WELCOME TO

Biochemistry | Chapter-8(2)

Chapter-8

Metabolism (Study of cycle/pathways without chemical structures)

(BIOCHEMISTRY & CLINICAL PATHOLOGY)

Metabolism (Study of cycle/pathways without chemical structures)

Unit-1

- **Metabolism of Carbohydrates:** Glycolysis, TCA cycle and glycogen metabolism, regulation of blood glucose level. Diseases related to abnormal metabolism of Carbohydrates.

Unit-2

- **Metabolism of lipids: Lipolysis, β-oxidation of Fatty acid (Palmitic acid) ketogenesis and ketolysis. Diseases related to abnormal metabolism of lipids such as Ketoacidosis, Fatty liver, Hypercholesterolemia**

Unit-3

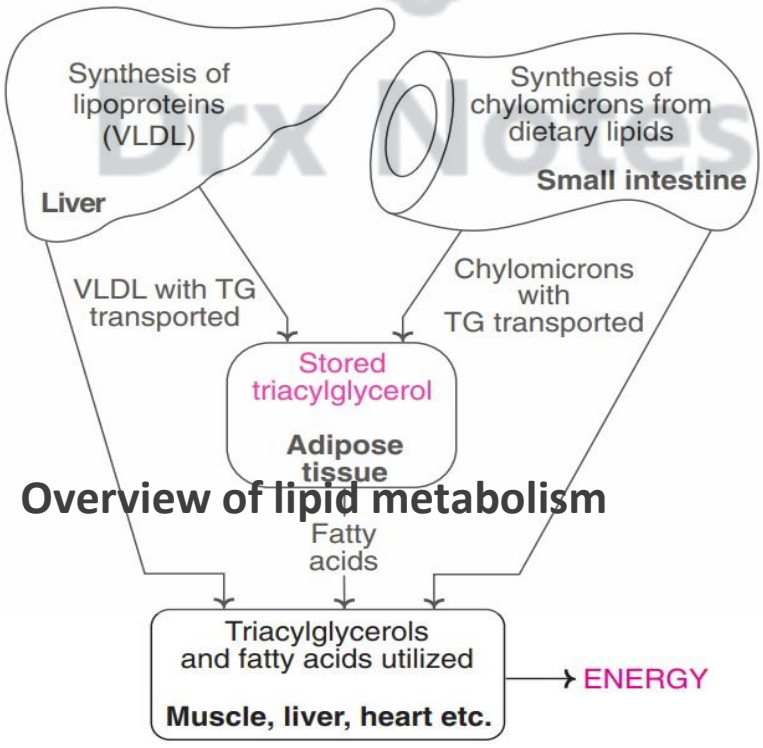
- **Metabolism of Amino acids (Proteins):** General reactions of amino acids and its significance– Transamination, deamination, Urea cycle and decarboxylation. Diseases related to abnormal metabolism of amino acids, Disorders of ammonia metabolism, phenylketonuria, alkaptonuria and Jaundice.
- **Biological oxidation:** Electron transport chain and Oxidative phosphorylation

Unit-2

Metabolism of lipid.

Lipids constitute about 15-20% of the body weight in humans. Triacylglycerols (formerly triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerols are stored in the adipose tissue and serve as energy reserve of the body. Triacylglycerols (TG) are highly concentrated form of energy, yielding 9 Cal/g, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form. Fat also acts as an insulating material for maintaining the body temperature of animals.

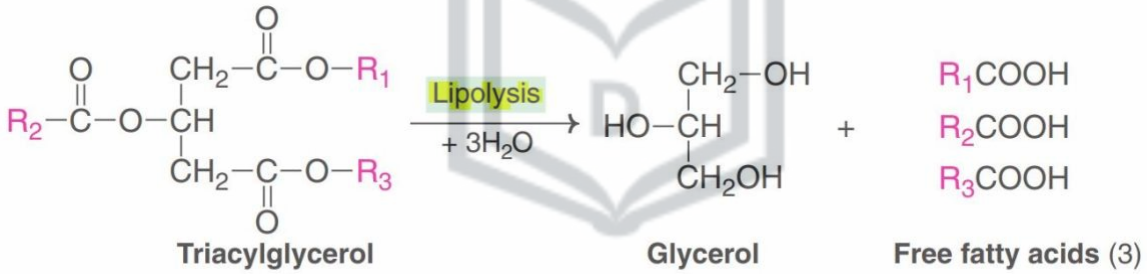
Transport of lipid— The insoluble lipids are solubilized in association with proteins to form lipoproteins in which form lipids are transported in the blood stream. Free lipids are undetectable in blood. Chylomicrons, very low-density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and albumin-free fatty acids are the different lipoprotein complexes that transport lipids in the blood stream.



Overview of lipid metabolism

Lipolysis

- Triacylglycerol (TG) is the stored fat in the adipose tissue. The enzyme, namely hormone sensitive triacylglycerol lipase, removes the fatty acid either from carbon 1 or 3 of the triacylglycerol to form diacylglycerol. The other two fatty acids of TG are cleaved by additional lipases specific for diacylglycerol and monoacylglycerol. The complete degradation of triacylglycerol to glycerol and free acids is known as lipolysis.
- Hormone-sensitive TG-lipase is so named because its activity is mostly controlled by hormones. Lipase is present in an inactive form 'b' and is activated (phosphorylated) by a cAMP dependent protein kinase to lipase 'a'. Several hormones—such as epinephrine (most effective), norepinephrine, glucagon, thyroxine, ACTH etc.— enhance the activity of



Complete hydrolysis (lipolysis) of triacylglycerol.

adenylate cyclase and, thus, increase lipolysis.

- **Fate of glycerol—** The adipose tissue lacks the enzyme glycerol kinase, hence glycerol produced in lipolysis cannot be phosphorylated here. It is transported to liver where it is activated to glycerol 3-phosphate. The latter may be used for the synthesis of triacylglycerols and phospholipids. Glycerol 3-phosphate may also enter glycolysis by getting converted to dihydroxyacetone phosphate.
- **Fate of free fatty acids—**The fatty acids released in the adipocytes enter the circulation and are transported in a bound form to albumin. The free fatty acids enter various tissues and are utilized for the energy. About 95% of the energy obtained from fat comes from the oxidation of fatty

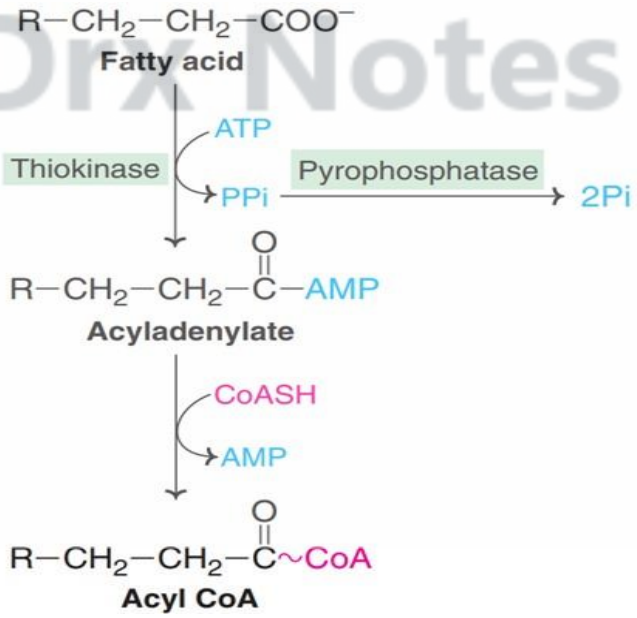
acids. Certain tissues, however, cannot oxidize fatty acids, e.g., brain, erythrocytes.

β-oxidation of Fatty acid (Palmitic acid).

Introduction—This process is known as beta-oxidation, because the oxidation and splitting of two carbon units occur at the beta-carbon atom. The oxidation of the hydrocarbon chain occurs by a sequential cleavage of two carbon atoms. Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement. The β-oxidation of fatty acids involves three stages.

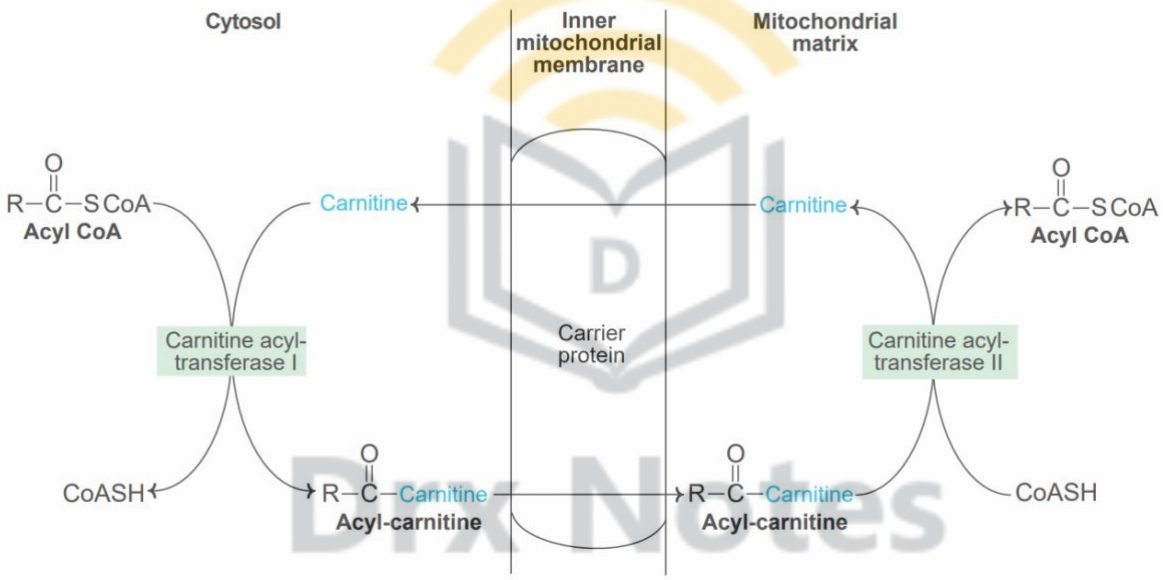
- Activation of fatty acids occurring in the cytosol.
- Transport of fatty acids into mitochondria.
- β-Oxidation proper in the mitochondrial matrix.

1. **Activation of fatty acids—** Fatty acids are activated to acyl CoA by thiokinase or acyl CoA synthetases. The reaction occurs in two steps and requires ATP, coenzyme A and Mg2+. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA. In the activation, two high energy phosphates are utilized, since ATP is converted to pyrophosphate (PPi). The enzyme inorganic pyrophosphatase hydrolyses PPi to phosphate (Pi). The immediate elimination of PPi makes this reaction totally irreversible.



Activation of fatty acid to acyl CoA by the enzyme thiokinase.

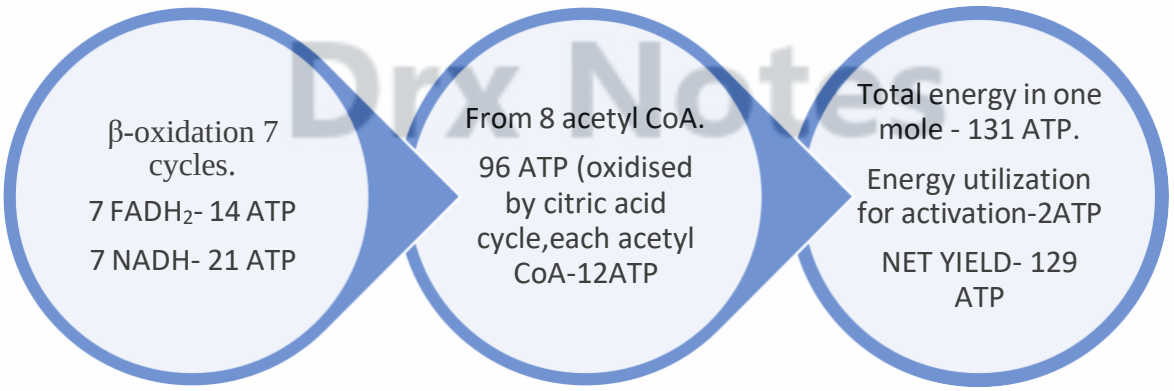
2. **Transport of fatty acids into mitochondria—** The inner mitochondrial membrane is impermeable to fatty acids. A specialized carnitine carrier system (carnitine shuttle) operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps.
    - Acyl group of acyl CoA is transferred to carnitine (β-hydroxy ϒ-trimethyl aminobutyrate), catalysed by carnitine acyltransferase I (present on the outer surface of inner mitochondrial membrane).
    - The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.
    - Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.
    - The carnitine released returns to cytosol for reuse



Carnitine shuttle for transport of activated fatty acid (acyl CoA) into mitochondria

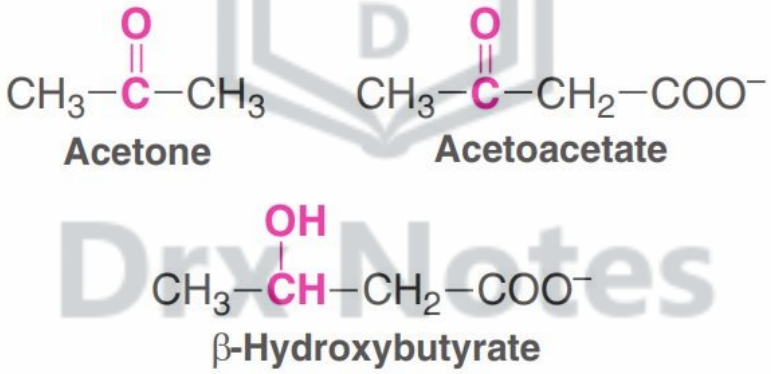
3. **β-Oxidation proper in the mitochondrial matrix—** Each cycle of β-oxidation, liberating a two-carbon unit-acetyl CoA, occurs in a sequence of four reactions.
    1. **Oxidation—**Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between α and β carbons (i.e., 2 and 3 carbons).
    2. **Hydration—** Enoyl CoA hydratase brings about the hydration of the double bond to form β-hydroxyacyl CoA.
    3. **Oxidation—** β-Hydroxyacyl CoA dehydrogenase catalyses the second oxidation and generates NADH. The product formed is β-ketoacyl CoA.
    4. **Cleavage—** The final reaction in β-oxidation is the liberation of a 2-carbon fragment, acetyl CoA from acyl CoA. This occurs by a thiolytic cleavage catalysed by β-ketoacyl CoA thiolase.

Energy of palmitic acid oxidation—



Ketogenesis

- The synthesis of ketone bodies occurs in the liver. The enzymes for ketone body synthesis are located in the mitochondrial matrix. Ketone bodies are water-soluble and energy yielding. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies.
- The three main types of ketone bodies produced are acetone, acetoacetate, and beta-hydroxybutyrate. Ketone bodies can be used by the brain and other tissues as an alternative energy source when glucose is scarce, and they are also involved in regulating blood glucose levels and reducing inflammation.
- However, excessive production of ketone bodies can lead to a condition known as ketoacidosis, which is a potentially life-threatening metabolic state characterized by high levels of ketone



bodies in the blood.

Ketogenesis occurs through the following reactions.

1. Two moles of acetyl CoA condense to form acetoacetyl CoA. This reaction is catalysed by thiolase, an enzyme involved in the final step of E-oxidation. Hence, acetoacetate synthesis is appropriately regarded as the reversal of thiolase reaction of fatty acid oxidation.
2. Acetoacetyl CoA combines with another molecule of acetyl CoA to produce β-hydroxy β-methyl glutaryl CoA (HMG CoA). HMG CoA

synthase, catalysing this reaction, regulates the synthesis of ketone bodies.

3. HMG CoA lyase cleaves HMG CoA to produce acetoacetate and acetyl CoA.
4. Acetoacetate can undergo spontaneous decarboxylation to form acetone.
5. Acetoacetate can be reduced by a dehydrogenase to β-hydroxybutyrate.

Regulation of ketogenesis—

The ketone body formation (particularly overproduction) occurs primarily due to nonavailability of carbohydrates to the tissues. This is an outcome of excessive utilization of fatty acids to meet the energy requirements of the cells. The hormone glucagon stimulates ketogenesis whereas insulin inhibits. The increased ratio of glucagon/insulin in diabetes mellitus promotes ketone body formation.

Ketolysis

- Ketolysis is the metabolic process by which ketone bodies are broken down and converted into energy in the body's cells. This process occurs primarily in the mitochondria of cells, where the ketone bodies are broken down into acetyl-CoA, which can then enter the citric acid cycle to produce ATP, the energy currency of cells.
- This process is important for individuals who rely on ketone bodies as their primary source of energy, such as those on a ketogenic diet or during periods of prolonged fasting.
- The rate of Ketolysis is influenced by several factors, including the availability of ketone bodies and the metabolic state of the cells.
- In some metabolic disorders, such as diabetes, there can be a disruption in the balance between ketone production and utilization, leading to an accumulation of ketone bodies in the blood and potentially causing ketoacidosis.

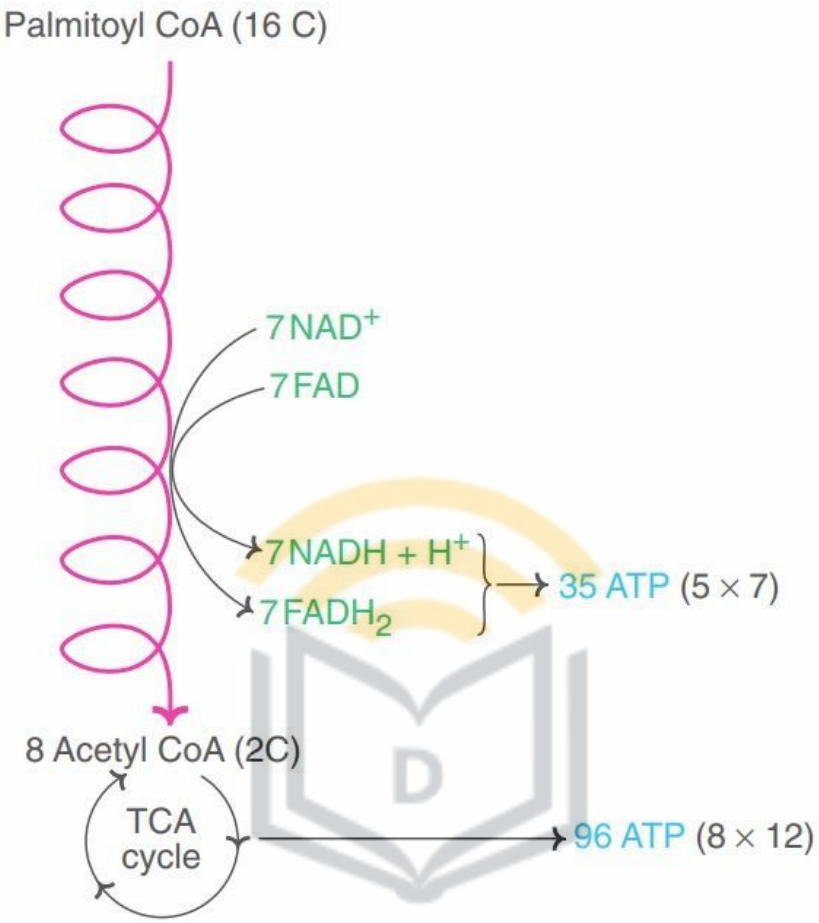
Diseases related to abnormal metabolism of lipids.

1. **Ketoacidosis—** Increase in concentration of both acetoacetate and β-hydroxybutyrate (strong acids) in blood would cause acidosis. The carboxyl group has a pKa around 4. Therefore, the ketone bodies in the blood dissociate and release H+ ions which lower the PH. Diabetic ketoacidosis is dangerous—may result in coma, and even death, if not treated. Ketosis due to starvation is not usually accompanied by ketoacidosis
2. **Hypercholesterolemia**—Increase in plasma cholesterol (> 200 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders
    - Diabetes mellitus— Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.
    - Hypothyroidism (myxoedema)— This is believed to be due to decrease in the HDL receptors on hepatocytes.
    - Obstructive jaundice— Due to an obstruction in the excretion of cholesterol through bile.
    - Nephrotic syndrome— Increase in plasma globulin concentration is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due to increase in plasma lipoprotein fractions in this disorder.
3. **Fatty liver—** The normal concentration of lipid in liver is around 5%. Liver is not a storage organ for fat, unlike adipose tissue. However, in certain conditions, lipids— especially the triacylglycerols—accumulate excessively in liver, resulting in fatty liver.

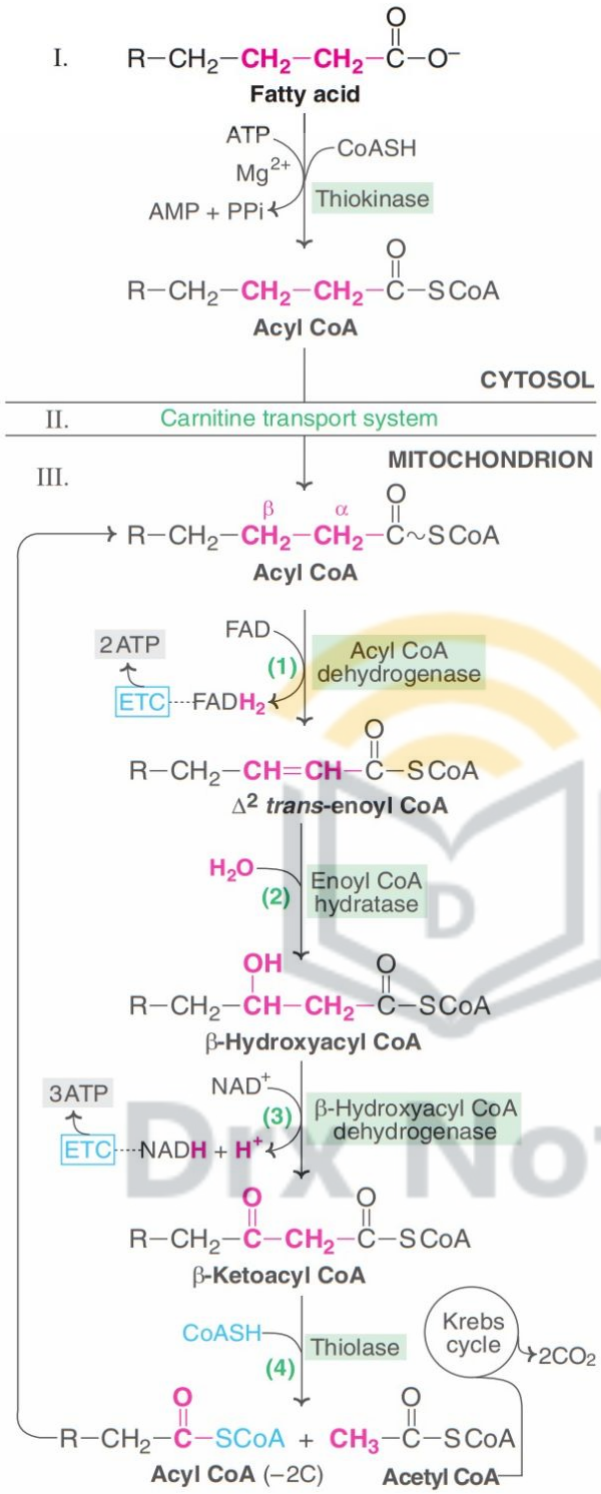
    In the normal liver, Kupffer cells contain lipids in the form of droplets. In fatty liver, droplets of triacylglycerols are found in the entire cytoplasm of hepatic cells. This causes impairment in metabolic functions of liver. Fatty liver is associated with fibrotic changes and cirrhosis, Fatty liver may occur due to two main causes

    - Increased synthesis of triacylglycerols
    - Impairment in lipoprotein synthesis.

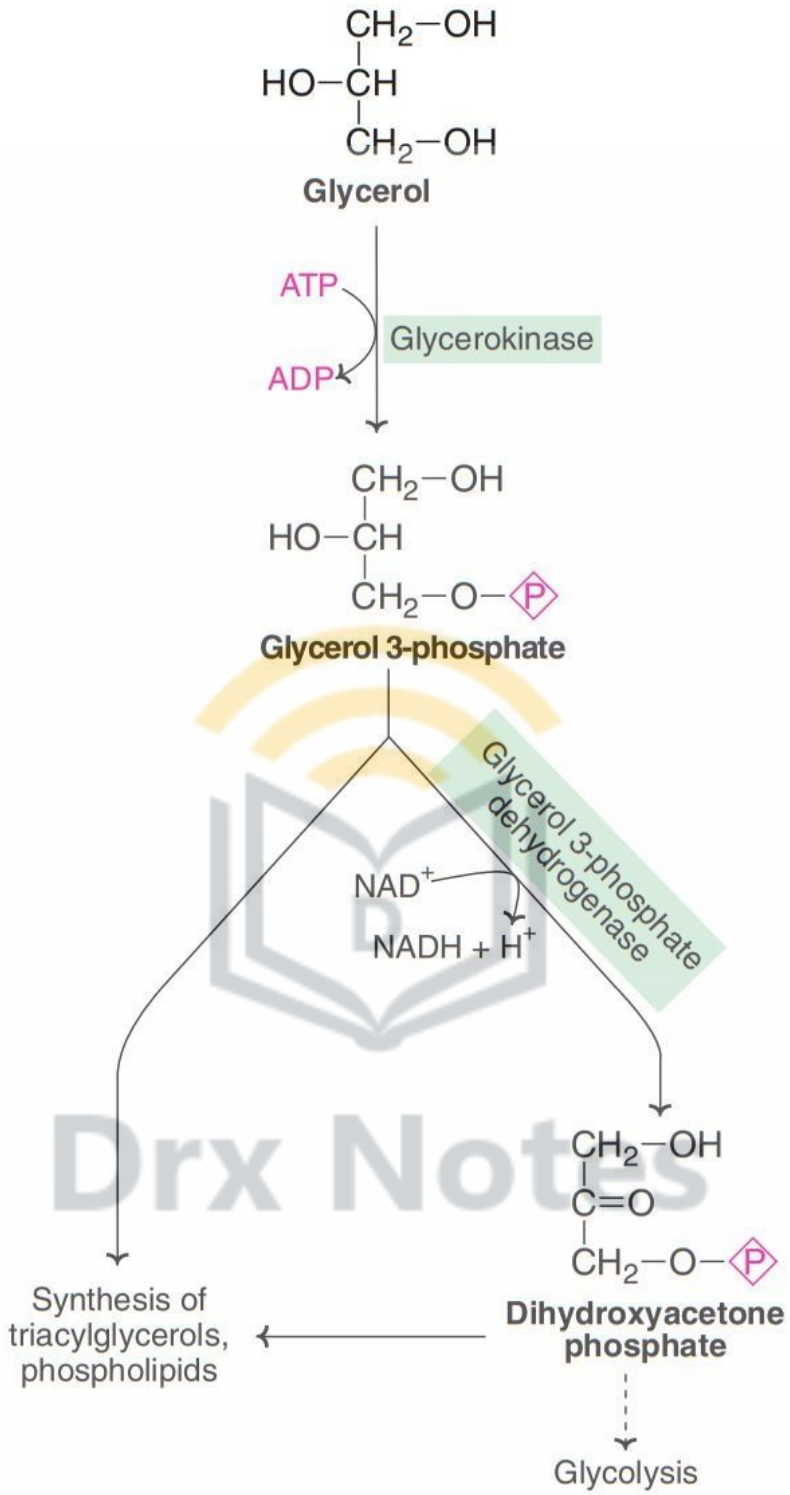
NOTE:



oxidation of palmitic acid.



β-Oxidation of fatty acids



Fate of glycerol