Workshop

Protective/preventive role of bioactive food components in human health

Overview on oxidative stress

What is it and how can it affect our health

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What are we speaking about?

The related terms **oxidative stress**, **oxidative damage**, **free radicals**, and **antioxidants** have become an integrated part of the scientific vocabulary and are often used in a variety of scientific discussions and issues by chemists, physicists, biologists, and many other researchers.

The scientific literature is repleted with articles concerning oxidative stress phenomena.

Number of reviewes in PuBMed : 2,313 in 2015; 2,048 in 2016

The objectives of this lesson are:

- to provide the terminology and definitions used in this field
- to describe the essence, distribution, causes, and importance of oxidative stress phenomena

A bit of history

Free radicals, known in chemistry since the beginning of the 20th century, were initially used to describe intermediate compounds in organic and inorganic chemistry

early 18th century - Since the discovery of oxygen_by Antoine Laurent Lavoisier, the necessity of controlling oxygen levels has been recognized

1775 - Priestly described the toxicity of the oxygen molecule to the organism and compared its effect on the body as similar to that of “burning a candle”

1954 - Daniel Gilbert and Rebecca Gersham (1) suggested free radicals as important players in biological environments and responsible for deleterious processes in the cell

1956- Herman Denham (2) suggested that these species might play a role in physiological events

1969- McCord and Fridovich discovered the role of the protein hemocuprein in the dismutation of superoxide radicals and described the existence of superoxide dismutase (SOD) in almost all aerobic cells (3). This discovery led to the description of the superoxide theory of oxygen toxicity

2,500 millions years ago…

In the Precambrian era, oxygen began to accumulate in the atmosphere as a result of evolution of the photosynthetic blu-green algae and the enormous benefits derived from its use for energy purposes led to the rapid growth of aerobic organisms.

The high reactivity of oxygen soon determined the onset of oxidative damage to important cellular structure. To combat the action of reactive oxygen species (ROS) an integrated system of both enzymatic both non-enzymatic antioxidants has been soon developed

Oxygen: the pros and the cons…

The pros: the increase in oxygen concentration in the atmosphere, today at 21%, and its derivative ozone (O3) has been beneficial, as it has allowed the absorbance of deleterious solar ultraviolet radiation (UVC, < 280 nm) and thereby enabled organisms to survive on dry land.

The cons: in its harmful role, oxygen itself has been toxic to anaerobic bacteria and forced them to develop a variety of mechanisms to cope with the increasing concentrations.

In the atmosphere oxygen concentration is a dynamic parameter that is constantly changing. There were periods when atmospheric oxygen reached a concentration of 35% and later stabilized at 21% (3).

Today, due to the massive cutting of rain forests, its concentration is decreasing again and probably will lead to changes in the biochemical response of the living cell.

Oxygen: the pros and the cons…

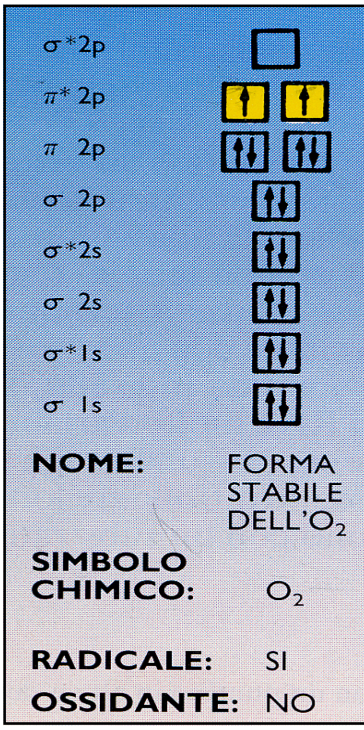
- **The Earth was originally anoxic**
- **Metabolism was anaerobic**
- **O_2 started appearing ~2.5 x 10^9 years ago**

Anaerobic metabolism-glycolysis

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \longrightarrow \text{Lactate} + 2\text{ATP} + 2H_2O$$

O_2 an electron acceptor in aerobic metabolism

$$\text{Glucose} + 6O_2 + 36\text{ADP} + 36\text{P}_i \longrightarrow 6CO_2 + 36\text{ATP} + 6H_2O$$



Oxygen chemistry

Ground-state oxygen has 2-unpaired electrons

The unpaired electrons have parallel spins

Oxygen is a paramagnetic molecule

The oxygen can not simultaneously acquire the 4 electrons for its reduction to water

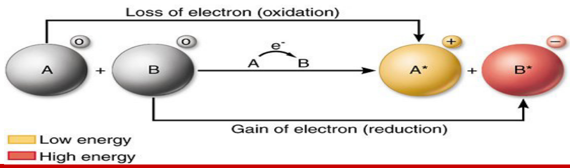
Oxygen molecule is minimally reactive due to spin restrictions which does not allow the donation or acceptance of another electron before rearrangement of the spin directions around the atom

Basic of Redox chemistry

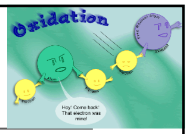
Term	Definition
Oxidation	Gain in oxygen Loss of hydrogen Loss of electrons
Reduction	Loss of oxygen Gain of hydrogen Gain of electrons
Oxidant	Oxidizes another chemical by taking electrons, hydrogen, or by adding oxygen
Reductant	Reduces another chemical by supplying electrons, hydrogen, or by removing oxygen

Basic of Redox chemistry

In biology, a reducing agent acts via donation of electrons, usually by donation of hydrogen or removal of oxygen.
An oxidation process is always accompanied by a reduction process in which there is usually a loss of oxygen, while in an oxidation process there is a gain in oxygen.
Such reactions, called redox reactions, are the basis for numerous biochemical pathways and their regulation (4).
They are also important for understanding biological oxidation and radical/ antioxidant effects.
While reductant and oxidant are chemical terms, in biological environments they should be termed **antioxidant and pro-oxidant**, respectively.



Pro-oxidants



In general pro-oxidants are referred to as reactive oxygen species (ROS) that can be classified into 2 groups of compounds, radicals and non-radicals.
The radical group is often incorrectly called free-radical (the term is not accurate, because a radical is always free)

Free Radicals:

- Any species capable of independent existence that contains one or more unpaired electrons
- A molecule with an unpaired electron in an outer valence shell

$R_3C\cdot$	Carbon-centered
$R_3N\cdot$	Nitrogen-centered
$R\text{-}O\cdot$	Oxygen-centered
$R\text{-}S\cdot$	Sulfur-centered

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Pro-oxidants

Non-Radicals:

- Species that have strong oxidizing potential
- Species that favor the formation of strong oxidants (e.g., transition metals)

H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite
Me^{n+}	Transition metals

Properties of free radicals

1. **Highly reactive**
2. **Very short half-life**
3. **Generate new radicals by chain reaction**
4. **Cause damage to biomolecules, cells and tissues**

Most free radicals in biological systems are derivatives of oxygen (Reactive Oxygen Species, ROS), but there are also derivatives of nitrogen (Reactive Nitrogen Species, RNS), Reactive Metabolites or Intermediates.

Reactive Oxygen Species (ROS)

Radicals:

$O_2^{\cdot-}$	Superoxide
$OH^{\cdot}$	Hydroxyl
$RO_2^{\cdot}$	Peroxyl
$RO^{\cdot}$	Alkoxyl
$HO_2^{\cdot}$	Hydroperoxyl

Non-Radicals:

H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

Reactive Nitrogen Species (RNS)

Radicals:

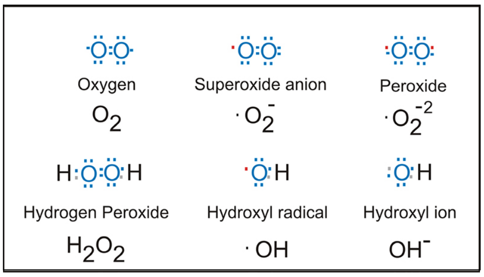
$NO^{\cdot}$	Nitric Oxide
$NO_2^{\cdot}$	Nitrogen dioxide

Non-Radicals:

$ONOO^-$	Peroxynitrite
ROONO	Alkyl peroxynitrites
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
HNO_2	Nitrous acid
NO_2^+	Nitronium anion
NO^-	Nitroxyl anion
NO^+	Nitrosyl cation
NO_2Cl	Nitryl chloride

Reactive Oxygen Species (ROS)

- **Superoxide ($O_2^{\cdot}$-)**
- **Hydrogen Peroxide (H_2O_2)**
- **Hydroxyl Radical ($OH^{\cdot}$)**
- **Singlet oxygen, $^1O_2$**



- **Reactive Oxygen Species is used in a broad sense for both free radicals ($O_2^{\cdot-}$, $OH^{\cdot}$) and non-free radicals (H_2O_2, $^1O_2$, which are extremely reactive) of the biological systems**

ROS production

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-,\ 2H^+} H_2O_2 \xrightarrow[\textstyle H_2O]{e^-,\ H^+} OH^- \xrightarrow{e^-,\ H^+} H_2O$$

Superoxide (O_2^-) — Hydrogen peroxide (H_2O_2) — Hydroxyl radical (OH^-)

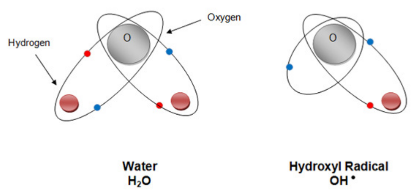
"Longevity" of ROS and RNS

Reactive Species	Half-life
Hydrogen peroxide Organic hydroperoxides Hypohalous acids	~ minutes
Peroxyl radicals Nitric oxide	~ seconds
Peroxynitrite	~ milliseconds
Superoxide anion Singlet oxygen Alcoxyl radicals	~ microsecond
Hydroxyl radical	~ nanosecond

Hydroxyl radical (·OH)

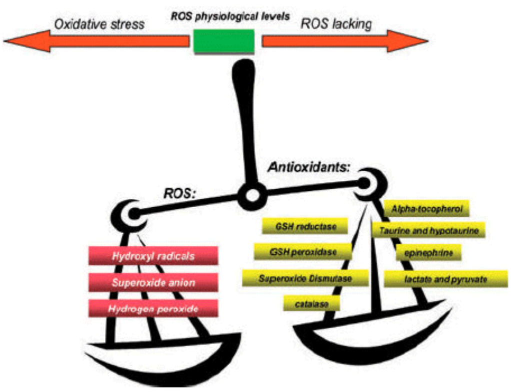
	$O_2^{\cdot -} + Fe^{3+}$	$\longrightarrow$	$O_2 + Fe^{2+}$ (ferrous)
Fenton	$H_2O_2 + Fe^{2+}$	$\longrightarrow$	$OH^- + \cdot OH + Fe^{3+}$ (ferric)
Haber-Weiss	$O_2^{\cdot -} + H_2O_2$	$\longrightarrow$	$OH^- + O_2 + \cdot OH$

- Transition metal catalyzed
- Fe2+ is an <u>extremely</u> reactive oxidant



Oxidative stress

The organism must control the presence of both pro-oxidants and antioxidants continuously.
The balance between these is tightly regulated and extremely important for maintaining vital cellular and biochemical functions.
This balance, often referred to as the redox potential, is specific for each organelle and biological site, and any interference of the balance might be deleterious for the cell and organism.
Changing the balance towards an increase in the pro-oxidant over the capacity of the antioxidant is defined as **oxidative stress** and might lead to oxidative damage.



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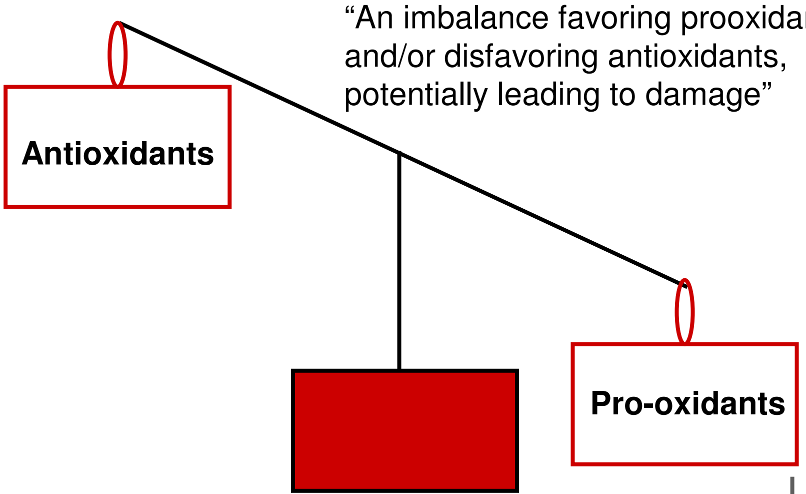
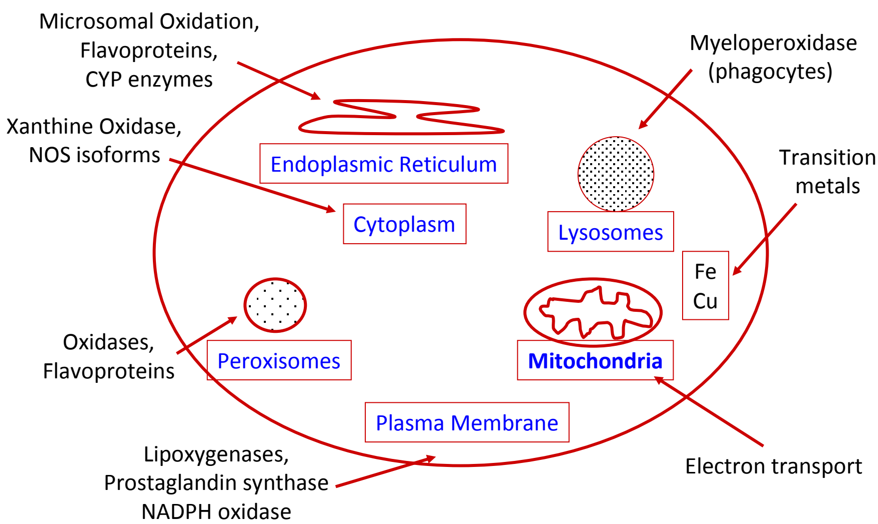
Oxidative stress

"An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage"



Sources of ROS

Endogenous sources of ROS and RNS



Mitochondria as Sources of ROS

Mitochondria are the largest contributors to intracellular oxidant production. Mitochondria generate ATP in an oxygen-dependent manner, during which the flow of electrons down the respiratory chain culminates at complex IV with the reduction of molecular oxygen to water.

Throughout this process, molecular oxygen can also undergo a one-electron reduction to generate a superoxide anion (5).

There are distinct molecular sites of superoxide production within the mitochondria (6)

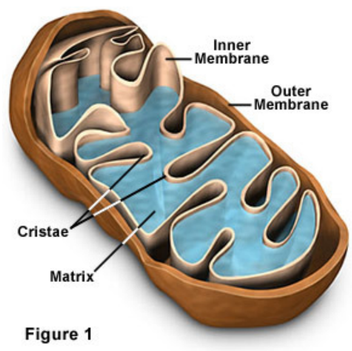
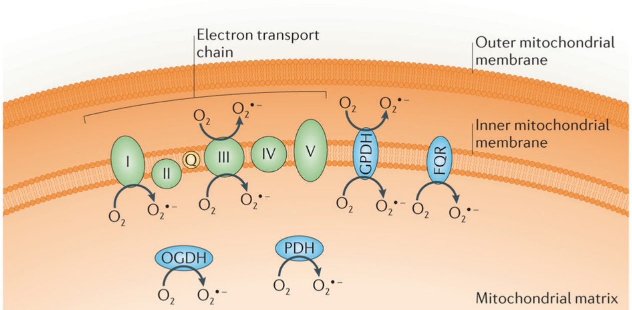


Figure 1

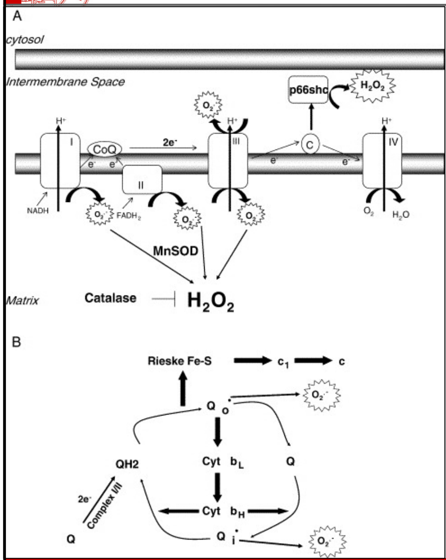
Mitochondria as Sources of ROS



Nature Reviews | Molecular Cell Biology

Multiple sites of mitochondrial superoxide anion (O2•–) production have been mapped. Quantitatively, complex I and complex III of the electron transport chain are the major sites of oxidant production, with the generation of superoxide anions occurring both on the matrix side and in the inner mitochondrial membrane space. Other contributors include metabolic enzymes in the mitochondrial matrix, such as OGDH (2-oxoglutarate dehydrogenase) and PDH (pyruvate dehydrogenase), and the mitochondrial membrane forms of GPDH (glycerol 3-phosphate dehydrogenase; also known as GPDM) and the FQR (electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial) system .

Mitochondria as Sources of ROS



Localization of the main mitochondrial sources of superoxide anion (7)

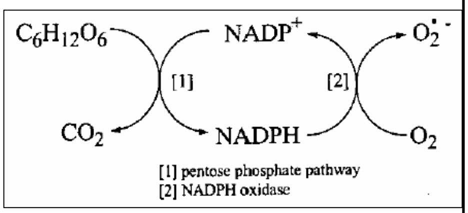
Component	Localization
Complex I (NADH dehydrogenase)	Inner membrane/ inner side
Complex II (succinate dehydrogenase)	Inner membrane/ inner side
Complex III (ubiquinol–cytochrome *c* reductase)	Inner membrane/ inner side
Complex III (ubiquinol–cytochrome *c* reductase)	Inner membrane/ outer side
External NADH dehydrogenase (yeast)	Inner membrane/ outer side
Glycerolphosphate dehydrogenase	Inner membrane/ outer side
Dehydroorotate dehydrogenase	Matrix
Mono amino oxidase	Outer membrane/ inner side

Complexes I and II generate superoxide into the mitochondrial matrix, whereas Complex III has the ability to generate superoxide into both the mitochondrial intermembrane space and the mitochondrial matrix . Complex III produces superoxide from the radical ubisemiquinone during the Q cycle which releases the superoxide into the matrix and the intermembrane space. ROS do not seem to be generated by Complex IV.

NADPH oxidase as a source of ROS

• Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are a class of enzyme whose primary function is the generation of ROS.
• Initially characterised in **neutrophils**, there are now seven oxidases recognised as part of the NADPH oxidase family, each distinctly expressed and regulated across a diverse range of cells and tissues.
• In addition to the originally established role of the phagocyte NADPH oxidase in host-defence, under normal conditions ROS generated by NADPH oxidase enzymes are now recognised as participating in a variety of fundamental physiological processes such as signal transduction (8)

NADPH oxidase catalyses the conversion of NADPH to NADP+ by liberating two electrons and one proton. The proton remains in the cytoplasm, while the two electrons are transported through the plasma membrane, binding to two oxygen molecules and forming two superoxide anions in the extracellular space.



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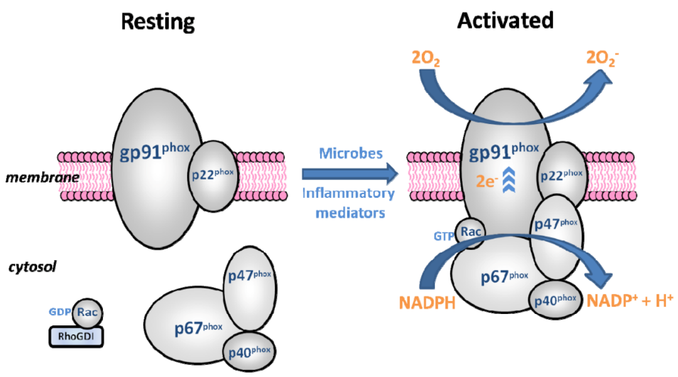


Resting and activated forms of NADPH oxidase.

The catalytic subunit, gp91phox, along with p22phox makes up flavocytochrome b558, the membrane-associated component of the enzyme. The three phox proteins, p47, p67 and p40 form a cytosolic complex in the resting cell, and upon activation translocate to the membrane, docking with flavocytochrome b558. The small G-protein Rac, in its GDP-bound form, is stabilised by RhoGDI in the resting state and also translocates to the membrane upon activation. When assembled, the enzyme generates the ROS superoxide (O2–) by accepting electrons (e–) from cytoplasmic NADPH and donating them to molecular oxygen (O2).

NADPH oxidase as a source of ROS

NADPH oxidase was originally thought to be unique to phagocytes. Following the expansion of the human genome sequence databases in the 1990s the NADPH oxidase family of ROS-generating oxidases was identified and characterised.

7 genes encoding gp91phox homologues have been identified:

Nox1 and Nox2 are most abundant in intestinal epithelium.

Nox3 was originally described in foetal kidney and is now thought to be expressed almost exclusively in the inner ear.

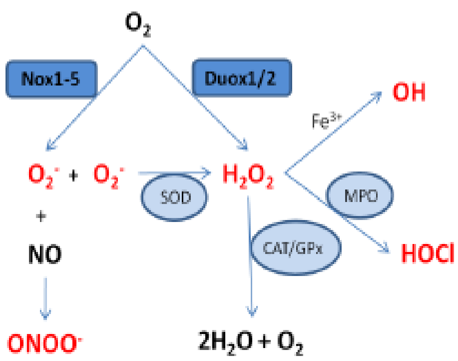
Nox4 was originally described as a renal oxidase (Renox) because of its high expression levels in the kidney but is now more recognised for its vascular expression.

Nox5 is found primarily in lymph node, spleen and testis.

The Duox proteins are highly expressed in the thyroid and airway epithelium, while Duox2 is also present in lung and gastrointestinal tract epithelium.The name Dual oxidase reflects the fact that these proteins possess a unique extracellular domain in addition to their Nox-like portions.

NADPH oxidase as a source of ROS

The major pathways for the formation of ROS and reactive nitrogen species (RNS) originating from the Nox/Duox enzymes



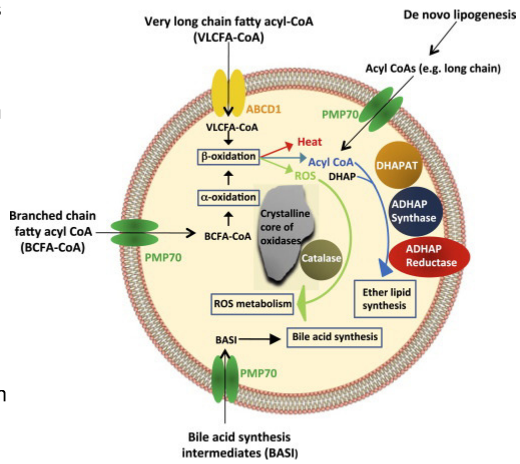
Superoxide (O2–) is generated by the Nox and can be dismutated to H2O2, either spontaneously or catalysed by superoxide dismutase (SOD), or can react with NO to form peroxynitrite (ONOO–). H2O2, generated by the Duox enzymes or by dismutation of O2– can be scavenged by the antioxidants catalase (CAT) or glutathione peroxidase (GPx) to form water (H2O) and oxygen (O2); be partially reduced to generate hydroxyl radical (OH) by the metal (Fe3+) catalysed Haber-Weiss and Fenton reactions; or react with chloride in a reaction catalysed by myeloperoxidase (MPO), resulting in formation of hypochlorous acid (HOCl).

Peroxisomes as a source of ROS

Peroxisomes are small, membrane-enclosed organelles that contain enzymes involved in a variety of metabolic reactions. Although peroxisomes do not contain their own genomes, they are similar to mitochondria and chloroplasts in that they replicate by division.

Peroxisomes were defined as organelles that carry out oxidation reactions leading to the production of hydrogen peroxide. A variety of substrates are broken down by such oxidative reactions in peroxisomes, including uric acid, amino acids, and fatty acids (9).

In animal cells, fatty acids are oxidized in both peroxisomes and mitochondria, but in yeasts and plants fatty acid oxidation is restricted to peroxisomes



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Peroxisomes as a source of ROS

$$R-CH_2-CH_2-\overset{O}{\overset{\|}{C}}-S-\mathbf{CoA} + O_2 \longrightarrow R-CH{=}CH-\overset{O}{\overset{\|}{C}}-S-\mathbf{CoA} + \boxed{H_2O_2}$$

$$2\,\boxed{H_2O_2} \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

or

$$\boxed{H_2O_2} + AH_2 \xrightarrow{\text{Catalase}} 2\,H_2O + A$$

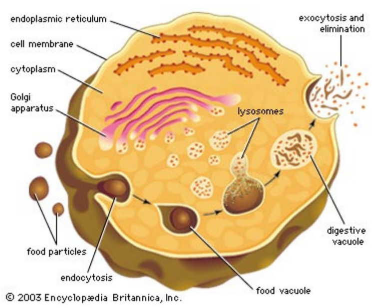
The oxidation of a fatty acid is accompanied by the production of hydrogen peroxide (H2O2) from oxygen. The hydrogen peroxide is decomposed by catalase, either by conversion to water or by oxidation of another organic compound (designated AH2).

Lysosome as a source of ROS

Lysosomes are membrane-enclosed organelles that contain an array of enzymes capable of breaking down all types of biological polymers—proteins, nucleic acids, carbohydrates, and lipids.

Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself.

In their simplest form, lysosomes are visualized as dense spherical vacuoles, but they can display considerable variation in size and shape as a result of differences in the materials that have been taken up for digestion.



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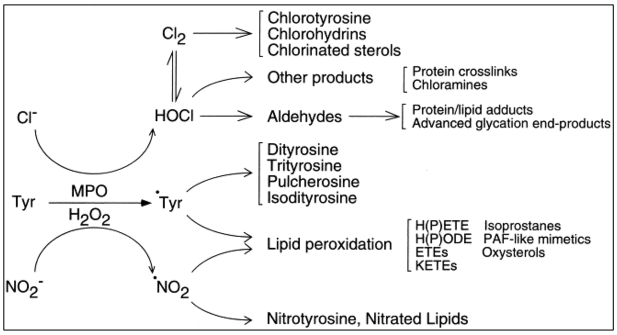
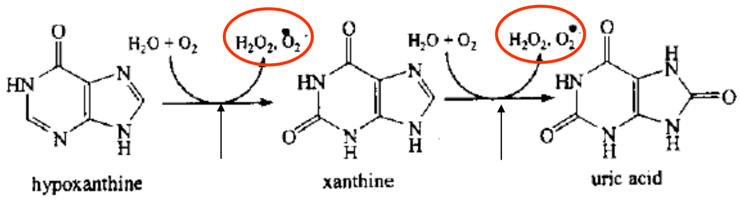
Lysosome as a source of ROS

$$Cl^- + H_2O_2 + H^+ \xrightarrow{MPO} HOCl + H_2O$$

Myeloperoxidase undergoes a complex array of redox transformations and produces HOCl, degrades H_2O_2 to oxygen and water, converts tyrosine and other phenols and anilines to free radicals, and hydroxylates aromatic substrates via a cytochrome P450-like activity



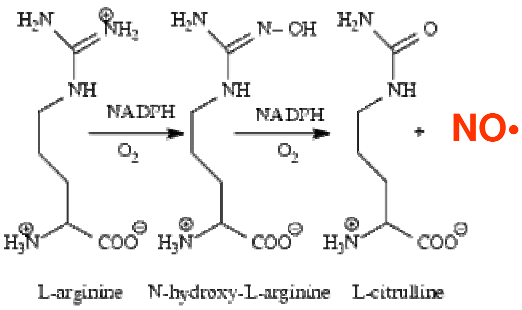
Cytoplasmic sources of ROS and RNS



xanthine oxidase xanthine oxidase

Nitric Oxide Synthases (NOS):

neuronal	nNOS (I)
endothelial	eNOS (III)
inducible	iNOS (II)

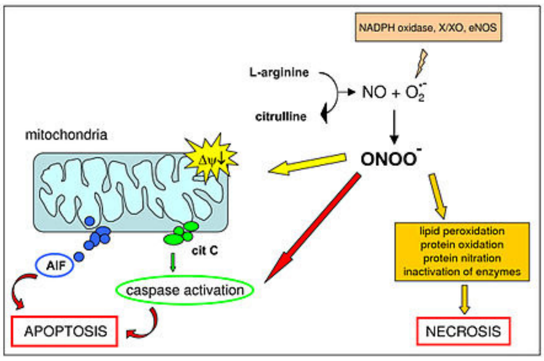


NO and peroxynitrite

Formation of peroxynitrite *in vivo* has been ascribed to the reaction of the free radical superoxide with the free radical nitric oxide.

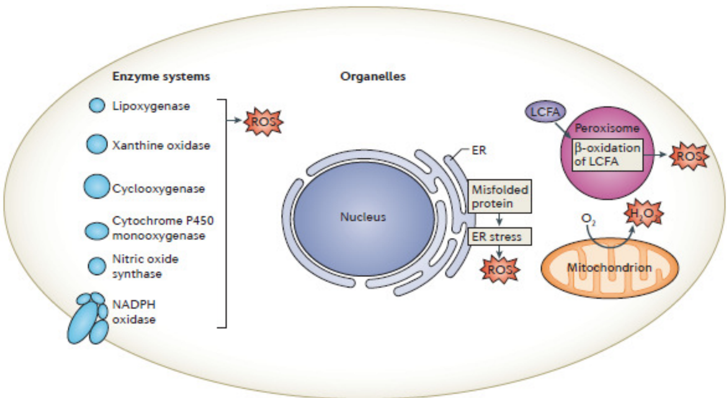
The resultant pairing of these two free radicals results in peroxynitrite, a molecule that is itself not a free radical, but that is a powerful oxidant.

Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. These reactions trigger cellular responses, committing cells to necrosis or apoptosis.



In vivo, peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders

Intracellular sources of ROS



Various organelles within the cell can generate ROS. These include mitochondria, the endoplasmic reticulum and peroxisomes. In addition, various enzymes, including oxidases and oxygenases, generate ROS as part of their enzymatic reaction cycles.

Exogenous sources of free radicals

- **Radiation**
  UV light, x-rays, gamma rays
- **Chemicals that react to form peroxides**
  Ozone and singlet oxygen
- **Chemicals that promote superoxide formation**
  Quinones, nitroaromatics, bipyrimidiulium herbicides
- **Chemicals that are metabolized to radicals**
  e.g., polyhalogenated alkanes, phenols, aminophenols
- **Chemicals that release iron**
  ferritin

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UV radiation

$$H_2O_2 \xrightarrow{UV_B} OH\cdot + OH\cdot$$

UV_A = 320-400 nm
UV_B = 290-320 nm
UV_C = 100-290 nm

- **Primarily a concern in skin and eye**
- **Can also cause DNA damage**
- **Can form singlet oxygen in presence of a sensitizer**

Ionizing radiation

$$2H_2O \xrightarrow{\gamma\text{-rays}} H_2O + e^- + H_2O^*$$

$$H_2O^* \longrightarrow H + \cdot OH$$

- **High energy radiation will result in ·OH**

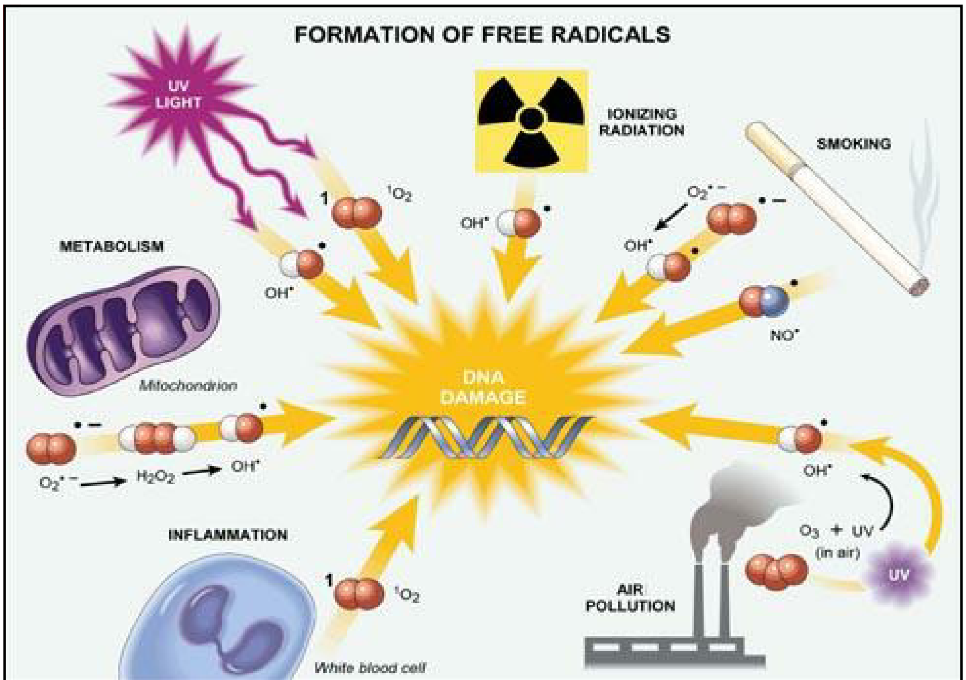
FREE RADICAL FORMATION

FREE RADICALS : THE CAUSE OF VIRTUALLY ALL DISEASES

Environmental pollution

Excessive Alcohol & smoking

High fat foods



ROS reactivity and stability

The reactivity of these various reactive species differs and is influenced by their ability to cross lipid membranes or diffuse in solution.

Superoxide is a short-lived, charged molecule that is believed to be only weakly toxic, as it reacts relatively slowly with different biomolecules. It modifies certain small molecules which may result in disruption of oxidative phosphorylation and cellular energy production and does not readily cross phospholipid membranes, acting primarily in proximity to its generation site.

Hydrogen peroxide is relatively stable under physiological conditions and reacts with a wide range of biologically important compounds.
It is readily diffusible within solution and across membranes and can therefore react locally or at a distance from its site of synthesis, depending on numerous factors including the presence of endogenous antioxidants or other ROS.

ROS reactivity and stability

Hydroxyl radical and peroxynitrite are highly reactive with a wide variety of biomolecules, causing oxidative damage to lipids, proteins, nucleic acids and free nucleotides.

Hydroxyl radical is unlikely to travel far from its source due to its high reactivity and peroxynitrite does not freely cross membranes.

Hypochlorous acid is a powerful oxidant and potent antibacterial substance that produces a variety of oxidative protein modifications, primarily in areas of inflammation where phagocytes are active. It is regarded by some as a critical killing mechanism for most invading pathogens.

Oxidative Damage in Biological Systems

Oxidative stress and cell damage

- **High doses:**
  directly damage/kill cells

- **Low doses/chronic overproduction of oxidants:**
  activation of cellular pathways
  stimulation of cell proliferation
  damage to cellular proteins, DNA and lipids

Activation of cellular pathways

For many years, the study of oxidants has centred on the never-ending battle between the production of ROS and the equally potent cellular antioxidant defence mechanisms.

This view has changed as a result of the growing appreciation that ROS reversibly modulate several important intracellular pathways.

Rather than being harmful, this rise in intracellular ROS level was shown to be required for downstream signalling.

There seems to be an increasing number of intracellular targets for redox-dependent signalling.

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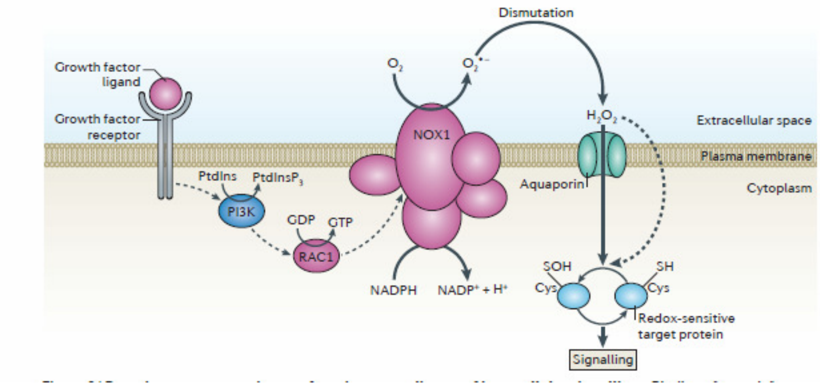


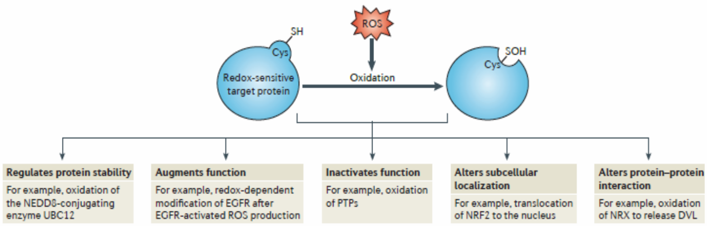
Reactive oxygen species can function as mediators of intracellular signalling. Binding of growth factors to their cell surface receptors leads to the activation of several downstream signalling events. One pathway involves the generation of ROS such as superoxide anion (O2•–) through the assembly and activation of the NADPH oxidase (NOX) superoxide-generating complex. After activation, NOX enzymes produce extracellular superoxide that can spontaneously dismutate to hydrogen peroxide (H2O2). Small amounts of hydrogen peroxide can diffuse back into the cell directly, but its entry is facilitated by aquaporin-dependent pathways. Once inside the cell, hydrogen peroxide alters the activity of redox-sensitive targets (such as, protein Tyr phosphatases) by modulating specific reactive Cys residues.

Harmful effects of free radicals

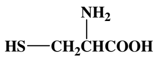
Free Radicals and biomolecules

1. <u>Proteins</u>

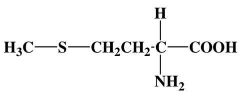
- Cause oxidation of ***sulfhydryl groups***, and modification of AA. ROS may damage protein by fragmentation, aggregation results in the loss of biological activity of proteins.



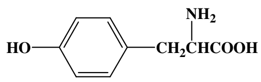
Protein targets for ROS



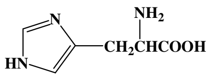
Cysteine



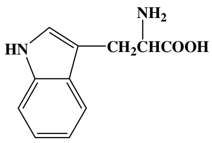
Methionine



Tyrosine



Histidine



Tryptophan

Oxidized proteins and amino acids found in biological systems

- 2-Oxohistidine
- 3-Chlorotyrosine
- 3-Nitrotyrosine
- 5-Hydroxy-2-aminovaleric acid
- Aminomalonic acid
- Dimers of hydroxylated aromatic amino acids
- Dopa
- Hydro(pero)xyleucine
- Hydro(pero)xyvalines
- *N*-Formylkynurenine; kynurenine
- *o*- and *m*-tyrosine
- *p*-Hydroxyphenylacetaldehyde
- Protein carbonyls

Consequences of protein thiol oxidation

Oxidation of catalytic sites on proteins

loss of function/abnormal function

BUT(!): sometimes it is gain in function!

Formation of mixed sulfide bonds

Protein-protein linkages (RS-SR)

Protein-GSH linkages (RS-SG)

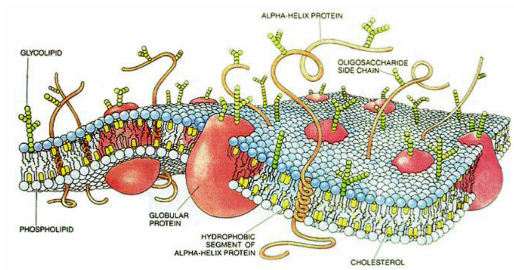
Alteration in 2° and 3° structure

Increased susceptibility to proteolysis

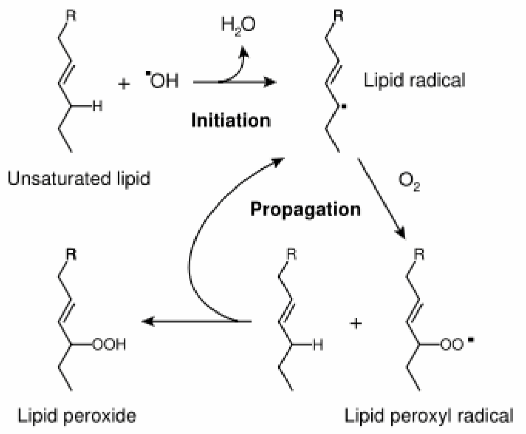
Harmful effects of free radicals

2. Lipids

The ***polyunsaturated lipid molecules of cell membranes*** are particularly susceptible to damaging free radicals process and contribute to the uncontrolled chain reaction (**lipid peroxidation**).



Lipid peroxidation



- **Lipid peroxidation refers to the oxidative degradation of lipids.**
- **It is the process whereby free radicals "steal" electrons *from the lipids in cell membranes, resulting in cell damage*.**
- **This process proceeds by a free radical chain reaction mechanism. It most often affects *polyunsaturated fatty acids (PUFA).***
- **In addition, end products of lipid peroxidation may be *mutagenic and carcinogenic***

Consequences of lipid peroxidation

- **Structural changes in membranes**
  - alter fluidity and channels
  - alter membrane-bound signaling proteins
  - increases ion permeability
- **Lipid peroxidation products form adducts/crosslinks with non lipids**
  - e.g., proteins and DNA
- **Cause direct toxicity of lipid peroxidation products**
  - e.g., 4-hydroxynonenal toxicity
- **Disruptions in membrane-dependent signaling**
- **DNA damage and mutagenesis**

Harmful effects of free radicals

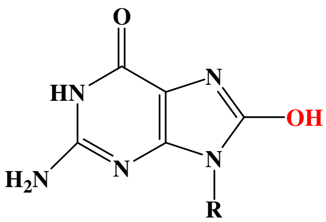
3. Carbohydrates

- Glycation increases the susceptibility of proteins to the attack by free radicals.

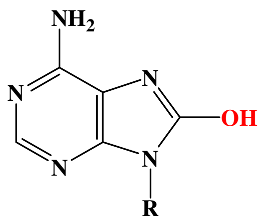
4. Nucleic acid

- cause ***DNA strand breaks***, fragmentation of bases and deoxyribose results in **cytotoxicity and mutations**.

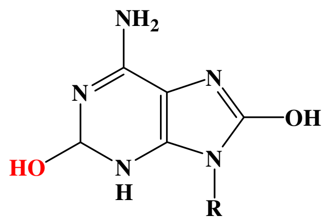
DNA oxidation products



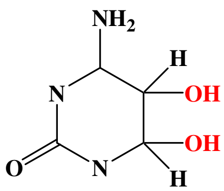
8-hydroxyguanine



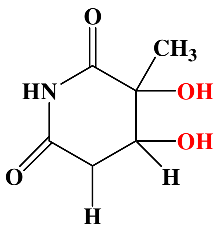
8-hydroxyadenine



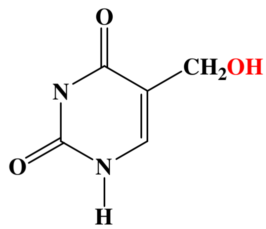
2-hydroxyadenine



5,8-dihydroxycytosine



thymidine glycol



5-hydroxymethyluracil

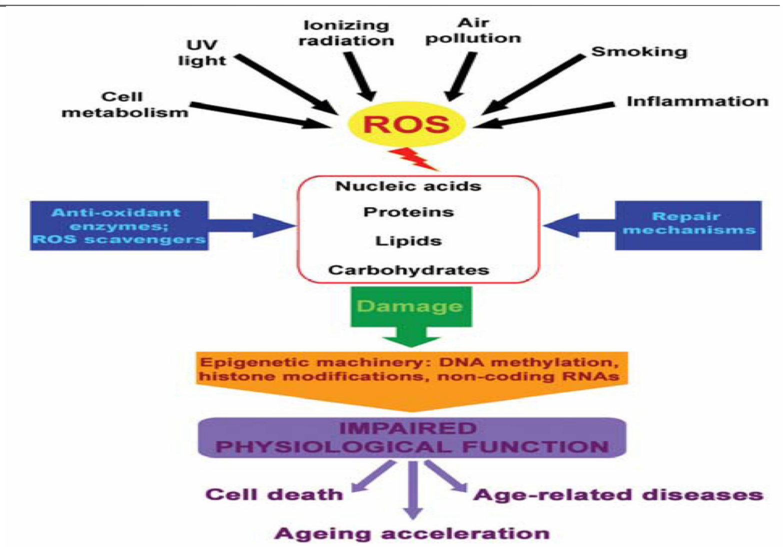
Consequences of DNA oxidation

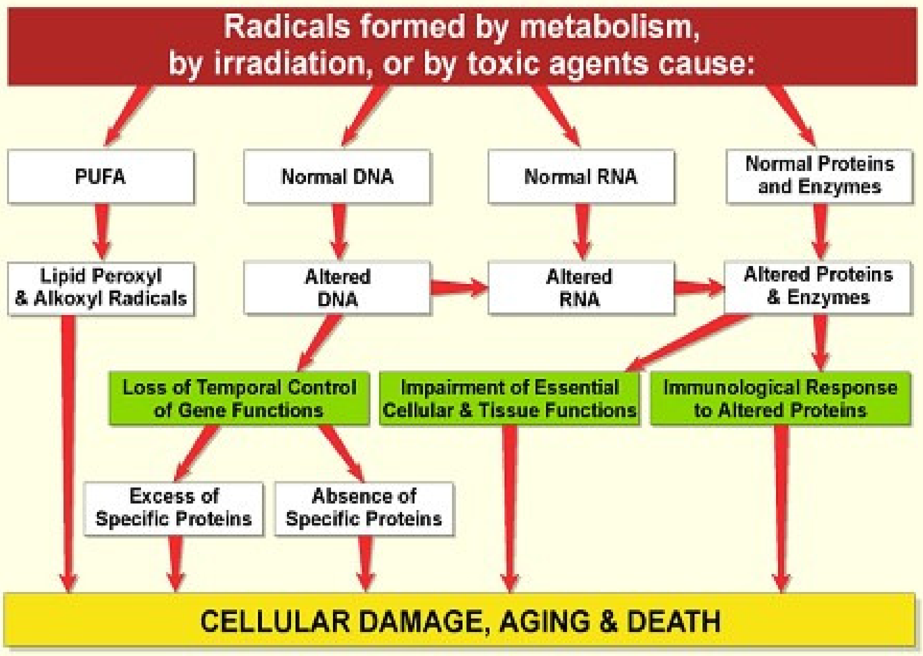
- **DNA adducts/Strand breaks**

  mutations
  initiation of cancer

- **Stimulation of DNA repair**

  can deplete energy reserves)
  imbalanced induction of DNA repair enzymes
  induction of error prone polymerases
  activation of other signaling pathways





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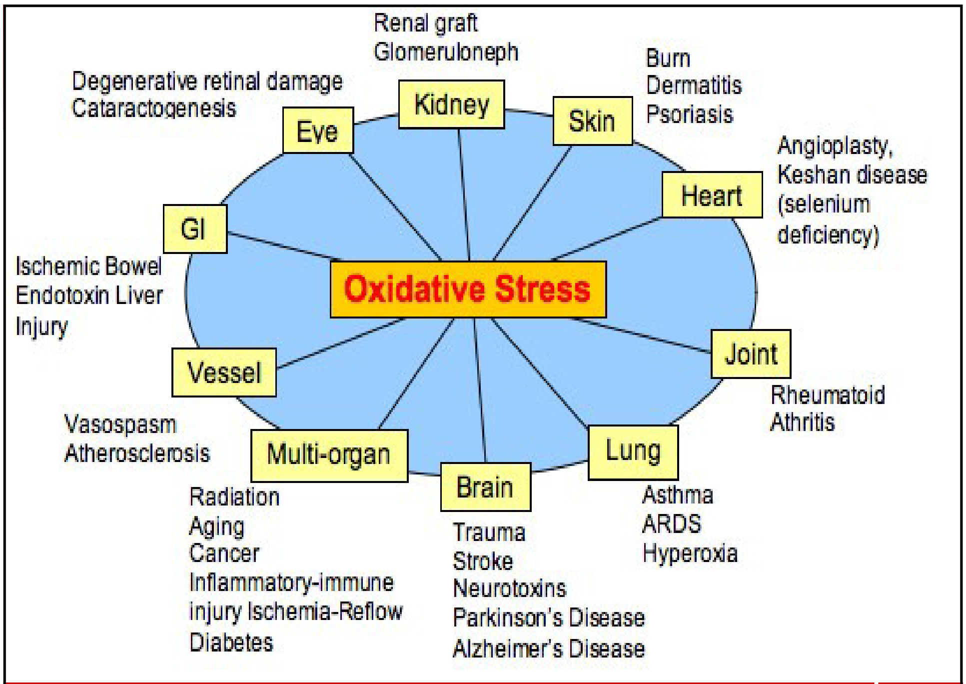
ROS mediated diseases

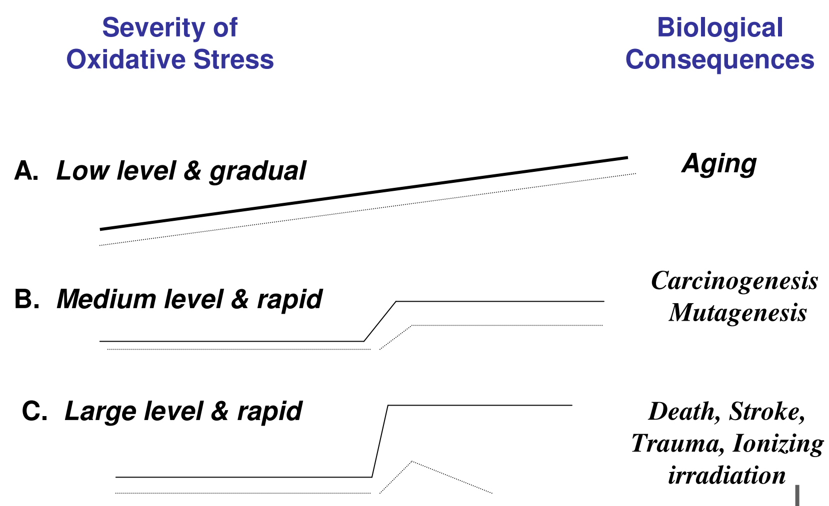
1. **Cardiovascular diseases (CHD): ox-LDL**, formed by the action of free radicals, promote CHD and atherosclerosis
2. **Cancers: damage DNA** and cause mutation and cytotoxicity, play a key role in carcinogenesis.
3. **Inflammatory diseases:** damage on the extracellular components such as collagen and hyaluronic acid, promote glomerulonephritis and ulcerative colitis.
4. **Respiratory diseases:** destroy endothelium. Cigarette smoke contains free radicals and promotes the production of more free radicals.

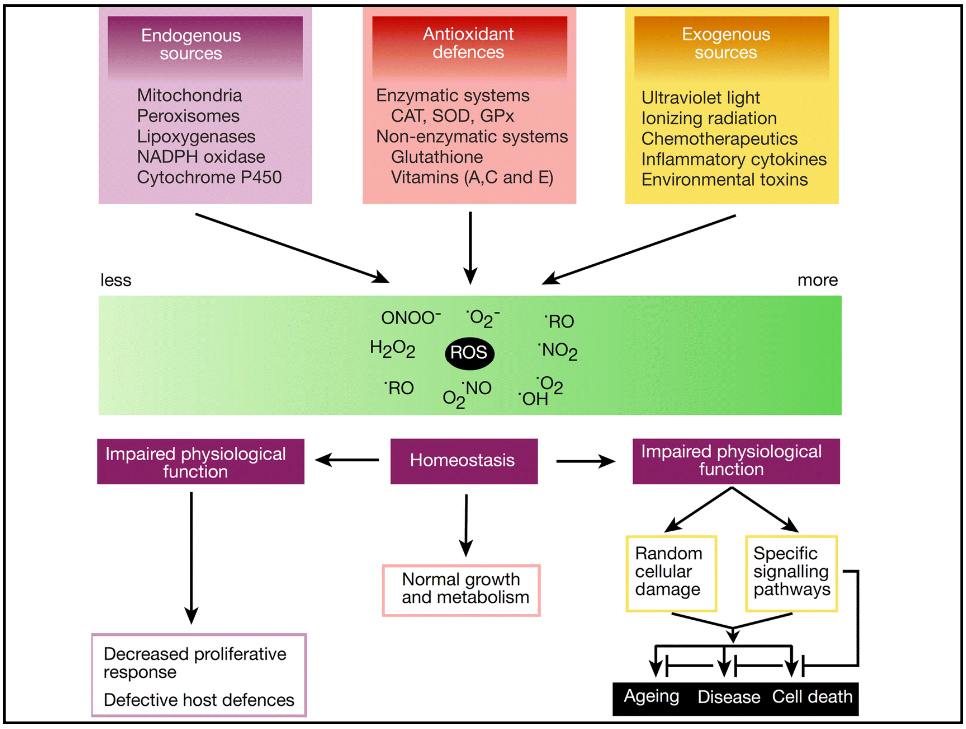
ROS mediated diseases

5. **Diabetes mellitus:** Destruction of islets results in pathogenesis.
6. **Cataract**
7. **Male infertility:** reduce sperm motility and viability.
8. **Aging process**
9. **Others:** such as Parkinson’s disease, Alzheimer’s disease, multiple sclerosis, liver cirrhosis, muscular dystrophy.

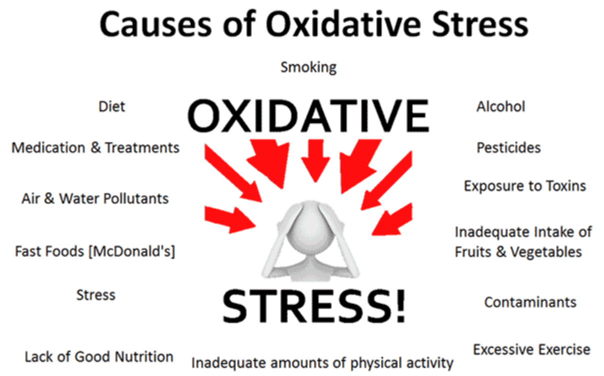


Severity of Oxidative Stress & Biological Consequences





In conclusion…..



Just about everything we do results in oxidation or inflammation producing potentially damaging free radicals

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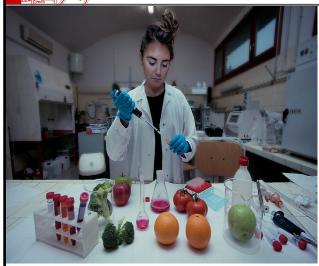

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Thank you!

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