**Learning Objectives**

1. Define lipoproteins and explain the rationale of their formation in blood.
2. List different types of plasma lipoproteins and describe their composition and features.
3. Explain the metabolism of individual lipoproteins.
4. Describe the biochemical sequence of events that lead to hyperlipidemic state.

**Reference:** Campbell Biochemistry and Lippincott’s Biochemistry
Lipids are insoluble in plasma. In order to be transported they are combined with specific proteins to form lipoproteins.

- **Lipoproteins**: Multicomponent complexes of proteins and lipids.
- Each type of lipoprotein has a characteristic molecular mass, size, composition, density and physiological role.
- The protein and lipid in each complex are held together by non covalent forces.
- The major function of LPs is to transport **TAG, Cholesterol** and **phospholipids** around the body.
<table>
<thead>
<tr>
<th>Lipoprotein class</th>
<th>Density (g mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>&lt;0.95</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.95–1.006</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006–1.019</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019–1.063</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063–1.210</td>
</tr>
</tbody>
</table>
Four Major Components of Lipoproteins

- **Phospholipids**
- **Cholesterol**
- **Cholesterol esters**
- **Triacylglycerols**

### Phospholipids

![Phospholipid Structure](image)

- **Glycerol**
- **Two Fatty acids**
- **PO₄**
- **Alcohol (Choline)**

**Lecithin**

### Cholesterol

![Cholesterol Structure](image)

- **Cholesterol esters**

### Triacylglycerols

![Triacylglycerol Structure](image)

- **Triacylglycerol**
General Structure of a Plasma Lipoprotein

Peripheral apoprotein
  (eg. Apo-C, E)

Cholesterol

Phospholipid

Integral apoprotein
  (eg. Apo-B)

Cholesterol ester

Triacylglycerol

Core of nonpolar lipids

Surface of polar lipids
**Separation Methods:**

A- **Ultra-centrifugation:**

Using the rate of floatation in NaCl solution. Chylomicrons, VLDL, LDL, HDL. and free fatty acids-albumin (depending on their density). FFA [carried on albumin].

B- **Electrophoresis:**

Chylomicrons, β-lipoproteins (LDL), pre-β-lipoproteins (VLDL), and α-lipoproteins (HDL). (depending on the net charge).

Separation of plasma lipoprotein by electrophoresis on agarose gel
They include **five types**

**Apolipoprotein A:** on HDL and chylomicron *(intestine / liver)*

Activates lecithin-cholesterol-acyltransferase *(LCAT)* and is the ligand for HDL receptor.

ApoAll: inhibits LCAT enzyme.

**Apolipoprotein B:**

B: 100 on VLDL and LDL and is ligand for LDL receptor *(liver).*

48 on chylomicrons *(intestine).*

**Apolipoprotein C:** on chylomicrons, HDL and VLDL *(Liver)*

ApoCII: possible activates LCAT.

Apo A: activates lipoprotein lipase.

ApoCIII inhibits apoCII.

**Apolipoprotein D:** on HDL and may act as lipid transfer protein.

**Apolipoprotein E:** on chylomicrons, VLDL and HDL and is the ligand for lipoprotein remnant and LDL receptors *(Liver)*
Apolipoproteins carry out several roles:

The distribution of apolipoproteins characterizes the lipoprotein

1. Act as structural components of lipoproteins

   e.g. apo B.

2. Activate enzymes involved in lipoprotein metabolism (enzyme cofactors),

   1. Apo A-I for LCAT
   2. Apo C-II for lipoprotein lipase

3. They act as ligands for interaction with lipoprotein receptors in tissues

   i.e. Recognize the cell membrane surface receptors.

   e.g. apo-B100 and apo –E for LDL receptor and apo-E for the lipoprotein remnant receptor and apo A-I for HDL receptor.
## Principal Enzymes in Lipoprotein Metabolism

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate(s)</th>
<th>Site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipoprotein lipase  ( \text{LPL(} + \text{apo C}[11 )</td>
<td>triacylglycerol in VLDL and chylomicron</td>
<td>capillary surfaces</td>
</tr>
<tr>
<td>hepatic lipase ( \text{HL(} )</td>
<td>triacylglycerol and phospholipids in IDL and HDL</td>
<td>liver and sinusoids</td>
</tr>
<tr>
<td>acid lipase</td>
<td>triacylglycerol and cholesteryl esters</td>
<td>lysosomes</td>
</tr>
<tr>
<td>lecithin: cholesterol acyltransferase ( \text{LCAT(} + \text{apo A}[1 )</td>
<td>phosphatidylcholine and cholesterol</td>
<td>lipoproteins, especially nascent HDL</td>
</tr>
</tbody>
</table>
Overview of Lipoprotein Functions

**Dietary lipid transport system**
- **EXOGENOUS LIPIDS**
  - Intestine
  - Chylomicrons
  - EXTRAHEPATIC TISSUES

**Triacylglycerol secretion system**
- **ENDOGENOUS LIPIDS**
  - Liver
  - LDL (70%)
  - VLDL
  - IDL
  - LDL (%30)
  - EXTRAHEPATIC TISSUES

**Reverse cholesterol transport system**
- **EXTRAHEPATIC TISSUES**
  - HDL
  - Liver
Chylomicrons

1. Assembled in intestinal mucosal cells.
2. They enter the lymphatic system and enter the blood via the thoracic duct.
3. They contain mostly TAG.
4. Nascent chylomicrons contain apoprotein B48 but pick up others apoproteins from HDL once they enter the circulation.

Function is to transport dietary TAG to the adipose tissues where it can be stored as fat or to muscles where the constituent fatty acids can be used for energy. Liver is responsible for the uptake of lipoproteins remnant. Chylomicrons remnants are taken up by the receptor-mediated endocytosis.
Metabolic fate of chylomicrons

1. Intestinal mucosal cells secrete nascent TAG-rich chylomicrons produced primarily from dietary (exogenous) lipids.

2. Apo C-II and apo E are transferred from HDL to the nascent chylomicron.

3. Extracellular lipoprotein lipase, activated by apo C-II, degrades TAG in CM.

4. Apo C-II is returned to HDL.

5. CE-rich CM remnants bind through apo E to specific receptors on the liver where they are endocytosed.
Very low density lipoproteins (VLDL)

- Synthesised in the liver. Contain mostly TAG but with a significant amount of cholesterol and cholesterol ester.
- Nascent VLDL contains apoprotein B100 but pick up others from HDL in the circulation

**Function:** Transport endogenously synthesised TAG to the extra hepatic tissues where it can be stored as fat or to muscles where the constituent fatty acids can be used for energy. The cholesterol is delivered to extra hepatic tissues once VLDL metabolised to LDL.
Metabolic fate of VLDL

1. Liver secretes nascent, endogenously synthesized, TAG-rich VLDL particles.

2. Apo C-II and apo E are transferred from HDL to nascent VLDL.

3. Extracellular lipoprotein lipase, activated by apo C-II, degrades TAG in VLDL.

4. Apo C-II and apo E are returned to HDL.

5. LDL binds to specific receptors on extrahepatic tissues and on the liver, where they are endocytosed.
**LDL**

- LDL is metabolized via the LDL receptor (apo-B100, apo E).
- It is formed from VLDL.
- 30% of LDL is degraded in extrahepatic tissues and 70% in the liver.

- **Mutations in LDL receptors** causes increased plasma LDL levels (i.e. increased cholesterol levels).
- This accelerates progress of atherosclerosis.
- The cholesterol in LDL is often called “bad cholesterol.”
Cellular uptake and degradation of LDL. ACAT = acyl CoA:cholesterol acyltransferase.
Lipoprotein (a) Lp(a) or Little‘a’

- **Lp(a)** is nearly identical in structure to an **LDL particle** that is disulphide-linked to **apo(a)**.
- Lp(a), is a particle that, when present in large quantities in the plasma, is associated with an increased risk of **coronary heart disease**.
- It has no known function and is absent in most animal species other than human.
- Normal value **20 mg %**, > 30 high risk.
- **Lp(a) is thought to promote atherosclerosis** by competing for plasminogen activation and subsequent lysis fibrin clots.

**Note:** Apo(a) is structurally homologous to plasminogen—the precursor of a blood protease whose target is fibrin, the main protein component of blood clots. It is hypothesized that elevated Lp(a) **slows the breakdown of blood clots that trigger heart attacks** because it competes with plasminogen for binding to fibrin.
HDL

- HDL is synthesized and secreted from both liver & intestine.
- HDL acts as repository for apo-C and apo-E that are required in the metabolism of chylomicrons and VLDL.

Nascent HDL consists of discoid phospholipids bilayers containing apoA and free cholesterol.

- LCAT- and its activator apo-A-1 bind to the disk.

lecithin + cholesterol $\rightarrow$ lysolecithin + cholesterol ester

- The liver is the final site of degradation of HDL cholesterol ester.
- An HDL cycle = transport of cholesterol from the tissues to the liver (reverse cholesterol transport).
LCAT is synthesized by the liver. LCAT binds to nascent HDL, and is activated by apo A-I.

**LCAT** transfers the fatty acid from carbon 2 of phosphatidylcholine to cholesterol. This produces a hydrophobic **cholesteryl ester**, which is sequestered in the core of the HDL, and **lyso-hosphatidylcholine**, which binds to albumin.
Metabolism of HDL. PC = phosphatidylcholine; lyso-PC = lysophosphatidylcholine. LCAT = Lecithin cholesterol transferase. CETP = cholesteryl ester transfer protein. ABCA1 = transport protein.

Note: For convenience the size of VLDLs are shown smaller than HDL, whereas VLDLs are larger than HDL.
FUNCTIONS OF HDL

1. HDLia a reservoir of apolipoprotein: apo C-II and apo E

2. HDL uptake of unesterified cholesterol: They take up cholesterol from non-hepatic (peripheral) tissues and return it to the liver as cholesteryl esters.

3. Esterification of cholesterol: When cholesterol is taken up by HDL, it is immediately esterified by the plasma enzyme LCAT. As the discoidal nascent HDL accumulates cholesteryl esters, it first becomes a spherical, relatively cholesteryl ester–poor HDL3 and, eventually, a cholesteryl ester–rich HDL2 particle that carries these esters to the liver. Cholesterol ester transfer protein (CETP) moves some of the cholesteryl esters from HDL to VLDL in exchange for triacylglycerol.
4. **Reverse cholesterol transport:** The selective transfer of cholesterol from peripheral cells to HDL, and from HDL to the liver for bile acid synthesis or disposal via the bile, and to steroidogenic cells for hormone synthesis. The efflux of cholesterol from peripheral cells is mediated, at least in part, by the transport protein, *ABCA1*. The uptake of cholesteryl esters by the liver is mediated by a cell-surface receptor, *SR-B1* (scavenger receptor class B type 1) that binds HDL.

[Note: **Hepatic lipase**, with its ability to degrade both TAG and phospholipids, also participates in the conversion of HDL2 to HDL3.]
HDL (cont.).

Act as a reservoir for apoproteins which can be donated or received from other lipoproteins. HDL particle serves as a circulating reservoir of apo C-II (it is transferred to VLDL and chylomicrons, and is an activator of lipoprotein lipase), and apo E (it is required for the receptor-mediated endocytosis of IDLs and chylomicron remnants.

HDL uptake of unesterified cholesterol:

Cholesterol (CE) in HDL is referred to as “good cholesterol.”

Diagram:

- Apoproteins: Apo A-I, CII, E
- HDL receptor mediated endocytosis by liver
- HDL receptor mediated endocytosis
- Peripheral tissues
- LDL receptor mediated endocytosis
- LDL
- VLDL
- Cholesterol can be converted to bile salts for excretion or repackaged in VLDL for redistribution
### Summary: Classes of Lipoprotein

(All contain characteristic amounts TAG, cholesterol, cholesterol esters, phospholipids and Apoproteins - NMR Spectroscopy)

<table>
<thead>
<tr>
<th>Class</th>
<th>Diameter (nm)</th>
<th>Source and function</th>
<th>Major apolipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons (CM)</td>
<td>500</td>
<td>Intestine. Transport of dietary TAG</td>
<td>A, B48, C(I, II, III) E</td>
</tr>
<tr>
<td>Very low density lipoproteins (VLDL)</td>
<td>43</td>
<td>Liver. Transport of endogenously synthesised TAG</td>
<td>B100, C(I, II, III), E</td>
</tr>
<tr>
<td>Low density lipoproteins (LDL)</td>
<td>22</td>
<td>Formed in circulation by partial breakdown of IDL. Delivers cholesterol to peripheral tissues</td>
<td>B100</td>
</tr>
<tr>
<td>High density lipoproteins (HDL)</td>
<td>8</td>
<td>Liver. Removes &quot;used&quot; cholesterol from tissues and takes it to liver. Donates apolipoproteins to CM and VLDL</td>
<td>A, C(I, II, III), D, E</td>
</tr>
</tbody>
</table>

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**Pie Charts**

- **Chylomicron (CM)**
  - 90% TAG
  - 8% Protein
  - 2% Phospholipids
  - 3% Cholesterol & Cholesteryl Esters

- **Very-Low-Density Lipoprotein (VLDL)**
  - 60% TAG
  - 20% Protein
  - 15% Phospholipids
  - 5% Cholesterol & Cholesteryl Esters

- **Low-Density Lipoprotein (LDL)**
  - 50% TAG
  - 22% Protein
  - 20% Phospholipids
  - 8% Cholesterol & Cholesteryl Esters

- **High-Density Lipoprotein (HDL)**
  - 40% TAG
  - 25% Protein
  - 30% Phospholipids
  - 5% Cholesterol & Cholesteryl Esters
Hyperlipidemias

Primary 5%
Familial & genetic

Secondary 95%
Role of LDL in atherosclerosis

- Damage to endothelium (hypertension, smoking etc.)
- LDLs penetrate vascular wall, deposit in the intima and with time are damaged by oxidation.
- **Oxidised LDLs** attract the attention of macrophages which ingest the LDL.
- Macrophages become overloaded with lipid and become “foam” cells which die and release pools of lipid in the vessel wall (plaques).
- A complex processes mediated by cytokines and growth factors causes smooth muscle cells to form a **collagenous cap over the lipid** (mature atherosclerotic plaque).
- Cap grows and can constrict the vessel (causing angina for example.)
- Macrophages can degrade the cap while T cells can inhibit collagen synthesis – the cap can rupture to expose collagen and lipids
- This leads to aggregation of platelets and blood clot formation.
- If the coronary artery is blocked by a **clot** – **heart attack**.

- Blocking of arteries in the **brain causes stroke**.
- **Antioxidants** (vitamin E and C) may protect LDL from oxidation
1. In response to endothelial injury—caused at least in part by oxidized LDL—monocytes adhere to endothelial cells, move to the subendothelium (intima), and are transformed into macrophages.

2. Macrophages consume excess modified (oxidized) lipoprotein, becoming foam cells.

3. Foam cells accumulate, releasing growth factors and cytokines that stimulate the migration of smooth muscle cells from the media to the intima. There, they proliferate, produce collagen, and take up lipid, potentially becoming foam cells.

LDL

Superoxide
Nitric oxide
Hydrogen peroxide
Other oxidants

Vitamin E
Ascorbic acid
β-Carotene
Other antioxidants

oxLDL

High-affinity receptors specific for LDL become down-regulated when the cell has sufficient cholesterol.

Low-affinity, nonspecific and nonregulated scavenger receptors take up modified LDL (oxLDL).

MACROPHAGE

EXTRACELLULAR MATRIX

MONOCYTES

SMOOTH MUSCLE CELL

ENDOTHELIAL CELL

FOAM CELL
LDL Oxidation and Atherosclerosis

- LDL-specific antioxidant action
  - Native LDL
  - Vessel lumen
  - Endothelial cells
  - Vessel wall
  - Antioxidants inhibit LDL oxidation by oxidants
  - Oxidized LDL
  - Antioxidants limit cellular responses to oxidized LDL

- Tissue-specific antioxidant action
  - Antioxidants impair cellular capacity to oxidize LDL
  - Oxidants
  - Vascular cells
  - Oxidants

Cellular responses to oxidized LDL:
- ↑ Monocyte adhesion
- ↑ Foam cell formation
- ↑ Cytotoxicity
- ↑ Vascular dysfunction

Formation and activation of atherosclerotic lesions

- Endothelial dysfunction
- Thrombogenicity
- Vascular inflammation
- Smooth muscle cell
- Foam cell
- Macrophage
- Free radicals
- Oxidized LDL
- Monocyte
- Thrombogenicity
- Vascular inflammation
•END Of Lipid Metabolism