◉ Sheet ○ Slides

Subject :	Cholesterol
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Number :	28

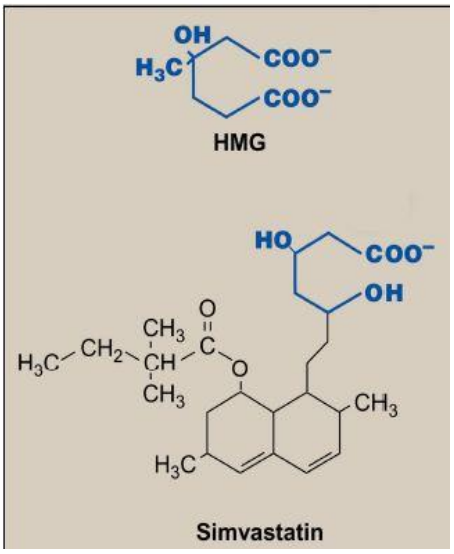
Cholesterol level in the plasma is normally 200 mg/100ml, high cholesterol is associated with increased risk of **cardiovascular disease** therefore we aim to lower the cholesterol level not to make it normal but below normal, in order to reduce the risk of cardiovascular disease and **atherosclerosis**. The ***First*** thing we think of is to reduce the dietary intake, but this reduction in intake will not decrease the rate of synthesis even if you make the intake zero, this will increase the synthesis and the overall effect will be a decrease in 5 or 10%. ***Second*** thing we can increase the PUSFA/SFA (PUSFA: Polyunsaturated Fatty Acids, SFA: Saturated Fatty Acids) and the PUSFA are found in the vegetable oils like corn or soy bean oil and decreasing the ratio PUSFA: SFA that are found in animal fat usually.

It is by an **unknown mechanism** by which PUSFA reduce cholesterol levels.

Third Increase the fiber as the amount of cholesterol and bile acids adsorbed to the fiber is increased so this will decrease the absorption and it will be excreted along with the fiber. ***Fourth*** Ingestion of plant steroid esters also decrease the amount of cholesterol as they are poorly absorbed; they are initially absorbed by intestinal cells and they are reexcreted with cholesterol so the overall increase ingestion of plant steroid will decrease cholesterol level that is absorbed.

-Decreasing cholesterol levels:

1. Inhibit the synthesis.
2. Increase bile salt (which are made from cholesterol) excretion.
3. Dietary (see above first, second, third and fourth).

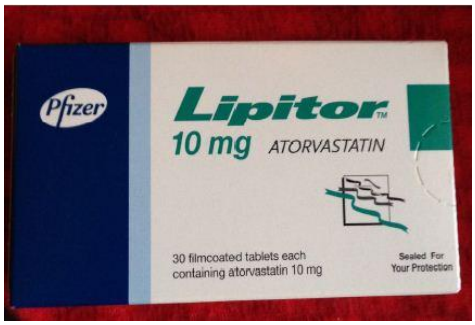


Now how we inhibit the synthesis?

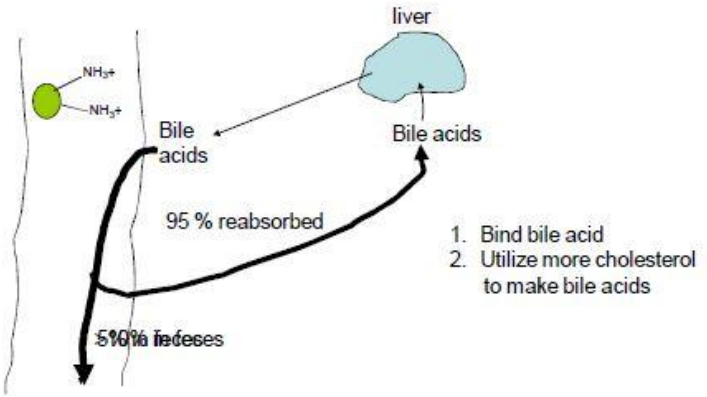
The answer is simple! Inhibit the enzyme that catalyses the rate limiting step (the slowest enzyme).

The enzyme is **<u>HMG CoA reductase</u>** (rate limiting enzyme) that catalyses the reduction of HMG CoA into mevalonic acid.

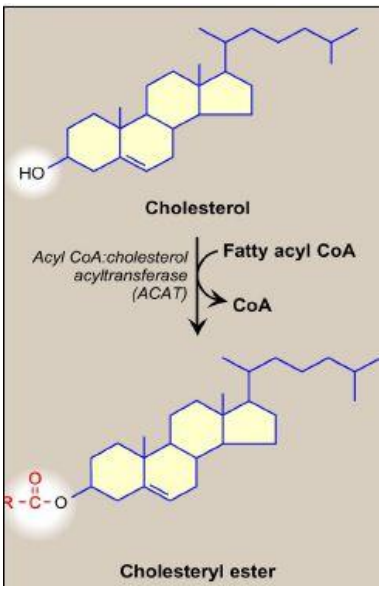
The compound you see similar to **simvastatin** that act as a competitive inhibitor, as part of the molecule is similar to the HMG CoA. This one is part of a group of drugs or medicines known as statins. So all of the statin drugs inhibit HMG CoA reductase you see an example **atorvastatin Lipitor**, some people take this drug to make the levels of cholesterol not normal but less than normal, most of the people that are above (55-60) years need to use the drug at some point especially if they diabetic because the diabetes itself increases the risk of coronary arteries disease so if you are diabetic and above 55 you ought to use a drug that lower the cholesterol level.



-The second method is by **bile sequestering agents**, cholesterol is converted to bile acids and they secreted to the small intestine as they are participate in fat digestion and they are reabsorbed. Only 5% of the daily bile production is excreted in the feces about 0.5 g a day. Bile acids themselves act as inhibitors for the conversion of cholesterol to bile acids, so what can we do to increase the rate for conversion to bile acids? (Meaning; I want to increase the rate of conversion in order to get rid of the cholesterol.) We can use agents that bind to bile acids and they prevent their reabsorption by using these agents the amount released into the intestine is more than 10% so the amount reabsorbed is less than 90% so we push more cholesterol to be converted into bile acids by this we can lower cholesterol.



Esterification of cholesterol in cells:



Notice the figure for the place of esterification. It occurs for the purpose of **storage**, if cholesterol is not immediately needed it has to be stored "converted to cholesterol esters". The form of the fatty acid that is esterified should be: "**Acyl CoA**", donor of the fatty acid. Now, what kind of reaction is this? **Acyl transfer reaction**, so the enzyme is transferase and the substrate is cholesterol and acyl CoA so the enzyme is **AcylCoA:Cholesterol acyl transferase (ACAT)** this is done in the **cell**.

Esterification can also occur in the **plasma** for the purpose of transport into the liver , in the plasma there is no CoA or acyl CoA synthetase but the cholesterol is found past of the lipoprotein (it is found in lipoprotein particles especially HDL and it has a lot of phospholipids) and the fatty acid can be obtained from phospholipids.

Fatty acids can be obtained (transferred) from lecithin "It is phosphatidylcholine an emulsifying agent present in the chocolate and powdered milk" to the cholesterol. The product is **Lysophosphatidylcholine and cholesterolester**. So this is how esterification can happen in the plasma inside the HDL which has lecithin. Q: What is the name of the enzyme that catalyses the reaction? First it is a transferase, the substrates are lecithin and cholesterol and the group that is transferred is acyl group, the name is lecithin:cholesterol acyl transferase (LCAT).

Q: Why not ACAT catalyze the reaction? Because there is no CoA in plasma.

Q: What is the location of the cholesterol? **Lipoproteins (HDL**), 25% the major lipid in the HDL is phospholipids, so the reaction can be done.

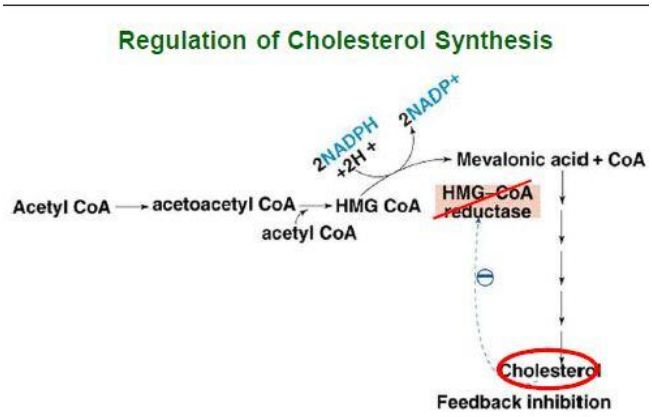
This reaction requires **apolipoprotein A1 which is activator **LCAT**

The regulation of cholesterol synthesis:

Cholesterol synthesis should be strictly regulated because the excess cholesterol can lead to atherosclerosis and death. However, cholesterol is required by all animal cells as component of plasma membrane. Thus, a balance must be preserved where the rate of synthesis is not very high leading to high cholesterol levels resulting in cardiovascular diseases, atherosclerosis,…etc.

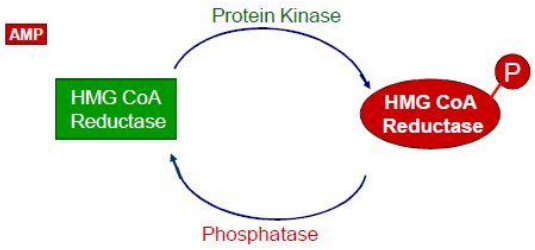
Regulation can happen at several levels:

1) **Regulation of gene expression;** the gene of the enzyme that synthesize cholesterol should be regulated so as not to be expressed.
   Q: what do we mean by gene expression?
   All the cells in your body contain your entire genome but no all cells produce all the products of the genome expression. When the gene is **transcribed** and translated (i.e. produce the **corresponding protein** of that gene) we say that the gene is expressed.
   Now we can modify the rate of transcription of the gene to increase the rate of enzyme synthesis or decrease it. If we decrease the rate of enzyme synthesis or the transcription of the gene this means we decrease the amount of the enzyme.
   (**Regulation of synthesis:** The rate limiting step in the pathway of cholesterol synthesis is HMGCoA to mevalonic acid. When the cholesterol level is increased in the cell the high cholesterol will bind to the HMG CoA reductase and inhibit it.)

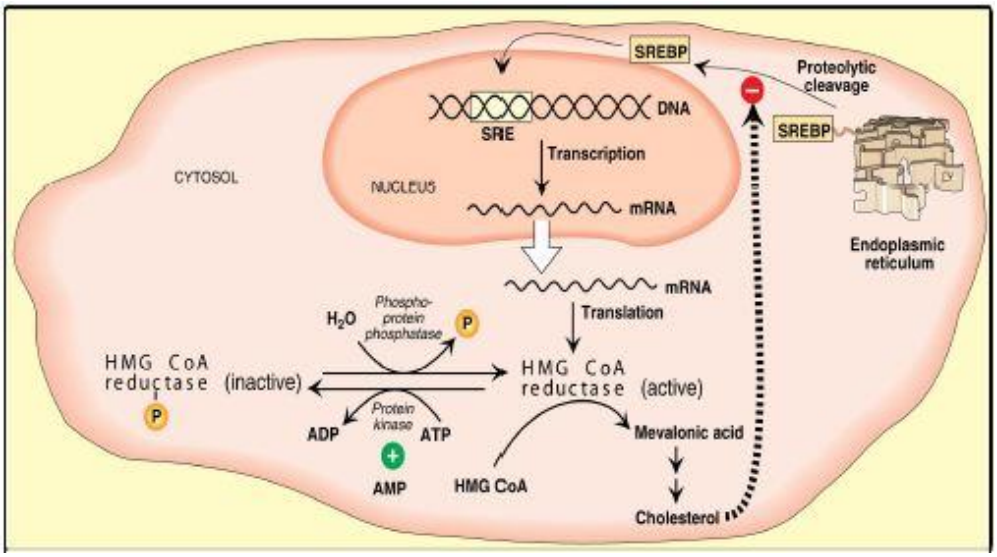


   Now a lot of the genes that are regulated their transcription needs some enzymes and factors, these factors are called transcriptional factors. Expression of HMG CoA reductase gene requires transcriptional factors, just before the sequence of the gene there is a sequence where the transcriptional factor can bind, and the sequence in this case is **SRE** (steroid regulatory

element) element that regulate the steroid synthesis. The transcription becomes active when it binds to **SREBP** (steroid regulatory element binding protein) this is the transcriptional factor of the gene and is usually attached to **ER**(endoplasmic reticulum) and you know that the DNA is present in nucleus when the cholesterol level is decreased in the cell, it will lead to the dissociation of the SREBP and binds to SRE, now the transcription of the gene becomes possible and DNA will give mRNA and the mRNA translation will give us the HMG CoA reductase.



2) **Covalent modification;** enzyme is there we can modify it by increasing or decreasing its rate. There are two forms the enzyme HMG CoA reductase and the **phosphorelated enzyme** HMG CoA reductase. The one which is active is the **non-phosphorelated** form. Remember! The synthesis of cholesterol starts from acetyl CoA , the glucose is the precursor of the carbon atoms of the cholesterol, i.e. from pyruvate dehydrogenase (glucose →"via glycolysis" pyruvate → "via pyruvate dehydrogenase" acetyl-coA will be used for cholesterol synthesis) if the blood glucose is low the enzymes that utilize glucose will be inhibited but the enzymes that produce glucose should become activated so like this idea and logic the phosphorelated one will be inactivated.[**Note**: Acetyl CoA can come from the fat degradation , but cholesterol is associated with growth as it is part of the cell membrane, and insulin goes with the growth and all of them work together.] The protein kinase that adds the phosphate group is **not** PKA. However, it is activated by **AMP** "low energy level" which binds with the protein kinase and makes it active, the AMP dependent protein kinase phosphorelates the HMG CoA reductase rendering it inactive. When the energy level is improved, the **phosphatase** will remove the phosphate group converting the enzyme to active form. **So the covalent modifications depend on the AMP levels**.



3) **Hormonal Regulation;** Glucagon and insulin. Glucagon stimulates the phosphorelated form and insulin the dephosphorelated form by protein phosphatases (they are activated by insulin) and its presence indicates plenty of sugar and growth conditions.
4) **Proteolytic regulation;** the levels of the enzyme itself can be changed by increasing protein degradation. As you all know the enzymes are constantly being degraded and made, by increasing the rate of degradation only the level of the enzyme is going to be reduced. **High cholesterol** levels stimulate the degradation of HMG CoA reductase (proteolysis)

   These are the ways for maintaining the cholesterol levels at appropriate conditions

-The two mechanisms are summarized by the figure from the book (look above).

**********past paper time**********

1-Which one of the following is **true** about esterification process in plasma:

A) It happens by the enzyme acyl CoA transferase.

B) It happens inside LDL in plasma.

c) Phosphatidylcholine is the second substrate.

d) It has the same mechanism that used in esterification in cells.

e) None of the above

2-which one of the following is **true** about HMG CoA reductase:

A) It is activated by high amount of cholesterol.

b) High insulin/glucagon ration inhibit it.

c) Its gene expression is more active when SREBP is bound to ER.

D) It is inhibited by phosphorylation.

E) None of the above.

(The third and fourth is not past paper question but test yourself as a doctor)

3-A 35 year old man present to his primary care physician intermitted chest discomfort. He had been entirely well until he experienced an episode of substernal tightness 3 months previously after enjoying a heavy meal in a restaurant. He noted similar substernal tightness on two occasions while raking leaves. His family shows a good history. His mom is healthy and has average lipid serum. Which one of the following is true about his case :

a) Peripheral tissue in this patient would show increase stores of cholesterol esters.

b) Most effective single therapy for him is reducing consumption of cholesterol.

c) Peripheral tissue in him would show decrease de novo cholesterol synthesis.

d) Most effective single therapy is to administrate HMG CoA reductase inhibitors.

e) None of the above.

4- Mice were genetically engineered to contain hydroxymethylglutaryl coenzyme A reductase in which serine 871, a phosphorylation site, was replaced by alanine. Which of the following statements concerning the modified form of the enzyme is most likely to be correct?

A. The enzyme is nonresponsive to adenosine triphosphate depletion.
B. The enzyme is nonresponsive to statin drugs.
C. The enzyme is nonresponsive to the sterol response element–sterol response element–binding protein system.

D. The enzyme is unable to be degraded by the ubiquitin–proteasome system.
E. none of the above

Transport of cholesterol:

-We said that the chylomicrons are synthesized in the small intestine they have dietary TAGs, cholesterol. When they are released into the plasma the TAG is removed and what we remains chylomicron remnants that ends up in the liver by endocytosis so the cholesterol is transported from the small intestine into liver through **chylomicron remnants**. This is dietary cholesterol.

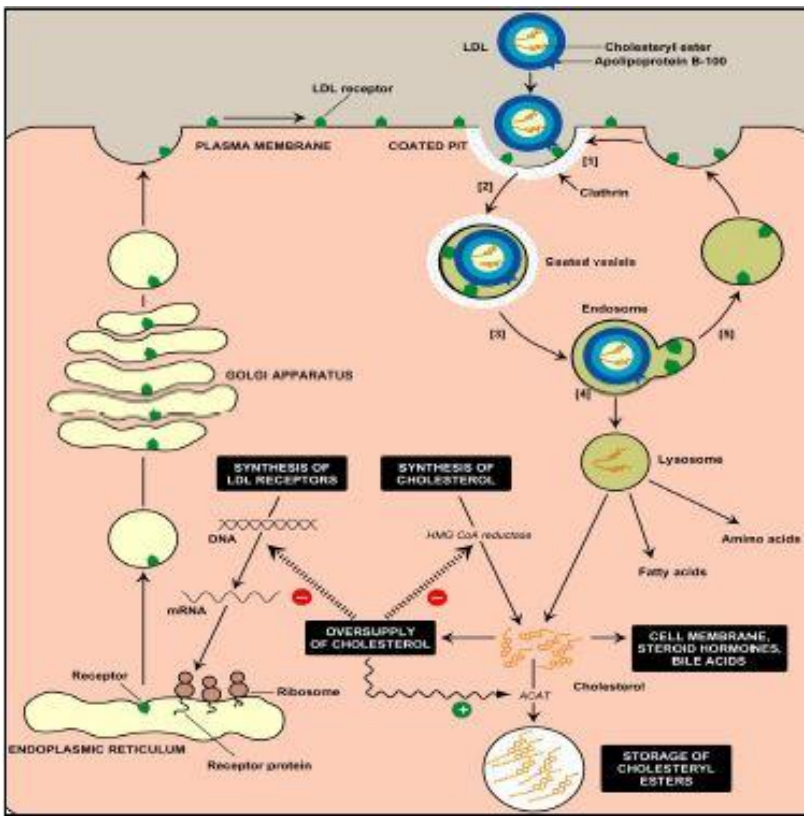
-The cholesterol synthesized in the liver is incorporated into the VLDL which is released into the blood and the TAG is removed in the capillaries converting the VLDL into IDL(intermediate between VLDL and LDL) is taken by the liver (50%) by endocytosis because it contains **apolipoprotein E** or it can continue with the removal of TAG and is converted into LDL so it mainly cholesterol and cholesterol esters it is taken by endocytosis by the liver or extra hepatic tissues. The extrahepatic tissues receive the cholesterol from LDL which is originally comes IDL which originally comes from VLDL "VLDL is the particle that transports cholesterol and TAG which are transported to the tissues and what remains is higher density particle LDL mainly from apolipoprotein B100 which can be recognized and taken by endocytosis on liver and extra hepatic tissues."

-VLDL is secreted acquires **apolipoprotein C2 and apolipoprotein E** from HDL and TAG hydrolyzed becomes IDL 50% in the liver and 50% continues LDL B100 apolipoprotein only and cholesterol and cholesterol esters are present which are taken by the liver and extrahepatic tissues.

-Remember:

(VLDL → IDL → LDL → HDL) the density increases.

-This figure shows how LDL is taken and LDL receptors has negative charge and can recognize apolipoprotein B100 that is the only one the LDL. There pits are coated by the protein clathrin upon binding LDL to the receptor the particle is taken up by endocytosis then what we have is a coated vesicle with clathrin which removed and the vesicles fuses with each other making a large vesicle called endosomes and they have proton pumps, they are pumped within the endosomes making the PH lower; this lowering in the PH causes the separation of the receptors from the particles, these will be recycled again. The particles that remain fuses with the lysosomes and LDL are degraded: the proteins give amino acids and the phospholipids give fatty acids and the cholesterol esters are hydrolyzed giving cholesterol.



-The hydrolyzed cholesterol can have several effects:

I. Inhibit the HMG CoA reductase to reduce cholesterol synthesis.
II. It can inhibit production of LDL receptors, notice! This inhibition reduces the amount of LDL particles introduced by the oversupply of cholesterol. This called **downregulation**.
III. It stimulates ACAT to promote storage of the cholesterol.
This is what happens in the endocytosis of the LDL particles. They can recognize apolipoprotein B100 but not B48.

-There are other receptors and are called macrophages scavenger receptors, these are non-specific it can recognize modified or damaged LDL, the damage either to the protein or phospholipids and the damaged will not be recognized by

the LDL receptors but by macrophage scavenger receptor and it does not undergo downregulation even if the cholesterol enters nothing will happen to them.

-When the macrophage is full of LDL it is converted into **foam cells** and the accumulation of foam cells in the sub-endothelial space is an early evidence of **atherosclerosis**.

-Foam cells are macrophages that are full with damaged or modified LDL.

-The damage can be by oxidants “notice the figure” and the antioxidants Vitamin C “ascorbic acid” and vitamin E, ß-carotene inhibit the damage of the LDL.

The exact details of the above are not included as they are going to be covered in pathology.

-Familial hyper Hypercholesterolemia: The level of cholesterol is high in the blood. The normal level is usually 200mg/dl and the familial hypercholesterolemia can reach levels that are high as 700mg/dl, the question is why?

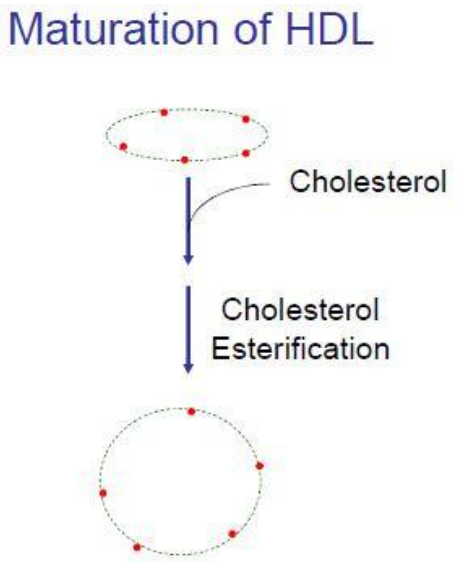
-This is a genetic disorder “familial” and can be inherited either from one parent “heterozygous 300mg/dl” or both “Homozygous 680 mg/dl”, the later has much higher levels and the reason is due to the absence of LDL receptors ,or the receptors are not functioning, or rapidly degraded abnormal receptors. If the disease is inherited from both parents this means that there is no receptors and the LDL will stay in the blood for longer periods of time and then they are taken by macrophages and results in atherosclerosis. [**Remember:** LDL does not carry TAGs, VLDL does that is delivered to the tissues.] This will also lead to accumulation of IDL which is converted to LDL leading to the accumulation. Moreover, cholesterol is abundant in the tissues, atherosclerosis, death in the childhood, also in their teen years they may suffer from myocardial infarctions and death as result. Measures should be done in order to decrease cholesterol.

HDL:

-The origin of HDL is not clear:

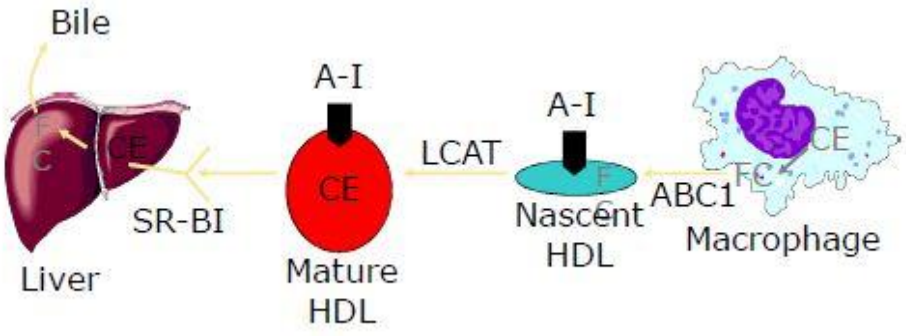
- ✓ May be it is produced in the liver.
- ✓ May be in the intestine.
- ✓ May be within the plasma from "free" apoA acquire phospholipid from other particles and it becomes HDL or other particles.

-When it is produced it is nascent HDL newly synthesized and the shape of the particle is discoid shape and the apolipoprotein a1 is the major apoprotein in the HDL.



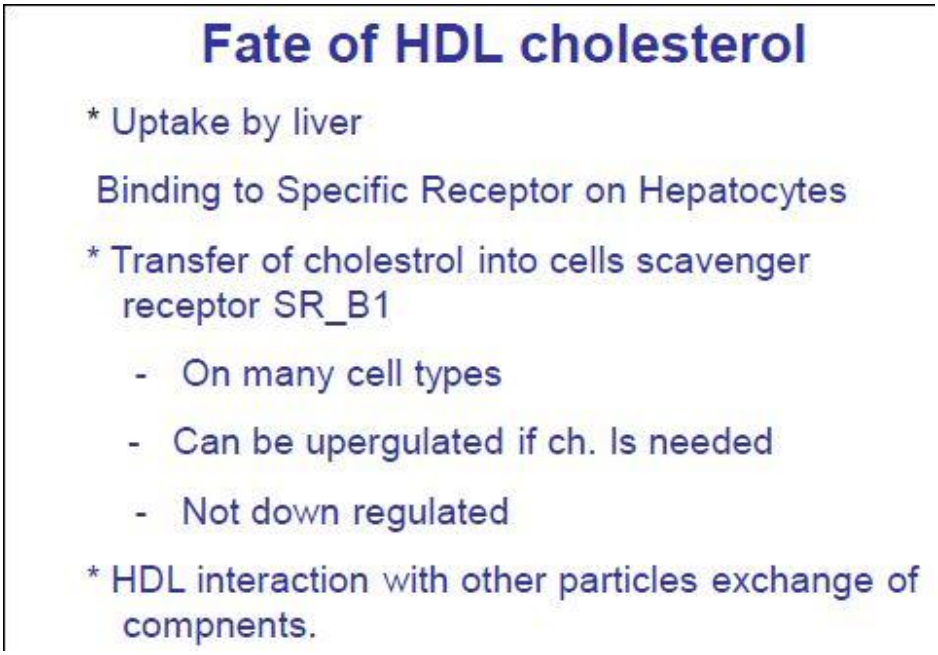
-The first step is to be esterified as we said to become cholesterol ester, the cholesterol is on the surface when it is esterified it goes into the interior. The discoid shape become sphere as it becomes filled with cholesterol esters. That's why it has a huge range of densities.

-HDL participates in the reverse transport of cholesterol, from cells to the liver, from foam cells in vascular tissues to the liver. We have a group of proteins called ABC (ATP binding cassette) the directional movement, in the cellular membrane the ABC1 change the orientation of the cholesterol from the interior leaflet to the exterior leaflet. Esterification of cholesterol by <u>LCAT</u> that is activated by apolipoprotein A1 we have cholesterol esters so the discoid becomes spherical shape. Peripheral tissues can move the cholesterol into the HDL.



HDL3 → HDL2 “larger more esters” also can transfer some of the cholesterol into VLDL.

That’s why HDL is good for as it moves cholesterol into the liver. In addition, when taking samples to the lab it is not enough to measure the total level of cholesterol we need to know the LDL and HDL amounts. LDL can cause atherosclerosis and the HDL take cholesterol from the tissues into the liver.



**********past paper time**********

5- Which one of the following is true about apolipoprotein c2:

A) It is important in the process of recognition of LDL on extrahepatic tissue.

b) If it is deficient, chylomicrons will persist in the blood.

c) Chylomicrons take it from LDL.

d) It is found only in HDL.

e) All of the above.

6-which one of the following is true about familial hypercholesterolemia:

a) It results from deficiency in LDL receptors.

b) It results in low concentrations of IDL in blood.

c) It must be inherited from both parents.

d) In it, phagocytes will be filled with VLDL and this may cause atherosclerosis.

e) None of the above.

7-which one of the following conditions will result in a decrease in both HMG reductase and LDL receptor activity: (This question is not from past paper but from **Lippincott**)

a) Increase ACAT activity.

b) Decrease scavenger receptor activity.

c) Increase intracellular unesterified cholesterol.

d) Decrease apolipoprotein E receptor activity.

e) None of the above.

8-which one of the following is responsible for reverse transport of cholesterol:

a)chylomicrons b)VLDL c)IDL
d)LDL e)HDL

Answers of questions: 1C /2d /3d /4a /5b /6a /7c /8e