Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Department

Biochemistry Lectures for 2nd Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

1. **Prof. Dr. Fadhil Jawad Al-Tu'ma, Ph.D., Professor of Clinical Biochemistry.**
2. **Dr. Dalya Shakir Obaida, Ph.D. Lecturer of Clinical Biochemistry.**

Second Semester

Lecture No. 7

Date: March, 16th, 2025

Lipid Metabolism
Lipoprotein Metabolism

Objectives:

1. Explain the types, biochemical constituents and the role of plasma lipoproteins, including chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), in the transport of cholesterol between tissues in the plasma.
2. Understand that the class of lipoprotein in which cholesterol is carried is important in determining the effects of plasma cholesterol on atherosclerosis development, with high levels of VLDL or LDL being deleterious and high levels of HDL being beneficial.
3. Give examples of inherited and non-inherited conditions affecting lipoprotein metabolism that cause hypo- or hyperlipoproteinemia.

Introduction:

Because lipids are hydrophobic and essentially insoluble in the water, therefore, they must be transported through the bloodstream (aqueous medium) packaged as lipoproteins as macromolecules which are more water soluble than fatty substances. Each lipoprotein particle is composed of a core of hydrophobic lipids such as cholesterol esters and TGs surrounded by a shell of polar lipids (the phospholipids), which allows a hydration shell to form around the lipoprotein. Free cholesterol molecules are dispersed throughout the lipoprotein shell to stabilize it in a way that allows it to maintain its spherical shape, **Figure 1.** The major carriers of lipids are chylomicrons, VLDL, and HDL. Metabolism of VLDL will lead to IDL and LDL, while metabolism of chylomicrons leads to chylomicron remnant formation.

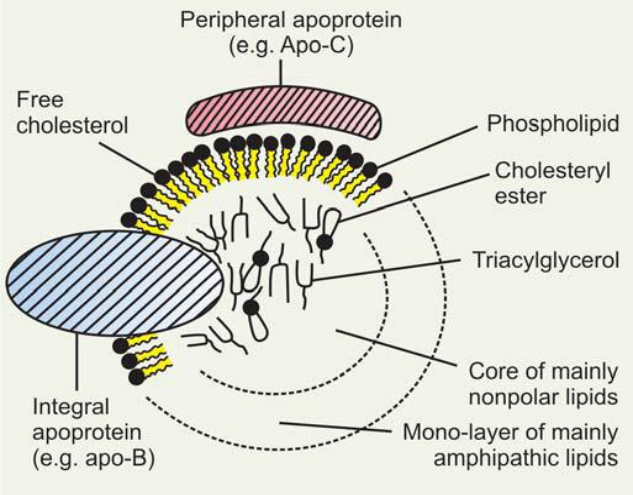

Fig. 1: Generalized structure of a plasma lipoprotein.
The similarities with the structure of the plasma membrane are to be noted. Small amounts of cholesteryl ester and triacylglycerol are found in the surface layer and a little free cholesterol in the core.

Through this carrier mechanism, lipids leave their tissue of origin, enter the bloodstream, and are transported to the tissues, where their components will be either used in synthetic or oxidative process or stored for later use. The apoproteins not only add structural stability of the particle but have other functions as well:

1. They activate certain enzymes required for normal lipoprotein metabolism
2. They act as ligands on the surface of the lipoprotein that target specific receptors on peripheral tissues that require lipoprotein delivery for their innate cellular function.

Classification of Lipoiproteins:

Lipoproteins can be classified according to their:

1. **Hydrated density**
2. **Electrophoretic mobility**
3. **Apo-lipoprotein content.**

1. Classification as per hydrated density: Pure fat is less dense than water. As the proportion of lipid to protein in lipoprotein complexes increases, the density of the macro-molecule decreases. Use of the above property has been made in separating various lipoproteins in plasma by ultracentrifugation which separate them into **four** major density classes:

a. ***Chylomicrons: Density lowest-floats***
b. ***Very low-density lipoproteins (VLDL or VLDLP)***
c. ***Low density lipoproteins (LDL)*** ***with their sub-classis*** LDL-1, IDL (intermediate density lipoprotein) and LDL-2.
d. ***High Density lipoproteins (HDL***) with their sub-classis HDL-2 and HDL-3.

2. Classification Based on Electrophoretic Mobility. The most widely used and simplest classification for lipoproteins is based on the separation of major four classes by electrophoresis. The most frequently employed electrophoretic media are “paper” and ‘agarose’. Plasma lipoproteins separated by this technique are classified in relation to comparable migration of serum proteins.

On electrophoresis, the different fractions according to mobility appear at:

a. The origin is ***chylomicrons,***
b. Migrating into β-globulin region is called β-***lipoproteins*** **(LDL)**
c. Migrating into Pre-β-globulin region, called as ***pre-***β- ***lipoproteins*** **(VLDL).**
d. Migrating to “α1-globulin region called α-***lipoproteins*** **(HDL)**

Migration in electrophoresis is shown diagrammatically in **Figure 2**.

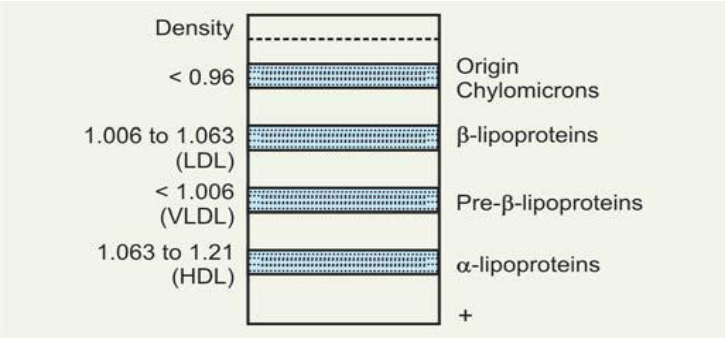

Fig. 2: Electrophoretic separation of plasma lipoproteins

3. Classification Based on Apo-lipoproteins. In this classification, Lipoproteins are designated by their "apo-lipoprotein" composition. At present, **five major** lipoprotein families have been identified and they are **Fig. 1:** Structure of lipoprotein molecule shown in **Table 1**.

Table 1: Lipoproteins classes as per apoprotein contents

Families	*Apolipoproteins*	*Density class*	*Mol. wt. range*	*Function*
LP A	A-I and A-II	HDL	17000 to 28000	• LCAT activator • 'Scavenger'
LP B	Apo-B (B_{48} and B_{100})	LDL and VLDL	250,000	• Cholesterol carrier to tissues
LP C	APO-C I, C II, C III	VLDL, LDL and HDL	6500 to 10,000	• *"Lipoprotein Lipase"* activator, CIII is lipase inhibitor
LP D	Apo-D	HDL_3	~ 20,000	• LCAT activator 3
LP E	Apo-E (Arginine rich)	VLDL, LDL, and HDL	32,000 to 39,000	• Cholesterol transport

Types of Apoproteins Present in Various Lipoprotein Fractions:

As stated above, lipoproteins are characterized by the presence of one or more proteins or polypeptides known as apoproteins. **According to ABC nomenclature:**

1. **HDL:** Two major apoproteins of HDL are designated as **apo-A-I** and **apo-A-II**. In addition to above, HDL also contains apo-C-I, C-II and C-III. HDL-3 is characterized by having apo-D and HDL may also acquire arginine-rich apo-E.
2. **LDL:** The main apoprotein of LDL is **apo-B100**, which is also present in VLDL.
3. **Chylomicrons:** Principal apoprotein of chylomicrons is **apo-B-48** (M.wt = 200 Kd). In addition, chylomicrons also contain apo-A (AI and AII) and apo-C (C-II and C-III), also arginine rich apo-E (34 Kd). ***Apo- C seems to be freely transferable between chylomicrons and VLDL on one hand and HDL on the other.***
4. **VLDL and LDL:** Principal apoproteins of VLDL, IDL and LDL is **apo-B-100** (350 Kd). They also contain apo-C (C-I, C-II and C-III), and apo-E. ***IDL carries some apo-E apoprotein.***

Apo-E: ***Arginine rich apo-E***, isolated from VLDL. It contains arginine to the extent of 10 per cent of the total amino acids and accounts for 5 -10% of

total VLDL apo-proteins in normal subjects but is present in excess in the "broad" β-VLDL of patients of type III hyperlipoproteinaemia.

Carbohydrate content: Carbohydrates account for approximately 5% Apo-B and include mannose, galactose, fucose, glucose, glucosamine and sialic acid. So, some of lipoproteins are glycoproteins.

Functions of Apolipoproteins:

Apolipoproteins carry out several roles:

1. By entering into the "polar" surface layer they make the lipoprotein molecules **water miscible (hydrophilic)**.
2. They can form part of the structure of the lipoprotein itself, e.g. Apo-B.
3. They are enzyme cofactors and can act as activator or inhibitor of the enzyme, e.g.
   - **Activators**—C-II for lipoprotein lipase A-1 for lecithin—cholesterol acyl transferase (LCAT)
   - **Inhibitors**—Apo-A-II and Apo-C III for lipoprotein lipase Apo-C1 for cholesteryl ester transferase protein.
4. They act as **ligands** for interaction with lipoprotein receptors in tissues, e.g. Apo B-100 and apo-E for the LDL receptor – Apo-E for the LDL receptor related protein (LRP), which has been identified as the **remnant receptor** and apo-Al for the HDL receptor.
5. Apo-D probably functions as "cholesteryl ester transfer protein" for transferring cholesteryl esters between different lipoproteins. Though the functions of apo-A IV and apo-D are yet not clearly defined, ***recently apo-D has been incriminated to be an important factor in 'human neuro-degenerative disorders"***.

Figure 3 **above** shows the characteristics of human plasma lipoproteins.

Chylomicrons:

Chylomicrons are assembled in the intestinal mucosa as a means to transport **dietary cholesterol and TGs** to the rest of the body. Chylomicrons are, therefore, the molecules formed to mobilize dietary **exogenous lipids**. The predominant lipids of chylomicrons are **TGs** (**which contain long chain fatty acids**).

The apolipoproteins that predominate before the chylomicrons enter the circulation include **apoB-48 and apoA-I, -A-II and IV**. **ApoB-48** combines only with chylomicrons, Fig. 4**.** The surface is a layer of phospholipids, with head groups facing the aqueous phase. Triacylglycerols sequestered in the interior (yellow) make up more than 80% of the mass. Several apolipoproteins that protrude from the surface (B-48, C-III, C-II) act as signals in the uptake and metabolism of chylomicron contents. The diameter of chylomicrons ranges from about 100 to 500 nm.)

Nascent chylomicrons formed in the intestinal mucosa are secreted into the lymphatic system and enter the circulation at the left subclavian vein through the thoracic duct. In the bloodstream, chylomicrons acquire **apoC-II and apoE** from plasma HDLs. In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the TG by the action of **lipoprotein lipase** (LPL), which is found on the surface of the endothelial cells of the capillaries. The **apoC-II** in the chylomicrons activates **LPL** in the presence of phospholipids and returns to HDL. The free fatty acids are then absorbed by the tissues and the glycerol backbone of the TG is returned, via the blood, to the liver and kidneys. Glycerol is converted to the glycolytic intermediate **dihydroxyacetone phosphate** (DHAP). During the removal of fatty acids, a substantial portion of phospholipid, **apoA** and **apoC** is transferred to HDLs. The loss of **apoC-II** prevents LPL from further degrading the chylomicron remnants.

Chylomicron remnants: containing primarily cholesterol, **apoE** and **apoB-48** are then delivered to, and taken up by, the liver through interaction with the **chylomicron remnant receptor**. The recognition of chylomicron remnants by the hepatic remnant receptor requires apoE. **Chylomicrons function to deliver dietary TG to adipose tissue and muscle and dietary cholesterol to the liver for bile acid biosynthesis and the excess amount is excreted in bile.** The action of lipoprotein lipase depletes the chylomicron of TAG. The process occurs rapidly; interaction of the chylomicron with the lipase results in loss of ~90% of the lipid before the particle dissociates. In addition, the action of lipoprotein lipase results in the dissociation of Apo-C-II from the particle, with the released Apo-C-II going back to HDL particles. Without Apo-C-II, the lipoprotein is no longer a substrate for lipase, and is called a chylomicron remnant. In contrast, the Apo-E remains with the remnant; Apo-E acts as the ligand for the chylomicron remnant receptor in liver.

Chylomicrons have a short half-life in circulation (less than 60 minutes in humans); note, however, that entry into circulation takes a long time, and chylomicron levels are elevated for ~12 hours after a meal. **(Apo-B-100 is a very large protein, containing 4536 amino acids. The "100" does not refer to the size in kD; instead, apo-B-48 is 48% of the size of Apo-B-100. Both Apo-B-100and Apo-B-48 are produced from the same gene. Apo-B-48 is produced in the intestine; it is shorter than ApoB-100 because of a differential editing of the mRNA).**

Lipoprotein Lipase:

Removal of fatty acids from chylomicrons and from VLDL requires ***lipoprotein lipase***, an enzyme located on the capillary walls. Lipoprotein lipase requires Apo-CII and phospholipid as activators; VLDL and chylomicrons have Apo-C-II, allowing the lipoprotein lipase to hydrolyze the TG in these particles. Heart lipoprotein lipase has a lower k_m for TG than does the adipose tissue isozyme; as a result, the heart enzyme is always active, while the rate of TG cleavage by adipose tissue depends on the level of substrate. Thus. the heart can always obtain substrate, while the adipose tissue only removes fatty acids from circulation when circulating lipid levels are elevated.

During lactation, the mammary gland lipoprotein lipase is highly active (due to both high levels of enzyme and low k_m) in order to support milk production at the expense of storing lipids in the adipose tissue. Insulin increases lipoprotein lipase levels in adipose tissue; this is one mechanism for increasing TG storage in adipose tissue.

Very Low Density Lipoprotein (VLDL):

The dietary intake of both fat and carbohydrate, in excess of the needs of the body, leads to their conversion into TGs in the liver. These TGs are packaged into VLDLs and released into the circulation for delivery to the various tissues (**primarily muscle and adipose tissue**) for storage or production of energy through oxidation. **VLDLs are, therefore, the molecules formed to transport endogenously derived TGs to extra-hepatic tissues**. In addition to TGs, VLDLs contain some cholesterol and cholesteryl esters and the apoproteins, apo-B-100, apo-C-I, apo-C-II, apo-C-III and apo-E. Like nascent chylomicrons, newly released VLDLs acquire apoCs and apoE from circulating HDLs.

The fatty acid portion of VLDLs is released to adipose tissue and muscle in the same way as for chylomicrons, through the action of lipoprotein lipase; glycerol is also released as mentioned above. The action of lipoprotein lipase coupled to a loss of certain apoproteins (the apo-Cs) converts VLDLs to **intermediate density lipoproteins (IDLs)**, also termed VLDL remnants. The apo-Cs are transferred to HDLs. The predominant remaining proteins are apo-B-100 and apo-E. Further loss of TGs converts IDLs to LDLs, Fig. 5.

Intermediate Density Lipoproteins, IDLs:

IDLs are formed as TGs are removed from VLDLs and are enriched in cholesterol (45%) which they deliver to peripheral tissues or to the liver. **The fate of IDLs is either conversion to LDLs or direct uptake by the liver**.

Conversion of IDLs to LDLs occurs as more TGs are removed. The liver takes up IDLs after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. For LDL receptors in the liver to recognize IDLs requires the presence of both apoB-100 and apoE (the LDL receptor is also called the apoB-100 / apoE receptor).

Low Density Lipoproteins, LDLs:

IDL is converted to LDL, largely by the liver, by removal of additional TGs. In addition to its formation from VLDL, some LDL is produced and released by the liver. LDL is a major transport form of cholesterol and cholesteryl esters. The relative rates of VLDL and LDL release by the liver depend on the availability of cholesterol. If the regulatory pathways signal the liver to increase its cholesterol output, then the liver increases its LDL production. LDL has specific cell surface receptors. The receptor-LDL complex is transported to lysosomes, for degradation of the particle, while most of the LDL receptors are recycled to the cell surface. The amount of LDL receptor is regulated by the cellular requirement for lipids, with the primary regulatory lipid being cholesterol. High levels of LDL-cholesterol are associated with elevated risk of heart disease; therefore LDL-cholesterol is the “bad cholesterol”.

The cellular requirement for cholesterol as a membrane component is satisfied in one of two ways: either it is synthesized *de novo* within the cell, or it is supplied from extra-cellular sources, namely, chylomicrons and LDLs.

The exclusive apolipoprotein of LDLs is apo-B-100. LDLs are taken up by cells via LDL receptor-mediated endocytosis for IDL uptake. The uptake of LDLs occurs predominantly in liver (75%), adrenals and adipose tissue. As with IDLs, the interaction of LDLs with LDL receptors requires the presence of apo-B-100. The endocytosed membrane vesicles (endosomes) fuse with lysosomes, in which the apoproteins are degraded and the cholesterol esters are hydrolyzed to yield free cholesterol. The cholesterol is then incorporated into the plasma membranes as necessary. Excess intracellular cholesterol is re-esterified by acyl-CoA-cholesterol acyltransferase (ACAT), for intracellular storage. The activity of ACAT is enhanced by the presence of intracellular cholesterol. Insulin and tri-iodothyronine (T3) increase the binding of LDLs to liver cells, whereas glucocorticoids such as dexamethasone have the opposite effect.

The precise mechanism for these effects is unclear but may be mediated through the regulation of apo-B degradation. The effects of insulin and T3 on hepatic LDL binding may explain the hypercholesterolemia and increased

risk of atherosclerosis that have been shown to be associated with uncontrolled diabetes or hypothyroidism. An abnormal form of LDL, identified as lipoprotein-X (Lp-X), predominates in the circulation of patients suffering from lecithin-cholesterol acyl transferase (**LCAT)** deficiency or cholestatic liver disease.

High Density Lipoproteins, HDLs:-

HDLs are synthesized *de novo* in the liver and small intestine, as primarily protein-rich disc-shaped particles. The primary apoproteins of HDLs are apo-A-I, apo-C-I, apo-C-II and apo-E. In fact, a major function of HDLs is to act as circulating stores of apo-C-I, apo-C-II and apo-E. HDLs are converted into spherical lipoprotein particles through the accumulation of cholesteryl esters. This accumulation converts nascent HDLs to HDL_2 and HDL_3. Any free cholesterol presents in chylomicron remnants and VLDL remnants (IDLs) can be esterified through the action of the HDL-associated enzyme, **lecithin: cholesterol acyltransferase**, LCAT. **LCAT is synthesized in the liver and so named because it transfers a fatty acid from the C-2 position of lecithin to the C-3-OH of cholesterol, generating a cholesteryl ester and lysolecithin**. The activity of LCAT requires interaction with apo-A-I, which is found on the surface of HDLs, Fig. 6.

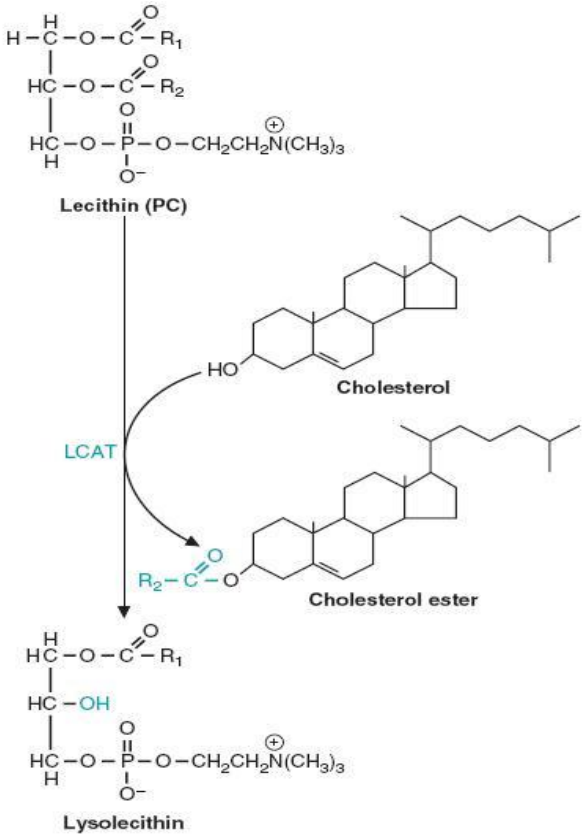

Fig. 6: Biochemical role of LCAT

Cholesterol-rich HDLs return to the liver, where they are endocytosed. Hepatic uptake of HDLs, or reverse cholesterol transport, may be mediated through an HDL-specific apo-A-I receptor or through lipid-lipid interactions. Macrophages also take up HDLs through apo-A-I receptor interaction. HDLs can then acquire cholesterol and apo-E from the macrophages; cholesterol-enriched HDLs are then secreted from the macrophages. The added apo-E in these HDLs leads to an increase in their uptake and catabolism by the liver. HDLs also acquire cholesterol by extracting it from cell surface membranes. This process has the effect of lowering the level of intracellular cholesterol, since the cholesterol stored within cells as cholesteryl esters will be mobilized to replace the cholesterol removed from the plasma membrane, Fig. 7.

The cholesterol esters of HDLs can also be transferred to VLDLs and LDLs through the action of the HDL-associated enzyme, cholesterol ester transfer protein (**CETP**). This has the added effect of allowing the excess cellular cholesterol to be returned to the liver through the LDL-receptor pathway as well as the HDL-receptor pathway.

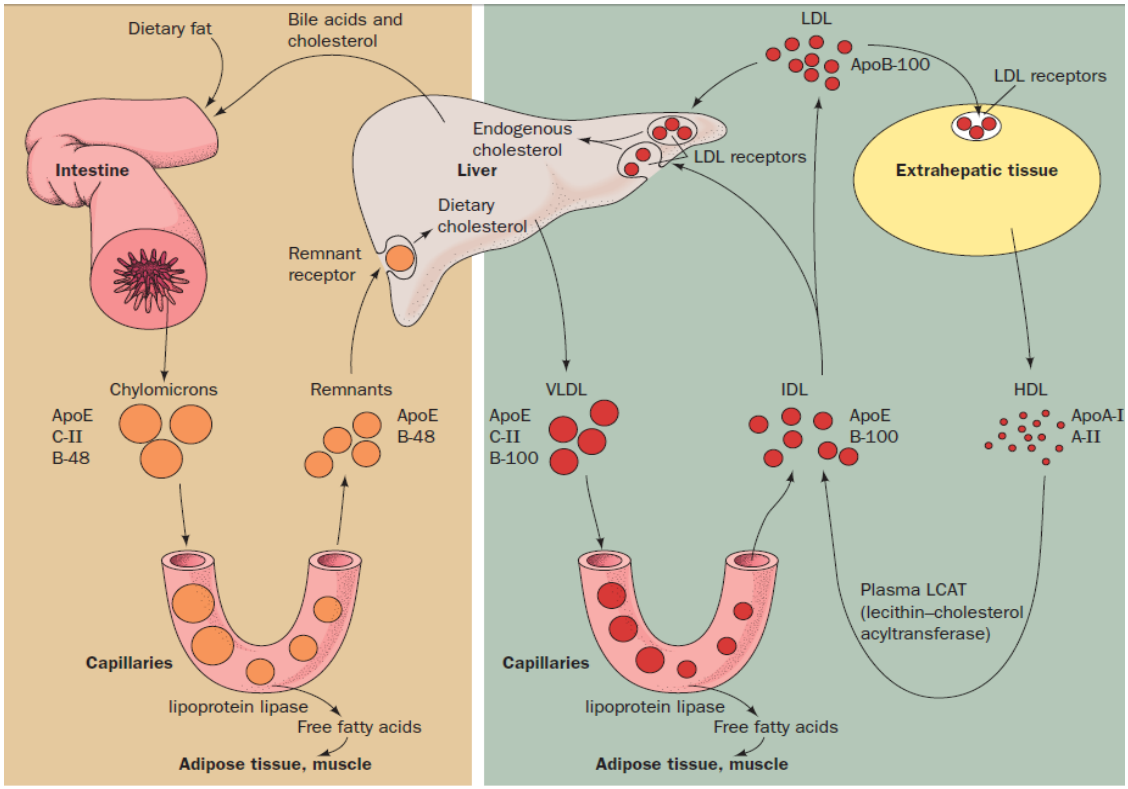

Fig. 7: Metabolism of lipoproteins

High levels of HDL are associated with reduced risk of heart disease, possibly due to increased cholesterol scavenging by HDL, and therefore lower LDL and total plasma cholesterol levels. (HDL-cholesterol is the “good cholesterol”). Exercise is associated with an increase in HDL levels. Females have higher HDL until menopause; this is strongly correlated with

lower risk of heart disease, and an increase in risk as HDL levels fall after menopause. The precise reason for this gender-based difference is poorly understood. Although estradiol levels have been proposed to be involved, a recent large clinical trial suggested that estrogen supplementation in post-menopausal women resulted in an increased incidence in heart disease.

LDL Receptors:

LDLs are the principal plasma carriers of cholesterol delivering cholesterol from the liver (via hepatic synthesis of VLDLs) to peripheral tissues, primarily the adrenals and adipose tissue. LDLs also return cholesterol to the liver. The cellular uptake of cholesterol from LDLs occurs following the interaction of LDLs with the LDL receptor (also called the **apo-B-100 / apo-E receptor**). The sole apoprotein present in LDLs is apo-B-100, which is required for interaction with the LDL receptor. The LDL receptor is a polypeptide of **839 amino acids** that spans the plasma membrane.

Once LDL binds the receptor, the complexes are rapidly internalized (endocytosed). ATP-dependent proton pumps lower the pH in the endosomes, which results in dissociation of the LDL from the receptor. The portion of the endosomal membranes harboring the receptor is then recycled to the plasma membrane and the LDL-containing endosomes fuse with lysosomes. Acid hydrolases of the lysosomes degrade the apoproteins and release free fatty acids and cholesterol. As indicated above, the free cholesterol is either incorporated into plasma membranes or esterified (by ACAT) and stored within the cell.

The level of intracellular cholesterol is regulated through cholesterol-induced suppression of LDL receptor synthesis and cholesterol-induced inhibition of cholesterol synthesis. The increased level of intracellular cholesterol that results from LDL uptake has the additional effect of activating ACAT, thereby allowing the storage of excess cholesterol within cells. However, the effect of cholesterol-induced suppression of LDL receptor synthesis is a decrease in the rate at which LDLs and IDLs are removed from the serum. This can lead to excess circulating levels of cholesterol and cholesteryl esters when the dietary intake of fat and cholesterol exceeds the needs of the body. The excess cholesterol tends to be deposited in the skin, tendons and (more gravely) within the arteries, leading to atherosclerosis. Free cholesterol in the cytosol has the following regulatory functions:

1. Activate ACAT
2. Suppresses HMG-CoA reductase; decrease de novo synthesis of cholesterol.

3. Suppresses further LDL receptor synthesis; decrease further hepatic uptake of LDL.

Lipoprotein (a) [LP(a)]

It is a special type of lipoprotein ***not present in all people***. In normal individuals it may be present only in 20 per cent of the population. In 40 per cent to 80 per cent of the population, normal LP (a) is not present in the serum in detectable amounts. In 20 per cent of normal individuals, if present, it is found to be more than 30 mg/dl. When present, ***it is attached to apo-B100 by a disulphide bond.***

Clinical Importance

The persons having LP (a) are ***more susceptible to heart attack at the younger age group of 30 to 40 years***. LP (a) ***inhibits fibrinolysis***. Levels more than 30 mg/dl increases the risk of myocardial infarction by 3 times and whenthe increased LP (a) level is ***associated with increased LDL***, the risk for myocardial infarction increases further.

Treatment of hyperlipoproteinemias:

1. **Control or correction :** Control of the cause in secondary hyperlipidemias **e.g.** control of diabetes mellitus, weight reduction in obesity and decreasing alcohol consumption.
2. **Dietary restriction:-** In cases of hypercholesterolemia, restriction of animal fats and eggs reduce the intake of cholesterol and saturated fat. The proportion of **PUFA** is to be increased in diet by supplementing vegetable oils and fish. Restriction of dietary fat decreases the chylomicrons while calories reduce the VLDL.
3. **Pharmacologic intervention:-**

Drug treatment to lower plasma lipoproteins and/or cholesterol is primarily aimed at reducing the risk of atherosclerosis and subsequent coronary artery disease that exists in patients with elevated circulating lipids. Drug therapy usually is considered as an option only if non-pharmacologic interventions (altered diet and exercise) have failed to lower plasma lipids, these are **called hypolipidemic agents** or drugs such as:

A. **Mevinolin, Mevastatin, Lovastatin:**
B. **Nicotinic acid:** Nicotinic acid reduces the plasma levels of both VLDLs and LDLs by inhibiting hepatic VLDL secretion, as well as suppressing the flux of FFA release from adipose tissue by inhibiting lipolysis. Because of its ability to cause large reductions in circulating levels of cholesterol, nicotinic acid is used to treat Type II, III, IV and V hyperlipoproteinemias.

Plasma Lipids:- Total plasma lipids are 400–600 mg/dl which include the following fractions and are complexes with apoproteins due to their water insolubility:

Lipid Parameters	mg/dl
Total cholesterol, TC	150-220
HDL – cholesterol, male (HDL-C)	30-60
HDL – cholesterol, female	35-75
LDL – cholesterol, 30-39 yr (LDL-C)	80-175
Triacylglycerol, male	50-200
Triacylglycerol, female	40-150
Phospholipids, PL	150-200
Free fatty acids, FFA	10-20

In peripheral laboratories, a **lipid profile or plasma lipids** is assessed by estimating the following fraction in plasma:

1. **Total cholesterol**
2. **HDL-cholesterol**
3. **LDL-cholesterol**
4. **Triglyceride, TG**
5. **Phospholipid**.

The sample of serum or plasma should be taken after 12-24 hours of fasting. In normal person, cholesterol level varies from **150-220 mg/dl**. It should be preferably below **200 mg/dl**. Concentration between **200-220 mg/dl** are deemed borderline ; between **220-240 mg/dl** is considered as elevated and **above 240 mg/dl** has definite risk for heart attack. According to previous table, HDL has a protective effect on the development of atherosclerosis. The opposite is true with regard to LDL.

Clinical Application: Serum cholesterol is increased in the following conditions:

1. **Diabetes mellitus**, because acetyl-CoA pool is increased and more molecules are channeled to cholesterol.
2. **Obstructive jaundice**, where excretion of cholesterol through bile is blocked .
3. **Hypothyroidism**, where the receptors for HDL on liver cells are decreased, and so excretion is not effective .
4. **Frederickson's type II hyperlipoproteinemia** which is a hereditary autosomal dominant condition.

5. N**ephritic syndrome**, where there is albumin loss through urine, globulins are increased as a compensatory mechanism. When lipoproteins are increased, cholesterol is correspondingly increased.

Risk factors for Coronary Artery Diseases:

1. **Serum cholesterol** value should be preferably below **200 mg/dl**. Values around **220 mg/dl will have moderate risk** and values **above 240 mg/dl indicate active treatment.**
2. **LDL-cholesterol level:** Blood levels under **130 mg/dl** are desirable. Levels between **130 and 159** are borderline; while **above 160 mg/dl** carry definite risk. Hence LDL is **Bad cholesterol**.
3. **HDL-cholesterol level:** Levels above 160 mg/dl protects against heart disease. Hence HDL-C is **good cholesterol**. A level below 35 mg/dl increases the risk of CAD. For every **1 mg/dl** drop in HDL, the risk of heart disease **rises 3%**. Predictive value is increased when **total cholesterol / HDL ratio** is considered; a ratio more than **3.5** is dangerous. Similarly, **LDL/HDL ratio** more than **3.5** is also deleterious.
4. **Cigarette smoking:** Nicotine of cigarette will cause lipolysis and thereby increases the acetyl-CoA and cholesterol synthesis. Nicotine also causes transient constriction of coronary and carotid arteries. Smoking tobacco causes damage to endothelial cells due to free radicals present in tobacco smoke. It is estimated that each puff of a cigarette produces 10^{14} free radicals. In addition, the resultant lack of oxygen causes damage or death to neurons, and nicotine and carbon monoxide, both present in tobacco smoke, cause an increase in blood pressure.
5. **Hypertension:** Systolic blood pressure more than 160 mm further increases the risk of CAD. Atherosclerosis may lead to hypertension. Hypertension causes damage to endothelial cells due to the shear stress as the blood flows through the arteries, especially at positions where arteries branch.
6. **Diabetes Mellitus:** In the absence of insulin, hormone sensitive lipase is depressed, more free fatty acids are formed, and these are catabolized to produce acetyl CoA. These cannot readily utilize, as the availability of oxaloacetate is reduced and citric acid cycle is sluggish. So acetyl-CoA pools are increased, and are channeled into cholesterol synthesis. Glycation of LDL prolonged its half-life. Oxidized LDL is taken up by non-specific endocytosis and further leading to atherosclerosis.
7. **Serum Triglyceride level:** Normal level is 50 – 200 mg/dl. Blood level more than 200 mg/dl is injurious to health.

8. **Homocysteine level:** An increase of 5 micromole/L of homocysteine in serum elevates coronary artery risk by as much as cholesterol increase of 20 mg/dl.

Biochemical Basis for Prevention of Atherosclerosis:

Protective factors for atherosclerosis are fish oils, antioxidants, physical activity and garlic. These are discussed below. The aim is to reduce total cholesterol below 200 mg/dl ; to decrease LDL-C below 130 mg/dl and to keep HDL-C above 35 mg/dl.

1. **Fish oils** These contain high levels of omega-3 fatty acids, which have a number of properties that could explain why fish oils or a diet high in oily fish have a protective effect:
   - They lower the blood level of VLDL and therefore they lower plasma level of LDL.
   - They decrease the formation of blood clots.
   - They decrease the formation of thromboxane A2 and prostacyclin I2 in favor of thromboxane A3 and prostacyclin I3, changes which protect against thrombosis. They have a hypotensive effect.
   - Vegetable oils (e.g. sunflower oil) contain polyunsaturated fatty acids (PUFA). They are required for the esterification and final excretion of cholesterol. So PUFA is helpful to reduce cholesterol level in blood. The optimum ratio of **ω-6 to ω-3** fatty acid is **4:1**. Very high intake of ω-6 oils will cause lowering HDL, elevation of plasma TGs, and will promote platelet aggregation. **ω-6 fatty acids** are found highly in sunflower oils , whereas fish oil are rich in **ω-3 fatty acids.**
2. In cases of moderate elevation of cholesterol, dietary restriction would be helpful. It may be noted that one egg yolk contains about 500 mg cholesterol. One double omelet increases the blood cholesterol, 15 mg more than the original level. Egg and meat contain high cholesterol.
3. **Antioxidants** These are naturally occurring compounds that have the ability to lower the levels of free radicals: they include vitamins C and E, the carotenoids and the flavonoids. Vitamin E and the carotenoids are particularly important in preventing oxidation of the unsaturated fatty acids within the LDL particle and within membranes of cells.
4. **Physical activity** Evidence for the beneficial effects of physical activity on the development of atherosclerosis first arose from a series of epidemiological studies. This activity is now known to cause several changes, all of which are beneficial in decreasing the risk of development of atherosclerosis. These are:

a. Fall in the total serum level of cholesterol;
b. Increase in the serum HDL-cholesterol level;
c. Fall in the serum LDL-cholesterol level;
d. Fall in the plasma TG level;
e. Loss of weight;
f. Reduction of blood pressure.

It also increases the sensitivity of tissues to insulin, which may provide better control of the blood glucose level to minimize the risk of damage to LDL by glycosylation.

5. **Garlic.** Ingestion of garlic, or other tubers, protects against coronary artery disease. The active agents in these tubers are the allyl sulphides. (e.g. allylcysteine sulphoxide, CH_2=CHSOCH_2CH(NH_2)COOH). Protection by the sulphides against coronary heart disease is due to a decrease in the blood levels of TG and cholesterol and decrease in platelet aggregation. However, the major effect is considered to be an increase in the NADH/ NAD^+ and NADPH/$NADP^+$ concentration ratios particularly in endothelial cells. This results in an increased level of reduced glutathione. This is important for a number of reasons but particularly since it can remove oxygen free radicals and hydrogen peroxide and can reduce the formation of the dangerous hydroxyl radical. In addition, it can repair damage caused by these radicals (especially damage to unsaturated fatty acids in membranes and to proteins).

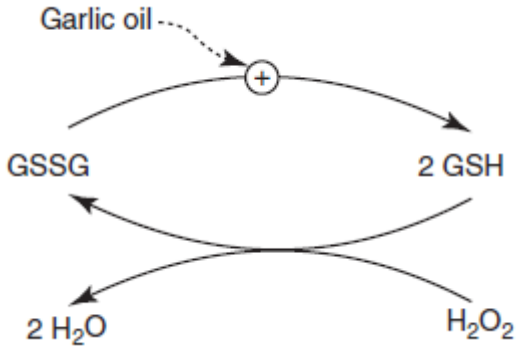

Fig. 8: Effect of garlic oil on the conversion of oxidised to reduced glutathione and degradation of hydrogen peroxide. GSSG represents oxidised glutathione, GSH represents reduced glutathione. The allyl sulphide in garlic oil reduces glutathione (i.e. GSSG → 2GSH). The latter is able to convert hydrogen peroxide to water in a reaction catalysed by glutathione peroxidase. Hydrogen peroxide can produce the dangerous hydroxyl free radical so that its removal is vitally important. Reduced glutathione is also important in restoring the normal structure to molecules damaged by free radicals (e.g. proteins).

6. Moderation in fat intake is necessary. The accepted standard is that less than 30% of total calories may be obtained from fat, out of which about 10% should be PUFA. The recommended daily allowance will

be about 20 – 25 g of oils and about 2 – 3 g of PUFA per day for normal adult.

7. Green leafy vegetables, due to its fiber content will increase the motility of bowel and reduce reabsorption of bile salts. Vegetable also contains plant sterols (sitosterol) which decrease the absorption of cholesterol.
8. Sucrose has a significant effect in raising plasma TGs. High carbohydrate diet, especially sucrose should be avoided by patients with hypercholesterolemia.
9. Keep diabetes under control.
10. Keep hypertension under control.
11. Avoid cigarettes.

HMG-CoA reductase inhibitors. The enzyme HMG-CoA reductase is the rate-limiting enzyme in synthesis of cholesterol and hence drugs that inhibit this enzyme are particularly effective in lowering the blood cholesterol level. These drugs are known as statins (e.g. simvastatin, provastatin).

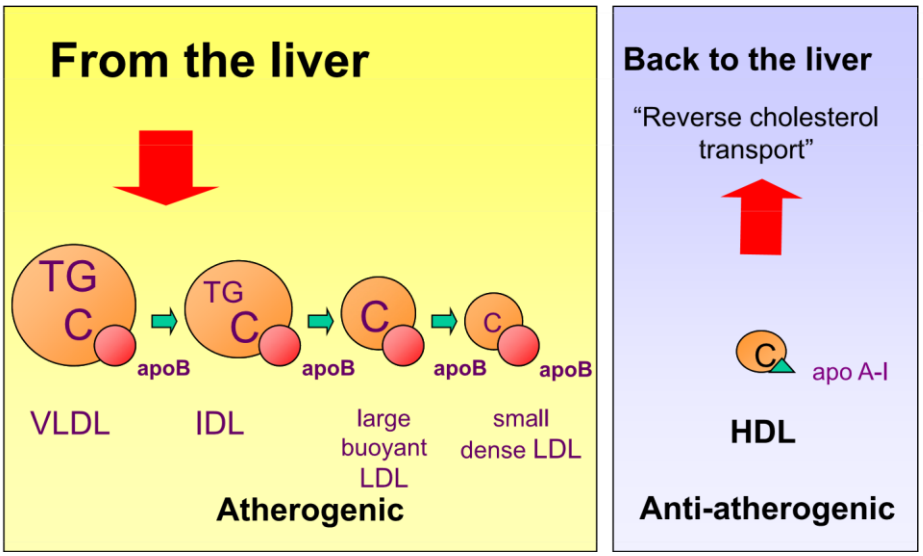

Fig. 9: Atherogenic and anti-atherogenic particles