Digestion and transport of tri Acylglycerol by plasma lipoproteins

- *slide* 1
The structures that you can see in this slide are:

1- The structure of glycerol (3 carbon compound with three hydroxyl group)
2- Fatty acid with 18 carbons

- Digestion involves hydrolysis of the ester bonds.
- Carboxyl group of the fatty acids can make ester bond with the hydroxyl group of glycerol
- Once the ester bond is formed the negative charge of the fatty acid is no longer existing (the ester bond doesn't have any charge)
- When three fatty acids are esterifies we get tri acyl glycerol (TAG), so it is a neutral fat (completely hydrophobic with no charges)
- TAG is oil so it can't be mixed with water, that's why it's transported in plasma, by lipoproteins (which makes TAG in a water soluble form), their whole transport depends on the presence of lipoproteins.
- The thing that aids in solubilizing the TAG is the phosphoacylglycerol because these are polar lipids and they can form micelles (phosphoacylglycerols are amphipathic molecules)

- What does lipoprotein mean?

As the word indicates, it's a protein that has the adjective of lipids (protein that have some lipid nature), it's a multimolecular (consists of more than one molecule –lipids & proteins- that join together) structure that is in a soluble form
-its function is to transport lipids in the plasma
- The lipids that are found in the lipoproteins include:
1- TAG (nonpolar lipids)
2- Free cholesterol (polar lipids).
3 -cholesterol ester (nonpolar lipids)
4- Phospholipids (polar lipids)
- 2 & 4 have polar functional groups (hydroxyl group in cholesterol, and we have negative and positive charges in the phospholipids)
- Neutral lipids are the non polar lipids

Apolipoproteins

- Apo as a suffix always means the protein part of the structure (ex. 1 Apoenzyme, which means the protein part of the enzyme 2 apotransferrin: it is the protein part of transferrin that transfers iron)
-so apolipoproteins mean the protein part of the lipoproteins, they are amphipathic molecules that have both polar and non polar regions
- they include several classes: Apolipoprotein: A, B, C and E... etc)

- Functions of apolipoproteins:
1- Structural role 2- they regulate some enzymes
3 -they are important for binding with some surface receptors

-** slide with representation of lipoprotein particle
- It shows that it has a peripheral region (the surface component) of the lipoprotein is formed from the phospholipids and unesterefied cholesterol (the polar lipids)

- The apolipoprotein is found also in the surface (being amphipathic, that it's hydrophilic components are found outside whereas it's hydrophobic components are found inside) and it's part of the surface component
- Whereas in the inner core (core component) of the lipoprotein, it’s formed from cholesterol ester and TAG

Classes of lipoproteins

-lipoproteins are found in plasma, and can be classified according to their density as:
*Density of water = 1 g/ml
1- Chylomicrones: their density is less than0. 95 which means it is less than water which means in turn that those molecules will float in water
2- VLDL (very low density lipoproteins): their density is almost like water density but still less than density of plasma (plasma density is higher than water density).
- Hydrolysis of TAG requires hydrolysis of the ester bond
3- IDL (intermediate density lipoproteins): their density is more
4- LDL (low density lipoproteins): their density is even more
5- HDL (high density lipoproteins): they have the highest density

- *What are the methods used to separate them (according to their densities)?*
1- Ultracentrifugation: centrifugation at very high speeds with force more the 100000 G's (100,000 times of ground gravity force)

- What determines the density and makes it high or low?

It depends on the percentage of protein component of the lipoprotein. As we all know the protein is more dense than water, the lipid is less dense than water.
-So, the higher the ratio of lipids to proteins, the lower is the density.
- so as the doctor shows in the slide : the percentage of protein in the chylomicrones is 2%, where as in the HDL this percentage is about 45 %, approximately half of HDL particle is protein, and the percentage in the rest of lipoprotein classes is in between.
- don't memorize the numbers/ figures in the slides, you just have to know the trend, for example, kno that there is an increase in the density as we go from chylomocrones to HDL " ya3ne e3raf enno kol ma yzeed % of protein in lipoprotein betzeed al density"
- You can notice from the slide that there is range of the density for each class, and that is because not all of the particles are similar within the same class, and those particles are continuously being changed as they undergo metabolism by water, that’s why density is continuously changing.
- memorize the trend of increasing protein component from 2%-45% from chylomicrones to HDL.

- *What are the major lipids in lipoproteins?*
-in chylomicrons: 90% of it are TAG
- VLDL: 55% of it are TAG
- IDL and LDL: 30%, 35% cholesterol ester respectively.
- HDL: 25 % phospholipids, 45 % proteins, so 70% are lipids and proteins, and 30% or less are other components.

-** slide shows apolipoproteins distribution on lipoproteins

- To memorize them, note this:

1- The only apolipoprotein in LDL is apolipoprotein B.
2- Apolipoprotein A is only found in HDL
2- Apolipoprotein B is found in all except the HDL
(The slides were not with me so forgive me u need to refer to them … ☺)

Functions of Apoproteins

Chylomicrons: transport of dietary lipids
- *VLDL*: transport of endogenous TAG from liver to other tissues, endogenous TAG means TAG that is synthesized inside the body. The major organ that synthesizes TAG is liver from excess carbohydrates.
- *The rest of lipoproteins* (IDL, LDL, HDL) their function is to transport cholesterol.
** The major lipids determine the function for each class of lipoproteins

"The doctor showed a slide that contains a picture showing the composition of the lipoproteins classes, he said that you might see these compositions varying from one book to another, but don't worry, you just have to understand the trend mentioned previously at this sheet"

The impact on the size

- *How can we predict which one of the lipoproteins is bigger in size?*
You can know this by knowing the ratio of surface component. Relatively, the higher the % of surface component, the lesser is the size.
- **For simplifying this**: let's suppose that we have one watermelon that weighs 12 KG, on the other hand, we have 4 watermelons each of weighs 3 KG (all together = 12) , so which one produces more peel?
- The answer is the 4 small watermelons
- The conclusion: the size and surface area are <u>inversely</u> related

The chylomicrons contain 95% TAG, it's the least in density and that means it's the biggest in size (1 micrometer as much as the bacterial size)
- Whereas the size HDL is very small (high density), chylomicrons are 100 times bigger.

Electrophoresis

- We talked previously in this sheet about separating the lipoproteins according their density by the ultra centrifugation, anyway this method is not convenient, and it's really expensive, so we need to use another method.

- We can use a routine, cheap & simple method to separate them, and this method is called electrophoresis which depends on the charge to mass ratio (as in plasma protein separation that we took previously and then we used a stain for proteins to visualize the separated plasma proteins).
- If we use *stains* specific for *lipids* then we can visualize lipoproteins, so the procedure of separating lipoproteins in electrophoresis is the same as separating plasma proteins, but instead of using stains specific for proteins, we use stains specific for lipids.

In electrophoresis of lipoproteins we note:

1) The fastest will be the HDL, since it is the smallest and the most negatively charged *why??* . Because it contains large percentage of proteins, and proteins contains a lot of negative charges, so HDL can move faster toward anode (positively charged electrode)
- HDL is also called alpha lipoproteins because migrates with alpha fraction of the plasma proteins
2) We can note that the chylomicrones don't move from the origin/ don't migrate in the electrical field, because of their large size and it has low percentage of proteins, only 1-2 %.
3) VLDL migrated faster than LDL and slower than HDL, they did not follow the trend as to be expected (HDL then LDL then VLDL); as the protein components differ from each other so the charge differs between them *"the migration/separation is not based on the density, it is based on the charge to mass ratio".*
- LDL is called beta lipoproteins because it migrates with the beta fraction of plasma proteins
- VLDL is called pre-beta (before beta) because it migrates faster than the beta fraction of the plasma proteins.
- So, the actual migration speed: HDL>VLDL>LDL>Chylomicrons

Digestion of dietary lipids

- Digestion here is hydrolysis of the ester bond between glycerol and fatty acid
-digestion is important for the absorption of the lipids, without it we can't absorb the lipids from the GI tract.
- Digestion is hydrolysis of two fatty acids (from TAG) to get two fatty acids and mono acyl glycerol
- Digestion of cholesterol ester includes hydrolysis of the ester bond between cholesterol and fatty acids
- Reaction of hydrolysis of ester bond requires attack of the ester bond by water, so it requires mixing water and oil for the digestion/ hydrolysis.
- But we have a problem with the solubility, so how can we make TAG soluble with water for digestion?

- We can do this with the **Emulsification**
**- slide shows the compounds that aid in the process of emulsification (solubilization of lipids)
- They are the cholic acid and the chenodeoxycholic acid: those structures look similar to the cholesterol, actually they are not cholesterol, but they are derived from cholesterol
- How do they differ from cholesterol?
*They have carboxyl group, which is not found in cholesterol.
*Cholic acid has 2 more hydroxyl groups, whereas chenodeoxycholic acid has 1 more hydroxyl group, both in comparison with cholesterol which has an original hydroxyl group on carbon #3 (-OH on the first ring to the left of the structure)
-They are converted to amphipathic molecules, and they are called bile acids
- Sometimes these bile acids are conjugated (added or linked) to another amino acid such as: glycine or taurine
When we mix a bile acid with glycine (pKa for glycine is lower than the bile acid's pKa) it becomes a stronger acid and the reason is between the brackets above, and it will exist mainly in the ionized form, now we call this conjugated bile acid: "Bile salt"
- We can use bile acids and bile salts interchangeably and it depends how it is - ionized or not ionized-
- So both of them are emulsifying agents. But they differ in their biochemical structure as shown above

As you can see in slide 8 the structure of the bile salt consist of two parts hydrophobic and hydrophilic part, so they can form micelles and within the interior of the micelles there are the TAG and cholesterol esters

- So, bile salts are able to form micelles with the phospholipids, these micelles are multimolecular complexes, that have hydrophilic surface and hydrophobic interior, within the interior are the TAG + CE.
- It's called mixed micelles: consist of TAG, cholesterol esters, phospholipids and bile salts.
- The benefit of making this (micelle) is: the size of these micelles is very small so there surface area will be relatively large, so large surface area allows the enzymes (lipase or esterase etc) to bind to the mixed micelles.
- Lipase is secreted from the pancreas as prolipase and will be activated in the small intestines, lipase also requires another protein that is also secreted from pancreas, this protein is called "co-lipase"
- the action of the lipase requires the formation of TAG in micelles form, and without the micelles form, the surface area will be small and the lipase will lose it's function and the contact between TAG and water will be less as well , and the result will be no digestion for TAG
- Recall, digestion of TAG by lipase with co-lipase includes hydrolysis of two fatty acids from the TAG, to form two fatty acids and MAG (mono acyl glycerol)

Slide # 9 (digestion of various lipids)

1) Digestion of cholesteryl ester: it's done by cholesteryl esterase to hydrolyze the ester bond between cholesterol and fatty acid to release free fatty acid and cholesterol
2) Phospholipids are digested by phospholipases to release two fatty acids and GlycerylPhosphorylCholine , then both of the products are absorbed
3) Digestion of TAG: catalyzed by the pancreatic lipase, it was mentioned above, note that the hydrolysis occurs on carbon #1 and carbon #3 and the product will be 2-mono acyl glycerol

- What if we inhibit the pancreatic lipase?
- there will be no more digestion for TAG , so it will not be absorbed , so they will get out with the feces and this will cause what is called " steatorrhea"; the feces/ stool will contain large amount of undigested fat so you can enjoy eating without worrying about getting fatter, because you are actually eating fat but not absorbing it ;)
- You can find this drug which inhibits the pancreatic lipase "anti obesity drug" and it costs about 40 JD, it's known as orlistat. "Though it's still better than the old ways of the Romans :P "

** The slide shows us where the digestion of dietary fats starts

- all the hydrolysis occurs in the small intestine, but the digestion of TAG with short and medium chain fatty acids (as those found in the diary milk) begins in the stomach due to the presence of gastric lipase & lingual lipase (secreted by lingual glands in the tongue, and it is acid stable and it will not be destroyed by the acidity of the stomach)
- Lingual lipase significance is in neonates who depend on the fat in the milk for their nourishment
- Digestion of fat in stomach doesn't require bile acids or pancreatic lipase, so the digestion can start early, so it is important in pancreatic insufficiency so the lingual lipase can digest some of fats in the milk

-** slide # 9 part two shows: after digestion, these are the mixed micelles that contain fatty acids, MAG and free cholesterol

- these micelles go along the small intestine , and when they come in touch with the intestinal mucosal cells , the components (products) of the digestion are absorbed by diffusion through the membrane , note that the convolutions are present to increase the surface area for the contact between the mixed micelles and the small intestinal cells for the absorption
- Now, once inside the cell, the next step is to transport the products into the blood, and for transport into the blood the TAG are re-synthesized
- So MAG are esterified to two fatty acids to form TAG, and the donor of the fatty acids is the fattyacyl-COA (the absorbed fatty acids are reactivated to fattyacyl-COA)
- So digestion is needed just for crossing the plasma membrane of the small intestinal mucosal cells and before they go to blood they get re-esterified to TAG

- Similarly cholesterol is converted to cholesterol ester before being transported into the blood.
- After that, these TAG and cholesterol ester with apolipoprotein B-48 will form the particles (chylomicrons) called nascent "new" chylomicron
- 90% of the chylomicrons size consists from TAG from dietary origin, these chylomicrons are so large (it's diameter is so close to the diameter of the cell that contains it), so they will be excreted by exocytosis – they can't cross the membrane- to the lymphatic system NOT to the blood
- Why to lymphatic system? And not to the blood vessels? Due to their large size & number they will block the capillaries of the small intestine, so instead, they will go to the lymphatic vessels, then to the lymph nodes, then to the thoracic duct, then they will enter the blood through entering the subclavical vein, which is a large vein and they will not block small capillaries in this way.
- What happens to them in the blood?
- chylomicron (after being secreted in blood) will acquire apolipoprotein C II & apolipoprotein E, which both are transferred from HDL to chylomicrons, so they will become mature chylomicrons (it contains TAG, cholesterol and apolipoproteins (B-48 ,C2 and E))
- Those micelles go into the capillaries, and in the capillaries, there is an extracellular enzyme called lipoprotein lipase, made by the endothelial cells. However it is not in the cells, it is secreted and remains attached to the endothelial cells. Therefore when the chylomicrons come in contact with it, It acts on the TAG in the lipoproteins (in the chylomicrons)
- So the lipoproteins (the chylomicrons) are digested by the action of lipoprotein lipase
- This lipase is not the same of pancreatic lipase, this lipoprotein lipase acts specially on chylomicrons and VLDL, it requires activation by apolipoprotein C-II "comes from HDL"
- This lipoprotein lipase is found in many tissues such as: adipose tissue, muscle and other tissues. However the enzyme of the adipose tissue is slightly different than the enzyme found in the muscle, that the muscle enzyme has lower KM (higher affinity) for TAG, so it gives us an indication that chylomicrons preferably go to the muscles rather than to the adipose tissues, and this makes sense; because the adipose tissue role is to store the TAG not for consumption, so whenever the lipoprotein concentration is low, it will go preferably to the muscles because they have different enzymes
- So after most of the TAG is removed, what happens to the size of the chylomicron? It will be greatly decreased; because of the removal of TAG
- The HDL will get back/ restore the apolipoprotein-C II, and what remains from chylomicrons will be a smaller particle that contain less TAG, cholesterol and cholesterol esters. There name will be then chylomicrons remnants, those have apolipoprotein-E which is important for binding to the cell surface of the liver where the particles will be taken by the endocytosis
- So the final place/site where the chylomicrons reach is the liver cells, after most of the TAG is removed and transferred to tissues.
-VLDL also has the same story but **The origin of VLDL is from the liver, why? Because VLDL takes the TAG that is synthesized in the liver from the excess carbohydrates **It is secreted directly to the blood not as the chylomicrons (which are secreted to lymphatics first) –because of it's smaller size in comparison to cholymicrons.-
- After removal of most TAG from the VLDL (by the same enzymes used with chylomicrons), the remnant of it will be IDL then LDL (smaller in size and more dense)
- The LDL origin is from the VLDL as we can see

"I am not sorry for any mistake you know how to find me o_O"