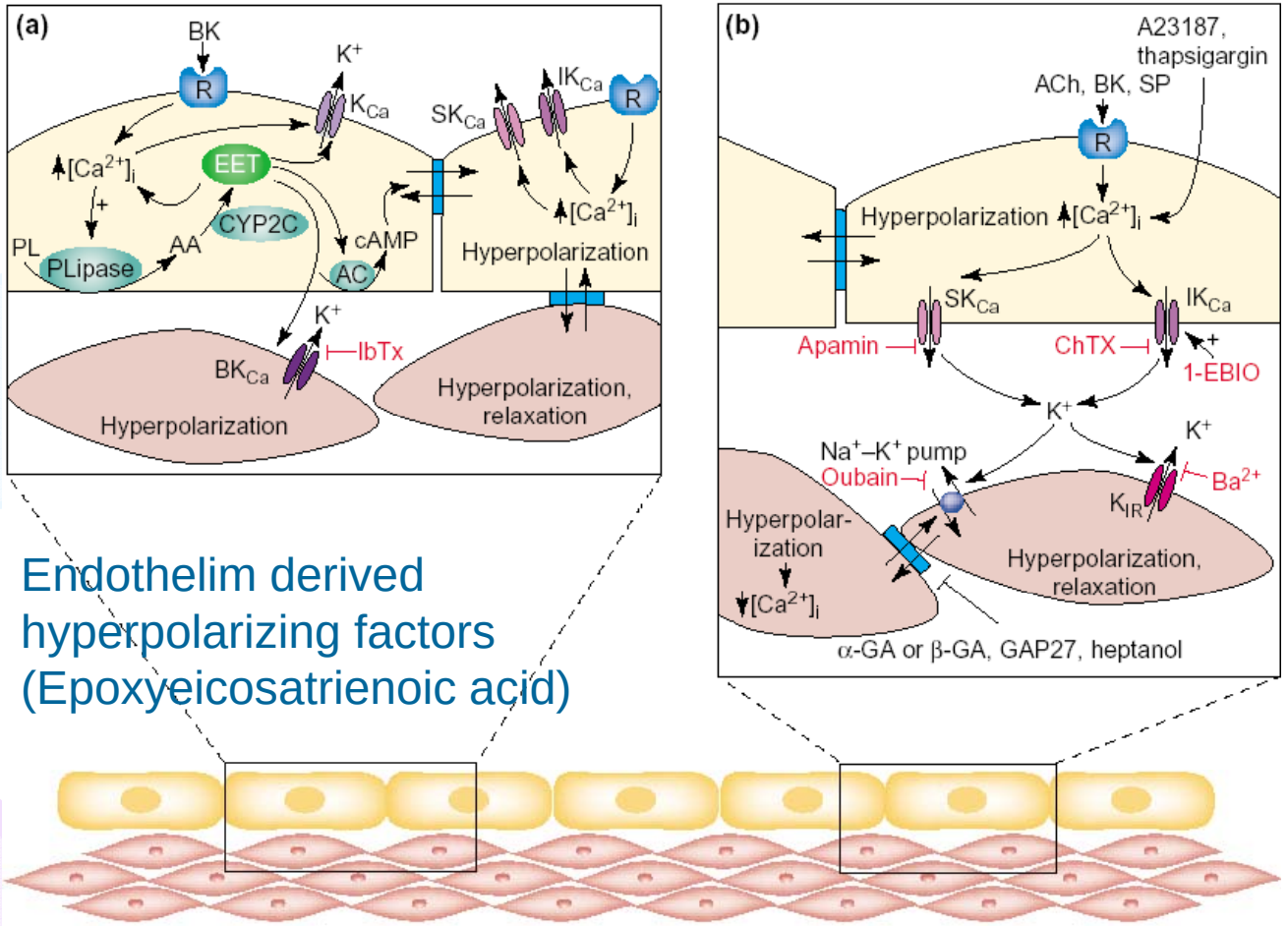
NO trigger Cell Survival or Death

Reduced supply of oxygen → **NO inhibit cytochrome oxidase**



Moncada S et al. Nat Rev Mol Cell Biol. 2002;3:214–220

Hyperpolarization of vascular smooth muscle cells
NO-independent pathway



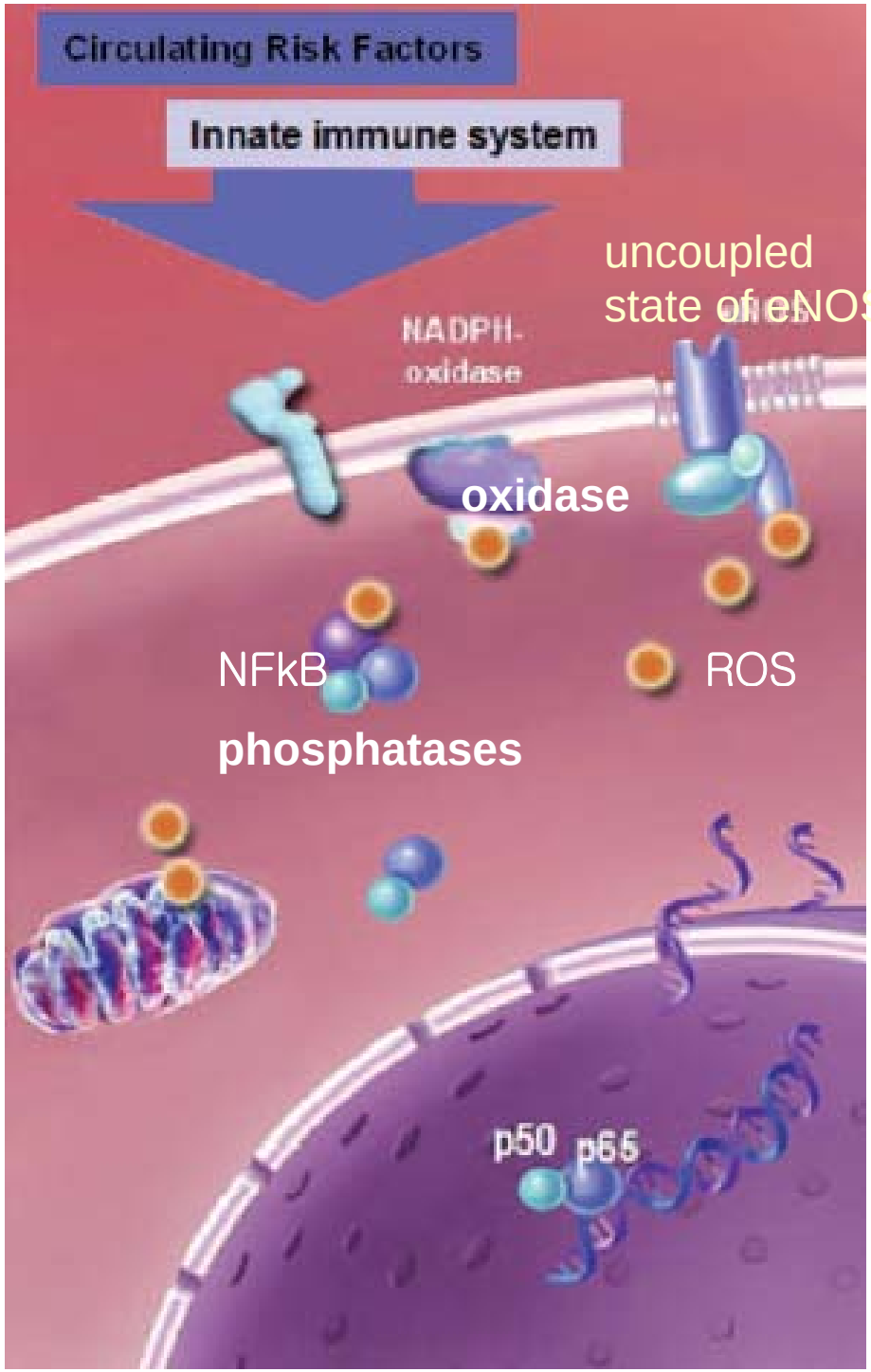
Endothelim derived hyperpolarizing factors (Epoxyeicosatrienoic acid)

increases potassium conductance

propagation of depolarization

maintain vasodilator tone

Busse R et al. Trends Pharmacol Sci. 2002;3:374–380



Oxidases
Uncoupled eNOS

↓

ROS

↓

Regulatory proteins

(NFkB, phosphatases)

↓

Expression of
Chemokines, Cytokines,
Adhesion molecules

↓

Endothelial Dysfunction
(Activation)

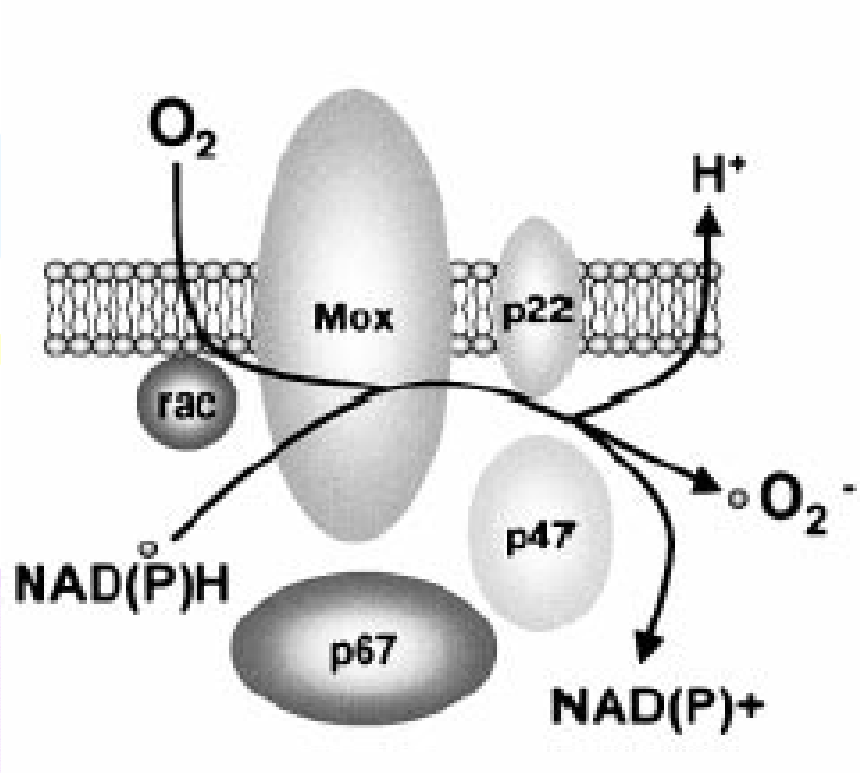
Deanfield JE et al. Circulation 2007;115:1285–95

NAD(P)H Oxidase

Role in Cardiovascular Biology and Disease

Kathy K. Griendling, Dan Sorescu, Masuko Ushio-Fukai

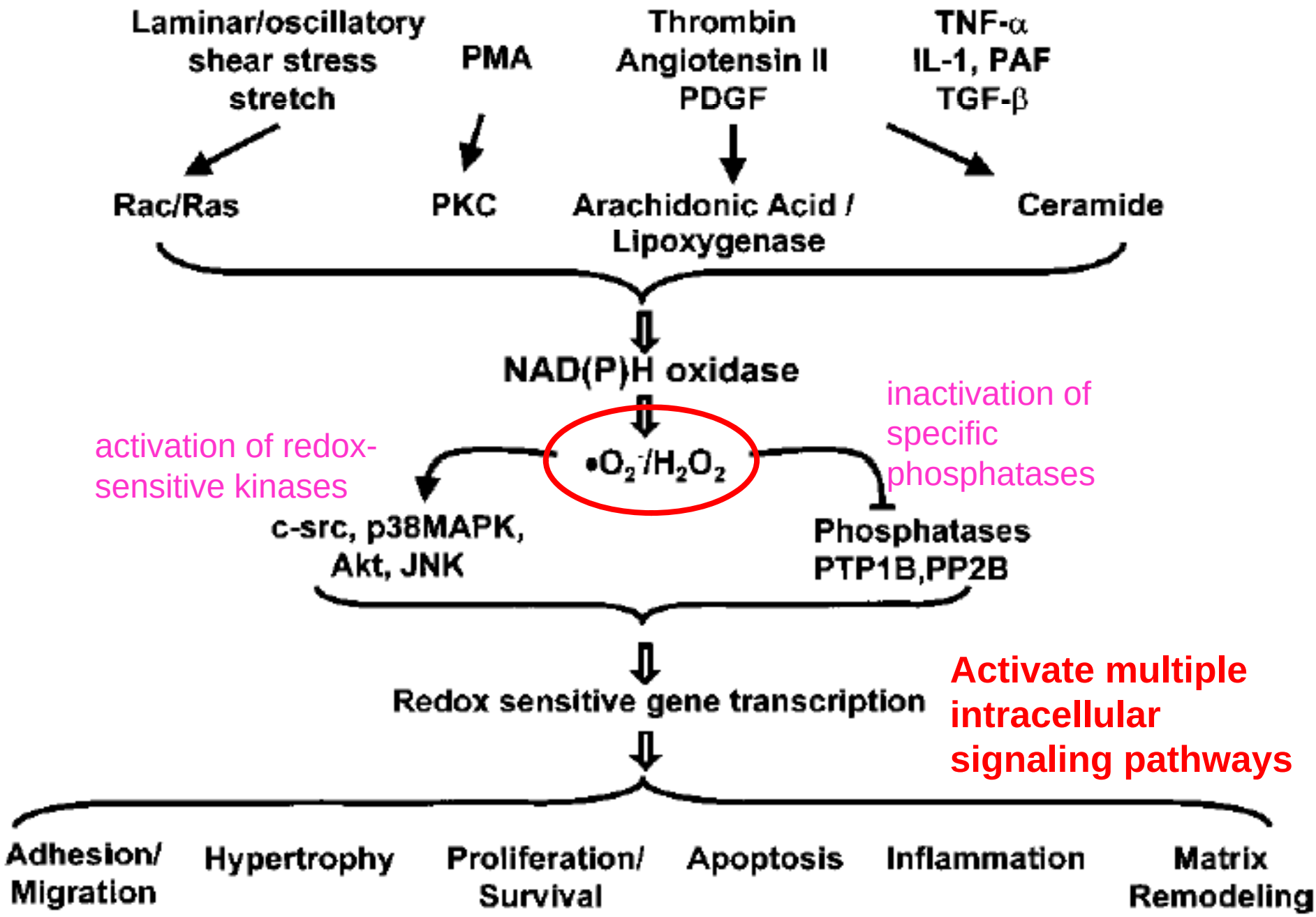
Circ Res. 2000;86:494–501



NADP oxidases

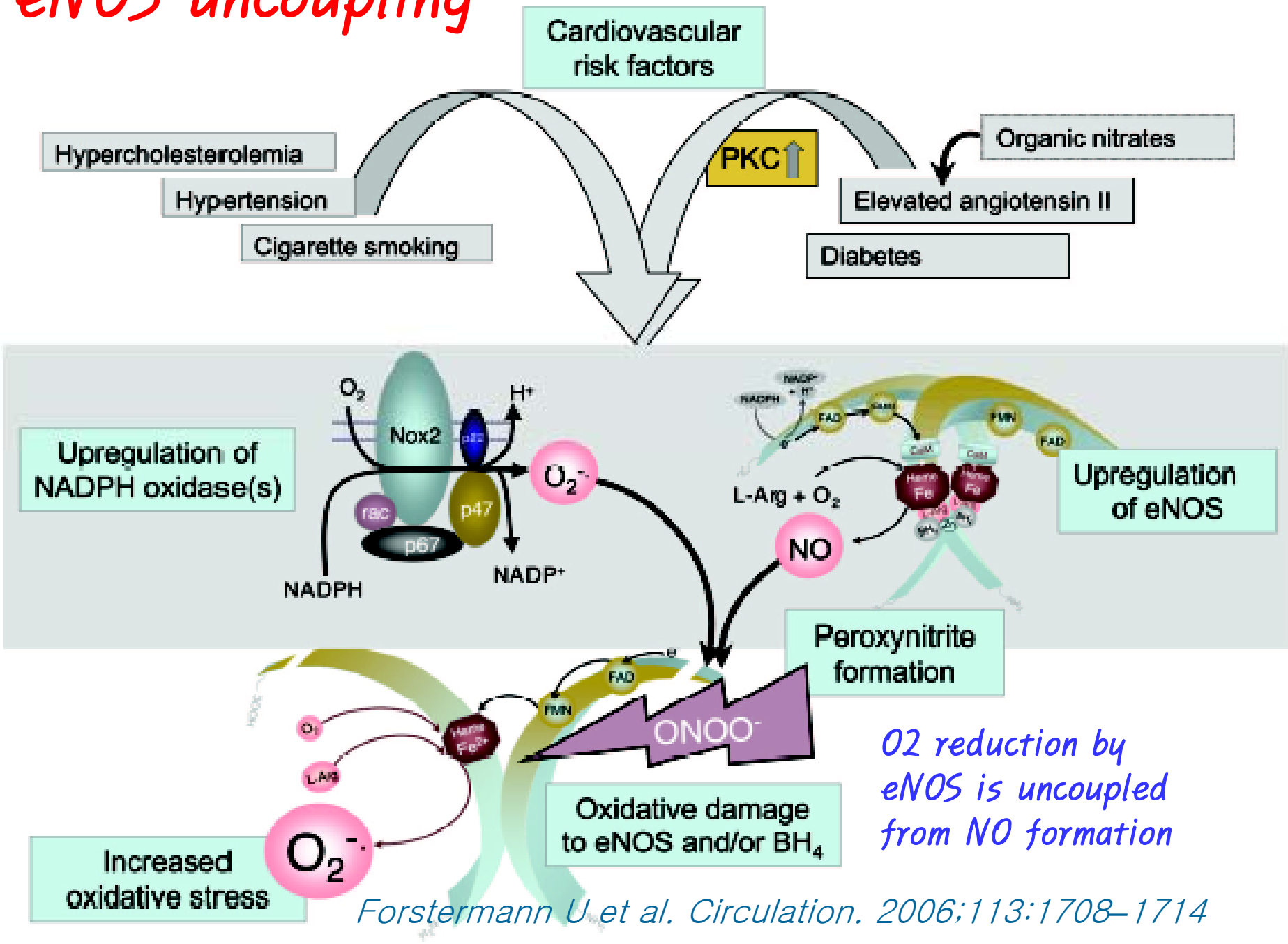
Xanthine oxidase

2 major **Superoxide Anion** producing enzyme systems.



Oxidase activation and consequences in CV cells *Circ Res. 2000;86:494–501*

eNOS uncoupling



O2 reduction by eNOS is uncoupled from NO formation

Forstermann U et al. Circulation. 2006;113:1708–1714

Activation by redox signaling

Protection

Inactivate peroxiredoxin

Cell signaling

Inactivating protein tyrosine phosphatases

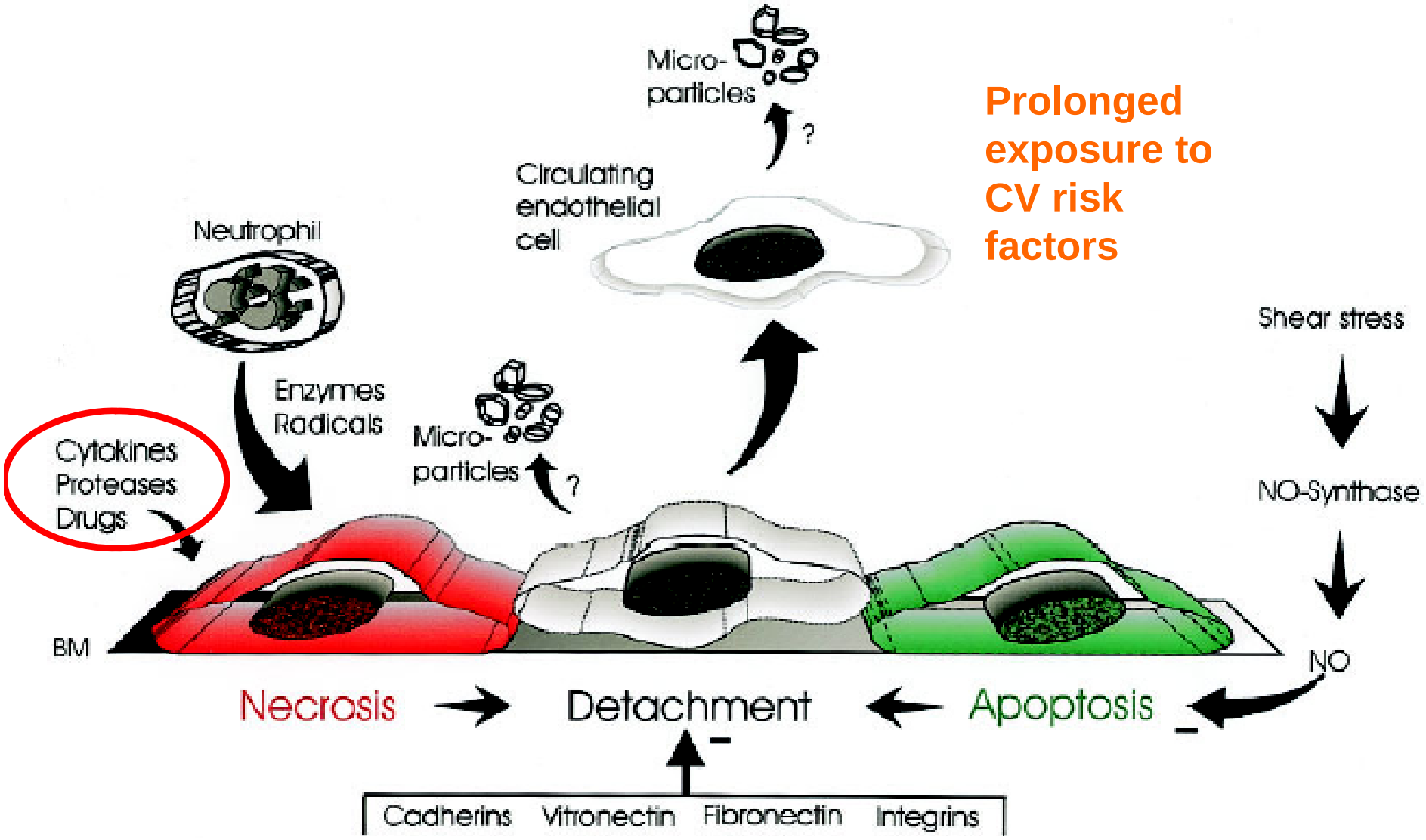
Activating protein tyrosine kinase

Phosphorylation of transcription factors

Induction of nuclear chromatin remodeling and transcription gene

Protease activation.

Rhee SG. Science. 2006;312:1882–1883



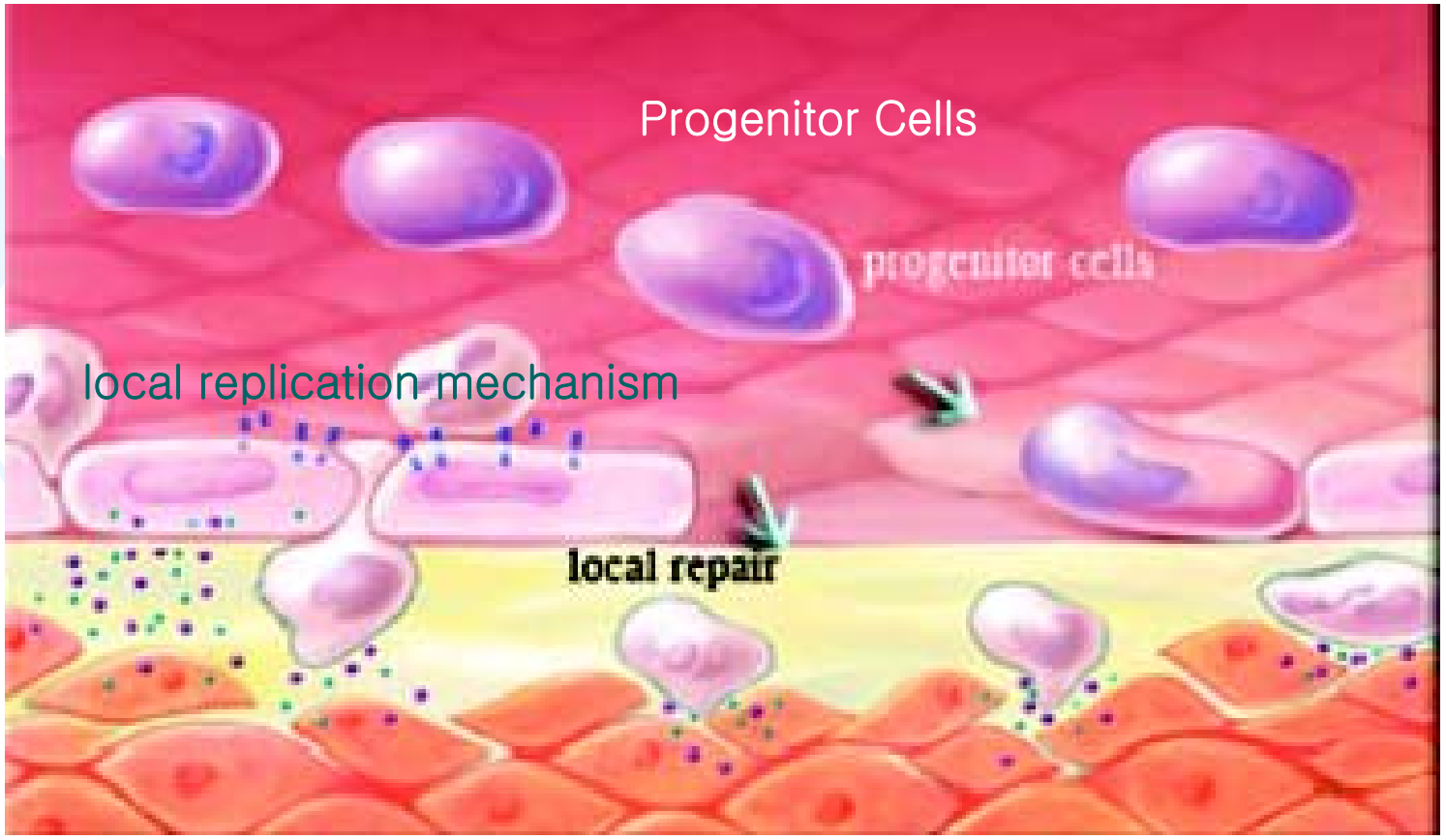
Prolonged exposure to CV risk factors

Fig. 1. Endothelial necrosis or apoptosis, and detachment. BM, basement membrane; NO, nitric oxide.

Woywodt A et al. Nephrol Dial Transplant. 2002;17:1728–1730

Re-endothelialisation

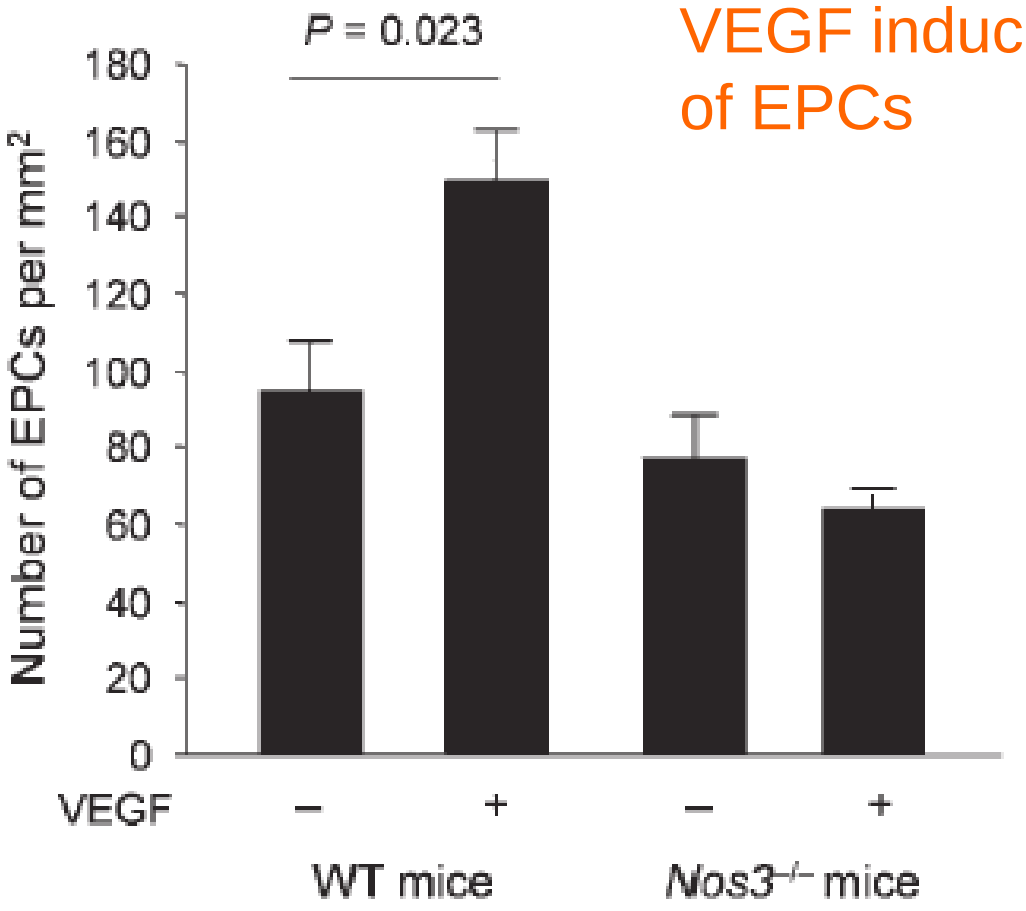
Bone marrow derived Progenitor Cells



Deanfield JE et al. Circulation 2007;115:1285–95

Incorporation of circulating progenitor cells

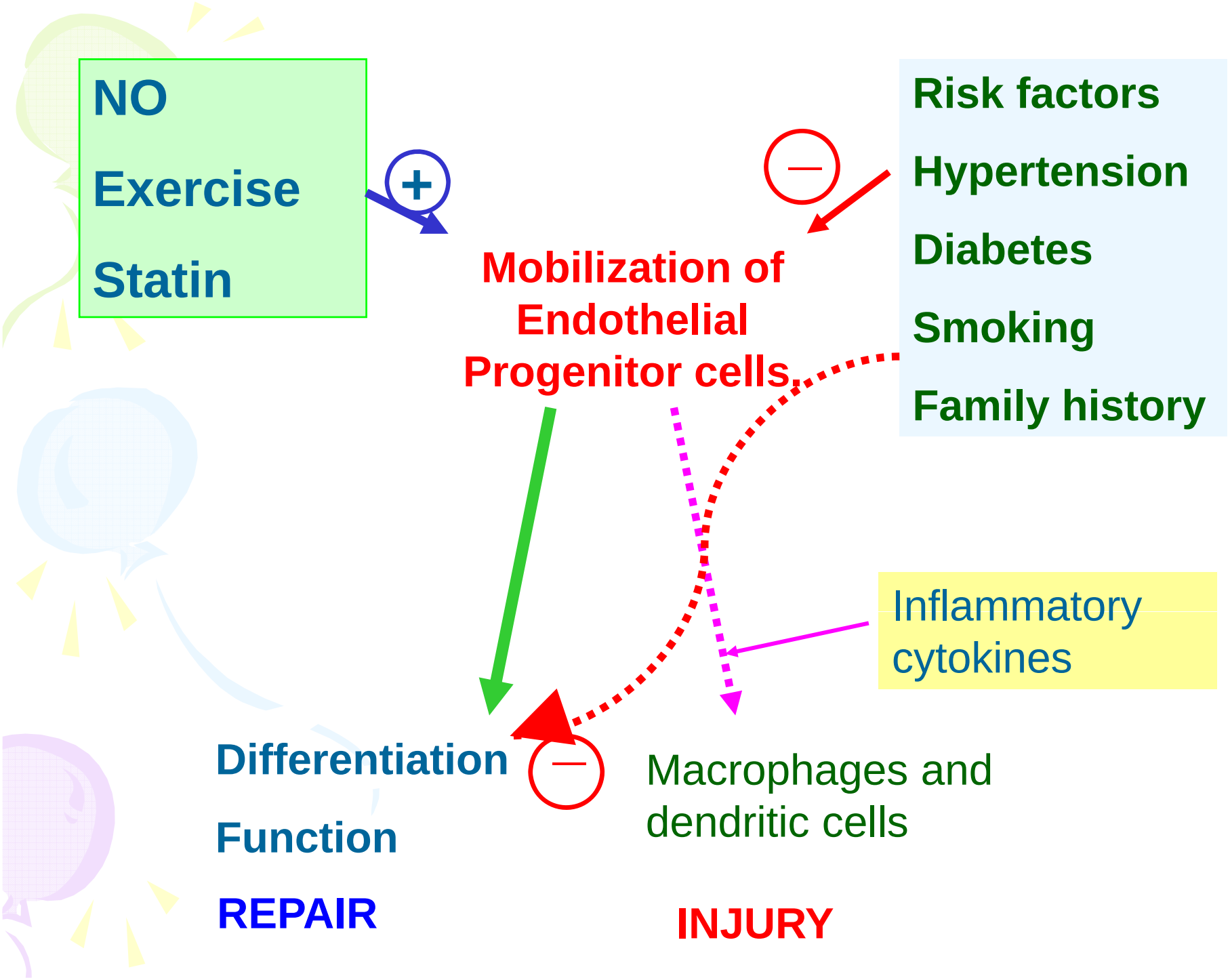
eNOS for Mobilization of Progenitor cells



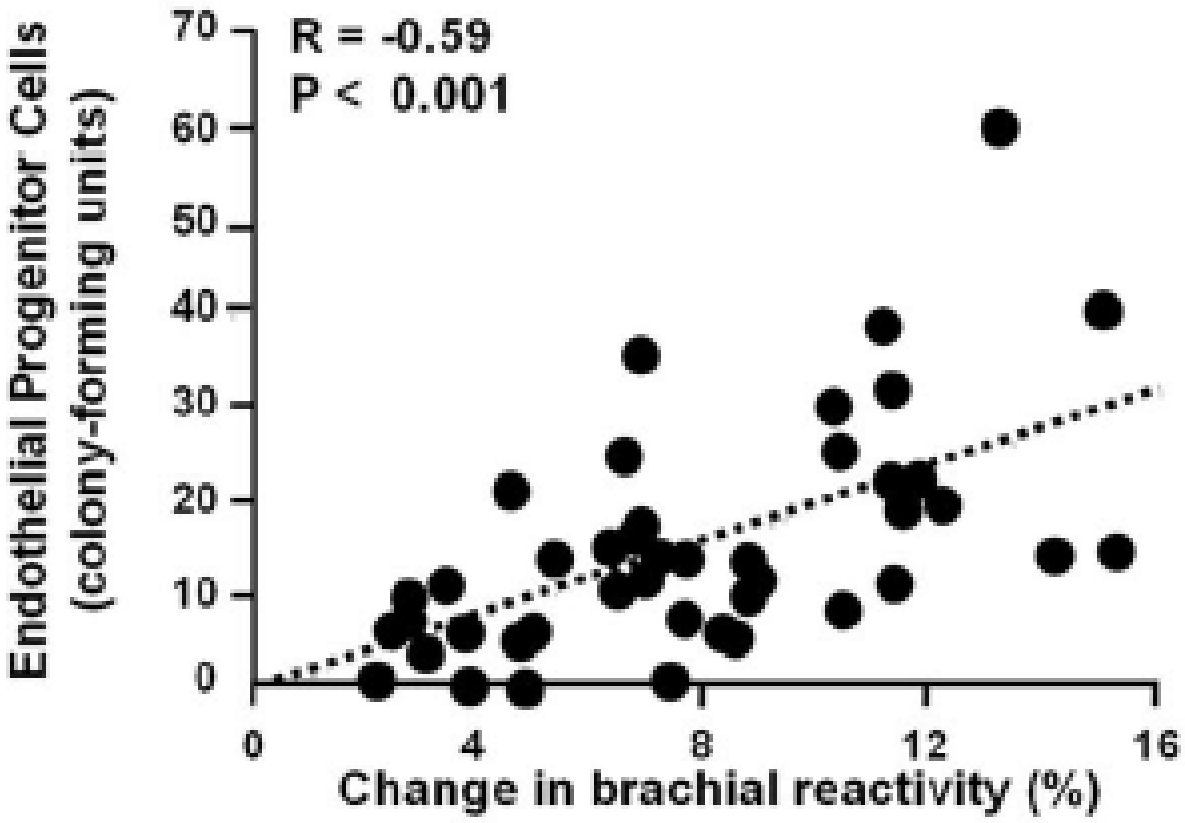
VEGF induced mobilization of EPCs

Impaired in patients with CV risk factors

Aicher A et al. Nat Med. 2003;9:1370–1376


NO
Exercise
Statin
+
Risk factors
Hypertension
Diabetes
Smoking
Family history
Mobilization of
Endothelial
Progenitor cells
Inflammatory
cytokines
Differentiation
Function
REPAIR
Macrophages and
dendritic cells
INJURY

Endothelial progenitor cells and FMD



in middle-aged men

Hill JM et al, N Engl J Med. 2003;348:593–600

Clinical Assessment of Endothelial Function

Evaluation of Endothelial Function

Detect early disease

Judge response to interventions

Quantify risk

Prevent progression

Reduce later adverse events

Clinical tests

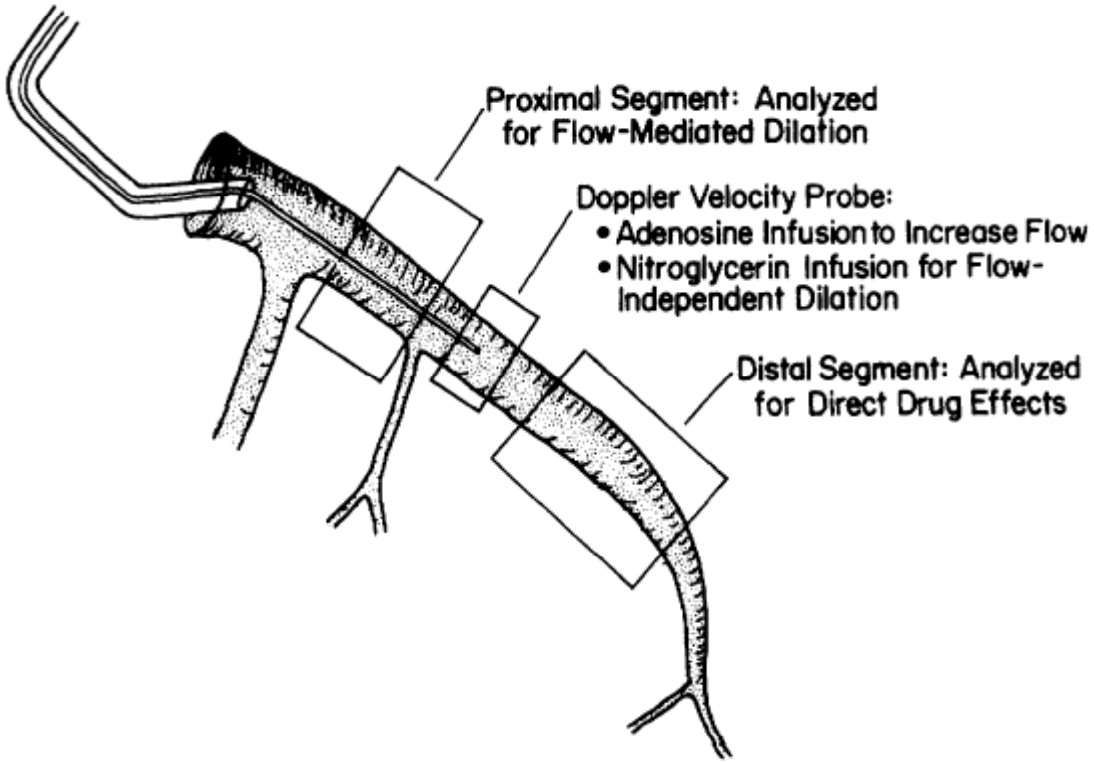
Evaluate functional properties of endothelium

- Safe
- Noninvasive
- Reproducible
- Repeatable
- Cheap
- Standardized
- Reflect dynamic biology
- Define subclinical disease processes
- Prognostic information

No single test

Flow mediated dilation of coronary arteries in humans

Cox DA et al. Circulation. 1989;80:458–465



Acetylcholine

Vessel Diameter

Coronary Angiography

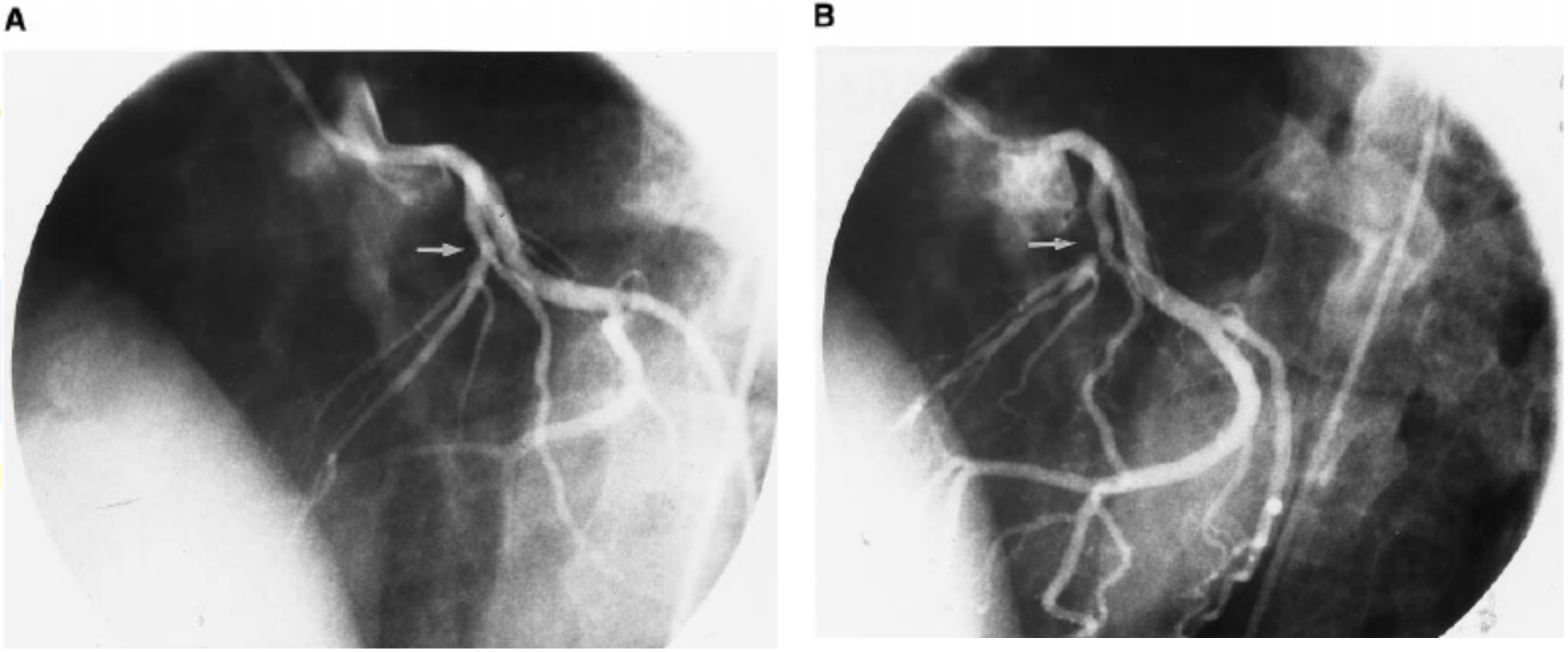
Acetylcholine ▶ Releases NO ▶ Vasodilatation.

Endothelial dysfunction

Vasoconstriction

Coronary angiogram

Endothelial function evaluation



20% diameter stenosis in mid-LAD

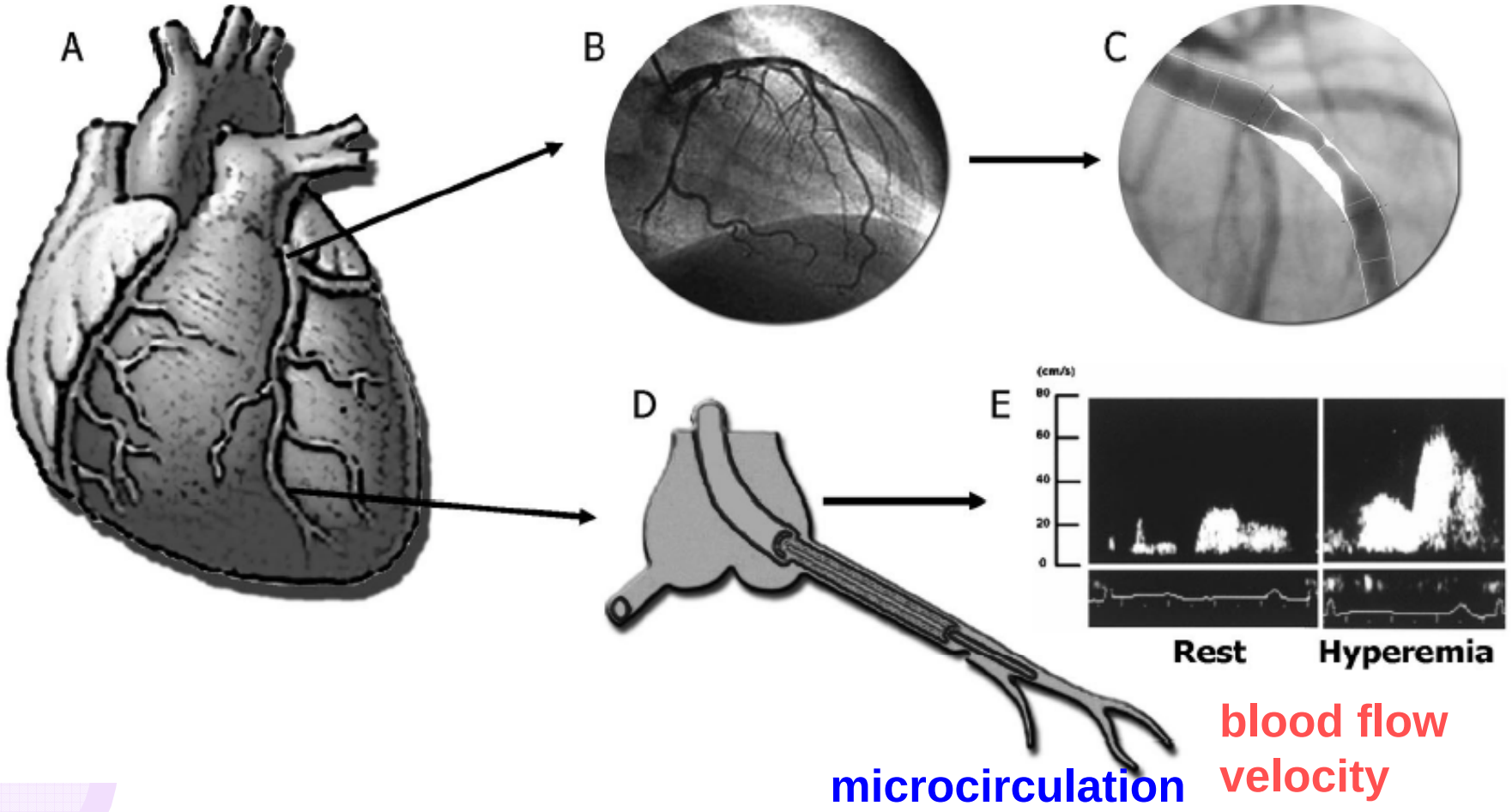
Mean percent change in CBF in response to acetylcholine was -50%.

95% diameter stenosis in mid-LAD

19 months later, progressive exertional angina and dyspnea

Suwaidi JA et al. Circulation. 2000;101:948–954

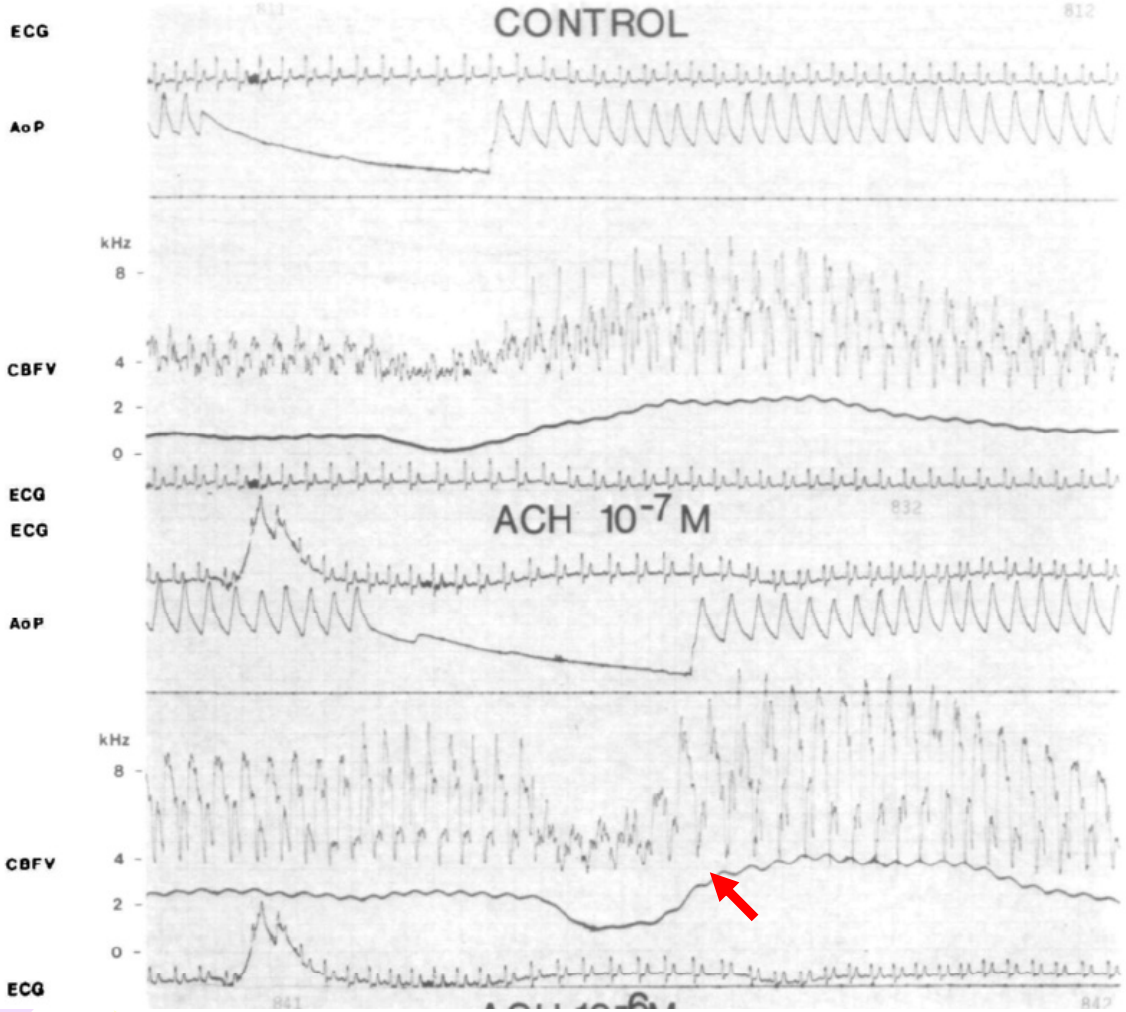
Methods for assessing endothelial regulation of vascular tone in coronary artery



Barac A et al. Hypertension. 2007;49:748-60

Doppler flow wires in coronary arteries

Resistance vessel function



Intracoronary infusion of ACH

Doppler catheter in LAD

Dilation of microcirculation

Coronary blood flow velocity

Drexler H et al. Hypertension. 1991;18:II90–II99

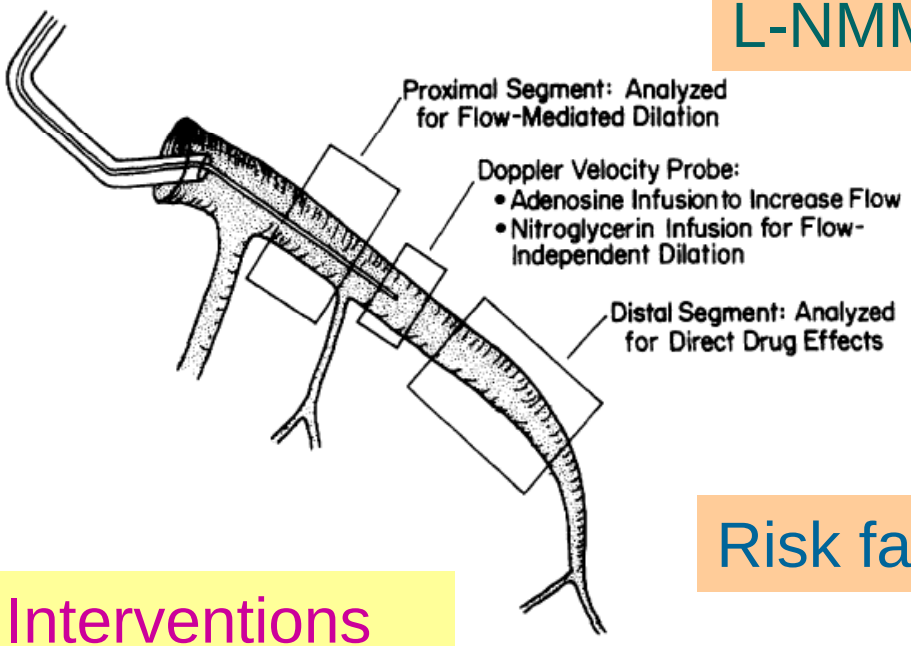
Invasive!

Endothelial agonists

Substance P

Adenosine

Bradykinin



Antagonists

L-NMMA

Risk factors

Interventions

Statins

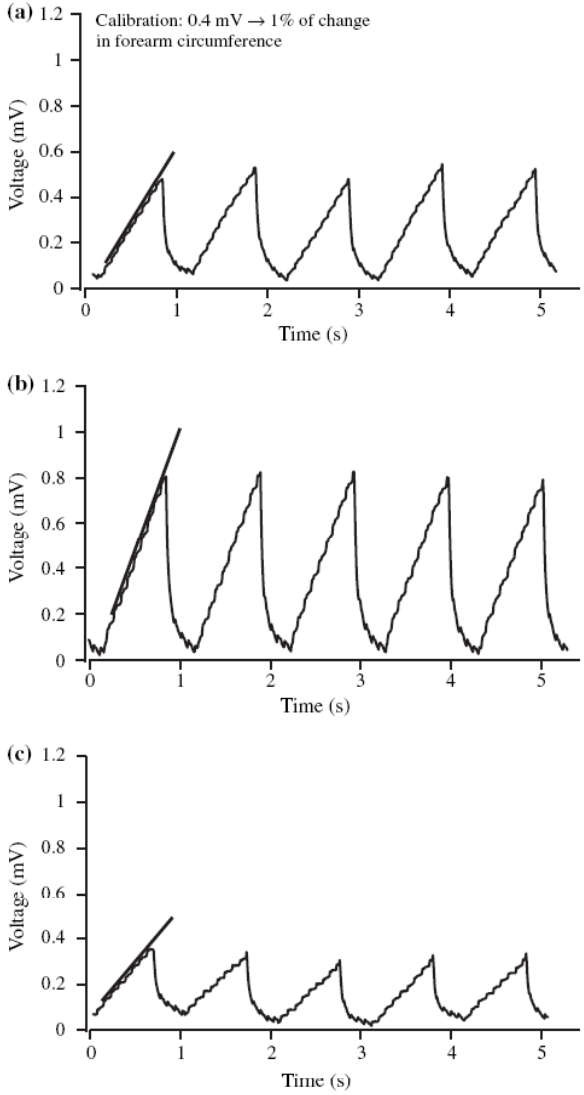
ACEi

Venous occlusion plethysmography

Local infusion of pharmacological probes

Forearm Resistance Vessel Tone

Correlation between acetylcholine responses in coronary circulation and forearm



Joannides R et al. Fundam Clin Pharmacol. 2006;20:311–320

Venous occlusion plethysmography

Invasive

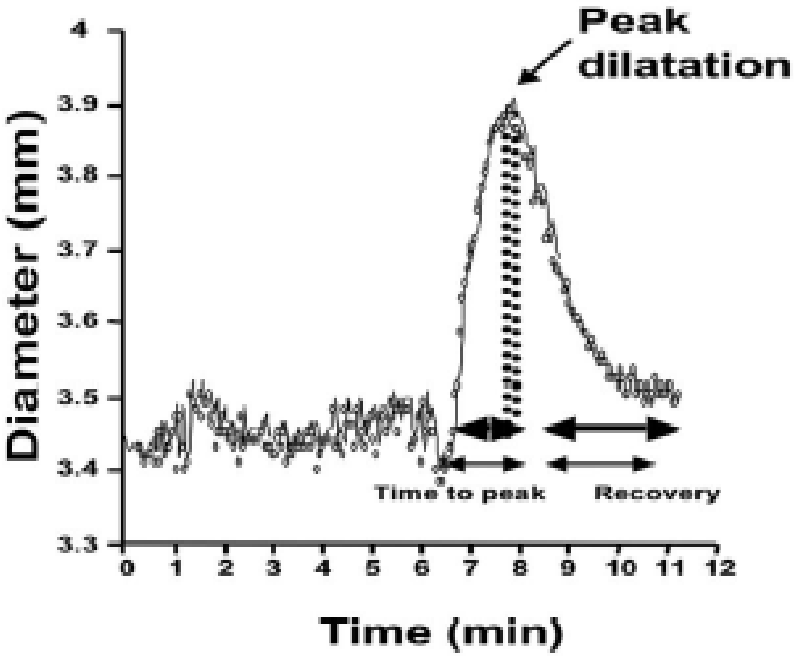
Requires arterial cannulation

Difficult to standardize

Joannides R et al. Fundam Clin Pharmacol. 2006;20:311–320

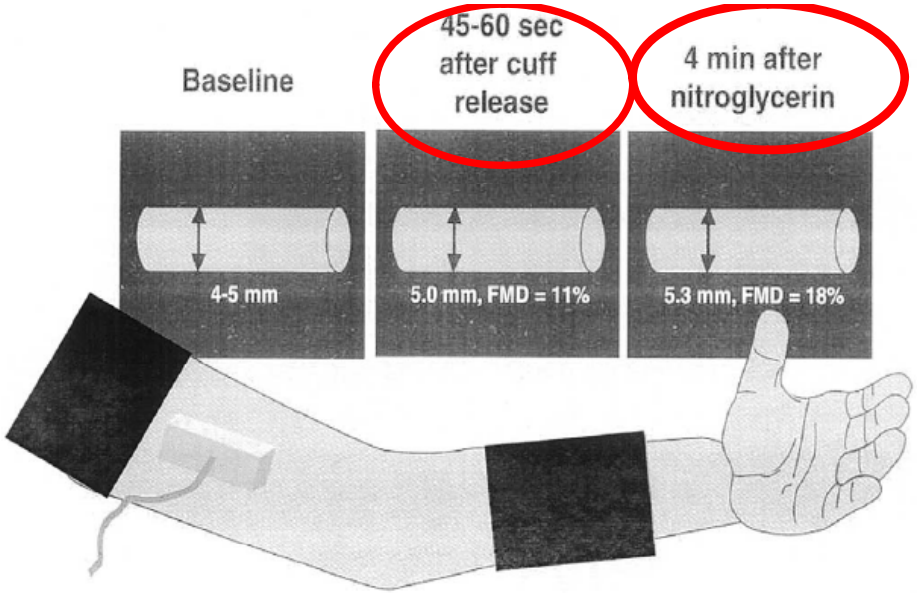
Flow Mediated Vasodilatation (FMD)
Brachial artery

60s after release of occlusive cuff

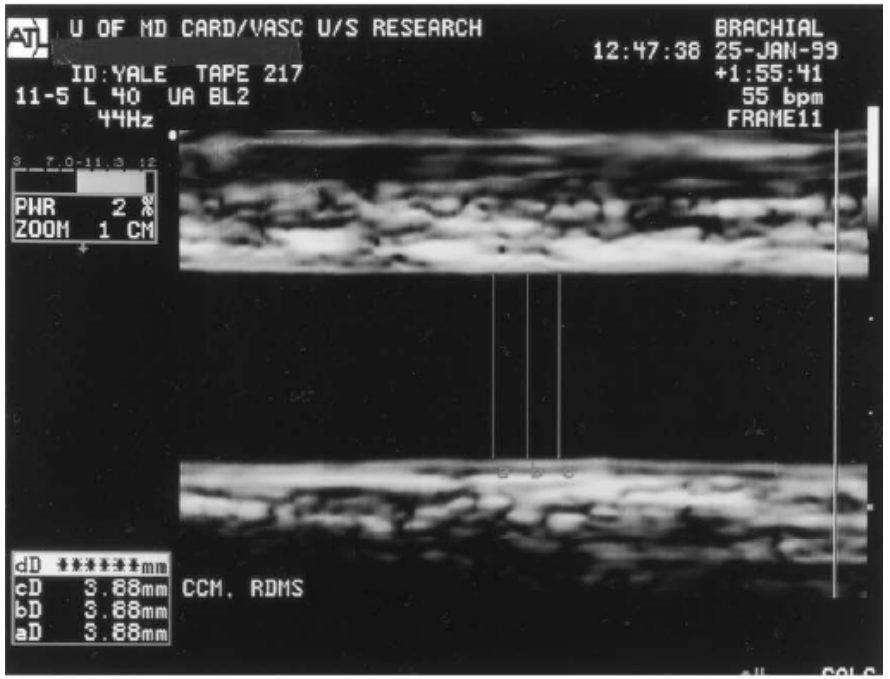


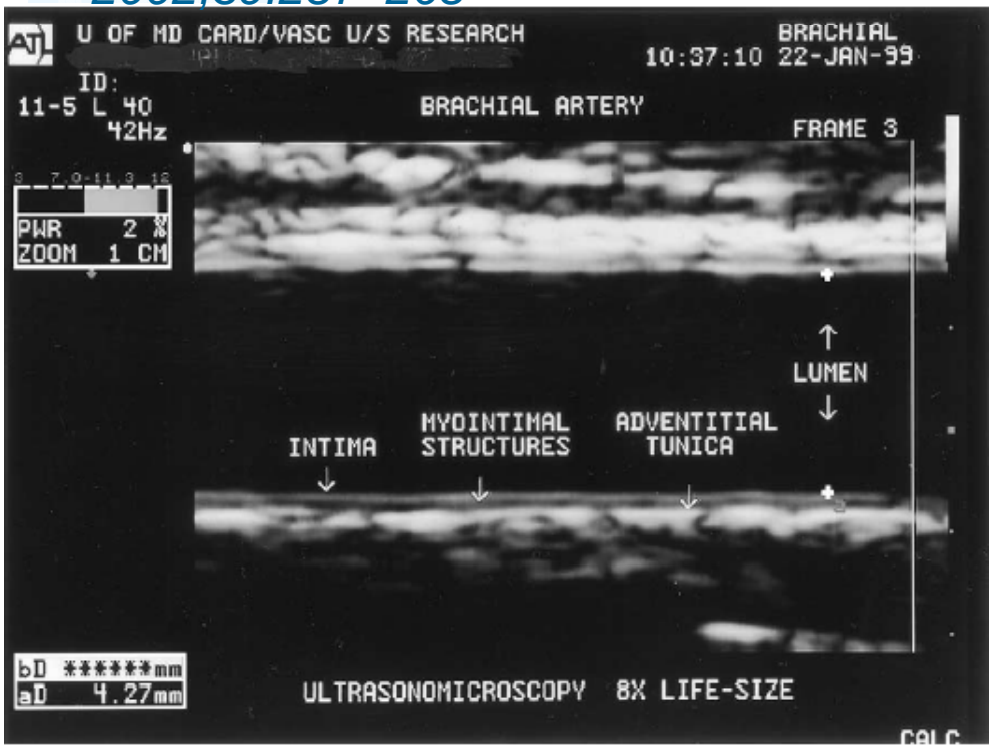
Brachial artery diameter

Deanfield JE et al. Circulation 2007;115:1285–95

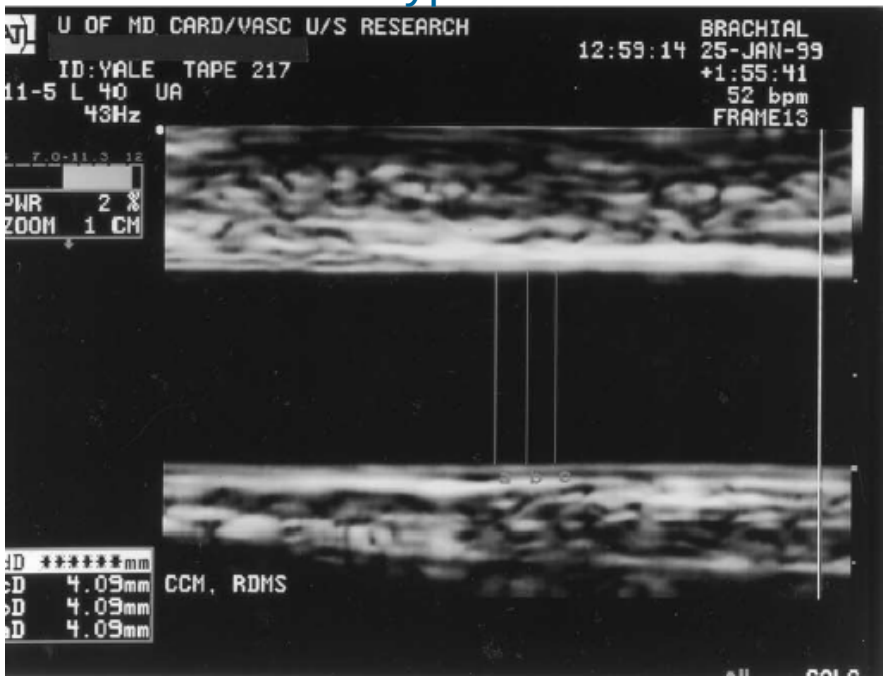


Corretti MC et al. J Am Coll Cardiol 2002;39:257–265

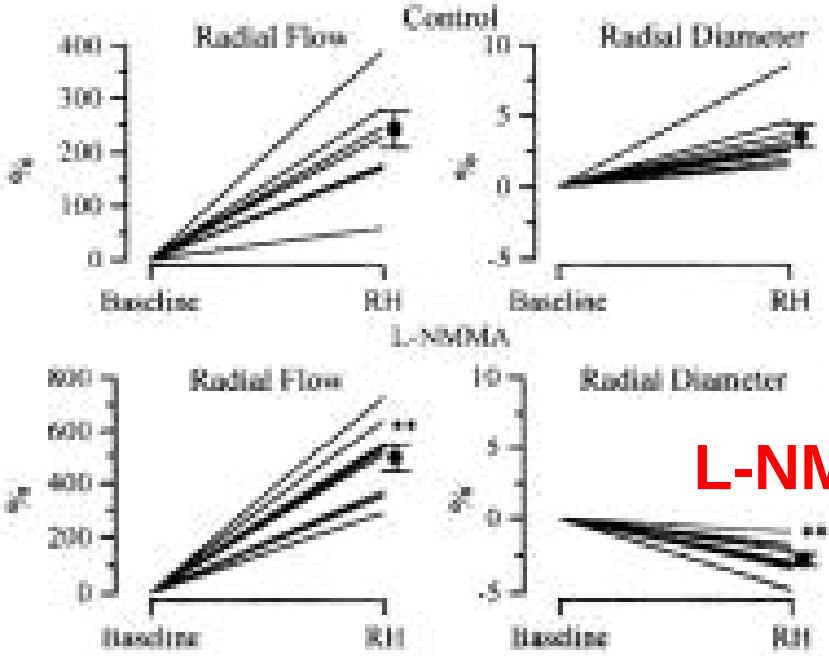




1 min after hyperemic stimulus



NO is essential for FMD



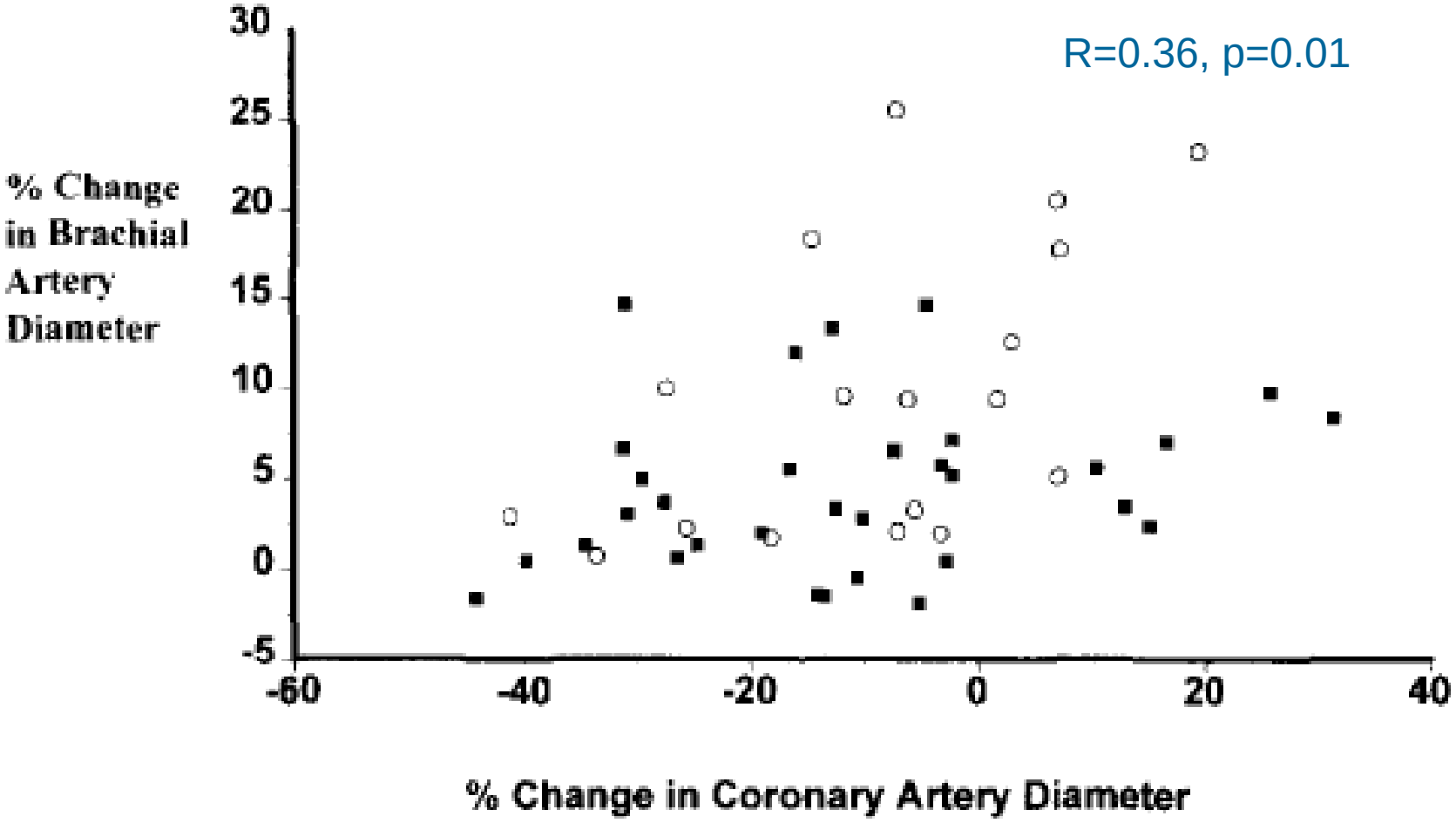
L-NMMA (NOS inhibitor)

FMD was abolished

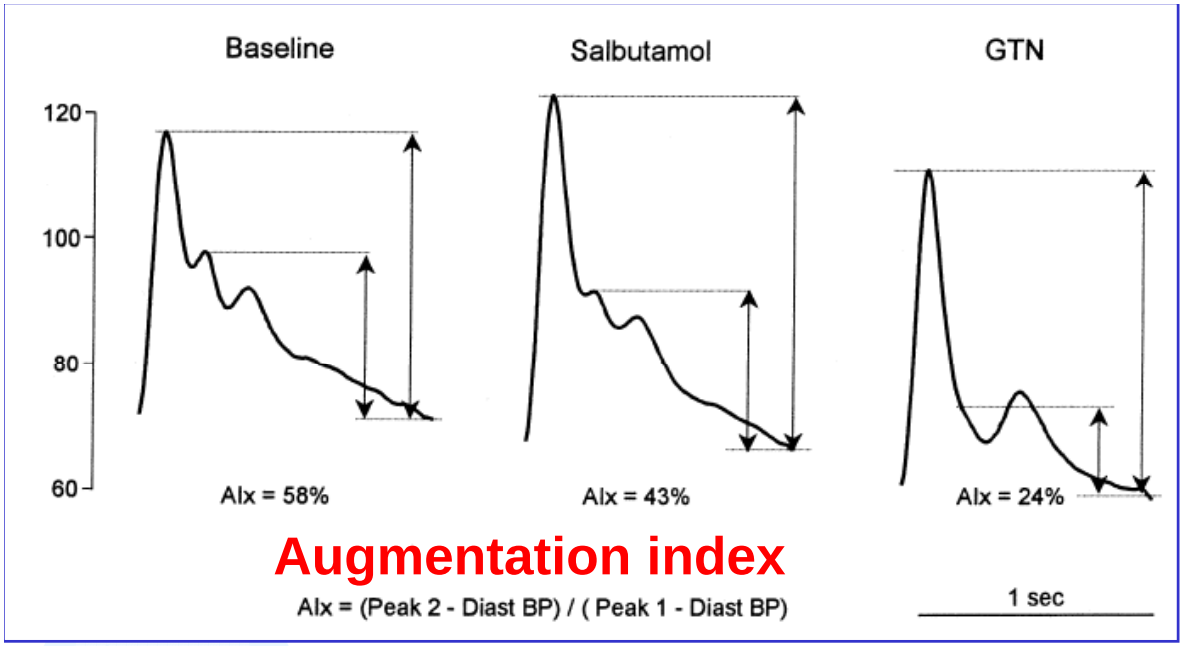
Not affected peak increase in flow during hyperemia

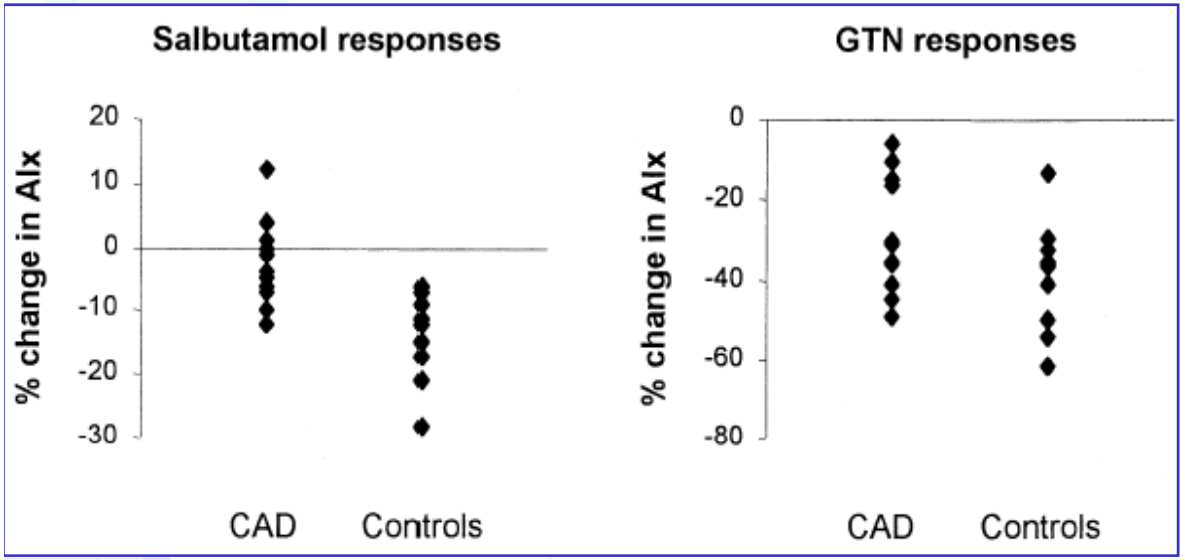
Joannides R et al. Circulation. 1995;91:1314–1319.

FMD in Brachial Artery correlate with Coronary Endothelial Function.



Anderson TJ et al. J Am Coll Cardiol. 1995;26:1235–1241





Peripheral Arterial Wave Form Analysis

Salbutamol reduce arterial stiffness in NO-dependent manner

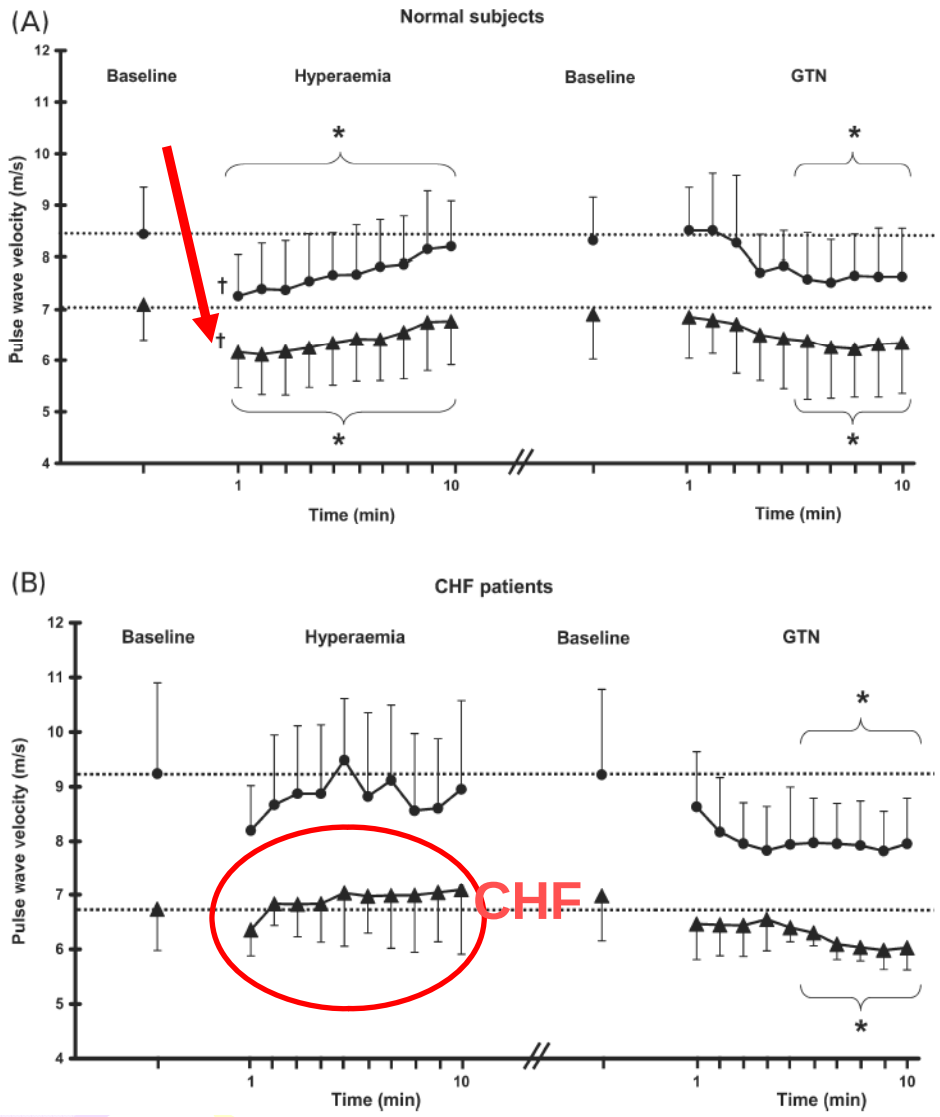
Arterial waveform changes following salbutamol

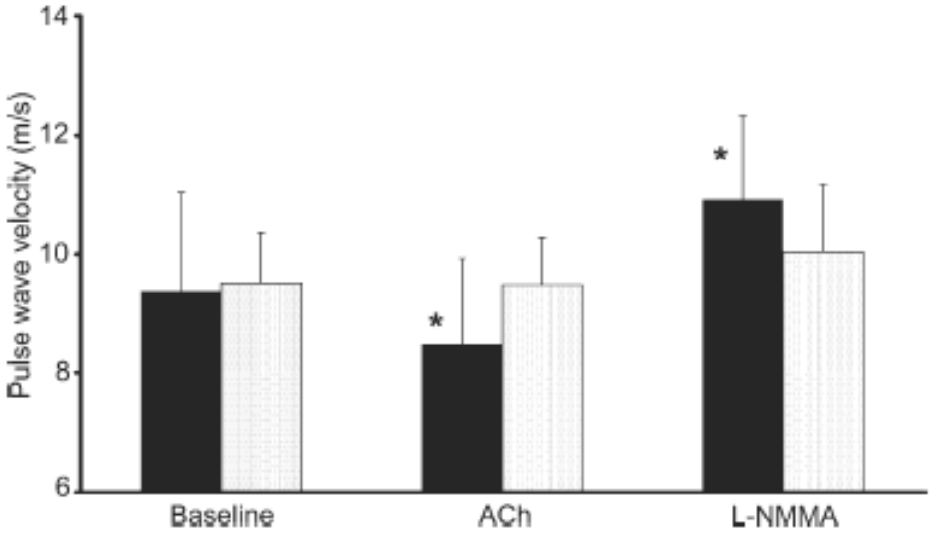
Endothelial function

Hayward CS et al. J Am Coll Cardiol. 2002;40:521–528

Flow mediated reduction of PWV

Pulse Wave Velocity by Oscillometry



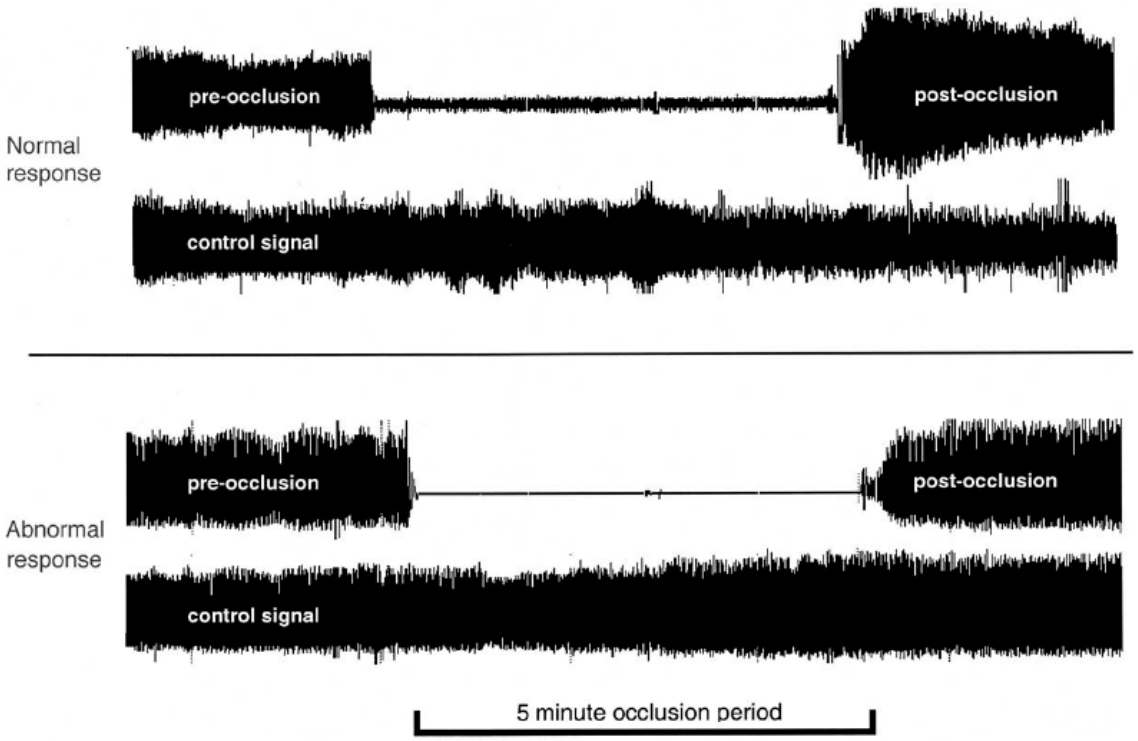


Abolished reduction of PWV

Naka KK et al. Eur Heart J. 2006;27:302–309

Reactive Hyperemia (RH)

Peripheral Artery Tonometry

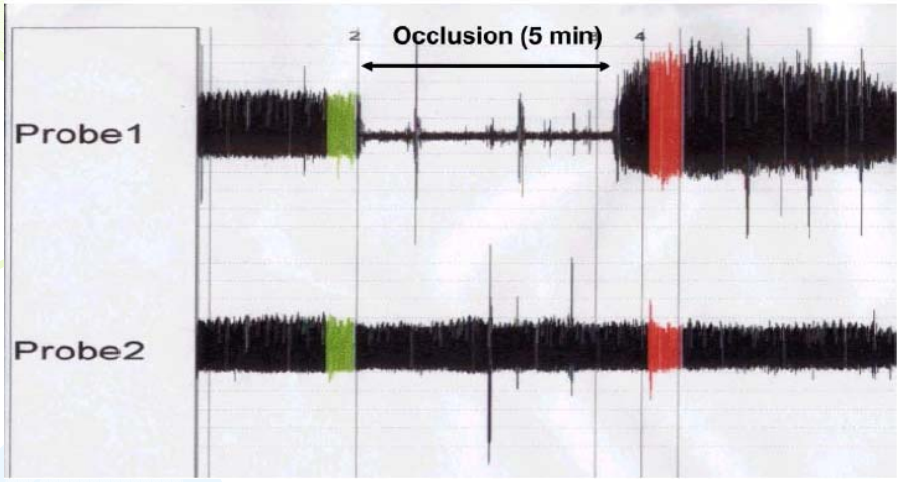


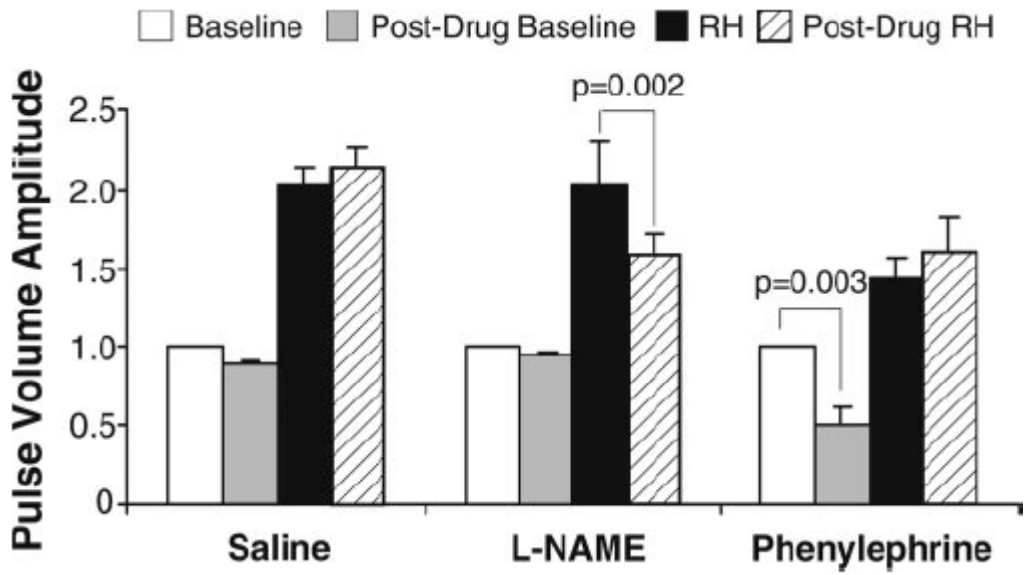
Patients with coronary microvascular endothelial dysfunction

Digital pulse volume change

Bonetti PO et al. J Am Coll Cardiol. 2004;44:2137–2141

Pulse Volume Amplitude and NO





Digital PVA–RH

Endothelial Function Test

Methods for Clinical Assessment of Endothelial Function

Technique (Outcome Measure)	Noninvasive	Repeatable	Reproducible*
Cardiac catheterization (change in diameter, change in coronary blood flow)	−	−	+/−
Venous occlusion plethysmography (change in forearm blood flow)	−	+/−	+/−
Ultrasound FMD (change in brachial artery diameter)	+	+	+/−
PWA (change in augmentation index)	+	+	+/−
PCA (change in reflective index)	+	+	+/−
PAT (change in pulse amplitude)	+	+	+/−

FMD, flow-mediated dilatation; PWA, pulse wave analysis; PCA, pulse contour analysis; and PAT, pulse amplitude tonometry.

Deanfield JE et al. Circulation 2007;115:1285–95

Methods for Clinical Assessment of Endothelial Function

Technique (Outcome Measure)	Reflects Biology	Reversible	Predicts Outcome†
Cardiac catheterization (change in diameter, change in coronary blood flow)	+	+	+
Venous occlusion plethysmography (change in forearm blood flow)	+	+	+
Ultrasound FMD (change in brachial artery diameter)	+	+	+↓
PWA (change in augmentation index)	+	−	−
PCA (change in reflective index)	+	−	−
PAT (change in pulse amplitude)	+	−	−

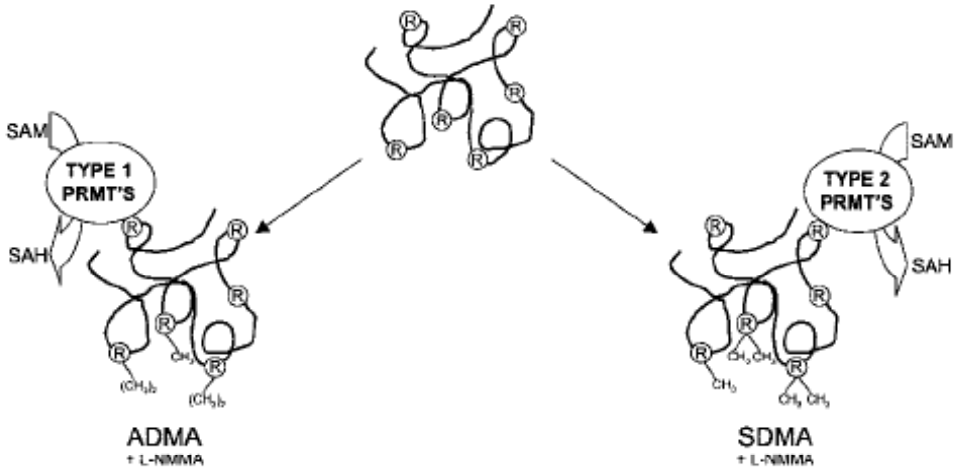
FMD, flow-mediated dilatation; PWA, pulse wave analysis; PCA, pulse contour analysis; and PAT, pulse amplitude tonometry.

Deanfield JE et al. Circulation 2007;115:1285–95

Circulating Markers

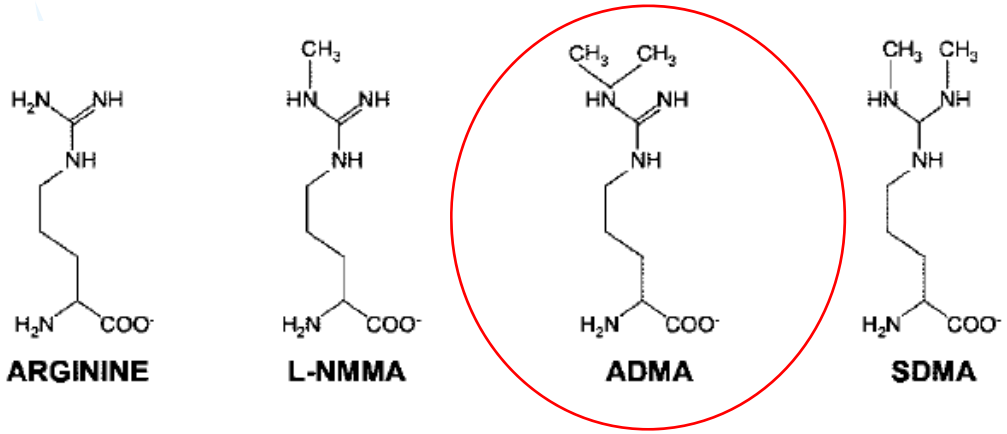
Protein arginine N- methyltransferase

S-adenosylmethionine

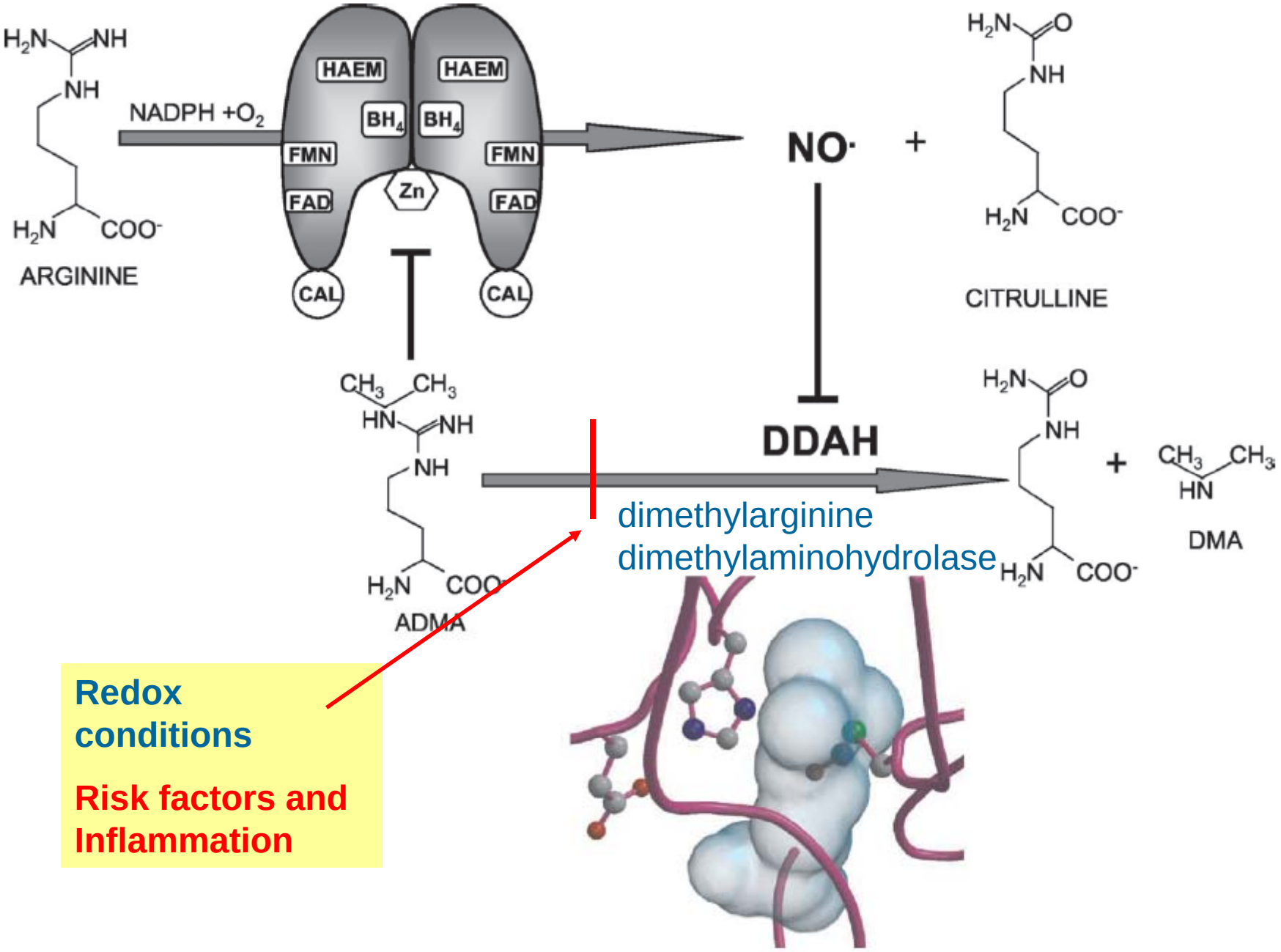


Asymmetric dimethylarginine

Endogenous Antagonist of NO Synthase



Vallance P et al. Arterioscler Thromb Vasc Biol. 2004;24:1023–1030



Vallance P et al. Arterioscler Thromb Vasc Biol. 2004;24:1023–1030

ADMA

Endothelial status

Expensive

Marker of CV risk

Reduction in NO bioavailability

Boger RH et al. Clin Chem Lab Med. 2005;43:1124–1129.

Endothelial cell activation increase expression of

Molecule
Adhesion molecules
ICAM-1
VCAM-1
E-selectin
P-selectin
sCD40L

Cytokines
- Interleukin 6
- Interleukin 18
- TNFα
- hs-CRP
- 8-iso-$PGF_{2\alpha}$
- ET-1
- Metalloproteinases

Adverse cardiovascular prognosis

Biochemical markers of endothelial dysfunction

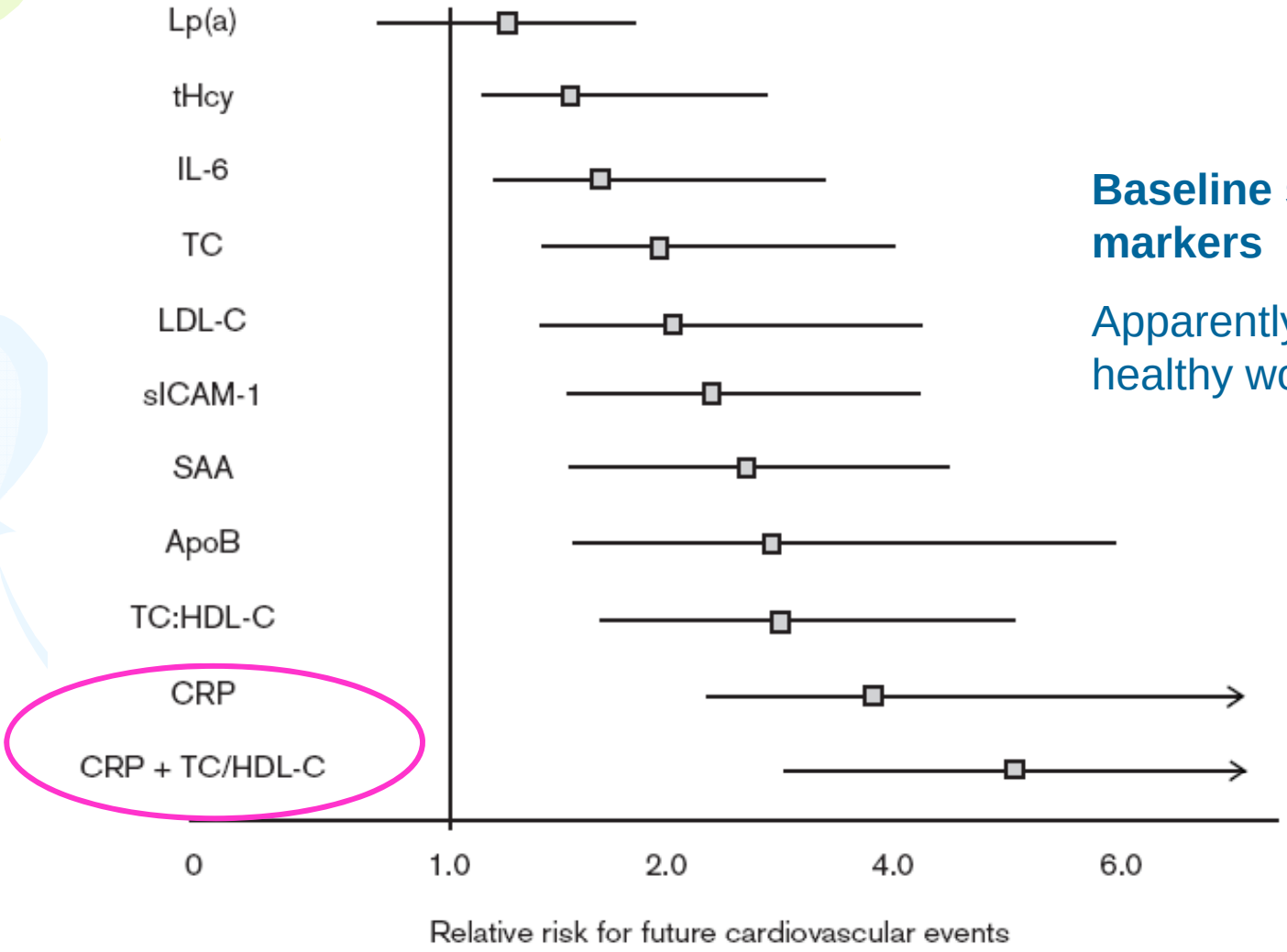
Molecule	Main sources
Adhesion molecules	
ICAM-1	ECs, circulating leukocytes
VCAM-1	ECs, VSMC
E-selectin	Activated ECs
P-selectin	ECs, (Weibel Palade bodies), platelets (α granules)
sCD40L	Activated platelets, T lymphocytes, ECs, VSMCs, macrophages, mast cells

Deanfield J et al. J Hypertens. 2005;23:7–17

Biomarker	Prospective Studies Convincing?	Standardized Commercial Assay Available?	Additive to Lipid Screening?	Additive to Framingham Risk Score?
Inflammation				
hsCRP	++++	+++	+++	++
sICAM-1	++	+/−	+	−
SAA	++	−	+	−
Interleukin-6	++	−	+	−
Interleukin-18	++	−	+	−
Myeloperoxidase	+	−	+/−	−
sCD40 ligand	+	−	−	−
Altered Thrombosis				
tPA/PAI-1	++	+/−	−	−
Fibrinogen	+++	+/−	++	−
Homocysteine	+++	+++	+/−	−
D-dimer	++	+	−	−
Oxidative Stress				
Oxidized LDL	+/−	−	−	−
Altered lipids				
Lipoprotein(a)	+++	+/−	+/−	−
LDL particle size	++	+/−	+/−	−

Ridker PM et al. Circulation. 2004;109:IV6–IV19

Relative risk for CV events

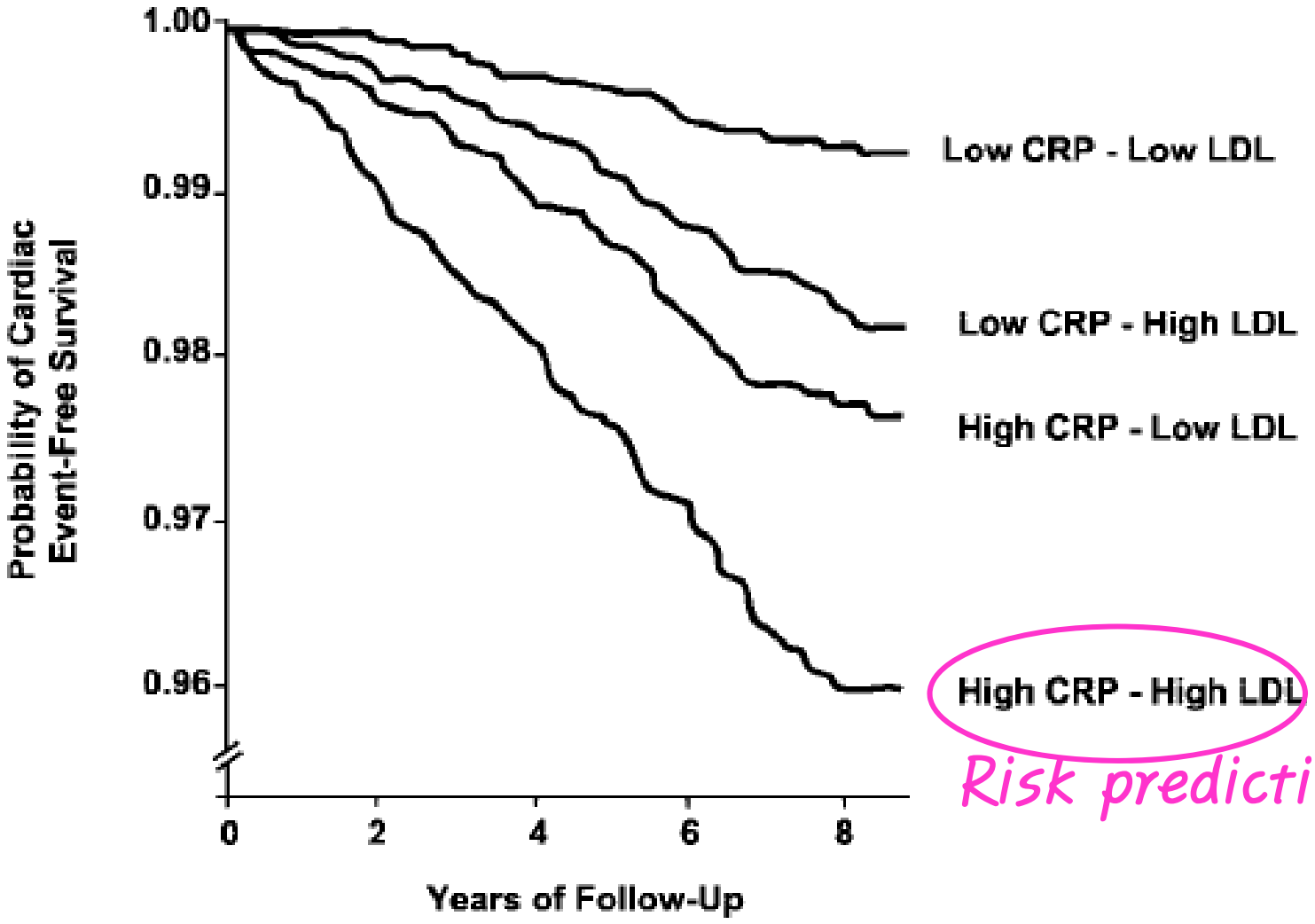


Baseline serum markers

Apparently healthy women

Rifai N et al. Curr Opin Lipidol. 2002;13:383–389

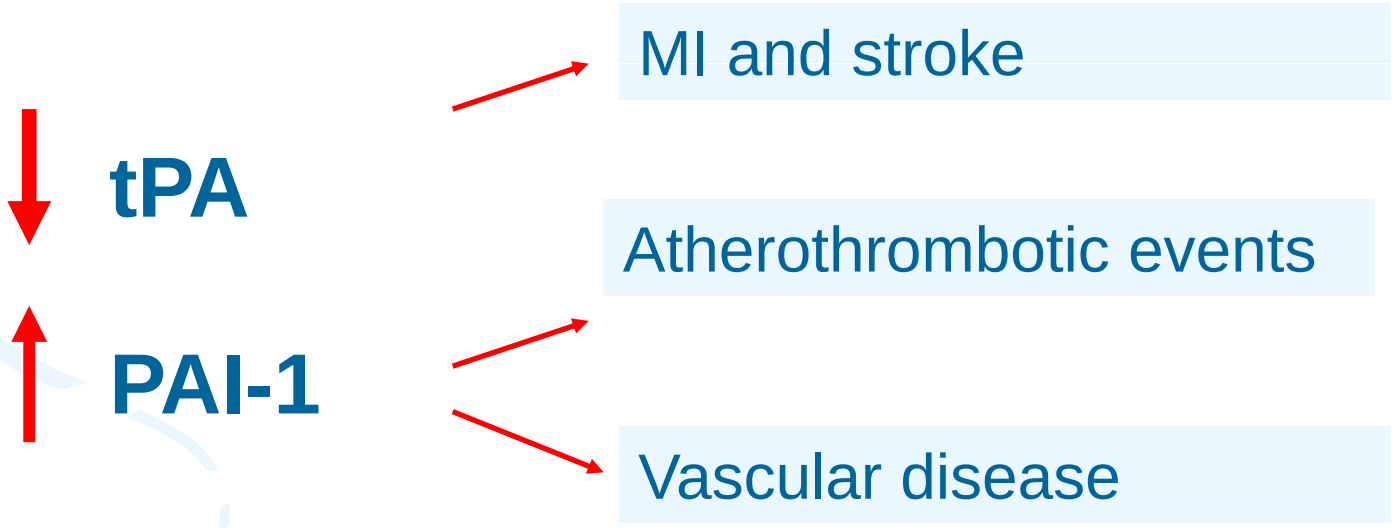
CV Event Free Survival



Risk prediction

Procoagulant consequences

Tissue plasminogen activator and Plasminogen activation inhibitor-1



Vaughan DE .J Thromb Haemost. 2005 3:1879–1883

von Willebrand factor

Released by

Activated Endothelial Cells

Activated platelets

Cellular activation

Coagulation

Platelet activation

Mannucci PM. Arterioscler Thromb Vasc Biol. 1998;18:1359–1362

TABLE 1. Triggers of In Vitro Secretion of vWF by Cultured Endothelial Cells

Mediators of Hemostasis	Mediators of Inflammation
Thrombin	Histamine
Fibrin	Complement components C5a and C5b-9
Plasmin	
Adenine nucleotides	Leukotrienes
	Superoxide anions
	Endotoxin
	Interleukin 1*
	Tumor necrosis factor*

*These cytokines do not directly secrete vWF, but they do enhance the activity of thrombin.[16]

"Stimulation and Perturbation" > > " damage or injury"

Not EC specific

Mannucci PM. Arterioscler Thromb Vasc Biol. 1998;18:1359–1362

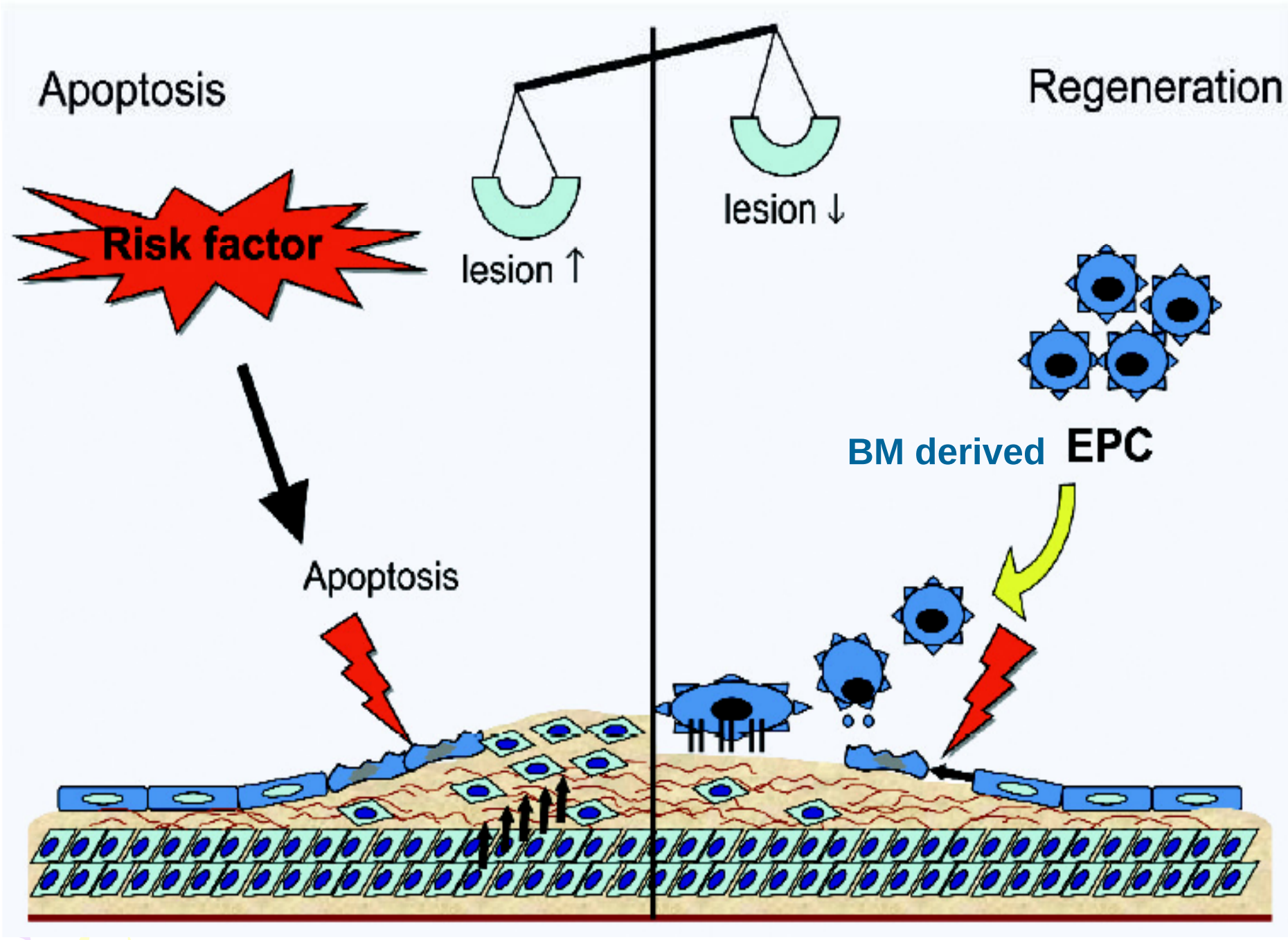
TABLE 2. Conditions Associated With the Increase of Plasma vWF in Humans

Rapid, Short-Term Increase	Slow, Long-Term Increase
Injection of	Liver cirrhosis
Epinephrine	Postoperative period
Vasopressin	Malignancy
Desmopressin	Pregnancy
Muscular exercise	Renal failure
Hypoglycemia	Acute coronary syndromes
CNS stimulation	Diabetes
Venous occlusion	Hemolytic anemias

As a marker of endothelial cell damage – Poor Specificity

Not Powerfully Predictive

Mannucci PM. Arterioscler Thromb Vasc Biol. 1998;18:1359–1362

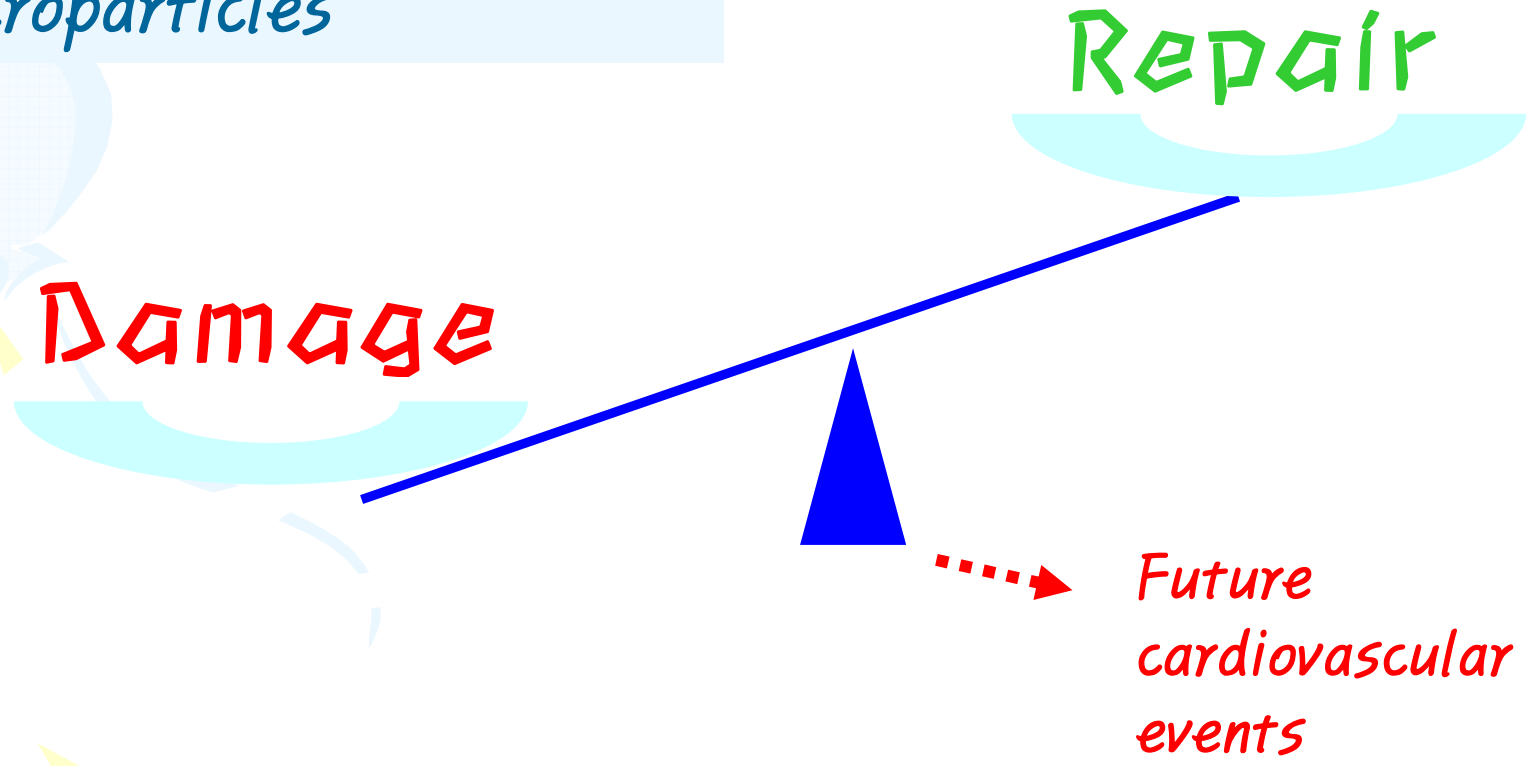


Werner N et al. J Cell Mol Med. 2006;10:318–332

Mature Endothelial Cells

Microparticles

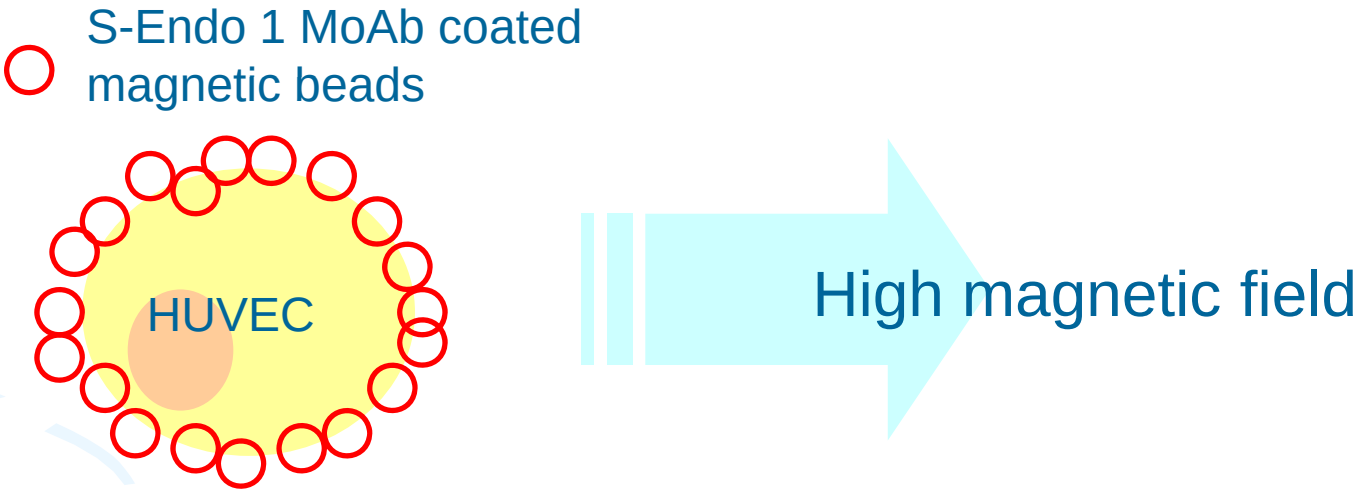
Endothelial Progenitor Cells



Flow cytometry

Magnetic bead selection & Fluorescent microscopy

S-Endo1 immuno-magnetic bead rosetted HUVEC → Cell sorting and isolation



Efficient and selective capture of EC from whole blood

George F et al. Thromb Haemost. 1992;67:147–153

Plasma marker	*Circulating endothelial cells*	*Endothelial progenitor cells*
Origin	Mature endothelium	Bone marrow, cord blood, mobilized MC
Morphology	Mature cells 20–50 micrometer diameter	Immature cells < 20 micrometer diameter
Phenotype	CD34, 146, KDR, VE-cadherin, TM, vWF	CD34, 38, 133, 117, 144 KDR
High proliferative potential	No	Yes
Pathophysiology	*Damage*	*Neovascularisation*

Size and Surface Markers

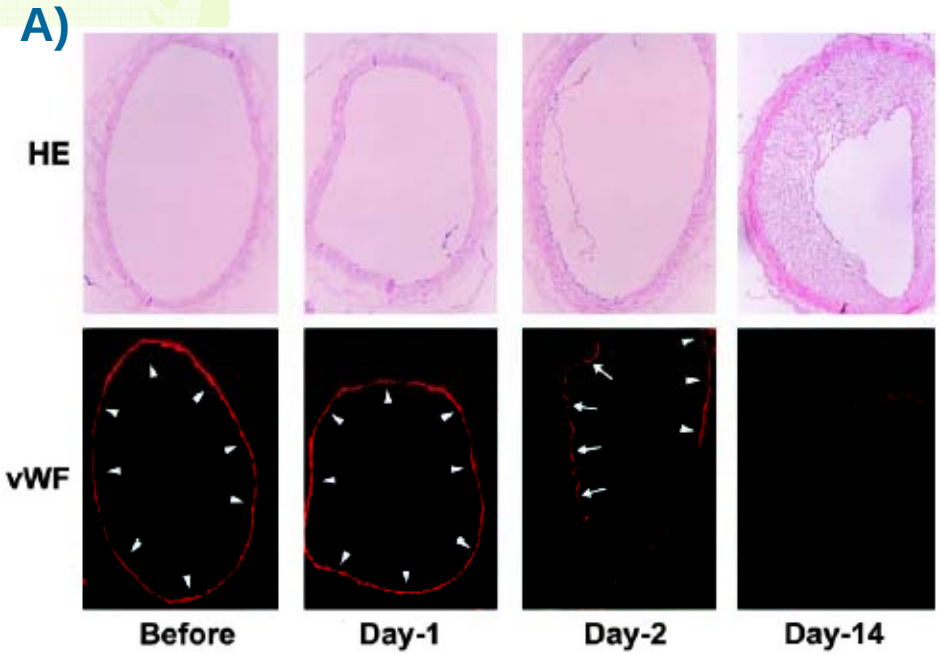
Goon PK et al. Clin Lab. 2005;51:531–538

Fujiyama S et al. Circ Res. 2003;93:980–989

Increased circulating endothelial cells

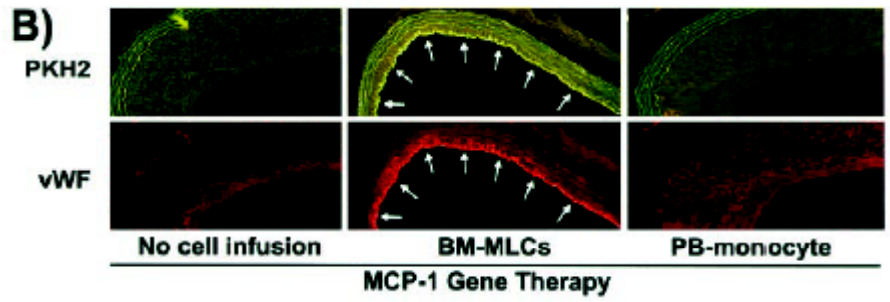
- Cancer
- Pulmonary hypertension
- Thalassemia
- Behcet's disease
- SLE
- Systemic sclerosis
- Angioplasty
- Rickettsial infection
- CMV infection
- Venous insufficiency
- Thrombotic thrombocytopenia
- Atherosclerotic disease
- Type 2 diabetes mellitus
- Acute coronary syndromes
- Septic shock
- Kawasaki disease
- Systemic lupus erythematosus
- Sickel cell anemia
- Renal transplantation
- Bone marrow transplantation
- ANCA vasculitis

Potential to differentiate into an EC phenotype



Endothelial detachment after balloon angioplasty

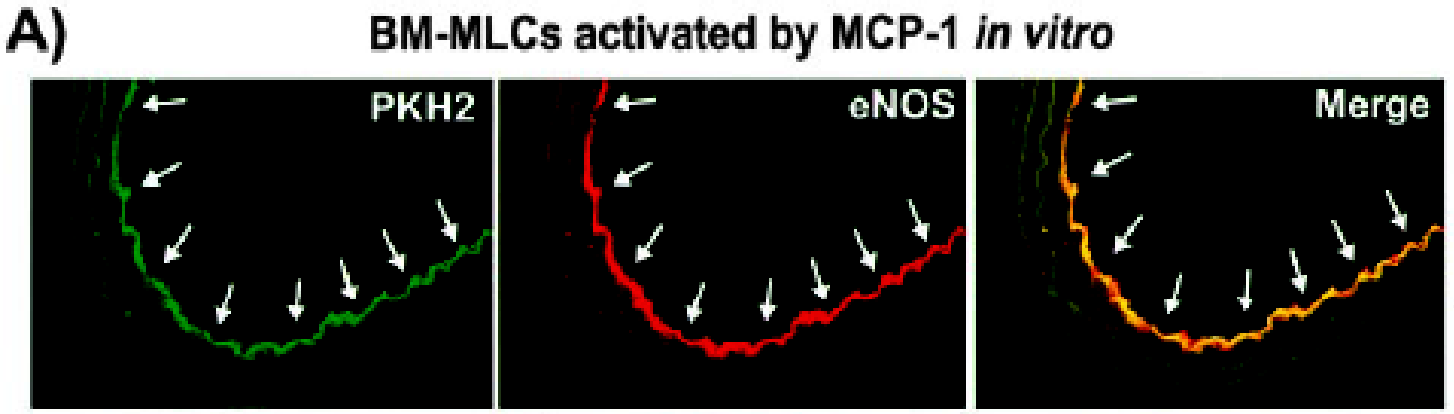
EC immunostained with anti-vWF antibody



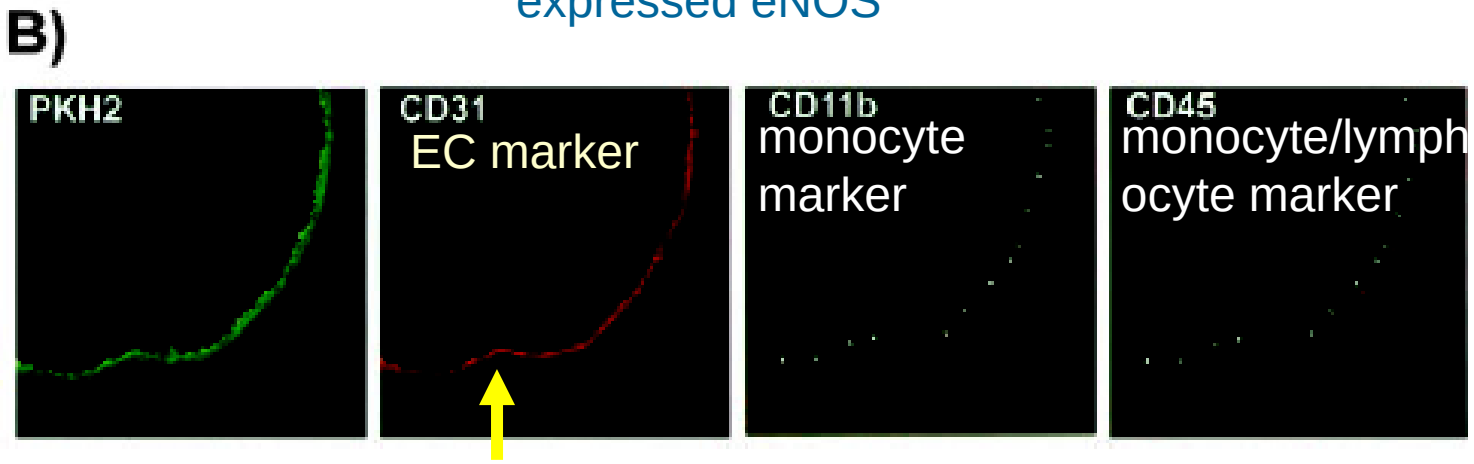
BM derived monocyte lineage cells.

Expressed EC marker vWF

Fujiyama S et al. Circ Res. 2003;93:980–989



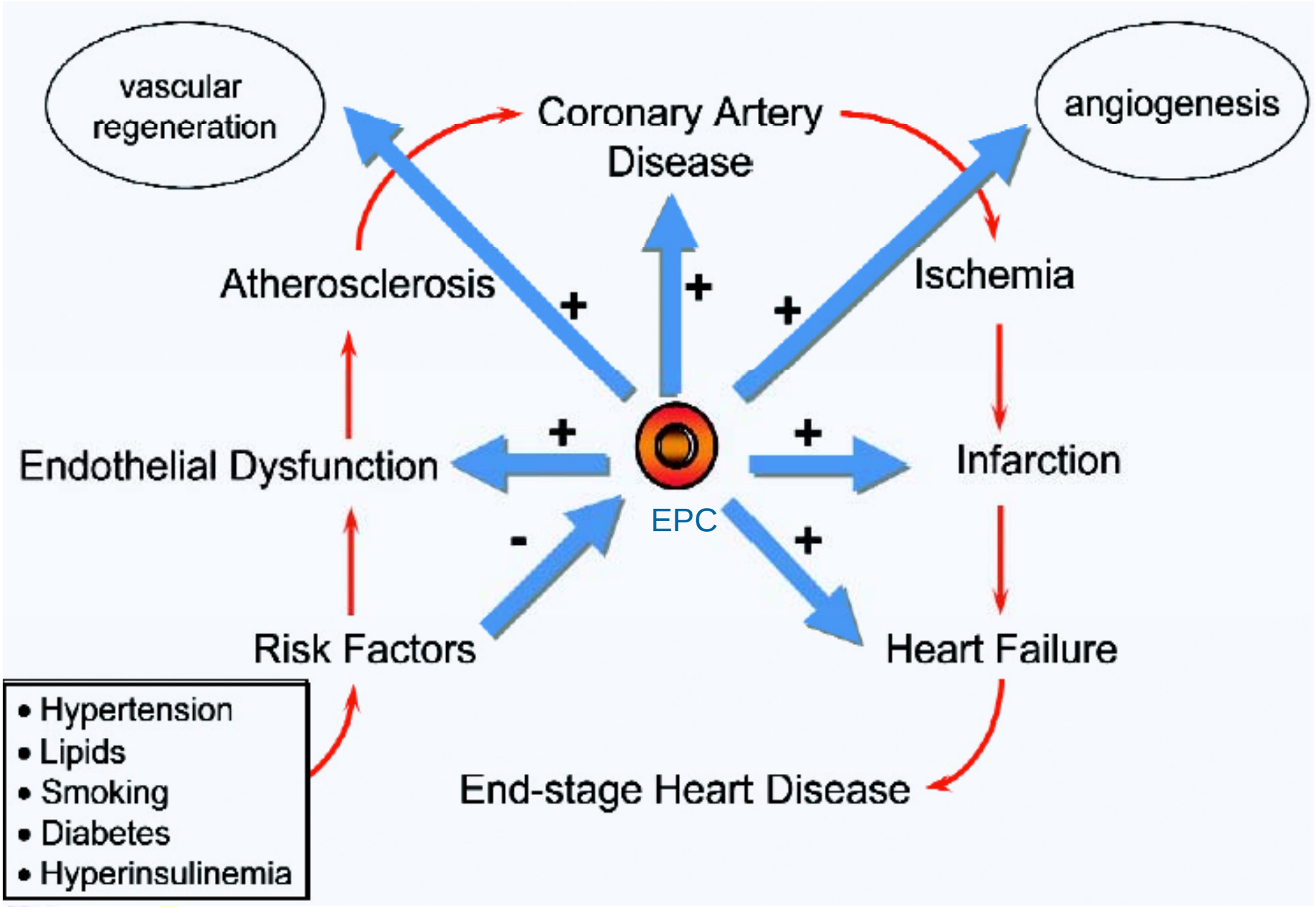
expressed eNOS



Potential to differentiate into an EC phenotype

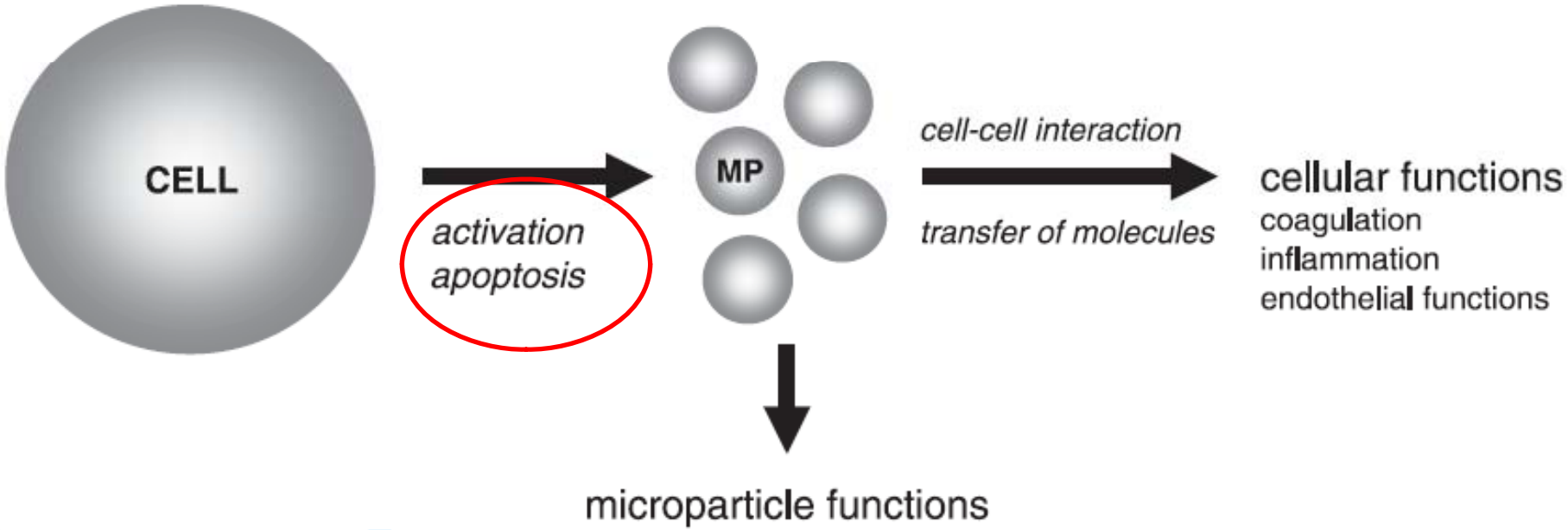
BM-MLCs function as EC progenitors

Fujiyama S et al. Circ Res. 2003;93:980–989



Werner N et al. J Cell Mol Med. 2006;10:318–332

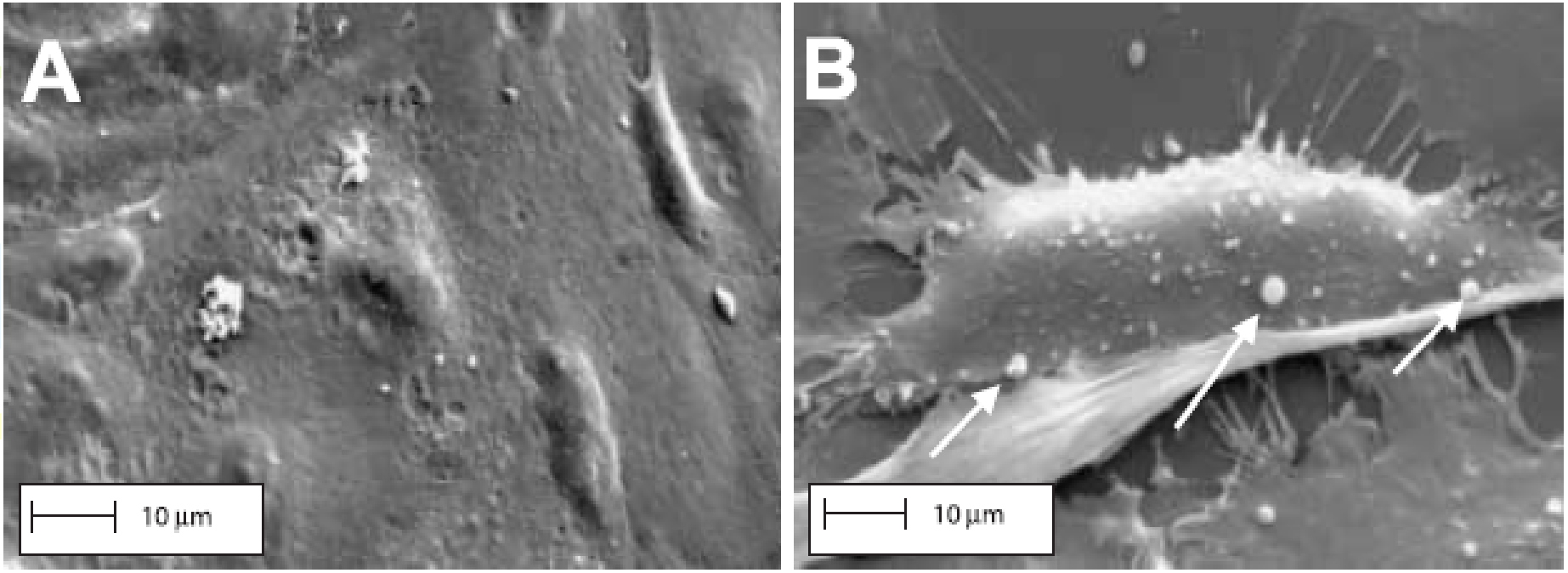
Circulating microparticles



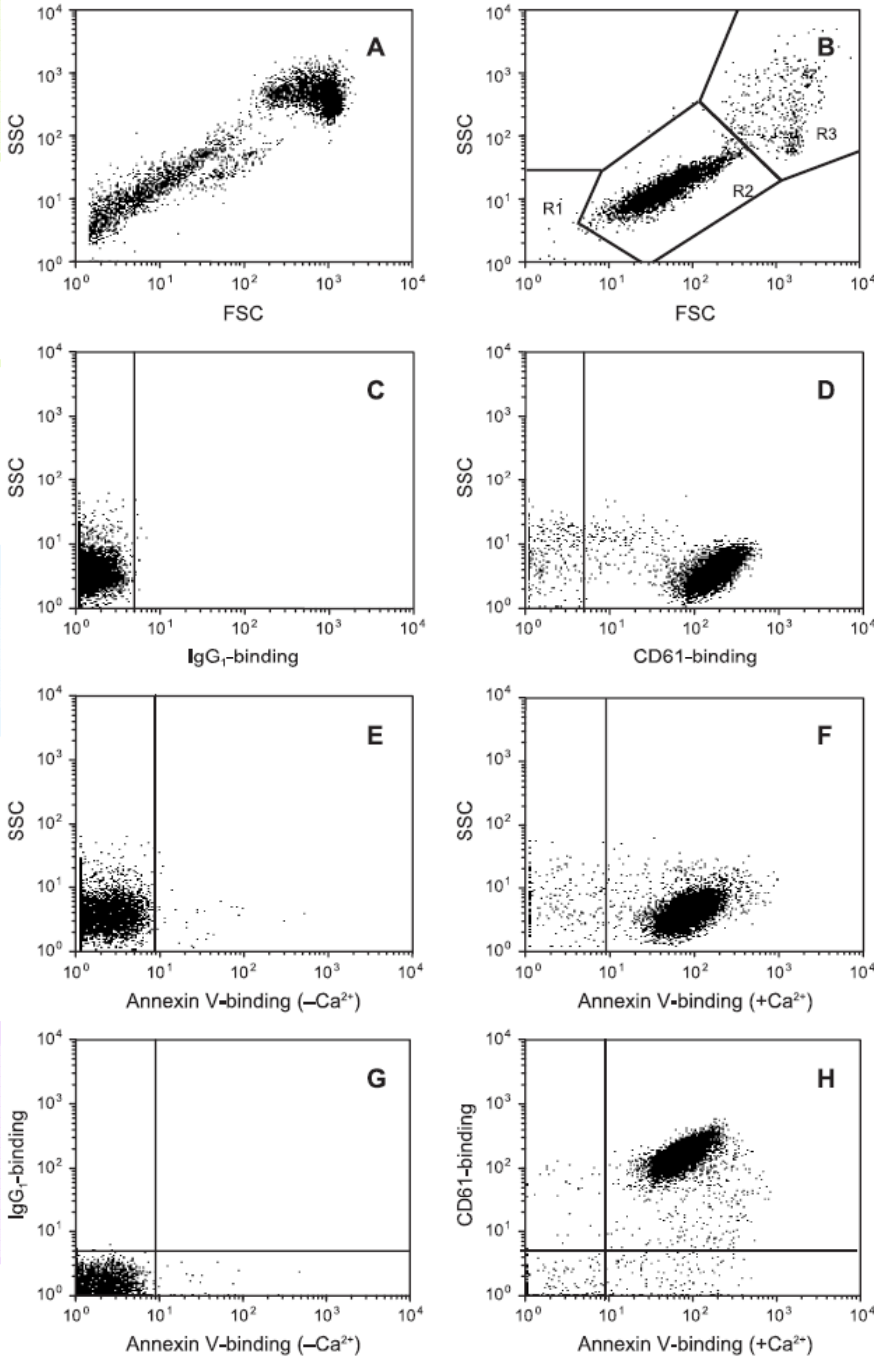
Diamant M et al. Eur J Clin Invest. 2004;34:392–401

Formation of Microparticles

Stimulation of HUVEC with IL-1 alpha



Diamant M et al. Eur J Clin Invest. 2004;34:392–401



Flowcytometry for Microparticle Analysis

Size and density

Fluorescence

(Ag on the cell surface)

Diamant M et al. Eur J Clin Invest. 2004;34:392–401

Risk factors

↓

Systemic Inflammation

↓

Endothelial Damage / Activation

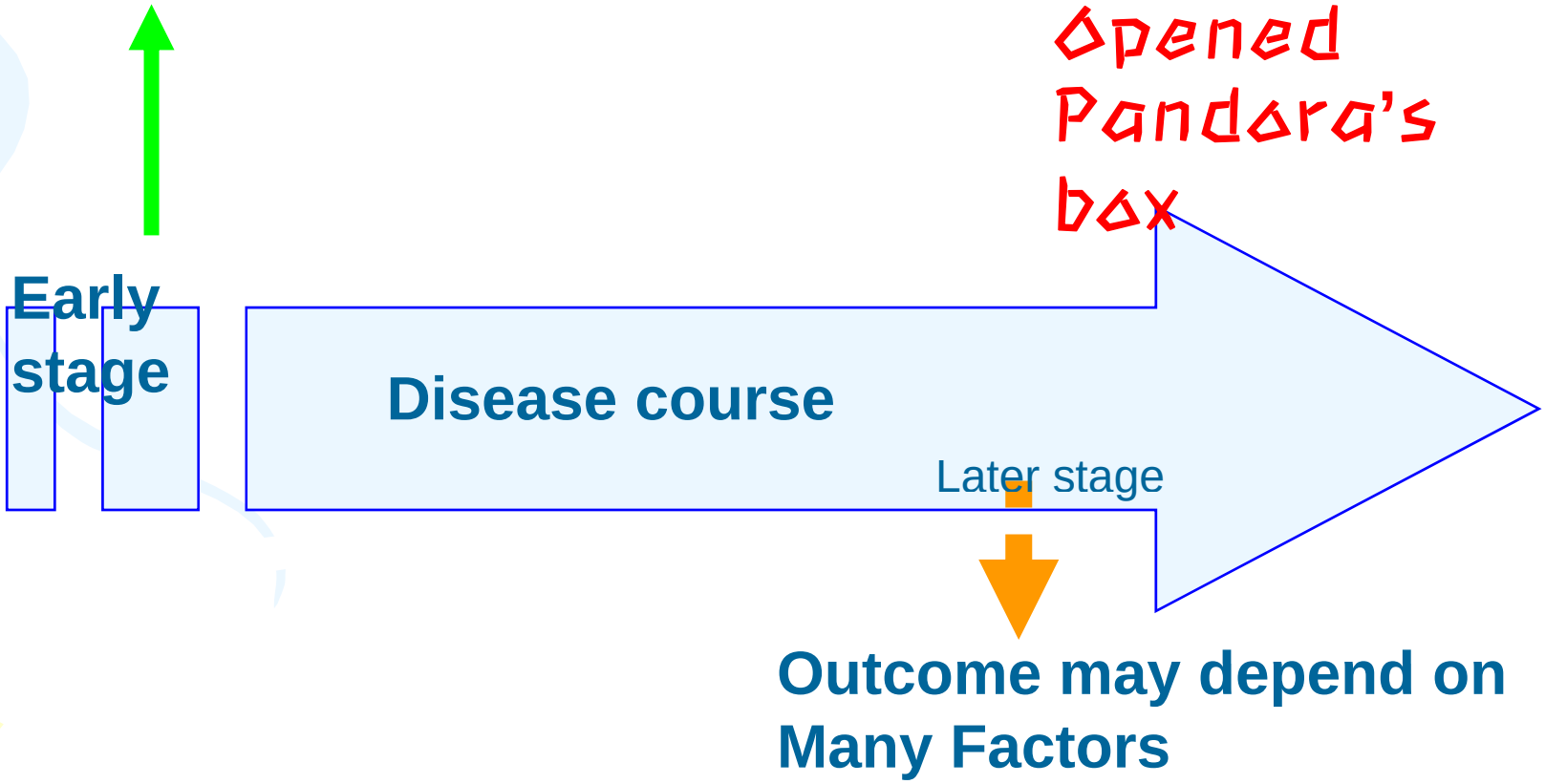
↓

Structural changes

Vita JA et al. Circulation. 2004;110:3604–3609

Juonala M et al. Circulation 2004;110:2918–2923

Prognostic Value of Endothelial Function Test



Comprehensive Approach

Evaluate new treatment strategies

Early preclinical phase