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carbohydratesisomersketone starchlipidproteinamine

Biochemistry

Doctor 2017 | Medicine | JU

◉ Sheet ○ Slides

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Doctor

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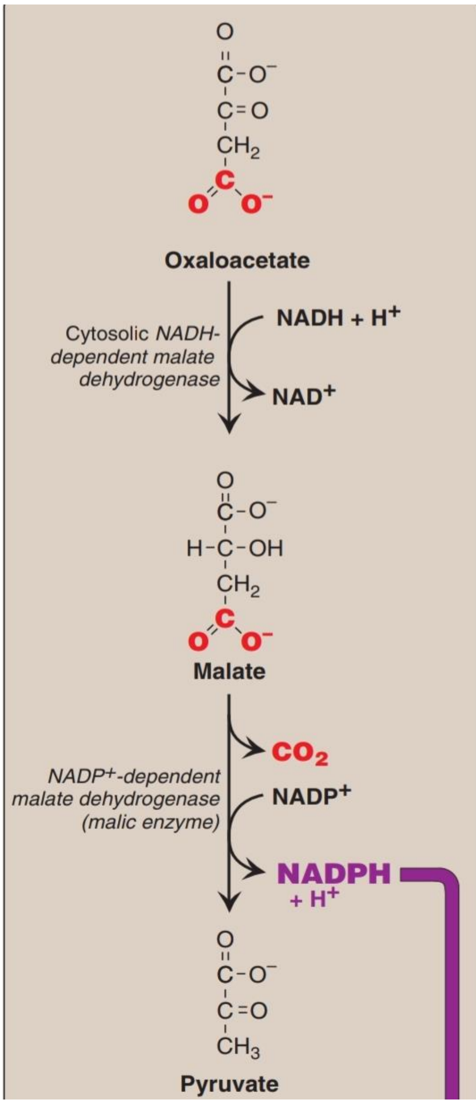
Sources of NADPH

- [The pentose phosphate pathway is the primary source of the NADPH and is the only source in RBC.]
- Cytosolic conversion of oxaloacetate to pyruvate with the generation of NADPH. It works in all tissues except RBCs. Two reactions are involved in this pathway :

1) **Oxaloacetate** is reduced into **malate**, while **NADH** is oxidized to **NAD+**, by the enzyme cytosolic NADH-dependent malate dehydrogenase.
2) **Malate** is oxidized and decarboxylated by cytosolic malic enzyme (NADP+ -dependent malate dehydrogenase) into **pyruvate**, **with producing NADPH and CO2.**



Remember that Oxaloacetate can arise from :

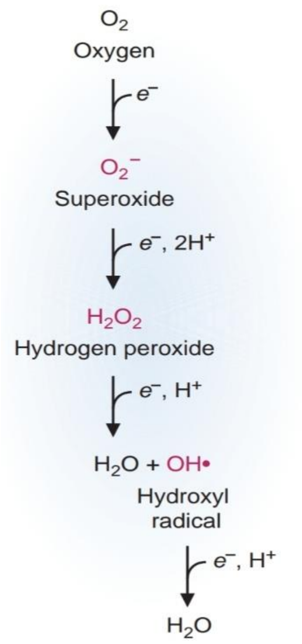
- Malate in Krebs Cycle.
- Cytosolic Citrate, that moves from the mitochondria into the cytosol, where it is cleaved into acetyl CoA and Oxaloacetate by ATP-citrate lyase.

Reactive Oxygen species (ROS)

Radicals are compounds that contain a single electron, usually in an outside orbital. Oxygen is a biradical, a molecule that has two unpaired electrons in separate orbitals. Through several enzymatic and non-enzymatic processes that routinely occur in cells, O2 accepts single electrons to form reactive oxygen species (ROS). ROS are highly reactive oxygen radicals or compounds that are readily converted in cells to these reactive radicals. The ROS formed by reduction of O2 are :

- The radical superoxide (•O2-).
- The nonradical hydrogen peroxide (H2O2), but it's the major oxidizing agent to produce OH•.
- The hydroxyl radical (OH•).

Oxygen Metabolism and Toxicity :



- ✓ More than 90% of consumed O2 is used in Respiratory Chain to produce ATP.
- ✓ 3-5% Of consumed O2, is converted to ROS, which are generated by :

1) Normal metabolism :

The generation of ROS from O2 in our cells is a natural during aerobic metabolism. The single electrons in ROS are usually derived from reduced electron carriers of the electron-transport chain (ETC).

2) Environmental factors :

- ➢ Exposure to Radiation, like : Ultraviolet light, X-ray, gamma radiation.
- ➢ Exposure Pollutants, like in the air, smoke, cigarette.
- ➢ Exposure to high oxygen pressure. Due to that, Patients who need oxygen masks must be under high control.
- ➢ Having Infection (during phagocytosis), or specific chemicals and drugs.
- ➢ Process of aging.

Some Of The Diseases Associated With ROS Injury :

- Atherosclerosis ● Respiratory Disease (Emphysema /Bronchitis) ● Diabetes
- Parkinson's disease ● Cancer ● Liver Damage ● Motor neuron disease ● Aging

Other Reactive Species (free radicals) :

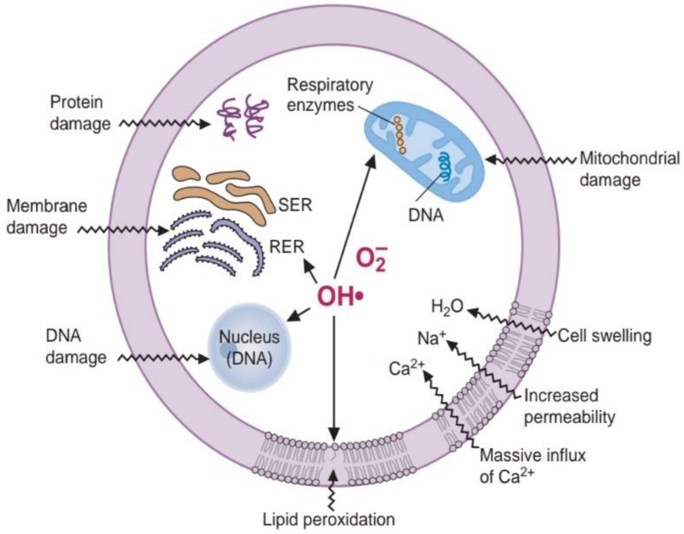
a) **RNOS** : contains nitrogen as well as oxygen. These radicals are derived principally from the free radical nitric oxide (NO), which is produced endogenously by the enzyme nitric oxide synthase. NO combines with O2 or superoxide to produce additional RNOS.

b) **Organic peroxides : RCOO•** : Organic radicals are generated when superoxide or the hydroxyl radical indiscriminately extract electrons from other molecules. Organic peroxy radicals are intermediates of chain reactions, such as lipid peroxidation

c) **Hypochlorous Acid : HOCl** : a powerful oxidizing agent that is produced ,from H2O2, endogenously and enzymatically by phagocytic cells.

ROS are related to Cellular Damage, they can :

- ✓ Cause many diseases.
- ✓ Contribute to complication of many chronic diseases.
- ✓ Affect Proteins , lipids, nucleic acids & Carbohydrates.



The main biological targets of ROS :

1- Proteins and Peptides :

In proteins, the amino acids proline, histidine, arginine, cysteine, and methionine are particularly susceptible to hydroxyl-radical attack, so the protein may fragment or residues cross-link with other residues, which increases the susceptibility of other proteins to proteolytic digestion.

2- DNA :

Binding of Fe2+ to DNA facilitates localized production of the hydroxyl radical, which can attack the deoxyribose backbone and cause strand breaks. “This DNA damage can be repaired to some extent by the cell or minimized by apoptosis of the cell”.

3- Polyunsaturated fatty acids : PUFA

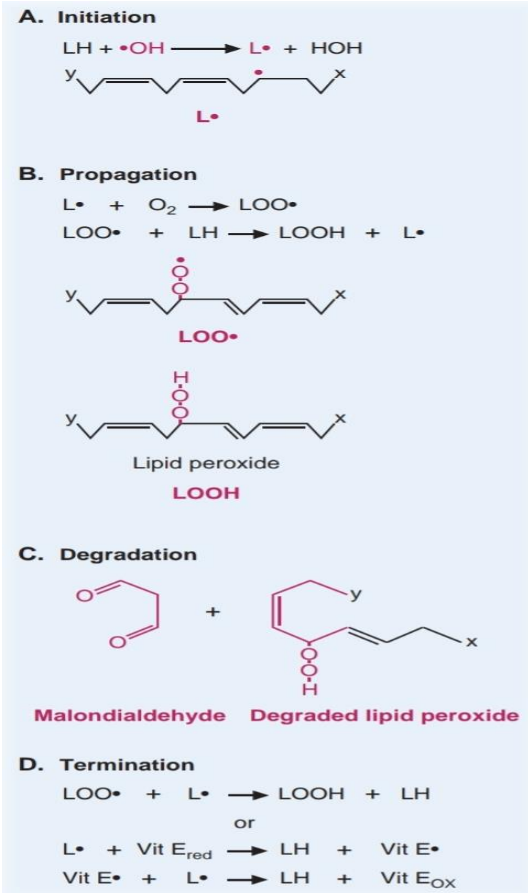
Free radicals target the lipid bilayer of the cell, mitochondria, endoplasmic reticulum and other organelles. That causes leaking of ions to the cell and swelling it, resulting in cell injury.

An initiator (such as a hydroxyl radical) begins the chain reaction. It extracts a hydrogen atom, preferably from the double bond of a polyunsaturated fatty acid in a membrane lipid forming a lipid free radical. The chain reaction is propagated when O2 adds to form lipid peroxyl radicals and lipid peroxides. Eventually, degradation of Lipohydroperoxide occurs, forming harmful such products as Malondialdehyde, which appears in the blood and urine and is used as an indicator of free radical damage.

- ➢ Initiation : Forming lipid free radical : LH + OH• → L• + H2O
- ➢ Prolongation : Chain reaction is set via lipid radicals in producing Lipohydroperoxide. These two reactions take place :

- Forming Peroxyl radical : LH• + O2 → LOO•
- Formation of Lipohydroperoxide : LH+ LOO• → LOOH + L•

➢ Termination : The chain reaction can be terminated by reduced vitamin E and other lipid-soluble antioxidants that donate single electrons. Two subsequent reduction steps form a stable, oxidized antioxidant.



Markers for ROS damage :

Many molecules from DNA, proteins and lipids can be used to determine this damage.

I. For proteins : carbonite.
II. For lipids : malondialdehyde, which is circulated in the blood then excreted in the urine. It's used to measure the oxidative stress in many diseases, such as : diabetes patients with G6PD deficiency, they have high level of malondialdehyde and carbonite, so that's a marker for high oxidative damage.

sources of ROS in the cell :

A) Electron Transport Chain :

The one-electron reduced form of CoQ (CoQH•) is free within the membrane and can accidentally transfer an electron to dissolved O2, thereby forming superoxide.

B) Oxidases & Oxygenases :

- Most of the oxidases, peroxidases, and oxygenases in the cell bind O2 and transfer single electrons to it via a metal : e- + O2 → H2O or H2O2.
- H2O2 can produce the hydroxyl free radical through the fenton rxn.
- Free radical intermediates of these reactions may be accidentally released before the reduction is complete.
- Monoxygenases : hydroxylases in the mitochondria and microsomal fractions.
- Dioxygenases : in the synthesis of prostaglandins, Thromboxane, leukotrienes

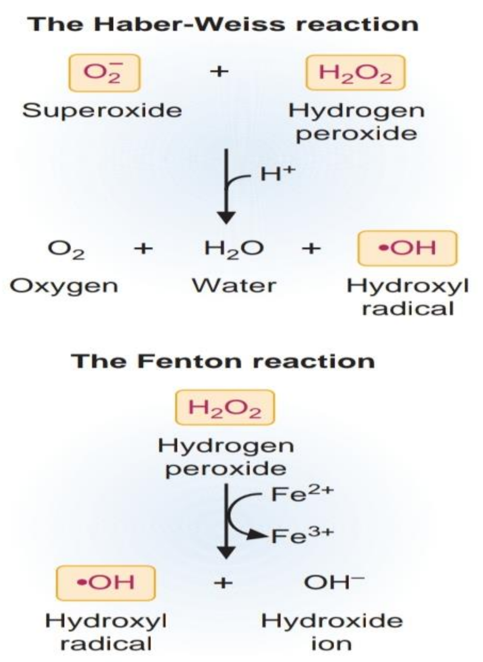
- Most oxidases generate H2O2, So Oxidases are confined to sites equipped with protective enzymes.

C) Cytochrome P450 :

enzymes are a major source of free radicals “leaked” from reactions. Because these enzymes catalyze reactions in which single electrons are transferred to O2 and an organic substrate, the possibility of accidentally generating and releasing free radical intermediates is high.

D) Fenton reaction:

Transition metals, such as Fe2+ or Cu+, catalyze formation of the hydroxyl radical from hydrogen peroxide in the non-enzymatic Fenton reaction. It occurs in the presence of excess iron, which is usually found in men or postmenopausal women. Adolescents have normal iron concentration, or sometimes iron deficiency.



Fe+2 + H2O2 → Fe+3 + HO• + OH-

E) Haber-Weiss reaction :

The superoxide anion can generate the more reactive hydroxyl and hydroperoxy radicals by reacting non-enzymatically with hydrogen peroxide.

O2• + H2O2 ← → O2 + HO• + OH-

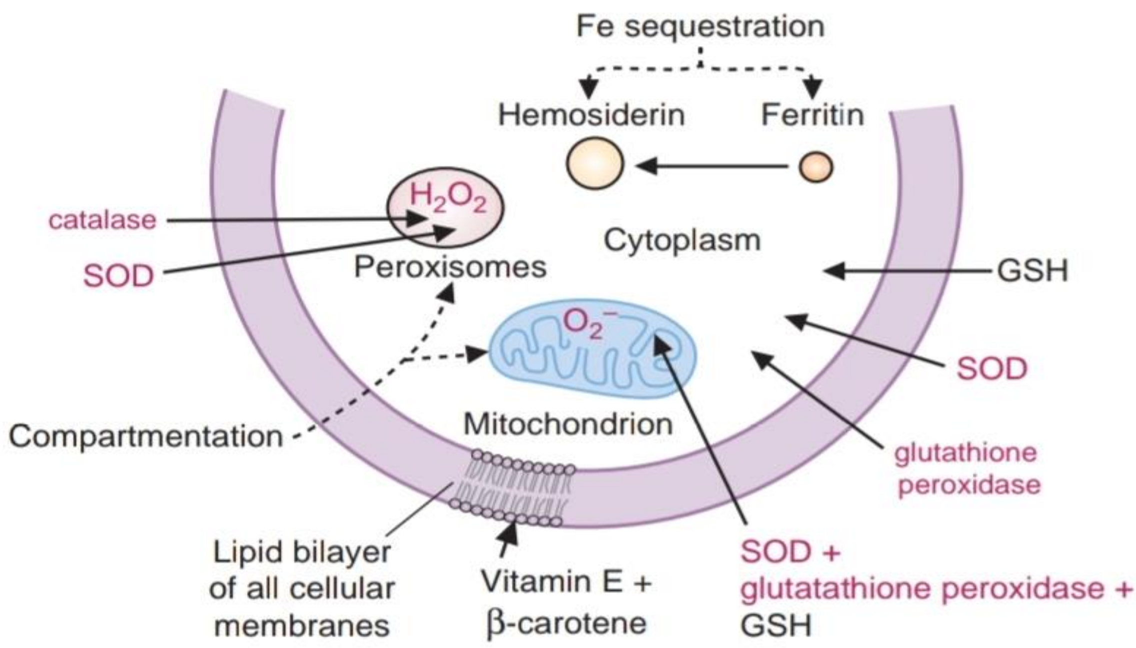
F) Ionizing Radiation :

It has a high enough energy level that it can split water into hydroxyl and hydrogen radicals, thus leading to radiation damage to the skin, mutations, cancer, and cell death. It also may generate organic radicals

G) Respiratory Burst :

During phagocytosis many radicals are generated such as : O2•, H2O2, OH●, NO ,HOCl.

Cellular Defense Against O2 Toxicity



A. Primary Antioxidants : Antioxidant Enzymes (AOEs) :

- SOD, Catalase, GSH peroxidase, GSH reductase. They are found in High concentration in liver , adrenal glands & kidney (high content of peroxisomes & mitochondria).
- SOD converts superoxide ion to hydrogen peroxide.
- Catalase (in peroxisomes) converts hydrogen peroxide to water and oxygen and water.
- GSH Peroxidase converts hydrogen peroxide to water, by oxidizing of Glutathione.
- GSH peroxidase can neutralize organic peroxides also. It needs Se as a catalyst.
- GSH Reductase converts GSSG (oxidized form of Glutathione) into GSH (reduced form), by oxidizing NADPH.

B. Secondary Antioxidants :

I. Dietary :

● Flavonoids ● Vitamins : such as Vitamin E (tocopherol), Vitamin C, β-Carotenes.

II. Endogenous antioxidants :

- Uric acid : the end product of purines degradation. ● GSH.
- Melatonin : is a secretory product of the pineal gland, which works as free radical scavenger to neutralize ROS and RNOS.

- Bilirubin : which is the end product of heme degradation. It is transported to the blood from different cells to reach the liver. Then it is excreted to the bile to reach the large intestine.
- Lipoic acid.
- Ubiquinone (Co , Q10).

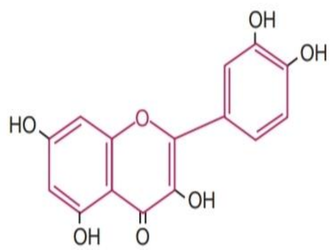
III. Repair Mechanism of DNA , oxidized fatty acids & membrane lipids and oxidized amino acids.

IV. Compartmentation of free radicals defenses :

- Peroxisomes protect the cell from Catalase and SOD.
- Mitochondria have GSH Peroxidase and SOD.
- Enzymes in smooth ER SOD and GSH peroxidase are present as isoenzymes
- Fe+2 in the cell is bound the ferritin protein.
- Liver, kidney and spleen have higher content of AOEs, because the have high amount of mitochondria and peroxisomes due to high metabolic activity.

Flavonoids

Flavonoids are a group of Polyphenolic compounds that contain two spatially separate aromatic rings, which are found in : Green tea, Chocolate, Fruits skin, Red wine, Vegetables as : onion, tomatoes, Broccoli. And Colored fruits as : Grapes, blueberries.



A flavonoid

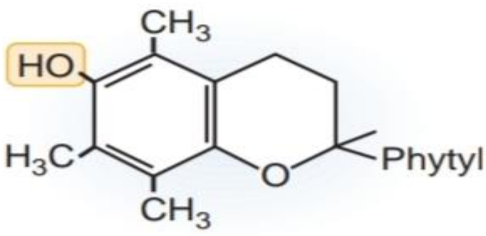
Possible Functions of Flavonoids :

a. *Inhibition of ROS production,* e.g. inhibition of X.O (xanthine oxidase).
   ⇨ Xanthine oxidase, an enzyme of purine degradation that can reduce O2 to O2- or H2O2 in the cytosol, is thought to be a major contributor to ischemia-reperfusion injury.
b. *Free radical scavengers* : converting free radicals to a nonradical, nontoxic form in non-enzymatic reactions. They neutralize free radicals by donating a hydrogen atom (with its one electron) to the radical. So they under go oxidation to reduce free radicals.
c. Chelate Fe & Cu: because they catalyze the production of ROS in Fenton reaction.
d. *Maintenance of Vitamin E*, because it’s an important antioxidant, it’s regenerated in the reduced form by flavonoids.

Some Flavonoids :

- **Catechins**, which is found in : strawberries , green & black tea.
- **Kaempferol**, which is found in : Brussel sprouts & apple.
- **Quercetin**, which is found in : beans , onions , apples and fruits skin.
- **Epicatechin**, which is found in : Cocoa , red wine.

Vitamin antioxidants



α-Tocopherol

A) Vitamin E

- ✓ The most widely distributed antioxidant in nature, is a lipid-soluble antioxidant vitamin that functions principally to protect against lipid peroxidation in membranes.
- ✓ It comprises several tocopherols that differ in their methylation pattern. Among these, a-tocopherol is the most potent antioxidant and is present in the largest amounts in our diet.
- ✓ It donates single e- to reduce free radicals , and terminates lipid peroxidation.

B) Carotenoids accept e- from lipid Peroxy radicals, so it's found in the membrane.

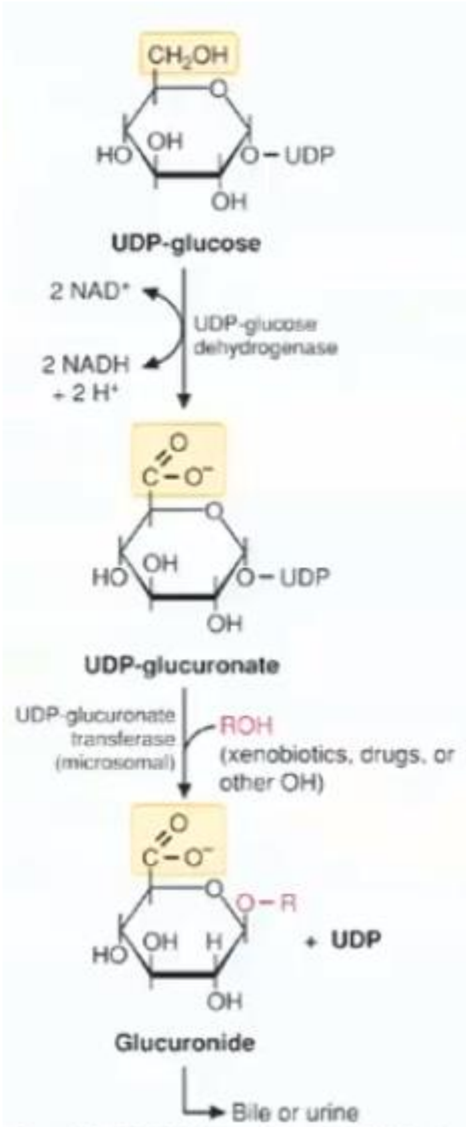
C) Vitamin C

- ✓ accepts single e- from O2-, H2O2, OH•, HOCl , and peroxyl radicals.
- ✓ It regenerates the reduced form of Vitamin E.
- ✓ It's water-soluble, found in the cytosol.

Formation of Glucouonate and its uses

- Glucuronic acid is a sugar acid which is glucose with oxidized form of carbon 6.
- The active form of Glucuronic Acid that donates the sugar in glycosaminoglycan synthesis and other glucuronylating reactions is UDP-Glucuronic acid.
- UDP-glucuronic acid is formed by the oxidation of UDP-glucose on carbon 6 (–CH2OH →–COOH) using one molecule of H2O, with reducing of two molecules of NAD+ into NADH.
- This reaction is catalyzed by UDP-glucose dehydrogenase.

❖ UDP-glucuronic acid donates glucuronic acid, which is required in detoxification reactions of a number of insoluble compounds, such as bilirubin, steroids, and several drugs, including morphine. These compounds are conjugated with glucuronic acid, to form glucuronide because it is more soluble, so it's easy to be excreted.



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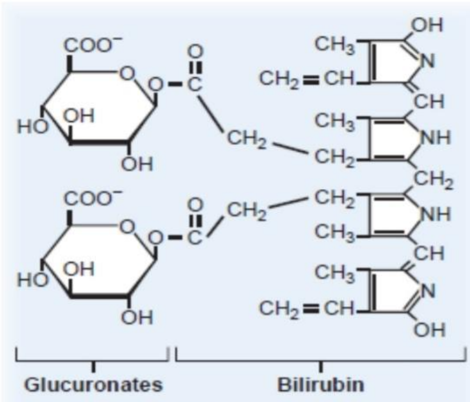
❖ Such as : bilirubin diglucuronide. Its formed when bilirubin, which is produced from heme metabolism, binds to glucuronic acid in the liver. Then it's transported to the large intestine, where it's reduced and then excreted from the body in urine and fecal matter. It gives urine its yellowish colour.



Bilirubin diglucuronide

❖ It is an essential components of glycosaminoglycans.

❖ In plants and mammals "including humans" Glucuronic acid serves as a precursor of ascorbic acid (vitamin C).

UDP-glucose in metabolism :

❖ Remember that UDP-glucose is involved in many pathways.