INTERMEDIARY METABOLISM

Lipid metabolism

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CONTENTS

Introduction
Mobilization of Triacylglycerol
Fat mobilization and oxidation of fatty acids
Biosynthesis of fatty acids
Formation of Malonyl-CoA
Fatty acid synthase complex
Regulation of lipogenesis
Storage of fatty acids as triacylglycerols
Starvation and certain pathological conditions lead to ketosis
Metabolism of acylglycerolipids and sphingolipids
Lipoproteins and lipid transport
Synthesis of cholesterol

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**Introduction**

Fats are generally the major source of energy in human tissues, mainly in the liver and in muscle, except for red cells and brain (which mainly depend on glucose for energy). Triacylglycerols (TG) are the storage and transport form of fats; fatty acids are the immediate source of energy. Fatty acids are released from the adipose tissue, transported bound to albumin in the blood plasma and delivered to cells for metabolism. Fatty acids are catabolised by an oxidative process called β oxidation in the mitochondria and peroxisomes. The major end products of β oxidation are acetyl- Coenzyme A (acetyl CoA) and the reduced forms of the nucleotides, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). Fat metabolism is primarily regulated by the rate of triacylglycerol hydrolysis (lipolysis) in adipose tissue, by the hormones insulin, glucagon, epinephrine and cortisol. The majority of fatty acids required by man are supplied in the diet; however, lipogenesis, their de novo synthesis pathway from two-carbon compounds, is present in several tissues such as liver, brain, kidney, mammary gland and adipose tissue. Excess of energy, especially a carbohydrate-rich diet is a trigger for lipogenesis and carbohydrates are converted to fatty acids in the liver and stored as TG in the adipose tissue. Liver is the major organ for lipogenesis. The pathway for lipogenesis is not simply the reverse of oxidation of fatty acids; and requires its separate set of enzymes located in the cytosol, distinct from those of lipolysis.

**Mobilization of Triacylglycerol**

Triacylglycerols are hydrolyzed by Lipases for release of fatty acids which are required for energy production. The specificity of the particular Lipases involved determines the sequence of hydrolysis of fatty acids from the three positions on glycerol.

Fatty acids and glycerol produced by adipose tissue lipases are released to circulating blood, where fatty acids are bound by serum albumin and transported to tissues to be utilized. Glycerol returns to liver, where it is converted to dihydroxyacetone phosphate and enters glycolytic or gluconeogenic pathways. The lipase that hydrolyzes the first fatty acid is regulated hormonally so that triacylglycerol hydrolysis is balanced with the process of triacylglycerol synthesis to assure adequate energy stores but at the same time avoids obesity.

![Diagram of triacylglycerol hydrolysis](Source: Concepts of Biochemistry 1st edition page No.201)
Different fates of triacylglycerols in the liver and adipose tissue

In adipose tissue triacylglycerols are stored as ‘fat depot’ in the cytosol of the adipocytes and are mobilized when the body requires energy. In the liver most of the triacylglycerols is utilized for production of very lowdensity lipoproteins (VLDL), which are released into the blood and deliver the newly synthesized lipids to the peripheral tissues. Very little triacylglycerol is stored in the liver.

Fat mobilization and oxidation of fatty acids

The triacylglycerol stored as fat is mobilized for energy purpose by the hydrolytic activity of hormone sensitive lipase, to release fatty acids (from carbon 1 or carbon 3 of the triacylglycerol) and glycerol. Other lipases, specific for mono or diacylglycerols, remove the remaining fatty acids. Hormone sensitive Lipase is activated when phosphorylated by cAMP-dependent protein kinase. Binding of the hormone (primarily epinephrine) to receptors on the adipocyte cell membrane activates adenyl cyclase, which converts ATP to cAMP. (In contrast, hormone mediated phosphorylation inactivates acetyl CoA carboxylase). Activation of the cascade switches off fatty acid synthesis whereas triacylglycerol mobilization and hydrolysis is stimulated. Hormone-sensitive lipase is dephosphorylated when plasma levels of insulin and glucose are high and thereby gets inactivated.

In plasma, free fatty acids (FFA) of long chain fatty acids are bound to albumin whereas in the cell they are attached to a fatty acid binding protein or Z protein. Short chain fatty acids are relatively more hydrophilic and are present as the unionized acid or as fatty acid anion.

β-Oxidation of fatty acids

In the mitochondria, β-oxidation of fatty acids takes place by sequential cleavage of two-carbon units from the carboxyl end of the fatty acid, after steps of dehydrogenation, hydration and oxidation to from a β-keto acid, which is split by thiolysis. All the above reactions occur while the fatty acid is activated in a thioester linkage to the 4'-phosphopantetheine of CoA.

Activation of fatty acids

Fatty acid oxidation requires the initial activation of the fatty acids in a reaction with ATP. This is the only step in the complete degradation of fatty acids that requires ATP. Acyl CoA Synthetase (Thiokinase), an enzyme present in the endoplasmic reticulum and inside and on the outer membrane of mitochondria, catalyzes the conversion of fatty acid to its activated form (acyl CoA), involving the expenditure of one high-energy phosphate.

Fatty acid + ATP + CoA $\rightarrow$ Acyl CoA + PPi + AMP

The activation reaction is brought to completion by utilizing the high-energy phosphate associated with pyrophosphate on hydrolysis. Thus two high-energy phosphates are utilized for activation of each fatty acid.
Carnitine transports long chain fatty acyl groups across the mitochondrial membrane (Carnitine shuttle)

Fatty acyl CoA is formed outside the mitochondria, whereas the fatty acid oxidizing machinery is present inside the inner membrane of mitochondria, which is impermeable to CoA and its derivatives (being polar molecules). Hence the fatty acyl CoA is transferred to carnitine (4-trimethylamino-3-hydroxy butyrate synthesized from lysine and methionine in liver and kidney), which act as the carrier of acyl groups across the mitochondrial membrane, and forms acyl carnitine. Carnitine acyl transferase 1 is an enzyme present in the outer mitochondrial membrane. It converts long-chain acyl CoA to acyl carnitine and is able to penetrate the inner mitochondrial membrane of mitochondria. Acyl carnitine then exchanges across the inner mitochondrial membrane with free carnitine by translocase, a carnitine acylcarnitine antiporter. The acyl carnitine then reacts with CoA, catalyzed by carnitine acyl Transferase II, located on the matrix side of the inner membrane. Acyl CoA is resynthesized in the mitochondrial matrix, liberating carnitine (Fig.1). The carnitine shuttle transports fatty acyl CoAs with chain lengths C_{12-18}. Oxidation of lower chain fatty acids within the mitochondria may occur independently of carnitine, since they cross the inner mitochondrial membrane directly and become activated to their CoA derivatives in the matrix.

Fig.1: Carnitine shuttle
Malonyl → CoA- Inhibitor of carnitine acyl transferase 1
(Source: Concepts of Biochemistry 1st edition)
Inhibitor of the carnitine shuttle: Malonyl CoA, formed during fatty acid synthesis in the cytosol is an inhibitor of carnitine acyl transferase 1, thus inhibiting the entry of acyl groups into the mitochondrial matrix. Thus the newly synthesized fatty acids in the cytosol are prevented from entering the mitochondria for β-oxidation.

**β-oxidation of fatty acids:** This process takes places in the mitochondrial matrix in a set of four reactions (Fig. 2). At the inner surface of the inner mitochondrial membrane, the fatty acyl CoA are oxidized by acyl CoA dehydrogenases by removal of hydrogen atoms to form enoyl CoA with a double bond between C-2 and C-3 atoms. These dehydrogenases are flavoproteins and the enzyme bound FADH$_2$ transfers electrons through other electron-transferring flavoproteins to ubiquinone in the electron transport system, forming two ATPs for each double bond formed.

**Fig.2:** Steps in β-oxidation of fatty acids.
(Source: Concepts of Biochemistry 1st ed.)
The second step in β-oxidation is hydration of the double bond to form 3-L-hydroxyacyl CoA. The third step is oxidation catalyzed by NAD-dependent dehydrogenase that produces NADH and the 4th and final step is thiolytic cleavage that releases a molecule of acetyl CoA. The acyl (minus 2 carbon units) group is ready for the next round of oxidation. Thus a long chain fatty acid is completely degraded to acetyl CoA (C-2 units). The acetyl CoA formed can enter the TCA cycle to be completely oxidized to CO₂ and water.

**Oxidation of odd chain fatty acids**

β-oxidation of fatty acids with odd number of carbon atoms proceeds by the same reaction steps as that of fatty acids with an even number of carbon atoms until the last three carbons are reached. This three carbon unit, propionyl CoA, is metabolized by a two step pathway, first it is carboxylated to methylmalonyl CoA and in the 2nd step it undergoes molecular rearrangement to form succinyl CoA (Fig. 3), an intermediate of the TCA cycle. Hence the (propionyl) residue formed by oxidation of odd-chain fatty acids is the only (glucogenic) moiety of fatty acid.

**Fig. 3. Metabolism of propionyl CoA**
(Source: Concepts of Biochemistry 1st ed.)

Very long chain fatty acids (e.g., C₂₀, C₂₂) are oxidized in the peroxisomes. The first dehydrogenation produces acetyl CoA, and H₂O₂ which is degraded by catalase. These
enzymes are induced by high fat diets. However, shorter chain fatty acids are not metabolized in peroxisomes. β-oxidation stops at octanoyl CoA. Octanoyl and acetyl groups are removed from the peroxisomes in the form of octanoyl and acetyl carnitine, and they are then oxidized in the mitochondria. Peroxisomes do not contain carnitine acyl transferase.

**Oxidation of unsaturated fatty acids**

This process provides lesser energy as compared to the oxidation of saturated fatty acids since they are relatively less reduced and therefore fewer reducing equivalents are produced.

**Energy yield from β-oxidation of fatty acids**

Each round of β-oxidation produces one molecule of acetyl CoA, one FADH and one NADH. For e.g. in oxidation of palmitoyl CoA, seven cleavages take place and in the last cleavage, two acetyl CoA units are formed. Thus at the end of β-oxidation of palmitate, 8 acetyl CoA, 7 FADH and 7 NADH are produced. Each FADH yields two ATP and each NADH yields 3 ATPs when oxidized by the electron transport chain, so the reduced nucleotides yield 35 ATP per palmitoyl CoA. Oxidation of acetyl CoA in the TCA cycle yields 12 ATP and therefore, oxidation of 8 acetyl CoA yield 96 ATP. However, 2 ATP equivalents (1 ATP going to 1 AMP) are utilized to activate palmitate to palmitoyl CoA. Thus complete oxidation of palmitic acid yield 129 ATP mol⁻¹.

**Biosynthesis of fatty acids**

Fatty acid synthesis (lipogenesis) takes place in the cytosol and is distinct from the oxidation fatty acids (Lipolysis) in the mitochondria. In humans, fatty acid synthesis occurs mainly in the liver and lactating mammary glands and, to a lesser extent in adipose tissue and kidney. The synthesis involves incorporation of carbons from acetyl CoA, the precursor molecule into the growing fatty acid chain, utilizing ATP, reduced nicotinamide adenine dinucleotide phosphate (NADPH), Mn²⁺, biotin and HCO₃⁻. Fatty acid chain elongation also occurs in the liver endoplasmic reticulum.

Cytosolic Acetyl CoA is derived from a number of sources viz. by transfer of acetate units, from mitochondrial acetyl CoA, which is produced by the oxidation of pyruvate or the degradation of fatty acids, from ketone bodies or amino acids, to the cytosol. The coenzyme A portion of acetyl CoA cannot pass through the mitochondrial membrane and only the acetyl group is transported to the cytosol by formation of citrate from a condensation of acetyl CoA and oxaloacetate (OAA) (Fig.4).

This translocation of citrate from the mitochondria to the cytosol is favoured at high concentration of citrate in the mitochondria and occurs when isocitrate dehydrogenase is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate. Since fatty acid synthesis requires large amounts of ATP, the increase in both ATP and citrate favours this pathway.
Formation of Malonyl-CoA: The initial and controlling step in fatty acid synthesis

The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by acetyl CoA carboxylase in the presence of ATP. Bicarbonate is the source of CO\textsubscript{2} for this reaction. The coenzyme is biotin (Fig.5). Acetyl CoA carboxylase is a multienzyme protein with a variable number of identical subunits, each containing biotin, biotin carboxylase, along with a regulatory allosteric site. The reaction involves first the carboxylation of biotin (requiring ATP) followed by transfer of carboxyl group to acetyl CoA to yield malonyl CoA.

\[
\text{O} \quad \text{CO}_2 \quad \text{Acetyl CoA} \quad \text{Carboxylase (biotin)} \quad \text{O}^- \quad \text{O} \\
\text{CH}_3 - \text{C} - \text{S} - \text{CoA} \quad \text{ATP ADP} + \text{Pi} \quad \text{C} - \text{CH}_2 - \text{C} - \text{S} - \text{CoA} \\
\text{Malonyl CoA}
\]

Regulation of acetyl CoA carboxylase

1. Allosteric: Fatty acid synthesis is regulated at the carboxylation step. Inactive acetyl CoA carboxylase consists of a protomer made of four subunits. Citrate activates this enzyme by causing the protomers to polymerize. On the other hand malonyl CoA (an intermediate of this pathway) or palmitoyl CoA (the end product of the pathway) inactivate the enzyme by depolymerization.
2. Covalent regulation: Epinephrine leads to phosphorylation of the enzyme and thereby its inactivation. Insulin leads to dephosphorylation of acetyl CoA carboxylase thereby activating it.

3. Long term regulation: Prolonged consumption of high-carbohydrate or fat free diet leads to an increased enzyme synthesis, thus increasing fatty acid synthesis. Fasting or high-fat diet leads to reduced fatty acid synthesis by decreasing the synthesis of acetyl CoA carboxylase.

**Fatty acid synthase complex**

This multienzyme protein is a polypeptide and consists of a dimer and each monomer with seven enzyme activities in the eukaryotes with the acyl carrier protein (ACP) as a part of this complex. In prokaryotes, the individual enzymes of the system are separate, and the acyl groups are combined with the ACP. In both these systems, ACP contains the vitamin pantothenic acid in the form 4'-phosphopantetheine. 4'-phosphopantetheine is also a component of Coenzyme A and carries acetyl and acyl groups on its terminal thiol (-SH) group during fatty acid synthesis.

First, a priming molecule of acetyl CoA combines with the cysteine-SH group catalyzed by acetyl transacylase (Fig. 6). Next the acetyl group is transferred to a cysteine residue on the enzyme temporarily, and the vacant ACP now accepts a three –carbon malonate unit from malonyl COA, catalyzed by malonyl transacylase, to form acetyl (acyl)-malonyl enzyme. The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase and liberates CO₂, forming acetoacetyl enzyme (3-ketoacyl enzyme). The loss of free energy during decarboxylation drives the reaction. The 3-ketoacyl group is reduced, dehydrated, and reduced again to form the corresponding saturated acyl-SH enzyme. The sequence of reactions involving the combining of a new molecule of malonyl CoA with the SH of 4'-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine –SH group is repeated 6 more times until a saturated 16-carbon acyl radical (palmityl) is formed. Palmitate is liberated from the enzyme complex by the seventh enzyme in the complex, thioesterase (deacylase). The equation for the overall synthesis of palmitate is:

$$8 \text{acetyl CoA} + 14\text{NADPH} + 14\text{H}^+ + 7\text{ATP} \rightarrow \text{palmitic acid} + 8\text{CoA} + 14\text{NADP}^+ + 7\text{ADP} + 7\text{Pi} + 7\text{H}_2\text{O}$$

The acetyl CoA used as the primer forms carbon atoms 15 and 16 of palmitate. Rest of the subsequent C₂ units added is via malonylCoA formation. Long chain fatty acids with odd number of carbon atoms are produced if propionyl-CoA acts as a primer.

**NADPH is the reducing equivalent in fatty acid synthesis**

The hexose monophosphate pathway is the major supplier of NADPH for fatty acid synthesis (2 NADPH produced for each glucose molecule entering the pathway). The cytosolic oxidation and decarboxylation of malate to pyruvate by a cytosolic NADP⁺ dependent malate dehydrogenase (Malic enzyme) also yields cytosolic NADPH.
Fig. 6: Biosynthesis of fatty acids (Source: Concepts of Biochemistry 1st ed.)
Malate can be formed by the reduction of oxaloacetate by cytosolic NAD$^+$-dependent malate dehydrogenase and the cytosolic NADH can be produced during glycolysis (Fig. 7). Oxaloacetate in turn can be formed from citrate that is moved from mitochondria into the cytosol, where it is cleaved into acetyl CoA and oxaloacetate by the citrate cleavage enzyme.

![Figure 7: Interrelationship between glucose metabolism and palmitate synthesis](Source: Concepts of Biochemistry 1st ed.)
Figure 7 shows the interrelationship between glucose metabolism and synthesis of palmitate. Pyruvate produced during glycolysis is the main source of the mitochondrial acetyl CoA required for fatty acid synthesis and the cytosolic NADH contributes to the reduction of NADP\(^+\) to NADPH needed for palmitoyl CoA synthesis. Oxaloacetate is produced in the mitochondria by pyruvate carboxylation (during gluconeogenesis). Acetyl CoA produced in the mitochondria condenses with oxaloacetate (OAA) to from citrate, the first step in the TCA cycle, citrate moves out of the mitochondria into the cytosol where it is cleaved to acetyl CoA (cytosolic). NADH produced in the cytosol during glycolysis in turn reduces NADP\(^+\) to NADPH required for palmitoyl CoA synthesis, Cytosolic acetyl CoA serves as a precursor for synthesis of palmitate.

**Chain elongation of fatty acids occurs in the endoplasmic reticulum**

Fatty acid elongase system of enzymes (Fig. 8) converts fatty acyl CoA to an acyl-CoA derivative with two carbons more. Malonyl CoA is the donor of acetyl groups and NADPH supplies the reducing equivalents. The acyl groups that may act as a starting molecule include the saturated fatty acids from C\(_{10}\) upward as well as unsaturated fatty acids. Humans lack the enzymes required to introduce double bonds beyond C\(_9\) and therefore, the polyunsaturated fatty acids linoleic and linolenic acids must be provided in the diet.

**Regulation of lipogenesis**

The reaction catalyzed by the enzyme acetyl-CoA carboxylase is the rate limiting step of lipogenesis. Acetyl-CoA carboxylase is an allosteric enzyme modulated in the following ways.

It is activated by citrate, which is increasingly formed from acetyl-CoA that in turn increases in the well-fed state. On the other hand this enzyme is inhibited by long chain acyl-CoA molecules formed either due to increased lipolysis or an influx of free fatty acids into the tissue. Acyl CoA inhibits the mitochondrial tricarboxylate transporter thereby inhibiting the transfer of citrate from the mitochondria to the cytosol. Acyl CoA also inhibits pyruvate dehydrogenase by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane leading to increased ratio of [ATP]/[ADP] in the inner mitochondrial membrane, and therefore converting the active dephospho from of pyruvate dehydrogenase to the phosphorylated inactive form. An increase in the ratio of [acetyl CoA]/[CoA] and [NADH]/[NAD\(^+\)] in the mitochondria due to oxidation of acyl CoA in the presence of increased free fatty acids also inhibits pyruvate dehydrogenase.

**Hormonal regulation of lipogenesis**

Insulin promotes lipogenesis by the following ways. It leads to increased uptake of glucose into the well which in turn increases pyruvate, a precursor for fatty acid biosynthesis and glycerol-3-phosphate which is required for esterification of the free fatty acids, insulin selectively converts the inactive pyruvate dehydrogenase to its active form in the adipose tissue but not in liver.

Insulin also activates acetyl-CoA carboxylase by dephosphorylating it. Insulin reduces the intracellular cAMP levels, by phosphorylating the enzyme adenylyl cyclase thus promoting lipogenesis.
Storage of fatty acids as triacylglycerols

Liver and adipose tissue are the major sites for synthesis of triacylglycerols from fatty acids although most tissues of the body are also capable of triglyceride synthesis. Synthesis, long
term storage and hydrolysis of triacylglycerols occur in adipose tissue, a specialized connective tissue designed for this purpose. In a homeostatic situation, there is continuous synthesis and breakdown of triacylglycerols in adipose tissue.

Monoacylglycerols, diacylglycerols and triacylglycerols (triglycerides) consist of one, two or three molecules of fatty acids esterified to a molecule of glycerol. Fatty acids are esterified at their carboxyl groups leading to a loss of negative charges thus forming neutral fat. The fatty acids come from the diet, from adipose tissue by blood transport or from de novo synthesis. Acetyl CoA for the biosynthesis comes mainly from glucose catabolism.

**Fatty acyl CoAs and glycerol-3-phosphate are precursors for triacylglycerols synthesis**

Triacylglycerols are synthesized from activated fatty acids and glycerol 3-phosphate or dihydroxyacetone phosphate. Glycerol-3-phosphate is formed either by reduction of dihydroxyacetone phosphate produced in glycolysis or by phosphorylation of glycerol by glycerol kinase. White adipose tissue lacks this enzyme so glycerolphosphate is produced from intermediates of glycolysis. Fatty acids are activated by conversion to their CoA esters by the following reaction:

\[
\text{R} - \text{C} - \text{O} + \text{ATP} + \text{CoASH} \rightarrow \text{R} - \text{C} - \text{SCoA} + \text{AMP} + \text{PPi} + \text{H}_2\text{O}
\]

This 2-step reaction has an acyl adenylate as intermediate and is driven by hydrolysis of pyrophosphate to Pi. Phosphatidic acid is a key intermediate in the synthesis of triacylglycerols from phosphorylated 3-carbon units and also in the synthesis of other lipids too. Phosphatidic acid is formed by two sequential acylations of glycerol-3-phosphate (Fig. 9). If dihydroxyacetone phosphate is the precursor it is acylated at C-1 followed by reduction at C-2. The lysophosphatidic acid is further esterified to form phosphatidic acid (Fig. 10). For synthesis of triacylglycerol, the phosphate group of phosphatidic acid is hydrolyzed by phosphatidate phosphatase to produce diacylglycerols, which is then acylated to triacylglycerol (Fig.11).

An exception to this is the intestinal mucosa where the synthesis of triacylglycerol does not require formation of phosphatidic acid. 2- monoacylglycerols, major product of intestinal lipid digestion, are directly absorbed into the mucosal cells. An enzyme in the mucosal cell, catalyses acylation of these monoacylglycerols with acyl CoA to form 1, 2-diacylglycerols, which is then further acylated.

Among the fatty acids, palmitic acid tends to be present in position 1 and oleic acid in positions 2 and 3 of human adipose tissue triacylglycerols. The specificity of acyl transferase involved and relative availability of different fatty acids in the fatty acyl CoA are the major determinants of the localization of the different fatty acids in triacylglycerol.

**Ketone bodies are formed from acetyl CoA: ketogenesis**

When the rate of fatty acid breakdown is high, liver mitochondria convert the excess acetyl CoA into ketone bodies acetoacetate, -3 hydroxybutyrate (β-hydroxy butyrate) and acetone, which is formed by spontaneous decarboxylation of acetoacetate. They are transported via blood to the peripheral tissues where they may be reconverted to acetyl CoA and oxidized.
by the TCA cycle. Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme 3-hydroxybutyrate dehydrogenase, the equilibrium being controlled by the mitochondrial ratio of [NAD$^+$] to [NADH]. Ketone bodies are important sources of energy for the peripheral tissues since they are soluble in aqueous medium and do not require transporters; they are synthesized by the liver when the amount of acetyl CoA in the liver exceeds its oxidative capacity and since they can be utilized by extra hepatic tissues in proportion to their concentration in blood. The brain too utilizes ketone bodies for fuel during prolonged starvation periods.

Fig. 9: Synthesis of Phosphatidic acid from glycerol 3-phosphate
(Source: Concepts of Biochemistry 1st ed.)
Liver is the primary site for production of ketone bodies

In the mitochondrial matrix, condensation of two acetyl CoA molecules catalyzed by the enzyme β-keto thiolase produces acetoacetyl CoA. Acetoacetyl CoA then condenses with another molecule of acetyl CoA to form β hydroxy-β-methyl glutaryl coenzyme A (HMG CoA). Cleavage of HMG CoA then yields acetoacetic acid and acetyl CoA (Fig. 12).
Some acetoacetate undergoes spontaneous decarboxylation to acetone.

\[ \text{Acetoacetate} \rightarrow \text{Acetone} \]

Under normal circumstances, acetone formation is very less, but in pathological conditions such as diabetic ketoacidosis, the acetone levels rise in blood and can be detected in the
patient’s breath. The reaction catalyzed by enzyme HMG-CoA synthase is the rate-limiting step in ketone body synthesis occurs in significant amount only in the liver.

**Fig. 12: Formation of acetoacetate**
(Source: Concepts of Biochemistry 1st ed.)

Utilization of ketone bodies by non-hepatic tissues requires formation of acetoacetyl CoA

Acetoacetate and β-hydroxybutyrate produced by the liver are major fuels for non-hepatic tissues such as cardiac and skeletal muscle, especially when glucose levels are low (starvation) or inefficiently used (insulin deficiency).
Acetoacetate is first reactivated to its CoA derivative, a mandatory step for utilization of ketone bodies. The enzyme required for this is a mitochondrial acetoacetate succinyl-CoA CoA transferase (thiophorase) present in most non-hepatic tissues, but absent in liver (Fig. 13)

\[
\text{Acetoacetate} + \text{succinyl CoA} \rightarrow \text{Acetoacetyl CoA} + \text{succinate}
\]

*Fig. 13: First step in utilizing acetoacetate in non-hepatic tissues*

Acetoacetyl CoA is then converted to acetyl CoA by the enzyme \( \beta \)-keto thiolase. Acetyl CoA in turn enters the TCA cycle with production of energy. The acetoacetate concentration is maintained by its formation from \( \beta \)-hydroxybutyrate by the mitochondrial enzyme \( \beta \)-hydroxybutyrate dehydrogenase.

**Starvation and certain pathological conditions lead to ketosis**

Hepatic production of acetoacetate and \( \beta \)-hydroxybutyrate is minimal (<=0.2 mM) under normal feeding conditions. However, in starvation, ketone bodies are synthesized at a high rate with circulating levels of acetoacetate plus \( \beta \)-hydroxybutyrate rising to 3-5 mM. During early steps of fasting, utilization of ketone bodies by heart and skeletal muscle conserves glucose for support of the central nervous system. During prolonged starvation, increased blood levels of acetoacetate and \( \beta \)-hydroxybutyrate lead to their efficient uptake by brain, further sparing glucose consumption.

However, in pathological conditions such as diabetic ketoacidosis, there is excessive accumulation of ketone bodies in blood (up to 20 mM) (ketonemia) and their excretion in the urine (ketouria). An elevation of ketone body concentration in the blood results in acidemia.

**Regulation of ketogenesis**

1. Factors regulating mobilization of free fatty acids that arise from lipolysis of triacylglycerol in adipose tissue are important in regulating ketogenesis (Fig. 14).

2. By carnitine acyl transferase 1
   Activity of carnitine acyl transferase 1 in the outer mitochondrial membrane regulates the entry of long-chain acyl groups into mitochondria before \( \beta \)-oxidation. Activity of this enzyme is low in the fed state, when fatty acid oxidation is depressed, and is high in starvation, when fatty acid oxidation increases. Concentration of malonyl CoA, the first intermediate in fatty acid biosynthesis increases during fed state. Malonyl CoA in turn inhibits carnitine acyl transferase 1, there by inhibiting \( \beta \)-oxidation. Most of the free fatty acids are esterified to acylglycerols and transported out of the liver in very low density lipoproteins (VLDL) during the fed state. However, during starvation, increase in free fatty acid concentration leads to increased formation of acetyl CoA. Decreased malonyl CoA concentration inhibits the carnitine acyl transferase 1 and allows more acyl CoA to
be $\beta$-oxidized. These events are further promoted by decrease in the [insulin]/[glucagon] ratio in starvation.

3. Acetyl CoA formed by $\beta$-oxidation is either oxidized in the TCA cycle or forms ketone bodies. The diversion of acetyl CoA to either the ketogenic pathway or to TCA cycle for complete oxidation is so regulated that the total free energy captured in ATP which results from the oxidation free fatty acid is maintained constant.

![Diagram of lipid metabolism](image)

**Fig. 14: Regulation of ketogenesis**
[Steps A to C are the important stages in the metabolic pathway of free fatty acids (FFA) that determines the extent of occurrence of ketogenesis]
(Source: *Concepts of Biochemistry 1st ed.*)
Hence, Lipogenesis involves two main enzyme systems in the cytosol of the cell; acetyl-CoA carboxylase and fatty acid synthase. The cofactors involved in this process are NADPH, ATP, Mn\(^{2+}\), biotin, pantothenic acid and HCO\(_3^-\). Elongation of long-chain fatty acids occurs in the endoplasmic reticulum by a microsomal elongase enzyme system. Lipogenesis is regulated at the acetyl-CoA carboxylase step by covalent, allosteric and hormonal modulation. Citrate activates the enzyme acetyl CoA carboxylase and long chain acyl-CoA inhibits it. Short term stimulation of the enzyme by insulin is by dephosphorylating it and long term stimulation is by increased synthesis of this enzyme. Glucagon and epinephrine on the other hand have antagonistic effects.

\(\beta\)-oxidation of fatty acids takes places in mitochondria and the process involves cleavage of 2-carbon units (acetyl CoA) sequentially from long chain fatty acyl CoA. The acetyl CoA units are oxidized in the TCA cycle, and the entire process produces large quantities of ATP. Oxidation of odd-chain fatty acids yield acetyl CoA units and in addition, a molecule of propionyl CoA, which is glucogenic. Very long chain fatty acids (C\(_{20}\), C\(_{22}\)) are oxidized in the peroxisomes up to octanoyl-CoA, which is then transferred to the mitochondria for further oxidation. Increased fatty acid oxidation leads to production of ketone bodies (acetoacetate, \(\beta\)-hydroxybutyrate and acetone) in the liver mitochondria. Ketogenesis occurs by synthesis and breakdown of 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) involving two main ketogenic enzymes, HMGCoA synthesis and HMG-CoA lyase. Ketogenesis is regulated at 3 main steps; (1) Lipolysis in adipose tissue producing free fatty acids, (2) activity of carnitine acyl transferase 1 in liver mitochondria which determines the proportion of fatty acid oxidized rather than esterified and (3) diversion of acetyl CoA between ketogenesis and TCA cycle. Ketone bodies serve as important fuels for extra hepatic tissues.

**Metabolism of acylglycerolipids and sphingolipids**

The two main classes of acylglycerolipids or glycerolipids are triacylglycerols and glycerophospholipids both having a glycerol backbone, but with different physical properties and functions. Triacylglycerols are the major constituents of storage fat and in diet. Phospholipids are the major components of the plasma membrane. Non-membrane bound phospholipids have additional roles in the body (e.g. components of plasma lipoproteins, components of lung surfactant etc). Sphingolipids are complex lipids with a sphingosine backbone in their structure. They account for 5-10% of the lipids of the plasma membrane. They are present in blood and nearly all body tissues. The highest concentrations are found in the white matter of the central nervous system.

**Biosynthesis of triacylglycerols**

The precursors for the synthesis of triacylglycerol, glycerol and fatty acids must be activated by ATP before they can be incorporated into acylglycerols. Activation of glycerol to sn-glycerol 3-phosphate is catalyzed by the enzyme glycerol kinase. Since the activity of this enzyme is low in adipose tissue or muscle, dihydroxyacetone phosphate an intermediate of the glycolytic pathway is reduced to glycerol -3- phosphate by glycerol-3-phosphate dehydrogenase, an NADH dependent enzyme (Fig. 15). Fatty acids are activated to acyl CoA by the enzyme acyl CoA-Synthetase, using ATP and CoA (described previously). Two molecules of acyl-CoA combine with glycerol-3-phosphate to form 1-2-diacylglycerol phosphate (phosphatidate). This is a 2-step process, glycerol 3-phosphate acyl transferase forming lysophosphatidate and then formation of phosphatidate by 1-acylglycerol-3-
phosphate acyl transferase. Phosphatidate is converted to 1, 2-diacylglycerol by phosphatidate phosphohydrolase. Finally, a 3rd molecule of acyl-CoA is esterified with the diacylglycerol forming triacylglycerol, catalyzed by the enzyme diacylglycerol acyltransferase. In intestinal mucosa, monoacyl glycerol is converted to 1, 2-diacylglycerol by monoacylglycerol acyltransferase.
**Fig. 15: Biosynthesis of acylglycerolipids**

**Catabolism of triacylglycerols**

Lipases hydrolyze triacylglycerols to their constituent fatty acids and glycerol by lipolysis, most of which occurs in the adipose tissue. The free fatty acids formed are released into the plasma where they are bound to albumin. The circulating free fatty acid is taken up by tissues and either utilized or re-esterified.

**Biosynthesis of glycerophospholipids**

Phosphatidate is the common precursor in the biosynthesis of triacylglycerols and glycerophospholipids such as phosphatidylserine or from 1,2-diacylglycerol, e.g. phosphatidyl choline and phosphatidyl ethanolamine. For synthesis of phosphatidyl inositol, cytidine triphosphate (CTP) combines with phosphatidate to form a cytidine diphosphatidate-diacylglycerol (CDP-diacylglycerol). This in turn forms phosphatidylinositol by combining with inositol, catalyzed by CDP-diacylglycerol inositol transferase. Successive phosphorylations convert phosphatidyl inositol first to phosphatidyl inositol-4-phosphate and then to phosphatidyl inositol 4, 5-bisphosphate which is broken down to 1-
2diacylglycerol (DAG) and inositol triphosphate (IP3) by hormones that elevate \([\text{Ca}^{+2}]\) e.g. vasopressin. DAG and IP3 are second messengers in a signal transduction pathway.

**Biosynthesis of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE)**

Phosphatidyl choline (PC) (Lecithin) and phosphatidyl ethanolamine (cephalin) are the most abundant phospholipids in most eukaryotic cells. They are mainly synthesized from choline and ethanolamine derived from diet or from the metabolic turnover of the body’s phospholipids. Phosphatidyl serine from membrane serves as a precursor of choline.

In the major biosynthesis pathway of phosphatidyl choline (Fig. 15) (lecinthin), sequential conversion of choline to phosphocholine, CDP-choline and phosphatidyl choline takes place. Phosphocholine cytidylytransferase converts phosphocholine to CDP-choline at the expense of CTP releasing inorganic pyrophosphate (PPi). CDP-choline having a high energy pyrophosphoryl bond is highly unstable and reactive and hence the phosphocholine is readily bound to the OH group at position 3 of 1, 2-diacylglycerol by choline phosphotranferase (Fig.17). This is the main pathway for dipalmitoyllecithin synthesis in lungs.

The cytidyl transferase reaction that forms CDP-choline is the rate-limiting step for phosphatidylcholine biosynthesis (Fig.16).

\[
\text{Choline} \quad \xrightarrow{\text{Choline kinase}} \quad \text{ATP} \quad \xrightarrow{\text{ADP}} \\
\text{O} \quad \xrightarrow{\text{O}^{-}} \quad \text{P} \quad \text{O}^{-} \quad \text{CH}_{2} \quad \text{CH}_{2} \quad \text{N} \quad (\text{CH}_{3})_{3} \\
\text{O}^{-} \quad \text{Phosphocholine} \\
\text{Phosphocholine cytidylyl transferase} \quad \xrightarrow{\text{CTP}} \quad \text{PPi} \\
\text{O} \quad \text{O} \quad \xrightarrow{\text{O}^{-}} \quad \text{O}^{-} \\
\text{Cytidine} \quad \text{O} \quad \text{P} \quad \text{O} \quad \text{P} \quad \text{O} \quad \text{CH}_{2} \quad \text{CH}_{2} \quad \text{N} \quad (\text{CH}_{3})_{3} \\
\text{O}^{-} \quad \text{O}^{-} \quad \text{CDP} \quad \text{Choline} \\
\text{Pyrophosphoryl linkage}
\]

**Fig.16: Biosynthesis of CDP-Choline from choline**

*(Source: Concepts of Biochemistry 1st ed.)*
Only in liver, phosphatidylcholine is formed from phosphatidylethanolamine by methylation catalyzed by phosphatidyl ethanolamine N-methyl transferase. S-adenosyl methionine is the methyl donor (Fig. 18).

Phosphatidyl serine is mainly formed from phosphatidylethanolamine in a reversible reaction (Fig. 19).

Phosphatidylinositol is formed via CDP-diacylglycerol and free myo-inositol (Fig. 20) catalyzed by phosphatidylinositol synthase. By successive phosphorylations, phosphatidyl inositol is converted first to phosphatidylinositol 4, 5-bisphosphate (PIP$_2$). PIP$_2$ is then broken down into diacylglycerol (DAG) and inositol triphosphate (IP$_3$), which act as second messengers to hormones that increase [Ca$^{2+}$].
Fig. 18: Biosynthesis of phosphatidyl choline from phosphatidyl ethanolamine
(Source: Concepts of Biochemistry 1st ed.)

Fig. 19: Biosynthesis of phosphatidyl serine
(Source: Concepts of Biochemistry 1st ed)

Diphosphatidylglycerol (cardiolipin) is a phospholipid present in mitochondria (Fig. 21) formed from phosphatidylglycerol, which in turn is synthesized from CDP-diacylglycerol and glycerol-3-phosphate.
**Fig. 20: Biosynthesis of phosphatidyl inositol**  
(Source: Concepts of Biochemistry 1st ed.)

**Fig. 21: Biosynthesis of cardiolipin**  
(Source: Concepts of Biochemistry 1st ed.)

**Biosynthesis of Plasmalogens (etherglycerolipid)**

Ether glycerolipids are synthesized from dihydroxy acetone phosphate (DHAP), long-chain fatty acids, and long chain fatty alcohols (Fig. 22). Dihydroxyacetone phosphate and long-chain fatty acyl CoA react to form acyldihydroxyacetone phosphate catalyzed by the enzyme acyl CoA: dihydroxyacetonephosphate with a long-chain fatty alcohol. Plasmalogen is ultimately formed by transfer of long-chain fatty acid from its respective CoA donor to the sn-2 position of alkyl-2 lyso-sn-glycerol-3-phosphate.
Degradation of phospholipids

Phospholipases are enzymes that act at specific sites of phospholipids thereby bringing about partial degradation followed by resynthesis (Fig. 23).

Phospholipases are present in all tissues and pancreatic juice (responsible for digestion of phospholipids). Snake and bee venoms and number of toxins have phospholipase activity. Phospholipases are involved in both degradation and also remodeling of phospholipases e.g. phospholipases A1 and A2 can remove specific fatty acids from membrane bound phospholipids which can be substituted with other fatty acids using fatty acyl CoA transferase.

Phospholipase A1 targets the ester bond in position 1. Phospholipase A2, catalyzes the hydrolysis of the ester bond in position 2 of glycerophospholipids forming lysophospholipid and a free fatty acid. Lysophospholipid in turn can be reacytated by acyl-CoA catalyzed by
the enzyme acyl transferase. On the other hand, the lysophospholipid (such as lysolecithin) is hydrolyzed by lysophospholipase B (phospholipase B), cleaving the residual-acyl group to form the corresponding glycerylphosphoryl base, which in turn is hydrolyzed by a hydrolase to yield glycerol-3-phosphate and the base.

**Fig. 23: Phospholipid hydrolysis by specific phospholipases**  
(Source: Concepts of Biochemistry 1st ed.)

Phospholipase C cleaves the ester bond at position 3 to give 1, 2-diacylglycerol and a phosphoryl base. Phospholipase D, mainly a plant enzyme hydrolyzes the nitrogenous base from phospholipids.

Lysolecithin is also formed when a fatty acid from the 2 position of lecithin is transferred to cholesterol to from cholesteryl ester by the enzyme lecithin: cholesterol acyltransferase (LCAT).

**Dipalmitoyl-lecithin is essential for normal lung function**

More than 80% of the phospholipids in the extra cellular lipid layer lining the lung alveoli are dipalmitoyl lecithin which act as surfactant and prevent atelectasis at the end of the expiration phase of breathing. Dipalmitoyl lecithin containing palmitic acid residues in both sn1 and sn2 positions decreases the surface tension of the aqueous surface layer of the lung. In addition surfactants also contain phosphatidyl diacylglycerol, phosphatidyl inositol and some surfactant proteins.

In the biomembrane, phospholipids serve as the major structural and functional components. In bile, they (mainly phosphatidyl choline) aid in solubilizing cholesterol stones and bile pigment gallstones. Phosphatidylinositol and phosphatidyl choline are sources of arachidonic acid for synthesis of prostaglandins, thromboxanes, leukotrienes and other eicosanoids. Phosphatidyl inositol 4 5-bisphosphate (PIP2) plays a major role in signal transduction by forming the second messengers IP3 and DAG.
**Biosynthesis of sphingolipids**

Sphingolipids are complex lipids, each having a backbone made up of a long chain amino alcohol called spingosine (Fig. 24).

![Fig. 24: Structure of spingosine](image)

The primary alcohol group at C-1 is a nucleophile that forms covalent bonds with sugars forming glycosphingolipids and with phosphocholine to form sphingomyelin. The amino group at C-2 bears a long-chain fatty acid (usually C\textsubscript{20} – C\textsubscript{26}) in amide linkage. The secondary alcohol at C-3 is free.

Sphingolipids are present in blood and most body tissues with highest concentration in the white matter of the central nervous system. Many Sphingolipids are components of plasma membrane of cells.

The three major types of Sphingolipids are phosphosphingosides, glycosphingolipids and sulfatides. All sphingolipids have the spingosine attached to a fatty acid in amide linkage to form a ceramide (Fig. 25). The fatty acid has a chain length varying from C\textsubscript{18} to C\textsubscript{24}. Ceramide is synthesized in the endoplasmic reticulum (Fig. 25).

**Sphingomyelin**, a structural lipid of membranes of nervous tissue, is the only sphingolipid that is a phospholipid. Sphingomyelins are formed when ceramide combines with phosphatidylcholine.

![Fig. 25: Ceramide](image)

![Fig. 26: Sphingomyelin](image)
Sphingomyelin is thus a ceramide phosphocholine. It is neutral at physiological pH. Excessive accumulation of sphingomyelin is seen in Niemann-Pick disease.

**Glycosphingolipids**

They are a combination of ceramide with one or more sugar residues. The chief glycosphingolipid classes are cerebrosides, sulfatides and gangliosides.

**Cerebrosides**

The most common cerebrosides are galactocerebroside (Fig. 27) and glucocerebroside. Galactocerebroside is mainly found in the brain.

Glucocerebroside (glucosylceramide) serves as an intermediate in the synthesis and degradation of complex glycosphingolipids. In Gaucher’s disease, a deficiency of the enzyme lysosomal glucocerebrosidase leads to excess accumulation of glucocerebroside in the spleen and liver. Uridine diphosphogalactose epimerase (Fig. 28) acts on the substrate uridine diphosphate glucose (UDP Glc) and leads to epimerization of the glucose moiety to galactose (UDP Gal). Galactosyl Ceramide is formed in a reaction between ceramide and UDP Gal. Further reaction with 3’-phosphoadenosine-5’-phosphosulfate (PAPS) forms sulfogalactosylceramide.

**Gangliosides** are sialic acid-containing glycosphingolipids present in large amounts in the ganglion cells of the central nervous system.

Glycosphingolipids are constituents of the outer leaflet of plasma membranes and probably involved in intercellular communication. Some gangliosides serve as receptors for bacterial toxins such as cholera toxin. Some of them are antigens e.g. the ABO blood group substances.

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toxins such as cholera toxin. Some of them are antigens e.g. the ABO blood group substances.

Gangliosides are synthesized from ceramide by sequential addition of UDP Glc, UDP Gal (activated sugars) and a sialic acid, N-acetylneuraminic acid (Fig. 29).
All sphingolipids are catabolized to the level of their starting precursor molecules i.e. sugars, sulfate, fatty acids, phosphocholine and sphingosine. However when the activity of one of the lysosomal hydrolytic enzyme is low due to a genetic defect, then the substrate for the defective or missing enzyme accumulates and is deposited within the lysosomes of the tissue where that particular sphingolipid is undergoing catabolism. These disorders are collectively called sphingolipidosis.

Assay of enzyme activity is used to confirm the diagnosis of a particular lipid storage disease. In Tay-Sachs disease (deficiency of enzyme Hexoseaminidase A) serum and tears are a source of enzyme for the diagnosis of the disorder. Sphingolipidoses are autosomal recessive with the disease occurring only in homozygotes with a defect in both alleles. Enzyme assays enable identification of carriers or heterozygotes. Few of the important lipidoses are described in Table 1.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme defect</th>
<th>Lipid Accumulating</th>
<th>Clinical Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay-Sachs Disease</td>
<td>Hexosaminidase</td>
<td>GM₂ ganglioside</td>
<td>Mental retardation, muscle weakness</td>
</tr>
<tr>
<td>Gaucher’s Diseases</td>
<td>β–Glucosidase</td>
<td>Glucosyl ceramide</td>
<td>Enlarged liver and spleen</td>
</tr>
<tr>
<td>Niemann-pick Disease</td>
<td>Sphingomyelinase</td>
<td>Sphingomyelin</td>
<td>Enlarged liver and spleen, mental retardation</td>
</tr>
<tr>
<td>Generalized Gangliosidosis</td>
<td>Gm₁-β-galactosidase</td>
<td>GM₁ Ganglioside</td>
<td>Mental retardation, enlargement of liver, skeletal deformation</td>
</tr>
</tbody>
</table>

Hence, triacylglycerols are the major lipids in fat deposits and in food. Phospholipids are the major components of the plasma membrane and other membranes and besides this; they play an important role in the metabolism of other lipids. All sphingolipids are formed from ceramide. Sphingomyelin is a phospholipid present in membranes of organelles such as Golgi. Glycosphingolipids are formed by addition of a ceramide with a sugar residue. Gangliosides are complex glycosphingolipids containing sialic acid. They serve as antigens and cell receptors. Lack of the phospholipids dipalmitoyl lecithin, lung surfactant in premature infants leads to respiratory distress syndrome of the newborn. Defective metabolism of phospholipids and sphingolipids also leads to multiple sclerosis (demyelination) and sphingolipidosis.

Lipoproteins and lipid transport

**Introduction**

The lipoproteins in plasma are complexes of lipids and specific proteins called apolipoproteins. The lipoproteins transport the fat absorbed from the diet and lipids synthesized in the liver and adipose tissue, to the various tissues and organs for consumption and storage of the excess. Lipids (triacyl glycerol and cholesteryl esters), being hydrophobic are transported across blood plasma (an aqueous medium) by
complexing them with amphipathic lipids (phospholipids and cholesterol) and proteins, thereby forming water miscible lipoproteins. Lipoproteins serve a dual function: (a) to keep the lipids in soluble form as they transport them in the plasma and (b) transport lipids from the intestines as chylomicrons and from the liver as very low density lipoproteins (VLDL) to most tissues for oxidation and adipose tissue for storage. Free fatty acids are mobilized from the adipose tissue and are bound to albumin in circulation. Defective production or utilization of lipoproteins leads to hypo-or-hyper- lipoproteinemias, e.g. In diabetes mellitus, deficiency of insulin leads to excessive mobilization of FFA from the adipose tissue and under utilization of chylomicrons and VLDL, causing hypertriacylglycerolemia.

Triacylglycerols, cholesterol, phospholipids and cholesteryl esters, are the major lipid components of lipoproteins (Table 2). Based on their differences in density lipoproteins can be separated by ultra centrifugation into four major groups (Table 3):
1. Chyomicrons
2. Very low density lipoproteins (VLDL or pre-β-lipoproteins)
3. Low density lipoproteins (LDL or β-lipoprotein) and
4. High-density lipoproteins (HDL or α-lipoproteins).

**Table 2: Composition and characteristics of the human plasma lipoproteins**

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% particle mass)</td>
<td>2</td>
<td>7</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Triacylglycerols (% particle mass)</td>
<td>83</td>
<td>50</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Cholesterol (% particle mass; free + esterified)</td>
<td>8</td>
<td>22</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>Phospholipids (% particle mass)</td>
<td>7</td>
<td>20</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>&lt;0.95</td>
<td>0.95 – 1.006</td>
<td>1.019 – 1.063</td>
<td>1.063 – 1.210</td>
</tr>
<tr>
<td>Apolipoproteins</td>
<td>AI, AII, AIV, B-48, CI, CII, CIII, E</td>
<td>B-100, CI, CII, CIII, E</td>
<td>B-100</td>
<td>AI, AII, AIV, CI, CII, CIII, D, E</td>
</tr>
</tbody>
</table>

**Table 3: Classes of plasma Lipoproteins based on their density**

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Density (g/ml)</th>
<th>Molecular Weight (Daltons)</th>
<th>Particle Diameter (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>1.063 – 1.210</td>
<td>HDL₂ 4 x 10⁵</td>
<td>70-130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDL₃ 2 x 10⁵</td>
<td>50-100</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019 – 1.063</td>
<td>2 x 10⁶</td>
<td>200-280</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006 – 1.019</td>
<td>4.5 x 10⁶</td>
<td>250</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.95 – 1.006</td>
<td>5 x 10⁶-10⁷</td>
<td>250-750</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>&lt;0.95</td>
<td>10⁸-10¹⁰</td>
<td>10³-10⁴</td>
</tr>
</tbody>
</table>
Each type of lipoprotein has a characteristic molecular mass, size, chemical composition, density and physiological role. The protein and lipid in the complex are held together by non-covalent forces.

Based on their electrophoretic properties, lipoproteins are resolved into α; β-and pre-β-lipoproteins and chylomicrons by agarose gel electrophoresis of plasma lipoproteins (Fig.30).

![Agarose gel electrophoresis of plasma lipoproteins](image)

**Fig.30: Agarose gel electrophoresis of plasma lipoproteins**

The protein components of lipoprotein molecules are the apolipoproteins. Some apolipoproteins are integral and cannot be removed whereas others are free to transfer to other lipoproteins (Fig. 31). Each type of lipoprotein has a characteristic apolipoprotein composition. Apolipoprotein A-1 (apoA-1) (Table. 4) is predominantly present in high density lipoproteins (HDLs) (α-lipoprotein) whereas Apo B is present in low density lipoproteins (LDL) (β-lipoprotein) and also in the intermediate density lipoproteins (IDL) and very low density lipoproteins (VLDL). Apo B of chylomicrons (B-48) is smaller than the apo B-100 of LDL and VLDL. The Apo C group of proteins is also present in IDLs and VLDLs.

Apolipoproteins CI, CII and CIII are small polypeptides, which can be exchanged freely between the different lipoproteins. The molecular weights of the apolipoproteins of the plasma lipoproteins vary from 6kDa (apo C-I) to 55 kDa for Apo B-100. Apo 100 is one of the longest single-chain polypeptides known with 4536 amino acids. Apo B-48 is formed from a part of the B-100 polypeptide by RNA editing, encoded by the same mRNA. In the intestine, a stop codon, which is not present in the genomic DNA is introduced at amino acid residue 2153 to release apo B-48. Apolipoproteins constitute nearly 60% of HDL and only 1% of chylomicrons.
Table 4: Apolipoproteins present in human plasma lipoproteins

<table>
<thead>
<tr>
<th>Apolipoproteins</th>
<th>Lipoproteins</th>
<th>Molecular mass (da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I</td>
<td>HDL, chylomicrons</td>
<td>28000</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>HDL, chylomicrons</td>
<td>17000</td>
</tr>
<tr>
<td>Apo A-IV</td>
<td>Secreted with chylomicrons, but transferred to HDL</td>
<td>46000</td>
</tr>
<tr>
<td>Apo B-100</td>
<td>LDL, VLDL, IDL</td>
<td>550000</td>
</tr>
<tr>
<td>Apo B-48</td>
<td>Chylomicrons, chylomicrons remnants</td>
<td>260000</td>
</tr>
<tr>
<td>Apo C-I</td>
<td>VLDL, HDL, chylomicrons</td>
<td>7600</td>
</tr>
<tr>
<td>Apo C-II</td>
<td>VLDL, HDL, chylomicrons</td>
<td>8916</td>
</tr>
<tr>
<td>Apo C-III</td>
<td>VLDL, HDL, chylomicrons</td>
<td>8750</td>
</tr>
<tr>
<td>Apo D</td>
<td>Subfraction of HDL</td>
<td>19300</td>
</tr>
<tr>
<td>Apo E</td>
<td>VLDL, HDL, chylomicrons, chylomicron remnants</td>
<td>34000</td>
</tr>
</tbody>
</table>

Some lipoproteins are glycoproteins, for e.g. about 5% of apo B consists of carbohydrate moieties. Apolipoprotein E (arginine-rich) is present in VLDL and HDL. It accounts for the broad β band in patients with type III hyperlipoproteinemia.

Apolipoproteins have several functions: (i) they aid in transport of lipids (ii) they are enzyme cofactors: e.g. apo A-I for lecithin cholesterol acyl transferase and C-II for lipoprotein lipase and (iii) they are ligands for binding of the lipoproteins to their receptors.
in tissues for e.g. apo B-100 and apo E for the LDL receptor, apo E for the remnant receptor, apo A-I from the HDL receptor.

**Metabolism of chylomicrons**

In the intestinal mucosal cells, the newly synthesized triacylglycerols and cholesteryl esters are packaged into chylomicrons. These chylomicrons carry dietary triacylglycerol, cholesterol, and cholesteryl esters and other lipids synthesized in the intestinal mucosal cells to the peripheral tissue. From the intestine they enter the lymphatic system, and the chylomicron-rich lymphatic fluid (found in the fed state) is called chyle.

Apolipoprotein-B (apo B) synthesized by ribosomes in the rough endoplasmic reticulum is incorporated into lipoproteins in the smooth endoplasmic reticulum, the site of triacylglycerol synthesis. Further lipid and carbohydrate moieties are added to the lipoproteins as they pass through the golgi. The chylomicrons are released from the intestinal cell by fusion of the secretory vacuole with the cell membrane (reverse pinocytosis). Chylomicrons enter the lacteals draining the intestine (the lymphatic system). These chylomicrons released by the intestinal mucosal cells are nascent in that they contain only apolipoprotein B-48. Only when they reach the plasma, they take up apo C and apo E polypeptides from HDL in circulation (Fig. 32).

![Fig. 32: Metabolism of chylomicrons](image)

[(A) apolipoprotein A; B-48, apolipoprotein B-48; (C), apolipoprotein C; (E), apolipoprotein E; HDL, high density lipoprotein; TG, triacylglycerol; C, cholesterol ester; P, phospholipids]

Chylomicrons are rapidly cleared from the circulation by hydrolysis of their triacylglycerols catalyzed by the enzyme lipoprotein lipase. This enzyme is located on the capillary walls of
the blood vessels, anchored to the endothelium by proteoglycan chains of heparan sulfate. Lipoprotein lipase is not significantly found in free blood circulation but an injection with heparin leads to its release into the circulation from its heparan sulfate binding. Hepatic lipase is an enzyme found on the endothelial cells of the liver. In contrast to lipoprotein lipase, hepatic lipase is nonreactive to chylomicrons but is involved in the metabolism of chylomicron remnant and HDL metabolism.

Phospholipids and apolipoprotein C-II are essential cofactors for lipoprotein activity. Apo C-II has a specific phospholipid binding site through which it is attached to the lipoprotein (chylomicrons and VLDL). On attachment of the lipoproteins to the lipoprotein lipase enzyme on the endothelial surface, the triacylglycerol is hydrolyzed to a diacylglycerol and further to a monoacylglycerol, which on complete hydrolysis yields free fatty acids and glycerol. Most of the free fatty acids are transported to the peripheral tissues and some remain in circulation bound to albumin (Fig. 33).

![Fig. 33: VLDL metabolism](source)
(A, apolipoprotein A; B-100, apolipoprotein B-100, C apolipoprotein C apolipoprotein C:E, apolipoprotein E; HDL, high density lipoprotein; TG, triacylglycerol; IDL, intermediate density lipoprotein; C, cholesterol and cholesterol esters; P, phospholipid. (Source: Concepts of Biochemistry 1st ed.)
In adipose tissue, insulin enhances the synthesis of lipoprotein lipase in adipocytes and its translocation to the endothelial surface. Lipoproteins lipase in the heart has a low Km for acylglycerol whereas the Km of the enzyme in adipose tissue is 10 times higher.

Lipoproteins lipase hydrolyzes about 90% of the triacylglycerol in chylomicrons followed by transfer of the apo C to HDL. The resultant lipoprotein called chylomicron remnant containing apo E is now relatively enriched in cholesterol and cholesteryl esters since most of its triacylglycerol is hydrolyzed (Fig. 32) The chylomicron remnants are taken up by the liver by receptor-mediated endocytosis by receptors specific for apo E and the cholesteryl esters and triacylglycerols are hydrolyzed and metabolized. Hepatic lipase acts as a ligand for the lipoprotein and also hydrolyses its triacylglycerol and phospholipids.

Metabolism of very low density lipoproteins (VLDLs)

VLDLs are formed in the liver and are involved in transport of hepatic triacylglycerol to the peripheral tissues. From the liver cells, VLDL is released by fusion of the secretary vacuoles with the cell membrane.

The nascent VLDL consists of apo-B-100 and A-1 and is rich in triacylglycerols. The VLDLs reach the hepatic sinusoids and thus enter the circulation where they take up apo C and apo E from HDL and are then metabolized (Fig. 33). The triacylglycerols in VLDLs are then hydrolyzed by lipoprotein lipase enzyme present on the endothelial surface of the peripheral tissues (similar to the chylomicron metabolism), finally forming VLDL remnants or intermediate density lipoproteins (IDL).

VLDL is the precursor of IDL, which in turn forms LDL. VLDL has a half-life of 3 hours in circulation. As VLDL passes through the circulation and reaches the peripheral tissues, its triacylglycerol is hydrolyzed rapidly by lipoprotein lipase which is activated by apo C-II, resulting in a decrease in its size and increase in density on release of fatty acids that are taken up by adipose tissue and muscle. Next, the apo C and apo E are transferred back to HDL. Cholesterol esters from HDL are now transferred to VLDL in exchange for phospholipids from the VLDL by cholesterol ester transfer protein (CETP). This protein is present in human plasma and also associated with HDL. CETP facilitates transfer of cholesteryl esters from HDL to VLDL, IDL and LDL, and thus enables the HDL to take up more and more cholesterol from the peripheral tissues.

The VLDL, after hydrolysis of its triacylglycerol after gaining cholesterol asters from HDL and transferring back the apo C to HDL, forms VLDL remnant or IDL (intermediate density lipoprotein) (Fig. .33). The main function of VLDL is to carry triglycerides from liver to the tissues for energy supply.

IDL is metabolized in two ways. It is either taken up directly by the liver via the LDL (apo B-100) receptor, or it is converted to LDL. LDL is taken up, via the LDL (B-100, E) receptor, by the extra hepatic tissues and liver.

Some of the main factors that enhance both the synthesis of triacylglycerol and VLDL secretion are (i) the fed state (ii) carbohydrate-rich diet (especially sucrose and fructose that lead to increased lipogenesis) (iii) alcohol consumption (iv) elevated levels of free fatty acids in circulation and (v) high insulin levels and low glucagon levels which promote fatty acids synthesis and esterification and inhibit fatty oxidation.
**LDL and LDL receptors**

Low-density lipoproteins (LDL), the main carriers of plasma cholesterol are mainly derived from VLDL, with only a small fraction released directly from liver. LDL has a half-life of approximately 2 days in blood. The main function of LDL is to transport cholesterol from the liver to the peripheral tissues. Elevated concentration of LDL predisposes an individual to cardiovascular disease.

LDL binds to the LDL receptors by the ligand apo B-100. The LDL receptors (apo B-100, E) are present on cell surfaces in specialized regions called clathrin-coated pits (Fig. 34). On binding of the ligand apo B-100 (in LDL) to the receptor, a transmembrane glycoprotein, the receptor-LDL complex is taken up by endocytosis. On fusion of these vesicles with lysosomes, the apoprotein and cholesterol esters in the LDL are hydrolyzed. The free cholesterol is retained in the cell and the LDL-receptors recycled back to the cell surface. This free cholesterol entering the cell (derived from diet) inhibits *de novo* synthesis of cholesterol by inhibiting the enzyme HMG CoA reductase. Esterification of cholesterol is enhanced by stimulating the activity of Acyl CoA: cholesterol acyl transferase (ACAT).

---

**Fig. 34:** Factors determining cholesterol homeostasis inside the cell  
(Source: Concepts of Biochemistry 1st ed.)

(LCAT, Lecithin: cholesterol acyl transferase; C cholesterol; CE, cholesterol; ester; A-1, apoprotein A-1; ACAT; acyl cholesterol acyl transferase)
The cholesterol transported inside the cell by LDL-cholesterol in the cell may enter one of these routes: (a) the free cholesterol is esterified into cholesterol esters (CE) catalyzed by the enzyme ACAT (b) it is incorporated into the cell membrane. (c) In adrenal cortex and gonads, the cholesterol is metabolized to steroid hormones (d) Formation of bile acids in liver (e) Removal of the cholesterol from the cell followed by its esterification with polyunsaturated fatty acids (PUFA) catalyzed by the enzyme lecithin cholesterol-acyl transferase (LACT), taken up by HDL\textsubscript{3} in circulation and finally, transported to liver. From the liver the cholesterol is either packaged into VLDL and enters circulation or is excreted as bile acids.

Role of liver in lipid transport and metabolism: The liver has a central role in regulating the plasma levels of LDL cholesterol, since a large number of LDL receptors are present in the liver. Both cholesterol synthesis (de novo) and retrieving free cholesterol from the lipoprotein remnants take place in the liver. Liver is the only organ, which excretes excess cholesterol via bile, thereby regulating the body cholesterol pool.

**Regulation of the LDL receptor**

The number of LDL (apo B-100, E) receptors on the cell surface is regulated by the requirement of cholesterol by the cell for various purposes such as synthesis of steroid hormones, incorporation in the cell membrane and bile acid synthesis. When the cholesterol level in a cell increases, the entry of further cholesterol leads to the down-regulation of the number of LDL receptors by inhibiting further synthesis of the receptors. Thus the apo B-100, E receptor when saturated is high regulated and its synthesis is inhibited by excess free cholesterol entering the cell but other low affinity LDL receptors exist which are not regulated. In addition, non-receptor mediated pathway or scavenger pathway also exists for entry of LDL cholesterol into the cell.

A deficiency of functional LDL receptors leads to significant elevation in plasma LDL, and thereby of plasma cholesterol, with triacylglycerol levels being normal, such as in familial type III hyperlipoproteinemia. Elevated LDL levels lead to the infiltration of LDL through arterial walls. Free radical induced oxidation of LDL leads to oxidative damage of LDL. The oxidized LDL is then scavenged by macrophages are laden which have high levels of scavenger receptor activity. When the macrophages are laden with cholesterol esters they are called foam cells. Unlike the native LDL entering the cell via the LDL receptor, the modified LDL taken up by macrophages does not regulate intracellular cholesterol levels hence cholesterol accumulates in these cells. The oxidized LDL disrupts normal endothelial function and also impairs nitric oxide synthesis and accelerates its degradation. Disruption of the endothelial lining leads to the entry of foam cells and their deposition in the connective tissue of the arterial walls. Lowering of LDL-cholesterol levels in the early stages enables reversal of atherosclerosis.

**Metabolism of HDL**

HDL is synthesized and secreted from two sources: liver and intestine (Fig. 35). The nascent HDL from intestine does not contain apo C or apo E but only apo A when it is freshly secreted. Liver being the site of synthesis of apo C and apo E, these apolipoproteins are transferred from liver HDL to intestinal HDL when the latter reaches the plasma. A major function of HDL, is to act as a repository for apo C and apo E that are essential for the metabolism of chylomicrons and VLDL (Fig. 32 and 33).
Fig 35: High-density lipoprotein (HDL) Metabolism
(Source: Concepts of Biochemistry 1st ed.)

(LCAT, Lecithin: cholesterol acyl transferase; C cholesterol; CE, cholesterol; ester, PL, phospholipids; FFA, free fatty acids; A-1 apoprotein A-1)

Newly secreted HDL (nascent) consists of discoid phospholipid bilayers containing apolipoproteins and free cholesterol. Lecithin: cholesterol acyl transferase (LCAT) and apo A-1 (the activator of LCAT) bind to the phospholipid disk. Enzymatic activity of LCAT converts surface phospholipid and free cholesterol into cholesteryl esters and lysolecithin. The cholesteryl esters being non-polar move into the interior of the bilayer, which is hydrophobic. Lysolecithin is bound to plasma albumin. This continuous process leads to the formation of a non-polar core that pushes the bilayer to generate a spherical HDL and the core is surrounded by a film of polar lipids and apolipoproteins. This process catalyzed by LCAT thus promotes removal of excess unesterified cholesterol from lipoproteins and from the tissues.

HDL cholesteryl ester is finally degraded in the liver and the apo A-1 is catabolized in the kidney. The cholesterol thus released may be repackaged into lipoprotein, from bile acids or secreted into bile for removal from the body.
HDL is involved in the transport of cholesterol from the peripheral tissues to the liver and the process is called reverse cholesterol transport (Fig. 35). HDL levels below 35mg/dl increases the risk for coronary artery disease.

HDL metabolism consists of the following sequence of reactions. HDL₃ takes up cholesterol and esterifies it, thus becoming bigger and less dense, forming HDL₂. The phospholipids and triacylglycerol in HDL₂ are hydrolyzed by hepatic lipase thereby releasing the cholesterol ester from HDL₂ in the liver. This leads to reformation of HDL₃, which enters the HDL cycle. Also, pre apo A-1 is released and enters the circulation forming pre β-HDL in association with phospholipid and cholesterol. Pre β-HDL is the most potent form of HDL in inducing cholesterol efflux from peripheral tissues to form discoidal HDL which on uptake of more cholesterol forms HDL₃. Excess of apo A-1 is degraded in the kidney. HDL₂ concentrations are inversely related to the incidence of coronary atherosclerosis, since higher the HDL₂ levels, greater the amount of cholesterol scavenged from the extra hepatic tissues thereby preventing cholesterol deposition in tissues which may lead to atherosclerosis. (HDL₃) or HDL₁ is observed in the blood of diet-induced hypercholesterolemic animals. HDL₁ is rich in cholesterol and has only apo E as its protein part. Its uptake is by the apo E remnant and also by the LDL receptor.

**Lipoprotein (a) [Lp(a)]:** Lipoprotein (a) is structurally identical to LDL except that it has an additions apolipoproteins moiety apolipoproteins (a), apo (a), which is covalently bound to apo B-100. The apo (a) of Lp (a) has 80% homology with plasminogen because of which it hinders the activation of plasminogen and impairs fibrinolysis thereby slowing down the dissolution of blood clots. This leads to intravascular thrombosis and predisposes the individual to heart attacks. Clinical studies are underway to determine the exact role of Lp(a) and to confirm its association with atherosclerosis.

**Hormonal regulation of fat mobilization**

In the adipose tissue, insulin primarily inhibits hormone-sensitive lipase, lowering the release of both free acids and glycerol. On the other hand, insulin promotes lipogenesis and acylglycerol synthesis and increases the complete oxidation of glucose to CO₂ via the HMP-shunt. Insulin increases the number of glucose transports on the plasma membrane by promoting their translocation from the golgi and thus increase the uptake of glucose in the adipocytes required for the hormonal action of insulin. Insulin also enhances the activity of the enzymes pyruvate dehydrogenase, acetyl-CoA carboxylase and glycerol phosphate acyltransferase, which are regulated by covalent modification (phosphorylation – dephosphorylation).

Lipolysis, i.e. the catabolism of triacylglycerols stores in the adipose tissue leads to release of free fatty acids from the adipose from the adipose tissue and elevated plasma fatty acid levels. Hormones promoting lipolysis are mainly epinephrine, glucagon, norepinophrine, growth hormone (GH), adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), thyroid stimulating hormone (TSH) and vasopressin. Most of these hormones activate the hormone-sensitive lipase. Lipolysis optimally occurs in presence of glucocorticoids and thyroid hormones.

Catecholamines (epinephrine and norepinephrine) activate adenylyl cylase, which converts ATP to cAMP. cAMP activates a cAMP dependent protein kinase which in turn activates the hormone-sensitive triacylglycerol lipase to its active form. Thus cAMP levels regulate
the extent of lipolysis. cAMP is inactivated to 5’-AMP by the enzyme cyclic 3’, 5’-nucleotide phosphodiesterase. The phosphodiesterase enzyme is inhibited by caffeine and theophylline and is stimulated by insulin. Thus insulin plays a major role in regulation of fat metabolism in the adipose tissue.

**Brown adipose tissue** is mainly involved in thermogenesis. This tissue is rich in mitochondria and cytochromes and has a well developed blood supply; ATP synthase activity is however low. Lipolysis is stimulated in the brown adipose tissue by norepinephrine. Oxidation of both glucose and fatty acids occurs but the process of oxidation is not coupled to phosphorylation in the mitochondria of this tissue with only substrate level phosphorylation taking place in glycolysis and at the succinate thiokinase step. Oxidation therefore leads to heat generation and almost no ATP production. Thermogenin is the uncoupling protein present in the brown adipose tissue, which is normally responsible for dissipating the proton gradient which is present across the mitochondria membrane in the white adipose tissue.

**Disorders of plasma lipoproteins**

Hyperlipoproteinemias are disturbances of lipid transport caused by abnormalities in the synthesis or degradation of plasma lipoproteins. The elevated levels of plasma lipoproteins may be predisposing factors for life-threatening diseases such as atherosclerosis and pancreatitis.

Primary hyperlipoproteinemias are caused due to defects in the synthesis or degradation of lipoprotein particles. The primary hyperlipoproteinemias occur very rarely.

Secondary hyperlipoproteinemias arise due to indirect effects of e.g. diet, use of drugs or alcohol, disease of metabolic, hormonal, infectious or malignant etiology. Most of the hyperlipoproteinemias are of the secondary type. Elevated levels of serum cholesterol and triglycerides are observed in diabetes, nephrotic syndrome, hypothyroidism etc. Normal levels of serum cholesterol but elevated serum triglycerides are found in pregnancy, alcoholism and individuals using oral contraceptives. According to Fredrickson’s classification there are five major types of hyperlipoproteinemia (Table 5).

**Hypolipoproteinemia**

Abeta lipoproteinemia (rare): caused by defect in triacylglycerol transfer protein due to which lipids are not added to apo B. Absence or very less of chylomicrons, VLDL and LDL leads to accumulation of acylglycerols in liver and intestine.

**Familial alpha-lipoprotein deficiency (Tangier’s disease)**

This defect was first observed in patients from Tangier island in Africa. It is an autosomal dominant condition, caused due to accelerated degradation of apo-A-1 and HDL. All patients have low or no HDL.

**Familial hypobetalipoproteinemia**

LDL concentrations are low, 10-60% of that observed is normal subjects.
Table 5: Frederickson’s classification of hyperlipoproteinemia

<table>
<thead>
<tr>
<th>Type</th>
<th>Lipoprotein fraction elevated</th>
<th>Electrophoresis pattern (Agarose or paper)</th>
<th>Metabolic defect</th>
<th>Example of primary hyperlipoproteinemia</th>
<th>Example of secondary hyperlipoproteinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Chylomicrons (triglycerides)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>Lipoprotein lipase deficiency or abnormal synthesis, or apo C-II deficiency</td>
<td>Familial hyperlipase deficiency (type I)</td>
<td>-</td>
</tr>
<tr>
<td>Type IIA</td>
<td>LDL (cholesterol)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>LDL receptor defect or mutation in ligand region of apo B-100</td>
<td>Familial hypercholesterolemia</td>
<td>Hypothyroidism, nephrotic syndrome Cushing’s disease</td>
</tr>
<tr>
<td>Type IIB</td>
<td>LDL and VLDL (cholesterol and triglycerides)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>Both VLDL and LDL elevated excess of apo B</td>
<td>Familial combined hyperlipidemia (Type II)</td>
<td>Same as those for type IIA</td>
</tr>
<tr>
<td>Type III</td>
<td>Chylomicrons and VLDL remnants (cholesterol and triglycerides)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>Abnormal apo E patients have only E2 of the three isoforms E2, E3, and E4 which has less affinity for E receptor</td>
<td>Familial type III hyperlipoproteinemia (broad beta disease)</td>
<td>Same as for type IIA</td>
</tr>
<tr>
<td>Type IV</td>
<td>VLDL (Triglycerides)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>Over production of VLDL probably caused by glucose intolerance and hyper-insulinemia</td>
<td>Familial hypertriglyceridemia</td>
<td>Von/Gierke’s disease, diabetes mellitus, alcohol, oral contraceptive induced</td>
</tr>
<tr>
<td>Type V</td>
<td>Chylomicrons and VLDL (Triglycerides and cholesterol)</td>
<td><img src="#" alt="Electrophoresis pattern" /></td>
<td>Secondary to other causes, or apoprotein CII deficiency</td>
<td>Familial type V hyperlipoproteinemia</td>
<td></td>
</tr>
</tbody>
</table>
Lipids being hydrophobic, non-polar lipids must be combined with polar (amphipathic) lipids such as phospholipids and proteins to form lipoproteins. Lipoproteins are water miscible and are vehicles for the transport of lipids between the tissues via blood plasma. There are four major classes of lipoproteins. Chylomicrons originate in the intestine and transport dietary lipids to the peripheral tissues and finally to the liver. Very low density (VLDL) lipoproteins are synthesized in liver and transport triacylglycerol from the liver. In plasma (circulation), after hydrolysis of most of their triglycerides, VLDL is converted to low density lipoproteins (LDL). LDLs are cholesterol-rich and are the major transporters of cholesterol to the tissues. High density lipoproteins (HDL) are synthesized both the liver and intestine and are involved in reverse-cholesterol transport from the peripheral tissues to the liver.

Lipoprotein lipase is the enzyme present in peripheral tissues, which is responsible for hydrolysis of the triacylglycerols in chylomicrons and VLDL. The remnants (both chylomicrons and VLDL remnants) are taken up the liver by receptor-mediated endocytosis. Part of the VLDL forms intermediate density lipoprotein (IDL), which either forms LDL or is taken up by the liver. LDL is the major transporter of cholesterol and is taken up by the tissues via the LDL (apo B-100, E) receptors.

Apolipoproteins are the protein part of the lipoproteins. They function as either enzyme activators (apo C-II for lipoprotein lipase enzyme and apo A-1, for LCAT) or as ligands for receptors on cell surface (such as apo A-1, apo E, and apo B-100).

Triacylglycerols are the main forms of storage lipids in the adipose tissue. Triacylglycerols are hydrolyzed by hormone-sensitive lipase in the adipose tissue to release free fatty acids and glycerol into circulation. Free fatty acids are transported to the tissues for fuel and are bound to albumin while in plasma. Insulin inhibits hormone sensitive lipase whereas this enzyme is stimulated by epinephrine and norepinephrine.

Brown adipose tissue is present mainly in hibernating animals and to a small extent in humans and is responsible for diet-induced thermogenesis. Thermogenin, a protein, conducts protons through the inner mitochondrial membrane and thus uncouples oxidation from phosphorylation causing thermogenesis.

Inherited defects in the metabolism of lipoproteins are rare but lead to hypo or hyperlipoproteinemia (primary). More common are the secondary hyperlipoproteinemias caused due to conditions such as hypothyroidism, Diabetes Mellitus, Nephrotic syndrome and atherosclerosis.

**Synthesis of cholesterol**

Cholesterol is present only in animals and is absent in prokaryotes and plants. All nucleated cells of the body synthesize cholesterol and synthesis is in the microsomal and cytosol fraction of the cell. The major sites of synthesis of cholesterol are liver, adrenal cortex, testis, ovaries and intestine. Cholesterol is the precursor of all other steroids, bile acids and Vitamin D in the body and is an important constituent of the plasma membrane. More than 50% of the cholesterol in the body is synthesized de novo, and rest is provided by diet.

Typical of a steroid, cholesterol has a cyclopentanoperhydrophenanthrene ring structure, made up of three cyclohexane rings designated A, B, C and ring D a cyclopentane (Fig. 36).
Cholesterol is a 27-carbon containing steroid with a hydroxyl group at third carbon. Most of the cholesterol in plasma is in an esterified form, with a fatty acid attached at the 3rd carbon. A double bond is present between carbons 5 and 6. An eight carbon side chain is attached to the 17th carbon of the ring.

**Fig. 36. Structure of Cholesterol**
(Source: Concepts of Biochemistry 1st ed.)

Acetate is the precursor from which all the carbon atoms in cholesterol are derived and NADPH provides the reducing equivalents. Cholesterol synthesis occurs in the cytoplasm and the enzymes required for this biosynthetic pathway are located in the cytosol and endoplasmic reticulum.

The biosynthetic pathway consists of the following steps (Fig. 37)

1. Formation of HMG-CoA and mevalonate: The formation of HMG CoA is similar to the steps involved in the production of ketone bodies, both resulting in the production of 3-hydroxy-3-methylglutaryl CoA (HMG CoA). However, ketone body formation takes place in the mitochondria whereas cholesterol biosynthetic pathway distinctly occurs in the cytoplasm.

2. Synthesis of mevalonic acid: HMG CoA is reduced by the enzyme HMG CoA reductase with NADPH serving as the reducing agent, two molecules of which are utilized. This reaction occurs in the endoplasmic reticulum.

3. Cholesterol synthesis: Mevalonic acid (mevalonate) is converted to 5-pyrophospho mevalonate, in a two step process requiring ATP, which in turn on decarboxylation and in the presence of ATP forms a five Carbon isoprene unit called isopentenyl pyrophosphate (IPP). IPP is isomerized to dimethylallyl pyrophosphate (DPP). IPP and DPP condense to form geranyl pyrophosphate (GPP). Another molecule of IPP condenses with GPP to form farnesyl pyrophosphate (FPP)
Two molecules of the farnesyl pyrophosphate (a 15 carbon compound) combine and are then reduced to form (30-carbon compound) squalene, with the release of pyrophosphate. Squalene then cyclizes to form lanosterol in the presence of molecular oxygen and NADPH. Lanosterol is finally converted to cholesterol in a multistep process involving shortening of the carbon chain from 30 to 27, removal of the two methyl groups at C-4, migration of the double bond from C-8 to C-5, and reduction of the double bond between C-24 and C-25. The enzymes involved in all these reactions are located in the endoplasmic reticulum.

Fig. 37: Biosynthesis of cholesterol
(Source: Concepts of Biochemistry 1st ed.)
**Regulation of cholesterol synthesis**

The reaction catalyzed by the enzyme HMG CoA reductase is the rate-limiting step of the cholesterol biosynthetic pathway. The regulatory enzyme is HMG CoA reductase, which is feed back regulated by cholesterol by causing decrease in transcription of the HMG CoA reductase gene leading to a decrease in de novo cholesterol synthesis. The amount of cholesterol derived from the diet determines the cholesterol synthesis. Excess of dietary cholesterol inhibits the HMG CoA reductase enzyme. This enzyme is also regulated hormonally. Glucagon and cortisol promote the formation of the phosphorylated inactive form of the HMG CoA reductase thereby reducing the rate of cholesterol synthesis. In contrast, insulin promotes the formation of the dephosphorylated active form of HMG CoA reductase thus stimulating increased synthesis of cholesterol.

During lipoprotein metabolism, chylomicrons remnants internalized by the hypatocytes, and LDL (low density lipoproteins) internalized by hepatocytes and peripheral tissues, provide cholesterol inside the cell, which causes decreased transcription of the HMG CoA reductase gene, leading to a decrease in de novo cholesterol synthesis. Drugs belonging to the statin group (Lovastatin, simvastatin) are reversible, competitive inhibitors of HMG CoA reductase. They are administered to hypercholesterolemic patients to decrease plasma cholesterol levels.

The concentration of cholesterol in tissues and body fluids is determined by a balance between the rate of the cholesterol synthesis and rate of metabolism and excretion. About 700mg cholesterol is synthesized daily in humans and the total body cholesterol content varies between 50-150gms. In blood cholesterol is transported by LDL from the liver to the peripheral tissues and HDL is involved in transporting cholesterol from the peripheral tissues to the liver. Cholesterol derived from the diet is packaged into chylomicrons in the intestinal cells and enters the lymphatics and reaches the liver as a part of chylomicrons remnants.

**Factors determining the cholesterol balance inside cells**

Cells of extrahepatic tissues take up the cholesterol from LDL. Once inside the cell, the free cholesterol has the following fates (a) It is esterified to form cholesterol esters (CE) catalyzed by the enzyme ACAT (Acyl cholesterol acyl transferase) utilizing monounsaturated fatty acids for esterification and deposition of cholesterol esters in the cell. Some of the cholesterol is taken up by HDL, esterified by LCAT (using PUFA) for transport to liver and thereby get excreted. (PUFA: poly unsaturated fatty acids). When the cholesterol content increases in the cell, the transcriptions of the gene for HMG CoA reductase is suppressed and do novo cholesterol biosynthesis inside the cell is reduced. Also elevated cholesterol inside the cell, downregulates the LDL receptor synthesis leading to reduced number of LDL receptors in the cell surface, thus preventing further uptake of circulating LDL-cholesterol. The level of cholesterol in blood is of primary importance due to its role in the development of atherosclerosis.

Cholesterol is eliminated from the body by the following ways (a) conversion to bile acids which get excreted in the feces and (b) secretion of cholesterol into bile, which transports it to the intestine for elimination. In the intestine some of the secreted cholesterol is acted upon by bacteria to form coprostanol and which along cholesterol make up the bulk of neutral fecal sterols.
Bile acids: The most common bile acids are cholic acid and chenodeoxycholic acid (Fig. 38). The bile acids are 24-carbon unit compounds with two to three hydroxyl groups and a side chain with a terminal carboxyl group. The carboxyl group with a pka of ~6 is not fully ionized at physiological pH. The bile acids are amphipathic with both polar and nonpolar groups and hence act as emulsifying agents in the intestine aiding in fat digestion.

Bile acid synthesis takes place in the liver via a multistep pathway involving introduction of hydroxyl groups at specific position on the steroid ring, reduction of the double bond between C-5 and C-6 and shortening of the hydrocarbon chain by the carbon and finally attaching a carboxyl group at the end of the chain.

The compounds formed are cholic and chenodeoxycholic acids also called the primary bile acids. The rate-limiting step in bile acid synthesis is the introductions of a hydroxyl group at carbon 7 of the sterol ring by the enzyme 7-α-hydroxylase (a monooxygenase) a microsomal enzyme, which requires NADPH, oxygen and cytochrome P450 for its activity. This enzyme is inhibited by cholic acid and deficiency of vitamin C.

The bile acids once formed in the liver are conjugated with either glycine or taurine by forming an amide bond between the carboxyl group of the bile acid and the amino group of glycine or taurine, resulting in the formation of the bile salts glycocholic and glycochenodeoxycholic acids, and taurocholic and taurochenodeoxycholic acid. Addition of glycine or taurine results in the presence of a carboxyl group with a lower pka (from glycine) or of the sulfate group (from taurine), both being fully ionized at physiological pH. Only the conjugated forms i.e. the bile salts are present in bile.

Secondary bile acids: In the intestine, bacteria cleave off glycine and taurine from the bile salts. They also form secondary bile acids by removal of a hydroxyl group from the primary
bile acids resulting in the formation of dixyloc acid from cholic and lithocholic acid from chenodeoxy cholic acid.

The bile salts from the liver, once secreted into the bile entering the intestine are reabsorbed from the ileum by active transport. This continuous process of secretion of bile salts into the bile, their passage through the duodenum where some are converted to bile acids, and their subsequent return to the liver via the portal blood are termed the enterohepatic circulation. All of the bile acids, except lithocholic acid, are effectively absorbed. Lithocholic acid is excreted in the feces.

Deficiency of bile salts leads to the formation of cholesterol gallstones-cholelithiasis. The secretion of cholesterol from the liver into bile should be accompanied by the simultaneous secretion of phospholipids and bile salts. If the concentration of phospholipids or bile salts in the bile is not appropriate and more cholesterol is secreted in the bile than can be solubilized by the bile salts in the gall bladder, initiating the formation of gall stones (cholelithiasis) takes places.

**Summary**

Cholesterol a 27-carbon steroid with a typical sterol ring (cyclipentanoperhydrophenanthrene nucleus) is present in all nucleated cells of animals but absent in plants. Cholesterol being amphipathic is an important component of the biological membrane and the outer surface of lipoproteins for maintaining the structural integrity. Cholesterol is the precursor of all other steroids in the body, i.e., sex hormones, corticosteroids, bile acids and Vitamin D. The amount of cholesterol derived from the diet (via cholesterol rich chylomicron remnants) determines to some extent, its de novo synthesis in the liver. In tissues, a cholesterol balance is maintained between the factors leading to cholesterol content inside the cell (such as synthesis, uptake via the LDL or scavenger receptors, hydrolysis of cholesteryl ester) and the factors reducing the cholesterol levels inside the cell (such as synthesis of steroids, formation of cholesteryl esters, reverse cholesterol transport by HDL). High concentration of cholesterol in the cell causes down regulation of the LDL-receptor (reducing its synthesis) and vice versa. Bile acids and salts are synthesized in the liver, and the hydroxylation step catalyzed by the monooxygenase enzyme 7-α-hydroxylase is the committing step in bile acid biosynthesis. The primary bile acids are cholic acid and chenodeoxycholic acid. Deoxycholic acid formed from colic acid and lithocholic acid formed from chenodeoxycholic acid is the secondary bile acids. The glycine or taurine conjugates of the bile acids are called bile salts.

Excess cholesterol is executed from the liver into the bile as cholesterol or bile salts. A large proportion of bile salts is absorbed at the ileum into the portal circulation and returned back to the liver (enterohepatic circulation).

**Suggested Reading**