



# **ADVANCES IN METABOLISM**

## **BCH 540**



# OVERVIEW OF LIPID & PROTEIN METABOLISM


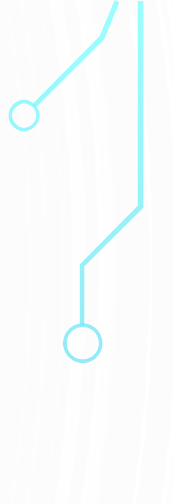
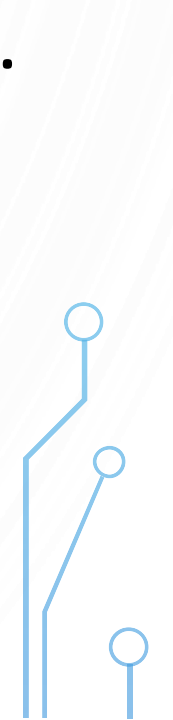
**Metabolism:** interconversion of chemical compounds in the body, the pathways taken by individual molecules, their interrelationships, and the mechanisms that regulate the flow of metabolites through the pathways





**Metabolic pathways :**

**Anabolic pathways**

- **Synthesis of larger and more complex compounds from smaller precursors (synthesis of protein from amino acids and synthesis of reserves of triacylglycerol and glycogen).**
  - **Endothermic pathways.**
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## **Catabolic pathways**

- **Breakdown of larger molecules, involving oxidative reactions; exothermic, producing reducing equivalents, and, mainly via the respiratory chain, ATP.**

## **Amphibolic pathways**

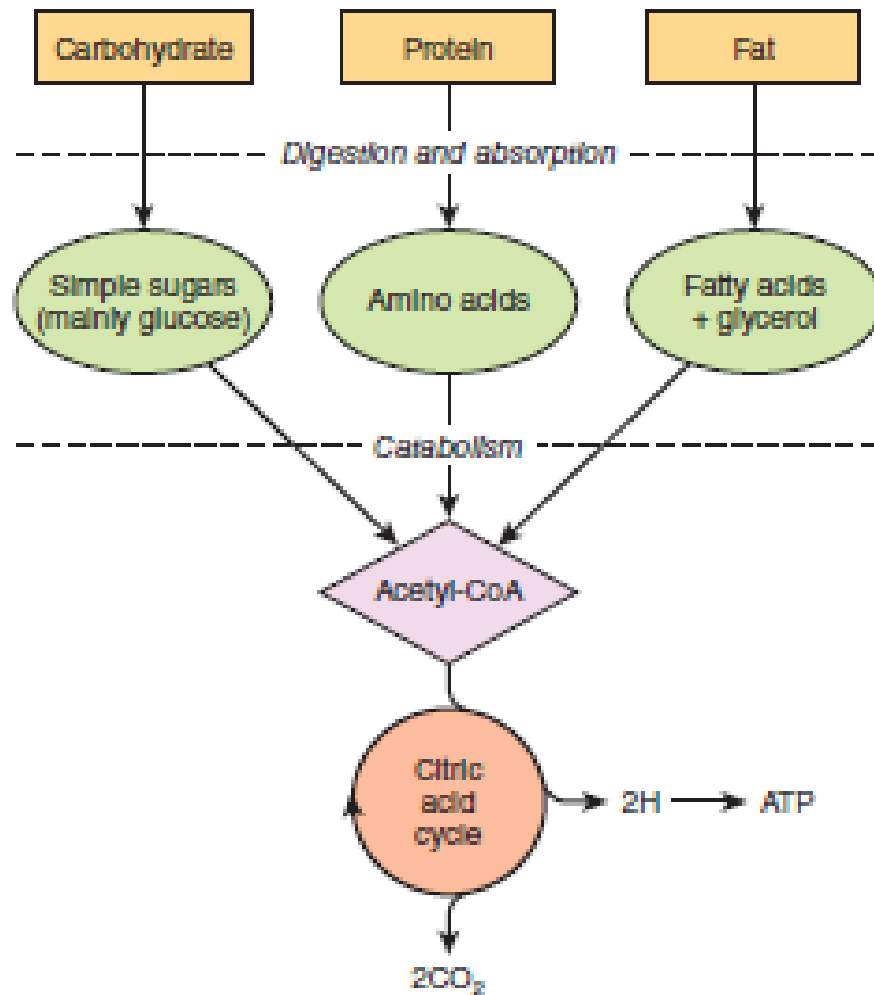
- **Occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, eg, the citric acid cycle.**



## **PATHWAYS THAT PROCESS THE MAJOR PRODUCTS OF DIGESTION**

The nature of the diet sets the basic pattern of metabolism. There is a need to process the products of digestion of dietary carbohydrate, lipid, and protein. These are mainly glucose, fatty acids and glycerol, and amino acids, respectively. All the products of digestion are metabolized to a common product, acetyl-CoA, which is then oxidized by the citric acid cycle, ultimately yielding ATP by the process of oxidative phosphorylation.





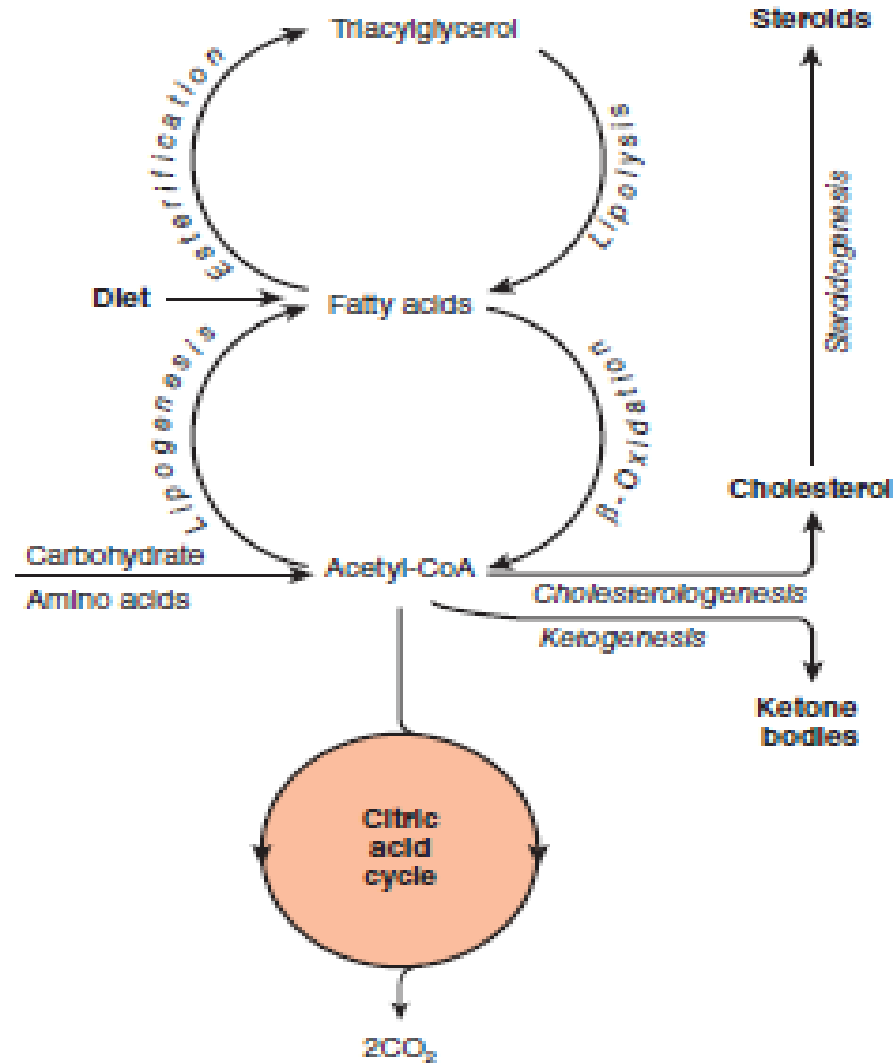
OUTLINE OF THE PATHWAYS FOR THE CATABOLISM OF DIETARY CARBOHYDRATE, PROTEIN, AND FAT. (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28<sup>TH</sup> EDITION).



## **LIPID METABOLISM IS CONCERNED MAINLY WITH FATTY ACIDS & CHOLESTEROL**

- The source of long-chain fatty acids is either dietary lipid or de novo synthesis from acetyl-CoA derived from carbohydrate or amino acids.
- Fatty acids may be oxidized to acetyl-CoA (-oxidation) or esterified with glycerol, forming triacylglycerol (fat) as the body's main fuel reserve.





OVERVIEW OF FATTY ACID METABOLISM SHOWING THE MAJOR PATHWAYS AND END PRODUCTS. (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28TH EDITION).



**Acetyl-CoA formed by  $\beta$ -oxidation may undergo three fates.**

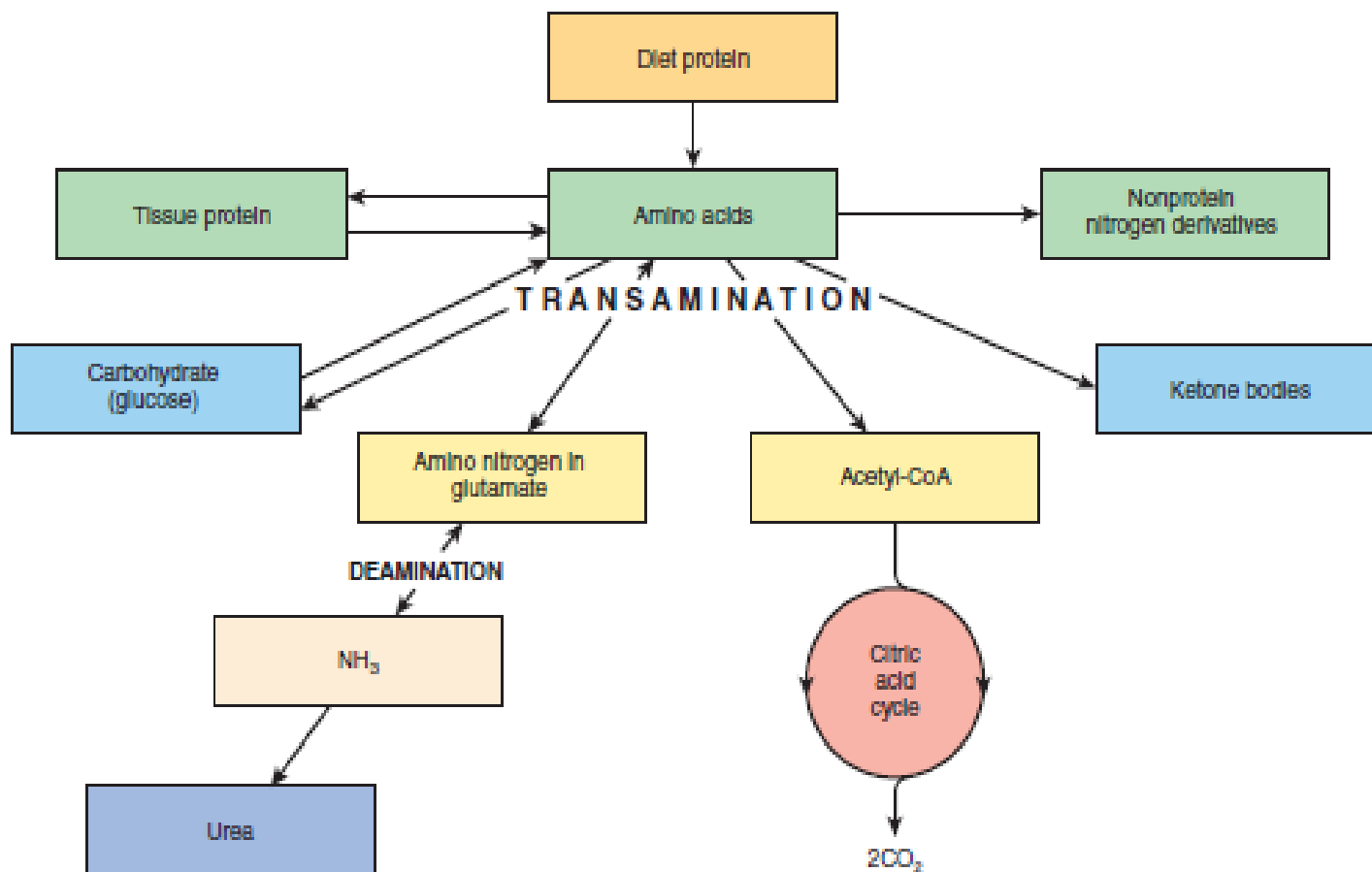
- **Acetyl-CoA arising from glycolysis oxidized to  $\text{CO}_2 + \text{H}_2\text{O}$  via the citric acid cycle.**
- **It is the precursor for synthesis of cholesterol and other steroids.**
- **In the liver, it is used to form ketone bodies (acetoacetate and 3-hydroxybutyrate) that are important fuels in prolonged fasting.**



## **MUCH OF AMINO ACID METABOLISM INVOLVES TRANSAMINATION**

- The amino acids are required for protein synthesis.
- The essential amino acids must be supplied in the diet, since they cannot be synthesized in the body.
- The nonessential amino acids are supplied in the diet, but can also be formed from metabolic intermediates by transamination using the amino nitrogen from other amino acids.





**OVERVIEW OF AMINO ACID METABOLISM SHOWING THE MAJOR PATHWAYS AND END PRODUCTS. (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28TH EDITION).**



- **After deamination, amino nitrogen is excreted as urea.**
- **The carbon skeletons that remain after transamination may:**
  - (1) be oxidized to CO<sub>2</sub> via the citric acid cycle.**
  - (2) be used to synthesize glucose (gluconeogenesis).**
  - (3) form ketone bodies, which may be oxidized or be used for synthesis of fatty acids.**



## **METABOLIC PATHWAYS STUDIED AT DIFFERENT LEVELS OF ORGANIZATION**

**(1) At the tissue and organ level the nature of the substrates entering and metabolites leaving tissues and organs is defined. (2) At the subcellular level each cell organelle (eg, the mitochondrion) or compartment (eg, the cytosol) has specific roles that form part of a subcellular pattern of metabolic pathways.**



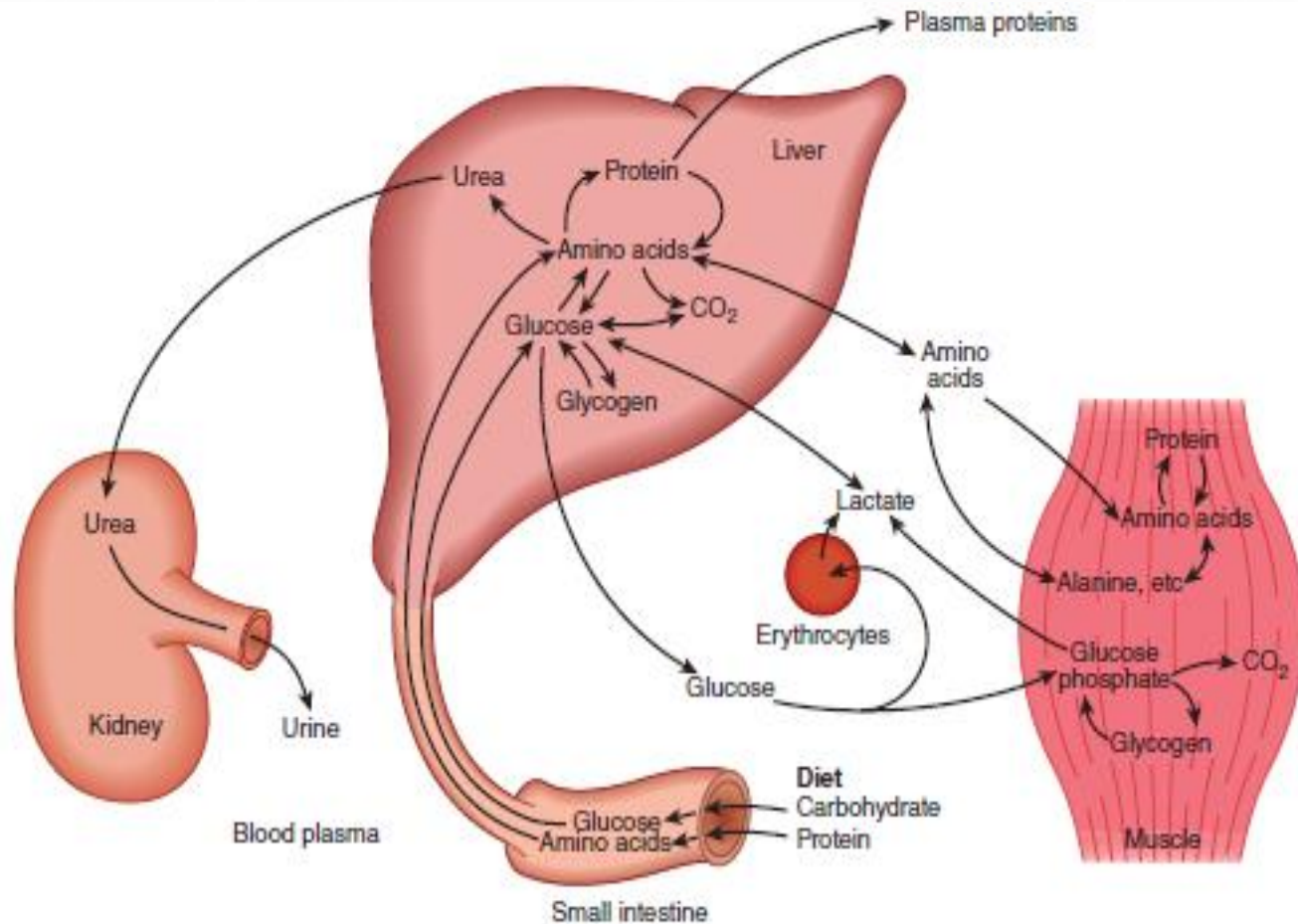
## **AT THE TISSUE & ORGAN LEVEL, THE BLOOD CIRCULATION INTEGRATES METABOLISM**

- **Amino acids resulting from the digestion of dietary protein are absorbed via the hepatic portal vein. The liver has the role of regulating the blood concentration of water-soluble metabolites. In the case of glucose, this is achieved by taking up glucose in excess of immediate requirements and converting it to glycogen (glycogenesis) or to fatty acids (lipogenesis).**



- **Between meals, the liver acts to maintain the blood glucose concentration by breaking down glycogen (glycogenolysis) and, together with the kidney, by converting noncarbohydrate metabolites such as lactate, glycerol, and amino acids to glucose (gluconeogenesis).**
- **The liver also synthesizes the major plasma proteins (eg, albumin) and deaminates amino acids that are in excess of requirements, forming urea, which is transported to the kidney and excreted.**





**TRANSPORT AND FATE OF MAJOR CARBOHYDRATE AND AMINO ACID SUBSTRATES AND METABOLITES. (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28TH EDITION).**



**Skeletal muscle** utilizes glucose as a fuel, both aerobically, forming CO<sub>2</sub>, and anaerobically, forming lactate. It stores glycogen as a fuel for use in muscle contraction and synthesizes muscle protein from plasma amino acids. Muscle accounts for approximately 50% of body mass and consequently represents a considerable store of protein that can be drawn upon to supply amino acids for gluconeogenesis in starvation.

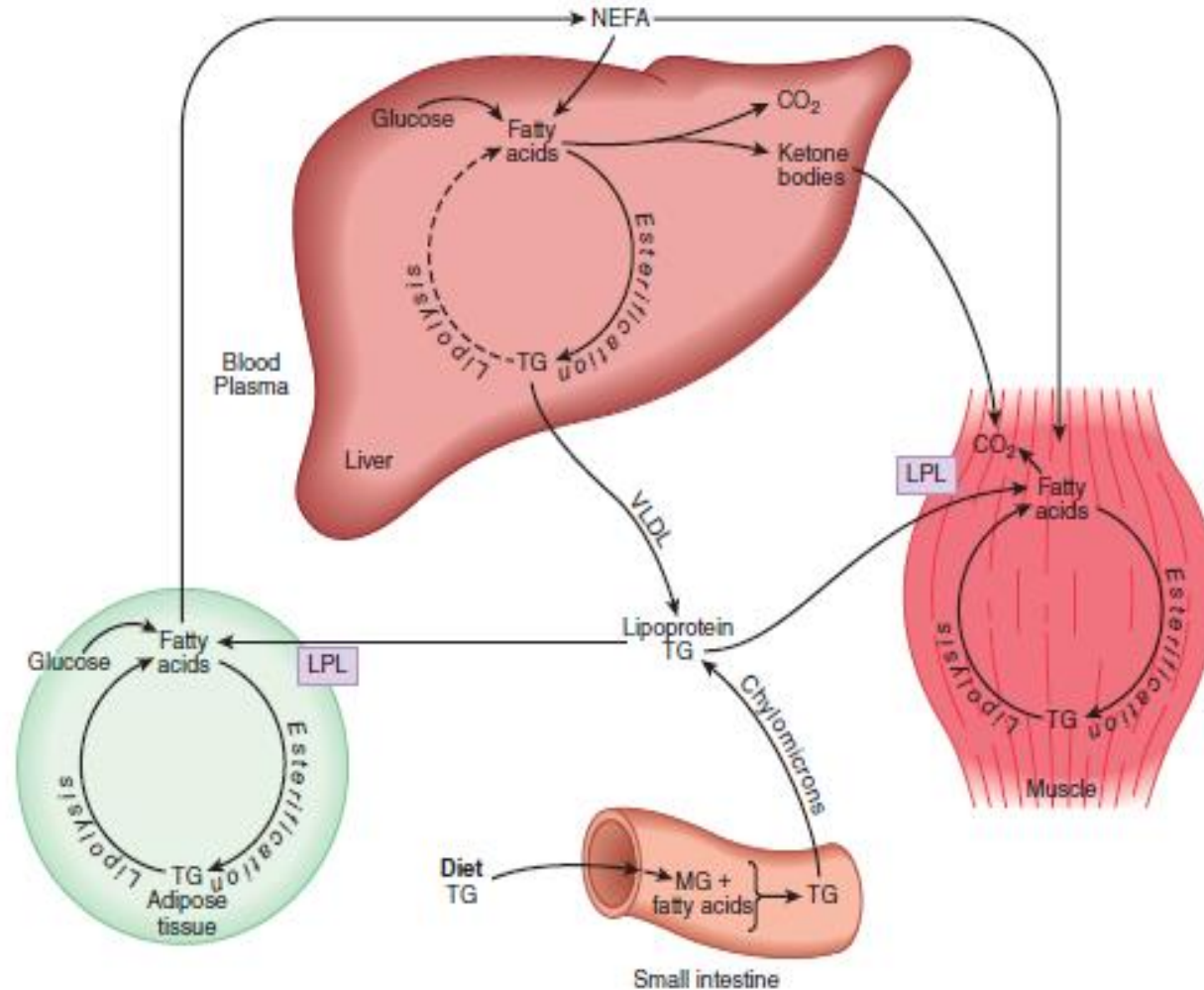


- **Lipids in the diet are mainly triacylglycerol, and are hydrolyzed to mono-acylglycerols and fatty acids in the gut, then re-esterified in the intestinal mucosa. Here they are packaged with protein and secreted into the lymphatic system and thence into the bloodstream as chylomicrons, the largest of the plasma lipoproteins. Chylomicrons also contain other lipid-soluble nutrients.**



- Unlike glucose and amino acids, **chylomicron triacylglycerol** is not taken up directly by the liver. It is first metabolized by tissues that have **lipoprotein lipase**, which hydrolyzes the triacylglycerol, releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel. The chylomicron remnants are cleared by the liver. The other major source of long-chain fatty acids is synthesis (lipogenesis) from carbohydrate, in adipose tissue and the liver.





**TRANSPORT AND FATE OF MAJOR LIPID SUBSTRATES AND METABOLITES. (FFA, FREE FATTY ACIDS; LPL, LIPOPROTEIN LIPASE; MG, MONO ACYLGLYCEROL; TG, TRIACYLGLYCEROL; VLDL, VERY LOW DENSITY LIPOPROTEIN.) (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28TH EDITION).**



- **Adipose tissue triacylglycerol is the main fuel reserve of the body. It is hydrolyzed (lipolysis) and glycerol and free fatty acids are released into the circulation. Glycerol is a substrate for gluconeogenesis. The fatty acids are transported bound to serum albumin; they are taken up by most tissues (but not brain or erythrocytes) and either esterified to triacylglycerols for storage or oxidized as a fuel.**



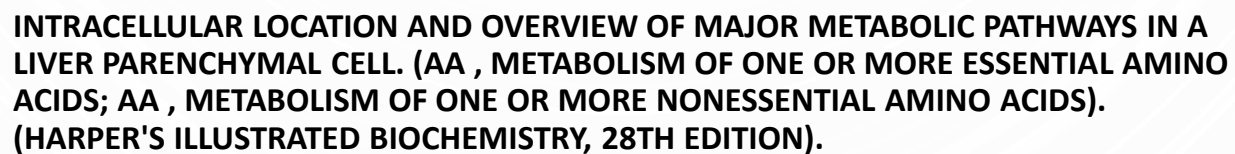
- **In the liver, triacylglycerol arising from lipogenesis, free fatty acids, and chylomicron remnants is secreted into the circulation in very low density lipoprotein (VLDL). This triacylglycerol undergoes a fate similar to that of chylomicrons. Partial oxidation of fatty acids in the liver leads to ketone body production. Ketone bodies are transported to extrahepatic tissues, where they act as a fuel in prolonged fasting and starvation.**



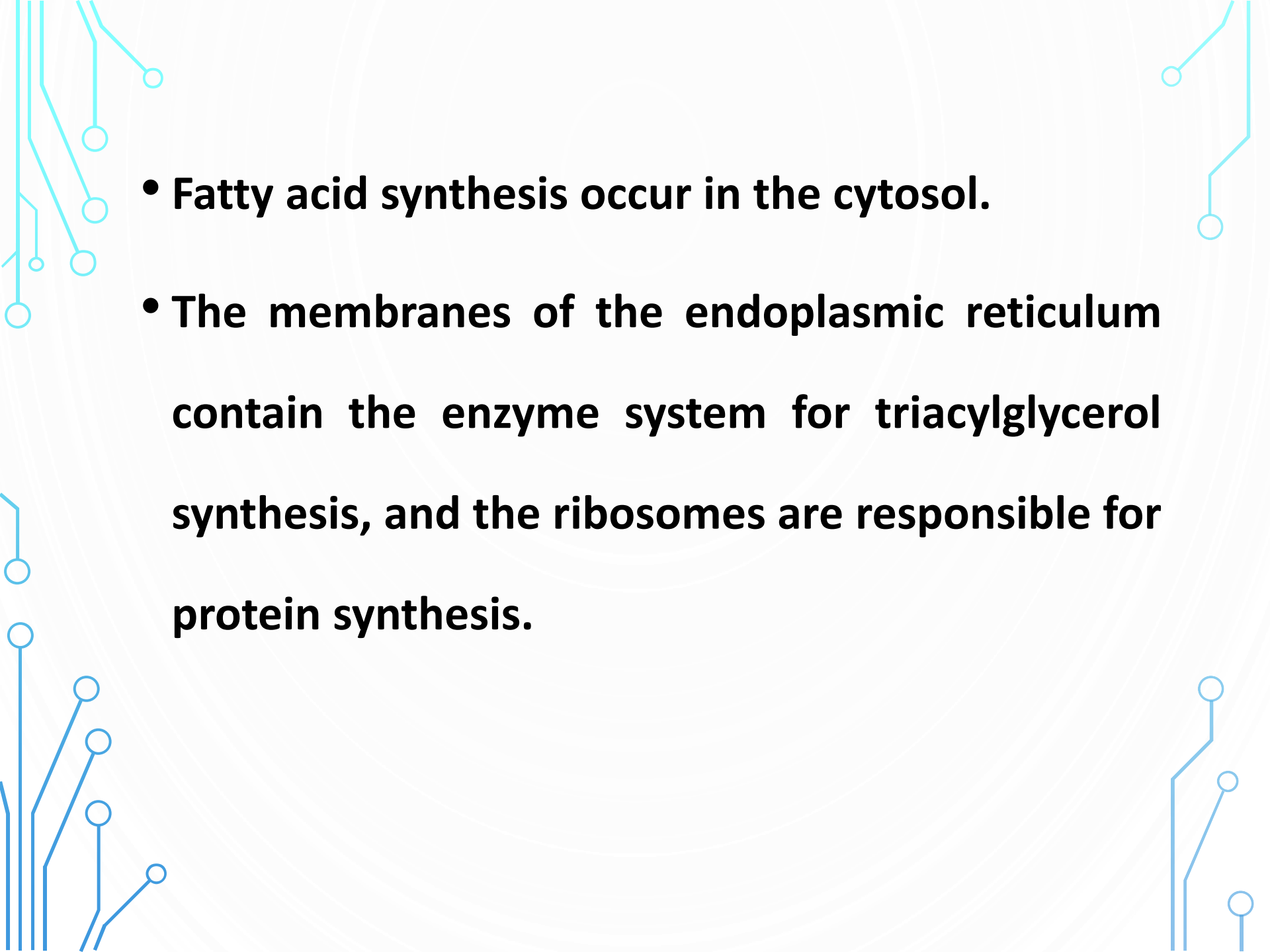
**AT THE SUBCELLULAR LEVEL, GLYCOLYSIS OCCURS IN THE CYTOSOL & THE CITRIC ACID CYCLE IN THE MITOCHONDRIA**

- **Compartmentation of pathways in separate subcellular compartments or organelles permits integration and regulation of metabolism. Not all pathways are of equal importance in all cells.**
- **The central role of the mitochondrion is immediately apparent, since it acts as the focus of carbohydrate, lipid, and amino acid metabolism. It contains the enzymes of the citric acid cycle, -oxidation of fatty acids and ketogenesis, as well as the respiratory chain and ATP synthase.**







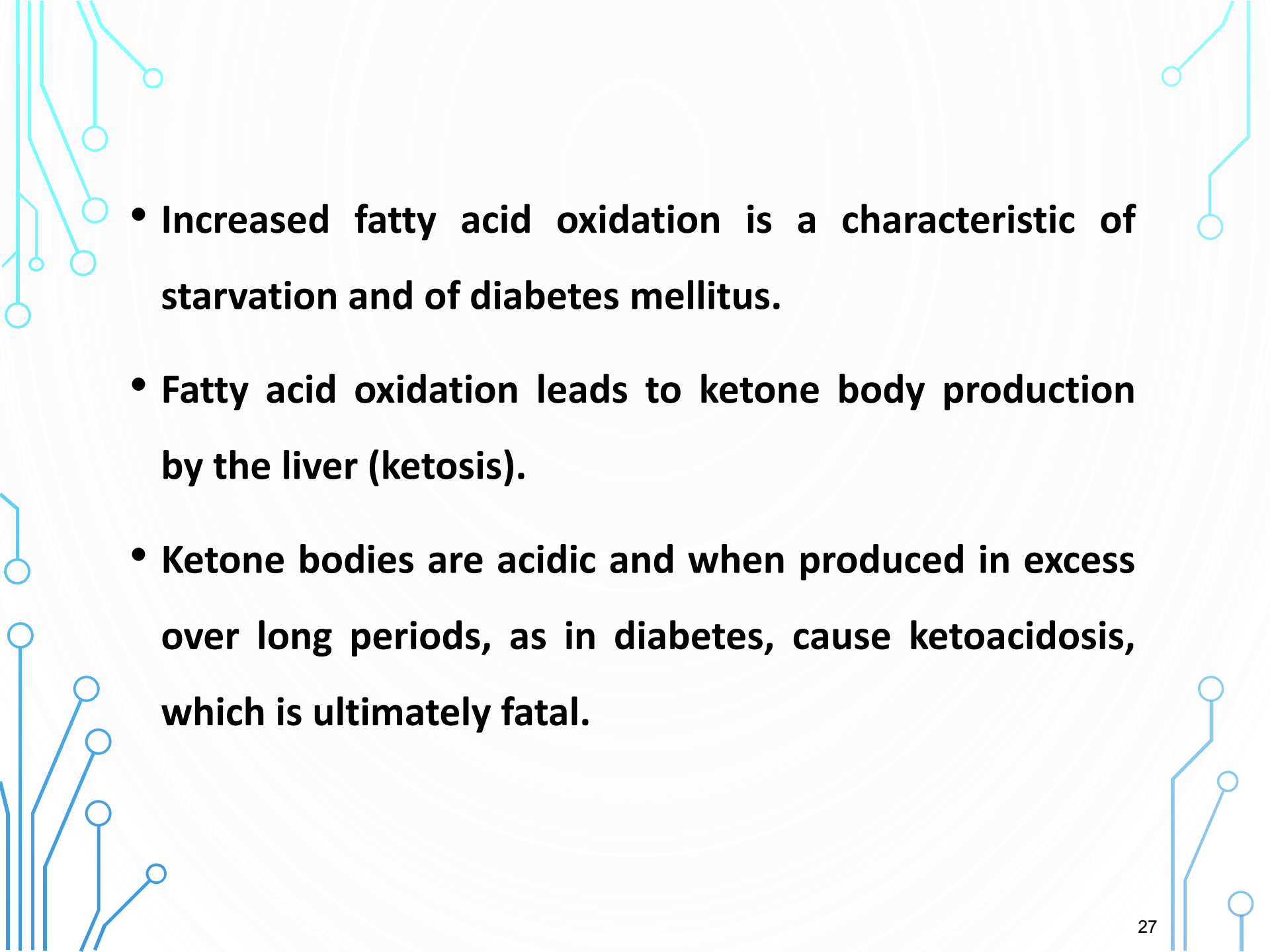
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- The image features a light blue background with faint, concentric circular lines. In the corners, there are decorative elements resembling circuit boards or neural networks, with blue lines and small circles.
- **Fatty acid synthesis occur in the cytosol.**
  - **The membranes of the endoplasmic reticulum contain the enzyme system for triacylglycerol synthesis, and the ribosomes are responsible for protein synthesis.**



## OXIDATION OF FATTY ACIDS

- Fatty acids are both oxidized to acetyl-CoA and synthesized from acetyl-CoA (different process taking place in a separate compartment of the cell).
- Each step in fatty acid oxidation involves acyl-CoA derivatives and is catalyzed by separate enzymes, utilizes NAD<sup>+</sup> and FAD as coenzymes, and generates ATP (an aerobic process).



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- **Increased fatty acid oxidation is a characteristic of starvation and of diabetes mellitus.**
  - **Fatty acid oxidation leads to ketone body production by the liver (ketosis).**
  - **Ketone bodies are acidic and when produced in excess over long periods, as in diabetes, cause ketoacidosis, which is ultimately fatal.**



## **Oxidation of Fatty Acids Occurs in Mitochondria**

### **Fatty Acids Are Transported in the Blood as Free Fatty Acids (FFA)**

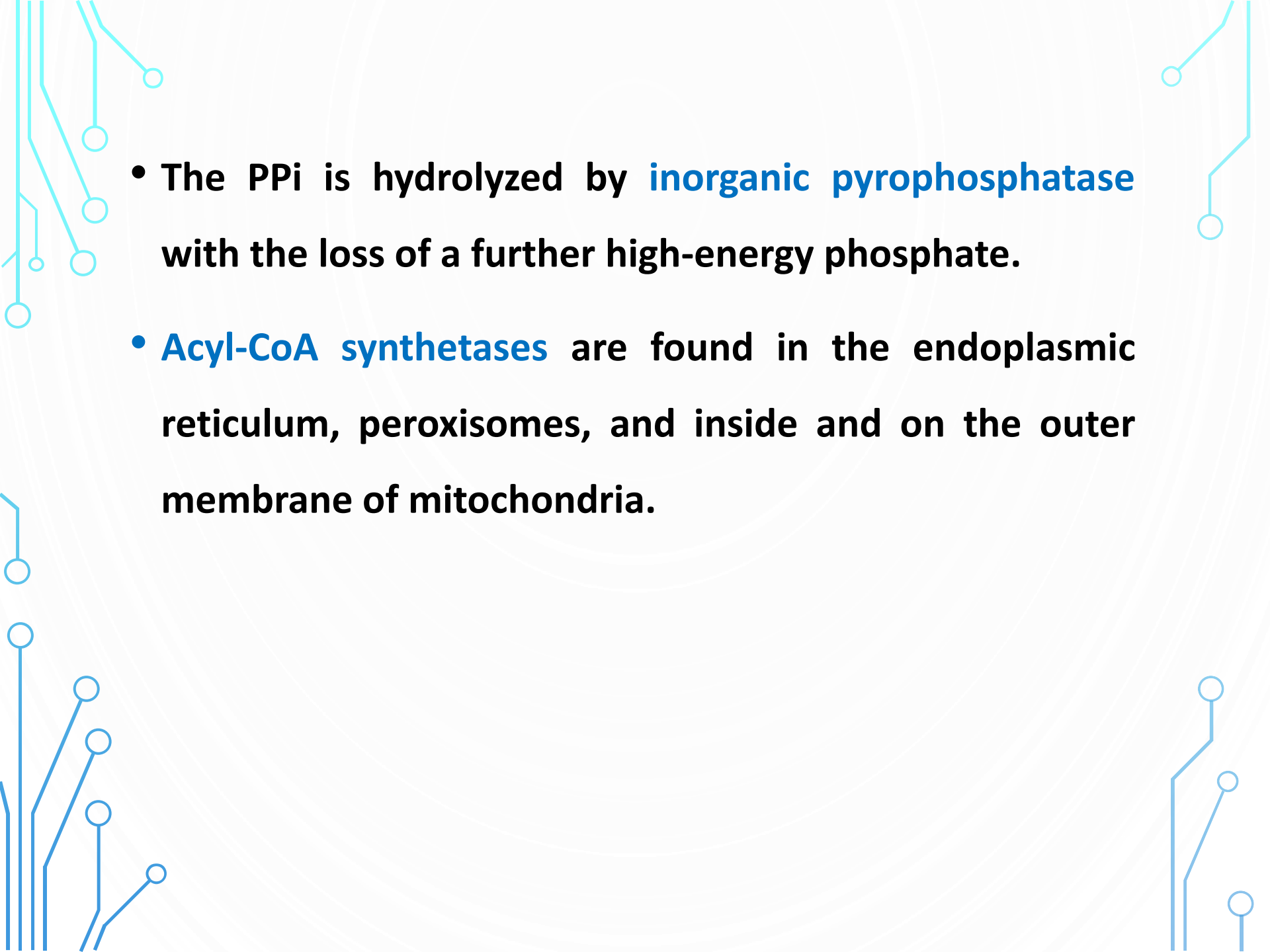
- **Unesterified (UFA) or nonesterified (NEFA) fatty acids—**  
**are fatty acids that are in the unesterified state.**
- **In plasma, longer-chain FFA are combined with**  
**albumin, and in the cell they are attached to a fatty**  
**acid-binding protein**



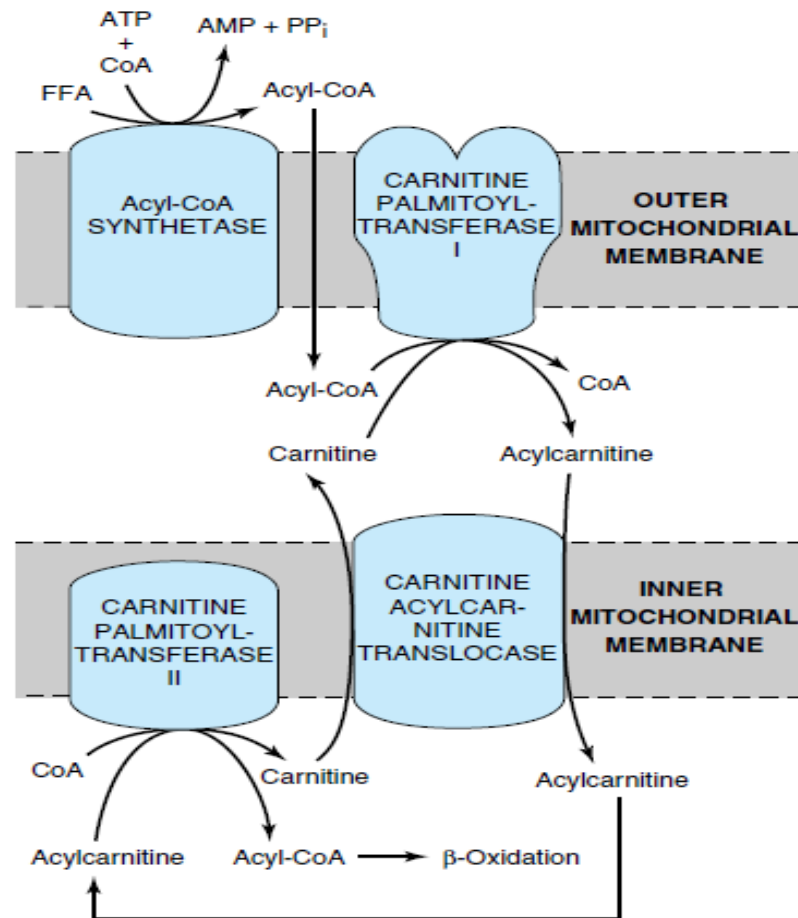
## FATTY ACIDS ARE ACTIVATED BEFORE BEING CATABOLIZED

- Fatty acids must first be converted to an active intermediate before they can be catabolized (only step required ATP).
- In the presence of ATP and coenzyme A, **the enzyme acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid (or free fatty acid) to an "active fatty acid" or acyl-CoA, which uses one high-energy phosphate with the formation of AMP and PPi



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- The slide features decorative circuit-like lines in the corners. The top-left and top-right corners have light blue lines, while the bottom-left and bottom-right corners have darker blue lines. These lines consist of straight segments connected by small circles, resembling a stylized circuit board or neural network.
- The PPI is hydrolyzed by **inorganic pyrophosphatase** with the loss of a further high-energy phosphate.
  - **Acyl-CoA synthetases** are found in the endoplasmic reticulum, peroxisomes, and inside and on the outer membrane of mitochondria.





**ROLE OF CARNITINE IN THE TRANSPORT OF LONG-CHAIN FATTY ACIDS THROUGH THE INNER MITOCHONDRIAL MEMBRANE. LONG-CHAIN ACYL-COA CANNOT PASS THROUGH THE INNER MITOCHONDRIAL MEMBRANE, BUT ITS METABOLIC PRODUCT, ACYLCARNITINE, CAN. (HARPER'S ILLUSTRATED BIOCHEMISTRY, 28TH EDITION).**



## LONG-CHAIN FATTY ACIDS PENETRATE THE INNER MITOCHONDRIAL MEMBRANE AS CARNITINE DERIVATIVES

- Carnitine (-hydroxy- $\gamma$ -trimethylammonium butyrate), is widely distributed and is particularly abundant in muscle. Long-chain acyl-CoA (or FFA) cannot penetrate the inner membrane of mitochondria.
- In the presence of carnitine, however, carnitine palmitoyltransferase-I, located in the outer mitochondrial membrane, converts long-chain acyl-CoA to acylcarnitine, which is able to penetrate the inner membrane and gain access to the  $\beta$ -oxidation system of enzymes.



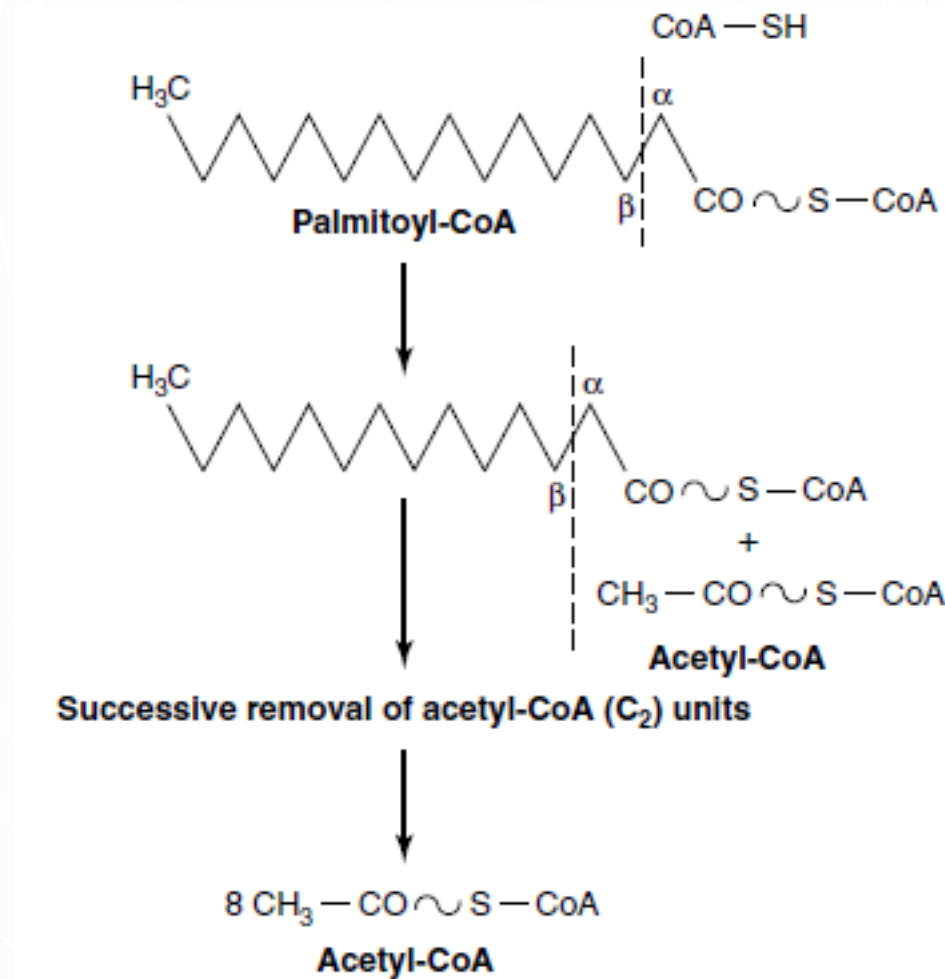
- Carnitine-acylcarnitine translocase acts as an inner membrane exchange transporter. Acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine.
- The acylcarnitine then reacts with CoA, catalyzed by carnitine palmitoyltransferase-II, located on the inside of the inner membrane, reforming acyl-CoA in the mitochondrial matrix, and carnitine is liberated.



## **B-OXIDATION OF FATTY ACIDS INVOLVES SUCCESSIVE CLEAVAGE WITH RELEASE OF ACETYL-COA**

- In  $\beta$ -oxidation, two carbons at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end. The chain is broken between the  $\alpha(2)$ - and  $\beta(3)$ -carbon atoms—hence the name  $\beta$ -oxidation. The two-carbon units formed are acetyl-CoA; thus, palmitoyl-CoA forms eight acetyl-CoA molecules.





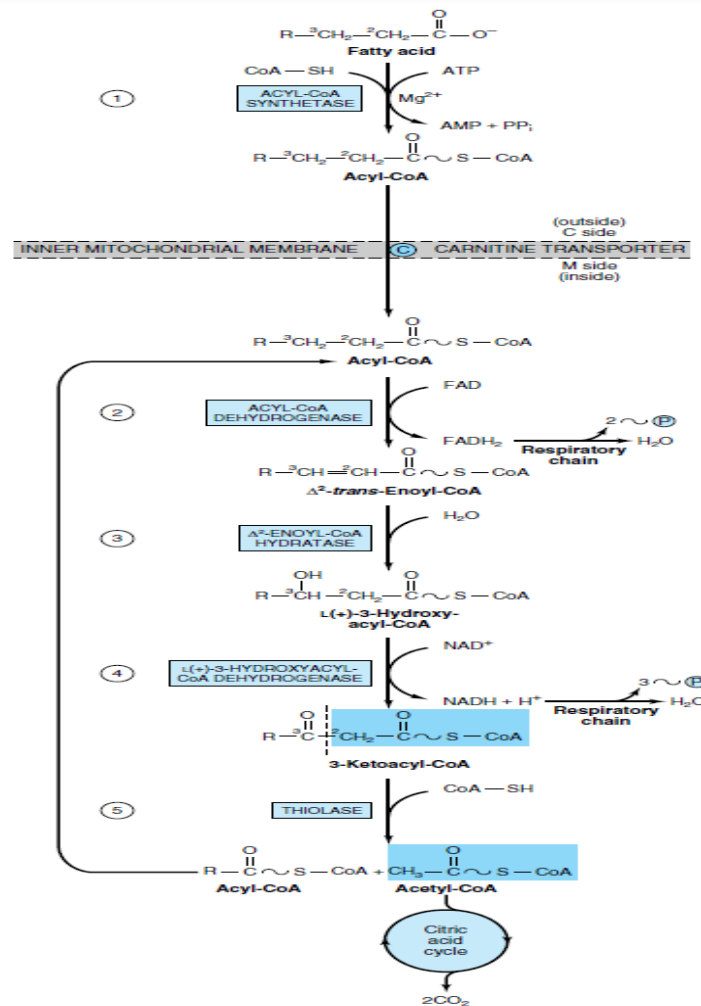
OVERVIEW OF -OXIDATION OF FATTY ACIDS.



## **THE CYCLIC REACTION SEQUENCE GENERATES $\text{FADH}_2$ & $\text{NADH}$**

**Several enzymes, known collectively as "fatty acid oxidase," are found in the mitochondrial matrix or inner membrane adjacent to the respiratory chain. These catalyze the oxidation of acyl-CoA to acetyl-CoA, the system being coupled with the phosphorylation of ADP to ATP**





**B-OXIDATION OF FATTY ACIDS. LONG-CHAIN ACYL-COA IS CYCLED THROUGH REACTIONS 2–5, ACETYL-COA BEING SPLIT OFF, EACH CYCLE, BY THIOLASE (REACTION 5). WHEN THE ACYL RADICAL IS ONLY FOUR CARBON ATOMS IN LENGTH, TWO ACETYL-COA MOLECULES ARE FORMED IN REACTION 5.**



- The first step is the removal of two hydrogen atoms from the 2( $\alpha$ )- and 3( $\beta$ )-carbon atoms, catalyzed by acyl-CoA dehydrogenase and requiring FAD (2-trans - enoyl-CoA and FADH<sub>2</sub> formed).
- The reoxidation of FADH<sub>2</sub> by the respiratory chain requires the mediation of another flavoprotein, termed electron-transferring flavoprotein.
- Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalyzed by  $\Delta^2$ -enoyl-CoA hydratase.



- The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalyzed by L-(+)-3-hydroxyacyl-CoA dehydrogenase to form the corresponding 3-ketoacyl-CoA compound.
- In this case, NAD<sup>+</sup> is the coenzyme involved. Finally, 3-ketoacyl-CoA is split at the 2,3-position by thiolase (3-ketoacyl-CoA-thiolase), forming acetyl-CoA and a new acyl-CoA two carbons shorter than the original acyl-CoA molecule.



- **The acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2. In this way, a long-chain fatty acid may be degraded completely to acetyl-CoA (C2 units). Since acetyl-CoA can be oxidized to  $\text{CO}_2$  and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.**



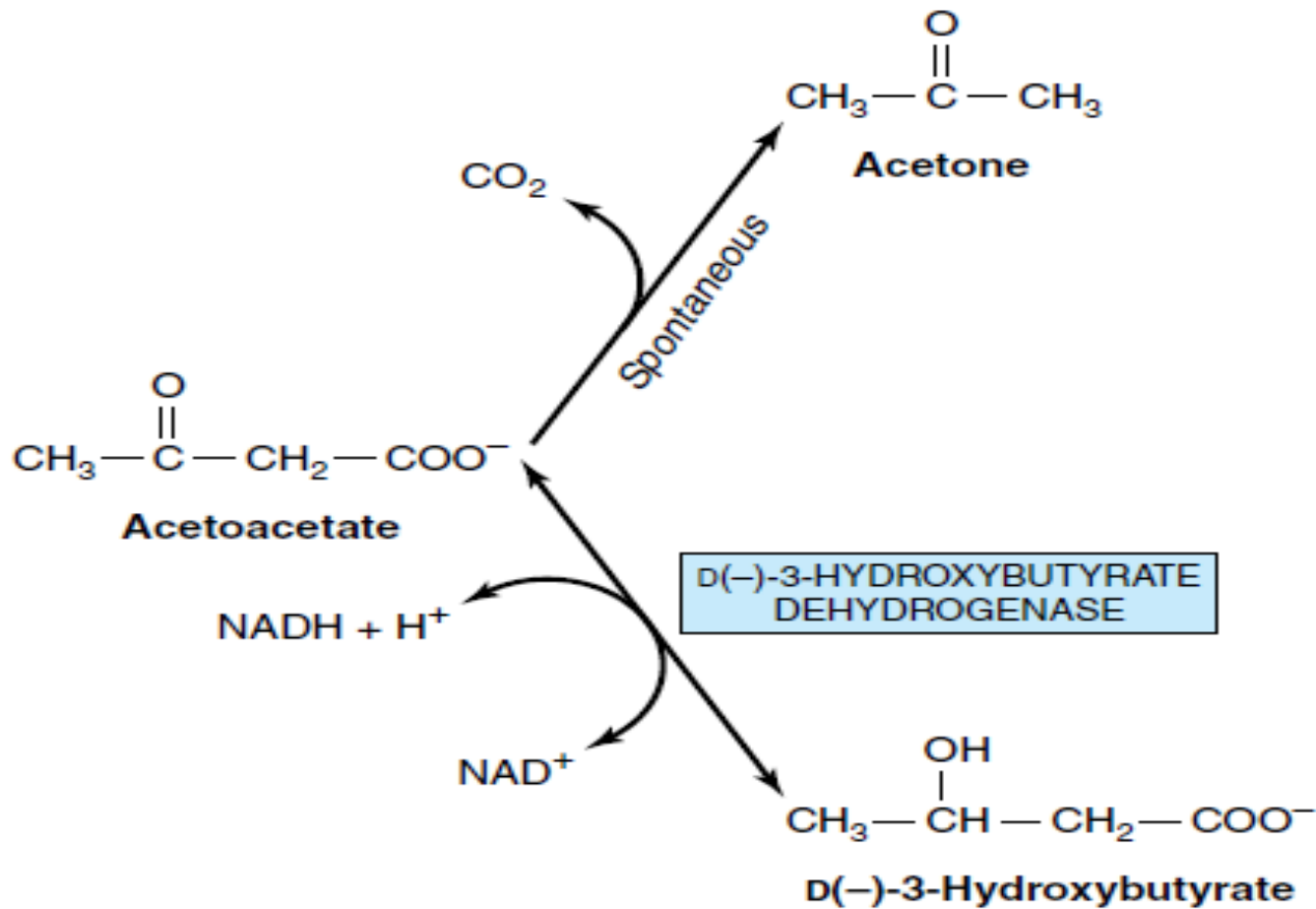
## **KETOGENESIS OCCURS WHEN THERE IS A HIGH RATE OF FATTY ACID OXIDATION IN THE LIVER**

- The oxidation of fatty acid in liver with high rate produce considerable quantities of acetoacetate and D(–)-3-hydroxybutyrate ( $\beta$ -hydroxybutyrate).
- Continually decarboxylation of acetoacetate yield acetone.
- Acetoacetate,  $\beta$ -hydroxybutyrate and acetone known as the ketone bodies.



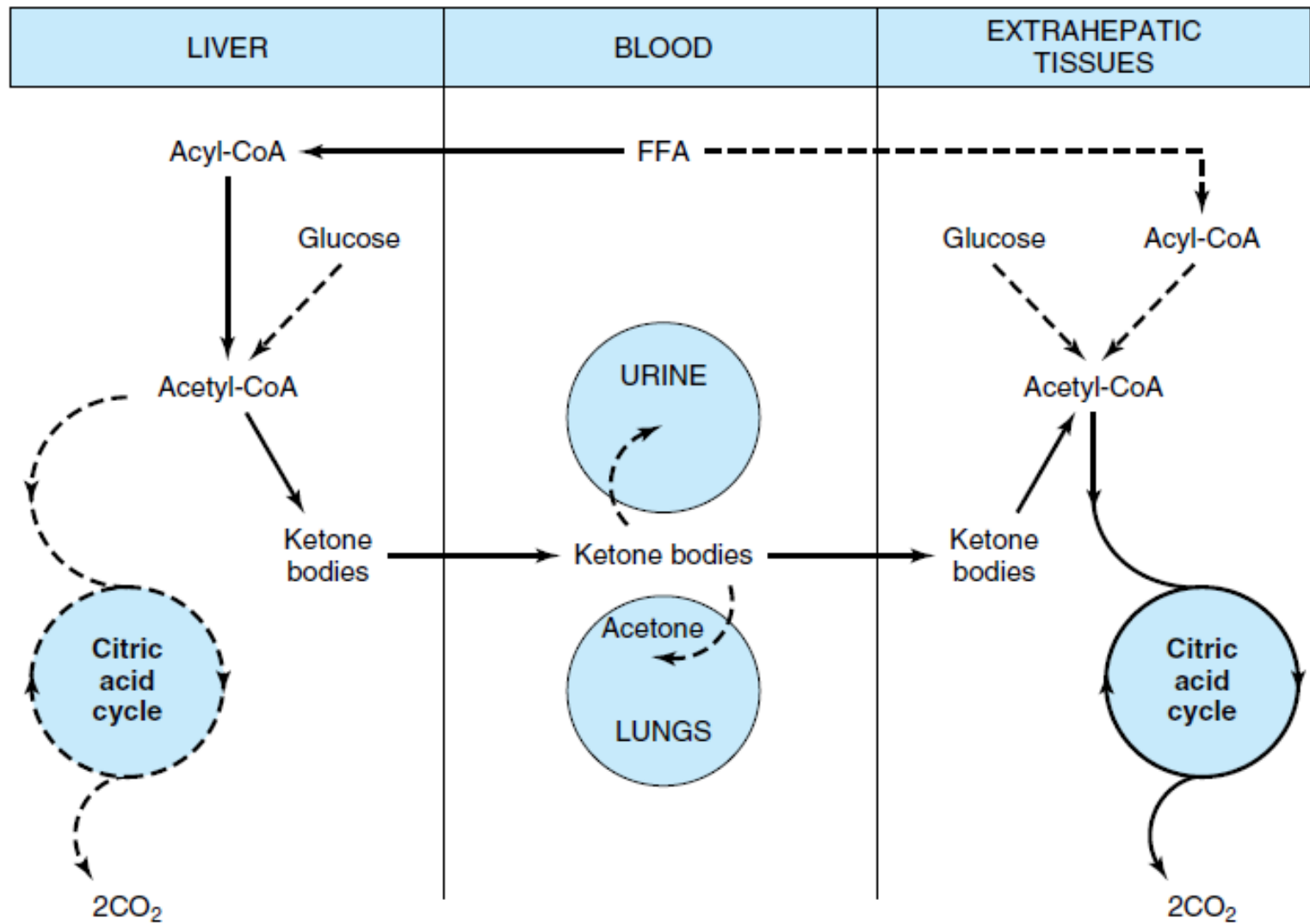
- **Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme D(–)-3-hydroxybutyrate dehydrogenase; the equilibrium is controlled by the mitochondrial  $[NAD^+]/[NADH]$  ratio.**





INTERRELATIONSHIPS OF THE KETONE BODIES. D(-)-3-HYDROXYBUTYRATE DEHYDROGENASE IS A MITOCHONDRIAL ENZYME.

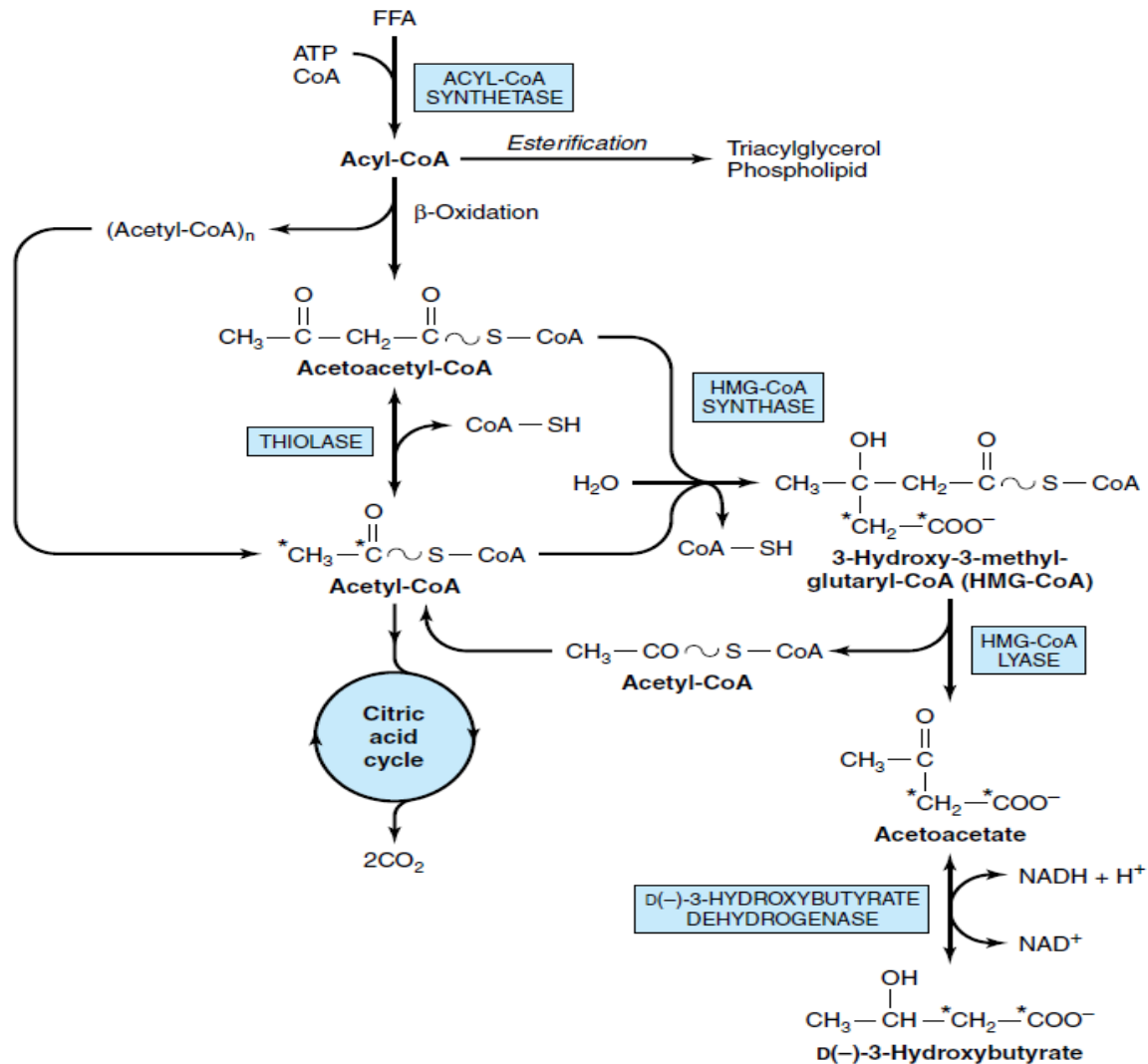




**FORMATION, UTILIZATION, AND EXCRETION OF KETONE BODIES.**



# PATHWAYS OF KETOGENESIS IN THE LIVER. (FFA, FREE FATTY ACIDS.)





## **3-HYDROXY-3-METHYLGLUTARYL-COA (HMG-COA) IS AN INTERMEDIATE IN THE PATHWAY OF KETOGENESIS**

- **Enzymes responsible for ketone body formation are associated mainly with the mitochondria.**
- **Two acetyl-CoA molecules formed in oxidation condense with one another to form acetoacetyl-CoA by a reversal of the thiolase reaction.**
- **Acetoacetyl-CoA, (starting material for ketogenesis).**
- **Acetoacetyl-CoA arises directly from the terminal four carbons of a fatty acid during  $\beta$ -oxidation.**



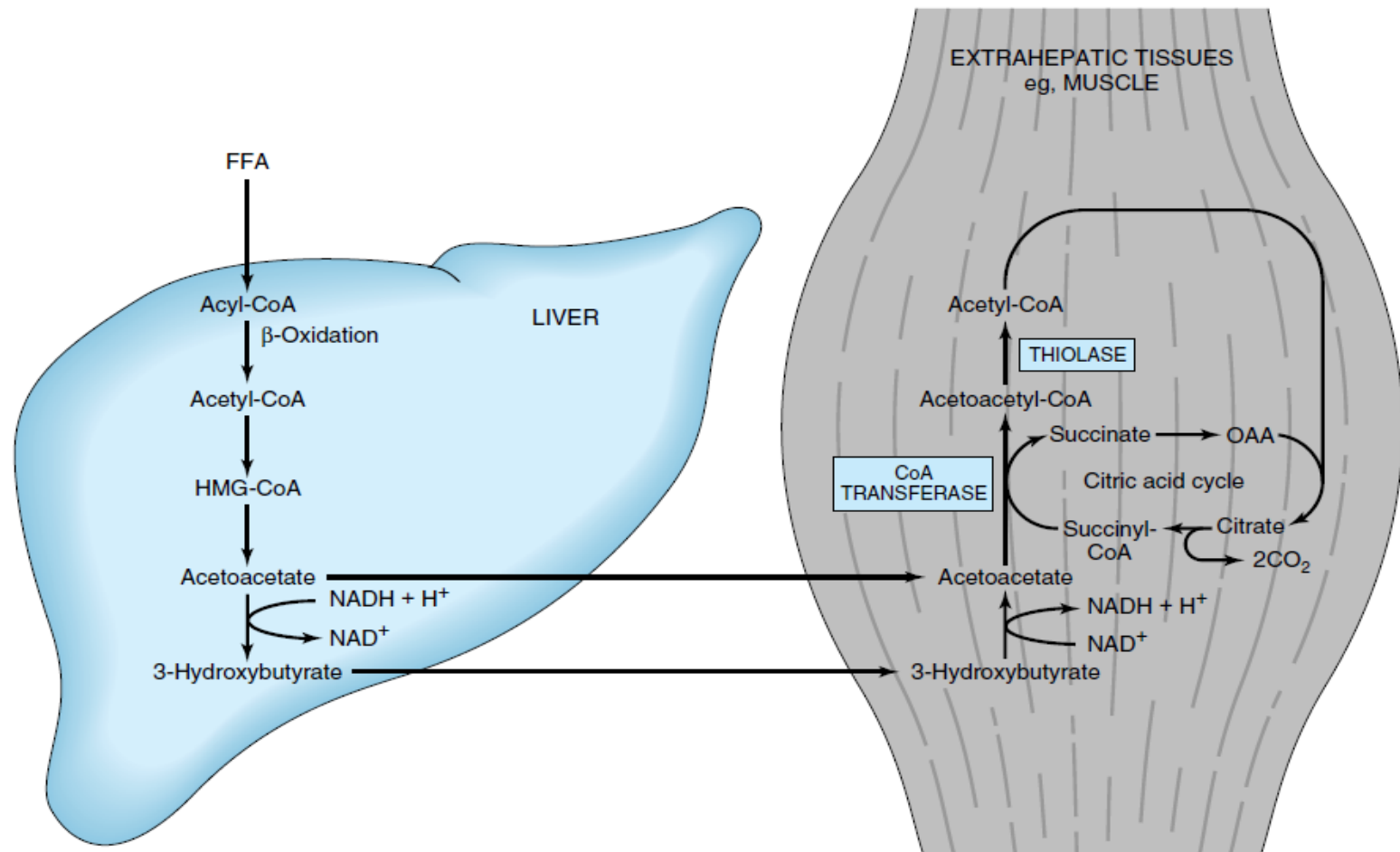
- **Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by 3-hydroxy-3-methylglutaryl-CoA synthase forms 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).**
- **3-Hydroxy-3-methylglutaryl-CoA lyase then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate.**



- **Both enzymes must be present in mitochondria for ketogenesis to take place. This occurs solely in liver and rumen epithelium.**
- **D(–)-3-Hydroxybutyrate is quantitatively the predominant ketone body present in the blood and urine in ketosis**



# KETONE BODIES SERVE AS A FUEL FOR EXTRAHEPATIC TISSUES





- **An active enzymatic mechanism produces acetoacetate from acetoacetyl-CoA in the liver.**
- **In extrahepatic tissues, acetoacetate is activated to acetoacetyl-CoA by succinyl-CoA-acetoacetate CoA transferase. CoA is transferred from succinyl-CoA to form acetoacetyl-CoA.**
- **With the addition of a CoA, the acetoacetyl-CoA is split into two acetyl-CoAs by thiolase and oxidized in the citric acid cycle.**



## **KETONEMIA**

- **ketonemia is due to increased production of ketone bodies by the liver rather than to a deficiency in their utilization by extrahepatic tissues.**
- **While acetoacetate and D(–)-3-hydroxybutyrate are readily oxidized by extrahepatic tissues, acetone is difficult to oxidize in vivo and to a large extent is volatilized in the lungs.**



- **In moderate ketonemia, the loss of ketone bodies via the urine is only a few percent of the total ketone body production and utilization**



## **KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS**

**1- Ketosis does not occur in vivo unless there is an increase in the level of circulating free fatty acids that arise from lipolysis of triacylglycerol in adipose tissue. (Free fatty acids are the precursors of ketone bodies in the liver). The liver, both in fed and in fasting conditions, extracts about 30% of the free fatty acids passing through it, so that at high concentrations the flux passing into the liver is substantial. Therefore, the factors regulating mobilization of free fatty acids from adipose tissue are important in controlling ketogenesis.**



**2- After uptake by the liver, free fatty acids are either  $\beta$ -oxidized to  $\text{CO}_2$  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.**



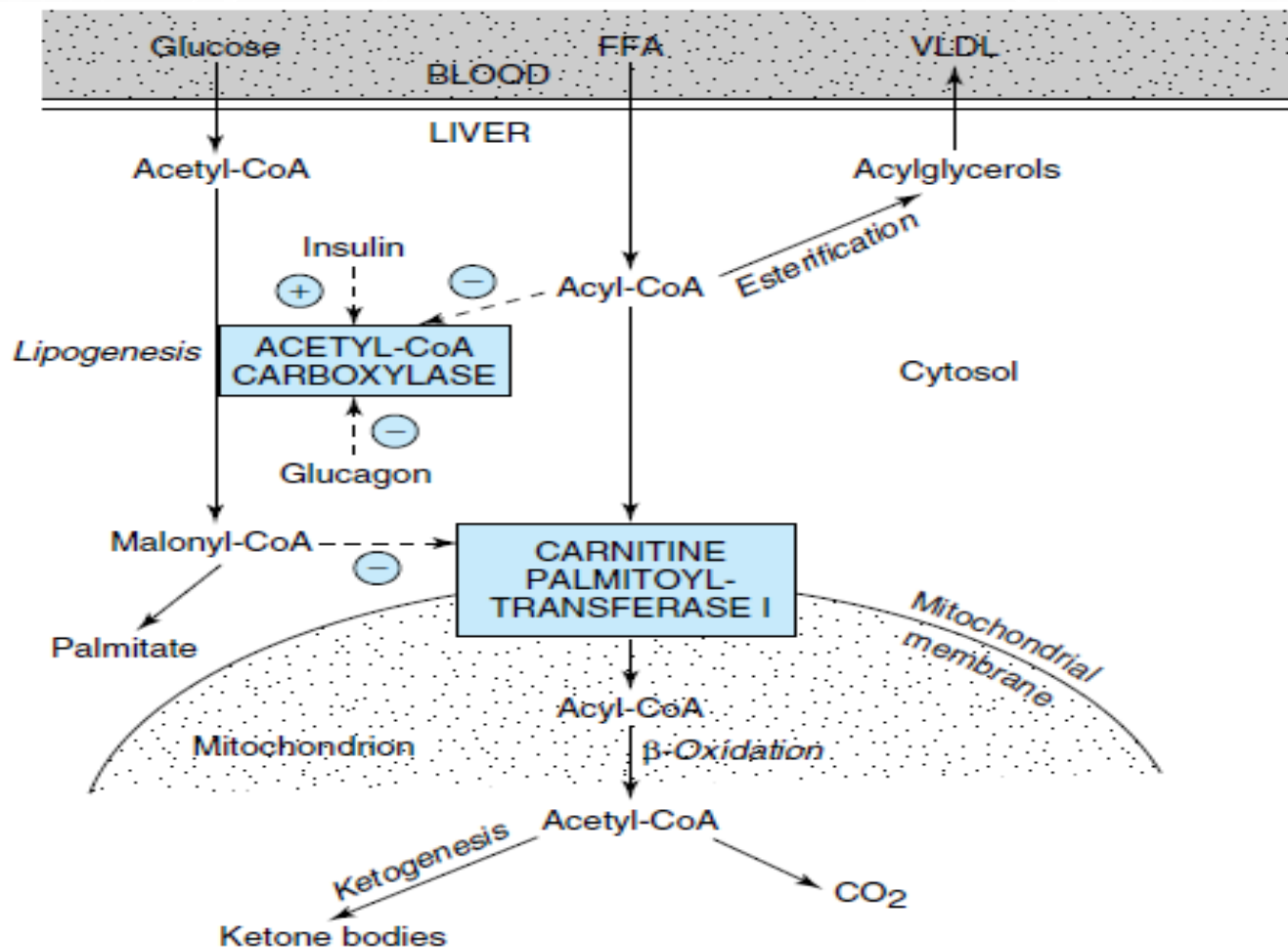
- **Malonyl-CoA, the initial intermediate in fatty acid biosynthesis formed by acetyl-CoA carboxylase in the fed state, is a potent inhibitor of CPT-I. Under these conditions, free fatty acids enter the liver cell in low concentrations and are nearly all esterified to acylglycerols and transported out of the liver in very low density lipoproteins (VLDL). However, as the concentration of free fatty acids 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  $\beta$ -oxidized.**



These events are reinforced in starvation by a decrease in the [insulin]/[glucagon] ratio. Thus,  $\beta$ -oxidation from free fatty acids is controlled by the CPT-I gateway into the mitochondria, and the balance of the free fatty acid uptake not oxidized is esterified.

3- In turn, the acetyl-CoA formed in  $\beta$ -oxidation is oxidized in the citric acid cycle, or it enters the pathway of ketogenesis to form ketone bodies. As the level of serum free fatty acids is raised, proportionately more free fatty acid is converted to ketone bodies and less is oxidized via the citric acid cycle to  $\text{CO}_2$ .





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.



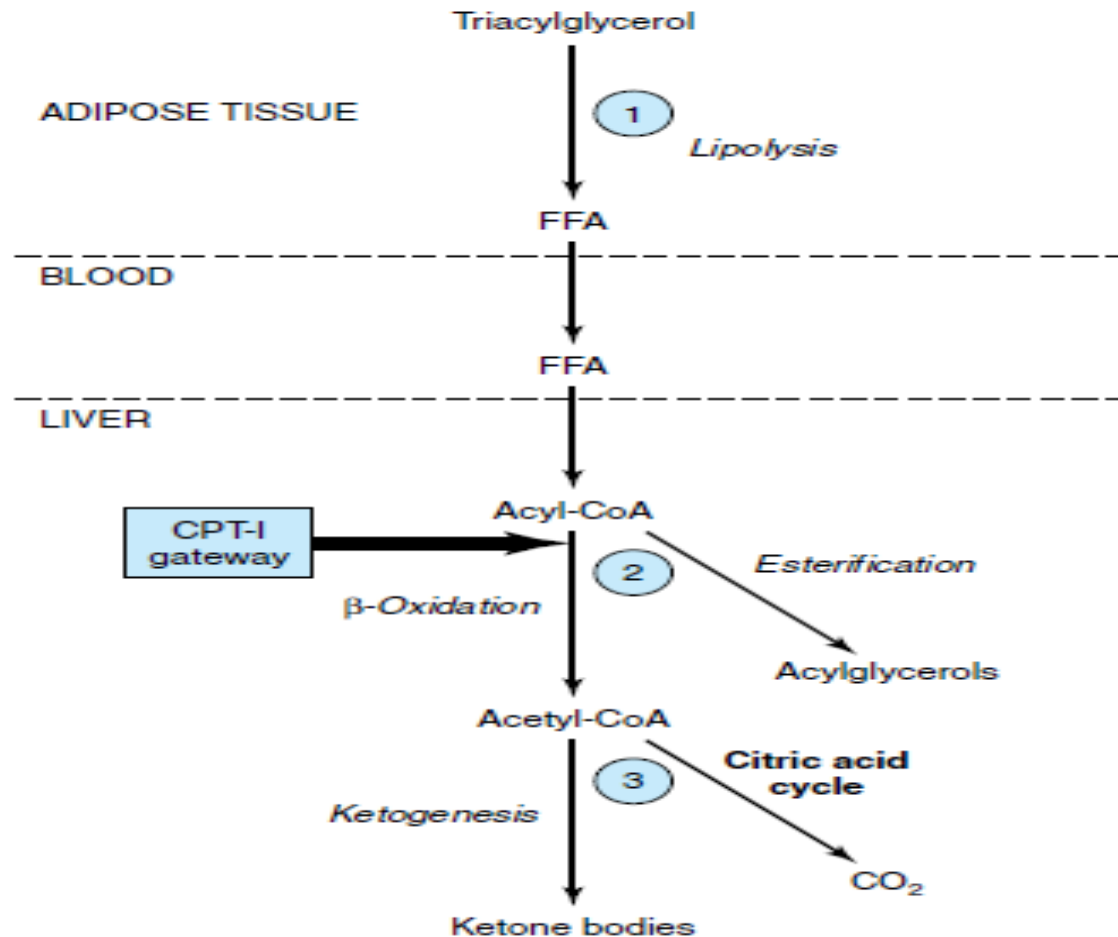
**The partition of acetyl-CoA between the ketogenic pathway and the pathway of oxidation to  $\text{CO}_2$  is regulated so that the total free energy captured in ATP which results from the oxidation of free fatty acids remains constant as their concentration in the serum changes.**

**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., ketone bodies are overproduced causing ketosis.**



Such a fall may occur because of an increase in the  $[NADH]/[NAD^+]$  ratio caused by increased  $\beta$ -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.





REGULATION OF KETOGENESIS. – 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.)



## **SYNTHESIS OF FATTY ACIDS (LIPOGENESIS)**

- Occurs in the Cytosol of liver, kidney, brain, lung, mammary gland, and adipose tissue.
- Need cofactors include NADPH, ATP,  $\text{Mn}^{2+}$ , biotin, and  $\text{HCO}_3^-$  (as a source of  $\text{CO}_2$ ).
- Acetyl-CoA is the immediate substrate, and free palmitate is the end product.

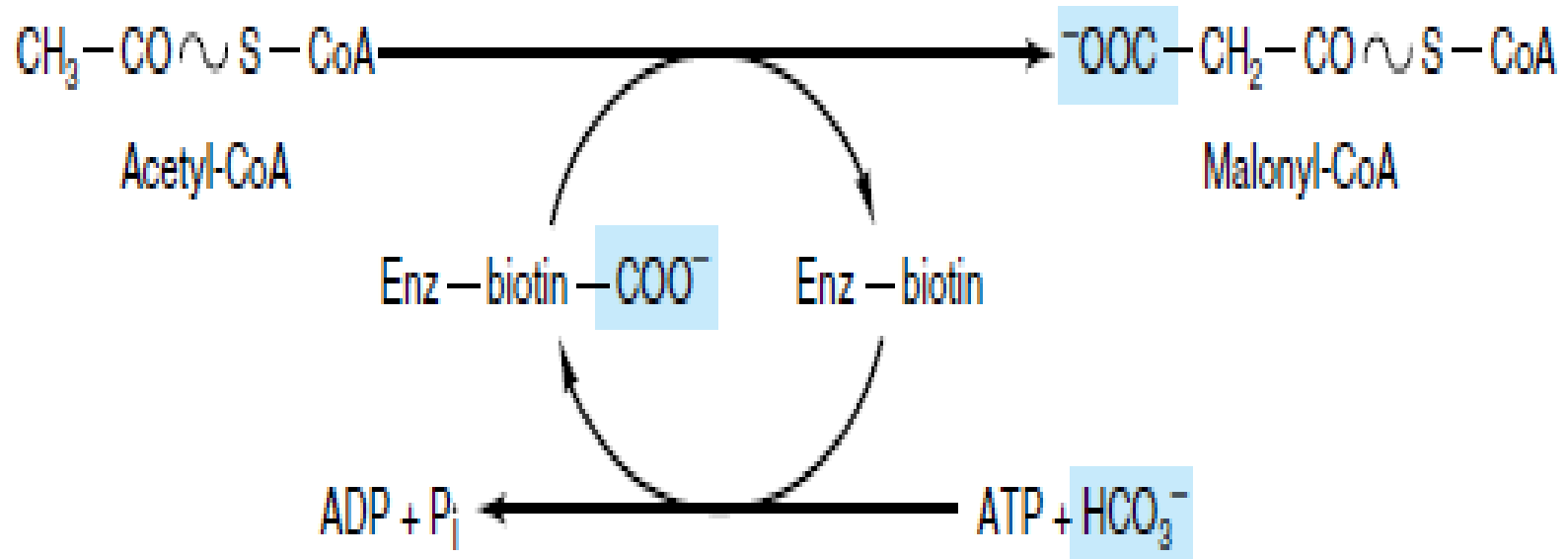


## **Production of Malonyl-CoA Is the Initial & Controlling Step in Fatty Acid Synthesis**

- **Bicarbonate as a source of  $\text{CO}_2$  is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and acetyl-CoA carboxylase (need biotin).**
- **Acetyl-CoA carboxylase is a multienzyme protein containing a variable number of identical subunits, each containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and transcarboxylase.**



- The reaction takes place in two steps: (1) carboxylation of biotin involving ATP and (2) transfer of the carboxyl group to acetyl-CoA to form malonyl-CoA.





## **The Fatty Acid Synthase Complex Is a Polypeptide Containing Seven Enzyme Activities**

- **The fatty acid synthase complex is a dimer (two identical monomers), each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain.**

**1- Initially, a priming molecule of acetyl-CoA combines with a cysteine ( $\text{—SH}$  group) catalyzed by acetyl transacylase.**

**2- Malonyl-CoA combines with the adjacent  $\text{—SH}$  on the 4'-phosphopantetheine of ACP of the other monomer, catalyzed by malonyl transacylase to form acetyl (acyl)-malonyl enzyme.**



**3- The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and liberates CO<sub>2</sub>, forming 3-ketoacyl enzyme (acetoacetyl enzyme), freeing the cysteine —SH group.**

**4- Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction.**

**5- The 3-ketoacyl group is reduced, dehydrated, and reduced again to form the corresponding saturated acyl-S-enzyme.**



- A new malonyl-CoA molecule combines with the —SH of 4'-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine —SH group.
- The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled.
- The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway.



## **THE MAIN SOURCE OF NADPH FOR LIPOGENESIS IS THE PENTOSE PHOSPHATE PATHWAY**

- **NADPH is involved as donor of reducing equivalents in both the reduction of the 3-ketoacyl and of the 2,3-unsaturated acyl derivatives. The oxidative reactions of the pentose phosphate pathway are the chief source of the hydrogen required for the reductive synthesis of fatty acids.**



## **The Nutritional State Regulates Lipogenesis**

- **Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc.**
- **Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat.**
- **The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed by restricted caloric intake, high-fat diet, or a deficiency of insulin, as in diabetes mellitus.**



## **Acetyl-CoA Carboxylase Is the Most Important Enzyme in the Regulation of Lipogenesis**

- **Acetyl-CoA carboxylase activated by citrate, which increases in concentration in the well-fed state.**
- **Citrate converts the enzyme from an inactive dimer to an active polymeric form.**
- **Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules, an example of negative feedback inhibition by a product of a reaction.**



- Thus, if acyl-CoA accumulates because it is not esterified quickly enough or because of increased lipolysis or an influx of free fatty acids into the tissue, it will automatically reduce the synthesis of new fatty acid. Acyl-CoA also inhibits the mitochondrial tricarboxylate transporter, thus preventing activation of the enzyme by egress of citrate from the mitochondria into the cytosol.
- Acetyl-CoA carboxylase is also regulated by hormones such as glucagon, epinephrine, and insulin via changes in its phosphorylation state



## **Pyruvate Dehydrogenase Is Regulated by Acyl-CoA**

**Acyl-CoA causes an inhibition of pyruvate dehydrogenase by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane, which leads to increased intramitochondrial  $[ATP]/[ADP]$  ratios and therefore to conversion of active to inactive pyruvate dehydrogenase thus regulating the availability of acetyl-CoA for lipogenesis. Furthermore, oxidation of acyl-CoA due to increased levels of free fatty acids may increase the ratios of  $[acetyl-CoA]/[CoA]$  and  $[NADH]/[NAD^+]$  in mitochondria, inhibiting pyruvate dehydrogenase.**



## **Insulin Also Regulates Lipogenesis by Other Mechanisms**

- **Insulin stimulates lipogenesis by several other mechanisms as well as by increasing acetyl-CoA carboxylase activity.**
- **It increases the transport of glucose into the cell (eg, in adipose tissue), increasing the availability of both pyruvate for fatty acid synthesis and glycerol 3-phosphate for esterification of the newly formed fatty acids, and also converts the inactive form of pyruvate dehydrogenase to the active form in adipose tissue, but not in liver.**



- **Insulin also—by its ability to depress the level of intracellular cAMP—inhibits lipolysis in adipose tissue and reducing the concentration of plasma free fatty acids and, therefore, long-chain acyl-CoA, which are inhibitors of lipogenesis.**



## **The Fatty Acid Synthase Complex & Acetyl-CoA Carboxylase Are Adaptive Enzymes**

**These enzymes adapt to the body's physiologic needs by increasing in total amount in the fed state and by decreasing in during intake of a high-fat diet and in conditions such as starvation, and diabetes mellitus. Insulin is an important hormone causing gene expression and induction of enzyme biosynthesis, and glucagon (via cAMP) antagonizes this effect.**



- **Feeding fats containing polyunsaturated fatty acids coordinately regulates the inhibition of expression of key enzymes of glycolysis and lipogenesis. These mechanisms for longer-term regulation of lipogenesis take several days to become fully manifested and augment the direct and immediate effect of free fatty acids and hormones such as insulin and glucagon.**



# BIOSYNTHESIS OF CHOLESTEROL (5 STEPS)

## Step 1: Biosynthesis of Mevalonate

HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) is formed in mitochondria to synthesize ketone bodies. However, since cholesterol synthesis is extramitochondrial. Initially, two molecules of acetyl-CoA condense to form acetoacetyl-CoA catalyzed by cytosolic thiolase. Acetoacetyl-CoA condenses with a further molecule of acetyl-CoA catalyzed by HMG-CoA synthase to form HMG-CoA, which is reduced to mevalonate by NADPH catalyzed by HMG-CoA reductase.



## **Step 2: Formation of Isoprenoid Units**

Mevalonate is phosphorylated sequentially by ATP by three kinases, and after decarboxylation the active isoprenoid unit, isopentenyl diphosphate, is formed.

## **Step 3: Six Isoprenoid Units Form Squalene**

Isopentenyl diphosphate is isomerized by a shift of the double bond to form dimethylallyl diphosphate, then condensed with another molecule of isopentenyl diphosphate to form the ten-carbon intermediate geranyl diphosphate.



**A further condensation with isopentenyl diphosphate forms farnesyl diphosphate. Two molecules of farnesyl diphosphate condense at the diphosphate end to form squalene. Initially, inorganic pyrophosphate is eliminated, forming presqualene diphosphate, which is then reduced by NADPH with elimination of a further inorganic pyrophosphate molecule.**



#### **Step 4: Formation of Lanosterol**

**Squalene can fold into a structure that closely resembles the steroid nucleus. Before ring closure occurs, squalene is converted to squalene 2,3-epoxide by a mixed-function oxidase in the endoplasmic reticulum, squalene epoxidase. The methyl group on C<sub>14</sub> is transferred to C<sub>13</sub> and that on C<sub>8</sub>–C<sub>14</sub> as cyclization occurs, catalyzed by oxidosqualene:lanosterol cyclase.**



## Step 5: Formation of Cholesterol

The formation of cholesterol from lanosterol takes place in the membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain. The methyl groups on  $C_{14}$  and  $C_4$  are removed to form 14-desmethyl lanosterol and then zymosterol. The double bond at  $C_8-C_9$  is subsequently moved to  $C_5-C_6$  in two steps, forming desmosterol. Finally, the double bond of the side chain is reduced, producing cholesterol.



## **CHOLESTEROL SYNTHESIS IS CONTROLLED BY REGULATION OF HMG-COA REDUCTASE**

- **The reduced synthesis of cholesterol in starving animals is accompanied by a decrease in the activity of the HMG-CoA reductase and by dietary cholesterol.**
- **HMG-CoA reductase in liver is inhibited by mevalonate, and by cholesterol.**



- **Cholesterol and metabolites repress transcription of the HMG-CoA reductase via activation of a sterol regulatory element-binding protein (SREBP) transcription factor (a family of proteins that regulate the transcription of a range of genes involved in the cellular uptake and metabolism of cholesterol and other lipids).**



- **Insulin or thyroid hormone increases HMG-CoA reductase activity, whereas glucagon or glucocorticoids decrease it. Activity is reversibly modified by phosphorylation-dephosphorylation mechanisms, some of which may be cAMP-dependent and therefore immediately responsive to glucagon.**



# CHOLESTEROL BALANCE REGULATION IN TISSUES

## Cell cholesterol increase is due to

- Uptake of cholesterol-containing lipoproteins by receptors, eg, the LDL receptor or the scavenger receptor.
- Uptake of free cholesterol from cholesterol-rich lipoproteins to the cell membrane.
- Cholesterol synthesis.
- Hydrolysis of cholesteryl esters by the enzyme cholesteryl ester hydrolase.



## **Cell cholesterol decrease is due to**

- **Efflux of cholesterol from the membrane to HDL via the ABCA1, ABCG1 or SR-B1.**
- **Esterification of cholesterol by ACAT (acyl-CoA:cholesterol acyltransferase).**
- **Utilization of cholesterol for synthesis of other steroids, such as hormones, or bile acids in the liver.**