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Chapter 25

Lipid Biosynthesis

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Chapter Topics

- Fatty Acid Biosynthesis
  - Chain elongation and unsaturated FA's.
- Complex Lipid Biosynthesis
  - Glycerolipids and Sphingolipids
- Eicosanoid Biosynthesis
- Cholesterol Biosynthesis
- Bile Acids and Steroids
- Lipoproteins

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Fatty Acid Biosynthesis

- Starting precursor is acetyl-CoA
- Occurs in cytoplasm, not in mitochondria
- Citrate is the “carrier” of acetyl-CoA formed in the mitochondria to the cytoplasm.
- See Fig. 25.1

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## Fatty Acid Biosynthesis, con't.

- Not simply the reverse of Fatty Acid oxidation, though the chemistry is similar.
- Energy difference from two reactions:
  - “Activation” of acetyl-CoA to make C-C bond formation irreversible.
  - NADPH as electron donor in double bond reduction (FAD was acceptor in oxidation)

The activation of acetyl-CoA requires an ATP for each C-C bond formed, whereas the breaking of C-C bonds by thiolase was a reversible reaction, near equilibrium. NADP has a lower reduction potential (-0.32 volts) than a flavoprotein (~0.0 volts), making the reduction of the double bond favored.

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## Malonyl-CoA an Intermediate

- “Activation Step”  
$$\text{Acetyl-CoA} + \text{ATP} + \text{CO}_2$$

↓ acetyl-CoA carboxylase

$$\text{Malonyl-CoA} + \text{ADP} + \text{P}_i$$
- A biotin enzyme.
- Mechanism similar to:
  - Pyruvate carboxylase
  - Propionyl-CoA carboxylase
  - (See Fig.'s 25.2 and 25.3)

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## Acetyl-CoA Carboxylase

- Bacterial enzyme contains three subunits:
  - Biotin carboxylase
  - Biotin carboxy carrier protein
  - Transcarboxylase
- Animal enzyme is a single **multifunctional** protein with three domains on one 230 kD polypeptide chain.
  - Protomer inactive, polymeric form active.

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## Regulation of Acetyl-CoA Carboxylase

- Good candidate for regulation.
  - First “committed” step to fatty acids.
  - Reaction far from equilibrium.
- Allosteric regulation:
  - Citrate activates (promotes polymeric form)
  - Fatty Acyl-CoA inhibits
- Covalent regulation by phosphorylation:
  - Increases  $K_m$  for citrate, lowers  $K_i$  for fatty acyl-CoA
  - Fig. 25.5

Note that citrate plays **two** roles: one as the activator of acetyl-CoA carboxylase, the other as the carrier of acetyl units across the mitochondrial membrane. Inhibition by fatty-acyl CoA is an example of **end-product inhibition**. Hormonally stimulated phosphorylation has the effect of turning off fatty acid synthesis by making the inhibitor more effective and the activator less effective.

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## Fatty Acid Synthase

- Overall reaction:  
$$\text{CH}_3\text{CO-SCoA} + n \text{HOOCCH}_2\text{CO-SCoA} + 2n \text{NADPH}$$

↓ Fatty Acid Synthase (FAS)

$$\text{CH}_3(\text{CH}_2\text{CH}_2)_n\text{CO-SCoA} + n \text{CO}_2 + 2n \text{NADP}$$
- Chemistry similar to oxidation spiral,  
**But**
- Intermediates bound to **acyl carrier protein (ACP)**, not CoASH (Fig. 25.6)

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## Fatty Acid Synthase

- Total of seven enzymatic reactions: (Fig. 25.7)
  - Acetyl transacylase (**AT**)
  - Malonyl transacylase (**MT**)
  - $\beta$ -Ketoacyl-ACP synthase (**KS**)
  - $\beta$ -Ketoacyl-ACP reductase (**KR**)
  - $\beta$ -Hydroxyacyl-ACP dehydratase (**DH**)
  - Enoyl-ACP reductase (**ER**)
  - Termination reaction (varies with organism)

**KS** is also referred to as the **condensing enzyme**.

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## Termination of Synthase Reaction

- Yeast and fungal enzyme
  - Palmitoyl transacylase (**PT**) transfers palmitoyl group back to CoA. (Same enzyme as **MT**).
- Animal enzyme
  - Thioesterase (**TE**) hydrolyses the thiol ester linkage to ACP to form the free fatty acid.
- Bacterial enzyme
  - Transfers acyl group to glycerol-phosphate in phospholipid biosynthesis.

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## Differences in Oxidation and Synthesis

- Mitochondria versus cytoplasm
- CoASH esters versus ACP-SH esters
- FAD acceptor versus NADPH donor
- NAD acceptor versus NADPH donor
- L- $\beta$ -hydroxy versus D- $\beta$ -hydroxy
- *Trans* double bond versus *cis* double bond

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## Organization of FAS *Bacteria and Plants*

- The seven proteins (**ACP, AT, MT, KS, KR, DH, ER**) are all separable proteins that can be isolated individually. There may be more than one of them (at least two forms of **KS**, for example) with different chain specificities.

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## Organization of FAS *Animals*

- All enzymes, plus thiolesterase (**TE**) are part of one polypeptide chain, a **multifunctional protein**.
  - MW ~ 250,000
- Enzyme functions as a **dimer** ( $\alpha_2$ )
  - See Fig. 25.9
- Interaction between cysteine SH of **KS** and phosphopantetheine SH of **ACP**
  - See Fig. 25.11

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## Organization of FAS *Yeast and Fungi*

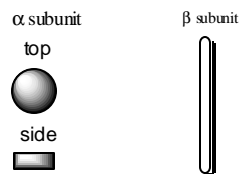
- Enzymes divided between 2 multifunctional proteins.
  - FAS-1 gene product =  $\alpha$  subunit
    - MW = 213,000
    - Contains **ACP**, **KS**, and **KR**
  - FAS-2 gene product =  $\beta$  subunit
    - MW = 203,000
    - Contains **AT**, **MT(PT)**, **ER**, and **DH**
- Overall structure is  $\alpha_6\beta_6$  (MW  $2.3 \times 10^6$ )

Early evidence of multifunctional proteins came from genetics in which there were two genes from complementation studies, FAS-1 and FAS-2.

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## Yeast and Fungi FAS, con't.

- $\alpha$  subunit is disc shaped, 30 Å x 80 Å
- $\beta$  subunit is elongated, 40 Å x 250 Å



$\alpha_6\beta_6$  organization is like a “MacDonald’s Arch, with the subunit forming arches above and below a hexagonal array of discs. (Illustrated on blackboard.)

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## Further Processing of FA's

### *Elongation*

- Mitochondria can use enzymes of oxidation with a special NADPH reductase to elongate by 2 carbons. (Fig 25.12)
- Endoplasmic reticulum contains elongation system involving malonyl-CoA
  - Longer chain FA's (C<sub>20</sub>, C<sub>22</sub>, C<sub>24</sub> made by this system)
  - Membrane enzymes

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## Further Processing of FA's

### *Unsaturated FA's*

- Bacteria add double bond as chain is being built. (Fig 25.13)
  - Process does not require oxygen.
  - Only monounsaturated FA's made.
- Eukaryotes introduce double bonds into pre-formed chains.
  - Process requires oxygen
  - "Desaturase" is a **mixed function** oxidase
  - Cytochrome b<sub>5</sub> and other proteins in ER membrane involved. (See Fig. 25.14)

Unsaturation and chain elongation enzymes are both found in the ER, and may be concurrent reactions.

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## Further Processing of FA's

### *Polyunsaturated FA's*

- Only in Eukaryotes.
- Requires oxygen.
- Susequent double bond is "methylene interrupted".
- Animals add double bonds only toward carboxyl end.
- Plants add double bonds toward methyl end.

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## Polyunsaturated Fatty Acid Structures

- Different “Families” according to distance of last double bond from methyl end:
  - $\omega$ -9 (oleic acid, 9-C<sub>18:1</sub>)
  - $\omega$ -7 (palmitic acid, 9-C<sub>16:1</sub>)
  - $\omega$ -6 (linoleic acid, 9,12-C<sub>18:2</sub>)—only from plants.
    - Arachidonic acid made from dietary linoleic acid (Fig 25.15)
  - $\omega$ -3 (alpha-linolenic acid, 9,12,15-C<sub>18:3</sub>), also only made in plants, but found in some fish.

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## Glycerolipid Biosynthesis

- Phosphatidic Acid is the common intermediate to both **triglycerides** and **glycerophospholipids**.
  - Starts with Dihydroxyacetone-phosphate
  - Reduction to L-glycerol-3-phosphate
  - Acylation with fatty-acyl CoA (or fatty acyl ACP in bacteria).
  - See Fig. 25.18

You should review the structures and names of the common glycerolipids: phosphatidic acid (PA), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl glycerol (PG), cardiolipin (diphosphatidyl glycerol, CL), and phosphatidyl inositol (PI). (Chapter 8, page 245).

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## Glycerolipid Biosynthesis, con't.

- Hydrolysis of Phosphatidic Acid produces **Diglyceride**.
  - Acylation yields triglyceride
  - **Salvage** pathways produce phosphatidyl ethanolamine and phosphatidyl choline
    - Choline and Ethanolamine activated as **CDP** derivative
  - Alternative activation of diglyceride with CTP for most other glycerolipid synthesis.
  - See Fig. 25.19

Remember that the synthesis of triglyceride requires DHAP or glycerol-3-P, and in adipose tissue there is no **glycerokinase**, so triglycerides can only be made if there is some carbohydrate to supply DHAP.

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## Glycerolipid Biosynthesis, con't.

- CDP diglyceride is precursor of
  - phosphatidyl inositol
  - phosphatidyl glycerol
  - cardiolipin
    - See Fig 25.22
  - Phosphatidyl serine
    - Not shown in book

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## PE—PS Exchange

- Phosphatidyl Serine can be decarboxylated to form phosphatidyl ethanolamine
- Serine can displace ethanolamine in an exchange reaction
$$\text{PS} \rightarrow \text{PE} + \text{CO}_2$$
$$\text{PE} + \text{serine} \rightarrow \text{PS} + \text{ethanolamine}$$
- Overall
$$\text{serine} \rightarrow \text{ethanolamine} + \text{CO}_2$$

This is the way that ethanolamine is synthesized—not by a direct decarboxylation of serine.

Note we are skipping discussion of **plasmalogens**, vinyl ether derivatives, as well as ether lipids. See Fig 25.23.

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## Sphingolipid Biosynthesis

- Sphingosine chain created by condensation of **palmitoyl-CoA** and **serine**.
- Reduction of the keto group, acylation of the nitrogen, then introduction of the double bond produces **ceramide**
  - See Fig 25.25
- Glycosphingolipids made by transferases using UDP-sugars or CMP-sialic acid.
  - See Fig 25.26



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## Sphingolipidoses

- Defects in biosynthesis of sphingolipids are very rare.
- A number of defects in **catabolism** are known.
- These are defects in specific **glycosidases** in the lysosomes, preventing degradation.
- Sphingolipids accumulate in lysosomes.
  - Hence these are **lysosomal storage diseases**.

There is only a brief discussion of sphingolipidoses on page 250. To learn more, you will need to consult another biochemistry text.

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## Eicosanoid Biosynthesis

- Made from arachidonic acid
  - Prostaglandins of the 2-series
    - Oxygen attack at positions 11 and 15.
    - See Fig. 25.28
  - Leukotrienes of the 4-series
    - See Fig 25.27 for some of the products.
- $\omega$ -3 fatty acids (5,8,11,14,17 eicosapentaenoic acid) produces prostaglandins of the 3 series and leukotrienes of the 5 series.

The numbers refer to the number of double bonds. PGE<sub>2</sub> has two double bonds, while PGE<sub>1</sub> has only one and PGE<sub>3</sub> has three. Leukotriene C<sub>4</sub> has four double bonds.

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## Eicosanoid Biosynthesis, con't.

- Aspirin inhibits cyclooxygenase
- Also site of action of other NSAID's
  - Two forms, COX-1 and COX-2 (Fig p. 834)
  - Cox-2 inhibitors more specific for inflammation
- Inhibits prostaglandins, not leukotrienes.
- Anti-inflammatory steroids block the phospholipase releasing arachidonic acid.

NSAID stands for “non-steroid anti-inflammatory drug”.

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## Aspirin and Blood Clotting

- Thromboxane is a prostaglandin made by platelets that promotes blood clotting.
- Prostacyclin is a prostaglandin made by endothelium cells that inhibits clotting.
- Aspirin blocks both, but
  - Low doses of aspirin preferentially knock out platelets COX, because endothelium cells can make more.
  - Therefore low doses of aspirin recommended to lower tendency for clot formation.

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## Cholesterol Biosynthesis.

Pathway subdivided into 5 parts:

1. acetate → mevalonic acid  
(first committed step to isoprenoids)
2. mevalonic acid → isopentenyl pyrophosphate
3. Isopentenyl pyrophosphate → squalene
4. squalene → lanosterol  
(first sterol)
5. lanosterol → cholesterol

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## Mevalonic Acid Synthesis

- **HMG-CoA** is an intermediate
  - Made from acetyl-CoA and the enzymes **thiolase** and **HMG-CoA synthase**.
  - Same intermediates as in ketone body formation, but synthesis occurs in **cytoplasm**, not in mitochondria.
- Cytoplasmic **HMG-CoA reductase** converts it to mevalonic acid.
  - See Fig. 25.31 and 25.32

Recall that in ketone body synthesis, a mitochondrial enzyme, **HMG-CoA lyase** converts HMG-CoA to acetoacetate.

Note that the reduction of HMG-CoA is a four electron process, requiring 2 NADPH. (Fig. 25.32)

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## HMG-CoA Reductase

- First committed step to isoprenoid synthesis
- Site of regulation of sterol synthesis.
  - Phosphorylation by a specific kinase inhibits
  - The kinase is activated by phosphorylation
    - (See Fig 25.33)
- Regulation also at level of enzyme synthesis and degradation.
  - Short half-life for degradation. Cholesterol decreases the half-life.
  - Cholesterol inhibits synthesis of new protein.

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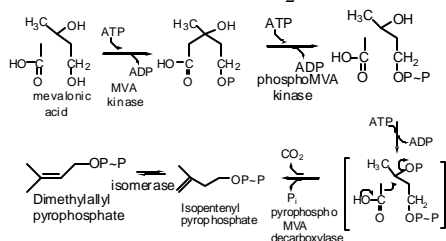
## HMG-CoA Reductase, con't.

- Site of action of the “statin” drugs.
  - Fig page 840
- Effect of drug more complicated than just blocking cholesterol synthesis:
  - Cells respond to drug by synthesizing more HMG-CoA reductase.
  - LDL receptor is co-regulated with reductase, so drug stimulates receptor synthesis.
  - Higher receptor levels help cells to get cholesterol from LDL rather than synthesize it.

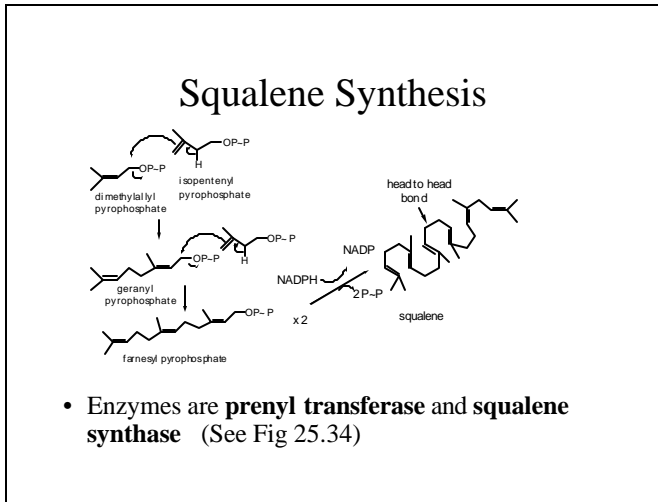
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## Isopentenyl Pyrophosphate The “isoprenoid” building block

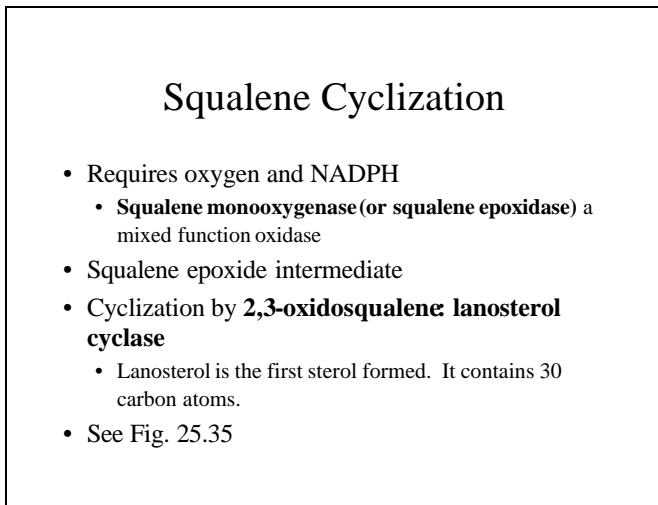
- Investment of 3 ATP's
- One carbon is lost as CO<sub>2</sub>



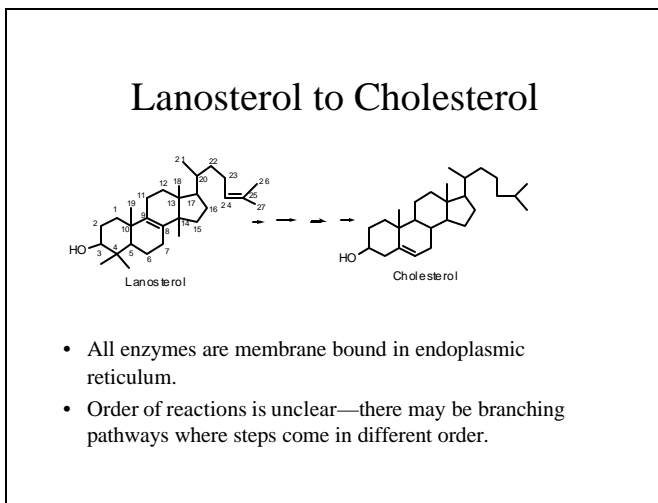
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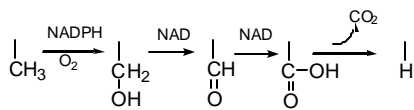
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## Lanosterol to Cholesterol, con't.

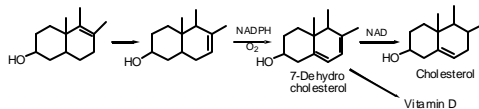
- Steps involve the following reactions:  
(order of steps unclear)
  - Reduction of 24, 25 double bond.
  - Removal of methyl groups at 4 and 14



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## Lanosterol to Cholesterol, con't.

- Steps, con't.
  - Migration of 8,9 double bond, introduction of 5,6 double bond.



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## Metabolic Fates of Cholesterol

- Ester formation—storage and transport
  - **ACAT** in cells
    - Acyl-CoA Cholesterol Acyl Transferase
    - (Acyl group from fatty acyl-CoA ester)
  - **LCAT** in blood
    - Lecithin Cholesterol Acyl Transferase
    - (Acyl group from phosphatidyl choline)
- Esterases liberate cholesterol from ester form.

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### Metabolic Fates of Cholesterol, con't.

- Bile Acid Synthesis (Fig 25.41)
- Note AB ring is *cis* fused, OH groups are all  $\alpha$  (towards back of ring).
- Major metabolites of cholesterol.
  - Made in liver, continuously recycled through enterohepatic circulation.
  - **Cholestyramine** is an ion exchange resin that binds bile acids and prevents resorption.
    - Used as a drug to lower serum cholesterol.

Bile acids excreted from liver to gallbladder, emptied into intestine where they mix with dietary lipids, forming dispersed micelles. Resorption by the intestine then results in return to liver.

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### Metabolic Fates of Cholesterol, con't.

- Vitamin D (See Fig 18.37)
  - 7-Dehydrocholesterol intermediate
  - Skin exposure to UV converts to Vitamin D<sub>3</sub> (aka **cholecalciferol**)
  - UV treatment of ergosterol produces vitamin D<sub>2</sub> **ergocalciferol**
  - Liver hydroxylates in 25 position
  - Kidney hydroxylates in 1 position
  - 1,25 dihydroxyvitamin D<sub>3</sub> is active agent

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### Metabolic Fates of Cholesterol, con't.

- Steroid Hormones(See Fig 25.43)
  - First step is by mitochondrial enzyme called **desmolase** that forms pregnenolone
  - Further conversion to **progesterone** occurs in endoplasmic reticulum
  - Progesterone is precursor of
    - Adrenal cortical hormones (C<sub>21</sub>)
    - Male sex hormones (androgens—C<sub>19</sub>)
    - Female sex hormones (estrogens—C<sub>18</sub>)

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## Plasma Lipoproteins

- Means of lipid transport in blood
- Several “classes”, each class probably heterogeneous in precise lipid composition
- One classification based on **density**
  - (HDL, LDL, VLDL)
- Another based on electrophoretic mobility
  - (alpha, beta, gamma classes of serum proteins)
- Final classification based on **apoprotein**

Early classifications based on experimental procedures for identification: ultracentrifugation for density class, electrophoresis for mobility class.

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## Plasma Lipoproteins

### Density Classification

(See Table 25.1)

<u>Class</u>	<u>Density</u>	<u>Particle Size</u>
• HDL	1.063-1.21	5-15 nm
• LDL	1.019-1.063	18-28 nm
• IDL	1.006-1.019	25-50 nm
• VLDL	0.95-1.006	30-80 nm
• Chylomicrons	<0.95	100-500 nm

Density decreases as percent protein decreases and percent lipid increases.

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## Plasma Lipoproteins

### Electrophoretic Classification

<u>Class</u>	<u>Mobility</u>	<u>Identity</u>
• <b>Alpha</b>	fastest	HDL
• <b>Beta</b>	slower	LDL
• <b>Pre-Beta</b>	in-between	VLDL

(chylomicrons don't migrate)

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## Plasma Lipoproteins Apoproteins (See Table 25.2)

<u>Apoprotein</u>	<u>Distribution</u>
• A-1	HDL
• A-2	HDL
• B-48	Chylomicrons
• B-100	VLDL, LDL
• C-1, C-2, C-3	VLDL, HDL
• E	Chylomicrons, VLDL, HDL

A-1 is a cofactor for **LCAT** (lecithin cholesterol acyl transferase). C-2 is a cofactor for **lipoprotein lipase**. Note the **A** and **B** apoprotein nomenclature was based on the **alpha** and **beta** designation of electrophoretic mobility.

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## HDL Metabolism

- Formed from phospholipid and cholesterol
- Picks up A-1
- LCAT forms some cholesterol esters
- Apo C-1, C-2, C-3 picked up
- Picks up more cholesterol and C.E. from tissues and from VLDL
- Exchanges C apoproteins with VLDL
- Taken up and degraded by liver

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## Chylomicron Metabolism

- Formed in intestinal cells from monoglyceride and fatty acids absorbed from diet.
- Excreted into lymphatic system.
- Lipoprotein lipase hydrolyzes triglyceride
- "Remnant particles" taken up and degraded by liver



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## VLDL Metabolism

- Made in liver
- Picks up C apoproteins by exchange with HDL, then returns them
- Triglyceride hydrolyzed by lipoprotein lipase
- Exchanges cholesterol and cholesterol ester with HDL
- Degraded to IDL (still has apo E), then to LDL

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## LDL Metabolism

- Binds to LDL receptor (Fig 25.40)
- Taken up by endocytosis
- Endosomes fuse with lysosomes
- Lowered pH dissociates from receptor
- Enzymes degrade protein and lipids
- Receptor recycled to plasma membrane
  - See Fig 25.39

“Statin” drugs treat elevated serum cholesterol by stimulating the synthesis of LDL receptors. Only works for heterozygotes because homozygotes have not receptor to stimulate. Elevated HDL seems to mitigate the plaque accumulation by acting to remove cholesterol from peripheral cells, returning it to the liver.

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## Hyperlipoproteinemias

- Familial Hypercholesterolemia
  - Defects in LDL receptor
  - LDL levels high, taken up by macrophage to form “foam cells”
    - Deposition causes atherosclerotic plaques
  - Heterozygotes treatable
  - Homozygotes have heart disease at early age
  - Atherosclerotic plaques from deposition

## Hyperlipoproteinemias, con't.

- Elevated chylomicrons and VLDL
  - Defect in lipoprotein lipase or C-2 apoprotein.
  - Blood triglycerides high.
  - Treat by lowering dietary lipid.
- Elevated VLDL
  - Associated with obesity and diabetes
  - Blood triglycerides high
  - Treat by lowering caloric intake

Defect in lipoprotein lipase or C-2 apoprotein was classified as "Type 1 hyperlipoproteinemia". Elevated VLDL alone was known as "Type IV hyperlipoproteinemia".