# Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

**College of Health and Medical Techniques**

**Medical Laboratories Techniques Department**

## Biochemistry Lectures for 2nd Year Students

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

**Academic Year: 2024 - 2025**

## Course Organizers:


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


**Second Semester**

**Lecture No. 4**

**Date: Feb., 16th, 2025**

# Lipid Metabolism

# Ketone Bodies Metabolism

**Objectives:** The student should understand the following subjects:

1. Identify the three compounds termed “ketone bodies” and describe the reactions by which they are formed in liver mitochondria with their roles.
2. Appreciate that ketone bodies are important fuels for extrahepatic tissues and indicate the conditions in which their biosynthesis and use are favored.
3. Understand that overproduction of ketone bodies leads to ketosis and, if prolonged, ketoacidosis, and identify pathological conditions when this occurs.

## Introduction:

Carbohydrates are essential for the metabolism of fat or fat is burned under the fire of carbohydrates. The acetyl-CoA formed from fatty acids can enter and get oxidized in TCA cycle only when carbohydrates are available.

During starvation and diabetes mellitus, acetyl-CoA takes the alternate route of formation of ketone bodies. Ketone bodies are **(acetoacetic acid ; β-hydroxy butyric acid and acetone).**

## Biological Importance:

1. The major purpose of ketone body formation in liver is to distribute excess fuel (acetyl-CoA) to other tissues.
2. Even number fatty acids are more ketogenic than odd number fatty acids.
3. Fat is more ketogenic than carbohydrate because fat generates more acetyl-CoA.

In humans and most other mammals, acetyl-CoA formed in the liver during β-oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the “ketone bodies,” for export to other tissues.

Acetoacetic acid is the primary ketone body. The other two ketone bodies are derived from acetoacetic acid. Ketone body metabolism consists of two phases.

1. Ketogenesis
2. Ketolysis

## Ketogenesis:

1. Biosynthesis of ketone bodies is called as ketogenesis.
2. Under certain conditions, production of acetyl-CoA either from β-oxidation or pyruvate oxidation is more rapid than it can be utilized for other metabolic processes.

3. Liver converts the excess acetyl-CoA to ketone bodies. Hence, liver mitochondria can be considered as net producer of ketone bodies.
4. Acetoacetate is the primary ketone body while β-hydroxy butyrate and acetone are secondary ketone bodies.

The steps involved are shown in **Figure1.** Beta hydroxyl butyrate does not contain a keto group and therefore strictly speaking is not a ketone body. It contains alcohol group.

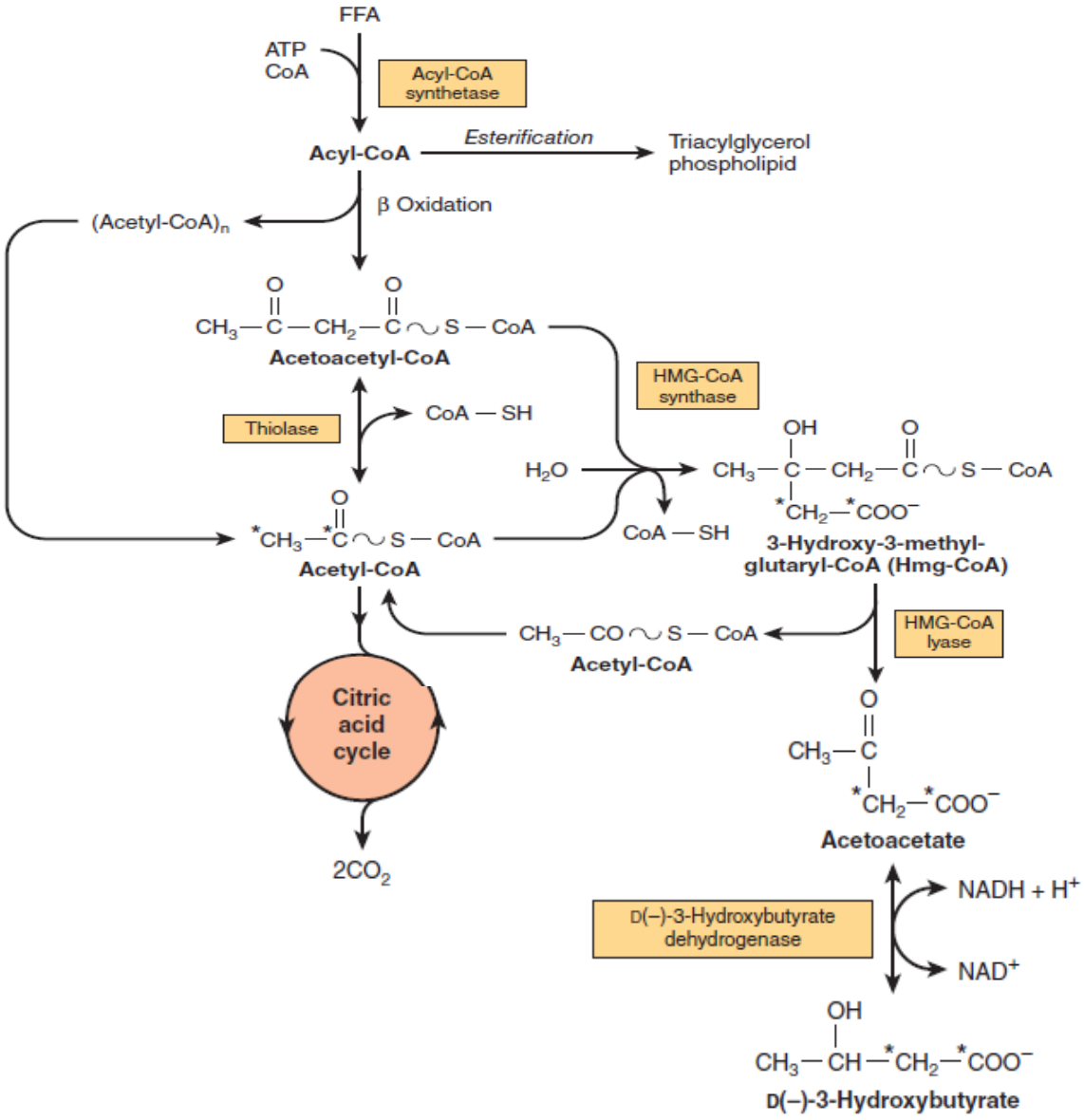


**Fig. 1: Ketone bodies biosynthesis in liver (Ketogenesis), FFA, free fatty acids**

## Step 1: Condensation

Two molecules of acetyl-CoA are condensed to form acetoacetyl-CoA. The first step in the formation of acetoacetate, occurring in the liver is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase; this is simply the reversal of the last step of β-oxidation.

## Step 2: Production of HMG-CoA

One more acetyl-CoA is added to acetoacetyl-CoA to form HMG-CoA (β-hydroxy-β-methylglutaryl-CoA). The enzyme is HMG-CoA synthase.

Mitochondrial HMG-CoA is used for ketogenesis, while cytosolic fraction is used for cholesterol synthesis. HMG-CoA is cleaved to free acetoacetate and acetyl-CoA. The acetoacetate is reversibly reduced by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-β-hydroxybutyrate. This enzyme is specific for the D-stereoisomer; it does not act on L-β hydroxyacyl-CoA and is not to be confused with L-β-hydroxyacyl-CoA dehydrogenase of the β-oxidation pathway.

**Step 3: Lysis**

Then HMG-CoA is lysed to form acetoacetate. Acetoacetate may also be formed by the degradation of carbon skeleton of ketogenic amino acids like leucine, lysine, phenylalanine and tyrosine. HMG-CoA lyase is present only in liver.

**Step 4: Reduction**

Beta-hydroxy butyrate is formed by reduction of acetoacetate. Ratio between acetoacetate and β-hydroxyl butyrate is decided by the cellular NAD : NADH ratio.

**Step 5: Spontaneous Decarboxylation**

Acetone, produced in smaller quantities than the other ketone bodies, is exhaled. In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of acetoacetate decarboxylase. Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic. Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.

Acetoacetate and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as the heart muscle and renal cortex prefer the ketone bodies to glucose as fuel. Other tissues like skeletal muscle and brain can also utilize the ketone bodies as alternate sources of energy, if glucose is not available. The brain, which preferentially uses glucose as fuel, can adapt to the use of acetoacetate or D-β-hydroxybutyrate under starvation conditions, when glucose is unavailable. The production and export of ketone bodies from the liver to extrahepatic tissues allow continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle, **Figure 2.**

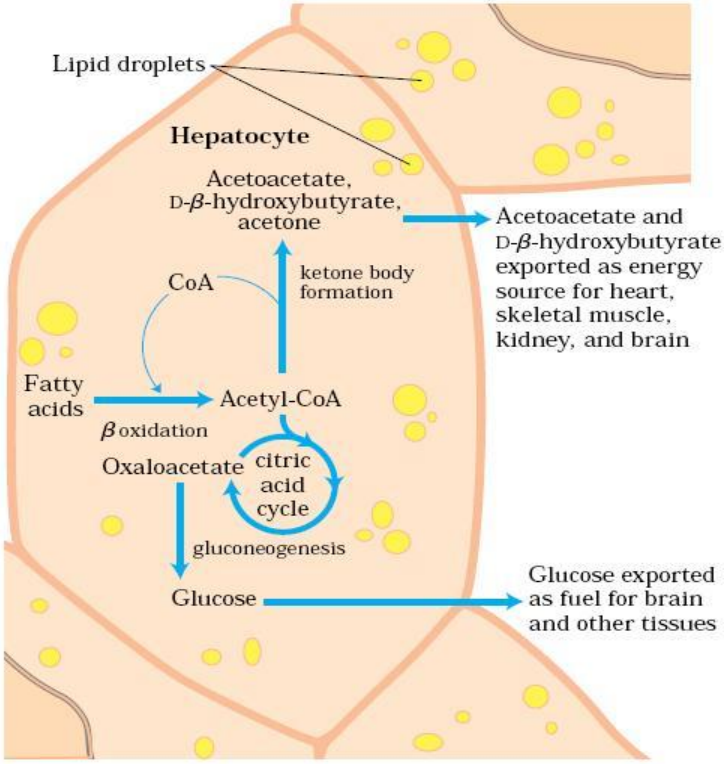


**Fig. 2: Ketone body formation and export from the liver. Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate. The released coenzyme A allows continued β- oxidation of fatty acids.**

## Explanation for Ketogenesis

1. During starvation and diabetes mellitus, the blood level of glucagon is increased. Glucagon inhibits glycolysis, activates gluconeogenesis, activates lipolysis, decreases malonyl-CoA level and stimulates ketogenesis. High glucagon/insulin ratio is potentially ketogenic.
2. Insulin has the opposite effect; it favors glycolysis, inhibits gluconeogenesis, depresses lipolysis, and increases malonyl-CoA level and decreases ketogenesis.

## Ketolysis:

Degradation of ketone bodies is called as ketolysis. The ketone bodies are formed in the liver; but they are utilized by extrahepatic tissues. Acetoacetate is converted to acetoacetyl-CoA by thiophorase enzyme, see below in **Figure 3.**

Almost all tissues and cell types can use ketone bodies as fuel, with the exception of liver and RBC. The placenta can use ketone body as fuel. Intestinal mucosal cells, brain and adipocytes use ketone bodies. Skeletal muscles, heart, liver, etc. primarily utilize fatty acids during starvation. Then acetoacetyl-CoA enters the β-oxidation pathway to produce energy. Summary of ketone body metabolism is shown in **Figure 4.**

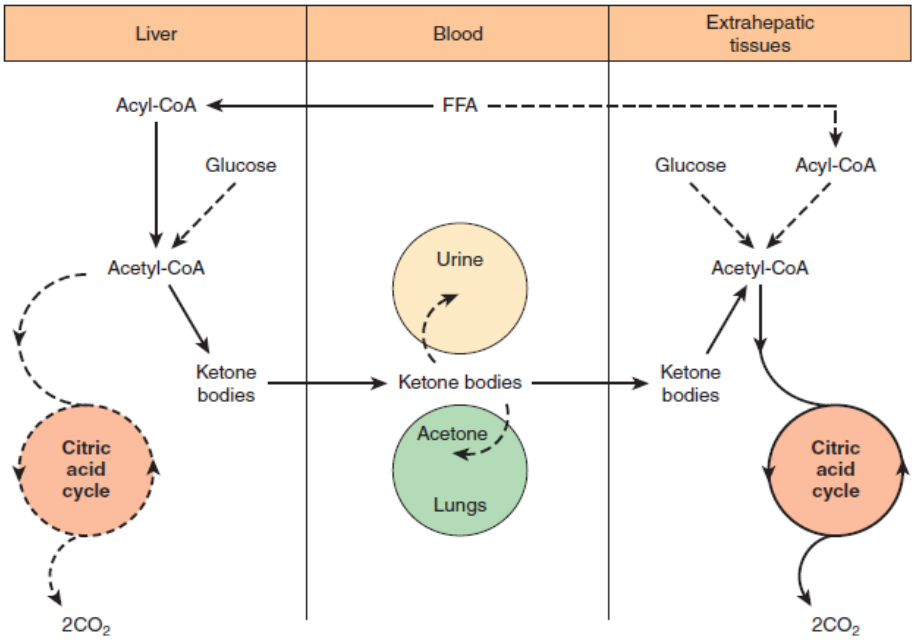


**Fig. 3: Formation, utilization, and excretion of ketone bodies. (The main pathway is indicated by the solid arrows.)**

## Biological Importance

1. Heart and kidney cortex prefers to use ketone bodies rather than glucose.
2. During prolonged starvation, brain derives most of energy from ketone bodies.
3. Liver is unable to use ketone bodies due to lack of enzymes.

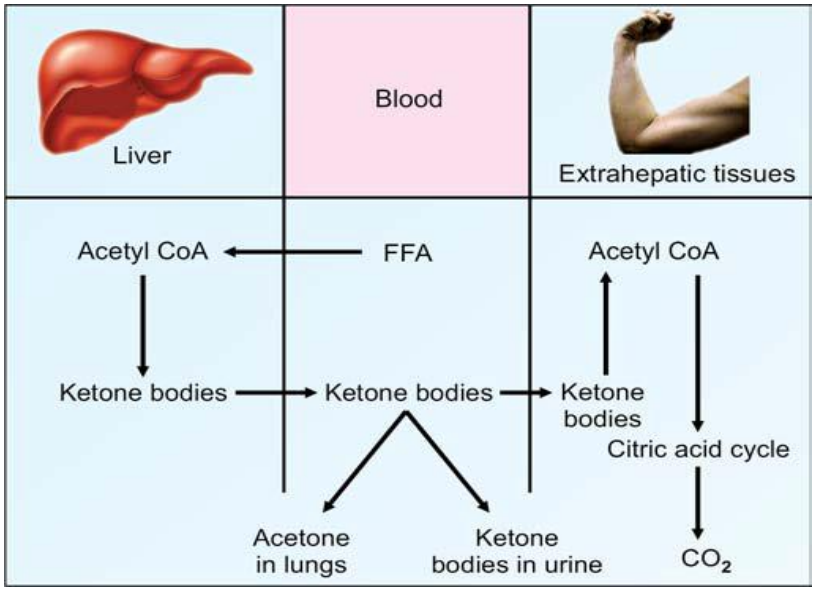


**Fig. 4: Summary of ketone body's metabolism (Formation, utilization and excretion)**

## Ketosis:

1. Normally the rate of synthesis of ketone bodies by the liver is such that they can be easily metabolized by the extrahepatic tissues. Hence, the blood level of ketone bodies is less than 1 mg/dL and only traces are excreted in urine (not detectable by usual tests).

2. But when the rate of synthesis exceeds the ability of extrahepatic tissues to utilize them, there will be accumulation of ketone bodies in blood.
3. This leads to ketonemia, excretion in urine (ketonuria) and smell of acetone in breath. All these three together constitute the condition known as ketosis.

## Causes for Ketosis:

1. Diabetes mellitus: Untreated diabetes mellitus is the most common cause for ketosis. Even though glucose is in plenty, the deficiency of insulin causes accelerated lipolysis and more fatty acids are released into circulation. Oxidation of these fatty acids increases the acetyl-CoA pool. Enhanced gluconeogenesis restricts the oxidation of acetyl-CoA by TCA cycle, since availability of oxaloacetate is less.
2. Starvation: In starvation, the dietary supply of glucose is decreased. Available oxaloacetate is channeled to gluconeogenesis. The increased rate of lipolysis is to provide alternate source of fuel. The excess acetyl CoA is converted to ketone bodies. The high glucagon favors ketogenesis. The brain derives 75% of energy from ketone bodies under conditions of fasting. Hyperemesis (vomiting) in early pregnancy may also lead to starvation-like condition and may lead to ketosis, **Figure 5.**

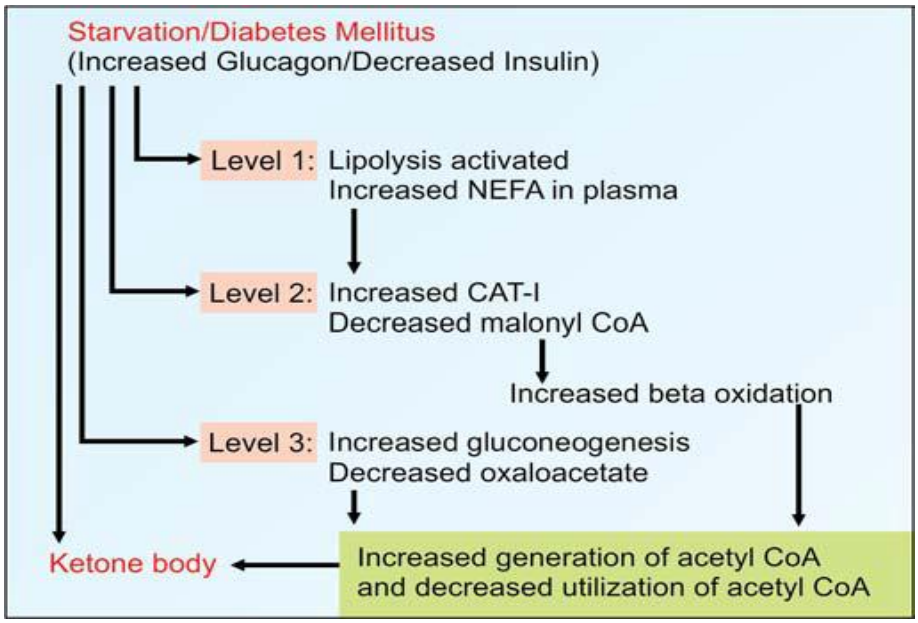


**Fig. 5: Summary of ketosis**

## Diagnosis of Ketosis

The presence of ketosis can be established by the detection of ketone bodies in urine by Rothera's test. Supportive evidence may be derived from estimation of serum electrolytes, acid-base parameters, glucose and urea estimation.

## Differential Diagnosis of Ketosis

The urine of a patient with diabetic keto acidosis will give positive Benedict's test as well as Rothera's test. But in starvation ketosis, Benedict's test is negative, but Rothera's test will be positive.

## Regulation of Ketogenesis:

These several ways for regulation of ketogenesis are summarized below: -

1. Mobilization of free fatty acids from adipose tissue controls ketogenesis. Any condition that increases mobilization of fat increase ketone body formation. Ketosis does not occur in vivo unless there is an increase in the level of circulating FFAs that arise from lipolysis of triacylglycerol in adipose tissue. FFAs are the precursors of ketone bodies in the liver.
2. Liver carnitine-acyl transferase-I activity determines rate of ketone body formation. Under fed conditions, CAT-I activity is inhibited by malonyl-CoA. Hence, ketogenesis is decreased due to less acetyl-CoA. During starvation, CAT-I activity is high due to low malonyl-CoA, Fig. 6. Hence, ketogenesis is more due to plenty of acetyl-CoA. After uptake by the liver, FFAs are either α-oxidized to $CO_2$ or ketone bodies or esterified to triacylglycerol and phospholipid. There is regulation of entry of fatty acids into the oxidative pathway by carnitine palmitoyltransferase- I (CPT-I), and the remainder of the fatty acid taken up is esterified. CPT-I activity is low in the fed state, leading to depression of fatty acid oxidation, and high in starvation, allowing fatty acid oxidation to increase.
3. Malonyl-CoA, the initial intermediate in fatty acid biosynthesis formed by acetyl-CoA carboxylase in the fed state, is a potent inhibitor of CPT-I. Under these conditions, FFA enter the liver cell in low concentrations and are nearly all esterified to triacylglycerols and transported out of the liver in very low-density lipoproteins (VLDL). However, as the concentration of FFA increases with the onset of starvation, acetyl-CoA carboxylase is inhibited directly by acyl-CoA, and (malonyl-CoA) decreases, releasing the inhibition of CPT-I and allowing more acyl-CoA to be β-oxidized. These events are reinforced in starvation by a decrease in the (insulin)/(glucagon) ratio. Thus, β-oxidation from FFA is controlled by the CPT-I gateway into the mitochondria, and the balance of the FFA uptake not oxidized is esterified.
4. ATP level in the cell controls ketogenesis. More ATP level favors ketogenesis whereas low ATP level prevents ketogenesis. In turn, the acetyl-CoA formed in β-oxidation is oxidized in the citric acid cycle, or it enters the pathway of ketogenesis to form ketone bodies. As the level of

serum FFA is raised, proportionately more FFA is converted to ketone bodies and less is oxidized via the citric acid cycle to $CO_2$.

**5.** Ketogenesis may be regarded as a mechanism that allows the liver to oxidize increasing quantities of fatty acids within the constraints of a tightly coupled system of oxidative phosphorylation.

**6.** A fall in the concentration of oxaloacetate, particularly within the mitochondria, can impair the ability of the citric acid cycle to metabolize acetyl-CoA and divert fatty acid oxidation toward ketogenesis. Such a fall may occur because of an increase in the (NADH)/(NAD+) ratio caused by increased β-oxidation of fatty acids affecting the equilibrium between oxaloacetate and malate, leading to a decrease in the concentration of oxaloacetate, and when gluconeogenesis is elevated, which occurs when blood glucose levels are low. The activation of pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, by acetyl-CoA partially, alleviates this problem, but in conditions such as starvation and untreated diabetes mellitus, ketone bodies are overproduced causing ketosis.

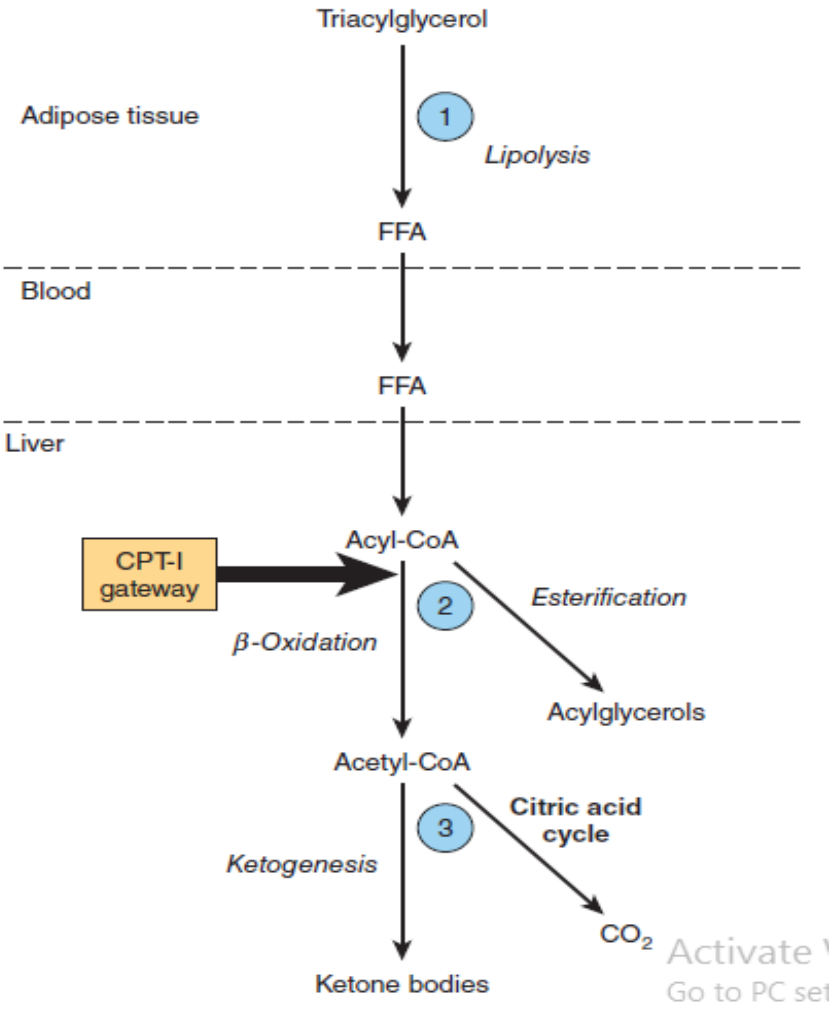


**Fig. 6: Regulation of ketogenesis. 1 to 3 show three crucial steps in the pathway of metabolism of free fatty acids (FFA) that determine the magnitude of ketogenesis. (CPT-I, carnitine palmitoyltransferase-I.)**

## Medical Importance:

1. Usually, the utilization of ketone bodies by peripheral tissues is proportional to their formation. Normal blood ketone bodies level is 1.0 mg/100ml.
2. Under certain metabolic conditions, the rate of ketone body formation exceeds the rate of their utilization by peripheral tissues. This results in accumulation of ketone bodies in blood (hyper ketonemia) and their excretion in urine (ketonuria).
3. Ketosis. Hyper ketonemia and ketonuria gives rise to ketosis. Main clinical symptoms of ketosis are headache, nausae, vomiting and finally coma. It occurs in starvation, uncontrolled diabetes mellitus, high fat diet, von Geirke's disease, fevers, severe muscular exercise and congenital propionyl-CoA carboxylase deficiency. Ketosis also occurs in ruminants. In cattle, it occurs during lactation. In sheep, it occurs due to toxemia of pregnancy.
4. Ketoacidosis. Under normal conditions, ketone bodies acetoacetate and β-hydroxybutyrate are neutralized by blood bicarbonate to maintain constant blood pH. Their formation in large quantities in starvation and diabetes causes depletion of blood bicarbonate. As a result, blood pH decreases and leads to condition known as acidosis. Since acidosis is due to over production of ketone bodies it is also called as ketoacidosis. Thus, over production of ketone bodies causes ketoacidosis.

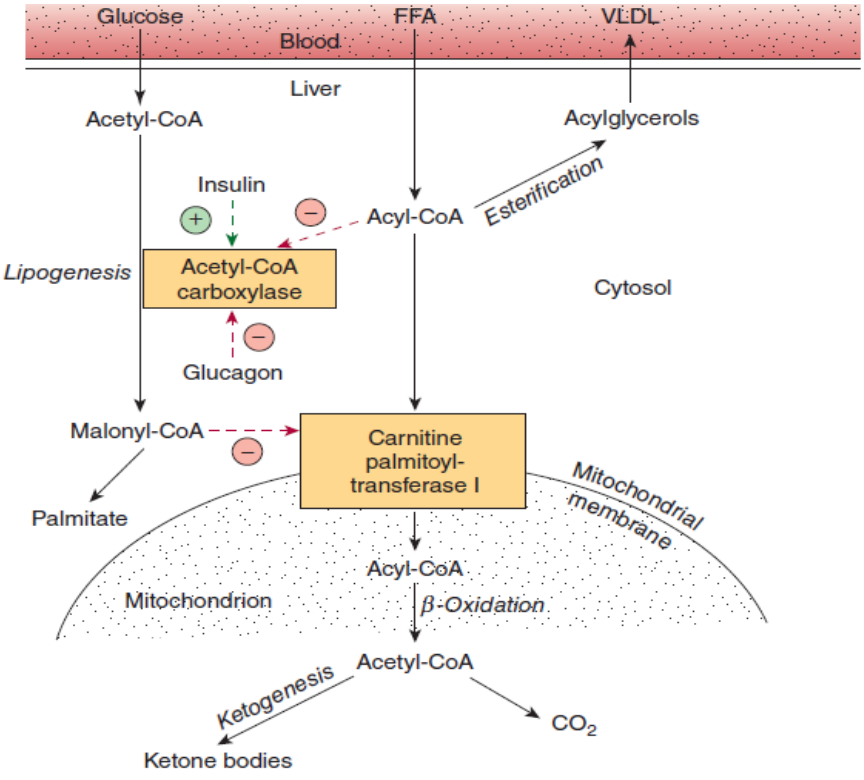


**Fig. 7: Regulation of long-chain fatty acid oxidation in the liver. (FFA, free fatty acids; VLDL, very low-density lipoprotein.) Positive and negative regulatory effects are represented by broken arrows and substrate flow by solid arrows.**