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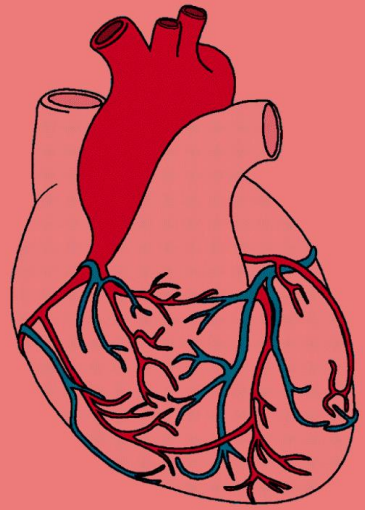
# Cholesterol metabolism



Color index :

Main text

IMPORTANT

Extra Info

Drs Notes

Cardiovascular Block - Biochemistry Team

Revised & Approved by: Shaqra Abdullah Alghamdi

Faisal Alotaibi

MED439

# Objectives:

- Understand the structure and functions of cholesterol.
- Discuss the regulation of cholesterol homeostasis in the body.
- Comprehend the important steps of cholesterol synthesis pathway.
- Identify different levels of regulation of cholesterol synthesis.
- Discuss the association of hypercholesterolemia with abnormal cholesterol metabolism.
- Understand the role of statins in the treatment of hypercholesterolemia.

# Cholesterol

- It's the most important animal steroid.
  because it is the structural component of all cell membranes

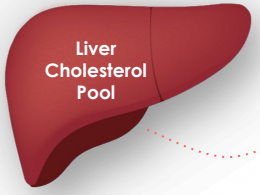
## Functions:

1. Maintains membrane fluidity.
   because at high temperatures, it stabilizes the membrane and raises its melting point, whereas at low temperatures it intercalates between the phospholipids and prevents them from clustering together and stiffening.
2. Insulating effect on nerve fibres.
   It's a component of the Myelin sheath
   "Low level of cholesterol lead to degradation of nerves "
3. Cholesterol is the parent molecule for bile acids and bile salts We need them for digestion and absorption of dietary fat , Steroid hormones e.g cortisol , Vitamin $D_3$ (1)

(1) cholesterol is not important only for Vit $D_3$ but it's important for all fat soluble vitamins.
(2) This is why we consider HDL a good cholesterol, because it brings all the excess cholesterol from the body to the liver to be metabolized.
*extra info : keep in mind extrahepatic tissues means anything formed outside the liver tissue like adipose tissue and muscles*

## Major sources of liver cholesterol

- Dietary cholesterol comes to liver in form of Chylomicron remnants "lipoprotein"
- De novo synthesis in the liver
- Cholesterol synthesized in extrahepatic tissues comes to liver in form of HDL (2)
  الزائد عن حاجتها بينتقل لـ liver




Liver plays a central role in the **regulation** of cholesterol homeostasis

## Major routes by which cholesterol leaves the liver

- Conversion to bile acids/salts
- Secretion of VLDL
  (Very low density lipoprotein )
  ينتقل لكل الأورقانز
- Free cholesterol secreted in the bile
  Excreted by stool

باختصار اللي يهمكم تعرفونه هنا هو ان الـ cholesterol ممكن يوصل للـ liver بثلاث طرق : من خلال الاكل او ممكن الـ Extrahepatic tissues تصنّعه بنفسها او اخر طريقه ان الـ liver تصنّعه بنفسها
بعدها لما يوصل لـ liver راح تتخلص منه برضو بثلاث طرق : يا انها ترسله للأعضاء الثانية على شكل VLDL أو جزء منه راح يُرسل مباشرة في الـ bile اما آخر طريقة أنها تحوله مباشرة إلى bile

# Cholesterol structure

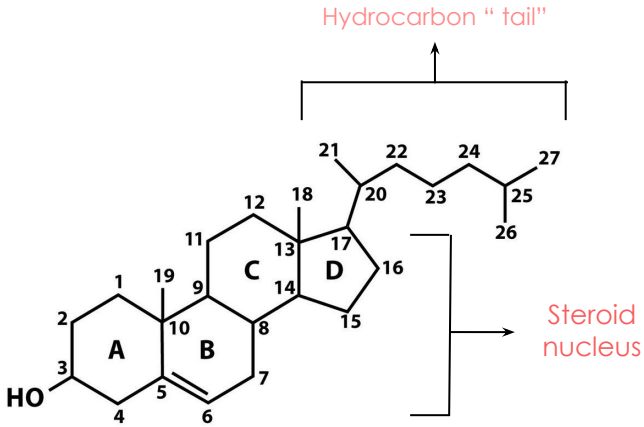
## ★ Free cholesterol

Cholesterol is made of steroid nucleus that has **4 hydrocarbon fused rings** and contain:

| Hydroxyl group at C3. | Double bond between C5 & C6. | Hydrocarbon "tail & 8-10 hydrocarbon long" at C17 |
|---|---|---|

The total number of carbon is 27C.

cholesterol is a type of sterol . all sterols are steroids, (but not all steroids are sterols)




**Cholesterol**

## cholesteryl ester

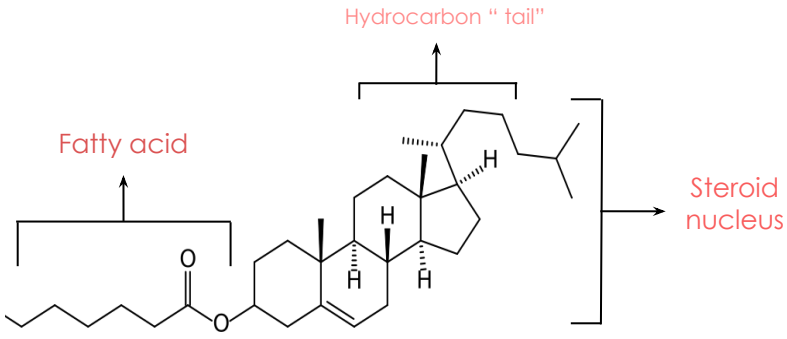
Cholesteryl ester has similar structure to cholesterol, **but added Fatty acid to the site of hydroxyl group (C3).**

Most plasma cholesterol is esterified with a fatty acid.

CEs (cholesteryl esters) are not present in membranes Due to absence of the hydrophilic part (hydroxyl group)& what present in the membrane is free cholesterol

Present in small amounts in most cells.

**More hydrophobic** than cholesterol
because a fatty acid group was added to it.




**Cholesteryl ester**

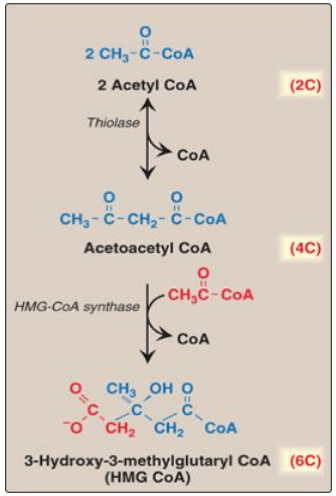
# Cholesterol synthesis

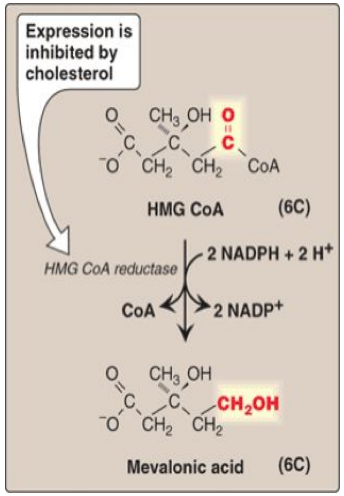
Synthesized in all tissues

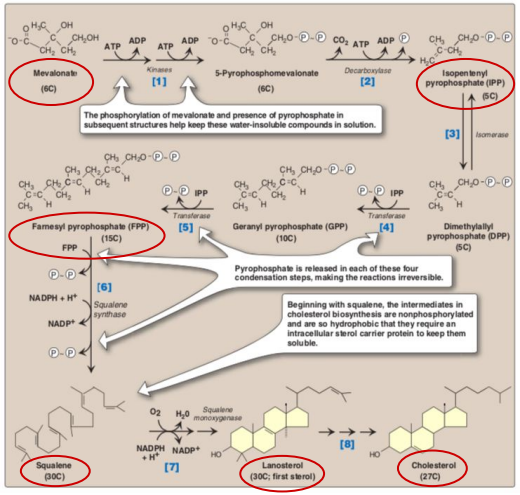
Major sites for synthesis: **liver**, **adrenal cortex**, **testes**, **ovaries** and **intestine**

All carbon atoms are derived from acetyl CoA (has 2 carbons)

Enzymes involved in biosynthesis are partly located in **ER** and partly in **cytoplasm**(1)




**We put the 3 pictures in case if they come as a SAQ , these 3 pictures explained in the next slides**

السلايدات اللي بعدها مريحه للعين اكثر لو تبون شرح ، تقدرون ترجعون لهالصور عشان تعرفون byproducts للي طلعت ودخلت زي NADPH ... الخ

(1)Even though the enzyme is present in the **ER**, synthesis only occurs in the **cytoplasm**, how? Because the catalytic domain of the enzyme that found in the cytoplasm.

A helpful video

# Cholesterol synthesis

reversible
irreversible

2C Acetyl coA + 2C Acetyl coA
Thiolase
coA
4C Acetoacetyl coA
Acetyl coA
coA
HMG- CoA synthase
3-Hydroxy-3-methylglutaryl-CoA ( HMG CoA )
6C
coA
HMG- CoA reductase
6C Mevalonic acid
Kinase
6C 5- Pyrophosphomevalonate
decarboxylase
5C Isopentenyl pyrophosphate (IPP)

★ **HMG CoA synthase** is present in both cytosol(cytoplasm) and mitochondria of liver:
- Mitochondrial - ketogenesis
  formation of ketone bodies
- Cytosolic – cholesterol synthesis

- **Rate limiting** and **key step**[1]
- Occurs in cytosol
- **HMG CoA reductase** is an **ER** membrane enzyme with catalytic unit hanging in the cytosol

The enzyme is present in **ER** but the site of action ( catalytic unit ) hanging in **cytosol**.


This step is not important

Production of a 5-carbon unit
patent sterol for all sterols, and the building block of cholesterol

★ Isomerase

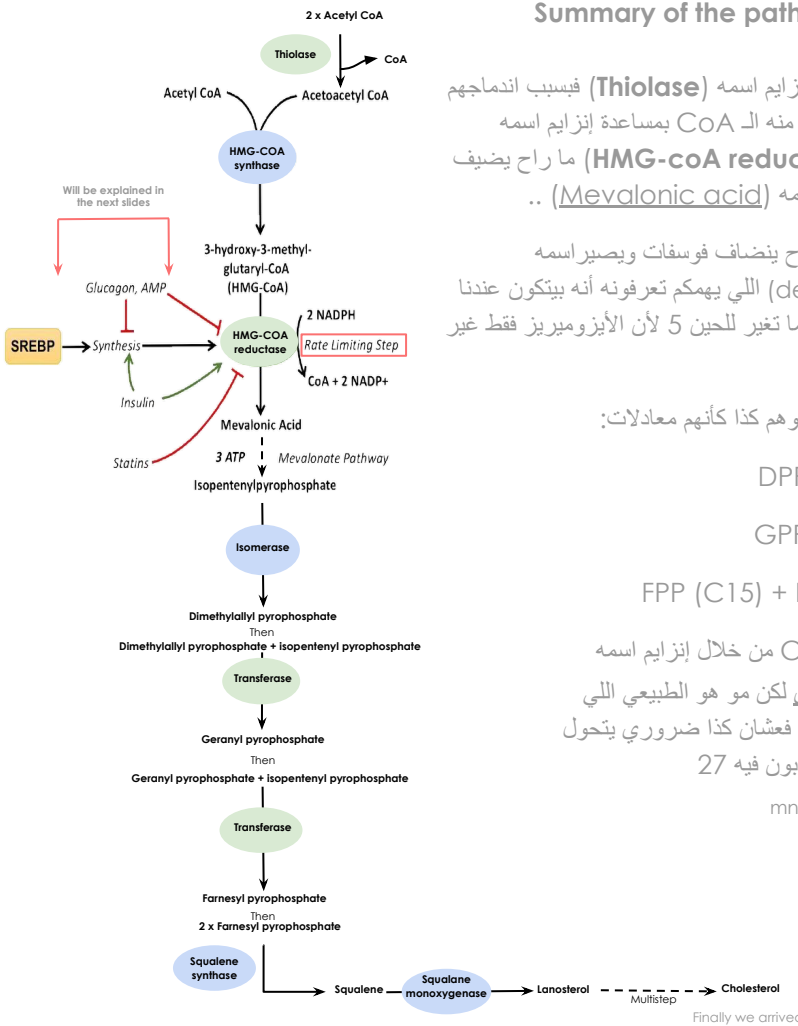
5C DPP
Transferase + IPP
10C GPP
Transferase + IPP
15C FPP
★ Squalene synthase + FPP
30C Squalene
★ Squalene monooxygenase
30C Lanosterol (First sterol)
Multiple steps
27C Cholesterol

Condensation to a 30C compound **(squalene)**
( which is an opened ring molecule )

Cyclization of squalene to 30C lanosterol[2]

Synthesis of 27-Carbon cholesterol **(defect in this leads to Smith-Lemli-Opitz Syndrome "SLOS" )**
There is an enzyme that has to transfer the double bond between C7&C8 to C5&C6, if the enzyme is absent that lead to **SLOS** because the lanosterol didn't not get converted to cholesterol.

(1) step that regulates the whole pathway, which is an irreversible and slow step, and is the target for inhibitors.
(2) to form a ring ( the first sterol that is made in the body ).




## Summary of the pathway In order for you to gain a better understanding

في البداية بنحتاج يكون عندنا اثنين Acetyl coA يندمجون مع بعض بعدين نحذف CoA من وحدة منهم بمساعدة إنزايم اسمه (**Thiolase**) فبسبب اندماجهم بيتكون عندنا مركب جديد اسمه Acetoacetyl coA بعدها برضو بنضيف Acetyl coA ثالث وبنحذف منه الـ CoA بمساعدة إنزايم اسمه (**HMG-coA synthase**) وبيتكون عندنا مركب مهم جدًا اسمه HMG coA بعدها بيجينا إنزايم اسمه (**HMG-coA reductase**) ما راح يضيف شيء مثل الإنزايمز اللي قبله لأن وظيفته فقط يغير المجموعة الوظيفية ويحذف coA وبيكون عندنا مركب اسمه (Mevalonic acid) ..

حاليًا صار عندنا الأسيد هذا اللي عدد الكربون فيه 6 بيصير له كم خطوه على الأغلب ما يهمكم تعرفونها ( راح ينضاف فوسفات ويصيراسمه Pyrophosphomevalonate عن طريق انزايم kinase وبعدها بنشيل منه $CO_2$ عن طريق انزايم (decarboxylase اللي يهمكم تعرفونه أنه بيتكون عندنا مركب جديد عدد الكربون فيه 5 واسمه (IPP) بعدها من خلال **Isomerase** راح يتحول إلى (DPP) عدد الكربون ما تغير للحين 5 لأن الأيزوميريز فقط غير شكله ما أضاف له شيء.

المراحل الجاية كلها عبارة عن إندماج مركبات مع بعض عشان ينتج لنا الكوليسترول في النهاية، احفظوهم كذا كأنهم معادلات:

DPP(C5) + IPP (C5) = GPP(C10).. The enzyme: **Transferase**

GPP (C10) + IPP(C5) = FPP(C15).. The enzyme: **Transferase**

FPP (C15) + FPP (C15) = Squalene (C30).. The enzyme is: **Squalene synthase**

لكن في هالحالة الـ Squalene بيكون linar وأحنا نحتاجه يكون rings فبنسوي له Cyclization من خلال إنزايم اسمه **Squalane monoxygenase** وبتغير اسم المركب وبيصير Lanosterol وهذا يعتبر أول ستيرول يتكون لكن مو هو الطبيعي اللي نحتاجه لأن عدد الكربون فيه 30 ولو بقى هذا بجسمنا وما تحول إلى كوليسترول فراح يسبب لنا مرض اسمه (SLOS) فعشان كذا ضروري يتحول لكن حنا ما يهمنا نعرف خطوات التحول المهم بالأخير بيتكون عندنا الكولسترول الطبيعي اللي عدد الكربون فيه 27

الخطوات الاولى لين ال pyrophosphomevalonate عليكم تحفظونها بس عشان تحفظون ال sequence of products بعدها عندكم هالـ mnemonics

"**I D**on't **G**et it why **F**atimah **S**tole **L**ama's **C**oat **or** **I D**o **G**et **F**rightened **S**tealing **L**uxurious"

**I** = **I**sopentenyl pyrophosphate
**D** = **D**imethylallyl pyrophosphate
**G** = **G**eranyl pyrophosphate
**F** = **F**arnesyl pyrophosphate
**S** = **S**qualene
**L** = **L**anosterol
**C** = **C**holesterol

★ extra explanation

## Summary of the pathway In order for you to gain a better understanding In tables

| Reaction 1 | |
|---|---|
| Reactant | 2 Acetyl coA |
| Product | Acetoacetyl coA |
| Enzyme | Thiolase |
| Action | Join 2 Acetyl coA together and remove CoA |
| Byproduct | COA |

| ★ Reaction 2 | |
|---|---|
| Reactant | Acetoacetyl coA + Acetyl coA |
| Product | HMG CoA |
| Enzyme | HMG- CoA synthase |
| Action | Join them together and remove CoA |
| Byproduct | COA |

| ★ Reaction 3 | |
|---|---|
| Reactant | HMG CoA |
| Product | Mevalonic acid |
| Enzyme | HMG- CoA reductase |
| Action | Join them together and remove CoA |
| Byproduct | Use 2NADPH + 2H+ and produce NADP+ |

| Reaction 4 | |
|---|---|
| Reactant | Mevalonic acid |
| Product | 5- Pyrophosphomevalonate |
| Enzyme | Kinase |
| Action | Add 2 phosphate groups to it |
| Consume | 2 ATP |

| ★ Reaction 5 | |
|---|---|
| Reactant | 5- Pyrophosphomevalonate |
| Product | Isopentenyl pyrophosphate (IPP) |
| Enzyme | decarboxylase |
| Action | Remove $CO_2$ group |
| Consume | 1 ATP |

| Reaction 6 | |
|---|---|
| Reactant | IPP |
| Product | Dimethylallyl pyrophosphate (DPP) |
| Enzyme | Isomerase |
| Action | Change the structure of the molecule |
| Byproduct | - |

★ extra explanation

# Summary of the pathway In order for you to gain a better understanding In tables ... contd

| Reaction 7 | |
|---|---|
| Reactant | DPP + IPP |
| Product | Geranyl pyrophosphate ( GPP ) |
| Enzyme | Transferase |
| Action | Join the two molecules together |
| Byproduct | 2 phosphate groups |

| Reaction 8 | |
|---|---|
| Reactant | GPP + IPP |
| Product | Farnesyl pyrophosphate ( FPP ) |
| Enzyme | Transferase |
| Action | Join the two molecules together |
| Byproduct | 2 phosphate groups |

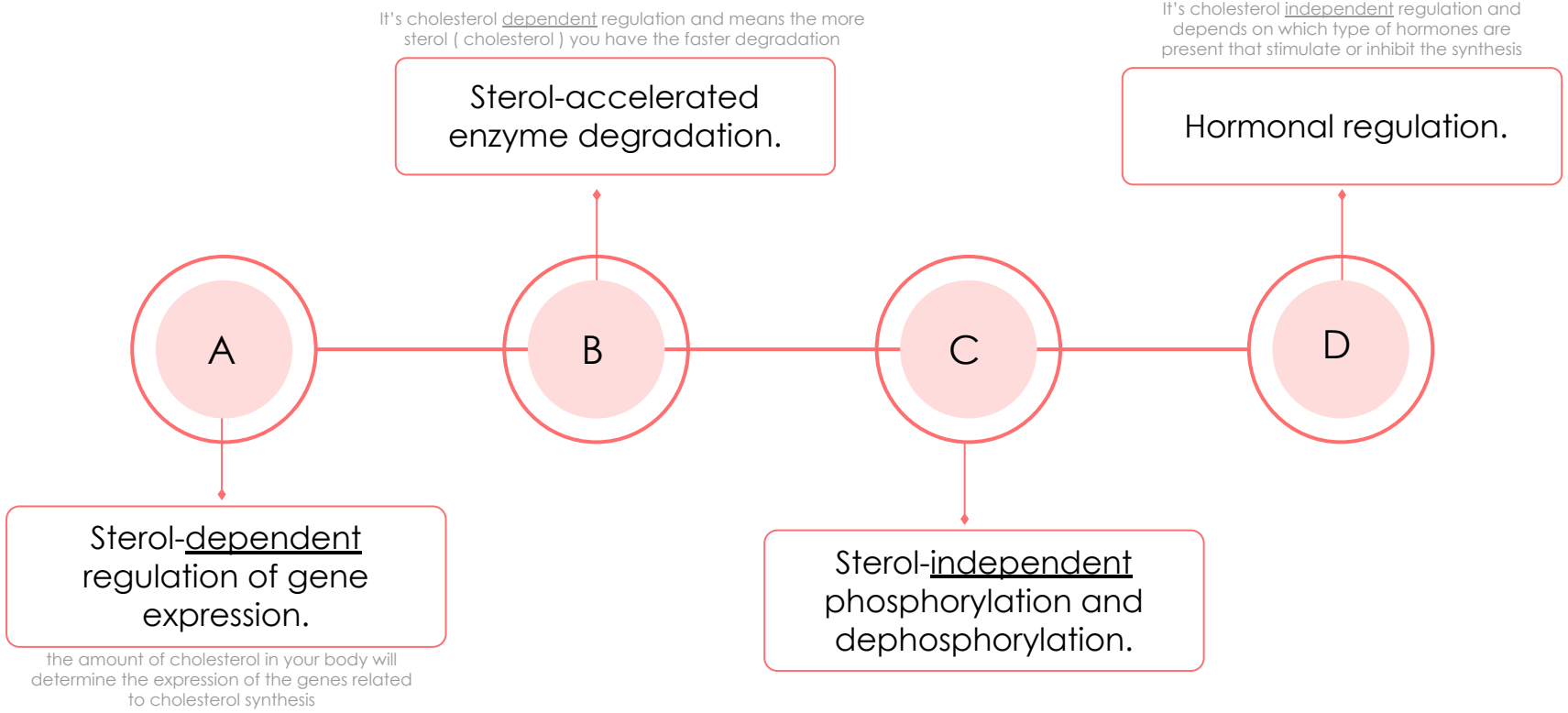
| ★ Reaction 9 | |
|---|---|
| Reactant | 2 FPP |
| Product | Squalene |
| Enzyme | Squalene synthase |
| Action | Join the two molecules together |
| Byproduct | 2 phosphate groups + Use NADPH + H+ and produce NADP+ |

| ★ Reaction 10 | |
|---|---|
| Reactant | Squalene |
| Product | Lanosterol |
| Enzyme | Squalene monooxygenase |
| Action | Cyclization of squalene ( close the rings ) |
| Byproduct | $H_2O$ + Use NADPH + H+ and produce NADP+ |

| Reaction 11 | |
|---|---|
| Reactant | Lanosterol |
| Product | Cholesterol |
| Enzyme | ( multiple steps not only one step ) |
| Action | Synthesis of 27-Carbon cholesterol |
| Byproduct | - |

A helpful video

# HMG CoA Reductase Regulation



# A) Sterol-dependent regulation of gene expression of HMG CoA

The goal of this regulation is to regulate transcription, how ? By transcription factors. These factors bind before the gene, and either activate or inhibit its transcription

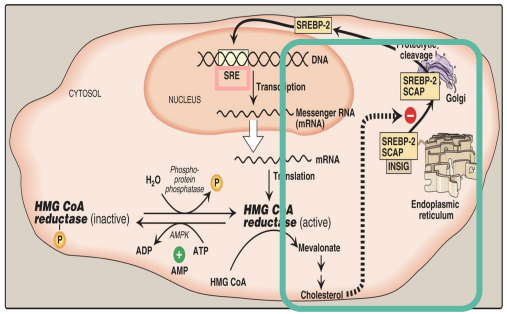
- When sufficient cholesterol is present, transcription is suppressed and vice versa.
- Sterol Regulatory Element (SRE) is a recognition sequence in the DNA. " the area that binds with the transcription factor "
  "نقدر نقول مكان موجود في DNA عشان يخلي ال SREBP اللي هو transcription factor ينتبه اذا جا ال nucleus ان من هنا يبدا ال transcription"
- SREBP(SRE binding protein) binding to SRE (Sterol Regulatory Element) is essential for transcription of this gene.
  SREBP is the transcription factor that present in the ER. "وعشانه موجود ب ER لازم يروح لل nucleus عشان يبدا ال transcription of the gene"
- SREBP cleavage-activating protein (SCAP) is an intracellular cholesterol sensor. so when the levels of cholesterol decrease, SCAP will take SREBP to the golgi bodies then in the Golgi bodies there are some enzyme that will cleave SCAP ( remove it from SREBP ) why ? Because SREBP wants to be free and able to bind with SRE in the DNA to synthesize more colesterol, and if cholesterol levels increase the cleavage will be stopped.

**Cholesterol level**

01 SCAP ( which already bound with SERBP ) will sense the raise in cholesterol and will bind to INSIG protein ( insulin induced protein ) in ER membrane.

02 SCAP-SREBP is retained in the ER because INSG prevents them from leaving to the golgi body.

03 Down regulation of cholesterol synthesis in other word the transcription of HMG CoA reductase enzyme will be inhibited.

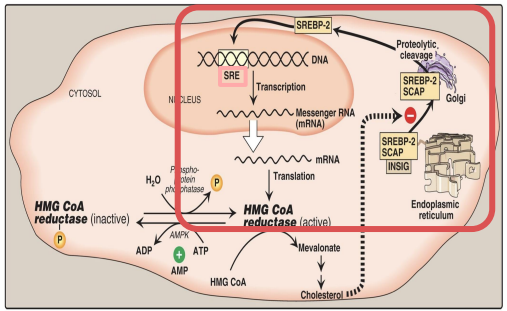



**Cholesterol level**

01 SCAP-SREBP moves to Golgi bodies.

02 SCAP is removed from SREBP because Inside the golgi bodies, there are enzymes that remove SCAP and release SREBP.

03 SREBP goes the the nucleus and binds to SRE in DNA and then HMG CoA gene is activated.



# B ) Sterol-“ dependent” accelerated enzyme degradation

“Dr : it’s a missing slide , you can find it in the reference “.

- The reductase itself is a sterol sensing integral protein of the Smooth ER membrane( SER ).
- When sterol levels in the Smooth ER are high, the enzyme binds to INSIG (insulin-induced protein) ➔ Binding leads to cytosolic transfer ubiquitination and proteasomal degradation of the reductase.

To make it clear the **HMG CoA reductase enzyme** has the ability to sense the levels of cholesterol in the cell if the cholesterol level is high in the SER the **HMG CoA reductase enzyme** will bind to **insig** and when the **HMG CoA reductase enzyme** binds to **insig** , the enzyme will go under degradation why ? To stop the synthesis of cholesterol since the **HMG CoA reductase enzyme** step is the rate limiting step that regulates the whole pathway

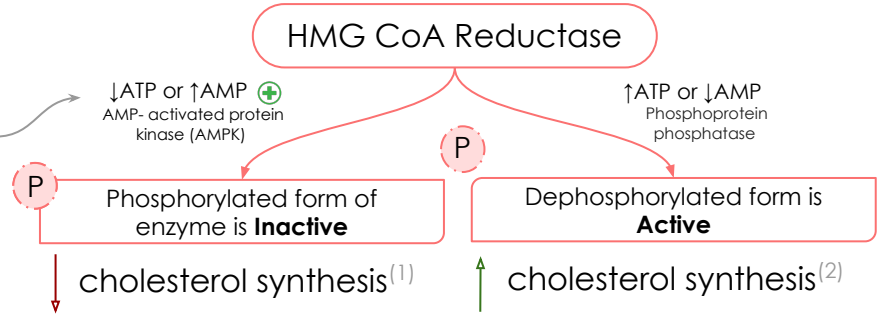
**Absence of HMG CoA reductase enzyme = low levels of cholesterol**

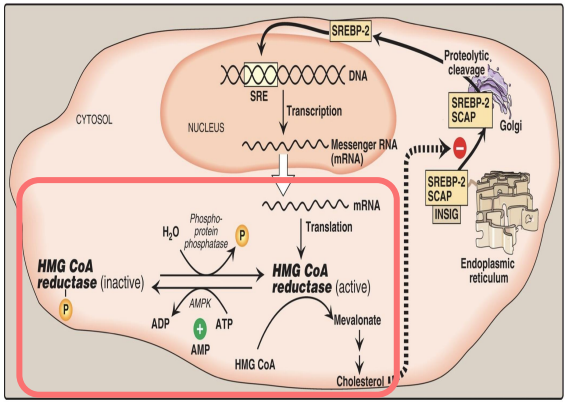
ايش هو upiquitin ؟ هو small protein لما يرتبط مع بروتين اخر راح يصير عندي Ubiquitination معناه ان البروتين الاخر معد يشتغل بسبب ارتباط upiquitin فيه ونتيجه لهذا الارتباط راح يصير degradation للانزايم
واخر شي ايش هو الـ proteasome ؟ هو 😂 protein complex مسؤول انه يسوي degradation لانزايمز ما نحتاجها ( لا ننسى ان الانزايم هو باصله بروتين يعني بيكسر بروتينات ما نحتاجها ) via the proteasome
to sum up : reductase+ INSIG → activitie the binding of upiquitin to them → this will active proteasome (to start degradation)→ finally leads to the degradation

# C) Sterol-independent phosphorylation and dephosphorylation

This regulation depend on the amount of energy presented in the cell “ **no enough ATP = no synthesis**”.

High AMP = Low ATP which means the cell is in need of energy. Cholesterol synthesis needs energy (anabolic reaction) and therefore when the cell is running low on energy it inhibits the synthesis of Cholesterol through AMPK




(1) When there’s high AMP, AMP kinase is activated and then the AMPK will phosphorylate the enzyme and make it inactive and the cholesterol synthesis will decrease.
(2) When there’s high ATP another enzyme which is called phosphoprotein phosphatase will remove a phosphate group to make the enzyme active and the cholesterol synthesis will increase .

# D ) Hormonal Regulation

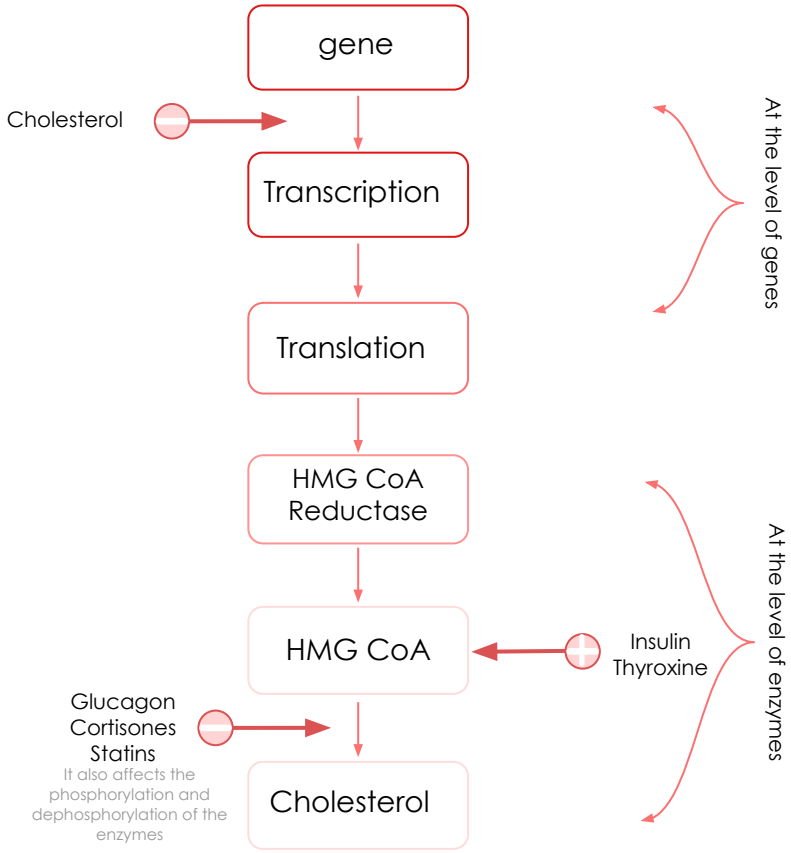
"independent"

- **Insulin** and **thyroxine** increase upregulation of enzyme expression.
  They increase enzyme concentration, thus increase cholesterol synthesis, because insulin has a major role in lipogenesis

- **Glucagon** and **cortisol** have opposite effect ( decease it ).

Cholesterol concentration itself controlling the gene, while hormones are controlling the enzymes

# HMG CoA Reductase Regulation



# Excretion of cholesterol

Normally most of the molecules (protein/carbohydrates) are broken down completely to $CO_2$ and $H_2O$ , but cholesterol can not be broken down completely because it has a big ring structure ) so the body will convert it to other molecules that can be easily excreted from the body.

- By conversion into **bile acids** and **bile salts** excreted in the **feces.** it's the only way for cholesterol excretion
  - Direct secretion of cholesterol in bile.
  - Transported to intestine for elimination.
- In the **intestine**, some cholesterol is converted by bacteria into **coprostanol** and **cholestanol** (which is reduced derivative of cholesterol) before excretion.
- Normally 5% of bile acids/salts that synthesized from cholesterol are excreted, so If we wanna remove the excess cholesterol we use **bile acids binding resin** that excrete more bile acids/sats " more than 5% of cholesterol is excreted ".
  Check the pharmacology team for more details ( Antihyperlipidemic drugs lecture )

**Hyper**cholesterol**emia** — High concentration of cholesterol in blood that leads to **atherosclerosis** or shock due to the loss of regulation of cholesterol synthesis

**Hyper** : high
**emia** : in the blood

**statin drugs** are used as a Treatment of hypercholesterolemia because they decrease plasma cholesterol levels

Check the pharmacology team for more details ( Antihyperlipidemic drugs lecture )

**β-Sitosterols/phytosterols*** are clinically useful in the dietary treatment of hypercholesterolemia(2)

*β-Sitosterol Is a type of several phytosterols that's why we say β-Sitosterol is used in the treatment of hypercholesterolemia not phytosterols

**What are statin drugs ?**
-Statins are structural analogs of ( structurally similar to ) HMG CoA reductase enzyme
**What is the M.O.A of statins drugs ?**
-inhibit enzyme activity by competitive inhibition(1)

**What are the β-Sitosterols ?**
-They're plant sterols like in avocados which are poorly absorbed by humans.
**What is the M.O.A of β-Sitosterols ?**
-They Block the absorption of dietary cholesterol.

(1) group of drugs that are structurally similar to HMG coA reductase enzyme that decrease the level of the cholesterol as a result competition will occur between them.
(2) substances similar to cholesterol found in the plants , poorly absorbed in in the intestine that's why they prevent the absorption of the cholesterol .

# Take Home Messages

- Cholesterol is important various body functions .
- Liver plays a major role in the cholesterol homeostasis in the body.
- HMG CoA reductase is a rate-limiting enzyme for cholesterol synthesis.

Numbering based on the cholesterol synthesis steps

# Summary

## Cholesterols

### Structure

Sterol: 4 ring with hydrocarbon tail and a hydroxyl group .
Cholesteryl ester: have fatty acid tail

### Function

- most important animal steroid.
- maintains membrane fluidity.
- insulating nerve fibers .
- parent molecule for bile acids, bile salt,steroid hormones, vitamin $D_3$.

### Synthesis

#### Location

In all tissues mainly in liver, intestines, adrenal cortex, testes , ovaries.

#### Cholesterol synthesis

##### 1-HMG CoA synthesis

Reactant: Acetoacetyle CoA ( from acetyl coA )

Product: HMG CoA

Location: cytosol

Enzyme : HMG-CoA synthase

##### 2-Mevalonic acid synthesis

Reactant: HMG CoA

Product: Mevalonic acid

Location: cytosol

Enzyme: HMG CoA reductase

##### 3-Synthesis IPP

Reactant: 5-Pyrophosphomevalonate ( which comes from Mevalonic acid )

Product: Isopentenyl pyrophosphate

Enzyme: decarboxylase

##### 4-synthesis FPP

Reactant: isopentenyl pyrophosphate + Geranyl pyrophosphate

Product: farnesyl pyrophosphate

Enzyme : Transferase

##### 5- condensation to squalene

Reactant: 2x farnesyl pyrophosphate

Product: Squalene

Enzyme: squalene synthase

##### 6- cyclization of squalene

Reactant: squalene

Product: lanosterol

Enzyme : Squalane monoxygenase

##### 7-synthesis of 27c cholesterol

Multiple steps & defect on these steps lead to Smith Lemli Opitz Syndrome

### HMG CoA reductase regulation:

1- sterol dependent regulation of HMG CoA gene expression

2- sterol accelerated enzyme degradation

3-sterol independent phosphorylation/dephos phorylttion

3- hormonal regulation

# Quiz

| Q1 : One of major routes by which cholesterol leaves the liver is : | | | |
|---|---|---|---|
| A ) Secretion of VLDL | B ) Secretion of LDL | C ) Secretion of HDL | D ) Secretion of Cholymicron |
| **Q2 : converting of HMG CoA to mevalonic acid done by :** | | | |
| A ) HMG CoA Reductase in cytosol | B ) HMG CoA dismutase enzyme in cytosol | C ) HMG CoA dismutase enzyme in ER | D ) HMG CoA Reductase in ER |
| **Q3 : When SCAP binds to INSIG protein that lead to …. ?** | | | |
| A ) SCAP-SREBP moves to golgi bodies | B ) SCAP-SREBP is retained in ER | C ) SCAP is removed from SREBP | D ) SREBP binds to SRE in DNA |
| **Q4 : Cholesterol synthesis increases when …… ?** | | | |
| A ) ATP is high | B ) ATP is low | C ) AMP is high | D ) B&C |
| **Q5 : Upregulation of enzyme expression of cholesterol increases by :** | | | |
| A ) Glucagon | B ) Insulin | C ) Cortisol | D ) None of them |
| **Q6 Which of one of the following is true when the cholesterol levels are high ?** | | | |
| A ) SREBP binds to SRE | B ) SREBP - SCAP binds to INSIG | C ) SREBP is free | D) go to the 3 choices and choose one 😈! |

## SAQs :

Q1: Enumerate the major sites for cholesterol synthesis :

Q2: Name the syndrome that result from defect in 27 carbon cholesterol synthesis

Q3: Which drug can you give to a patient with Hypercholesterolemia ? & mention the MOA of it

★ **MCQs Answer key:**

1) A 2) A 3) B 4) A 5) B 6) B

★ **SAQs Answer key:**

1) Liver , adrenal cortex , ovaries , testes and intestine

2) Smith-Lemli- Opitz Syndrome “SLOS”

3) statin drugs ,it’s structural analogs of HMG CoA reductase enzyme and they inhibit the enzyme activity by competitive inhibition

## Girls team:

Manal Altwaim
Duaa Alhumoudi
Rania Almutiri
Alia Zawawi
Noura Alshathri
Reem Alamri
Renad Alhomaidi
Fatimah Alhelal

## Boys team:

Omar Alsuliman
Abdullaziz Alomar
Hamad Almousa
Homoud Algadheb
Abdullah Alanzan
Abdullah Almazro
Ahmad Alkhayatt
Abdullaziz Alrabiah

just wash your hands

Revised by

Made by

Shatha Aldhohair

Abdulaziz Alsalem

Biochemistry439@gmail.com

@Biochemistry439