جامعة الملك سعود
King Saud University

Biochemistry437

Oxidative Stress

Biochemistry Team 437

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Revised by

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Doctors slides
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Highlights

Cardiovascular block

EDITING FILE

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Objectives:

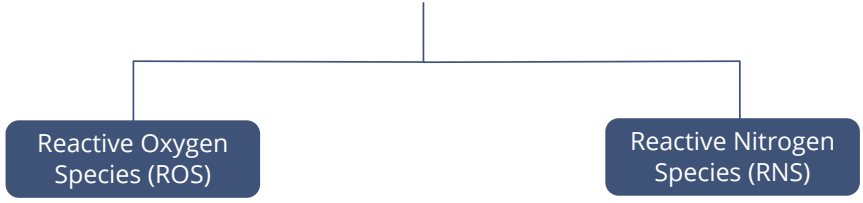
- Define oxidative stress.
- Understand the harmful effects of oxidative stress to the cell and its diseases.
- List the types, sources and effects of Reactive Oxygen Species (ROS).
- List various antioxidants in the body.
- Understand the role of glutathione system in detoxifying oxidants in the body.
- Discuss how G6PD deficiency leads to oxidative stress.
- Understand the role of Reactive Nitrogen Species (RNS) in contributing to oxidative stress.
- Correlate the role of oxidative stress to pathogenesis of atherosclerosis.

Overview

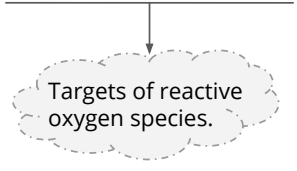
- Oxidative stress
- Reactive Oxygen Species (ROS): types, sources, effects
- Antioxidants
- Glutathione system
- G6PD deficiency
- Nitric oxide (NO): Reactive Nitrogen Species (RNS)
- Oxidative stress and atherosclerosis

Oxidative Stress

A condition in which cells are exposed to excessive levels of:



- Cells are unable to neutralize their deleterious effects with antioxidants.
- Oxidative stress is implicated in: **Atherosclerosis**, **CAD**, **ageing**.
- Cellular imbalance of oxidants* and antioxidants damages: **DNA**, **Proteins**, **Lipids**.
- **Diseases due to oxidative stress:**
  - Inflammatory diseases (Rheumatoid arthritis)
  - Atherosclerosis
  - CAD
  - Obesity
  - Cancer
  - G6PD deficiency hemolytic anemia



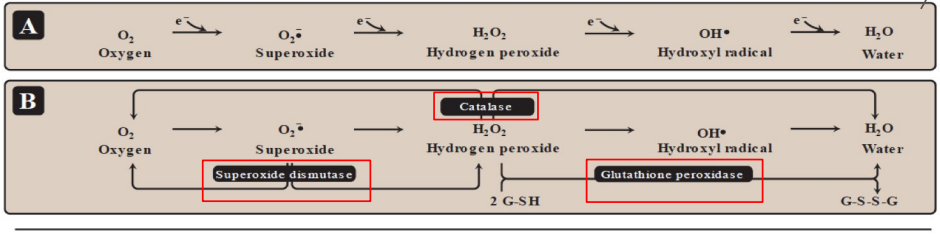
- Reactive Oxygen Species and Reactive Nitrogen Species are produced normally in the cells, and we have a system to take care of these species which is (Antioxidant Machinery).

- If the production of these Reactive species is too much, our antioxidant machinery is not working sufficiently, it leads to oxidative stress.

* oxidants are oxidizing agents, meaning they oxidise the compounds they come across
- oxidation is losing an electron

Reactive Oxygen Species (ROS)

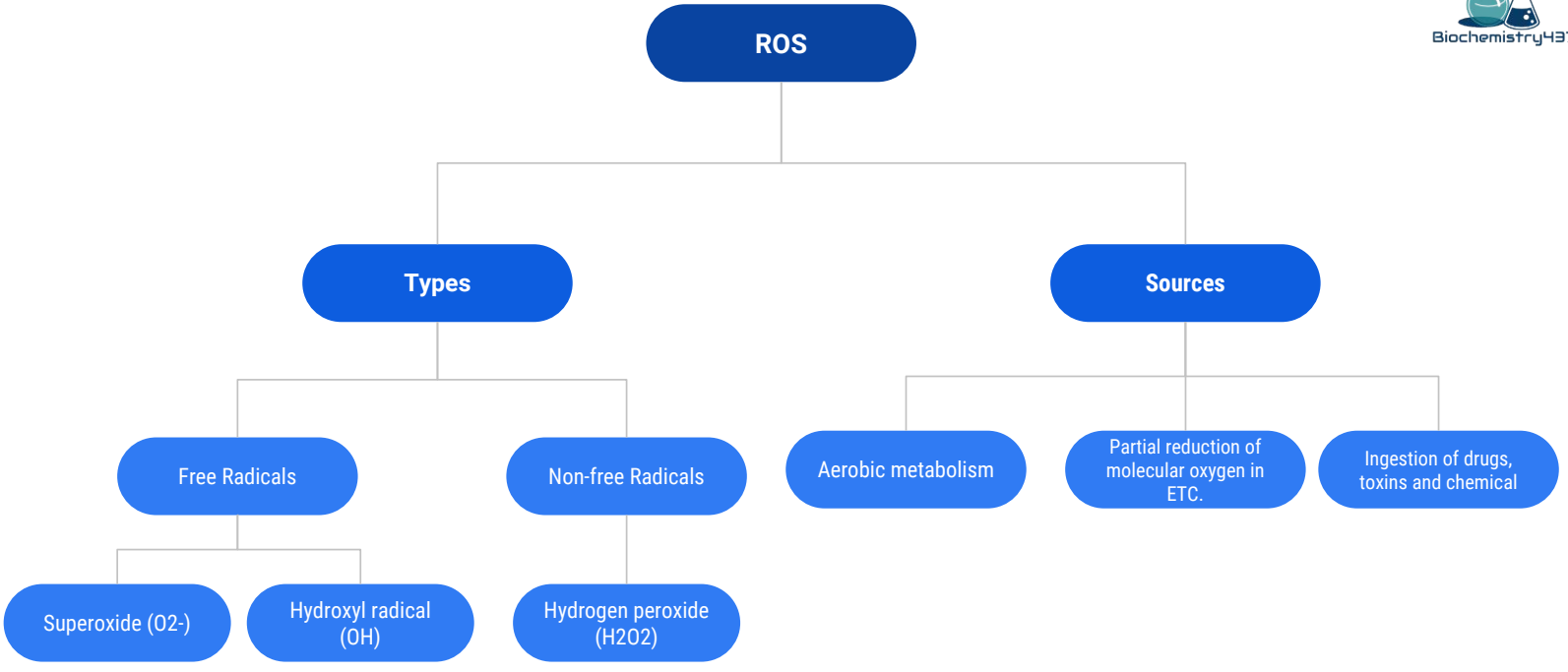
- Incomplete (partial) reduction of oxygen to water produces ROS.
  -e.g. at the end of ETC-
- ROS are continuously formed:
  - As byproducts of aerobic metabolism. [1]
  - Through reactions with drugs and toxins.
  - When cellular antioxidant level is low.
  - Creating oxidative stress in cell.
  - radiation
- ROS can damage DNA, proteins, unsaturated lipids → cell death.
- Cells have protective antioxidant mechanisms that neutralize ROS.

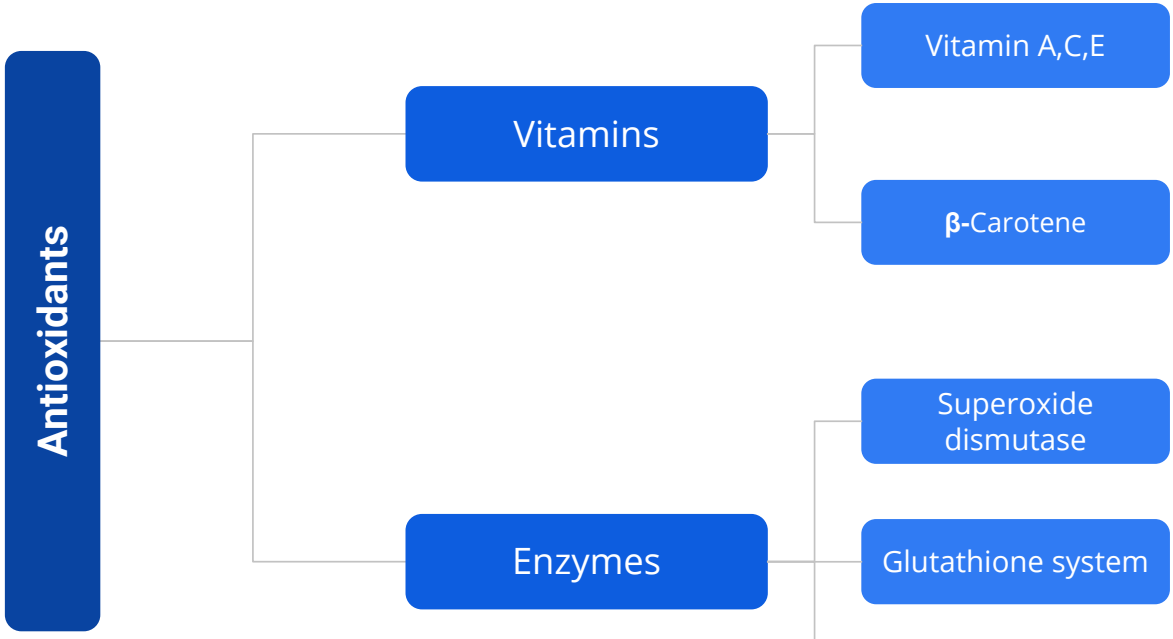


1- These ROS are usually neutralized in the mitochondria by antioxidant machinery

- Oxygen takes electrons and becomes superoxide which is an anion with an unpaired electron [unpaired electron = free radical]
- The superoxide takes an electron and becomes hydrogen peroxide which is not a free radical but a ROS and can cause damage, although free radicals are more active than hydrogen peroxide.
-another electron is given to hydrogen peroxide to give hydroxyl radical
- Hydroxyl radical is highly reactive but short lived.

Antioxidant machinery depends on a number of enzymes:
- Superoxide dismutase turns superoxide to hydrogen peroxide and a molecule of oxygen.
- then the enzyme Catalase converts H2O2 to oxygen or water.
- Glutathione peroxidase transfers hydrogen peroxide into water "the specific mechanism is explained later"
- Hydroxyl radical has no enzyme to break it only antioxidants and it is short lived.


ROS
Types
Sources
Free Radicals
Non-free Radicals
Aerobic metabolism
Partial reduction of molecular oxygen in ETC.
Ingestion of drugs, toxins and chemical
Superoxide (O2-)
Hydroxyl radical (OH)
Hydrogen peroxide (H2O2)



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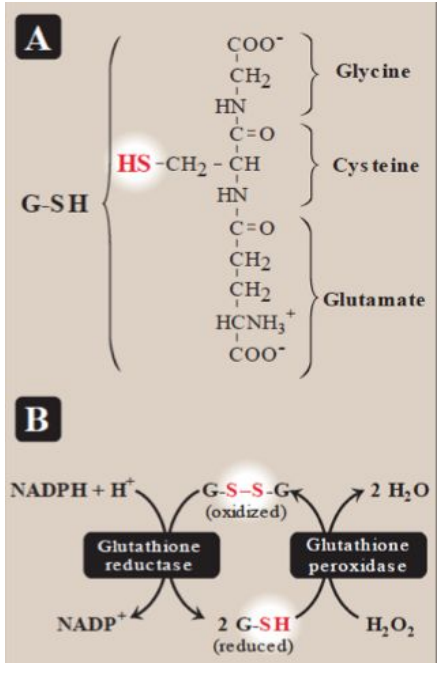
Imbalance between oxidants and antioxidants in the cells can result in the development of many diseases including atherosclerosis.

Very useful video

Glutathione System

- Present in most cells.
- Chemically detoxifies H_2O_2.
- Catalyzed by glutathione reductase.
- Produces NADPH that reduces H_2O_2
- The goal of this system is to detoxify hydrogen peroxide and restore NADPH



- GSH " reduced glutathione" will converte H2O2 => H2O by glutathione peroxidase and become GSSG " oxidized glutathione".
- GSSG will be reduced again with NADPH to GSH by **glutathione reductase**. To be able to do another round of neutralizing, NADPH will be reduced to NADP+ in this process
- Lastly we need to restore NADPH, we do this by the enzyme G6PD "next slide"

Very useful video

G6PD Deficiency

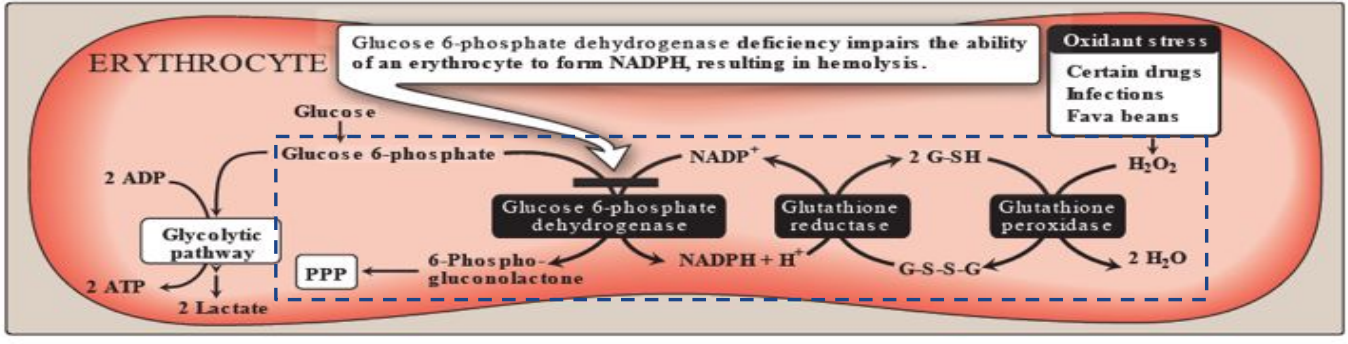


Figure 13.10
Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathionine; G-S-S-G = oxidized glutathionine; PPP = pentose phosphate pathway.

- Glucose - 6- phosphate dehydrogenase (G6PD) converts NADP+ into NADPH
- A deficiency in G6PD Leads to:
  - NADPH deficiency.
  - Cells are unable to reduce free radicals. " we need NADPH to properly reduce them in the glutathione system"
  - Oxidation of cellular proteins is increased causing impaired cell functions.

Interesting video,
2:25 minutes only.

Free radicals and its
effect on the skin,
2:23 minutes only.

Effects of ROS

"Radical Oxygen Species"

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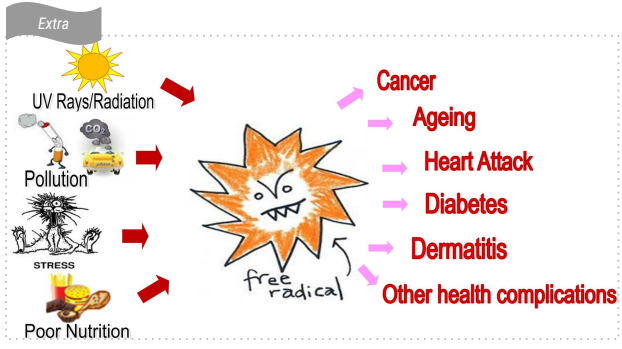
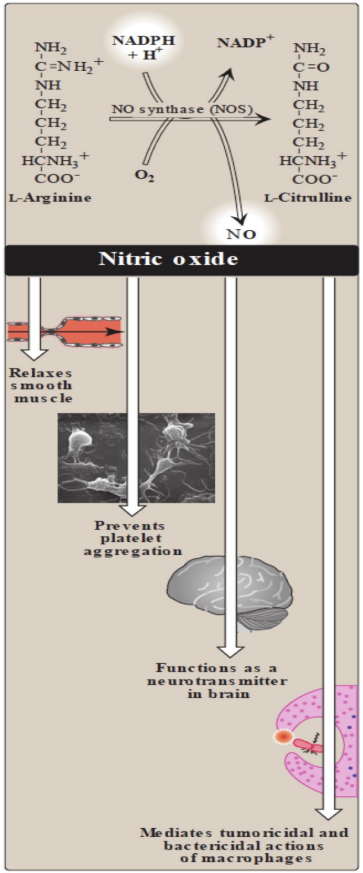
- Lipid peroxidation (polyunsaturated* fatty acids)
- DNA damage
- Protein denaturation
- Cytoskeletal damage
- Chemotaxis**
- Cell signaling effects
- Release of Ca2+ from Intracellular stores
- Altered vascular tone
- Increased endothelial cell permeability



*unsaturated are most likely to undergo peroxidation because they have weak double bonds.
** recruitment of inflammatory cells.

Nitric Oxide (NO)



- NO is produced by nitric oxide synthase

  arginin + NADPH +O2 gives NO and citrulline
- Endothelial-derived relaxing factor.
- Causes vasodilation by relaxing vascular smooth Muscles.
- NO is a gas with short half-life (3-10 sec).
- NO + Oxygen/Superoxide Nitrates, Nitrites, Peroxynitrite (O=NOO-).
- Peroxynitrite is a Reactive Nitrogen Species (RNS).
- Functions of NO:
  - Relaxes smooth muscle
  - Prevents platelet aggregation
  - Neurotransmitter in the brain
  - Mediates tumoricidal and bactericidal actions of macrophages

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Interesting animation
1:15 minutes only.

Synthesis and some of the actions of nitric oxide (NO). NADPH = reduced nicotinamide adenine dinucleotide phosphate. [Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by NOS.]

Nitric Oxide (NO)

bNOS — Bacterial[1]

eNOS — In the endothelium (vaso-relaxation)[2]

nNOS — In the neural tissue (neurotransmission)[2]

iNOS — In macrophages, neutrophils (infection)
- iNOS activity (normally low) increased by infection and pro-inflammatory cytokines
- Increased iNOS activity → free radicals → oxidative stress

Activated macrophages produce O_2^{*-} radical + NO → OH* radical → highly bactericidal

The NO will have a different function depending on where it's produced.

[1]Not produced by humans, produced by bacteria so if you target bacteria you target these molecules.
[2]eNOS and nNOS are constitutive, they're produced at constant rates but low amounts all the time because they're required for physiological functions.

More production of iNOS lead to more oxidative stress.

Oxidative Stress and Atherosclerosis

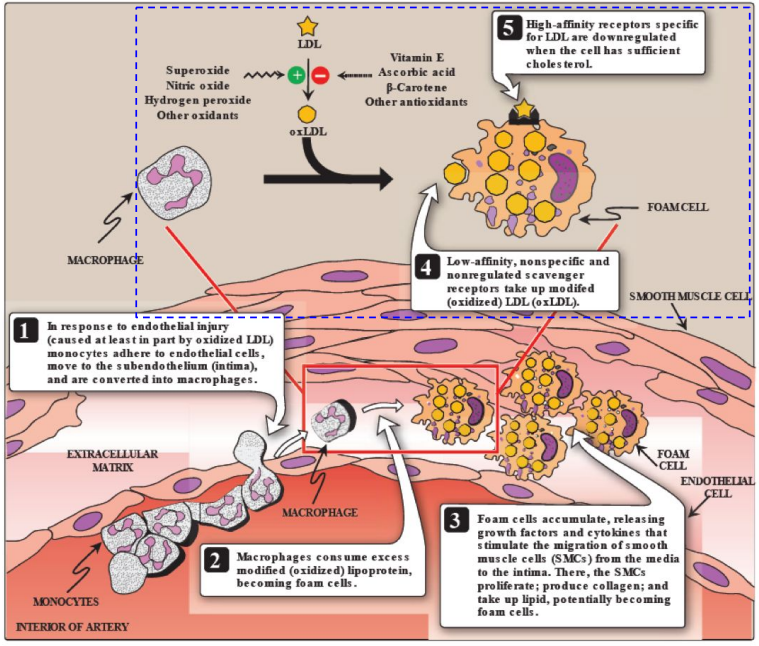

Figure 18.22
Role of oxidized lipoproteins in plaque formation in an arterial wall. LDL = low-density lipoprotein.

- Superoxide, nitric oxide, hydrogen peroxide, or any other oxidant oxidize LDL to oxLDL, and this oxLDL contributes to the formation of foam cells and ultimately atherosclerosis
- Antioxidants responsible for neutralizing oxLDL are: Vitamin E, ascorbic acid, B-carotene, and others.
- Guathionie system doesn't work here, because it is only present in the mitochondria

Take Home Messages

- Oxidative stress is due to excessive production of ROS and NOS in the cells.
- Cells neutralize these oxidants by a number of antioxidant processes.
- Imbalance between oxidants and antioxidants in the cells can result in the development of many diseases including atherosclerosis.

MCQs:

1- Which of the following Supplements would be best to combat free radicals, knowing the vitamins and minerals they contain.

A- Iron, Vitamin K, and Potassium, vitamin E
B- B- carotene, Vitamin A, Vitamin, folic acid
C- Folic acid, Vitamin A, Vitamin E, B carotene
D- B- carotene, Vitamin A, Vitamin, E, Vitamin C

2- Which type of nitric oxide has a role in potentially causing edema?

A-Type 1 n-NOS
B- Type 2 e-NOS
C- Type 3 i-NOS
D- Type 4 b-NOS

3- If a patient is deficient in G6PD what problems might he encounter?

A- Insufficient cellular energy
B- Edema
C- Impaired cells
D- Heart palpitations
E- A and C

4- Which of the following deficiency could cause decreased Nitric Oxide production?

A- D-argineine
B- L-arginine
C- L-Citrulline
D- Citrulline

5- Which of the following is a reactive Nitrogen species?

A- Hydrogen Peroxide
B- Peroxynitrite
D- Citruline
C- Vitamin C

6- Which of the following enzymes has no effect against free radicals

A-Superoxide dismutase
B-Catalase
C-Superoxide demutase
D-Glutathione

D,B,E,B,A,C

Summary

Oxidative stress is when cells are exposed to high levels of reactive species, which they cannot neutralize leading to cell injury and eventual death.

Reactive species, cause cell injury by injuring the, DNA, proteins, or lipids, eventually leading to cell death

Types of Reactive species (**1-Oxygen based** ROS [Hydroxyl radical, superoxide, hydrogen peroxide] , **2-Nitrogen based** NOS [n-NOS, e-NOS, i-NOS, b-NOS])

Antioxidants (anti-oxidation) are the molecules which counteract the effect of reactive species neutralizing their effects (types of antioxidants **1-Enzymes**: [Superoxide dismutase, Catalase, Glutathione system] **2-Vitamins**: [A, C, E, β-Carotene])

Glutathione system (reduces hydrogen peroxide via two methods, 1-Either by converting H2O2 into H2O, or 2-By forming NADPH which will then convert H2O2 into H2O)

G6PD deficiency, leads to
1-NADPH deficiency 2-Cells are unable to release free radicals 3-Impaired cell function due to increased oxidation

Oxidative Stress-Mediated Atherosclerosis (the destruction of well-balanced homeostatic mechanisms, which causes oxidative stress. And the oxidative stress causes cell injury accelerating atherogenesis)

(The oxidation of LDL (due to any reactive species) to oxLDL, and this oxLDL contributes to the formation of foam cells.)

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