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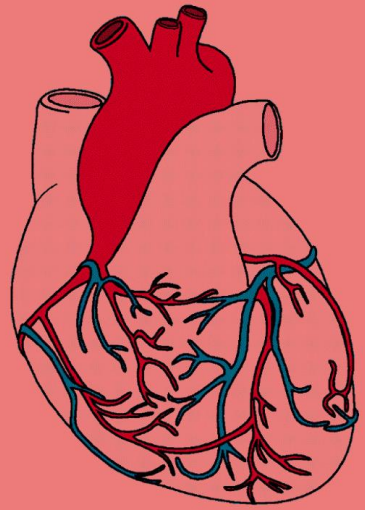
Oxidative stress



Color index :

Main text

IMPORTANT

Extra Info

Drs Notes

Cardiovascular Block - Biochemistry Team

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MED439

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.

★ Introduction (important explanation)

A helpful video

A helpful video

- At the electron transport chain particularly in complex IV, **4** electrons are transferred into the oxygen to form water "complete reduction of oxygen to water". Which is harmless to the cell. This is occur under normal conditions.

$$O_2 + 4e^- + 4H^+ \xrightarrow{\text{reduction}} H_2O \text{ "harmless"}$$

- However, when the oxygen gain **less than 4** electron it will form harmful molecules that can damage the cell and cause atherosclerosis, coronary artery disease, ageing and so on.

Examples of harmful molecules:

when oxygen gain only one electron: $O_2 + 1e^- \xrightarrow{\text{reduction}} O_2^{-1}$ superoxide "harmful"

when oxygen gain only two electron: $O_2 + 2e^- \xrightarrow{\text{reduction}} O_2^{-2}$ peroxides "harmful"

These molecules called reactive oxygen species (ROS) when there is "Incomplete reduction of oxygen to water".

- Note that this is not the only way to form ROS. Creating oxidative stress, drugs and toxins can do so.

How does our body fight them?

- Our body has antioxidants (enzymes or vitamins) that can convert ROS into harmless molecules.
  Such as: H_2O, O_2, hydrogen peroxide.

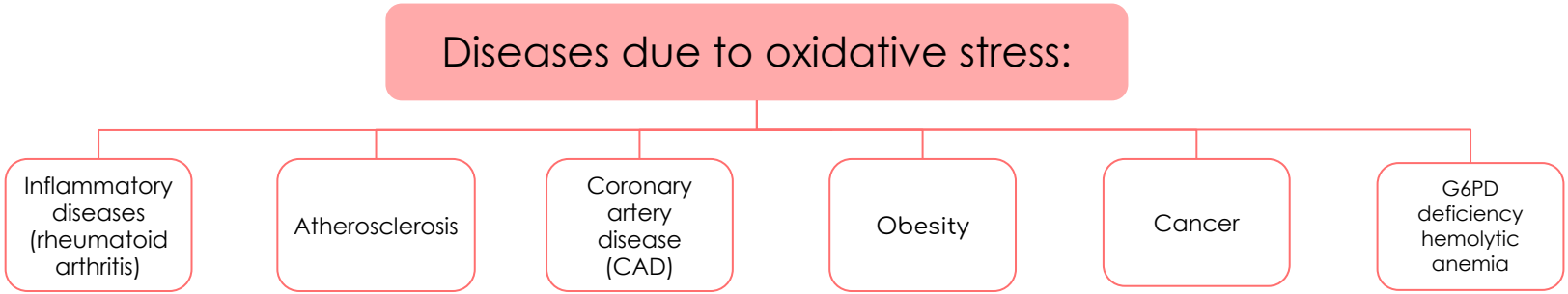
These enzymes are : Superoxide dismutase, Catalase, Glutathione system.

Remember :
- **Oxidation** :
the loss of electrons
- **Reduction** :
the gain of electrons

Oxidative stress

they're produced normally in the cells, and we have a system to take care of these species which is (Antioxidant Machinery) but if the production of these reactive species is too much , our antioxidant machinery will not work sufficiently , also If there's inflammation in the body it will lead to oxidative stress

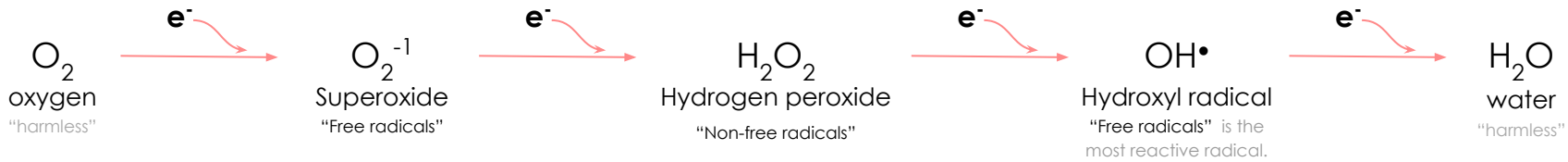
- A condition in which cells are exposed to excessive levels of:
  - Reactive Oxygen Species (ROS).
  - Reactive Nitrogen Species (RNS).
  - Both of them have unpaired electrons, that's why they are "Extremely reactive".
- Cells are **unable** to neutralize their deleterious "harmful" effects with antioxidants.
- Oxidative stress is implicated (can appear) in atherosclerosis ,coronary artery disease (CAD) and ageing.
- Cellular **imbalance** of oxidants and antioxidants damages (in other word the targets) : DNA, proteins, lipids.
- Oxidant: is a substance that has the ability to oxidize other substances.
- Antioxidants: substances that inhibits oxidation, they give electrons to the species to become stable.



Reactive Oxygen Species (ROS)

- **Incomplete** reduction of oxygen to water produces ROS. normally they produced by the body and then utilized after doing their job
- ROS are continuously formed "sources" :
  - As byproducts of aerobic metabolism.
  - Partial reduction of molecular oxygen in ETC.
  - Ingestion of drugs, toxins, chemicals or exposure to radiation.
  - When cellular antioxidant level is low. "imbalance between oxidants and antioxidants"
  - Creating oxidative stress in cell.
- ROS can damage DNA, proteins, **unsaturated** lipids → cell death.
- Cells have protective antioxidant mechanisms that neutralize ROS.

Formation of reactive intermediate from molecular oxygen : "when the O_2 is receiving electrons (reduction)"



Types of ROS :

1) **Free radicals:** Superoxide (O_2^{-1}), Hydroxyl radical (OH•). unpaired electrons
2) **Non-free radical:** Hydrogen peroxide (H_2O_2).

NOTE: The dot (•) means an electron

original pic

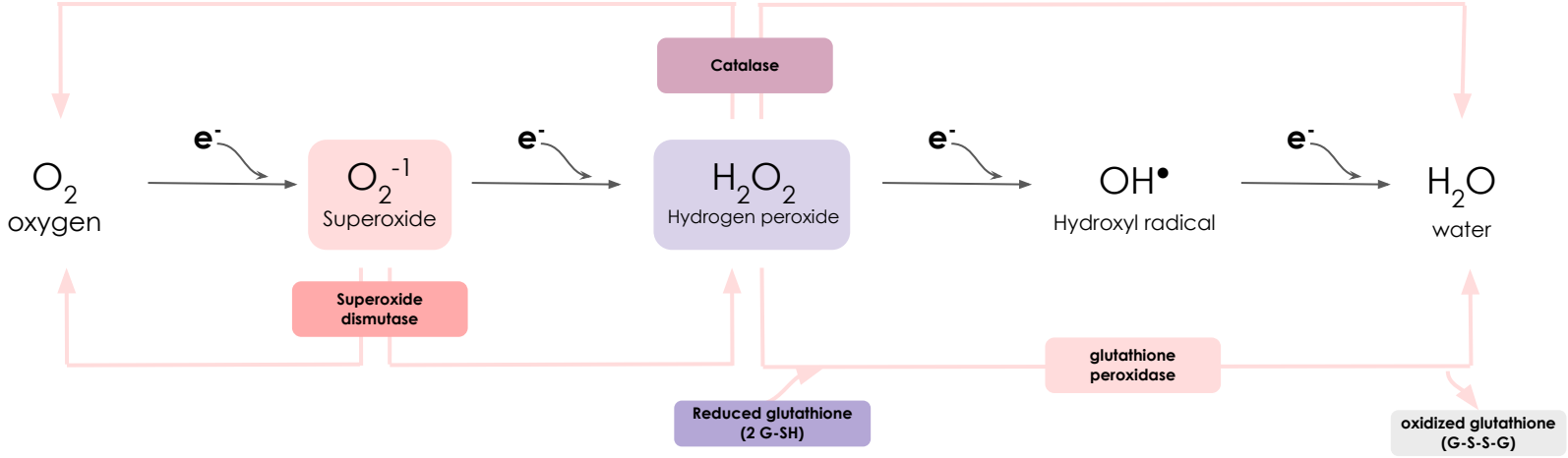
Actions of antioxidant enzymes

- The problem begins when the reduction stops in Superoxide, Hydrogen peroxide or Hydroxyl radical. one or two of these ROS will accumulate in our body and cause many problems and diseases
- Glutathione peroxidase converts H_2O_2 directly into H_2O. that's why when we have a problem in the glutathione system the H_2O_2 accumulates in our body

as we know from the previous slides, peroxide are ROS.

Here with the help of antioxidant, **superoxide** and **Hydrogen** will be converted into harmless molecules, either oxygen or water

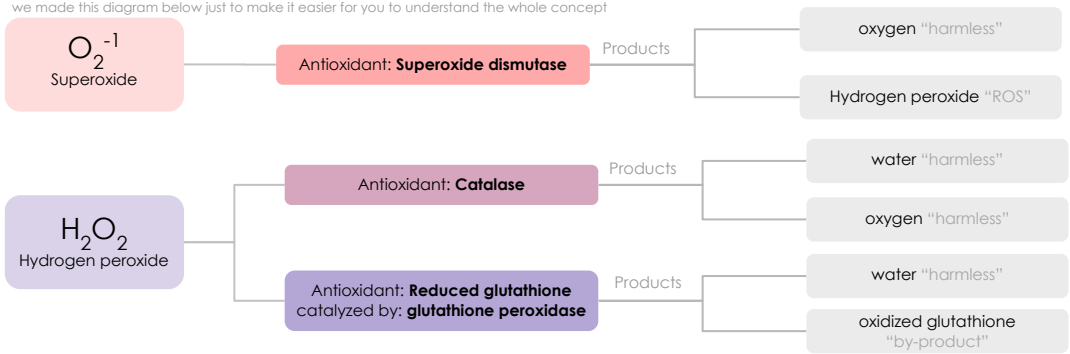
Antioxidant ال إضافة عليها هنا لكن ، قبل اللي بالسلايد الموجود التفاعل نفس



Antioxidants :

1) **Enzymes:**
   - Superoxide dismutase.
   - Catalase.
   - Glutathione system.
2) **Vitamins:**
   - Vitamins A, C, E. ♠
   - β-Carotene. : yellow/orange pigment that gives vegetables and fruits their rich colors

we made this diagram below just to make it easier for you to understand the whole concept

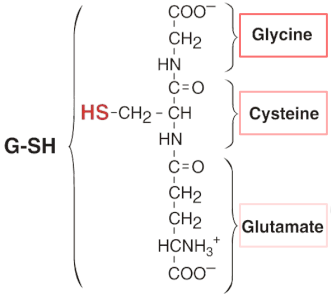


Effects of ROS :

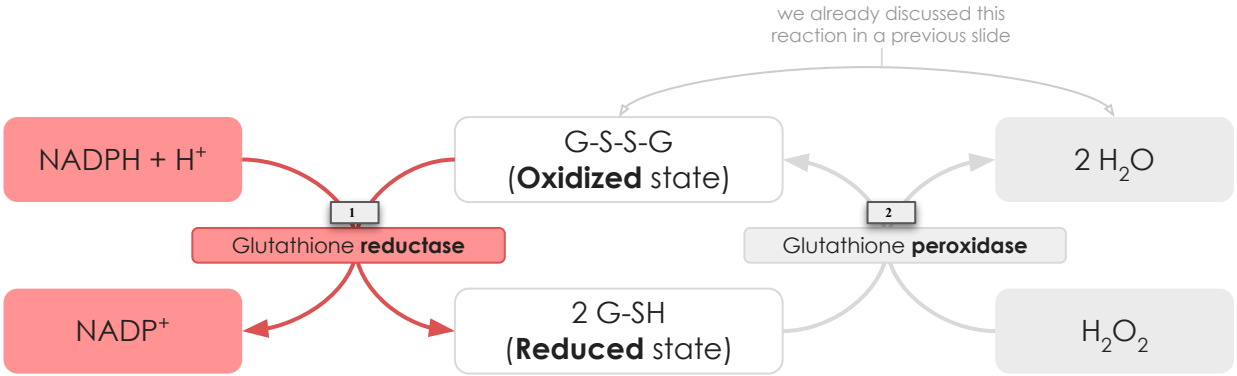
1. Lipid peroxidation (polyunsaturated fatty acids).
   - Lipid peroxidation: is the oxidative degradation of lipids. I.e the process in which free radicals "steal" electrons from the lipids.
   - unsaturated are most likely to undergo peroxidation because they have weak double bonds.
2. Cytoskeletal damage.
   - cytoskeleton is a complex, dynamic network of interlinking protein filaments present in the cytoplasm of all cells and Its function to give the cell its shape , when it's damaged the cell will lose its shape and materials causing damage to the cell.
3. Cell signaling effects.
   - Release of Ca^{2+} from intracellular stores.
4. Chemotaxis.
   - recruitment of inflammatory cells.
5. Increased endothelial cell permeability.
   - causing edema along with the inflammation.
6. DNA damage.
7. Protein denaturation.
8. Altered vascular tone.

Glutathione system

- Present in most cells. where in the cell exactly ? mitochondria
- Chemically detoxifies H_2O_2 into water.
- Catalyzed by **glutathione reductase**.
- Uses NADPH that reduces glutathione which reduces H_2O_2.
- ★ **Reduced glutathione** consist of: glycine, cysteine , glutamate .



Structure of reduced glutathione (G-SH)



In the original slide you can only find the diagram above, here we wrote it down for a better understanding.

1. Reduction of **oxidized glutathione** (G-S-S-G) into **reduced glutathione** (2 G-SH) by receiving an electron from NADPH + H^+ with the help of **Glutathione reductase** enzyme.
2. Now **reduced glutathione** (2 G-SH) will complete the cycle by converting (detoxify "remove toxic substances") hydrogen peroxide into water with the help of **Glutathione peroxidase** enzyme.
3. Lastly we need to restore NADPH, HOW? by the enzyme glucose-6-phosphate dehydrogenase (G6PD). "it's the only way to restore NAPH , if you recall in foundation block G6PD enzyme is part of hexose monophosphate shunt (HMP) " more details in the next slide

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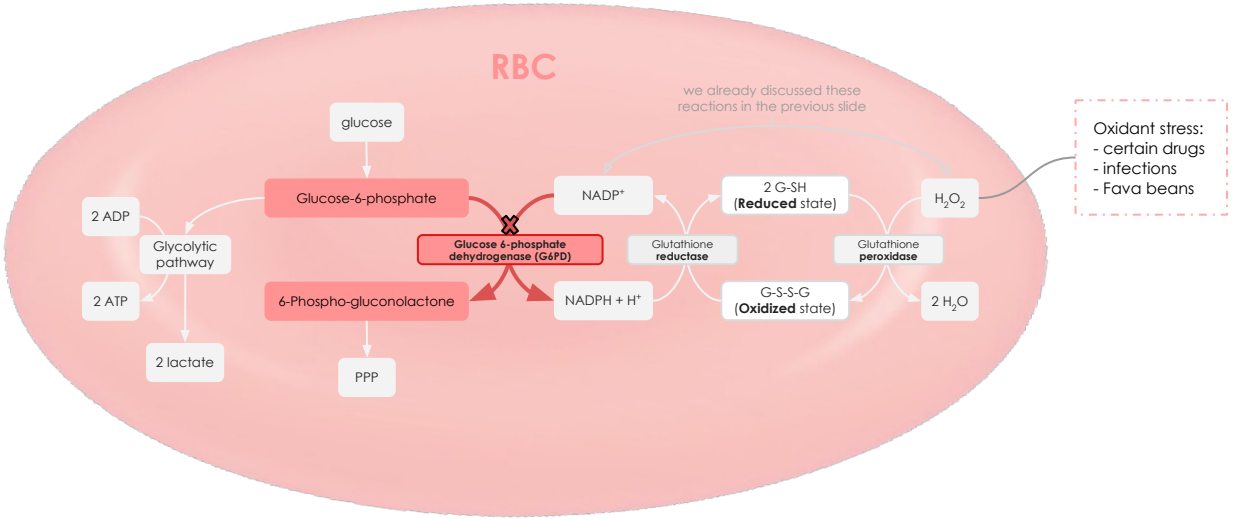
G6PD deficiency

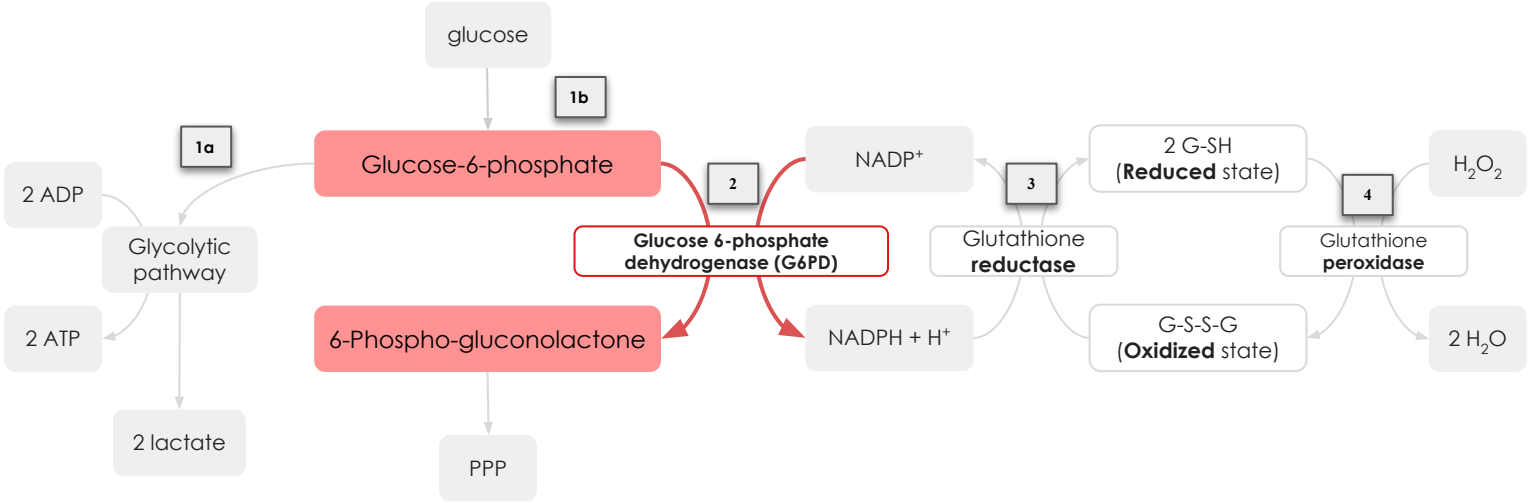
Learn more about G6PD deficiency



- Glucose-6-phosphate dehydrogenase **deficiency** impairs the ability of an erythrocyte to form NADPH, **resulting in hemolysis**.
  - In normal red blood cells, Glucose 6-phosphate dehydrogenase (G6PD) is responsible of the conversion of NADP+ into NADPH.
  - BUT here G6PD has been blocked. So as a result many ROS will accumulate inside the RBCs. Why? because it doesn't have enough NADPH + H^+ to complete the glutathione system.

- G6PD deficiency can leads to :
  - NADPH deficiency. because it's the only enzyme that can convert NADP+ to NADPH
  - Cells are unable to reduce free radicals. RBCs will be under oxidative damage (due to accumulation of H_2O_2) that leads to the death of RBCs
  - Oxidation of cellular proteins is increased causing impaired cell function. as mentioned before oxidants will cause damage to proteins

Summary of the pathway In order for you to gain a better understanding



1. To make it clear, glucose will go under two pathways 90% of glucoses will go to glycolysis as you see in step **1a** and it will generate 2ATP as we learnt in ..... and 10% will go to HMP shunt step **1b**, the goal of the shunt is **not** to generate ATP from the glucose but to restore NADPH and provide pentose (ribose) that useful for the synthesis of nucleic acids .

2. the enzyme glucose 6-phosphate dehydrogenase (G6PD) which is an **important** enzyme in HMP shunt will take an electron (e^-) and proton (H+) from glucose-6-phosphate and give it to NADP+ , as a result 6-phospho-gluconolacton will be produced (keep in mind it's oxidized because it lost an electron).

3. Now NADPH is formed , the importance of glutathione reductase enzyme is to convert glutathione to its **reduced state** , why ? The body needs it in the reduced state because it will be able to give an electron to H_2O_2 and convert it to H_2O so the enzyme will take the electron from NADPH and give it to glutathione "oxidized state" to become in reduced state and as a result NADP+ will be produced.

4. glutathione "reduced state"now is ready to give an electron to H_2O_2 by the help of glutathione peroxidase enzyme and convert H_2O_2 to H_2O and glutathione will go back to its **oxidized state**.

Note that " glutathione will not stay in its oxidized state and it will gain an electron from NADPH again , but NADPH will become NADP+ so? , yes you figure it out ! NADP+ will gain an electron from glucose 6-phosphate by the enzyme glucose 6-phosphate dehydrogenase , that's why deficiency in this enzyme will cause a lot of problems as we discussed since its the rate limiting step for HMP shunt ! ".

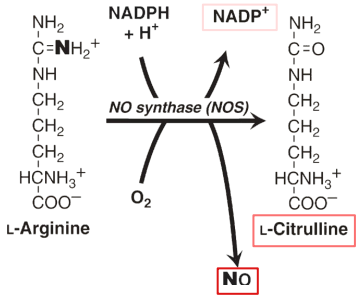
Nitric Oxide (NO)

- Endothelial-derived relaxing factor (EDRF).
- Causes: vasodilation, by: relaxing vascular smooth muscle. Due to stimulation of cGMP
- is a gas with short half-life (3-10 sec).
- NO with **Oxygen** or **Superoxide** will produce :
  1. Nitrates.
  2. Nitrites.
  3. Peroxynitrite ($O=NOO^-$), is a highly Reactive Nitrogen Species (RNS).
- The nitric oxide itself does not cause harm but its by-products are harmful.
- NO is produced by nitric oxide synthase.

Synthesis of nitric Oxide (NO)

In the original slide you can only find some pics like the one on the right, here we wrote it down for a better understanding.

1. ★**N** (Nitrogen) has been taken from **L-Arginine** with the addition of $\mathbf{O_2}$ and **NADPH.**
2. **NO synthase** (NOS) is the enzyme involved in synthesis of Nitric Oxide.
   - The main coenzyme that required for NO synthesis is **NADPH.**
   - Additional coenzymes : FAD , Fe^{+2}, FMN (flavin mononucleotide) , BH_4 (tetrahydrobiopterin)
3. The results :
   - **NO** (Nitric Oxide) is produced.
   - L-Arginine got converted into L-Citrulline.
   - $NADP^+$.

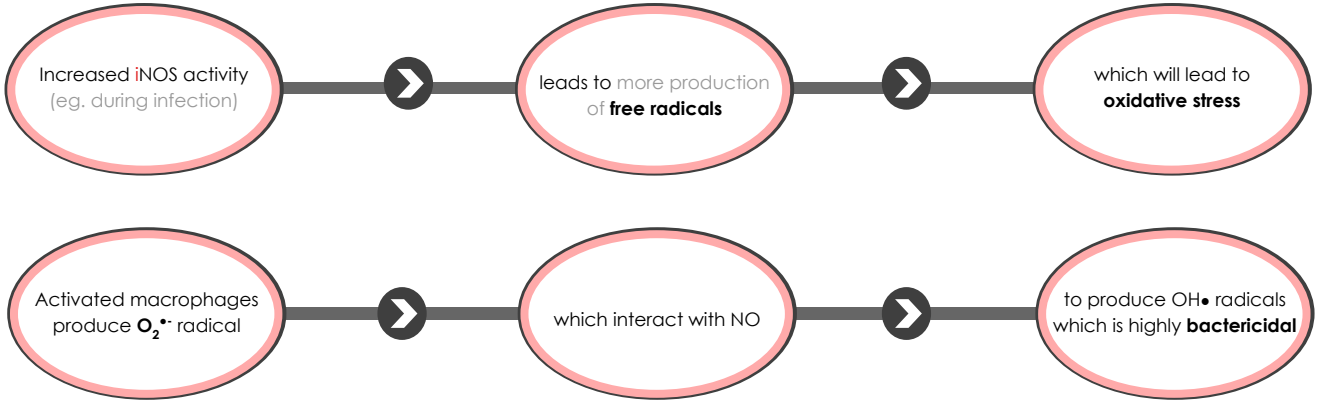


Nitric Oxide (NO)

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

Types of nitric oxide synthase (NOS)	eNOS	nNOS	iNOS	bNOS
Location	endothelium	neural tissue	macrophages, neutrophils	bacteria
Function	vaso-relaxation	neurotransmission	infection iNOS activity (normally low) increased by : - infection - pro-inflammatory cytokines	cytoprotective agent against oxidative stress in the bacteria like Staphylococcus aureus

eNOS and **nNOS** are constitutive (Dr: even if there is no inflammation it's there) they're produced at constant rates but in low amounts all the time because they're required for physiological functions. So we don't worry about them, what we worry about are **iNOS** and **bNOS** (inducible)

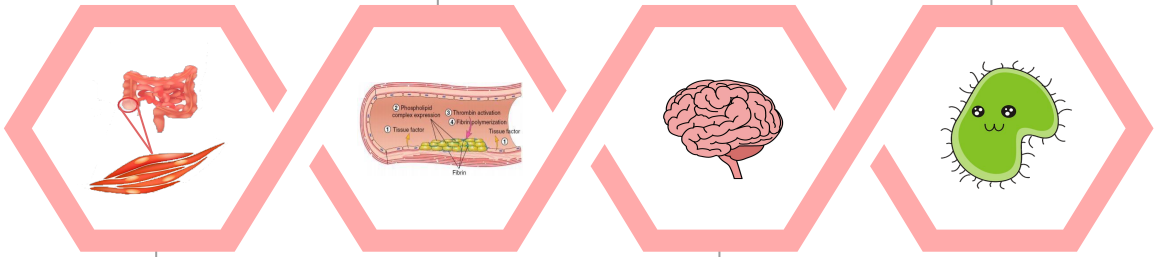


Nitric oxide functions

Prevent platelet aggregation

Via the stimulation of cGMP

★ Mediate tumoricidal "destroy the tumor cells" and bactericidal actions of macrophage



Smooth muscle relaxation

It will stimulate the cGMP

Keep in mind that ↑ cGMP = ↑ vasodilation

Function as neurotransmitter in the brain

There it allows the brain blood flow and also has important roles in intracellular signaling

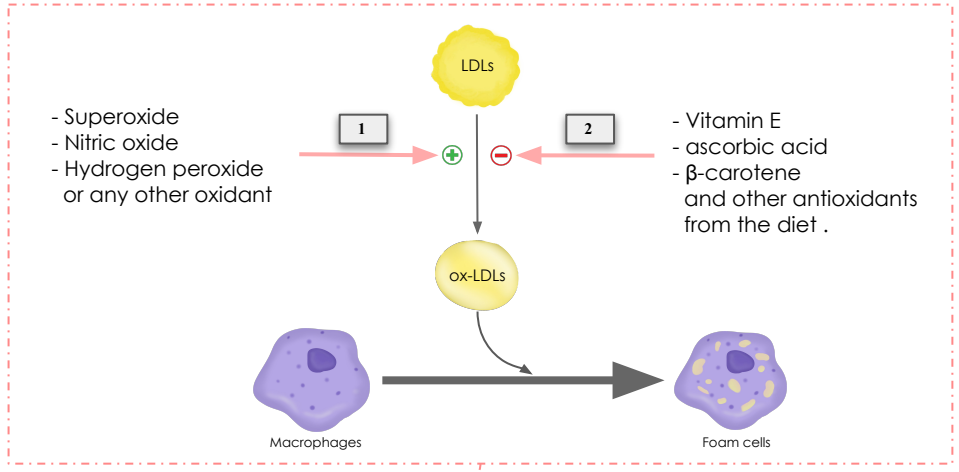
original pic

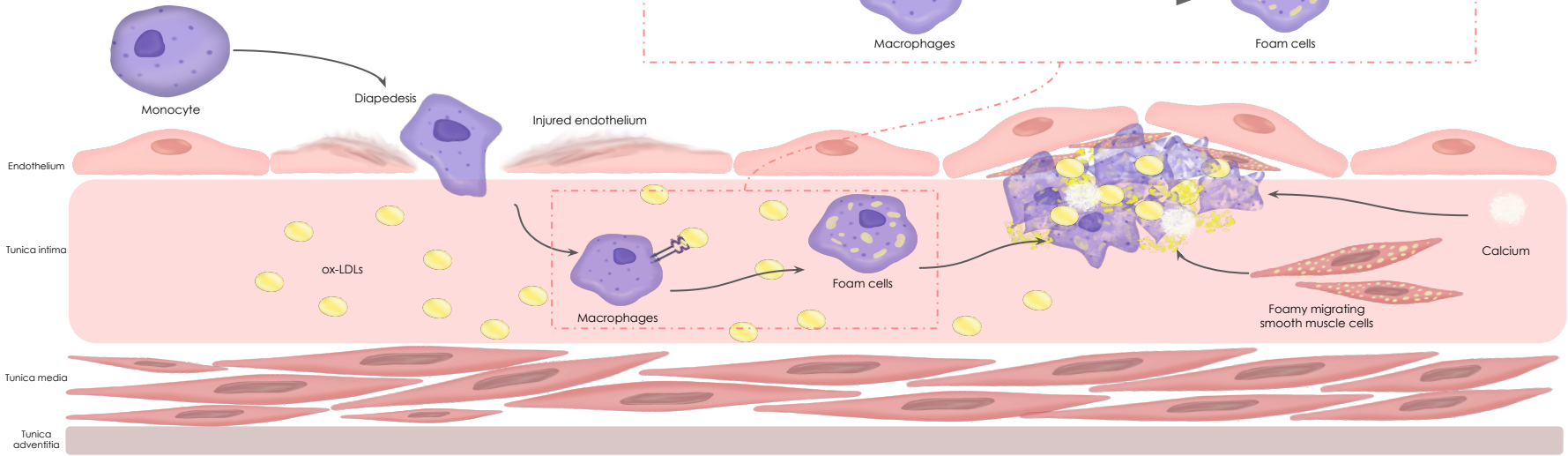
This illustration is drawn by the **AMAZING** Duaa ♡♡♡

Oxidative stress and atherosclerosis

1 **Oxidants** will oxidized LDLs to become oxidized-LDLs which contributes to the formation of foam cells and ultimately atherosclerosis. More details? Check lipoprotein & atherosclerosis lecture

2 **antioxidants** on the other hand are responsible for neutralizing oxLDL





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.

Summary

Oxidative stress

Reactive **Oxygen** Species

Source

- byproducts of aerobic metabolism
- Ingestion of drugs, toxins and chemicals
- antioxidant level is low
- Creating oxidative stress in cell

Types

- Free Radicals
  - Superoxide (O^{2-})
  - Hydroxyl radical (OH)
- Non-free Radicals peroxide
  - Hydrogen peroxide (H_2O_2)

Effects

- Lipid peroxidation
- Chemotaxis
- DNA damage
- Cell signaling effects
- Protein denaturation
- Altered vascular tone
- Cytoskeletal Damage
- Increased endothelial cell permeability

Reactive **Nitrogen** Species

Source

Nitric oxide + Oxygen or superoxide

Synthesis

By NOS in our body

Type

- Produced by human
  - eNOS
  - nNOS
  - iNOS
- Produced by bacteria
  - bNOS

Function

- **NO** Have major role in cardiovascular system
  - **endothelium :** Relaxes smooth muscle= vasodilation = reduce blood pressure
  - Prevent platelet aggregation
- Neural tissue
  - neurotransmitters in brain
- Immune defense

Diseases due to Oxidative stress

- rheumatoid arthritis
- atherosclerosis
- CAD
- obesity
- cancer
- G6PD deficiency hemolytic anemia

Defense mechanism against oxidative stress

Antioxidant

- Vitamins A,C,E
- β-Carotene
- Superoxide dismutase
- Catalase
- Glutathione system

Quiz

Q1 : Which of the following is a Free radical ?			
A) Water	B) Hydrogen peroxide	C) Oxygen	D) Superoxide
Q2 : Superoxide dismutase is used to convert Superoxide into ?			
A) O_2 + water	B) water + Hydrogen peroxide	C) O_2 + Superoxide	D) O_2 + H_2O_2
Q3 : Which one of these enzymes helps with the oxidation of 2 G-SH and forming H_2O ?			
A) Glutathione reductase	B) Glutathione Peroxidase	C) G6PD	D) Both A&B
Q4 : Which one of these types of nitric oxide synthase is increased during infection ?			
A) eNOS	B) nNOS	C) iNOS	D) bNOS
Q5 : Which one of the following is the main coenzyme that's required for NO synthesis ?			
A) FMN	B) FAD	C) NADPH	D) BH_4
Q6 : Deficiency in which of the following will cause hemolysis ?			
A) Glutathione peroxidase	B) Glutathione reductase	C) G6PD	D) Catalase

SAQs :

Q1: Enumerate antioxidants.

Q2: Mention 4 diseases caused by oxidative stress.

Q3: List 4 ROS effects.

Q4: What causes LDL to get oxidized, then forming a foam cell ?

★ **MCQs Answer key:**

1) D 2) D 3) B 4) C 5) C 6) C

★ **SAQs Answer key:**

1) Superoxide dismutase, Catalase, Glutathione system, Vitamins A, C, E and β-Carotene
2) Atherosclerosis, CAD, cancer, rheumatoid arthritis
3) DNA damage, Protein denaturation, cytoskeleton damage, and chemotaxis
4) ROS

Girls team:

- Manal Altwaim
- Duaa Alhumoudi
- Rania Almutiri
- Alia Zawawi
- Noura Alshathri
- Reem Alamri
- Renad Alhomaidi
- Fatimah Alhelal

Boys team:

- Omar Alsuliman
- Abdullaziz Alomar
- Hamad Almousa
- Homoud Algadheb
- Abdullah Alanzan
- Abdullah Almazro
- Ahmad Alkhayatt
- Abdullaziz Alrabiah

" Nothing in this world that's worth having comes easy"

Revised by Shatha Aldhohair

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