Free Radical and Antioxidants Lecture 3

Lecture Outline

- Free Radical Chemistry
- Antioxidant's action
- Repair mechanisms
- Little bit of aging ….here and there

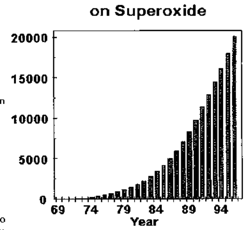
A Brief History of Free Radical Biology

A free radical is a molecule containing an unpaired electron. Free radicals are unstable and reactive. In 1969 **Joe M. McCord, Ph.D.** and Irwin Fridovich, Ph.D. discovered an enzyme that catalyzes the elimination of a free radical form of oxygen, $O_2^{\cdot -}$, called *superoxide*. The enzyme was named *superoxide dismutase*, or SOD:

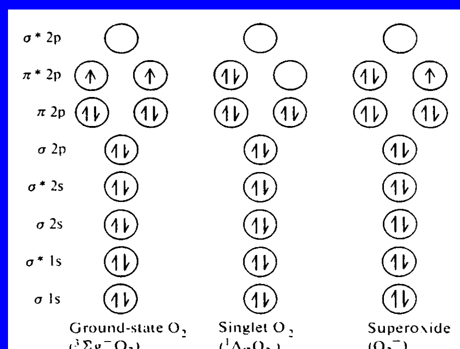
$$O_2^{\cdot -} + O_2^{\cdot -} \xrightarrow{SOD} H_2O_2 + O_2$$

Until this discovery it was assumed that biological systems were too mild-mannered to produce such reactive chemicals. The existence of an enzyme to get rid of free radicals implied that cells must be able to produce the radicals. The only cells found not to contain SOD were anaerobic bacteria, which cannot grow in the presence of oxygen. Humans have three separate forms of SOD: one in the mitochondria, one in the cytosol, and one secreted outside the cells. SOD provided a very useful tool for the study of oxygen-derived free radicals. Since its discovery in 1969 more than 22,000 scientific articles have been written on the subject.

Cumulative Publications on Superoxide



When cells are diseased or injured, the normal metabolism of oxygen goes awry, leading to the increased production of superoxide. In addition, white blood cells intentionally produce superoxide in order to kill microorganisms. These white cells are activated by trauma and inflammation, in addition to infection. Hence, nearly all diseases involve the production of increased amounts of free radicals. Diseases of primary interest include heart attack, stroke, arthritis, cancer, AIDS, and neurological diseases such as ALS.



I. Introduction

A. What is a Free Radical?

"It is an atom or group of atoms possessing <u>one or more unpaired</u> electrons".

The word "free" in front of "radical" is considered unnecessary.

II. Notation

A. Superscript dot to the right, usually

B. Examples (Note: dot, then charge)

$H^{\bullet}$ $O_2^{\bullet -}$ $CO_2^{\bullet -}$

$HO^{\bullet}$ $^{\bullet}CH_3$

$O_2^{\bullet\bullet}$ or $O_2^{2\bullet}$

$Acs^{\bullet -}$ $PQ^{\bullet +}$

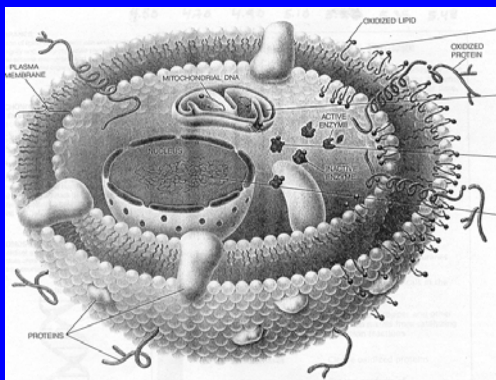
C. Noncommital

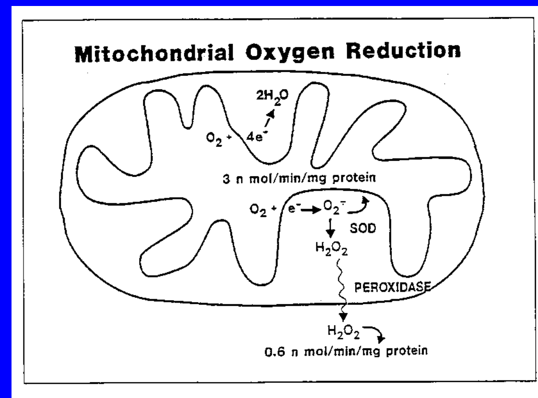
$(HO)^{\bullet}$ $(CH_3)^{\bullet}$

(See Koppenol WH. (1990) What is in a name? Rules for radicals. *Free Radic. Biol. Med.* **9**:225-227.)

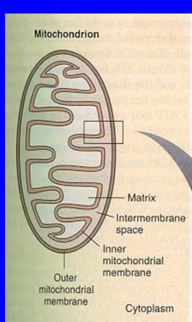
Question?

- Where are radicals produced ?





MITOCHONDRIA RESPIRATION



$4H^+ + O_2 \rightarrow 2H_2O$
(~95% of the time)

$O_2 \rightarrow {}^*O_2^{\bullet-} \rightarrow H_2O_2$

*Only 1-5% generate free radicals

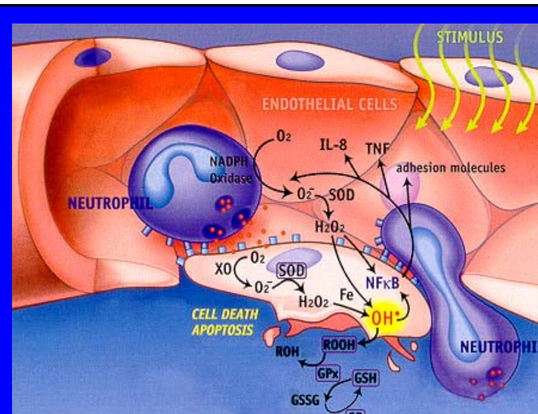
Reactive Oxygen Species

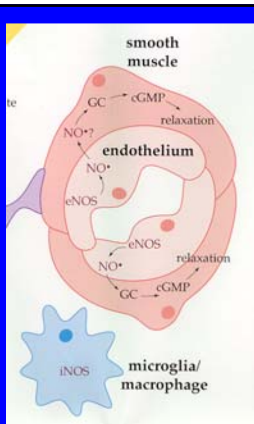
- Approximately 1-5% of oxygen consumed by cells is reduced to the superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)
- H_2O_2 permeates cellular membranes, entering nearly all cellular compartments

What is the oxidants production in a day?

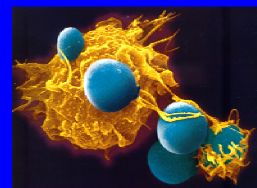
In theory by B. Halliwell

- Using 3.5ml O_2/kg/min gives at Rest
- O_2 consumption of 352.8L/day (70kg; male)
- 14.7 moles oxygen a day
- If 1% of oxygen becomes a superoxide radical
- 0.147 moles of superoxide is produced.





White blood cells use myeloperoxidase to kill invading microbes



O_2 Oxygen

NADPH Oxidase

$O_2^{\bullet -}$ Superoxide

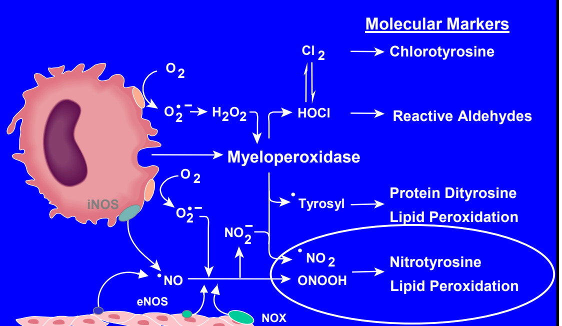
H_2O_2 Hydrogen peroxide

Peroxidase

Cl^-, Br^-, I^-, or SCN^-

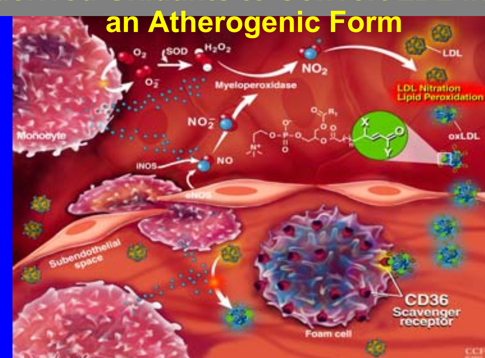
HOX Hypohalous Acid

Phagocyte-generated Oxidants Implicated in Host Defense and Inflammatory Disease



Endothelium

Monocytes Use MPO–generated NO-derived Oxidants to Convert LDL into an Atherogenic Form



Podrez et al (2002) *J. Biol. Chem.* 277:38503-16
Podrez et al (2002) *J. Biol. Chem.* 277:38517-23

Control of Extracellular Levels of $O_2^{\bullet -}$ and NO·

$O_2^{\bullet -}$ NO·

EC-SOD

$$O_2^{\bullet -} + NO\cdot \longrightarrow ONOO^-$$

$$\geq 10^9\ m^{-1} \cdot s^{-1}$$

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 273, No. 18, Issue of May 1, pp. 11038–11043, 1998
Printed in U.S.A.

Production of Nitric Oxide by Mitochondria*

(Received for publication, October 25, 1996, and in revised form, December 10, 1997)

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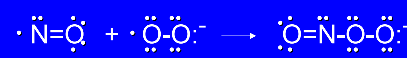
Peroxynitrite

- $O_2^{\bullet-}$ reacts extremely rapidly with **NO•** to form Peroxynitrite (**ONOO**$^-$)

  - **$O_2^{\bullet-}$ + NO• → ONOO$^-$**

- This is a non-radical species that is a potent oxidizing and nitrating intermediate.

•NO reacts very rapidly with the free $O_2^{\bullet-}$ to form $ONOO^-$.



"Radical" Reactions

$O_2 + e \Leftarrow\Rightarrow O_2^{\bullet-}$	Equation 1
$O_2 + 2e + 2H^+ \rightarrow H_2O_2$	Equation 2
$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow SOD \rightarrow H_2O_2 + O_2$	Equation 3
$H_2O_2 + 2GSH \rightarrow GPX \rightarrow H_2O + GSSG + ROH$	Equation 4
$2H_2O_2 + \rightarrow Catalase \rightarrow 2H_2O + O_2$	Equation 5
$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$	Equation 6
$O_2^{\bullet-} + M^{n+} \rightarrow O_2 + M^{(n-1)+}$	Equation 7
$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + HO^- + Fe^{3+}$	Equation 8

Hydroxyl Radical

First YOU need REDOX active Iron

- **Fe^3 + $O_2^{\bullet-}$ → Fe^{2+}** (Free or low molecular weight chelates of transition metals such as Fe^{3+} are reduced by $O_2^{\bullet-}$ to Fe^{2+})

Hydroxyl radical Continue

- The Haber-Weiss reaction (also known as superoxide-driven Fenton chemistry).

- **$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + HO^- + Fe^{3+}$**
  - The reduced metal ion then reacts with H_2O_2 to generate the extremely reactive HO•.

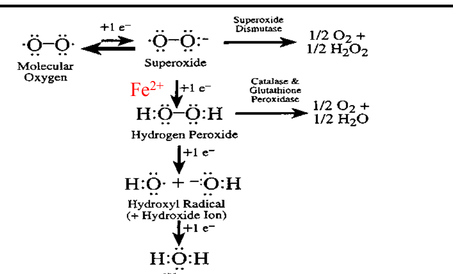


Figure 2. The reductive chemistry of oxygen illustrated by Lewis dot diagrams. However, the representation is not accurate for oxygen, failing to accound for the two net bonds between the oxygens, and should be only viewed as a illustrative teaching aid. Because molecular oxygen contains two unpaired electrons, its reduction to water proceeds most readily through a series of four single electron reduction steps. The lifetimes of superoxide and hydrogen peroxide are sufficiently long that they can be scavenged by the specific antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase.

Hydroxyl Radical

- Transition metals (such as Fe^{2+}) catalyze the cleavage of H_2O_2 to •OH, the hydroxyl free radical
- •OH is believed to be the primary agent of protein, DNA, and lipid damage

Reactive oxygen, reactive nitrogen, and reactive chlorinating species have many origins

- Mitochondria, NO•, $O_2^{-\bullet}$, H_2O_2
- Endothelial Cells, NO•, $O_2^{\bullet-}$, H_2O_2
- Immune cells (anti-bactericidal)
  - Hypochlorous acid (bleach)
  - NO•, $O_2^{\bullet-}$, H_2O_2

Many Functions

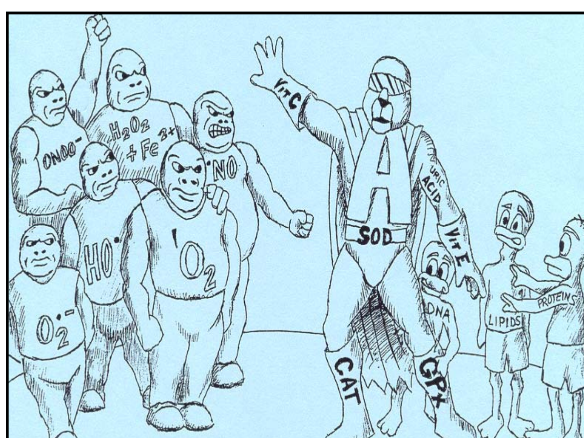
- Modulate metabolism
- Modulation of cellular redox state
- Anti-bactericidal
- Cell signaling
- Activation of gene-transcription factors

Question?

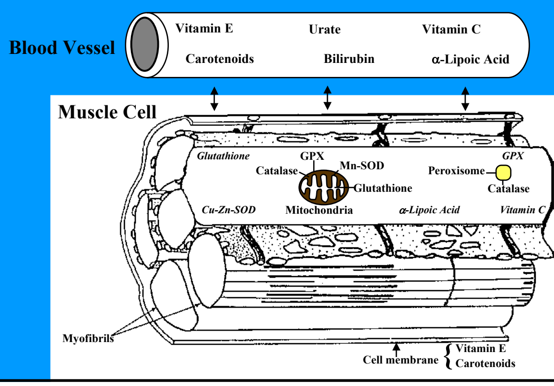
- How do we protect ourselves from these reactive intermediates?
- Do we need to protect us under normal healthy conditions?

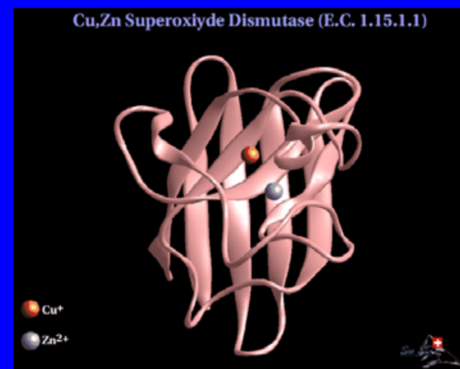
Antioxidant Defense Systems

- 1) Enzymatic defenses
- 2) Non-enzymatic defenses

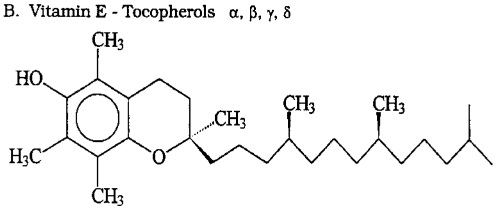


Muscle-Antioxidant Defenses





B. Vitamin E - Tocopherols α, β, γ, δ



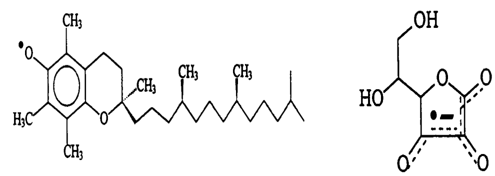
Chromane head

Phytyl Tail

C. The Antioxidant Reaction

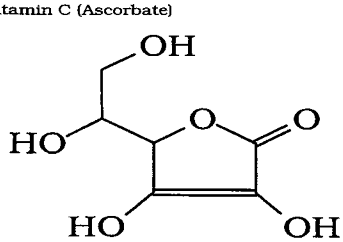
$R^{\bullet} + TOH \rightarrow RH + TO^{\bullet}$

$R^{\bullet} + AscH^{-} \rightarrow RH + Asc^{\bullet -}$



VI. Vitamins C & E. (Donor Antioxidants)

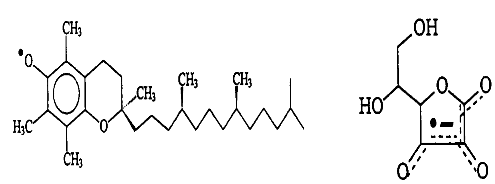
A. Vitamin C (Ascorbate)



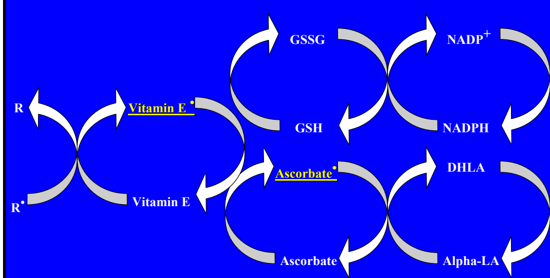
$pK_1 = 4.2$; $pK_2 = 11.6$

Principal, water-soluable antioxidant, i.e. small molecule - chain breaking.

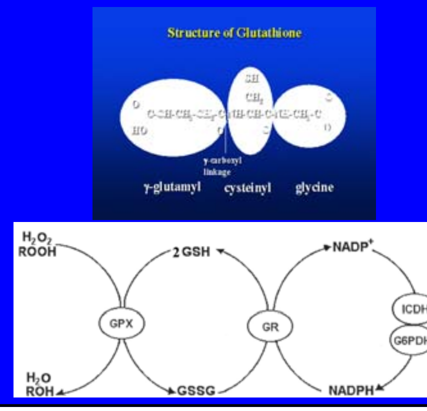
$AscH^{-} + R^{\bullet} \rightarrow Asc^{\bullet -} + R\text{-}H$



Antioxidant-Network to Maintain Redox Balance

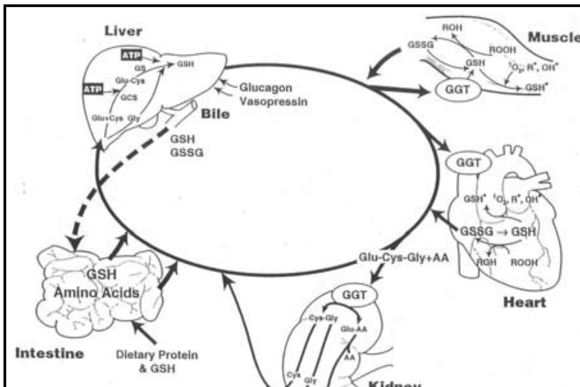


Glutathione Redox Cycle



What is an good Antioxidant?

- What is an antioxidant?
- A substance when present in trace (small) amounts inhibits the oxidation of the bulk
- What are considered good antioxidants ?
  - **Relatively un-reactive (antioxidant•)**
  - **Repaired Rapidly**
  - **Decays to harmless products**

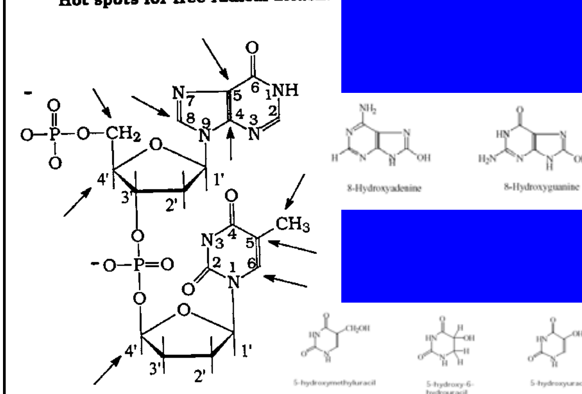


Leeuwenburgh, C., and Ji, L. L. (1995) Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. *Arch Biochem Biophys* 316, 941-949.

Oxidative Stress

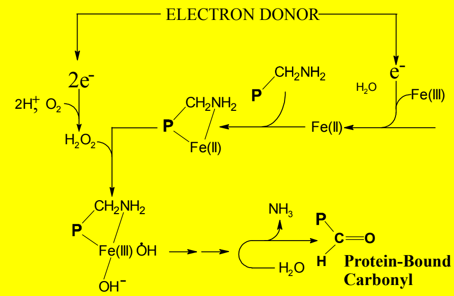
- What happens when oxidants and antioxidants are not in balance?
  - Protein damage
  - DNA damage
  - Lipid Damage

Hot spots for free radical attack:



- "Protein" Oxidation determined by carbonyl Formation.
- Adams, S., Green, P., Claxton, R., Simcox, S., Williams, M. V., Walsh, K., and Leeuwenburgh, C. (2001) Reactive carbonyl formation by oxidative and non-oxidative pathways. *Front Biosci* 6, A17-24.

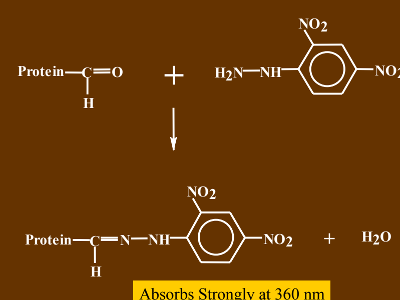
Potential Mechanism for Protein-bound Carbonyls Generated by Metal Catalyzed Oxidation



Aging and Carbonyl's

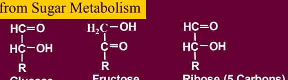
1) Increases protein oxidation is associated with disease and aging
2) Protein oxidation often is assessed by reactivity with 2,4-dinitrophenylhydrazine
3) These observations implicate oxidative stress in the pathogenesis of disease and aging
4) Dr. Stadtman proposed that hydroxyl radicals are the likely radical to produce damage

Reaction of 2,4-Dinitrophenylhydrazine with Protein-bound Carbonyls



Biochemical Pathways that Generate Reactive Aldehydes

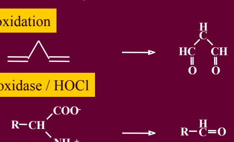
1) Products from Sugar Metabolism



2) Glyoxydation Reactions

3) Lipid Peroxidation

4) Myeloperoxidase / HOCl

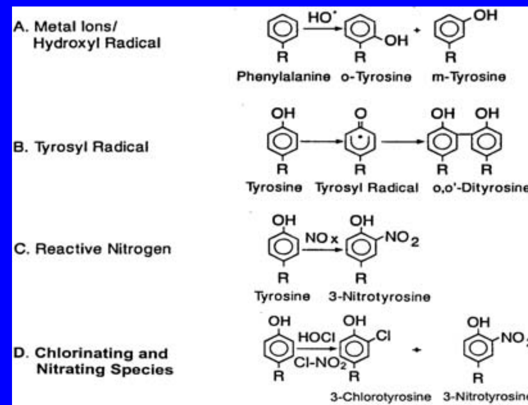


2, 4 Dinitrophenylhydrazine will React with Any Carbonyl Group

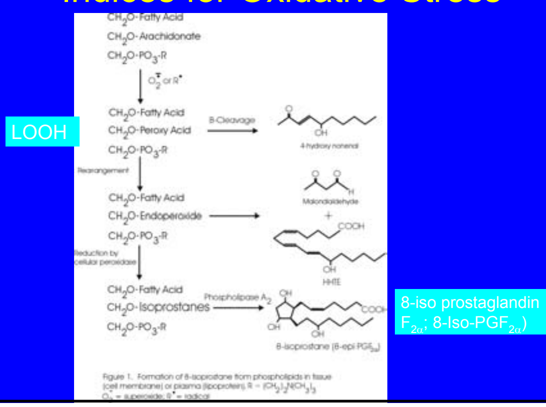
1) Reducing Sugars
2) Aldehydes
3) Lipid Peroxidation Products

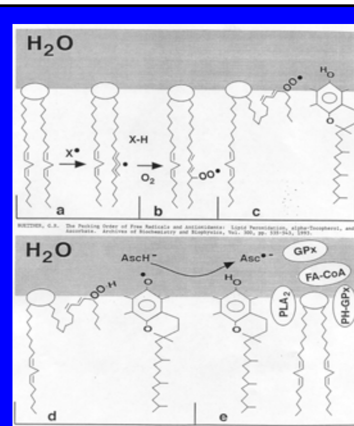
Many Forms of Oxidation Might Generate Protein Carbonyls

1) Peroxynitrite ($ONOO^-$)
2) Hypochlorous Acid (HOCl)
3) Metal Catalyzed Oxidation (H_2O_2/Metal)



Indices for Oxidative Stress





IV. Lipid Peroxidation

A. Classic Lipid Peroxidation (The Basics)

1. Initiation

$LH + X^\bullet \xrightarrow{R_i} L^\bullet + XH$

2. Propagation

$L^\bullet + O_2 \xrightarrow{k_o \approx 3\times10^8\ M^{-1}s^{-1}} LOO^\bullet$

$LOO^\bullet + LH \xrightarrow{k_p \approx 10\text{-}50\ M^{-1}s^{-1}} L^\bullet + LOOH$ →

3. Termination

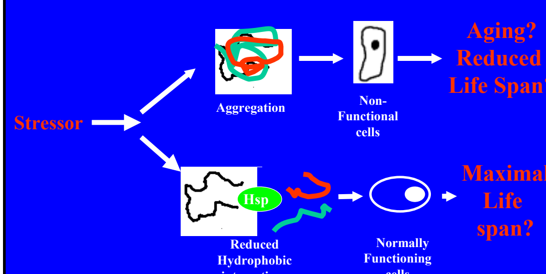
$2\ LOO^\bullet \xrightarrow{k_t \approx 10^6\text{-}10^7\ M^{-1}s^{-1}}$ non-radical products

$L^\bullet + LOO^\bullet \longrightarrow$ "

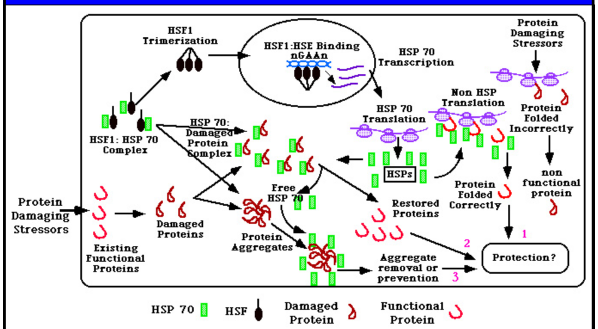
$L^\bullet + L^\bullet \longrightarrow$ "

4. Chain Branching: Alkoxyl Radicals

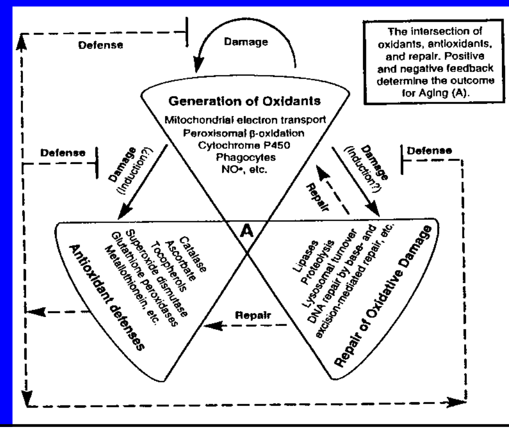
Aging And Protein Aggregation

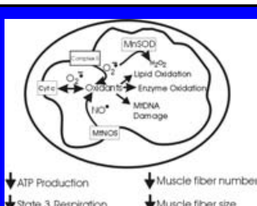


Regulation of The Heat Shock Response

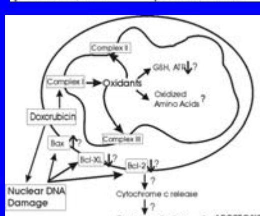


(modified from Locke, 1997)



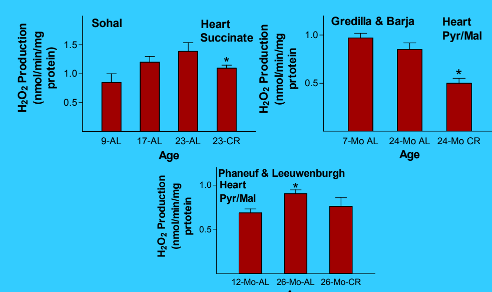


With Aging there is oxidative Stress



With drug use there is oxidative stress

There is a chronic exposure to oxidants during a life-span

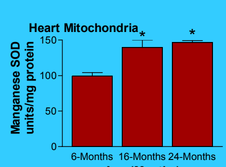


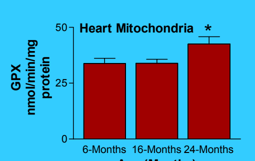
Sohal et al. Mech Ageing Dev 74 (1-2):121-33, **1994.**

Gredilla R, Sanz A, Lopez-Torres M, **Barja** G. FASEB J 15(9):1589-91, **2001.**

Phaneuf & **Leeuwenburgh** (AJP)

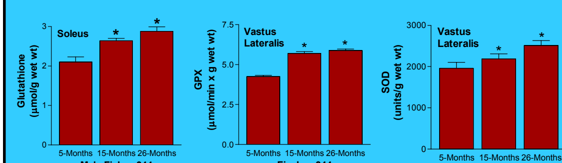
Cardiac mitochondrial SOD and GPX are up-regulated with age



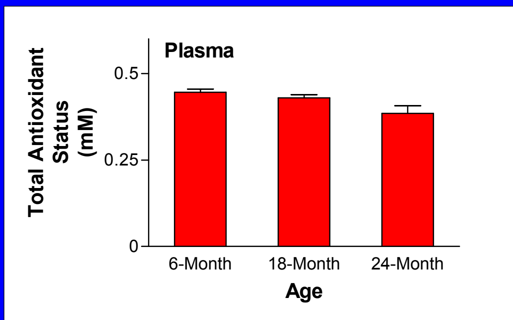


Phaneuf, S, and Leeuwenburgh, C Cytochrome c release from the mitochondria in the aging heart: Role for apoptosis. ***Am J. Physiol. 2001.***

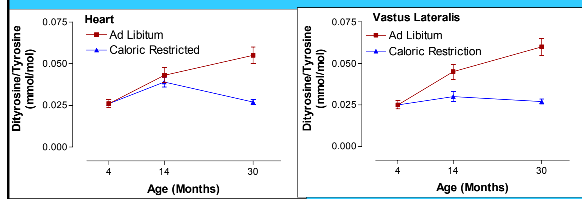
Antioxidant defenses are not compromised in aged skeletal muscle



Leeuwenburgh, C. et al. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. ***Am J Physiol.*** **267:R439-45, 1994.**

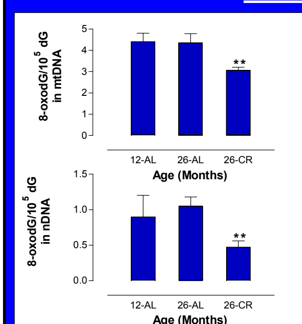


Oxidative Damage in Heart and Skeletal Muscle is Increased with Aging and is Reduced by Caloric Restriction

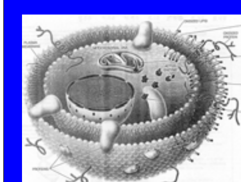


Leeuwenburgh, C. et al. Caloric restriction attenuates dityrosine cross-linking of cardiac and skeletal muscle proteins in aging mice. ***Arch Biochem Biophys.* 346:74-80. 1997.**

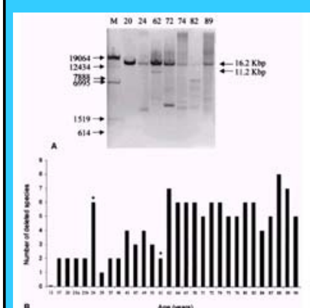
There is a chronic exposure to oxidants during a life-span and life-long caloric restriction reduces oxidant production and oxidative stress

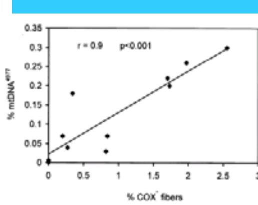


Drew, B., Phaneuf, S., Dirks, A., Selman, C., Gredilla, R., Lezza, A., Barja, G., and Leeuwenburgh, C. (2003) Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol* 284, R474-R480



Mitochondrial Deletions





Pesce V, Cormio A, Fracasso F, Vecchiet J, Felzani G, **Lezza AM**, Cantatore P, **Gadaleta MN**. Free Radic Biol Med,1;30(11):1223-33, 2001.

Reactive Oxygen Species and adverse effects

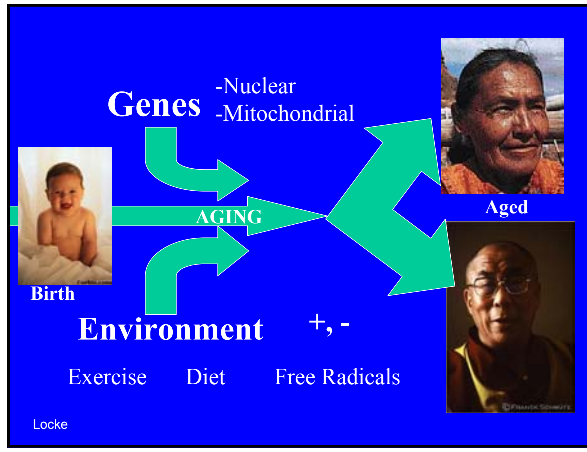
- **Oxidative Stress**: ROS can alter the activities of enzymes, induce mutations, and damage cell membranes "mitochondrial dysfunction"
- **Apoptosis:** Programmed cell Death. Radicals can mediate cell to die.

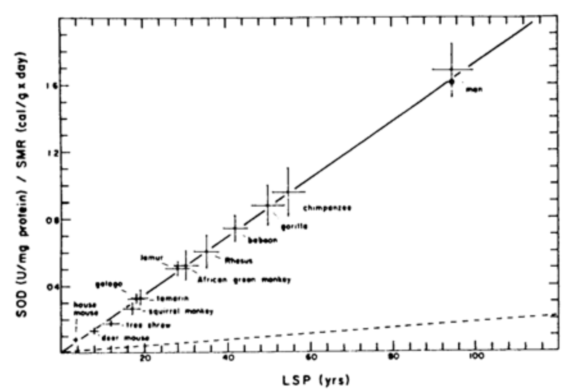
Conclusions

- Aging results in variable changes of oxidant production (heart, brain, muscle)
- Antioxidant enzymes (SOD, GPX) increase in response to the chronic oxidative stress
- GSH (non-enzymatic increases in muscle)
- Despite this there is oxidative damage to lipid, DNA, and proteins.
- In addition, Mt-DNA deletions increase in several tissues.
- ATP production?

Summary

- Decreased efficiency of antioxidant defense mechanisms? NO, most tissues maintain or up regulate their defenses.
- Concentrations of oxidatively damaged proteins, DNA, and lipids increase with age. Generally speaking, Yes.





SOD Levels and Aging

- Long lived animals have more SOD
  - **This does not seem to be correct!!!!**
- Cutler et al. 1980