Sheet # 22

Cholesterol metabolism I

→*Today is the first lecture in the **Cholesterol** metabolism and you can refer to chapter 18 in Lippincott illustrated review*

Q: Why **Cholesterol** was written in 3 different colors?
To show you the origin of the name of **Cholesterol**
OL: alcohol (contain hydroxyl group)
Ster: steroid
Chole: refer to **Gallbladder** (because it is the steroidal alcohol that was isolated first from the **Gallbladder**) it was isolated 200 years ago. And since then many people work on **Cholesterol** to understand its synthesis, regulation and its role in the atherosclerosis
12 scientists awarded a noble prize for their work on **Cholesterol**
it's a substance that has been attracting many researchers in the past till now .

SLIDE #2

Steroid nucleus as you see in the slide is formed of **(4 rings)**
3 of them are six-membered ring and the 4^{th} one is 5-membered ring
They are fused together in this manner to give the steroid nucleus
steroid nucleus is made of:
1) 17 carbons
2) 4 Rings (3 of them are six-membered ring)
Note: you should be familiar with the numbers on each carbon atom; because they will be repeated again and again several times but at least each carbon atom has a specific number..

SLIDE #3

Showing the **Cholesterol** structure:-

1) Hydroxyl group (OH) at carbon 3... The rest of the molecule is just carbon and hydrogen atoms

2) Double boned between carbon (5 ' 6)

3) 2 methyl groups (CH3) attached to carbon (10 ' 13)
they are carbon (18 ' 19)

4) '8 carbons' side chain attached to carbon (17)

"27 carbons" are the total number of carbons in the **Cholesterol**
all atoms are (H&C) but only a one (O) atom at carbon #3

SLIDE #4

These look like stones!? What are these?! What do these have to do with **Cholesterol**?!
This structure is a **Gallbladder** (مرارة) with stones "disease state"
But these stone actually precipitated **Cholesterol** which is **hydrophobic**
And **Non-polar "allowing** it to precipitate in the **Gallbladder".**

→**How** can we handle this kind of disease?!
Removal of **Gallbladder**.

→→**When** did they isolate **Cholesterol**?**!**
Cholesterol was isolated from **Gallbladder** Stones in **1774.**

SLIDE #5

this is **Cholesterol** esterified to (**F.A**) a 16 carbon (**F.A**)
It's called a **cholesterol-ester** which is **more hydrophobic, less soluble** because it loses its polar group (OH) group
another form of Cholesterol which is found in tissues.

SLIDE #6

Sources and Elimination of **Cholesterol**

Cholesterol is important for the function of the cell membrane
it's required by all animal cells. It's synthesized by all cells but mainly in the liver, small intestine and adrenal cortex.
The total amount usually synthesis per day 1000 mg

Cholesterol is obtained from our diet. But not everything we eat contains Cholesterol.
If your diet is from an animal source it contains **Cholesterol**
any food from vegetable origin it doesn't contain **Cholesterol**, why?!
Simply plant cells can't synthesis **Cholesterol**

Note: any type of vegetable oil we use it does not contain Cholesterol because it is not found in food from a plant origin

Note: A low **Cholesterol** diet contains 300 mg
some food are rich in **Cholesterol** for example: 1 egg contains about 250 mg of cholesterol.

(IF we want to reduce the **Cholesterol** intake it's very hard to do with a dietary regime but it's possible if you eat only plant origin food)

Even though cholesterol is synthesized in all cells not all cells can degrade cholesterol.

We **can't** use cholesterol **as source of energy** we do not degrade or oxidize **Cholesterol** to get energy (ATP).

Cholesterol should be eliminated as (free cholesterol) OR (after conversion to bile salts in the liver) this how we get rid of cholesterol.
Besides the fact that "**small portion**" of **Cholesterol** is converted to steroids hormones this another way to get rid of daily intake and synthesized **Cholesterol**

SLIDE #7

What is this? this is not **Cholesterol** … its called Ergosterol "it's a plant sterol", plants do not make **Cholesterol** but they do make Plant sterol
they are an important dietary components because they are poorly absorbed by human, our cells do not absorb plant sterols which help to reduce the amount of **Cholesterol** absorbed because there is reveres secretion of sterols in to small intestine, if some sterols are absorbed they are re-excreted with some **Cholesterol** also.

→"**Plant sterols are important for reducing the cholesterol intake by human.**"

SLIDE #8

this **slide** illustrate the homeostasis of **Cholesterol**, how **Cholesterol** pool is obtained , U can notice that the liver is the center in the **Cholesterol** pool in the body the dietary CH is

transferred to the liver by **Chylomicron remnants .**
Cholesterol that is produced, that excess in cells, or that comes from dying or dead cells is carried to liver. De-novo synthesis of **Cholesterol** in the body occurs in the liver

● On the other hand, liver **Cholesterol** is transported "secreted" out of the liver by:

1. VLDL
2. Free cholesterol (secreted in the bile)
3. Converted to bile (acids/salts).

"So the liver is the main organ involved in the cholesterol regulation, synthesis, transport ..."

Q: what is the food which the most rich in cholesterol?! Liver " كبده "
Because it is the main organ involved in **Cholesterol** metabolism, **BUT**
it's not significantly contributing in the dietary **Cholesterol** because we don't eat liver every day "" كما قال الدكتور على الأضحى.
EGGS are the major contributor of dietary cholesterol, eaten daily.

Note : its hard to take low **Cholesterol** diet but u can make it by lowering the amount of egg you eat " minimum **Cholesterol** intake 300mg "
which roughly equal 1 egg

SLIDE #9

Cholesterol synthesis requirements:

1. Carbon source (Acetyl CoA) all carbons of **Cholesterol** "the 27 carbons" all of them came from (Acetyl CoA). The scientists were able to determine each carbon whether it comes from:
1) Methyl carbon of the acetyl group
2) The carbonyl carbon of the acetyl group
by tracing elements of carbon 14 it was possible to tell which carbon comes from methyl group and which carbon comes from the carbonyl group.

2. Energy (ATP) linking many acetyl group together (2 carbons)
requires energy SO energy is needed to join groups together

3. Reducing power (NADPH) WHY?!
Cholesterol doesn't have more than 1 oxygen where as the acetyl contains many oxygen, during the processing "synthesis" of **Cholesterol** oxygen is removed so we need a reducing agent that removes the oxygen. **"Reducing power is required for the synthesis."**

4. Oxygen (O2)

So for the synthesis we need ...
1) a lot of **carbon** atoms.
2) A lot of **ATP.**
3) A lot of **NADPH.**
4) **One** Oxygen Atoms.

SLIDE #10

As an overview stages of **Cholesterol** synthesis:

We start with **acetyl CoA** (2 carbons) that condense together in several reaction in 3 reactions to form **Mevalonate (6 carbons)** is converted to activate isoprene **units** (5 carbons) which are condensed together to from **Squalene** (30 carbons) then **lanosterol** Steroid (30 carbons) Finally **Cholesterol** (27 Carbons).

Acetyl CoA → Mevalonate → isoprene unit's → Squalene→ Lanosterol→ Cholesterol.

These stages help you to determine how the reaction occurs and how many Acetyl CoA do we need.
Note: You have to remember these names.

**** Isoprene units what are they?!**

→ Simply it's a 5 carbon compound (pentane) but with a double bond (pentene) Actually **Isopentene** Only (C&H)

***Note**: the Dr Said that we are not going to study all the reaction*
(Some of them are important we are going to study them for their molecular importance)

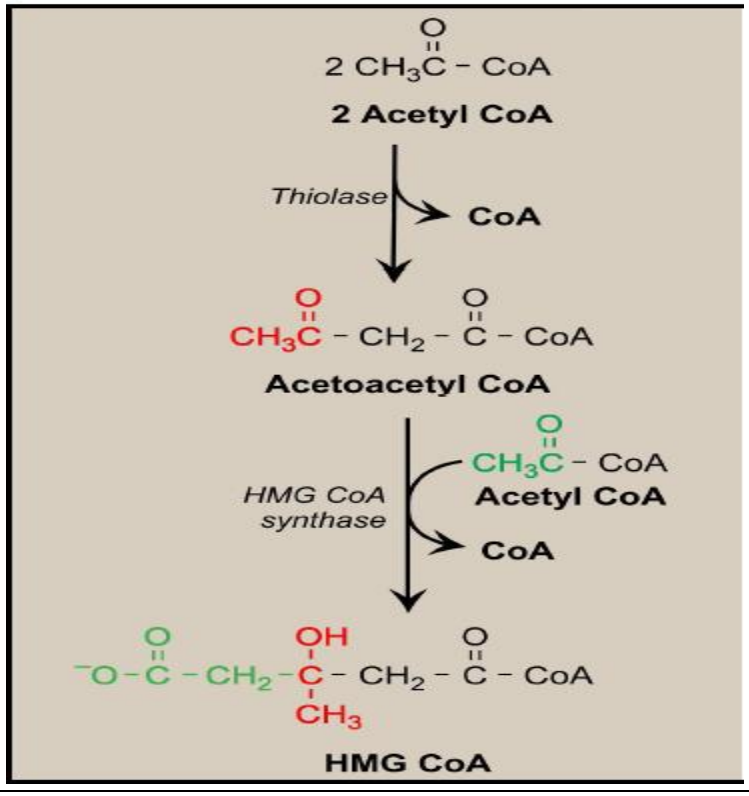
SLIDE #11

The 1st reaction involves condensation of **(2 Acetyl CoA groups)** to form **Acetoacetyl CoA** Which accepts 1 more **Acetyl CoA** to form **HMG CoA**

Note : we see this sequence of reactions in the synthesis of Ketone bodies and the enzyme catalyzes this step is HMG CoA synthase

But there is a difference between them :
that **Ketone bodies** synthesis occurs in the **mitochondria** but **cholesterol** occurs in **cytoplasm**

****** The same 2 step in Ketone and **Cholesterol** synthesis



SLIDE #12

The 2nd step is reduction OF HMG CoA "the 1st reduction reaction"
you may notice that **2 NADPH** are required per HMG CoA molecule which means 2 reduction steps For Carboxyl group when you reduce a carboxyl group it is reduced to aldehyde then to alcohol (2 reduction steps) are involved here, with elimination of CoA. Eventually ***HMG CoA will be converted to Mevalonate or (mevalonic acid)***

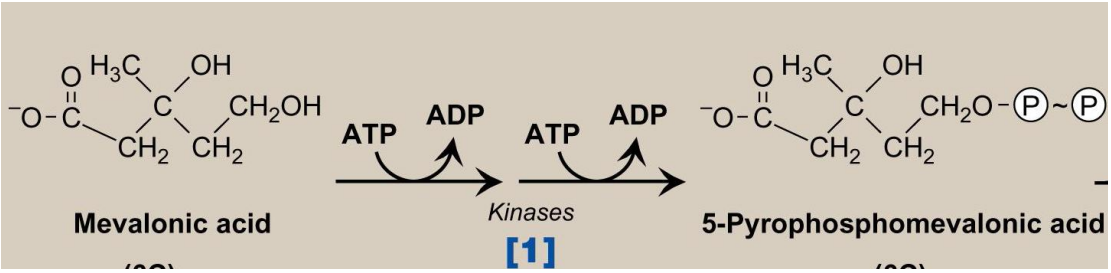
This step is considered as a **rate limiting step** (committed step) HMG CoA is used only for **Cholesterol** synthesis (polyIsoprene synthesis) **So** it must be regulated (to regulate the amount of **Cholesterol** synthesized)
Enzyme catalyzes this reaction called **"HMG CoA Reductase"** (very important enzyme) in the next lecture the Dr will talk about it.

Q: *Why Reductase?! Reduction step.*

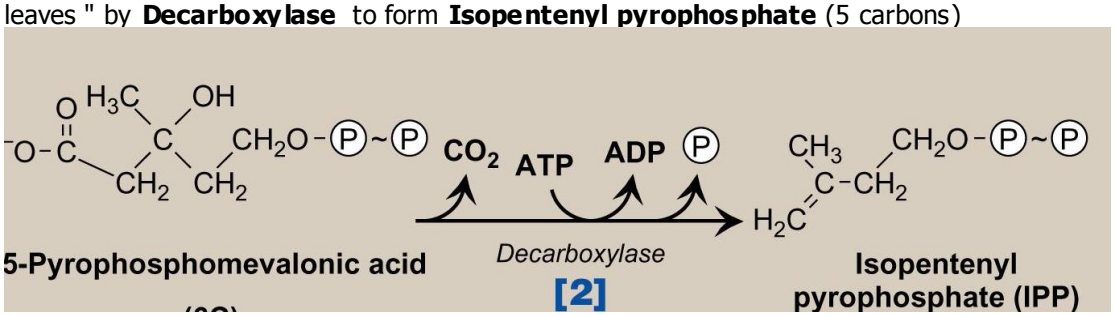
***SLIDE** #13*

This **slide** shows the reaction occur to ***Mevalonate or (mevalonic acid)***

1. mevalonic acid (6 carbons)has to be activated 1st activation happens by adding 2 (phosphate group) 2 phosphorylation steps which give pyrophosphomevalonic acid (6 carbons) "we use 2 ATP in this step "



2. **pyrophsophomevalonic acid** (6 carbons) undergoes decarboxylation " COO- leaves " by **Decarboxylase** to form **Isopentenyl pyrophosphate** (5 carbons)



3. **Isopentenyl pyrophosphate (**5 carbons**)** it's converted to "Isomerize to" **Dimethylallayl pyrophosphate** (5 carbons**)** by (Isomerase) "not important to memorize.

4. Both **Isopentenyl pyrophosphate** and **Dimethylallayl pyrophosphate** are condensed together to form **Genaryl pyrophosphate** (10 carbons) { 5+5} By (*Transferase*)

5. **Genaryl pyrophosphate** (10 carbon) + **Isopentenyl pyrophosphate** (15 carbons) Will give **Farnesyl pyrophosphate** (15 Carbons)

Q: What derives the condensation?! And why ATP is not required directly here?!
Because there is a release of pyrophosphate. The pyrophosphate was added for condensation, and once the condensation occurs the pyrophosphate released and rapidly hydrolyzed to (2 phosphate). And the same for the 2nd condensation which is **Farnesyl pyrophosphate**

SLIDE #14

The next step is condensation of 2 **Farnesyl pyrophosphate** (15 carbon) DR colored one with red color and the another one with blue color showing how does the condensation occurs, They are joined together with a release of 2 pyrophosphates which will give **Squalene** (30Carbons)
{15+15}.
Notice that **Squalene** is composed of { C & H } hydrocarbon it is (Something like petrol derivatives) it's synthesized in our bodies in many cells and it's produced even in Bactria and found in (Co Enzyme Q) Its lipid soluble because of the hydrocarbon tale.

Natural Rubber tree uses a pathway to make rubber similar to the pathway used for **Cholesterol** synthesis.

Q: Why Vitamins (A as example) are fat soluble?!
Because of the hydrocarbon tale.

SLIDE #15

It's **Squalene** (It was written in this pattern to show that we are close to our goal "Cholesterol."

SLIDE #16

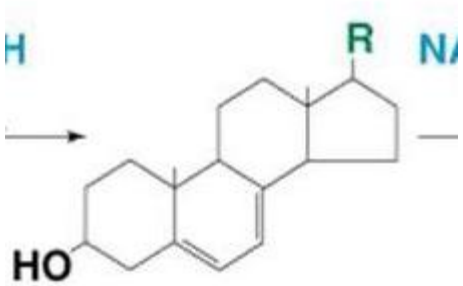
The next step involves addition of Oxygen. Here The Oxygen is added "terminal Double bond" to Carbon(2 and 3) " only 1 oxygen atom is added" to form **Squalene 2,3-epioxide.**

Notice that (carbon and oxygen and carbon) form a triangle which is not an stable compound, in a complex reaction "many step reaction"
converts **Squalene 2,3-epioxide** to **Lanosterol** " which is a steroid "
here we can notice that there is a steroid nucleus by just 1 reaction involving **Squalene 2,3-epioxide** this reaction is called concerted reaction all steps work together to form a 4 rings compound (**Lanosterol)** it is the 1st compound have "ster" in it which means its is the 1st steroid intermediate.

SLIDE #17&18
"DO NOT REMMBER ANYTHING FROM THEM EXEPT"

Lanosterol → **Cholesterol** (about 10-20 steps) are involved in this conversion "from **Lanosterol to Cholesterol"**

1. removal of 3 methyl groups
2. shifting of the backbone

1. it can be converted to Vitamin D In the skin upon exposure to sunlight. 2. We can obtain Vitamin D from plants because they make different steroids that give different V (D) 3. V(D2) From plant V(D3) From animal	 7-Dehydrocholesterol

U have to know all these intermediates "the most common intermediates" In the right order and the number of carbon atoms for each one

SILDE #20

Synthesis of Bile acids
How does **Cholesterol eliminated?!**

1. VLDL
2. Free cholesterol
3. Converted to bile (acids/salts).

How is **Cholesterol** converted to bile acids *"the Dr said that he shows us in previous lecture the 1º and 2º Bile acids and what do we mean by each one."*

This **slide** shows how to convert **Cholesterol** to Cholic Acid:

●Notice that the difference between these 2 compounds is that Cholic acid contain 2 more hydroxyl groups at carbon (7 ,12) in the steroid nucleus .

The conversion of **Cholesterol to Cholic acid "Bile acid"** requires "**Cholesterol 7- α-hydroxylase"** the first step in this conversion is hydroxylation at carbon 7 (this step is a rate limiting step "regulated step") because the cholic acid the end product of this step works as inhibitor and Cholesterol acts as stimulator

Note: The inhibition by Cholic acid is **Feedback inhibition**.

Note: hydroxylation at carbon **7** is the **rate limiting step**.

SLIDE #21

This step is conjugation of Cholic acid with **Glycine OR Taurine**
which converts bile acid to bile salts. This conjugation increases the strength of the acids which makes them in slat form "Ionized Form"

Taurine: it is an amino acid that has sulfate group rather than carboxylate group. It has no important role in energy metabolism because we find in small amounts

SLIDE #22

This Figure shows the production of **Bile acid** from **cholesterol** once they are produced they will be conjugated .Then they will be excreted from the liver through the **Bile Duct**.

They are stored in **Gallbladder** then at time of food "food intake" contraction of the bladder will excrete the bile in to the small intestine.

Note: in the small intestine the newly synthesized **bile acid** are know as primary Bile Acids because they are newly formed. Then they are deconjugated by removal of **Glycine** and **Taurine** .Then bacterial enzyme will convert this 1 º to 2º **Bile Acids**.

→What is the difference between the 1º and 2º ?!
The 2º lost one or tow hydroxyl groups.

Note: 95% of the bile acid whether 1º or 2º are reabsorbed through the portal vein and get back to liver to be excreted again. "Liver contains 1º and 2º Bile acids"

Q: Why 95%?!?!
Because 5% (0.5 gram/day) is not reabsorbed/lost (eliminated through feces)

We call this circulation → Enterohepatic cycle (From Liver to Intestine)

SLIDE #23

How do we Lower **Cholesterol** level?! And why we need to lower its level?!

We lower it to avoid myocardial infarction or atherosclerosis

HOW?!

1. Dietary management

A) Reduce the intake of **Cholesterol** in food "but it is not effective and it is very hard" because any animal food contains **Cholesterol**.
*To reduce the **Cholesterol** intake to less than 300 mg, will only affect the **Cholesterol** level by (5-10) % "**not effective**"*

B) Increase the ration of (PUSFA/SFA). PUSFA are known to decrease the level of **Cholesterol** in the plasma.
PUSFA:" polyunsaturated fatty acids"

C) Increase the fiber "الألياف" they are found in vegetables, fruits, whole grains. They help in decreasing the **Cholesterol** by binding to bile acids and binding to **Cholesterol** and decrease its absorption**.**
It is ***not effective*** only 10%

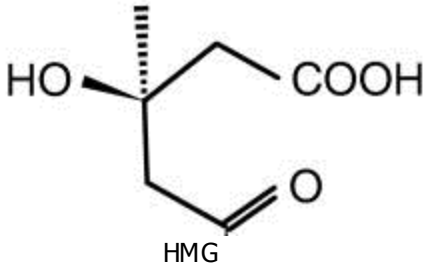
D) Daily Ingestion of Plant Steroid Esters maybe helpful.

2. Inhibition of synthesis

Q: which enzyme is involved in this inhibition?!

HMG CoA Reductase. Because it catalyzes the rate limiting step SO if we inhibited we will inhibit the synthesis

SLIDE #24

This is the structure of HMG (hydroxymethylglutaryl)	 HMG **NOTE**: the Figure in the slid is wrong!

Now look the to the 2nd molecule the large molecule "**Simvastatin"**
It contains this blue portion which is similar to HMG ; which will work on ***HMG CoA Reductase*** as a **competitive inhibitor** because it has the same structural features which is similar to substrate.
Note: **Simvastatin** is one of family of drugs called ***Statin***

SLIDE #25

Lipitor "***Atrorvastatin***" It is one of the most frequent prescribed drug. It is part of a family of drugs called ***Statin.*** Any **male** patient (above 50) or female patient (above 55 years) and has a high level of cholesterol he/she should take this drug.

SLIDE #26

other way to lower **Cholesterol**:

We talked earlier about reabsorbing of bile acids normally 95% of bile acids are reabsorbed, 5 % are secreted in the feces.
If we use a substance that can bind **specifically** or **selectively** or **tightly** to bile acids like "**cholestyramine**". When these substances bind to bile acids, Bile acids will not be reabsorbed …. So instead of 5% that secreted in the feces, 10 % will be secreted. Doubling the amount we are losing daily.

● In this case **HOW** this will act on **Cholesterol** level?!
Remember that we said that bile acid is inhibitor for the "Cholesterol **7- α-hydroxylase**" So low level of bile acids will push more **Cholesterol** to
Be converted to Bile acids

→ **Cholestyramine**: is a substance that binds selectively to bile acids and prevent their absorption. **SO** bind to bile acids and utilize more **Cholesterol** to make the Bile acids.

SLIDE #27

Esterification of Cholesterol means that **Cholesterol** converted to **Cholesteryl-ester** BY binding to F.A by ester bond

Q: How Fatty acids can be utilized?!What do we need?! Do we need energy?! What is the source of F.A for Esterification of **Cholesterol?**
In the **cells**!?

→**Acyl CoA.** In the cells the F.A are activated by joining to CoA
Acyl CoA *is the active donor of the F.*A

→The enzyme that catalyses this reaction is **"Acyl-CoA : cholesterol acyltransferase".** Is abbreviated (**ACAT**)
U can remember what this enzyme does by its name:
1.Acyl-CoA/Cholesterol: Substrate.
2. Acyl: the group that transferred. **"ACAT"**
3. Transferase: transfer the Acyl group

SLIDE #28
Cholesterol is esterified in the plasma as well "In the next lecture will take it in details"

Note: in the plasma there is no "Acyl CoA" and there is no Thiokinase" or any enzyme that produce "Acyl CoA" So the source of F.A for Esterification Is **phospholipids:** " **Lecithin**: **Phosphatidylcholines"**

→**Lecithin** is the donor of fatty acids.

If the **Lecithin** donates the F.A to Cholesterol; **Lecithin** will be converted to **Lysolecithin** "Lysophosphatidylcholines".

If we want to name the enzyme catalyzes this step, we use the same method used in **"ACAT"**

1. Lysthin/cholesterol: substrates
2. Acyl: the group that transferred "LCAT"
3. Transferase transfer the Acyl group

→The enzyme is called "Lecithin ***Cholesterol Acyltransferas***"

U should know these 2 enzymes (LCAT & ACAT)

Q : why we can't make acyl CoA in the plasma ?

→In plasma u can't synthesis Acyl CoA because u need CoA and it is not found there. CoA it's a cellular Coenzyme .

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