DOCTOR 2020 | JU

METABOLISM

WRITER : Ahmad Zaidan & Lana Khabbas

CORRECTOR : Ali Almahrook

DOCTOR: Mamoun Ahram

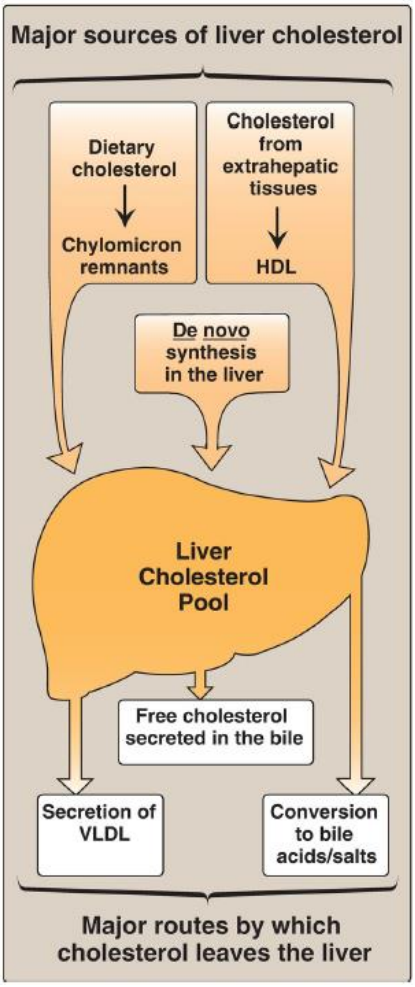
Cholesterol in the body

There are major sources of liver cholesterol:

1.Diet 2.HDL (which transport cholesterol from peripheral tissues to the liver)

3. De novo synthesis in the liver (from the scratch)

(Point 3) It is important to people who wants to lower their cholesterol. Accordingly, managing diet is NOT enough for these people because the body can synthesize it to keep its level balanced.

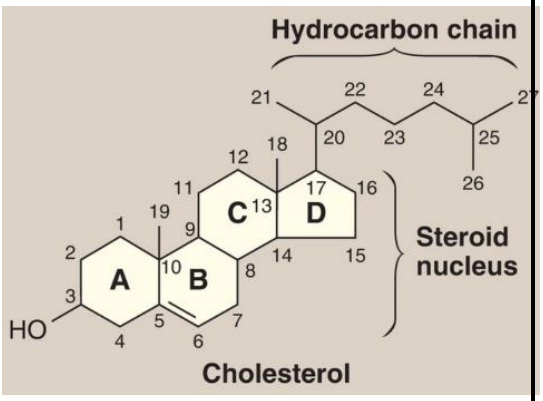


There are major routes by which cholesterol leaves the liver:

1. **VLDL (which transport cholesterol from the liver then it will be converted to IDL then LDL which carries a lot of cholesterol)**
2. **Releasing cholesterol into the bile**
3. **Converting cholesterol to bile acids/salts**

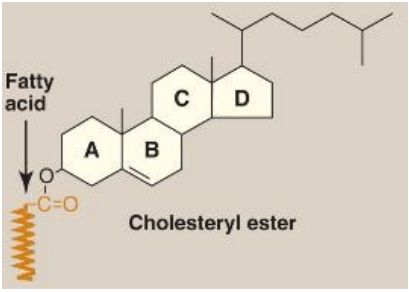
The balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels.

Structure of cholesterol



- **Cholesterol is an amphipathic compound. (it has a large hydrophobic region but with small hydoxyl group which gives it polarity (hydrophilicity))**
- **It is a 27-carbon molecule that consists of:**
- **Four fused hydrocarbon rings (A–D) of 17 carbons called the steroid nucleus (these rings make up the nucleus of steroid molecules, so they are all derived from cholesterol)**
- **Steroid nucleus is common between all steroid, but it can be modified by adding some groups**
- **Vitamin D is an exception, it is derrived from cholesterol but it lacks this nucleus**
- **Two methyl groups (C18 and 19)**
- **Eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring.**
- **Ring A has a hydroxyl group at carbon 3.**
- **Ring B has a double bond between carbon 5 and carbon 6.**

- **Generally, cholesterol doesn't really exist as free cholesterol. Most plasma cholesterol is esterified with a fatty acid attached at carbon 3.**



BY esterifying the hydroxyl group, the cholesterol will become fully hydrophopic

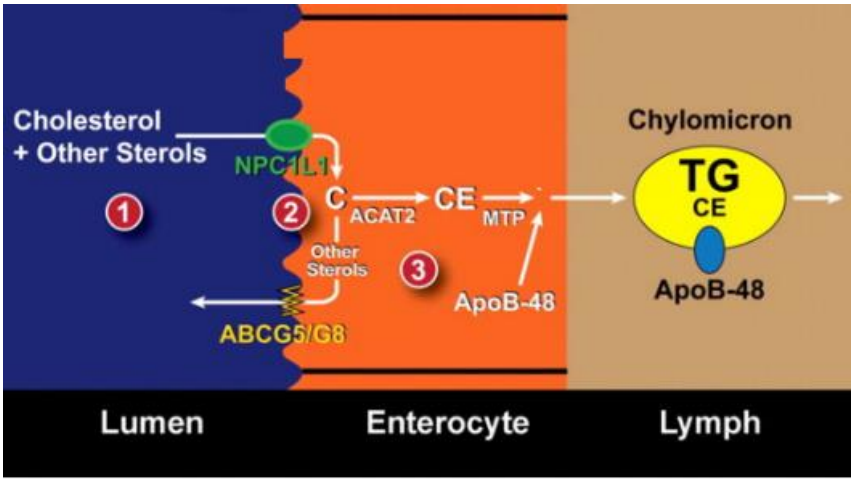
Intestinal uptake of cholesterol is mediated by the Niemann-Pick C1-like 1 protein (NPC1L1), which can be targeted by ezetimibe (drug used to reduce cholesterol absorbtion).

Remember: Niemann-Pick disease is one of the lysosomal storage dieases results from sphingomyelinase dificiency

ABCG5/8 protein is an efflux transporter (carries cholesterol from enterocytes to the lumen)

Defects in the efflux transporter (ABCG5/8) result in the rare condition of sitosterolemia.

Cholestelol is well-absorbed by intestines, then it will be carried by chylomicrons



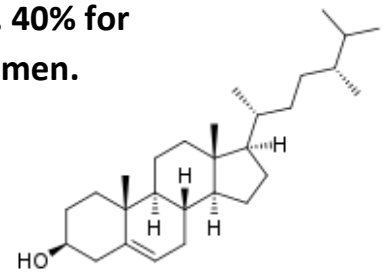
Mamalians are exclusively the only organisms that have cholesterol in their bodies while other organisms have other steroids that is similar to cholesterol

Plant sterols (phytosterols) are poorly absorbed by humans (5% vs. 40% for cholesterol) and are actively transported back into the intestinal lumen.

However, Plant sterols can compete cholesterol in absorbrion thus reduce the absorption of dietary cholesterol.

Having plants sterols is considered one of dietary strategies to reduce plasma cholesterol levels.

NOTE: it is not really that efficient to control your cholesteol level through diet because the body can synthesize it.



(phytosterols)

NOTES regarding cholesterol synthesis:

- All the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA).
- NADPH is the reducing agent (source of electrons).
- The pathway is endergonic (anabolism), and energy is provided by the hydrolysis of
  1. The thioester bond of acetyl CoA
  2. ATP
- Synthesis requires enzymes in the cytosol, the membrane of the smooth endoplasmic reticulum (SER), and the peroxisome.
- The pathway is regulated to balance the rate of cholesterol synthesis/excretion.

The first reaction:

2 Acytel CoA get condensed producing acetoacytel CoA (4C) by Thiolase enzyme

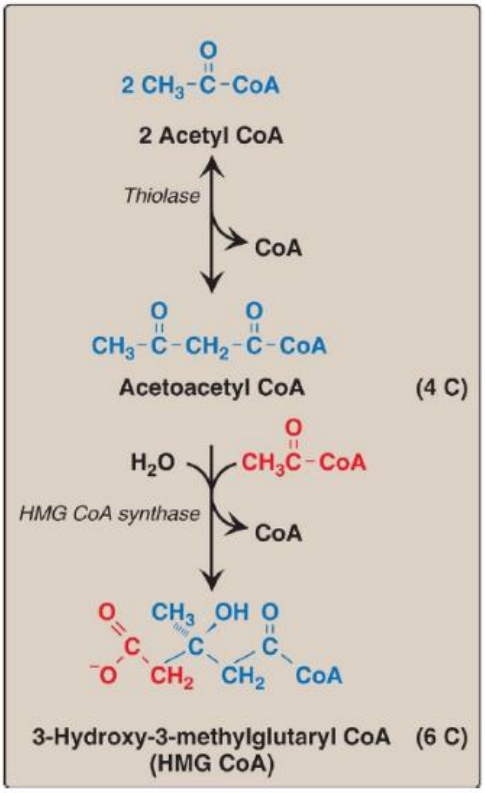
The reaction is provided by energy which comes from breaking the thioester bond

Then the product of the first reaction will react with another acytel CoA producing 3-hydroxy-3-methylglutaryl CoA (HMG CoA 6C) by HMG CoA synthase

These two steps are the same as the first two reactions in ketone bodies synthesis

Liver parenchymal cells contain two isoenzymes of the HMG CoA synthase.

1. A cytosolic enzyme participates in cholesterol synthesis.
2. A mitochondrial enzyme functions in the pathway for ketone body synthesis.



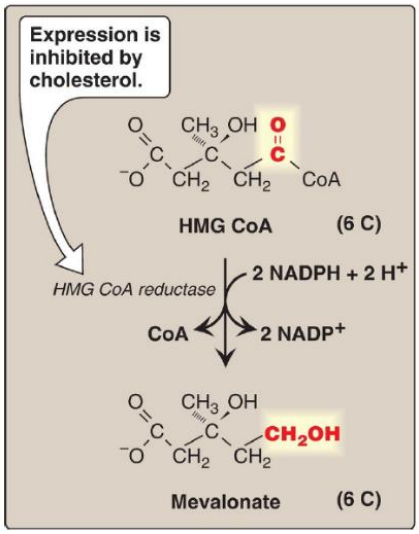
Synthesis of mevalonate (6C):

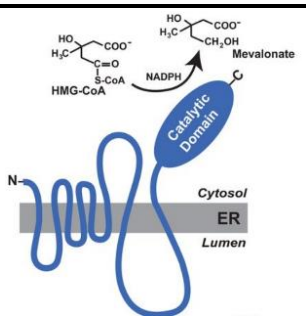
HMG CoA is reduced to mevalonate by HMG CoA reductase.

The reaction is rate-limiting. It means that it is the slowest step, highly regulated and it requires energy

Two molecules of NADPH are oxidized.

CoA is released making the reaction irreversible.
(It is the commited step)





HMG CoA reductase is an integral membrane protein of the SER, with its catalytic domain projecting into the cytosol.

So the reaction happens in the cytosol

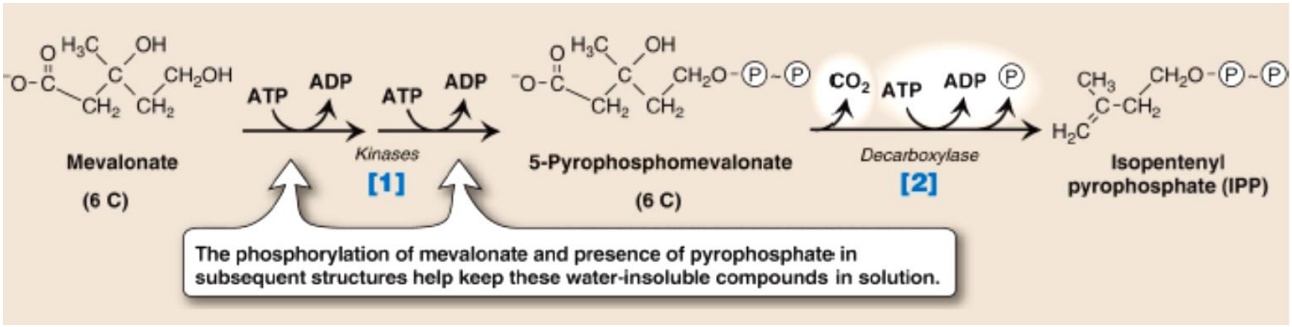
(1) Then, Mevalonate is activated by transferring 2 phosphate groups (pyrophosphate) from ATP in two sequential reactions each one requires one ATP. Don't worry about the name of the product

(2) The product from (1) will be decarboxylated and it will consume 1 ATP forming isopentenyl pyrophosphate (IPP) (5C)

IPP is the precursor of a family of molecules with diverse functions, the isoprenoids.

Cholesterol is a sterol isoprenoid.

Nonsterol isoprenoids include ubiquinone (or, coenzyme Q).



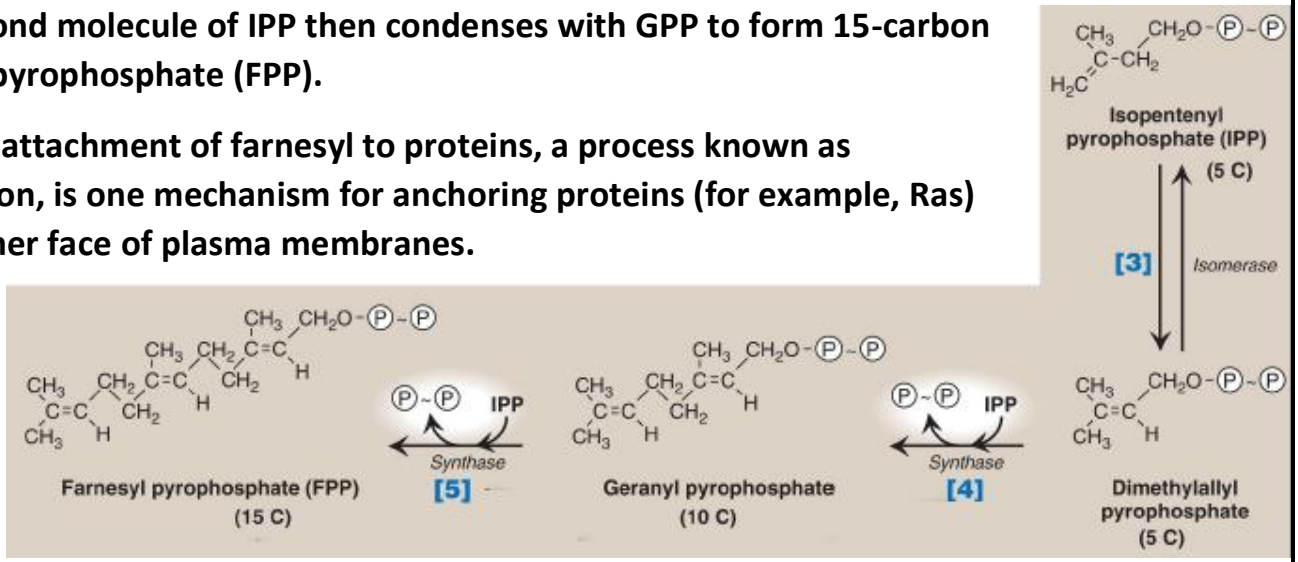
[3] IPP is isomerized to DPP (5C)

[4] Another IPP comes and it will combine with DPP to form 10-carbon geranyl pyrophosphate (GPP).

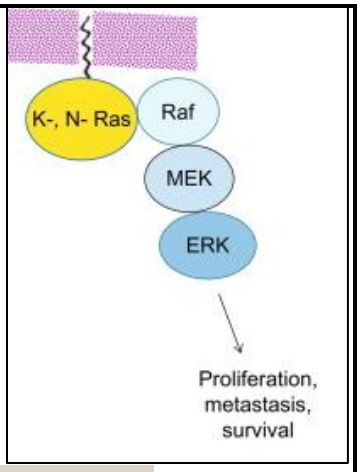
We get the required energy by releasing pyrophosphate which will get cleaved to 2 inorganic phosphates

[5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP).

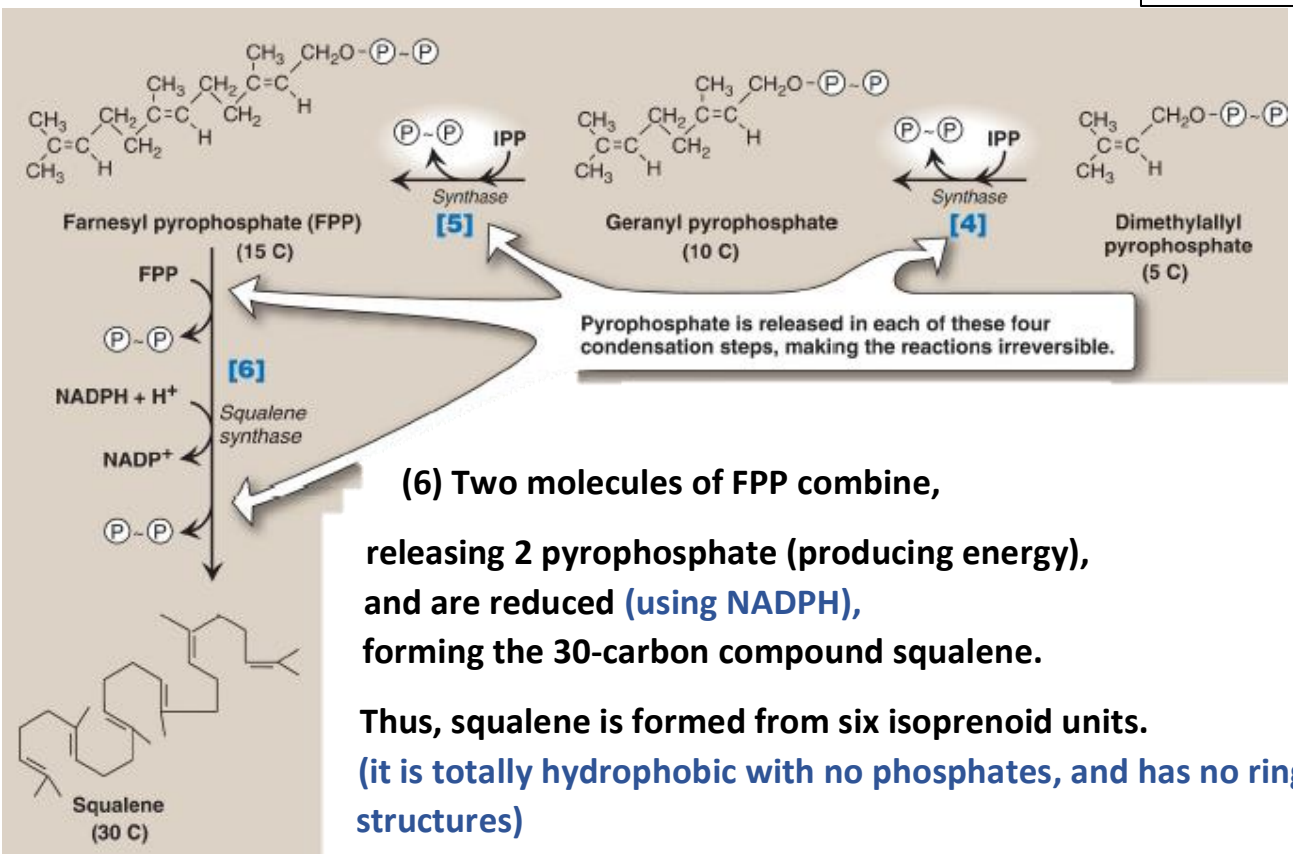
Covalent attachment of farnesyl to proteins, a process known as prenylation, is one mechanism for anchoring proteins (for example, Ras) to the inner face of plasma membranes.



Extra information: Ras is proto-oncogene which means that if it is mutated, it will cause cancer, Ras is a protein that is responsible for growth factor signal cascades, when it binds to its receptor, it will become active and able to activate another proteins like Raf



Inhibition of farnesyl transferase (which is the enzyme that is resposnsible to prenylation of Ras) was a hot topic of research to kill cancer cells because Ras (whether it is normal or oncogene) must be anchored to plasma membrane to be functional



(6) Two molecules of FPP combine,

releasing 2 pyrophosphate (producing energy),
and are reduced (using NADPH),
forming the 30-carbon compound squalene.

Thus, squalene is formed from six isoprenoid units.
(it is totally hydrophobic with no phosphates, and has no ring structures)

Because 3 ATP are hydrolyzed per mevalonate residue converted to IPP a total of 18 ATP are required to make the polyisoprenoid squalene.

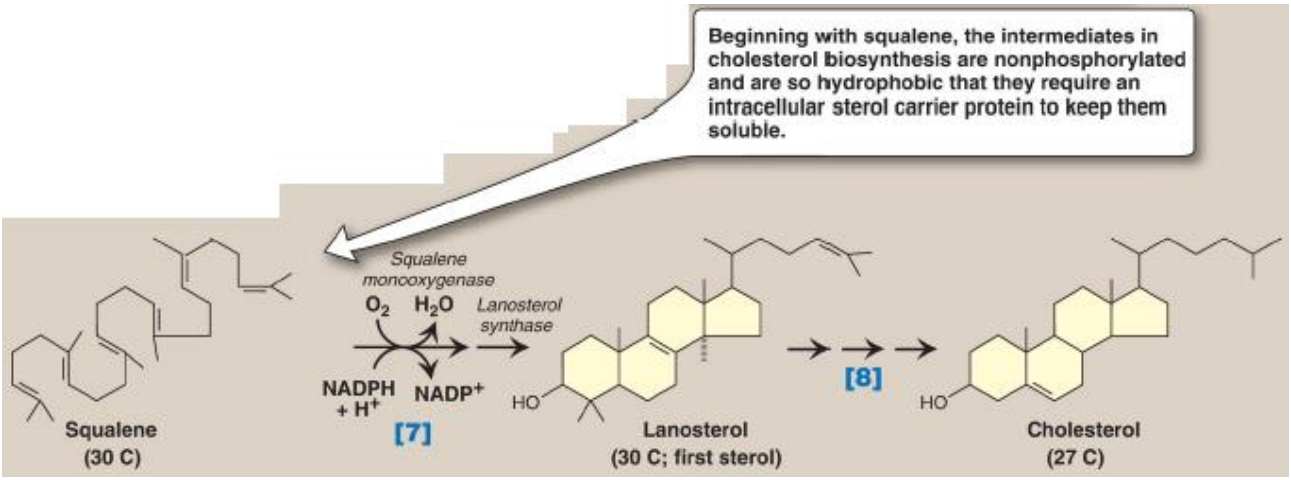
IPP is 5C → we need 6 IPP to produce squalene (30C) → each IPP requires 3 ATP

Squalene is hydrophobic and insoluble in the cytosol, so it needs a carrier and it bind to an intracellular sterol carrier protein to keep them soluble.

[7] Then Squalene gets hydroxylated (which drives the formation of rings),so it is converted to the sterol lanosterol by SER-associated enzymes that use molecular oxygen (O2) and NADPH.

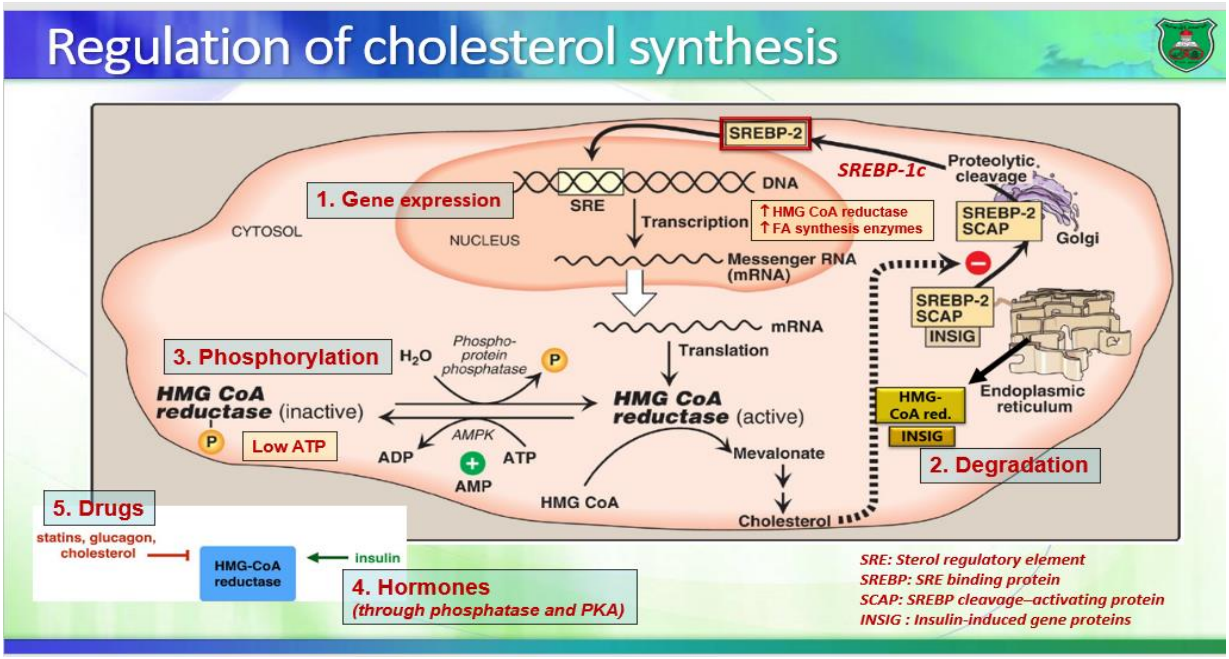
REMEMBER: The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.

[8] Then many reactions occur. The side chain of lanosterol is shortened, the methyl groups are removed (Squalene has 30C, CH is 27), and a double bond is re-located (make it between carbon 5&6), and cholesterol is formed.



Regulation

Level of Choleserol must remain balanced in the body, so it must be regulated. This regulation is multi-levels which tells you how important cholesterol is. It is regulated at the level of gene expression, transcription, post translation and signalling as well which are innate (done by the body itself). Also there is pharmacological regulation (by using drug)



At the transcriptional level, there is *Sterol regulatory element (SRE)* (element = DNA sequence) which is located in the promoter region of HMG CoA reductase gene and it is the binding site of SREBP-2 which located in the ER bound to SCAP. When the sterol level is low, this complex (SREBP-2&SCAP) moves from ER to golgi which has proteases that cleave the SREBP-2 then it will bind to SRE thus activate trasncription (synthesis of reductase)

When the level of sterol is high, sterol molecules will bind to protein called INSIG which will bind SREBP-2 & SCAP complex retaining it in the ER

The second mechanism is done by INSIG, it will bind with reductase enzyme and degrade it.

The third one, when there is high level of AMP (which indicate low energy state), AMP kinase or AMPK will become active and it will phosphorylate the reductase enzyme (inactivating it)

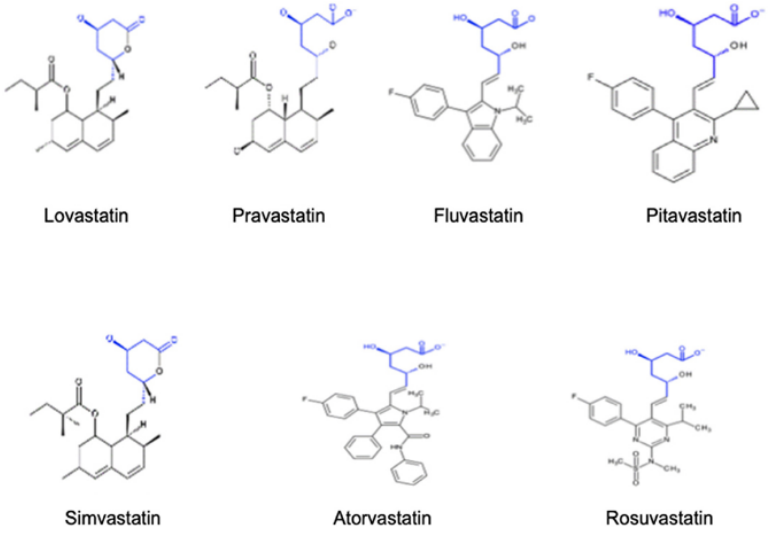
The fourth one is hormonal regulation (by glucagon and insulin), when glucagon is high (which indicates that the cell in starvaion and we don't need to synthesize cholesterol), so glucagon will bind to its receptor (G-protein coupled receptor) and activating adenylyl cyclase which will produce AMP molecules which will activate PKA. PKA will phosphorylate reductase (inactivating it)

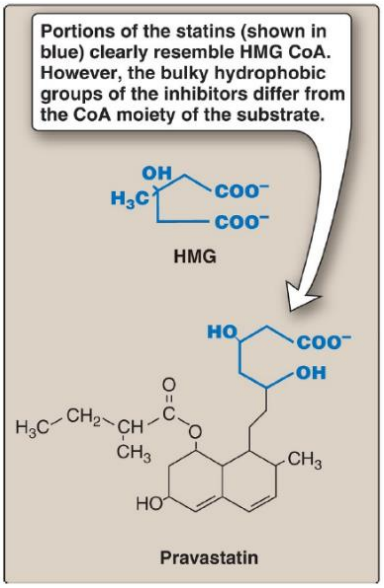
Insulin will activate the phosphatase that will dephosphorylate reductase (activating it)

The fifth mechanism is pharmacological by drugs which are called statins (they contain group that looks like 3-hydroxy-3-methylglutaryl (HMG) which is the substrate of reductase, so they will bind to the active site of the enzyme and blocking it (inhibition cholesterol synthesis))

There are many statins each one its advantages, beside managinging cholesterol level they have a protective role from cancer cells

NOTE: inhibition cholesterol synthesis is better way than managing cholesterol by diet





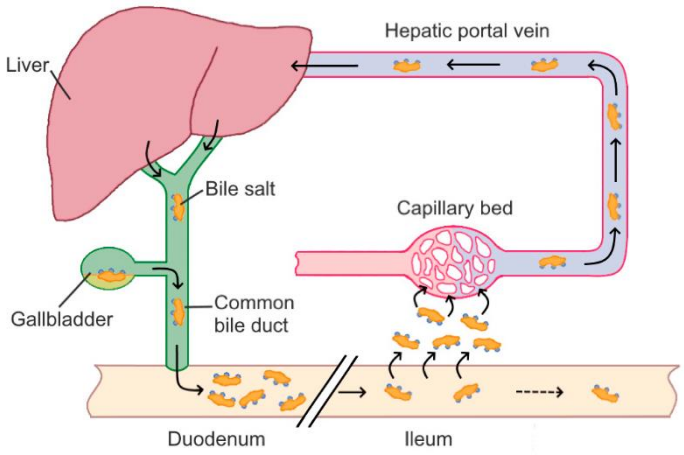
Elimination of cholesterol

The intact steroid nucleus is eliminated from the body by:

1.conversion to bile acids and bile salts (conjugate base form), a small percentage of which is excreted in the feces 2.secretion of cholesterol into the bile, which transports it to the intestine for elimination.

Note: The terms bile acid and bile salt are frequently used interchangeably.

What is bile?



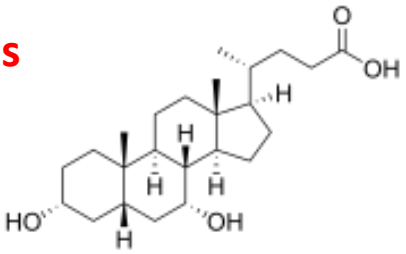
Bile consists of a watery mixture of organic and inorganic compounds.

Bile is synthesized in the liver and it gets pumped out through common bile duct to the duodensem (small intestines) then it get reabsorbed

Some is stored in the gallblader

Phosphatidylcholine (PC) and conjugated bile salts are the most important organic components of bile.

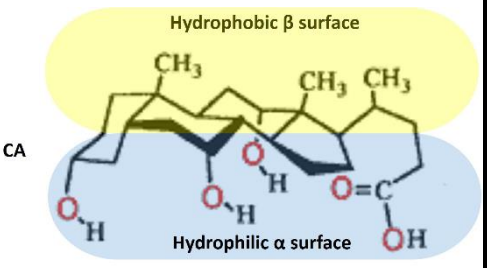
Structure and protonation states of bile acids



The bile acid (are modified from cholesterol) it is an amphipathic molecule that contains 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group

The carboxyl group has a pKa of ~6.

In the duodenum (pH ~6), this group will be protonated in half of the molecules (the bile acids) and deprotonated in the rest (the bile salts).



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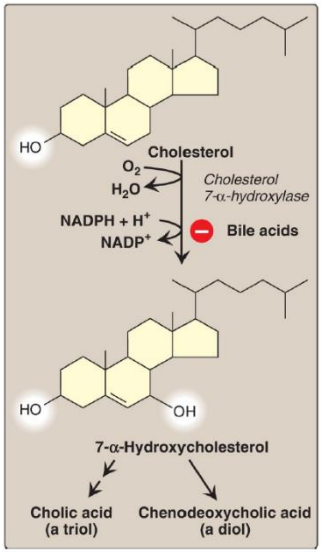
Synthesis of primary bile acids

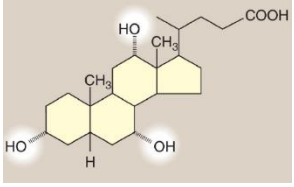
One of the main differences between cholesterol and bile acids is the presence of hydroxyl groups, so there is an enzyme (cholesterol 7-alpha-hydroxylase) that inserts hydroxyl groups into cholesterol.

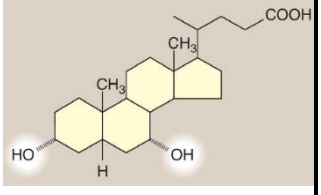


This reactios is the commited step (rate limiting step)

Expression of the enzyme is downregulated by bile acids.

The *7-α-hydroxylase is* a SER-associated *cytochrome P450 monooxygenase* found only in liver.

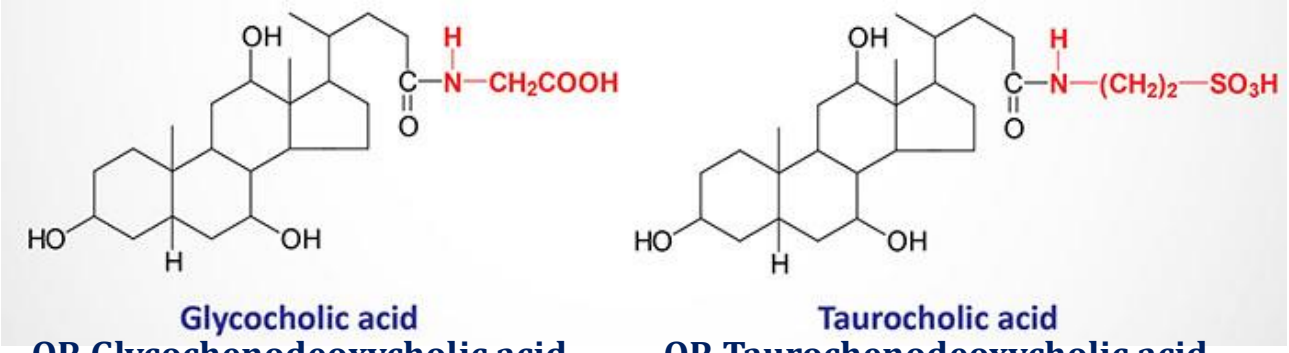




Then it will be modified by many things such as 1.The double bond of the cholesterol B ring is reduced 2.The hydrocarbon chain is shortened by three carbons 3. Introducing a carboxyl group at the end of the chain. Producing one of the 2 forms of bile acids →→

In the liver, they are conjugated to either glycine or taurine (an end product of cysteine metabolism) forming more amphipathic and ionized compounds, better emulsifiers for lipids, and the only ones found in bile

The ratio of glycine forms (glycocholic acid) to taurine forms (taurocholic acids)in the bile is~3/1



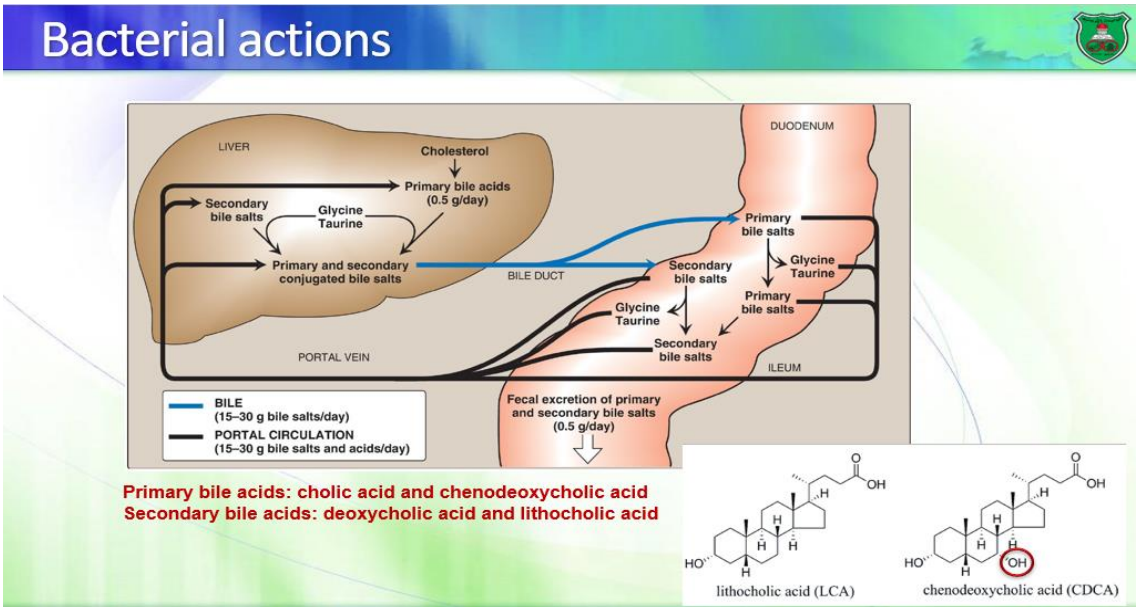
Glycocholic acid
OR Glycochenodeoxycholic acid

Taurocholic acid
OR Taurochenodeoxycholic acid

Liver releases bile into intestines through bile duct

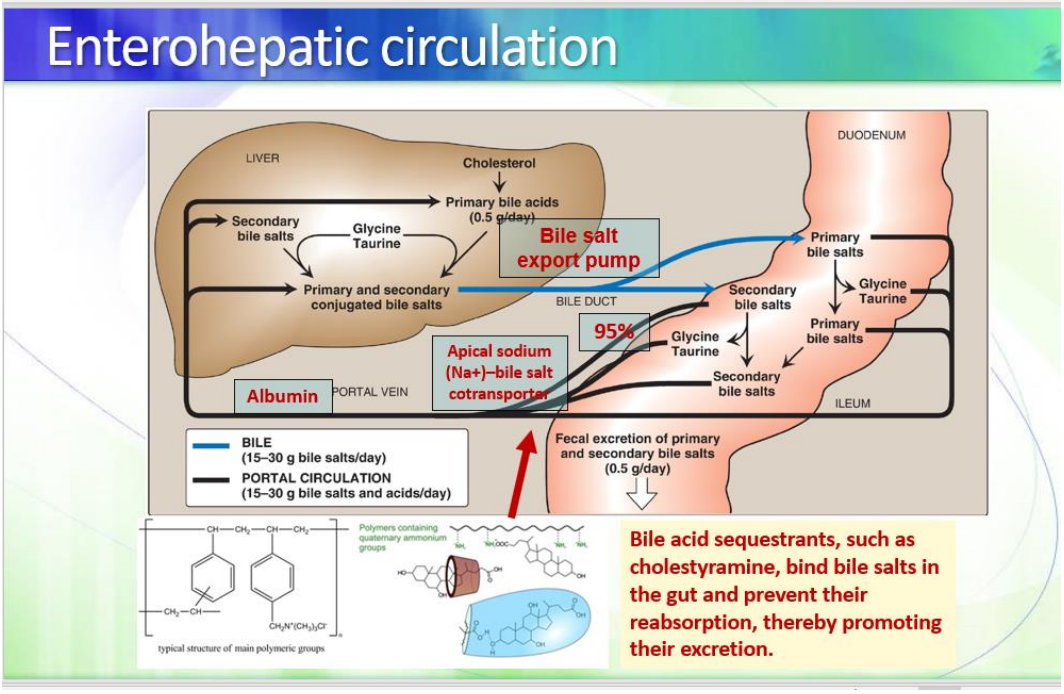
These conjugated bile acids are called primary bile acids. However, they will get modified (deconjugation (releasing glycine or taurine) & dehydroxylation by bacteria) in the intestines producing what we call secondary bile acids

Primary and secondary are reabsorbed efficiently by intestinal cells into the portal vein, so they are transprted back to the liver.



Enterohepatic circulation: the movement of bile acids from liver to intestines using bile salt export pump via bile duct then they will be modified in the intestines tand reabsorbed using cotransporter called apical Na+-bile salt cotransporter, so they are moving it the blood via portal vein to the liver. However, they are hydrophobic molecules (dehydroxylated), so they need a carrier which is albumin

95% are reabsorbed and 5% are excreted with stool



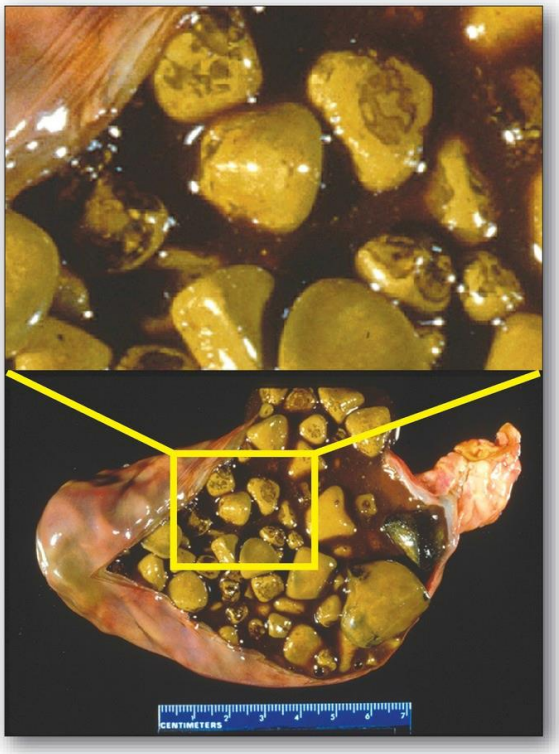
Scientists have targeted reabsorbtion of the bile acids from intestines to reduce cholesterol level in the body, so they made some drugs (bile acid sequestrants) that targets Na+-bile salts cotransporter and block reabsorbtion, so high amount of bile acids will be excreted and more cholesterol will be converted to bile acids to compensate thus reducing cholesterol level in the body.

Bile Salt Deficiency: Cholelithiasis

If the cholesterol level is high or bile acids level is low, the cholesterol will become insoluble which will induce the formation of gallbladder stones (cholelithiasis)

Treatment: cholecystectomy (removing gallbladder surgically)

Alternatively (if the patients can't be treated surgically): oral administration of chenodeoxycholic acid results in a gradual (months to years) dissolution of the gallstones.



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