Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

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

Academic Year: 2024 - 2025

Course Organizers:

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

Second Semester

Lecture No. 3

Date: Feb., 3rd, 2025

Lipid Metabolism

Metabolism of Fatty Acids

Objectives: The student should understand the following subjects:

1. Describe the fatty acids, types, saturated and unsaturated.
2. Digestion and absorption of lipids and transported in the blood and activated of fatty acids and then transported into the matrix of the mitochondria for breakdown to obtain energy.
3. Outline the β-oxidation of fatty acid pathway by which fatty acids are metabolized to acetyl-CoA.

Fatty acids, are included in the group of derived lipids. It is the most common component of lipids in the body. They are generally found in ester linkage in different classes of lipids. In the human body, free fatty acids are formed only during metabolism. Fatty acids are aliphatic carboxylic acids and have the general formula, $CH_3–(CH_2)_n$-COOH, where n = 0,1,2,3 ….etc. The fatty acids are either saturated (without double bonds or unsaturated (with one or more double bonds, and the number of carbon atoms is either even or odd carbons.

Examples: **Acetic acid** CH_3 —COOH; **Butyric acid** $CH_3(CH_2)_2$—COOH; **Palmitic acid** CH_3—$(CH_2)_{14}$—COOH; **Stearic acid** CH_3 —$(CH_2)_{16}$—COOH.

The carbon atoms of fatty acids are numbered as C1, C2, etc. starting from the COOH group, or, starting from the methyl end, the carbon atoms may be numbered as omega (ω)—1,2,3 ……. etc.

1 2 3 4 5
CH_3—CH_2—CH_2—CH_2—CH_2—COOH
ω1 ω2 ω3 ω4 ω5

The oxidation of long-chain fatty acids to acetyl-CoA is a central energy-yielding pathway in many organisms and tissues. The electrons removed from fatty acids during oxidation pass through the respiratory chain, driving ATP synthesis; the acetyl-CoA produced from the fatty acids may be completely oxidized to CO_2 in the citric acid cycle, resulting in further energy conservation. Although the biological role of fatty acid oxidation differs from organism to organism, the mechanism is essentially the same which is called **β- oxidation**.

The long alkyl chains of their constituent fatty acids are essentially hydrocarbons, highly reduced structures with an energy of complete oxidation (~38 kJ/g) more than twice that for the same weight of carbohydrate or protein. The TGs can be stored in large quantity in cells without the risk of undesired chemical reactions with other cellular constituents due to its water insolubility, whereas, storage of poly-

saccharides as glycogen is water of solvation can account for two-thirds of the overall weight of the stored molecules. Because TG molecules are insoluble in water, ingested TGs must be emulsified before they can be digested by water-soluble enzymes in the intestine, and TGs absorbed in the intestine or mobilized from storage tissues must be carried in the blood bound to proteins that counteract their insolubility.

Digestion of Lipids:

The major dietary lipids are triacylglycerol, cholesterol and phospholipids. The **lingual lipase** from the mouth enters stomach along with the food. It has an optimum pH of 2.5 – 5. The enzyme therefore continues to be active in the stomach. It acts on short chain triglycerides (SCT) present in milk and butter. The action of lingual lipase is observed to be more significant in the newborn infants. **Gastric lipase** is acid stable, with an optimum pH about 5.4 and is secreted by Chief cells, the secretion is stimulated by Gastrin. Up to 30% digestion of TG occurs in stomach.

Role of Bile Salts in Lipids Digestion:

The bile salts (sodium glycol-cholate and sodium-taurocholate) **lower the surface tension.** They emulsify the fat droplets and increases the surface area of the particles for enhanced activity of enzymes, **Figure 1.** The hydrophobic portions of bile salts intercalate into the large aggregated lipid, with the hydrophilic domains remaining at the surface. This leads to breakdown of large aggregates into smaller and smaller droplets. Thus, the surface area for action of lipase is increased.

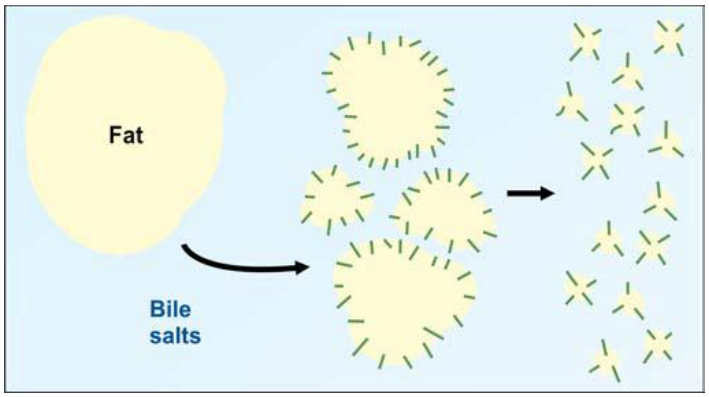

Fig. 1: Action of bile salts.

Digestion in Intestines

The lipids are dispersed into smaller droplets; surface tension is reduced; and surface area of droplets is increased. This process is favored by:

1. Bile salts (detergent action)
2. Peristalsis (mechanical mixing)
3. Phospholipids.

A list of physiologically important lipases used for dietary lipid digestion is shown in Table 1.

Table 1: Physiologically important lipases

Lipase	Site of action	Preferred substrate	Product(s)
Lingual / acid stable lipase	Mouth, stomach	TAGs with short and medium chain FAs	FFA + DAG
Pancreatic lipase + colipase	Small Intestine	TAGs with long chain FAs	FFA + 2MAG
Intestinal lipase with bile acids	Small Intestine	TAGs with medium chain FAs	FFA+ glycerol
Phospholipase A2 + bile acids	Small intestine	PLs with unsaturated FA on position 2	Unsaturated FFA Lysolecithin
Lipoprotein lipase	Capillary wall	TAGs in chylomicron or VLDL	FFA + glycerol
Hormone sensitive lipase	Adipocytes	TAG stored in adipose tissue	FFA+ DAG

Dietary Fats are Absorbed in the Small Intestine

In vertebrates, before ingested TG can be absorbed through the intestinal wall they must be converted from insoluble macroscopic fat particles to finely dispersed microscopic micelles. This solubilization is carried out by bile salts, such as taurocholic acid, which are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of a fatty meal. Bile salts are amphipathic compounds that act as biological detergents, converting dietary fats into mixed micelles of bile salts and TGs (Fig. 2, step 1).

Micelle formation increases the fraction of lipid molecules accessible to the action of water-soluble lipases in the intestine, and lipase action converts TGs to monoacylglycerols and diacylglycerols, free fatty acids, and glycerol (step 2). These products of lipase action diffuse into the epithelial cells lining the intestinal surface (the intestinal mucosa) (step 3), where they are reconverted to TGs and packaged with dietary cholesterol and specific proteins into lipoprotein aggregates called **chylomicrons** (Fig.2, step 4).

Apolipoproteins are lipid-binding proteins in the blood, responsible for the transport of TGs, phospholipids, cholesterol, and cholesteryl esters between organs. Apolipoproteins combine with lipids to form several classes of lipoprotein particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface. Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons and very low-density lipoproteins (VLDL) to very-high-density lipoproteins (VHDL), which can be separated by ultra or high-speed centrifugation.

In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II), move from the intestinal mucosa into the lymphatic system, and then enter the blood, which carries them to muscle and adipose tissue (Fig. 2, step 5). In the capillaries of these tissues, the extracellular enzyme **lipoprotein lipase,** activated by apoC-II, hydrolyzes TGs to fatty acids and glycerol (step 6), which are taken up by cells in the target tissues (step 7). In muscle, the fatty acids are oxidized for energy; in adipose tissue, they are re-esterified for storage as triacylglycerols (step 8).

TGs that enter the liver by this route may be oxidized to provide energy or to provide precursors for the synthesis of ketone bodies. When the diet contains more fatty acids than are needed immediately for fuel or as precursors, the liver converts them to triacylglycerols, which are packaged with specific apolipoproteins into VLDLs. The VLDLs are transported in the blood to adipose tissues, where the triacylglycerols are removed and stored in lipid droplets within adipocytes.

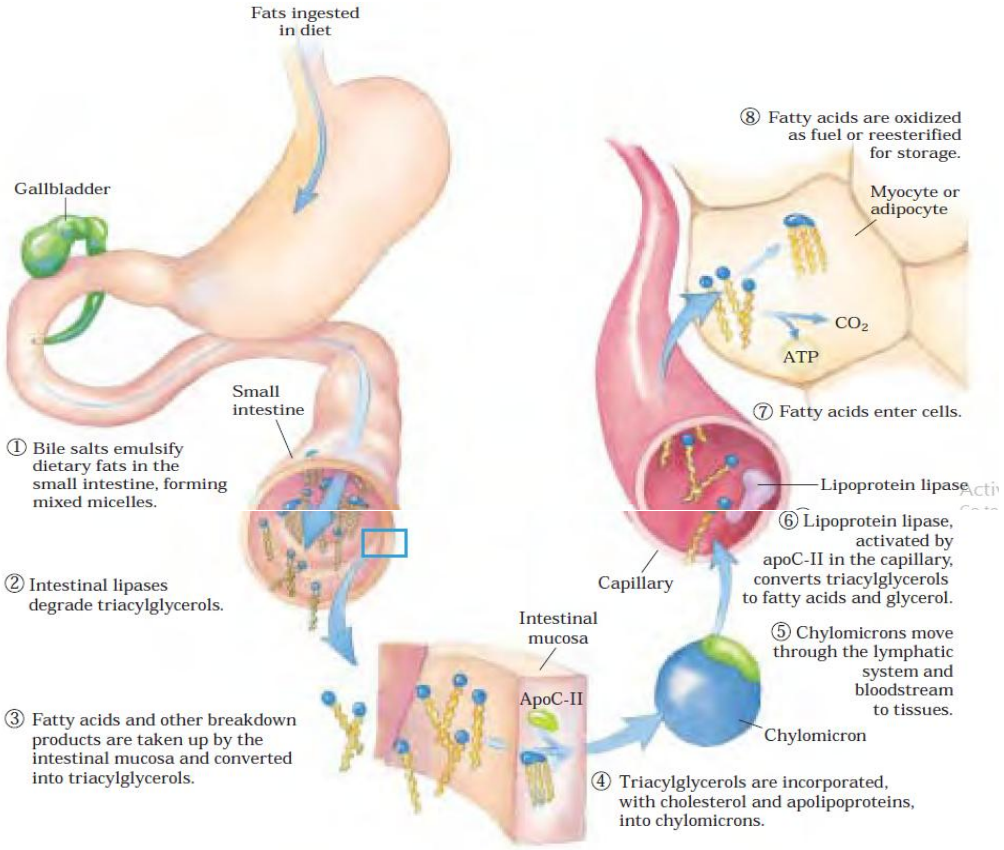

Fig. 2: Processing of dietary lipids in vertebrates. Digestion and absorption of dietary lipids occur in the small intestine, and the fatty acids released from triacylglycerols are packaged and delivered to muscle and adipose tissues.

Digestion of Triacylglycerols

1. **Pancreatic lipase** can easily hydrolyze the fatty acids esterified to the 1st and 3rd carbon atoms of glycerol forming 2-monoacylglycerol and two molecules of fatty acid (Fig. 2).

2. Then an **isomerase** shifts the ester bond from position 2 to 1. The bond in the 1st position is then hydrolyzed by the **lipase** to form free glycerol and fatty acid (Fig. 2).
3. The major end products of the digestion of TAG are 2-MAG (78%), 1-MAG (6%), glycerol and fatty acids (14%). Thus, digestion of TAG is partial (incomplete).
4. **Cholesterol** ester may be hydrolyzed to free cholesterol and fatty acid. The action of **phospholipase** A2 produces lysophospholipid and a fatty acid.

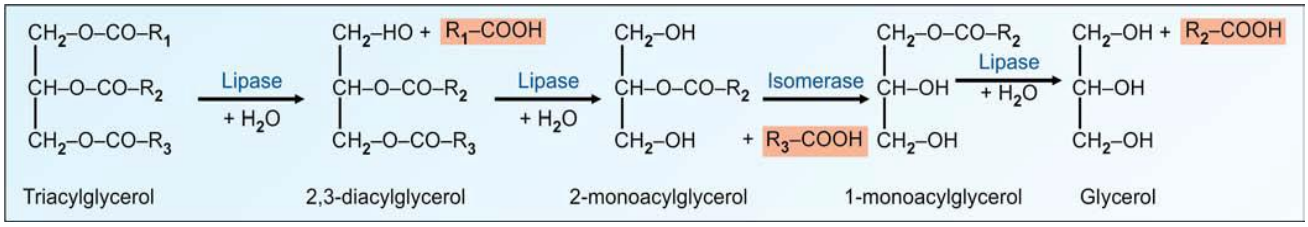

Fig. 3: Complete hydrolysis of triglyceride.

The binding of **co-lipase** to the triacylglycerol molecules at the oil water interface is obligatory for the action of lipase. The co-lipase is secreted by the pancreas as an inactive zymogen (molecular weight 11,000). It is activated by trypsin.

Absorption of Lipids:

There are six steps of lipid absorption:

1. **Minor digestion** of triacylglycerols in mouth and stomach by lingual (acid-stable) lipase
2. **Major digestion** of all lipids in the lumen of the duodenum/ jejunum by pancreatic lipolytic enzymes
3. **Bile acid** facilitates formation of mixed micelles
4. **Passive absorption** of the products of lipolysis from the mixed micelle into the intestinal epithelial cell
5. **Re-esterification** of 2-monoacylglycerol with free fatty acids inside the intestinal enterocyte

Assembly of chylomicrons containing Apo B48, TGs, cholesterol esters and phospholipids **and export** from intestinal cells to the lymphatics.

Small Chain Fatty Acid Absorption is Different

i. **Short chain** fatty acids (SCFA) (seen in milk, butter, ghee) and **medium chain** fatty acids (MCFA) (in coconut oil and mother's milk) do not need re-esterification.
ii. They can directly enter into blood vessels, then to portal vein, finally to liver where they are immediately utilized for energy. Their absorption is rapid. They are better absorbed than long chain fatty acids, see Table 1.

Abnormalities in Absorption of Lipids

1. **Defective digestion:** In steatorrhea, daily excretion of fat in feces is more than 6 g per day. It is due to chronic diseases of pancreas. In such cases, unsplit fat is seen in feces.
2. **Defective absorption:** On the other hand, if the absorption alone is defective, most of the fat in feces may be split fat, i.e. fatty acids and monoglycerides. Defective absorption may be due to diseases:
   A. Celiac disease or Crohn's disease.
   B. Surgical removal of intestine.
3. **Obstruction of bile duct: Chyluria.** There is an abnormal connection between the urinary tract and lymphatic drainage system of the intestine. Urine appears milky due to lipid droplets.

Beta Oxidation of Fatty Acids

Neutral lipids are stored in adipocytes (and in steroid synthesizing cells of the adrenal cortex, ovary, and testes) in the form of lipid droplets, with a core of sterol esters and TGs surrounded by a monolayer of phospholipids. When hormones signal the need for metabolic energy, TGs stored in adipose tissue are mobilized (brought out of storage) and transported to tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be oxidized for energy production. The hormones epinephrine and glucagon, secreted in response to low blood glucose levels, activate the enzyme adenylyl cyclase in the adipocyte plasma membrane, which produces the intracellular second messenger cyclic AMP (cAMP). Cyclic AMP–dependent protein kinase (PKA) phosphorylates perilipin A, and the phosphorylated perilipin causes **hormone-sensitive lipase** in the cytosol to move to the lipid droplet surface, where it can begin hydrolyzing TGs to free fatty acids and glycerol as shown in **Figure 3.** Cells with defective perilipin genes have almost no response to increases in cAMP concentration; their hormone-sensitive lipase does not associate with lipid droplets. As hormone-sensitive lipase hydrolyzes TG in adipocytes, the fatty acids thus released **(free fatty acids, FFA)** pass from the adipocyte into the blood, where they bind to the blood protein **serum albumin, see Figure 4.**

This protein (*M*r 66,000), which makes up about half of the total serum protein, non-covalently binds as many as 10 fatty acids per protein monomer. Bound to this soluble protein, the otherwise insoluble fatty acids are carried to tissues such as skeletal muscle, heart, and renal cortex. In these target tissues, fatty acids dissociate from albumin and are moved by plasma membrane transporters into cells to serve as fuel.

About 95% of the biologically available energy of TGs resides in their three long-chain fatty acids; only 5% is contributed by the glycerol moiety.

The glycerol released by lipase action is phosphorylated by **glycerol kinase**, and the resulting glycerol-3-phosphate is oxidized to dihydroxy acetone phosphate. The glycolytic enzyme triose phosphate isomerase converts this compound to glyceraldehyde-3-phosphate, which is oxidized via glycolysis.

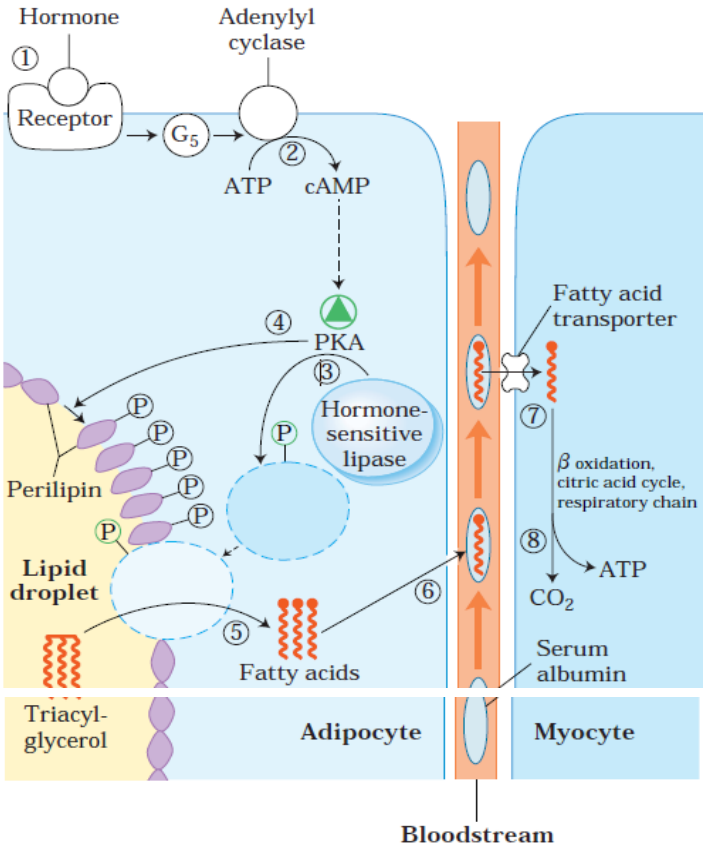

Fig. 4: Mobilization of triacylglycerols stored in adipose tissue.

When low levels of glucose in the blood trigger the release of glucagon, (1) the hormone binds its receptor in the adipocyte membrane and thus (2) stimulates adenylyl cyclase, via a G protein, to produce cAMP. This activates PKA, which phosphorylates (3) the hormone-sensitive lipase and (4) perilipin molecules on the surface of the lipid droplet. Phosphorylation of perilipin permits hormone-sensitive lipase access to the surface of the lipid droplet, where (5) it hydrolyzes TGs to free fatty acids. (6) Fatty acids leave the adipocyte, bind serum albumin in the blood, and are carried in the blood; they are released from the albumin and (7) enter a myocyte via a specific fatty acid transporter. (8) In the myocyte, fatty acids are oxidized to CO_2, and the energy of oxidation is conserved in ATP, which fuels muscle contraction and other energy requiring metabolism in the myocyte.

Activation and Transportation of Fatty Acids into Mitochondria:

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. The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix.

Preparative Steps for Beta Oxidation

The co-enzyme A is a complex molecule containing B complex vitamin pantothenic acid and a molecule of beta mercapto-ethanolamine; this SH group is a reactive center of CoA-SH and forms thioester bond in acyl-CoA. To emphasize the function of the SH group, the CoA is sometimes written as CoA-SH, **Figure 5.**

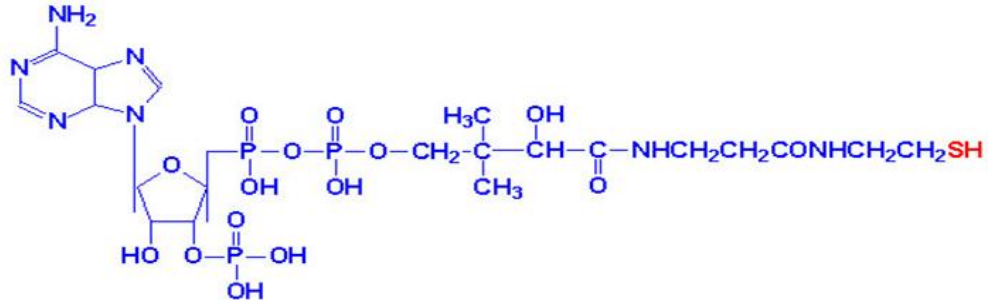

Fig. 5: Structure of co-enzyme A (CoA) (CoA-SH)

Preparative Step 1: Activation of Fatty Acids

Fatty acids are activated to their co-enzyme A (CoA) derivative. This activation is taking place in **cytoplasm**. ATP is hydrolyzed to AMP and PP_i and the energy from hydrolysis of PP_i drives the reaction forward. Thus **two high energy bonds** are utilized in this reaction. The enzyme is a **thiokinase** or fatty acyl-CoA **synthetase, Figure 6**.

Acetyl group and acyl groups are different. Three different enzymes, one each for short chain, medium chain and long chain fatty acids have been identified. Small chain fatty acids may also be activated by thiophorase enzyme, using succinyl-CoA.

Activated fatty acid (fatty acyl-CoA) is formed outside the mitochondria. Hence the fatty acyl-CoA is transferred to carnitine which acts as the carrier of acyl groups across the mitochondrial membrane, and forms acyl-carnitine. 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 mitochondria.

The **acyl-CoA synthetases** catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a **fatty acyl–CoA,** coupled to the cleavage of ATP to AMP and PP_i as shown below**:**

Fatty acid + CoA + ATP ---------► Fatty acyl–CoA + AMP + PP_i

The reaction occurs in two steps and involves a fatty acyl–adenylate intermediate. Fatty acyl–CoAs, like acetyl-CoA, are high-energy compounds; their hydrolysis to FFA and CoA has a large, negative standard free-energy change (ΔG^o = 31 KJ/mol), **Figure 6,**

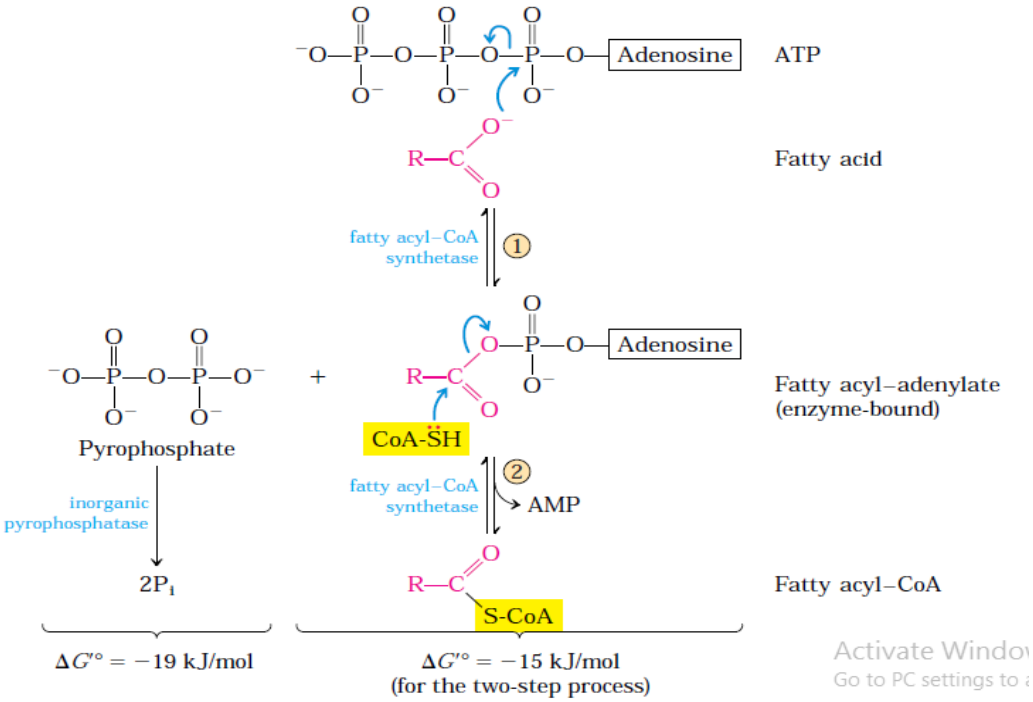

Fig. 6: Conversion of a fatty acid to a fatty acyl–CoA.

The overall reaction is:

Fatty acid + CoA + ATP ------► Fatty acyl – CoA + AMP + 2P$_i$ ΔG^o = 34 kJ/mol

Preparative Step 2: Role of Carnitine

The activated fatty acyl-CoA esters formed at the cytosolic side of the outer mitochondrial membrane can be transported into the mitochondrion and oxidized to produce ATP, or they can be used in the cytosol to synthesize membrane lipids. Fatty acids are transiently attached to the hydroxyl group of **carnitine** to form **fatty acyl-carnitine**, **Figure 7**. The long chain fatty acyl-CoA cannot pass through the inner mitochondrial membrane.

Carnitine is β-hydroxy-γ-trimethyl ammonium butyrate is synthesized from lysine and methionine in liver and kidney, vitamin C (ascorbic acid) is essential for the synthesis of carnitine. During growth or pregnancy, the requirement of carnitine might exceed its natural production. Human genetic disorders affect different steps of this process.

$(CH_3)_3N^+–CH_2–CHOH–CH_2–COOH$.

Carnitine

Oral carnitine reduces fat mass; an increase muscle mass, reducand reduces weight loss. Carnitine protects against lipid peroxidation of phospholipid membranes and oxidative stress in myocardium and endothelium. The potential pharmaceutical roles in heart diseases, kidney diseases, male infertility, as a weight loss supplement, and to improve fatigue in cancer chemotherapy are being explored.

The highest concentrations of carnitine are found in red meat and dairy products. Other natural sources of carnitine include nuts and seeds (e.g. sunflower), legumes or pulses (beans, peas), vegetables (broccoli, garlic, mustard greens), fruits (bananas), cereals (corn, rice bran, rye, wheat).

The symptoms of carnitine deficiency disease range from mild muscle cramping to severe weakness and even death. The muscle, kidney, and heart are the tissues primarily affected. Muscle weakness during prolonged exercise is an important characteristic of a deficiency of carnitine acyl transferases because muscle relies on fatty acids as a long-term source of energy.

Preparative Step 3: Carnitine Acyl Transferase

The fatty acids with 14 or more carbons, which constitute the majority of the free fatty acids, FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes, they must first undergo the three enzymatic reactions of the **carnitine shuttle.**

The shuttle begins with the transfer of activated fatty acid **(acyl-CoA)** to the hydroxyl group carnitine to form **acyl-carnitine**. This reaction is catalyzed by **carnitine-acyl transferase I** (CAT-I) (also called ***carnitine palmitoyl transferase I***) which is bound to the outer mitochondrial membrane and it regulates entry of fatty acids into mitochondria, **Fig. 12.**

Preparative Step 4: Translocase

A protein **translocase** will carry the acyl-carnitine across the membrane to the matrix of mitochondria. On the matrix side of the membrane another enzyme, carnitine acyltransferase-II (**CAT-II**) will transfer the acyl group back to co-enzyme A molecule **(Fig. 7).** Carnitine is returned to the cytosolic side by the translocase.

The fatty acyl-carnitine ester then enters the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter** of the inner mitochondrial membrane. Acyl carnitine is translocated into matrix of mitochondria by **carnitine-acyl carnitine translocase** present in inner mitochondrial membrane and is a carrier protein involved in facilitated transport.

Acyl-carnitine is then shuttled across the inner mitochondrial membrane by a translocase. The acyl group is transferred back to CoA on the matrix side of the membrane. This reaction, which is catalyzed by ***carnitine acyl transferase II (carnitine palmitoyl transferase II)*****,** is simply the reverse of the reaction that takes place in the cytosol and liberates acyl group from acyl carnitine as acyl-CoA.

The three-step for transferring fatty acids into the mitochondrion:

a. Esterification to CoA

b. Trans-esterification to carnitine followed by transport

c. Trans-esterification back to CoA) links two separate pools of coenzyme A and of fatty acyl-CoA, one in the cytosol, the other in mitochondria.

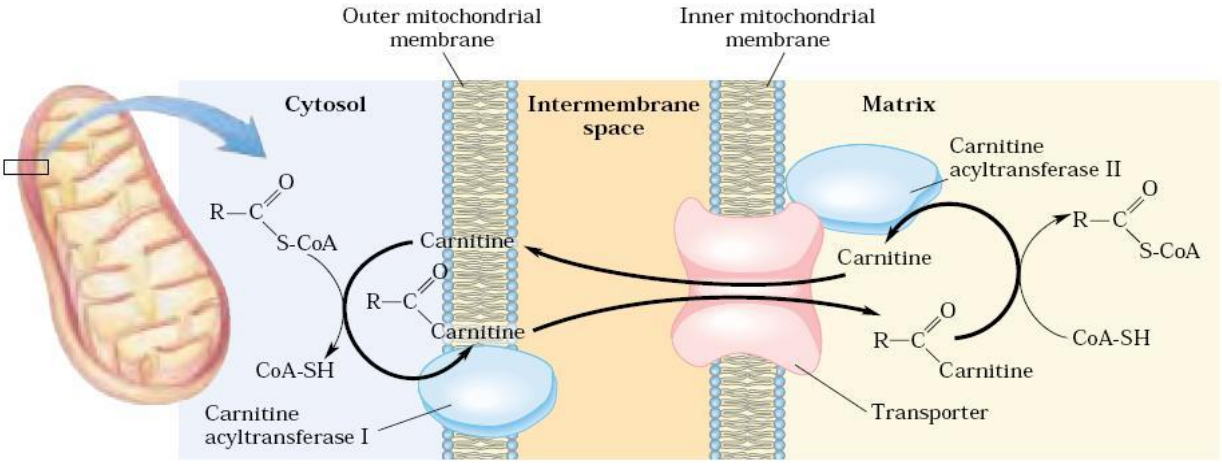

Fig. 7. Carnitine shuttle

These pools have different functions. Coenzyme A in the mitochondrial matrix is largely used in oxidative degradation of pyruvate, fatty acids, and some amino acids, whereas cytosolic coenzyme A is used in the biosynthesis of fatty acids. Fatty acyl-CoA in the cytosolic pool can be used for membrane lipid synthesis or can be moved into the mitochondrial matrix for oxidation and ATP production. The carnitine-mediated entry process is the rate-limiting step for oxidation of fatty acids in mitochondria.

To complete the shuttle, carnitine is sent back to outside of inner mitochondrial membrane by carnitine-acyl carnitine translocase. The carnitine shuttle transports fatty acyl-CoAs with chain lengths C12-18.

In conclusion, carnitine shuttle transfers acyl-CoA from outside of inner mitochondrial membrane into matrix of mitochondria.

Clinical Applications

1. Medium chain and short chain fatty acids do not require carnitine for transport across the inner mitochondrial membrane. So, medium chain and short chain fatty acids are easily oxidized.
2. **Carnitine deficiency** is reported in preterm infants, in whom impaired fatty acid oxidation is noticed. So more glucose is utilized, resulting in episodes of hypoglycemia.
3. **Deficiency of translocase:** It leads to defective metabolism of long chain fatty acids. In this condition, muscle cramps are precipitated by fasting, exercise and high fat diet.

Stages of Fatty Acids Utilization :

Peripheral tissues gain access to the lipid energy reserves stored in adipose tissue through three stages of processing, see **Figure 8.**

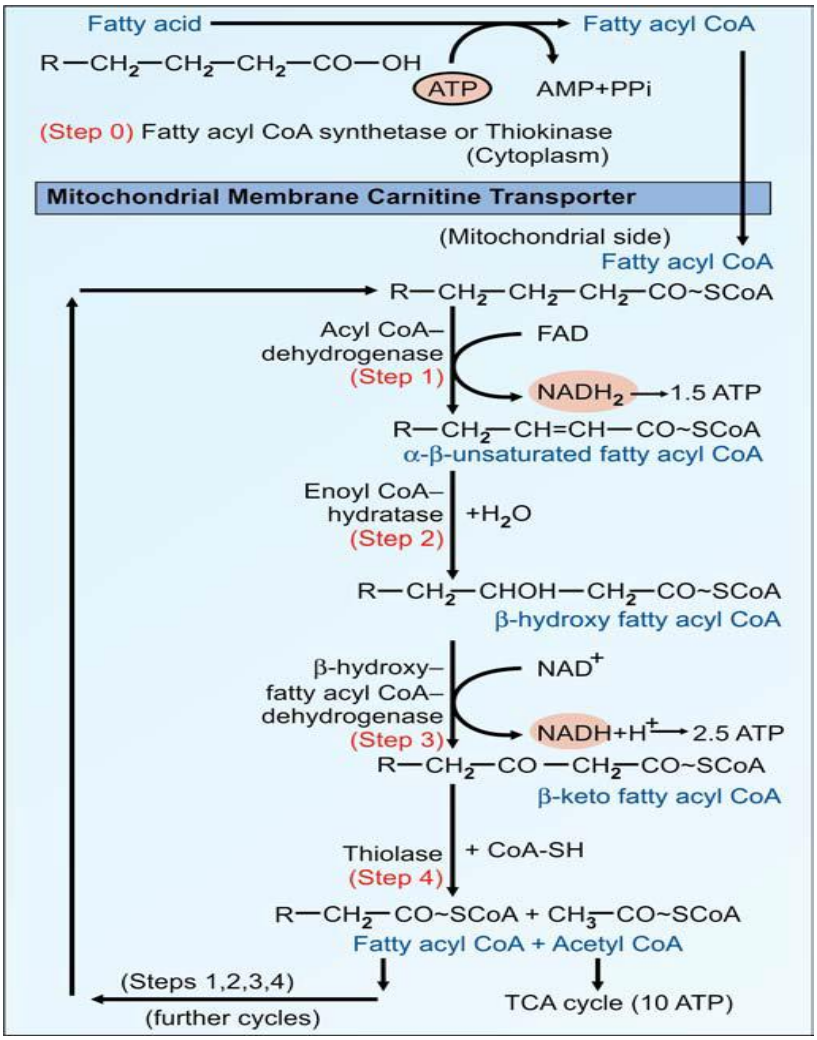

Fig. 8: Beta oxidation of fatty acids. Important to remember that the first step is FAD dependent and the third step is NAD^+ dependent.

1. The lipids must be mobilized. TGs are degraded to fatty acids and glycerol, which are released from the adipose tissue and transported to the energy-requiring tissues.
2. The fatty acids must be activated and transported into mitochondria for degradation.
3. The fatty acids are broken down step-by step into acetyl-CoA, which is then processed in the citric acid cycle.

Stages of Fatty Acid β-Oxidation :

Takes place in three stages.

1. Fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16-carbon palmitic acid undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two carbons of palmitate (originally C15 and C16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-

CoA requires removal of four hydrogen atoms from the fatty acyl moiety by dehydrogenases.

2. The acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation.
3. The reduced electron carriers NADH and $FADH_2$ produced in the two stages donate their electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP.

Reactions of β-Oxidation Steps

The next 4 reactions are sequentially repeated for complete oxidation of fatty acids. After one round of four metabolic steps, one acetyl-CoA unit is split off and acyl-CoA with 2 carbon atoms less is generated. This would undergo the same series of reactions again until the fatty acid is completely oxidized.

Step 1: FAD Linked Dehydrogenase

Step 2: Hydration

Step 3: NAD^+ Dependent Dehydrogenase

Step 4: Cleavage:

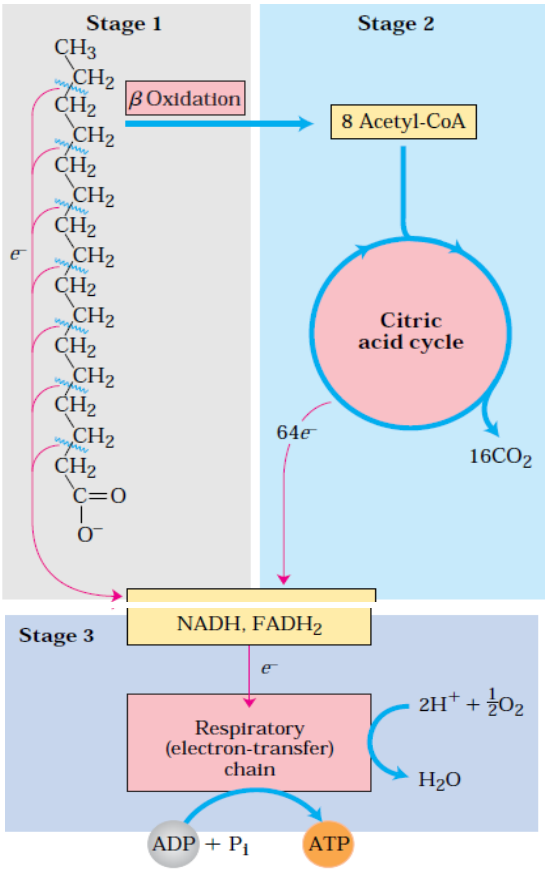

Fig. 9: Stages of fatty acid oxidation.

Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl- CoA. This process is called *β*-oxidation. Stage 2: The acetyl groups are oxidized to CO_2 via the citric acid cycle. Stage 3: Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

Further Cycles

The newly formed fatty acyl-CoA will sequentially undergo further cycles of steps 1, 2, 3 and 4 of beta-oxidation until the fatty acid is completely converted to acetyl-CoA, **Figure 9.**

Box 2: Summary of beta oxidation

When one molecule of palmitate undergoes beta-oxidation, the net reaction is:

Palmitoyl-CoA		8 Acetyl-CoA
+ 7 FAD		+ 7 FADH2
+ 7 NAD^+	==========▶	+ 7 NADH
+ 7 H_2O		+ 7 H^+
+ 7 HSCoA		

Energetics of Beta Oxidation (ATP Yield):

Palmitic acid (16 C) needs 7 cycles of beta oxidation **(Fig. 9).** So, it gives rise to 8 molecules of acetyl-CoA (Box 2). Every molecule of acetyl-CoA when oxidized in the TCA cycle gives 10 (or 12 as indicated previously) molecules of ATP. Each molecule of FADH2 produces 1.5 molecules of ATP and each NADH generates 2.5 molecules of ATP, when oxidized in the electron transport chain. Hence, the energy yield from one molecule of palmitate may be calculated as:

8 acetyl-CoA × 10 = 80 ATP

7 FADH2 × 1.5 = 10.5 ATP

7 NADH × 2.5 = 17.5 ATP

Gross total = 108 ATP

Net yield = 108 minus 2 =106 ATPs

Breakdown of a fatty acid requires activation to the acyl-CoA, a process that costs two ATP equivalents. For stearic acid (18 C), the activation step is followed by eight cycles of the β-oxidation pathway, resulting in nine acetyl-CoA, eight NADH, and eight $FADH_2$. The nine cycles of the TCA cycle required to consume the acetyl-CoA produced result in formation of nine ATP, 27 NADH, and nine $FADH_2$. Thus, the complete breakdown of stearic acids results in a net production of seven ATP + 35 NADH + 17 $FADH_2$, Table -2.

Table 2: Comparison of Energetics of Metabolism for Glucose and Stearic Acid

Energetic Molecule	Glucose	Stearate ↓ Acetyl-CoA	9Acetyl-CoA ↓ CO_2	Stearate (Total)
Product				
ATP	4 → 4 ATP	−2	9	7→7 ATP
NADH + H^+	10 → 30 ATP	8	27	35 → 105 ATP
FADH2	2 → 4 ATP	8	9	17→34 ATP
Total	38 ATP			146 ATP

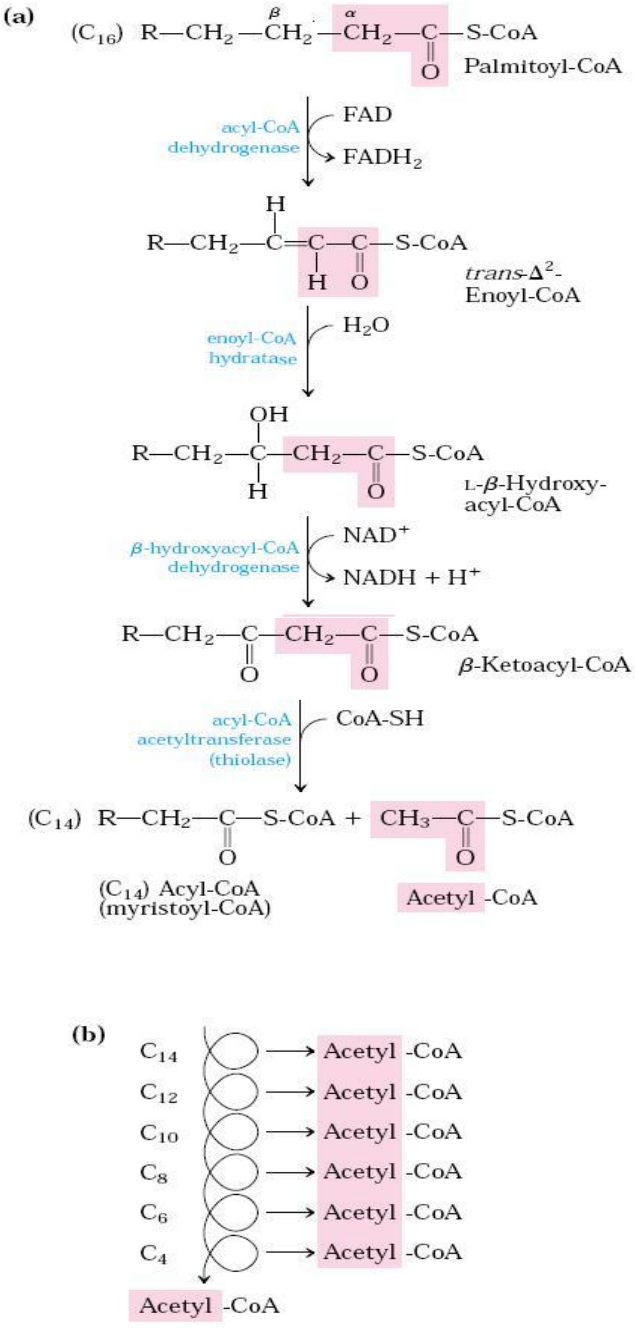

Fig. 10 : Summary of β-oxidation pathway of palmitic acid (16 C) It undergoes 7 cycles, which give rise to 8 molecules of acetyl CoA.

If we use the same values for ATP production from the reduced cofactors, this results in a total of 146 ATP. Glucose contains only six carbons, while stearic acid contains 18 carbons. A fairer comparison involves consideration of the amount of ATP produced per carbon. Dividing the ATP production by

the number of carbons in the compound reveals that glucose yields 6.3 ATP per carbon, while stearic acid yields 8.1 ATP per carbon. Thus, the fatty acid results in slightly more ATP than does glucose.

An even more useful comparison, however, takes molecular weight into account. Glucose has a molecular weight of 180 g/mol, while stearic acid has molecular weight of 284 g/mol. Dividing ATP produced by the molecular weight of the compound reveals yields of 0.2 ATP/gram (dry weight) of glucose compared to 0.5ATP/gram (dry weight) of stearic acid. Thus, on a dry weight basis, fatty acids have a higher energy density than do carbohydrates. The energy density difference of fatty acids and glucose is even more striking when the hydration of the compound *in vivo* is taken into account; in aqueous solution, glucose is associated with roughly three times its weight in water, while fatty acids are stored as hydrophobic (and therefore nearly totally dehydrated) TGs. This means that, physiologically, fatty acids contain roughly eight-times the energy per unit mass.

Table-3 summarizes the yields of NADH, $FADH_2$, and ATP in the successive steps of palmitoyl-CoA oxidation. Note that because the activation of palmitate to palmitoyl-CoA breaks both phosphor anhydride bonds in ATP, the energetic cost of activating a fatty acid is equivalent to two ATP, and the net gain per molecule of palmitate is 106 ATP. The standard free-energy change for the oxidation of palmitate to CO_2 and H_2O is about 9,800 kJ/mol. Under standard conditions, the energy recovered as the phosphate bond energy of ATP is 106 x 30.5 kJ/mol = 3,230 kJ/mol, about 33% of the theoretical maximum. However, when the free-energy changes are calculated from actual concentrations of reactants and products under intracellular conditions, the free-energy recovery is more than 60%; the energy conservation is remarkably efficient.

Table 3: Bioenergetic enzymatic reactions of palmitic acid oxidation

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed	
		New	Previous
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5	14
β-Hydroxyacyl-CoA dehydrogenase	7 NADH + 7H^+	17.5	21
Isocitrate dehydrogenase	8NADH + 8H^+	20	24
α-Ketoglutarate dehydrogenase	8 NADH + 8H^+	20	24
Succinyl-CoA synthetase		8	8
Succinate dehydrogenase	8FADH2	12	16
Malate dehydrogenase	8NADH + 8H^+	20	24
Total		108	131

The β-oxidation pathway discussed above applies to nearly all fatty acids and their derivatives. However, some fatty acids contain odd-numbers of

carbons or sites of unsaturation. These compounds require additional reactions to complete their breakdown.

Energy output of stearic acid oxidation :

1. Number of ATPs generated by complete oxidation of 9 Acetyl-CoAs × 12 = 108
2. Number of ATPs generated by respiratory chain oxidation of 8 NADH × 3 = 24
3. Number of ATPs generated by respiratory chain oxidation of 8 FADH2 × 2 = 16
4. Total number of ATP molecules = 148

Stearyl-CoA oxidation and coupled phosphorylation is expressed as equation below.

Stearyl-CoA + 148ADP + 148Pi+ $26O_2$ -----► 148 ATP + 165 H_2O + 18CO_2 + CoA-SH

Since two high energy bonds are needed for stearyl-CoA formation. The net yield of ATP per molecule of stearic acid is obtained by modifying above equation.

Stearic acid + $26O_2$ + 146ADP + 146Pi ------► 146ATP + 18CO_2 + 165H_2O

Thus, β-oxidation of stearic acid produces 146 ATP. Note that large amount of H_2O is also produced along with ATP.

Efficiency of β- Oxidation :

It has been estimated that stearic acid oxidation in calorimeter produces 1120 KJ of energy β-oxidation of stearic acid in the body generates 146 ATPs. Since 51.6 KJ of energy is needed for one ATP formation about 7280 KJ of energy is used for ATP formation. Thus, only 65% of energy is conserved and remainder is lost as heat. Therefore, efficiency of β-oxidation system is 65%.

Regulation of Beta Oxidation:

i. The availability of free fatty acid (FFA) regulates the net utilization through beta oxidation.
ii. The level of FFA, in turn, is controlled by glucagon:insulin ratio. Glucagon increases FFA level and insulin has the opposite effect.
iii. CAT-I is the regulator of entry of fatty acid into mitochondria. Malonyl CoA inhibits CAT-I activity.

Thus, during de novo synthesis of fatty acid, beta oxidation is inhibited.

Oxidation of Odd-Numbered Fatty Acids:

Fatty acids with odd-numbers of carbons are found in some marine animals, in many herbivores, in microorganisms, and in plants. These fatty acids are subjected to β-oxidation in the same way as fatty acids with even-numbers of carbons. However, the final β-oxidation spiral results in the production of the three-carbon compound (propionyl-CoA), which cannot be metabolized in the same way as acetyl-CoA, **Figure 11**.

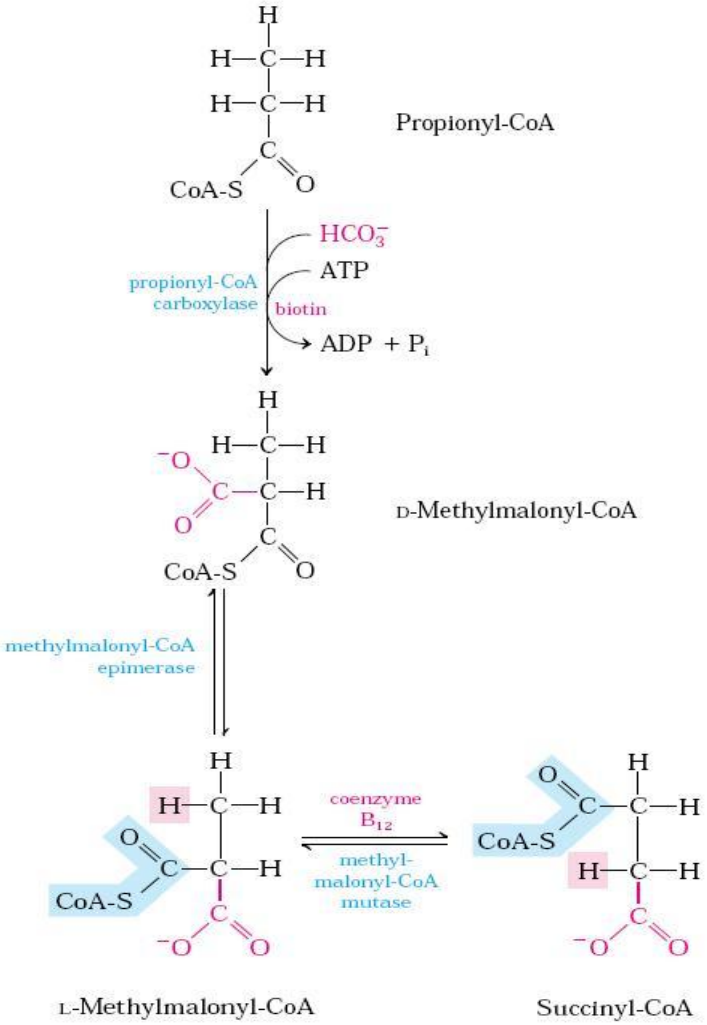

Fig. 11. Oxidation of propionyl-CoA produced by β-oxidation of odd-number fatty acids

Propionyl-CoA is a substrate for the biotin-dependent enzyme propionyl-CoA carboxylase, which uses the energy in ATP to add a carbon, resulting in the four-carbon compound D-methyl malonyl-CoA. The next reaction, catalyzed by **methyl malonyl-CoA epimerase**, reverses the stereochemistry at the chiral carbon of the substrate, resulting in L-methyl malonyl-CoA.

The final reaction in the pathway, catalyzed by **methyl malonyl-CoA mutase**, converts the branched chain compound L-methyl malonyl-CoA into succinyl-CoA, a TCA cycle intermediate. Unlike acetyl-CoA, succinyl-CoA can be used as a gluconeogenic substrate. Succinyl-CoA production can also be used to increase TCA capacity.

The reactions involved in converting propionyl-CoA to succinyl-CoA are useful for more than merely completing the metabolism of odd chain fatty acids; metabolism of some amino acids and of some other compounds also results in propionyl-CoA production.

Methyl malonyl-CoA mutase is one of two known vitamin B_{12}-dependent enzymes in humans (the other is methionine synthase). Most vitamin B_{12}-

dependent enzymes catalyze carbon-transfer reactions, where a group is moved from a first atom to second atom in exchange for a hydrogen derived from the second atom. In the case of methylmalonyl-CoA mutase, the carbonyl of the thioester is moved from the branched α-carbon to the methyl carbon. Vitamin B_{12} (cobalamin) is used only in animals and some microorganisms; because plants do not use this compound, strict vegetarians are at some risk for developing pernicious anemia, the disorder associated with vitamin B_{12} deficiency.

Although the cobalamin ring structure similar in general appearance to the porphyrin structure of heme and chlorophyll, cobalamin contains a corrin ring, not a porphyrin. In addition, the cobalamin contains a cobalt ion rather than the iron typically present in heme or the magnesium found in most chlorophyll derivatives.

Vitamin B_{12} is frequently called cyanocobalamin, although the cyanide group is actually an artifact of the purification procedure.

Short-Chain Fatty Acids:

Fatty acids smaller than about 10 carbons can enter the mitochondrial matrix without needing assistance from carnitine pathway. Otherwise, they are metabolized normally.

Long-Chain Fatty Acids:

Fatty acids above a certain size (greater than 22 carbons) cannot enter the mitochondria. Instead, these compounds are metabolized in another type of subcellular organelle, the peroxisome. The peroxisomal β-oxidation pathways are basically similar to those of the mitochondria, except that the peroxisomal acyl-CoA dehydrogenase releases its electrons by forming hydrogen peroxide rather than by donation to the electron transport chain, because the electron transport pathway does not exist in the peroxisomes. Peroxisomal β-oxidation stops with short acyl chains (4 to 8-carbons) because the peroxisomal thiolase will not cleave shorter acyl chains. The short chain acyl-CoA is converted to acyl-carnitine, which then goes to mitochondria to be metabolized to acetyl-CoA.

Disorders of Fatty Acids Oxidation:

Fatty acid oxidation is impaired in many diseases.

1. **Carnitine deficiency:**

   It occurs in premature infants and in new-borns. It is due to inadequate formation or loss in urine due to renal leakage. Lack of carnitine results in impaired transport of acyl-CoAs into mitochondria. The plasma-free fatty acid level raises due to decreased β-oxidation. Main symptom is hypoglycemia, because all tissues use glucose for energy production, other symptoms is lipid accumulation, muscle weakness and

hypoketonemia. Oral supplementation of carnitine results in disappearance of symptoms.

2. **Carnitine acyl transferase deficiencies**
   a. **Hepatic carnitine acyl transferase deficiency.** Deficiency of CAT-I in the liver leads to impaired fatty acid oxidation. As a result, hypoketonemia and hypoglycemia develops.
   b. **Muscle carnitine acyl transferase-II deficiency** Due to deficiency of CAT-II fatty acid oxidation is impaired in muscle. Muscle weakness and myoglobinuria are the main symptoms.
   c. **Hypoglycemic agents** like sulfonylureas particularly glyburide and tolbutamide used in diabetics inhibit transferases.