

**DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR
ATHEROGENESIS (VASCULAR ENDOTHELIUM): An Overview**

{SEE DIAGRAMS IN HANDOUT WITH CLASS REP}

What is the general structure of Lipoprotein (Fig 1a & b)?

- ❑ Lipoprotein is a globular, micelle-like particle that has a Hydrophobic Core wrapped in a Hydrophilic Coating.
- ❑ Hydrophobic Core contains **Triacylglycerols** and **Cholesteryl esters**
- ❑ Hydrophilic Coating is made up of Phospholipids, Free Cholesterol and Proteins.
- ❑ Protein components of lipoproteins are called **Apolipoproteins** (or **Apoproteins**).
- ❑ About 10 different Apoproteins are found in human Lipoproteins.
- ❑ Apoproteins are usually located on the surface of Lipoproteins.
- ❑ **Apoproteins help to solubilize the Hydrophobic Lipids and to target the lipoproteins to the correct tissues.**
- ❑ **Most lipoproteins are assembled in the liver or small intestine.**

What are the general functions of Lipoproteins?

- ❑ Lipoproteins transport fats and lipids (Triacylglycerols, Cholesterol and Phospholipids) around the body in the aqueous environment of blood plasma.

What are the major types of Lipoproteins (Fig 2a & b)?

- ❑ Lipoproteins are most commonly isolated by Ultra-centrifugation, thus they are named (classified) according to their Density and Physical properties.
- ❑ The different types of lipoproteins are.
 - ❑ Chylomicrons
 - ❑ Very Low Density Lipoproteins (VLDL).
 - ❑ Intermediate Density Lipoproteins (IDL).
 - ❑ Low Density Lipoproteins (LDL).
 - ❑ High Density Lipoproteins (HDL).
 - ❑ Lipoprotein little a {Lp(a)}

What are the functions of Chylomicrons?

- ❑ **Chylomicrons:**
 - ❑ Largest and least dense of the lipoproteins.
 - ❑ Synthesized in the intestine.
 - ❑ Principal form in which dietary Triglycerides are carried to tissues
 - ❑ Major functions include:
 - ❑ Transport of dietary (exogenous) triacylglycerols and cholesterol from the intestine to other tissues in the body.
 - ❑ Transport of ingested Triacylglycerols to skeletal muscle and adipose tissue,
 - ❑ Transport of ingested cholesterol to the liver.
 - ❑ At target tissues Triacylglycerols are hydrolyzed by Lipoprotein lipase, an enzyme located on the surface of the cells.
 - ❑ Released fatty acids and mono-acylglycerols are taken up by the tissues, and either used for energy production or re-esterified to triacylglycerol for storage.

- As their Triacylglycerol content is depleted, the Chylomicrons shrink and form cholesterol-rich chylomicron remnants, which are then transported in the blood to the liver.

What are the functions of VLDL?

- **VLDL:**
 - Synthesized in the liver and transport Triacylglycerols, Cholesterols and Phospholipids from the Liver to other tissues, such as Skeletal Muscle and Adipose Tissue.
 - Lipoprotein Lipase hydrolyzes Triacylglycerols in VLDL and releases fatty acids, which are taken up by the tissues.
 - VLDL-remnants are then transformed to IDL.

What are the functions of IDL and how is IDL transformed to LDL?

- **IDL (also called VLDL remnants):**
 - IDL are the VLDL-remnants remaining in the blood after depletion of Triacylglycerol in VLDL by the action of Lipoprotein Lipase.
 - IDL transports Triacylglycerols, Cholesterol and Phospholipids in blood.
 - IDL is transformed to LDL in the blood.
 - In the transformation of IDL to LDL:
 - Lipoprotein Lipase hydrolyzes the remaining Triacylglycerols,
 - Cholesterol is esterified at the C-3 position by the addition of a fatty acid chain obtained from **Phosphatidylcholine (Lecithin)**.
 - Esterification reaction is catalyzed by **Lecithin-Cholesterol Acyl Transferase (LCAT)**.
 - In addition, all the Apoproteins other than **apoB-100** are removed.

What are the functions of LDL?

- LDL is formed from IDL in blood plasma
- LDL transports mainly Cholesterol and Phospholipids to the Target tissues and Liver.
- LDL receptors located on the surface of target cells specifically binds to apoB-100 in the LDL coat.
- LDL is taken in target cells by a process known as **Receptor-Mediated Endocytosis**.
- Lysosomal enzymes in target cells metabolizes LDL:
- Cholesterol esters in LDL are metabolized by a Lysosomal Lipase to release cholesterol, which is either incorporated into the cell membrane or re-esterified by **Acyl-CoA Cholesterol Acyl-Transferase (ACAT)** for storage (**Fig 3**).
- In order to prevent the build up of Cholesterol and its ester derivatives in the cells, high levels of Cholesterol has the following effects:

- **Decrease the synthesis of LDL receptors, thereby reducing the rate of uptake of cholesterol by receptor-mediated endocytosis,**
- **Inhibit cellular biosynthesis of cholesterol via inhibition of HMG-CoA Reductase.**

What are the functions of HDL?

- **HDL (HDL₂ and HDL₃):**
 - Synthesized in the blood mainly from components derived from the degradation of other Lipoproteins.
 - **Main function of HDL: Removal of Cholesterol from cell membranes to the Liver for excretion.**
 - HDL has a protective effect by preventing cellular uptake of cholesterol and lipids.
 - **Function of HDL is opposite to that of LDL.**
 - Cholesterol molecules in HDL are extracted from cell membranes and converted into Cholesterol esters by the action of **Lecithin-Cholesterol Acyl Transferase (LCAT)**
 - HDL is then either taken up directly by the liver or transfers their cholesterol esters to VLDL in the blood.
 - Liver is the only organ that can dispose of significant quantities of Cholesterol, primarily in the form of Bile Salts.

How does Cholesterol occur in blood plasma?

- Cholesterol occurs in plasma lipoproteins in two forms:
 - Free Cholesterol also called Un-esterified Cholesterol
 - Cholesterol Ester (Cholesterol esterified to Long-chain Fatty Acid)
 - About 70 to 75% of plasma cholesterol is esterified to long-chain fatty acids.
 - **Un-esterified (Free) Cholesterol can be deposited on plasma membrane of cells.**
 - **Cholesterol esters (Esterified Cholesterol) are not deposited on plasma cell membranes.**
- Cholesterol in blood plasma is in a dynamic state. This is because:
 - Cholesterol enters the blood in complex with Lipoproteins that keep it in solution.
 - Cholesterol leaves the blood as tissues remove it from Lipoproteins.

What is the function of LCAT?

- HDL and LCAT play important roles in the transport and elimination of Cholesterol from the body.
- LCAT catalyzes the reversible formation of Cholesterol esters.
- The LCAT reaction:



- **LCAT-HDL system** functions to protect cells, especially their plasma membranes, from damaging effects of excessive deposits of Unesterified Cholesterol.
- Cholesterol ester generated in the LCAT reaction diffuses into the core of HDL particle
- HDL then transports the Cholesterol esters from the tissues and plasma to the liver, where the cholesterol is converted to Bile Salts and excreted.
- This process is referred to as **Reverse Transport of Cholesterol**,
- LCAT acting on HDL provides a vehicle for transportation of cholesterol from peripheral tissues to the liver.

What is Atherosclerosis?

Atherosclerosis: Greek words Athero (gruel or paste) and Sclerosis (hardness)

- Atherosclerosis is a slow, progressive disease that may start in childhood
- Atherosclerosis is the hardening and narrowing of the arteries due to the slow build-up of Plaque on the inner lining of the wall of arteries.
- It is a disease of the Arterial Intima leading to the formation of Fibrous (Atheromatous) Plaques and to Stenosis/ Occlusion of the Lumen.
- Formation of Plaque reduces the Inside Diameter of the Artery, which can later result in drastic reduction in blood flow
- Plaque is made up of Fat, Cholesterol, Calcium, and other substances in blood.
- Atherosclerosis affects large and medium-sized arteries.
- Atherosclerosis can affect the arteries of the brain, heart, kidneys, other vital organs, and the arms and legs.

What are the different types of Plaques?

- There are two types of plaque: Hard and Stable; Soft and Unstable
- Hard plaque causes artery walls to thicken and harden.
- Soft plaque is more likely to break apart from the walls and enter the bloodstream, causing a blood clot that can partially or totally block the flow of blood in the artery (partial or total Ischemia).

What is the suggest role of Platelets in Atherosclerosis?

- Platelets are involved in forming a group of substances called Prostaglandins, one of which may damage arteries.
- Platelets also contain a substance called "Platelet Growth Factor," which can stimulate the growth of Smooth Muscle cells.
- Smooth muscle cells are normally present in the artery wall.
- However, abnormal growth and increase in smooth muscle formation by the Platelet Growth Factor is believed to be one of the earliest events in the Atherosclerosis process.

What are some of the factors associated with onset and development of Atherosclerosis?

- High plasma Cholesterol concentration
- Cholesteryl ester is contained in Atheromatous Plaques;
- Oxidised LDL is a characteristic component in Atherosclerotic lesions because it encourages recruitment of Macrophages and stimulates the release of various Growth Factors.

- ❑ Risk of developing Atherosclerosis is Directly Related to plasma concentration of LDL and Inversely Related to plasma concentration of HDL.
- ❑ High levels of plasma LDL result in increase risk
- ❑ Low levels of plasma HDL result in increase risk
 - That is the reason why **LDL-cholesterol** is frequently called “**Bad**” **Cholesterol** and **HDL-cholesterol** is “**Good**” **Cholesterol**, despite the fact that chemically the cholesterol is the same.
- ❑ Variety of other risk factors such as: Hypertension, Obesity, Diabetes Mellitus and Smoking.

THEORIES OF ATHEROSCLEROSIS

- ❑ Exactly how Atherosclerosis begins or what causes it is not fully known, but several theories have been proposed.
- ❑ **SEE LEARNING ISSUE P & C 22.6**

General Theory: 1

- ❑ Atherosclerosis begins when the Endothelium of an artery is damaged
- ❑ As time goes on, Fats, Cholesteryl esters, Fibrin, Platelets, Calcium and Cellular debris are deposited in the artery wall
- ❑ These substances stimulate the cells of the artery wall to produce substances that causes division and accumulation of cells in the innermost layer of the artery wall where the Atherosclerotic lesions form.
- ❑ Further accumulation of Fats, Cholesteryl esters and Oxidized Cholesterol within these cells and around them continues forming Atheromatous plaque
- ❑ Accumulating cells and surrounding materials markedly thicken the innermost layer and reducing the diameter of the artery
- ❑ Resistance increases as friction of blood flow against vessel wall increases
- ❑ Circulation of blood flow is reduced, and cells may be deprived of oxygen or experience toxic accumulation of metabolic wastes.
- ❑ Development of a plaque also deforms the endothelial wall, increasing turbulent flow and increasing resistance.
- ❑ Hardening of the arterial walls increases resistance to flow, as vessel walls lose their ability to distend
- ❑ If the wall is thickened sufficiently, the diameter of the artery will be reduced and the amount of blood decreased, thus decreasing the Oxygen supply.
- ❑ If the Oxygen supply to the heart muscle is reduced, a heart attack can occur.
- ❑ If the Oxygen supply to the brain is cut off, a stroke can occur.
- ❑ And if the Oxygen supply to the extremities occurs, gangrene can result.

General Theory 2: (Fig 4)

- ❑ Earliest abnormality is migration of Monocytes to the Sub-endothelium of the Artery,
- ❑ Monocytes then differentiate into Macrophages
- ❑ These cells accumulate Cholesterol esters derived from plasma LDL
- ❑ **Distortion of Sub-endothelium leads to Platelet Aggregation on the Endothelial surface and release of Platelet-Derived Mitogens such as Platelet-Derived Growth Factor (PDGF)**

- ❑ PDGF stimulate Smooth Muscle Cell Growth and formation of Foam Cells
- ❑ **Death of Foam cells results in the accumulation of a cellular lipid that can stimulate Fibrosis, leading to formation of Atheromatous plaque**
- ❑ Resulting **Atherosclerotic plaque narrows the blood vessel and serves as the site of Thrombus Formation**
- ❑ Myocardial Infarction occurs if it occurs in Coronary Vessel

What is the theory relating Hyper-Homocysteinemia to Atherogenesis

- ❑ Hyperhomocysteinemia is the accumulation of Homocysteine in blood
- ❑ It may be due to deficiency of **Cystathionine Synthase**, the enzyme that catalyzes the conversion of Homocysteine and Serine to Cystathionine
- ❑ Re-Methylation of Homocysteine leads to production of Methionine
- ❑ Excess plasma Homocysteine forms **Homocysteine Thiolactone**, a high reactive intermediate, which **Thiolates Free Amino groups in LDL** and causes them to aggregate
- ❑ Aggregates can be endocytosed (taken in) by Macrophages
- ❑ Cholesteryl esters and other lipids in LDL are used to form Atheromathous plaque.
- ❑ Accumulated Homocysteine causes Lipid Oxidation and Platelet Aggregation, which in turn lead to Fibrosis and Calcification of Atherosclerotic Plaques.
- ❑ Some patients with Atherosclerosis, who exhibit none of the other risk factors, such as, Smoking or Oral Contraceptive therapy, have been found to be deficient of Cytathionine Synthase activity.

What is the theory relating Lp(a) and Atherosclerosis?

- ❑ Lp(a) is made up of two polypeptide components: **apo(a)** and an **LDL-like protein containing apo B-100**.
- ❑ **Increased levels of Lp(a) is an Independent Risk Factor for Atherosclerosis.**
- ❑ The **apo(a)** is a **deformed relative of Plasminogen (Plasmin)** that is responsible for dissolving Fiber Clots.
- ❑ **Strong resemblance between apo (a) and Plasminogen suggests a link between Lipids, Clotting mechanism and Atherogenesis.**
- ❑ Microthrombi containing Fibrin on the vessel wall become incorporated into the Atherosclerotic plaque.
- ❑ Lp(a) may insinuate itself into the arterial wall following endothelial damage, inhibiting the cleavage of Fibrin in microthrombi by competing with Plasminogen for access to Fibrin.
- ❑ Atherosclerptic damage of the arterial wall soon follows, leading to occlusive disease.

Theory on Genetic link of Atherosclerosis:

- ❑ A gene found on Chromosome 19 codes for Apolipoprotein E, which is a protein that can exist in several different forms.
- ❑ Apolipoprotein E is important for removing excess cholesterol from the blood, and does so by carrying cholesterol to receptors on the surface of liver cells.
- ❑ Defects in Apolipoprotein E sometimes result in its inability to bind to the receptors, which leads to increase cholesterol level in blood and consequently leads to risk of developing Atherosclerosis.

What laboratory investigations are used in diagnosis of Atherosclerosis?

Significance of Laboratory Investigations:

Estimation of Lipoproteins, Cholesterol and Lp(a).

- Lipoproteins are considered to be an accurate predictor of heart disease.
- Lipid profile Test:
 - HDL, LDL, and VLDL levels are a part of the Lipid Profile Test that also evaluates Cholesterol and Triacylglycerols.
- **Electrophoresis of blood plasma for lipoproteins gives the following:**
 - Chylomicrons, which are primarily Triacylglycerols;
 - VLDL (Prebeta Lipoproteins), which are mainly Triacylglycerols;
 - LDL (Beta Lipoproteins), which are primarily Cholesterol;
 - HDL (Alpha Lipoproteins), which are predominantly Protein with a small amount of Cholesterol.

Lipoprotein test is used to access the risk of coronary artery disease.

- High levels of the “Protective” HDL are associated with a decreased risk fo coronary disease, whereas
 - High levels of LDL and VLDL are associated with an increased risk of coronary occlusive disease.
- **Cholesterol Testing:**
 - Commonly used to determine Risk for coronary heart disease (CHD)
 - Used for evaluation of Hyperlipidemias.
 - Cholesterol is the main lipid associated with arteriosclerotic vascular disease.
 - Cholesterol is transported in the bloodstream by lipoproteins.
 - Nearly 75% of the Cholesterol is bound to LDL, and
 - About 25% is bound to HDL.
 - The purpose of Cholesterol testing is to identify patients at risk for arteriosclerotic heart disease.
 - By itself cholesterol is not a totally accurate predictor of heart disease.
 - Both HDL and Total Cholesterol are Independent variables of Risk of CHD.
 - **Thus when combined in a Ratio Fashion, the accuracy of prediction is increased.**
 - **Total Cholesterol/HDL Ratio should be at least 5:1, the Ratio 3:1 is Ideal.**

Apolipoprotein Testing:

- Quantification of **apolipoproteins** is beginning to be performed as a routine clinical procedure.
- Generally **apo A-1** (HDL) and apo B (LDL) are better determinants of Atherosclerotic disease than are Lipid or Lipoprotein determinants.
- **Decrease levels of apo A-1 and Increased levels of apo B-100 are associated with Increased Risk of coronary heart disease.**
- **Low Ratio of apo A-1 to apo B may be a good predictor of coronary heart disease.**
- Individuals with increased concentrations of Lp(a) appear to have a significantly higher risk for coronary heart disease.

Fig 2: Schematic diagrams of Lipoproteins

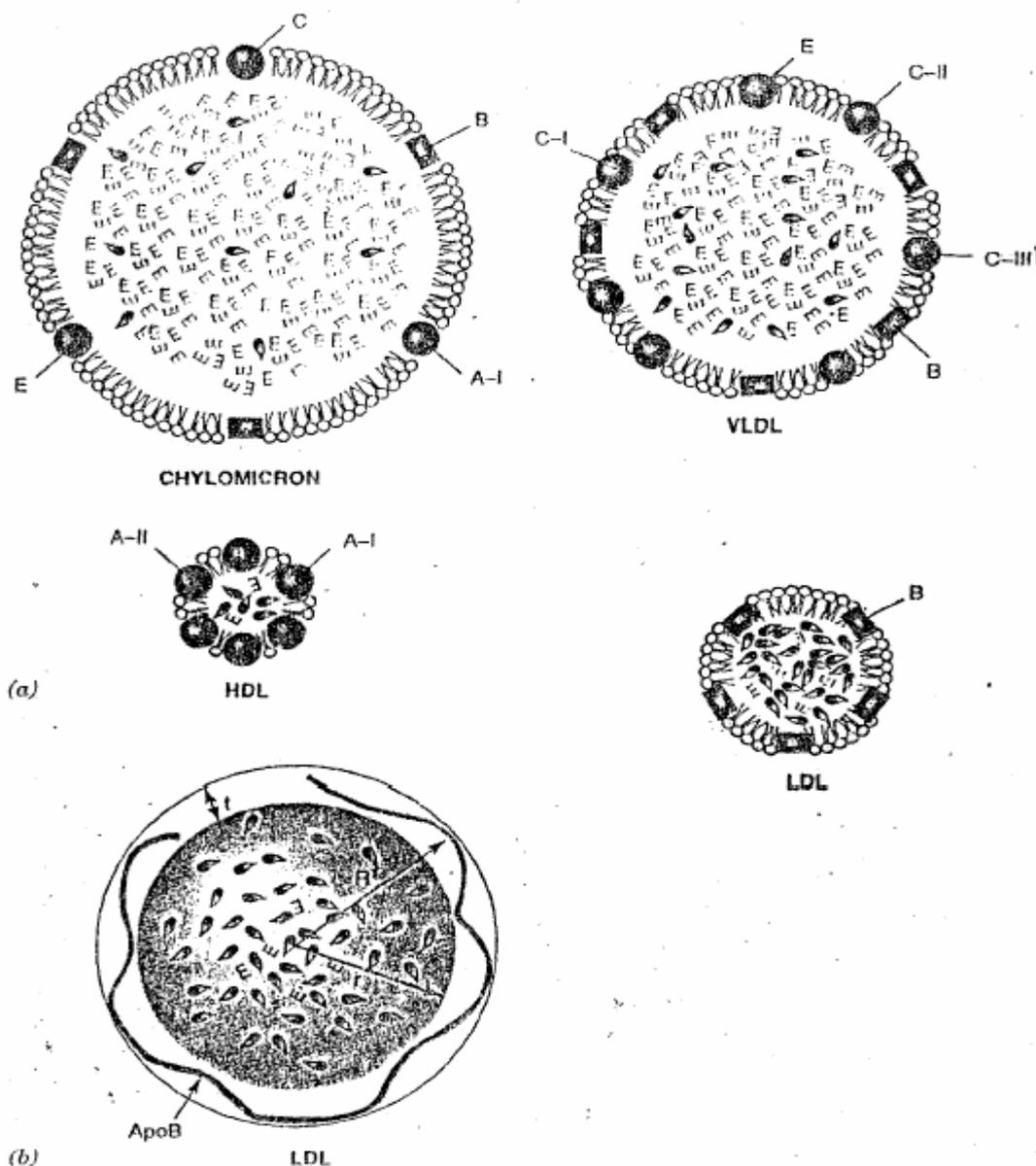


FIGURE 2.43

Generalized structure of plasma lipoproteins.

(a) Spherical particle model consisting of a core of triacylglycerols (yellow E's) and cholesterol esters (orange drops) with a shell ~ 20 Å thick of apolipoproteins (lettered), phospholipids, and unesterified cholesterol. Apolipoproteins are embedded with their hydrophobic edges oriented toward the core and their hydrophilic edges toward the outside.

From Segrest, J. P., et al. *Adv. Protein Chem.* 45:303, 1994.

(b) LDL particle showing ApoB-100 imbedded in outer shell of particle.

From Schumaker, V. J., et al. *Adv. Protein Chem.* 45:205, 1994.