UNIT – II: Lipid metabolism 1 ***Lipid metabolism:*** *Oxidation of saturated (□-oxidation); Ketogenesis and ketolysis; biosynthesis of fatty acids, lipids; metabolism of cholesterol; Hormonal regulation of lipid metabolism. Defective metabolism of lipids (Atheroslerosis, fatty liver, hypercholesterolmiea).*

- Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by nonpolar solvents, because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles or on albumin. Lipids are a major source of energy for the body, and they provide the hydrophobic barrier. Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.
- **Classification of lipids:**

1. *Simple lipids*: Esters of fatty acids with various alcohols.

a. *Fats:* Esters of fatty acids with glycerol. **Oils** are fats in the liquid state.
b. *Waxes:* Esters of fatty acids with higher molecular weight monohydric alcohols.

2. *Complex lipids*: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

a. *Phospholipids:* Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, eg, in **glycerophospholipids** the alcohol is glycerol and in **sphingophospholipids** the alcohol is sphingosine.
b. *Glycolipids (glycosphingolipids):* Lipids containing a fatty acid, sphingosine, and carbohydrate.
c. *Other complex lipids:* Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

3. *Precursor and derived lipids:* These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins and hormones.

- Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as **free fatty acids,** a transport form found in the plasma. Fatty acids that occur in natural fats are usually straight-chain derivatives containing an even number of carbon atoms. The chain may be **saturated** (containing no double bonds) or **unsaturated** (containing one or more double bonds).

- *Saturated Fatty Acids* may base on acetic acid (CH_3COOH) as the first member of the series in which **-CH_2-** is progressively added between the terminals **-CH_3-** and **-COOH-** groups.
- *Unsaturated Fatty Acids* contain one or more double bonds and it may be further subdivided as follows:

(1) Monounsaturated (monoethenoid, monoenoic) acids, containing one double bond.
(2) Polyunsaturated (polyethenoid, polyenoic) acids, containing two or more double bonds.
(3) Eicosanoids: These compounds, derived from eicosa- (20-carbon) polyenoic fatty acids, comprise the **prostanoids, leukotrienes (LTs),** and **lipoxins (LXs).** Prostanoids include **prostaglandins (PGs), prostacyclins (PGIs),** and **thromboxanes (TXs).**

UNIT – II: Lipid metabolism

- ***Saturated fatty acids.***
- ***Unsaturated Fatty Acids***

- ***Chemistry of Fatty acids***

- The carbon chains of saturated fatty acids form a zigzag pattern when extended, as at low temperatures. At higher temperatures, some bonds rotate, causing chain shortening,
- A type of **geometric isomerism** occurs in unsaturated fatty acids, depending on the orientation of atoms or groups around the axes of double bonds, which do not allow rotation. If the acyl chains are on the same side of the bond, it is ***cis-***, as in **oleic acid**; if on opposite sides, it is ***trans-***, as in **elaidic acid**, the ***trans*** isomer of **oleic acid**.
- Naturally occurring unsaturated long-chain fatty acids are nearly all of the ***cis*** configuration, the molecules being **"bent"** 120 degrees at the double bond. Thus, **oleic acid** has an **L shape**, whereas **elaidic acid** remains **"straight."**
- Increase in the number of *cis* double bonds in a fatty acid leads to a variety of possible like **arachidonic acid**, with four ***cis*** double bonds, has **"kinks"** or a **U shape**.
- ***Trans*** double bonds alter these spatial relationships.
- The melting points of even-numbered-carbon fatty acids increase with chain length and decrease according to unsaturation.

Trans and Cis- Fatty acid
Saturated Fatty acid Unsaturated Fatty acid

UNIT – II: Lipid metabolism

- ***Structures of some common classes of lipids***

Note By: ► Naturally, occurring unsaturated vegetable oils have almost all **Cis** bonds, but using oil for frying causes some of the **Cis** bonds to convert to **Trans** bonds.
► Fatty acids with **Tran's** bonds are **carcinogenic**.

UNIT – II: Lipid metabolism

- ***Digestion, Absorption, Secretion, and Utilization of Dietary Lipids***

The average daily intake of lipids for an adult is about 81 g, of which more than 90% is normally triacylglycerol (TAG-triglyceride). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and unesterified ("free") fatty acids. **UNIT – II: Lipid metabolism**

Emulsification of lipid in the small intestine

- The critical process of emulsification of lipids occurs in the duodenum. Emulsification is accomplished by the bile salts, and mechanical mixing due to peristalsis. Bile salts, made in the liver and stored in the gallbladder, are derivatives of cholesterol.
- Bile salts, produce in the liver and stored in the gallbladder, are derivatives of cholesterol. They consist of a sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage. These emulsifying agents interact with the dietary lipid particles and the aqueous duodenal contents, thereby stabilizing the particles as they become smaller, and preventing them from coalescing.

- ***Use of dietary lipids by the tissues***

- Triacylglycerol contained in chylomicrons is broken down primarily in the capillaries of skeletal muscle and adipose tissues, but also those of the heart, lung, kidney, and liver. Triacylglycerol in chylomicrons is degraded to free fatty acids and glycerol by *lipoprotein lipase*. Primarily adipocytes and muscle cells synthesize this enzyme. The free fatty acids derived from the hydrolysis of TAG be transported in the blood in association with serum albumin and they are taken up by cells where it oxidize fatty acids to produce energy.

- Glycerol that is released from TAG is used almost exclusively by the liver to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate.

Note By: ***Chylomicrons*** are large lipoprotein particles that transport dietary lipids from the intestines to other locations in the body. Chylomicrons are one of the 5 major groups of lipoproteins which enable fats and cholesterol to move within the water based solution of the blood stream. Chylomicrons transport exogenous lipids to liver, adipose, cardiac and skeletal muscle tissue.

- ***Essential fatty acids***

- Two fatty acids are dietary essentials in humans

i. **Linoleic acid**, which is the precursor of arachidonic acid, the substrate for prostaglandin synthesis.
ii. **α-linolenic** acid is the precursor for growth and development.

- Essential fatty acid deficiency can result in a scaly dermatitis, as well as visual and neurologic abnormalities.

Linolenic acid Linoleic acid

UNIT – II: Lipid metabolism

- ***Fatty Acid and Triacylglycerol Metabolism***

- Fatty acids exist "free" in the body and are found as fatty acyl esters in more complex molecules, such as triacylglycerols. Low levels of free fatty acids occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting.
- Free fatty acids can be oxidized by many tissues particularly liver and muscle to provide energy. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids.
- Fatty acids are also precursors of the hormone-like prostaglandins.
- Esterified fatty acids, in the form of triacylglycerols stored in adipose cells, serve as the major energy reserve of the body.
- Triglyceride breakdown is facilitated by three enzymes:

i. Triacylglycerol lipase (which is activated by epinephrine)
ii. Diacyclglycerol lipase
iii. Monoacylglycerol lipase

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- ***Release of fatty acids from TAG***

- The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process is initiated by hormone-sensitive lipase, which removes a fatty acid from carbon 1 and/or carbon 3 of the TAG. Additional lipases specific for diacylglycerol or monoacylglycerol remove the remaining fatty acids.
- *Hormonal control of lipolysis:* The breakdown of triglycerides by lipases is under hormonal control. The main enzymes involved are epinephrine, glucagon and insulin. Epinephrine and glucagon promote the breakdown of fat (lipolysis) while insulin inhibits fat breakdown.

FAT
Epinephrine (+) ↓↑ (+) Insulin
FATTY ACID

Hormonal regulation of triacylglycerol degradation

- Hormone-sensitive lipase (HSL) is activated when phosphorylated by a 3',5'-cyclic AMP(cAMP)–dependent protein kinase.
- 3',5'-Cyclic AMP is produced in the adipocyte by several hormones like as epinephrine or glucagon binds to receptors on the cell membrane, and activates *adenylyl cyclase*.

- Fatty acid synthesis is turned off when TAG degradation is turned on. In the presence of high plasma levels of insulin and glucose, HSL is dephosphorylated, and becomes inactive.
- The glycerol released during TAG degradation cannot be metabolized by adipocytes because they apparently lack *glycerol kinase*. Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated. The resulting glycerol phosphate can be used to form **TAG** in the liver, or can be converted to **DHAP** by the *glycerol phosphate dehydrogenase*.

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- ***Various pathway generation during triacylglycerol degradation***

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- ***β-Oxidation of fatty acids***

- The major pathway for catabolism of even-numbered saturated fatty acids is a mitochondrial pathway called β-oxidation. In which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and $FADH_2$.
- In β-oxidation, the fatty acid is broken down to release acetyl-CoA. The process involves 4 main steps:

i. Dehydrogenation
ii. Hydration
iii. Oxidation
iv. Thiolysis

- Beta-oxidation of fatty acids takes place in the mitochondrial matrix for the most part. However, fatty acids have to be activated for degradation by ***coenzyme A*** by forming a **fatty acyl-CoA thioester**. For short and medium length fatty acids, they undergo this reaction in the mitochondria. The long chain fatty acids can't go through the membrane though, so this reaction occurs at the outer mitochondrial membrane.
- The final fatty acid products are **acetyl-CoA** for the even numbered fatty acids (without double bonds), and for those with an odd number of carbons, it is **3-carbon propionyl-CoA**.

A. Beta-Oxidation of Fatty Acids (even chain)

1. *Dehydrogenation* (Acyl-CoA Dehydrogenase): This first reaction is the oxidation of the **C_a-C_b** bond. It is catalyzed by *acyl-CoA dehydrogenases*. This catalyst is a family of three soluble matrix enzymes. These enzymes carry noncovalently bound FAD that is reduced during the oxidation of the fatty acid.

2. *Hydration* (Enoyl-CoA Hydratase): In this pathway is one in which water is added across the new double bond to make **hydroacyl-CoA**. The catalyst in this reaction is *Enoyl-CoA hydratase*. This is also called a *crotonase* and it converts **trans-enoyl-CoA** to **L-B-Hydroxyacyl-CoA**. This reaction would be classified as a hydration reaction because you are adding water.

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3. *Oxidation* (L-Hydroxyacyl-CoA Dehydrogenase): Here the oxidation of the hydroxyl group at the beta position which forms a **beta-ketoacyl-CoA** derivative and it is catalyzed by *L-Hydroxyacyl-CoA Dehydrogenase*. This enzyme needs to have **NAD^+** as a coenzyme and the **NADH** produced represents metabolic energy because for every NADH produced, it drives the synthesis of 2.5 molecules of ATP in the electron transport pathway. So, this reaction is classified as an oxidation reaction.

Mechanism of L-Hydroxyacyl-CoA Dehydrogenase

4. *Thiolysis:* This is the final reaction of this pathway and *thiolase* catalyzed this reaction. This reaction cleaves the **β-ketoacyl-CoA**. The products of this reaction are an **acetyl-CoA** and a **fatty acid** that has been shortened by two carbons. So, this reaction is classified as a **cleavage reaction**.

- Repetition of the Beta Oxidation Cycle: The shortened fatty acyl-CoA that was the product of the last reaction now goes through another beta-oxidation cycle. This keeps happening until eventually you wind up with two molecules of acetyl-CoA in the final step. This acetyl-CoA is then available to be further metabolized in the TCA cycle, or it can be used as a substrate in amino acid biosynthesis. It cannot be used as a substrate for gluconeogenesis.

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B. Beta oxidation of fatty acid with an odd number of carbons

- Chains with an odd-number of carbons are oxidized in the same manner as even-numbered chains, but the final products are **propionyl CoA** and **acetyl CoA**.

- **Propionyl CoA** is converted into **succinyl CoA** (which is an intermediate in the citric acid cycle) in a reaction that involves **Vitamin B_{12}**.

- **Succinyl CoA** can then enter the citric acid cycle. Because it cannot be completely metabolized in the citric acid cycle, the products of its partial reaction must be removed in a process called **cataplerosis**. This allows regeneration of the citric acid cycle intermediates, possibly an important process in certain metabolic diseases.

- Animals *cannot* make glucose from even carbon fatty acids. The only scope for glucose synthesis from **fatty acids** is from the **propionyl CoA** left behind after the beta-oxidation of odd carbon fatty acids.

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

The ATP yield for every oxidation cycle is 14 ATP, broken down as follows:
1 $FADH_2$ x 1.5 ATP = 1.5 ATP
1 NADH x 2.5 ATP = 2.5 ATP
1 acetyl CoA x 10 ATP = 10 ATP
For an even-numbered saturated fat **(C_{2n}), n - 1** oxidations are necessary and the final process yields an additional acetyl CoA. In addition, two equivalents of ATP are lost during the activation of the fatty acid. Therefore, the total ATP yield can be stated as: **(n - 1) * 14 + 10 – 2= No. of ATP**
For instance, the ATP yield of **Palmitate** (C_{16}, $n = 8$) is:
(8 - 1) * 14 + 10 – 2=**106 ATP**
Or
7 $FADH_2$ x 1.5 ATP = 10.5 ATP
7 NADH x 2.5 ATP = 17.5 ATP
8 acetyl CoA x 10 ATP = 80 ATP
ATP equivalent used during activation = -2

- ***BIOSYNTHESIS OF FATTY ACIDS***

- **Fatty acid synthesis** is the creating of fatty acids from **acetyl-CoA** and **malonyl-CoA** precursors through action of enzymes called ***fatty acid synthases***. It is an important part of the lipogenesis process, which - together with **glycolysis** stands behind creating fats from blood sugar in living organisms.
- Synthesis takes place in the cytosol
- In humans, fatty acids are predominantly formed in the **liver** and **lactating mammary glands**, and, to a lesser extent, the **adipose tissue**.
- Most **acetyl-CoA** is formed from **pyruvate** by *pyruvate dehydrogenase* in the mitochondria. **Acetyl-CoA** produced in the mitochondria is condensed with **oxaloacetate** by *citrate synthase* to form **citrate**, which is then transported into the cytosol and broken down to yield **acetyl-CoA** and **oxaloacetate** by *ATP citrate lyase*. **Oxaloacetate** in the cytosol is reduced to **malate** by cytoplasmic *malate dehydrogenase*, and **malate** is transported back into the mitochondria to participate in the **Citric acid cycle**.
- The process involves 4 main steps: 1. Condensation, 2. Reduction, 3. Dehydration & 4. Reduction

***Acyl carrier protein* (ACP):** The acyl carrier protein (ACP) is an important component in both fatty acid and polyketide biosynthesis

- ***DIFFERENCES BETWEEN FATTY ACID DEGRADATION AND SYNTHESIS***

Characteristic	***Degradation***	***Synthesis***
Location	Mitochondrial Matrix	Cytosol
Activated intermediates	Thioesters of CoA	Thioesters of ACP
Process	2-Carbon fragments removed as	2-Carbon elongation using malonyl

	acetyl CoA	CoA
Direction	Starts at carboxyl end	Starts at methyl end
Redox reaction cofactors	FAD/$FADH_2$ and NAD^+/NADH	NADP+/NADPH
Major tissue site	Muscle and liver	Liver
Hormonal regulation	Low insulin / glucagon ratio	High insulin/glucagon ratio
Activator	FFA generated by hormone-sensitive lipase	Citrate
Inhibitor	Malonyl CoA (inhibits carnitine acyl transferase)	Fatty acyl CoA (inhibits acetyl CoA carboxylase)