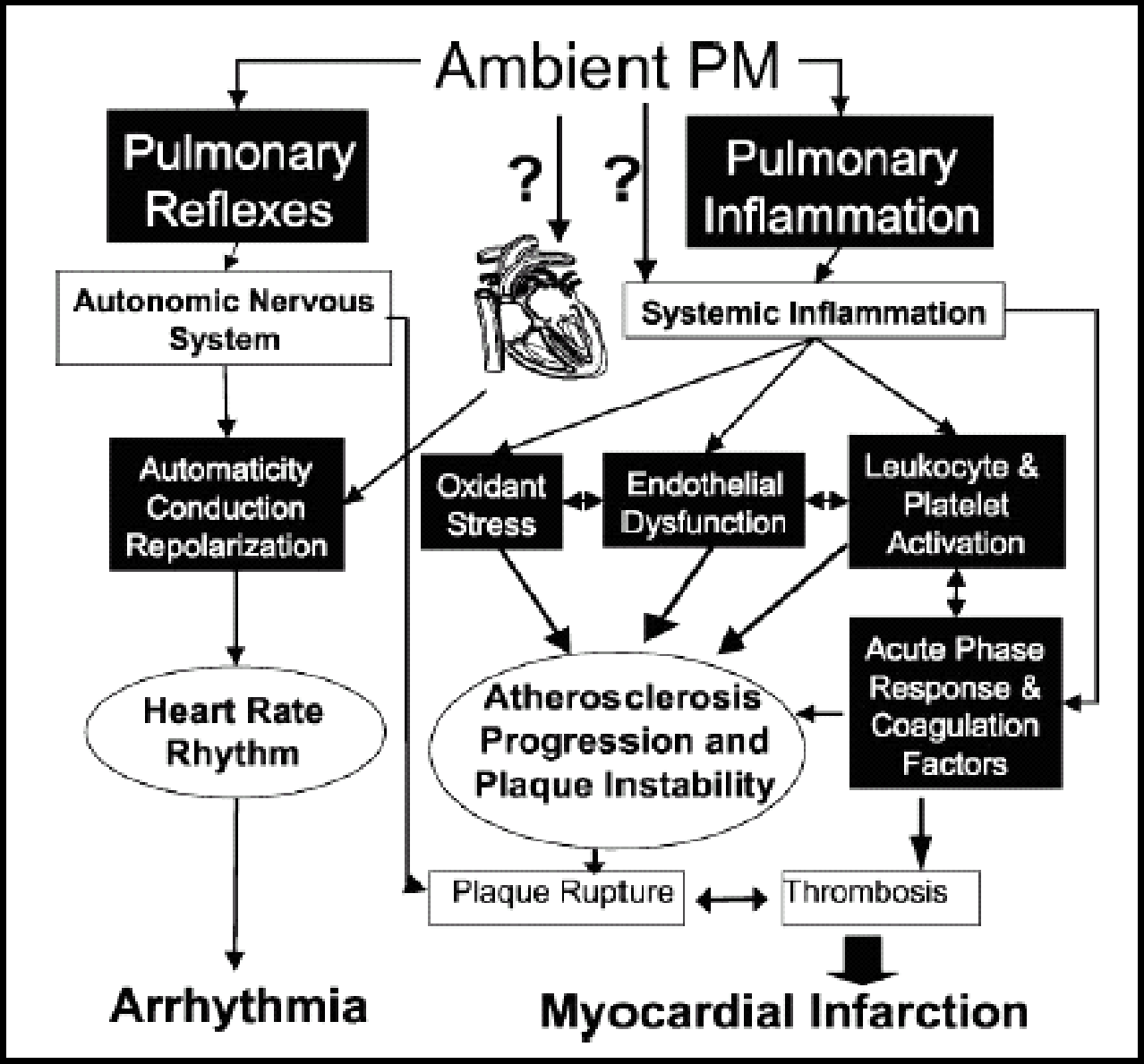


Brook et al. *Circulation*. 2004. 109:2655-2671.

RO1 ES013432

"Atherogenic effects of Ambient PM in Susceptible Animals"

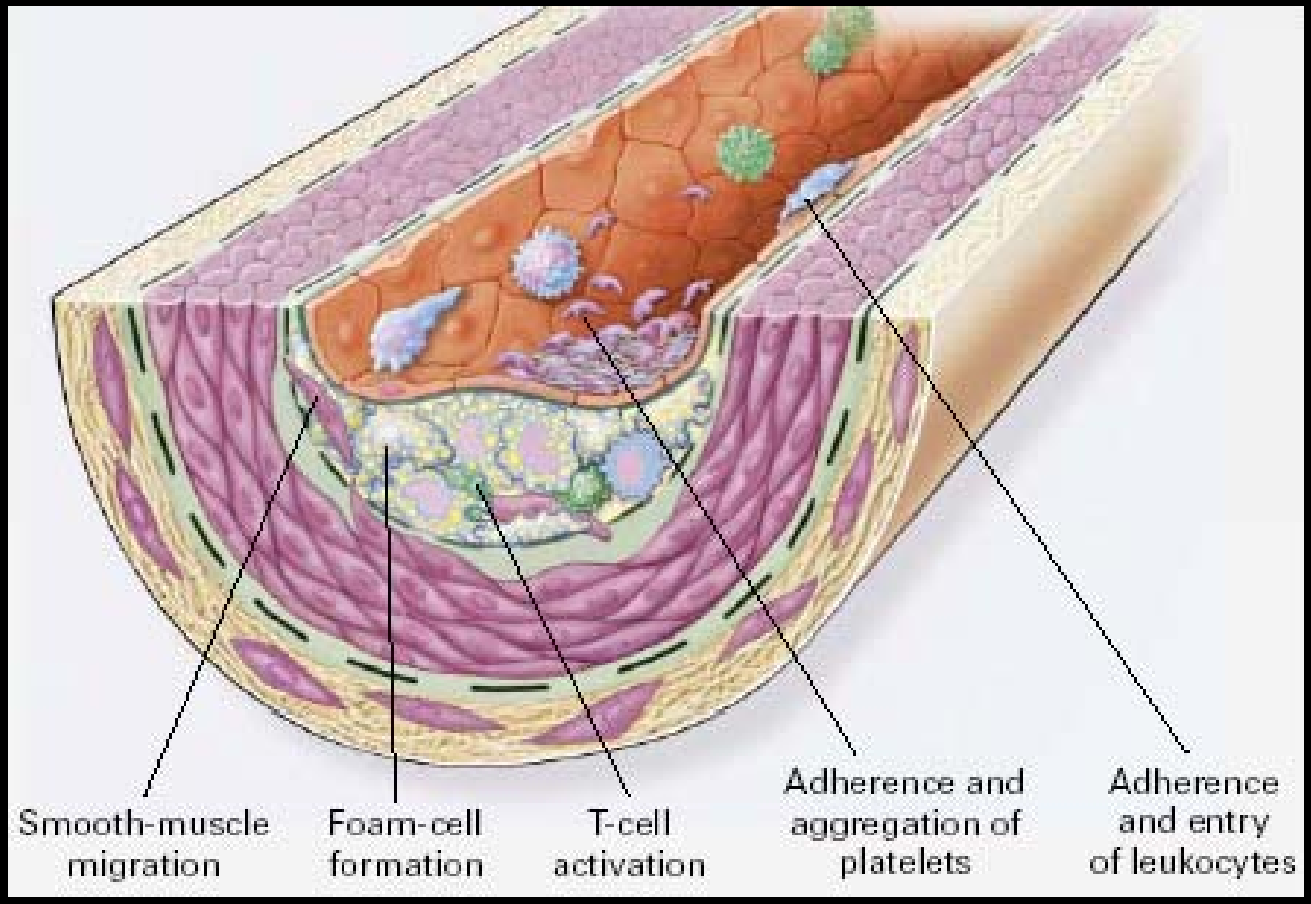
PI: Andre Nel, MD/PhD*

Co-PI: Costas Sioutas, PhD+
Aldons Lusis, PhD*
Jesus Araujo, MD/PhD*
Joseph Loo, PhD*

* UCLA
+ USC

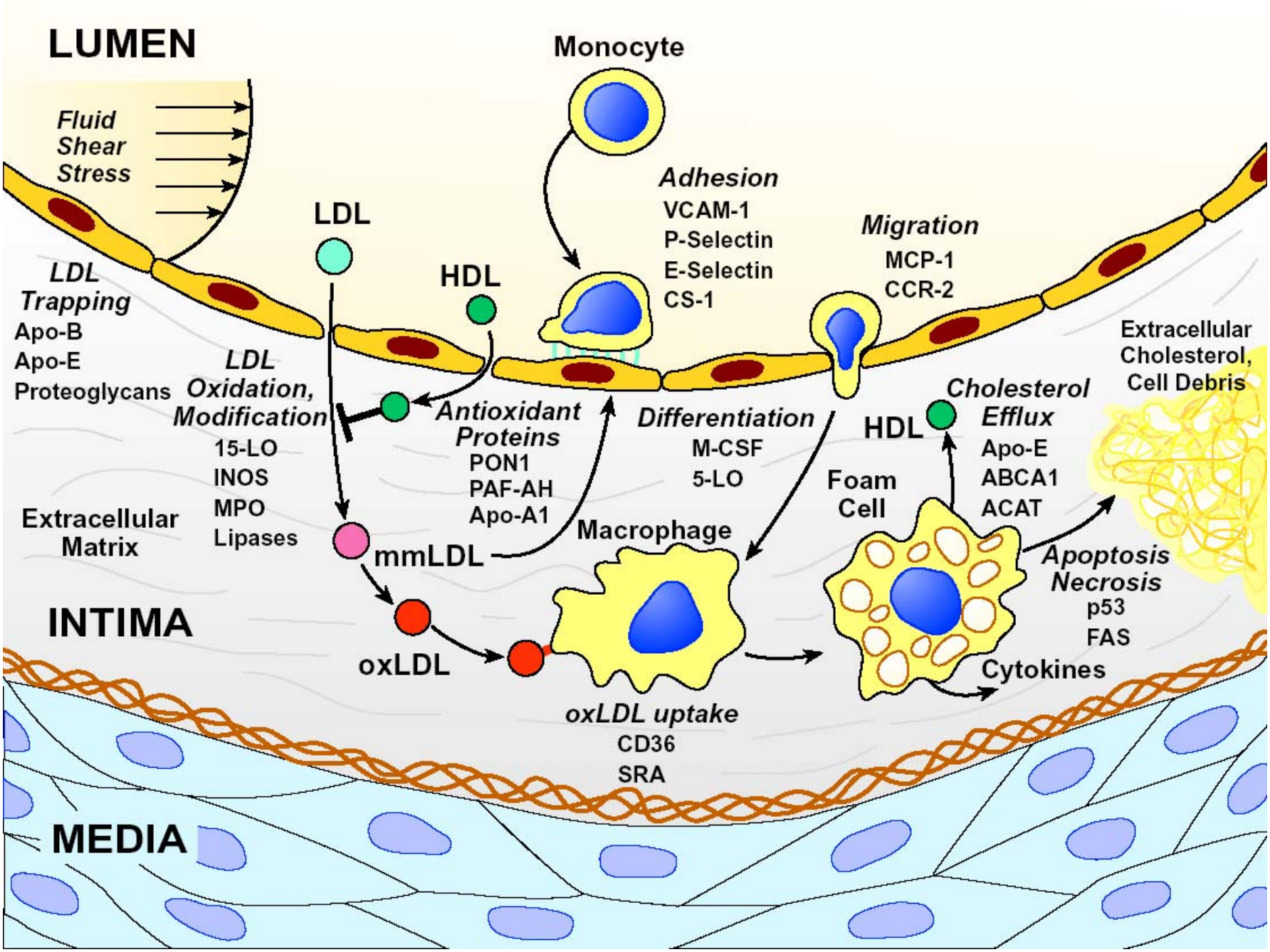
Major Hypothesis: PM-induced oxidative stress synergizes with oxidized lipid components to enhance inflammation and apoptosis in atherosclerotic lesions

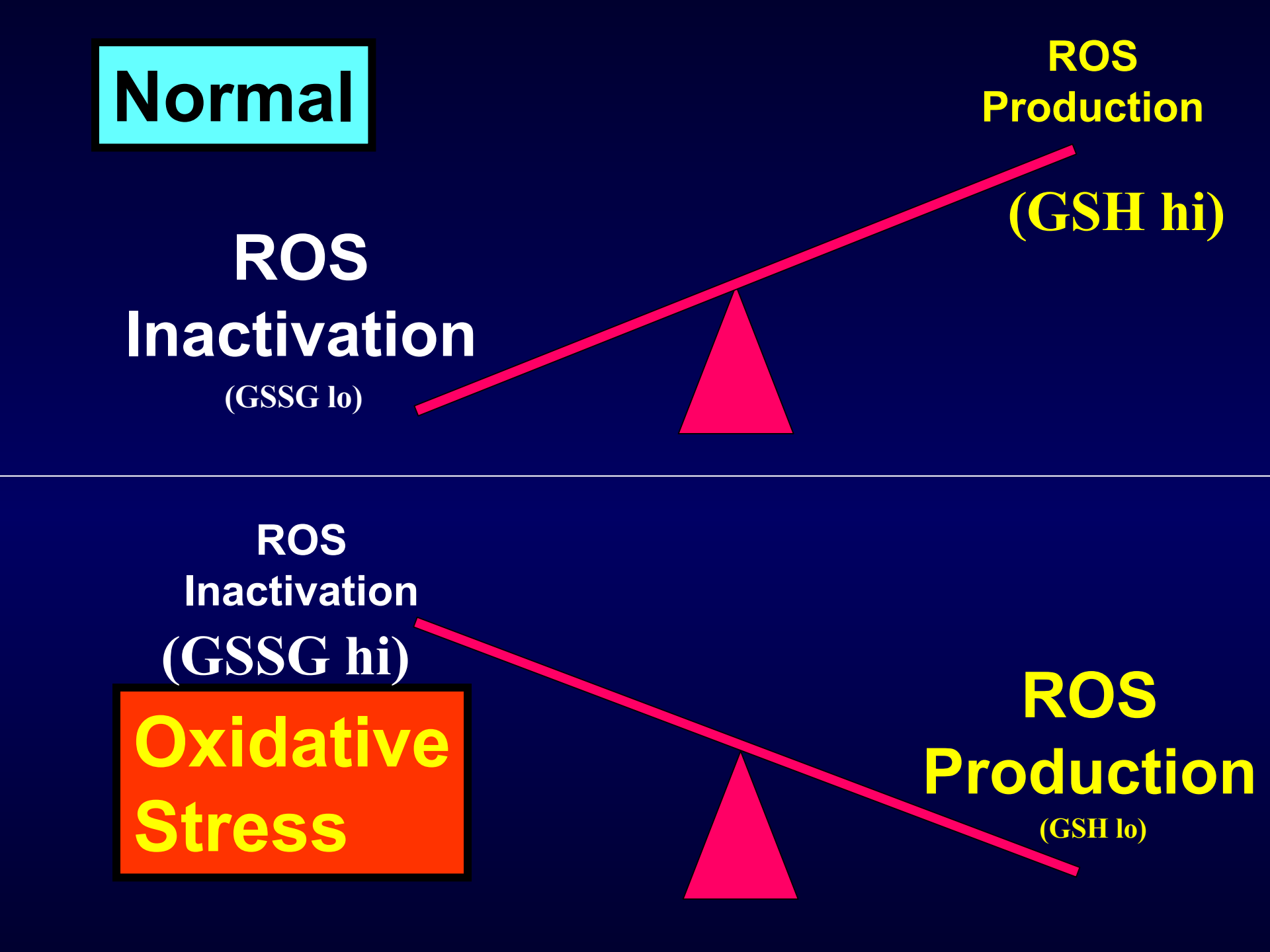
PM is now a recognized risk factor for Atherosclerosis



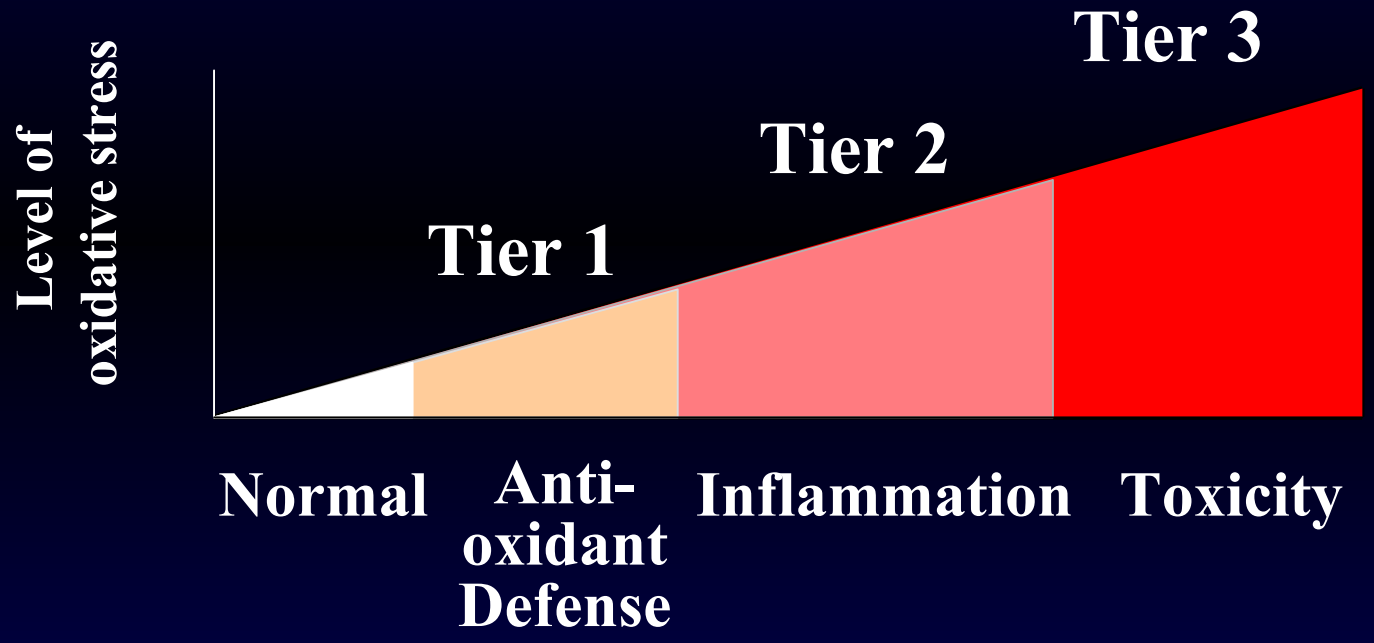
A disease of oxidative stress, chronic inflammation and apoptosis…..

Synergy between oxidized LDL and ambient PM ?


LUMEN
Monocyte
Fluid
Shear
Stress
LDL
HDL
Adhesion
VCAM-1
P-Selectin
E-Selectin
CS-1
Migration
MCP-1
CCR-2
LDL
Trapping
Apo-B
Apo-E
Proteoglycans
LDL
Oxidation,
Modification
15-LO
INOS
MPO
Lipases
Antioxidant
Proteins
PON1
PAF-AH
Apo-A1
Differentiation
M-CSF
5-LO
Extracellular
Cholesterol,
Cell Debris
Cholesterol
Efflux
HDL
Apo-E
ABCA1
ACAT
Foam
Cell
Extracellular
Matrix
mmLDL
Macrophage
Apoptosis
Necrosis
p53
FAS
INTIMA
oxLDL
Cytokines
oxLDL uptake
CD36
SRA
MEDIA


Normal
ROS
Production
(GSH hi)
ROS
Inactivation
(GSSG lo)
ROS
Inactivation
(GSSG hi)
Oxidative
Stress
ROS
Production
(GSH lo)

Stratified Oxidative Stress Hypothesis



Nel et al. JBC. 2003; 278:50781

Specific Aims:

Aim 1: To determine whether exposure to ambient PM exacerbate the rate and magnitude of atherosclerosis in apoE and LDL-R knockout mice.

Aim 2: To determine the role of PM-induced oxidative stress in the development of inflammation and apoptosis in atherosclerotic lesions in apoE and LDL-R deficient mice.

Aim 3: To determine whether modified antioxidant defense mechanisms affect the induction of atherosclerotic lesions by ambient PM in apoE deficient mice.

Aim 1

Particle concentrators (VACES): 1. Fine; 2. Ultrafine; 3. Filtered Air

Inhalation chambers

↓

In vivo exposures

Animal strains: ApoE $^{-/-}$ & LDL-R $^{-/-}$

Exp 1 : Early lesion development

Exp 2 : Late lesion development

↓

Endpoints

Lesion scoring

Histology

Immunocytochemistry (inflammation, & apoptosis)

Impinger samples

↓

In vitro cellular exposures

Cell types: Endothelial, macrophage, & smooth muscle cells

Exp 3 : Stimulation by CAPs ±Ox PAPC

↓

Endpoints

Inflammation

Signaling : MAPK

Apoptosis assays

Proteomics

Aim 2

In vivo Endpoints: Animal sacrifice

- Lesional HO-1 expression, phospholipid oxidation
- MAPK activation
- Apoptosis
- Serum SAA, fibrinogen, IL-6, GM-CSF
- HDL antioxidant activity (oxidative PON modification)
- *In vivo* luciferase imaging HO-1 promoter

In vitro Endpoints for oxidative stress

- ROS generation: DCF & HE
- GSH/GSSG ratios
- Hierarchical oxidative: HO-1, cytokines, MAPK, apoptosis
- 2D gel electrophoresis and protein MS

Aim 3

Particle concentrators (VACES): 1. Fine; 2. Ultrafine; 3. Filtered Air

↓

In vivo exposures

Exp. 1 Tg HO-1/apoE $^{-/-}$

Exp. 2 Nrf2 x apoE double KO

Exp. 3 apoE /PON1 double KO

↓

Endpoints:

Atherosclerotic scores & markers inflammation & apoptosis (Aim 1)

Lesional markers for oxidative stress, including ARE-dependant genes (Aim 2)

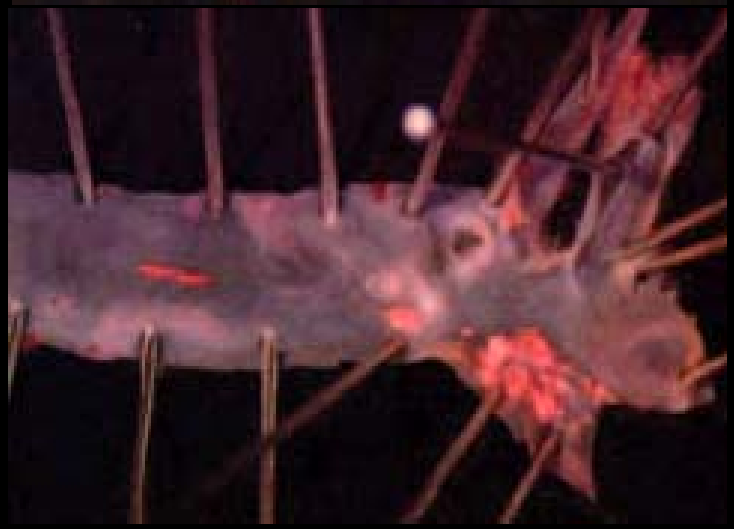
HDL antioxidant activity

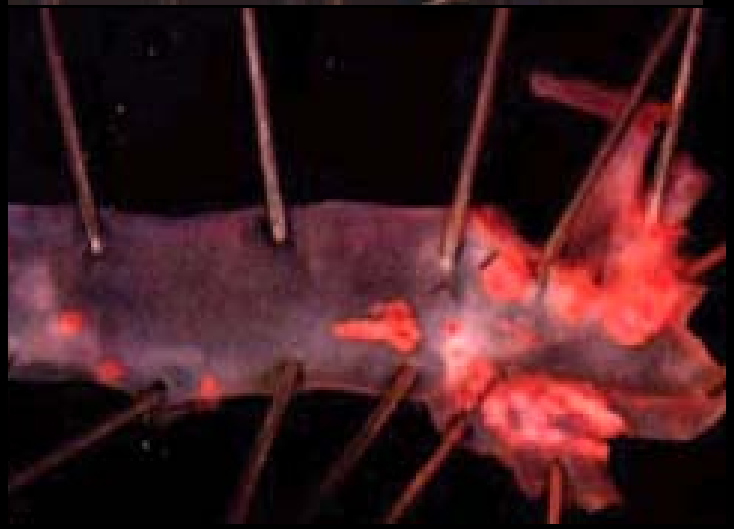
High Chol diet/ No Arsenite

High Chol diet/Arsenite

C57Bl/6 wildtype

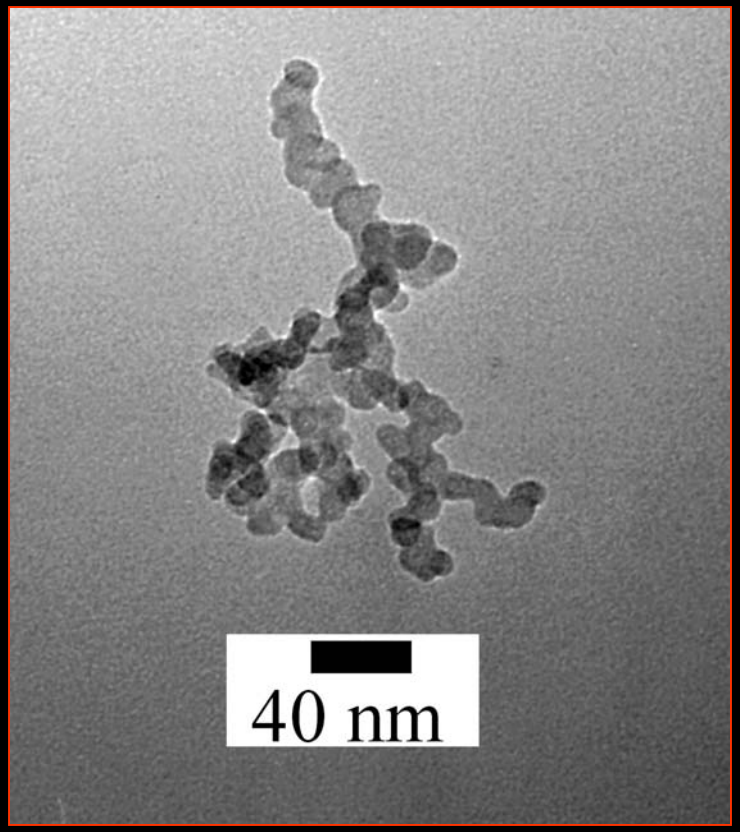
ApoE knockout





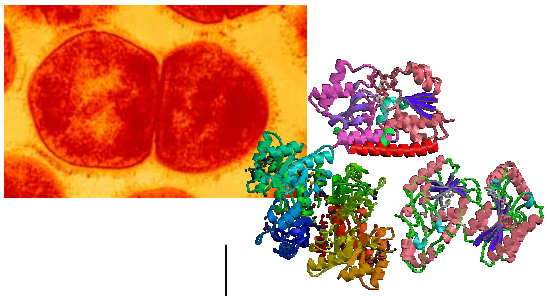
Petia P. Simeonova et al. Environ Health Perspect 111:1744–1748 (2003).

Passage of Inhaled Particles Into the Blood Circulation in Humans

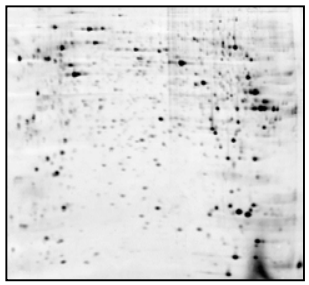


Nemmar et al
***Circulation*. 2002;105:411-414.**

Proteomics Aids Biological Research

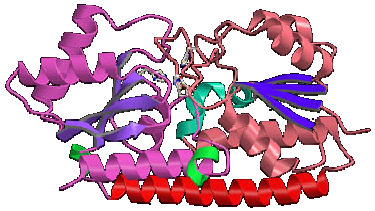


complex
protein
mixture



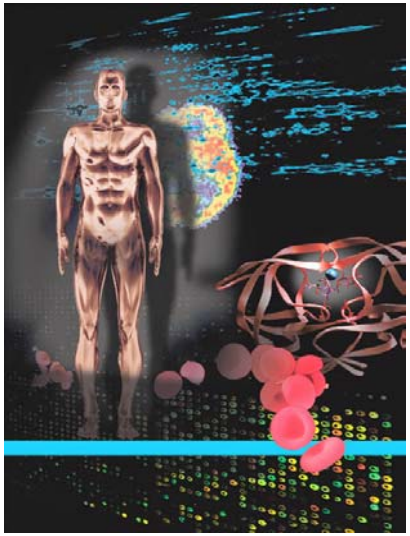
protein
separation

mass spectrometry

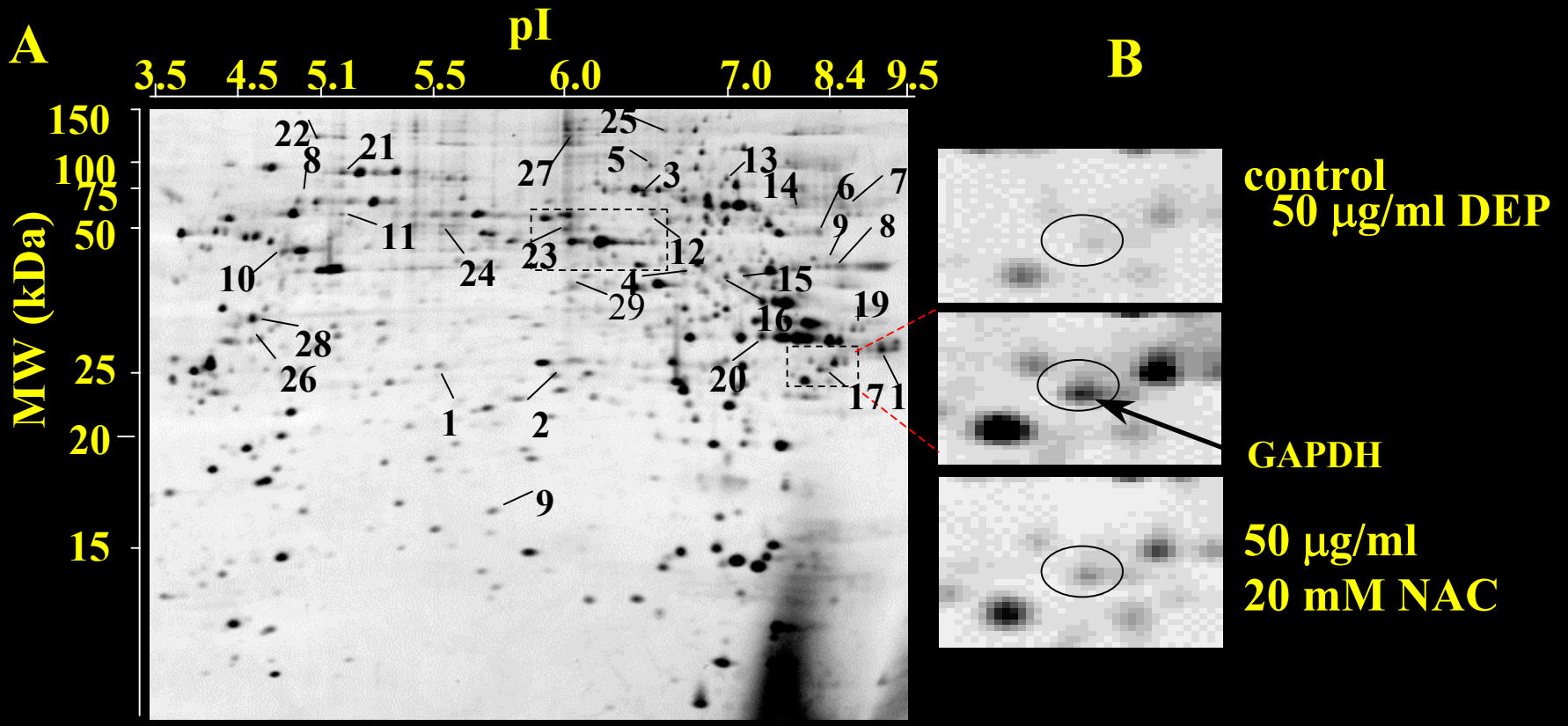


protein identification
protein modification
protein abundance

Biology



Exploration of the role of Oxidative Stress through the use of Proteomics

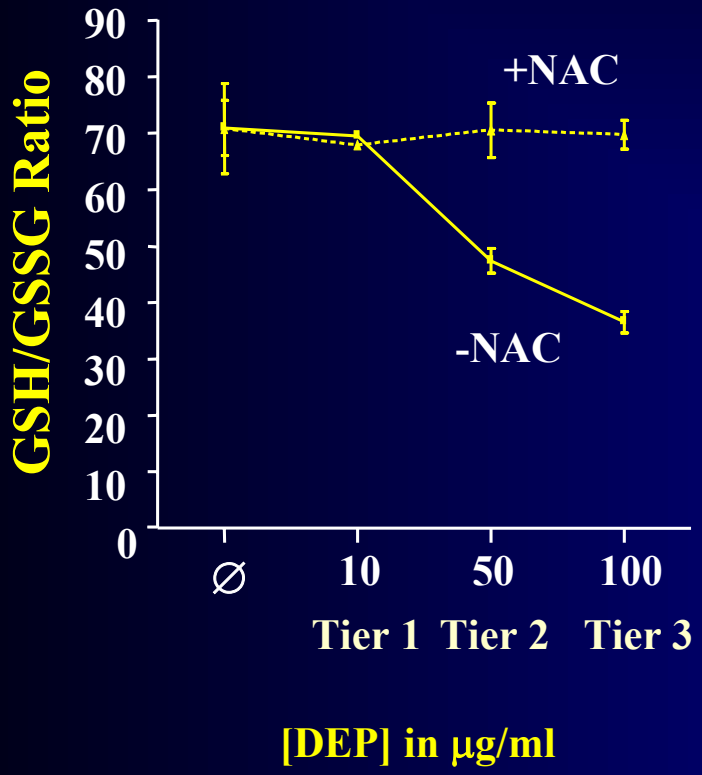


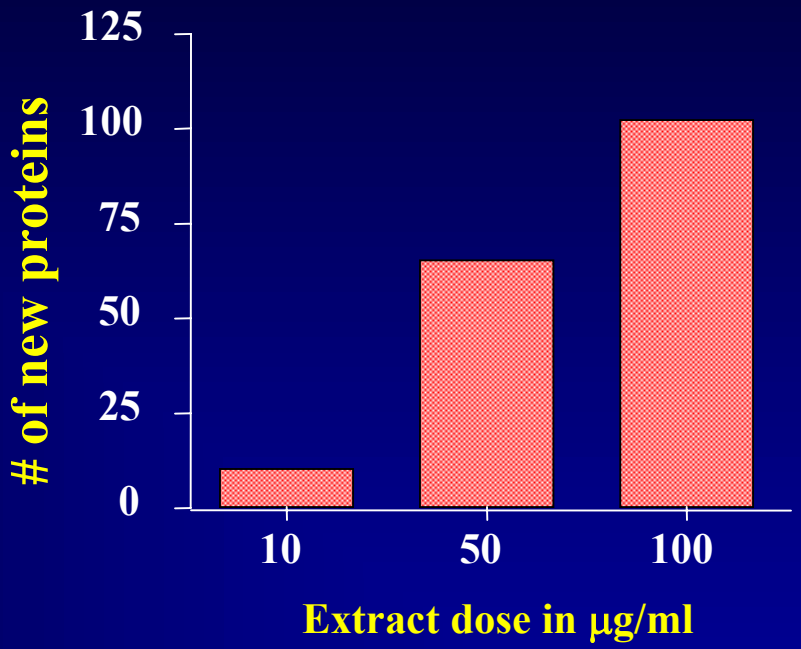
Oxidative stress response ?

Inflammatory markers ?

Nel, Xiao, Loo, et al. JBC. 2003; 278:50781

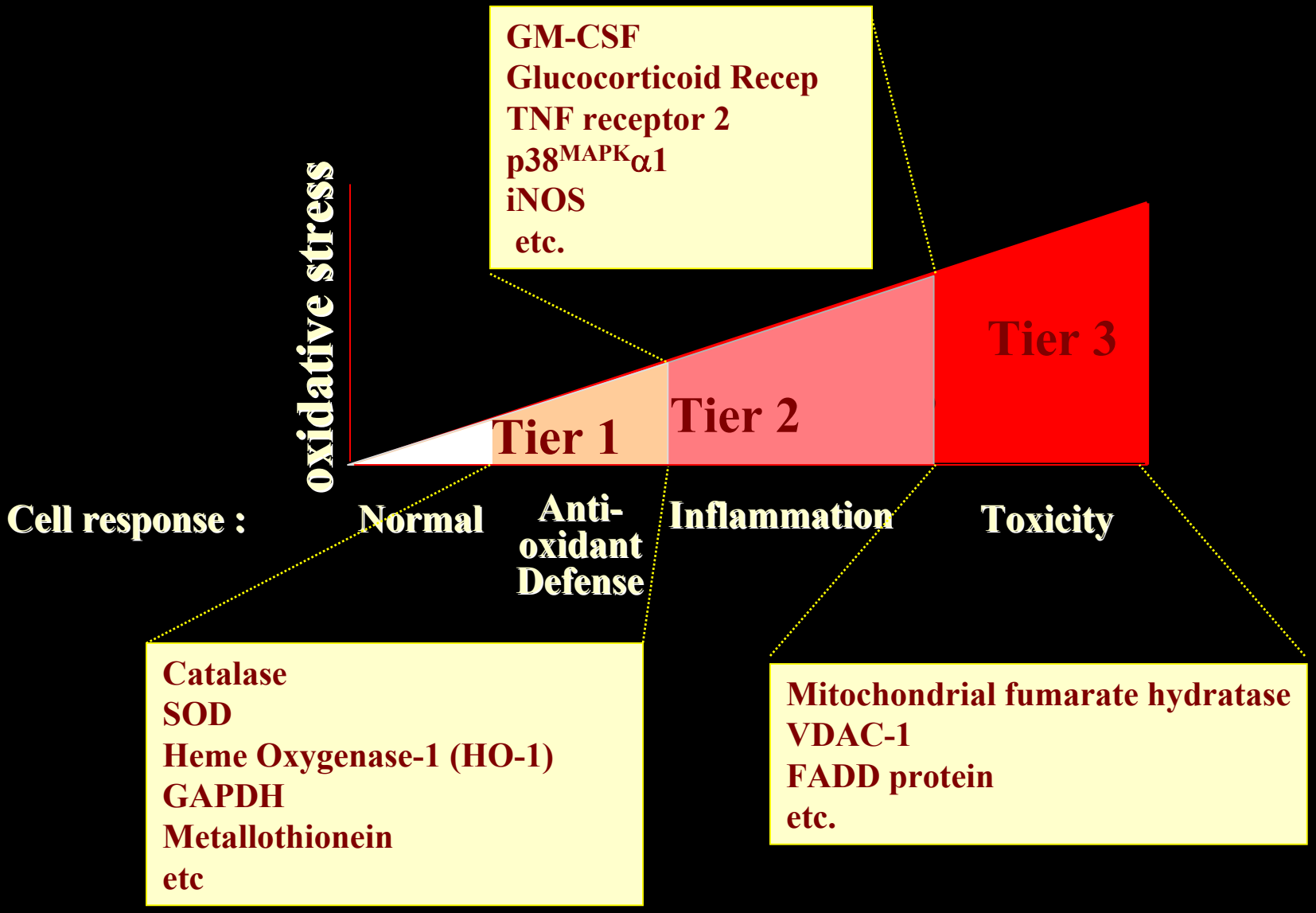
Dose-dependant induction of new Oxidative Stress Proteins by DEP



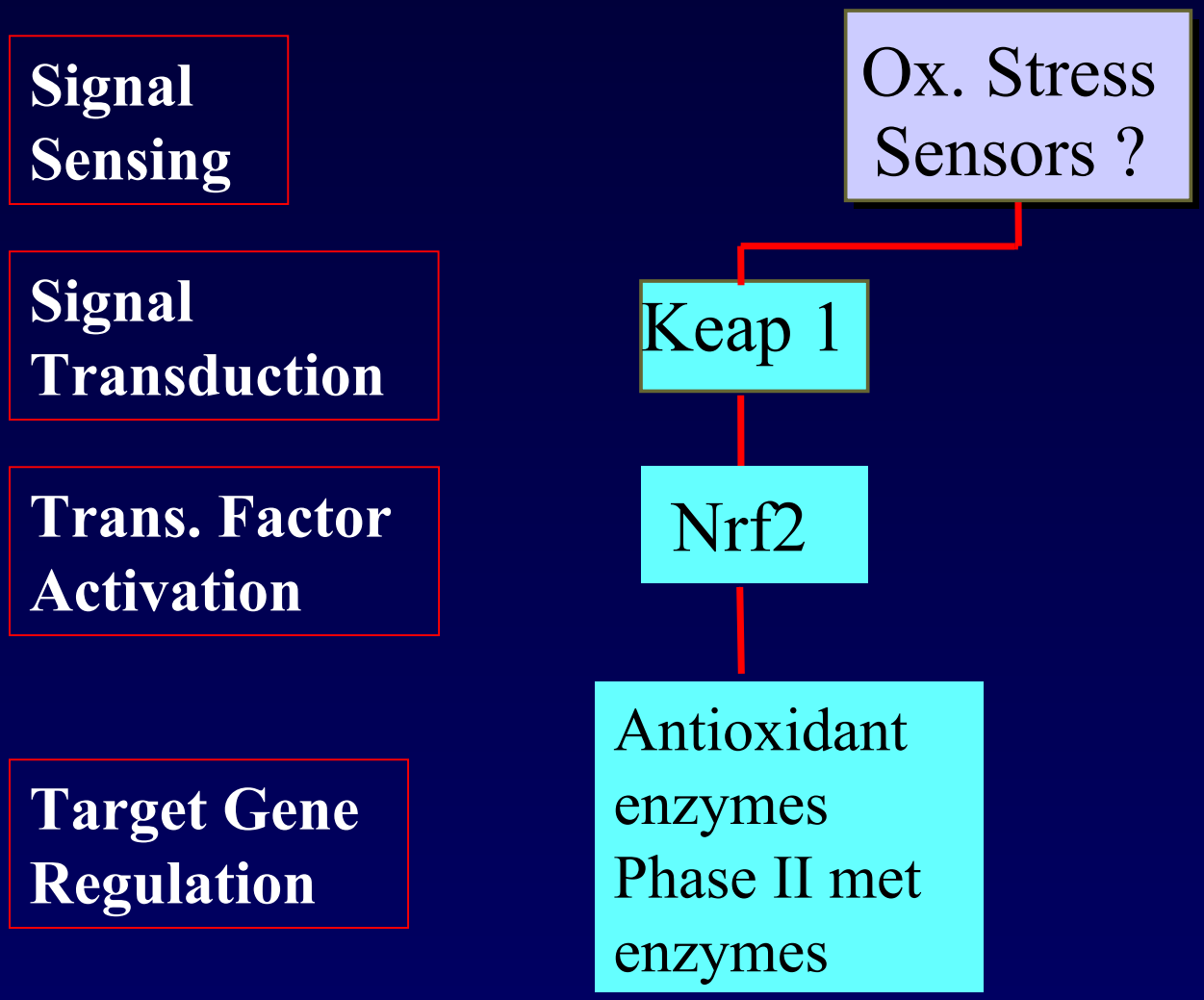


Xiao, Loo, Nel et al. JBC. 2003; 278:50781

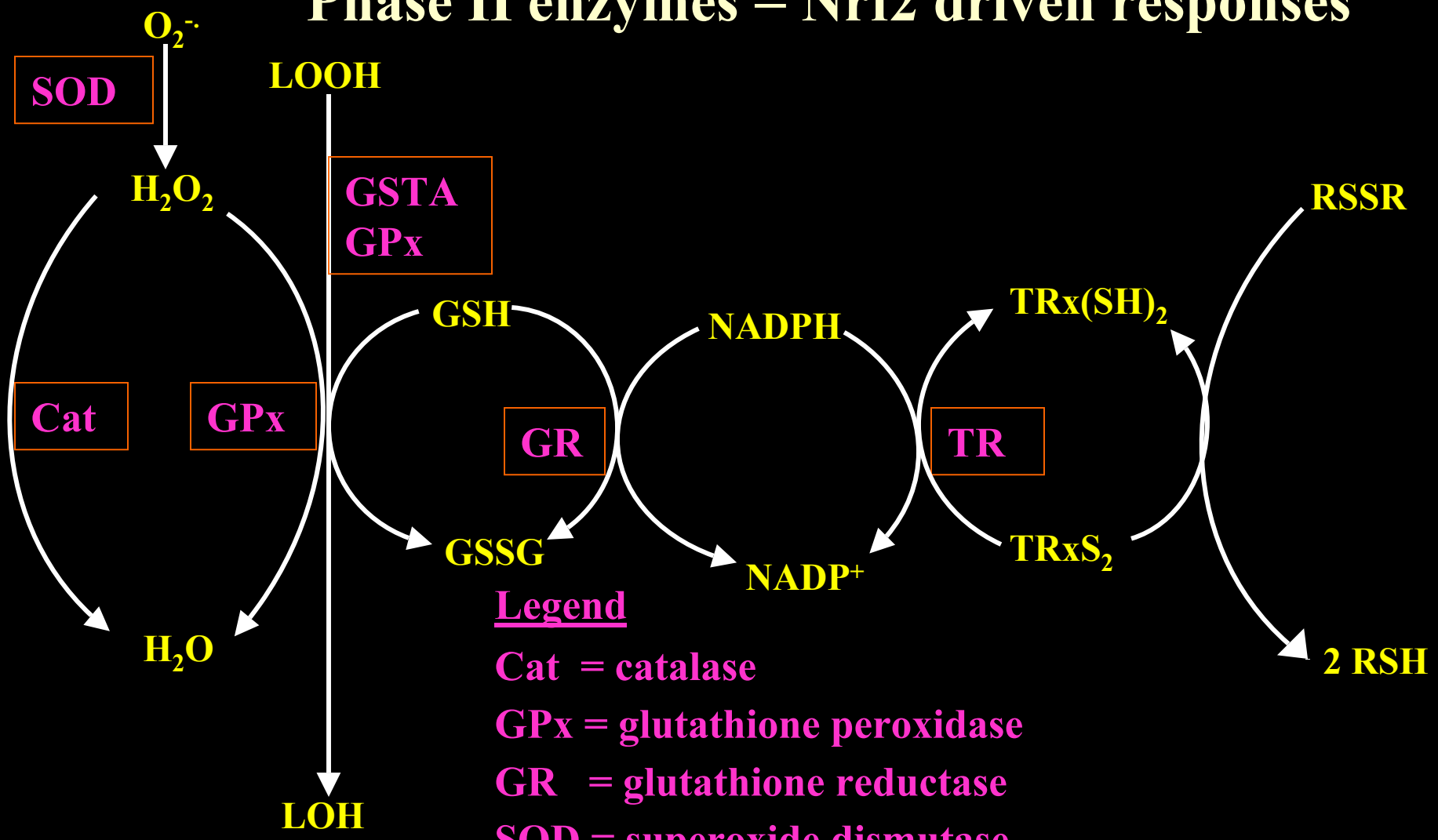
Macrophage/epithelial oxidative stress analysis (in vitro)



Adaptive Genetic Programming



Phase II enzymes = Nrf2 driven responses



Legend

Cat = catalase

GPx = glutathione peroxidase

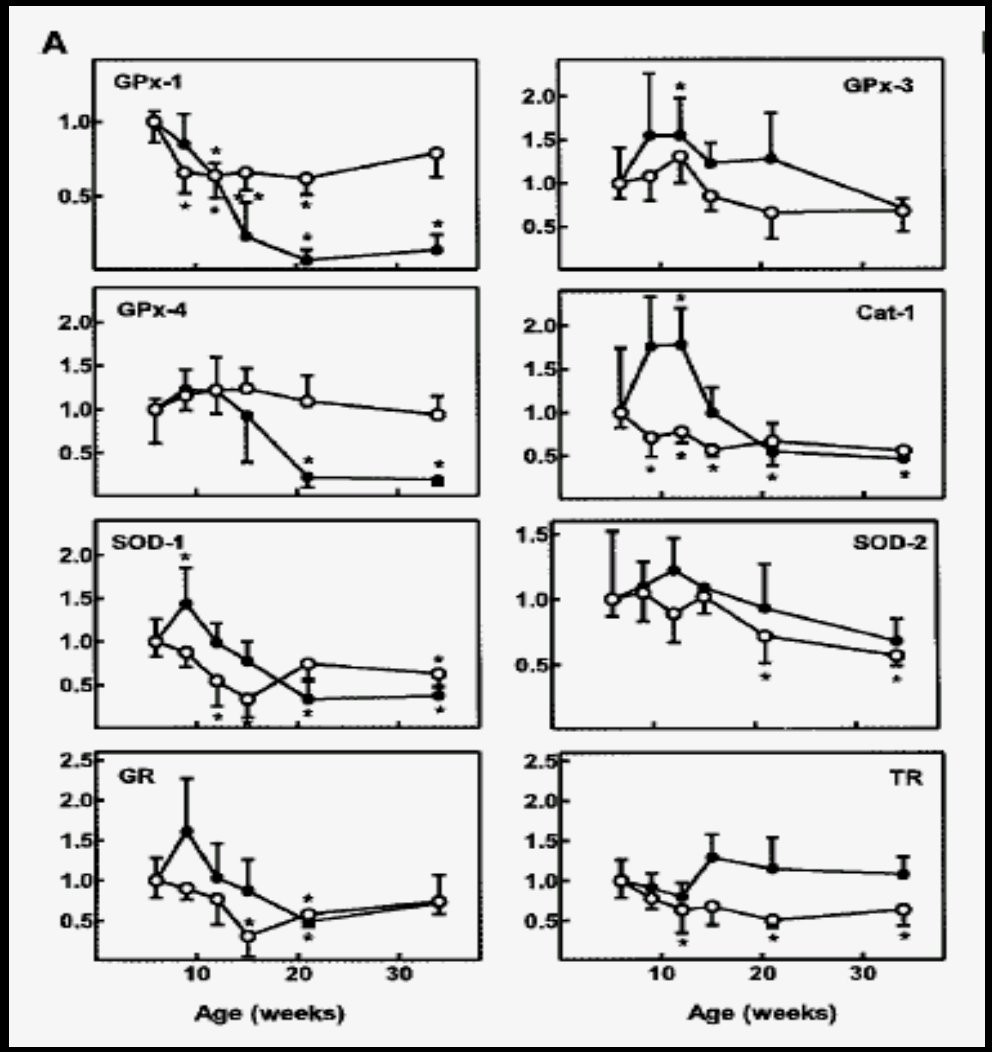
GR = glutathione reductase

SOD = superoxide dismutase

GST = glutathione S transferase

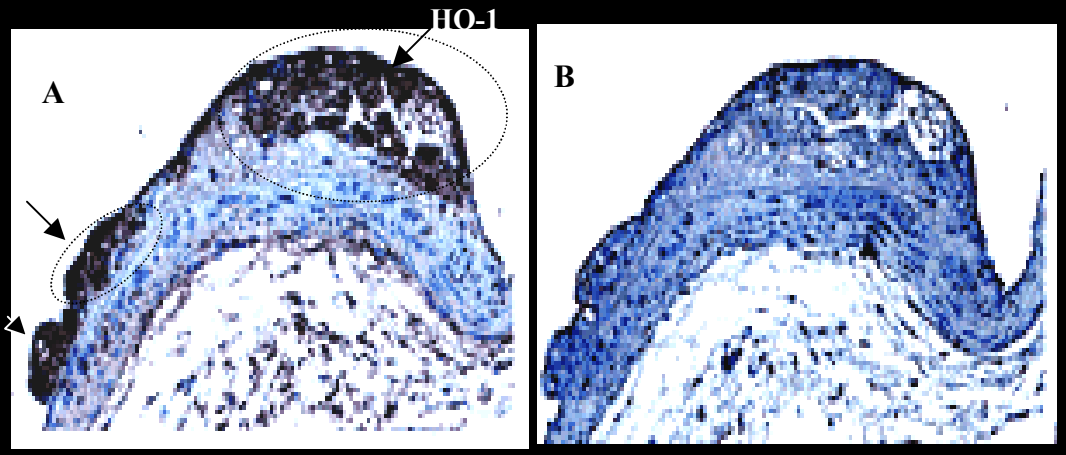
TR = thioredoxin reductase

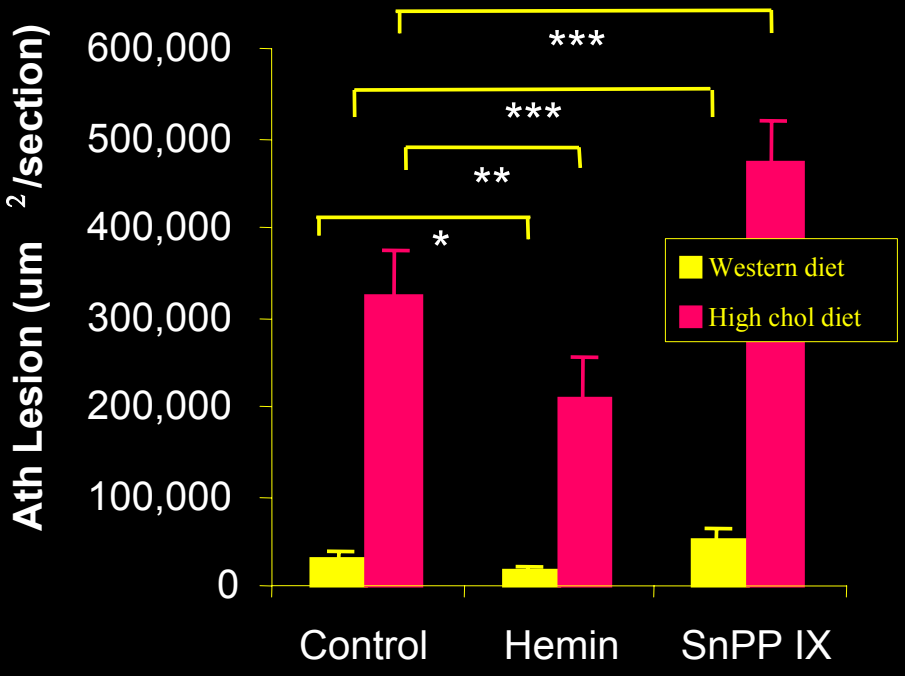
Aorta of ApoE-Deficient Mice Responds to Atherogenic Stimuli by a Prelesional Increase and Subsequent Decrease in the Expression of Phase II Antioxidant Enzymes



Peter t' Hoen et al
Circ Res. 2003;93:262-269

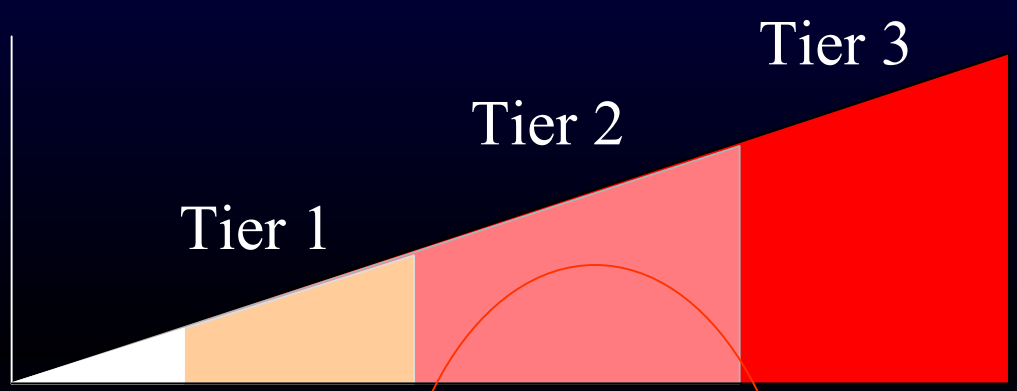
HO-1 expression in atherosclerotic lesions in LDL-R null mice.





Hemin-induced HO-1 expression inhibits while SnPP Rx accelerates atherosclerosis in LDL-R$^{-/-}$ mice

Xiao, et al.

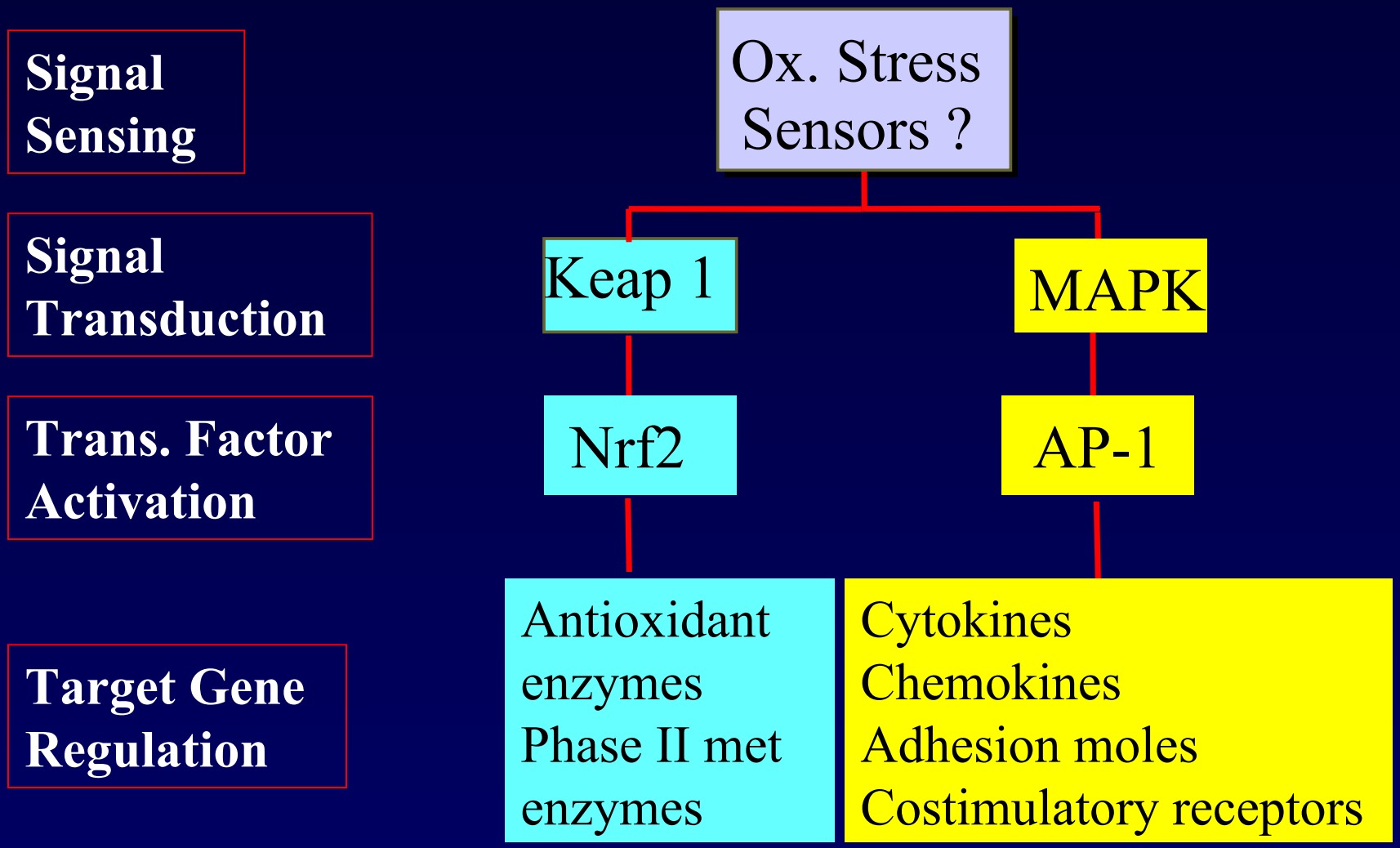


Cell response pathway: Normal | **Anti-oxidant Defense** | **Inflammation** | **Toxicity**

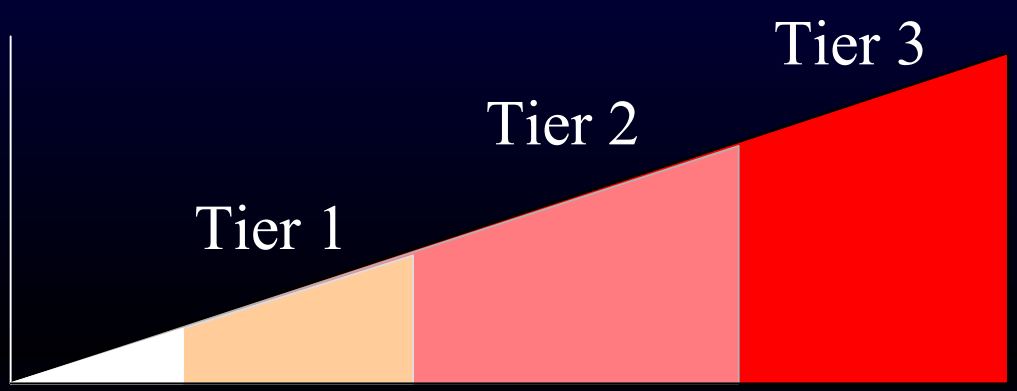
Signaling pathway: **MAP Kinases**

Genetic response: **AP-1**

Adaptive Genetic Programming

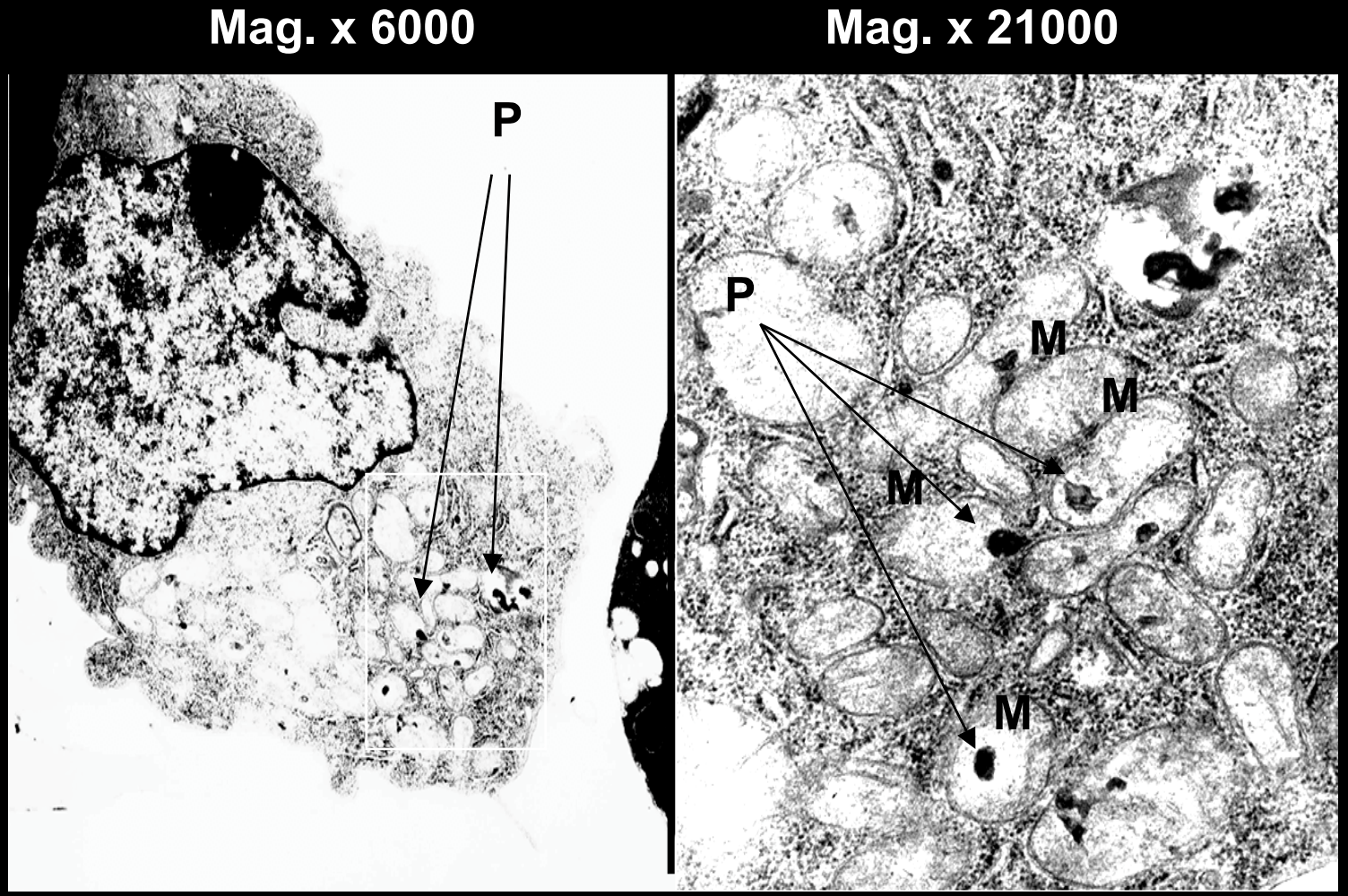


Xiao, et al.



Cell response pathway:	**Normal**	**Anti-oxidant Defense**	**Inflammation**	**Toxicity**
Signaling pathway:	**___**	**Nrf-2**	**MAPK**	**Mitochondrial perturbation**
Genetic response:	**——**	**ARE**	**AP-1**	**PT pore**

Ultrafines lodge in and destroy mitochondria



Li et al