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In the last lecture we talked about cholesterol synthesis, today we will continue talking about cholesterol and this sheet will cover the following:

A) ESTERIFICATION OF CHOLESTEROL

B) RELATIONSHIP BETWEEN CHOLESTEROL LEVELS AND DEATH PER 1000 MEN

C) REGULATION OF CHOLESTROL SYNTHESIS

D) TRANSPORT OF CHOLESTEROL IN THE BLOOD

E) REVERSE TRANSPORT OF CHOLESETROL

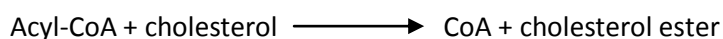
F) NOTES ABOUT MEASURMENTS OF CHOLESTEROL

G) MODIFIABLE AND NON MODIFIABLE CAD RISK FACTORS

I) NOTES

A) ESTERIFICATION OF CHOLESTEROL:

Esterification of cholesterol involves addition of fatty acid by ester bond to hydroxyl group at carbon no.3. The reaction occurs in 2 different ways: 1- in the cells; by donation of acyl group from acyl coa and the enzyme is known as **acyl coA:cholesterol acyltransferase (ACAT)**; this enzyme will transfer Acyl group to produce cholesteryl ester.



2- in the plasma; but there is no acyl coA in the plasma (no active fatty acids); esterification occurs in the lipoproteins which are rich with phospholipids, especially phosphatidylcholine (lecithin); which is the donor (source) of the fatty acid, it donates the fatty acid that is attached to carbon no. 2 leaving lysolecithin and cholesteryl ester will be formed. The enzyme has a similar name; **lecithin:cholesterol acyl transferase (LCAT)**.

B) RELATIONSHIP BETWEEN CHOLESTEROL LEVELS AND DEATH PER 1000 MEN:

Why did cholesterol have the attention of many scientists over the years?

Because it was found that high cholesterol is associated with increased risk of coronary artery disease; this study shows that the death rate (after admission to hospitals because of myocardial infarction) is increased if the total cholesterol is increased, the higher the

cholesterol concentration the higher is the death rate per 1000 men who were admitted to the hospital with myocardial infarction (myocardial infarction is not always fatal) but if the cholesterol concentration was high the death rate will greatly increase, which means that it is not only associated with increased risk of having myocardial infarction through atherosclerosis, but with increased risk of fatal myocardial infarction that ends with death. That's why cholesterol was studied thoroughly by many scientists.

Check the curve (slide #29) ; it is showing death rate per 1000 men in relation to cholesterol levels ; high cholesterol levels may increase the morbidity and mortality of Myocardial infarction (some patients may live with it others will die.) . High levels of cholesterol will lead to **exponentially** increase the death rate per 1000 men leading to increase mortality & risks of CHD (coronary heart disease) or atherosclerosis.

C) REGULATION OF CHOLESTROL SYNTHESIS:

Although cholesterol is very important for the cell function, from our realization of dangers of heart disease and their increased risks in relation with cholesterol levels, the body regulates its rate of synthesis strictly. The regulatory enzyme is **HMG coA reductase** that catalyzes rate limiting step in cholesterol synthesis (The step that convert HMG coA to Mevalonic acid) , how is it regulated?

In several ways, this reflects the importance of the regulation. The regulation happens by: 1- at level of gene expression of the enzyme, the enzyme synthesis is increased or decreased according to the needs of the cell.

2- Covalent modification it can be activated and deactivated.

3- Hormonal regulation.

4- Proteolytic regulation.

This enzyme; HMG coA reductase's activity is ultimately regulated by the level of cholesterol, when the level of cholesterol is increased this will lead to the inactivation of the enzyme HMG coA reductase, this is a kind of negative feedback regulation.

MODES OF REGULATION:

1) Gene expression (long term regulation < for days >):

Gene expression is the synthesis of mRNA from the genes in some cells but not in others, genes are found in all cells. All genes are found in all cells, but which gene is regulated and which gene is synthesized is determined by different cells (not all cells will produce all proteins and transcribe all genes), every cell has specific genes that are expressed or their messenger RNA is synthesized. Expression of HMG coA reductase or the synthesis of mRNA

for the enzyme (transcription) requires a transcriptional factor (which is a protein factor), this factor is required in the synthesis, **SREBP (sterol regulatory element binding protein)** only after binding of this protein transcription can occur. At the beginning of the gene there is a sequence of nucleotide **SRE (sterol regulatory element)**, if the level of cholesterol is decreased, this will increase the rate of transcription or expression of the gene; after binding of SREBP to SRE, the synthesis of mRNA occurs. On the other hand, if the level was high, there will be no synthesis of mRNA, and no synthesis of the enzyme.

2) Covalent modifications (short term regulation < only for few minutes >):

HMG coA reductase can exist in two forms: phosphorylated (inactive form) shown in red and dephosphorylated (active form) shown in green (slide 33). Phosphorylation of the enzyme depends on the presence of AMP, when AMP is found in high concentration it binds to a protein kinase (which adds phosphate group to proteins) in this case it is the enzyme HMG coA reductase converting it into the dephosphorylated form. Always the addition of phosphate spares glucose. And the synthesis of cholesterol starts from acetyl coA which comes from glucose and fatty acids, if there is need for glucose\energy, it is not the time for synthesis of cholesterol, that's why phosphorylated form in inactive form, also it is activated by AMP. What does the increased level of AMP signify?

It signifies that the energy level of the cell is very low, because if most ATP is converted to ADP, how do we get energy from ADP?

We transfer phosphate group from one ADP to another ADP forming AMP and ATP. That is why high AMP shows very low energy in the cell, so it's not the time for synthesis pathways; it's time for degradation pathways. Getting Energy is more important than synthesis of molecules that require a lot of energy as you saw.

Note: HMG coA is found in 10 mitochondria for the ketone bodies 2) cytosol for cholesterol synthesis.

When energy level is good and insulin is high, well fed state insulin activates phosphatase to remove phosphate group activating the enzyme, presence of insulin indicates well fed state, it's time for growth, development and differentiation of cells extra.

We call it covalent modification because phosphate group is covalently added usually to serine or threonine residues.

3) Hormonal regulation: (glucagon Vs insulin):

Glucagon: the phosphorylated form is increased. Glucagon indicates low glucose (low energy) , so it's not the perfect time so synthesizes cholesterol , it will increase phosphorylated form of HMG coA reductase .

Insulin (well fed state): the dephosphorylate active form is increased by stimulating phosphatase which in turn will dephosphorylate the inactive form making it active again.

4) Proteolytic regulation:

The level of many enzymes in the cell is not constant over the time, they may increase and decrease. The regulation of the amount of enzymes is done by 1-regulation of synthesis (transcription amount of mRNA) or by 2-the amount of degradation.

So, each enzyme is in a continuous synthesis and degradation, to regulate quantity of the enzyme, even if it's a slow rate process, the amount of enzyme is regulated by changing the rates of synthesis and degradation.

If the cholesterol level is high this will stimulate the degradation or proteolysis of HMG coA reductase, the rate of proteolysis is increased which means that the level of the enzyme is decreased, a lot of enzymes are regulated at the level of transcription and at the level of the degradation of the enzyme.

SREBP is usually attached to endoplasmic reticulum (it didn't reach the nucleus of the cell). When cholesterol is high it inhibits the cleavage of the bond connecting SREBP to ER, and it is removed by proteolysis so low cholesterol level will cleave this bond allowing the protein to migrate to nucleus binding to the DNA, and stimulate transcription of messenger RNA of the enzyme.

::: these 4 modes work together to maintain level of cholesterol :::

D) TRANSPORT OF CHOLESTEROL IN THE BLOOD:

Cholesterol is found in all five classes of lipoproteins from chylomicrons which carry dietary cholesterol and TAG, to VLDL which is synthesized in the liver. Chylomicrons mainly carry TAG but when it is removed by the action of lipoprotein lipase what remain are the chylomicron remnants which are rich in cholesterol and they are taken by the liver.

VLDL also is converted to IDL and LDL; IDL can be removed by the liver cells or converted to LDL, which is removed by liver and extra hepatic tissues.

:::CHYLOMICRONS :::

In the picture below, after a fatty meal TAG after its digestion and absorption, it is synthesized in intestinal cells, they are incorporated in large particles chylomicron (they contain more TAG than Cholesteryl ester (CE) as ratio) which go to lymph then blood, when reaching blood they acquire two apolipoproteins (**apo C-II, apo E**) from HDL, these mature chylomicrons (Now the mature chylomicrons contain APO B-48, APO C-II & APO E as surface component), are acted upon by lipoprotein lipase that removes TAG and converts it into free fatty acids taken by the cell, and glycerol which is sent back to the liver. After that

the size of chylomicrons decreases gradually, the apolipoprotein C-II is transferred back to HDL; by their removal, **Chylomicron remnant** are produced!

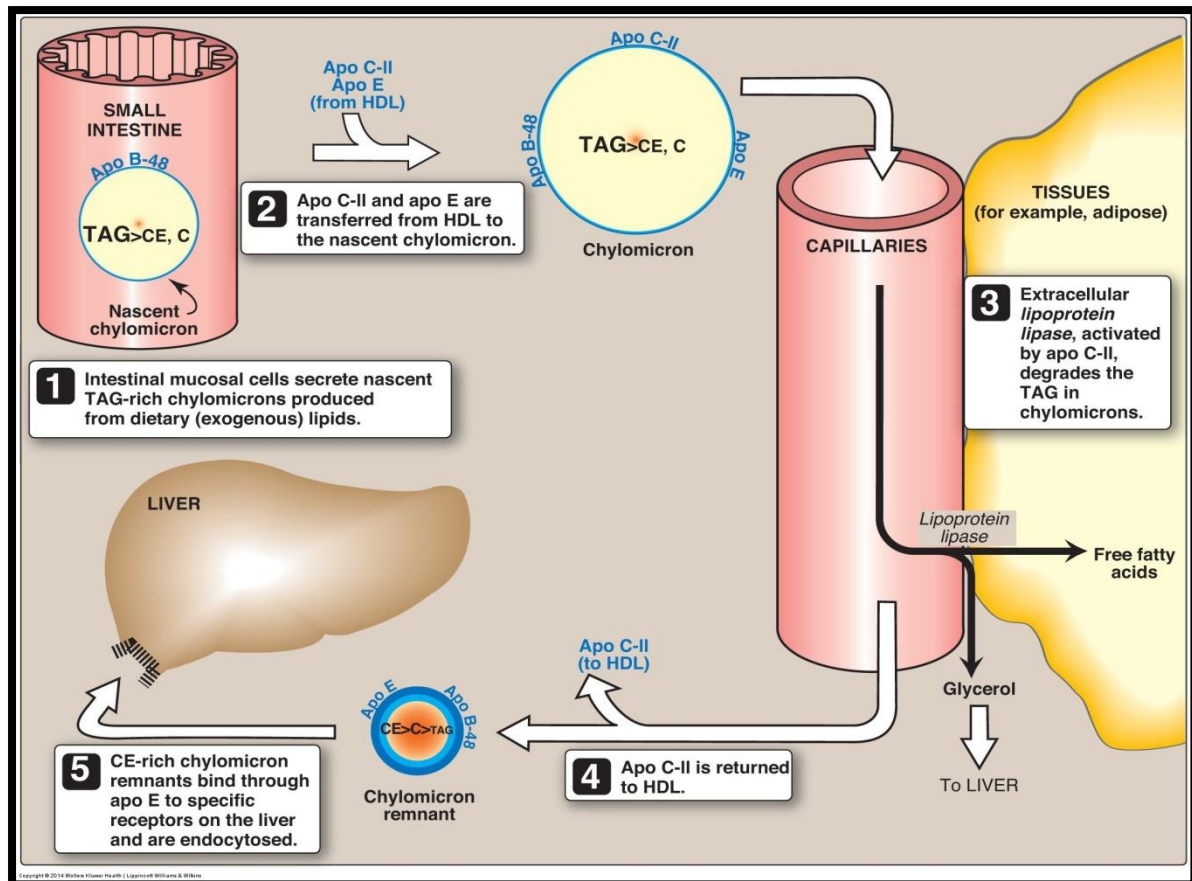
Chylomicron remnants are smaller in size and denser, they contain apo B-48 and apo E and now it contains surface components and more CE than TAGs.

Apolipoprotein E is important for the binding of the particles on the plasma membrane of hepatic cells which have receptors for apolipoprotein E, so, the remnants are taken by endocytosis into hepatic tissues. So, the cholesterol eaten in food is transferred to chylomicrons and ultimately goes to liver.

When we want to measure cholesterol, we should avoid the time after meals, because they may contain different amounts of cholesterol, so it's not true to measure it right after the meal or even after 3-4 hours; because cholesterol is still circulating in the chylomicrons and chylomicron remnants. These steps (this process) it will remain for **10-12 hours** after a fat-rich meal; that's why we ask the patients to fast for **14 hours** before we measure cholesterol level .

If we measure it during this process then measurements will indicate the amount of cholesterol in the last meal! Which can be variable, so we take the baseline (that is: after this process is finished).

We should wait for 12-14 hours until the chylomicrons and their remnants are almost totally removed from the circulation. LDL, IDL, VLDL are left.



These steps are well illustrated in the figure above.

Now I will mention some points about the **LPL**: (Lipoprotein Lipase):

- 1- Extracellular enzyme attached to the capillary's walls of most tissues including (Adipose , Muscle , Cardiac tissue)
- 2- It has different **Isomers**, with different **K_m** for each isomer toward TAGs.

Notes about this point:

- The enzyme found in muscles, especially **Cardiac muscle** have **low K_m** (that is; higher affinity), this means that it will take **TAGs** from chylomicrons **even when its concentration is low** (first priority). So cardiac muscle is especially dependent on energy provided by fatty acids.
- The enzyme found in **Adipose tissue** have **high K_m** (that is ; lower affinity) , meaning that it will take Fatty acids for storage only **when TAGs concentration is increased , because it takes them for storage only, not for energy use.**
- Synthesis and transfer to the luminal surface of capillary is stimulated by insulin, this makes sense because after eating the level of insulin and chylomicrons is high, the enzyme should be ready to take care of the TAG in these particles, so insulin

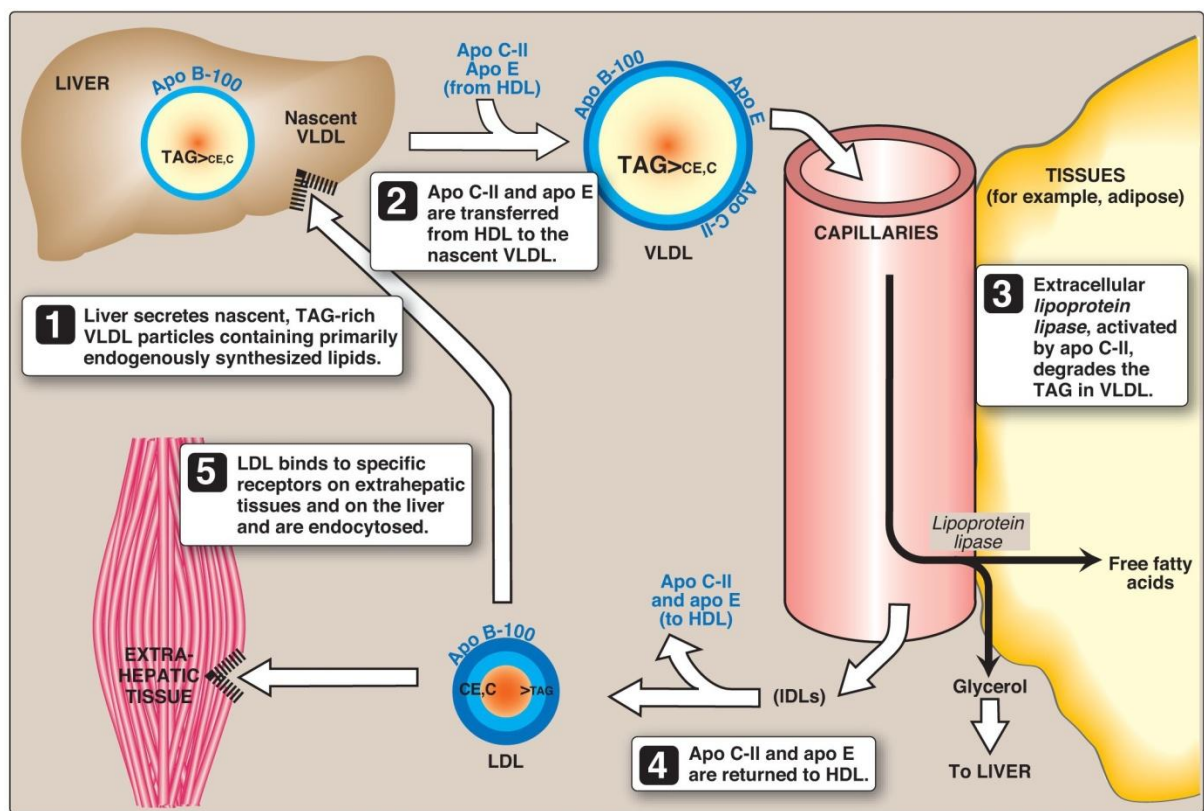
stimulates the entrance of fatty acids as well as synthesis of TAG in the adipose tissues (as we have seen before).

- It is activated by Apolipoprotein C-II that's why it is transferred from HDL to chylomicrons because chylomicrons require this apolipoprotein for activation, deficiency of apolipoprotein C-II or LPL leads to the accumulation of these chylomicrons in the plasma, chylomicrons won't be cleared from the circulation.
- If you examine plasma after hours of food, even after 12 hours you can use your naked eye to see that the plasma is rich with chylomicrons; the plasma is normally yellowish clear solution, but here it will be filled with these large particles giving it emulsion or milky appearance. And if you leave it the refrigerator over the night the top of it you will see a creamy layer in patients with this **enzyme deficiency**.

Extra notes about this enzyme from Lippincott:

- Elevated levels of insulin will increase the synthesis of LPL in Adipose tissue but will be decreased in Muscle.
- Fasting(decreased Insulin) favours LPL synthesis in Muscle
- LPL's highest concentration is found in Cardiac muscle ; reflecting the use of fatty acids to provide much of energy needed for cardiac function .

::: End of extra notes:::



1-This applies to VLDL which is first secreted from the liver (The liver synthesizes **Nascent VLDL** which contain about 60% TAGs, with **APO B-100**); they are released to the blood directly (not to pass through lymph). at the beginning they contain large amounts of TAG than cholesterol and cholesterol ester

2- It will acquire **APO E & APO C-II** , now its **Mature VLDL** .

3- It will go to capillaries with the action of the same LPL releasing fatty acids & Glycerol to the liver. Now the density of VLDL increased thus it's converted from VLDL (**Very Low Density**) to IDL (**Intermediate Density**) Lipoproteins.

4- IDL will release **APO C-II & APO E** . So the density is decreased, thus it's converted from IDL (**Intermediate Low Density**) to LDL (**LOW Density**) Lipoproteins.

Now it has only **APO B-100** as surface component which we began with, with **CE & C more the TAGs**.

4- IDL will release **APO C-II & APO E** . So the density is decreased, thus it's converted from IDL (**Intermediate Low Density**) to LDL (**LOW Density**) Lipoproteins.

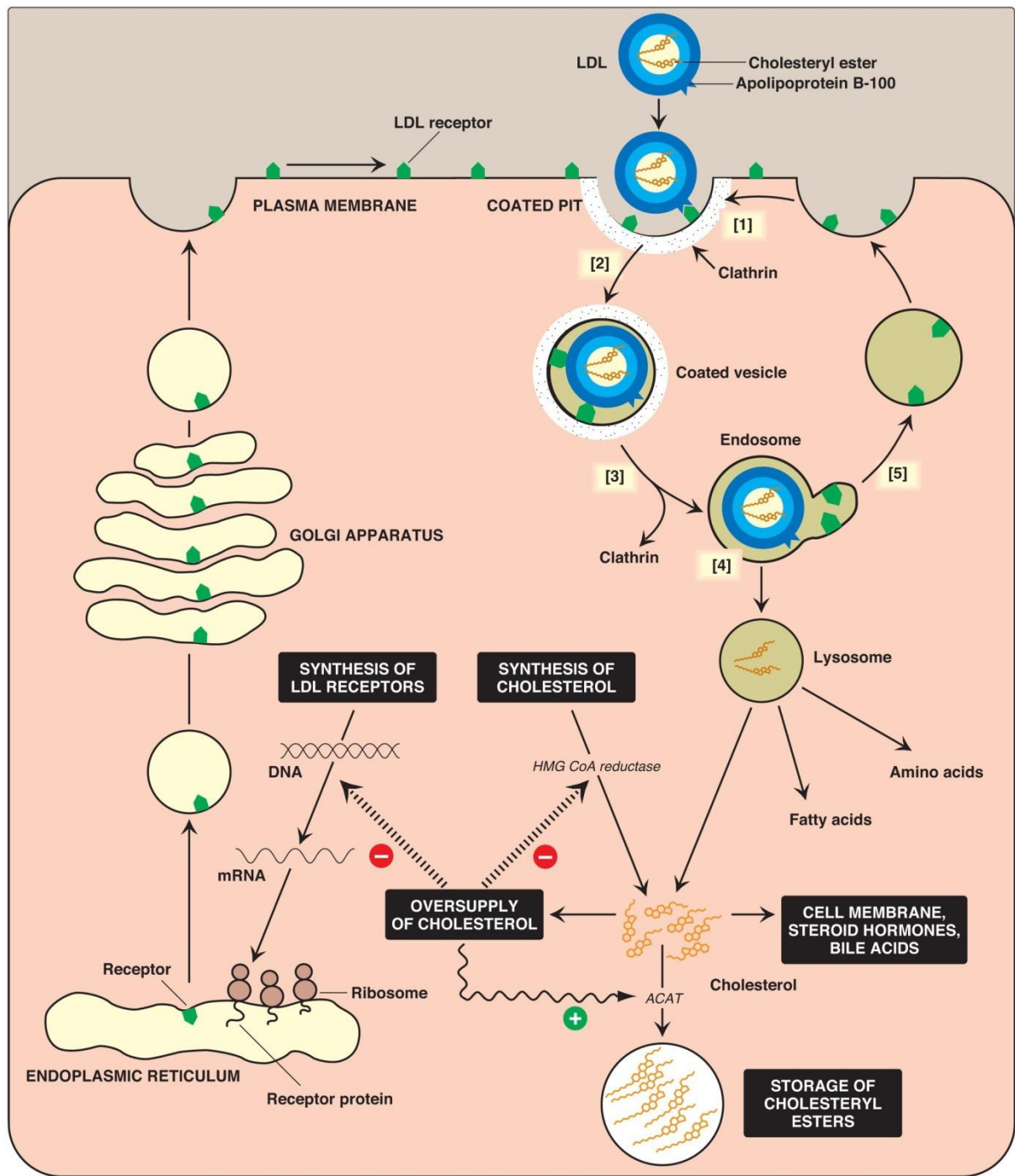
Now it has only **APO B-100** as surface component, with **CE & C more the TAGs**.

APO B-100 is important in **LDL** for its binding to extracellular receptors on muscles (extra hepatic tissues) and liver. Then LDL particles are endocytosed. (the uptake happens by endocytosis).

Note: **not all IDLs are converted to LDL ! , 50% of them are taken by endocytosis.**

These steps are well illustrated in the figure above.

HOW LDL ENDOCYTOSIS OCCURS?



endocytosis requires binding of LDL to the receptors, In liver cells (for example) there are LDL Receptors found on cell surface clustered in regions in cells called **PIT**, LDL receptors which are found clustered in a specific region of the cell called “**coated pit**”, the plasma membrane is coated on the inner leaflet with a protein called clathrin, that is important For endocytosis, and that is where it occurs.

- 1- after binding to LDL receptors, invagination of the membrane, LDL particles are taken by endocytosis, coated vesicle is formed, it contains plasma membrane coated with clathrin, in the inside LDL particle bound to LDL receptors.
- 2- The clathrin is removed
- 3- The vesicle will turn to **Endosome**.
- 4- Pumping of protons in endosomes will lead to change in the **pH** of it , thus leading to dissociation of **LDL** from **LDL receptor** .
- 5- Then the receptors are aggregated in a separate part of the endosome, and recycled back to the plasma membrane in order to bring more LDL particles, they are not used once, but several times, introducing more LDL particles into the cell, whereas LDL particles that are left will fuse with **Lysosomes** (membranous organelles in the cell with proteolytic digestive enzymes where degradation of molecules in cells occurs).

6-Degradation in Lysosomes will convert LDL components as following;

1') PROTEINE PART (**APO B-100**) >>>> converted to **AMINO ACIDS**.

2') **CHOLESTERYL ESTERS** >>>> converted to **FATTY ACIDS and FREE CHOLESTEROL**.

what should be done to the **free cholesterol** in the cell?

we have 2 things: 1- introduced in to membrane but it will take certain amounts only 2- excess cholesterol is stored, to be stored it has to be esterified, in the cells this is done by **ACAT**, which amount is increased, oversupply of cholesterol lead to increasing the amount of ACA. This facilitates the storage of cholesterol in the form of cholesterol esters.

These steps are well illustrated in the figure above.

OVER SUPPLY OF CHOLESTEROL WILL LEAD TO :

- 1-The oversupply of cholesterol inhibits HMG coA reductase, decreasing the synthesis of new cholesterol.
- 2- But to stop LDL receptor bringing more and more into the cell, the receptor synthesis itself is decreased, oversupply of cholesterol stops the synthesis of mRNA of the LDL receptor, decreasing its amount and this (decreasing the amount of receptors in response to high cholesterol) is known as down regulation. Preventing the cell from being totally engorged with cholesterol, because of the regulation of the entering amount. Even if the LDL receptors are used again an again but to some extent maybe 10 or 20 times, then with each time some of the receptors will be lost, so the synthesis of new receptor molecules in inhibited by the cholesterol.

Macrophage Scavenger Receptor A (MSRA) :

They are non specific receptors; so it will take modified and damaged LDL, if LDL is modified or damaged by oxidation of its Lipids (especially fatty acids) or proteins it **can be taken by MSRA** , but **not by LDL receptors** (the LDL receptor is specific for the LDL, actually it is specific for apolipoprotein B-100, , the LDL should be intact LDL not damaged or modified to be able to bind to this receptor)

MSRA is not down regulated **as LDL receptors** which are down regulated .So **MSRA** will continue taking **LDL** until it get engorged with LDL and blows up !(the doctor used the word **تنفجر**) ; producing **Foam Cells** **مثل الرغوة** .Histologically when we want to see these cells under the microscope we stain(v) them with a stain(n) that will give it an **empty appearance** .

Accumulation of Foam cells in the sub endothelial space is an early evidence of Atherosclerosis plaque, Producing atherosclerosis.

FAMILIAL HYPERCHOLESTEROLEMIA:

Familial : indication that it's an inherited genetic disease with a familial pattern. **Hyper**: increase. **Emia** : in blood

(Some families have high cholesterol levels, in these certain families, the members die from early atherosclerosis (in their 20's, 40's, sometimes childhood)) .

When family members are **Homozygous** (that is both parents have the disease inherit both defective genes), cholesterol level may reach **680 – 700 mg/dl!** , while **heterozygous** (one defective gene) **cholesterol** level may reach **300 mg/dl**. (**Normal** people have **200 mg/dl**).

It happens due to absence of LDL receptors or abnormal receptors, or the events following the uptake (endocytosis) are defected, or recycling is impaired, or down regulation. So LDL won't enter the cells; that's not the problem! Because cells are capable of synthesizing cholesterol .But LDL remaining in the plasma will be oxidized, since its oxidized it won't be taken by **LDL RECEPTOR BUT WITH MSRA !** :S

In homozygous: they found that there was no receptor at all, so, the LDL level in the plasma increases to a large extent, it will be oxidized, accumulation of IDL (which is also taken by the LDL receptor, so more IDL will be converted to LDL which in turn will not be oxidized, it will be taken by “macrophage scavenger receptor”, and cholesterol deposited in the tissues leading to atherosclerosis and death in the childhood. Death occurs from myocardial infarction before the age of 20. Even young children (less than 10 yrs.) can die from atherosclerosis or myocardial infarction if they were homozygous, if heterozygous they will

live for a longer period than that. Patients may die before the age of 20 from a stroke , some **Homozygous** children (2 years old) died from a stroke .

While in heterozygous: they found half the normal no. because there is at least one normal allele that is able to produce half of the no. of receptors usually found.

::: HDL:::

Sources:

How it starts? Its origin is not clear. But it can be found in plasma in several ways;

1-it may be excreted from the liver and intestine as discoid shape particle (disc like), because it is mainly formed of phospholipids and proteins. No core components like TAG or cholesterol esters, it is only made of surface components.

2- Or budding from other lipoprotein particles; like when chylomicrons are getting smaller and smaller, part of the surface component of the chylomicron particle will bud (pieces of it will be released) and acquire apolipoprotein A-1, thus becoming a particle.

3- Or from free apo A (secreted by the liver), when it is in the circulation, it accepts phospholipids from lipoprotein particles forming HDL.

So the origin of HDL is not clear; it could be one of these or more than one of these 3 mechanisms.

Maturation of HDL :

After secretion, it is found in discoid shape, cholesterol can be transferred from other particles, or from the outside leaflet of the plasma membrane, from dying cells or any cell with excess cholesterol (foam cells) is delivered to HDL. While in HDL it undergoes esterification by LCAT, once esterified, it won't remain on the surface (on the surface no free cholesterol), it enters from periphery to the inside, so now it has a core, so, it transforms from discoid shape into spherical shape, and once inside, it won't go back in the reverse direction (from HDL to cells again; this won't happen, only from cells to HDL). The idea of esterification is to trap the cholesterol in HDL .

HDL interaction with other particle, exchange of other components. The HDL can interact with other particles; can transfer cholesterol to VLDL and TAG comes in its place, so LDL VLDL HDL, can exchange their components. So, the trapped cholesterol (mentioned before) with the presence of this protein (**cholesteryl ester transfer protein**), the cholesterol can be transferred from HDL to VLDL and IDL.

A summary for the cholesterol in the HDL: starts from liver or small intestine (not known) as discoid shape, free cholesterol taken from peripheral tissues, they are esterified, apolipoprotein A is a stimulator for (LCAT), for the esterification. The HDL3 became HDL2 when it got bigger, it can interact with HDL receptors or exchange in the component as we said, getting back to HDL3, so we have several forms of HDL. The new forms (discoid shape are HDL3 and HDL2 which differ in their size).

F) NOTES ABOUT MEASUREMENTS OF CHOLESTEROL :

When we measure the level of cholesterol in plasma **it's not enough to know the total cholesterol level** , we should know where is this cholesterol : is it found in LDL ? how much found in HDL ? . On the other hands we are not concerned about chylomicrons ; because we do measurements after chylomicrons are cleared (after 14 hours fasting as previously mentioned) . **We are concerned about HDL, LDL more.**

LDL: if it's not taken by the receptors it will be oxidized leading to Atherosclerosis .

HDL: it will transfer cholesterol to liver for Elimination.

Hence , it's much better to have high cholesterol in HDL than LDL ! .

G) MODIFIABLE AND NON MODIFIABLE CAD RISK FACTORS :

Modifiable and non-modifiable CAD risk factors

Cigarette smoking	Males > 45 years Females > 55 years
Obesity	Males
Hypertension (blood pressure \geq 140 / 90 mmHg)	Family history of coronary artery disease
Physical inactivity	
Kidney disease	
Diabetes mellitus	
Alcohol consumption	
Stress	
Elevated LDL	
Reduced HDL	

The risk factors for coronary heart disease: we always ask what is the cholesterol level that we should aim at? If a man came (45 yrs.), we measured his cholesterol, when do we tell him it's high? The same level of cholesterol can be high in one person and normal/acceptable in another person.

If people have risk factors (2 or more), their cholesterol level should be decreased, what are these risk factors? (If you have them the risk of myocardial infarction will increase).

Those are modifiable: you can decrease LDL, increase HDL, stop smoking, lose some weight, you can treat hypertension, diabetes etc.

The non-modifiable: like age and sex; if you are a male, 48 yrs. Old, you can't that. (Females>55, males>45, those are risk factors, being male, family history of artery disease).

If someone have high risk factors , then cholesterol levels should be **reduced** , for example if it was acceptable to have 130 g/dl in LDL it wouldn't be accepted the same for a person whose age more than (45 years) assuming he's male or a person with high risk factors

I) NOTES

There was a past Question: which of the following food is the most rich in cholesterol :::: the answer was **LIVER**.

Eggs are rich in cholesterol but not the most.

But if the Question indicates that (daily **intake food**) or, the **most significance source of cholesterol** >>>> the answer is **EGGS**. (Because they are consumed on daily basis)

(The doctor said that mostly he will not repeat it ..Unless he wanted to discover who's attending the lectures).

All foods of animal origin contain cholesterol (beef liver cooked badly: 3ounce. Egg: 225)

Units that we should be familiar with:

1 OZ أوقية = 28 gram

1 pound = 16 OZ = 450 G

1 g of fat = 9 cal

Past question: 1 gram of fat has 9 calories, one ounce how much?

9x28=252 calories

بعتذر عن أي خطأ ! ^_^
علاء الدين دحبور