Cholesterol Synthesis

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The structure of cholesterol
Cholesterol represented by a formula in (A), by a schematic drawing in (B), and as a space-filling model in (C)
Cholesterol structure

FA for esterification

cholesterol
Cholesterol

- Basic structure is composed of 4 fused nonaromatic rings (hydrogenated phenantrene + cyclopentane), to which is attached 8C branched linear chain

- It contains –CH₃ groups at position C-18 and C-19, -OH group at position 3

- It is structural components of animal cell membranes and animal cells cannot survive without it

- It is the major sterol found in humans, but it is not found in plants or in prokaryotes to any extent
Cholesterol synthesis

- Similar to ketogenic pathway
- Occurs in cytosol of all nucleated cells, mainly in the liver and intestinal epithelium
- It is taken up by cells by receptor-mediated endocytosis
- Requires NADPH and ATP
- Highly regulated
Cholesterol

- Synthesized in all tissues
- Primary site: liver (~1g/day)
- Secondary sites:
  - intestine,
  - adrenal cortex,
  - reproductive tissues (ovaries, testes)
- 80% in liver, ~10% intestine, ~5% skin
Fecal bile acids and neutral sterols (≈500 mg/day)

Extrahepatic tissues

Liver Synthesis* (≈1000 mg/day)

Dietary cholesterol (≈300–700 mg/day)

Biliary Cholesterol (≈1000 mg/day)

Absorption (≈700 mg/day)

Intestine

Fecal bile acids and neutral sterols (≈500 mg/day)

Two sources of cholesterol: Synthesis and Absorption
Sources of hepatic cholesterol

- **Dietary cholesterol**
  - From chylomicron remnants

- **Cholesterol from extra-hepatic tissues**
  - Reverse cholesterol transport via HDL
  - Chylomicron remnants
  - IDL

- **De novo synthesis**
Dietary cholesterol

- Animal products – eggs (yolk), liver, brain, meat ...
- Absorbed about 50%
- Increased intake = decreased absorption
- Excreted – 500 mg/daily (bile acids)
The pathway for cholesterol synthesis occurs in the cytoplasm.

Synthesis utilizes an activated two carbon intermediate acetyl-CoA.

Acetyl-CoA must be first transported out of the mitochondria using citrate shuttle transport system.

The total energy requirement for converting mitochondrial acetyl-CoA into cytoplasmic acetyl-CoA is 1 ATP.
Cholesterol is synthesized “de novo” from acetyl-CoA in 4 stages

1. Synthesis of mevalonic acid

2. Conversion of mevalonic acid to isopentenyl pyrophosphate (IPP), an activated isoprenoid unit

3. Condensation of 6 molecule of IPP to form squalene

4. Squalene cyclizes and the tetracyclic product is converted to cholesterol
De novo synthesis of cholesterol

**Overall equation:**

18 Acetyl CoA + 18 ATP + 16 NADPH + 4 O₂ → cholesterol + 9 CO₂ + 16 NADP⁺ + 18 ADP + 18 Pi
1. Synthesis of acetoacetyl-CoA

- 2 acetyl-CoA are condensed to form acetoacetyl-CoA

- Thiolase, is the enzyme, which is usually associated with degradation
Hydroxymethylglutaryl-CoA (HMG-CoA):

- The precursor for cholesterol synthesis
- Also an intermediate on the pathway for synthesis of ketone bodies from acetyl-CoA (the enzymes for ketone body production are located in the mitochondrial matrix)

HMG-CoA destined for cholesterol synthesis is made by equivalent, but different, enzymes in the cytosol.
Mevalonate synthesis

- **HMG-CoA** is synthesised by condensation of acetyl-CoA + acetoacetyl-CoA, catalysed by HMG-CoA Synthase

- **HMG-CoA Reductase**, located in the smooth ER, reduces mevalonate with the need of NADPH to form HMG-CoA

- Rate-limiting (controlling) enzyme and site of inhibition by statins
- HMG-CoA Reductase is an integral protein of endoplasmatic reticulum membranes.

- The HMG-CoA Reductase reaction, in which mevalonate is formed from HMG-CoA, is rate-limiting for cholesterol synthesis.
2. **Isopentenyl pyrophosphate synthesis**

- **Mevalonate** is phosphorylated (using ATP), to 5-carbon active isoprenoids, pyrophosphate derivatives.

- ATP-dependent decarboxylation, with dehydration yields **isopentenyl pyrophosphate**.
3. Squalene synthesis

- **Isopentenyl Pyrophosphate Isomerase** inter-converts **Isopentenyl pyrophosphate** and **Dimethylallyl pyrophosphate** required for **Geranyl pyrophosphate synthesis**.
Farnesyl pyrophosphate synthesis

- **Prenyl Transferase** catalyzes "head-to-tail" condensations

- Dimethylallyl pyrophosphate and isopentenyl pyrophosphate react to form geranyl pyrophosphate

- Condensation with another isopentenyl pyrophosphate yields farnesyl pyrophosphate

![Diagram showing the synthesis of farnesyl pyrophosphate](image)

- Dimethylallyl pyrophosphate and isopentenyl pyrophosphate react to form geranyl pyrophosphate.
- Geranyl pyrophosphate condenses with another isopentenyl pyrophosphate to form farnesyl pyrophosphate.
- Farnesyl pyrophosphate is a sesquiterpene.
- Condensation with another isopentenyl pyrophosphate yields farnesyl pyrophosphate.
Squalene synthesis

- Squalene synthase: “Head-to-head” condensation of 2 farnesylpyrophosphates with reduction by NADPH, produces squalene, a triterpene.
**Lanosterol synthesis**

- **Squalene epoxidase** catalyzes conversion of squalene to 2,3-epoxid.

  - This mixed function oxidation requires **NADPH** as reductant and **O_2** as oxidant.

- **Squalene oxidocyclase** catalyzes a series of e-shifts, initiated by donation of a proton to the epoxide, that lead to **cyclization**.
In the process of cyclation, or squalene conversion to lanosterol:

- **Squalene epoxidase** forms 2,3 oxidosqualene
- **Lanosterol synthase** brings about cyclization by protonation of the epoxide
- The cyclization involves a series of hydride and methyl shifts, -\(\text{CH}_3\) group from C-14 is shifted to C-13 and from C-8 to C-14
- Enzyme protonates the epoxide, forming a cation and then -\(\text{OH}\) group
4. Conversion of lanosterol to cholesterol involves 19 reactions, catalyzed by enzymes in ER membranes

These changes are in gonane backbone and in site chain of lanosterol

- $\text{CH}_3$ groups of C-14 and C-4 (2) are oxidized to CO$_2$ and these way are removed
- = bonds of C-8 and C-9 are shifted to C-5 and C-6
- = bond of site chain between C-24 and C-25 undergoes hydrogenation
After these series of reactions, lanosterol is oxidized ($O_2$) and reduced (NADPH) with the loss of 3 carbons to cholesterol ($C_{27}$).
Alternative pathway is demethylation forming desmosterol, which is reduced to cholesterol.

Sites of ACAT inhibitors

Acyl-CoA cholesterol acyltransferase (ACAT)
Stage 1
Acetyl CoA (C₂)

HMG-CoA

NADPH

NADP⁺

Mevalonate (C₅)

Stage 2
Mevalonate

3ATP

3ADP

Active Isoprenoids (C₅)

NADPH

Several Condensation Steps

Squalene (C₃₀)

Stage 3
Squalene (C₃₀)

O₂

Cyclization

NADPH

Squalene epoxidase/cyclase

NADP⁺

Lanosterol (C₃₀)

(4-ring structure)

Stage 4
Lanosterol (C₃₀)

O₂

(19 steps)

NADPH

NADP⁺

3 CH₃

Cholesterol (C₂₇)

4 stages cholesterol synthesis

rate-determining step
cholesterol activates proteolytic degradation
amount controlled by induction/repression
hormonally controlled via phosphorylation
Farnesyl pyrophosphate, an intermediate on the pathway for cholesterol synthesis, also serves as precursor for synthesis of various isoprenoids:

- **Dolichol**

  serves as a carrier of saccharide, which is attached to N-asparagine at oligosaccharide chain synthesis of glycoproteins.
Coenzyme Q (ubiquinone), which has an isoprenoid side-chain, functions in the electron transfer chain.

The number of isoprenoid units can vary, in mammals it is usually 10.
HMG CoA reductase

2 different regulatory mechanisms are involved:

◇ Short-term regulation
◇ Long-term regulation
Short-term regulation includes effect of many factors on HMG CoA reductase activity involving:

c-AMP
Cholesterol,
Glucagon
Glucocorticoids
Thyroid hormones
Insulin
Epinephrine
Long-term regulation includes:

- Proteolysis
- Degradation stimulated by cholesterol and cholesterol metabolites
- Sterol-sensing domain
HMG-CoA reductase can be present in 2 forms:
- Phosphorylated is non-active
- Dephosphorylated is active

The equilibrium between active and non-active form is maintained by protein kinase and phosphoproteinphosphatase.

- Phosphorylation by cAMP-dependent protein kinases inactivates the reductase
  (This kinase is active when cellular AMP is ↑, corresponding to when ATP is ↓)
- Inactivation can be reversed by 2 specific phosphatases

The process is called covalent modification of enzyme.
HMG CoA reductase - Phosphorylation

HMG CoA reductase – OH  
(active)

HMG CoA reductase – P  
(inactive)

AMP-Activated  
Protein Kinase (high activity)

AMP-Activated  
Protein Kinase (low activity)

AMP

phosphatase

kinase

insulin

(+)

Glucagon/epi

increase cAMP

(+)

(+)

HMG CoA reductase – OH

(+)
Covalent Modification of HMG-CoA Reductase

- **Insulin** (and thyroid hormones):
  - Induces protein phosphatase
  - Activates HMG-CoA reductase
  - Stimulates synthesis of enzyme by affecting the rate of transcription
- Glucagon, glucokorticoids and starvation have the opposite effects
Long-term regulation

Regulated proteolysis of HMG-CoA Reductase:

- HMG-CoA Reductase includes a transmembrane **sterol-sensing domain** that activates degradation of the enzyme via the proteasome

- Degradation of HMG-CoA Reductase is stimulated by cholesterol, oxidized derivatives of cholesterol, mevalonate, farnesol (dephosphorylated farnesyl pyrophosphate)

at the level of **gene expression**: cholesterol levels control the amount of mRNA
Induction of HMG-CoA reductase enzyme and the LDL receptor requires binding of SREBP-1 to the promoter regions of the genes that encode these proteins.
A. Normal/High cellular free cholesterol

Protease is inhibited

Sterol Regulatory Element Binding Protein trapped in ER as precursor

Repressed Genes:
1. HMG CoA reductase
2. LDL receptor

Levels of free cellular cholesterol keep the protease inactive so that pre-SREBP-1 remains intact and the genes for HMG-CoA reductase and the LDL receptor are repressed.
B. Low cellular free cholesterol
Protease is active to cleave pre-SREBP-1

Low cholesterol permits release of SREBP-1 from the ER by activation of a protease that proteolytically removes SREBP-1 from pre-SREBP-1, the inactive form

Induced Genes:
1. HMG CoA reductase
2. LDL receptor
Lowering cholesterol

Key principle of cholesterol

♦ Biosynthesis, rather than diet,

contributes the majority
of body cholesterol,
which can deposit
in artery walls to cause atherosclerosis
Lowering cholesterol - history

During the past 5 decades, a number of drugs representing various strategies for the management of hyperlipidemia have been developed:

- **Niacin, 1955**
- **Bile acid sequestrants, 1961**
- **Fibrates, 1967**
- **Statins (HMG-CoA reductase inhibitors), 1987**
- **Cholesterol absorption inhibitor: First New Therapy for Hyperlipidemia in 15 years, 2002**
Therapies for high cholesterol

♣ Statins
  ▪ Block HMG-CoA reductase, necessary for cholesterol synthesis in the liver

♣ Fibrates
  ▪ Activate Peroxisome proliferator-activated receptors (PPAR), promotes FA oxidation

♣ Niacin
  ▪ Block cholesterol uptake from intestines

♣ Estrogen
  ▪ Elevates HDL
Drugs used to inhibit cholesterol synthesis include competitive inhibitors of HMG-CoA Reductase. They include various "statin drugs," a portion of each is analogous in structure to mevalonate or to the postulated mevaldehyde intermediate.
Statins, anti-hyperlipidemic drugs, decrease HMG CoA reductase activity.
Energy requirement

- 3 molecules of ATP are required for synthesis of any isoprenoid unit
- 6 isoprenoid units are required for synthesis of 1 mole of cholesterol

Total ATP requirement for 1 molecule cholesterol synthesis is

\[6 \times 3 = 18 \text{ ATP}\]
Reverse Cholesterol Transport (RCT)

Is the process whereby excess cholesterol in peripheral cells, especially foam cells, is returned to the liver for degradation and excretion.

RCT involves apoA-I, ABCA1 and LCAT as well as receptors on the liver for uptake of the excess cholesterol.
Reverse Cholesterol Transport
Delivery of peripheral tissue cholesterol to the liver for catabolism
Requires HDL, apoA-I and LCAT

UC = unesterified cholesterol
CE = esterified cholesterol
PL = phospholipid
LDLr = LDL receptor
apoA-I
CHOLESTEROL DISORDERS

Cholesterol: in serum transported in lipoproteins

In metabolism disorders

- Primary, genetic
- Secondary} hypercholesterolemia

primary disorder is always in lipoproteins
Accumulation of cholesterol without hypercholesterolemia, xanthomas formation

- Primary proliferation of RES system probably occurs, cells (from unknown reason) accumulate cholesterol

- Similar symptoms:
  - Histiosis X
  - Hand-Schuller-Christian’s disease
Xanthomas

- Raised, waxy appearing, often yellow skin lesions (shown here on knee)

- Associated with hyperlipidemia

- Tendon xanthomas common on Achilles and hand extensor tendons
Xanthomas
raised lesions related to hyperlipidemia

Eruptive Xanthomas
- generally associated with hypertriglyceridemia

Xanthomas of the eyelid
- generally associated with hypercholesterolemia
Cholesterol values

- **Total cholesterol**
  
  **Desirable range:**
  
  2,8-5,2 mmol/L

- **Optimal values for LDL and HDL**
  
  < 3,4 mmol/L LDL
  
  > 1,6 mmol/L HDL
Atherosclerosis occurs when:

- Cholesterol and its esters are deposited in arterial walls
- Blood flow is restricted and clots are formed

Reduction of the amount of cholesterol in the diet:

- Has met with some success in slowing this process
- The limited success is due to the fact that much of cholesterol found in circulation comes from biosynthesis, not from the diet
Atherosclerosis, cont.

- Hardening of the arteries due to the deposition of atheromas
- Heart disease is the leading cause of death
- Caused by the deposition of CEs on the walls of arteries
- Atherosclerosis is correlated with ↑ LDL and ↓ HDL
Other factors that influence the tendency toward atherosclerosis include:

- Advancing age
- Male sex
- Limited exercise
- Presence of hypertension
- Above-average levels of insulin
- Thyroid hormone
- Smoking and family history
Atherosclerosis - development

Endothelial cells normally produce:
- Prostaglandin \( I_2(\text{PGI}_2) \)
- Prostacyclin (inhibits platelet aggregation)

When endothelial cells are damaged, platelet release:
- Tromboxane \( A_2 \) (platelets aggregate)
- Platelet-derived growth factor
  (cause proliferation of smooth muscle cells, which migrate from the medial to the intimal layer of the arterial wall)

Cells within the intimal layer release:
- Lipids (TAG, cholesterol), which accumulate in the developing plaque
- LDL enter the area, contribute to the lipid building
• Cells in the lesion secrete collagen, elastin and glycosaminoglycans, forming fibrous cap and cholesterol crystals appear in the core of the plaque

• Cells are trapped in the plaque, they die, forming the debris

• Calcification also occurs

• Rupture and hemorrhage of the encapsulated plaque in a coronary vessel may cause the acute formation of a clot (thrombus), which further occludes the vessel, causing a myocardial infarction
Atherosclerosis: Progression

- Tear in endothelium
- Fatty streak
- Atheroma
- Raised plaque with fibrous cap
- Unstable plaque
- Ruptured plaque with large thrombus
(a) Normal artery wall

White blood cells adhere and migrate into artery wall to fight infection

Endothelium

Rolling white blood cell

Blood flow through lumen

Intima

Media (smooth muscle cells)

Adventitia

(b) Fatty streak stage

Macrophage foam cell formation

(c) Atheroslerotic plaque stage

Fibrous cap formation

Macrophage foam cell accumulation

Formation of necrotic core

(d) Rupture of endothelium and occlusive blood clot formation

Occlusive blood clot
Turbulence in atherosclerosis
Early and late atherosclerotic lesions

Fatty streak

Thrombotic athero lesion, myocardial infarct
METABOLIC FATE OF CHOLESTEROL

Introducing into structures (membrane component)

7-dehydrocholesterol → CALCIOL

Cholesterol esters

BILE → Cholestanol, koprostanol (feces)

BILE

Progesterone → Corticoids

Primary bile acids

Sex hormones
THE END