Overview of Oxidative Stress in Toxicology

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Oxidative stress: background

- Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) and the ability of a cell to detoxify them.
- Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.
- ROS (such as superoxide) can be beneficial by acting as cellular messengers in redox signaling or as a way to attack and kill pathogens
- Over-production of ROS and RNS can cause disruptions in normal mechanisms involved in cellular signaling

Exogenous and endogenous sources of oxidative stress

Exogenous sources

Oxygen
γ-irrradiation
UV-irradiation
Drugs
Environmental chemicals
Food
Tobacco smoke
Xenobiotics

Endogenous sources

Cells (e.g. mitochondria)
Enzymes (e.g. NO synt., CYP2E1)
Metabolism (e.g. mitochondria)
Diseases (e.g. ischemia)

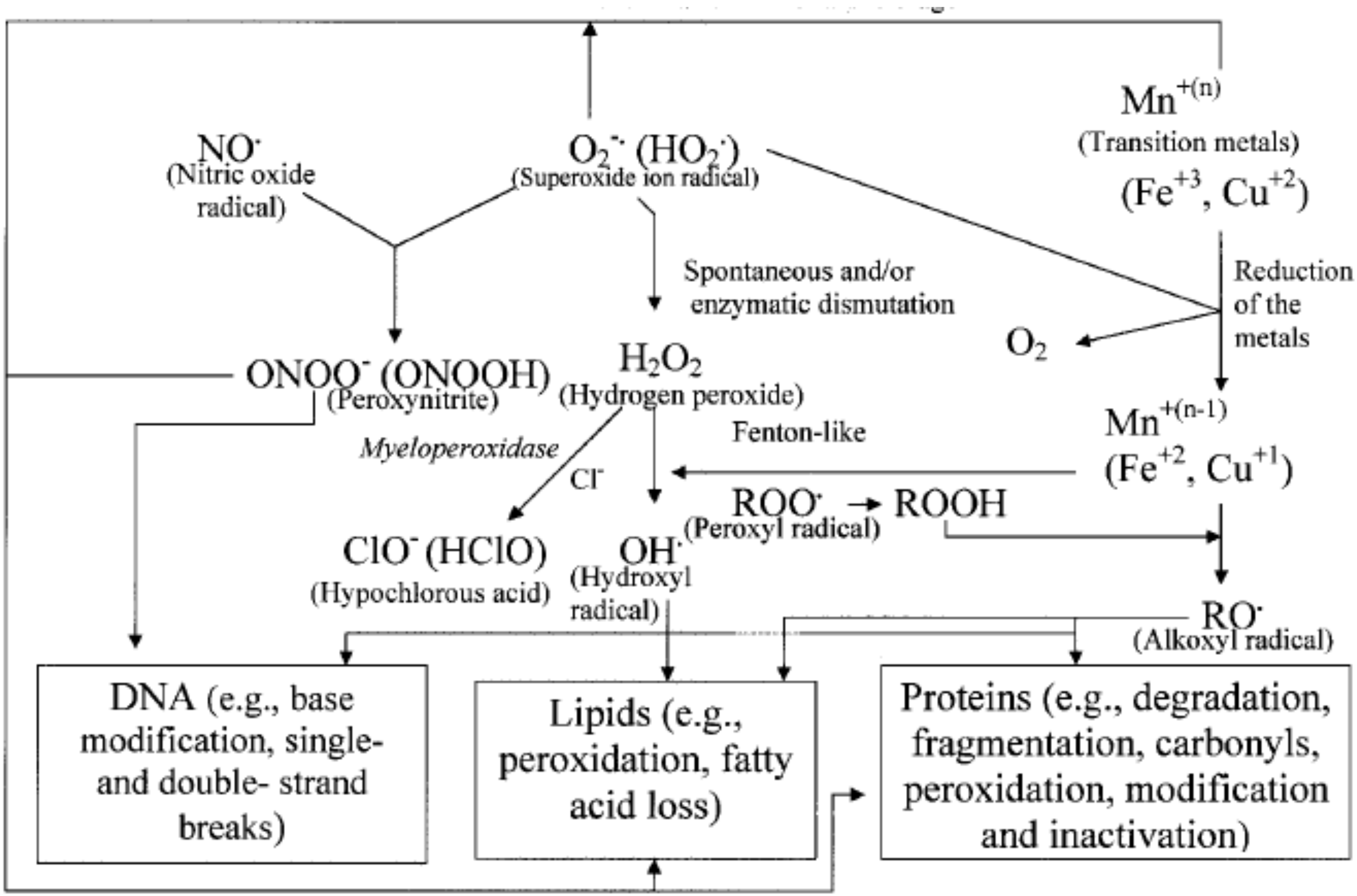
↓

Reactive oxygen species (ROS) and/or Reactive nitrogen species (RNS)

↓

Oxidative stress

Consequences of ROS and RNS generation

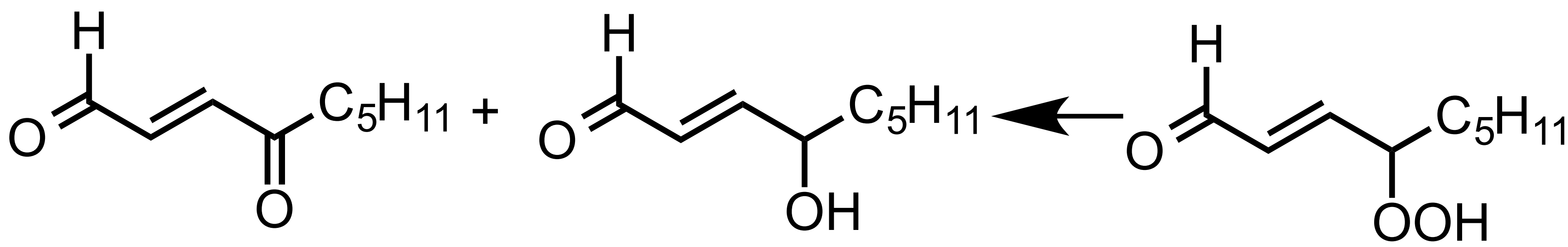


Kohen R, Nyska A. *Toxicol Pathol*. 2002;30:620

Electrophilic aldehydes and oxidative stress

·OH + Polyunsaturated fatty acids → Lipid hydroperoxides
(ROS)

↓ Homolytic decomposition



4-oxo-
2(*E*)-nonenal
(ONE)

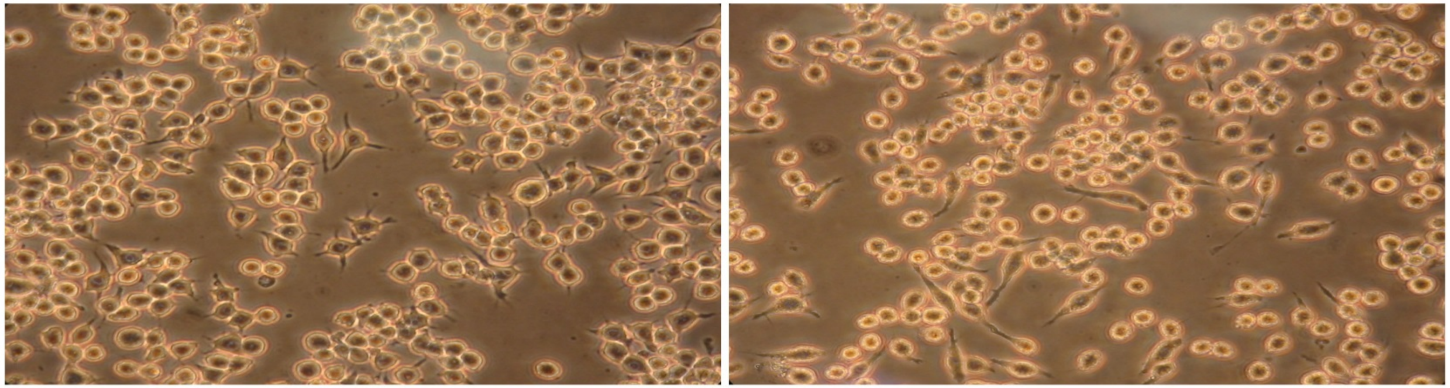
4-hydroxy-
2(*E*)-nonenal
(HNE)

4-hydroperoxy-
2(*E*)-nonenal
(HPNE)

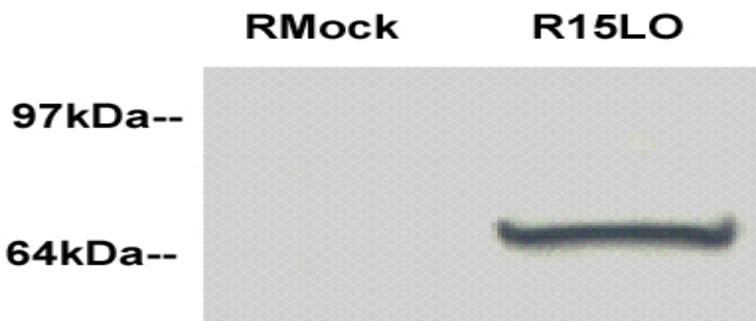
Blair IA. *J Biol Chem*. 2008;283:15545

15-Lipoxygenase (LOX)-1 transfected cell (R15LO)

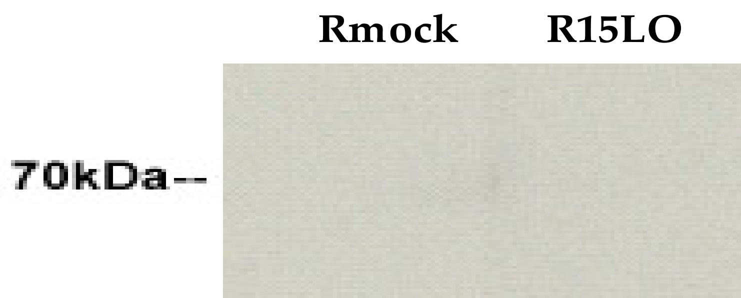
Rmock R15LO



Western blot for 15-LOX-1



Western blot for COX-2

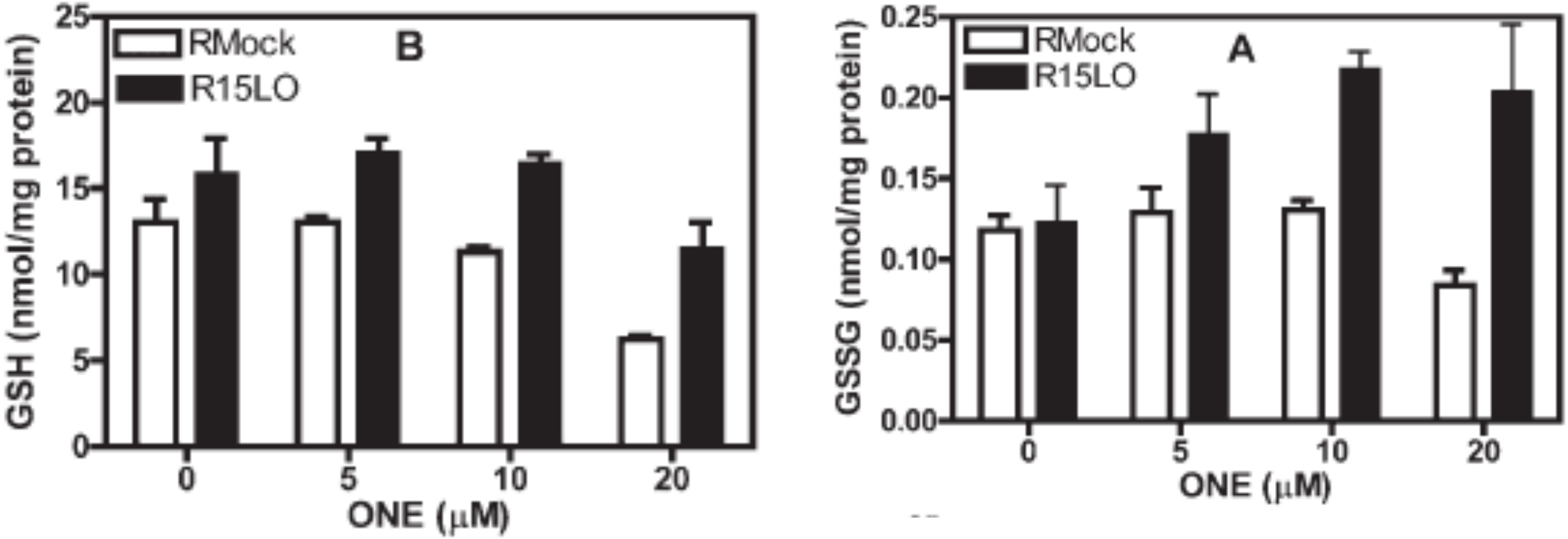


Model system to study 15-LOX-1-mediated lipid peroxidation to **15(*S*)-HPETE**

Oxidative stress and glutathione (GSH)

- Intracellular GSH provides a major defense against oxidative stress
- During oxidative stress reduced GSH is converted to glutathione disulfide (GSSG)
- The high abundance of GSH and low abundance of GSSG helps to maintain cells in a reducing environment helps to prevents oxidative damage to cellular macromolecules.
- Hydrogen peroxide and lipid hydroperoxides (eg 15-HPETE) undergo GSH peroxidase-mediated reduction to water or lipid hydroxides, respectively

Oxidation of glutathione and oxidative stress



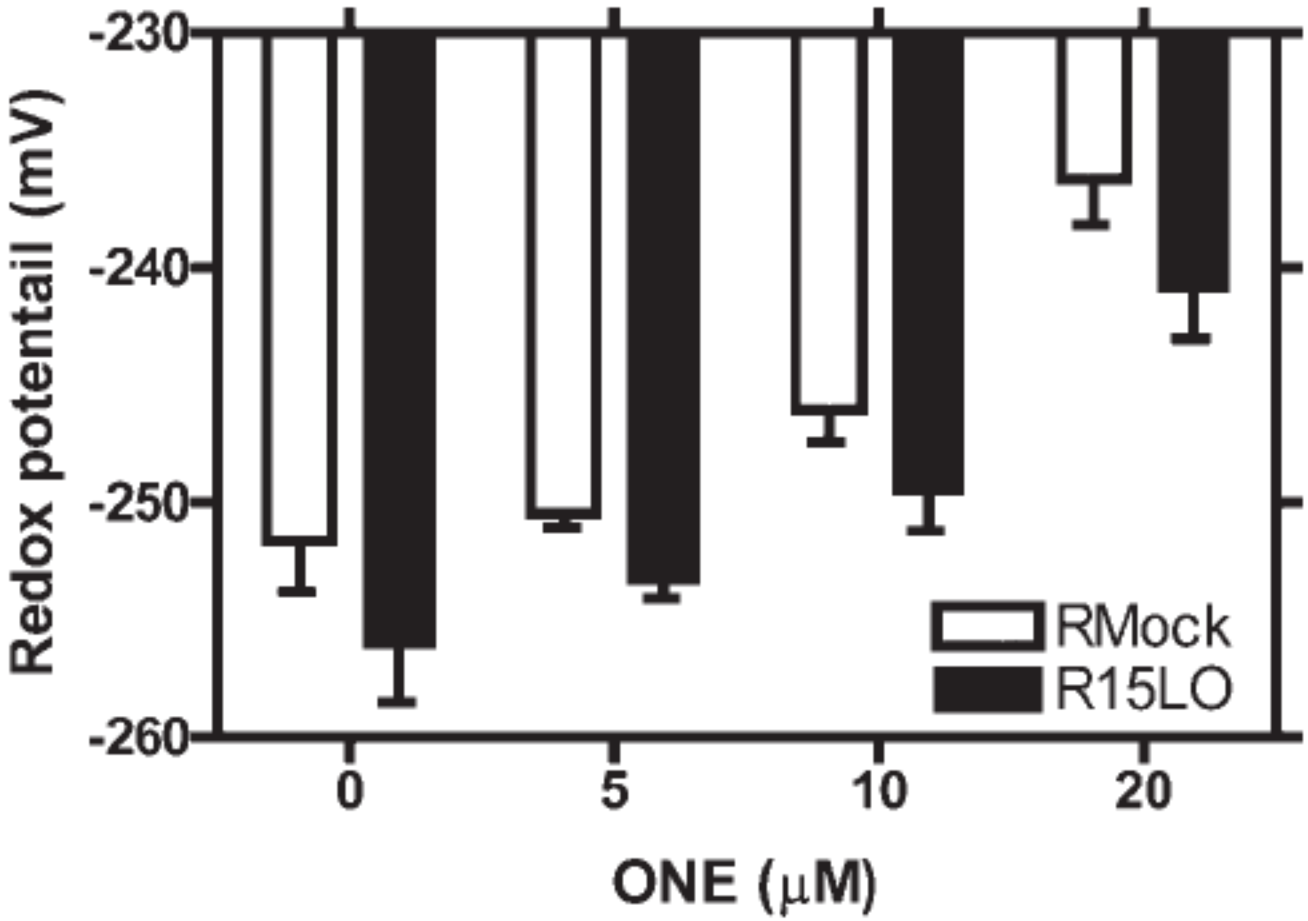
R15LO = Monocyte/macrophage cells transfected with 15-lipoxygensase gene
RMock = Monocyte/macrophage cells transfected with empty plasmid

Zhu P, Oe, T, and Blair IA. *Rapid Commun Mass Spectrom* . 2008;30:620

GSH homeostasis and cellular redox status

- GSH forms adducts with a great variety of both endogenous and exogenous reactive electrophilic intermediates, generally facilitated by GSH S-transferases (GSTs)
- This is considered to normally represent a detoxification of the relevant reactive intermediate
- Changes of the half-cell reduction potential of the 2GSH/GSSG couple correlate with the biological status of the cell
- GSH/GSSG homeostasis plays an important role in maintaining cellular redox status. Determinations of the reduction potential (Nernst equation) can be used to monitor oxidative stress.

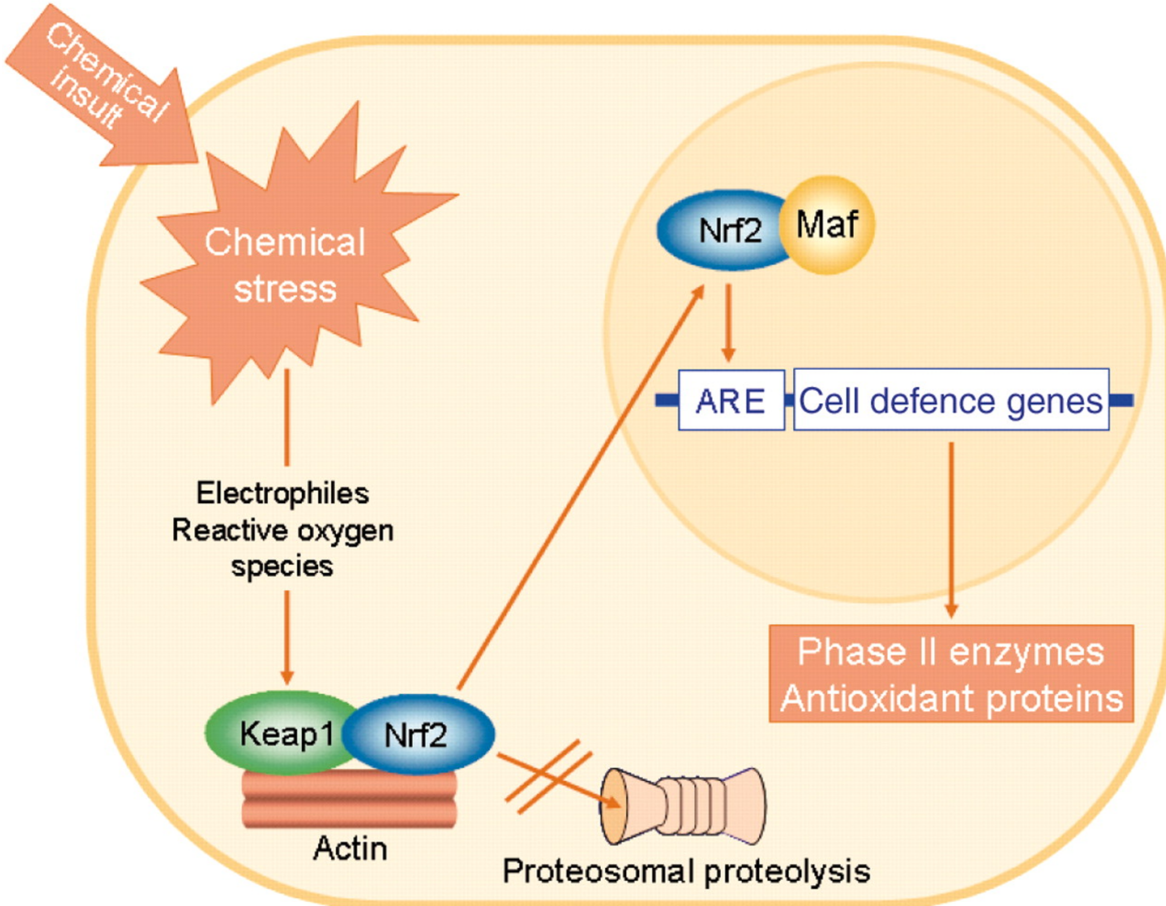
Redox status determined from GSSG/GSH ratio



Expression of 15(S)-hydroperoxy-eicosatetraenoic acid up-regulated anti-oxidant defense

Zhu P, Oe, T, and Blair IA. *Rapid Commun Mass Spectrom* . 2008;30:620

Nrf2 mediated antioxidant response



Binding of electrophiles such as ONE to Keap1 SH-groups releases Nrf2, which up-regulates GSH biosynthesis and other anti-oxidant responses

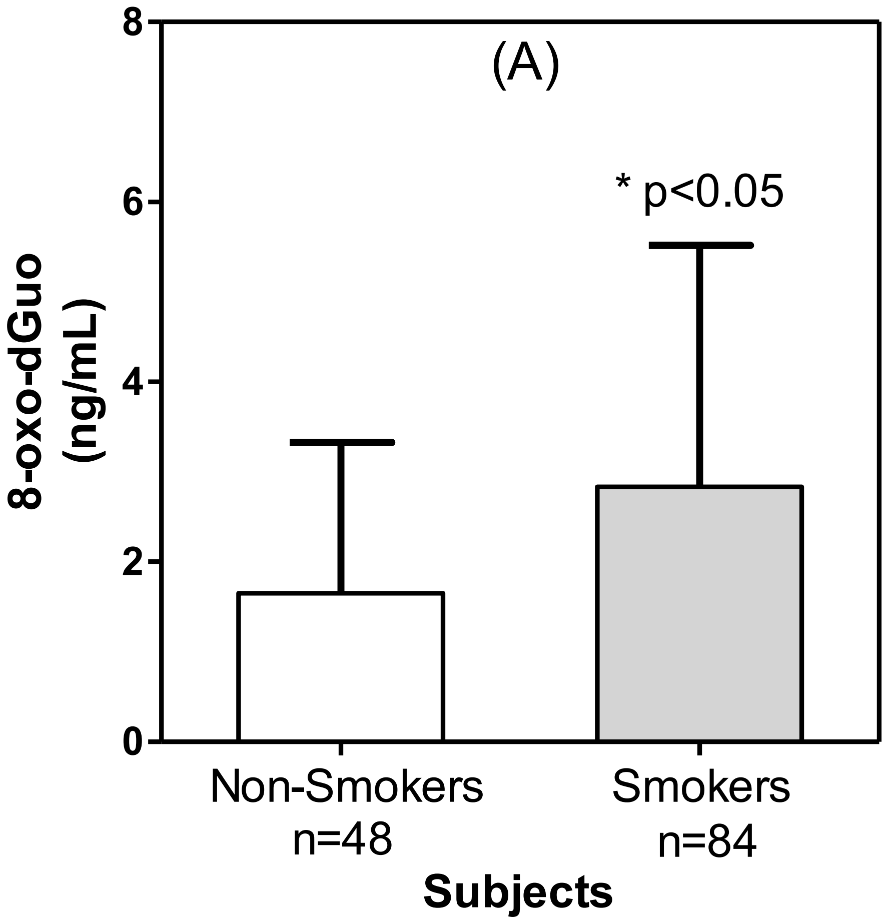
J Exp Biol **2006;209:2337**

Biomarkers of oxidative stress: background

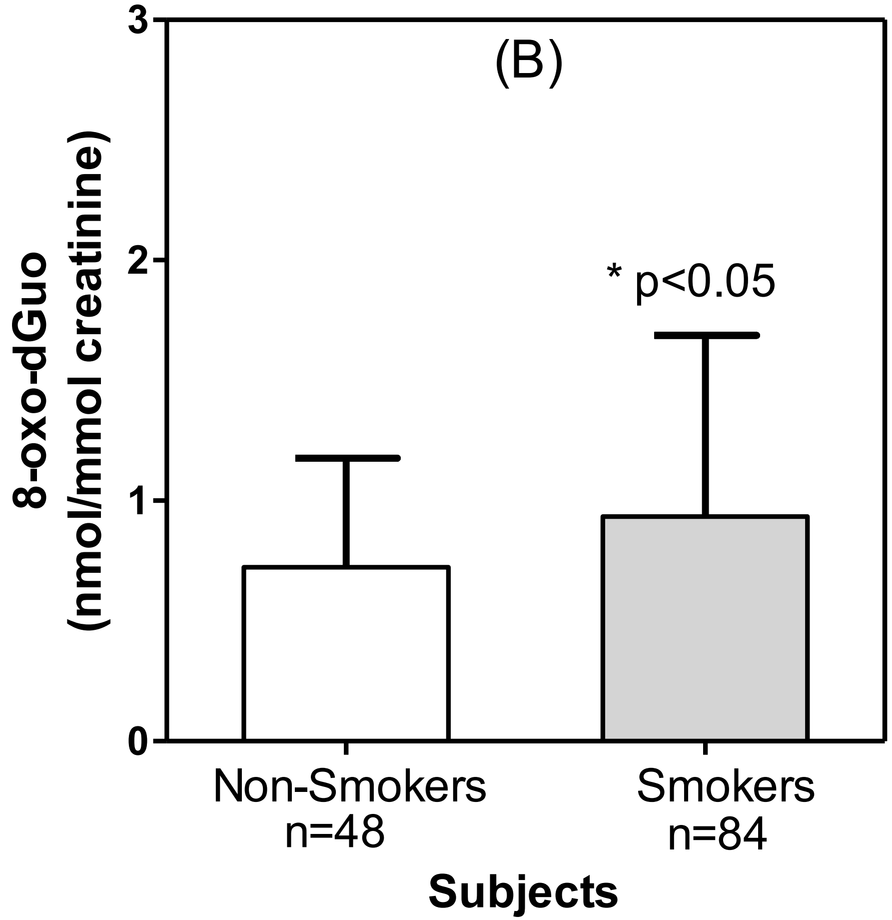
Type	Biomarker	Reference
DNA	8-oxo-2'-deoxyguanosine (8-oxo-dGuo)	Mesaros et al. Free Radic Biol Med. 2012;53:610
DNA	8-oxo-2'-deoxyguanosine (8-oxo-dAdo)	Cooke et al. FASEB J. 2003;17:1195
DNA	Cyclo-dA and Cyclo-dG	Yuan Nucleic Acids Res. 2011;14:5945
DNA	Etheno DNA-aducts (etheno-Ade)	Chen and Kao Tox Lett. 2007;169:72
DNA	Malondialdehyde dGuo adduct (M1G)	Jeong at al. Chem Res Tox. 2005;18:51
lipid	Malondialdehyde (MDA)	Lee et al. Curr Med Chem. 2012;19:2504
lipid	Isoprostanes	Milne at al. Chem Rev. 2011;111:5973
lipid	Acrolein	Wang et al. Biomarkers 2011;16:144
protein	Nitrotyrosine	Tsikas D. Amino Acids. 2012;42:45

Urinary 8-oxo-dGuo: non-smokers vs smokers

Concentration (ng/mL)

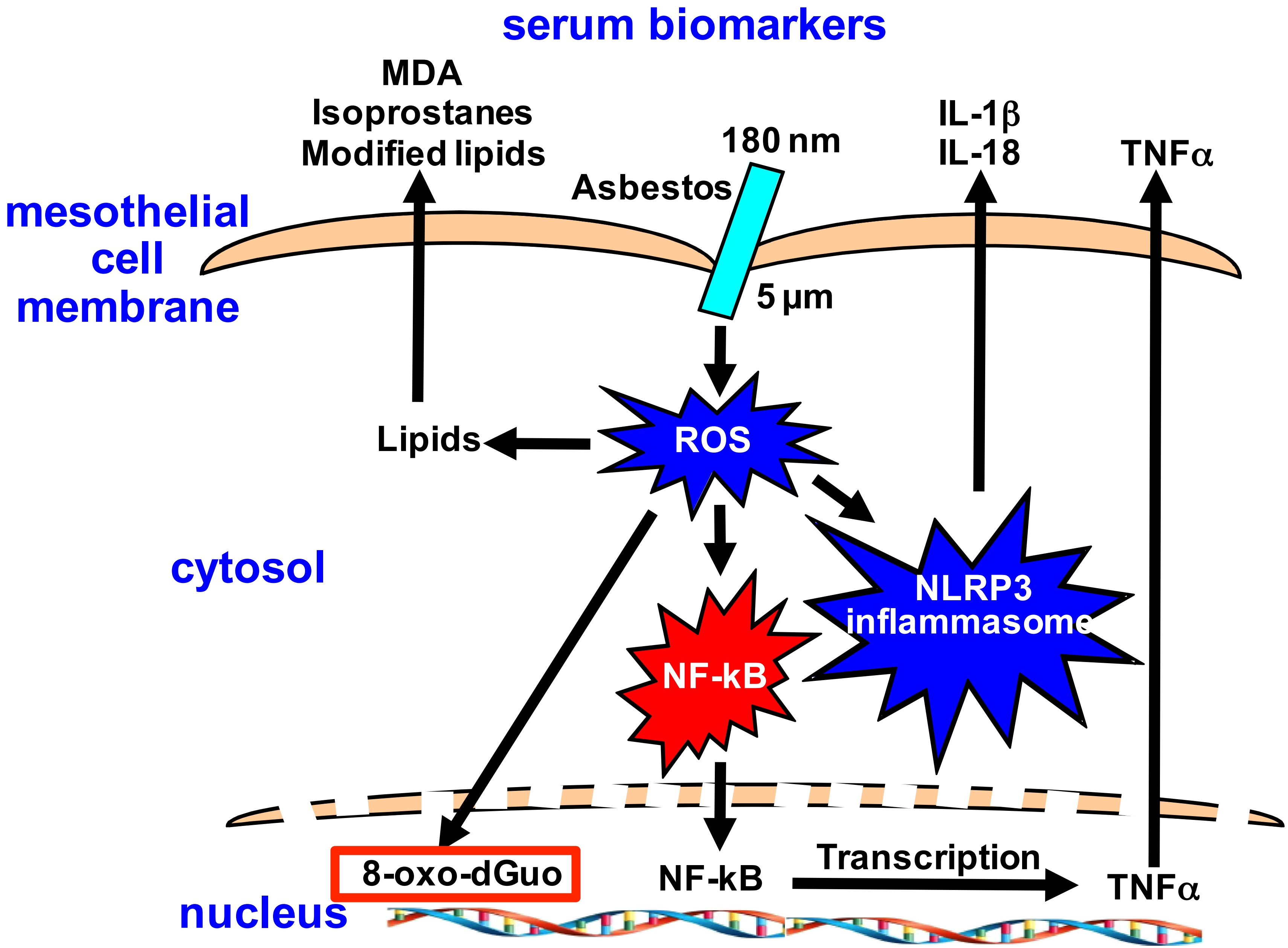


Normalized to creatinine

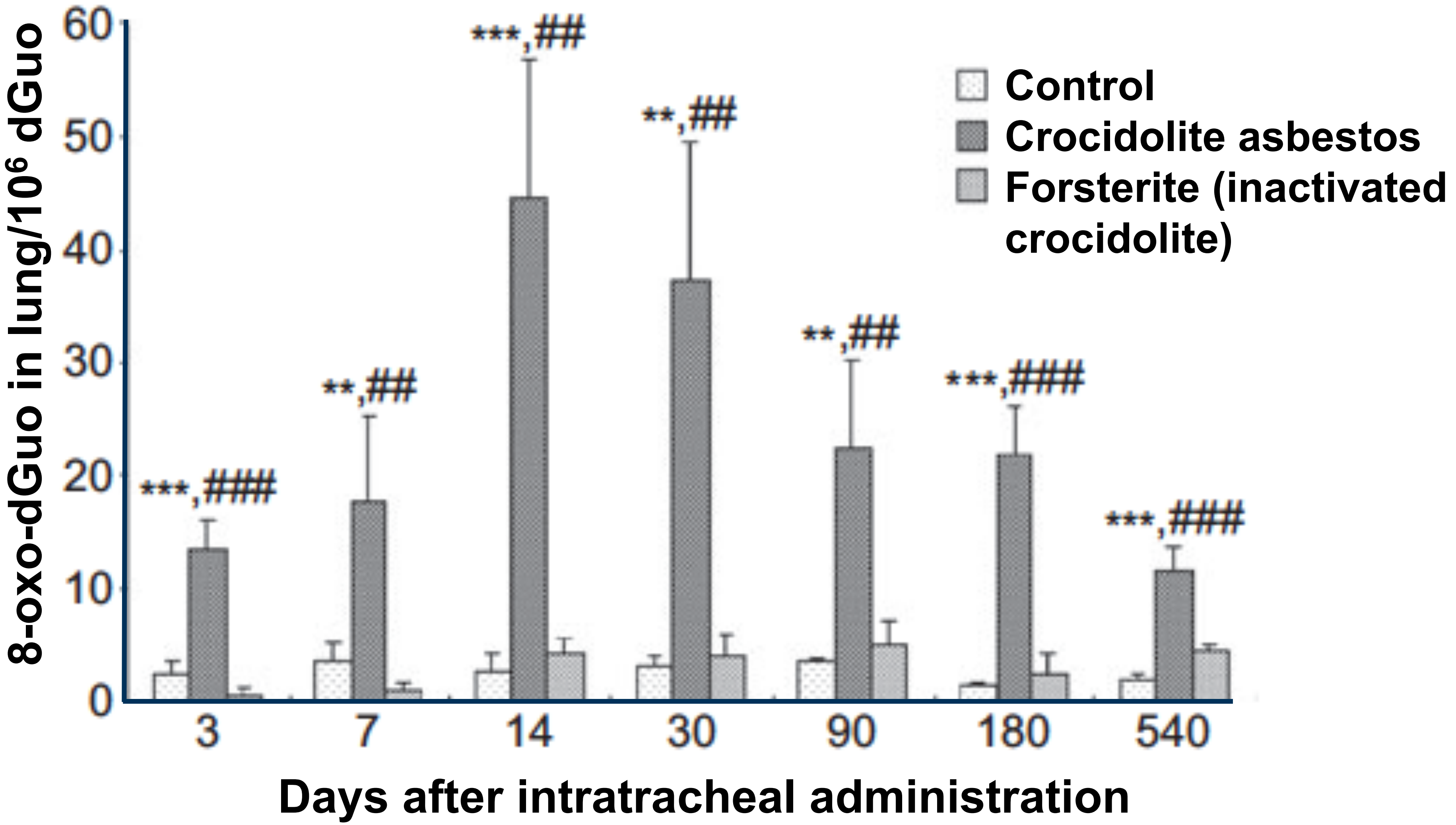


Mesaros *et al. Free Radic Biol Med*. 2012;53:610

Asbestos-induced oxidative stress



Asbestos-induced formation of lung 8-oxo-dGuo

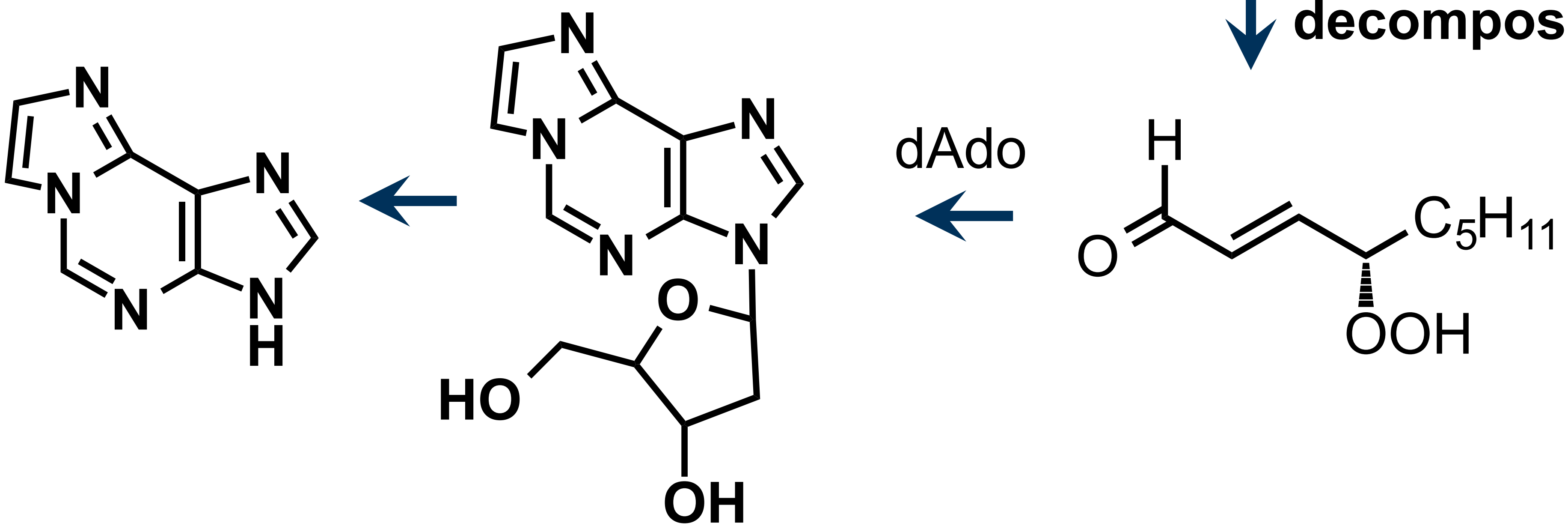


Takata A. et al *Inhal. Toxic*. 2009;9:739

Formation of urinary etheno-adenine

·OH + Polyunsaturated fatty acids → Lipid hydroperoxides
(ROS)

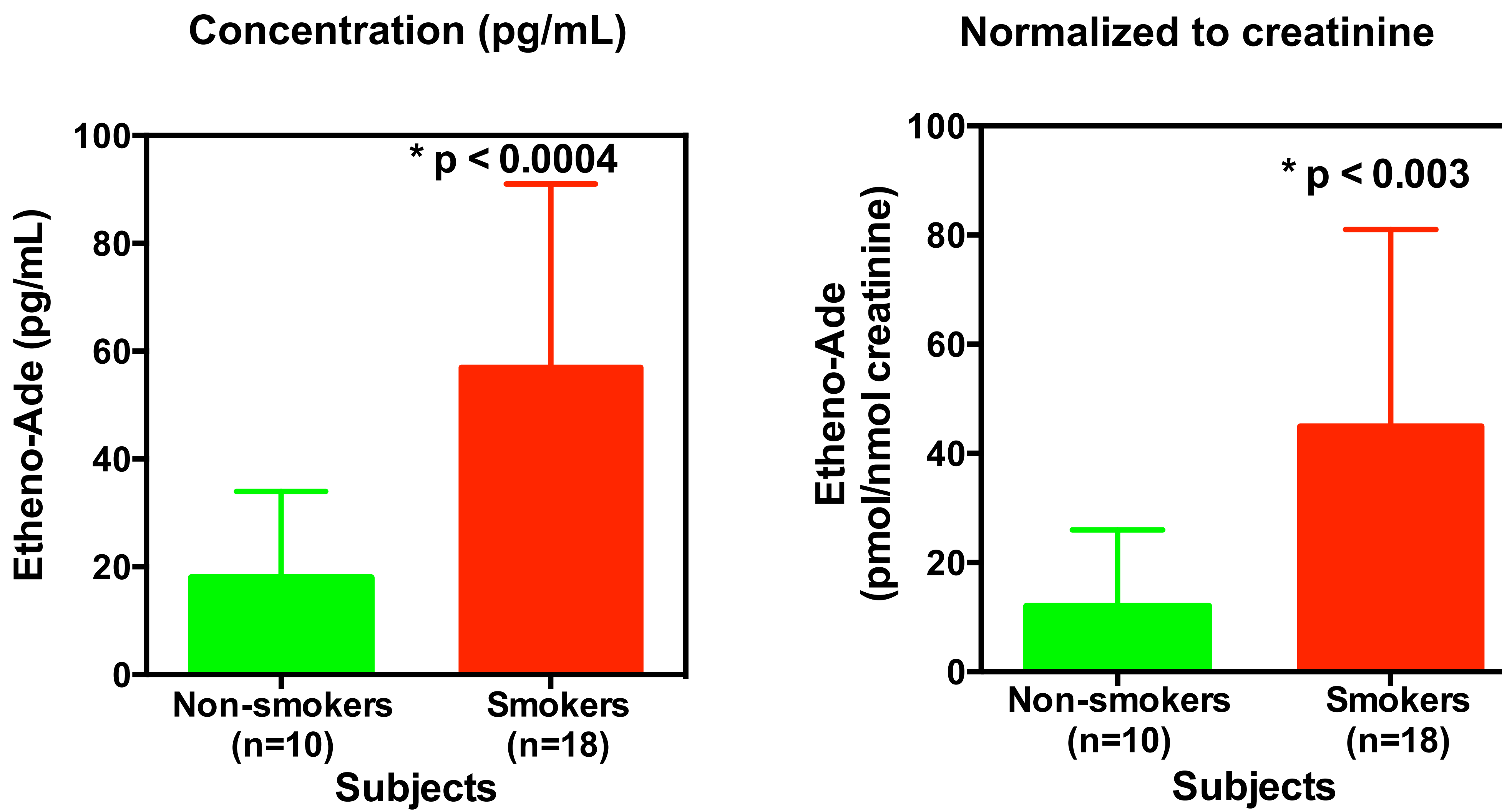
↓ Homolytic decomposition



etheno-Ade **etheno-dAdo** **4-hydroperoxy-2(*E*)-nonenal**

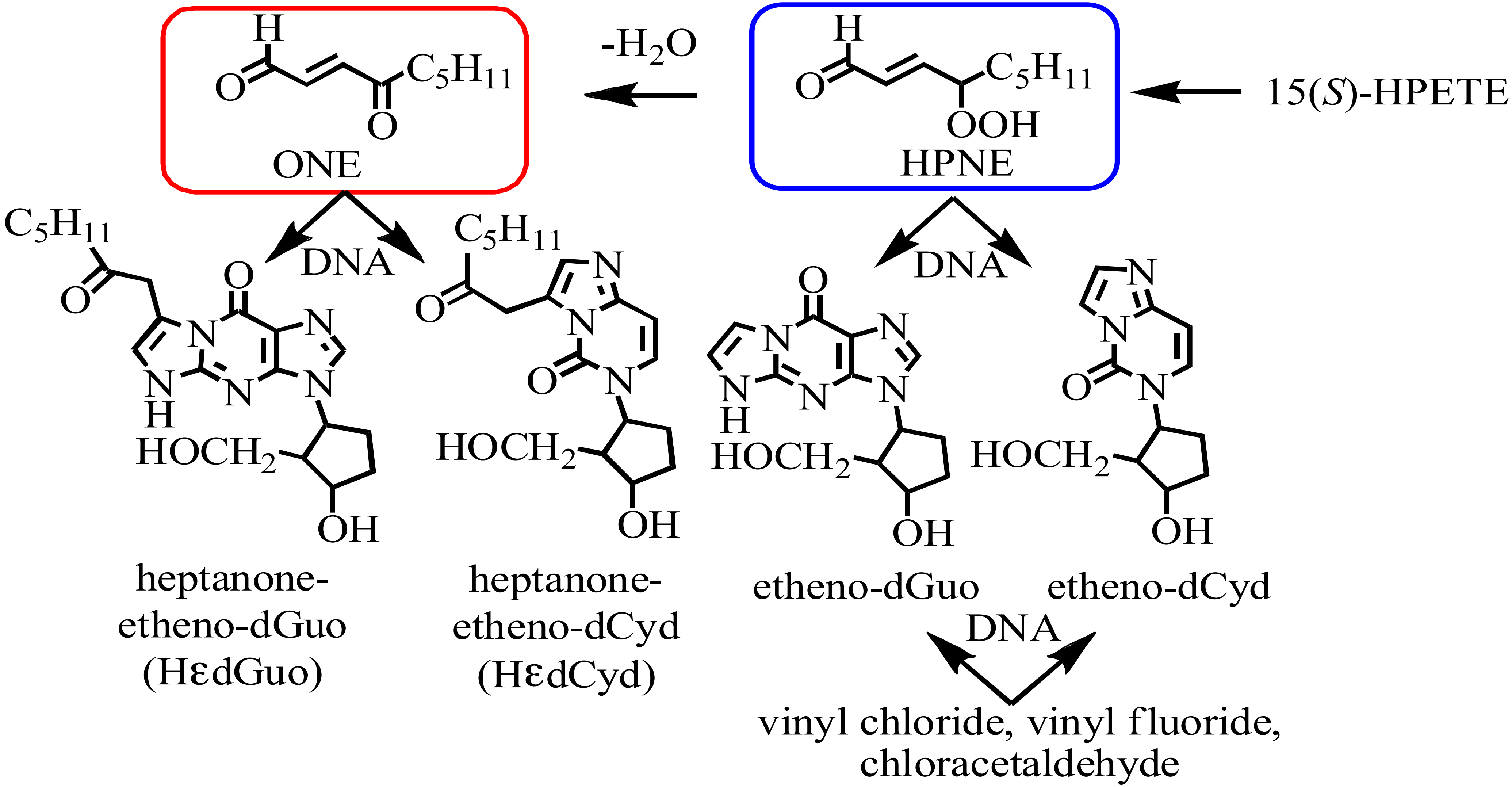
Lee et al. *Chem Res Toxicol*. 2005;18:780

Urinary etheno-Ade: non-smokers vs smokers



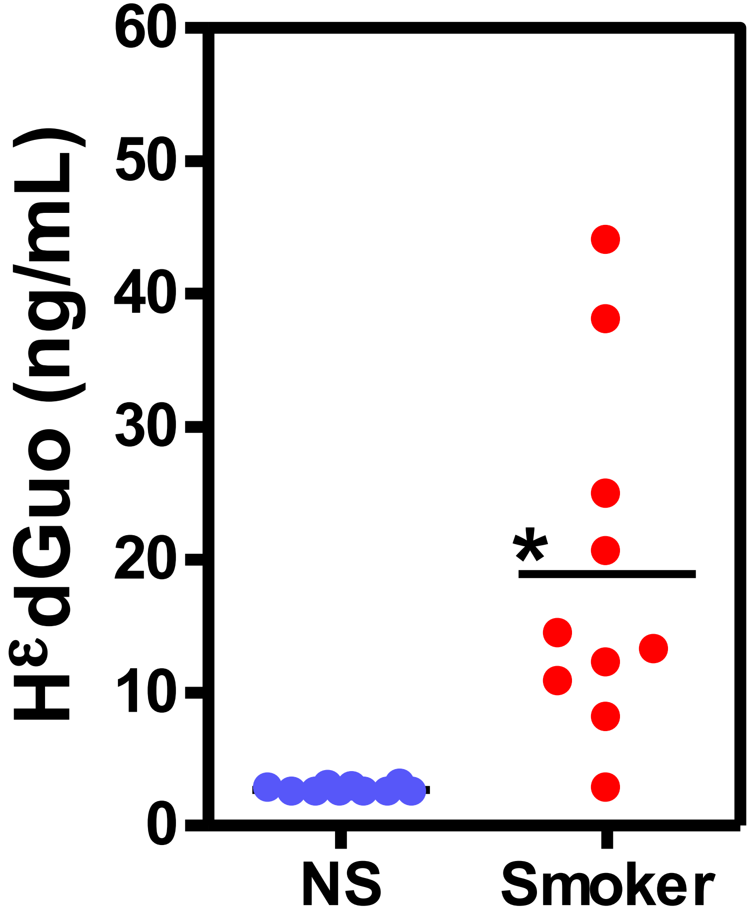
Chen HJ, Kao CF. *Tox Lett*. 2007;169:72

Formation of urinary heptanone-etheno-dGuo

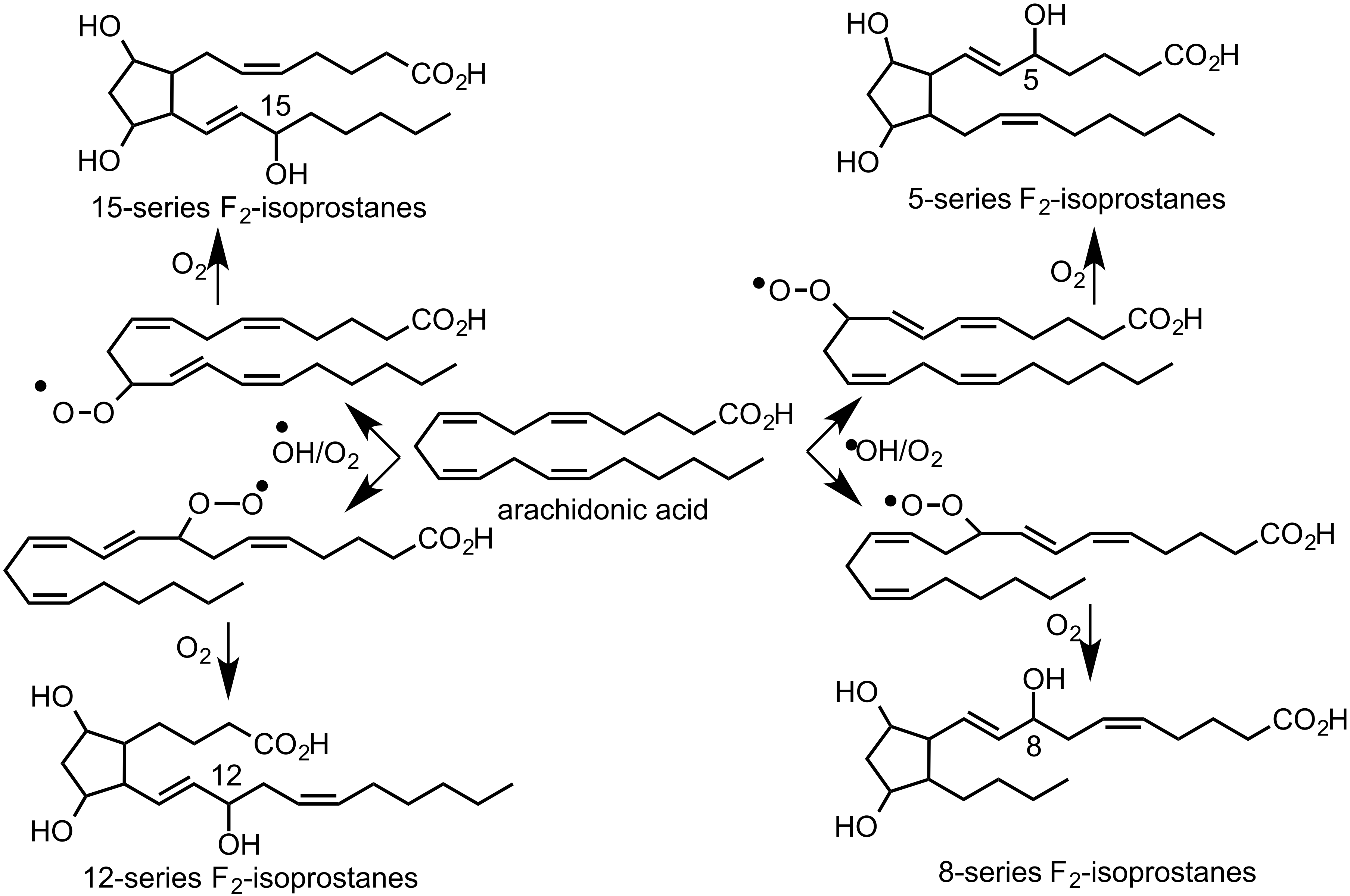


Pollack M, Yang IY, Kim HY, Blair IA, Moriya M. *Chem Res Toxicol*. 2006;19:1074

Urinary heptanone-etheno-dGuo (HεdGuo)



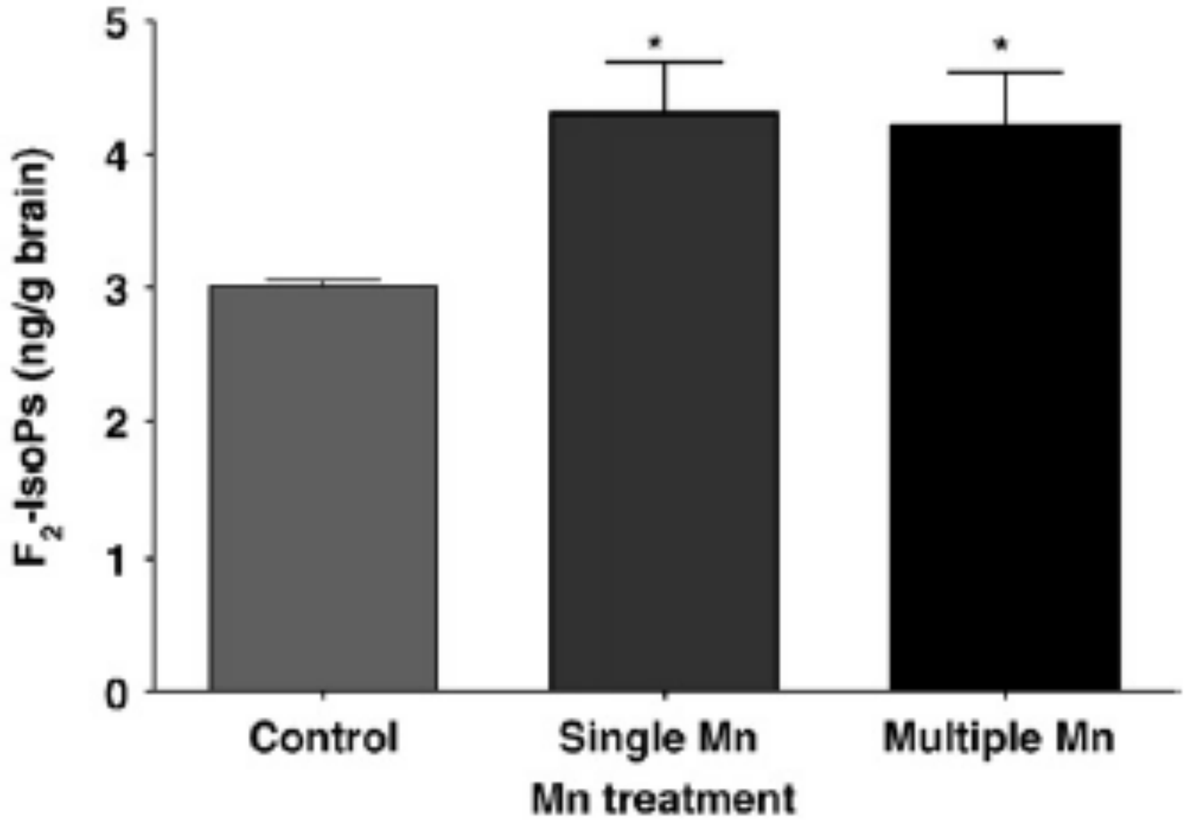
Isoprostane formation during oxidative stress



Milne *et al. Chem Rev*. 2011;111:5973

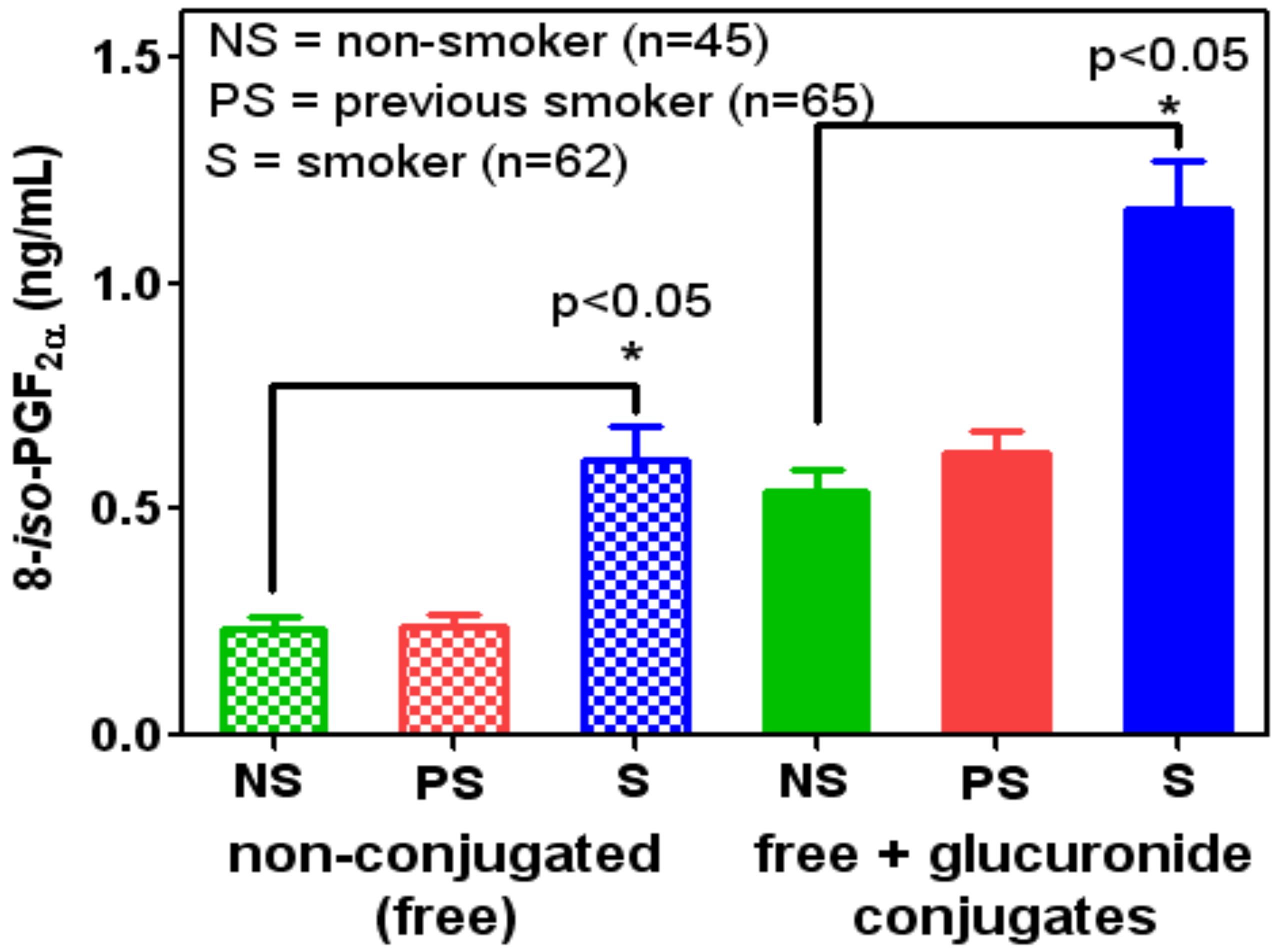
Manganese-induced isoprostane formation

Cerebral F_2-isoprostane formation in mice



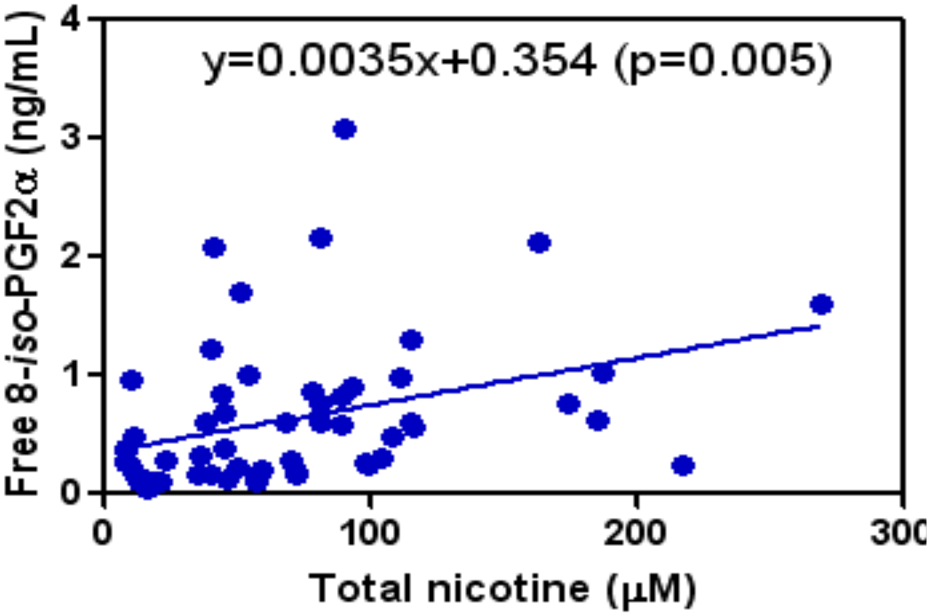
Milatovic *et al. Toxicol Appl Pharmacol*. 2009;240:219

Urinary 8-iso-$PGF_{2\alpha}$: non-smokers vs. smokers

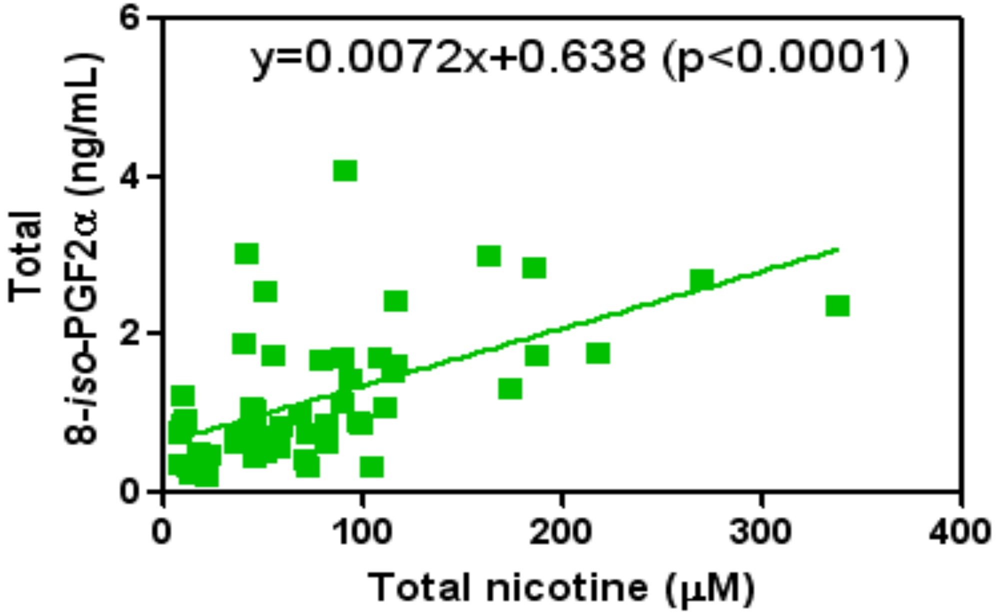


Urinary 8-iso-$PGF_{2\alpha}$: relationship with nicotine

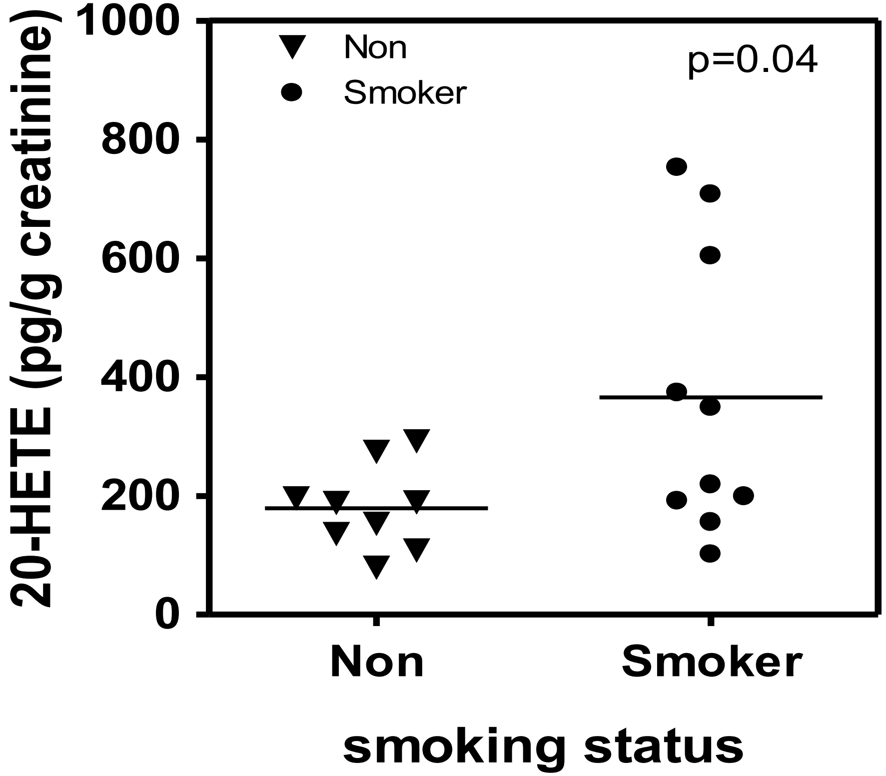
Free 8-iso-$PGF_{2\alpha}$



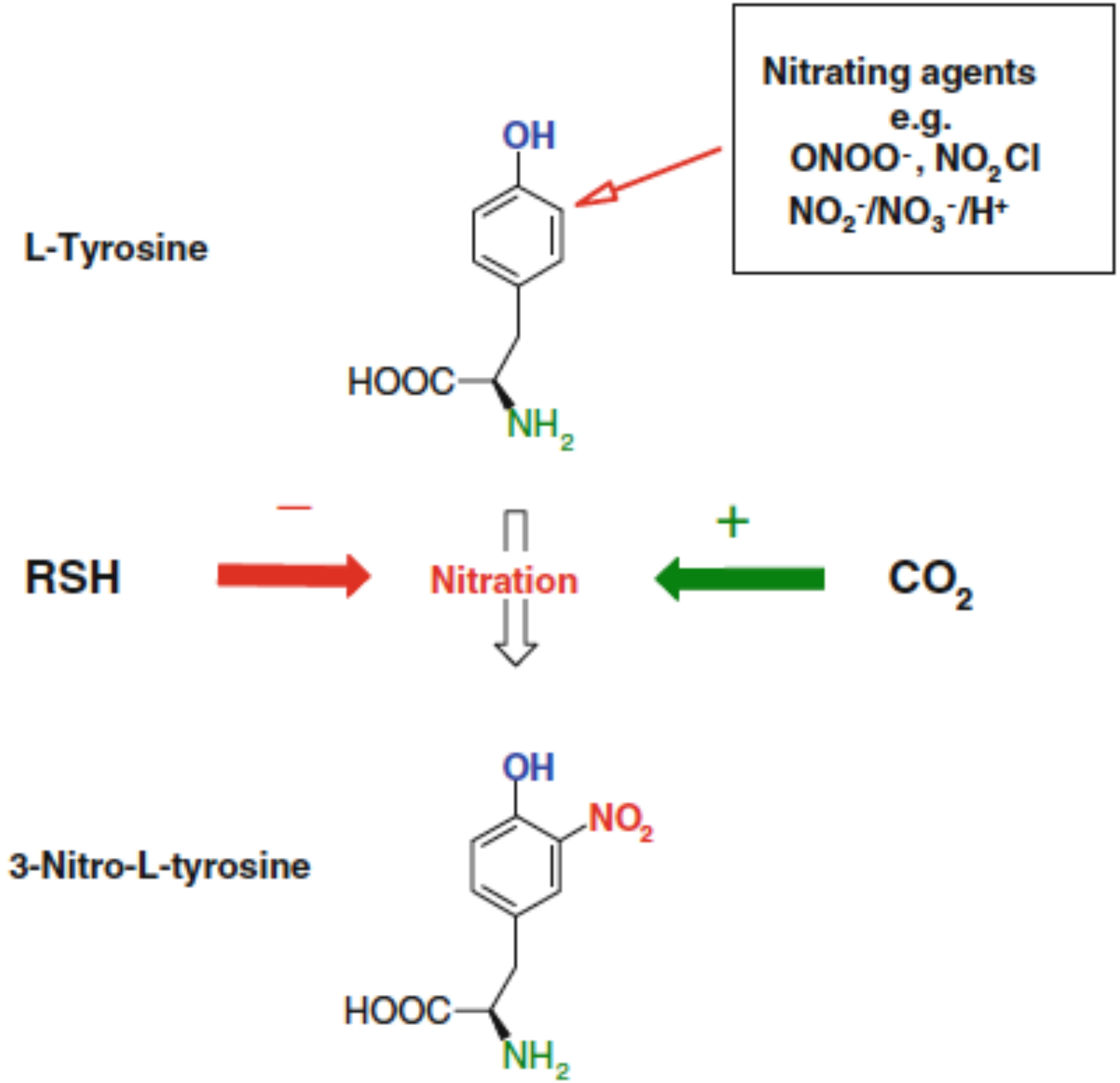
Total 8-iso-$PGF_{2\alpha}$



20-HETE levels in urine in smoker vs. non-smokers



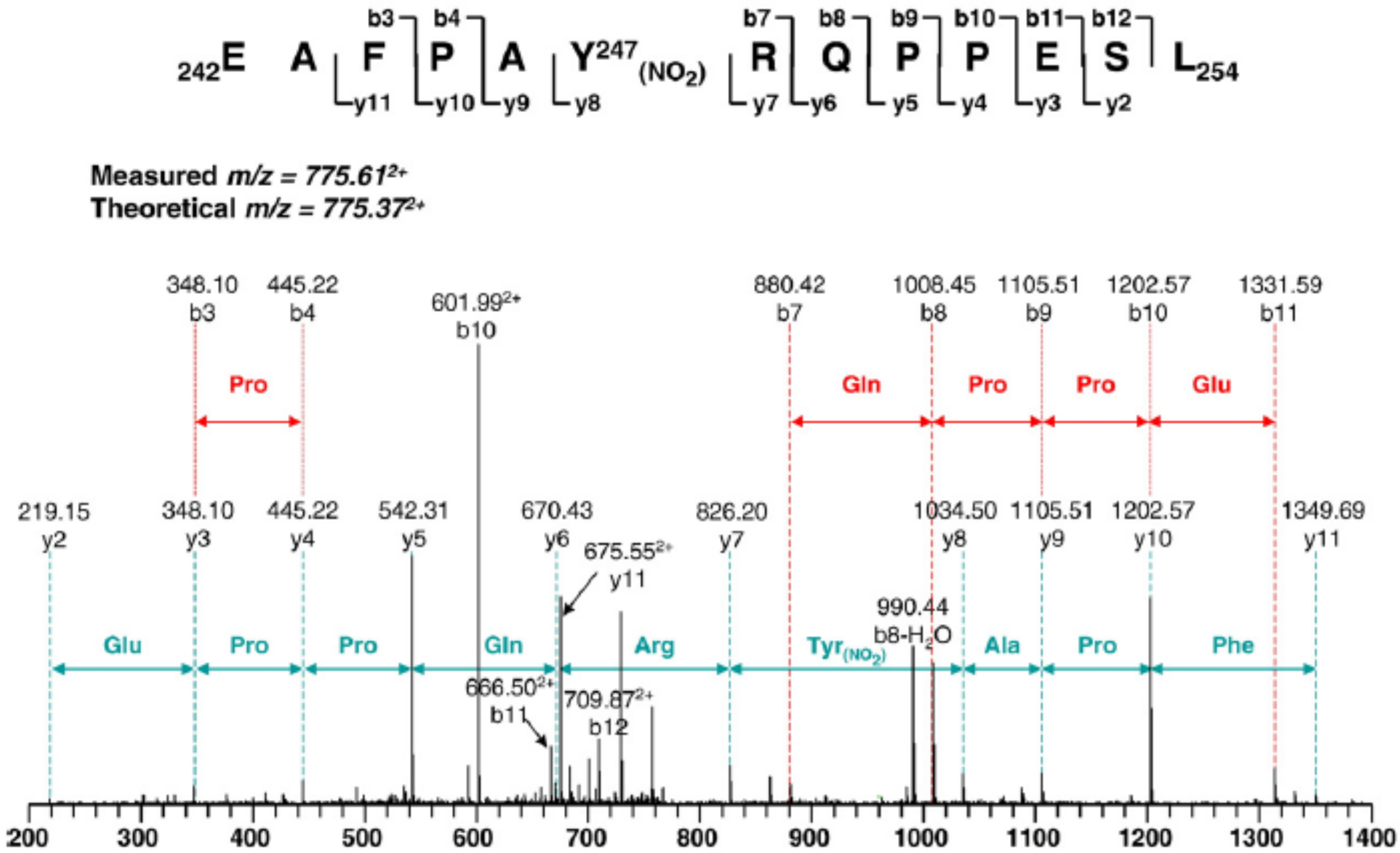
Formation of nitrotyrosine during oxidative stress



Tsikis D. *Amino Acids*. 2012;42:45

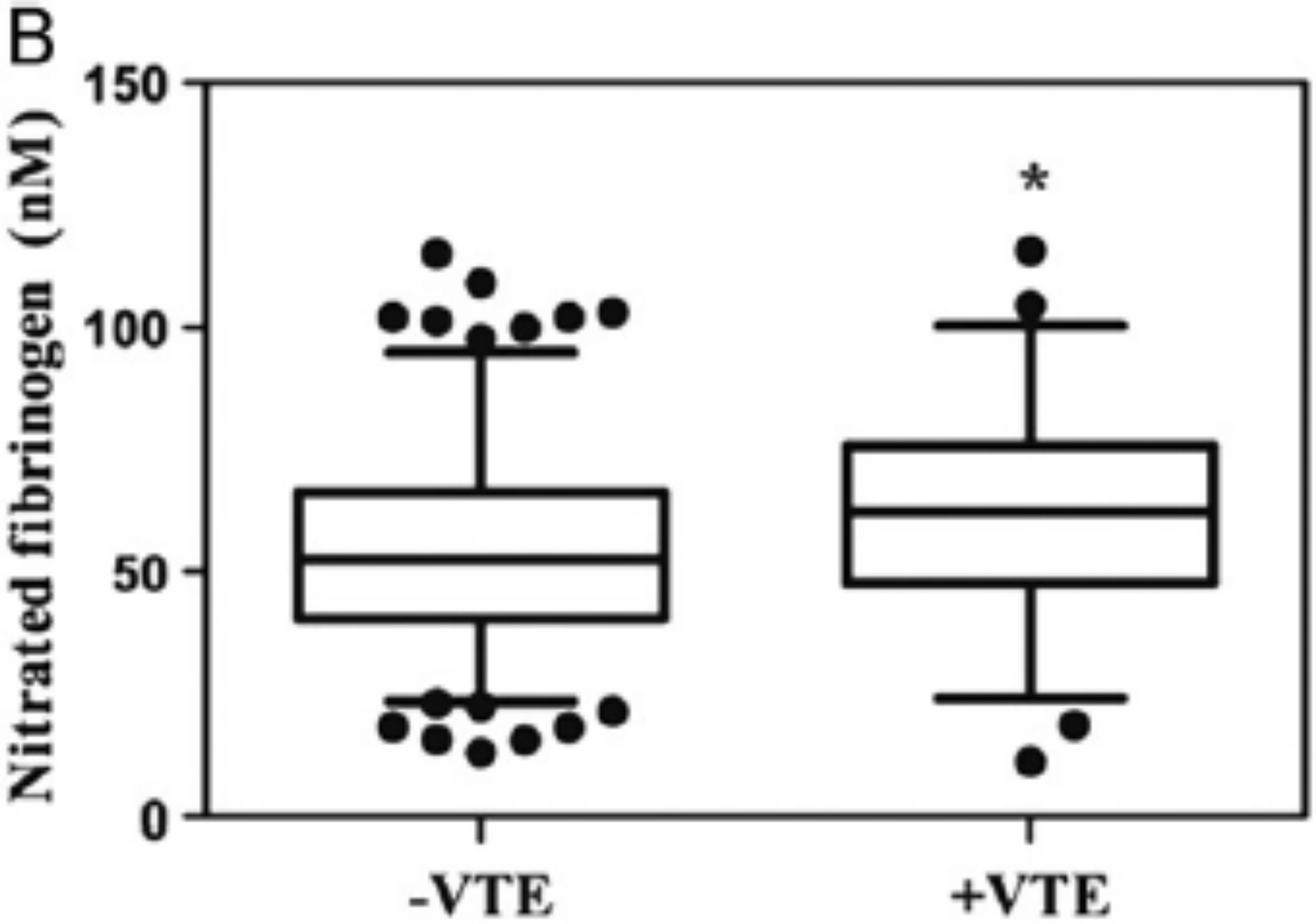
Nitrotyrosine formation during reperfusion injury

Nitrated peptide from mitochondrial complex I sub-unit isolated from mouse heart



Liu *et al. Biochim Biophys Acta*. 2009;1794:476

Nitrated fibrinogen in venous thromboembolism



(n= 178) **(n= 48)**

VTE = venous thromboembolism

Martinez *et al. Free Radic Biol Med.* 2012;53:230

Oxidative stress symposium

1:45 pm Quantitative analysis of thiol electrophile and redox modifications by LC-MS/MS
Daniel C Liebler, Jing Yang, Keri A Tallman, Vinayak Gupta, Kate S Carroll, Ned A Porter

2:25 pm HDL oxidation in the pathogenesis of cardiovascular disease
Baohai Shao, **Jay W Heinecke**

3:05 pm Intermission

3:20 pm Cyclo-dA and Cyclo-dG as biomarkers of oxidative stress
Yinsheng Wang

4:00 pm Antimalarial drug artesunate ameliorates oxidative lung damage in experimental allergic asthma
W E Ho, C Cheng, H Y Peh, F Xu, C N Ong, W S Wong, J. S. Wishnok, Ravindra Kodihalli, **Steven R Tannenbaum**