1st International Meeting on Nitric Oxide:
From Basic Science to Clinical Evidence

Sociedad Espanola de Hipertension (SEH)
Menarini International

Auditorium Hospital Universitari San Juan de Dios, May 9, 2003, BARCELONA

Pathogenic Mechanisms of Arterial Damage and New Therapeutical Insights

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- Depts. of Medicine and Clinical Pathology, Univ. of Naples, Italy
- Dept. of Medicine, San Diego Univ., California
- e-mail: claunap@tin.it

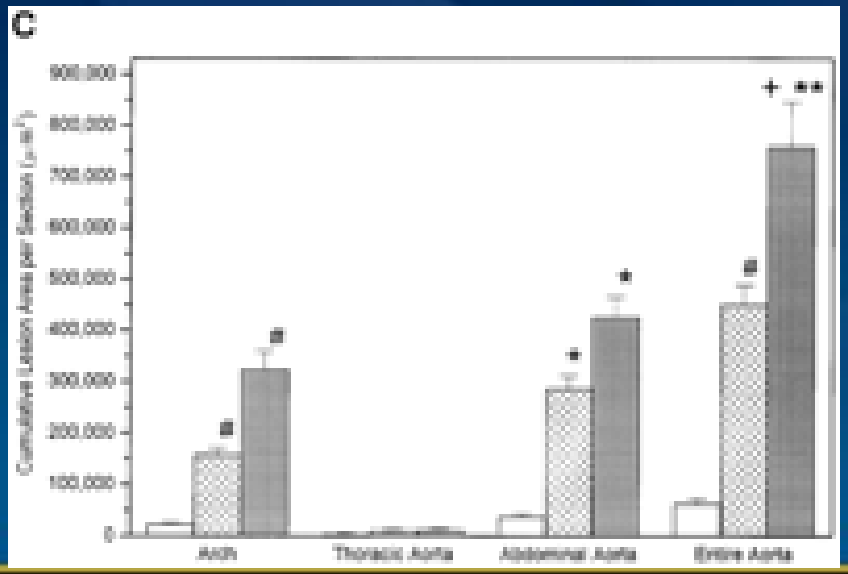
When arterial damage begins in humans?

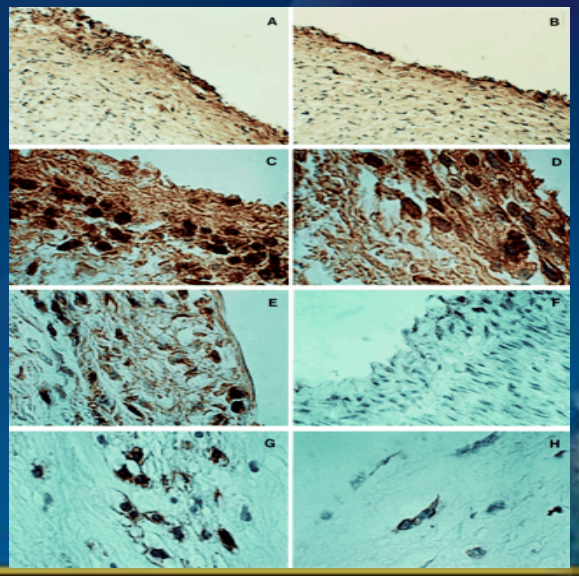
- In adult age?

Fatty Streak Formation Occurs in Human Fetal Aortas and is Greatly Enhanced by Maternal Hypercholesterolemia: Intimal Accumulation of Low Density Lipoprotein and its Oxidation Precede Monocyte Recruitment into Early Atherosclerotic Lesions

C. Napoli, F. P. D'Armiento, F. P. Mancini, J. L. Witztum, G. Palumbo, W. Palinski

J. Clin. Invest. Volume 100, December 1997, pp 2680-2690.





The New England Journal of Medicine

New England Journal of Medicine Volume 340 January 14, 1999

Mechanisms of Disease: Atherosclerosis - An Inflammatory Disease

Russell Ross

"...it is established that atherogenesis begins in fetal age (8)…"

(8) C. Napoli, F. P. D'Armiento, F. P. Mancini, et al. ***J. Clin. Invest.*** Volume 100, December 1997, pp 2680-2690.

THE LANCET

Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study.

C. Napoli, CK Glass, JL Witztum, R Deutsch, FP D'Armiento, W Palinski.
***Lancet* 1999 Oct 9;354:1234-1241.** THE LANCET PUBLISHING GROUP

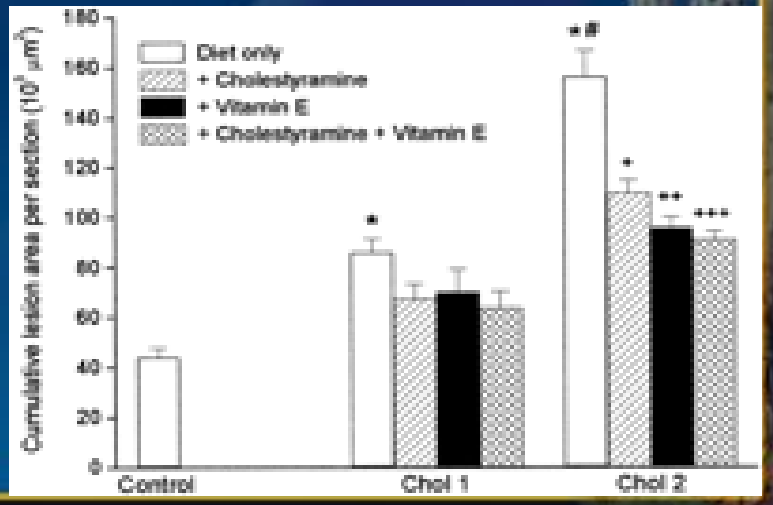
PDAY Study (1994), BOGALUSA (1998)

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association.
Fighting Heart Disease and Stroke

Maternal Hypercholesterolemia Enhances Atherogenesis in Normocholesterolemic Rabbits, Which Is Inhibited by Antioxidant or Lipid-Lowering Intervention During Pregnancy: An Experimental Model of Atherogenic Mechanisms in Human Fetuses

C. Napoli, J. L. Witztum, F. Calara, F. de Nigris, W. Palinski

(*Circulation Research*. 2000;87:946-954)



European Heart Journal
W.B. SAUNDERS

European Heart Journal
Vol. 22, pp 4-9 January 1, 2001

Maternal hypercholesterolemia during pregnancy influences the later devolopment of atherosclerosis: clinical and pathogenic implications

C. Napoli, and W. Palinski

Endothelium dysfunction and arterial damage are different entities in intracranial arteries

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association
Fighting Heart Disease and Stroke

Volume 99, April 20, 1999, pp 2003-2010.

Intracranial Arteries of Human Fetuses Are More Resistant to Hypercholesterolemia-Induced Fatty Streak Formation Than Extracranial Arteries

C. Napoli, MD, PhD; J. L. Witztum, MD; F. de Nigris, BiolD, PhD; G. Palumbo, PhD; F. P. D'Armiento, MD; W. Palinski, MD

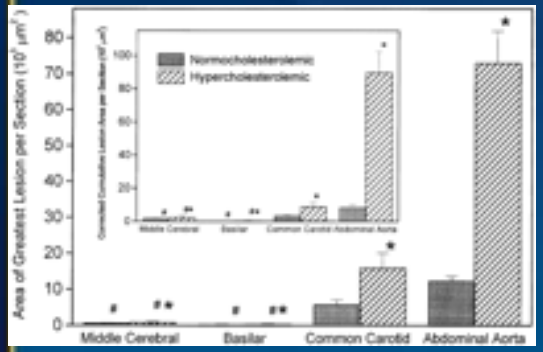


Table 3. Comparison of Total Activity of Antioxidant Enzymes in Homogenates of Intracranial and Extracranial Arteries

	Abdominal Aorta	Carotid Arter	Middle Cerebral Artery	Basilar Artery
No. of segments	12	15	15	12
Glutathione peroxidase, mU/mg protein	70.6±11.5	67.5 ±14.5	84.8±16.5	80.2±11.5
Catalase, IU/mg protein	15.4±8.5	13.6±7.5	19.1±6.5	18.4±5.9
Cu/Zn-SOD, IU/mg protein	6.8±0.9	7.2±1.0	6.5 ±0.9	6.6±0.9
Mn-SOD, IU/mg protein	2.0±0.4	1.6 ±0.4	3.2±0.6[1]	3.1±0.6[1]

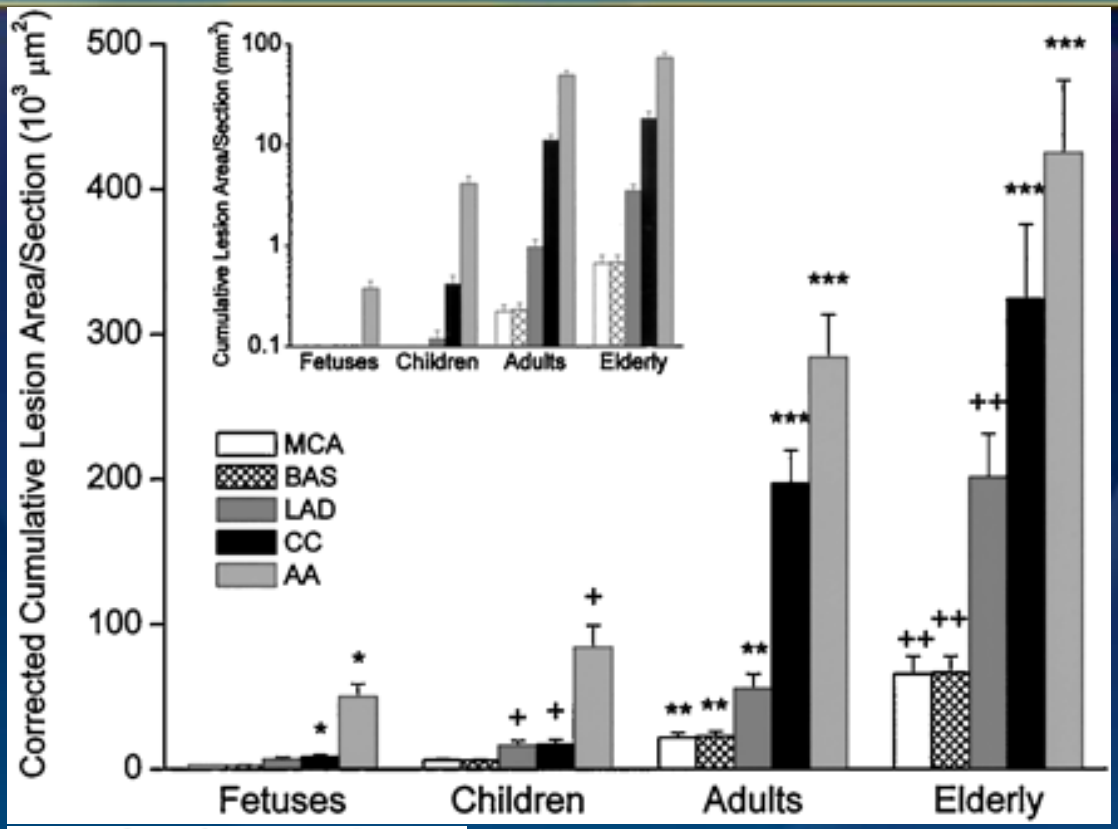
Stroke
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Stroke Association
A Division of American Heart Association

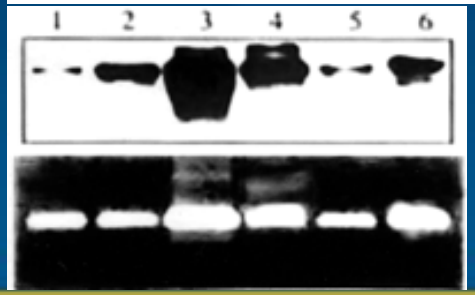
Volume 32, November, 2001

Age-Related Effects on Atherogenesis and Scavenger Enzymes of Intracranial and Extracranial Arteries in Men Without Classic Risk Factors for Atherosclerosis

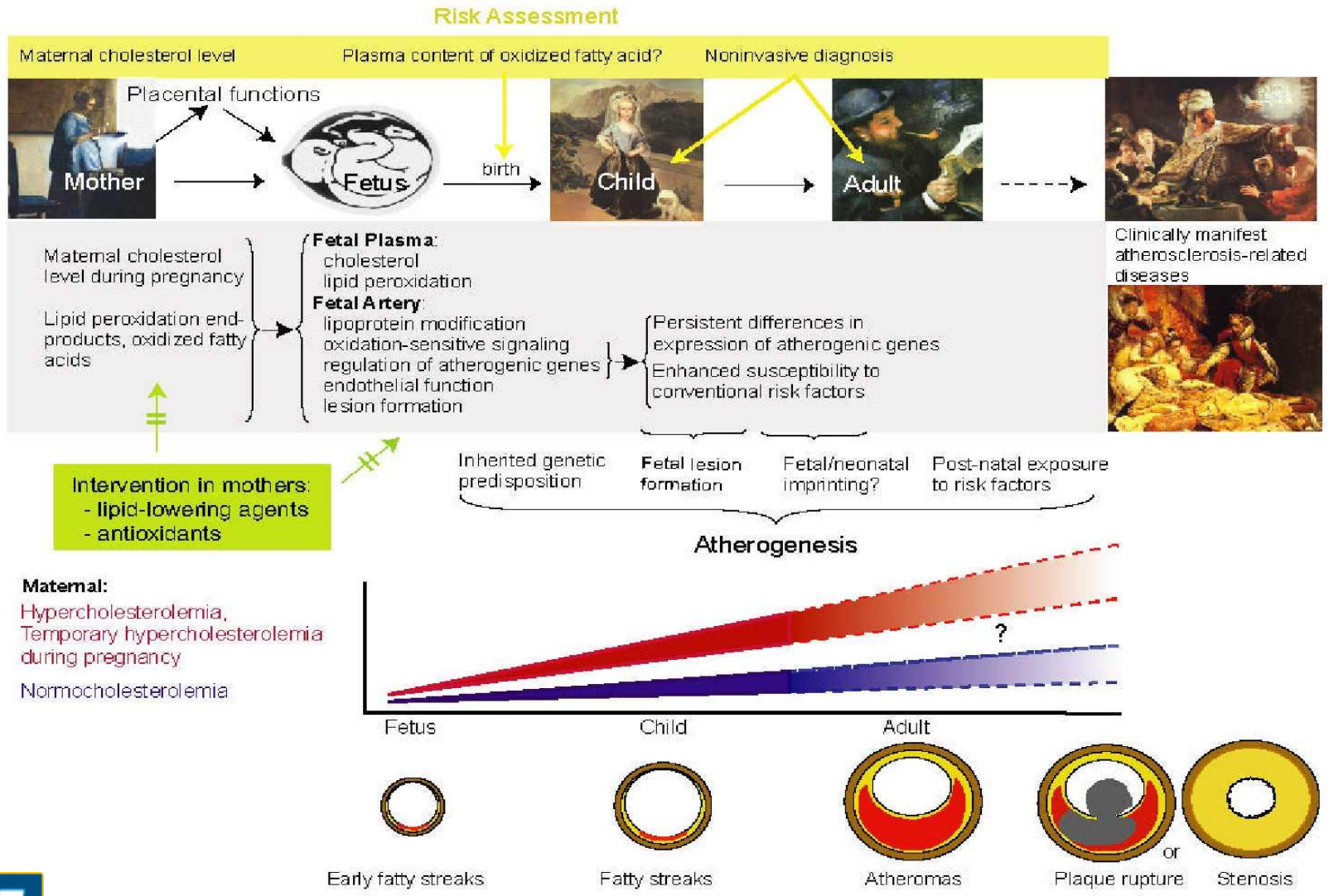
F. P. D'Armiento, MD; A. Bianchi, MD; F. de Nigris, PhD; D. M. Capuzzi, MD; M.R. D'Armiento, MD; G. Crimi, MD; P. Abete, MD; W. Palinski, MD; M. Condorelli, MD; and C. Napoli, MD, PhD

(Stroke. 2001;32:2472-2480)





(Stroke. 2001;32:2472-2480)



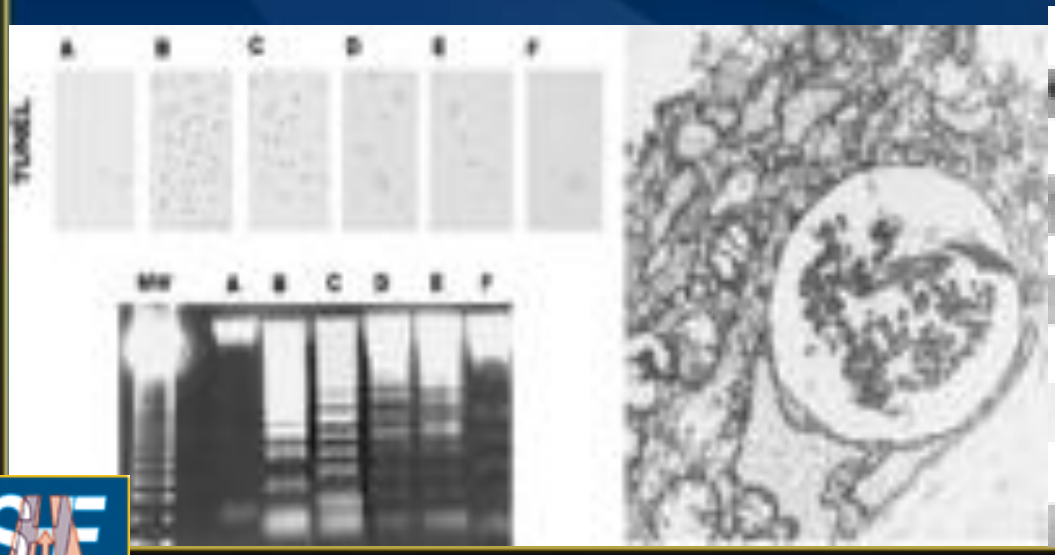
Napoli et al. FASEB J 2002; 16:1348-1360.

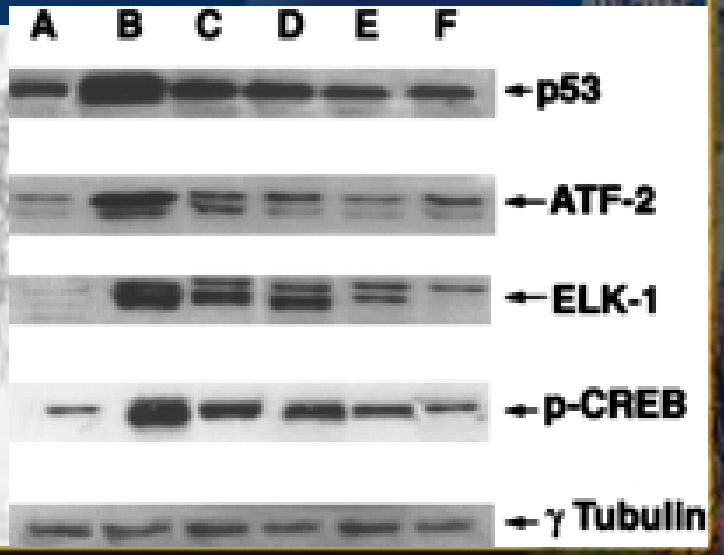
Oxidative stress interferes with coronary apoptosis

FASEB J Volume 14, pp 1996-2007, October 2000

Mildly oxidized low density lipoprotein activates multiple apoptotic signaling pathways in human coronary cells

C. NAPOLI, O. QUEHENBERGER, F. DE NIGRIS,
C. K. GLASS, W. PALINSKI





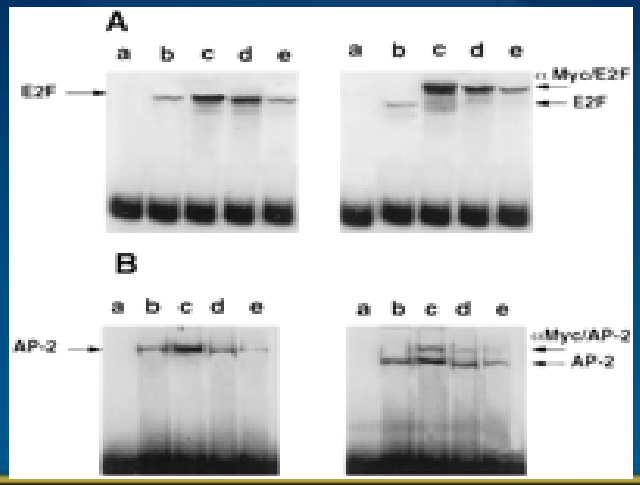
Oxidative stress may activate oncogenes in the arterial wall

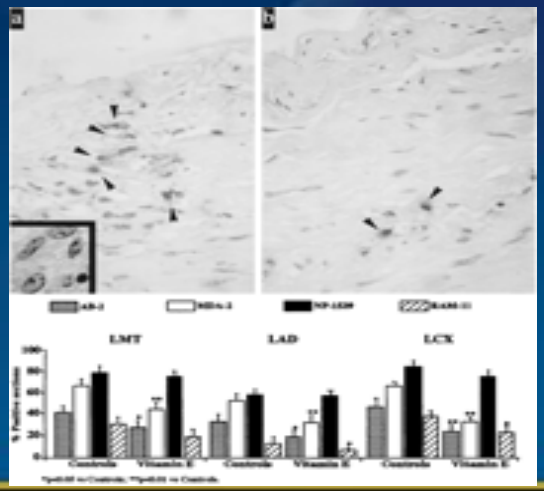
Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association
Fighting Heart Disease and Stroke

Circulation **2000; 102: 2111-2117.**

Evidence for Oxidative Activation of c-Myc–Dependent Nuclear Signaling in Human Coronary Smooth Muscle Cells and in Early Lesions of Watanabe Heritable Hyperlipidemic Rabbits: Protective Effects of Vitamin E

F. de Nigris, T. Youssef, S.A. Ciafré, G.L. Condorelli, W. Palinski, and C. Napoli

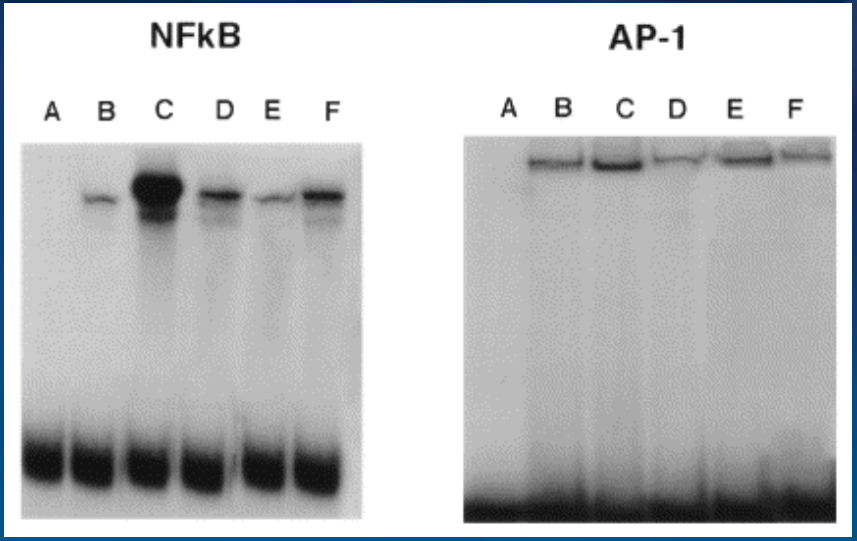




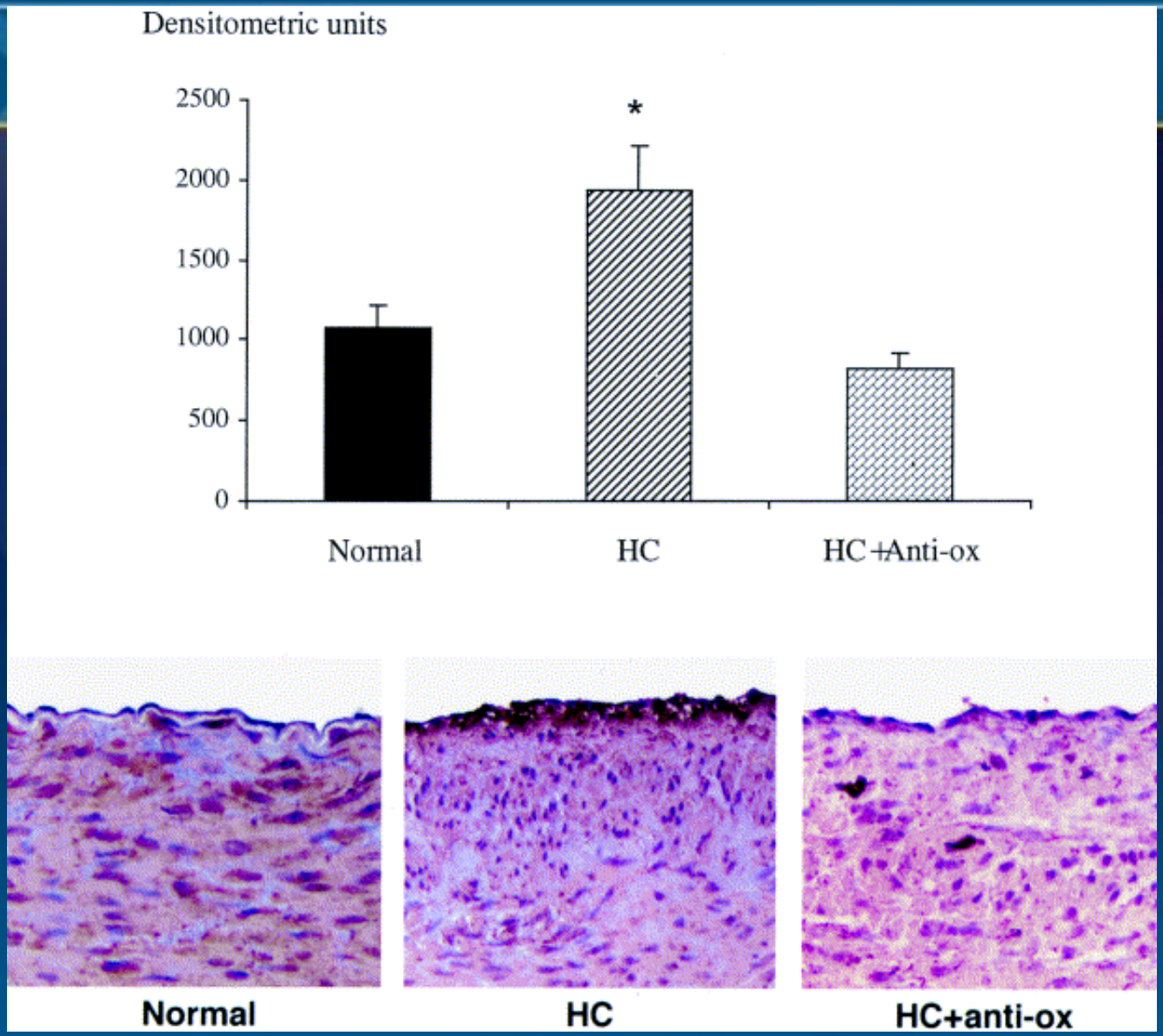
Oxidative stress may promote NFkβ activation in the cardiovascular system

Binding shift analysis for assessing activity of NFK- B (left panel) and of the AP-1 complex (right panel) in nuclear extracts from human coronary SMC exposed to oxLDL and both tocopherols added before LDL oxidation.

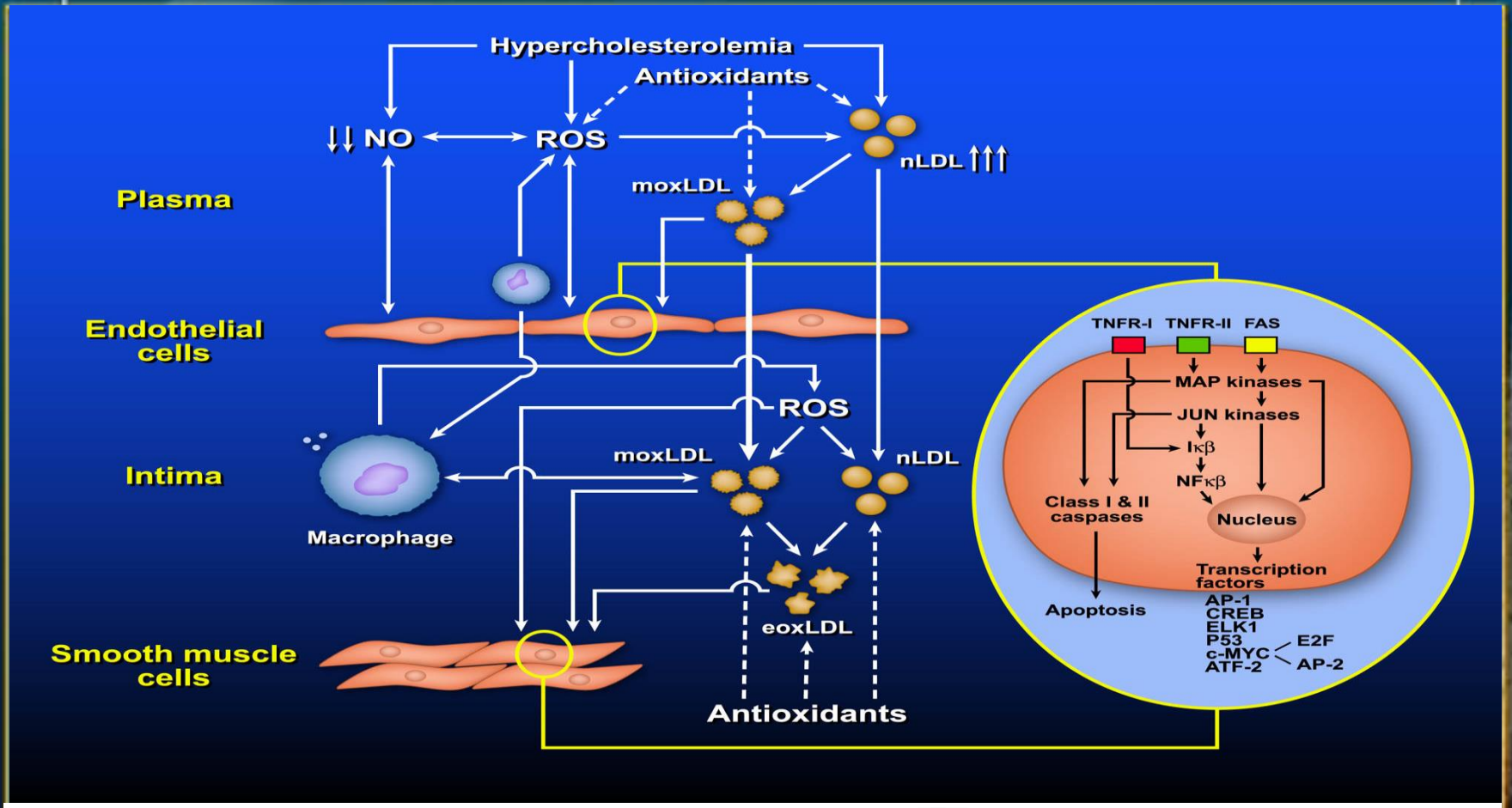
A: sham; B: LDL; C: m-oxLDL; D: m-oxLDL + 10μM α-tocopherol; E: m-oxLDL + 50μM α-tocopherol; F: m-oxLDL + 50μM γ-tocopherol.



de Nigris et al. *Biochemical Pharmacology* 59: 1477-1487, 2000



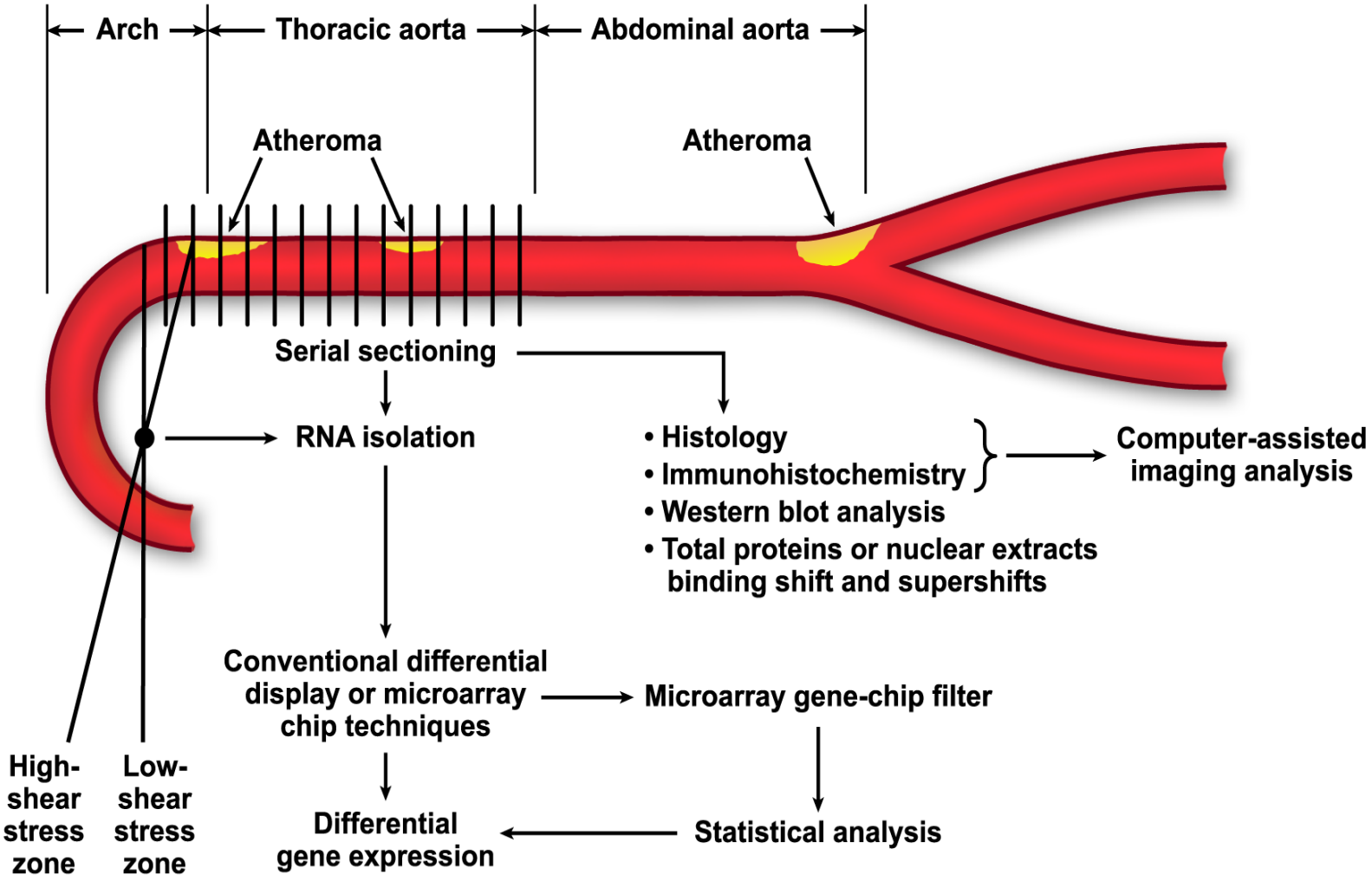
Rodriguez-Porcel et al. Cardiovasc Res 2002; 53:1010-8.



Mayo Clinic Proceedings

Napoli C and Lerman LO,
2001;76:619-633.

The road ahead…

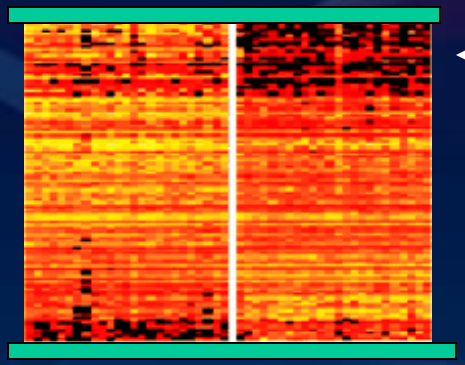


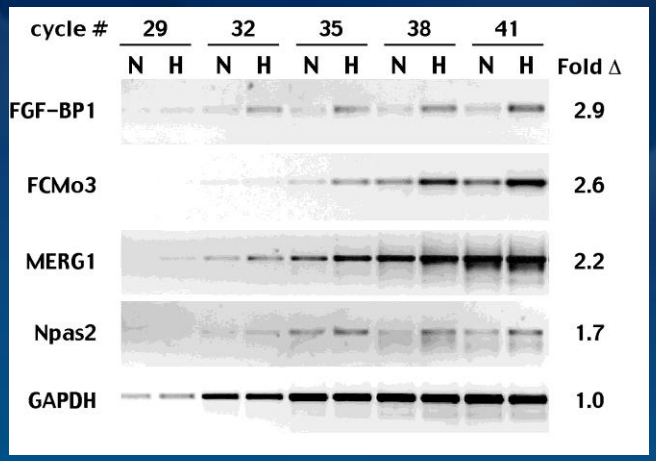
de Nigris F & Napoli C. *Antioxidants & Redox Signaling* 2001; 3:1119-1130.

SAMPLES (laser capturing)

Healthy tissue ↙ ↘ Atherosclerotic Lesion

MICROARRAY CHIP GENE ANALYSIS





Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association.
Fighting Heart Disease and Stroke

Napoli C et al *Circulation* 2002; 105:1360-1367.

MICROARRAY GENE ANALYSIS

Upregulated

- SOD3 (extracellular superoxide dismutase)
- Beta-galactoside alpha 2,6-sialyltransferase
- ERR1 (estrogen related receptor alpha)
- p140mDia
- NPAS2 (member of the bHLH-PAS family)
- DM-PK (myotonic dystrophy protein kinase)
- IL-6 signal transducer (gp130)
- Plakoglobin
- FMO3 (flavin-containing monooxygenase 3)
- MERG1 (Mouse potassium channel; similar to HERG)
- GDI-1 (GDP-dissociation inhibitor 1)
- Lamin A/C and C2
- McaBP (mouse 57-KD calcium-binding protein)
- Tryptophanyl-tRNA synthetase, WRS alpha-2 subunit
- Fibroblast growth factor binding protein (FGF-BP)
- T-cell receptor germline beta-chain J-beta-2 gene cluster
- Vacuolar adenosine triphosphatase subunit A
- MMMH (mouse 8-hydroxyguanine glycosylase)
- Rhotekin
- EXT2 (multiple exostosis protein)
- MHR a-1 (type I keratin)
- T cell transcription factor NFAT1 isoform C
- MUP2 (mouse uroplakin 2)

Downregulated

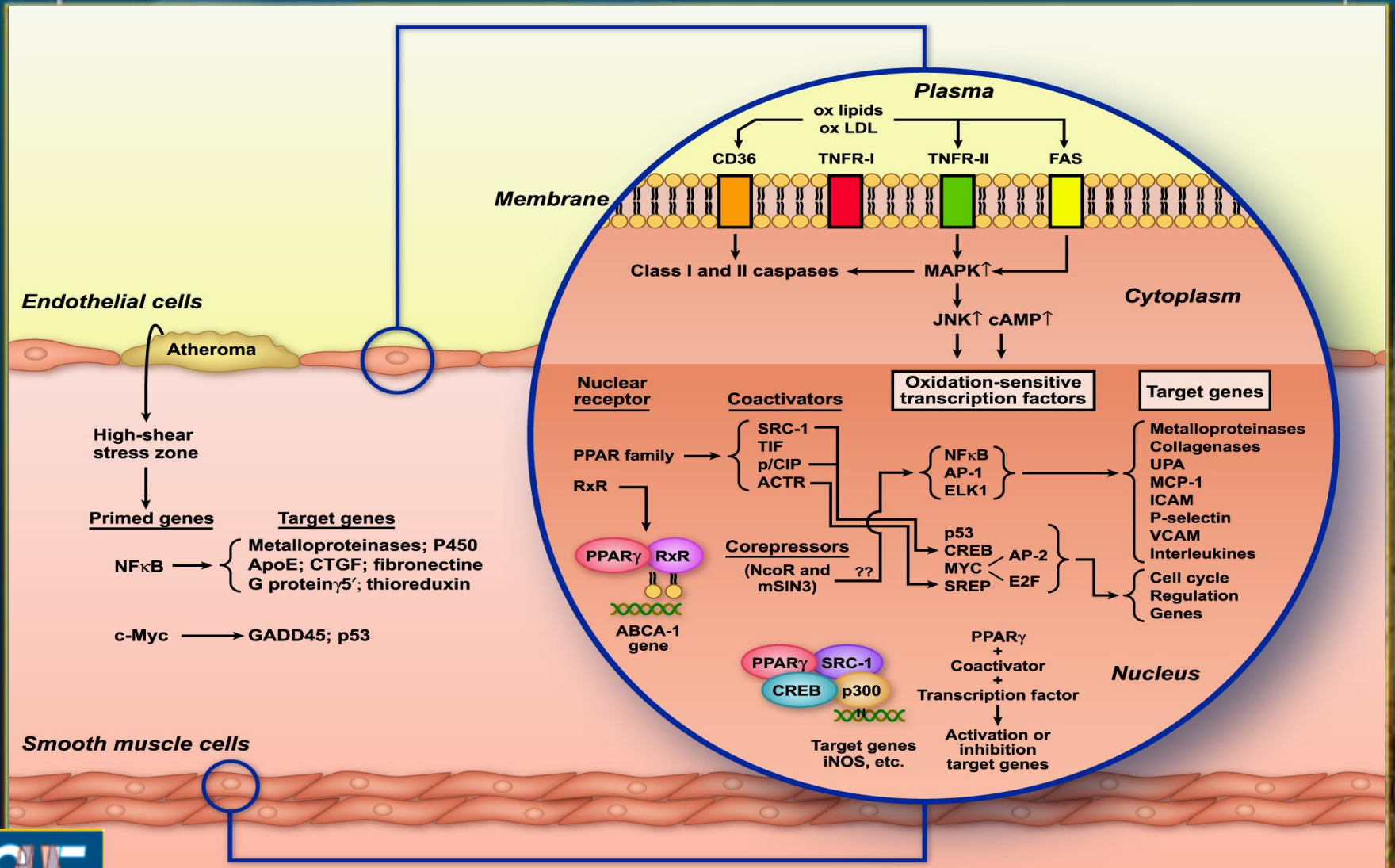
- Cytochrome c oxidase subunit VIaH
- Zinc finger protein (AA 1-580)
- SAP (serum amyloid P)
- C57BL/6J OB/OB haptoglobin
- FAH (fumarylacetoacetate hydrolase)
- MHR23B
- Alpha-7 integrin
- TFIIH, 62 kD subunit (transcription factor)
- P58 (protein kinase inhibitor)
- JUMONJI protein
- Calpactin 1 light chain (P11)
- ALDH2 (mitochondrial aldehyde dehydrogenase 2)
- BAD
- Neuronal intermediate filament protein
- Fas antigen
- MTF-2A (mouse basic transcription factor)
- Mouse C10-like chemokine
- ATPS F6 (mitochondrial ATP synthase coupling factor 6)
- pp105 Rb (retinoblastoma susceptibility protein)
- APO-C2 (apolipoprotein C2)
- Retinoic acid-responsive proteine (MK)
- GKL (gut-enriched Kruppel-like factor; zinc finger protein)
- calpactin I light chain
- Non-pancreatic secreted type II phospholipase A2
- GALT (galactose-1-phosphate uridyl transferase)
- Asparagine synthetase
- IgG variable region PIR-S276746 (Ig heavy chain J region JH3)

Heart Online BMJ Publishing Group

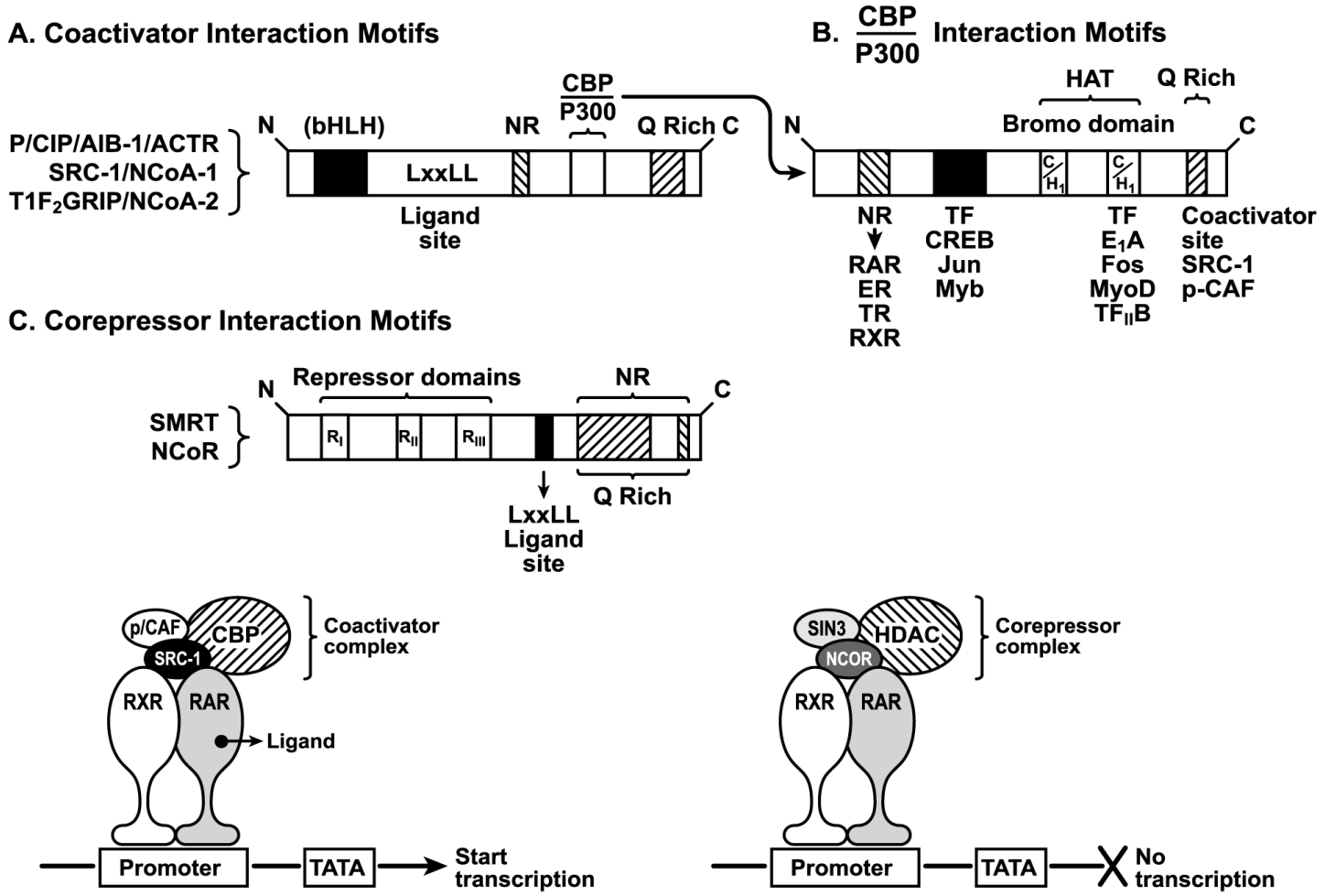
Napoli C, Lerman A, Sica V, & de Nigris F.

- MICROARRAY ANALYSIS: A NOVEL RESEARCH TOOL FOR CARDIOVASCULAR SCIENTISTS AND PHYSICIANS.
- ***HEART 2003; 89:597-604.***

New pathogenic mechanisms depend from coregulator transcriptional events in the arterial wall

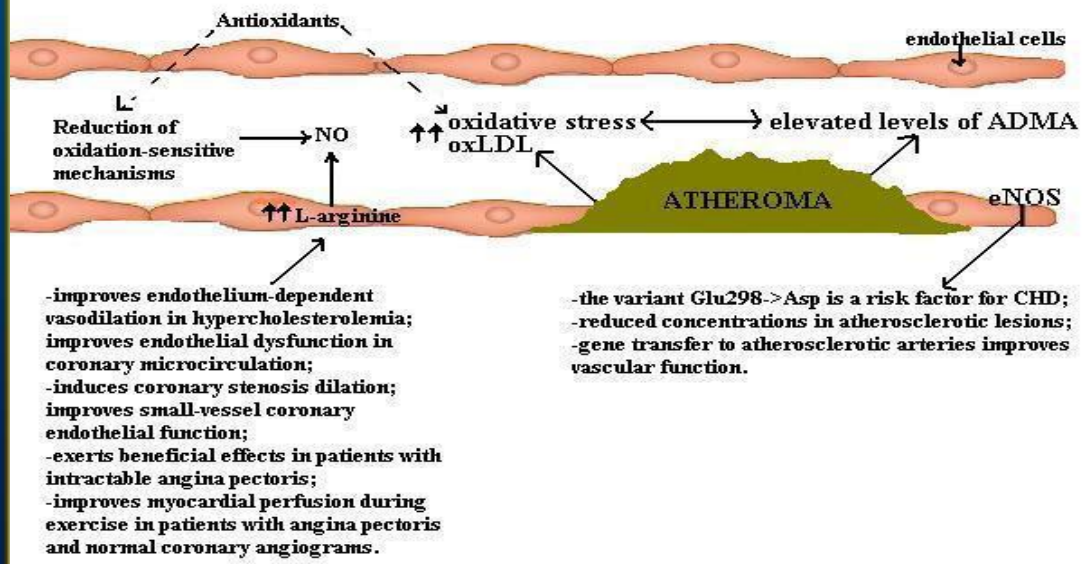


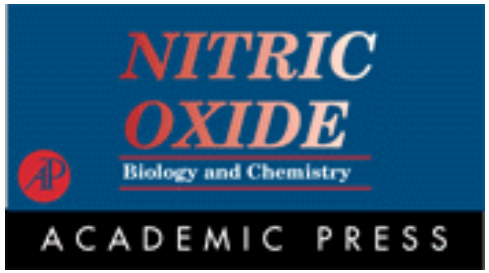
Napoli C et al. J Cell Biochem 2001; 82: 674-682



de Nigris F, Lerman LO and Napoli C. *Int J Cardiol* 2002; 86:153-168.

New therapeutical insights



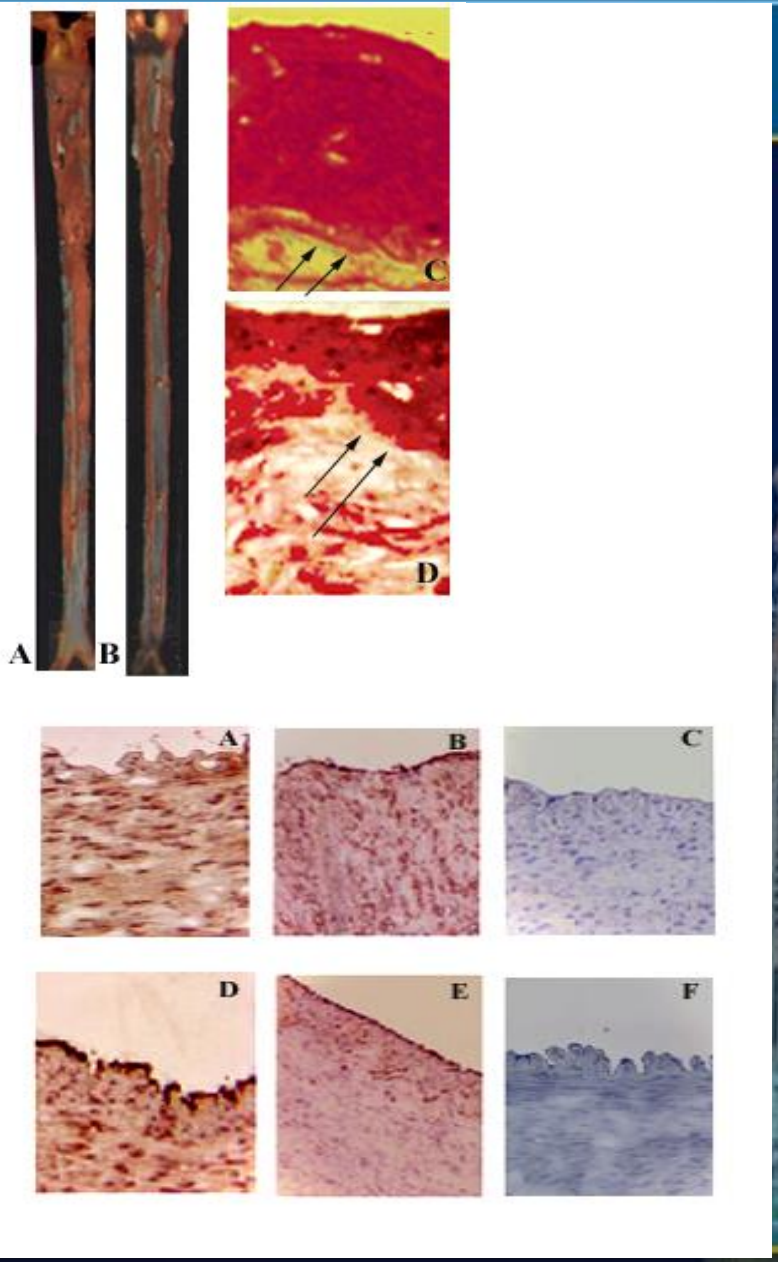


Claudio Napoli, & Louis J. Ignarro
Nitric Oxide*: *Biology and Chemistry
Vol. 5, April 1, 2001
pp 88-97

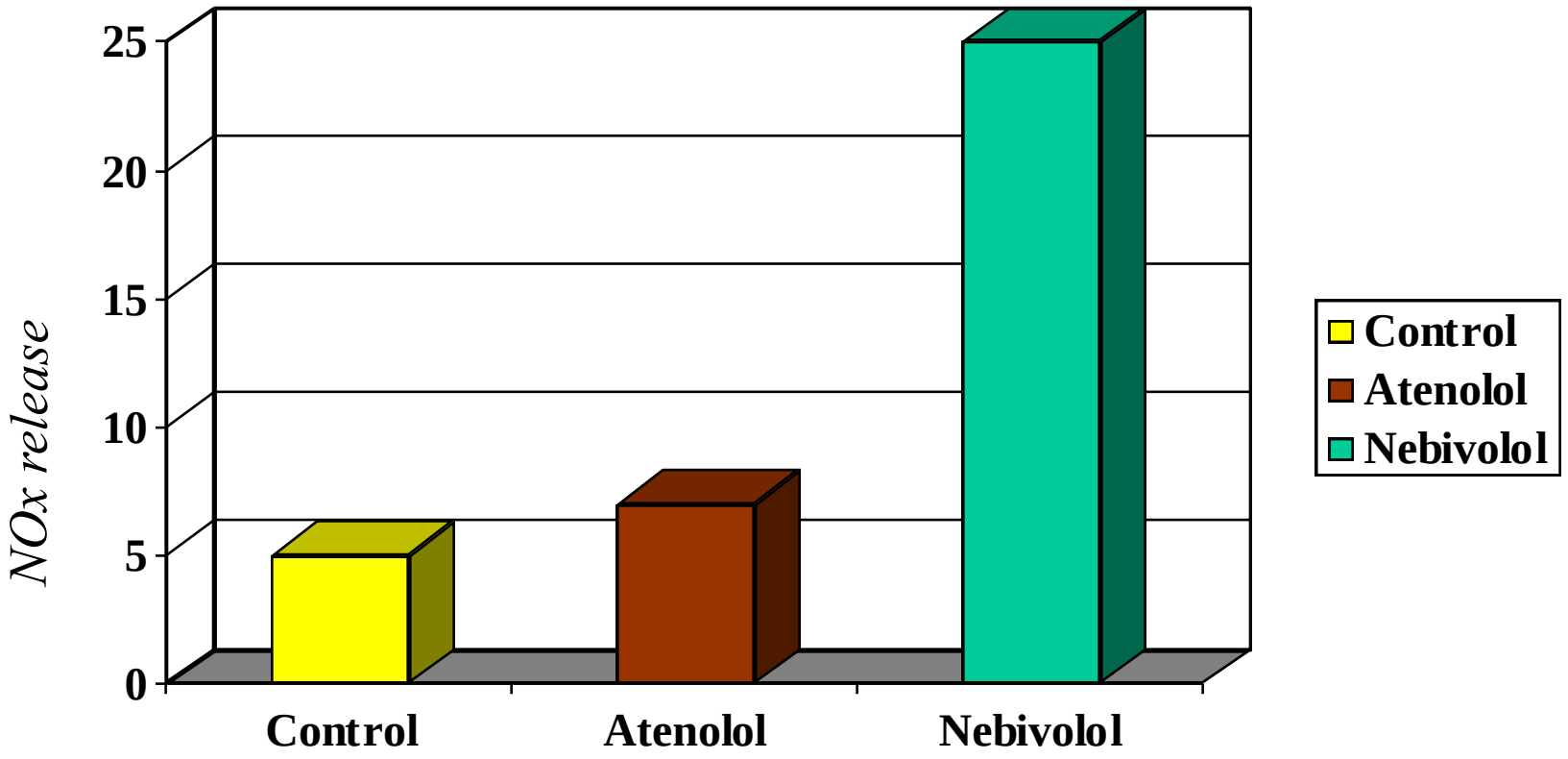
PNAS Online

- Computer-assisted imaging analysis of oil red-O sections revealed a significant mean percent reduction of lesions of 39.8±12.3% in NO-releasing aspirin treated mice ($p<0.01$ vs controls).
- This effect was coupled to reduction of macrophage deposition of 28.3±10.2% ($p<0.05$ vs controls).

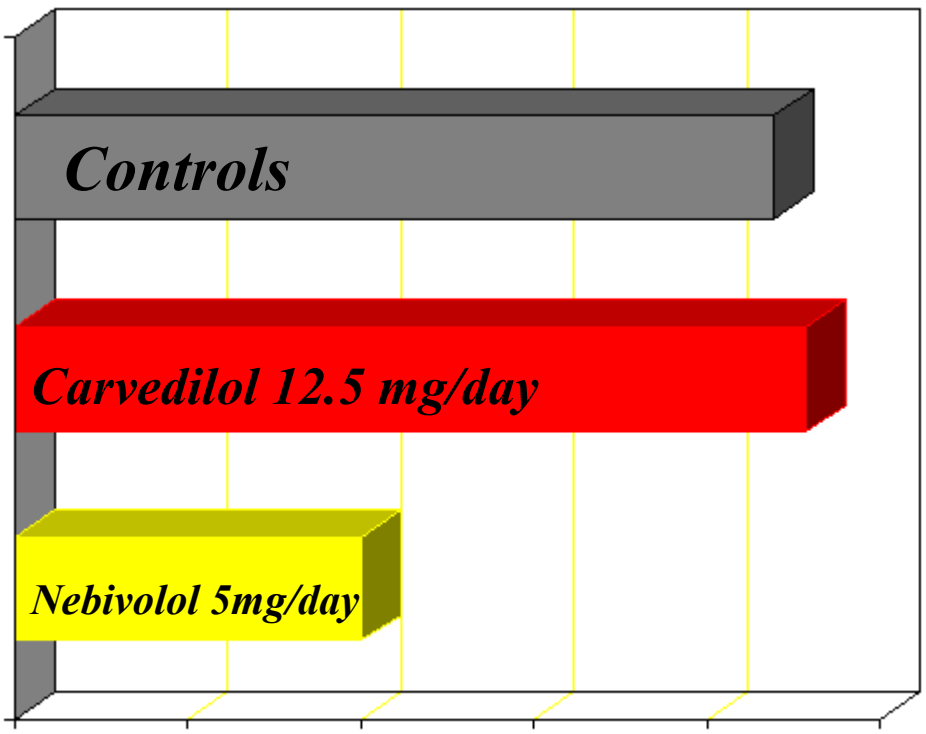
Napoli C et al.
Proc Natl Acad Sci USA 2002; 99:12467-12470.



Nebivolol releases NO in cultured cells



Antiatherosclerotic effect of Nebivolol, a new NO-releasing beta-blocker, in cholesterol-fed rabbits



Napoli C, et al. (2003), unpublished data.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association.
Fighting Heart Disease and Stroke

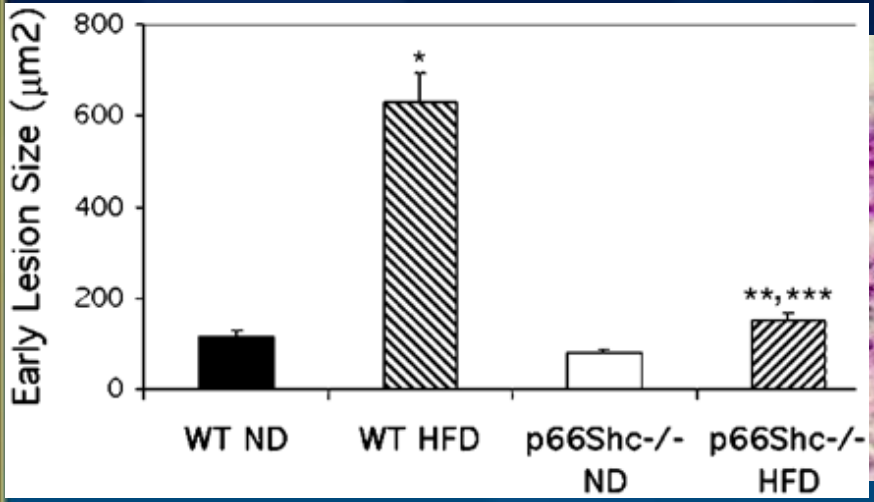
- Ignarro LJ, Napoli C, Loscalzo J.
- Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: an overview.
- ***Circ Res.* 2002; 90:21-28.**

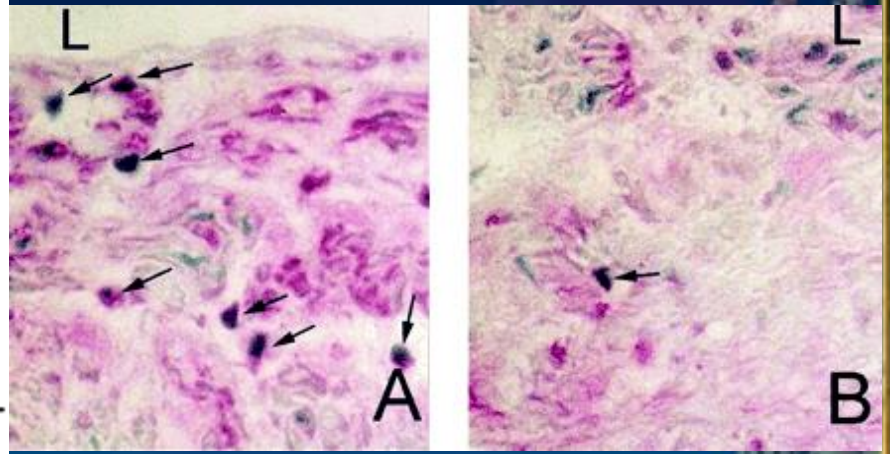
ANNUAL REVIEWS
Pharmacology & Toxicology

Napoli C and Ignarro LJ
Nitric Oxide-Releasing Drugs.
Annu Rev Pharmacol Toxicol 2003;43:97-123.

PNAS Online

Napoli C, Martin-Padura I, de Nigris F, Giorgio M, Condorelli M, Sica G, de Rosa G, & Pelicci PG.
Deletion of the p66Sch longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed high-fat diet.
***Proc Natl Acad Sci USA* 2003; 100:2112-16.**





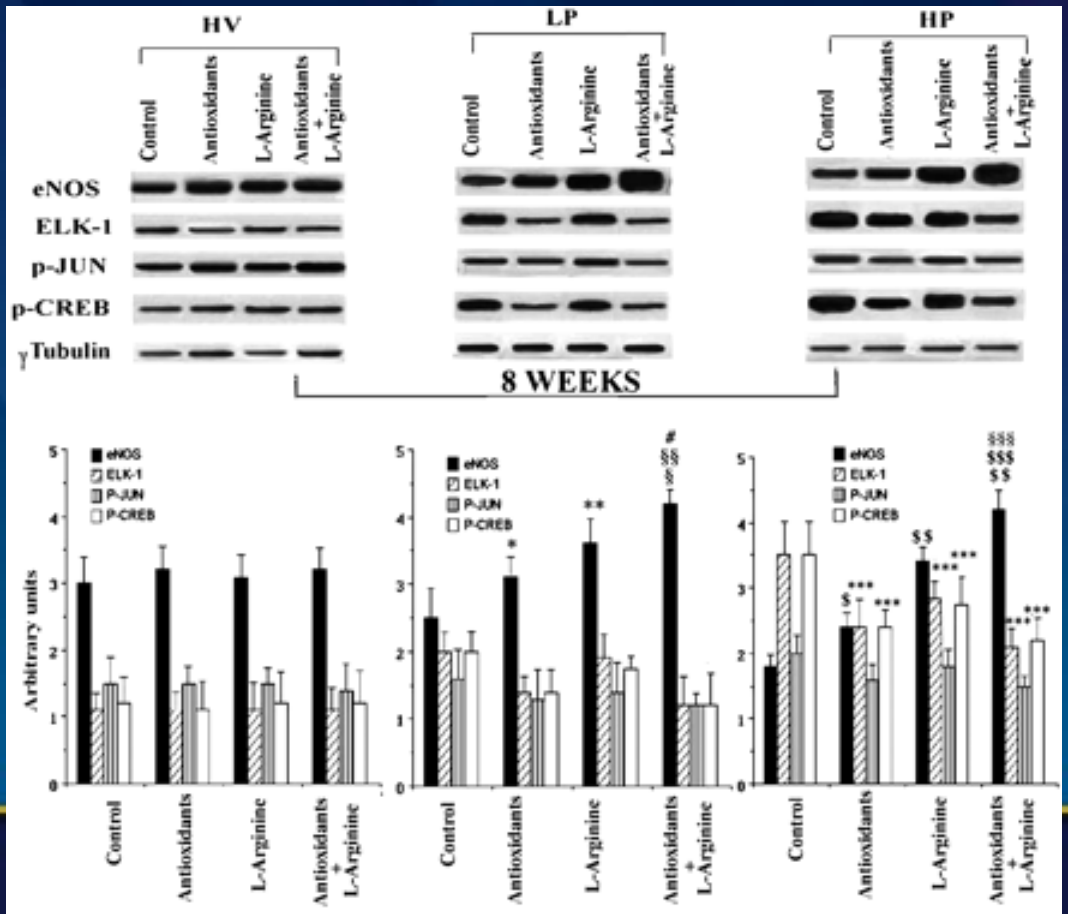
Science *SAGE KE, Ramsayer K, Life extending genetic alteration prevents Arterial damage (February 12, 2003)*

PNAS Online

de Nigris F, Lerman LO, Williams-Ignarro S, Sica G, Palinski W, Ignarro LJ, & Napoli C

Beneficial effects of antioxidants and L-arginine on oxidation-sensitive gene expression and endothelial NO synthase activity at sites of disturbed shear stress.

***Proc Natl Acad Sci USA* 2003; 100:1420-25.**



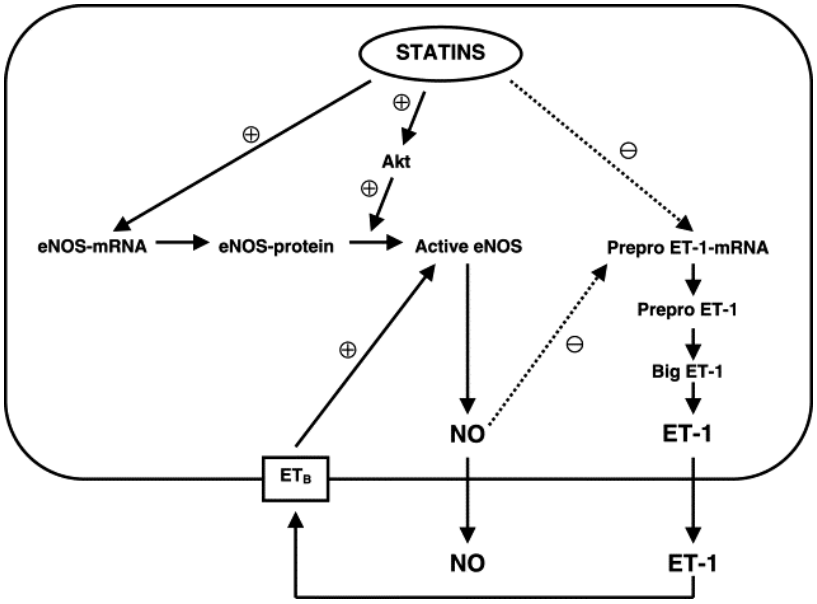
Matrix Metalloproteinase Inhibitors?

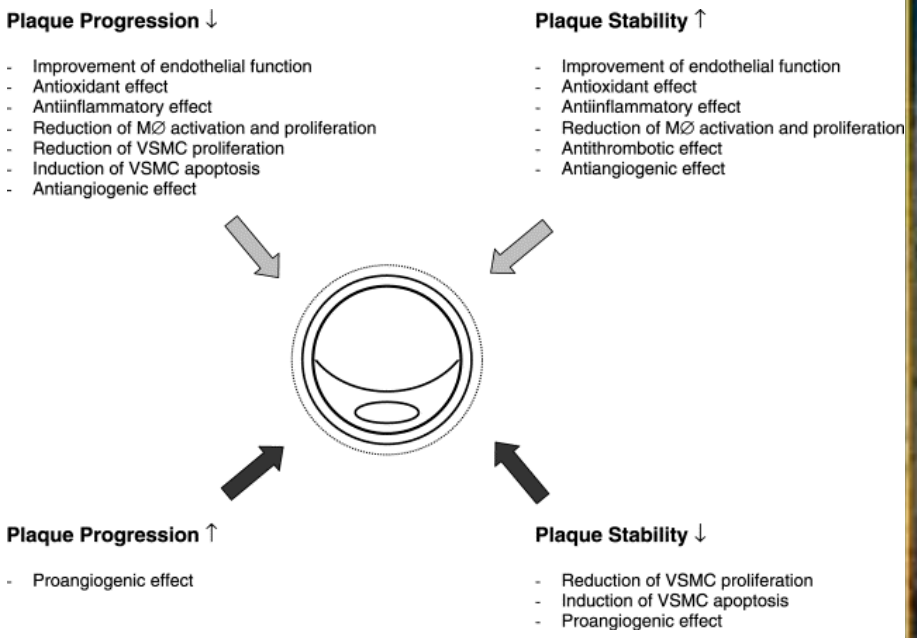
- The constitution of the cardiac extracellular matrix depends on the balance in activity of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases. Advanced atherosclerosis is associated with increased collagenase activity (increased expression of MMP-1 and MMP-9) and reduced expression of tissue inhibitors of metalloproteinases.

- Experimentally, MMP inhibition reduces vascular inflammation, although theoretical concerns remain about this therapeutic approach in patients.

European Heart Journal
W.B. SAUNDERS

Bonetti PO, Napoli C, Lerman A. Statin effects beyond Lipid lowering-are they clinically relevant?
Eur Heart J 2003; 24:225-248.





Arteriosclerosis, Thrombosis, and Vascular Biology

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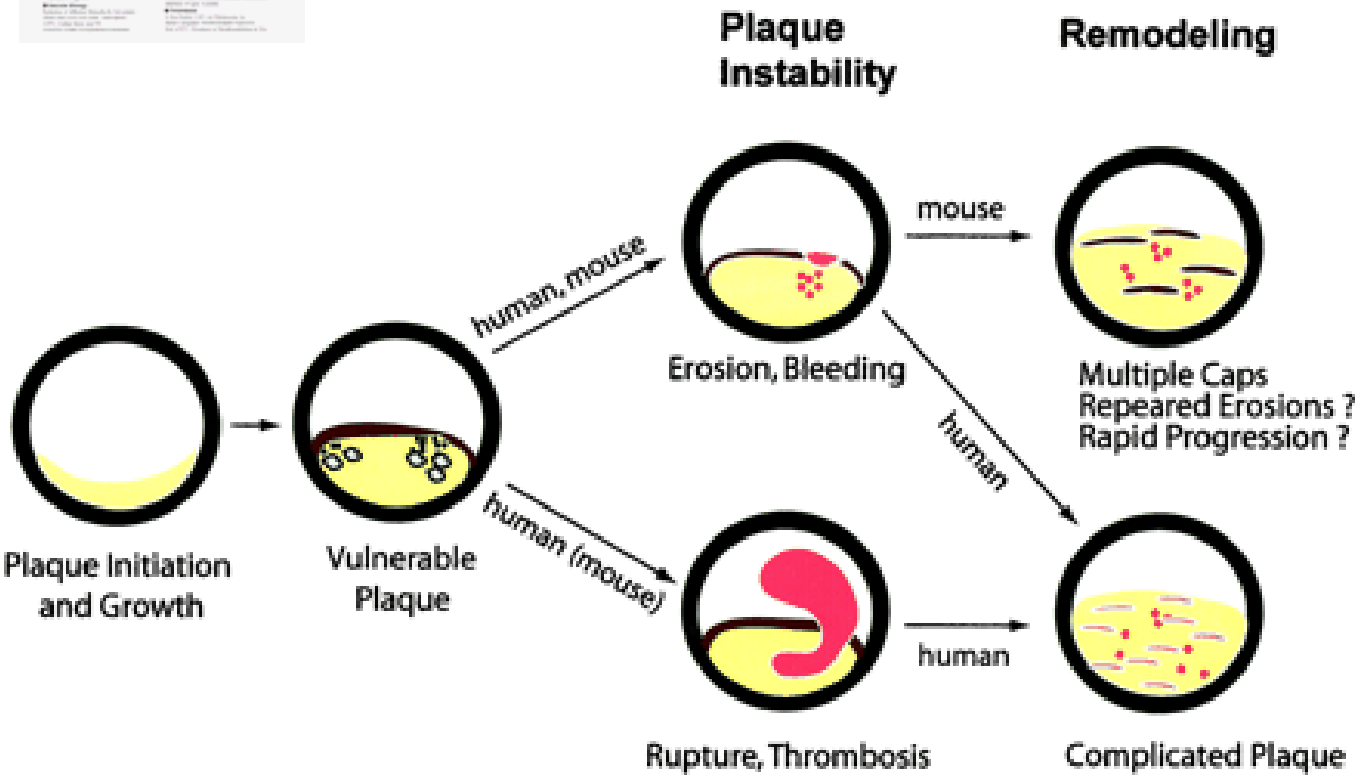
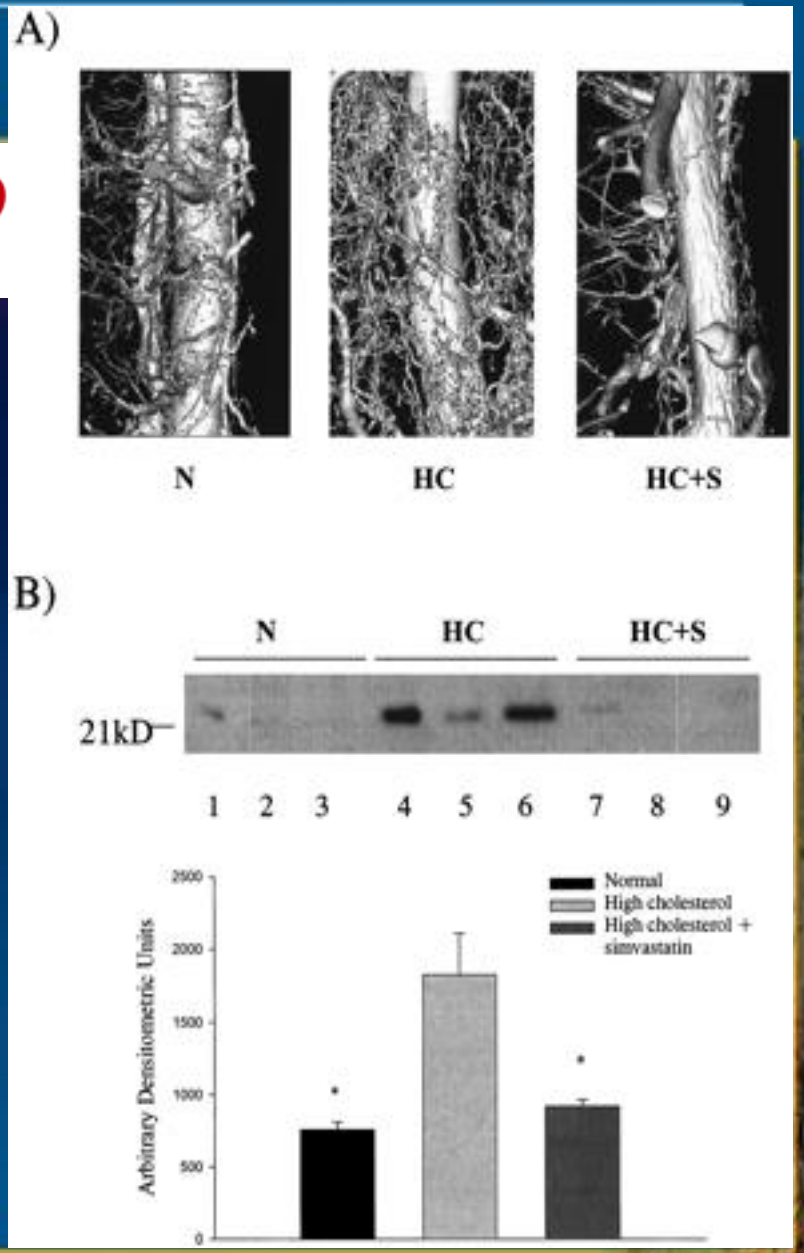
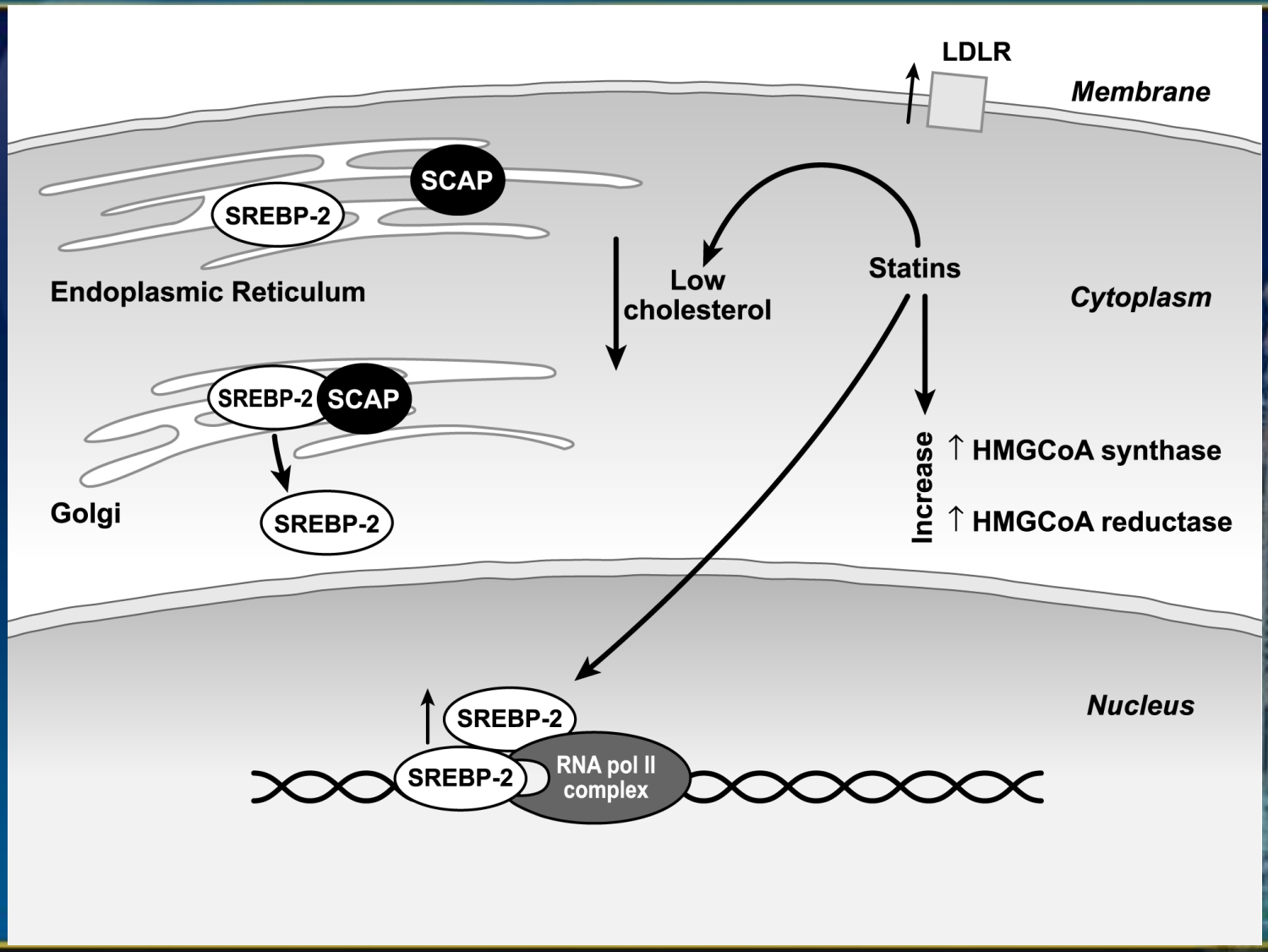


Palinski W, and Napoli C. Unraveling pleiotropic effects of statins on plaque rupture. *Arterioscler Thromb Vasc Biol* 2002; 22:1745-50.

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association.
Fighting Heart Disease and Stroke

Wilson SH, Herrmann J, Holmes DR jr, Napoli C, & Lerman A.
Circulation 2002; 105:415-420.





de Nigris et al, Int J Cardiol 2002