Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 22

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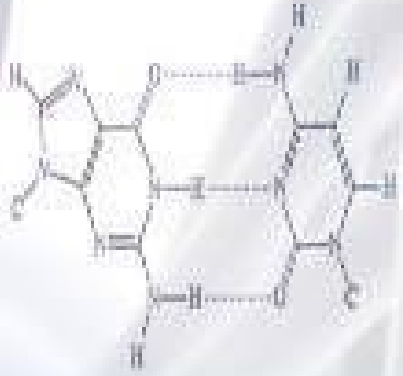
Biochemistry



Majida Al-Foqaraa'

Cholesterol and Bile Acid Synthesis

This sheet was done using Section 3 record … Enjoy !

- The Content :

1- Cholesterol synthesis requires

2- Synthesis of bile Acids

3- Lowering cholesterol level

4- Esterification of cholesterol

5- Regulation

6- Transport

The Doctor said that this lecture is the last one in the lipid metabolism and there will be 6 lectures for amino acids and nucleic acid metabolism

First : cholesterol synthesis requires

As we talked in the previous lecture, cholesterol requires carbon source from Acetyl CoA which provides carbon of ALL carbon atoms in the cholesterol, and for joining molecules together (synthesis) we need energy from ATP, and also we need reducing power (NADPH) because cholesterol almost have 27 carbon atoms and one oxygen atom so oxygen atoms originally present are removed during the synthesis and this is reduction so you need reducing power as well as oxygen itself.

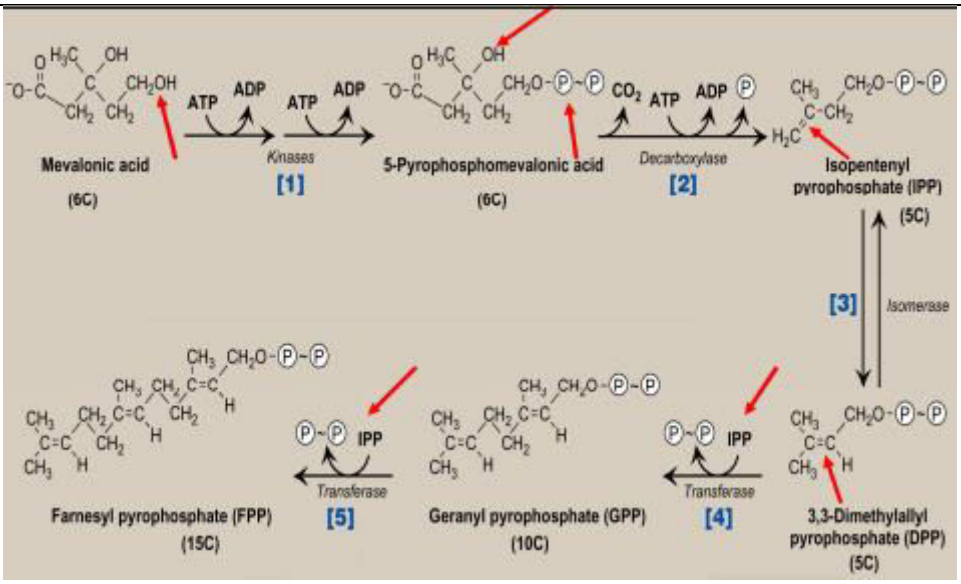
The pathway of synthesis starts with Acetyl CoA as an only source to condensate with another Acetyl CoA to give Mevalonate (in more than one step) then Mevalonate is converted to isoprene units, 6 of these isoprene units then condense to give squalene also in multiple steps, and squalene is converted to lanosterol then to cholesterol.

This pathway is long ; more than 35 steps that we don't need to know all of them but we should know the strategies of synthesis and some important intermediates. Synthesis starts with two molecules of Acetyl CoA by condensation to form Acetoacetate which is common in ketone bodies synthesis that occurs in the mitochondria but cholesterol synthesis occurs in the cytosol. Another condensation with Acetyl CoA to form HMG CoA catalyzed by HMG CoA synthase. Note that we have isoenzymes; one acting in the mitochondria and the other in the cytosol.

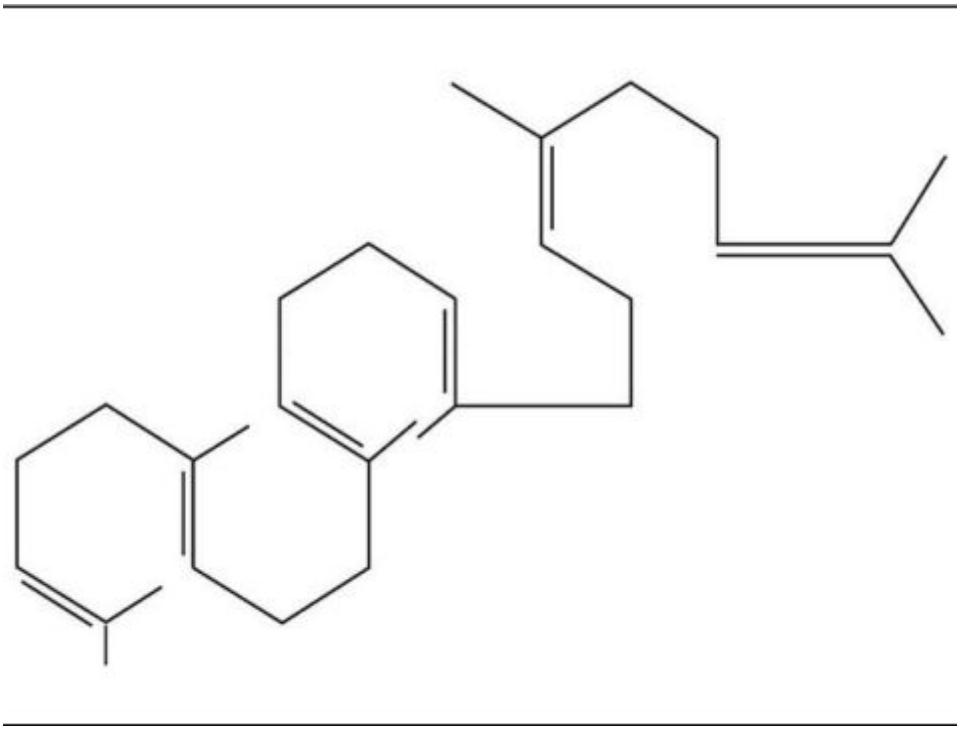
Next, this HMG CoA is reduced to give Mevalonate (6 carbon) by the enzyme HMG CoA reductase; an important enzyme for biosynthesis of cholesterol and regulation , and this is the rate limiting step considering that this is an early step (the third step) .Note that HMG has a carboxyl group which is reduced to aldehyde then to alcohol

Now , Mevalonate (or Mevalonic acid) has two OH groups, one of them will attach to two phosphate groups from 2ATP to form **5-pyrophosphomevalonate** and the purpose of this is to activate this compound for condensation . this compound will be dehydrated and decarboxylated (6 carbons become 5) and the result to have a branch so we call it Isopentenyl pyrophosphate (called like this because it has OH group and double bond) then the enzyme isomerase will catalyze the formation of the isomer form which is called **dimethylallyl pyrophosphate** and this compound will condense with the isomer to form **Geranyl PP** (10 carbons) and by releasing of PP we make the reaction move to the forward direction. This Geranyl PP will condense with another Isopentenyl PP to form **Farnesyl PP** (15 carbons) also this reaction is driven to the forward direction by releasing of PP.

By condensing of two farnesyl PP and releasing 2 PP forming Squalene (30 carbons) , we remove the 2 PP because its like a head to head reaction . This Squalene is only made from C and H so it's a hydrocarbon compound and this structure is known as poly-isoprene compound (isoprene is a 5 carbons compound) , we can see this structure in many molecules like Co-Q in the ETC, this Co-Q has 10 isoprene units which make it fat soluble having 50 carbon atoms . Also, Vitamins A, E, and K are examples of fat soluble because of these poly-isoprene units. Dolicol involved in the synthesis of glycoproteins is also fat soluble due to these isoprene units.



The doctor mentioned the rubber band as an example; he said that rubber can be artificial or natural and the natural rubber, which is hydrocarbons, is made in the rubber trees in same mechanism our liver makes cholesterol.



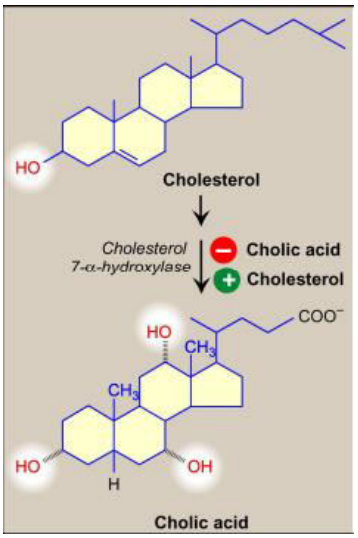
Squalene,which is a linear compound, can be in a structure that is very close to cholesterol as shown in the figure above . Next step is addition of Oxygen at carbon 2 and 3 in the form of epoxide which is very unstable so this will be converted to Lanosterol as the first intermediate to have the

steroid nucleus. The enzyme to catalyze this reaction is Squalene mono-oxygenase (or cyclase as the doctor said) , So this single reaction produces 4 rings !

Lanosterol has 30 carbons so the next steps is the removal of the extra carbons to form cholesterol and this process has many reactions (15 steps) that aren't required from us EXCEPT the intermediate **7-dehydrocholesterol** that is the precursor of Vitamin D . When we expose our skin to sunlight, this intermediate is cleaved at the second ring to synthesize Vitamin D. This vitamin we can get it from food supplements or by addition to milk –as an example- but the major source is sun light effect so if we don't expose our skin to sun light, Vitamin D deficiency would progress .

Until now we talked about cholesterol synthesis; reactions and intermediates, we should know those intermediates we have discussed previously and the number of their carbon atoms

Second : Synthesis of Bile Acids



Synthesis of Bile Acids occurs from cholesterol ,we can see in the figure the cholic acid and cholesterol , the main difference between them is the carboxyl group at carbon 24 because we remove the 25,26,27 carbons and the remained carbon is oxidized to carboxyl group, OH groups in the cholic acid are added at carbon 7 and 12 , we can see a dash line meaning that these OH groups are below the "plane" in one side and the methyl groups are on the other side –above the plane- so they are **amphipathic** molecules as they have <u>hydrophilic</u> and <u>hydrophobic</u> sites .

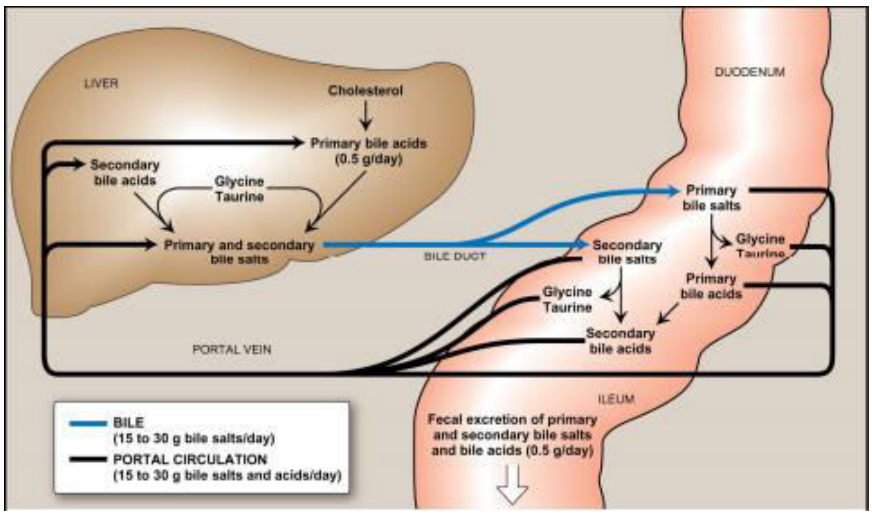
The hydroxylation at carbon 7 is the **rate limiting step ,** this means that the bile acid containing OH group at 7 may or may Not contain OH at 12 . The enzyme Hydroxylase catalyzes the addition of OH groups , we have two things regulating this enzyme ; firstly, its **inhibited** by **cholic acid** (high levels of cholic acid inhibit more conversion) and secondly, its **activated** by **cholesterol** (higher levels of cholesterol → more bile acids produced) and this is important when talking about lowering cholesterol level as we will see later .

Bile Acids are conjugated by addition of :

1- Glycine : it's a stronger acid with a carboxyl group than cholic acid , when added its called **Glycocholic acid (bile salt)**
2- Tuarine : a sulfur containing amino acid , again when its conjugated with cholic acid, this cholic acid will be stronger in acidity (also bile salt)

#theyre both mainly found in the ionized form so we call them bile salt, and when conjugating the Pka becomes lower and higher acidity.

The Figure shows the pathway of bile acids , cholesterol is converted to primary bile acid (primary means they still never reach to intestine) in the liver , the amount is 0.5 gram/day ,then this primary is conjugated to glycine or taurine as we said before then released to the small intestine where they do their function in solublization and then glycine and tuarine are removed becoming acids again, then these primary acids are acted upon by bacterial enzymatic action to remove some of the OH groups then we call them **Secondary bile acids** (secondary because they are produced later on after having passed through the intestines).



After that, secondary bile acids are reabsorbed back to the liver then conjugated again in the liver and so on , we call this cycle Enterohepatic cycle . Bile acids are circulating several times per day , almost 30 gram /day , not all bile acids are reabsorbed; some are excreted in the feces (about 0.5 g/day which is same about the synthesized one i.e each day liver synthesize 0.5 g of bile to compensate for that excreted in the feces)

At this point the doctor answered two questions from the students and the summary was : secondary bile acids have LESS OH groups and LESS solubility than primary bile acids.

Third: lowering Cholesterol Level

We do care about lowering cholesterol level because the increased level in cholesterol gives a high risk for atherosclerosis and myocardial infarctions . We can reduce cholesterol levels by :

Dietary restriction: decrease cholesterol intake but it may not be the most effective way because of the problem that if we reduce the intake, synthesis will increase because there is a regulation between them , so its effective only by 10%.

Increase the ratio of poly unsaturated fatty acid PUFA to saturated fatty acid because the saturated FA increase the cholesterol level. PUFA found in omega 3 and 6 essential FA

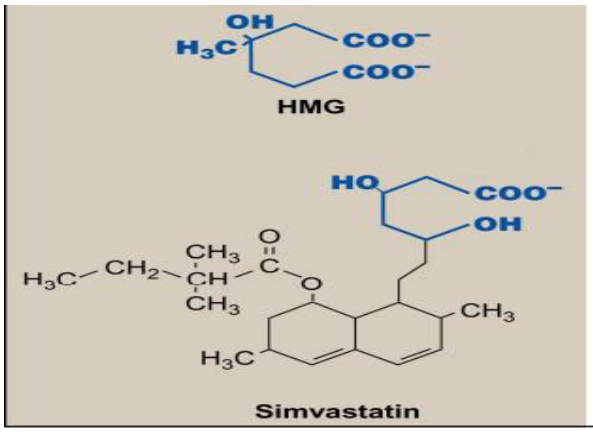
Increase the dietary fiber

Increase daily ingestion of plant steroid esters – plants make steroids but NOT cholesterol. These steroids are poorly absorbed so they decrease the absorption of cholesterol.

- ***Inhibition of synthesis :***

It's the most effective way , we can do it by:

1- inhibiting the enzyme **HMG CoA Reductase** because it catalyzes the rate limiting step ; One way to inhibit the enzyme is by drugs, as we see in the figure we have a drug that's similar-part of it- in structure with HMG and we call these group of drugs "STATIN" and they inhibit the enzyme by Competitive Inhibition (by looking at the structure you should be able to deduce this) , this is done by knowing the mechanism and steps of the reaction and designing a chemicals for inhibition . Another drug called LIPITOR (atorvastatin) it is used widely as an inhibitor for HMG CoA reductase , so myocardial infractions and CHD starts to decline …

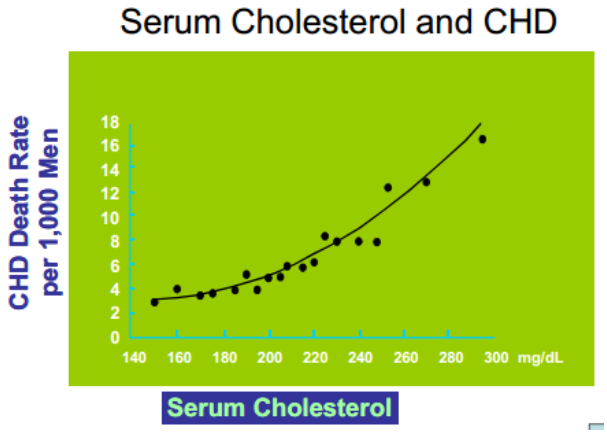


Inhibitors of HMG CoA reductase

2- decrease the enterohepatic cycle : we said that liver makes the bile acids and secrets them to intestine then 95% of them are reabsorbed BUT bile acids inhibit bile acids synthesis, So if we decrease the bile acid reabsorption to liver we will increase the conversion of cholesterol to bile acids. There is a substance called cholesteramine (polyamine + charged) that binds to bile acids and inhibits its reabsorption so instead of having 5% of this bile acids in feces , we will increase it to 10% or more and by this we are removing the feed back inhibition and lowering cholesterol level. This method is less used than drugs such as statin.

Fourth : Esterification of cholesterol

- Esterification is done in the ***cell*** by adding Acyl group from the Donor (fatty Acyl CoA) at OH at carbon 3 to make it a **Cholesterol ester** and this done in the cell for the purpose of the STORAGE, to name the enzyme we see the type of the reaction ; transfer reaction so the enzyme is transferase , and we see the substrate , then the enzyme is Acyl CoA Acyl Transferase [ACAT] .
- Esterification is done in the **Plasma** , especially in the lipoprotein ; HDL particles which is formed mainly from Phospholipids and protein .Note : in the plasma there is **No CoA** so there is another source which is **lecithin** – as a phospholipid found in HDL- . This Lecithin donates its Fatty Acyl group and lysolecithin is remained from lecithin. the enzyme is Lecithin Cholesterol Acyl Transferase [LCAT] .



The diagram shows the relation between cholesterol level and the mortality from Myocardial infraction , we see that it's a high risk for these abnormality when the cholesterol is high .

Fifth : Regulation

Cholesterol level should be regulated strictly because high cholesterol level is bad and cholesterol is also required for normal functions of cells (so we cant live without it) . We have many mechanisms for that and all of them target the enzyme **HMG CoA reductase.** High amount of cholesterol inhibited cholesterol synthesis by negative feedback. Mechanisms :

1- Regulation of transcription (Gene expression) : any gene is found in all cell But not all cells will make mRNA for that gene . Formation of mRNA is a Transcription. What happens is that there is a sequence before the gene called **SRE (Sterol Regulatory Element)** . there is a transcriptional factor protein called SREBP (SRE Binding Protein) that regulates the transcription of SRE. Now, when cholesterol is low, this protein will be cleaved from the endoplasmic Reticulum ER reaching the nucleus and binding to SRE and stimulate the expression of mRNA leading to the production of cholesterol. SREBP isn't cleaved **unless the cholesterol is low** and then synthesis But if the cell is reaching enough amount of cholesterol then no need for this process (note: cells such as muscle cells which receive already-produced cholesterol don't have this SRE transcription factor because they don't need to produce cholesterol)

2- Covalent modification : by addition of phosphate group converting the enzyme from active form to inactive form . This is done in the presence of high AMP (meaning low ATP) which activates of AMP-dependant Kinase that adds P group to the enzyme (HMG CoA reductase) so that the cell won't use any more energy on cholesterol synthesis (save its energy). this process can be reactivated by activity of phosphatase removing of P group

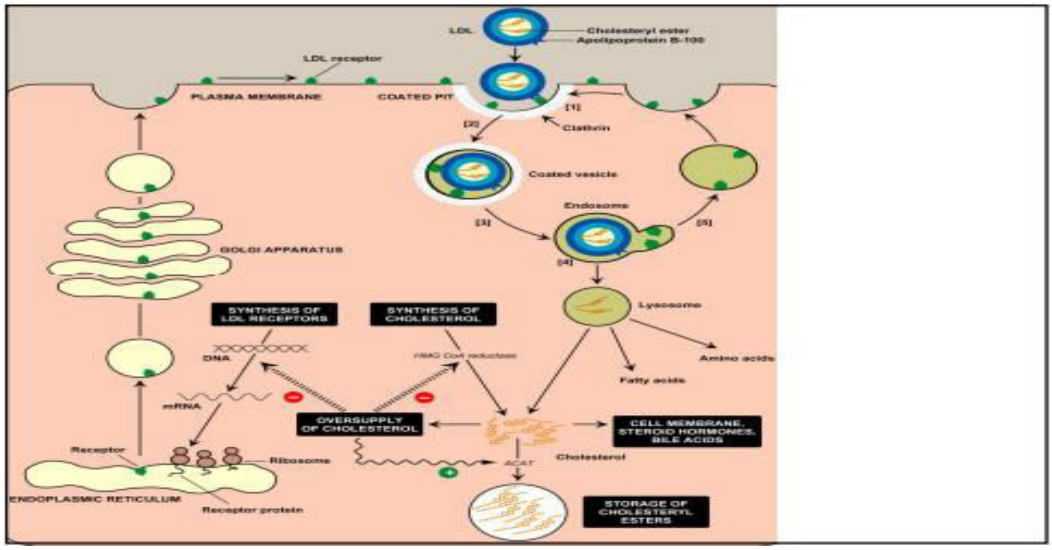
(As a general rule ; addition of P group means low blood glucose and low energy)

3- Hormonal Regulation : it was found that glucagon enhances the phosphorylated form whereas Insulin enhances the Dephosphorylated form of the HMG CoA reductase. High insulin---high glucose → cholesterol, High glucagon ----low glucose ; no need for cholesterol synthesis .
4- Proteolytic regulation : any enzyme in the cell has a balance between synthesis and degradation (turnover) .High level of cholesterol increase the activity of HMG CoA reductase proteolysis (less enzyme = less synthesis).

Sixth : Transport of Cholesterol in the Blood

5- First it comes from the intestines in the form of chylomicrons , chylomicrons after losing TAG they become remnants and these remnants are taken by the liver by endocytosis. Also , VLDL which is synthesized in the liver taking TAG and cholesterol to circulate . In the circulation, VLDL is converted to IDL which either taken by liver or continue as LDL . LDL , originally from VLDL, is taken by the liver(endocytosis) or by extra hepatic tissue . So , Transport of cholesterol in chylomicrons in the intestines to the liver or from the liver in the form of VLDL then IDL then LDL .

A figure in the book shows the endocytosis ; how the LDL enters the cell .by LDL receptors ,which are found in the cell membrane in clathrin coated pit , the whole LDL particle will bind and this is followed by endocytosis, then the coated vesicle will fuse with other vesicle to form the Endosome. the receptors then will be separated by the decreasing of the PH and they will go back to the membrane (cycling) Not used just for one



time !. Next , fusing with lysosome then degradation of protein to amino acids , TAG to fatty acids, and cholesterol ester to free cholesterol . These free cholesterol either added to cell membrane or the cholesterol oversupply doing three things :

1-inhibition of HMG CoA reductase (decrease it's synthesis)
2-esterification for storage (by conjugating with acyl groups)
3-inhibition Of LDL receptors; down regulation ,to reduce the LDL entering to the cell .

- LDL Must be intact (Normal) for binding to the receptors But LDL can be modified or damaged by oxidation . Damaged or modified LDL are taken by Macrophages to produce **foam cells** and this uptake doesn't undergo down regulation - meaning that macrophages constantly taking the LDL to form a foam cells that's the initial of **atherosclerosis.**

The doctor Stopped here and he said that we have to complete the rest from the slide and from the book and , most importantly, to see the figures …

I would like to thank all who have written any sheet because really it's hard „ Thank You All
*A Great Dedication To Mohammad Habes Al-Zghool
Made By : Mahmoud WK Shehab