Metabolism

فريق طوفان الأقصى

Modified N: 13

Writer : سلطان غيث
صهيب زعيتر

Corrector: سلطان غيث
صهيب زعيتر

Metabolism of lipids IX:

Plasma lipoproteins

Prof. Mamoun Ahram

NOTE: The topic of lipoproteins is clinically important

Resources

- This lecture
- Lippincott's Biochemistry, Ch. 18

Characteristics of lipoproteins

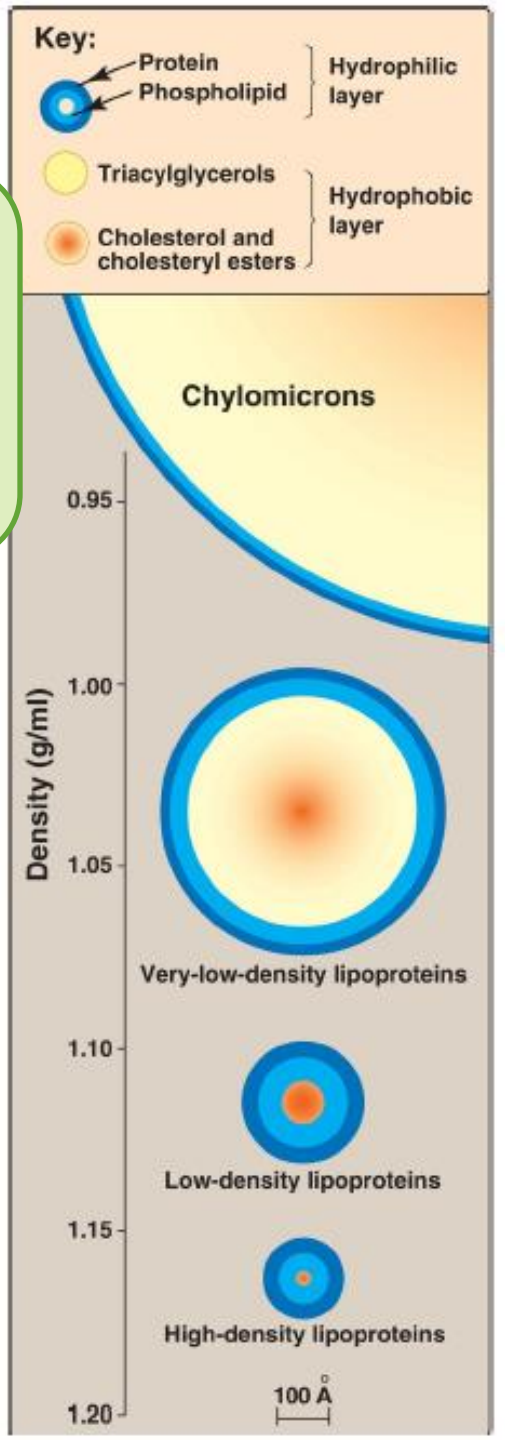
- Lipoproteins function to
  - Solubilize and carry plasma lipids
  - Transport lipids to (and from) the tissues

NOTE: lipoproteins also differ in the function ,route and the way they are transported

- They range in size and density and have variable purposes and lipid and protein composition.

The higher the protein:lipid ratio, the higher the density

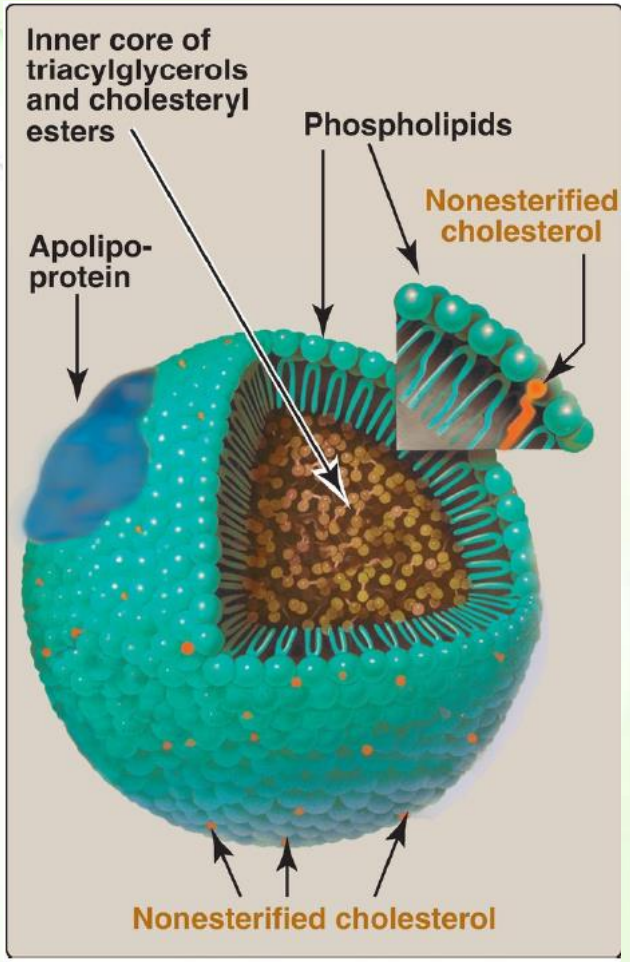
Note : About the function (solubilize and carry plasma lipids).....lipids aren't found freely in the blood because they would tend to cluster and aggregate so, they need a molecule to carry them in the blood –make them soluble- .... that's exactly what lipoproteins do



Lipid composition of lipoproteins

- A neutral lipid core (containing TAG and cholesteryl esters) surrounded by a shell of amphipathic apolipoproteins, phospholipid, and non-esterified (free) cholesterol.
  - These amphipathic compounds are oriented such that their polar portions are exposed on the surface of the lipoprotein.
- Sources of the lipid cargo: diet (exogenous source) or de novo synthesis (endogenous source).

- Total cholesterol=LDL-C + HDL-C + VLDL-C
  - VLDL-C is calculated by dividing TAG by 5 because the TAG/cholesterol ratio is 5/1 in VLDL.
    - The goal value for total cholesterol is <200 mg/dl.



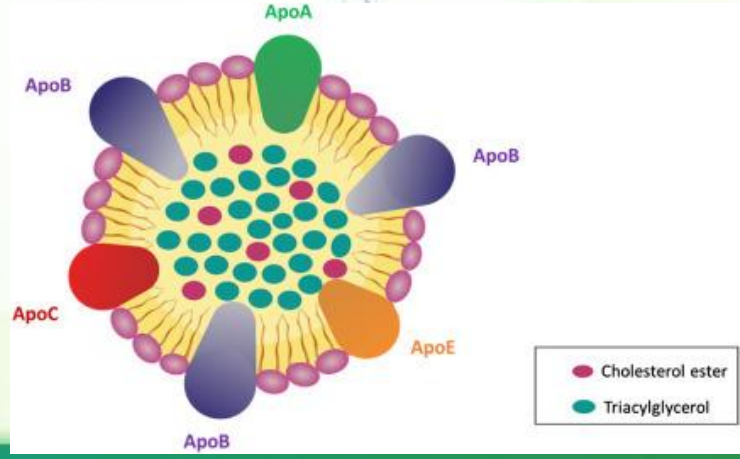
- **The complement in this slide:** As we said , among the lipoproteins, the lipid composition is different but in general, they have similar structure…..they look like (Micelles) as the hydrophobic region is embedded inside while the hydrophilic region and charged group such as phosphate are outside

- **NOTE:** Scientists said that there are two routes that lipoproteins take: **exogenous route** (transporting dietary lipids –lipids we eat- from intestine to liver… and **endogenous route** (transporting lipids that are synthesized internally… specifically in the liver , and how they are transported from the liver to peripheral tissue and from peripheral tissue back to the liver

- **NOTE:** Again , as we said that the lipid composition is differ among lipoproteins, example…for **chylomicrons** (from intestine, contain a huge amount of TAG and contain cholesterol esters)…..for **VLDL** (from liver, contain TAG and cholesterol esters…ratio of TAG/cholesterol esters is 5:1)…for **LDL &HDL** (contain TAG and huge amount of cholesterol esters)….the source of each is different but all contain a lot cholesterol esters so, in order to calculate the amount of cholesterol that are found in the system , we must take into consideration the amount of HDL & LDL & VLDL…**except for VLDL** **(calculated by dividing TAG by 5 because the TAG/cholesterol ratio is 5/1 in VLDL)**

Protein composition of lipoproteins (Apolipoproteins)

- Functions:
  - Structural (cannot be removed).
  - Recognition sites for cell-surface receptors
  - Activators or coenzymes for enzymes involved in lipoprotein metabolism.
- some are exchanged freely among lipoproteins.
- Classes of apolipoproteins are denoted by letters, and subclasses are designated by Roman numbers.
  - Example: apoC-I, apoC-II, and apoC-III.

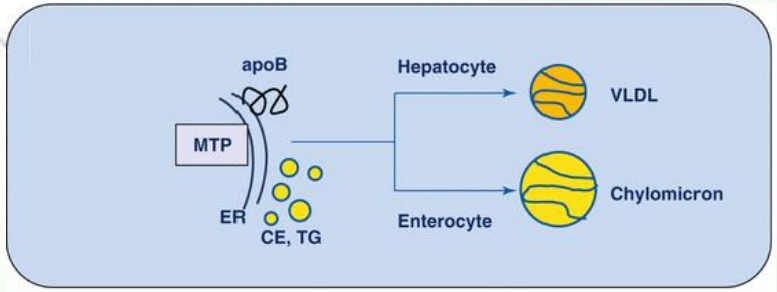


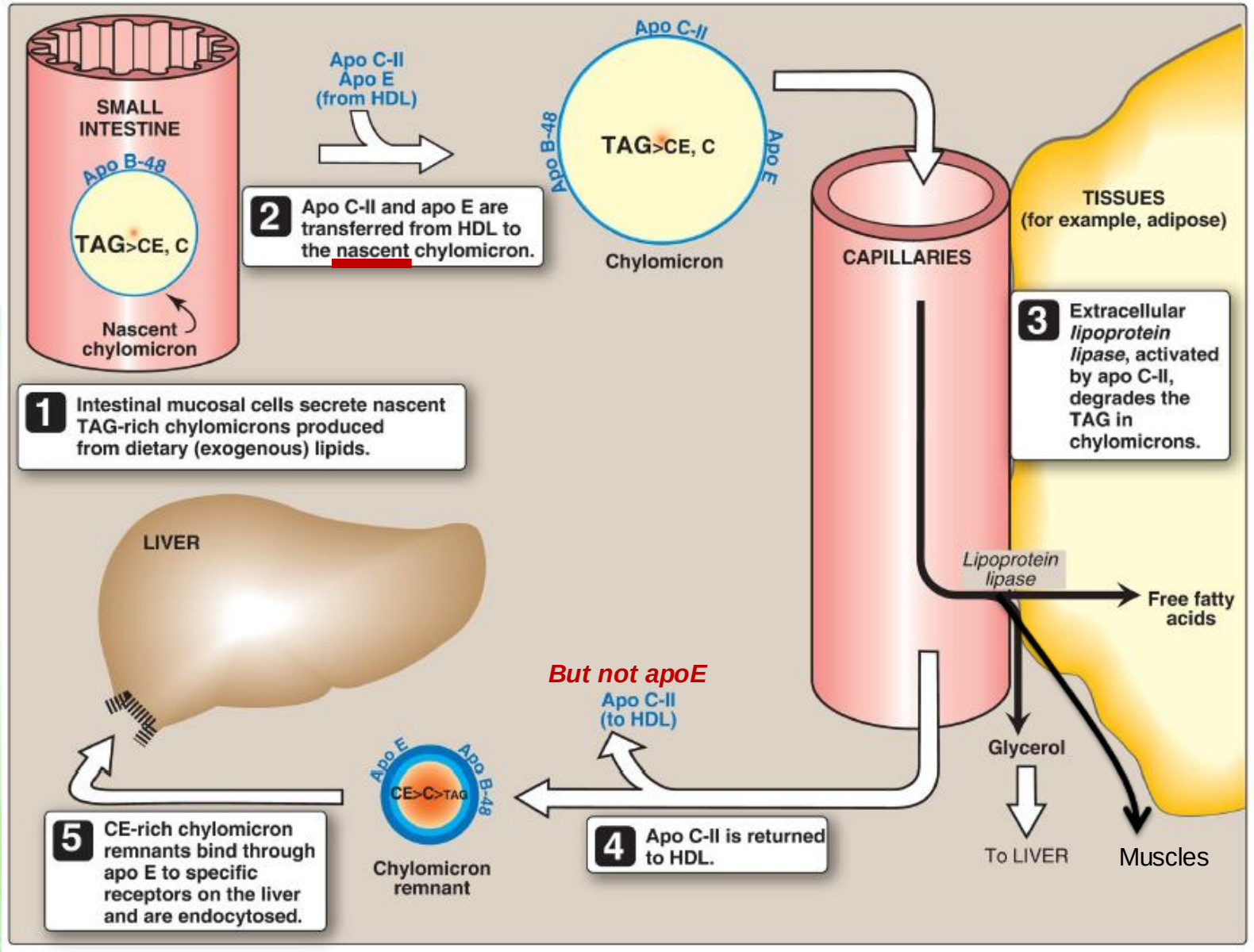
- **NOTE:** Apolipoproteins differ in their function..**structural function** (form the lipoprotein) such as ApoB-48 that form the chylomicron , without it there will be no chylomicron…another example is ApoB-100 that form the LDL & VLDL and so on.

- **NOTE:** another function of apolipoprotein is to be a ligand to receptor….they bind to receptor on cell surface and allowing the lipoprotein to bo internalized
- Don’t focus on the (subclasses that are designated by Roman number) of lipoprotein…there will be no question in the exam for the differences between them….we will focus on the classes (A/B/C)

Chylomicrons

Microsomal triglyceride transfer protein (MTP) assembles the apoB protein with the lipids in the ER before transition to the Golgi, where the particles are packaged in secretory vesicles.





Nice story

- **The complement in this slide:** chylomicrons are formed in the intestinal cells by a protein called **(MTP)- Microsomal triglyceride transfer protein –** the function of it is to assemble ApoB-48 (the structural protein) with different dietary lipids (cholesterol , TAG , lipid soluble vitamin….etc) forming what is called a **(nascent / original / immature) chylomicron** and releasing it outside the intestinal cell. Once it released into the capillaries and lymphatic system , it gains two other proteins (ApoC-II & ApoE) from HDL , these two proteins are transferred to the immature chylomicron to become a **(mature) chylomicron.** Now remember that the mature chylomicron contain (ApoB-48 /ApoC-II /ApoE)….let's continue our story…..as long as the mature chylomicron traveling in the capillaries, it plays sports (become thinner & thinner) how is that happening? Let me tell you……there is a lipoprotein called a lipoprotein lipase found on the surface of endothelial cells ,this lipase will interact with ApoC in the mature chylomicron……ApoC will activate the lipoprotein lipase, once it is activated , it will degrade the TAG and release the fatty acid from it , these FA will go to tissue…muscle tissue will use them as a source of energy (Beta oxidation)….Adipocytes will store them in form of TAG….so, as the mature chylomicron travels in the capillaries….interaction with lipoprotein lipase will happen and the chylomicron will be thinner and thinner……then ApoC will be released from the mature chylomicron going back to HDL…….in this case the mature chylomicron will become a (**chylomicron remnants**) containing (ApoE & ApoB-48)….. So chylomicron remnants are formed due to the removal of TAG by lipases ……..the story will be continued in the next slide

- **The complement in this slide:** when the chylomicron remnants arrive to liver , the ApoE in the chylomicron remnants react with the ApoE receptor on the surface of hepatocytes which leads to **receptor- mediated endocytosis** , endocytosis means(cellular process where a cell engulfs substances by wrapping its cell membrane around them , forming a vesicle) this vesicle containing (ApE in the chylomicron remnants that interact with ApE receptor) will enter the cell and transform to endosome which will fuse with lysosome….the PH within endosome & lysosome is low , it's about 6 , 5.5 respectively, as a consequence of this low PH , ApoE receptor will be released and returns back to cell surface so that it can interact with another chylomicron remnants…let's continue the story, now we have a lysosome containing hydrolytic enzymes (proteases / lipases / esterases….etc) which will degrade the chylomicron remnants , which will release the cholesterol and Fatty acid and others to the cytosol…..then cells take them and do a lot of processes (storage/ hormones/oxidation….etc) . The end of the story

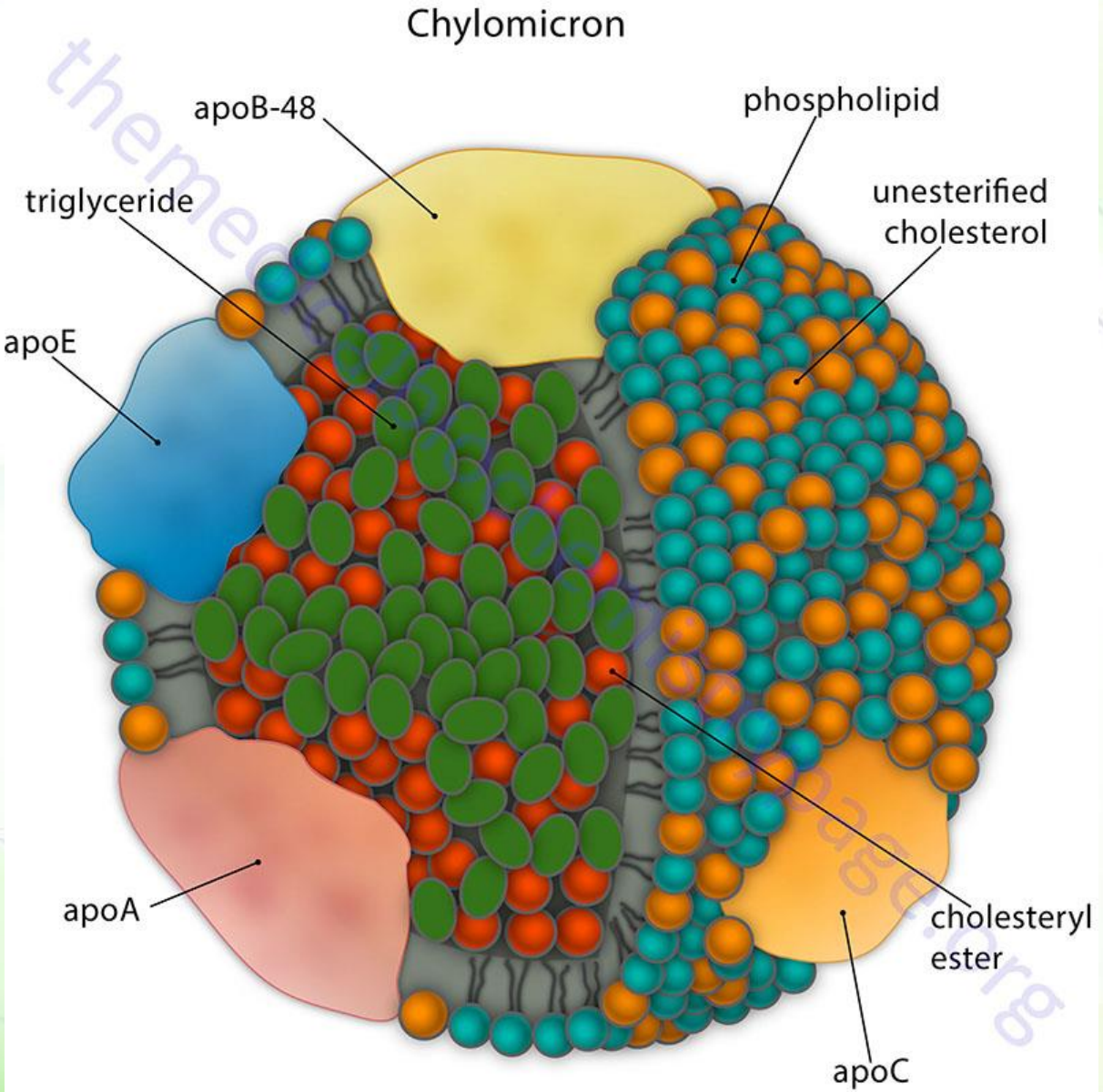
- **NOTE:** Quick Overview of the story….Assembly by MTP→ forming immature chylomicron→ adding (ApoC-II & ApoE) from HDL → forming mature chylomicron →(ApoC – lipase) interaction → removal of TAG → forming chylomicron remnants → receptor mediated endocytosis → Release of ApoE receptor →cells take the degraded lipids and process them .

Apolipoproteins

Apolipo-protein	Molecular Weight	Chylomicron (CM)	VLDL	IDL/CM remnants	LDL	HDL
AI	28,016	Ex	Ex			St
AII	17,414	Ex	Ex			Ex
B100	515,000		St	St	St	
B48	241,000	St*		St*		
CI	6600	Ex	Ex			Ex
CII	8800	Ex	Ex			
CIII	8750	Ex	Ex	Ex		Ex
E	34,100	Ex	Ex	Ex		Ex

*B48 is exclusive to chylomicrons and chylomicrons remnants. St, structural apolipoprotein; Ex, exchangeable apolipoprotein. Other apolipoproteins (AIV, AV, D, F, G, H, J, (a)) are beyond the scope of this review.

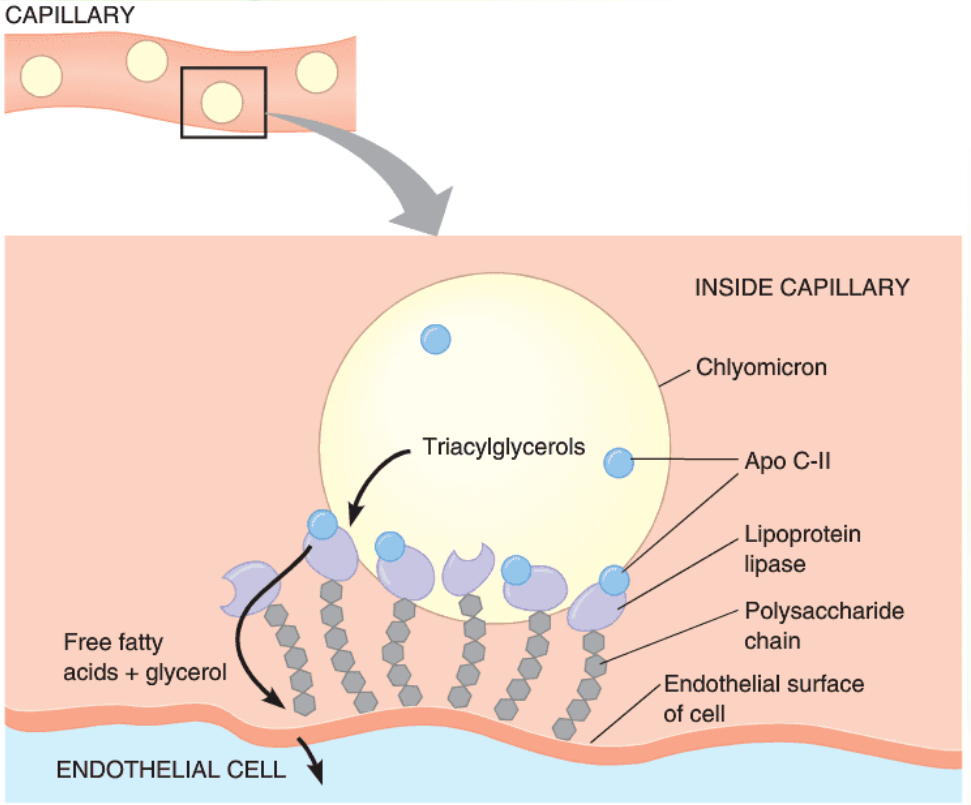
Structure of chylomicrons



Function of apo CII

NOTE: we talked about this slide in the previous story…except the RED paragraph

- ApoCII interacts with the lipoprotein lipase, which exists on the cell surface of endothelial cells, activating it.
- Lipoprotein lipase degrades TAG releasing fatty acids and glycerol, which enter the tissues.
- When TAGs are removed, chylomicron remnants are formed, which contain cholesteryl esters, phospholipids, apolipoproteins, fat-soluble vitamins, and a small amount of TAGs.



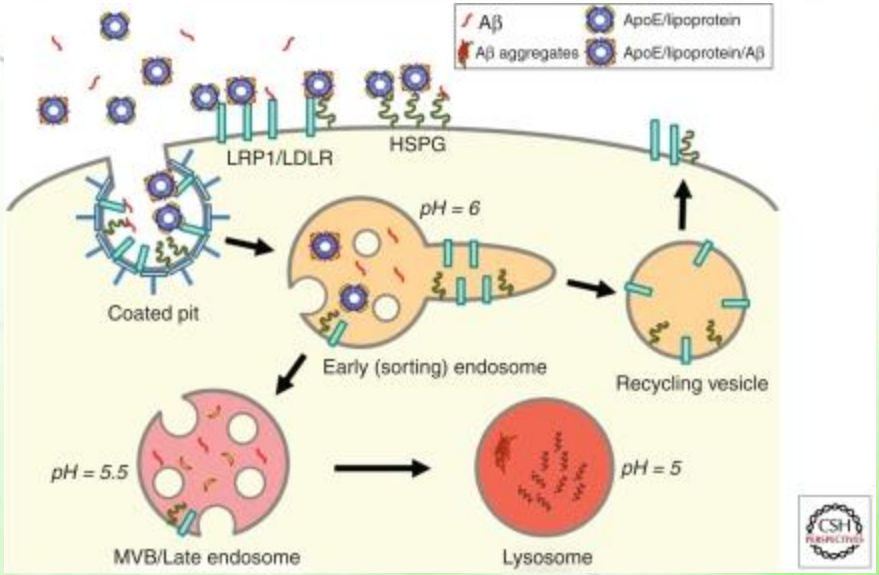
- **Type I hyperlipoproteinemia, familial chylomicronemia, hypertriacylglycerolemia: Deficiency of LPL or apo C-II leading to the accumulation of chylomicron-TAG in the Plasma.**

NOTE: people with type 1 hyperlipoproteinemia will be susceptible to myocardial infarction

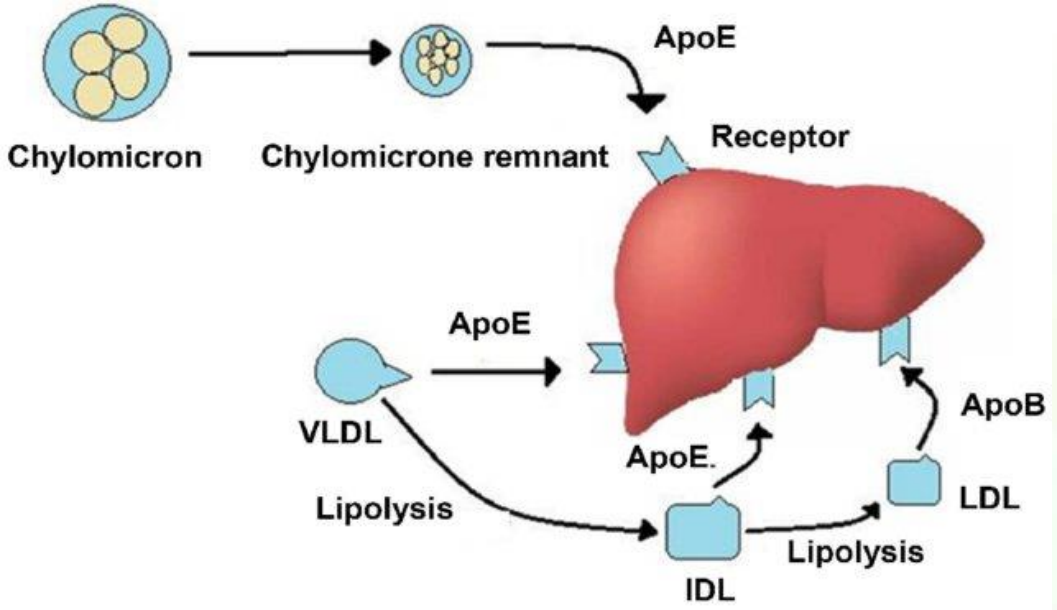
High level of LDL accompanied with type 1 hyper lipoproteinemia

Fate of chylomicron remnant

- Chylomicron remnants bind to apoE receptors on the cell surface of hepatocytes and are taken into the by receptor-mediated endocytosis.
- The intracellular remnants are hydrolyzed to their component parts.



NOTE: we talked about this slide in the previous story…**except the RED paragraph**



Type III hyperlipoproteinemia: mutations in apoE gene leading to decreased clearance of chylomicron remnants.

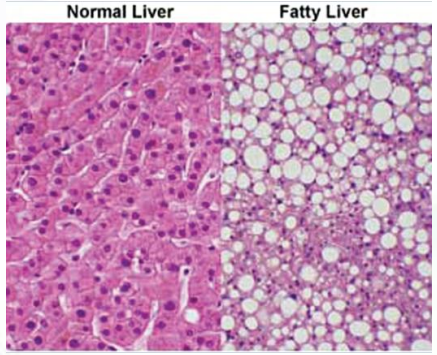
NOTE: the mutation could also be in ApoE receptor gene

Very-low-density lipoprotein

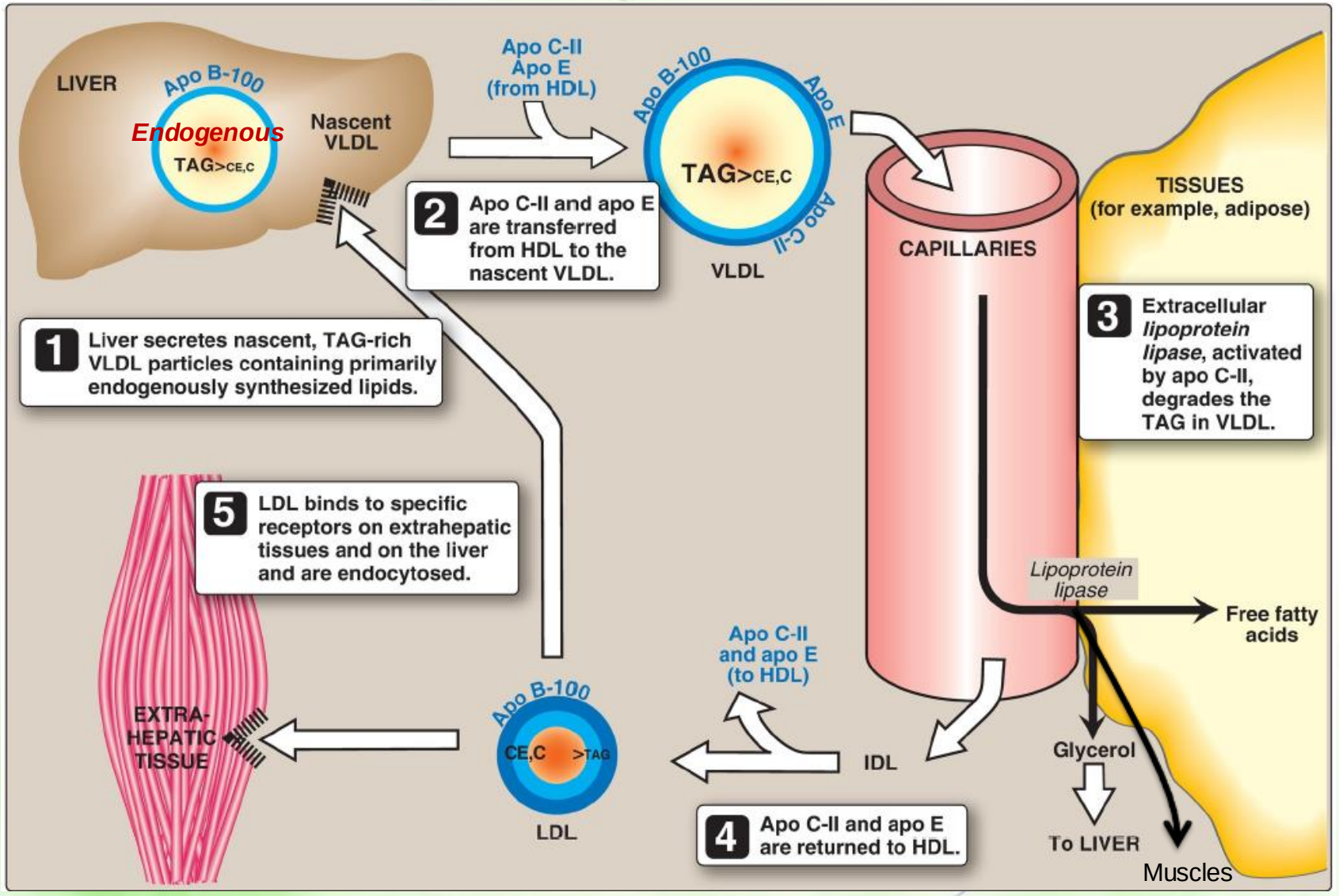
- Nonalcoholic fatty liver (hepatic steatosis):

<u>hepatic TAG synthesis >> VLDL release</u>

- Examples: obesity and type 2 DM



- **Abetalipoproteinemia**: a rare hypolipoproteinemia caused by defective MTP, leading to low VLDL or chylomicrons and TAG accumulates in the liver and intestine.
  - Deficient fat-soluble vitamins



- **The complement in this slide:** When the signals come from the tissues to the liver and say (hey liver , we need energy)…the liver responds by releasing TAG & cholesterol esters in VLDL(contain huge amount of TAG & also contain cholesterol esters & **ApoB-100- which is the structural protein of VLDL-**) this form of VLDL is called**(immature/nascent)** VLDL…And Just like chylomicron , VLDL gains (ApoC-II & ApoE) from HDL….turning into **mature VLDL** ,(ApoC –lipase) interaction will take place , releasing TAG from the VLDL becoming thinner ..thinner & thinner until it turns to **Intermediate density lipoprotein(IDL)** containing (cholesterol esters/ApoB-100/ ApoC–II /ApoE) , ApoC & ApoE will be released from intermediate density lipoprotein(return to HDL) turns into LDL containing (ApoB-100 & cholesterol esters)….take a look at the picture above

- **NOTE:** remember that, ApoB-100 & ApoB-48 (structural) are came from the same gene BUT in the liver , RNA editing will take place , stop codon in the middle of mRNA will be changed to sense codon that will make the whole mRNA translated into 100kD polypeptide for ApoB-100 and 48kD polypeptide for ApoB-48

تشمّع الكبد

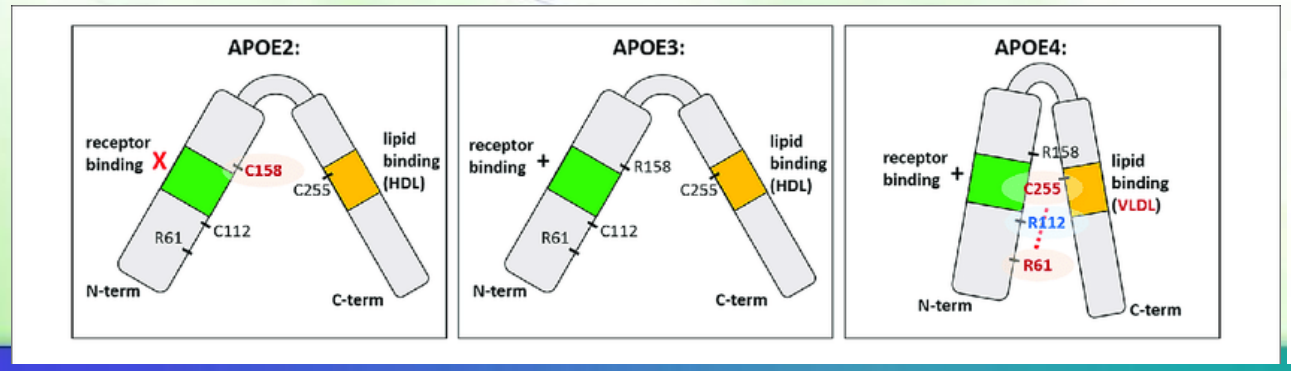
- **The complement in this slide:** Nonalcoholic fatty liver (hepatic steatosis) , the amount of TAG produced by liver is much higher than the amount of VLDL produced by the liver , as a result , liver cells will contain high amount of TAG that aren’t capable to be loaded into VLDL (look at the Histo picture in the slide above)

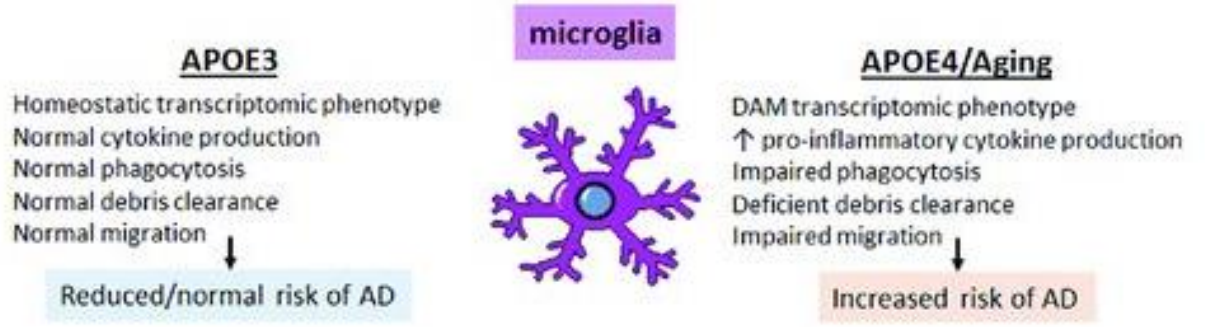
A note about apoE

isoenzymes: produced from different genes but have the same function.

NOTE: ApoE are found to have three isoforms that are divided into(2/3/4) depending on the amino acid composition. **Isoforms** means (a proteins that are **produced from the same gene** but they differ sometimes the difference is amino acid/extra domain…etc)

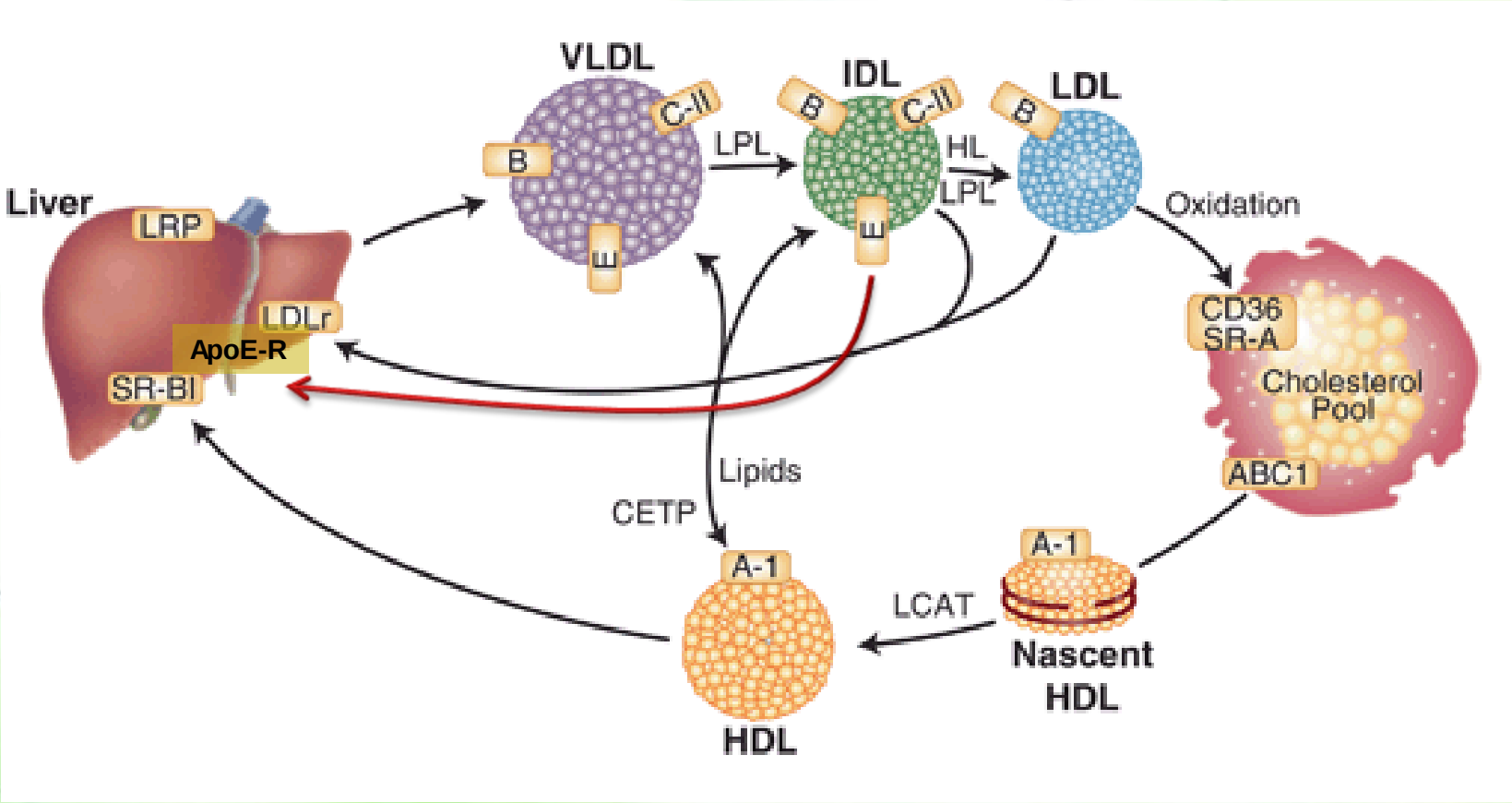
- ApoE is present in three isoforms, E-2 (the least common), E-3 (the most common), and E-4.
  - ApoE-2 binds poorly to receptors.
    - patients who are homozygotic for apoE-2 are deficient in the clearance of IDL and chylomicron remnants. **Those people will have high levels of IDL and chylomicron remnants in their system.**
    - These individuals have familial type III hyperlipoproteinemia (familial dysbetalipoproteinemia or broad beta disease), with hypercholesterolemia and premature atherosclerosis.
  - The apoE-4 isoform confers increased susceptibility to an earlier age of onset of the late-onset form of Alzheimer's disease.
    - Homozygotes are at the greatest risk.





Relation of VLDL to HDL, IDL, and LDL

- **NOTE:** we already talked about this picture.....remember that the HDL is a donor of (ApoC-II & ApoE)



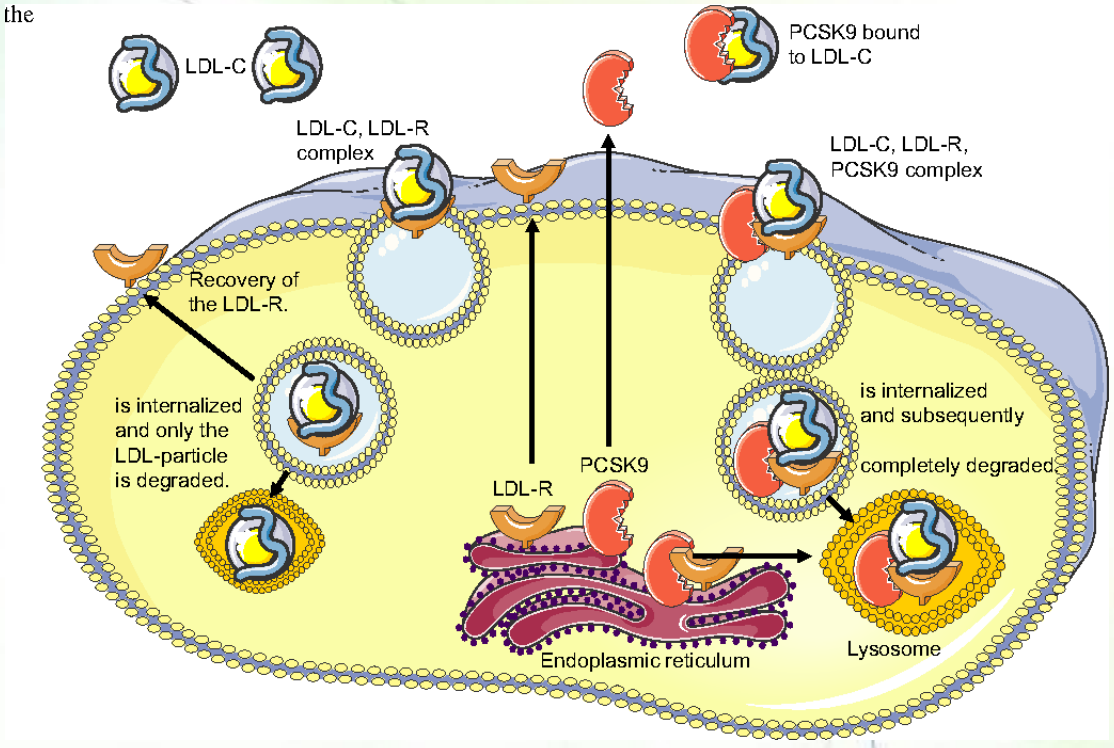
Regulation of lipoprotein lipase

- LPL is synthesized by adipose tissue and by cardiac and skeletal muscle.
  - The highest concentration of LPL is in the cardiac muscle.
- Expression of the tissue-specific isozymes is regulated by nutritional state and hormonal level.
  - In the fed state (elevated insulin levels), LPL synthesis is increased in adipose tissue **(to storage it)** but decreased in muscle tissue.
  - Fasting (decreased insulin) favors LPL synthesis in muscle.**(to generate energy)**

NOTE: **remember that , LPL is found on the surface of endothelial cells but , the source of LPL isn't the endothelial cells rather, it is synthesized by adipocytes , and muscle cells…..and they release it to be presented on the endothelial cells**

Low density lipoprotein

- Primary lipoprotein is B-100.
- Plasma cholesterol, ~70% of LDL content, is taken to peripheral tissues.
- Receptor-mediated endocytosis
- Type IIa hyperlipidemia (familial hypercholesterolemia [FH]): reduced synthesis of functional LDL receptor leading to premature atherosclerosis. **high cholesterol in their system.**
- Defective apo B-100: autosomal dominant hypercholesterolemia with reduced binding to LDL receptor.



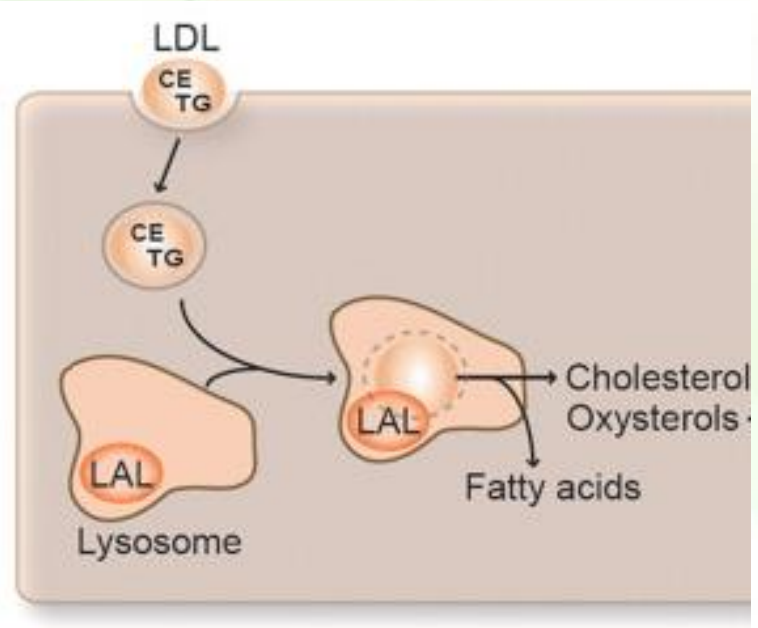
- Proprotein convertase subtilisin/kexin type 9 (PCSK9) promotes internalization and lysosomal degradation of the receptor.
  - PCSK9 inhibitors are now available for the treatment of hypercholesterolemia.

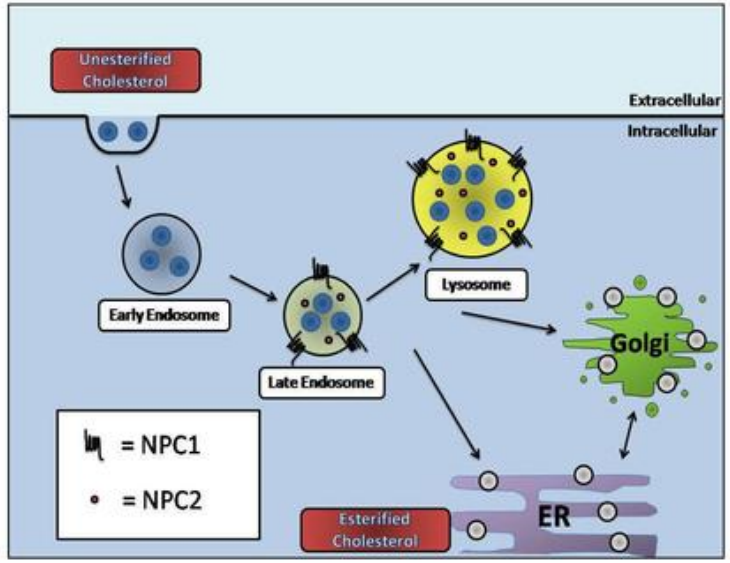
- **The complement in this slide:** remember that LDL came from VLDL & IDL
- LDL contain ApoB-100 that are structural & functional protein….functional mmmmm how ? When LDL reaches the peripheral tissues , ApoB-100 will bind to LDL receptor and (such as ApoE) receptor –mediated endocytosis will take place , vesicle containing LDL bind to its LDL receptor is internalized into the cell…then turns to endosome that will fuse with lysosome , then the LDL receptor will be released to the surface so that it can bind another LDL and endocytosed it…the remaining lysosome (containing hydrolytic enzymes) will degrade the LDL….and so on

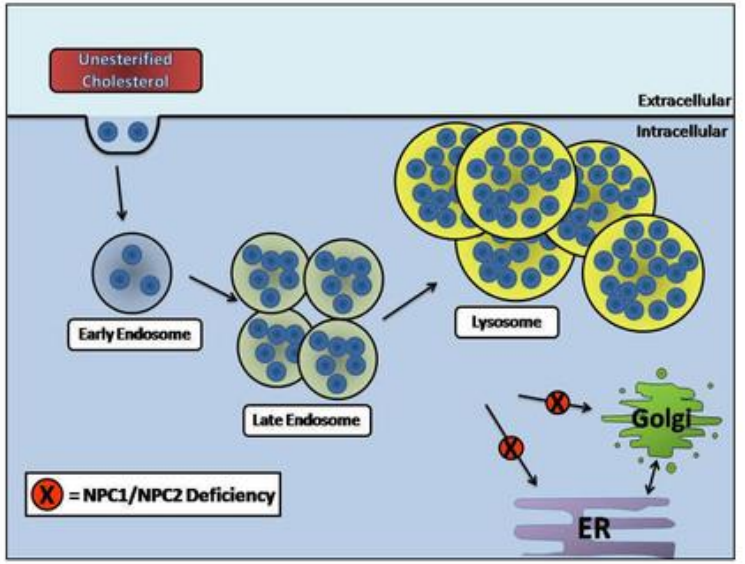
- **NOTE:** PCSK-9 is found inside the vesicle and its function is to bind the LDL receptor , preventing it from going back to cell surface (trapping it inside the lysosome….so it will be degraded by the hydrolytic enzymes)…….**WHY WE DON'T INHIBIT PCSK-9**…..so that the LDL receptor will go back and expressed on the cell surface leads to more clearance of LDL , helping in treatment of hypercholesterolemia…..**that's the magic of biochemistry**

Lysosomal storage diseases

- Lysosomal acid lipase hydrolyzes cholesterol esters and TAG, which are then transported out.
- Wolman disease: a severe, autosomal-recessive deficiency of lysosomal acid lipase leading to massive intracellular accumulation of cholesteryl esters and triglycerides.
- Niemann-Pick disease, type C: autosomal-recessive deficiency in the transport of free cholesterol out of the lysosome.





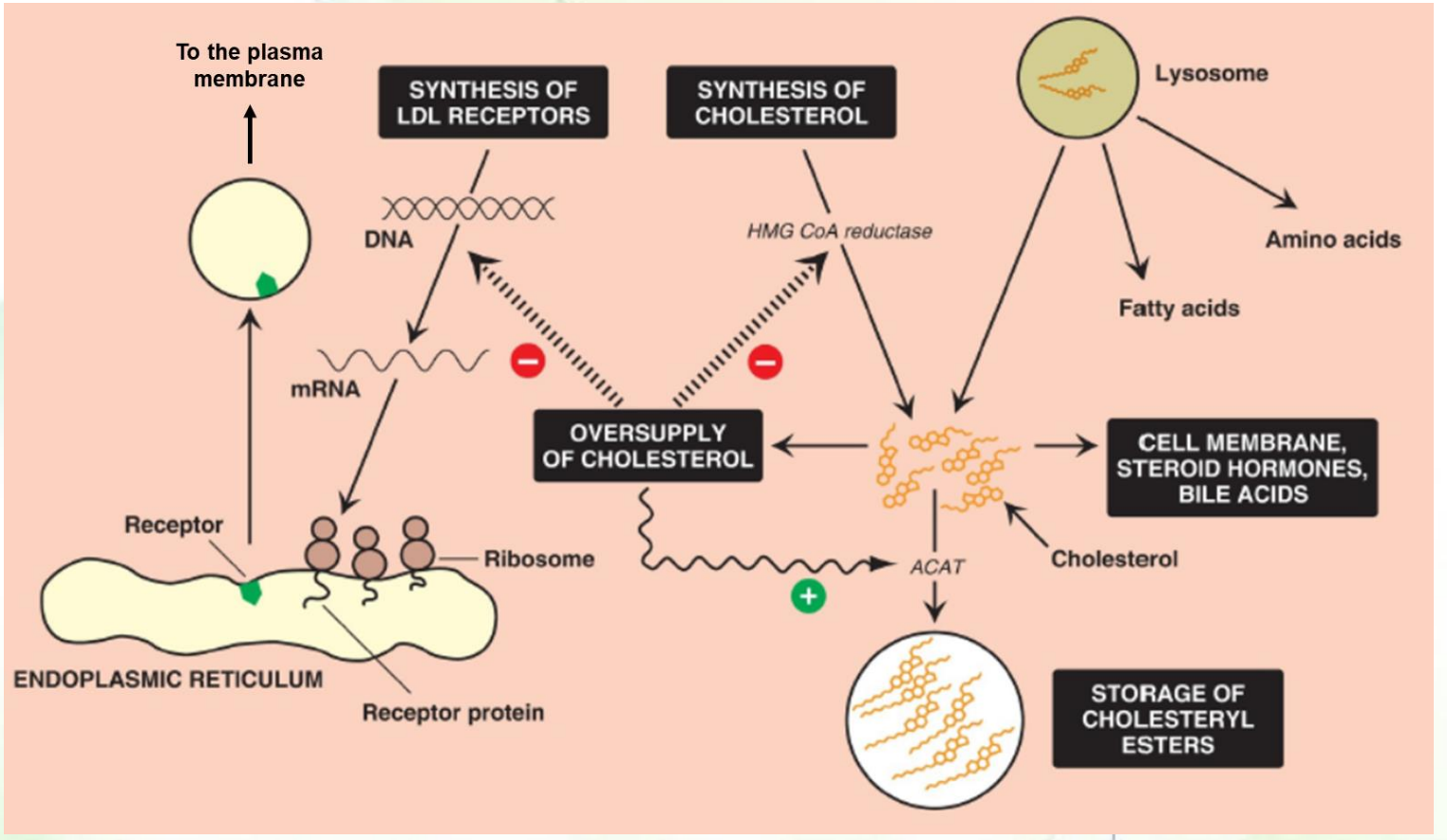


- **The complement in this slide:** when LDL is inside the lysosome, we have enzymes that will start working on it like: Lysosomal acid lipase.(acidic enzyme that is stable at low PH)
- It releases the FAs that are associated with TAGs and CEs (Cholesterol ester).
- Then the free fatty acids (FFA) and cholesterol will be transported outside the lysosome.
- The cell now have cholesterol and FFA, it will use cholesterol in like synthesis of (hormones, bile acid..)
- FFA will be used in (storage, Beta oxidation....)

- **The complement in this slide:** in diseases that are associated with Lysosomal acid lipase deficiency , I will have an accumulation of TAG and CE inside the lysosome, and it would be enlarged. **Wolman disease**
- Remember NPC1L1 that is found in the intestine, we have an isoform of it in the lysosome **(Niemann-Pick protein)**, and it is responsible for the transport of free cholesterol when it is not associated with FA outside the lysosome, so if I have deficiency in it there will be an accumulation of cholesterol inside the lysosome. **Niemann-Pick disease type C**

Fate and effects of cholesterol

- High intracellular cholesterol levels
  - inhibit de novo cholesterol synthesis
  - induce the degradation of HMG CoA reductase.
  - decrease the synthesis of LDL receptor through the negative regulation of SREBP-2.
- Excess cholesterol is esterified by *acyl CoA:cholesterol acyltransferase* (*ACAT)* and stored in the cells as cholesterol ester.
  - The activity of ACAT is enhanced by the increased intracellular cholesterol.



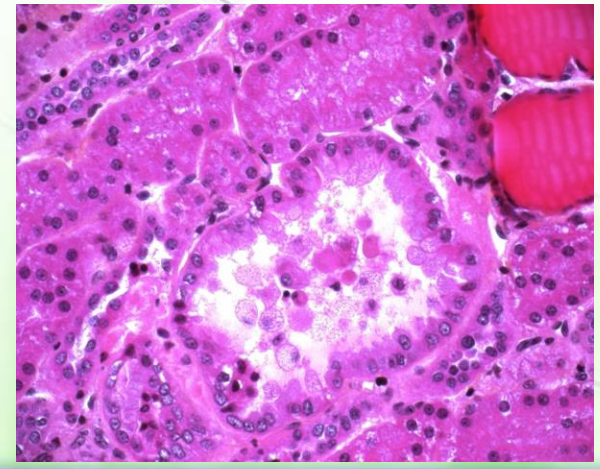
NOTE: The enzyme responsible for esterification, which connects cholesterol with fatty acids, is called acyl CoA: cholesterol acyltransferase. This enzyme transfers an acyl CoA to cholesterol.

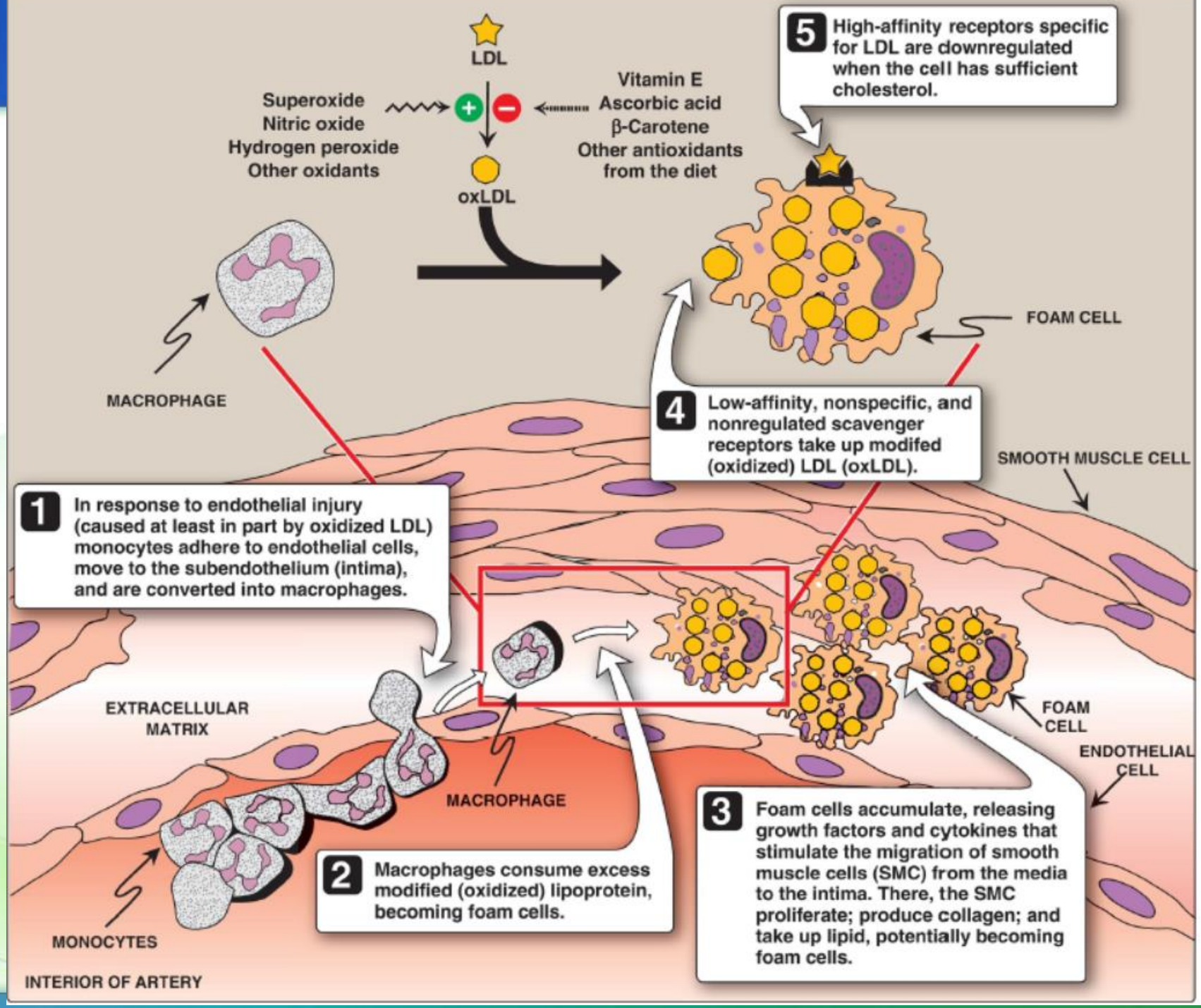
We have mentioned every single word here in cholesterol Lec so u can read it fast 😉

- **The complement in this slide:** As we said in cholesterol Lec , we said that we have regulation at different levels, HMG reductase enzyme that is responsible for making the committed step then eventually making cholesterol.
- This enzyme is found in the ER and attached to INSIG protein (trapped) when cholesterol level is high HMG reductase will not get out of the ER and the cell will degrade it.
- This INSIG that trapped reductase inside the ER to get degradation, also binds to transcription factors(SREBP-2) inside the ER and to get activated it travels to the Golgi apparatus (cleavage of it takes place here) then from Golgi to the nucleus it binds on the element and stimulate gene expressions to the genes that make the enzymes that are responsible for FA synthesis and cholesterol synthesis
- When I have a high concentration of cholesterol, SREBP-2 will stay in the ER and it won't get out.
- So, the enzymes, proteins and genes(the gene that makes the LDL receptor) will not be transcribed.

Foam cells

- Macrophages possess high levels of unregulated scavenger receptor class A (SR-A) that can bind and endocytose LDL particles carrying oxidized lipids.
- Cholesteryl esters accumulate in macrophages, which transform into “foam” cells that form atherosclerotic plaque.
- LDL-Cholesterol is the primary cause of atherosclerosis.





The complement in this slide: LDL is known as bad cholesterol why? Because it carries cholesterol from the liver to peripheral tissues.

- But there is another reason, when I have a high concentration of LDL in the system(increase the level of cholesterol), the oxidation of LDL will increase.
- I will have a thing called oxidized LDL, The levels of oxidized LDL increase with elevated nitric oxide, free radicals, and ROS.
- Oxidized LDL is harmful because it tends to aggregate with each other, and there is another reason that involves macrophages.
- When there is damage in blood vessels, immune cells will intervene (specifically macrophages), these macrophages function is clearance of the system(eating dead cells and debris), they will release cytokines and inflammatory molecule .
- These macrophages will start eating the things that are in the site of injury, eating oxidized LDL as well, HOW?? Because they have a very important receptor called **scavenger receptor class A**, and this receptor is not regulated so it will eat as much oxidized LDL as is present 😛
- These macrophages will turn into "**foam cells**" فقاعات look at the histo image in the previous slide (so big and the white thing is the oxidized LDL)

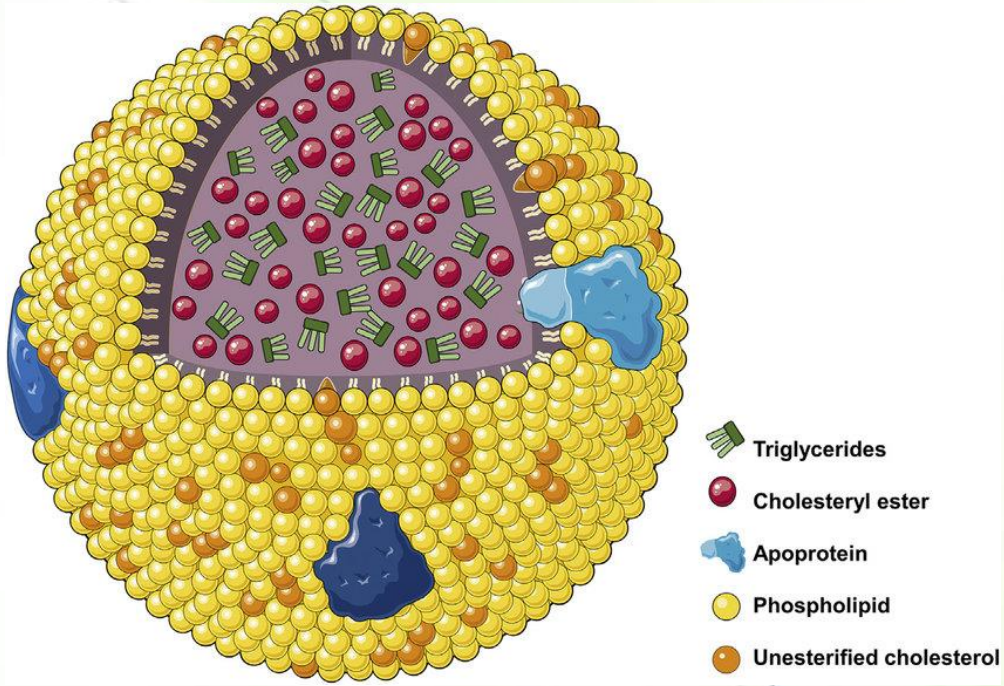
The complement in this slide: These foam cells tend to aggregate also, so I will have MI, Heart attack , and these foam cells will cause atherosclerosis as well why? Because these macrophages will release cytokines, these cytokines will increase the growth and proliferation of the smooth muscle cells and endothelial cells, so instead of having one layer of smooth muscle cells then an endothelial layer above it, **I will have LAYERS of smooth muscles and endothelial cells.**

So, who caused atherosclerosis? Foam cells and inflammatory cells because of the release of cytokines.

This is why LDL is known as bad cholesterol 😀 (it tends to cluster)

High-density lipoprotein

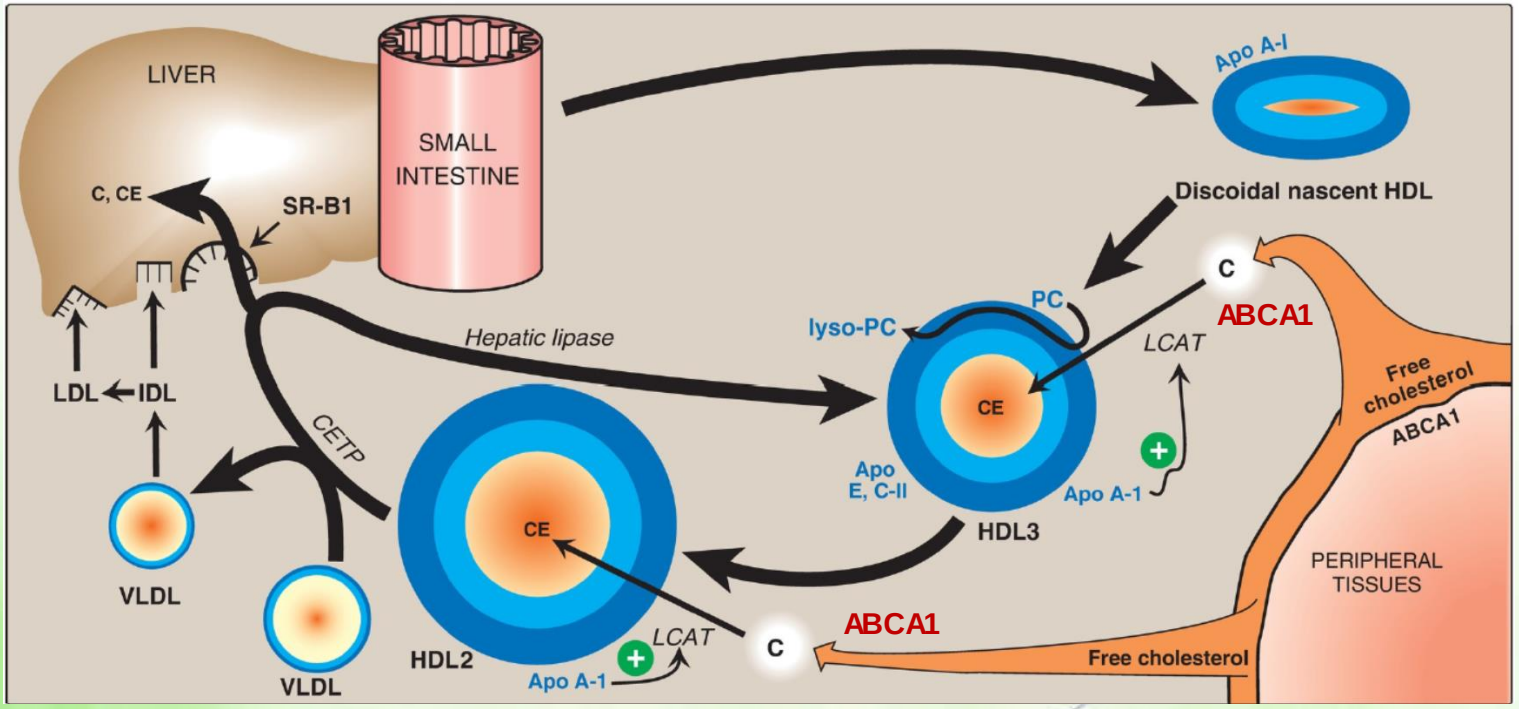
- HDL particles are formed by the addition of lipid to apo A-1 (~70% of lipoproteins in HDL), which is synthesized by the liver and intestine.
- Functions:
  - HDL provides apo CII and E to VLDL and chylomicron remnants.
  - They take up non-esterified cholesterol from peripheral tissues and return it to the liver as cholesteryl esters. That’s why it's called good cholesterol



Transport of cholesterol by HDL

- The liver-synthesized, nascent, HDL-bound plasma enzyme lecithin:cholesterol acyltransferase (LCAT or PCAT) esterifies the HDL-carried cholesterol by transferring the FA of carbon 2 of PC and the CE is sequestered in the HDL core.
- Lysophosphatidylcholine is carried by albumin.
- LCAT is activated by apo A-I and inhibited by cholesterol ester.
- Hepatic lipase, which degrades TAG and phospholipids, participates in the conversion of HDL2 to HDL3.

Note:
Lecithin = phosphatidylcholine



The complement in this slide: HDL is synthesised in the liver, and it is released as nascent (immature) HDL

- HDL travels to the peripheral tissues and what it does is interacting on the cell surface with protein called ABC A1
- This is a transporter found on the cells of the peripheral tissues.
- HDL come by unknown mechanism and interact with ABC A1, both of them open its mouth and the pumping of cholesterol start from the cell toward HDL, now we have cholesterol inside HDL.
- This cholesterol inside HDL will get through esterification and become cholesterol ester (CE).
- The source of FA is **phosphatidylcholine**.
- The FA on Carbon number 2 is degraded from phosphatidylcholine, and it is given to cholesterol to become CE.
- There is an enzyme found in HDL called **Lecithin cholesterol coenzyme A acyltransferase** (LCAT)
- This enzyme takes FA from **lecithin** (phosphatidylcholine) and gives it to cholesterol converting it to cholesterol ester.
- HDL now has a good amount of CE, this CE will inhibit LCAT.

Lecithin = phosphatidylcholine

- There will be no more CE formation so what is the solution 😭?

in the next slide xD

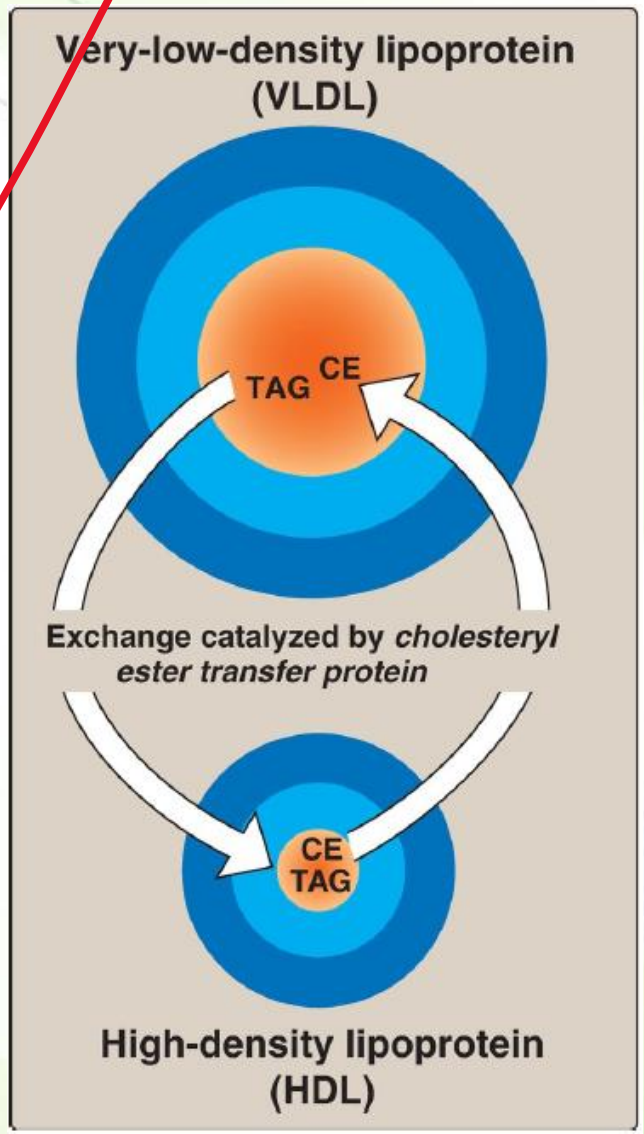
VLDL ↔ HDL through CETP

Nice exam question: **What is the function of CETP ?**

NOTE: While HDL is backing to the liver it meets VLDL (talking to each other making the Lec longer):

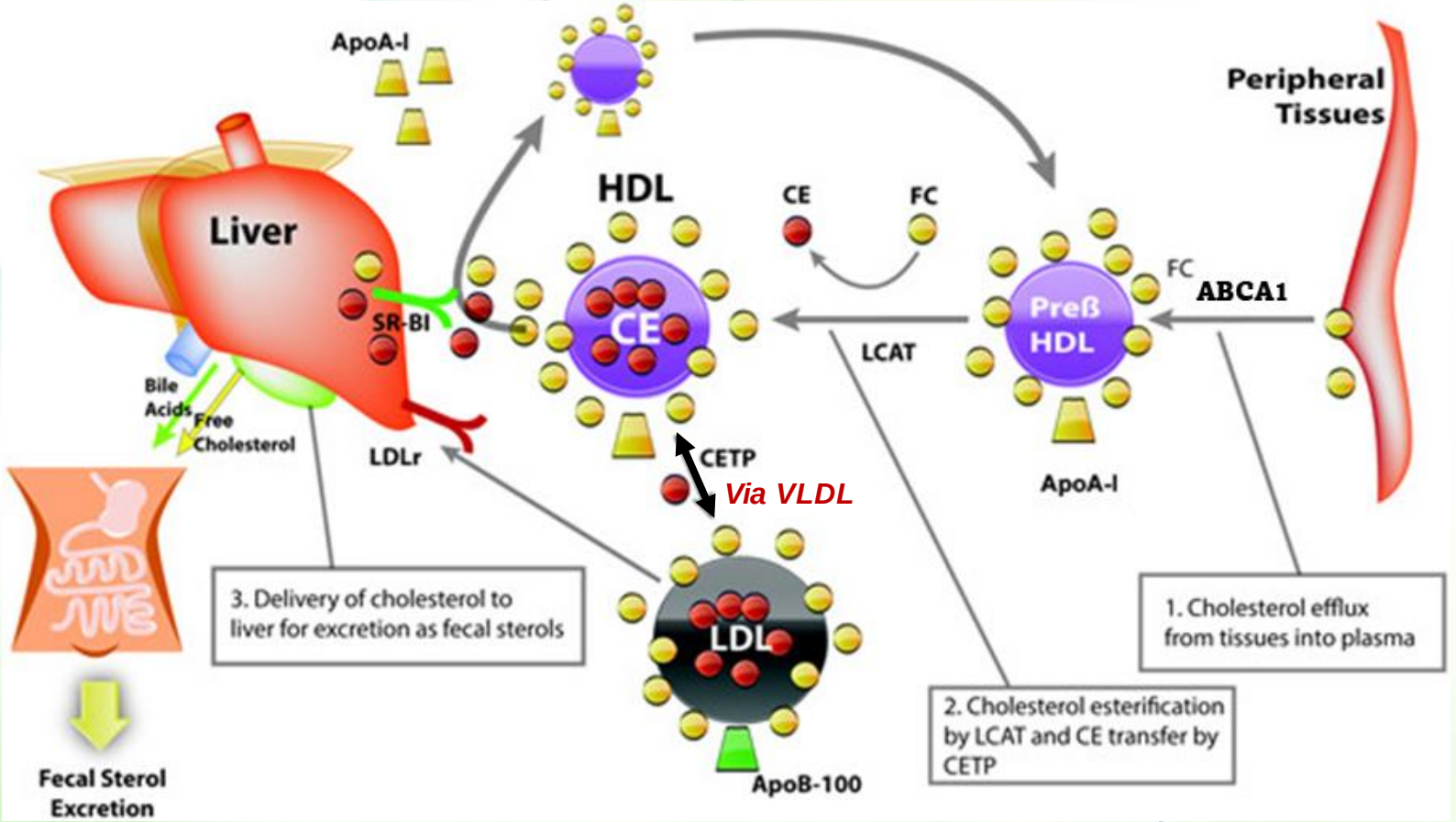
- Some TAGs are transferred from VLDL to HDL in an exchange reaction that concomitantly transfers cholesteryl esters from HDL to VLDL. both are happy 😁
- This exchange is accomplished by cholesteryl ester transfer protein (CETP).
- End-result is relieving product inhibition of LCAT. **(LCAT is active again)**
- VLDL can then be converted to IDL and LDL.

Answer: transfer of TAGs from VLDL to HDL and transfer of CE from HDL to VLDL .



Reverse cholesterol transport

- The efflux of cholesterol from peripheral cells is mediated primarily by the transport protein ABCA1.
  - Tangier disease: no ABCA1, no HDL particles, degradation of apo A-1.
- Cholesteryl ester uptake by the liver is mediated by scavenger receptor class B type 1 (SR-B1).



Defective ABCA1 causes sitosterolemia, cystic fibrosis, X-linked adrenoleukodystrophy, respiratory distress syndrome, and liver disease.

The complement in this slide:

- When HDL reaches the liver after going through everything we Mentioned previously (don't forget the donation of apo C and apo E) there will be interaction between HDL with liver cells via protein called **scavenger receptor class B**(this one is regulated unlike the one in the macrophages) this will take CE from HDL to the inside of liver cells.
- ABC A1 function : pumping cholesterol out of cells of peripheral tissues to the inside of HDL, if this ABC A1 is defective there will be no HDL and it will stay nascent (immature) **because the gaining of cholesterol from peripheral tissues makes HDL mature.**
- This Disease is called Tangier disease. **(Defective in ABC A1)**