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Biochemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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In the name of God, the Most Merciful, the most Kind

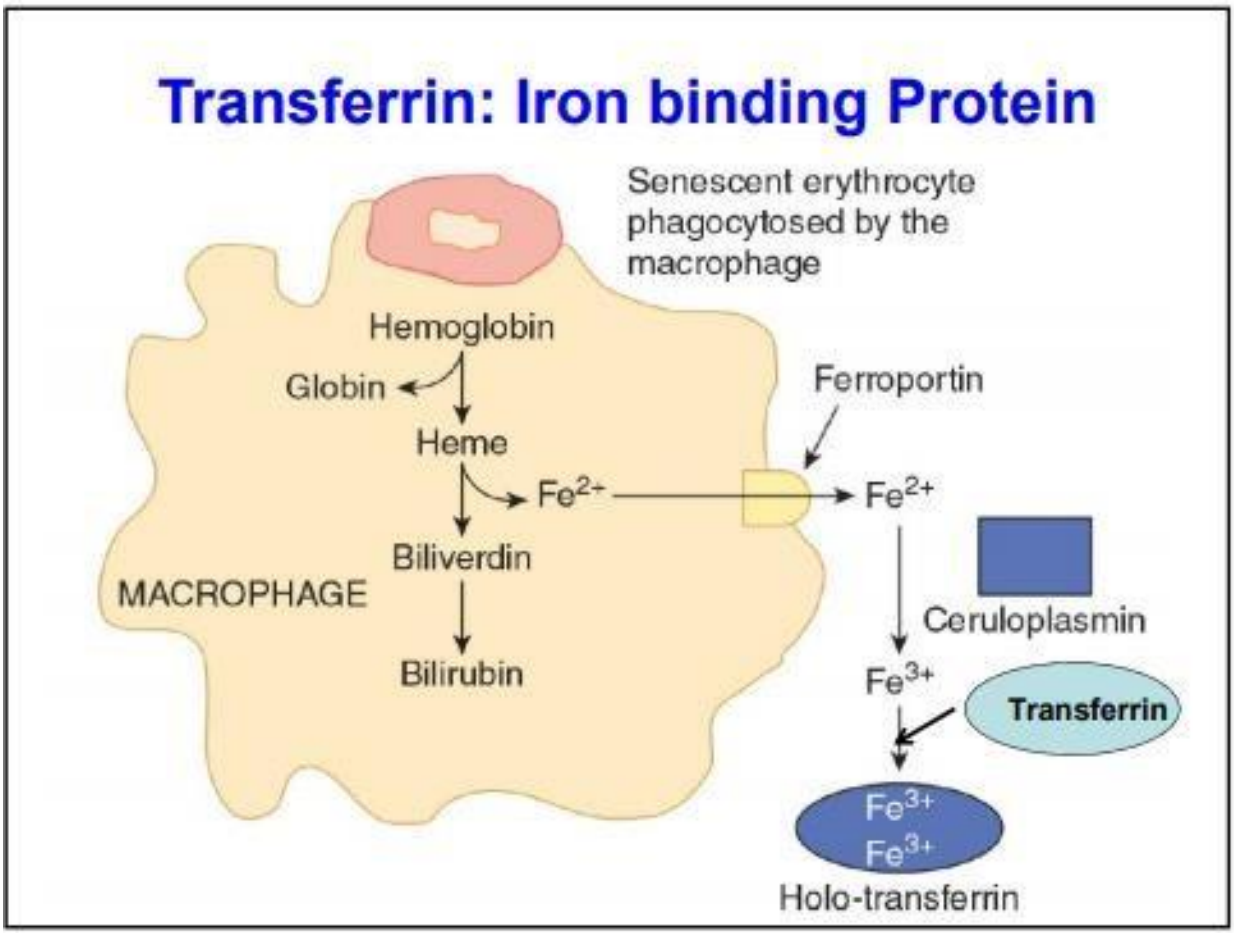
P.s.: Doctor Faisal explained the unfinished part of Plasma Proteins at the beginning of this lecture so it is included in this sheet and then comes the introduction to Lipids. Good luck!

Ceruloplasmin:

- ***Ceruloplasmin is one of the plasma proteins and it's an α2-globulin (remember that plasma proteins are divided into: α1, α2, β and γ)***
- ***160 kDa***
- ***Binds to 90% of the plasma copper (6 atoms/molecule) and albumin binds the remaining 10%***
- ***It's the major copper binding protein in plasma by which copper tightly binds to Ceruloplasmin. Pay attention that Ceruloplasmin only binds to copper, it doesn't transfer it from a tissue to another (e.g.: from small to intestine to somewhere else)***
- ***It was also found that it has oxidase activity acting as a ferroxidase and oxidizing iron***
- ***There are some genetic disorders related to the synthesis of Ceruloplasmin which may lead to:***
  - ***Hypoceruloplasminemia: it is compatible with life and the individual having it may live a normal life***
  - ***Aceruloplasminemia: complete absence of the Ceruloplasmin (no ceruloplasmin in the blood), a severe disease that results in very bad consequences.***
  - ***Wilson disease: accompanied by Ceruloplasmin metabolism where the synthesis of the protein is normal but the corporation of copper in ceruloplasmin is impaired (low incorporation of copper into newly synthesized ceruloplasmin polypeptides). So, the level of ceruloplasmin (complexed with copper) in the plasma is decreased even though the level of copper in the liver may be high and the liver is not able to excrete the access copper, so it accumulates instead and that's where the problem happens. Thus, it's not a problem in the protein but in other proteins involved in the complete synthesis of Ceruloplasmin.***

    This disease can be treated by:

-limiting the intake of copper in food and by using agents that bind to copper, make it soluble thus facilitating its secretion in the bile.



The figure above shows the function of Ceruloplasmin as a Ferroxidase; in which it oxidizes the iron that results from Heme degradation.

- ***When Heme is degraded by macrophages, Iron (Fe^{+2}) is secreted into the plasma and since the Fe^{+2} form (Ferrous) is toxic, it gets converted into the fe^{+3} form (Ferric) by the action of **Ceruloplasmin**. After that, it can be attached to the protein **transferrin** and form **Holo-transferrin** (Fe^{+3} + transferrin).***
- ***This way Iron gets recycled/reused after Heme degradation.***

Copper:

- ***An essential trace element***
- ***Found in food***
- ***Average daily intake is: 2-4 mg***
- ***Body contains ~100 mg***

- *Can alternate between Cu^{+2} and Cu^{+1} and this is how it does its function in oxidation-reduction reactions*
- ***Metallothioneins**, a group of small proteins regulate tissue level of copper (even though it's an essential element for life and no life without copper but excess copper can be harmful)*
- *A cofactor for a number of enzymes such as:*
  - *Cytochrome oxidase, amine oxidase*

Haptoglobin:

- *α2 glycoprotein (90kDa)*
- *Synthesized mainly by hepatocytes*
- *Two kinds of polypeptide chains:*

  Two α chains, One β chain

- *Three polymorphic forms (its gene can synthesize polymorphic forms)*

  Hp 1-1 (homozygote; inheriting the same form), Hp 2-1 (heterozygote; inheriting two different forms), Hp 2-2 (homozygote)

  Polymorphism is associated with many inflammatory diseases

- *It is an acute phase protein*

  ↑ Level in a variety of inflammatory states, burn, and nephrotic syndrome

Haptoglobin main function is binding free hemoglobin:

➔ *Usually hemoglobin doesn't reach the plasma as (90%) of the hemoglobin (from aged RBCs) is taken by the reticuloendothelial system/phagocytes and gets degraded there.*

➔ *But the other (10%) of hemoglobin is degraded each day and gets released into the plasma where it binds to the plasma protein **Haptoglobin**. The molecular weight of hemoglobin is 65kDa which is small and easy to be filtered by the kidney. if so, iron will be lost through urine and the hemoglobin by time will precipitate in the kidney leading to the damage of the kidney.*

*To prevent the previously discussed complications, **Haptoglobin** binds to Hemoglobin forming a large complex (90 kDa + 65 kDa = 155 kDa) which can't be excreted in urine and will be soon taken by the liver **thus iron won't be lost but taken by the liver.***

This is a protection mechanism for both iron and kidneys.

- *The Hp level can be measured as **Hb binding capacity** (how much Hemoglobin is bound to Haptoglobin) and normally it's 40-180 mg.*
- *Haptoglobin level is greatly decreased in Hemolytic anemia.*

If there is a disease that causes rupture of RBCs (e.g: Hemolytic Anemia), more amount of hemoglobin will be released into the circulation and it will bind to Haptoglobin making the capacity completely saturated.

- *Hb-Hp complex has shorter half-life than that of Hp (the complex is rapidly taken by the liver) // Hb-Hp complex is cleared 80 times faster than Hp*

*$t_{1/2}$ **of Hp is 5 days** while $t_{1/2}$ **of Hb-Hp is 90 minutes***

α1-fetoprotein (AFP):

- *First discovered in the serum of the fetus (NOT IN ADULTS; we don't have it normally // **ps: I know it's written that is available in very low levels in adults but the doctor said the above sentence so it had to be added**)*
- *Detectable in the maternal blood in pregnancy*
- *Elevated level in some congenital defects*
- *Low level indicates increased risk of Down's syndrome*
- *High level in many cases of liver cancer (tumor marker)*

α2-Macroglobulin:

- *Large protein (720 kDa)*
- *Tetramer of 4 identical chains*
- *8-10 % of the total plasma proteins (the most abundant after albumins, globulins)*
- *Synthesized by: **Monocytes, Hepatocytes, Astrocytes***
- *Level varies with age, gender*

- *Increased level in nephrotic syndrome (a disease that affects the kidney making it more permeable usually due to increased synthesis by monocytes and other proteins)*
- *It is a pan proteinase inhibitor (just like α1-antitrypsin discussed earlier)*

Proteinases include trypsin, pepsin, plasmin, thrombin
The proteolytic enzymes in plasma are important but should be controlled and that's done by α2-Macroglobulin

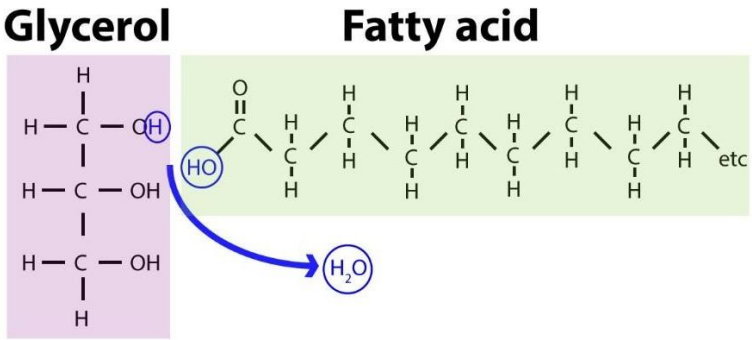
- *Inhibitor of Coagulation, Fibrinolysis*
- *Forms a complex with the proteinase followed by clearance; "Venus flytrap" mechanism:*

(Binding α2-Macroglobulin to protease where the protease cleaves one of the peptide bonds in the protein: this protein has an internal cyclic thioester bond (a high energy bond found between two A.As) linking a cysteine and a glutamine residue and once it gets cleaved, the protein is able now to bind covalently to the protease inhibiting it.

*In other words, the protease degrades one bond in the α2-Macroglobulin so this protein then traps the protease and binds to it. (*Flytrap: a plant that traps flies)*

- *Binding to many cytokines (signal molecules) and directing them to their target cells*

Lipids Metabolism



Glycerol is a three carbons compound with three hydroxyl groups bound to each carbon, each hydroxyl group forms an ester bond with a fatty acid (these three fatty acids can be different) forming a Fat molecule.

This ester bond can be cleaved by attacking water and generating a fatty acid and the OH of glycerol.

**NOTE: usually, the second (middle) fatty acid is unsaturated.*

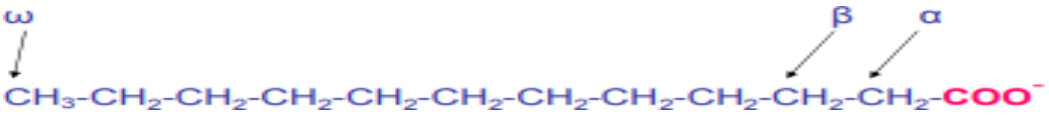
Fatty acids have a long hydrocarbon chain that contains a carboxyl group at the end of it and doesn't contain oxygen so this long side chain is completely hydrophobic/non polar.

But the pKa of the carboxyl group in fatty acids is ≈ 4.8, so, at physiological pH fatty acids exist as anions (as COO- not COOH). The ionized form is slightly more soluble than the protonated form due to the presence of the negative charge at the carboxyl group.

The structure is usually written as an abbreviated form $(CH3(CH2)_nCOO-)$ rather than the long formula.

The number of carbons is usually even and this reflects the fact that they are built of the condensation of two carbon units (Fatty acids synthesis usually starts from acetates: two carbons + two carbons + two carbons….= even number of carbons)

- ➔ *We have to number the carbon atoms and we usually start from the carboxyl group carbon so it's considered as carbon 1. We can also designate the carbon atoms using Greek letters (α, β, γ….) so the first carbon after the carbon of the carboxyl group is Cα, the third carbon of the chain is β and so on.. the last carbon is called Omega (ω) regardless of the length of the fatty acid (because omega is the last letter of the Greek alphabet).*



In unsaturated fatty acids, we have to identify the carbons involved in the double bond(s).

In this example: $CH3-CH2-CH2-CH2-CH2-CH=CH-CH2-CH=CH-(CH2)_7-COO^-$

Unsaturated Fatty Acid 18:2Δ9,12 or 18:(9,12)

The first double bond is between carbons (9:10) and the second one is between carbons (12:13), so we address the double bonds location by writing the carbons numbers which are before the double bond and in this case they are carbons 9 and 12. So the double

bonds are at carbons (9,12) and the fatty acid length is 18 carbons so the fatty acid description can be written as 18:2Δ9,12 or 18:(9,12).

**This is an example of a poly unsaturated fatty acid and in humans' body we can't synthesize it, although we need it as an essential fatty acid and we get it from food.*

- ➔ *Notice that we don't need to write the complete structure of FA, a simple abbreviated form which describes how many carbon atoms are in the structure, the number of double bonds and their location is enough to give you the complete information about the fatty acid. But you have to imagine the structure yourself.*

The hydrocarbon chain can be saturated or it may contain one or more double bonds.

- ➔ *Another way to refer to the double bonds location is **counting from the omega end.***

For the example above: if you count this way, you will find out that the first double bond is at carbon 6 so it's called an omega-6 fatty acid (ω6).

If you would like to know the type of the fatty acid mathematically: **you subtract the number of carbon which is at the second double bond (counting from the carboxyl side) from the number of carbons this fatty acid contains.

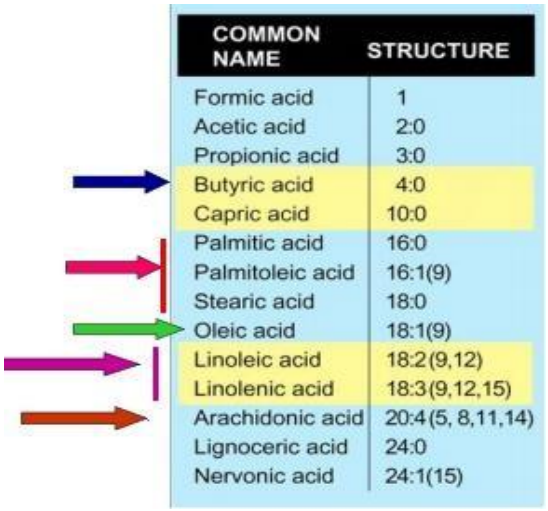
*Applying for the example above: it's an 18 carbons fatty acid and the second double bond is at the twelfth carbon so (18-12=6), this way we conclude that it's an **ω-6 fatty acid.** Notice that we don't need to write the whole structure to know the type of the fatty acid, this can be done just by knowing the number of carbons and double bonds available.*

Some Physiologically Important Fatty Acids

**Formic acid, Acetic acid and Propionic acid are not fatty acids, they are carboxylic acids and mentioned here just for comparison.*

NOTICE that fatty acids start with four carbons.

**Butyric acid is a 4 carbons fatty acid and it is an example of a short chain fatty acid. It's called butyric because it was isolated first from the butter.*



COMMON NAME	STRUCTURE
Formic acid	1
Acetic acid	2:0
Propionic acid	3:0
Butyric acid	4:0
Capric acid	10:0
Palmitic acid	16:0
Palmitoleic acid	16:1(9)
Stearic acid	18:0
Oleic acid	18:1(9)
Linoleic acid	18:2(9,12)
Linolenic acid	18:3(9,12,15)
Arachidonic acid	20:4(5, 8,11,14)
Lignoceric acid	24:0
Nervonic acid	24:1(15)

Butter and dairy products contain large quantities of butyric acid therefore it is named according to its source.

Most of the fatty acids are given names according to their source (which they were isolated from at first).

Butter is rich with butyric acid and diary milks are usually rich with short chained fatty acids.

**Palmitic acid: a saturated 16-carbons fatty acid found in the palm trees.*

**Palmitoleic acid: a monounsaturated omega-7 fatty acid.*

**Stearic acid: found in wax.*

**Oleic acid: a monounsaturated omega-9 fatty acid found in olive oil.*

-Palmitoleic acid and Oleic acid are monounsaturated fatty acids.

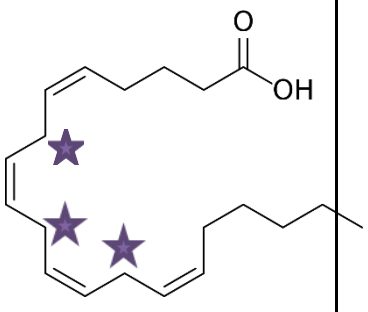
-Notice that Linoleic acid and linolenic acid (both polyunsaturated fatty acids derived from linen), their names are very close so we have to distinguish between them:

**Linoleic acid: contains two double bonds and their locations are at 9,12.*

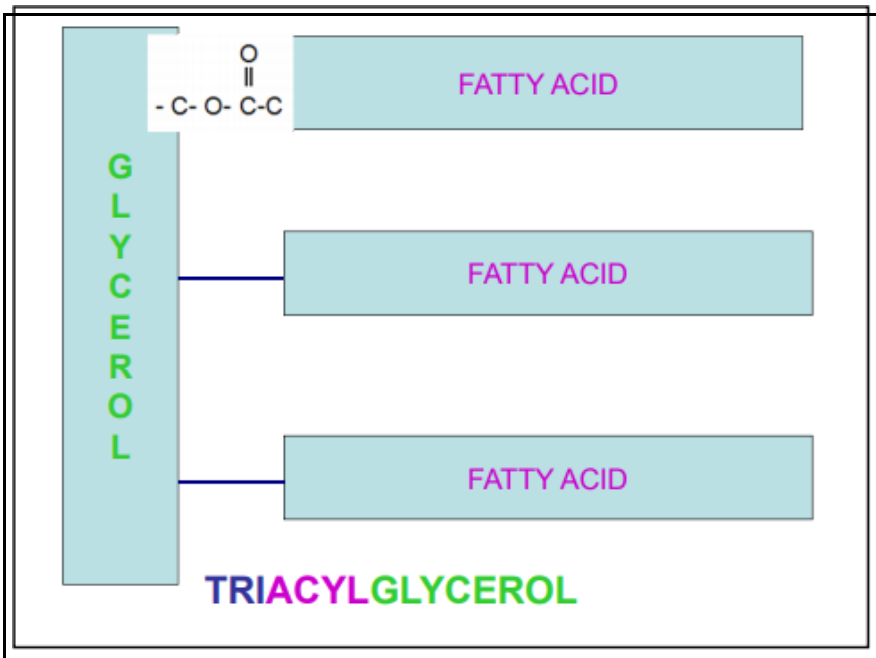
**Linolenic acid: contains three double bonds and their locations are at 9,12,15.*

**Arachidonic acid: a 20 carbons polyunsaturated fatty acid which contains 4 double bonds at carbons (5,8,11,14) and is found in peanuts.*

-Notice that only one CH2 separates between double bonds so we add 3 for each double bond location. Double bonds are always non-conjugated and separated by a CH2.



An Example for clarification:



Triacylglycerol (TAG) (chemical name) or FAT (in animals) or oil (in plants). Whether it's oil or fat, they are all Triacylglycerol and it's the major energy reserve in the body.

The difference between fats and oils is the melting point; the melting point for oils is low so they are found liquid in the room temperature while it's high in fats and that's why they are found solid at room temperature. The degree of unsaturation affects their state (liquid or solid) at room temperature. The presence of double bonds/unsaturated fatty acids greatly decreases the melting point, so plants oils contain polyunsaturated fatty acids. (The cis double bonds in the unsaturated fatty acids cause "kinks" in the structure of the carbon chain. As a consequence, unsaturated fat molecules cannot pack together very well and are liquids at room temperature)

Why Fats not Carbohydrates are used as a source of energy:

** Because they are more reduced (less oxygen; fats contain less oxygen than carbohydrates) so they are able to be oxidized more than carbohydrates thus produce more energy (twice as much).*

9 kcal per gram compared with 4 kcal per gram of carbohydrates

** Hydrophobic: can't form hydrogen bonds with water and can't adsorb water while carbohydrates can due to the presence of large number of hydroxyl groups. So, if you put fats in an environment full of water, they are separated. While a paper (cellulose) becomes wet in water, plastics don't.*

-Fats can be stored without H2O while carbohydrates are hydrophilic (stored in the cells with water; unless the cell is very dry)

-1 gram of carbohydrates: 2 grams of H2O (e.g. with each 100 grams of carbs, there are 200 grams of water because carbohydrates are very hydrophilic, absorb water, get stored with water and form hydrogen bonds with it)

Average adult has 10 Kg of Fat

How many calories can be obtained by the complete oxidation of fats?

-90,000 kcal in an average (not fat or slim) 70-kg male adult.

****90,000 kcal is a huge amount of energy. For example: you may raise the temperature of a one cube meter of water from 1C to 91C by using 90000 kcal.***

How much do we eat daily? About 2000 kcal.

So if we don't eat anything for 45 days, our fat storage can be sufficient for these 45 days.

-If carbohydrates were the form of energy storage, it would be 22.5 Kg. And because for each gram of carbohydrates, there are two grams of water, 45 Kg of water must accompany the carbohydrates. This is not advantageous so the storage of energy as fats is more efficient for us.

-Fatty Acids are not just the storage form but also the preferred fuel between meals (after 3-4 hours of food intake, absorption, and transition are over, the preferred fuel is fatty acids). Fatty acids are the major fuel used by tissues (less found in plasma) but Glucose is the major Fuel in extracellular fluids (blood, ICF, plasma). Yet fatty acids which are found in much less amount are preferred to be used as an energy source except for the brain.

Fuel Type	*Amount in Fluids (g)*	*Amount used in 12 hours (g)*
FA	*0.5*	*60 (540 kcal) 'continuously recycled/rapid turnover and which tells that adipose tissue is not a static tissue'*
Glucose	*20*	*70 (280 kcal)*

****Fat is stored in Adipose tissue and when it is needed by any other tissue, it gets transported to that tissue when hormonal signal reaches the adipocytes.***

Please pay attention that when we are talking about the rapid turnover of fats, we don't talk about how fast it is but how often it happens.