LDL Targets vs Pleiotropic Effects

Does Pathophysiology Matter?

The opinions expressed today are my own and do not reflect those of any private organization or government (unfortunately)

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LDL Targets vs Pleiotrophic Effects

"Treating the number" vs Pleiotropic Effects:

Events directly related to reducing a number:

Lipids - Atherosclerosis (MI, Stroke, PVD)

BP - Stroke, Cardiomyopathy, MI, Nephropathy (Prot)

Glucose - Triopathy, CV events?

Pleio: Those drug effects not related to reducing lipids, BP, Gluc

Direct modulation of enzymes or receptors

Not necessarily class effects

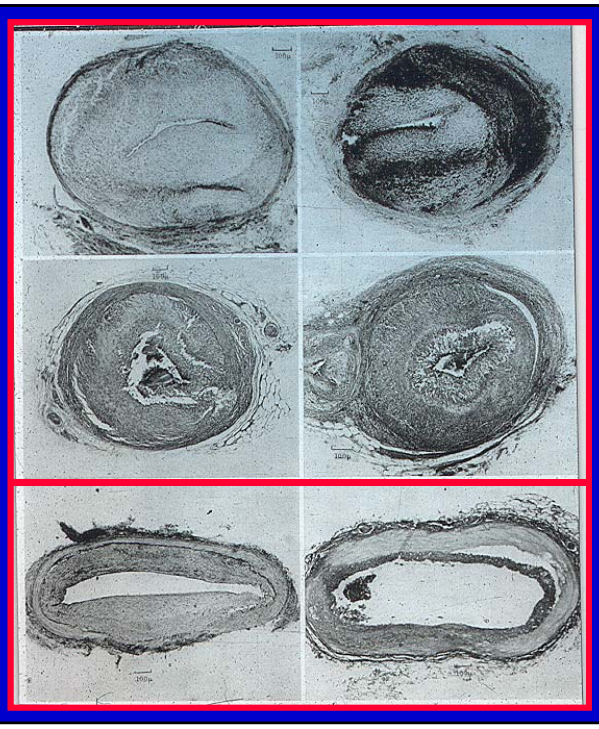
Experimental Atherosclerosis

Rhesis Monkeys
(Normal chol 140 mg/dl)

High fat diet 18 mths:
chol – 700 mg/dl

followed by
Low fat diet 24 mths:
chol – 140 mg/dl

Circ Res 27:59, 1970



LDL Targets vs Pleiotrophic Effects

Early CVD Disease:

Remodeling of the vascular system in response to excess FFA's, cytokines, lipoproteins, intravascular pressure, &/or glucose

Advanced CVD Disease:

Fibrosis and acute decompensation of a vascular structure (rupture of an artery or cardiac failure) which activates coagulation pathway → interrupts blood flow

→ **CV event**

LDL Targets vs Pleiotrophic Effects

Indicators of Advanced CVD Lesions:

- Any cardiac event (MI, stroke, angina, TIA, claudication)
- Left ventricular hypertrophy or high pulse pressure
- Type 2 Diabetes or Type 1 DM for 15 years (post-puberty)

- LDL: >160 or >130 for 15 yrs or >100 for 30 yrs
- BP: >160 or >140 mmHg for 10 years
- Smoking > 20 pack-years

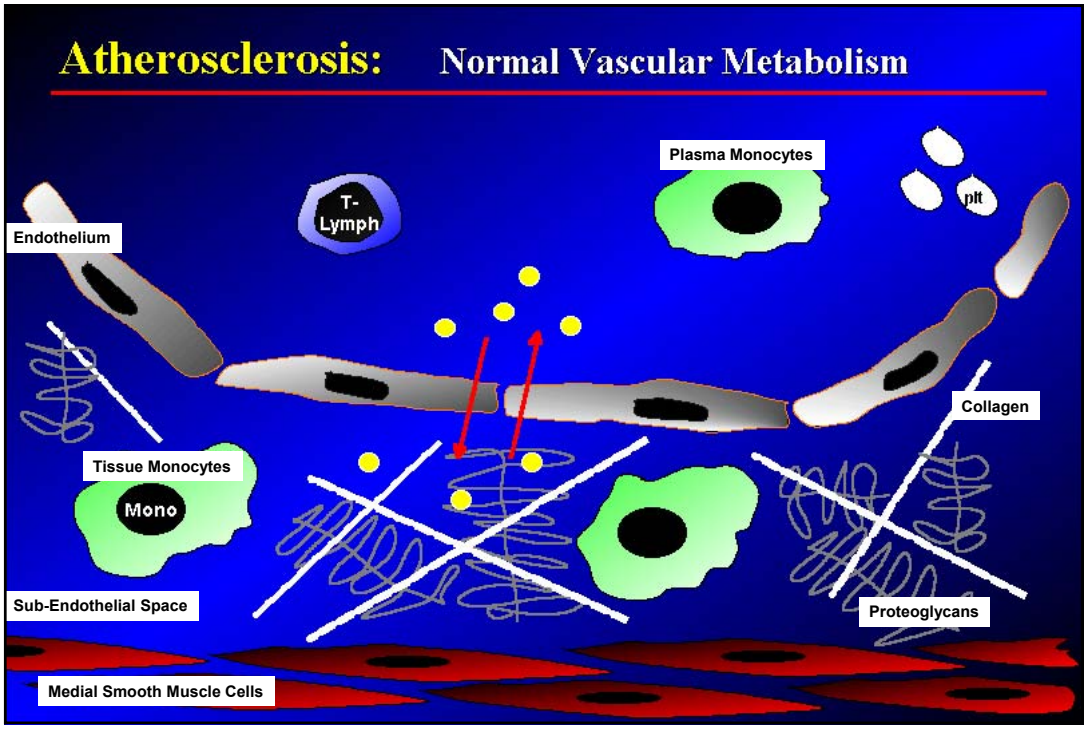
LDL Targets vs Pleiotrophic Effects

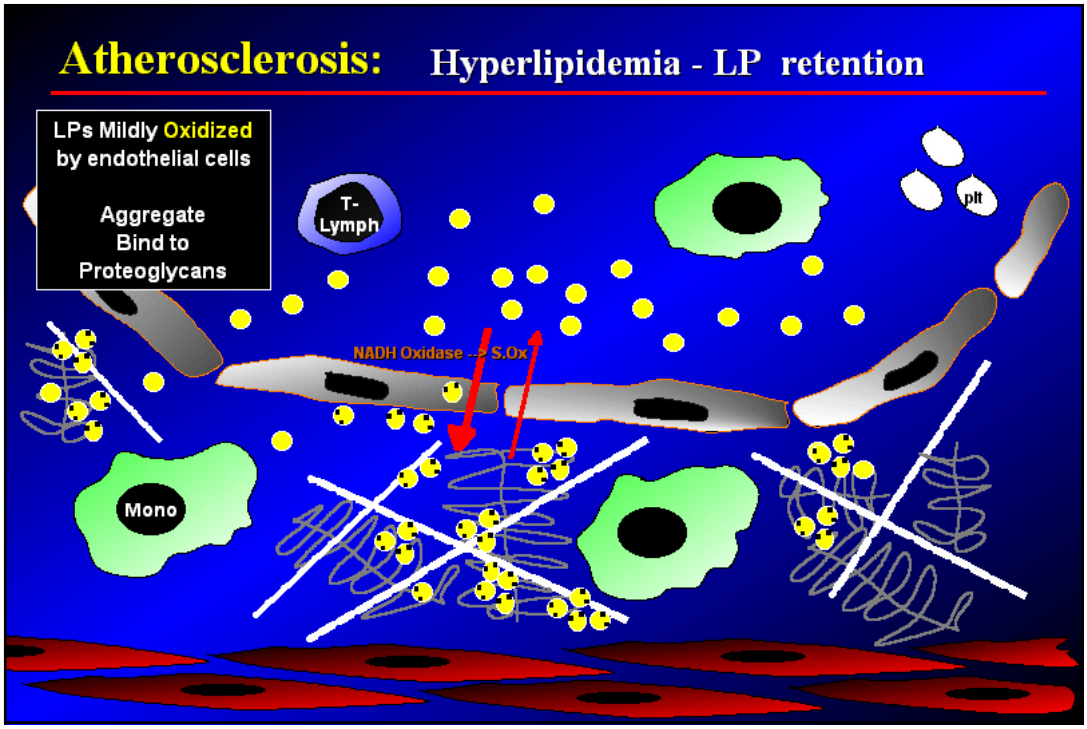
Lipids HBP Insulin Resist

Oxidation

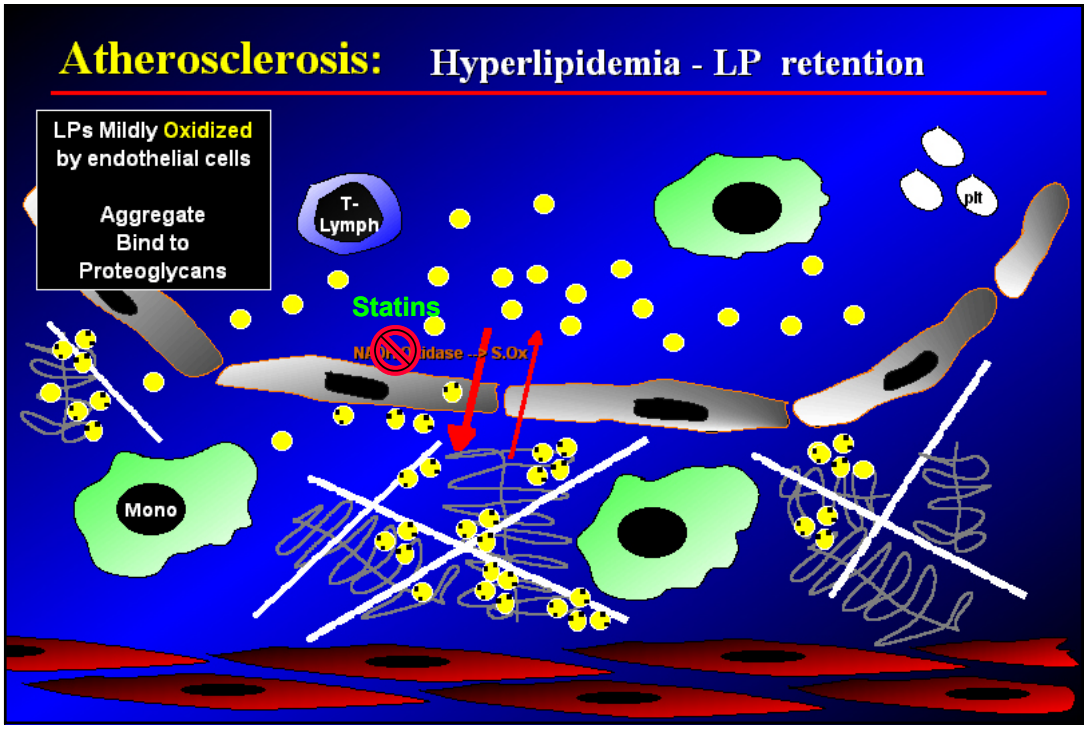
Inflammation

Innate Immunity


Atherosclerosis: Normal Vascular Metabolism
Plasma Monocytes
T-Lymph
plt
Endothelium
Collagen
Tissue Monocytes
Mono
Sub-Endothelial Space
Proteoglycans
Medial Smooth Muscle Cells


Atherosclerosis: Hyperlipidemia - LP retention
LPs Mildly Oxidized by endothelial cells
Aggregate
Bind to
Proteoglycans
T-Lymph
plt
NADH Oxidase --> S.Ox
Mono

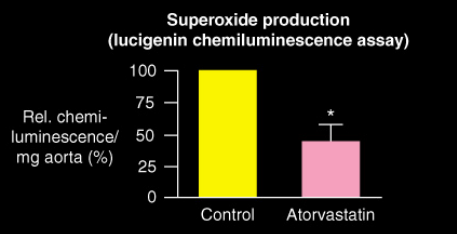
Atherosclerosis: Hyperlipidemia - LP retention

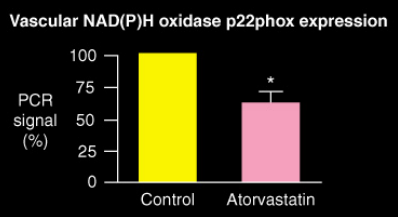


VBWG

Statins downregulate AT_1 receptors and reduce ROS production in normocholesterolemic SHR

"In vivo" statin effect: Dependent on change in LDL or Pleiotropic?



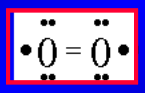


*$P < 0.05$ vs control
ROS = reactive oxygen species
SHR = spontaneously hypertensive rats

Wassmann S, et al. *Hypertension*. 2001;37:1450-1457.

Atherosclerosis: LP Oxidation - Free Radicals

Oxygen:



Biradical - can accept a pair of electrons
Spin restriction of these electrons slows the reaction
This allows one-electron transfer ---> Free Radical Formation

Superoxide:

$$O_2 + e^- \longrightarrow O_2^-\bullet$$

$$O_2^-\bullet \xrightarrow{Dismutase} H_2O_2$$

Stimulated by TZDs

First intermediate in reduction of oxygen to water
Can lead to formation of many other reactive species
(hydrogen peroxide [diffusion], hydroxyl, perhydroxyl)

Catalase → H_2O

H_2O_2 → Vasc prolif & hypertrophy

Hydroxyl Radical:

Most potent oxidant
Extremely short half-life
Metals required in biol.

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + \bullet OH$$
$$H_2O_2 + Cu^+ \longrightarrow Cu^{2+} + OH^- + \bullet OH$$
$$H_2O_2 + O_2^-\bullet \longrightarrow O_2 + H_2O + \bullet OH$$

Atherosclerosis: LP Oxidation - Free Radicals

Biological Sources of Super Oxide (SO):

H_2O_2 Diffuses Away

→ NADH oxidation in mitochondrial electron transport
→ NADPH oxidation by microsomal cytochrome P-450
Phagocytic cells: glucose/FFA --> ↑ H_2O_2 + Myeloperoxidase
MPX generates Free Radicals from H_2O_2

Oxidation of Organic Molecules:

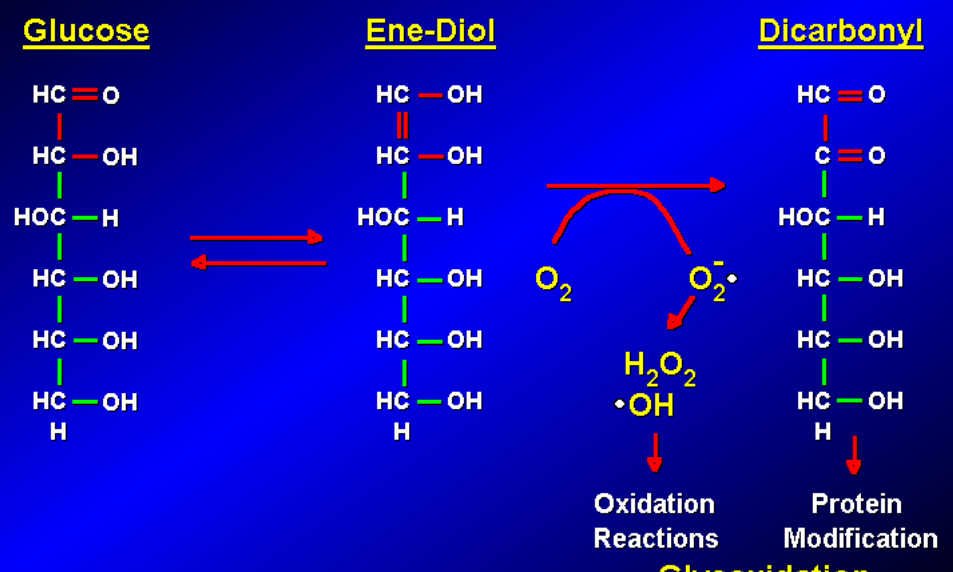
Proteins: proline; histidine, arginine (metal binders)--->
fragmentation, aggregation, cross-linking, degradation

Fatty acids: linoleic (18:2), arachidonic (20:4), DHA (22:6) --->
peroxidation, aldehyde formation ---> cytotoxic, mutagenic

Carbohydrates: glucose --->
auto-oxidation

Yu BP; Physiol Reviews 74:139, 1994

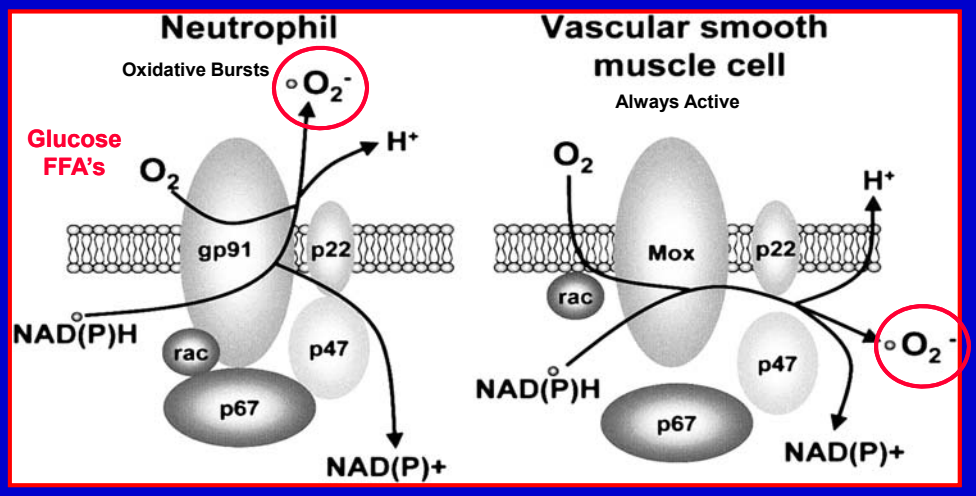
Atherosclerosis: Glucose Auto-oxidation



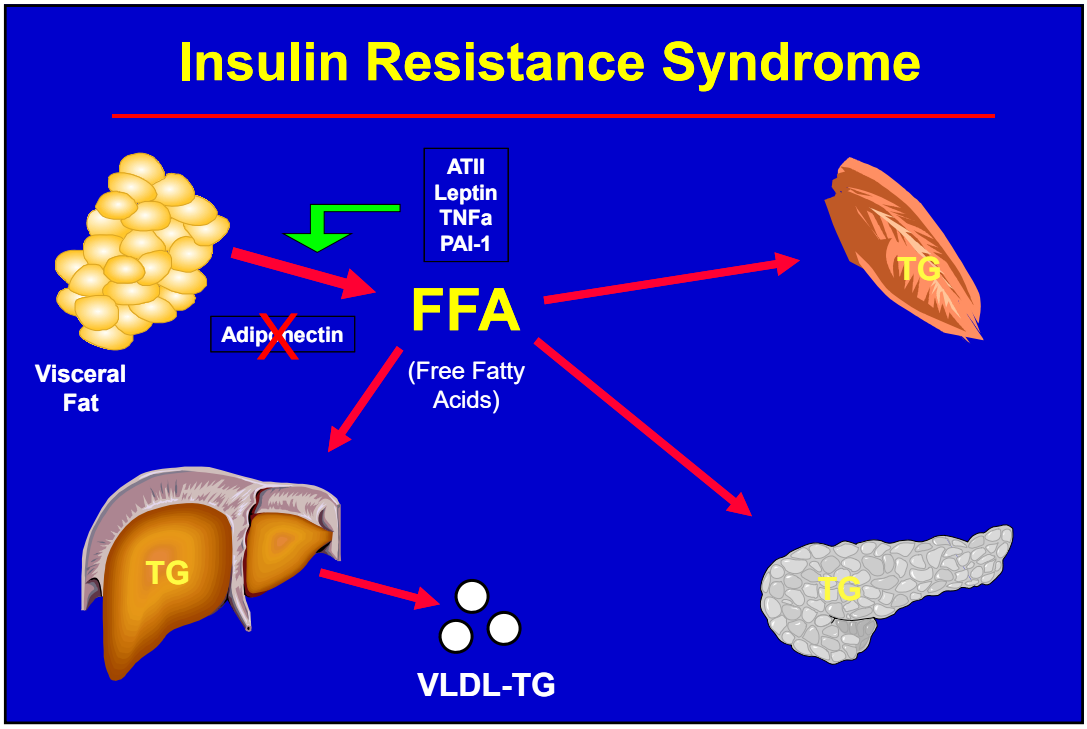
Glycoxidation

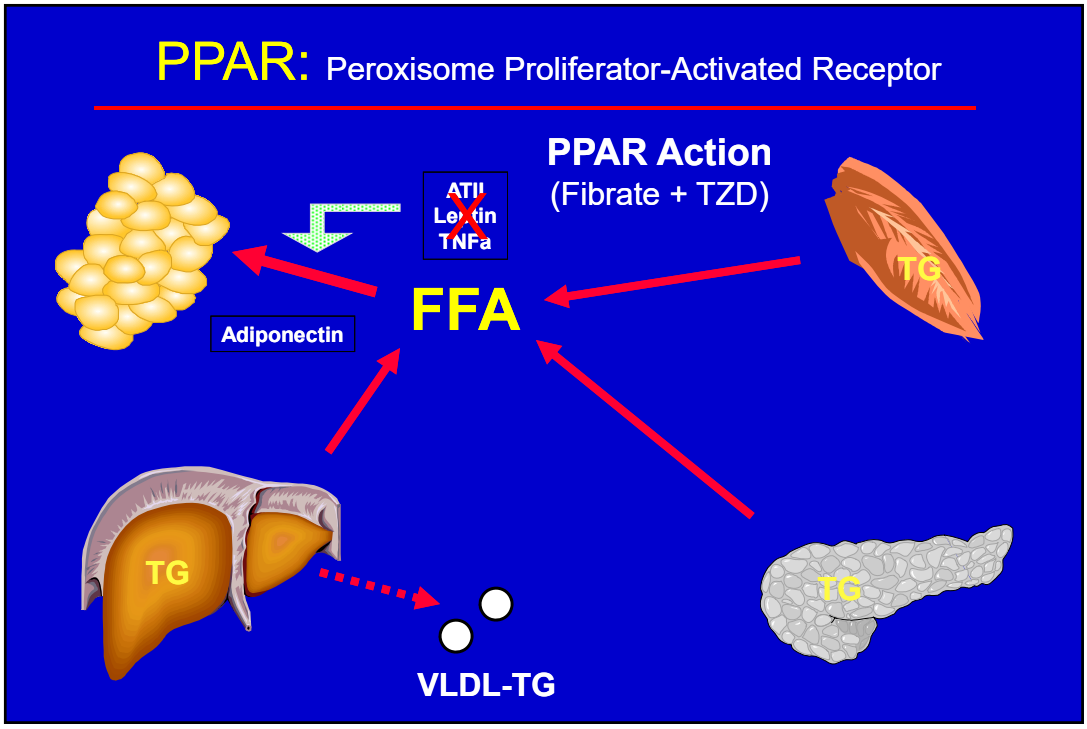
Semenkovich and Heinecke; Diabetes 46:327, 1997

Structure of the NAD(P)H Oxidase

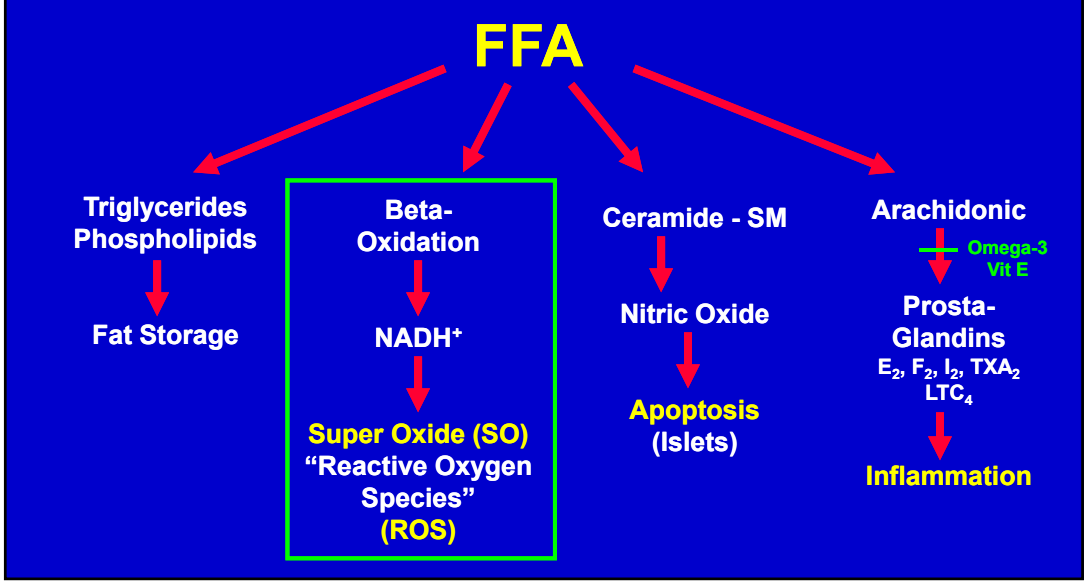


Vascular Oxidase stimulated by: PK-C, ATII, TNFa, Aldosterone, Thrombin, & Turbulent Flow
Inhibited by: Nitric Oxide - TZD's, Statins, Spironolactone, (Amlodip → scavenger)

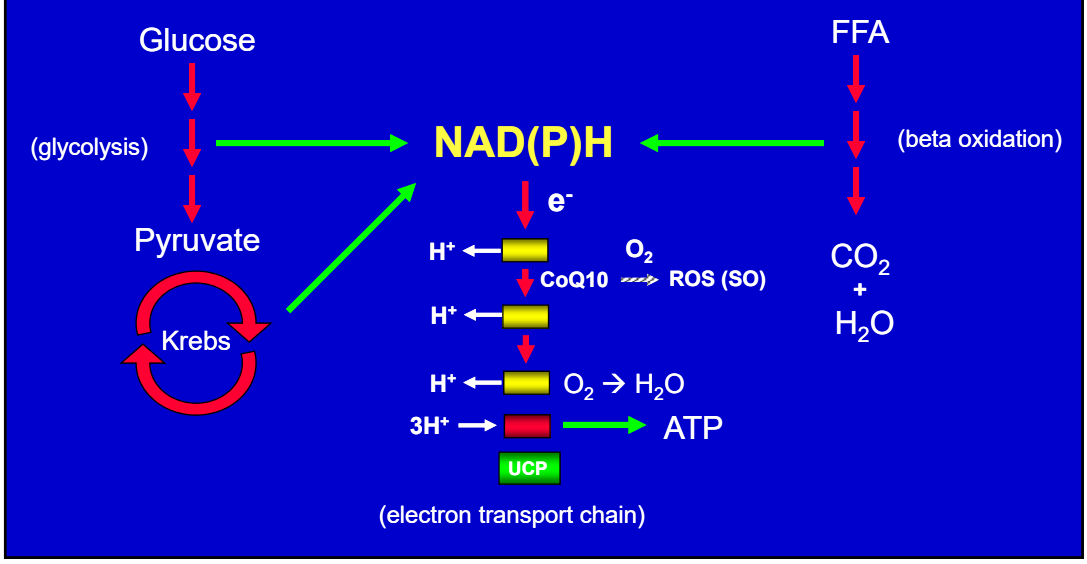

Insulin Resistance Syndrome
ATII
Leptin
TNFa
PAI-1
FFA
(Free Fatty Acids)
Adiponectin
Visceral Fat
TG
TG
TG
VLDL-TG


PPAR: Peroxisome Proliferator-Activated Receptor
PPAR Action
(Fibrate + TZD)
ATII
Leptin
TNFa
FFA
Adiponectin
TG
TG
TG
VLDL-TG

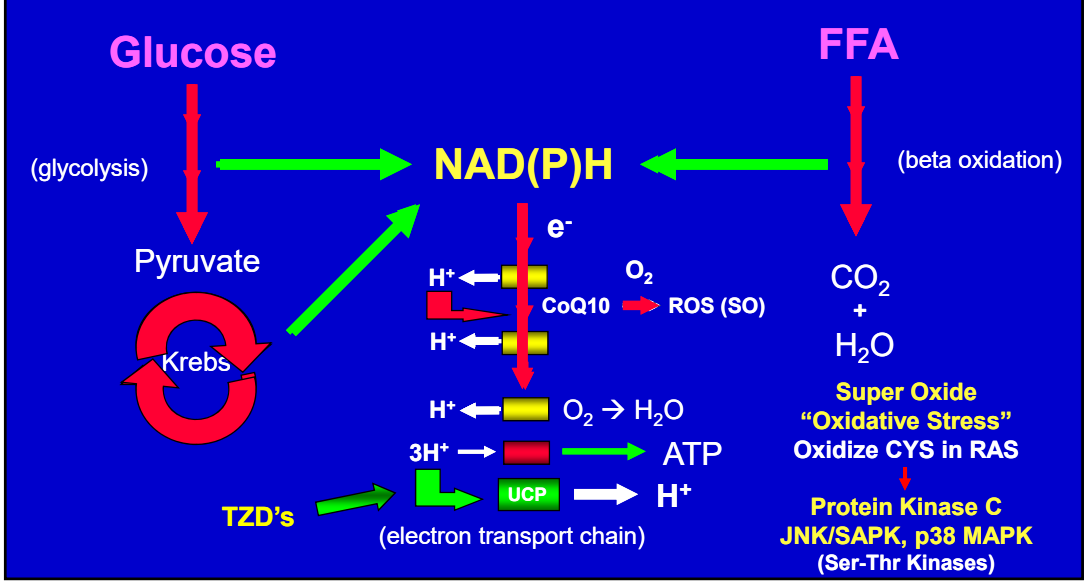
Insulin Resistance Syndrome



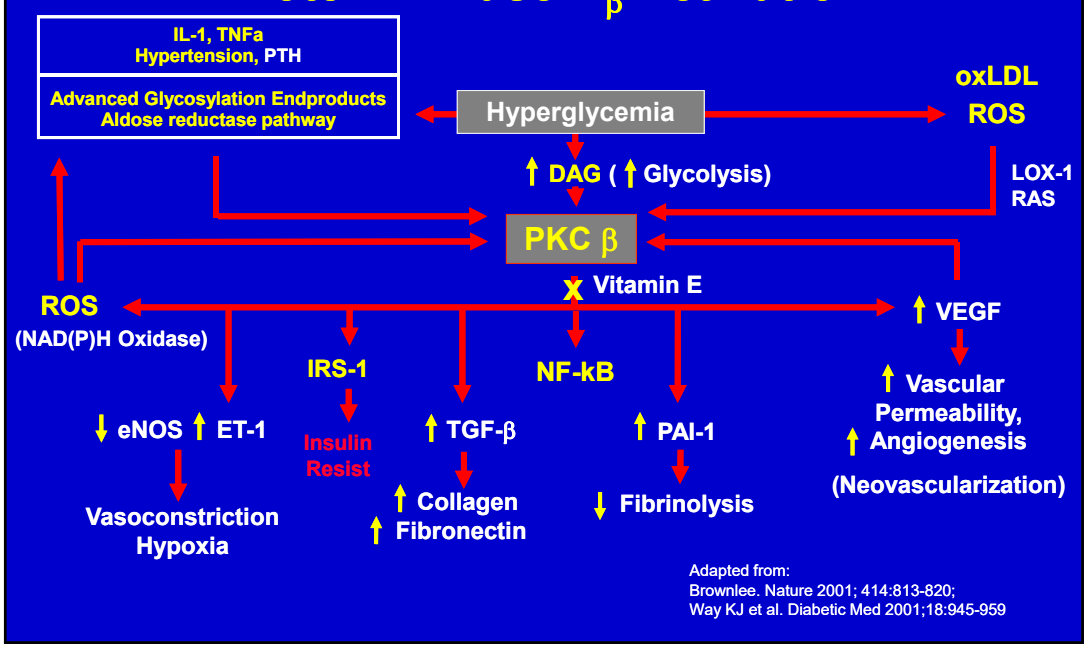
Glucose and Fatty Acid Oxidation



Glucose and Fatty Acid Oxidation

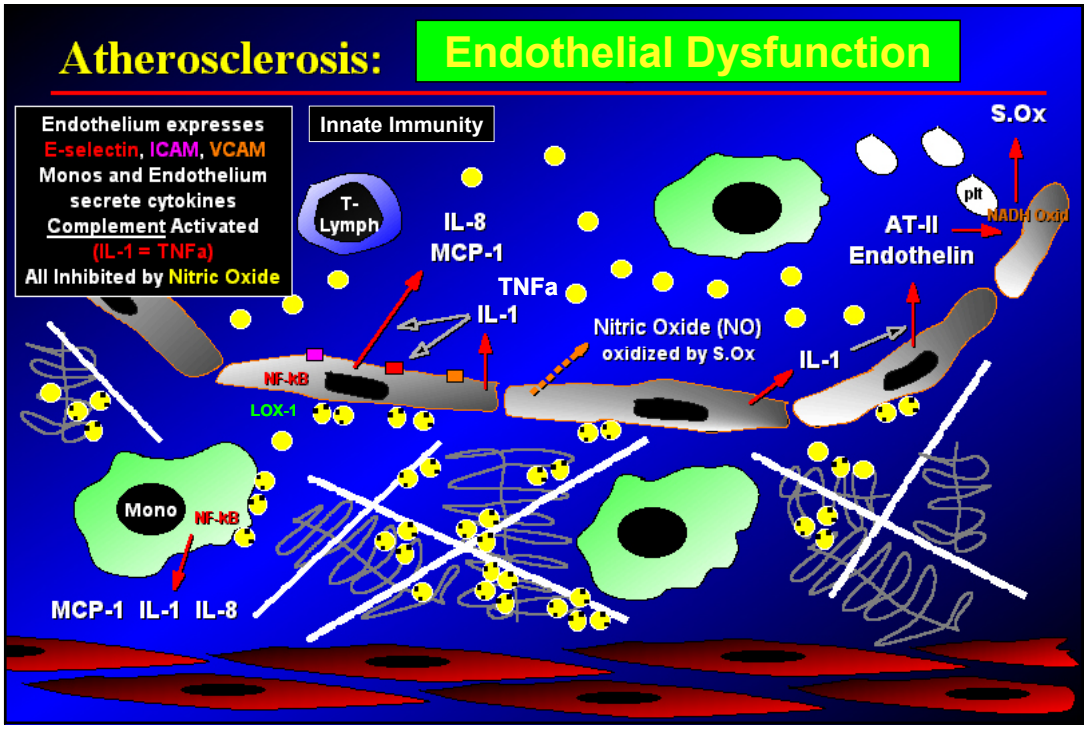


Protein Kinase C_β Activation



Adapted from:
Brownlee. Nature 2001; 414:813-820;
Way KJ et al. Diabetic Med 2001;18:945-959

Atherosclerosis: Endothelial Dysfunction

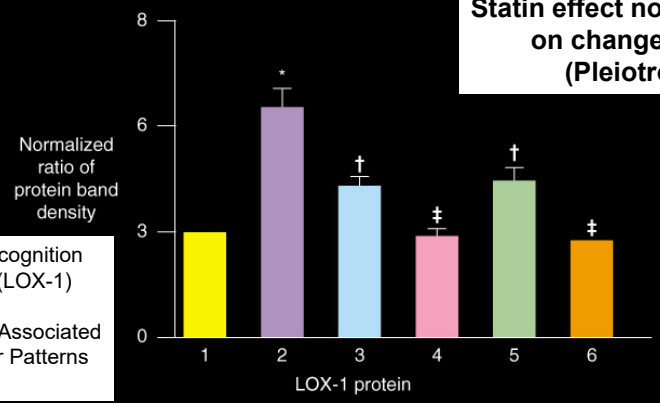


VBWG

Statins decrease LOX-1 expression

Dose-dependent effects

Statin effect not dependent on change in LDL (Pleiotropic)



PRR – Pattern Recognition Receptors (LOX-1)

DAMP – Damage Associated Molecular Patterns (ox-LDL)

1 Control
2 ox-LDL (40 g/mL)
3 Simv (1 µM) + ox-LDL
4 Simv (10 µM) + ox-LDL
5 Atorv (1 µM) + ox-LDL
6 Atorv (10 µM) + ox-LDL

*$P < 0.01$ vs control; †$P < 0.05$ vs ox-LDL; ‡$P < 0.05$ vs low conc
LOX-1 = Lectin-like receptor for oxidized LDL-C

Lui DY, et al. *Cardiovasc Res.* 2001;52:130-135.

VBWG

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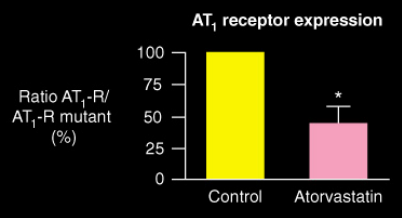

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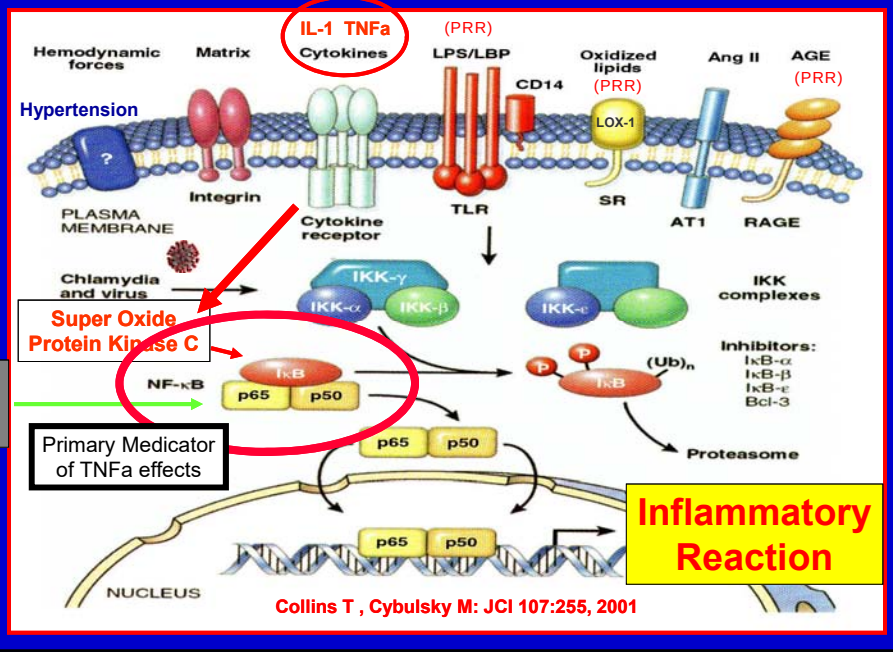
Wassmann S, et al. *Hypertension.* 2001;37:1450-1457.

Nuclear Factor kB (NF-kB) Activation



Collins T , Cybulsky M: JCI 107:255, 2001

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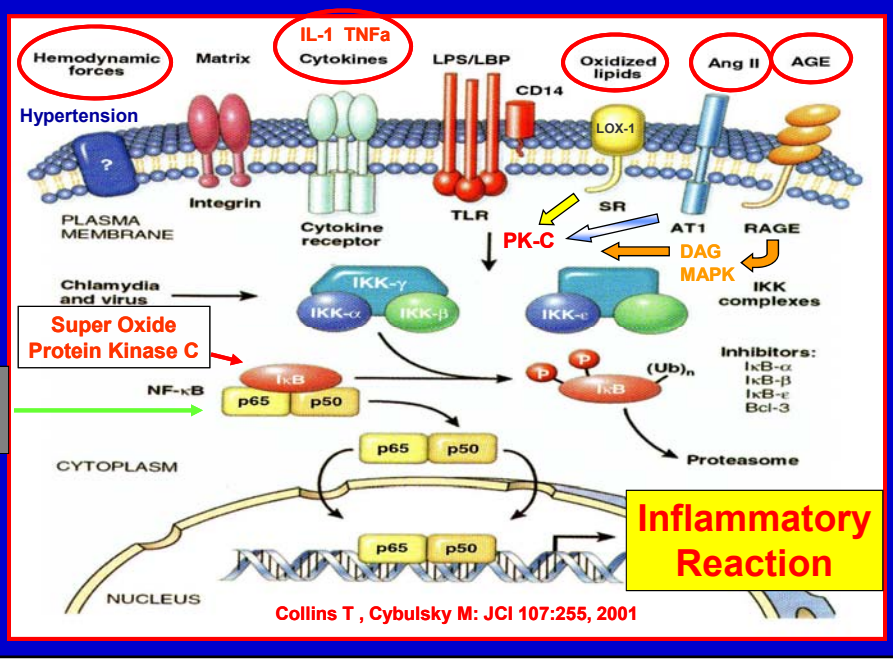


Collins T , Cybulsky M: JCI 107:255, 2001

Nitric Oxide and Super Oxide

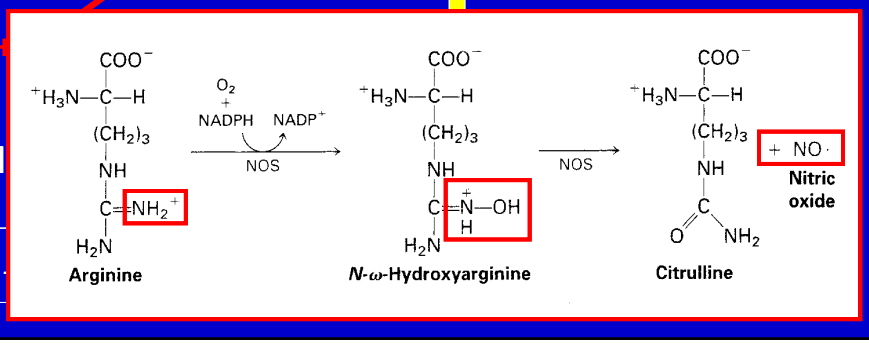
Nitric Oxide Synthase **(NOS)** activated by **bradykinin (ACE-I, Amlo)**, acetylcholine, histamine, serotonin, thrombin, **estrogen,** substance P, shear stress, and **insulin**
Inhibited by asym dimethylarginine **(Prot → ADMA → DDAH (-S.Ox) & Urine)**

L-ARG —(**NOS**, **NADPH**, **BH4**)→ **L-Citrullin + NO** → **$ONOO^-$** **(peroxynitrite)**

NOS **NADPH** **BH**

L-Citrull

SO converts



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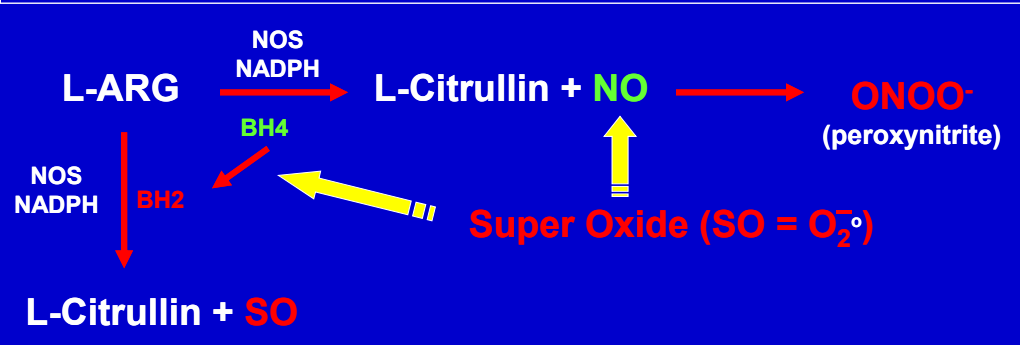
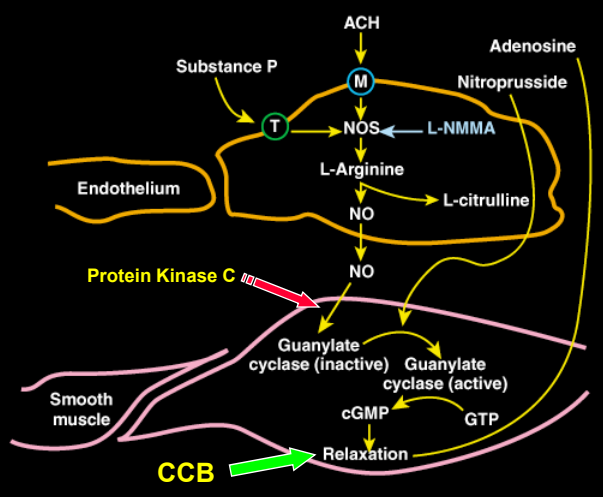
Nitric Oxide Synthase **(NOS)** activated by **bradykinin (ACEI, Amlo)**, acetylcholine, histamine, serotonin, thrombin, **estrogen,** substance P, shear stress, and **insulin**
Inhibited by asymmetric dimethylarginine **(ADMA - CRI)**



BH4 is a required cofactor for NOS
SO converts Tetrahydrobiopterin **(BH4)** to Dihydrobiopterin **(BH2)** → BH4 deficiency

VBWG

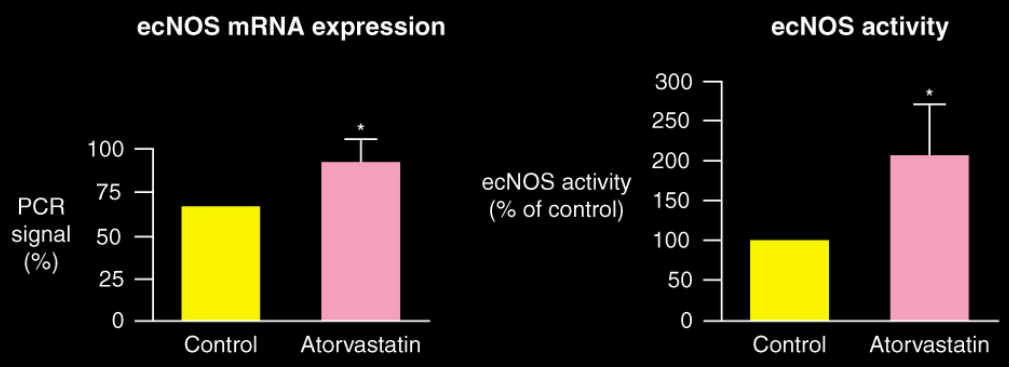
Physiology of NO in the human coronary and peripheral vasculature



Adapted from Quyyumi AA. *Am J Med.* 1998;105(1A):32S-39S.

VBWG

Statins upregulate ecNOS expression and increase ecNOS activity in normocholesterolemic SHR



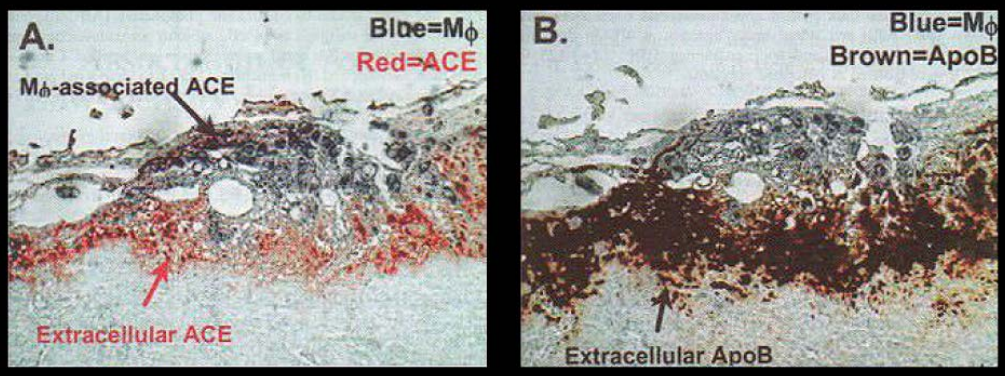
*$P < 0.05$ vs control
ecNOS = endothelial cell nitric oxide synthase
SHR = spontaneously hypertensive rats

Wassmann

"In vivo" statin effect: Dependent on change in LDL or Pleiotropic?

VBWG

Colocalization of ACE and LDL in aortic valvular lesions

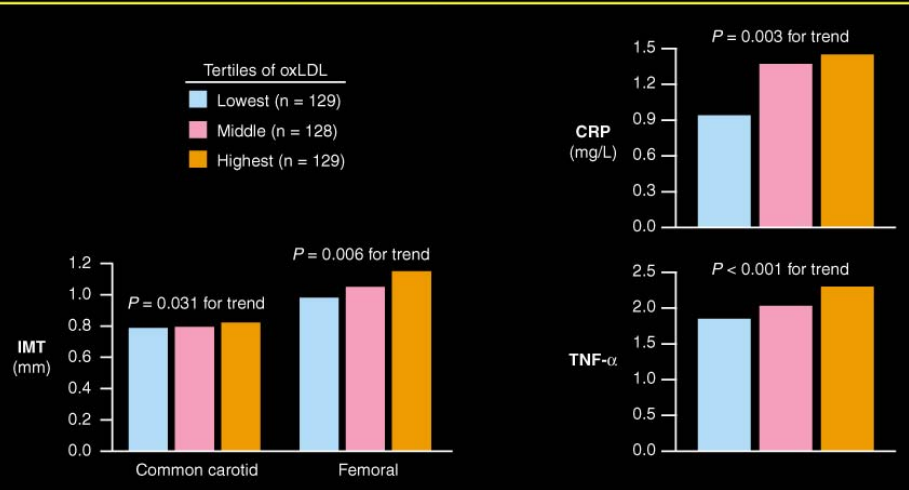


Hyperlipidemia → HBP
Statins lower BP

O'Brien KD, et al. *Circulation*. 2002;106:2224-2230.

VBWG

OxLDL correlates with subclinical atherosclerosis and inflammatory activity

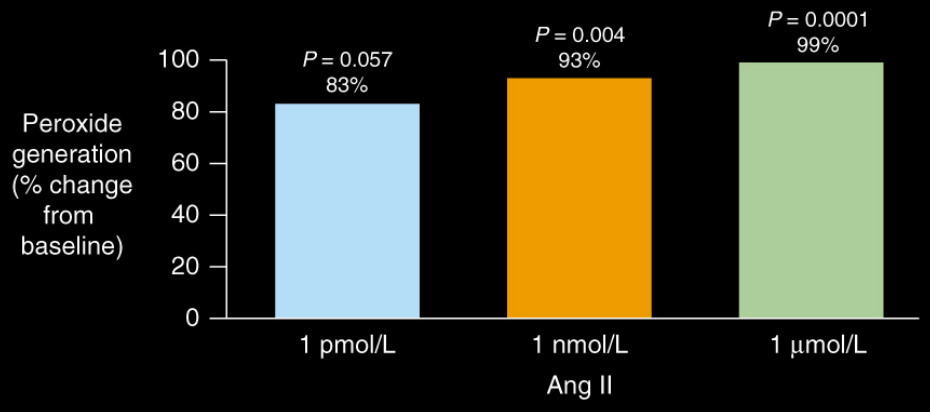


Hulthe J, Fagerberg B. *Atheroscler Thromb Vasc Biol.* 2002;22:1162-1167.

VBWG

Ang II induces superoxide production in human vascular tissue

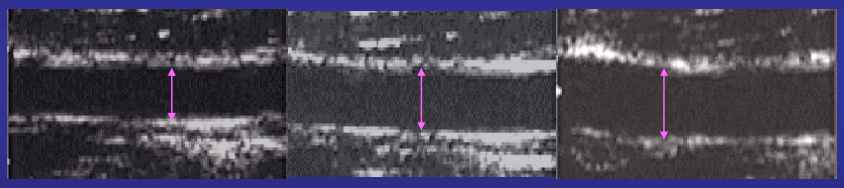
Human internal mammary artery segments incubated with Ang II for 4 hours



Berry C, et al. *Circulation.* 2000;101:2206-2212.

Flow-Mediated Vasodilation (FMD) in the Brachial Artery

Baseline Diameter	Hyperemic Flow	Nitroglycerin



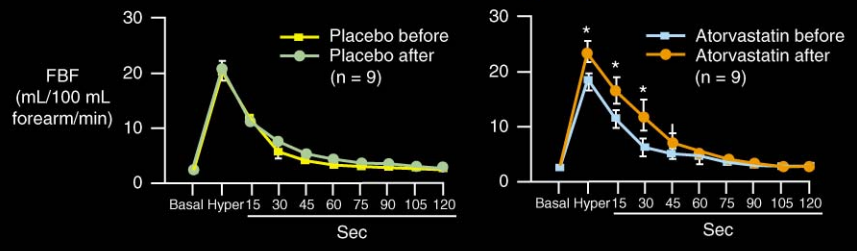
3.02 mm	3.34 mm	3.57 mm
Resting	10.6%	18.2%

Francois Charbonneau, 1996.

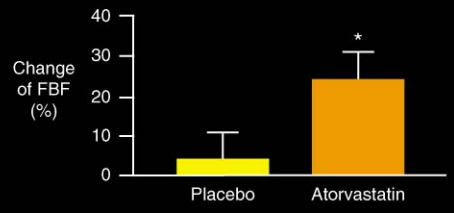
VBWG

Statin improves endothelium-dependent blood flow in patients with LDL <130 mg/dL

18 Patients treated with atorvastatin 80 mg or placebo for 6 wks



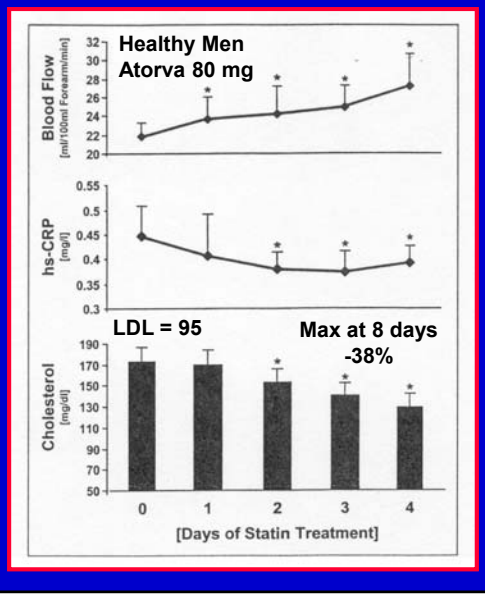
Surrogate Measure

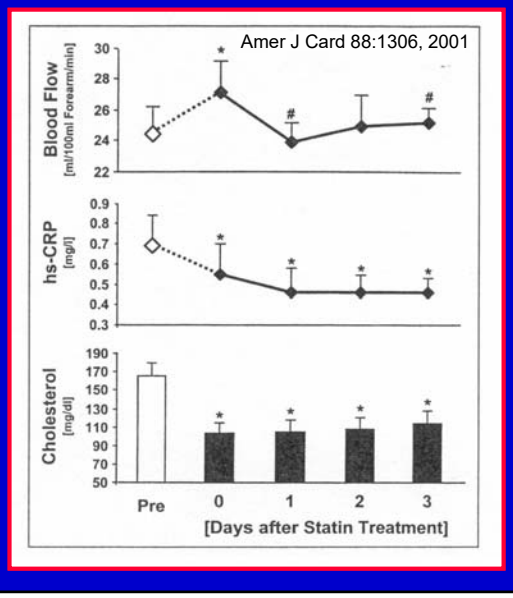


FBF = forearm blood flow
*P < 0.05 vs placebo group

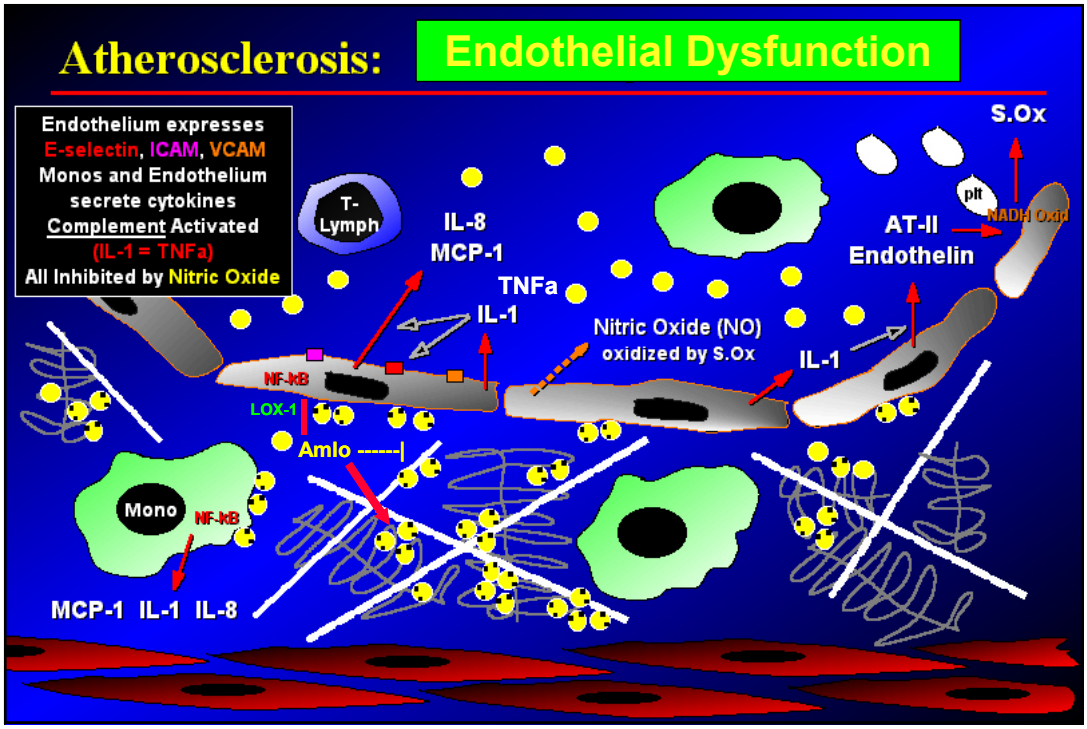
Wassman et al. *Am J Cardiol.* 2004:93:84-88.

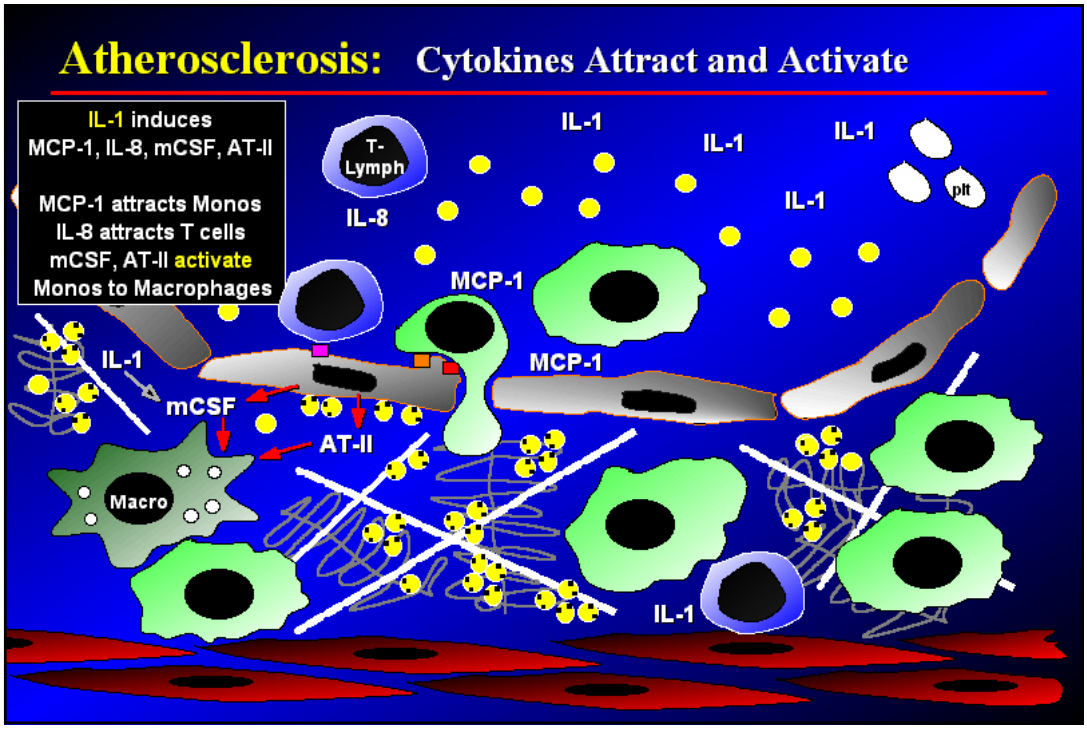
Atorvastatin: Rapid & Prolonged Action

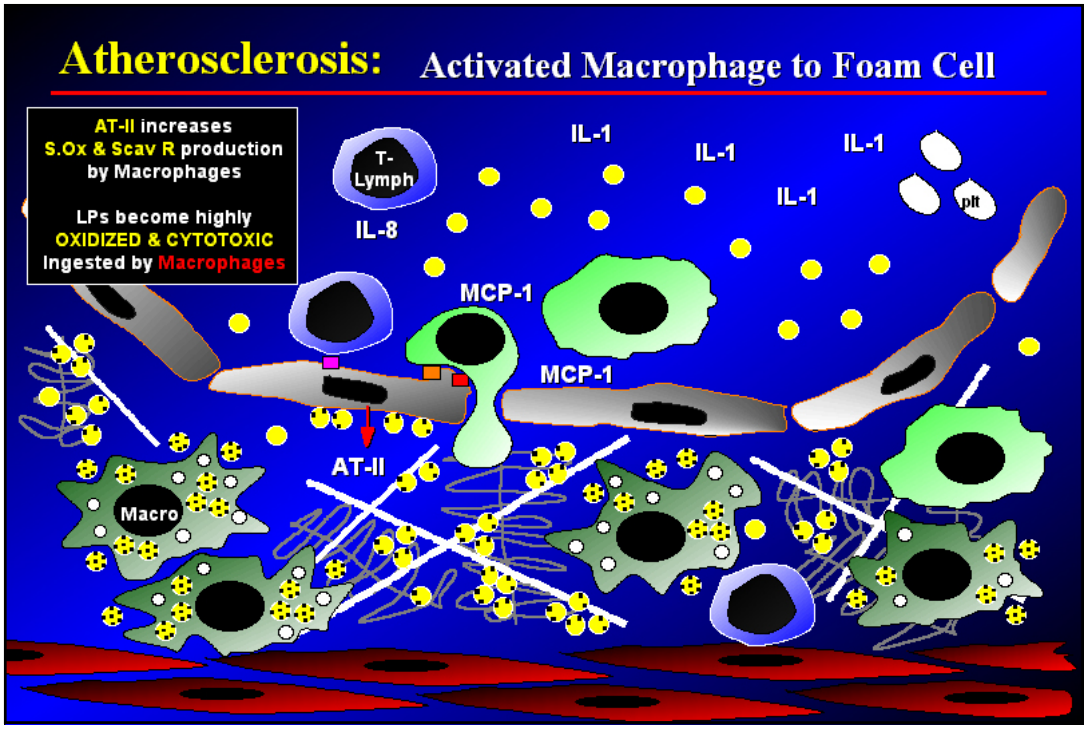


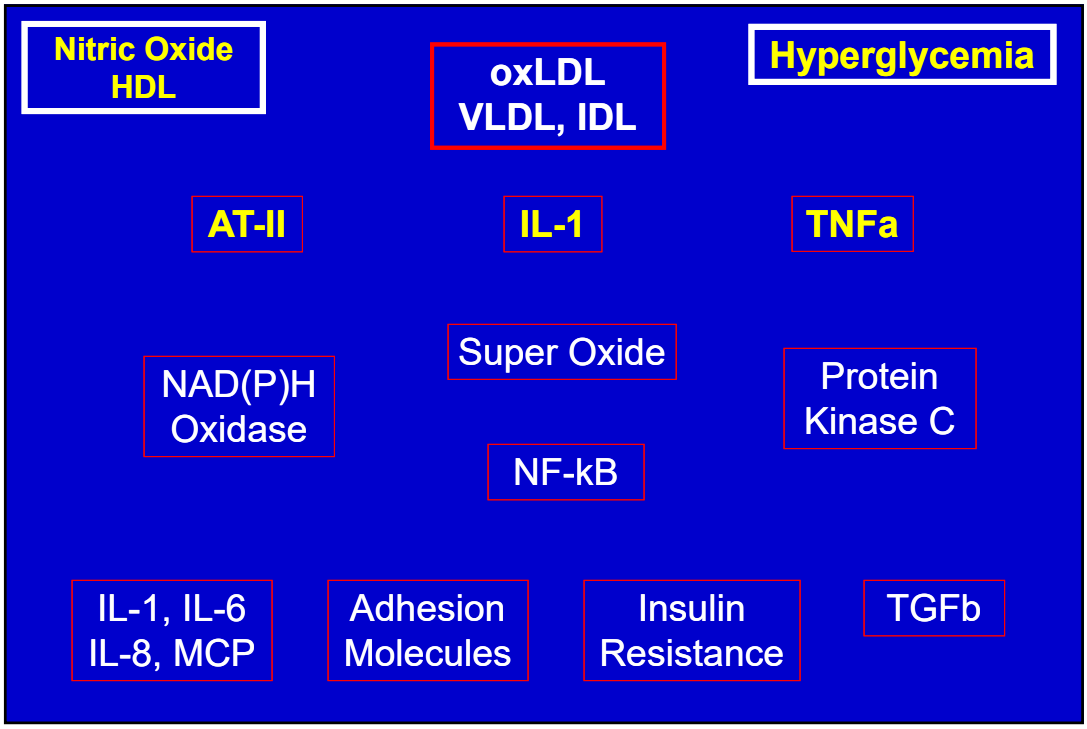


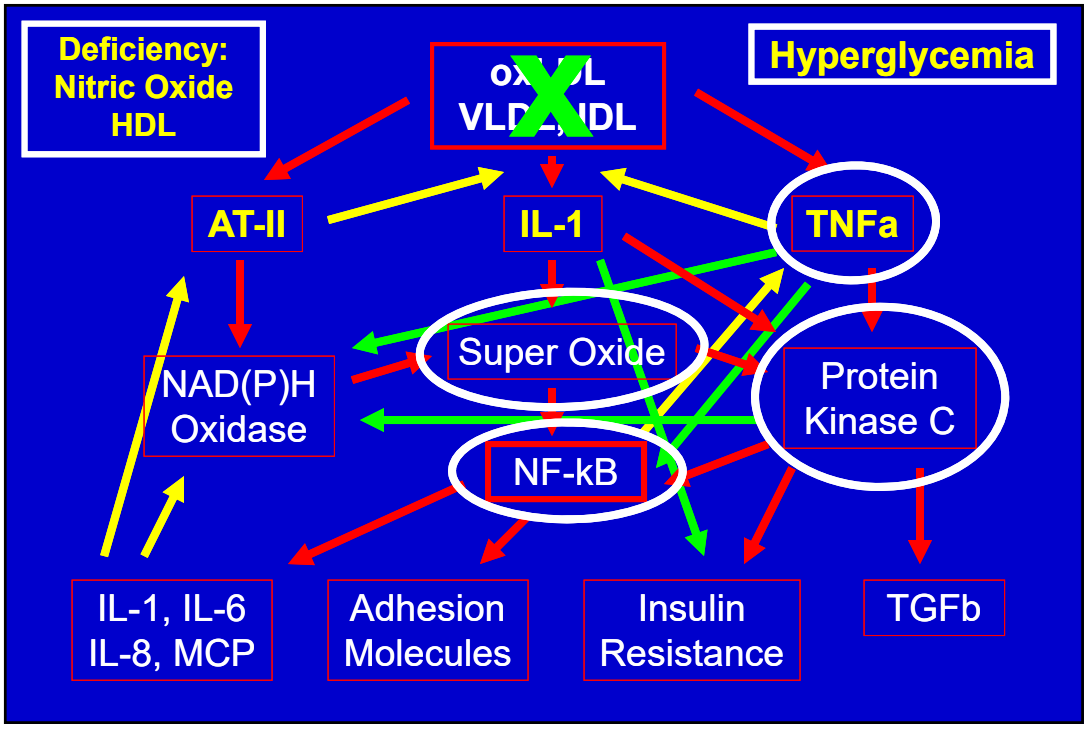
Atherosclerosis: Endothelial Dysfunction

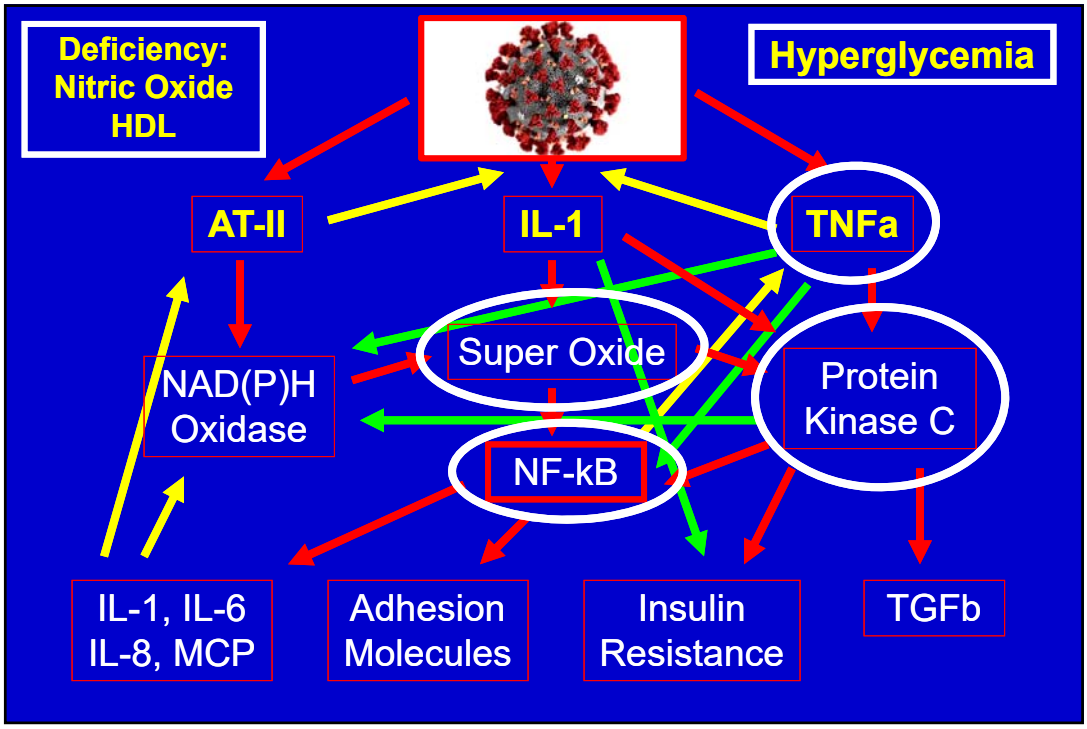


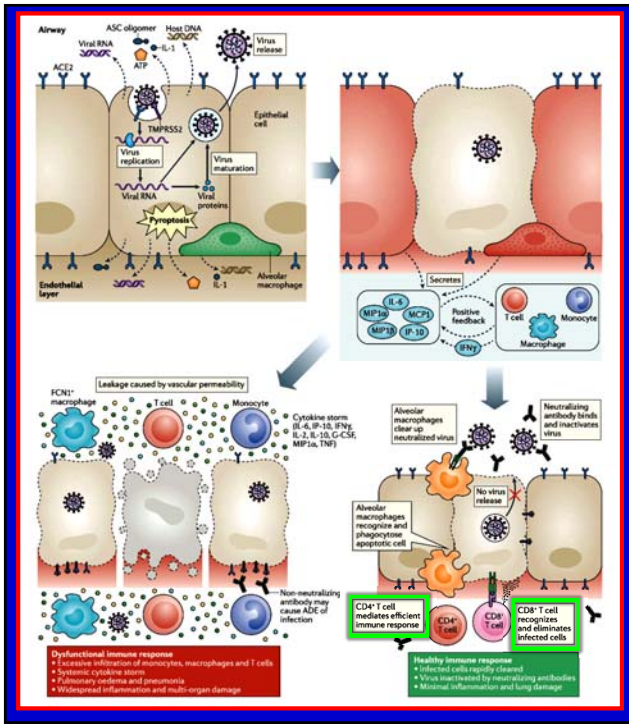

Atherosclerosis: Cytokines Attract and Activate
IL-1 induces
MCP-1, IL-8, mCSF, AT-II
MCP-1 attracts Monos
IL-8 attracts T cells
mCSF, AT-II activate
Monos to Macrophages
T-Lymph
IL-1
IL-1
IL-1
IL-1
plt
IL-8
MCP-1
MCP-1
IL-1
mCSF
AT-II
Macro
IL-1


Atherosclerosis: Activated Macrophage to Foam Cell
AT-II increases
S.Ox & Scav R production
by Macrophages
LPs become highly
OXIDIZED & CYTOTOXIC
Ingested by Macrophages
T-Lymph
IL-1
IL-1
IL-1
IL-1
plt
IL-8
MCP-1
MCP-1
AT-II
Macro


Nitric Oxide
HDL
oxLDL
VLDL, IDL
Hyperglycemia
AT-II
IL-1
TNFa
Super Oxide
NAD(P)H
Oxidase
Protein
Kinase C
NF-kB
IL-1, IL-6
IL-8, MCP
Adhesion
Molecules
Insulin
Resistance
TGFb


Deficiency:
Nitric Oxide
HDL
Hyperglycemia
AT-II
IL-1
TNFa
Super Oxide
NAD(P)H
Oxidase
Protein
Kinase C
NF-kB
IL-1, IL-6
IL-8, MCP
Adhesion
Molecules
Insulin
Resistance
TGFb





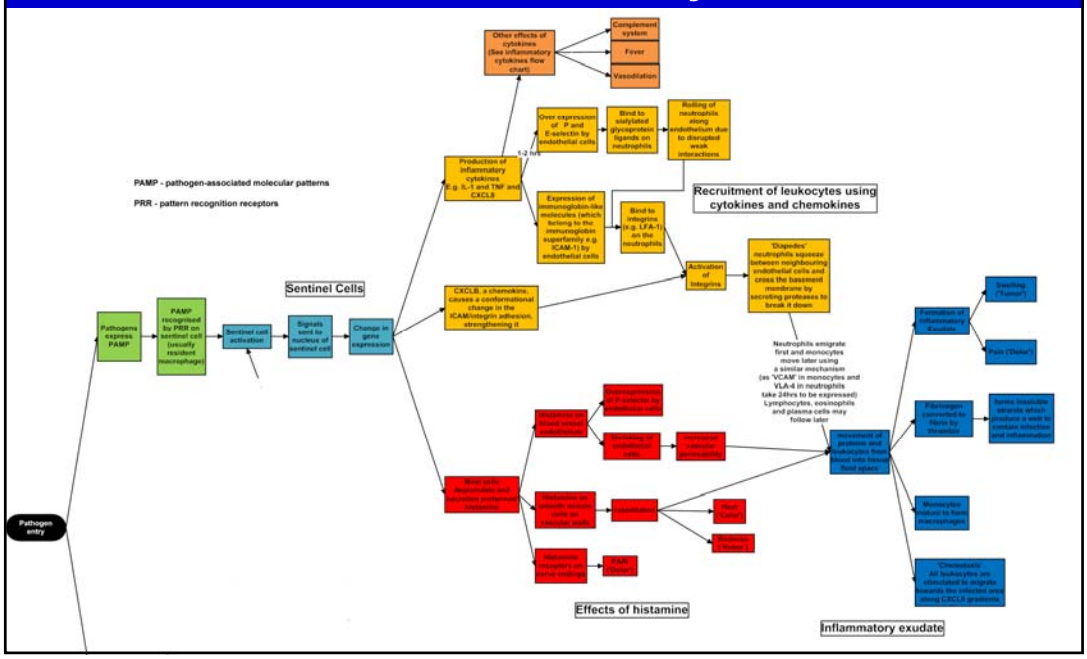
The Trinity of COVID-19: Immunity, Inflammation, and Intervention

Tay MZ et al:
Nature Reviews – Immunology
6:363-374, 2020

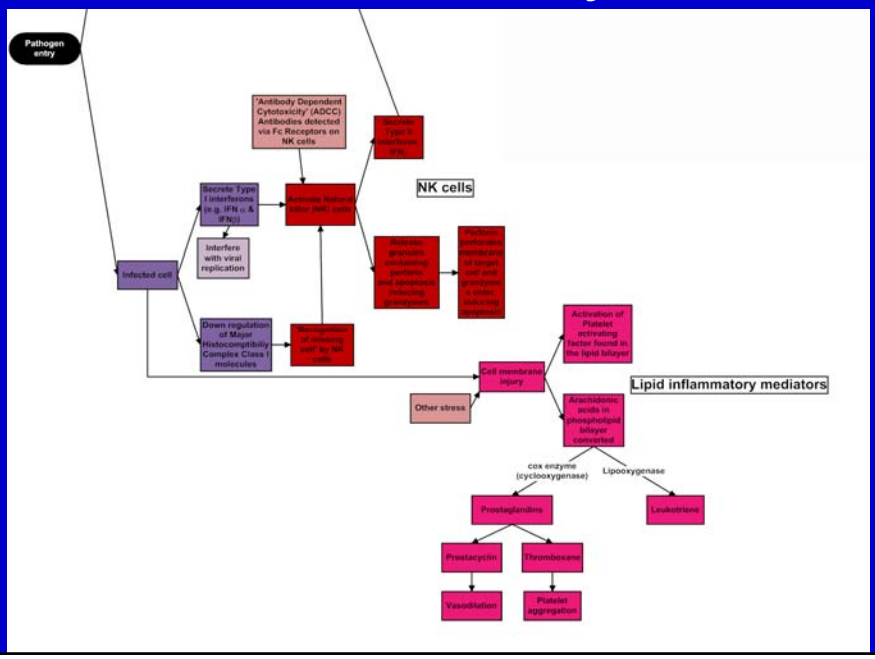
Fig. 1 | **Chronology of events during SARS-CoV-2 infection.** When severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects cells expressing the surface receptors angiotensin-converting enzyme 2 (ACE2) and TMPRSS2, the active replication and release of the virus cause the host cell to undergo pyroptosis and release damage-associated molecular patterns, including ATP, nucleic acids and ASC oligomers. These are recognized by neighbouring epithelial cells, endothelial cells and alveolar macrophages, triggering the generation of pro-inflammatory cytokines and chemokines (including IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1). These proteins attract monocytes, macrophages and T cells to the site of infection, promoting further inflammation (with the addition of IFNγ produced by T cells) and establishing a pro-inflammatory feedback loop. In a defective immune response (left side) this may lead to further accumulation of immune cells in the lungs, causing overproduction of pro-inflammatory cytokines, which eventually damages the lung infrastructure. The resulting cytokine storm circulates to other organs, leading to multi-organ damage. In addition, non-neutralizing antibodies produced by B cells may enhance SARS-CoV-2 infection through antibody-dependent enhancement (ADE), further exacerbating organ damage. Alternatively, in a healthy immune response (right side), the initial inflammation attracts virus-specific T cells to the site of infection, where they can eliminate the infected cells before the virus spreads. Neutralizing antibodies in these individuals can block viral infection, and alveolar macrophages recognize neutralized viruses and apoptotic cells and clear them by phagocytosis. Altogether, these processes lead to clearance of the virus and minimal lung damage, resulting in recovery. G-CSF, granulocyte colony-stimulating factor; TNF, tumour necrosis factor.

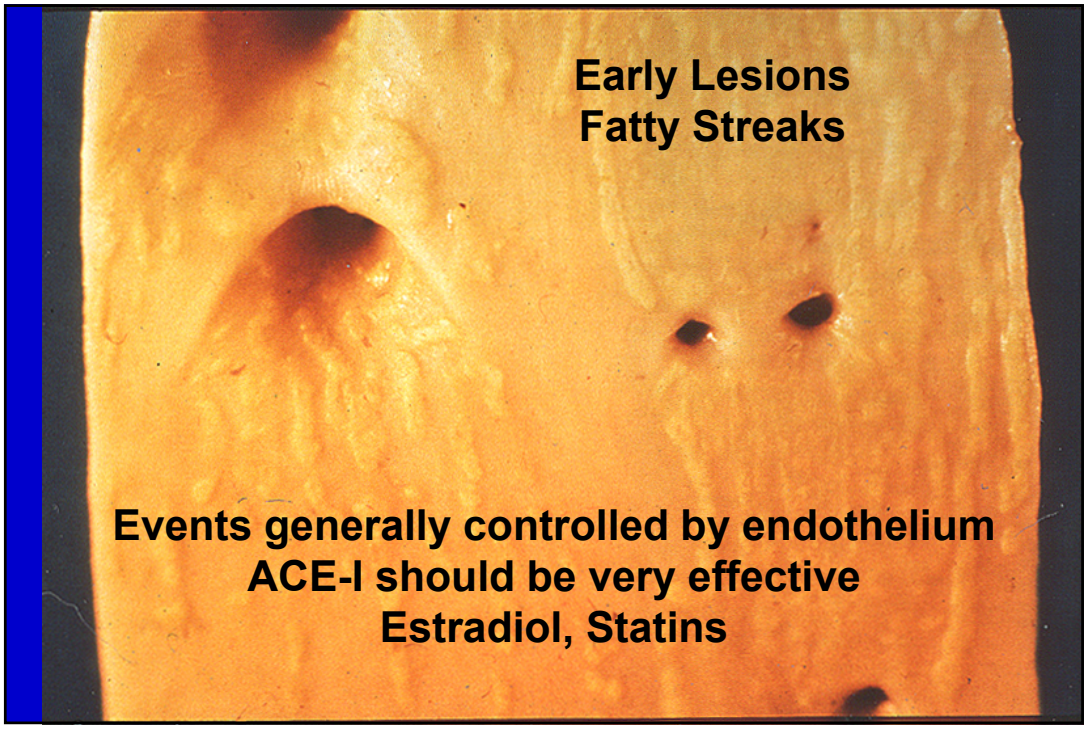
RBD – Receptor binding domain
TMPRSS2 – cellular serine protease
PRR – pattern recognition receptors
PAMP – pathogen associated molecular patterns
DAMP – damage associated molecular patterns
MIP – macrophage inflammatory proteins
ADE – antibody dependent enhancement

Innate Immunity



Innate Immunity

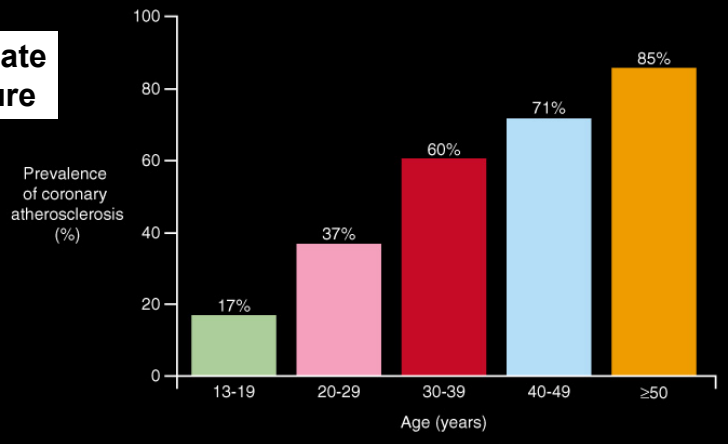




VBWG

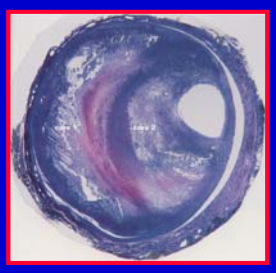
High prevalence of coronary atherosclerosis in teens and young adults

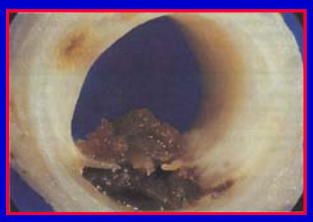
IVUS measurement of intimal thickness: 0.5 mm threshold



Tuzcu EM, et al. *Circulation*. 2001;103:2705-2710.

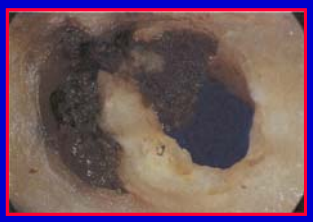
Atherosclerosis: Plaque Rupture → Thrombosis





Intraluminal

Intramural



Occlusive → Infarct

Atherosclerosis: Smooth Muscle Cell Recruitment

SMCs attempt to <u>contain</u> the inflammatory response:

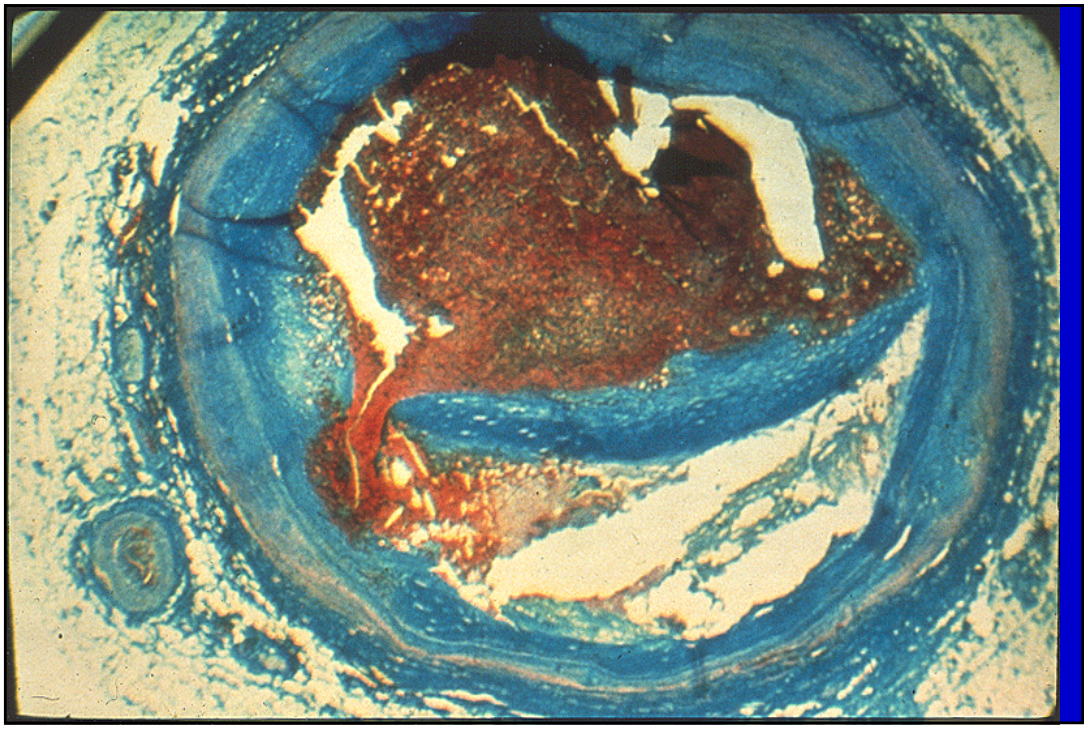
activated by: Endothelin, PDGF, TGFb inhibited by: IL-1

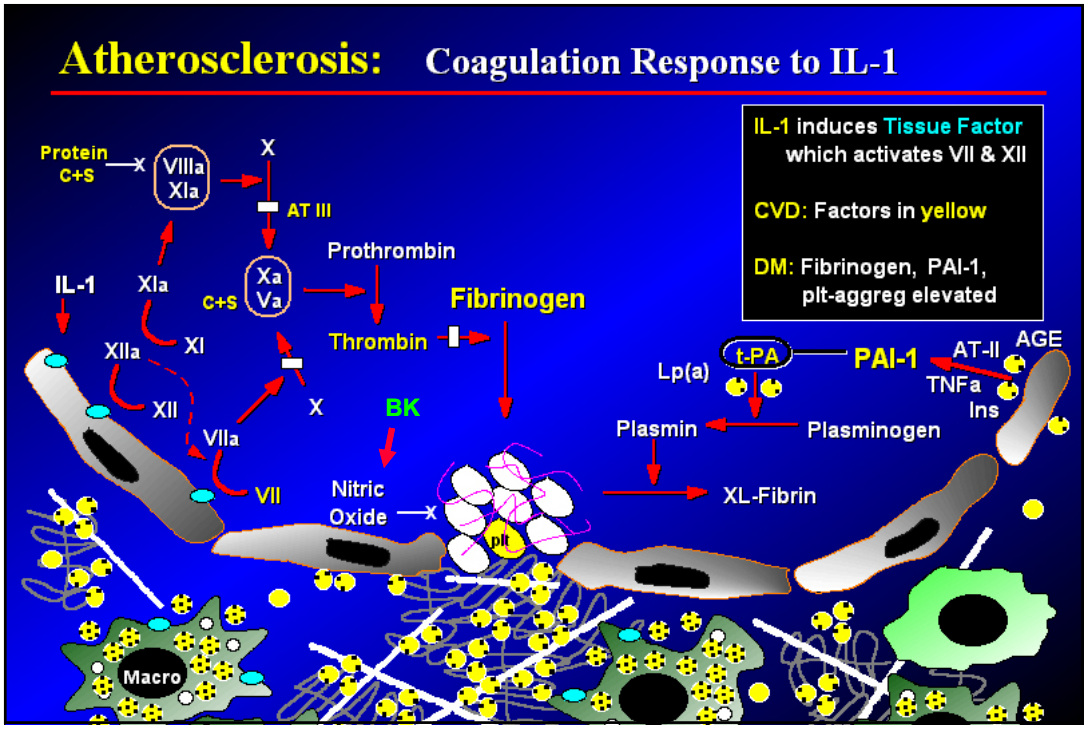
SMCs begin to take control of plaque metabolism (Dominate pathology in HT CVD)

IL-1 → ICAM, VCAM, LOX-1, VEGF, collagenase (MMP), apoptosis

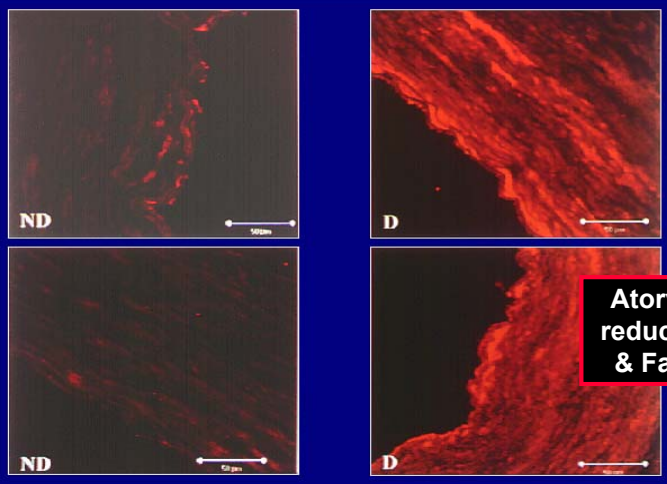
AT-II → superoxide, PAI-1, matrix synthesis (fibrosis)

Amlodip ----| Migration, prolif, pro-collagen, MMP-1, E-apoptosis, S.Ox




Atherosclerosis: Coagulation Response to IL-1
IL-1 induces Tissue Factor which activates VII & XII
CVD: Factors in yellow
DM: Fibrinogen, PAI-1, plt-aggreg elevated
Protein C+S
VIIIa XIa
X
AT III
Prothrombin
IL-1
XIa
C+S
Xa Va
Fibrinogen
XIIa
XI
Thrombin
XII
X
BK
VIIa
VII
Nitric Oxide
plt
Lp(a)
t-PA
PAI-1
AT-II
AGE
TNFa
Ins
Plasmin
Plasminogen
XL-Fibrin
Macro

PAI-1 in Internal Mammary Arteries of People With and Without Diabetes

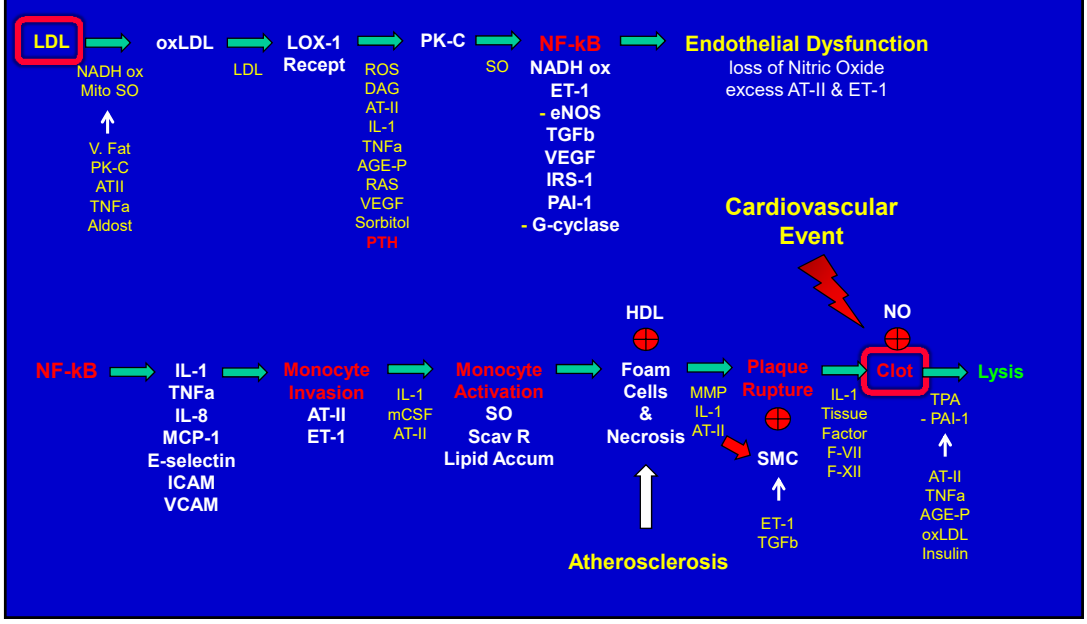


Atorvastatin reduces PAI-1 & Factor VII

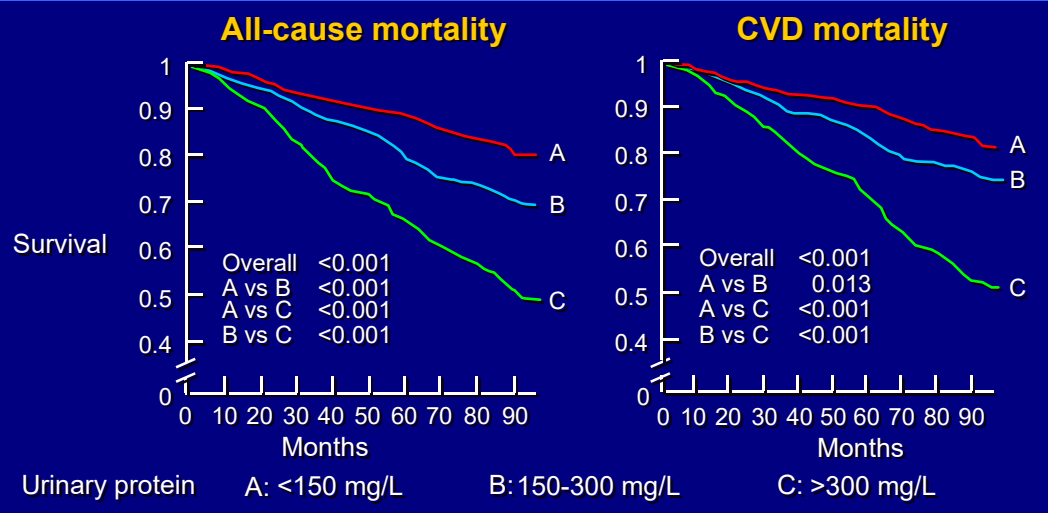
ND = no diabetes; D = diabetes.

Pandolfi A et al. *Arterioscler Thromb Vasc Biol*. 2001;21:1378-1382.

Atherogenesis



Effect of Proteinuria on All-Cause and CVD Mortality in Patients With Type 2 Diabetes

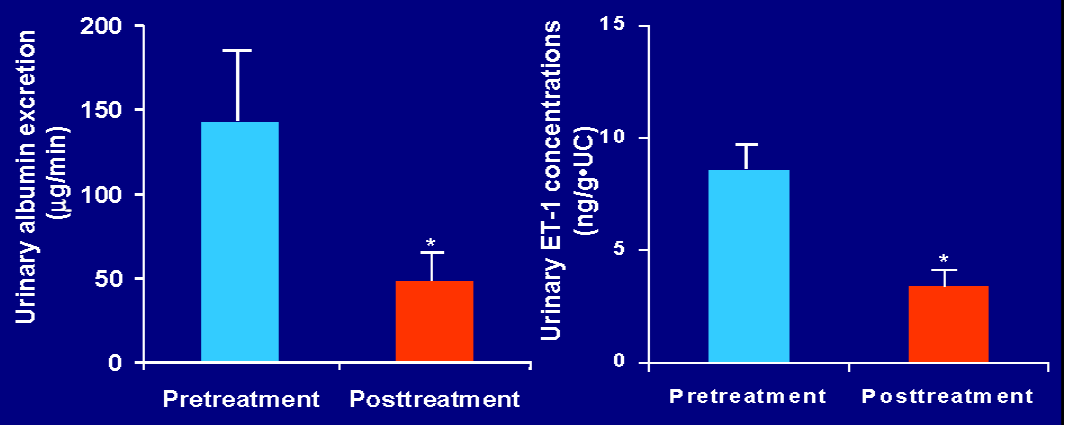


Urinary protein A: <150 mg/L B: 150-300 mg/L C: >300 mg/L

II.17 ©1998 PPS

Miettinen H et al. *Stroke*. 1996;27:2033-2039.

Effect of Pioglitazone on Urinary Albumin and Endothelin-1 Excretion in Patients With Microalbuminuria



*$P<0.01$.

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Nakamura T et al. *J Diabetes Complications*. 2000;14:250-254.

Effects of Atorvastatin on Proteinurea & Progression of Renal D.

Glomerulonephritis (n = 56)

Age	55.6 yrs
CrCl	50.4 ml/min
UPE	2.2 g/day
LDL	198 mg/dl → 121
HDL	36 mg/dl
Trig	174 mg/dl → 132
Album	3.3 g/dl

One year Rx HBP

ACEI	96%
CCB	45%
ASA	66%

Goal:
LDL < 120 mg/dl or ↓ 40%
Dose: 10 - 40 mg/d

Bianchi et al;
Amer J Kid D 41:565-570, 2003