29

Biochemistry

Doctor 2017 | Medicine | JU

Sheet ○ Slides

DONE BY

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DOCTOR

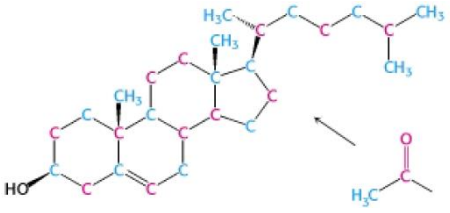
Faisal

Note : the sheet covers the last few minutes of lecture 28 in addition to lecture 29 , just in case you want to check the record ☺.

Cholesterol Synthesis

Cholesterol can be synthesized by all tissues in humans, mainly in the liver, small intestine and adrenal cortex. Its synthesis requires:

1. Carbon source: As with fatty acids, all carbon atoms are obtained from **acetyl CoA** which is composed of two carbons.
   Notice that scientists have determined the exact origin of each carbon; whether it originates from CH_3 or COO- of acetyl CoA. (the different colors in the picture beside)



2. Reducing power: Also similar to fatty acids**, NADPH** is the reducing power.
3. Energy: Since it is an anabolism pathway; it is endergonic, using **ATP** as an energy source.
4. **O_2** (not required in fatty acid synthesis).

Stages of cholesterol synthesis:

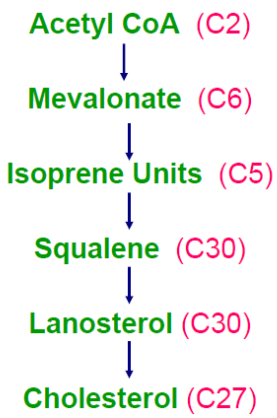
Synthesis of cholesterol is a long pathway, but we should only know and understand the steps the doctor mentioned.

Overview:

Firstly, 3 **acetyl CoA** molecules will be joined together forming a 6-carbon molecule called **mevalonate**. Mevalonate is activated and then decarboxylated forming an **isoprene unit** (5 carbons).

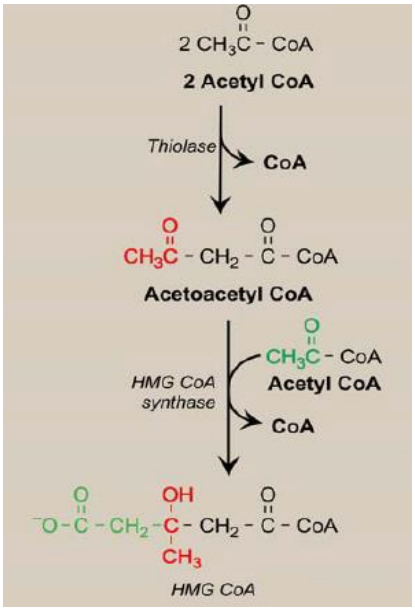
Note: isoprene has some similarities with isopentane.

6 isoprene units condense together forming a 30-carbon compound called **squalene**. Squalene is converted to **lanosterol** (30 carbons). And then during synthesis some carbons are lost converting it to 27-carbon compound, which is the final product "**cholesterol**".



Now, let's discuss the the steps thoroughly:

The first two reactions in the cholesterol synthetic pathway are similar to those in the pathway that produces ketone bodies. They result in the production of **3-hydroxy-3-methylglutaryl CoA (HMG CoA)**.



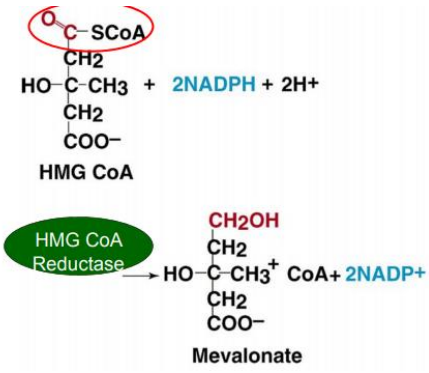
First, two acetyl CoA molecules condense to form **acetoacetyl CoA** with the catalysis of thiolase enzyme.

Next, a third molecule of acetyl CoA is added by HMG synthase, producing **HMG CoA**, a six-carbon compound.

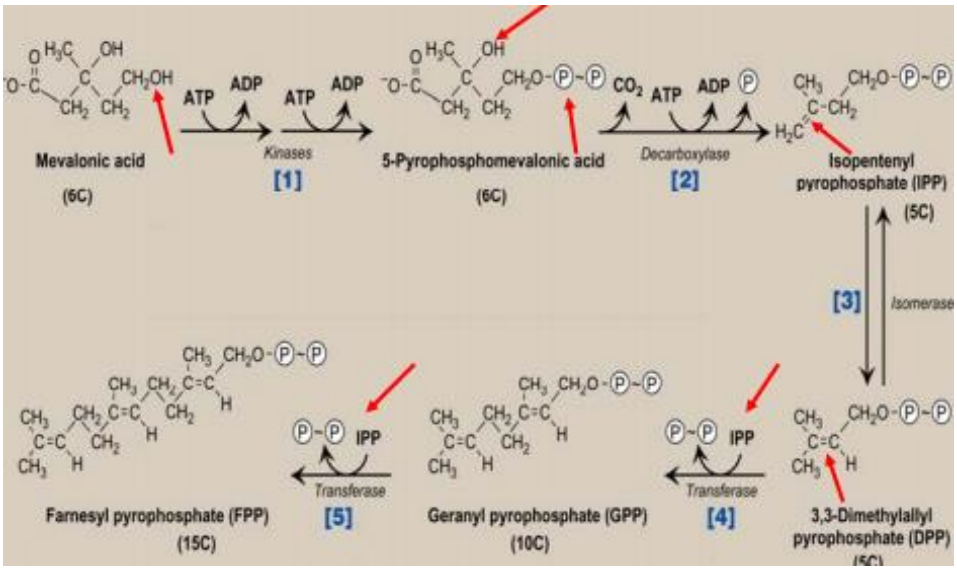
Extra note: liver parenchymal cells contain two isozymes of the synthase:

- Cytosolic isozyme: cholesterol synthesis
- Mitochondrial isozyme: ketone bodies synthesis.

The next step is the reduction of HMG CoA to **mevalonate** which is catalyzed by NADPH dependent enzyme (HMG CoA reductase. In this step one carboxyl group of HMG CoA is reduced in two steps(carboxyl [1]→ aldehyde [2]→hydroxyl). This is the most important step in cholesterol synthesis; since it is the rate limiting and key regulated step in this pathway.



Notice that mevalonate contains both carboxyl and hydroxyl groups.



Then, mevalonate is prepared for condensation (activated) in two steps; each of which transfers a phosphate group from ATP, forming **5-pyrophosphomevalonic acid**.

5-pyrophosphomevalonic acid undergoes decarboxylation; the carboxyl group is released as CO_2 and a double bond is introduced instead, forming **isopentenyl pyrophosphate (IPP).**

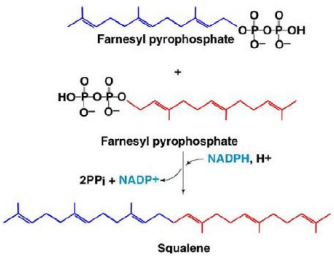
From its name we can conclude that: isopentenyl → it is derived from isopentane, -enyl→ it contains a double bond, pyrophosphate → it is found in its active form.

Then, IPP undergoes isomerization by changing the location of the double bond forming 3,3-dimethylallyl pyrophosphate or **DPP** (the doctor said that its full name is not important; just know that it's an isomer of IPP).

Then, IPP and DPP are condensed together to form ten-carbon **geranyl pyrophosphate (GPP)**. This reaction is driven in the forward direction because of the release of pyrophosphate which will be rapidly hydrolyzed to two inorganic phosphates (P_i); and that's why an activated form of IPP is required.

A second molecule of IPP then condenses with GPP to form 15-carbon **farnesyl pyrophosphate (FPP)** and again the hydrolysis of the released pyrophosphate drives the reaction in the forward direction.

Two molecules of FPP are condensed together head-to-head (in the previous condensation reactions, the molecules were condensed head-to-tail) forming **squalene (30 carbon)** and two pyrophosphates are released, again driving the reaction in the forward direction.



Note: squalene is the first intermediate in cholesterol biosynthesis that is nonphosphorylated and so, is highly hydrophobic containing hydrogens and carbons only (similar to oil products). So, hydrocarbons can be produced in living cells with squalene as their first product.

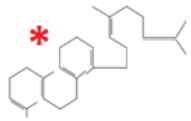
Notice that each five carbons in squalene form an **"isoprene unit"** so it is called polyisoprene.

Polyisoprenes are not only found in cholesterol synthesis but in many other hydrophobic compounds such as:

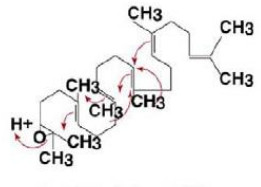
1. **Ubiquinone or coenzyme Q:** which has a long isoprene tail that is composed of around 50 carbons making it highly hydrophobic; that's why it's found in the inner mitochondrial membrane as part of the electron transport chain.
2. **Carotenes** in plants.
3. **Natural rubber**.

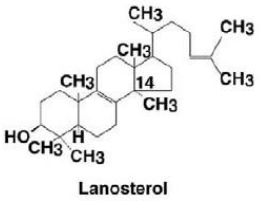
So, it is a universal way of synthesizing highly hydrophobic compounds.
Note: since squalene is composed of six isoprene units and 3 ATP units are hydrolyzed per mevalonate residue converted to IPP, a total of **18 ATP** molecules are required to make squalene.

*Here, squalene is drawn in a different way to show its similarities with the cholesterol.



The next step is adding one oxygen to squalene forming **squalene-2,3-epioxide** by squalene peroxidase but this oxygen is bound to two adjacent carbons making the epoxide highly unstable (the angle of the bond is 60° while normally in stable compounds it is around 104°). So, by an enzyme called cyclase a complex reaction occurs through which the rings get closed forming **lanosterol**. (The presence of double bonds also helps in the closure of the rings).





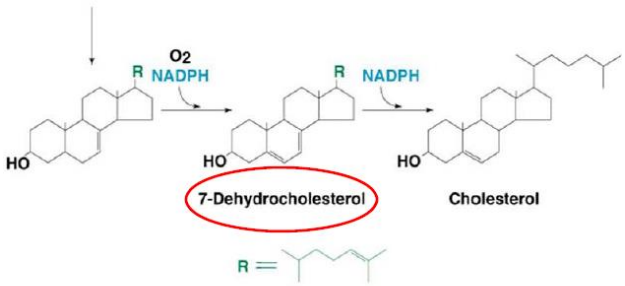
Lanosterol:

- Notice from its name that it contains steroid nucleus; so it is the first steroid intermediate in cholesterol synthesis.
- It is a 30-carbon compound containing three extra methyl groups than cholesterol.

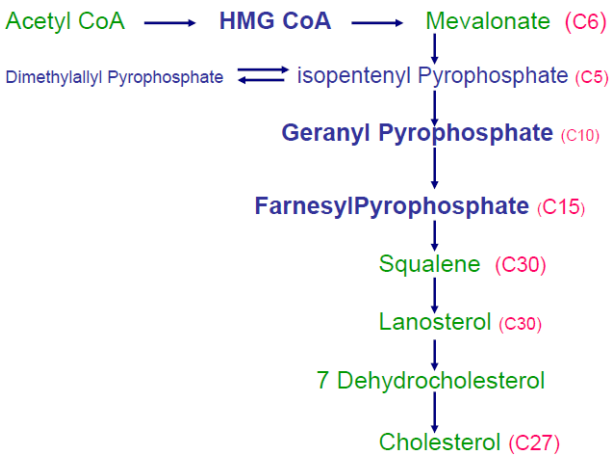
Then, lanosterol is modified through several steps (the doctor said that they are not included) producing **cholesterol**.

The last intermediate in these steps is **7-dehydrocholesterol** (which is cholesterol with a double bond between carbons 7 and 8).

7-dehydrocholesterol is the precursor of vitamin D. So, upon exposure to sunlight vitamin D will be produced from it.

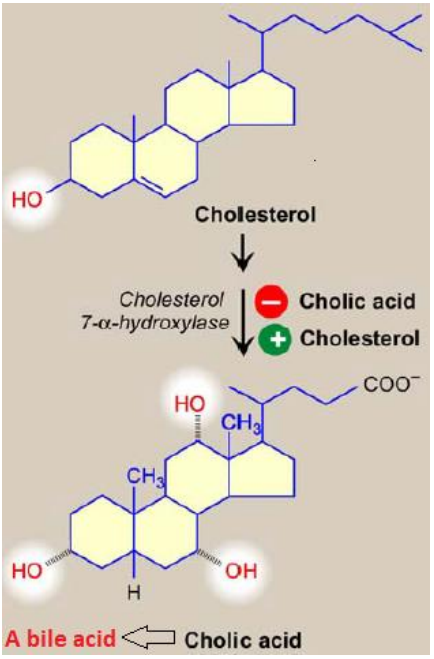


This figure provides a quick recap to cholesterol synthesis pathway, and the doctor said that it is important to memorize the first few steps till reaching mevalonate **in detail**. But from mevalonate to cholesterol focus on **the intermediates and how many of carbons they contain.**



Bile acids synthesis

look at the picture beside, what are the differences in structure between bile acid and cholesterol?



➔ The bile acid has additional hydroxyl groups, doesn't have double bonds in the ring, and its side chain is three carbons less (contains five carbons instead of eight) with an additional carboxyl at its end.

Notice that the hydroxyl groups are connected via dashed lines, what does that indicate?

➔ It indicates that the hydroxyl groups lie "below" the plane of the rings and the methyl groups lie "above"; therefore, the molecule have both a polar and a nonpolar face; so, bile acids can act as emulsifying agents (solubilizers) for nonpolar agents as TAG.

So, the function of bile acids is to solubilize fats, making them suitable substrates for lipases during digestion, which means that digestion won't occur without the help of bile acids.

So, from the structure we can conclude that bile acids are synthesized in the liver by a multistep pathway in which hydroxyl groups are inserted at specific positions on the steroid structure, the double bond of cholesterol ring is reduced and the hydrocarbon chain is shortened by three carbons introducing a carboxyl group at the end of the chain.

The rate limiting step in this pathway is the first step at which a hydroxyl group is introduced at carbon 7 of the steroid nucleus by **cholesterol 7-α-hydroxylase**.

Regulation of this step:

1. It is inhibited by the end product of the pathway **(cholic acid; a bile acid)**
   →Feedback inhibition.
2. It is activated by its substrate **(cholesterol)**.

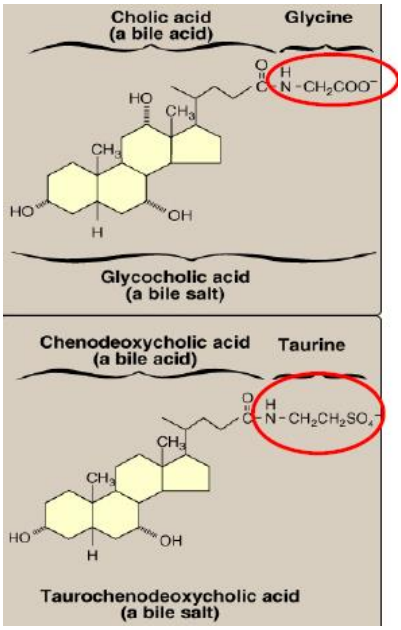
Note: either one or more hydroxyl groups are added so that different bile acids can be produced.

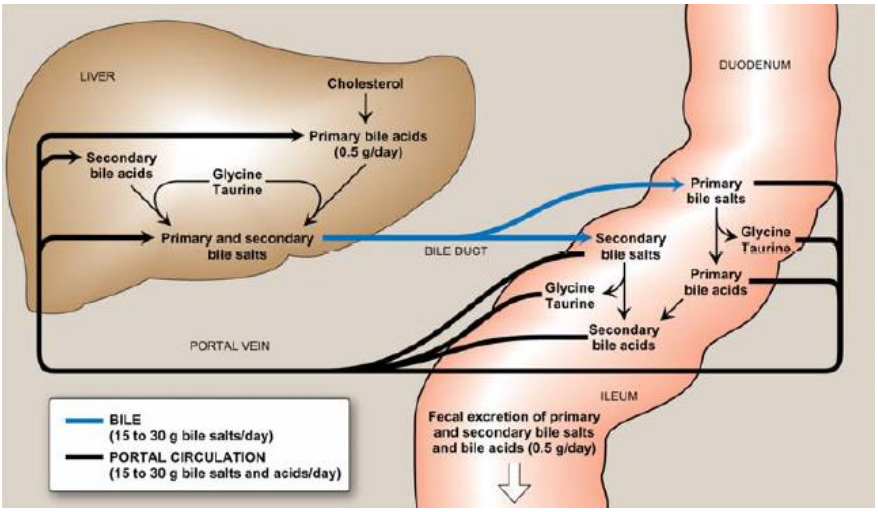
Synthesis of conjugated bile acids (bile salts):

Since bile acids are weak acids (weak carboxyl group), they can get stronger if conjugated to either **glycine or taurine** by an amide bond between the carboxyl of the bile acid and the amine of the added compound.

1. Glycine: its carboxyl group has a lower pka, therefore it is a stronger acid and it will be found in the ionized form mainly; that's why it's called a "bile salt".
2. Taurine: it is the end product of cysteine metabolism, containing a sulfonate group that is also a stronger acid (lower pka) that the original carboxyl, therefore it will be found in the ionized form mainly and so called a "bile salt".

Note: the terms "bile acid" and "bile salt" can be used interchangeably but it depends on their ionized state.





When cholesterol is converted to bile acids, they are called **primary** bile acids (newly synthesized); meaning that they have never reached the small intestine. Then, when glycine or taurine are added, they are converted to bile salts.

Bile salts are secreted with the bile, stored in the gall bladder so that after a fatty meal, gall bladder will contract, releasing the primary bile salts via the bile duct into the small intestine. And in the small intestine, they participate in fat digestion.

Note: if the bile duct gets closed, bile acids won't reach the small intestine, so, fat won't be digested and will be excreted in feces.

Bacteria in the intestine can then deconjugate bile salts (remove glycine and taurine). They can also remove the hydroxyl group at carbon 7 converting the primary bile acids into secondary bile acids.

Bile salts secreted into the intestine are efficiently reabsorbed (about 95%) through the portal vein, returning back to the liver where they can get conjugated and secreted again.

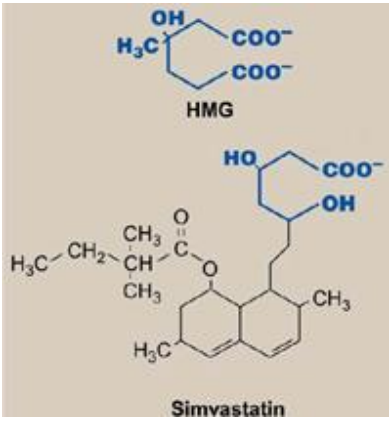
Only 5% are not reabsorbed (0.5 g/day) and those will be excreted in the feces. So, each day 0.5 gram is synthesized and 0.5 gram is lost.

This continuous process of secretion of bile salts into the bile, their passage in the small intestine where some are deconjugated then dehydroxylated to secondary bile acids, their subsequent return to the liver as a mixture of primary and secondary forms is termed **the enterohepatic circulation**.

Lowering cholesterol level

Since high cholesterol level is associated with increased risk of cardiovascular diseases; there are several ways to lower its level:

1. **Dietary:**
   A. **Decreasing cholesterol intake** (decreasing the intake of food that contains cholesterol).
   → This is not effective by itself; it lowers only about 5-10%.
   B. **Increasing the ratio of PUSFA/SFA** (since high amounts of SFA in food increase cholesterol level)
   *PUFSA=polyunsaturated fatty acids, SFA= saturated fatty acids.
   → SFA are usually found in animal fat and PUSFA are usually found in vegetable oil.
   C. Increasing the fiber in the diet.
   D. Daily ingestion of plant steroid esters
   → since they reduce the reabsorption of cholesterol as we took in the previous lecture.
   However, all dietary manipulations won't lower the cholesterol level more than 20% which is not enough especially for elderly to protect them from myocardial infarction and arthrosclerosis.
2. **Inhibition of synthesis:**
   Inhibition is done by using drugs, and the best enzyme to be targeted by drugs is HMG CoA reductase.
   An example is **the statin drugs** which are structural analogs of HMG CoA; so by **competitive inhibition** these drugs can inhibit the synthesis of cholesterol.



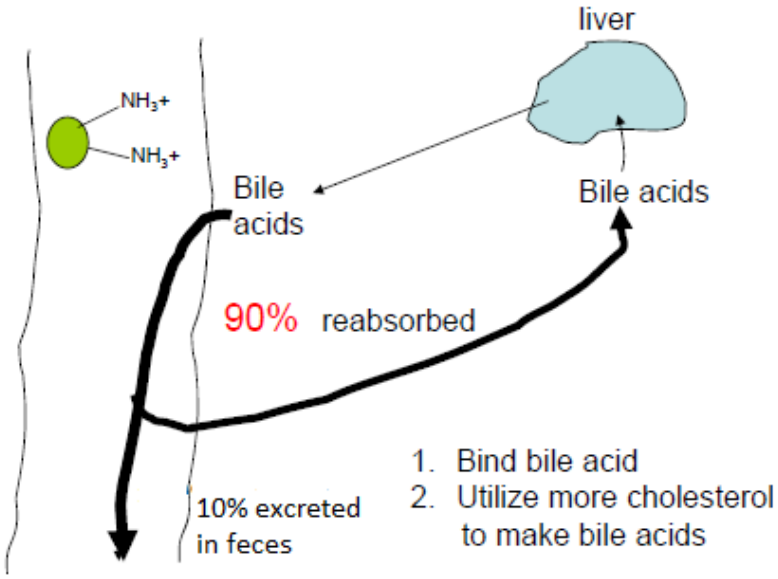
Simvastatin is one of the statin drugs, notice the group it contains that is very similar to HMG CoA.

Lipitor is the trade name of another statin called **Atorvastatin**, it's used to decrease cholesterol level.

3. Decreasing the enterohepatic circulation of bile acids:

By using **bile sequestering agents**, which are chemicals that bind to bile acids and prevent their reabsorption.

An example is **cholestyramine**, which is a polyamine that cannot be absorbed in the small intestine. Its amine groups (positively charged) bind to the bile acids (negatively charged); thus preventing their reabsorption. So, more than 10% of the bile acids will be excreted in the feces and around 90% will be reabsorbed. Therefore, the level of bile acids in the liver will decrease, and this will relieve the inhibition on bile acids synthesis since they are negative feedback inhibitors for this pathway, thereby diverting additional cholesterol into that pathway.



Esterification of cholesterol

This occurs if cholesterol is not required immediately for some structural or synthetic purpose. So, by transferring a fatty acid to the hydroxyl group of cholesterol forming an ester bond, cholesterol will lose its amphipathic nature making it suitable for storage purposes. This occurs in the cells and in the plasma.

A. In the cells:

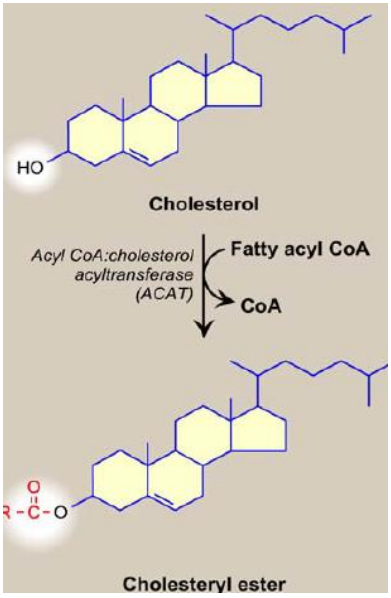
Cholesterol is esterified by the enzyme **acyl CoA:cholesterol acyltransferase (ACAT)** which transfers a fatty acid from **fatty acyl CoA** to cholesterol producing cholesterol ester that can be stored in the cell.

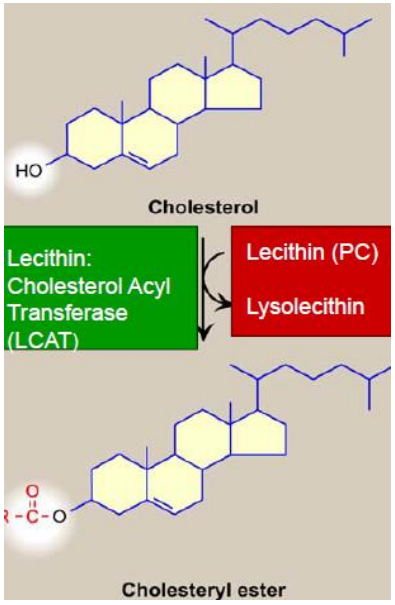
B. In the plasma:

in the plasma, cholesterol is found in the lipoproteins, but there is no CoA (it is a cellular component). Therefore, the donor of fatty acids **is phosphatidylcholine (lecithin)** which is also found in the lipoproteins; so, it's easy to transfer fatty acids from it to cholesterol.

This reaction is catalyzed by **lecithin:cholesterol acyltransferase (LCAT).**

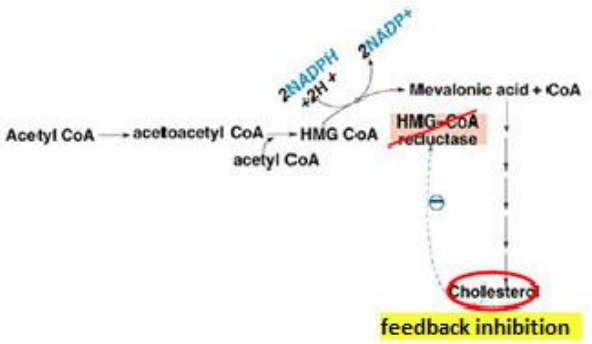
After a fatty acid is transferred from lecithin to cholesterol, lysolecithin is produced in addition to the cholesterol ester.





Regulation of cholesterol synthesis

Cholesterol synthesis is regulated at many levels (strictly regulated; because cholesterol is important for function of cells. However, large amount of cholesterol can be lethal). And in almost all of the regulatory mechanisms the target enzyme is HMG CoA reductase, which catalyzes the third step (an early step in the pathway) that is also the rate limiting step.

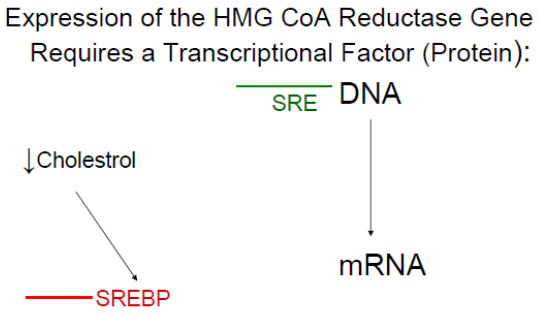


And in general it is regulated by cholesterol itself; as high cholesterol level will lead to inhibition of HMG CoA reductase. This is called **feedback inhibition**.

1. **Regulation of gene expression:**

   It means whether to synthesize the mRNA or not, since all genes are found in all cells but not all genes are expressed.

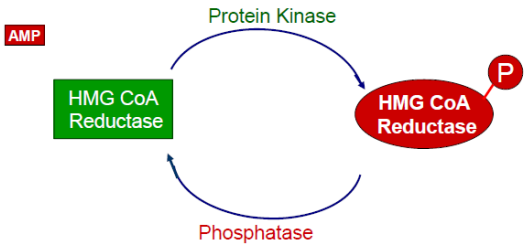
   Expression of the gene for HMG CoA reductase requires **a transcriptional factor; SREBP** (sterol regulatory element binding protein) that should bind to DNA at the sterol regulatory element **(SRE)** found before (upstream) the reductase gene. This protein (SREBP) is covalently bound to the ER in the cytoplasm. And when cholesterol level is low, it will lead to cleavage of this protein from the ER and it migrates to the DNA. After binding to the DNA, the reductase gene will be transcribed producing mRNA, this results in increased synthesis of HMG CoA reductase and therefore, increased cholesterol synthesis.



2. **Covalent modification:**

   HMG CoA reductase can exist in two forms:

   A. **Phosphorylated inactive form**
   B. **Dephosphorylated active form**



Important rule: always phosphorylating an enzyme will spare glucose (reduces the consumption of glucose and makes it more available). Meaning that the

products of glucose catabolism shouldn't be used and since HMG CoA reductase utilizes one of these products (acetyl CoA), then phosphorylation will inactivate it.

HMG CoA reductase is phosphorylated by an **AMP dependent protein kinase** (active when the level of AMP is high; low energy level) and so cholesterol synthesis is inhibited.

When insulin is available (high energy level), **protein phosphatase** is activated, hydrolyzing the phosphate from HMG CoA reductase therefore, converting it to its active form.

Note: this is the only **short term** regulatory mechanism (regulates the synthesis quickly).

3. Hormonal regulation:

Glucagon: ↑Phosphorylated Form

Insulin:↑ **Dephosphorylated** Form (↑Phosphatase)

From the book: an increase in insulin and thyroxine favors the upregulation of the expression of the gene for the reductase. Glucagon and glucocorticoids have the opposite effect.

4. Proteolytic regulation:

The doctor didn't explain this point but it is mentioned in the slides so here is some extra explanation:

HMG CoA reductase is embedded to the ER membrane and so it is composed of a cytosolic domain that carries out catalysis and a membranous domain that contains **a sterol sensing protein** that senses the sterol levels.

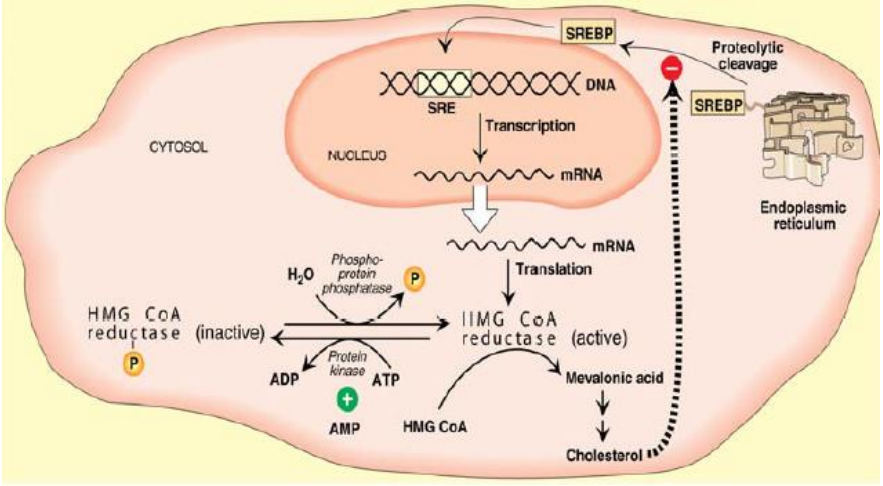
So, if sterol increases too much, HMG CoA reductase is ubiquitinated and proteolyzed.

↑ Cholesterol

↑↑HMG CoA Reductase Proteolysis

↓ HMG CoA Reductase

Summary:



Best of luck ☺