Biosynthesis of Fatty Acids Eicosanoids

BIOMEDICAL IMPORTANCE

Fatty acids are synthesized by an **extramitochondrial system**, which is responsible for the complete synthesis of palmitate from acetyl-CoA in the **cytosol**.

In most **mammals, glucose is the primary substrate** for lipogenesis, but in ruminants it is **acetate,** the main fuel molecule they obtain from the diet.

inhibition of lipogenesis occurs in type 1 (insulin-dependent) diabetes mellitus, and variations in the activity of the process affect the nature and extent of **obesity**.

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity .

A high ratio of polyunsaturated fatty acids to saturated fatty acids (P:S ratio) in the diet is considered to be beneficial in preventing coronary heart disease.

Prostaglandins mediate inflammation, **pain**, induce **sleep**, and also regulate **blood coagulation** and **reproduction**. **Nonsteroidal anti-inflammatory drugs (NSAIDs)** such as **aspirin and ibuprofen** act by inhibiting prostaglandin synthesis.

THE MAIN PATHWAY FOR DE NOVO SYNTHESIS OF FATTY ACIDS (LIPOGENESIS) OCCURS IN THE CYTOSOL

This system is present in **many tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue.**

 Its cofactor requirements include NADPH, ATP, Mn2+, biotin, and HCO3. 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 CO2** is required in the initial reaction **for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and acetyl-CoA carboxylase.**
**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
(**Figure 1**).

FIGURE 1 Biosynthesis of malonyl-CoA by acetyl carboxylase.
Acetyl carboxylase is a multienzyme complex containing two enzymes,
biotin carboxylase (E1) and a **carboxyltransferase (E2)** and the **biotin** carrier protein (BCP). The reaction proceeds **in two steps.** In step 1, catalysed by E1, biotin is carboxylated as it accepts a COO– group from HCO3 –
 and ATP is used. In step 2, catalyzed by E2, the COO– is transferred to acetyl-CoA forming malonyl- CoA.
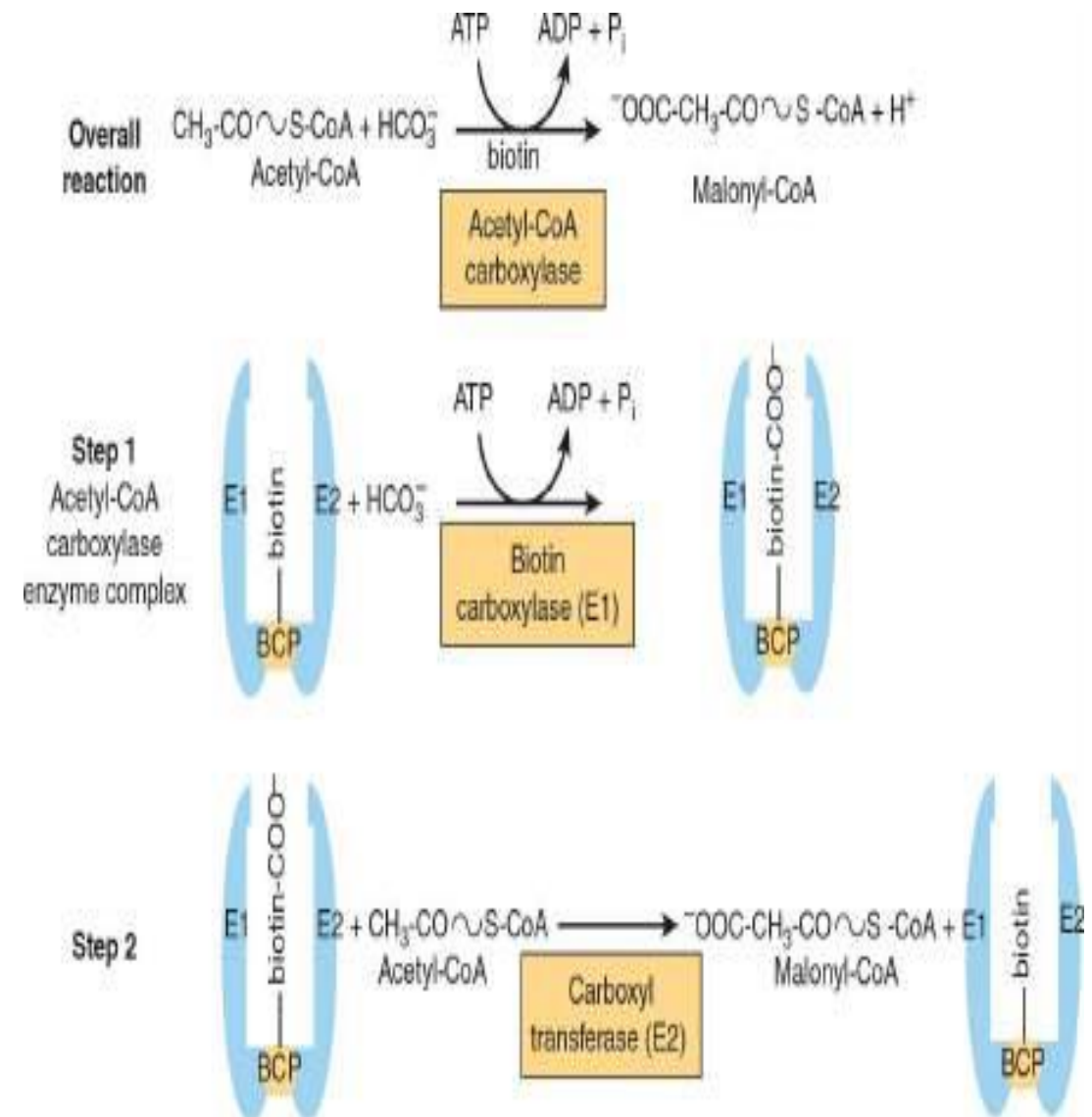
The Fatty Acid Synthase Complex Is a Homodimer of Two Polypeptide Chains Containing Six Enzyme Activities and the Acyl Carrier Protein

After the formation of malonyl-CoA,
fatty acids are formed by the **fatty acid synthase enzyme complex**. It contains the vitamin **pantothenic acid Figure 2**.

Sequence of enzyme domains in primary structure of fatty acid synthase monomer

FIGURE 2 Fatty acid synthase multienzyme complex.

The complex is a dimer of two identical polypeptide monomers in which six enzymes and the acyl carrier protein (ACP) are linked in the primary structure in the sequence shown.
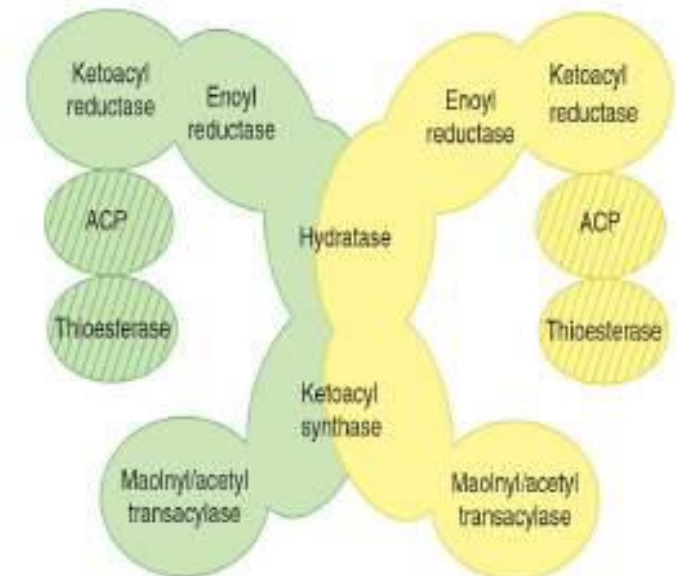
Fatty acid synthase homodimer

1- Initially, a priming molecule of **acetyl-CoA** combines with a cysteine —SH group (**Figure 3**, reaction **1a), while malonyl-CoA** combines with the adjacent —SH on the 4'-phosphopantetheine of ACP of the other monomer (**reaction 1b**). These reactions are catalyzed by **malonyl acetyl transacylase**, to form **acetyl (acyl)-malonyl enzyme**.

2- The acetyl group attacks the methylene group of the malonyl residue, catalyzed by **3- ketoacyl synthase**, and liberates CO_2, forming **3-ketoacyl enzyme** (acetoacetyl enzyme) (reaction 2

3- **The 3-ketoacyl group** is **reduced**, **dehydrated**, and reduced **again** (reactions **3-5**) to form the corresponding **saturated acyl enzyme** (product of reaction 5).

4- 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 (palmitoyl) has been assembled**. It is liberated from the enzyme complex by the activity of the **sixth enzyme** in the complex, **thioesterase (deacylase).**

The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway.

Its **possible fates** are **esterification into acylglycerols**, **chain elongation**, **desaturation**, or **esterification into cholesteryl ester**. In mammary gland, there is a separate thioesterase specific for acyl residues of C8, C10, or C12, which are subsequently found in milk lipids.

FIGURE 3 Biosynthesis of long-chain fatty acids. After the initial priming step in which acetyl-CoA is bound to a cysteine-SH group on the fatty acid synthase enzyme (reaction 1a) in each cycle the addition of a malonyl residue causes the acyl chain to grow by two carbon atoms. (Cys, cysteine residue; Pan, 4'-phosphopantetheine).
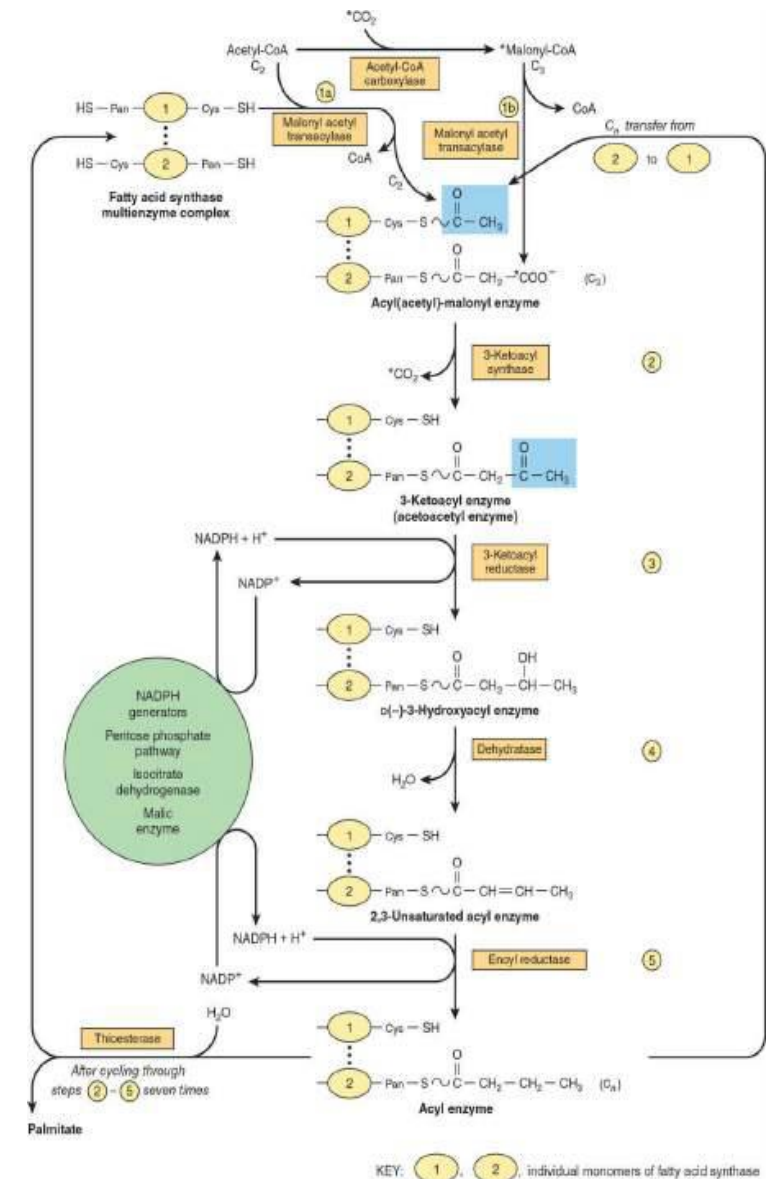
Acetyl CoA is produced in **the mitochondria by the oxidation of pyruvate**, **fatty acids degradation** of **carbon skeleton of certain amino acids** and from **ketone bodies.**

Mitochondria are not permeable to acetyl Co A.

Alternate:

Acetyl CoA is condenses with oxaloacetate in mitochondria to form **citrate**.

Citrate is freely transported to cytosol by tricarboxylic acid transporter.

In the cytosol it is cleaved by ATP citrate lyase to **liberate acetyl co A and oxaloacetate.**

Oxaloacetate in the cytosol is converted to **malate.**

Malic enzyme converts **malate to pyruvate.** and **the pyruvate** can be used to regenerate **acetyl-CoA** after transport into the **mitochondrion.**

Alternatively, **malate itself can be transported into the mitochondrion**, where it is able to reform **oxaloacetate**.

NADPH and CO2 are generated in this reaction. Both of them are utilized for fatty acid synthesis.

The equation for the overall synthesis of palmitate from acetyl-CoA a malonyl-CoA is

$$CH_3CO—S—CoA + 7HOOCCHCO—S—CoA + 14NADPH + 14H^+$$
$$→ CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA—SH + 14NADP^+$$

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C2 units is via malonyl-CoA. **Propionyl-CoA instead of acetyl-CoA** is used as the primer for the synthesis of long-chain fatty acids with an **odd number** of carbon atoms, which are found particularly in ruminant fat and milk.

The Main Source of NADPH for Lipogenesis Is the Pentose Phosphate Pathway

NADPH is involved as a donor of reducing equivalents. The oxidative reactions of the **pentose phosphate pathway are the chief source of the hydrogen required for the synthesis of fatty acids.**

Other **sources of NADPH include** the reaction that converts **malate to pyruvate** catalyzed by the **NADP malate dehydrogenase (malic enzyme)** (**Figure 4**) **and** the extramitochondrial **isocitrate dehydrogenase** reaction (a substantial source in ruminants).

FIGURE 4 The provision of acetyl-CoA and NADPH for lipogenesis. (K, α-ketoglutarate transporter; P, pyruvate transporter; PPP, pentose phosphate pathway; T, tricarboxylate transporter.)
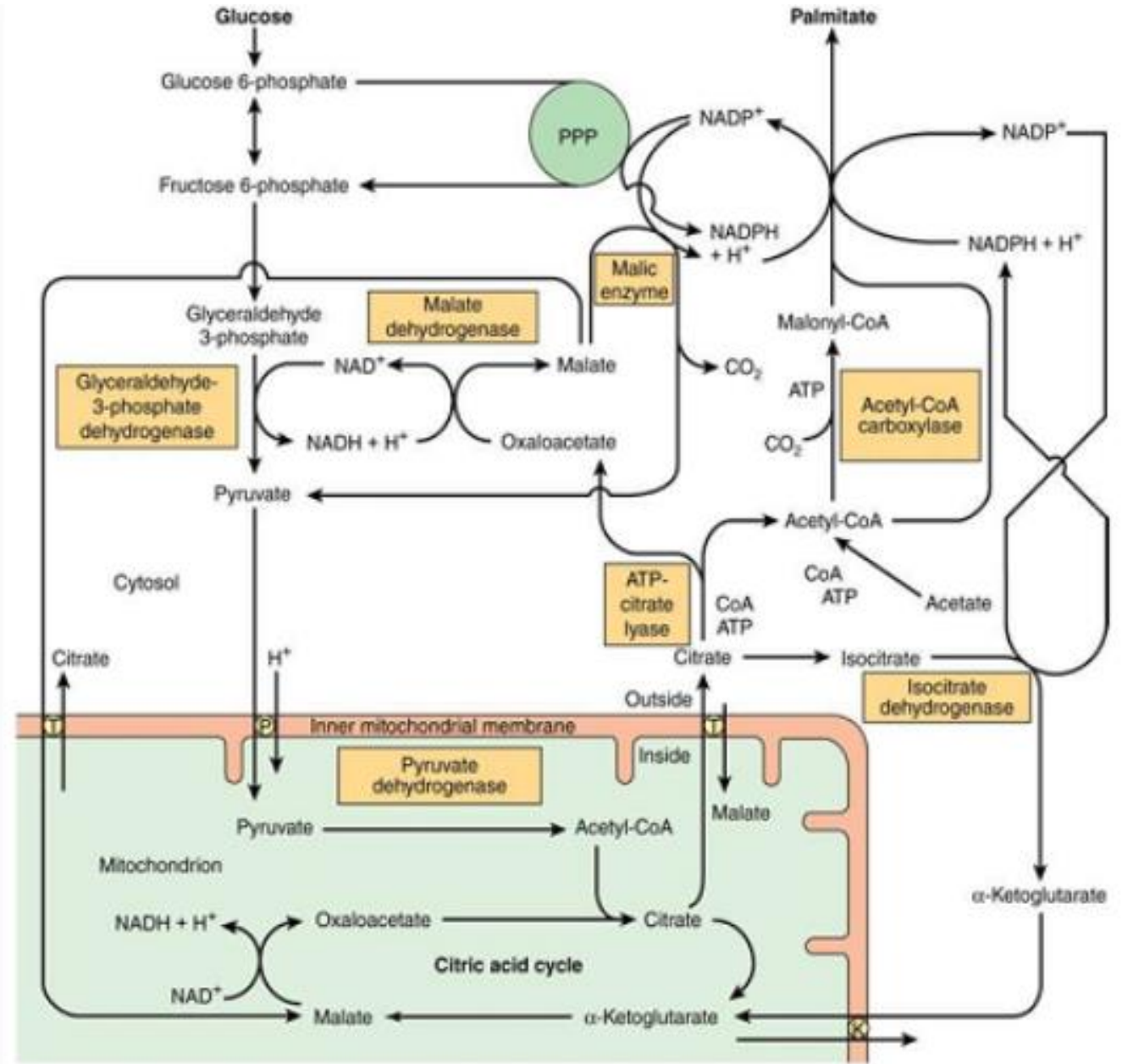
Acetyl-CoA Is the Principal Building Block of Fatty Acids

Acetyl-CoA is formed from glucose via the oxidation of pyruvate in the matrix of the mitochondria .However, as it does not diffuse readily across the mitochondrial membranes, its transport into the cytosol, the principal site of fatty acid synthesis, requires a special mechanism involving **citrate**. After condensation **of acetyl-CoA with oxaloacetate** in the citric acid cycle within mitochondria**, the citrate produced** can be translocated into the **extramitochondrial** compartment via the tricarboxylate transporter, where in the presence of CoA and ATP, it undergoes **cleavage to acetyl-CoA and oxaloacetate by ATP-citrate lyase**, which increases in activity in the well-fed state. **The acetyl-CoA is then available for malonyl-CoA formation and synthesis of fatty acids (Figures 1 and 3),**
 and the **oxaloacetate** can form **malate** via NADH-linked **malate dehydrogenase,** followed by the **generation of NADPH** and pyruvate via the **malic enzyme**. The NADPH becomes available for lipogenesis, and the pyruvate can be used to regenerate acetyl-CoA after transport into the mitochondrion (Figure 4).

Alternatively, **malate itself can be transported into the mitochondrion**, where it is able to reform oxaloacetate. **Acetate (derived from carbohydrate digestion in the rumen and activated to acetyl-CoA extramitochondrially) is the main source of acetyl- CoA.**

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "**microsomal system**") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as the acetyl donor and NADPH as the reductant, and is catalyzed by the microsomal **fatty acid elongase** system of enzymes (**Figure 5**).

 Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide **C22 and C24 fatty acids for sphingolipids.**

FIGURE 5 Microsomal elongase system for fatty acid chain elongation. NADH may also be used by the reductases, but NADPH is preferred.
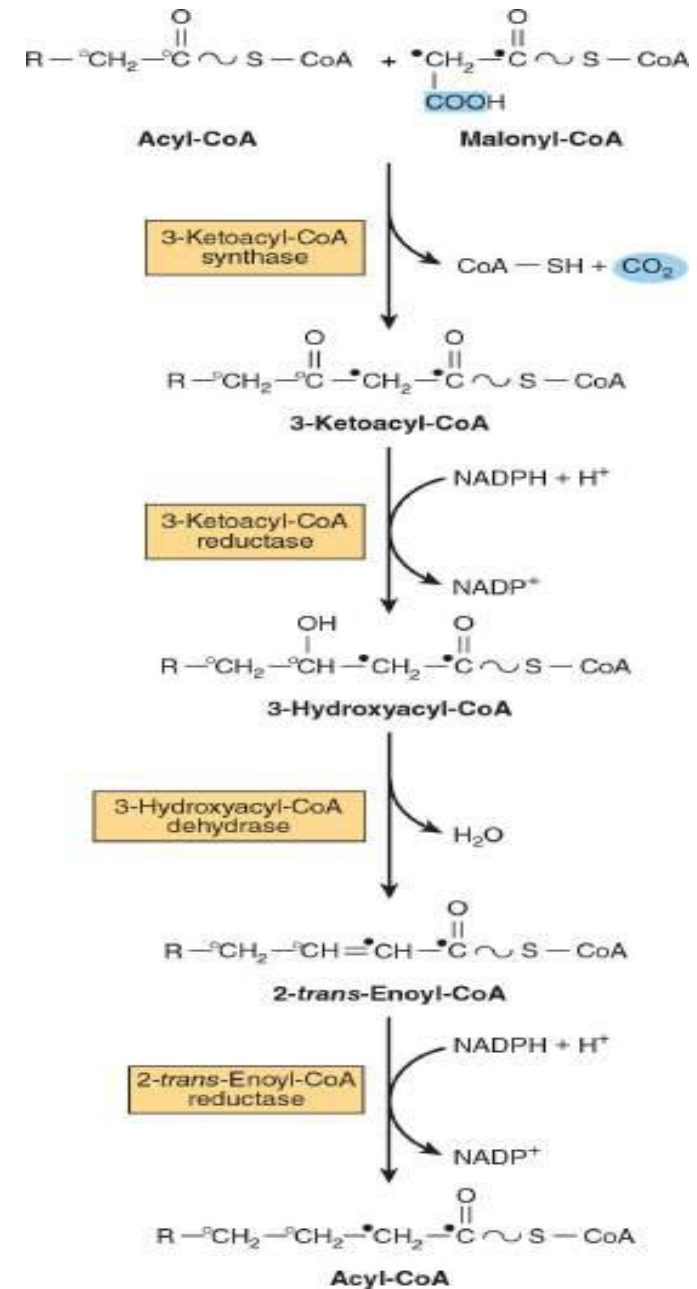
THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored **as fat in** many animals in anticipation of periods of caloric deficiency such as starvation, etc., and to provide energy for use between meals.

 Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat,

 It is **depressed** by **restricted caloric** intake, **high-fat diet**, or a **deficiency of insulin**, as in diabetes mellitus. An **inverse relationship** has been demonstrated between **hepatic lipogenesis** and the concentratio**n of serum-free fatty acids**.

 Lipogenesis is increased when **sucrose is fed instead of glucose.**

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

Long-chain fatty acid synthesis is controlled in **the short** term by **allosteric and covalent modification of enzymes** and in **the long term** by changes in **gene expression governing rates of synthesis of** enzymes.

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

Acetyl-CoA carboxylase is an allosteric enzyme and is activated by **citrate**, An example of negative feedback **inhibition by a product of a reaction** (**Figure 6**). 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**.

FIGURE 6 Regulation of acetyl-CoA carboxylase. Acetyl-CoA carboxylase is **activated** by **citrate.**

In addition, acyl-CoA inhibits **the tricarboxylate transporter,** which transports citrate out of mitochondria into the cytosol, thus decreasing the citrate concentration in the cytosol and favoring inactivation of the enzyme.

Acetyl-CoA carboxylase is also **regulated** by **hormones** such as **glucagon**, **epinephrine**, and **insulin** via changes in its phosphorylation state (details in **Figure 7**).
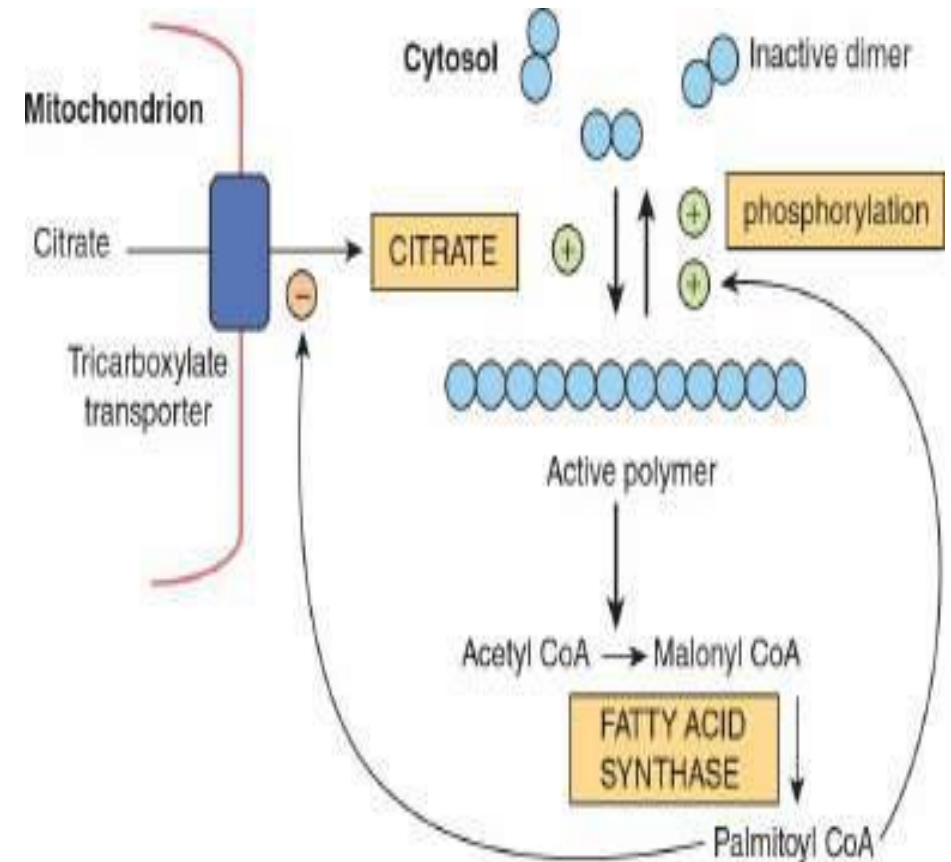
FIGURE 7 Regulation of acetyl-CoA carboxylase by phosphorylation/dephosphorylation. The enzyme is **inactivated by phosphorylation by AMP-activated protein kinase** (AMPK), which in turn **is phosphorylated and activated by AMP-activated protein kinase kinase (AMPKK).** Glucagon and epinephrine increase cAMP, and thus activate this latter enzyme via cAMP-dependent protein kinase. The kinase kinase enzyme is also believed to be activated by acyl-CoA. **Insulin activates acetyl-CoA carboxylase via dephosphorylation of AMPK.**
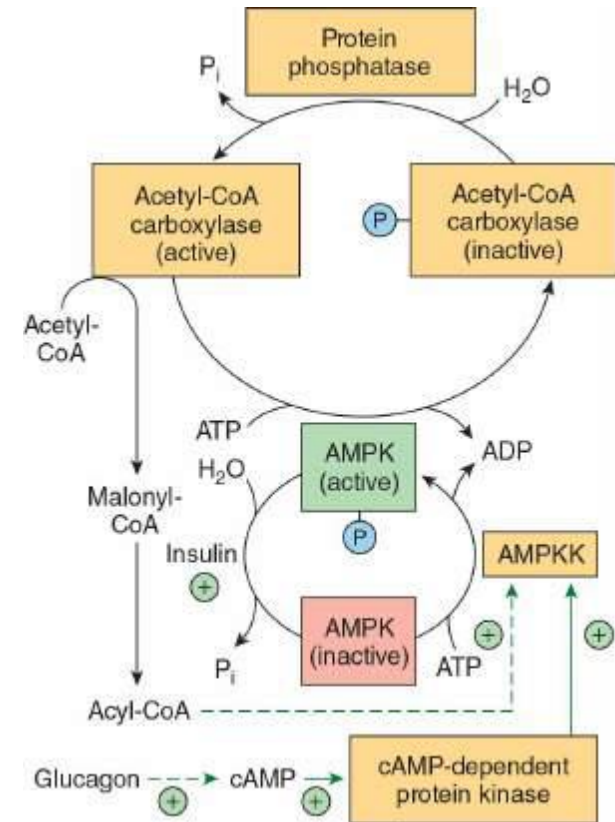
Pyruvate Dehydrogenase Is Also Regulated by Acyl- CoA

Acyl-CoA causes an inhibition of pyruvate dehydrogenase . 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:

1-increasing acetyl-CoA carboxylase activity.

2- **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 triacylglycerol synthesis via esterification of the newly formed fatty acids

3- and also **converts the inactive form of pyruvate dehydrogenase to the active form in adipose tissue**, but not in liver.

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

SOME POLYUNSATURATED FATTY ACIDS CANNOT BE SYNTHESIZED BY MAMMALS & ARE NUTRITIONALLY ESSENTIAL

Certain long-chain unsaturated fatty acids of metabolic significance in mammals are shown in **Figure 8**. Other C20, C22, and C24 polyenoic fatty acids may be derived from oleic, linoleic, and α-linolenic acids by chain elongation.
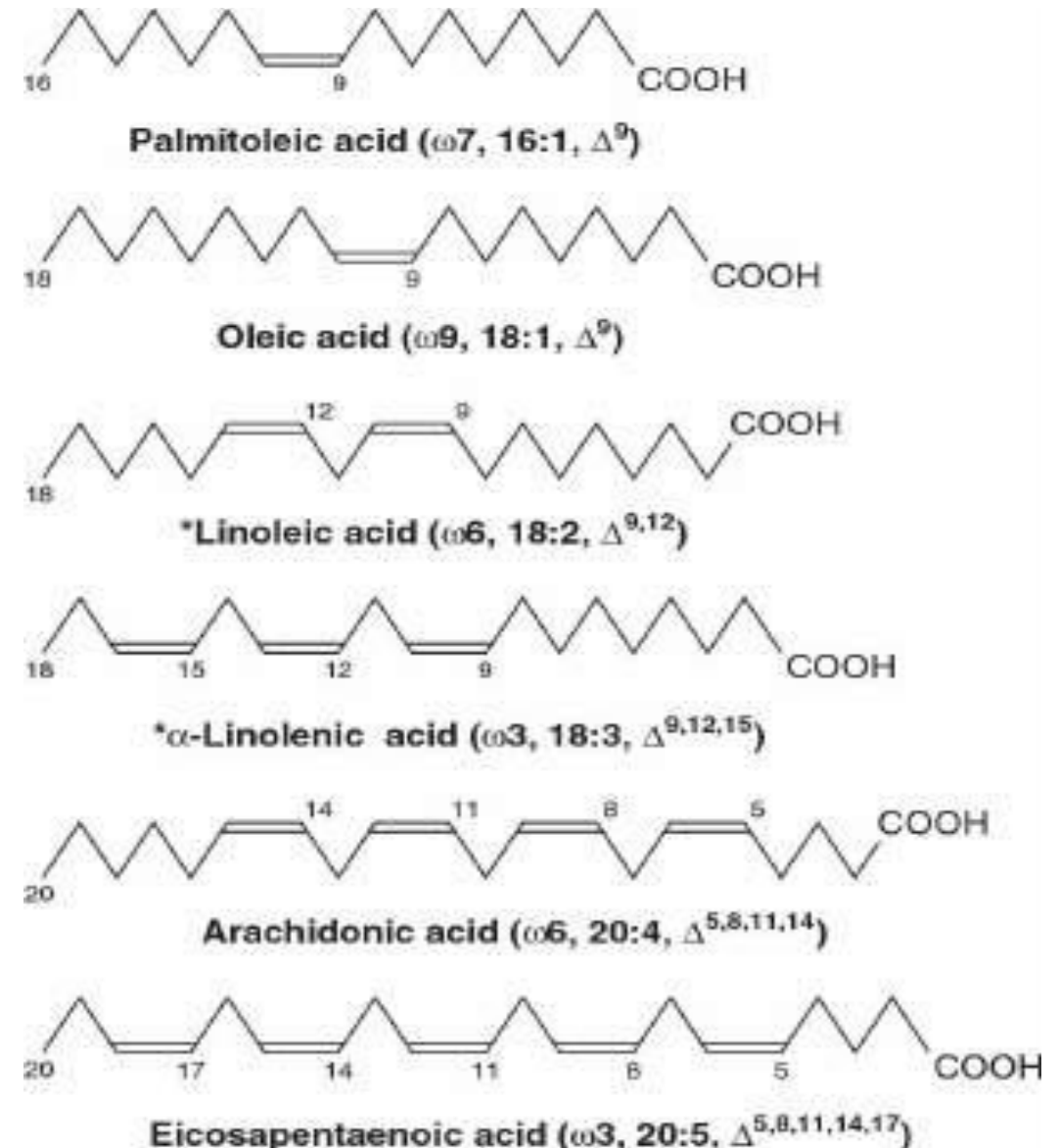
Palmitoleic and oleic acids **are not essential** in the diet because the tissues can introduce a double bond at the Δ9 position of a saturated fatty acid.

Linoleic and **α-linolenic acids** are the only fatty acids known to be **essential** for the complete nutrition of many species of animals, including humans, and are termed the **nutritionally essential fatty acids**.

In humans and most other mammals, **arachidonic acid** can be formed from linoleic acid. **Double bonds can be introduced at the Δ4, Δ5, Δ6, and Δ9 positions** in most animals, **but never beyond the Δ9 position**. In contrast, plants are able to synthesize the nutritionally essential fatty acids by introducing double bonds at the Δ12 and Δ15 positions.

FIGURE 8 Structure of some unsaturated fatty acids. Although the carbon atoms in the molecules are conventionally numbered—that is, numbered from the carboxyl terminal—the ω numbers (eg, ω7 in palmitoleic acid) are calculated from the reverse end (the methyl terminal) of the molecules. The information in parentheses shows, for instance, that α-linolenic acid contains double bonds starting at the third carbon from the methyl terminal, has 18 carbons and 3 double bonds, and has these double bonds at the 9th, 12th, and 15th carbons from the carboxyl terminal. *Nutritionally essential fatty acids in humans.

Palmitoleic acid (ω7, 16:1, Δ^9)

Oleic acid (ω9, 18:1, Δ^9)

*Linoleic acid (ω6, 18:2, $\Delta^{9,12}$)

*α-Linolenic acid (ω3, 18:3, $\Delta^{9,12,15}$)

Arachidonic acid (ω6, 20:4, $\Delta^{5,8,11,14}$)

Eicosapentaenoic acid (ω3, 20:5, $\Delta^{5,8,11,14,17}$)

MONOUNSATURATED FATTY ACIDS ARE SYNTHESIZED BY A Δ9 DESATURASE SYSTEM

Several tissues including the liver are considered to be responsible for the formation of **nonessential monounsaturated fatty acids from saturated fatty acids.** The first double bond introduced into a saturated fatty acid is nearly always in **the Δ9 position**. An enzyme system—Δ9 **desaturase** (**Figure 9**)—in the endoplasmic reticulum catalyzes the conversion of palmitoyl-CoA or stearoyl-CoA to palmitoleoyl-CoA or oleoyl-CoA, respectively. Oxygen and either NADH or NADPH are necessary for the reaction
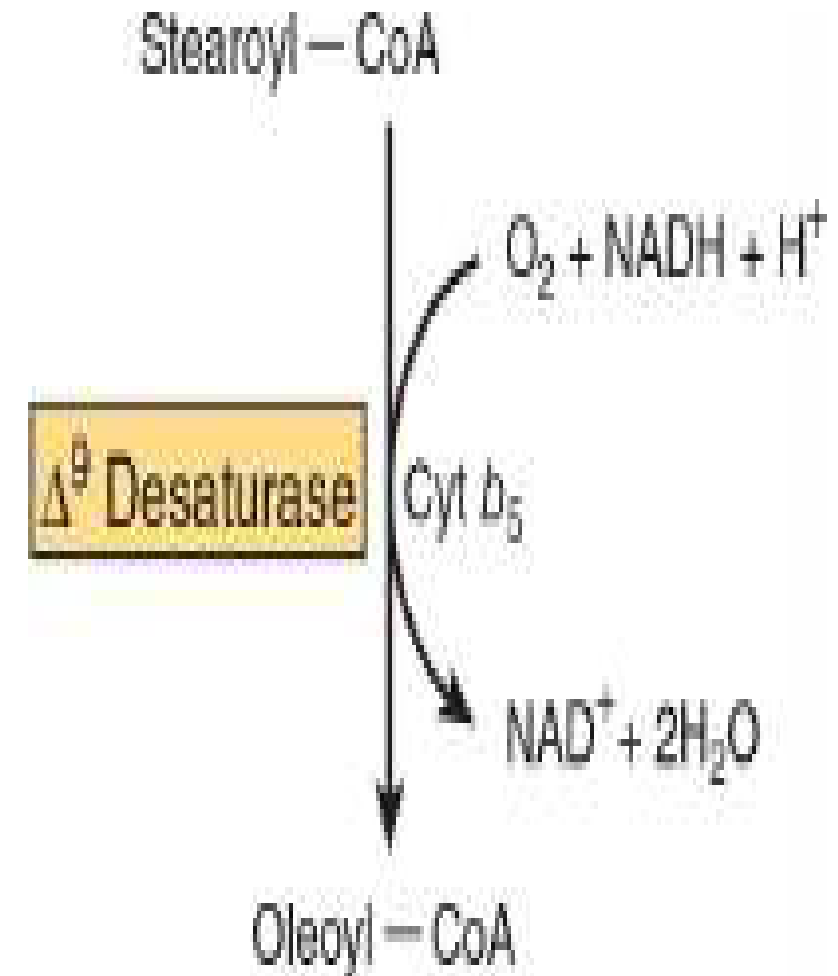

Stearoyl — CoA

O_2 + NADH + H^+

Δ^9 Desaturase | Cyt b_5

NAD^+ + $2H_2O$

Oleoyl — CoA

FIGURE 9 Microsomal δ9 desaturase.

SYNTHESIS OF POLYUNSATURATED FATTY ACIDS INVOLVES DESATURASE & ELONGASE ENZYME SYSTEMS

Additional double bonds introduced into existing monounsaturated fatty acids are always separated from each other by a methylene group .

Since animals have a Δ9 desaturase, they are able to synthesize the ω9 (oleic acid) family of unsaturated fatty acids completely by a combination of chain elongation and desaturation (Figures 9 and **10**) after the formation of saturated fatty acids by the pathways described in this chapter. However, as indicated above, linoleic (ω6) or α-linolenic (ω3) acids are required for the synthesis of the other members of the ω6 or ω3 families (pathways shown in Figure 10) and must be supplied in the diet.

Linoleic acid is converted to arachidonic acid (20:4 ω6) via **γ-linolenic acid (18:3 ω6)**. The nutritional requirement for arachidonate may thus be dispensed with if there is adequate linoleate in the diet. Cats, however, cannot carry out this conversion owing to the absence of Δ6 desaturase and must obtain arachidonate in their diet. The desaturation and chain elongation system are greatly diminished in the starving state, in response to glucagon and epinephrine administration, and in the absence of insulin as in type 1 diabetes mellitus.

FIGURE 10 Biosynthesis of the ω9, ω6, and ω3 families of polyunsaturated fatty acids.
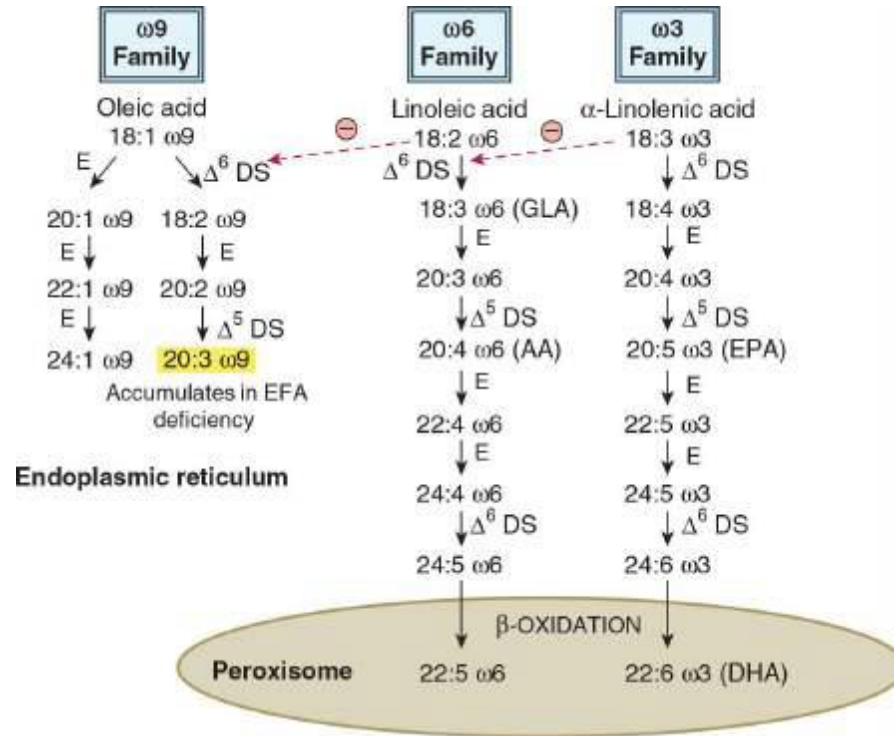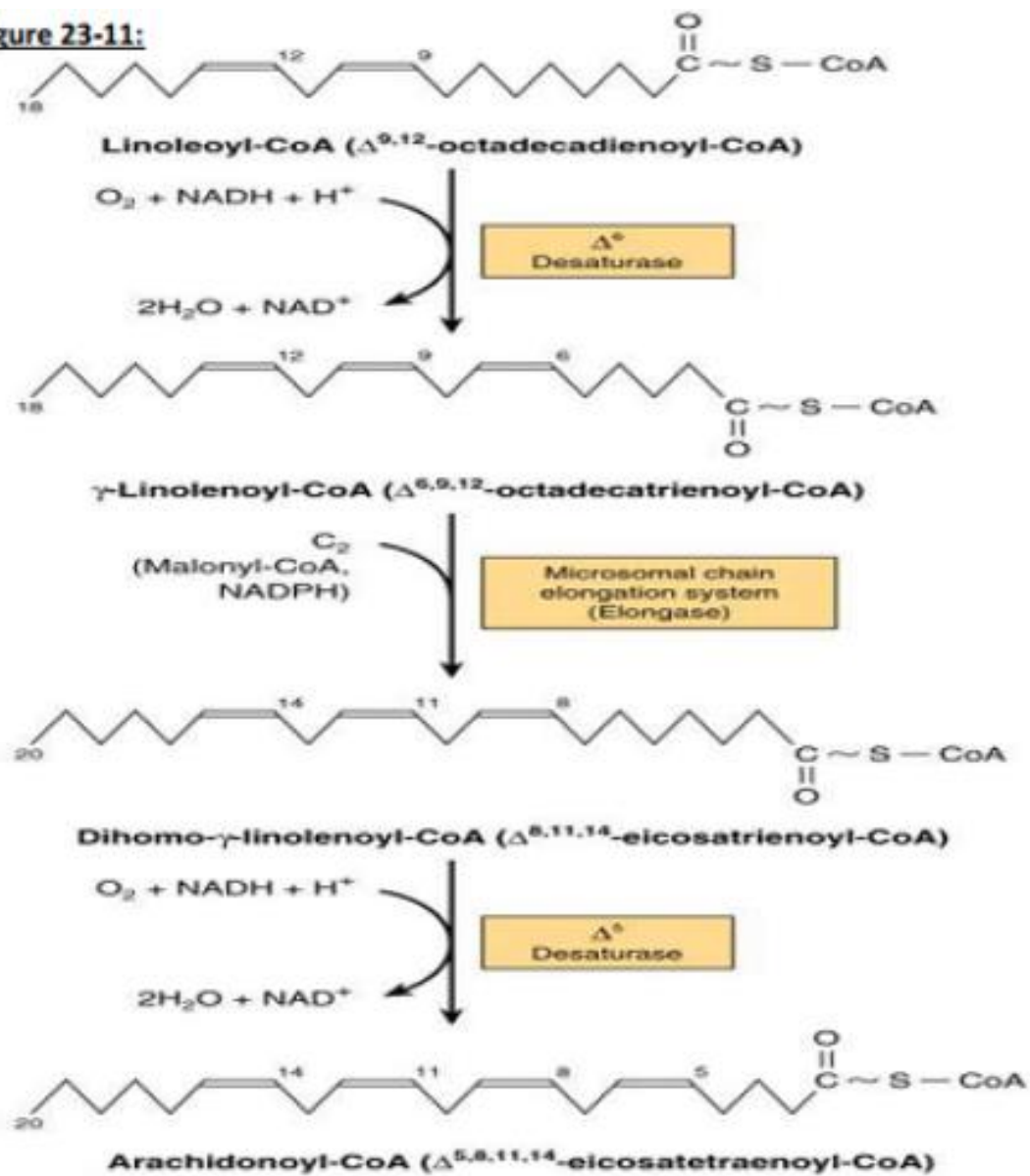
Figure 23-11:

Linoleoyl-CoA ($\Delta^{9,12}$-octadecadienoyl-CoA)

O_2 + NADH + H$^+$

Δ^6 Desaturase

$2H_2O$ + NAD$^+$

γ-Linolenoyl-CoA ($\Delta^{6,9,12}$-octadecatrienoyl-CoA)

C_2
(Malonyl-CoA, NADPH)

Microsomal chain elongation system (Elongase)

Dihomo-γ-linolenoyl-CoA ($\Delta^{8,11,14}$-eicosatrienoyl-CoA)

O_2 + NADH + H$^+$

Δ^5 Desaturase

$2H_2O$ + NAD$^+$

Arachidonoyl-CoA ($\Delta^{5,8,11,14}$-eicosatetraenoyl-CoA)

In animals, the ω9, ω6, and ω3 families of polyunsaturated fatty acids are synthesized in the endoplasmic reticulum from oleic, linoleic, and β-linolenic acids, respectively, by a series of elongation and desaturation reactions. The production of 22:5 ω6 (osbond acid) or 22:6 ω3 (docosahexanoic acid [DHA]), however, requires one cycle of β-oxidation, which takes place inside peroxisomes after the formation of 24:5 ω6 or 24:6 ω3. (AA, arachidonic acid; E, elongase; DS, desaturase; EFA, essential fatty acids; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; Minu red(-) , inhibition.)

Oxidation of Fatty Acids: Ketogenesis

BIOMEDICAL IMPORTANCE

Fatty acids are broken down in mitochondria by oxidation to acetyl-CoA in a process that generates large amounts of energy. When this pathway is proceeding at a high rate, three compounds, **acetoacetate, D-3- hydroxybutyrate**, and **acetone**, known collectively as the **ketone bodies**, are produced by the liver. Acetoacetate and D-3-hydroxybutyrate are used as fuels by extrahepatic tissues in normal metabolism, but overproduction of ketone bodies causes **ketosis**.

Increased fatty acid oxidation and consequently ketosis is a characteristic of starvation and of diabetes mellitus. Since ketone bodies are acidic, when they are produced in excess over long periods, as in diabetes, they cause **ketoacidosis**, which is ultimately fatal.

Because gluconeogenesis is dependent on fatty acid oxidation, any impairment in fatty acid oxidation leads to **hypoglycemia**. This occurs in various states of **carnitine deficiency** or deficiency of essential enzymes in fatty acid oxidation, for example, **carnitine palmitoyltransferase**, or inhibition of fatty acid oxidation by poisons, for example, **hypoglycin**.

OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

Although **acetyl-CoA** is both an end point of **fatty acid catabolism** and the starting substrate for **fatty acid synthesis**, breakdown is not simply the reverse of the biosynthetic pathway, but an entirely separate process taking place in a different compartment of the cell. The separation of **fatty acid oxidation in mitochondria** from **biosynthesis in the cytosol** allows each process to be individually controlled and integrated with tissue requirements.

Each step-in fatty acid oxidation involves acyl-CoA derivatives, is catalyzed by separate enzymes, utilizes **NAD+ and FAD** as coenzymes, and **generates ATP**. It is an **aerobic process**, requiring the presence of oxygen.

Fatty Acids Are Transported in the Blood as Free Fatty Acids

Free fatty acids (FFAs)—also called 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**, so that in fact they are never really "free.

Fatty Acids Are Activated Before Being Catabolized

Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the o**nly step** in the complete degradation **of a fatty acid that requires energy from ATP**. In the presence of **ATP and coenzyme A**, the enzyme **acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid (or FFA) to an "active fatty acid" or **acyl-CoA**, using one high-energy phosphate and forming AMP and PPi (**Figure 1).**

The PPi is hydrolyzed by **inorganic pyrophosphatase** with the loss of a further high-energy phosphate, ensuring that the overall reaction goes to completion. **Acyl-CoA synthetases** are found in the **endoplasmic reticulum**, **peroxisomes**, and on the **outer membrane of mitochondria.**

FIGURE 1 Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acyl- CoA formed by acyl-CoA synthetase enters the intermembrane space. For transport across the inner membrane, acyl groups must be transferred from CoA to carnitine by **carnitine palmitoyltransferase-I.** The acylcarnitine formed is then carried into the matrix by a translocase enzyme in exchange for a free carnitine and acyl-CoA is reformed by **carnitine palmitoyltransferase-II.**
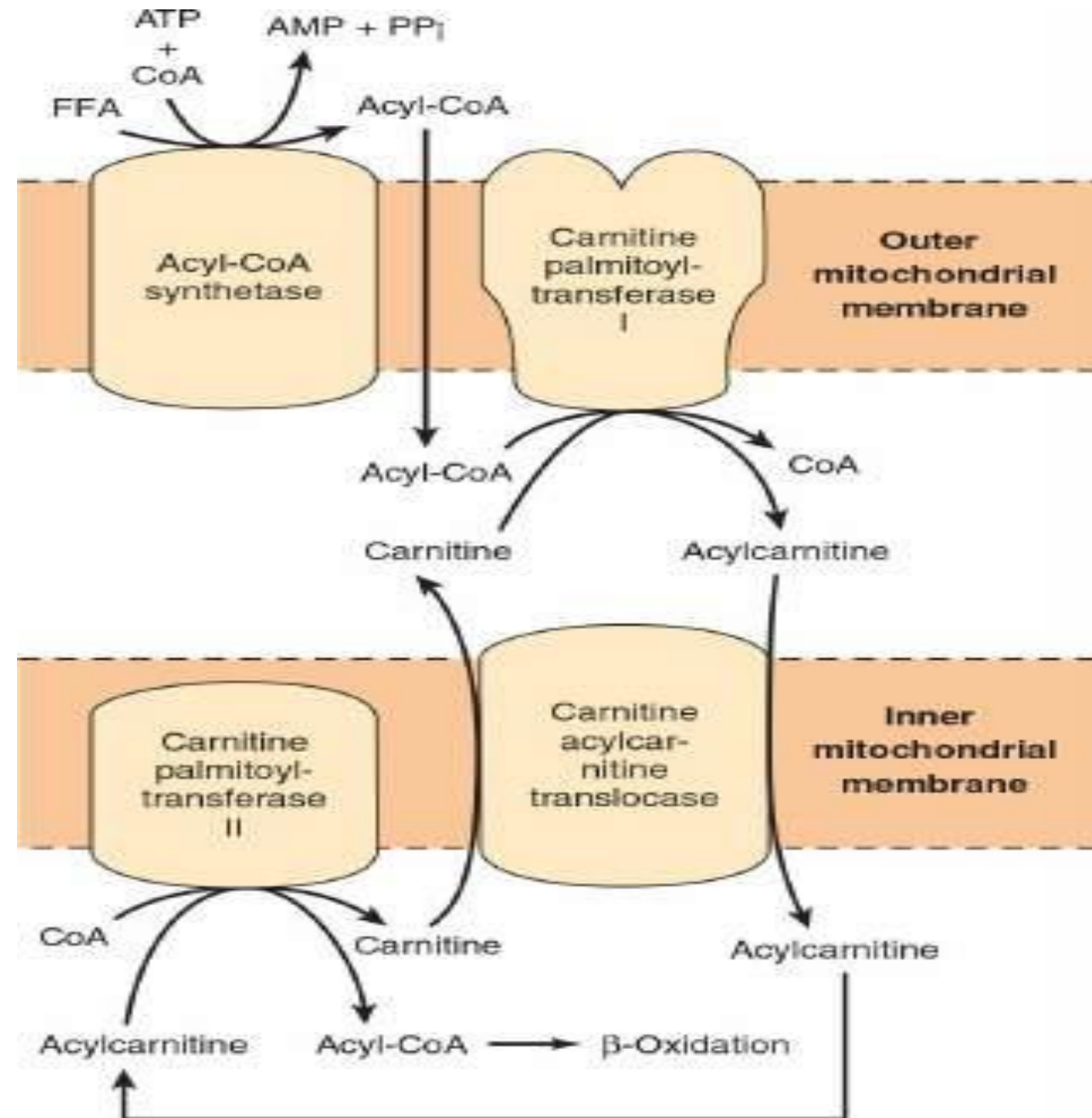
Long-Chain Fatty Acids Cross the Inner Mitochondrial Membrane as Carnitine Derivatives

Carnitine 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, transfers the long-chain acyl group from CoA to carnitine, forming **acylcarnitine** and releasing CoA. **Acylcarnitine is able to penetrate the** inner membrane and gain access to the β-oxidation system of enzymes via the inner membrane exchange transporter **carnitine-acylcarnitine translocase**. The transporter binds acylcarnitine and transports it across the membrane in exchange for carnitine. The acyl group is then transferred to CoA so that acyl-CoA is reformed and carnitine is liberated. This reaction is catalyzed by **carnitine palmitoyltransferase-II,** which is located on the inside of the inner membrane .

β-OXIDATION OF FATTY ACIDS INVOLVES SUCCESSIVE CLEAVAGE WITH RELEASE OF ACETYL-COA

In the **Figure 2**, pathway for the oxidation of fatty acids, two carbons at a time are cleaved from acyl-CoA molecules, starting at the carboxyl end. The chain is broken between the α(2)- and β(3)-carbon atoms—hence the the process is termed **β-oxidation**. The two-carbon units formed are acetyl-CoA; thus, palmitoyl(C16)-CoA forms eight acetyl-CoA molecules.
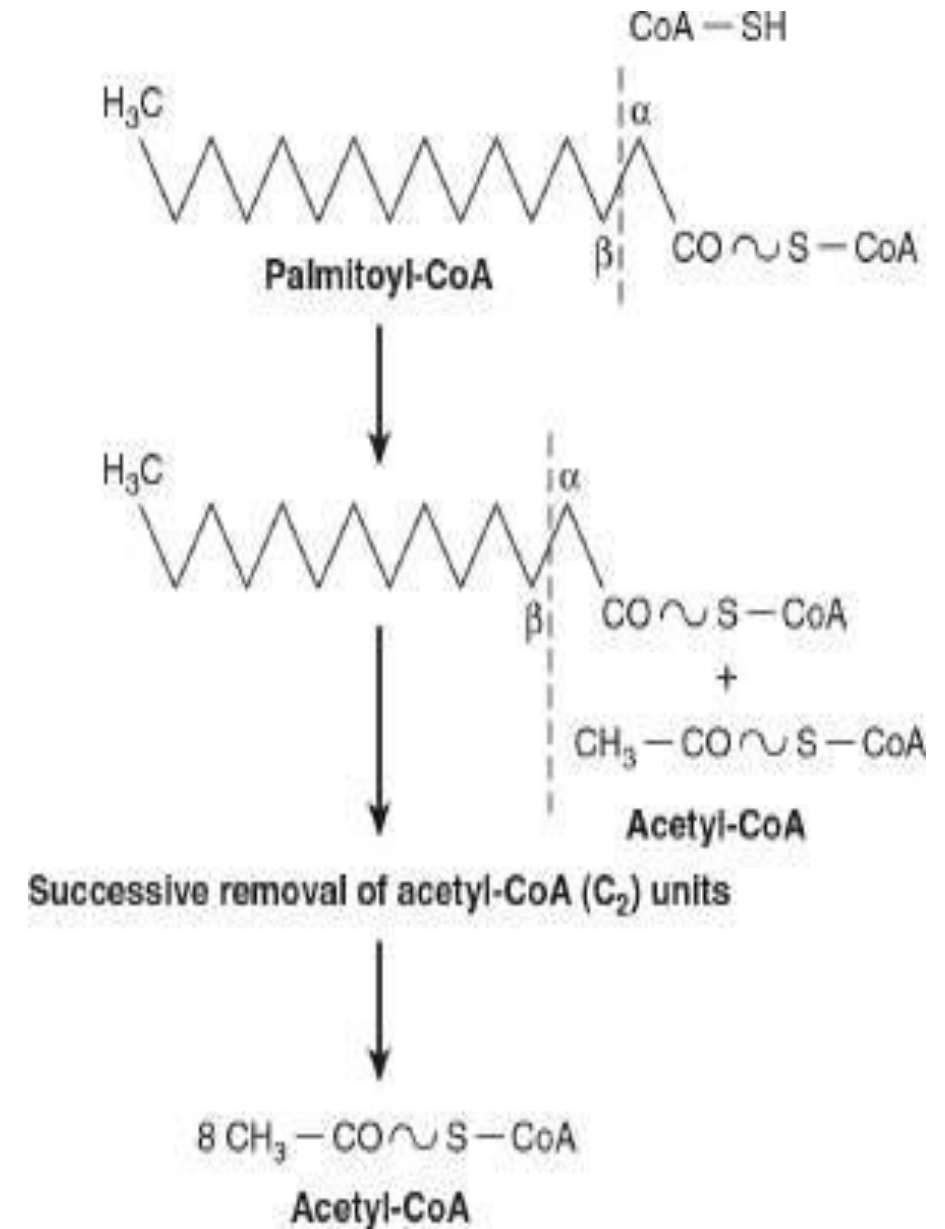

FIGURE 2 Overview of β-oxidation of fatty acids.

The β-Oxidation Cycle Generates FADH2 & 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** via the β- oxidation pathway. The system proceeds in cyclic fashion which results in the degradation of long fatty acids to acetyl-CoA. In the process, **large quantities of the reducing equivalents FADH2 and NADH are generated** and are used to form ATP by oxidative phosphorylation (**Figure 3**).

FIGURE 3 β-Oxidation of fatty acids. Long-chain acyl-CoA is cycled through reactions Symbol(2) to Symbol(5) , acetyl-CoA being split off, each cycle, by thiolase (reaction Symbol(5)). When the acyl radical is only four carbon atoms in length, two acetyl-CoA molecules are formed in reaction Symbol (5).
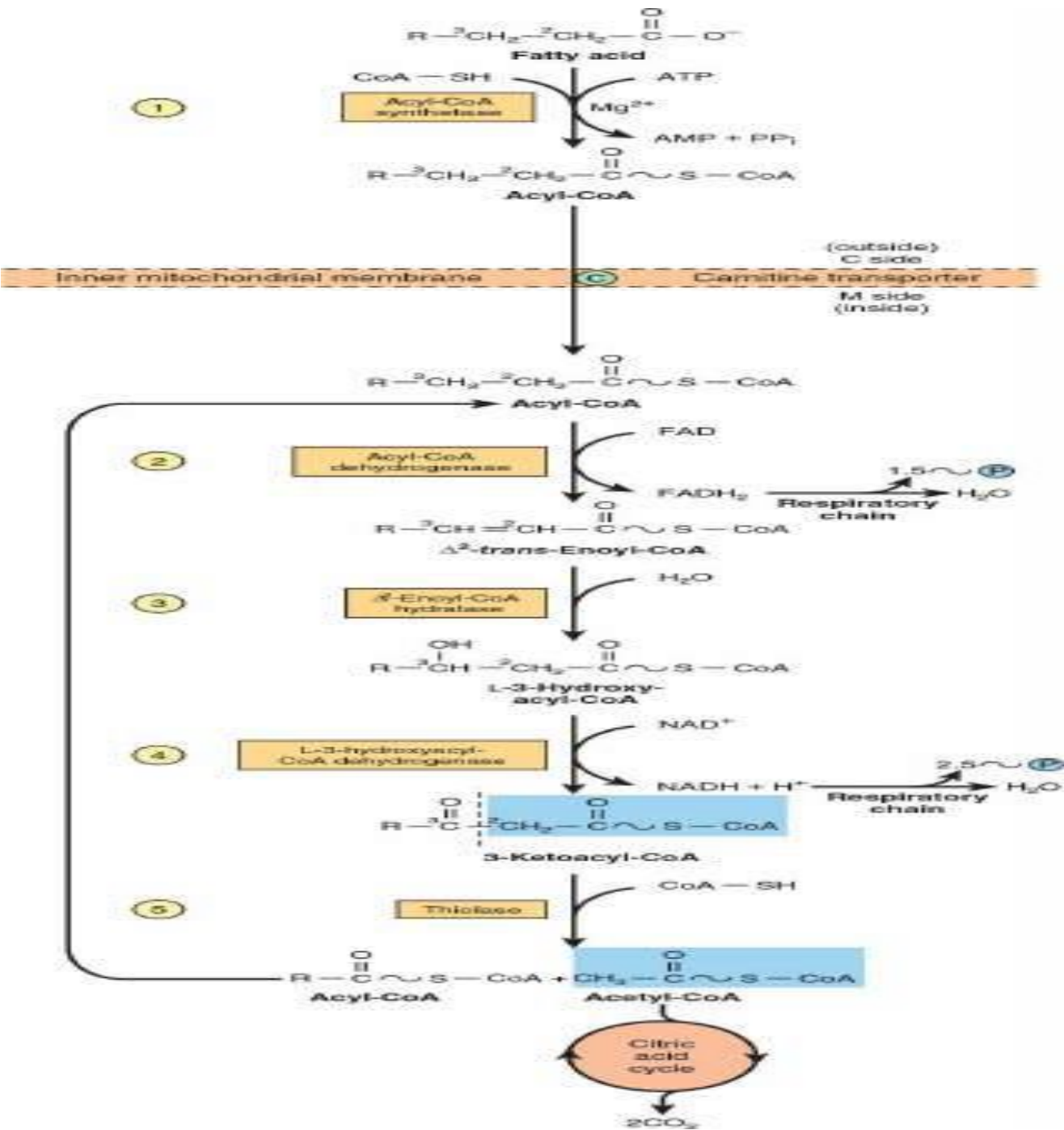
The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)-carbon atoms, catalyzed by **acyl-CoA dehydrogenase** and requiring flavin adenine dinucleotide (FAD). This results in the formation **of Δ2-** *trans***-enoyl-CoA** and **FADH2**. Water is added to saturate the double bond and form **3-hydroxyacyl-CoA**, catalyzed by **Δ2-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+ 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 shorter acyl-CoA formed in the cleavage reaction **reenters the oxidative pathway** at reaction 2 (Figure 3). In this way, **a long-chain fatty acid** with an even number of carbons may be **degraded completely** to **acetyl-CoA (C2 units).** For example, after seven cycles, the C16 fatty acid, palmitate, would be converted to eight acetyl-CoA molecules. Since acetyl-CoA can be oxidized to CO_2 and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.

Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation described above producing acetyl-CoA until a three-carbon (propionyl-CoA) residue remains. This compound is converted to succinyl-CoA, a constituent of the citric acid cycle.

Hence, **the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic**.

Oxidation of Fatty Acids Produces a Large Quantity of ATP

Each cycle of β-oxidation generates one molecule of FADH2 and one of NADH. The breakdown of 1 mol of the **C16 fatty acid, palmitate, requires seven cycles and produces 8 mol of acetyl-CoA.** Oxidation of the reducing equivalents via the respiratory chain leads to the synthesis **of 28 mol of ATP** (Table 1) and oxidation of **acetyl-CoA via the citric acid cycle produces 80 mol of ATP** .

The breakdown of 1 mol of palmitate, therefore, yields a gross total of **108** mol of ATP. However, **two** high-energy phosphates **are used** in the initial activation step (Figure 3), thus there is a net gain of **106** mol of ATP per mole of palmitate used (Table 1), or **106 × 30.5* = 3233 kJ.** This represents 33% of the free energy of combustion of palmitic acid.

TABLE 1 **Generation of ATP from the Complete Oxidation of a C16 Fatty Acid**

Step	Product	Amount Product Formed (mol)/mol Palmitate	ATP Formed (mol)/ mol Product	Total ATP Formed (mol)/mol Palmitate	ATP Used (mol)/ mol Palmitate
Activation		–			2
β-Oxidation	FADH$_2$	7	1.5	10.5	–
β-Oxidation	NADH	7	2.5	17.5	–
Citric acid cycle	Acetyl-CoA	8	10	80	–
	Total ATP formed (mol)/mol palmitate			108	
	Total ATP used (mol)/mol palmitate				2

The table shows how the oxidation of 1 mol of the C16 fatty acid, palmitate, generates 106 mol of ATP (108 formed in total—2 used in the activation step).

Peroxisomes Oxidize Very-Long-Chain Fatty Acids

A modified form of β-oxidation is found in peroxisomes and leads to the breakdown of very-long-chain fatty acids (eg, C20, C22) with the formation of acetyl-CoA and H2O2, which is broken down by catalase. This sytem is **not** linked directly to phosphorylation and the **generation of ATP**, and **also does not attack shorter-chain f**atty acids; the β-oxidation sequence ends at octanoyl-CoA. Octanoyl and acetyl groups are both further oxidized in mitochondria. The peroxisomal enzymes are induced by high-fat diets and in some species by hypolipidemic drugs such as clofibrate. **Another role of peroxisomal β-oxidation is to shorten the side chain of cholesterol in bile acid formation**.

Peroxisomes **also take part** in the synthesis of ether glycerolipids, cholesterol, and dolichol .

Oxidation of Unsaturated Fatty Acids Occurs by a Modified β-Oxidation Pathway

The CoA esters of unsaturated fatty acids are degraded by the enzymes normally responsible for β-oxidation until **either a Δ3-*cis*-acyl-CoA compound** or **a Δ4-*cis*-acyl-CoA compound is formed**, depending on the position of the double bonds (**Figure 4**). The former compound is isomerized **(Δ3cis → Δ2-*trans*-enoyl-CoA isomerase)** to the corresponding Δ2-*trans*-CoA stage of β-oxidation for subsequent hydration and oxidation. Any Δ4-*cis*-acyl-CoA either remaining, as in the case of linoleic acid, or entering the pathway at this point after conversion by acyl- CoA dehydrogenase to Δ2-*trans*-Δ4-*cis*-dienoyl-CoA, is then metabolized as indicated in Figure 4.

FIGURE 4 Sequence of reactions in the oxidation of unsaturated fatty acids, for example, linoleic acid. Δ4-*cis*-fatty acids or fatty acids forming Δ4-*cis*-enoyl-CoA enter the pathway at the position shown. NADPH for the dienoyl-CoA reductase step is supplied by intramitochondrial sources such as glutamate dehydrogenase, isocitrate dehydrogenase, and NAD(P)H transhydrogenase.
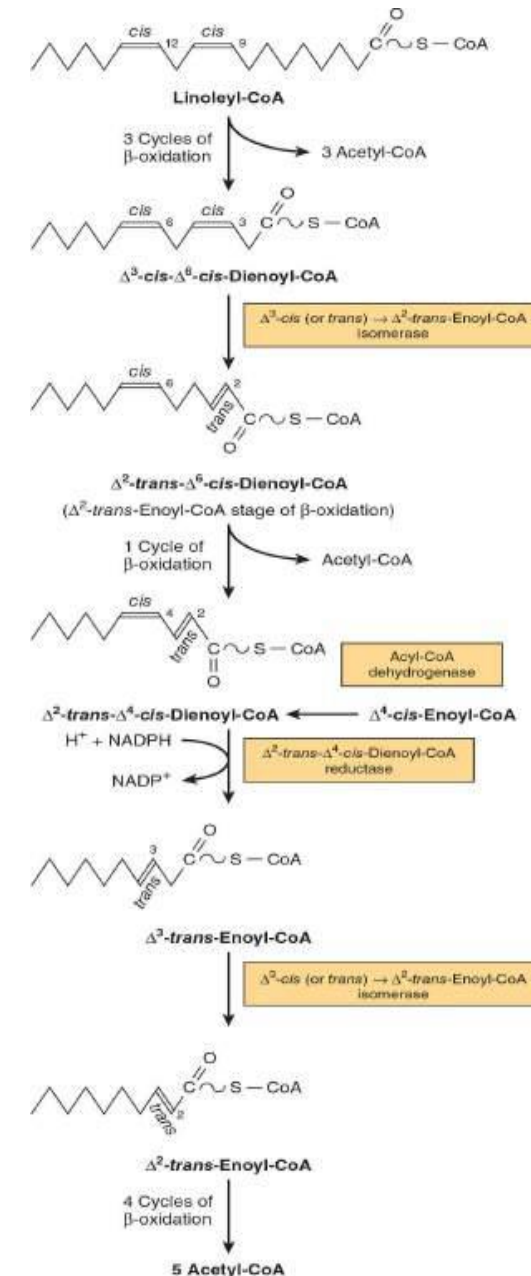
KETOGENESIS OCCURS WHEN THERE IS A HIGH RATE OF FATTY ACID OXIDATION IN THE LIVER

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of **acetoacetate** and D-**3-hydroxybutyrate** (β-hydroxybutyrate). **Acetoacetate** continually undergoes spontaneous decarboxylation to yield **acetone**. These three substances are collectively known as the **ketone bodies** (**Figure 5**). Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme D-**3-hydroxybutyrate dehydrogenase**; the equilibrium is controlled by the mitochondrial [NAD+]/[NADH] ratio, that is, the **redox state**. The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L. However**, in ruminants, 3- hydroxybutyrate is formed** continuously from **butyric acid** (a product **of ruminal fermentation**) in the rumen wall. In **nonruminants, the liver appears to be the only organ that adds significant quantities of ketone bodies to the blood. Extrahepatic tissues utilize acetoacetate and 3- hydroxybutyrate as respiratory substrates** (**Figure 6**).

FIGURE 5 Interrelationships of the ketone bodies. D-3-Hydroxybutyrate dehydrogenase is a mitochondrial enzyme.
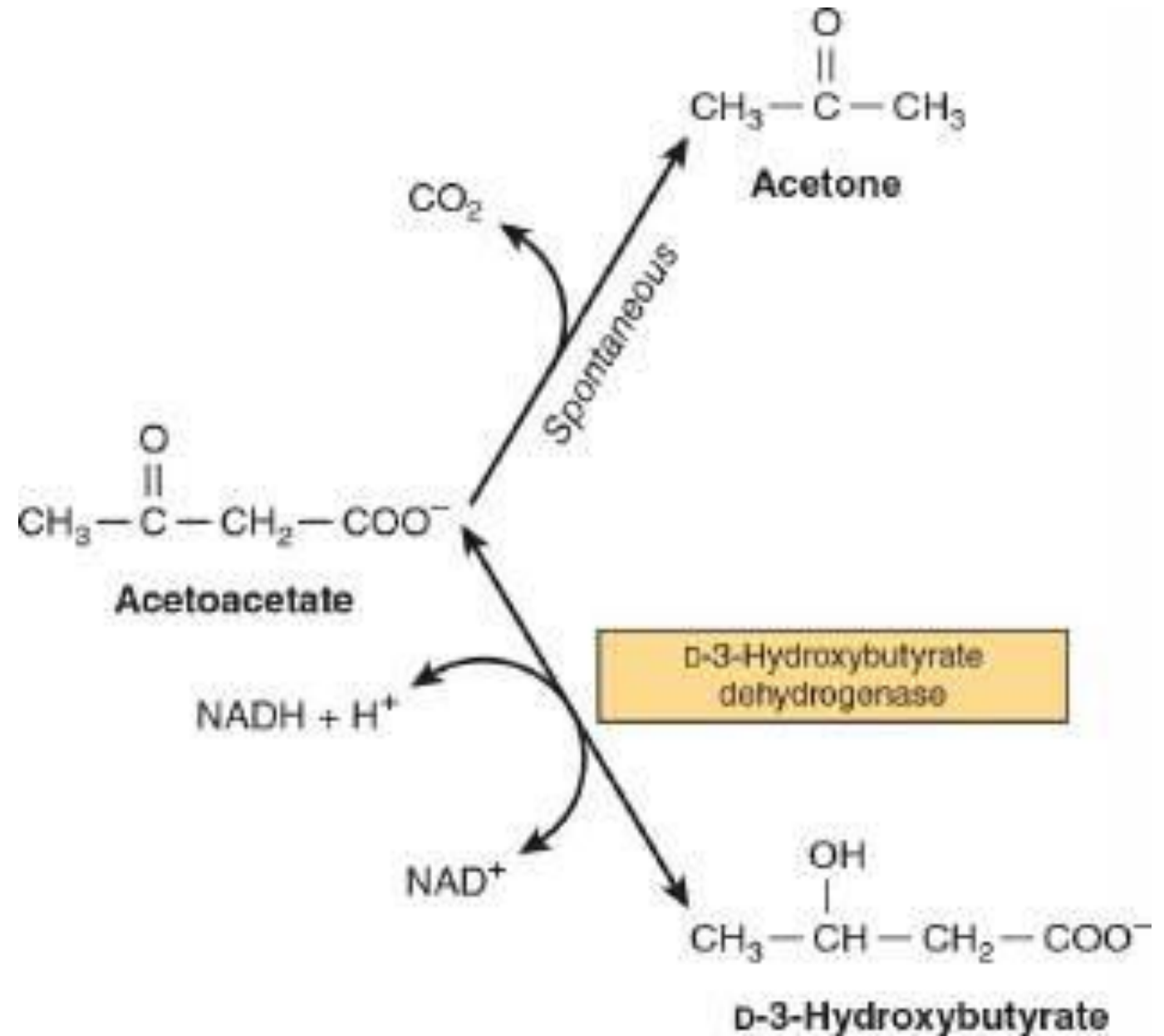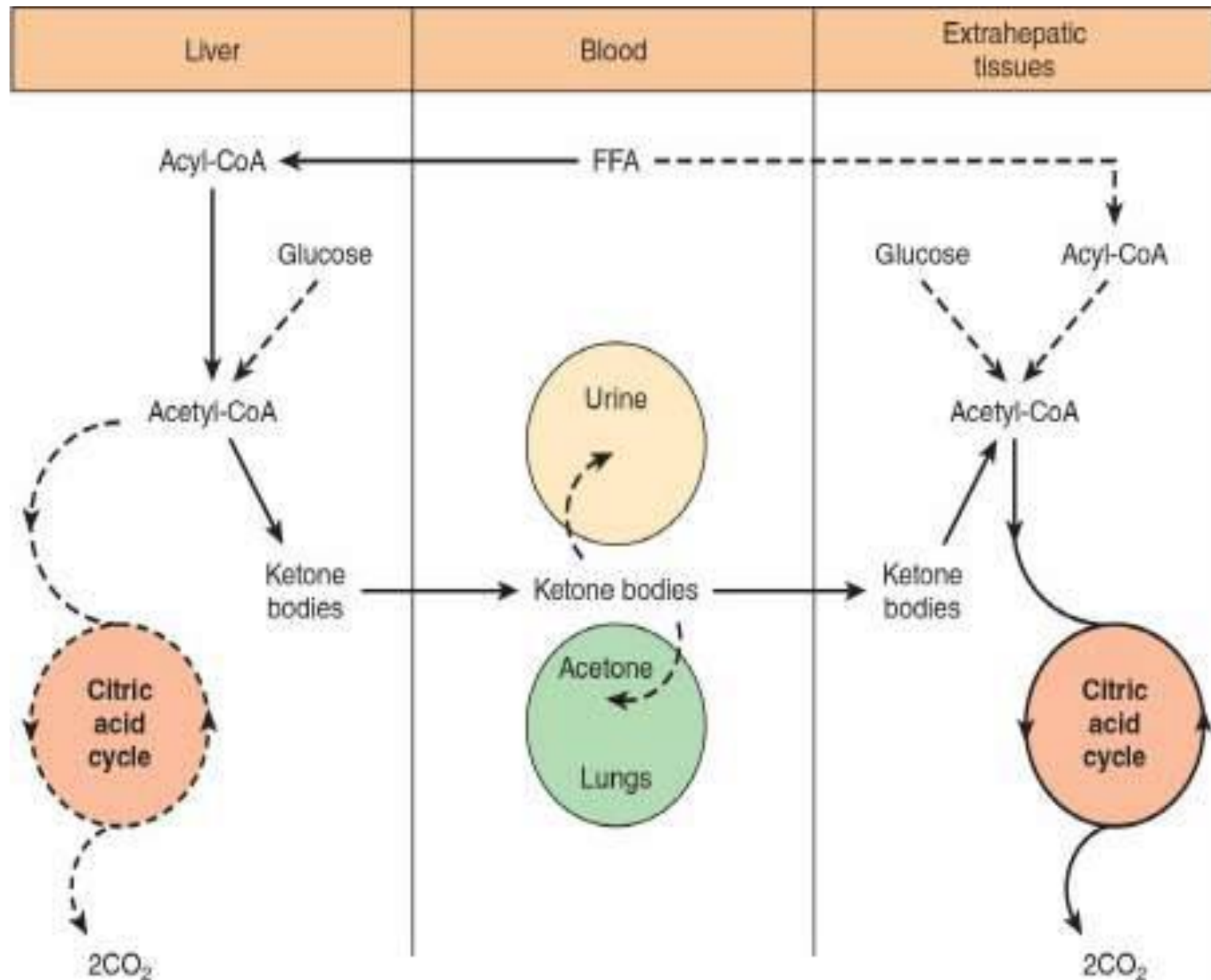
FIGURE 6 Formation, utilization, and excretion of ketone bodies.
(The main pathway is indicated by the solid arrows.)

Acetoacetyl-CoA Is the Substrate for Ketogenesis

The enzymes responsible for ketone body formation (ketogenesis) are associated mainly with the mitochondria. **Acetoacetyl-CoA** is formed w**hen two acetyl-CoA molecules** produced via fatty acid breakdown condense to form a**cetoacetyl-CoA** by a reversal of the **thiolase** reaction, and may also arise directly from the **terminal four carbons of a fatty acid during β-oxidation** (**Figure 7**). Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by **3-hydroxy-3- methylglutaryl-CoA (HMG-CoA) synthase** forms **HMG-CoA**. **HMGCoA 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**.

D-3- Hydroxybutyrate is quantitatively the predominant ketone body present in **the blood and urine** in ketosis.

FIGURE 7 Pathways of ketogenesis in the liver. (FFA, free fatty acids.)
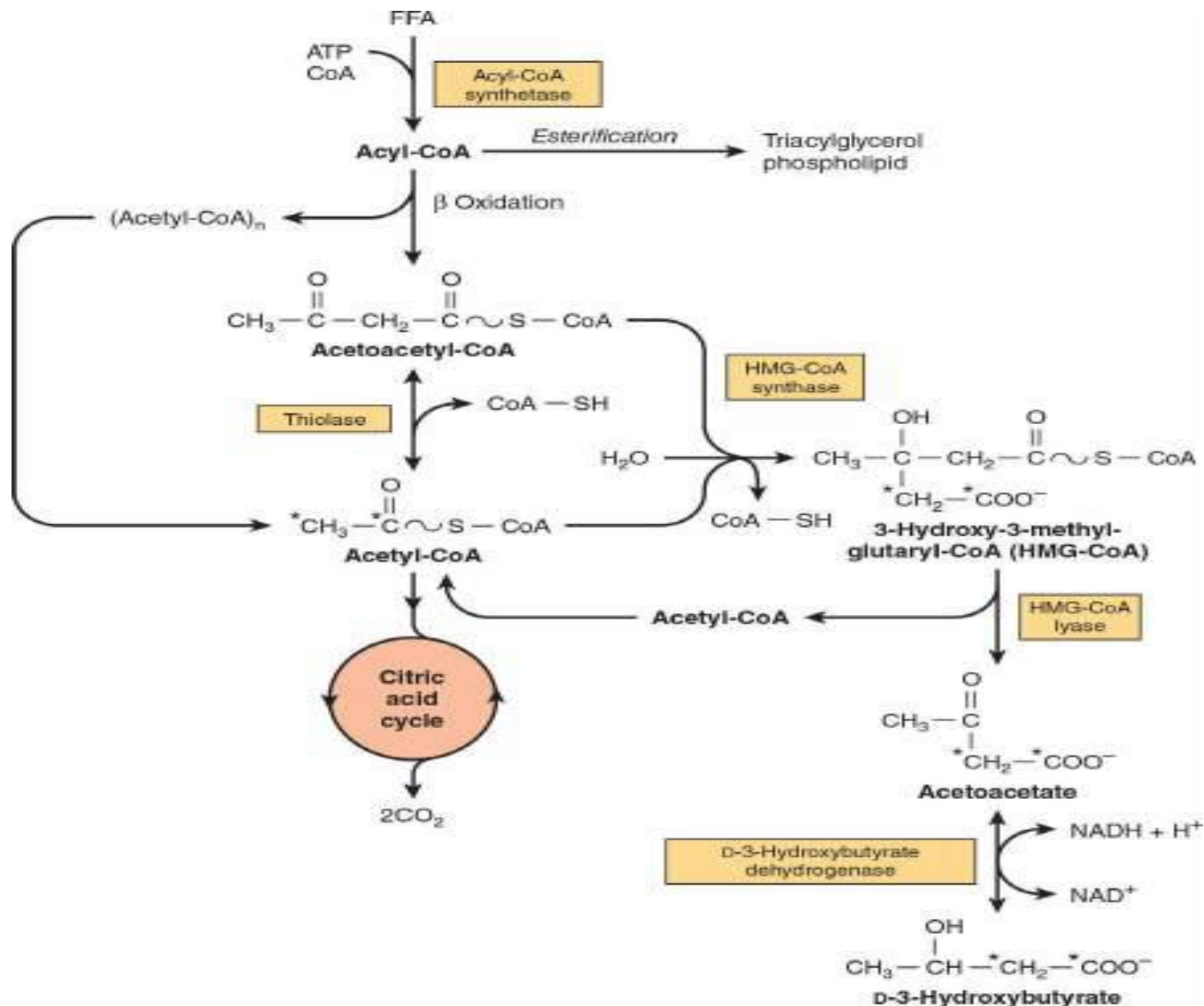
Ketone Bodies Serve as a Fuel for Extrahepatic Tissues

While an active enzymatic mechanism produces acetoacetate from acetoacetyl-CoA in the liver, **acetoacetate once formed can only be reactivated directly in the cytosol**, where it is used in a different, much less active pathway as **cholesterol synthesis** . This accounts for the net production of ketone bodies by 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 (**Figure 8**). In a reaction requiring the addition of a CoA, **two acetyl-CoA molecules are formed by the splitting of acetoacetyl-CoA by thiolase and these are oxidized in the citric acid cycle**. 1 mol of acetoacetate or 3-hydroxbutyrate yields **19** or **21.5** mol of **ATP,** respectively, by these pathways. If the blood level of ketone bodies rises to a concentration of ~12 mmol/L, the **oxidative machinery** becomes saturated and at this stage, **a large proportion of oxygen consumption may be accounted for by their oxidation.**

FIGURE 8 Transport of ketone bodies from the liver and pathways of utilization and oxidation in extrahepatic tissues.
**In moderate ketonemia**, the loss of ketone bodies via the urine is only **a few** percent of the total ketone body production and utilization. **Since there are renal threshold-like effects** (there is not a true threshold) that vary between species and individuals, measurement of the **ketonemia**, not the **ketonuria,** is the preferred method of assessing the severity of ketosis.
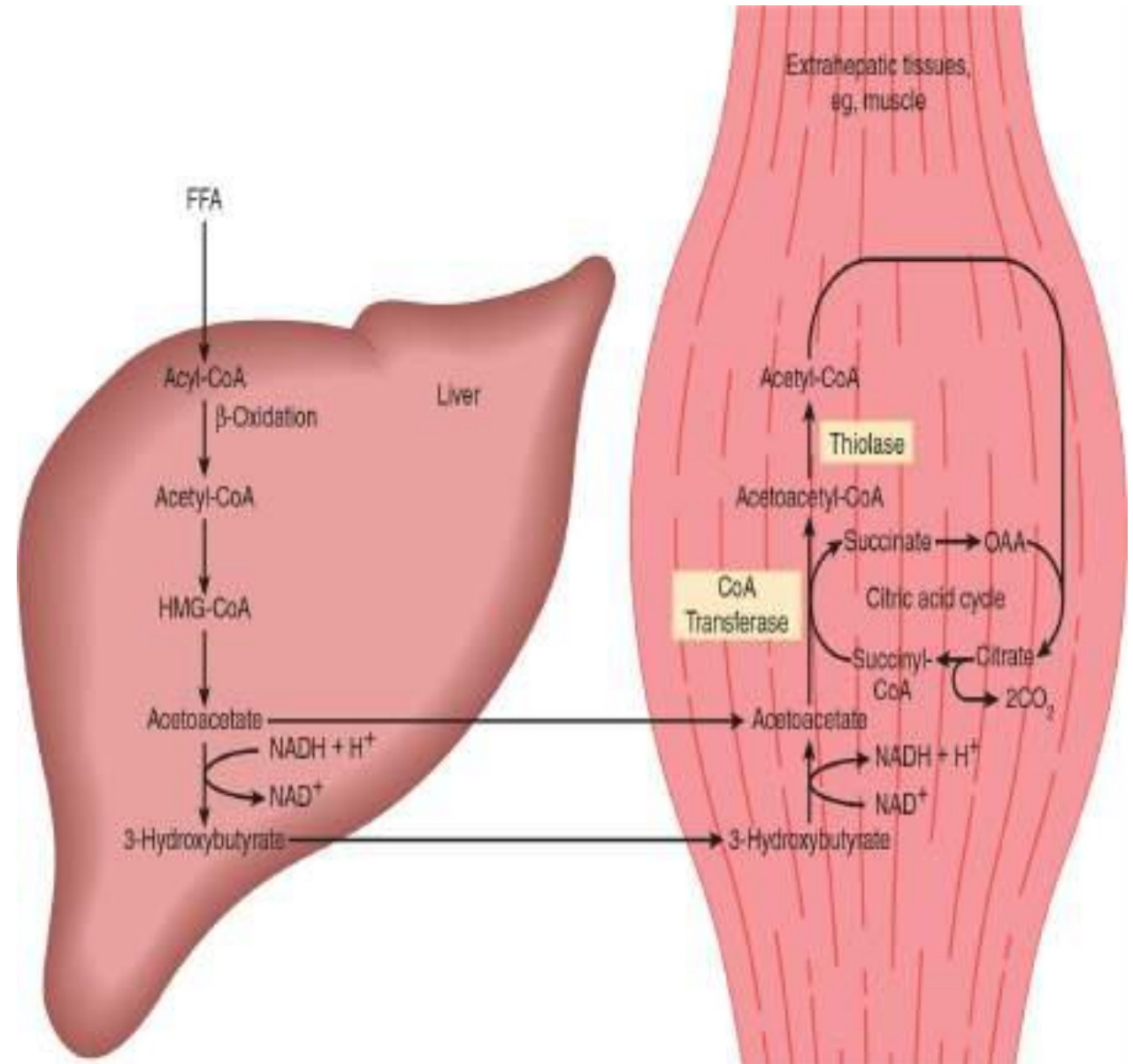
KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS

1. Ketosis does not occur in vivo unless there is an increase in the level of circulating FFAs arising from lipolysis of triacylglycerol in adipose tissue. **FFAs are the precursors of ketone bodies in the liver**. Both in fed and in fasting conditions, the liver extracts ~30% of the FFAs passing through it, so that at high concentrations the flux passing into the organ is substantial. **Thus, the factors regulating mobilization of FFA from adipose tissue are important in controlling ketogenesis (Figures 9).**

FIGURE 9 Regulation of ketogenesis. Symbol(1) to Symbol (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.)
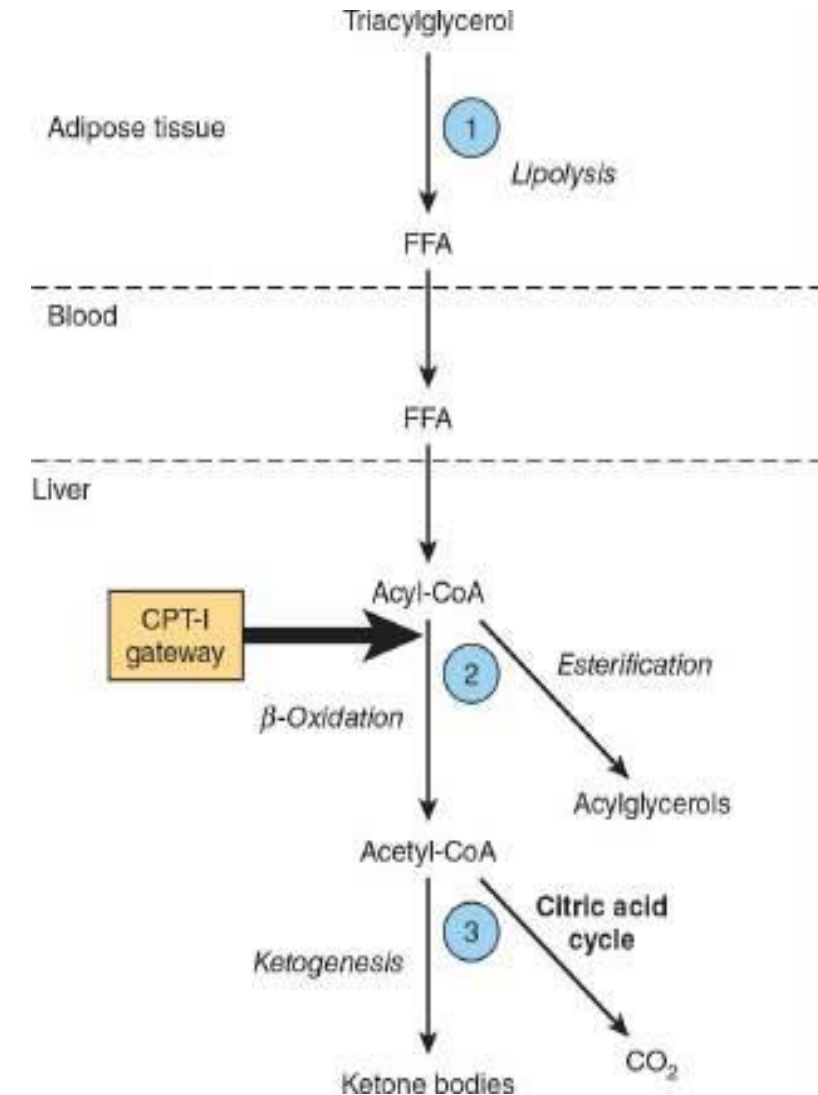
2. After uptake by the liver, FFAs are either **oxidized** to CO_2 or ketone bodies or **esterified** to triacylglycerol and phospholipid. There is regulation of entry of fatty acids into the oxidative pathway by **carnitine palmitoyltransferase-I** (CPT-I) (Figure 1), and the remainder of the fatty 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 **is a potent inhibitor of CPT-I (Figure 10). In the fed state**, therefore, FFAs 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** (VLDLs).

 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.

FIGURE 10 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.
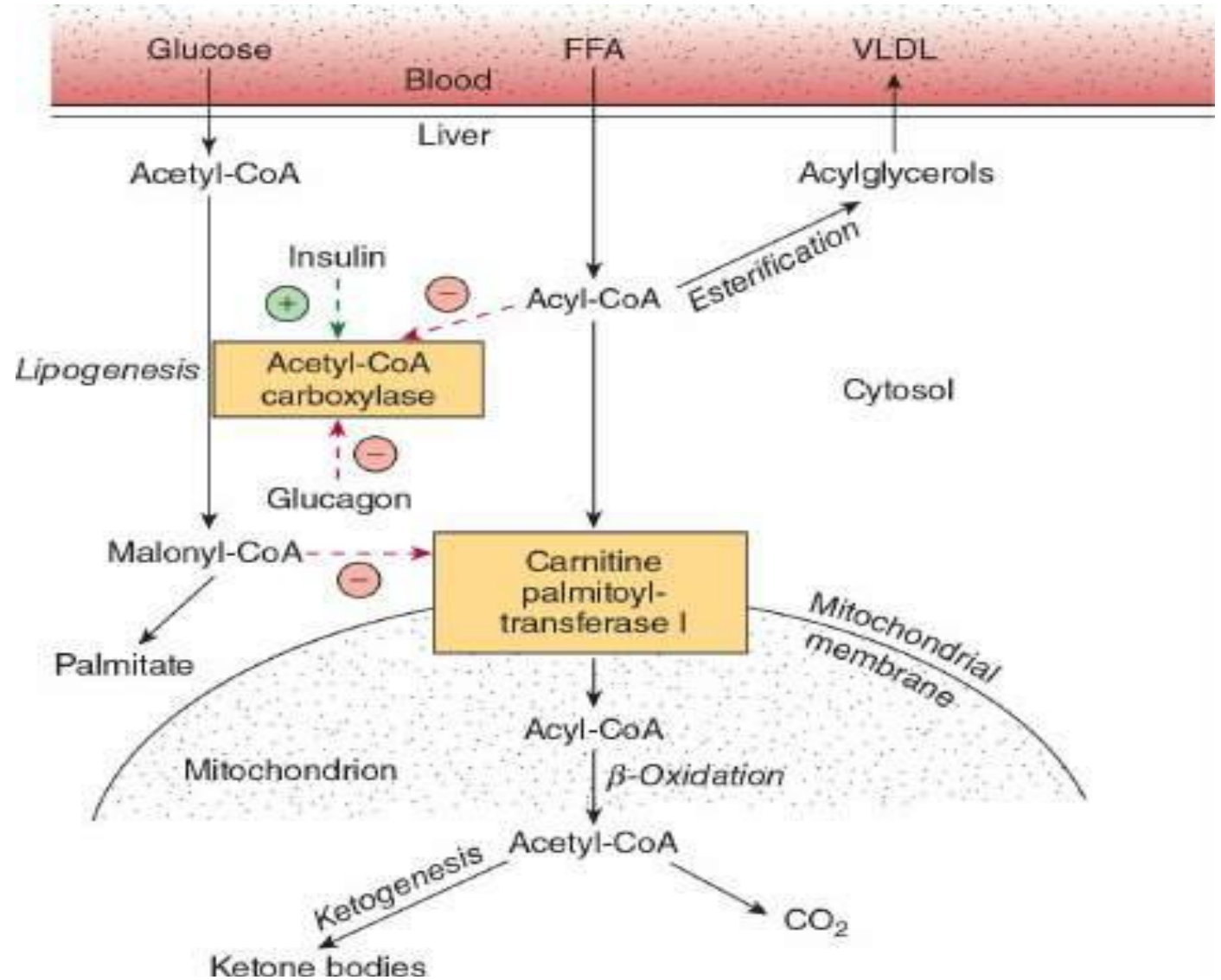
3. In turn, the **acetyl-CoA formed in β-oxidation** is **oxidized in the citric acid cycle**, or it enters the pathway of **ketogenesis** via aetoacetyl-CoA to form **ketone bodies**. As **the level of serum FFA is raised, proportionately more** of **the acetyl-CoA produced** from their breakdown **is converted to ketone bodies** and **less is oxidized via the citric acid** cycle to CO_2. The partition of acetyl-CoA between the ketogenic pathway and the pathway of oxidation to CO_2 is regulated so that the total free energy captured in ATP which results from the oxidation of FFA 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**. Such a fall may occur because of an increase in the (NADH)/(NAD+) ratio caused when increased β-oxidation alters the equilibrium between oxaloacetate and malate so that the concentration of oxaloacetate is decreased, and also when gluconeogenesis is elevated due to low blood glucose levels. The activation by acetyl-CoA of pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, partially alleviates this problem,

but in conditions such **as starvation** and untreated **diabetes mellitus**, ketone bodies are overproduced and cause **ketosis**.

Metabolism of Acylglycerols & Sphingolipids

BIOMEDICAL IMPORTANCE

Acylglycerols constitute the **majority of lipids** in the body. **Triacylglycerols** are the **major lipids** in fat deposits and in food, and their roles in lipid **transport and storage** and in various **diseases** such as **obesity**, **diabetes**, and hyperlipoproteinemia .

The **amphipathic nature** of phospholipids and sphingolipids makes them ideally suitable as the main lipid **component of cell membranes.**

Phospholipids also take part in the metabolism of many other lipids. Some phospholipids have **specialized functions**; for example, dipalmitoyl lecithin is a major component of **lung surfactant**, which is lacking in **respiratory distress syndrome** of the newborn. **Inositol phospholipids** in the cell membrane act as precursors of **hormone second messengers**, and **platelet-activating factor** (PAF) is an **alkylphospholipid.**

Glycosphingolipids, which contain **sphingosine** and **sugar** residues as well as a **fatty acid** are found in the outer leaflet of the plasma membrane with their oligosaccharide chains facing outward. They form part of the **glycocalyx** of the cell surface and are **important** (1) in cell adhesion and cell recognition, (2) as receptors for bacterial toxins (eg, the toxin that causes cholera), and (3) as ABO blood group substances. A dozen or so **glycolipid storage diseases** have been described (eg, Gaucher disease and Tay-Sachs disease), each due to **a genetic defect** in the pathway for **glycolipid** degradation in the lysosomes.

HYDROLYSIS INITIATES CATABOLISM OF TRIACYLGLYCEROLS

**Triacylglycerols** must be hydrolyzed by a **lipase** to their constituent **fatty acids** and **glycerol** before further catabolism can proceed. Much of this **hydrolysis** (lipolysis) occurs in **adipose tissue** with release **of free fatty acids into the plasma**, where they are found combined with **serum albumin**. This is followed by free fatty acid **uptake into tissues** (including liver, heart, kidney, muscle, lung, testis, and adipose tissue, but not readily by brain), where they are **oxidized to obtain energy** or **reesterified**. The **utilization of glycerol** depends on whether such tissues have the enzyme **glycerol kinase**, which is found in **significant** amounts in **liver**, **kidney**, **intestine**, brown **adipose tissue**, and the **lactating mammary gland**.

TRIACYLGLYCEROLS & PHOSPHOGLYCEROLS ARE FORMED BY ACYLATION OF TRIOSE PHOSPHATES

The major pathways of triacylglycerol and phosphoglycerol biosynthesis are outlined in **Figure 1**. Important substances such as **triacylglycerols, phosphatidylcholine**, **phosphatidylethanolamine**, **phosphatidylinositol**, and **cardiolipin**, a constituent of mitochondrial membranes, **are formed from glycerol-3-phosphate**. Significant branch points in the pathway occur at the **phosphatidate** and **diacylglycerol** steps. Phosphoglycerols containing an ether link (—C—O—C—), the best known of which are **plasmalogens** and **PAF**, are derived from **dihydroxyacetone phosphate.**
 Glycerol-3- phosphate and dihydroxyacetone phosphate are intermediates in glycolysis, making a very important **connection between carbohydrate and lipid metabolism .**

FIGURE 1 Overview of acylglycerol biosynthesis. (PAF, platelet activating factor.)
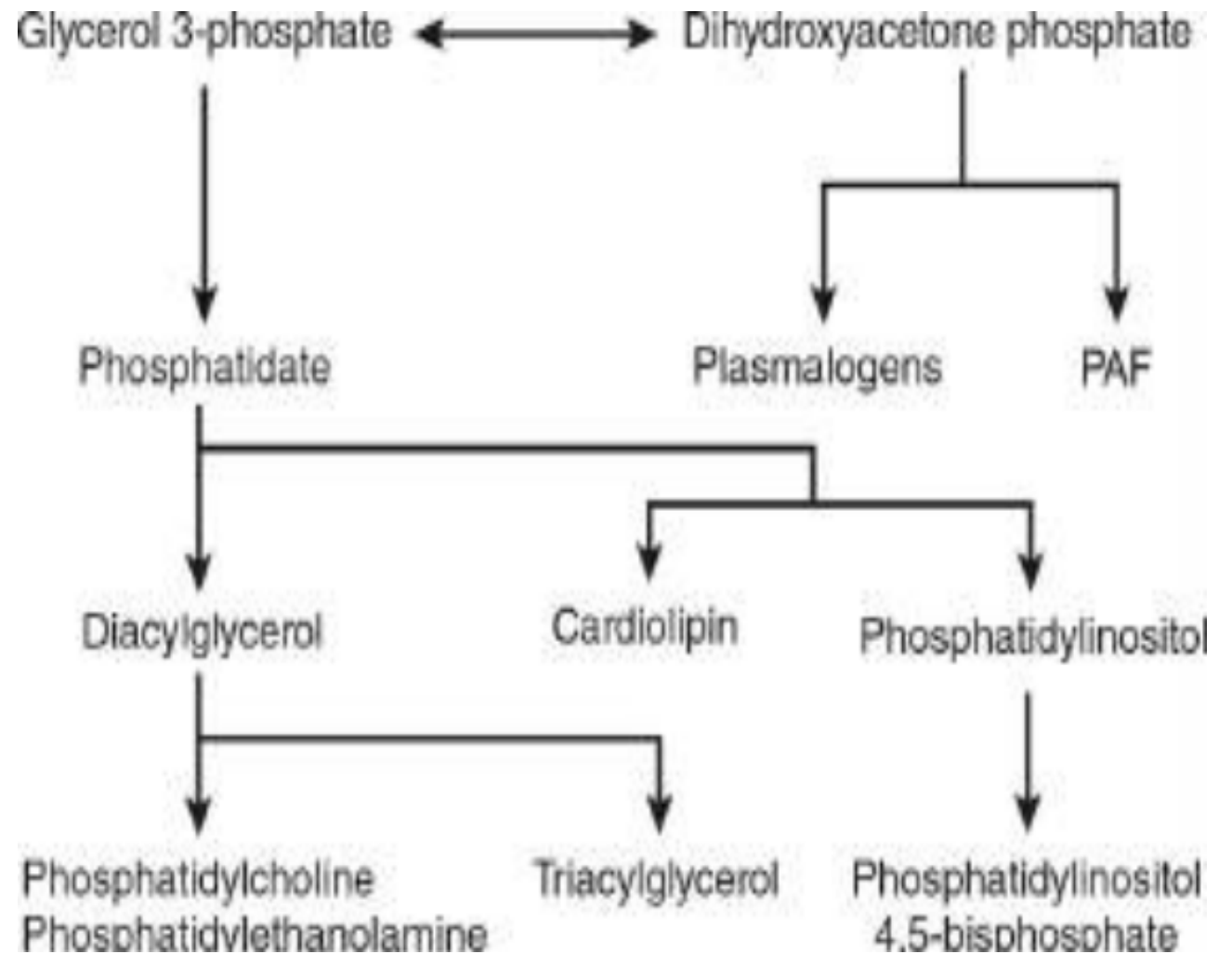
Phosphatidate Is the Common Precursor in the Biosynthesis of Triacylglycerols, Many Phosphoglycerols, & Cardiolipin

Both **glycerol** and **fatty acids** must be activated by **ATP** before they can be incorporated into acylglycerols. **Glycerol kinase** catalyzes the activation of glycerol to *sn*-glycerol 3-phosphate. If the **activity** of this enzyme is **absent or low**, as in **muscle** or **adipose tissue,** most of the glycerol-3- phosphate is formed from dihydroxyacetone phosphate by **glycerol-3- phosphate dehydrogenase** (**Figure 2**).

FIGURE 2 Biosynthesis of triacylglycerol and phospholipids. ,
monoacylglycerol pathway; , glycerol phosphate pathway.
Phosphatidylethanolamine may be formed from ethanolamine by a pathway similar to that shown
for the formation of phosphatidylcholine from choline.
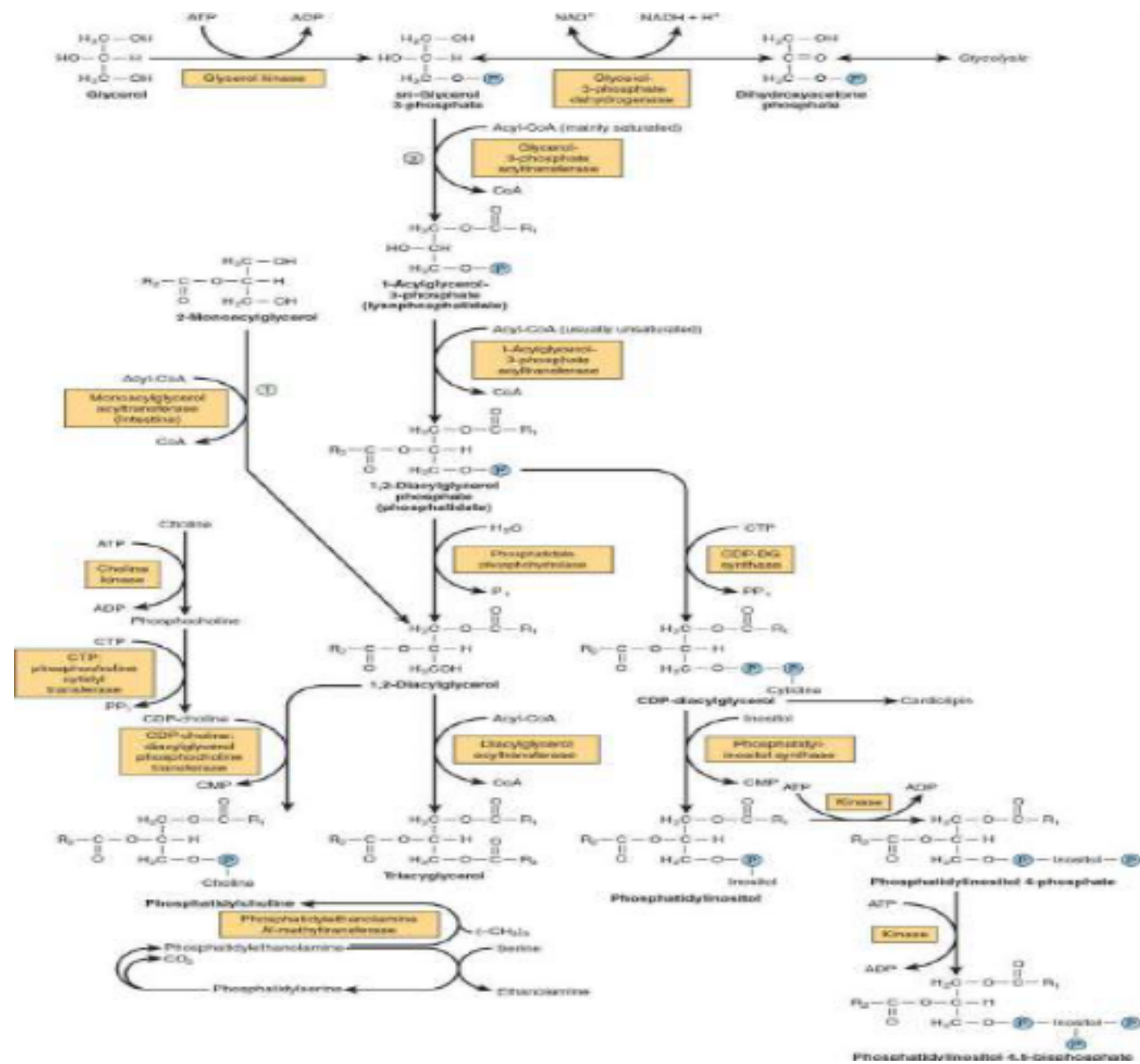
Biosynthesis of Triacylglycerols

Two molecules of acyl-CoA, formed by the activation of fatty acids by **acyl-CoA synthetase** , **combine with glycerol-3- phosphate** to form **phosphatidate** (**1,2-diacylglycerol phosphate).**

This takes place in two stages, catalyzed by **glycerol-3-phosphate acyltransferase** and **1-acylglycerol-3-phosphate acyltransferase**.
Phosphatidate is converted by **phosphatidate phosphohydrolase** (also called **phosphatidate phosphatase** [PAP]) and **diacylglycerol acyltransferase (DGAT)** to **1,2-diacylglycerol** and then **triacylglycerol**. **Lipins**, a family of three proteins, have **PAP activity** and they also act as transcription factors which regulate the expression of genes involved in lipid metabolism.
 Diacylglycerol acyltransferase (DGAT) catalyzes the only step specific for triacylglycerol synthesis and is thought to be **rate limiting** in most circumstances.
 In intestinal mucosa, **monoacylglycerol acyltransferase** converts **monoacylglycerol** to **1,2-diacylglycerol** in the **monoacylglycerol pathway**.

Most of the activity of these enzymes resides in **the endoplasmic reticulum**, but **some is found in mitochondria**. Although **phosphatidate phosphohydrolase** protein is found mainly in the **cytoso**l, the active form of the enzyme is **membrane bound**

Biosynthesis of Phospholipids

In the biosynthesis of **phosphatidylcholine** and **phosphatidylethanolamine**, **choline or ethanolam**ine must first be activated by **phosphorylation by ATP** followed by linkage to CDP. The **resulting CDP-choline** or **CDP-ethanolamine** reacts with 1,2-diacylglycerol to form either **phosphatidylcholine** or **phosphatidylethanolamine**, respectively. **Phosphatidylserine** is formed from phosphatidylethanolamine directly by reaction with serine (Figure 2).

Phosphatidylserine may reform **phosphatidylethanolamine** by **decarboxylation**. An alternative pathway in liver enables **phosphatidylethanolamin**e to give rise directly to **phosphatidylcholin**e by progressive **methylation of the ethanolamine residue**.

 The **regulation of triacylglycerol, phosphatidylcholine**, and **phosphatidylethanolamin**e **biosynthesis** is driven by the **availability of free fatty acids.** Those **that escape oxidation** are preferentially converted to phospholipids, and when this requirement is satisfied, they are used for triacylglycerol synthesis.

Cardiolipin (**diphosphatidylglycerol**;) is a **phospholipid present in mitochondria**. It is formed from phosphatidylglycerol, which in turn is synthesized from **CDP diacylglycerol** (Figure 2) and **glycerol-3-phosphate**, has a key **role in mitochondrial structure** and function, and is also thought to be involved in programmed cell death (**apoptosis**).

Biosynthesis of Glycerol Ether Phospholipids

In **glycerol ether phospholipids**, one or more of the glycerol carbons is attached to **a hydrocarbon chain** by an **ether linkage** rather than an **ester bond. Plasmalogens and PAF(Platelet activating factor)** are **important examples** of this type of lipid. The biosynthetic pathway is located in **peroxisomes.**

 Dihydroxyacetone phosphate is the precursor of the glycerol moiety (**Figure 3**). It combines with **acyl-CoA** to give **1-acyldihydroxyacetone phosphate**, and the ether link is formed in the next reaction, producing **1- alkyldihydroxyacetone phosphate**, which is then converted to **1- alkylglycerol 3-phosphate**. After further acylation in the 2 position, the resulting **1-alkyl-2-acylglycerol 3-phosphate** (analogous to phosphatidate in Figure 2) **is hydrolyzed to give the free glycerol derivative.**

Plasmalogens, which comprise much of the phospholipid in mitochondria, are formed by **desaturation of the analogous 3-phosphoethanolamine derivative** (Figure 3).

PAF (1-alkyl-2-acetyl-*sn*-glycerol-3- phosphocholine) is synthesized from the corresponding 3-phosphocholine derivative. It is formed by many **blood cells** and **other tissues and aggregates platelets** . It also has **hypotensive** and **ulcerogenic** properties and is involved in a variety of biologic responses, including **inflammation,** chemotaxis, and **protein phosphorylation**.

FIGURE 3 Biosynthesis of ether lipids, including plasmalogens, and platelet-activating factor (PAF). In the de novo pathway for PAF synthesis, acetyl-CoA is incorporated at stage*, avoiding the last two steps in the pathway shown here.
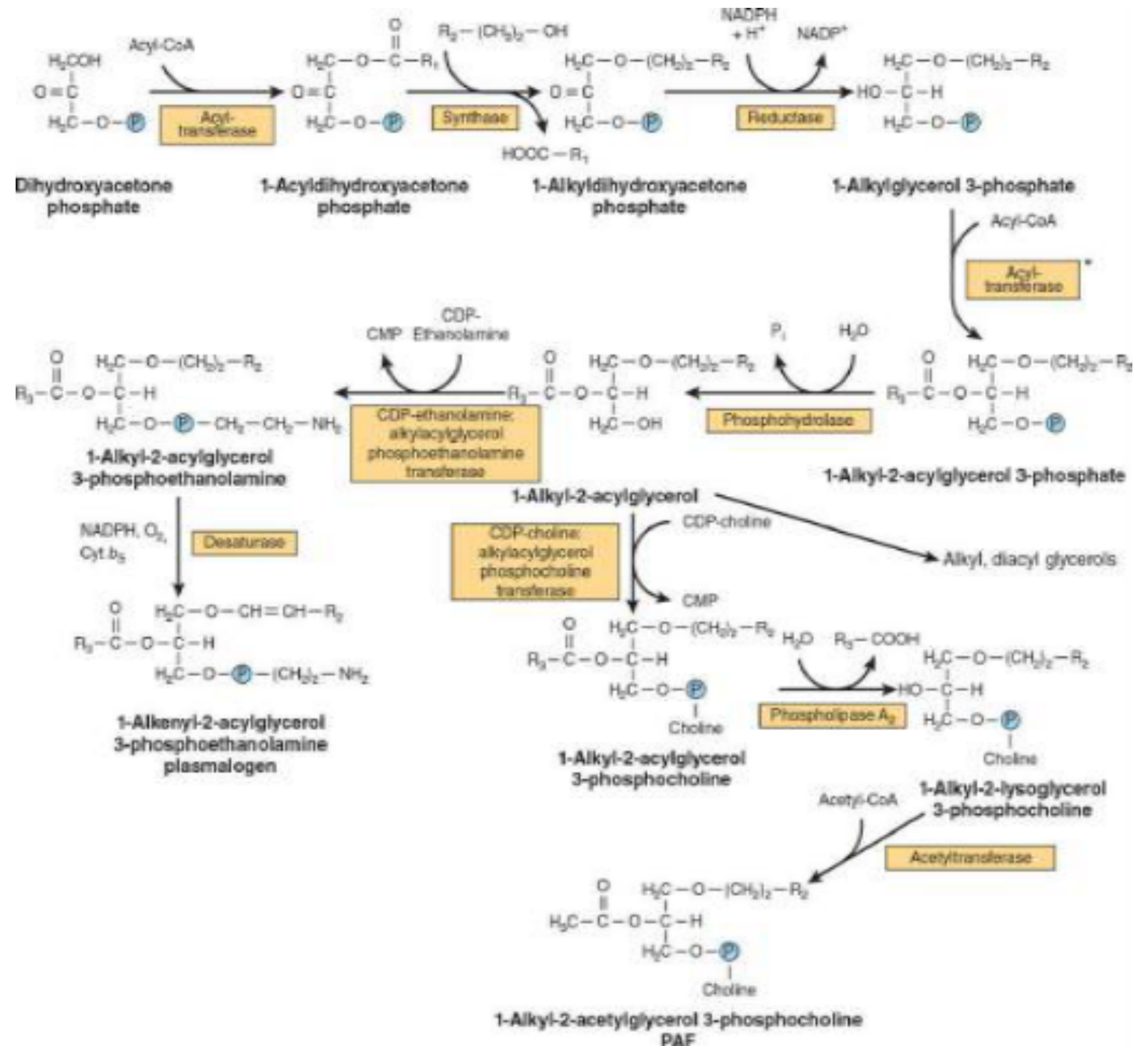
Phospholipases Allow Degradation & Remodeling of Phosphoglycerols

Although **phospholipids are actively degraded**, **each portion of the molecule turns over at a different rate—for** example, the turnover time of the **phosphate group is different** from that of the **1-acyl group**. This is due to the **presence of enzymes** that allow **partial degradation** followed by **resynthesis** (**Figure 24–4**). **Phospholipase A2** catalyzes the hydrolysis of **glycerophospholipids** to form a free fatty acid and **lysophospholipid,** which in turn may be reacylated by acyl-CoA in the presence of an acyltransferase. Alternatively, **lysophospholipid** (eg, lysolecithin) is attacked by **lysophospholipase**, forming the corresponding glyceryl phosphoryl base, which may then be split by a hydrolase liberating **glycerol-3-phosphate** plus base. **Phospholipases A1, A2, B, C,** and **D** attack the bonds indicated in **Figure 5**. **Phospholipase A2** is found in pancreatic fluid and snake venom as well as in many types of cells; **phospholipase C** is one of the major toxins secreted by bacteria; and **phospholipase D** is known to be involved in mammalian signal transduction.

FIGURE 24–4
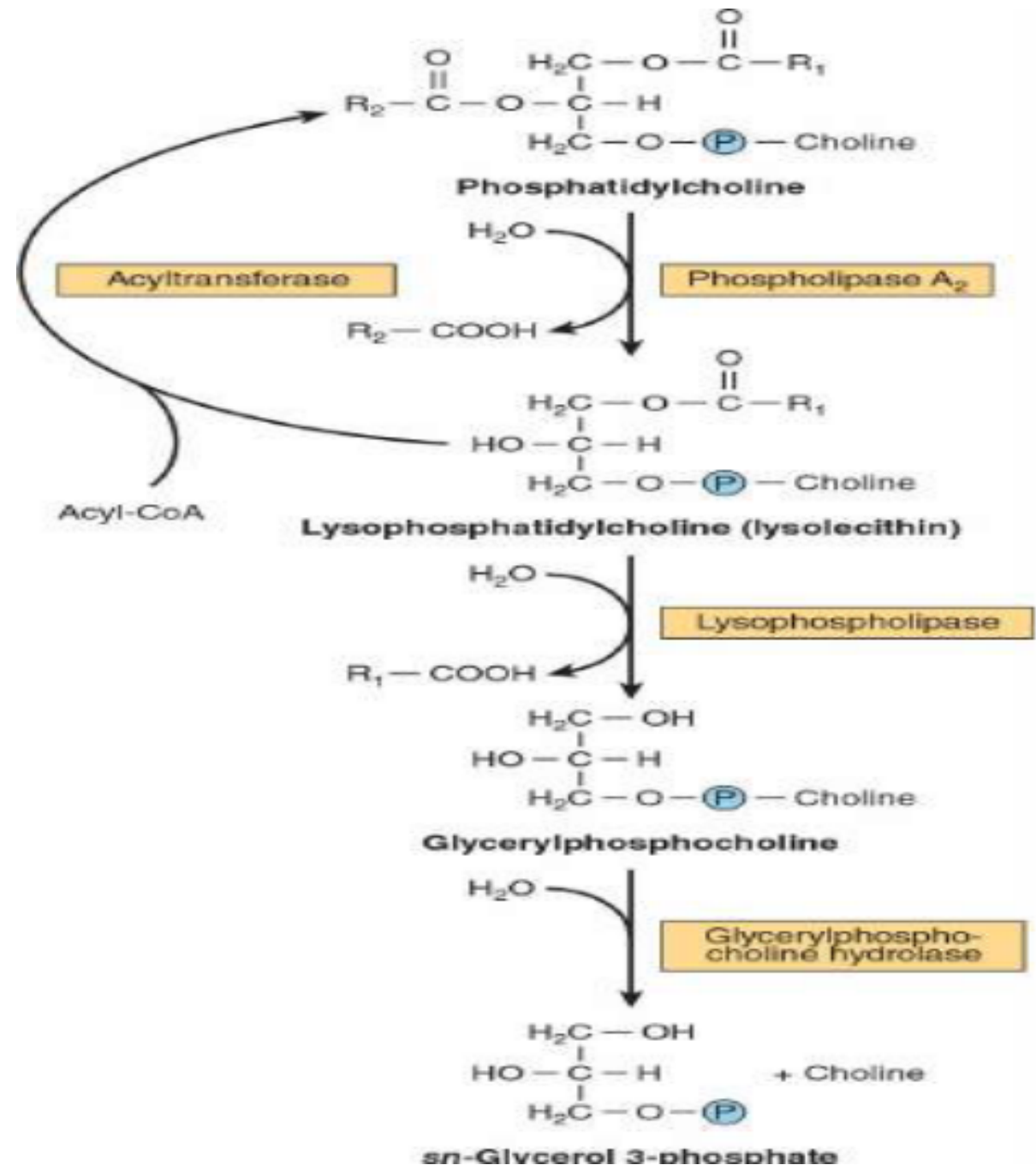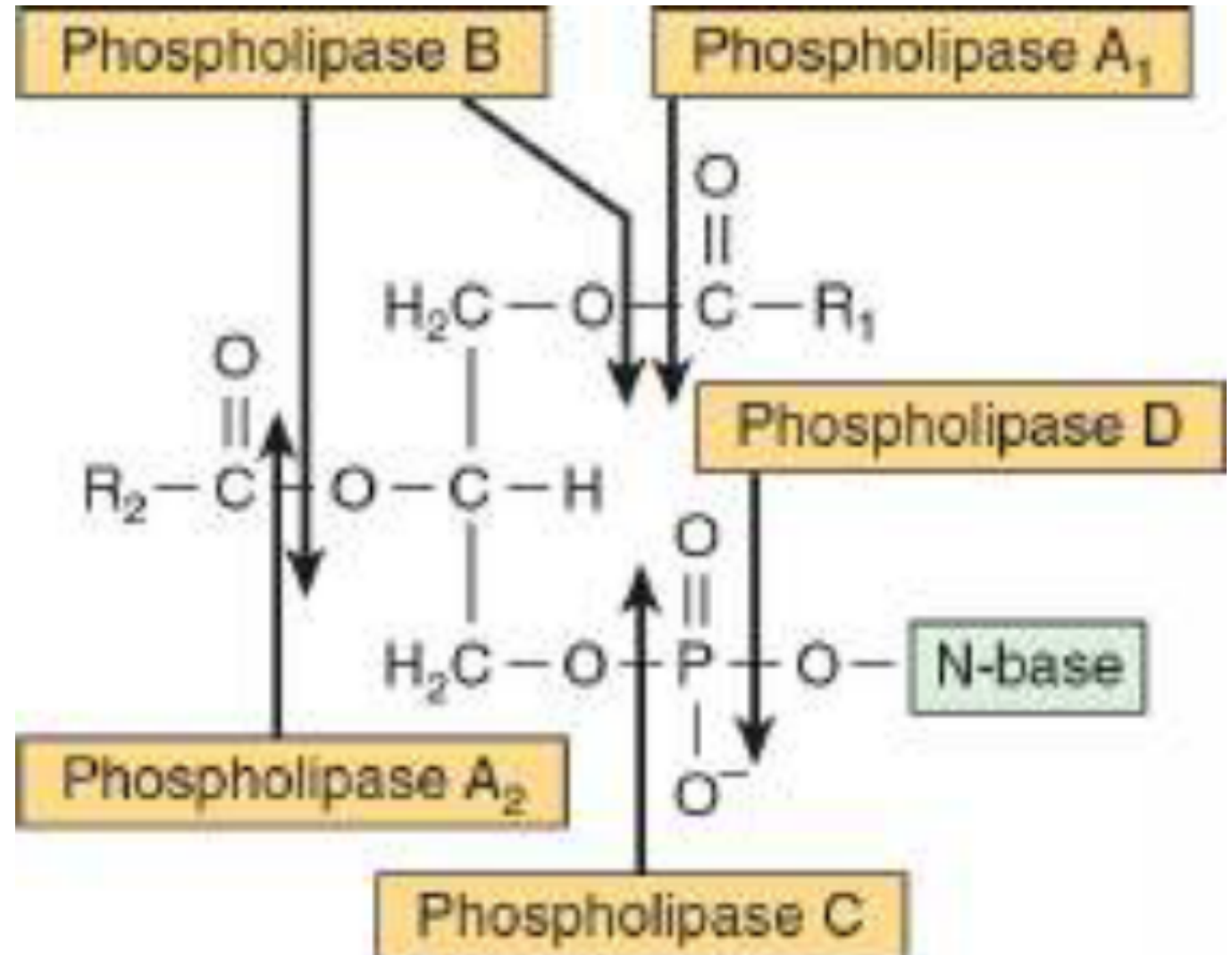**Metabolism of phosphatidylcholine (lecithin).**

FIGURE 5 Sites of the hydrolytic activity of phospholipases on a phospholipid substrate.

Lysolecithin (lysophosphatidylcholine) may be formed by an alternative route that involves **lecithin: cholesterol acyltransferase (LCAT)**. This enzyme, found in plasma, catalyzes the **transfer of a fatty acid residue from the 2 position of lecithin** to **cholesterol to form cholesteryl ester** and **lysolecithin**, and is considered to be responsible for much of the cholesteryl ester in plasma lipoproteins .

Long-chain saturated fatty acids are **found predominantly in the 1 position of phospholipids**, whereas **the polyunsaturated fatty acids (eg, the precursors of prostaglandins) are incorporated more frequently into the 2 position.**

 The incorporation of fatty acids into lecithin occurs in three ways; by **complete synthesis of the phospholipid**; by **transacylation between cholesteryl ester and lysolecithin**; and by **direct acylation of lysolecithin by acyl-CoA**. Thus, a continuous exchange of the fatty acids is possible, particularly with regard to introducing essential fatty acids into phospholipid molecules.

ALL SPHINGOLIPIDS ARE FORMED FROM CERAMIDE

Ceramide is synthesized in the **endoplasmic reticulum** from the amino acid **serine** as shown in **Figure 6**. **Ceramide** is **an important signaling** molecule (second messenger) **regulating pathways** including programmed cell death **(apoptosis)**, the **cell cycle**, and **cell differentiation** and **senescence**.

FIGURE 6 Biosynthesis of ceramide.
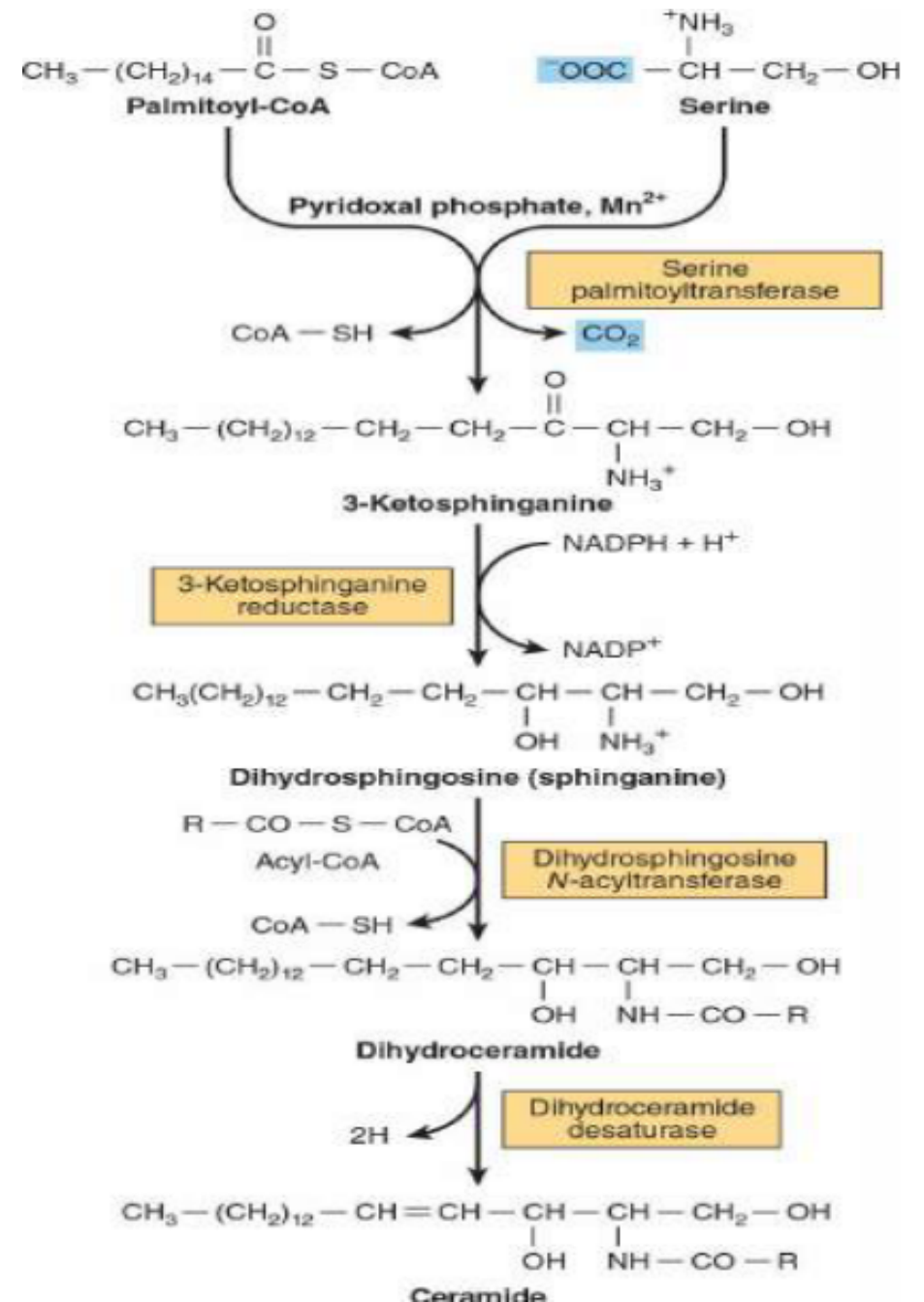
Sphingomyelins are **phospholipids** and are formed when **ceramide reacts** with **phosphatidylcholine** to form **sphingomyelin** plus **diacylglycerol** (**Figure 7A**). This occurs mainly in the **Golgi apparatus** and to a lesser extent in the **plasma membrane.**
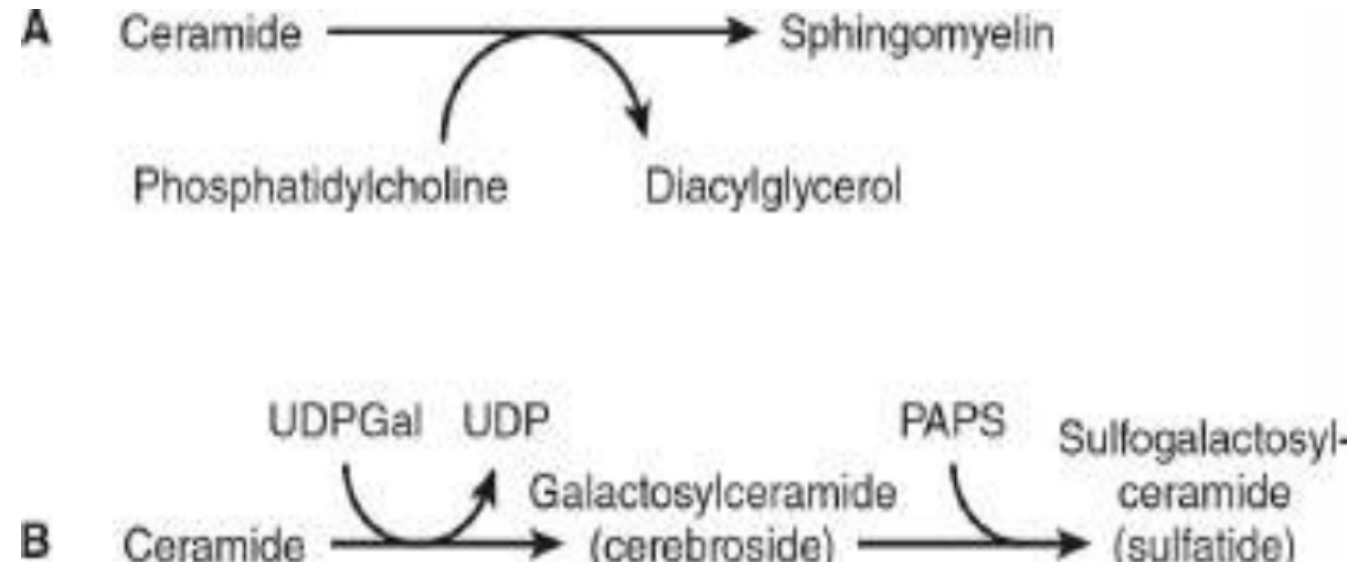

FIGURE 24–7 Biosynthesis of (A) sphingomyelin, (B) galactosylceramide and its sulfo derivative. (PAPS, (3′-phosphoadenosine-5′-phosphosulfate)

Glycosphingolipids Are a Combination of Ceramide With One or More Sugar Residues

The simplest glycosphingolipids **(cerebrosides)** are **galactosylceramide (GalCer)** and **glucosylceramide (GlcCer)**. **GalCer** is a major lipid of **myelin**, whereas GlcCer is the major glycosphingolipid of **extraneural tissues** and a precursor of most of the more complex glycosphingolipids. GalCer (**Figure 7B**) is formed in a reaction between ceramide and uridine diphosphate galactose (UDPGal) (formed by epimerization from UDPGlc— **Sulfogalactosylceramide** and other sulfolipids such as the **sulfo(galacto)-glycerolipids** and the **steroid sulfates** are formed after further reactions involving **3′-phosphoadenosine-5′-phosphosulfate (PAPS; "active sulfate").**

 Gangliosides are synthesized from ceramide by the stepwise addition of activated sugars (eg, UDPGlc and UDPGal) and a **sialic acid**, usually *N*-acetylneuraminic acid (**Figure 8**). A large number of gangliosides of increasing molecular weight may be formed. Most of the enzymes transferring sugars from nucleotide sugars (glycosyl transferases) are found in the **Golgi apparatus.**

FIGURE 8
Biosynthesis of gangliosides.
(NeuAc, *N*acetylneuraminic acid.)
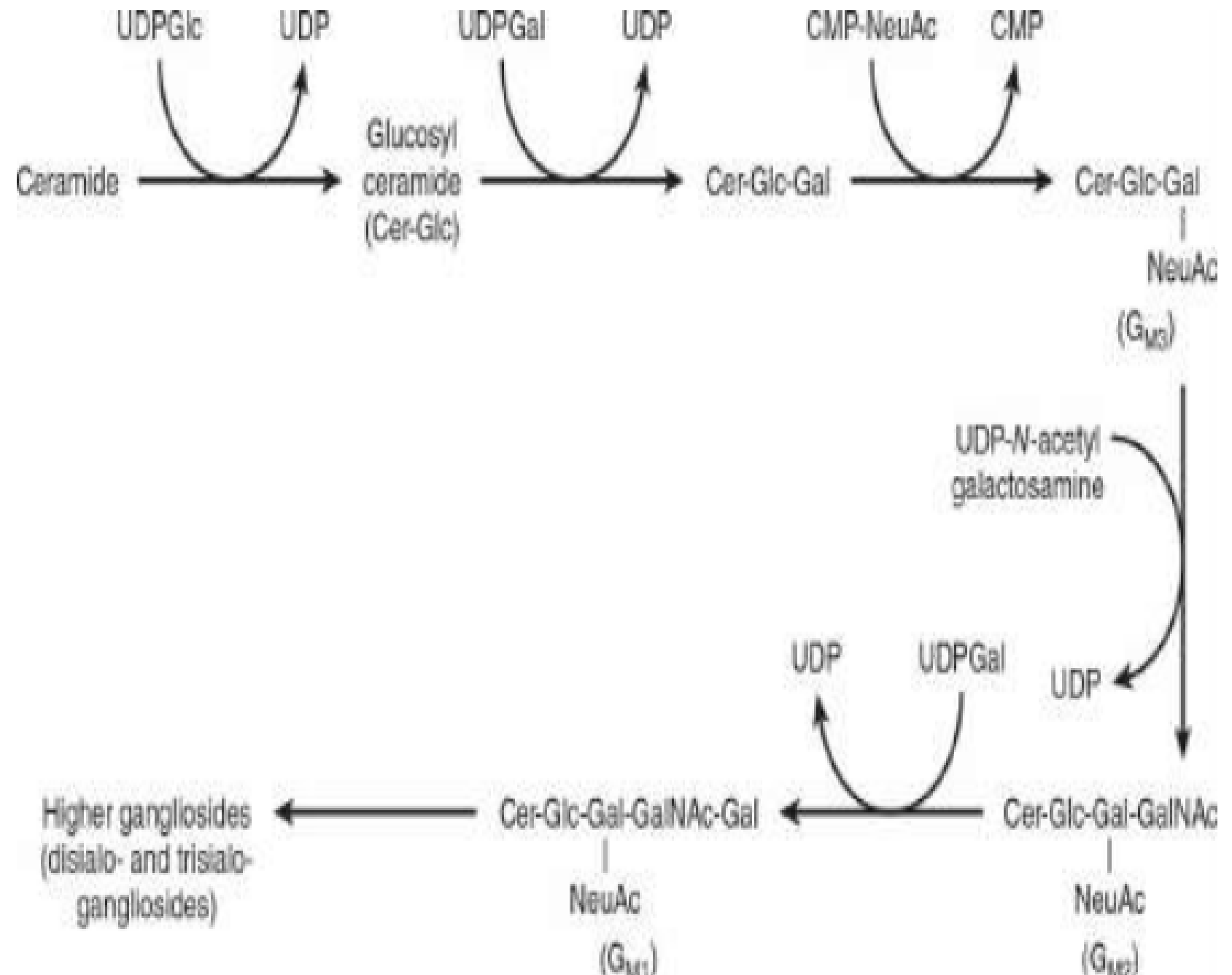
Glycosphingolipids are constituents of the outer leaflet of plasma membranes and are important in **cell adhesion** and **cell recognition**. Some are **antigens**, for example, **ABO blood group substances**. Certain gangliosides function as **receptors for bacterial toxins** (eg, for **cholera toxin**, which subsequently activates adenylyl cyclase).

Bioenergetics: The Role of ATP

Biomedical Importance

Bioenergetics or biochemical thermodynamics: is the study of the energy changes accompanying biochemical reactions.

Biologic systems are essentially isothermic and use chemical energy to power living processes.

Some problems related to energy:

1. Death from **starvation** occurs when available energy reserves are depleted, and certain forms of malnutrition are associated with energy imbalance (**marasmus**).

2. Thyroid hormones control the **metabolic rate** (rate of energy release), and

disease results if they malfunction.

3. Excess storage of surplus energy causes **obesity**, an increasingly common disease of Western society which predisposes to many diseases, including **cardiovascular disease** and **diabetes mellitus type 2**, and lowers life expectancy.

How do we define amount of energy?

The energy actually available to do work known as free energy Energy (ΔG)

The quantity of usable energy in a reaction is called Gibbs Free Energy (ΔG)

ΔG : is the difference between the energy contained in the products of a reaction and the reactants:

ΔG = (energy of products) - (energy of reactants)

Free Energy is the Useful Energy in a System

Gibbs change in **free energy (ΔG)** is that portion of the total energy change in a system that is available for doing work—that is, the useful energy, also known as the **chemical potential.**

Entropy S: is a quantitative expression for the randomness or disorder in a system.

Enthalpy H: is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products.

General Laws of Thermodynamics

The first law of thermodynamics states that **the total energy of a system, including its surroundings, remains constant**.

- It implies that within the total system, energy is neither **lost** nor **gained** during any change.
-Energy may be **transferred** from one part of the system to another, or may be **transformed** into another form of energy.

- In living systems, chemical energy may be transformed into heat or into

electrical, radiant, or mechanical energy.

The second law of thermodynamics states that **the total entropy of a system must increase if a process is to occur spontaneously.**

Entropy is the extent of disorder or randomness of the system and becomes maximum as equilibrium is approached.

Under conditions of constant temperature and pressure, the relationship between the free-energy change (ΔG) of a reacting system and the change in entropy (ΔS) is expressed by the following equation, which combines the two laws of thermodynamics:

ΔG =ΔH −TΔS
Where ΔH is the change in enthalpy (heat) and T is the absolute temperature.

In biochemical reactions, since ΔH is approximately equal to the total change in internal energy of the reaction or ΔE, the above relationship may be expressed in the following way:

$\Delta G = \Delta E - T\Delta S$

If ΔG is negative, the reaction proceeds spontaneously with loss of free energy, that is, it is **exergonic**. If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

On the other hand, **if ΔG is positive**, the reaction proceeds only if free energy can be gained, that is, it is **endergonic**. If, in addition, the magnitude of ΔG is great, the system is stable, with little or no tendency for a reaction to occur.

If ΔG is zero, the system is at equilibrium and no net change takes place.

When the reactants are present in concentrations of 1.0 mol/L, ΔG_0 is the standard free-energy change. For biochemical reactions, a standard state is defined as having a pH of 7.0. The standard free-energy change at this standard state is denoted by $\Delta G_0'$.
The standard free-energy change can be calculated from the equilibrium constant Keq.

$\Delta G_0' = -RT K\ln eq'$
where R is the gas constant and T is the absolute temperature.

$$\Delta G = \Delta G^0 + R\,T \ln \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

It is important to note that the actual ΔG may be larger or smaller than $\Delta G_0'$ depending on the concentrations of the various reactants, including the solvent, various ions, and proteins.

In a biochemical system, an enzyme only speeds up the attainment of equilibrium; it never alters the final concentrations of the reactants at equilibrium.

Endergonic Processes Proceed by Coupling to Exergonic Processes

The vital processes—for example, synthetic reactions, muscular contraction, nerve impulse conduction, and active transport—obtain energy by chemical linkage, or coupling, to oxidative reactions. In its simplest form, this type of coupling may be represented as shown in Figure 1
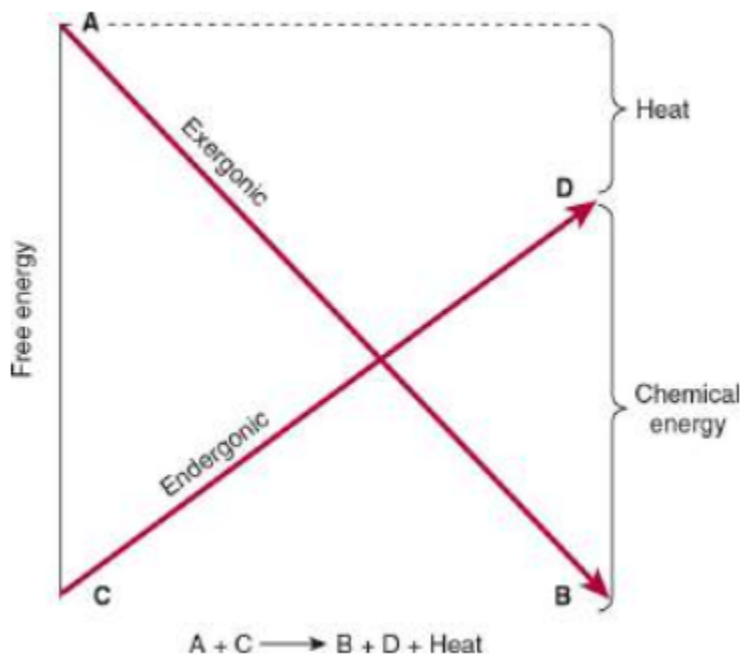

FIGURE 1 Coupling of an exergonic to an endergonic reaction.

The conversion of metabolite **A** to metabolite **B** occurs with **release of free energy** and is coupled to another reaction in which free energy is **required to** convert metabolite **C** to metabolite **D**.

 The terms **exergonic** and **endergonic**, rather than the normal chemical terms "**exothermic**" and "**endothermic**," are used to indicate that a process is accompanied by loss or gain, respectively, of free energy in any form, not necessarily as heat.

In practice, an endergonic process **cannot exist independently**, but must be a component of a coupled **exergonic– endergonic system** where the overall net change is exergonic. The exergonic reactions are termed **catabolism** (generally, the breakdown or oxidation of fuel molecules), whereas the synthetic reactions that build up substances are termed **anabolism**. The combined catabolic and anabolic processes constitute **metabolism**.

Metabolism: term used to describe the inter-conversion of chemical compounds in

the body.

Metabolic pathways fall into **three categories**

1- Anabolic pathways: which are those involved in the synthesis of larger and more complex compounds from smaller precursors—for example, the synthesis of protein from amino acids and the synthesis of reserves of triacylglycerol and glycogen. Anabolic pathways are endothermic.

2- **Catabolic pathways**, which are involved in the breakdown of larger molecules, commonly involving oxidative reactions; they are exothermic, producing reducing equivalents, and, mainly via the respiratory chain, ATP.

3- Amphibolic pathways, which occur at the "crossroads" of metabolism, acting as links between the anabolic and catabolic pathways, for example, the citric acid cycle

If the reaction shown in Figure 1 is to go from left to right, then the overall process must be accompanied by loss of free energy as heat. One possible mechanism of coupling could be envisaged if a common obligatory intermediate (I) took part in both reactions, that is,

$$A + C \rightarrow I \rightarrow B + D$$
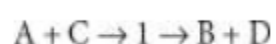
Some exergonic and endergonic reactions in biologic systems are coupled in this way. This type of system has a built-in mechanism for biologic control of the rate of oxidative processes since the common obligatory intermediate allows the rate of utilization of the product of the synthetic path (D) to determine by mass action the rate at which A is oxidized. Indeed, these relationships supply a basis for the concept of **respiratory control,** the process that prevents an organism from burning out of control. An extension of the coupling concept is provided by dehydrogenation reactions, which are coupled to hydrogenations by an intermediate carrier
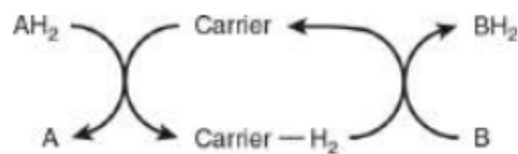(Figure 2).

FIGURE 2 Coupling of dehydrogenation and hydrogenation reactions by an intermediate carrier.

An alternative method of coupling an exergonic to an endergonic process is to synthesize a compound of high-energy potential in the exergonic reaction and to incorporate this new compound into the endergonic reaction, thus effecting a transference of free energy from the exergonic to the endergonic pathway. The biologic advantage of this mechanism is that the compound of high potential energy, $\sim$Ⓔ , unlike I in the previous system, need not be structurally related to A, B, C, or D, allowing Ⓔ to serve as a transducer of energy from a wide range of exergonic reactions to an equally wide range of endergonic

reactions or processes, such as biosynthesis, muscular contraction, nervous excitation,
and active transport. In the living cell, the principal high-energy intermediate or carrier compound is ATP (Figure 3)
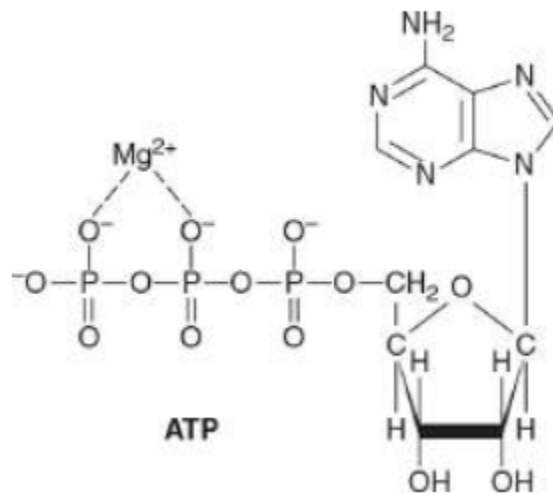
FIGURE 3: Adenosine triphosphate (ATP) is shown as the magnesium complex.

High-Energy Phosphates Play a Central Role in Energy Capture and Transfer

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment.

 Autotrophic organisms utilize simple exergonic processes; for example, the energy of sunlight (green plants), the reaction $Fe^{2+} \rightarrow Fe^{3+}$ (some bacteria).

On the other hand, **Heterotrophic** organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment. In all these organisms, ATP plays a central role in the transference of free energy from the exergonic to the endergonic processes.

ATP is a nucleotide consisting of the nucleoside adenosine (adenine linked to ribose) and three phosphate groups. In its reactions in the cell, it functions as the Mg2+ complex (Figure 3).

The importance of phosphates in intermediary metabolism became evident with the discovery of the role of ATP, adenosine diphosphate (ADP), and inorganic phosphate (Pi) in glycolysis.

The Intermediate Value for the Free Energy of Hydrolysis of ATP Has Important Bioenergetic Significance

The standard free energy of hydrolysis of a number of biochemically important phosphates is shown in **Table 1**. An estimate of the comparative tendency of each of the phosphate groups to transfer to a suitable acceptor may be obtained from the $\Delta G0'$ of hydrolysis at 37°C. This is termed the **group transfer potential**. The value for the hydrolysis of the terminal phosphate of ATP divides the list into two groups. **Low energy phosphates**, having a low group transfer potential, exemplified by the ester phosphates found in the intermediates of glycolysis, have $G0'$ values smaller than that of ATP, while in **high-energy phosphates**, with a more negative $G0'$, the value is higher than that of ATP. The components of this latter group, including ATP, are usually anhydrides (eg, the 1- phosphate of 1,3-bisphosphoglycerate), enol phosphates (eg, phosphoenolpyruvate), and phosphoguanidines (eg, creatine phosphate, arginine phosphate).

TABLE 1 Standard Free Energy of Hydrolysis of Some Organophosphates of Biochemical Importance

Compound	$\Delta G^{0\prime}$	
	kJ/mol	kcal/mol
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate (to 3-phosphoglycerate)	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP → AMP + PP$_i$	−32.2	−7.7
ATP → ADP + P$_i$	−30.5	−7.3
Glucose-1-phosphate	−20.9	−5.0
PP$_i$	−19.2	−4.6
Fructose-6-phosphate	−15.9	−3.8
Glucose-6-phosphate	−13.8	−3.3
Glycerol-3-phosphate	−9.2	−2.2

Abbreviations: PP$_i$, pyrophosphate; P$_i$, inorganic orthophosphate.
Note: All values are taken from Jencks WP: Free energies of hydrolysis and decarboxylation. *In: Handbook of Biochemistry and Molecular Biology*, vol 1. *Physical and Chemical Data*. Fasman GD (editor). CRC Press, 1976:296–304, except that for PP$_i$ which is from Frey PA, Arabshahi A: Standard free-energy change for the hydrolysis of the alpha, beta-phosphoanhydride bridge in ATP. Biochemistry 1995;34:11307. Values differ between investigators, depending on the precise conditions under which the measurements were made.

The symbol $\sim\textcircled{P}$ indicates that the group attached to the bond, on transfer to an appropriate acceptor, results in transfer of the larger quantity of free energy. Thus, ATP has a high group transfer potential, whereas the phosphate in adenosine monophosphate (AMP) is of the low-energy type since it is a normal ester link (**Figure 4**). In energy transfer reactions, ATP may be converted to ADP and P$_i$ or, in reactions requiring a greater energy input, to AMP + PP$_i$ (Table 1).
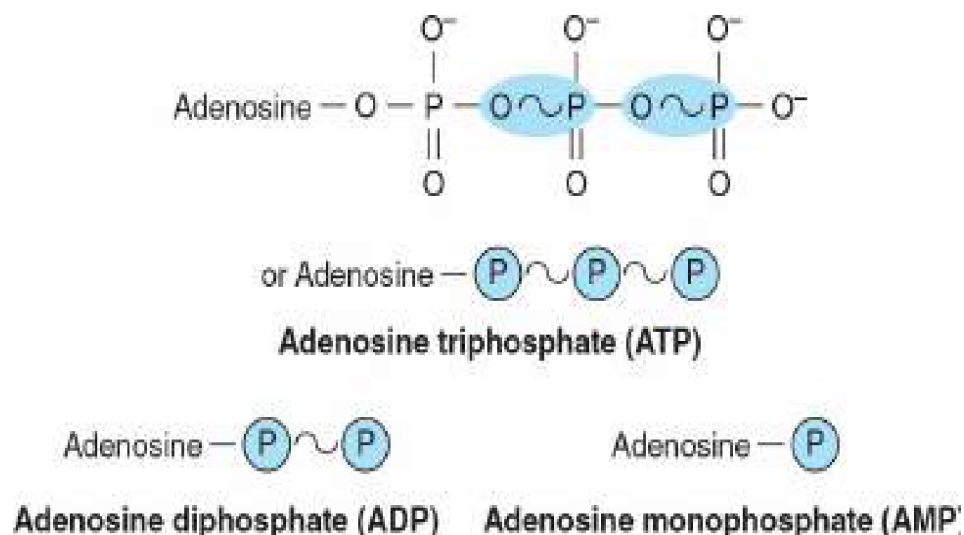
FIGURE 4 Structure of ATP, ADP, and AMP showing the position and the number of high-energy phosphates $(\sim\!P)$.

The intermediate position of ATP allows it to play an important role in energy transfer. The high free-energy change on hydrolysis of ATP is due to relief of charge repulsion of adjacent negatively charged oxygen atoms and to stabilization of the reaction products, especially phosphate, as resonance hybrids (**Figure 5**) Other "high-energy compounds" are thiol esters involving coenzyme A (eg, acetyl-CoA), acyl carrier protein, amino acid esters involved in protein synthesis.
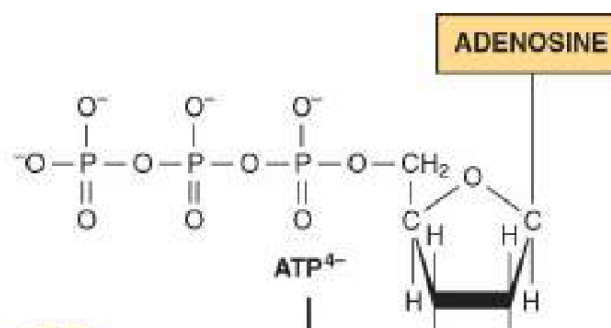
FIGURE 5 The free-energy change on hydrolysis of ATP to ADP.

ATP ACTS AS THE "ENERGY CURRENCY" OF THE CELL

The high group transfer potential of ATP enables it to act as a donor of high-energy phosphate to form those compounds below it in Table 1. Likewise, with the necessary enzymes, ADP can accept phosphate groups to form ATP from those compounds above ATP in the table. In effect, an

ATP/ADP cycle connects those processes that generate ~Ⓟ to those processes that utilize ~Ⓟ (**Figure 6**), continuously consuming and regenerating ATP. This occurs at a very rapid rate since the total ATP/ADP pool is extremely small and sufficient to maintain an active tissue for only a few seconds.
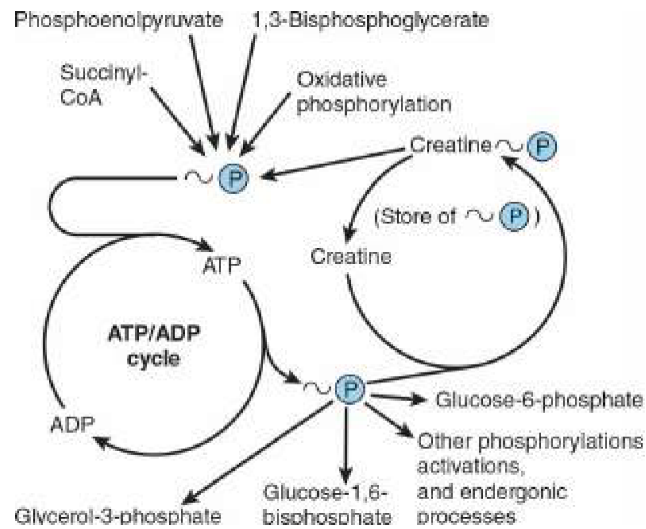
FIGURE 6 Role of ATP/ADP cycle in transfer of high-energy phosphate.

There are three major sources of taking part in **energy conservation** or **energy capture**:

1. Oxidative phosphorylation is the greatest quantitative source of $\sim$℗ in aerobic organisms. ATP is generated in the mitochondrial matrix as O2 is reduced to H2O by electrons passing down the respiratory chain

2. Glycolysis. A net formation of two $\sim$℗ results from the formation of lactate from one molecule of glucose, generated in two reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, respectively.

3. The citric acid cycle. One $\sim$℗ is generated directly in the cycle at the succinate thiokinase step.

Phosphagens act as storage forms of group transfer potential and include **creatine phosphate**, which occurs in vertebrate skeletal muscle, heart, spermatozoa, and brain, and **arginine phosphate**, which occurs in invertebrate muscle. When ATP is rapidly being utilized as a source of energy for muscular contraction, phosphagens permit its concentrations to be maintained, but when the ATP/ADP ratio is high, their concentration can increase to act as an energy store (**Figure 7**)
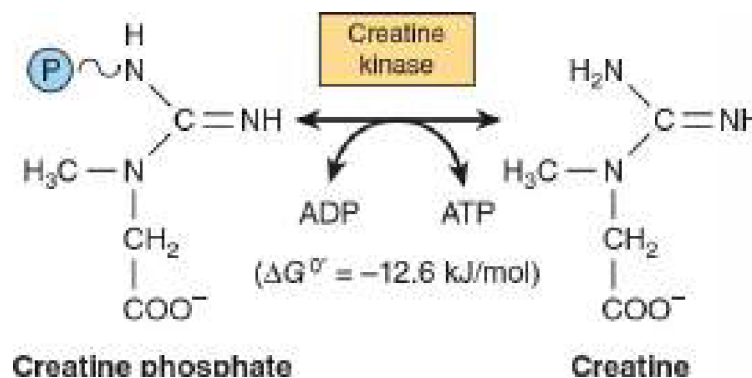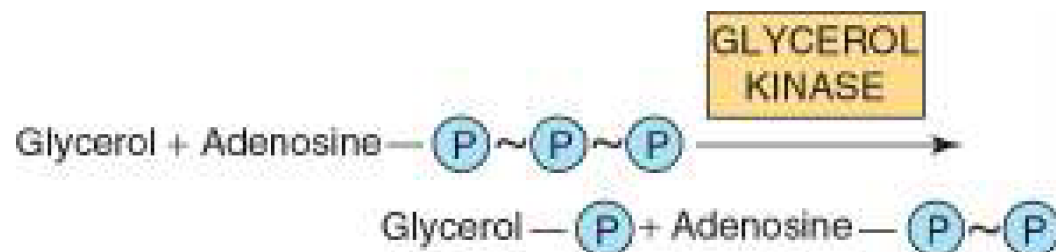

FIGURE 7 Transfer of high-energy phosphate between ATP and creatine. When ATP acts as a phosphate donor to form compounds of lower free energy of hydrolysis (Table 1), the phosphate group is invariably converted to one of low energy. For example, the phosphorylation of glycerol to form glycerol-3-phosphate:

ATP Allows the Coupling of Thermodynamically Unfavorable Reactions to Favorable Ones

Endergonic reactions cannot proceed without an input of free energy. For example, the phosphorylation of glucose to glucose-6-phosphate, the first reaction of glycolysis:

$$\text{Glucose} + P_i \rightarrow \text{Glucose-6-phosphate} + H_2O$$
$$(\Delta G^{0'}) = +13.8 \text{ kJ/mol} \qquad (1)$$
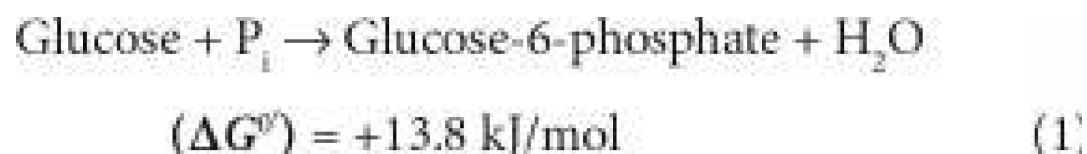
is highly endergonic and cannot proceed under physiologic conditions. Thus, in order to take place, the reaction must be coupled with another— more exergonic—reaction such as the hydrolysis of the terminal phosphate of ATP.

$$\text{ATP} \rightarrow \text{ADP} + P_i \ (\Delta G^{0'} = -30.5 \text{ kJ/mol}) \qquad (2)$$

When (1) and (2) are coupled in a reaction catalyzed by hexokinase, phosphorylation of glucose readily proceeds in a highly exergonic reaction that under physiologic conditions is irreversible. Many "activations" reactions follow this pattern.

Adenylyl Kinase (Myokinase) Interconverts Adenine Nucleotides

This enzyme is present in most cells. It catalyzes the following reaction:

ADENYLYL
KINASE

ATP + AMP ⇌ 2ADP

Adenylyl kinase is important for the maintenance of energy homeostasis in cells because it allows:

1. The group transfer potential in ADP to be used in the synthesis of ATP.

2. The AMP formed as a consequence of activating reactions involving ATP to rephosphorylated to ADP.

3. AMP to increase in concentration when ATP becomes depleted so that it is able to act as a metabolic (allosteric) signal to increase the rate of catabolic reactions, which in turn lead to the generation of more ATP

When ATP Forms AMP, Inorganic Pyrophosphate (PPi) Is Produced

ATP can also be hydrolyzed directly to AMP, with the release of PPi (Table–1). This occurs, for example, in the activation of long-chain fatty acids.

ACYL-CoA
SYNTHETASE

ATP + CoA • SH + R • COOH ⟶ AMP + PPi + R • CO — SCoA

This reaction is accompanied by loss of free energy as heat, which ensures that the activation reaction will go to the right, and is further aided by the hydrolytic splitting of PPi, catalyzed by **inorganic pyrophosphatase**, a reaction that itself has a large $\Delta G_0'$ of −19.2 kJ/mol. Note that activations via the pyrophosphate pathway result in the loss of two ~ⓅP rather than one, as occurs when ADP and Pi are formed.

INORGANIC
PYROPHOSPHATASE

PPi + H₂O ⟶ 2P
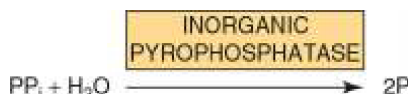
A combination of the above reactions makes it possible for phosphate to be recycled and the adenine nucleotides to interchange (**Figure 8**).
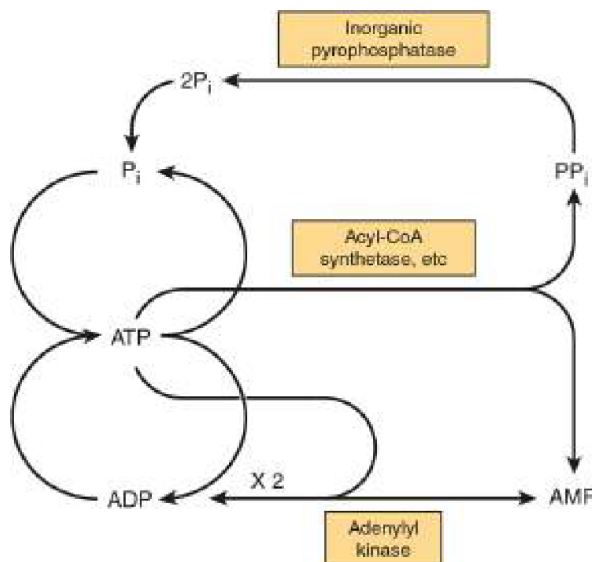

FIGURE 8 **Phosphate cycles and interchange of adenine nucleotides.**

Other Nucleoside Triphosphates Participate in Group Transfer Potential

By means of the **nucleoside diphosphate (NDP) kinases**, UTP, GTP, and CTP can be synthesized from their diphosphates, for example, UDP reacts with ATP to form UTP.

NUCLEOSIDE DIPHOSPHATE KINASE

ATP + UDP ⟷ ADP + UTP (uridine triphosphate)
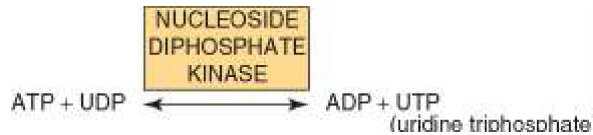
All of these triphosphates take part in phosphorylations in the cell. Similarly, specific **nucleoside monophosphate (NMP) kinases** catalyze the formation of NDP from the corresponding monophosphates. Thus, Adenylyl kinase is a specialized NMP kinase.

SUMMARY

--Biologic systems use chemical energy to power living processes.

--Exergonic reactions take place spontaneously with loss of free energy (ΔG is negative). Endergonic reactions require the gain of free energy (ΔG is positive) and occur only when coupled to exergonic reactions.

--ATP acts as the "energy currency" of the cell, transferring free energy derived from substances of higher energy potential to those of lower energy potential.

Catabolism of Proteins & of Amino Acid Nitrogen

PROTEIN TURNOVER

The continuous degradation and synthesis (turnover) of **cellular proteins** occur in **all forms of life**. **Each day**, humans **turn over 1 to 2%** of their total **body protein**, principally **muscle protein**. High rates of **protein degradation occur** in tissues that are undergoing structural rearrangement, for example, **uterine tissue during pregnancy**, **skeletal muscle in starvation,** and tadpole tail tissue during metamorphosis. While approximately **75% of the amino acids liberated** by protein degradation are **reutilized,** the remaining excess free amino acids are not stored for future use. **Amino acids not immediately incorporated** into new protein are **rapidly degraded.** The major portion of the carbon skeletons of the amino acids is converted to **amphibolic intermediates**, while in humans **the amino nitrogen** is **converted** to **urea and excreted in the urine.**

PROTEASES & PEPTIDASES DEGRADE PROTEINS TO AMINO ACIDS

The relative susceptibility of a protein to degradation is expressed as its **half-life ($t\frac{1}{2}$)**, the **time required to lower its concentration to half of its initial value**. Half-lives of liver proteins **range** from **under 30 minutes** to **over 150 hours**. Typical "housekeeping" **enzymes such as those of glycolysis**, have $t\frac{1}{2}$ values of **over 100 hours**. By contrast, **key regulatory enzymes** may have $t\frac{1}{2}$ values as low as **0.5 to 2 hours**. PEST sequences, **regions rich in proline (P), glutamate (E), serine (S), and threonine (T)**, target some proteins for **rapid** degradation. **Intracellular proteases hydrolyze internal peptide bonds.** The **resulting peptides** are then degraded to amino acids by **endopeptidases that hydrolyze internal peptide bonds**, and **by aminopeptidases** and **carboxypeptidases** that remove amino acids sequentially from the **amino-** and **carboxyl-termini**, respectively.

ATP-Independent Degradation

Degradation of blood glycoproteins follows loss of a sialic acid moiety from the nonreducing ends of their oligosaccharide chains. Asialoglycoproteins are then internalized by liver-cell asialoglycoprotein receptors and degraded by lysosomal proteases. **Extracellular**, **membrane-associated**, **and long-lived intracellular proteins** are also **degraded** in **lysosomes by ATP-independent** processes

ATP & Ubiquitin-Dependent Degradation

Degradation of regulatory proteins with short half-lives and of **abnormal** or **misfolded proteins** occurs in the **cytoso**l, and **requires ATP and ubiquitin**. Named based on its presence in all eukaryotic cells, **ubiquitin is a small** (76 residue) **polypeptide that targets many intracellular proteins for degradation.** **Figure 1** illustrates the three-dimensional structure of ubiquitin. Ubiquitin molecules are attached by **non–α-peptide bonds** formed between the carboxyl terminal of ubiquitin and the ε-amino groups of lysyl residues in the target protein (**Figure 2**). **The residue** present at **its amino terminus affects whether a protein is ubiquitinated**.

Amino terminal **Met** or **Ser** residues **retard**, whereas **Asp** or **Arg accelerate ubiquitination**. Attachment of a single ubiquitin molecule to transmembrane proteins alters their subcellular localization and targets them for degradation. Soluble proteins undergo **polyubiquitination**, the ligase-catalyzed attachment of four or more additional ubiquitin molecules (Figure 28–1). Subsequent degradation of ubiquitin-tagged proteins takes place in the **proteasome**, a macromolecule that also is ubiquitous in eukaryotic cells. The proteasome consists of a **macromolecular**, **cylindrical complex of proteins**, whose stacked rings form a central pore that harbors the active sites of proteolytic enzymes. For degradation, a protein thus must first enter the central pore. Entry into the core is regulated by the two outer rings that recognize polyubiquitinated proteins (**Figures 3** and **4**).

FIGURE 1 Three-dimensional structure of ubiquitin. Shown are α- helices (blue), β-strands (green), and the R-groups of lysyl residues (orange). Lys48 & Lys63 are sites for attachment of additional ubiquitin molecules during polyubiquitination. Created by Rogerdodd at Wikipedia using PyMOL, PDB id 1ubi, and crediting the European Bioinformatics Institute.
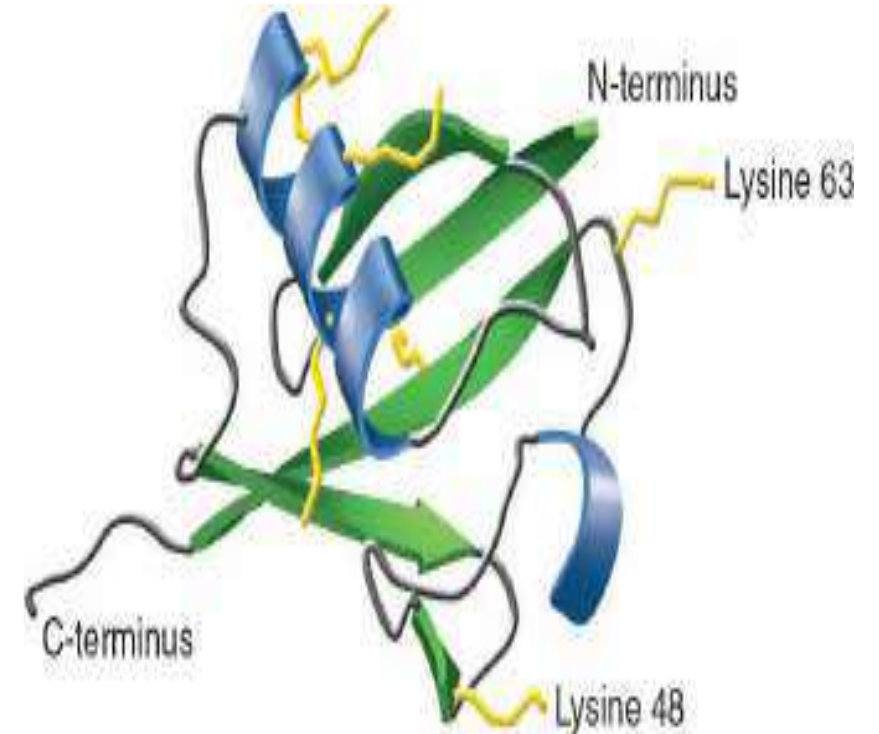
FIGURE 2 Reactions involved in the attachment of ubiquitin (Ub) to proteins. Three enzymes are involved. E1 is an activating enzyme, E2 a transferase, and E3 a ligase. While depicted as single entities, there are several types of E1, and over 500 types of E2. The terminal COOH of ubiquitin first forms a thioester. The coupled hydrolysis of PPi by pyrophosphatase ensures that the reaction will proceed readily. A thioester exchange reaction now transfers activated ubiquitin to E2. E3 then catalyzes the transfer of ubiquitin to the ε-amino group of a lysyl residue of the target protein. Additional rounds of ubiquitination result in subsequent polyubiquitination.

FIGURE 2 Reactions involved in the attachment of ubiquitin (Ub) to proteins.
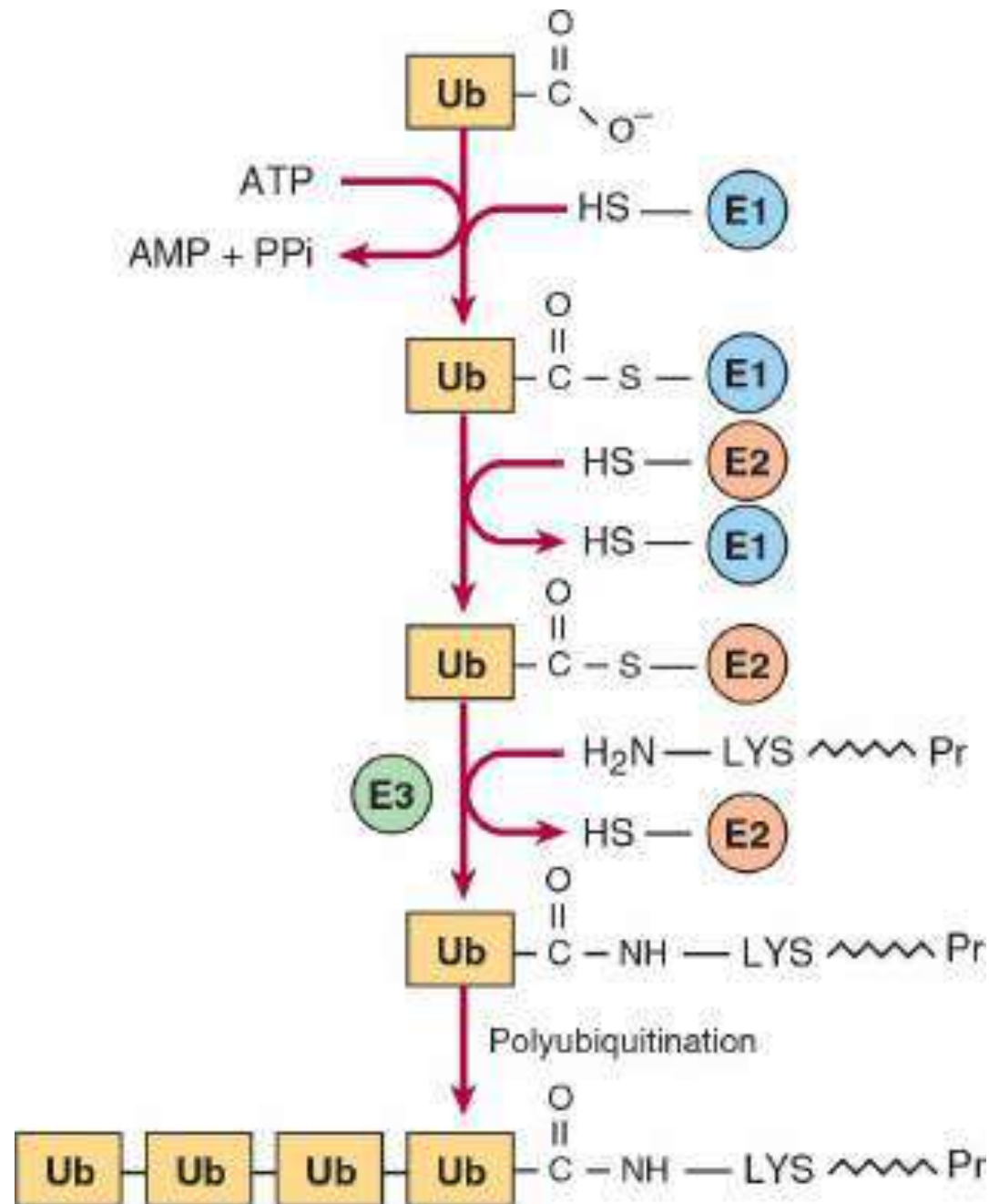
FIGURE 3 Representation of the structure of a proteasome. The upper ring is gated to permit only polyubiquitinated proteins to enter the proteosome, where **immobilized internal proteases degrade them to peptides.**
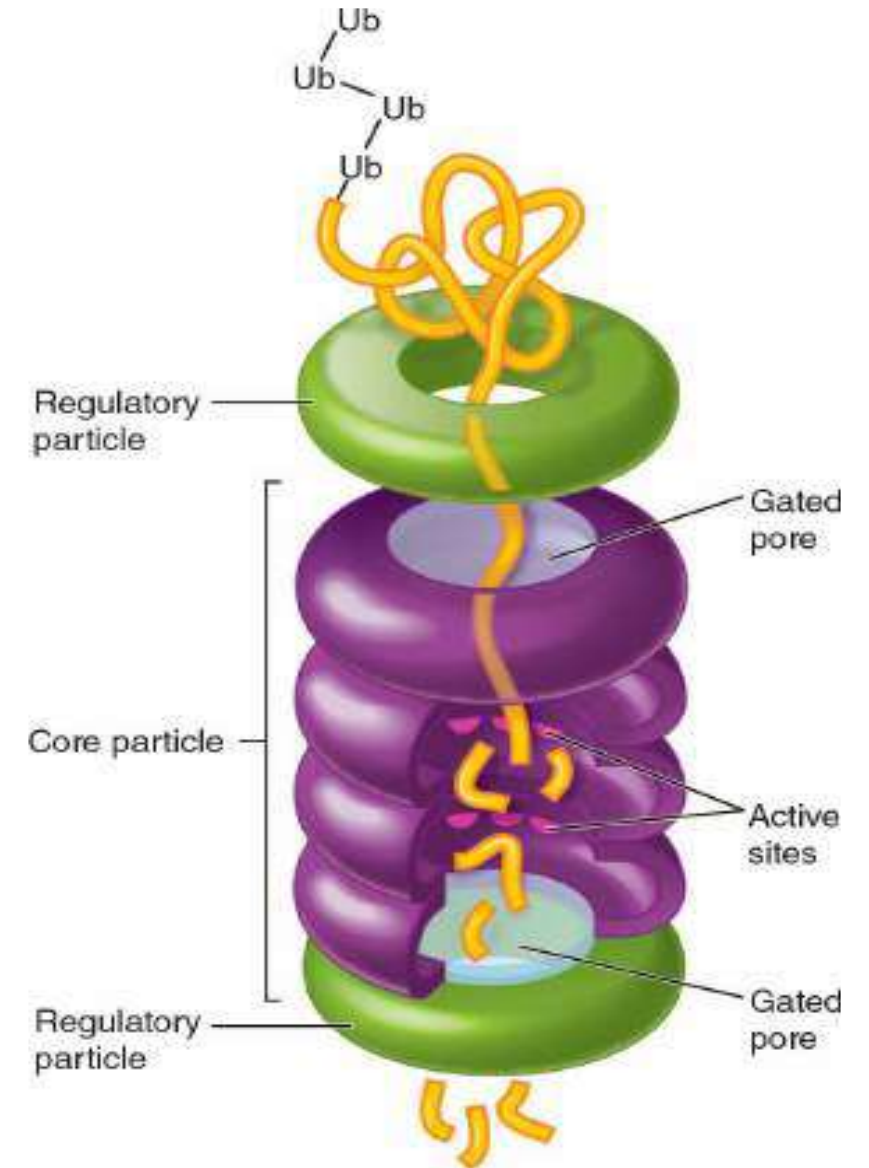
FIGURE 4 An end-on view of a proteasome. Created by Rogerdodd at Wikipedia and crediting the European Bioinformatics Institute. For the discovery of ubiquitin-mediated protein degradation, Aaron Ciechanover and Avram Hershko of Israel and Irwin Rose of the United States were awarded the 2004 Nobel Prize in Chemistry. Genetic disorders that result from defects in the genes that encode ubiquitin, ubiquitin ligases, or deubiquitinating enzymes include Angelman syndrome, autosomal recessive juvenile Parkinson disease, von Hippel-Lindau syndrome, and congenital polycythemia. For additional aspects of protein degradation and of ubiquitination, including its role in the cell cycle.
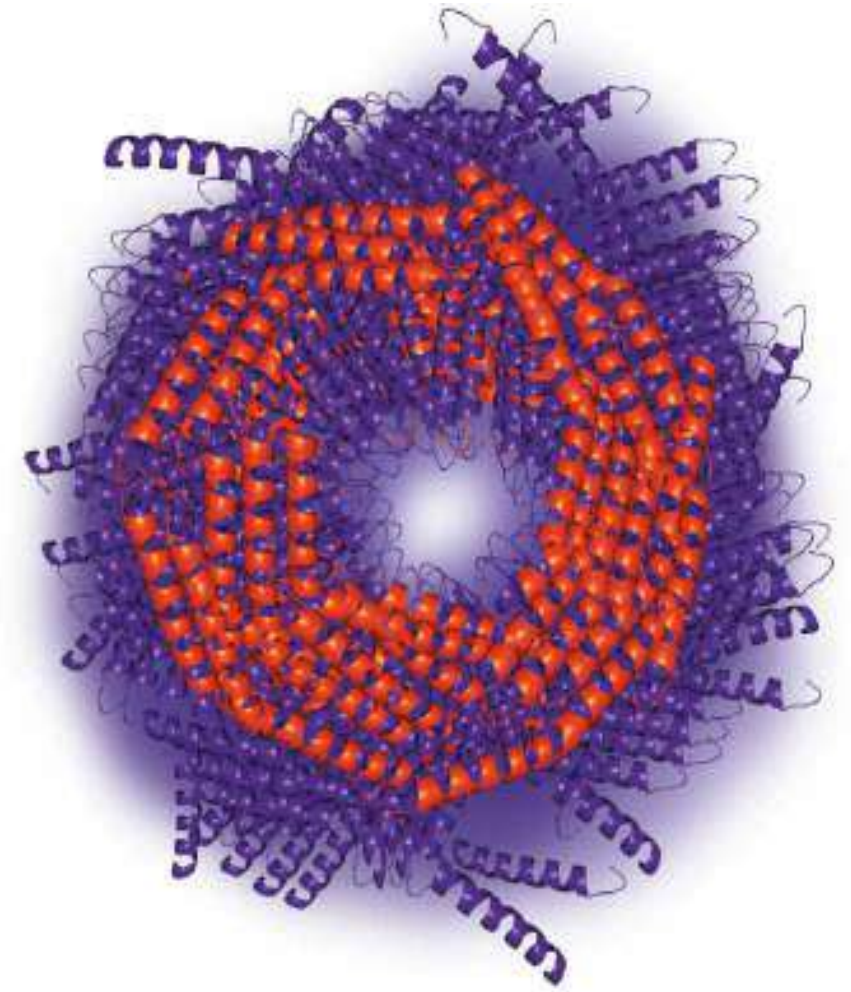
INTERORGAN EXCHANGE MAINTAINS CIRCULATING LEVELS OF AMINO ACIDS

The maintenance of **steady-state concentrations of circulating plasma amino acids** between **meals depends** on the net **balance between release from endogenous** protein stores and **utilization by various tissues**. Muscle generates over half of the total body pool of free amino acids, and liver is the site of the urea cycle enzymes necessary for disposal of excess nitrogen. **Muscle and liver thus play major roles in maintaining circulating amino acid levels.**

Figure 5 summarizes the postabsorptive state. Free amino acids, particularly **alanine and glutamine**, are **released from muscle** into the circulation. **Alanine** is **extracted** primarily by the **liver**, and **glutamine is extracted by the gut** and the **kidney**, both of which convert a significant portion to alanine. **Glutamine** also serves as **a source of ammonia** for excretion by the kidney. The **kidney** provides a major source of **serine** for uptake by peripheral tissues, including liver and muscle. Branched-chain amino acids, particularly **valine**, are released by **muscle** and taken up predominantly by the brain. The key role of alanine in amino acid output from muscle and gut and uptake by the liver is shown.

FIGURE 5 **Interorgan amino acid exchange in normal postabsorptive humans.** The key role of alanine in amino acid output from muscle and gut and uptake by the liver is shown.
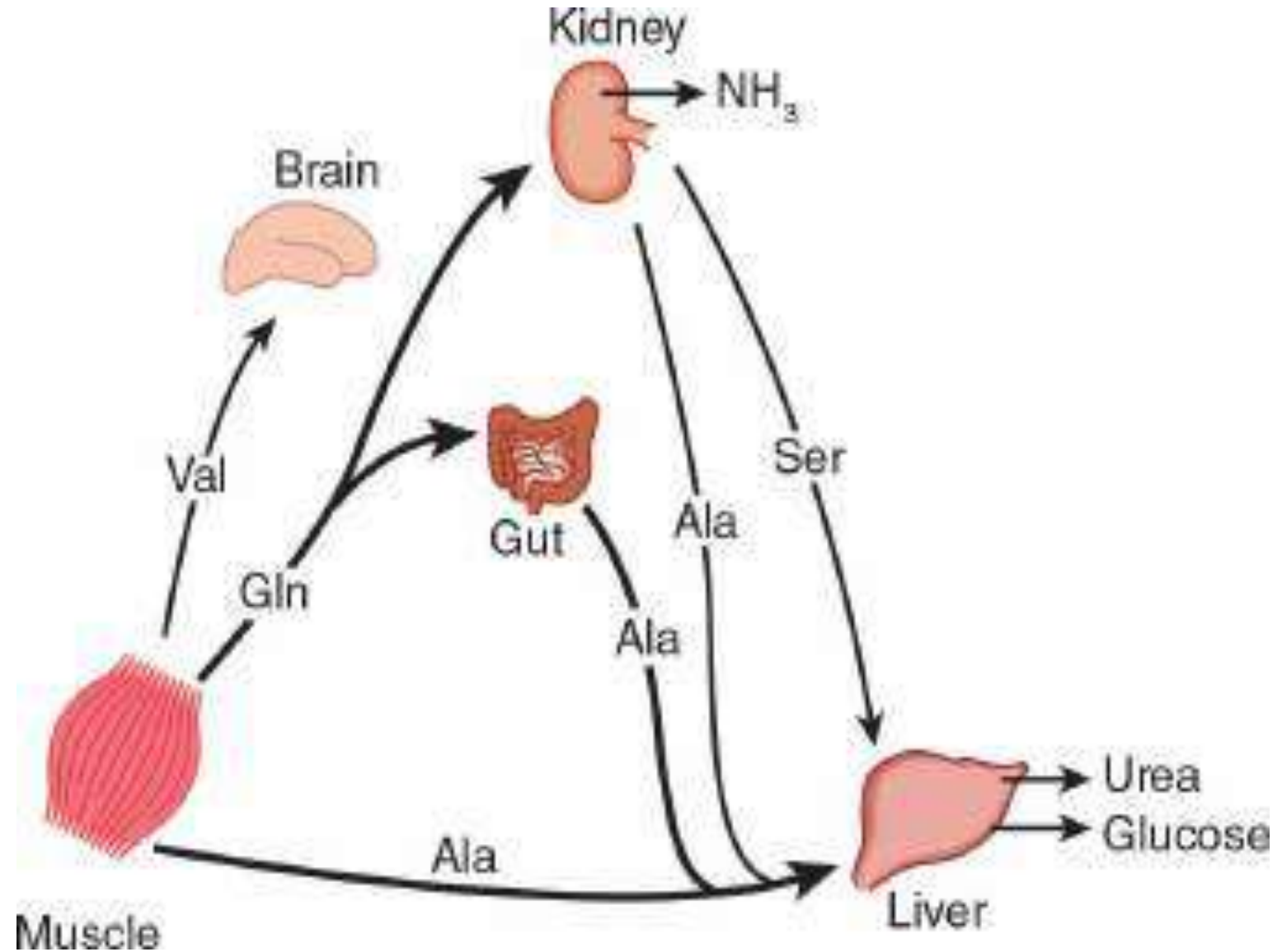
Alanine is a key **gluconeogenic amino acid** (**Figure 6**). The rate of hepatic gluconeogenesis from alanine is far higher than from all other amino acids. The capacity of the liver for gluconeogenesis from alanine does not reach saturation until the alanine concentration reaches 20 to 30 times its normal physiologic level. Following a protein-rich meal, the splanchnic tissues release amino acids (**Figure 7**) while the peripheral muscles extract amino acids, in both instances predominantly branched chain amino acids. Branched-chain amino acids thus serve a special role in nitrogen metabolism. **In the fasting state**, they provide the **brain with an energy source**, and postprandially they are extracted predominantly by muscle, having been spared by the liver.

FIGURE 6 The glucose-alanine cycle. Alanine is synthesized in muscle by transamination of glucose-derived pyruvate, released into the bloodstream, and taken up by the liver. In the liver, the carbon skeleton of alanine is reconverted to glucose and released into the bloodstream, where it is available for uptake by muscle and resynthesis of alanine.
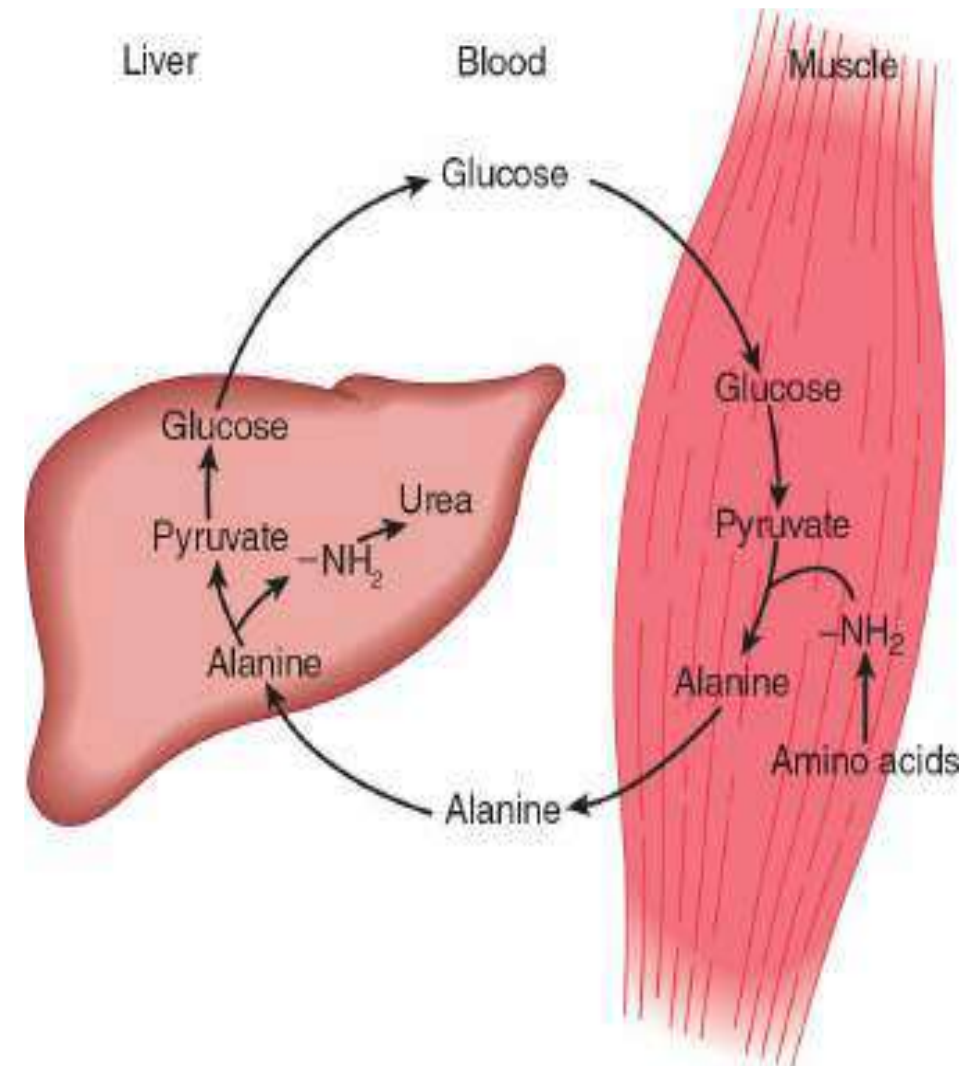
FIGURE 7 Summary of amino acid exchange between organs immediately after feeding.
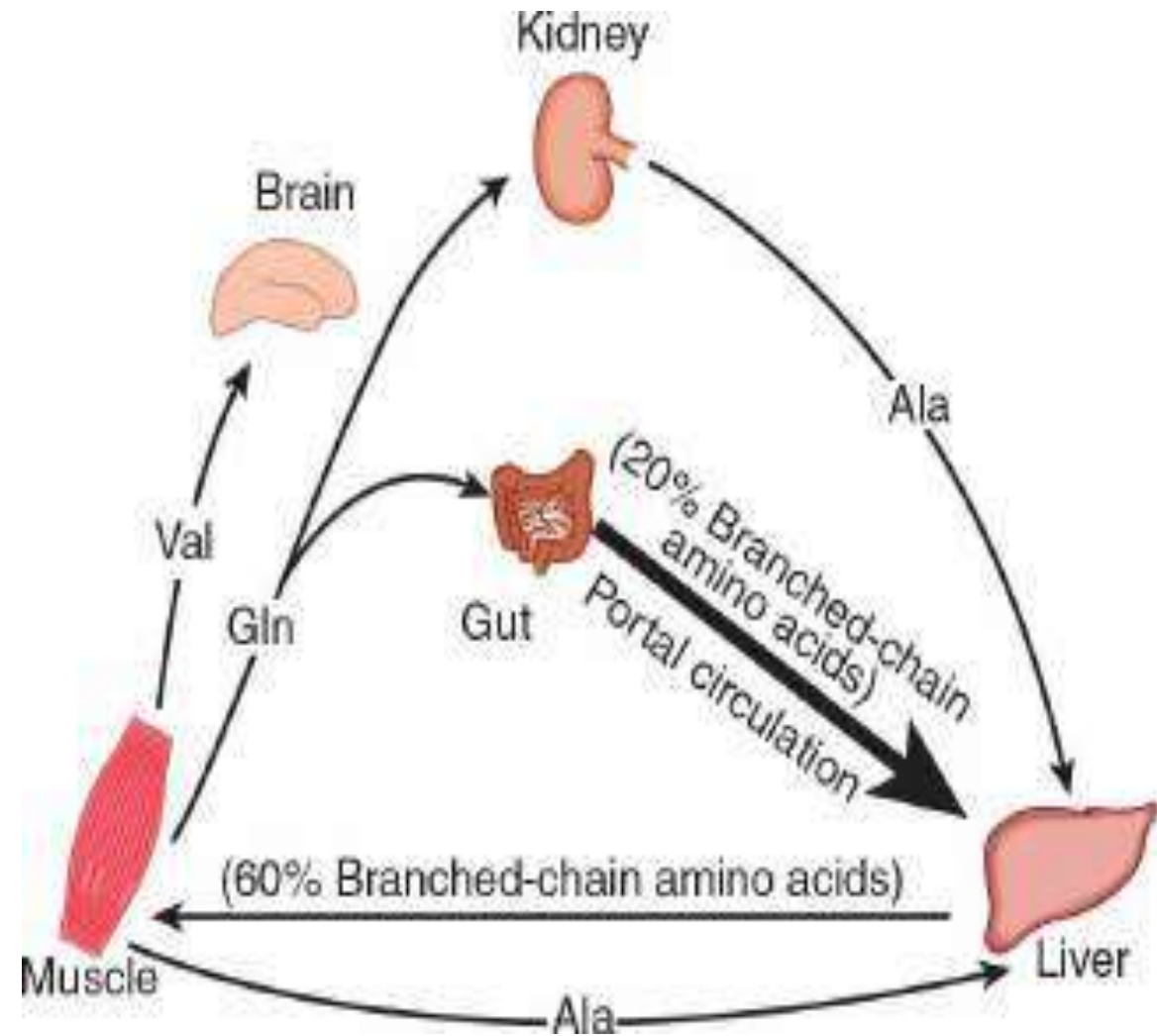
ANIMALS CONVERT α-AMINO NITROGEN TO VARIED END PRODUCTS

Depending on their ecological niche and physiology, **different animals** excrete excess **nitrogen as ammonia**, **uric acid**, or **urea**. The aqueous environment of teleostean fish, which are **ammonotelic** (excrete ammonia), permits them to excrete water continuously to facilitate excretion of ammonia, which is highly toxic. While this approach is appropriate for an aquatic animal, birds must both conserve water and maintain low weight. **Birds, which are uricotelic**, address both problems by excreting nitrogen-rich uric acid (see Figure 11) as semisolid guano.

Many land animals, including humans, are **ureotelic** and excrete nontoxic, highly water-soluble urea. Since **urea is nontoxic** to humans, high blood levels in renal disease are a consequence, not a cause, of impaired renal function.

BIOSYNTHESIS OF UREA

Urea biosynthesis occurs in four stages: (1) **transamination**, (2) **oxidative deamination of glutamate,** (3) **ammonia transport**, and (4) reactions of the **urea cycle** (**Figure 8**). The expression in liver of the RNAs for all the enzymes of the urea cycle increases severalfold in starvation, probably secondary to enhanced protein degradation to provide energy.

FIGURE 8 Overall flow of nitrogen in amino acid catabolism.
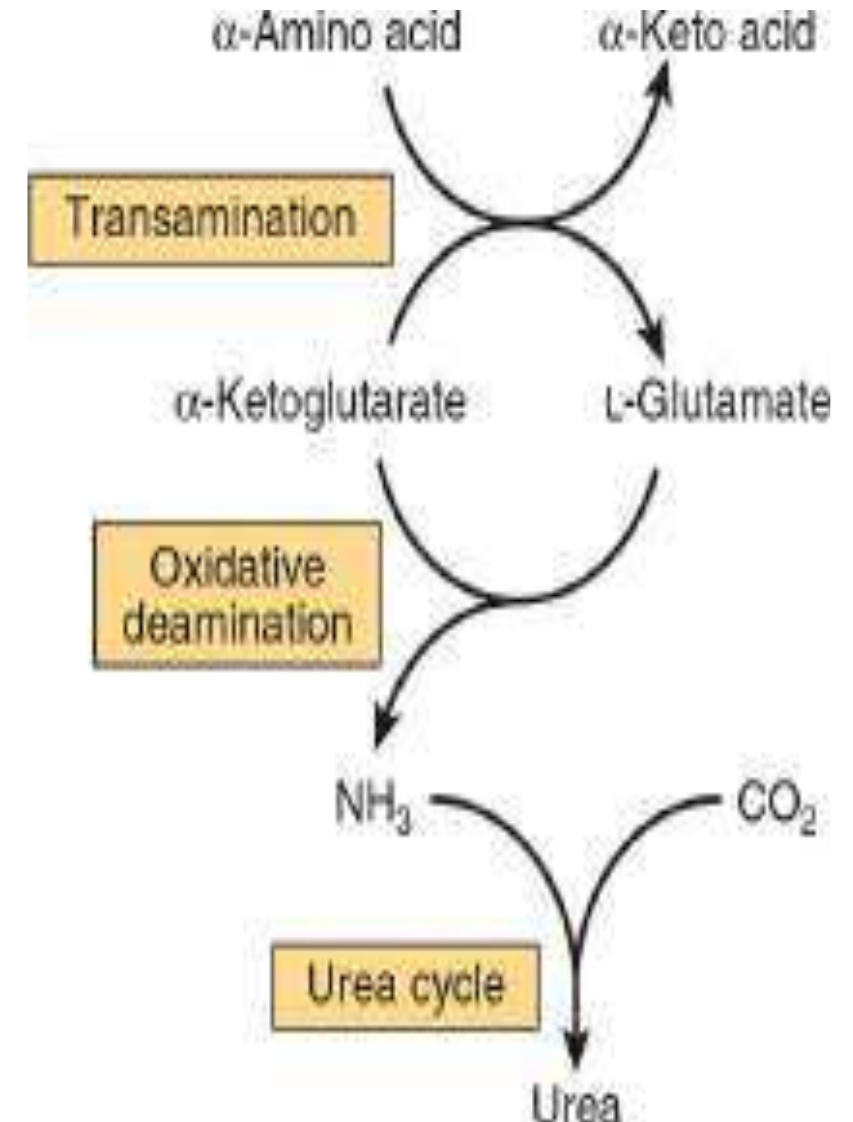
Transamination Transfers α-Amino Nitrogen to α- Ketoglutarate, Forming Glutamate

Transamination reactions interconvert pairs of α-amino acids and α-keto acids (**Figure 9**). **Transamination reactions, which are freely reversible, also function in amino acid biosynthesis** .**All of the common amino** acids **except lysine, threonine, proline, and hydroxyproline** participate in **transamination**. Transamination is **not restricted** to **α-amino** groups. The **δ-amino** group of **ornithine** (but not the ε-amino group of lysine) readily undergoes **transamination**.
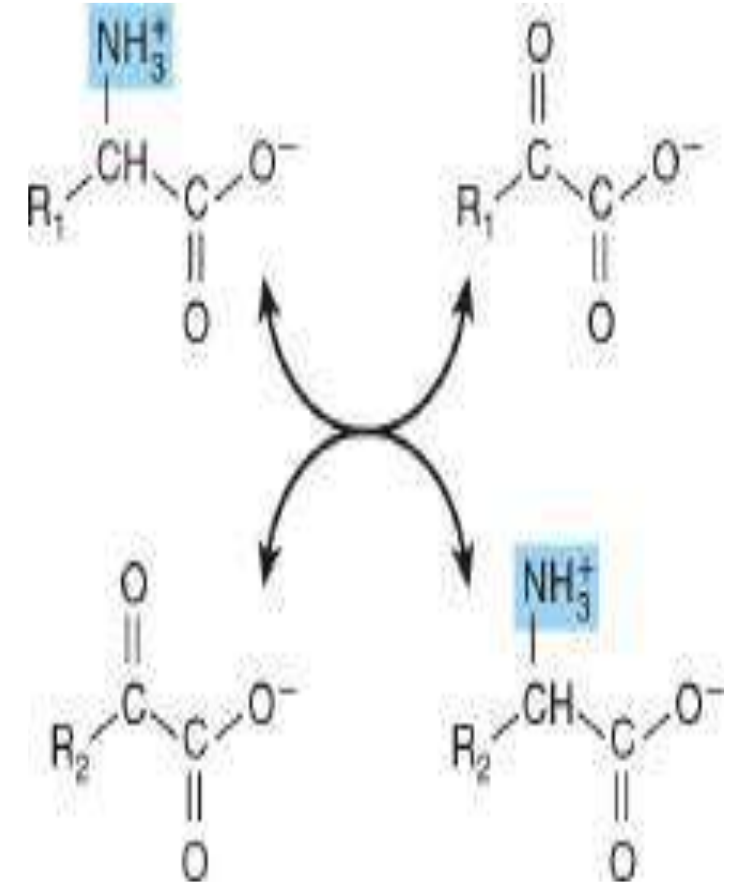
FIGURE 28–9 Transamination.

The reaction is freely reversible with an equilibrium constant close to unity. **Alanine-pyruvate aminotransferase** (alanine aminotransferase, EC 2.6.1.2) and **glutamate-α-ketoglutarate aminotransferase** (glutamate aminotransferase, EC 2.6.1.1) catalyze the **transfer of amino groups to pyruvate** (forming alanine) or to α-ketoglutarate (forming glutamate). **Each aminotransferase** is **specific for one pair** of substrates, but nonspecific for the other pair. Since alanine is also a substrate for glutamate aminotransferase, the α-amino nitrogen from all amino acids that undergo transamination can be concentrated in glutamate. This is **important because L-glutamate** is the only amino acid that undergoes **oxidative deamination** at an appreciable rate in mammalian tissues. The **formation of ammonia** from α-amino groups thus occurs mainly via the α- amino **nitrogen of L-glutamate**.

Transamination occurs via a "ping-pong" mechanism characterized by the alternate addition of a substrate and release of a product (**Figure 10**). Following removal of its α-amino nitrogen by transamination, the remaining carbon "skeleton" of an amino acid is degraded by pathways.
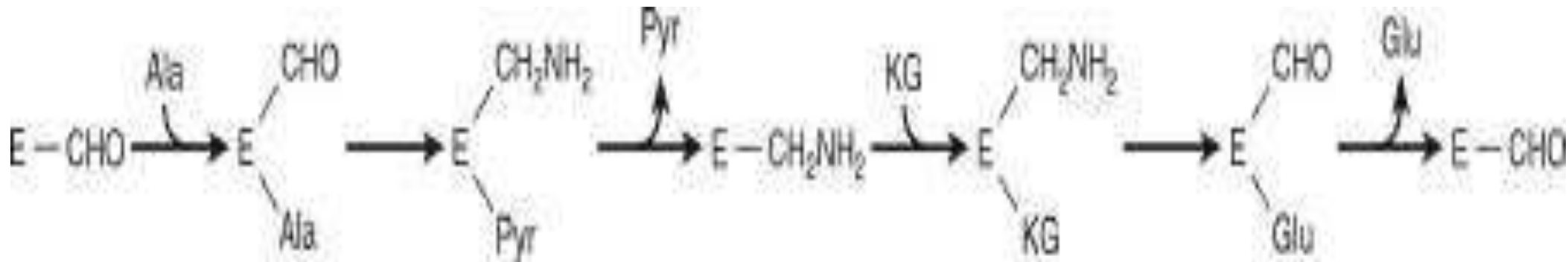
FIGURE 10 " **Ping-pong" mechanism for transamination.** E— CHO and E—CH2NH2 represent enzyme-bound pyridoxal phosphate and pyridoxamine phosphate, respectively. (Ala, alanine; Glu, glutamate; KG, α-ketoglutarate; Pyr, pyruvate.)

Pyridoxal phosphate (PLP), a derivative of vitamin B6, is present at the **catalytic site** of all **aminotransferase**s, and plays a key role in **catalysis**. During transamination, **PLP serves as a "carrier"** of amino groups. An enzyme-bound Schiff base (**Figure 1 11**) is formed between the oxo group of enzyme-bound PLP and the α-amino group of an α-amino acid. The Schiff base can rearrange in various ways. In transamination, rearrangement forms an α-keto acid and enzyme-bound pyridoxamine phosphate. As noted earlier, certain diseases are associated with elevated serum levels of aminotransferases

FIGURE1 1 Structure of a Schiff base formed between pyridoxal phosphate and an amino acid.
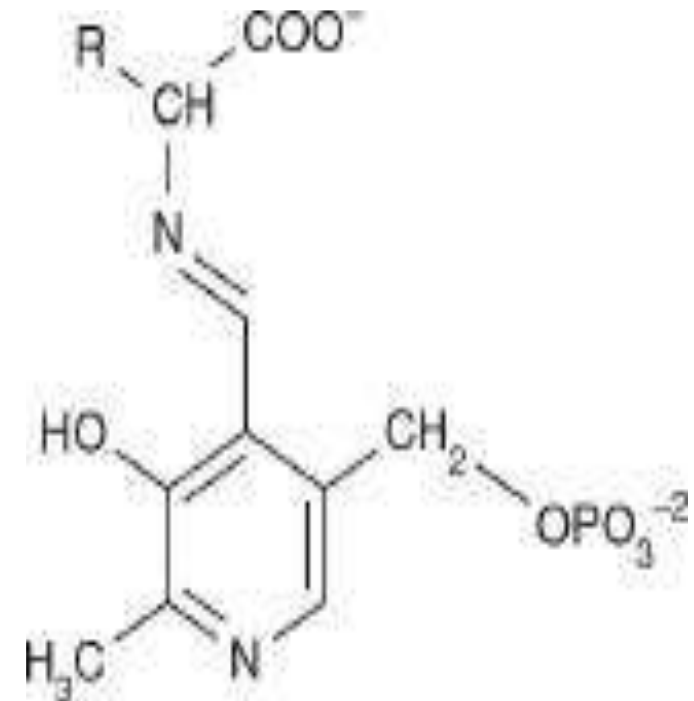
L-GLUTAMATE DEHYDROGENASE OCCUPIES A CENTRAL POSITION IN NITROGEN METABOLISM

Transfer of amino nitrogen to α-ketoglutarate forms L-glutamate. Hepatic **L-glutamate dehydrogenase** (GDH), which can use either NAD+ or NADP+, releases this nitrogen as ammonia (**Figure 12**). Conversion of α-amino nitrogen to ammonia by the concerted action of glutamate aminotransferase and GDH is often termed "transdeamination." Liver GDH activity is allosterically inhibited by ATP, GTP, and NADH, and is activated by ADP. The GDH reaction is freely reversible, and also functions in amino acid biosynthesis

FIGURE 12 The reaction catalyzed by glutamate dehydrogenase,
EC 1.4.1.2. NAD(P)+ means that either NAD+ or NADP+ can serve as the oxidoreductant. The reaction is reversible, but strongly favors glutamate formation.
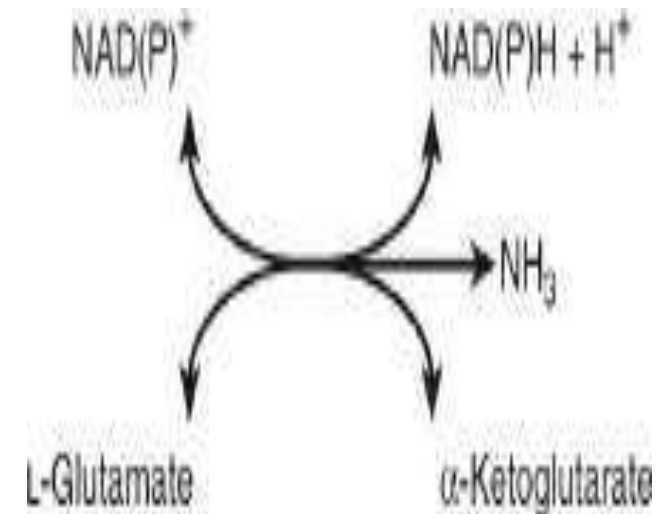
AMINO ACID OXIDASES REMOVE NITROGEN AS AMMONIA

L-Amino acid oxidase of liver and kidney convert an amino acid to an α- imino acid that decomposes to an α-keto acid with release of ammonium ion (**Figure 13**). The reduced flavin is reoxidized by molecular oxygen, forming hydrogen peroxide (H2O2), which then is split to O2 and H2O by **catalase**, EC 1.11.1.6.

FIGURE 13 Oxidative deamination catalyzed by L-amino acid oxidase (L-α-amino acid:O2 oxidoreductase, EC 1.4.3.2). The α-imino acid, shown in brackets, is not a stable intermediate.
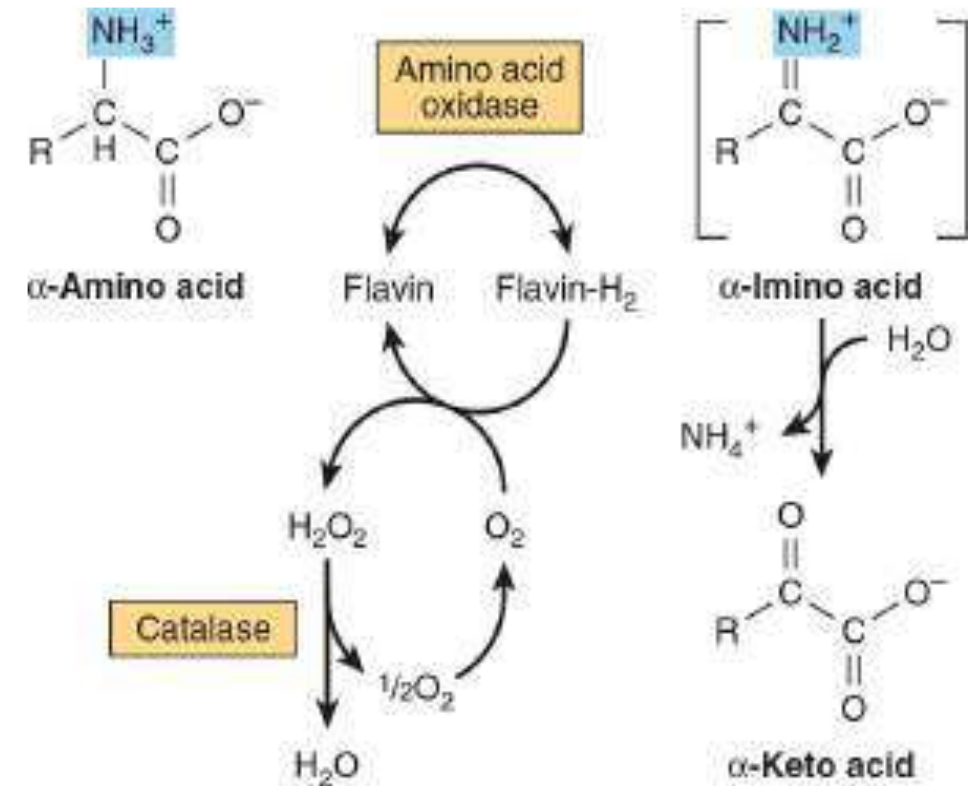
Ammonia Intoxication Is Life-Threatening

The ammonia produced by enteric bacteria and absorbed into portal venous blood and the **ammonia produced by tissues** are rapidly **removed from circulation** by the **liver and converted to urea**. Thus, normally, only traces (10-20 µg/dL) are present in peripheral blood. This is essential, since ammonia **is toxic to the central nervous system**. Should portal blood bypass the liver, systemic blood ammonia may reach **toxic levels**. This occurs in severely **impaired hepatic function** or the development of collateral links between the portal and systemic veins in cirrhosis.

Symptoms of **ammonia intoxication** include **tremor**, **slurred speech**, blurred vision, coma, and ultimately death. Ammonia may be toxic to the brain in part because it reacts with α ketoglutarate to form glutamate. The resulting **depletion of α-ketoglutarate** then impairs **function of the tricarboxylic acid (TCA) cycle in** neurons.

Glutamine Synthetase Fixes Ammonia as Glutamine

Formation of glutamine is catalyzed by mitochondrial **glutamine synthetase** (**Figure 14**). Since amide bond synthesis is coupled to the hydrolysis of **ATP to ADP** and Pi, the reaction strongly favors glutamine synthesis. During catalysis, glutamate attacks the γ-phosphoryl group of ATP, forming **γ-glutamyl phosphate** and **ADP.** Following deprotonation of , NH3 attacks γ-glutamyl phosphate, and **glutamine and Pi** are released. In addition to providing glutamine to serve as a carrier of nitrogen, carbon and energy between organs (Figure 28–5), **glutamine synthetase plays** a major role both in **ammonia detoxification** and in **acid– base homeostasis**. A rare **deficiency in neonate glutamine synthetas**e results in severe **brain damage**, **multiorgan failure**, and **death.**

FIGURE 14 Formation of glutamine, catalyzed by glutamine synthetase, EC 6.3.1.2.
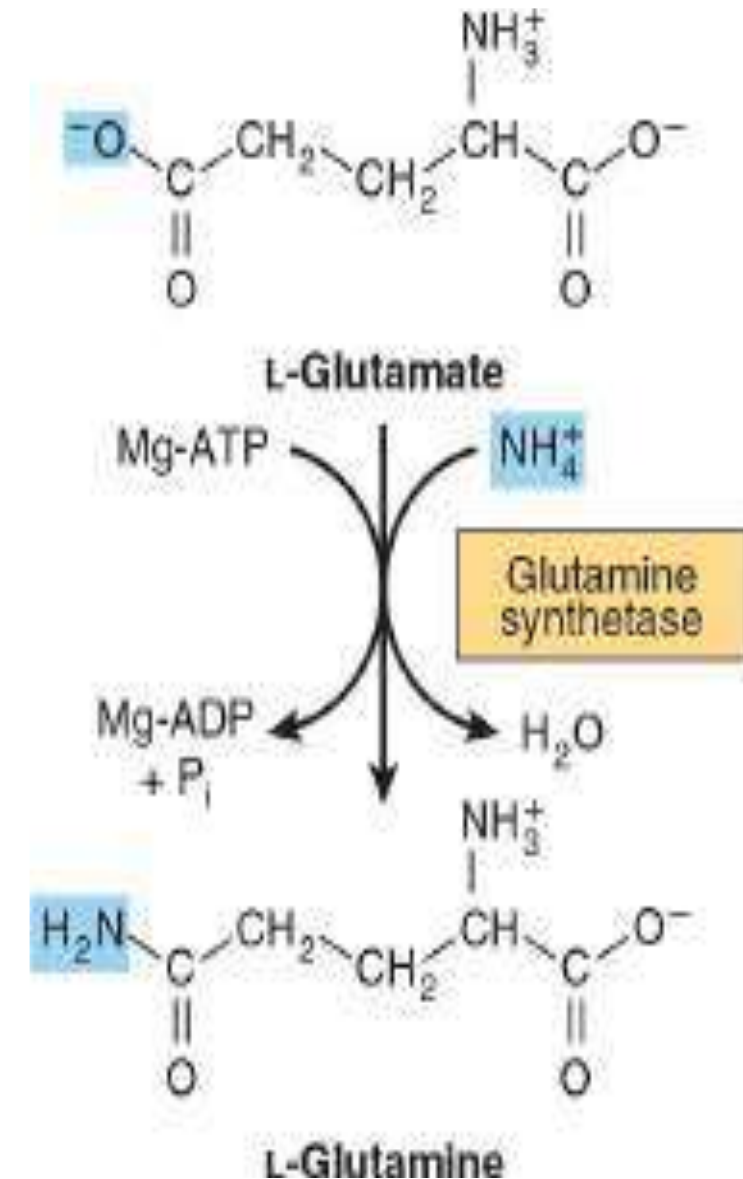
Glutaminase & Asparaginase Deamidate Glutamine & Asparagine

There are two human isoforms of mitochondrial **glutaminase**, termed liver-type and renal-type glutaminase. Products of different genes, the glutaminases differ with respect to their structure, kinetics, and regulation. Hepatic glutaminase levels rise in response to high protein intake while renal kidney-type glutaminase increases in metabolic acidosis. Hydrolytic release of the amide nitrogen of glutamine as ammonia, catalyzed by glutaminase (**Figure 15**), strongly favors glutamate formation. An analogous reaction is catalyzed by L-asparaginase .The concerted action **of glutamine synthetase** and **glutaminase** thus **catalyzes the interconversion of** free ammonium ion and glutamine.

L-Glutamine

Glutaminase

L-Glutamate

FIGURE 15 The reaction catalyzed by glutaminase, EC 3.5.1.2.
The reaction proceeds essentially irreversibly in the direction of glutamate and formation. Note that the *amide* nitrogen, not the α-amino nitrogen, is removed.
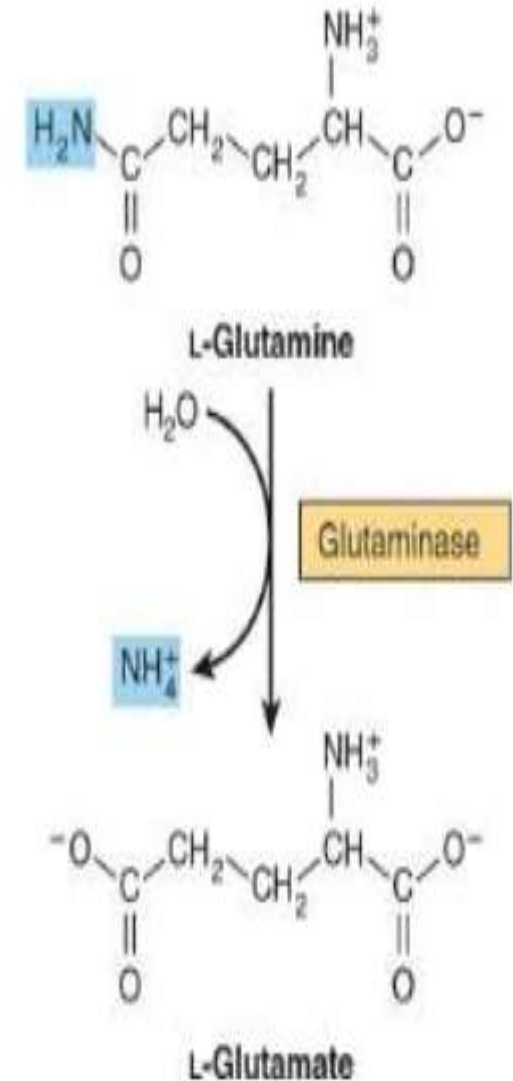
Formation & Secretion of Ammonia Maintains Acid–Base Balance

Excretion into urine of ammonia produced by renal tubular cells facilitates cation conservation and **regulation of acid–base balance**. Ammonia production from intracellular renal amino acids, especially glutamine, increases in **metabolic acidosis** and decreases in **metabolic alkalosis**.

Urea is the Major End Product of Nitrogen Catabolism in Humans

Synthesis of 1 mol of urea requires 3 mol of ATP, 1 mol each of ammonium ion and of aspartate, and employs five enzymes (**Figure 16**). Of the six participating amino acids, *N*-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea. The major metabolic role of **ornithine**, **citrulline**, and **argininosuccinate** in mammals is **urea synthesis**. Urea synthesis is a cyclic process. While ammonium ion, CO_2, ATP, and aspartate are consumed, the ornithine consumed in reaction 2 is regenerated in reaction 5. Thus, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. As indicated in Figure 16, some **reactions of urea synthesis** occur in the **matrix of the mitochondrion**, and other **reactions in the cytosol.**

FIGURE 16 Reactions and intermediates of urea biosynthesis. The nitrogen-containing groups that contribute to the formation of urea are shaded. Reactions (1) and (2) occur in the matrix of liver mitochondria and reactions 3 ,4 , and 5 in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers (see red dots) present in the inner membrane of liver mitochondria.
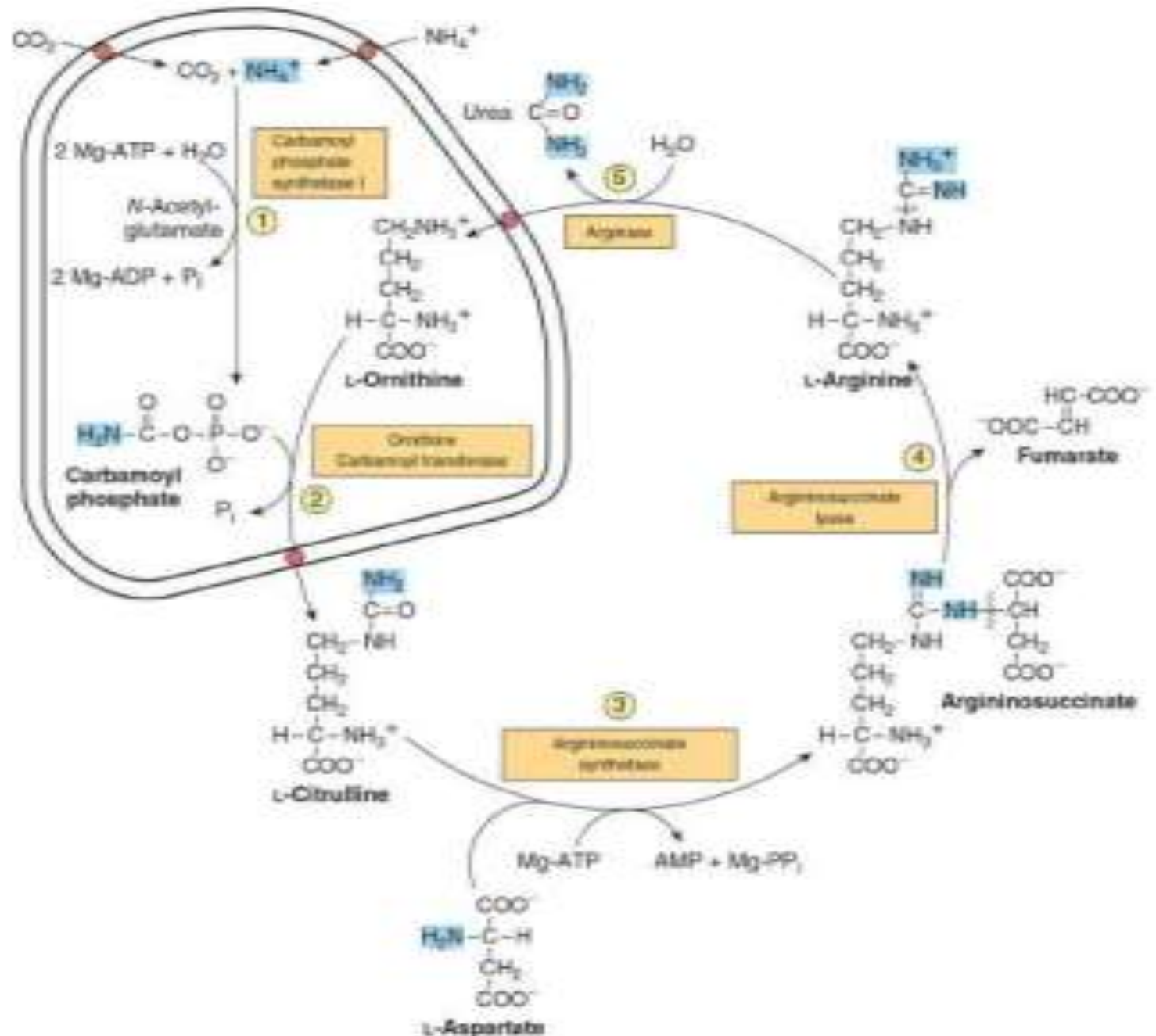
Carbamoyl Phosphate Synthetase I Initiates Urea Biosynthesis

Condensation of CO_2, ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthetase I** (EC 6.3.4.16). A cytosolic form of this enzyme, carbamoyl phosphate synthetase II, uses glutamine rather than ammonia as the nitrogen donor and functions in pyrimidine biosynthesis . The concerted action of glutamate dehydrogenase and carbamoyl phosphate synthetase I thus shuttles amino nitrogen into carbamoyl phosphate, a compound with high group transfer potential. Carbamoyl phosphate synthetase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of *N*-**acetylglutamate**, an allosteric activator that enhances the affinity of the synthetase for ATP. Synthesis of 1 mol of carbamoyl phosphate requires **2 mol of ATP.** One ATP serves as the phosphoryl donor for formation of the mixed acid **anhydride bond of carbamoyl phosphate**. The **second ATP provides** the driving force for synthesis of the amide bond of carbamoyl phosphate. The other products are **2 mol of ADP and 1 mol of Pi** (reaction 1, Figure 16). The reaction proceeds stepwise. Reaction of bicarbonate with ATP forms carbonyl phosphate and ADP. Ammonia then displaces ADP, forming carbamate and orthophosphate. Phosphorylation of carbamate by the second ATP then forms carbamoyl phosphate.

Carbamoyl Phosphate Plus Ornithine Forms Citrulline

L-**Ornithine transcarbamoylase** (EC 2.1.3.3) catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate (reaction 2, Figure 16). While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol. Entry of ornithine into mitochondria and exodus of citrulline from mitochondria therefore involve mitochondrial inner membrane permeases (Figure 16).

Citrulline Plus Aspartate Forms Argininosuccinate

Argininosuccinate synthetase (EC 6.3.4.5) **links aspartate** and **citrulline** via the amino group of aspartate (reaction 3, Figure 16) and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms **argininosuccinate**.

Cleavage of Argininosuccinate Forms Arginine & Fumarate

Cleavage of argininosuccinate is catalyzed by **argininosuccinate lyase** (EC 4.3.2.1). The reaction proceeds with retention of all three nitrogens in arginine and release of the aspartate skeleton as fumarate (reaction 4, Figure 16). Subsequent **addition of water** to fumarate forms L-malate, whose subsequent NAD+-dependent oxidation forms oxaloacetate. These two reactions **are analogous** to **reactions of the citric acid cycle**, but are catalyzed by *cytosolic* **fumarase** and **malate dehydrogenase**. Transamination of oxaloacetate by glutamate aminotransferase then reforms aspartate. The carbon skeleton of aspartate-fumarate thus acts as a carrier of the nitrogen of glutamate into a precursor of urea.

Cleavage of Arginine Releases Urea & Reforms Ornithine

Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver **arginase** (EC 3.5.3.1), **releases urea** (reaction 5, Figure 16). The other product, ornithine, reenters liver mitochondria and participates in additional rounds of urea synthesis. **Ornithine and lysine** are potent **inhibitors** of **arginase**, and compete with arginine. **Arginine** also serves as **the precursor** of the potent muscle relaxant **nitric oxide (NO)** in a Ca2+- dependent reaction **catalyzed** by **NO synthetase.**

Carbamoyl Phosphate Synthetase I Is the Pacemaker Enzyme of the Urea Cycle

The activity of carbamoyl phosphate synthetase I is determined by *N*acetylglutamate, whose steady-state level is dictated by the balance between its **rate of synthesis from acetyl-CoA and glutamate** and its rate of **hydrolysis to acetate and glutamate**, reactions catalyzed by *N*- **acetylglutamate synthetase** (NAGS) and *N*-acetylglutamate deacylase (hydrolase), respectively.

Acetyl-CoA + L-glutamate → N-acetyl-L-glutamate + CoASH

N-acetyl-L-glutamate + H₂O → L-glutamate + acetate
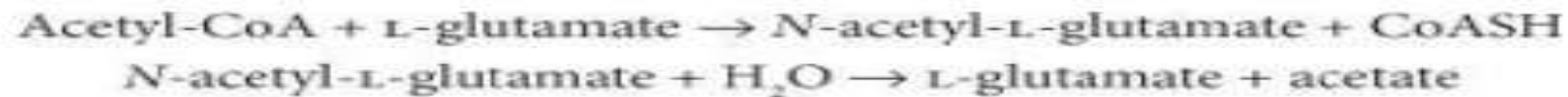
Major changes in diet can increase the concentrations of individual urea cycle enzymes 10- to 20-fold. For example, **starvation elevates enzyme levels,** presumably to cope with the increased production of ammonia that accompanies enhanced **starvation-induced degradation of protein.**

GENERAL FEATURES OF METABOLIC DISORDERS

The comparatively rare, but well-characterized and medically devastating metabolic disorders associated with the enzymes of urea biosynthesis illustrate the following general principles of inherited metabolic diseases.

1. Similar or identical clinical signs and symptoms can accompany various genetic mutations in a gene that encodes a given enzyme or in enzymes that catalyze successive reactions in a metabolic pathway.

2. Rational therapy is based on an understanding of the relevant biochemical enzyme-catalyzed reactions in both normal and impaired individuals.

 3. The identification of intermediates and of ancillary products that accumulate prior to a metabolic block provides the basis for metabolic screening tests that can implicate the reaction that is impaired.

4. Definitive diagnosis involves quantitative assay of the activity of the enzyme suspected to be defective.

5. The DNA sequence of the gene that encodes a given mutant enzyme is compared to that of the wild-type gene to identify the specific mutation(s) that cause the disease.

6. The exponential increase in DNA sequencing of human genes has identified dozens of mutations of an affected gene that are benign or are associated with symptoms of varying severity of a given metabolic disorder.

METABOLIC DISORDERS ARE ASSOCIATED WITH EACH REACTION OF THE UREA CYCLE

Five well-documented diseases represent defects in the biosynthesis of enzymes of the urea cycle. Molecular genetic analysis has pinpointed the loci of mutations associated with each deficiency, each of which exhibits considerable genetic and phenotypic variability (**Table 1**).

TABLE 1 Enzymes of Inherited Metabolic Disorders of the Urea Cycle

Enzyme	Enzyme Catalog Number	OMIM* Reference	Figure and Reaction
Carbamoyl-phosphate synthetase 1	6.3.4.16	237300	28–13①
Ornithine carbamoyl transferase	2.1.3.3	311250	28–13②
Argininosuccinate synthetase	6.3.4.5	215700	28–13③
Argininosuccinate lyase	4.3.2.1	608310	28–13④
Arginase	3.5.3.1	608313	28–13⑤

*Online Mendelian inheritance in man database: ncbi.nlm.nih.gov/omim/

Urea cycle disorders are characterized by **hyperammonemia**, **encephalopathy,** and **respiratory alkalosis**. Four of the five metabolic diseases, deficiencies of carbamoyl phosphate synthetase I, ornithine carbamoyl transferase, argininosuccinate synthetase, and argininosuccinate lyase, result in the **accumulation of precursors of urea**, principally **ammonia** and **glutamine**. Ammonia intoxication is most **severe** when the metabolic **block occurs at reactions 1 or 2** (Figure 16), for if citrulline can be synthesized, some ammonia has already been removed by being covalently linked to an organic metabolite. **Clinical symptoms** common to all **urea cycle disorders** include **vomiting**, avoidance of high-protein foods, **intermittent ataxia**, **irritability**, **lethargy**, and **severe mental retardation**. The most dramatic clinical presentation occurs in full-term infants who initially appear normal, then exhibit progressive lethargy, hypothermia, and apnea due to high plasma ammonia levels. The clinical features and treatment of all five disorders are similar. Significant improvement and minimization of **brain damage** can accompany a low-protein diet ingested as frequent small meals to avoid sudden increases in blood ammonia levels. The goal of dietary **therapy** is to **provide sufficient protein, arginine**, and **energy** to promote growth and development while simultaneously minimizing the metabolic perturbations.

Carbamoyl Phosphate Synthetase I

N-Acetylglutamate is essential for **the activity** of carbamoyl phosphate synthetase I, EC 6.3.4.16 (reaction 1, Figure 16). Defects in **carbamoyl phosphate synthetase I** are responsible for the relatively rare (estimated frequency 1:62,000) metabolic disease termed "**hyperammonemia type 1.**"

N-Acetylglutamate Synthetase

N-Acetylglutamate synthetase, EC 2.3.1.1 (NAGS), catalyzes the formation from acetyl-CoA and glutamate of the *N*-acetylglutamate **essential for carbamoyl phosphate synthetase I activity.**

$$\text{L-Glutamate} + \text{acetyl-CoA} \rightarrow N\text{-acetyl-L-glutamate} + \text{CoASH}$$

  While the clinical and biochemical features of NAGS deficiency are indistinguishable from those arising from a defect in carbamoyl phosphate synthetase I, a deficiency in NAGS may respond to administered *N*acetylglutamate.

Ornithine Permease

The **hyperornithinemia**, **hyperammonemia**, and **homocitrullinuria** (**HHH**) syndrome results from **mutation** of the *ORNT1* **gene** that encodes the mitochondrial **membrane ornithine permease.** The **failure to import cytosolic ornithine into the mitochondrial** matrix renders the **urea cycle inoperable**, with consequent **hyperammonemia**, and **hyperornithinemia** due to the accompanying accumulation of cytosolic ornithine. In the absence of its normal acceptor (ornithine), mitochondrial carbamoyl phosphate carbamoylates lysine to homocitrulline, resulting in homocitrullinuria.

Ornithine Transcarbamoylase

The X-chromosome–linked deficiency termed **"hyperammonemia type 2" reflects a defect in ornithine transcarbamoylase** (reaction 2, Figure 16).The mothers also exhibit hyperammonemia and an aversion to highprotein foods. Levels of **glutamine are elevated in blood**, **cerebrospinal fluid**, and **urine**, probably as a result of **enhanced glutamine synthesis** in response to **elevated** levels of tissue **ammonia.**

Argininosuccinate Synthetase

In addition to patients who **lack** detectable **argininosuccinate synthetase** activity (reaction 3, Figure 16), a 25-fold elevated K_m for citrulline has been reported. In the resulting citrullinemia, plasma and cerebrospinal fluid citrulline levels are elevated, and 1 to 2 g of citrulline are excreted daily.

Argininosuccinate Lyase

Argininosuccinic aciduria, accompanied by **elevated levels of argininosuccinate** in **blood**, **cerebrospinal fluid,** and **urine**, is associated with friable, tufted hair (trichorrhexis nodosa). Both early- and late-onset types are known. The metabolic **defect is in argininosuccinate lyase** (reaction 4, Figure16). Diagnosis by the **measurement of erythrocyte** argininosuccinate lyase activity can be performed on **umbilical cord blood** or **amniotic fluid cells**.

Arginase

Hyperargininemia is an autosomal recessive defect in the gene for **arginase** (reaction 5, Figure 16). Unlike other urea cycle disorders, the first symptoms of **hyperargininemia typically do not appear until age 2 to 4 years**. Blood and cerebrospinal fluid levels of arginine are elevated. The urinary amino acid pattern, which resembles that of lysine-cystinuria, may reflect competition by arginine with lysine and cysteine for reabsorption in the renal tubule.

Analysis of Neonate Blood by Tandem Mass Spectrometry Can Detect Metabolic Diseases

Metabolic diseases caused by the absence or functional impairment of metabolic enzymes can be devastating. Early dietary intervention, however, can in many instances ameliorate the otherwise inevitable dire effects. The early detection of such metabolic diseases is thus is of primary importance. Since the initiation in the United States of newborn screening programs in the 1960s, all states now conduct metabolic screening of newborn infants. The powerful and sensitive technique of **tandem mass spectrometry** (MS) can in a few minutes detect over 40 analytes of significance in the detection of metabolic disorders. Most states employ tandem MS to screen newborns to detect metabolic disorders such as organic acidemias, aminoacidemias, disorders of fatty acid oxidation, and defects in the enzymes of the urea cycle. An article in *ClinicalChemistry* 2006 39:315 reviews the theory of tandem MS, its application to the detection of metabolic disorders, and situations that can yield false positives, and includes a lengthy table of detectable analytes and the relevant metabolic diseases.

Can Metabolic Disorders Be Rectified by Gene or Protein Modification

Despite results in animal models using an adenoviral vector to treat citrullinemia, at present gene therapy provides no effective solution for human subjects. However, direct CRISPR/Cas9-based modification of a defective enzyme can restore functional enzyme activity of cultured human pluripotent stem cells.

SUMMARY

---Human subjects degrade 1 to 2% of their body protein daily at ratesthat vary widely between proteins and with physiologic state. Key regulatory enzymes often have short half-lives.

---Proteins are degraded by both ATP-dependent and ATP-independent pathways. UbAiquitin targets many intracellular proteins for degradation. Liver cell surface receptors bind and internalize circulating asialoglycoproteins destined for lysosomal degradation.

---Polyubiquitinated proteins are degraded by proteases on the inner surface of a cylindrical macromolecule, the proteasome. Entry into the proteasome is gated by a donut-shaped protein pore that rejects entry to all but polyubiquitinated proteins.

---Fishes excrete highly toxic NH3 directly. Birds convert NH3 to uric acid. Higher vertebrates convert NH3 to urea.

---Transamination channels amino acid nitrogen into glutamate. GDH occupies a central position in nitrogen metabolism.

---Glutamine synthetase converts NH_3 to nontoxic glutamine. Glutaminase releases NH_3 for use in urea synthesis.

---NH_3, CO_2, and the amide nitrogen of aspartate provide the atoms of urea.

---Hepatic urea synthesis takes place in part in the mitochondrial matrix and in part in the cytosol.

---Changes in enzyme levels and allosteric regulation of carbamoyl phosphate synthetase I by N-acetylglutamate regulate urea biosynthesis.

---Metabolic diseases are associated with defects in each enzyme of the urea cycle, of the membrane-associated ornithine permease, and of NAGS.

---The metabolic disorders of urea biosynthesis illustrate six general principles of all metabolic disorders.

---Tandem mass spectrometry is the technique of choice for screening neonates for inherited metabolic diseases

Biosynthesis of the Nutritionally Nonessential Amino Acids

NUTRITIONALLY ESSENTIAL & NUTRITIONALLY NONESSENTIAL AMINO ACIDS

While often employed with reference to amino acids**, the terms "essential" and "nonessential" are misleading since all 20 common amino acids are essential to ensure health.** Of these 20 amino acids, **8** *must* be present in the human diet, and thus are best termed "***nutritionally essential.***"

 The other **12 amino acids are "*nutritionally nonessential*** " since they need not be present in the diet (**Table 27–1**).

This suggests a survival advantage in retaining the **ability** to **manufacture "easy" amino acids** while **losing the ability to make "difficult" amino acids.** The metabolic pathways that form the **nutritionally essential amino acids occur** in **plants and bacteria**, but not in **humans**.

TABLE 1 Amino Acid Requirements of Humans

Nutritionally Essential	Nutritionally Nonessential
Arginine[a]	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Hydroxyproline[b]
Tryptophan	Hydroxylysine[b]
Valine	Proline
	Serine
	Tyrosine

[a]Nutritionally "semiessential." Synthesized at rates inadequate to support growth of children.
[b]Not necessary for protein synthesis, but is formed during posttranslational processing of collagen.

TABLE 2 Enzymes Required for the Synthesis of Amino Acids From Amphibolic Intermediates

	Number of Enzymes Required to Synthesize		
Nutritionally Essential		**Nutritionally Nonessential**	
Arg[a]	7	Ala	1
His	6	Asp	1
Thr	6	Asn[b]	1
Met	5 (4 shared)	Glu	1
Lys	8	Gln[a]	1
Ile	8 (6 shared)	Hyl[c]	1
Val	6 (all shared)	Hyp[d]	1
Leu	7 (5 shared)	Pro[a]	3
Phe	10	Ser	3
Trp	5 (8 shared)	Gly[e]	1
	59 (total)	Cys[f]	2
		Tyr[g]	1
			17 (total)

[a]From Glu.
[b]From Asp.
[c]From Lys.
[d]From Pro.
[e]From Ser.
[f]From Ser plus sulfate.
[g]From Phe.

BIOSYNTHESIS OF THE NUTRITIONALLY NONESSENTIAL AMINO ACIDS

Glutamate

Glutamate, the precursor of the so-called "glutamate family" of amino acids, **is formed by the reductive amidation of the citric acid cycle α- ketoglutarate, a reaction catalyzed by mitochondrial glutamate dehydrogenase (Figure 1).** The reaction strongly favors glutamate synthesis, which **lowers the concentration of cytotoxic ammonium ion.**
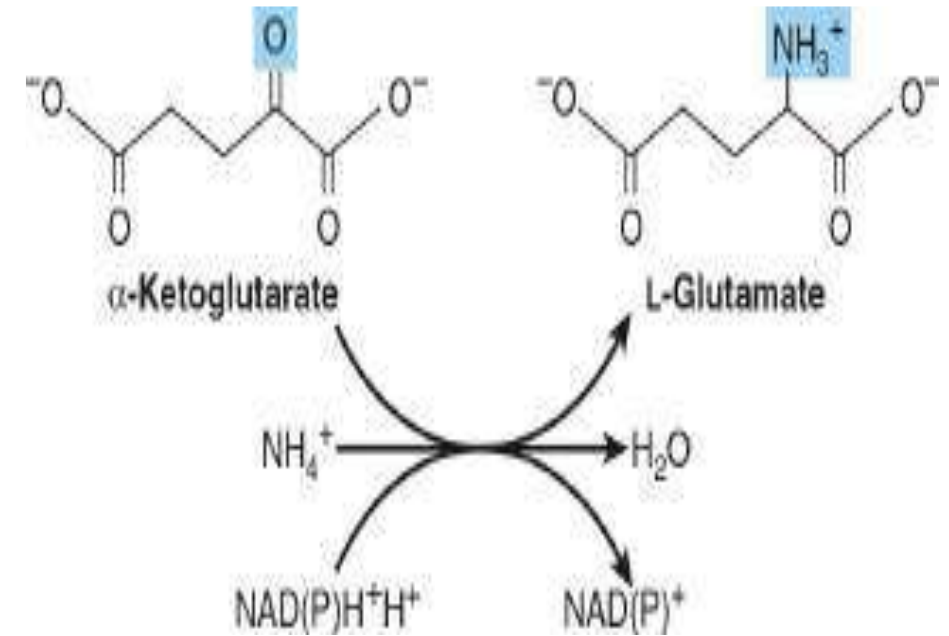

FIGURE 1 The reaction catalyzed by glutamate dehydrogenase (EC 1.4.1.3).

Glutamine

The amidation of glutamate to **glutamine** catalyzed by **glutamine synthetase** (**Figure 2**) involves the intermediate formation of γ- glutamyl phosphate (**Figure 3**). Following the ordered binding of **glutamate and ATP,** glutamate attacks the γ-phosphorus of ATP, forming **γ-glutamyl phosphate and ADP. NH4 + then binds**, and uncharged **NH3 attacks** γ-glutamyl phosphate. Release of Pi and of a proton from the γ- amino group of the tetrahedral intermediate then allows release of the **product, glutamine.**

L-Glutamate

L-Glutamine

NH$_4^+$

Mg-ATP

Mg-ADP + P$_i$
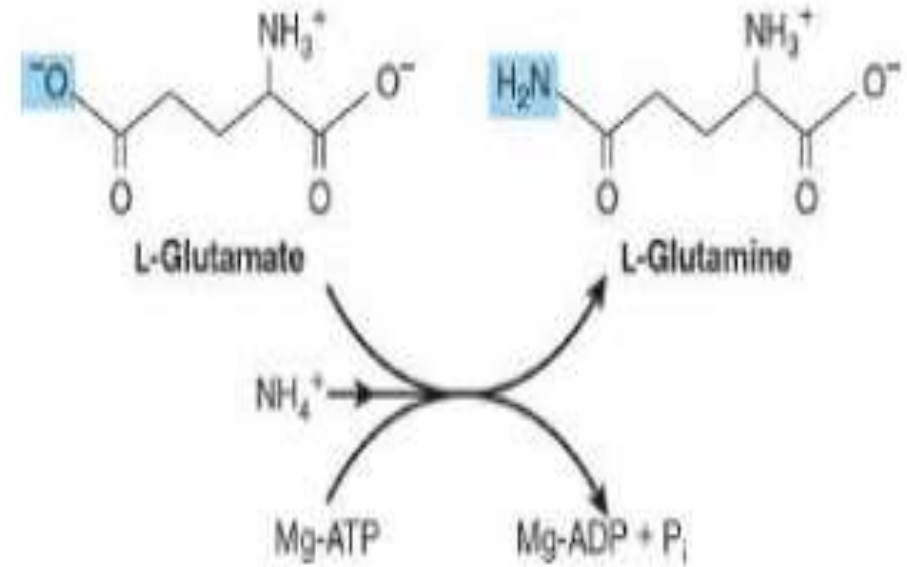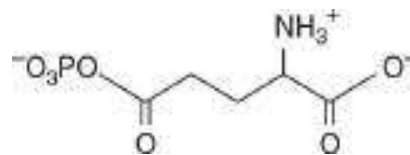
FIGURE 2 The reaction catalyzed by glutamine synthetase (EC 6.3.1.2).

FIGURE 3 γ-Glutamyl phosphate.

Alanine & Aspartate

Transamination of **pyruvate** forms **alanine** (**Figure 4**).

Similarly,

transamination of **oxaloacetate** forms **aspartate.**

FIGURE 4 Formation of alanine by transamination of pyruvate.
The amino donor may be glutamate or aspartate. The other product thus is α-ketoglutarate or oxaloacetate.

Glutamate Dehydrogenase, Glutamine Synthetase, & Aminotransferases Play Central Roles in Amino Acid Biosynthesis

The combined action of the enzymes glutamate dehydrogenase, glutamine synthetase, and the aminotransferases (Figures 1, 2 and 4) results **in conversion of inorganic ammonium ion** into the **α-amino nitrogen of amino acids.**

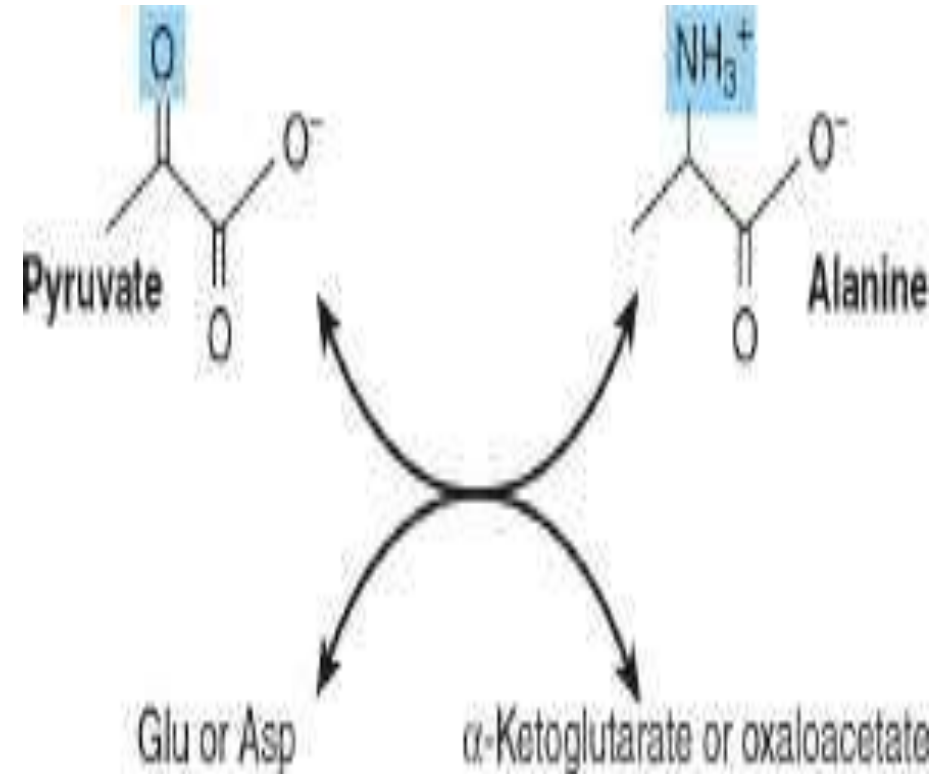# Asparagine

The conversion of **aspartate** to **asparagine**, catalyzed by **asparagine synthetase** (**Figure 5**), resembles the glutamine synthetase reaction (Figure 2), but **glutamine**, rather than **ammonium ion**, provides **the nitrogen**. **Bacterial** asparagine synthetases can, however, also use **ammonium ion**. The reaction involves the intermediate formation of **aspartyl phosphate** (**Figure 6**). The coupled hydrolysis of PPi to Pi by pyrophosphatase, EC 3.6.1.1, ensures that the reaction is strongly favored.

FIGURE 5 The reaction catalyzed by asparagine synthetase (EC 6.3.5.4). Note similarities to and differences from the glutamine synthetase reaction
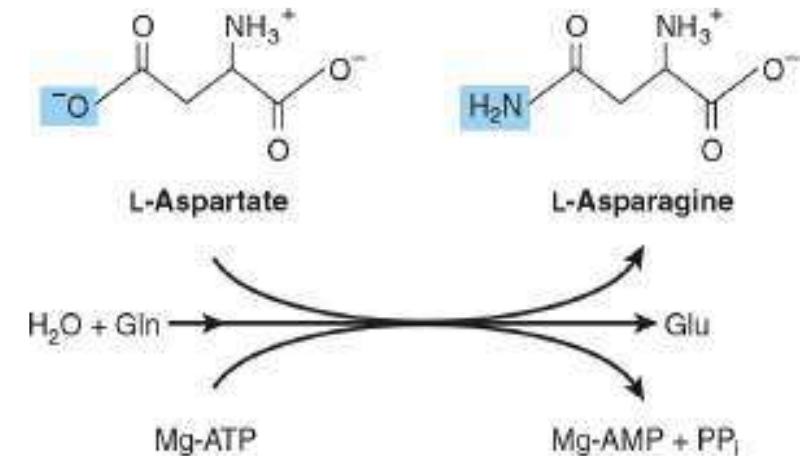
FIGURE 6 Aspartyl phosphate.
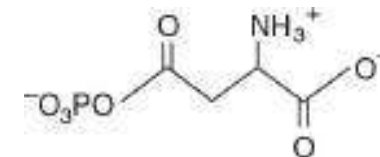
Serine

Oxidation of the α-hydroxyl group of the glycolytic intermediate 3- phosphoglycerate, catalyzed by **3-phosphoglycerate dehydrogenase, converts it to 3-phosphohydroxypyruvate. Transamination and subsequent dephosphorylation then form serine (Figure 7).**

FIGURE 7 Serine biosynthesis. Oxidation of 3-phosphoglycerate is catalyzed by 3-phosphoglycerate dehydrogenase .Transamination converts phosphohydroxypyruvate to phosphoserine. Hydrolytic removal of the phosphoryl group catalyzed by phosphoserine hydrolase then forms L-serine.
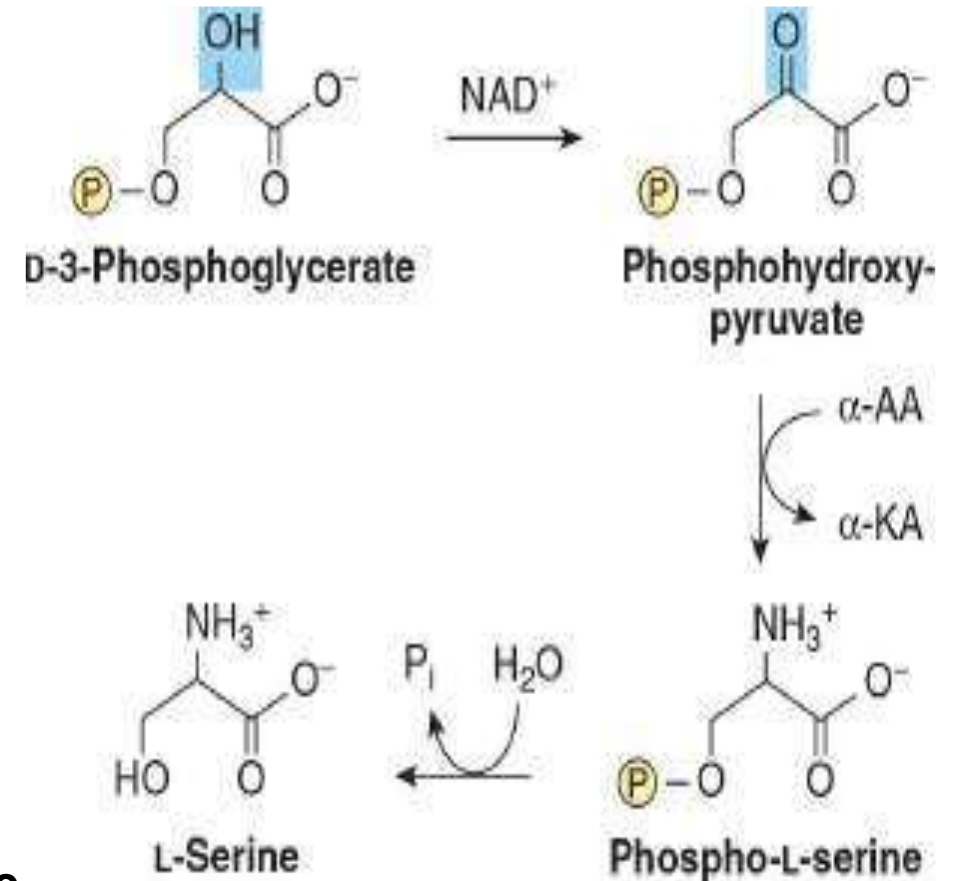
Glycine

Glycine aminotransferases can catalyze the synthesis of glycine from glyoxylate and glutamate or alanine. Unlike most aminotransferase reactions, these strongly favor glycine synthesis. Additional important mammalian routes for glycine formation are from choline (Figure 8) and from serine (Figure 9).
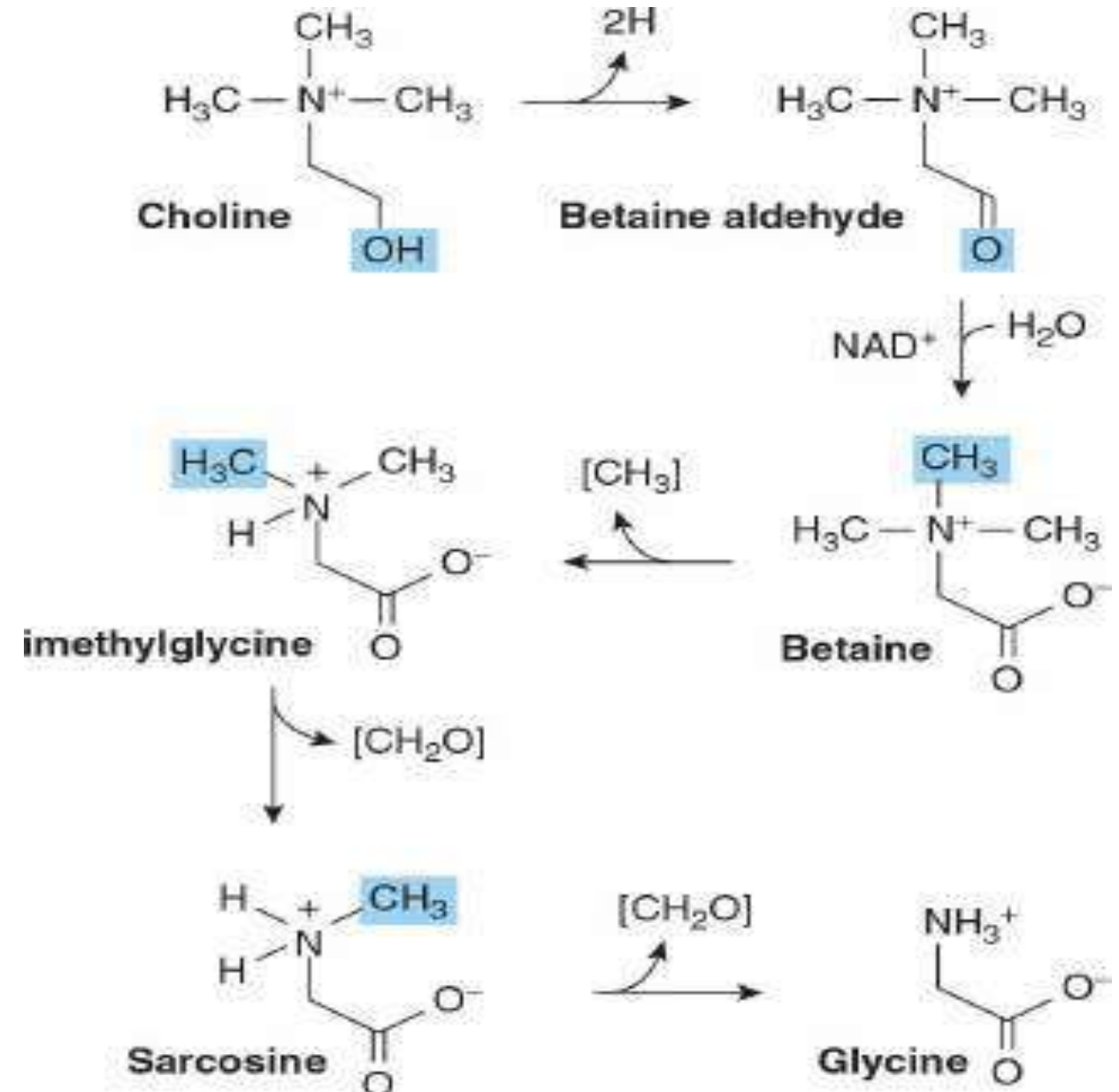
FIGURE 8 Formation of glycine from choline. Catalysts include choline dehydrogenase (EC 1.1.3.17), betaine aldehyde dehydrogenase (EC 1.2.1.8), betaine-homocysteine N-methyltransferase (EC 2.1.1.157), sarcosine dehydrogenase (EC 1.5.8.3), and dimethylglycine dehydrogenase (EC 1.5.8.4).

FIGURE 9 Interconversion of serine and glycine, catalyzed by serine hydroxymethyltransferase .The reaction is freely reversible. (H4 folate, tetrahydrofolate.)
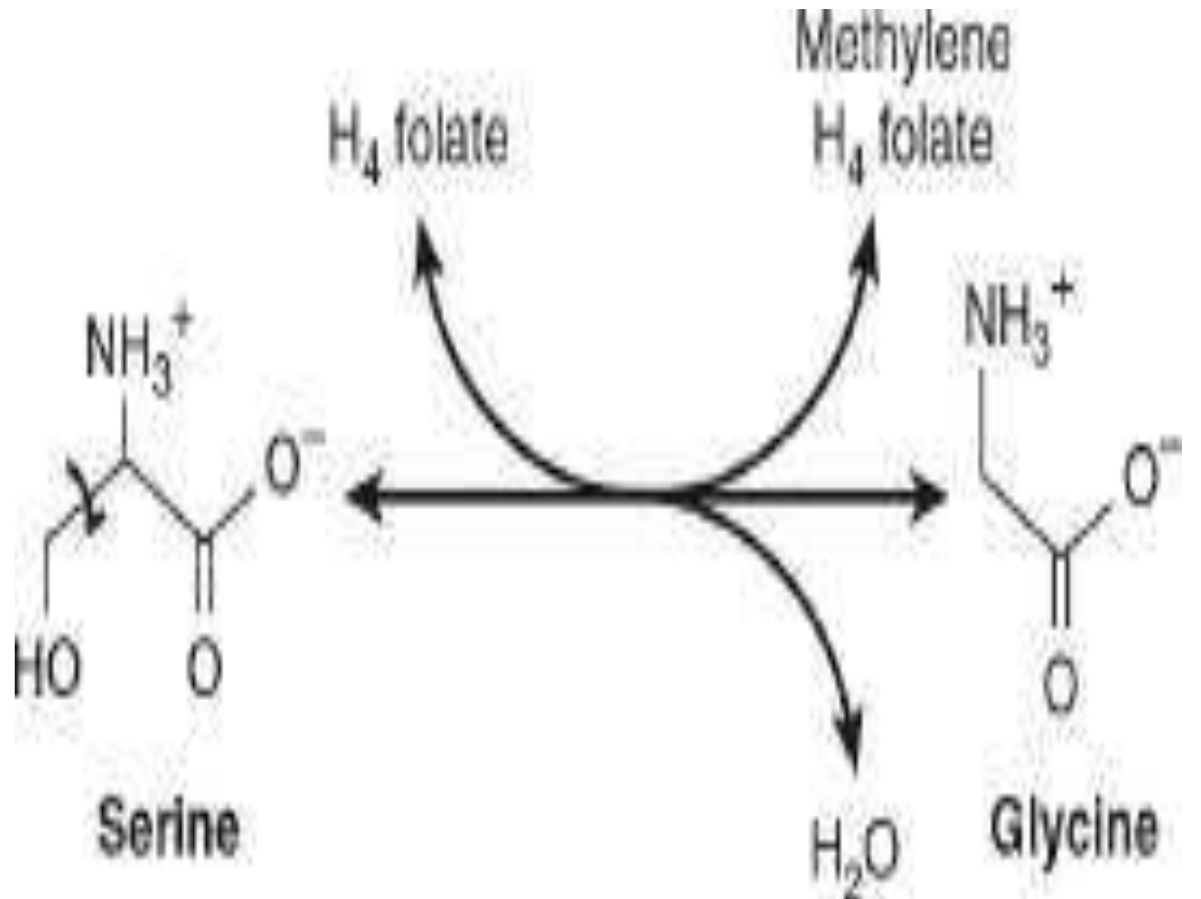
Proline

The initial reaction of proline biosynthesis converts the **γ-carboxyl group of glutamate** to the mixed acid **anhydride of glutamate γ-phosphate** (Figure 3). Subsequent reduction forms glutamate γ-semialdehyde, which following spontaneous cyclization is reduced to **L-proline** (Figure 10).

FIGURE 10 Biosynthesis of proline from glutamate. Catalysts for these reactions are **glutamate-5-kinase**, **glutamate-5-semialdehyde dehydrogenase** ,and **pyrroline-5-carboxylate reductase**. Ring closure of glutamate semialdehyde is spontaneous.
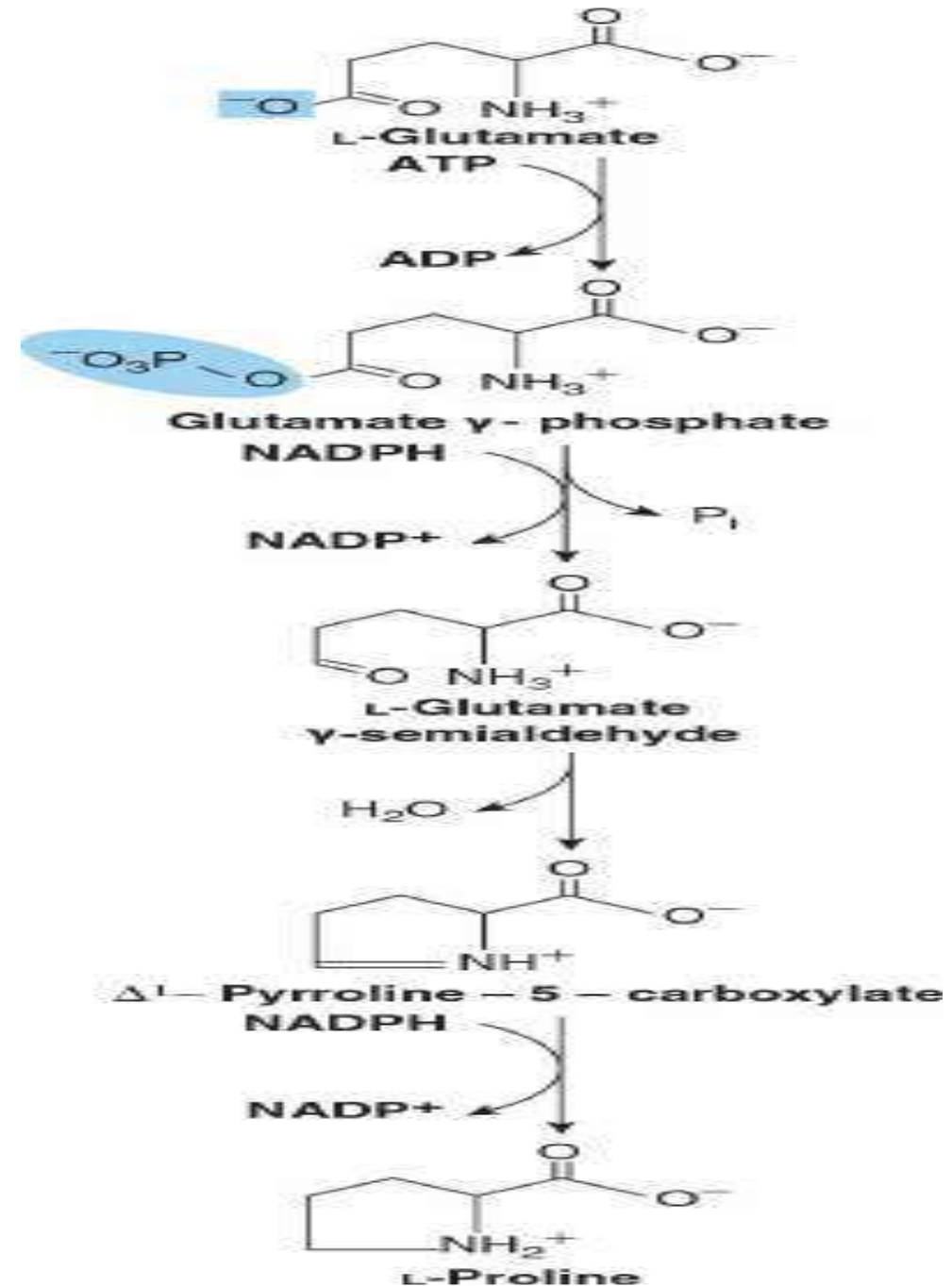
Cysteine

While **not nutritionally essential,** cysteine is formed from **methionine, which is nutritionally essential.** Following conversion of methionine to homocysteine , homocysteine and serine form cystathionine, whose hydrolysis forms cysteine and homoserine (**Figure 11**).

FIGURE 11 Conversion of homocysteine and serine to homoserine and cysteine. The sulfur of cysteine derives from methionine and the carbon skeleton from serine. The catalysts are **cystathionine β-synthase) and cystathionine γ-lyase .**
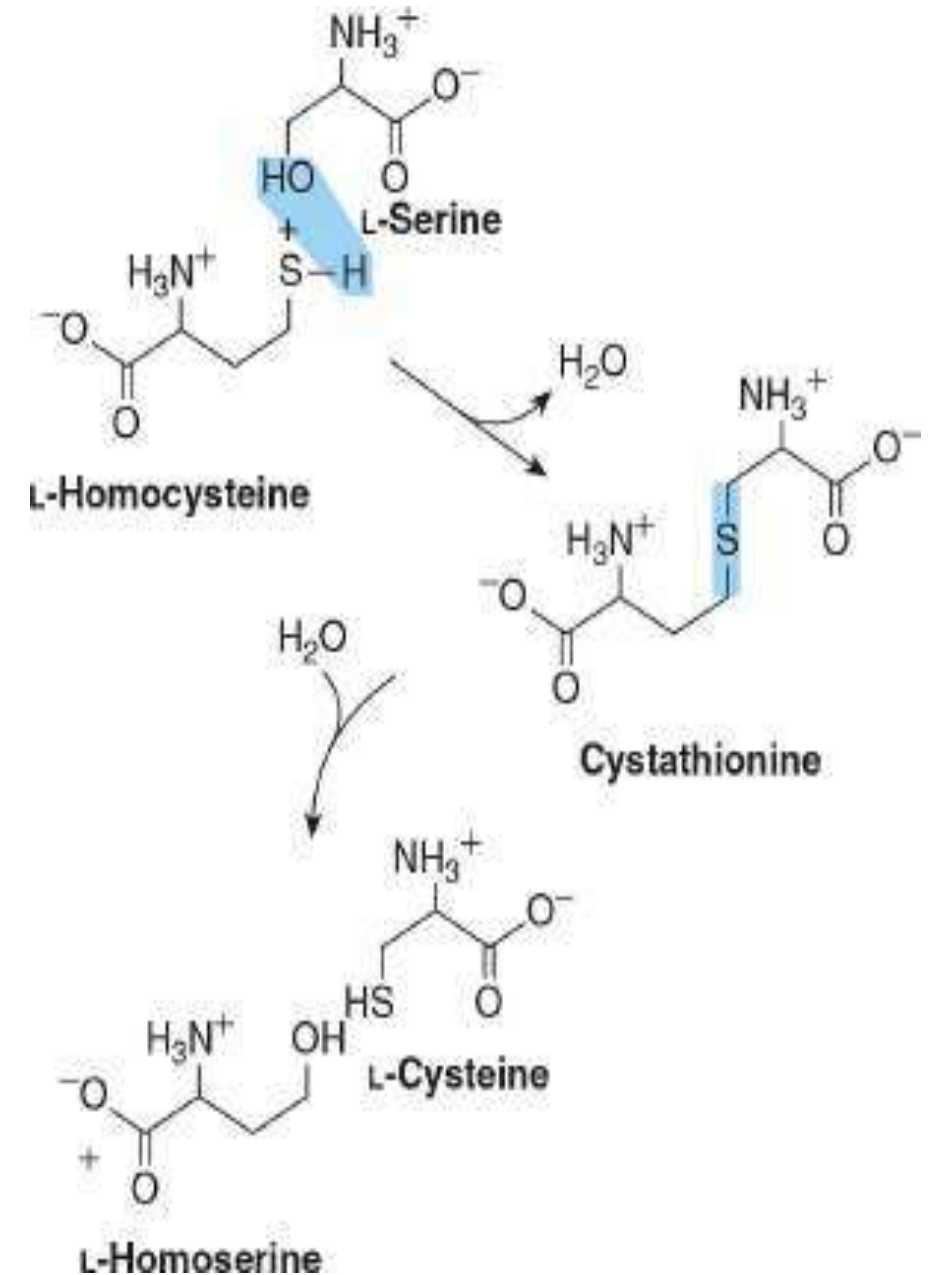
Tyrosine

Phenylalanine hydroxylase converts **phenylalanine to tyrosine** (**Figure 12**). If the diet contains adequate quantities of the nutritionally **essential amino acid phenylalanine,** tyrosine is nutritionally nonessential. However, since the phenylalanine hydroxylase reaction is **irreversible**, **dietary tyrosine cannot replace phenylalanine**. Catalysis by this mixed-function oxidase incorporates one atom of O2 into the *para* position of **phenylalanine** and reduces the other atom to **water**. **Reducing power, provided as tetrahydrobiopterin, derives ultimately from NADPH**.

FIGURE 12 Conversion of phenylalanine to tyrosine by phenylalanine hydroxylase (EC 1.14.16.1). Two distinct enzymatic activities are involved. **Activity II** catalyzes reduction of **dihydrobiopterin** by NADPH, and **activity I** the **reduction of O2 to H2O and of phenylalanine to tyrosine.** This reaction is associated with several defects of phenylalanine metabolism.
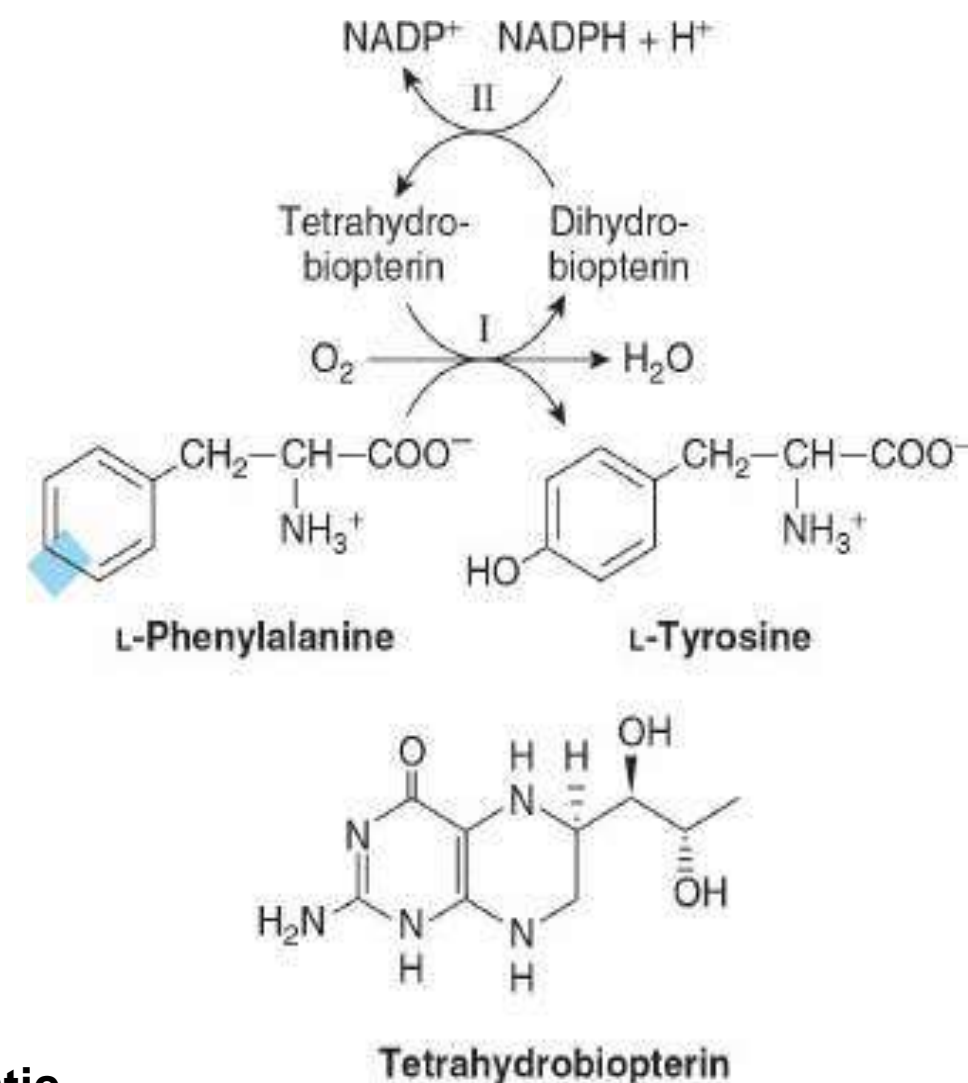
Hydroxyproline & Hydroxylysine

Hydroxyproline and **hydroxylysine** occur principally **in collagen**. Since there is **no tRNA** for either hydroxylated amino acid, neither dietary hydroxyproline **nor** dietary hydroxylysine is incorporated **during protein synthesis**. Peptidyl **hydroxyproline and hydroxylysine** arise from **proline** and **lysine**, but only after these amino acids have been incorporated into peptides. Hydroxylation of peptidyl prolyl and peptidyl lysyl residues, catalyzed by **prolyl hydroxylase** and **lysyl hydroxylase** of **skin**, **skeletal muscle**, and **granulating wounds requires**, in addition to the substrate, molecular **O2, ascorbate**, **Fe2+,** and **α ketoglutarate** (**Figure 13**). For every mole of proline or lysine hydroxylated, one mole of α-ketoglutarate is decarboxylated to succinate. The hydroxylases are mixed-function oxidases. **One atom of O2** is incorporated into **proline** or **lysine**, the **other into succinate** (Figure 13). A **deficiency of the vitamin C required for these two hydroxylases** results in **scurvy**, in which **bleeding gums, swelling joints**, and **impaired wound healing** result from the impaired stability of collagen .

FIGURE 13 Hydroxylation of a proline-rich peptide. Molecular oxygen is incorporated into both succinate and proline. **Procollagen proline 4-hydroxylase** thus is a mixed-function oxidase. **Procollagen-lysine 5-hydroxylase** catalyzes an analogous reaction.
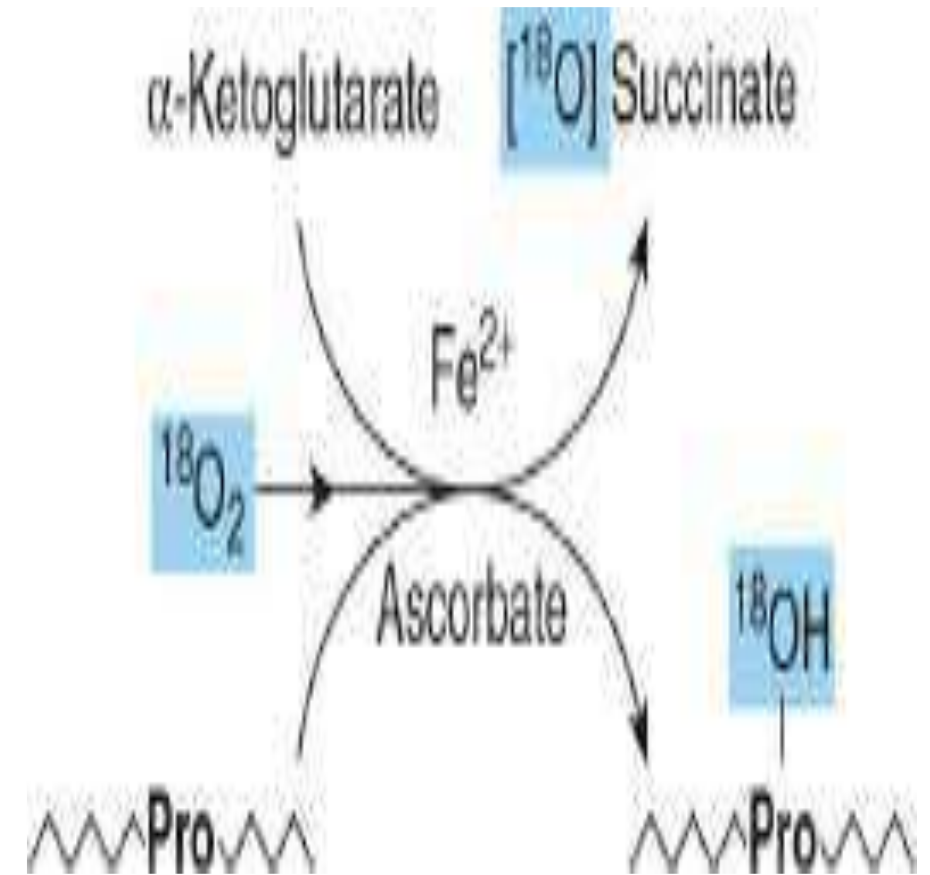
Valine, Leucine, & Isoleucine

While leucine, valine, and isoleucine are all nutritionally essential amino acids, tissue aminotransferases reversibly interconvert all three amino acids and their corresponding α-keto acids. These α-keto acids thus can replace their amino acids in the diet.

Selenocysteine, the 21st Amino Acid

While the occurrence of selenocysteine (**Figure 14**) **in proteins is uncommon**, at least **25** human **selenoproteins** are known. **Selenocysteine is present at the active site** of several human **enzymes that catalyze redox reactions**. Examples include **thioredoxin reductase, glutathione peroxidase**, and the **deiodinase** that converts **thyroxine** to **triiodothyronine**. Where present, **selenocysteine** participates in the **catalytic mechanism of these enzymes**. Significantly, the replacement of **selenocysteine** by **cysteine** can actually *reduce* **catalytic** activity. **Impairments in human selenoproteins** have been implicated in **tumorigenesis** and **atherosclerosis**, and are associated with selenium deficiency **cardiomyopathy** (Keshan disease).

FIGURE 14 Selenocysteine (top) and the reaction catalyzed by selenophosphate synthetase (EC 2.7.9.3) (bottom).

$$H-Se-CH_2-\underset{\underset{NH_3^+}{|}}{\overset{\overset{H}{|}}{C}}-COO^-$$

$$Se + ATP + H_2O \longrightarrow AMP + P_i + H-Se-\underset{\underset{O^-}{|}}{\overset{\overset{O}{\parallel}}{P}}-O^-$$
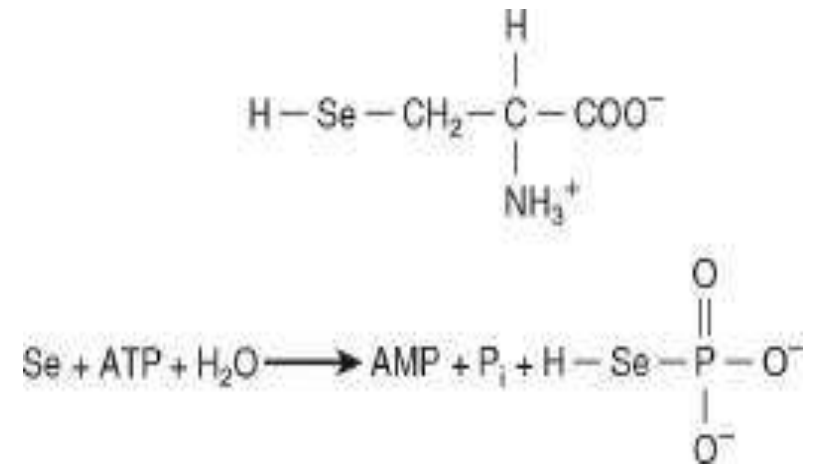
Biosynthesis of selenocysteine requires serine, selenate (SeO_4^{2-}), ATP, a specific tRNA, and several enzymes.

Serine provides the carbon skeleton of selenocysteine.

Selenophosphate, formed from ATP and selenite (Figure 14), serves as the **selenium donor**. Unlike 4-hydroxyproline or 5-hydroxylysine, selenocysteine arises *cotranslationally* during its incorporation into peptides. **The UGA anticodon of the unusual tRNA called tRNASec normally signals STOP.**

The ability of the protein synthetic apparatus to identify a selenocysteine-specific UGA codon involves the selenocysteine insertion element, a stem-loop structure in the untranslated region of the mRNA. **tRNASec is first charged with serine by the ligase that charges tRNASer.** Subsequent **replacemen**t of **the serine oxygen** by **selenium** involves **selenophosphate** formed by **selenophosphate synthetase** (Figure 14). Successive enzyme-catalyzed reactions convert **cysteyl-tRNASec** to **aminoacrylyl-tRNASec** and then **to selenocysteyltRNASec**. In the presence of a specific **elongation factor** that recognizes selenocysteyl-tRNASec, **selenocysteine can then be incorporated into proteins**.

Gluconeogenesis & the Control of Blood Glucose

BIOMEDICAL IMPORTANCE

Gluconeogenesis is the process of synthesizing glucose from noncarbohydrate precursors. The major substrates are the glucogenic amino acids, lactate, glycerol, and propionate. Liver and kidney are the major gluconeogenic tissues; the kidney may contribute up to 40% of total glucose synthesis in the fasting state and more in starvation.

The key gluconeogenic enzymes are expressed in the small intestine, but it is unclear whether or not there is significant glucose production by the intestine in the fasting state, although propionate arising from intestinal bacterial fermentation of carbohydrates is a substrate for gluconeogenesis in enterocytes.

A supply of glucose is necessary especially for the nervous system and erythrocytes. After an overnight fast, glycogenolysis and gluconeogenesis make approximately equal contributions to blood glucose; as glycogen reserves are depleted, so gluconeogenesis becomes progressively more important. Failure of gluconeogenesis is usually fatal. **Hypoglycemia** causes brain dysfunction, which can lead to coma and death. Glucose is also important in maintaining adequate concentrations of intermediates of the citric acid cycle even when fatty acids are the main source of acetylCoA in the tissues. In addition, gluconeogenesis clears lactate produced by muscle and erythrocytes, and glycerol produced by adipose tissue. In ruminants, propionate is a product of rumen metabolism of carbohydrates, and is a major substrate for gluconeogenesis.

Excessive gluconeogenesis occurs in **critically ill patients** in response to injury and infection, contributing to **hyperglycemia** which is associated with a poor outcome. Hyperglycemia leads to changes in osmolality of body fluids, impaired blood flow, intracellular acidosis, and increased superoxide radical production , resulting in deranged endothelial and immune system function and impaired blood coagulation. Excessive gluconeogenesis is also a contributory factor to hyperglycemia in **type 2 diabetes** because of impaired downregulation in response to insulin.

GLUCONEOGENESIS INVOLVES GLYCOLYSIS, THE CITRIC ACID CYCLE, PLUS SOME SPECIAL REACTIONS

Thermodynamic Barriers Prevent a Simple Reversal of Glycolysis

Three nonequilibrium reactions in glycolysis , catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase, prevent simple reversal of glycolysis for glucose synthesis (Figure 1). They are circumvented as follows.
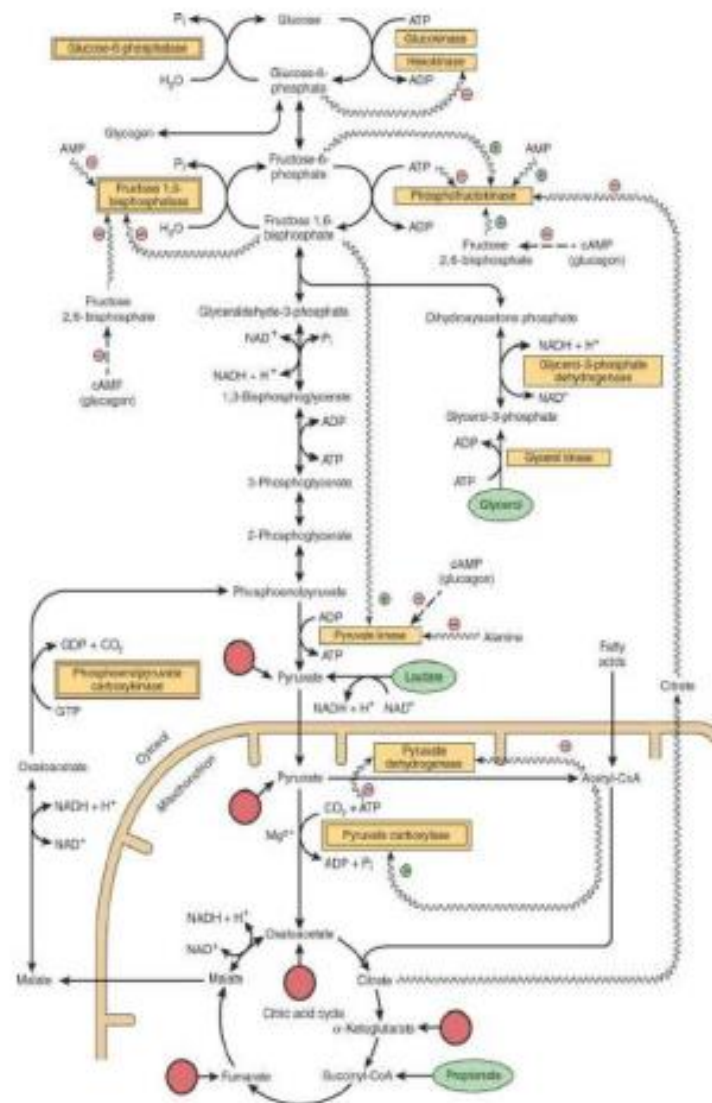
FIGURE 1 Major pathways and regulation of gluconeogenesis and glycolysis in the liver.

Entry points of glucogenic amino acids after transamination are indicated by arrows extended from circles. The key gluconeogenic enzymes are shown in double bordered boxes. The ATP required for gluconeogenesis is supplied by the oxidation of fatty acids. Propionate is important only in ruminants. Arrows with wavy shafts signify allosteric effects; dash-shafted arrows, covalent modification by reversible phosphorylation. High concentrations of alanine act as a "gluconeogenic signal" by inhibiting glycolysis at the pyruvate kinase step.

Pyruvate & Phosphoenolpyruvate

Reversal of the reaction catalyzed by pyruvate kinase in glycolysis involves two endothermic reactions. Mitochondrial **pyruvate carboxylase** catalyzes the carboxylation of pyruvate to oxaloacetate, an ATP-requiring reaction in which the vitamin biotin is the coenzyme. Biotin binds CO_2 from bicarbonate as carboxybiotin prior to the addition of the CO_2 to pyruvate .The resultant oxaloacetate is reduced to malate, exported from the mitochondrion into the cytosol and there oxidized back to oxaloacetate. A second enzyme, **phosphoenolpyruvate carboxykinase**, catalyzes the decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate using GTP as the phosphate donor. In liver and kidney, the reaction of succinate thiokinase in the citric acid cycle produces GTP (rather than ATP as in other tissues), and this GTP is used for the reaction of phosphoenolpyruvate carboxykinase, thus providing a link between citric acid cycle activity and gluconeogenesis, to prevent excessive removal of oxaloacetate for gluconeogenesis, which would impair citric acid cycle activity.

Fructose 1,6-Bisphosphate & Fructose-6-Phosphate

The conversion of fructose 1,6-bisphosphate to fructose-6-phosphate,

for the reversal of glycolysis, is catalyzed by **fructose 1,6-bisphosphatase**. Its presence determines whether a tissue is capable of synthesizing glucose (or glycogen) not only from pyruvate but also from triose phosphates. It is present in liver, kidney, and skeletal muscle, but is probably absent from heart and smooth muscle.

Glucose-6-Phosphate & Glucose The conversion of glucose-6-phosphate to glucose is catalyzed by **glucose- 6-phosphatase**. It is present in liver and kidney, but absent from muscle, which, therefore, cannot export glucose into the bloodstream.

Glucose-1-Phosphate & Glycogen
The breakdown of glycogen to glucose-1-phosphate is catalyzed by phosphorylase. Glycogen synthesis involves a different pathway via uridine diphosphate glucose and **glycogen synthase** .The relationships between gluconeogenesis and the glycolytic pathway are shown in Figure 1. After transamination or deamination, glucogenic amino acids yield either pyruvate or intermediates of the citric acid cycle. Therefore, the reactions described above can account for the conversion of both lactate and glucogenic amino acids to glucose or glycogen. Propionate is a major precursor of glucose in ruminants; it enters gluconeogenesis via the citric acid cycle. After esterification with CoA, propionyl-CoA is carboxylated to D-methylmalonyl-CoA, catalyzed by **propionyl-CoA carboxylase**, a biotin-dependent enzyme (Figure 2). **Methylmalonyl-CoA racemase** catalyzes the conversion of Dmethylmalonyl-CoA to L-methylmalonyl-CoA, which then undergoes isomerization to succinyl-CoA catalyzed by **methylmalonyl-CoA mutase**. In nonruminants, including human beings, propionate arises from the β - oxidation of odd-chain fatty acids that occur in ruminant lipids , as well as the oxidation of isoleucine and the side chain of cholesterol, and is a (relatively minor) substrate for gluconeogenesis. Methylmalonyl-CoA mutase is a vitamin B12-dependent

enzyme, and in deficiency, methylmalonic acid is excreted in the urine (methylmalonic aciduria).
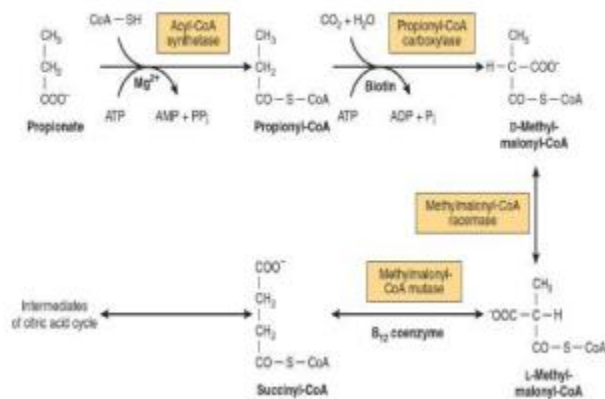
FIGURE 2 Metabolism of propionate.

Glycerol is released from adipose tissue as a result of lipolysis of lipoprotein triacylglycerol in the fed state; it may be used for reesterification of free fatty acids to triacylglycerol, or may be a substrate for gluconeogenesis in the liver. In the fasting state, glycerol released from lipolysis of adipose tissue triacylglycerol is used as a substrate for gluconeogenesis in the liver and kidneys.

GLYCOLYSIS & GLUCONEOGENESIS SHARE THE SAME PATHWAY BUT IN OPPOSITE DIRECTIONS, AND ARE RECIPROCALLY REGULATED

Changes in the availability of substrates are responsible for most changes

in metabolism either directly or indirectly acting via changes in hormone secretion. Three mechanisms are responsible for regulating the activity of enzymes concerned in carbohydrate metabolism: (1) changes in the rate of enzyme synthesis, (2) covalent modification by reversible phosphorylation, and (3) allosteric effects.

Induction & Repression of Key Enzymes Require Several Hours

The changes in enzyme activity in the liver that occur under various metabolic conditions are listed in Table 1. The enzymes involved catalyze physiologically irreversible nonequilibrium reactions. The effects are generally reinforced because the activity of the enzymes catalyzing the reactions in the opposite direction varies reciprocally (see Figure 1). The enzymes involved in the utilization of glucose (ie, those of glycolysis and lipogenesis) become more active when there is a superfluity of glucose, and under these conditions the enzymes of gluconeogenesis have low activity. Insulin, secreted in response to increased blood glucose, enhances the synthesis of the key enzymes in glycolysis. It also antagonizes the effect of the glucocorticoids and glucagon-stimulated cAMP, which induce synthesis of the key enzymes of gluconeogenesis.

	Activity in					
	Carbohydrate Feeding	Fasting and Diabetes	Inducer	Repressor	Activator	Inhibitor
Glycogenolysis, glycolysis, and pyruvate oxidation						
Glycogen synthase	↑	↓			Insulin, glucose-6-phosphate	Glucagon
Hexokinase						Glucose-6-phosphate
Glucokinase	↑	↓	Insulin	Glucagon		
Phosphofructokinase-1	↑	↓	Insulin	Glucagon	5'AMP, fructose-6-phosphate, fructose 2,6-bisphosphate, P_i	Citrate, ATP, glucagon
Pyruvate kinase	↑	↓	Insulin, fructose	Glucagon	Fructose 1,6-bisphosphate, insulin	ATP, alanine, glucagon, norepinephrine
Pyruvate dehydrogenase	↑	↓			CoA, NAD+, insulin, ADP, pyruvate	Acetyl-CoA, NADH, ATP (fatty acids, ketone bodies)
Gluconeogenesis						
Pyruvate carboxylase	↓	↑	Glucocorticoids, glucagon, epinephrine	Insulin	Acetyl-CoA	ADP
Phosphoenolpyruvate carboxykinase	↓	↑	Glucocorticoids, glucagon, epinephrine	Insulin	Glucagon	
Glucose-6-phosphatase	↓	↑	Glucocorticoids, glucagon, epinephrine	Insulin		

TABLE 1 Regulatory and Adaptive Enzymes Associated With Carbohydrate Metabolism

Covalent Modification by Reversible Phosphorylation Is Rapid

Glucagon and **epinephrine**, hormones that are responsive to a decrease in blood glucose, inhibit glycolysis and stimulate gluconeogenesis in the liver by increasing the concentration of cAMP. This in turn activates cAMPdependent protein kinase, leading to the phosphorylation

and inactivation of **pyruvate kinase**. They also affect the concentration of fructose 2,6- bisphosphate and therefore glycolysis and gluconeogenesis, as described below.

Allosteric Modification Is Instantaneous

In gluconeogenesis, pyruvate carboxylase, which catalyzes the synthesis of oxaloacetate from pyruvate, requires acetyl-CoA as an **allosteric activator**. The addition of acetyl-CoA results in a change in the tertiary structure of the protein, lowering the K_m for bicarbonate. This means that as acetyl-CoA is formed from pyruvate, it automatically ensures the provision of oxaloacetate, by activating pyruvate carboxylase. The activation of pyruvate carboxylase and the reciprocal inhibition of pyruvate dehydrogenase by acetyl-CoA derived from the oxidation of fatty acids explain the action of fatty acid oxidation in sparing the oxidation of pyruvate (and hence glucose) and stimulating gluconeogenesis. The reciprocal relationship between these two enzymes alters the metabolic fate of pyruvate as the tissue changes from carbohydrate oxidation (glycolysis) to gluconeogenesis during the transition from the fed to fasting state (see Figure 1). A major role of fatty acid oxidation in promoting gluconeogenesis is to supply the ATP that is required. **Phosphofructokinase (phosphofructokinase-1)** occupies a key position in regulating glycolysis and is also subject to feedback control. It is inhibited by citrate and by normal intracellular concentrations of ATP and is activated by 5′ AMP. At the normal intracellular [ATP] the enzymeis about 90% inhibited; this inhibition is reversed by 5′ AMP (Figure 3)
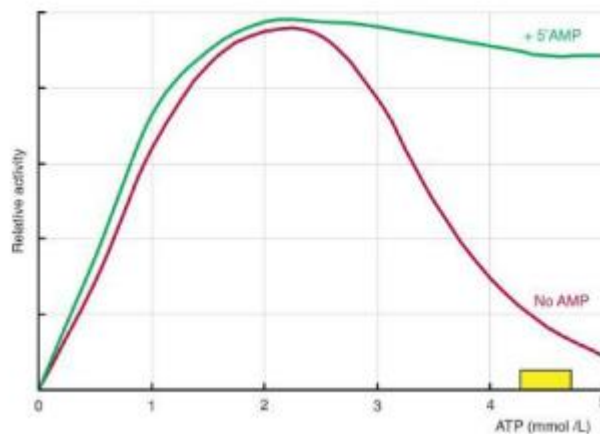
FIGURE 3 The inhibition of phosphofructokinase-1 by ATP and relief of inhibition by ATP. The yellow bar shows the normal range of the intracellular concentration of ATP.

5′ AMP acts as an indicator of the energy status of the cell. The presence of **adenylyl kinase** in liver and many other tissues allows rapid equilibration of the reaction

2ADP ↔ ATP + 5′ AMP

Thus, when ATP is used in energy-requiring processes, resulting in the formation of ADP, [AMP] increases. A relatively small decrease in [ATP] causes a several fold increase in [AMP], so that [AMP] acts as a metabolic amplifier of a small change in [ATP], and hence a sensitive signal of the energy state of the cell. The activity of phosphofructokinase-1 is thus regulated in response to the energy status of the cell to control the quantity of carbohydrate undergoing glycolysis prior to its entry into the citric acid cycle. At the same time, AMP activates glycogen phosphorylase, so increasing glycogenolysis. A consequence of the inhibition ofphosphofructokinase-

1 by ATP is an accumulation of glucose-6- phosphate, which in turn inhibits further uptake of glucose in extrahepatic tissues by inhibition of hexokinase.

Fructose 2,6-Bisphosphate Plays a Unique Role in the Regulation of Glycolysis & Gluconeogenesis in Liver

The most potent positive allosteric activator of phosphofructokinase-1 and inhibitor of fructose 1,6-bisphosphatase in liver is **fructose 2,6- bisphosphate**. It relieves inhibition of phosphofructokinase-1 by ATP and increases the affinity for fructose-6-phosphate. It inhibits fructose 1,6- bisphosphatase by increasing the K_m for fructose 1,6-bisphosphate. Its concentration is under both substrate (allosteric) and hormonal control (covalent modification) (Figure 4).
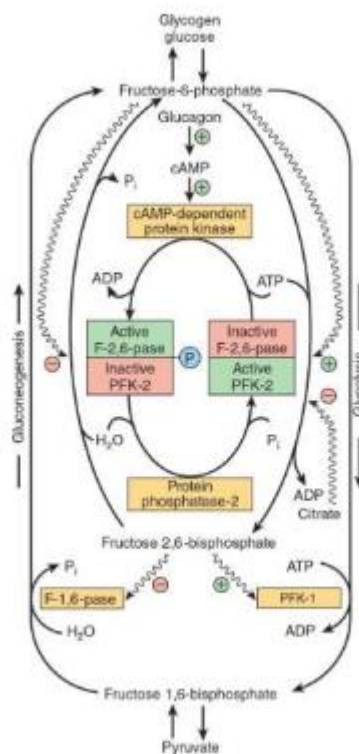
FIGURE 4 Control of glycolysis and gluconeogenesis in the liver by fructose 2,6-bisphosphate and the bifunctional enzyme PFK-2/F-2,6-Pase (6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase). (F-1,6-Pase, fructose 1,6-bisphosphatase; PFK-1, phosphofructokinase-1 [6- phosphofructo-1-kinase].) Arrows with wavy shafts indicate allosteric effects.

Fructose 2,6-bisphosphate is formed by phosphorylation of fructose-6-phosphate by **phosphofructokinase-2**. The same enzyme protein is also responsible for its breakdown, since it has **fructose 2,6-bisphosphatase** activity. This **bifunctional enzyme** is under the allosteric control of fructose-6-phosphate, which stimulates the kinase and inhibits the phosphatase. Hence, when there is an abundant

supply of glucose, the concentration of fructose 2,6-bisphosphate increases, stimulating glycolysis by activating phosphofructokinase-1 and inhibiting fructose 1,6- bisphosphatase. In the fasting state, glucagon stimulates the production of cAMP, activating cAMP-dependent protein kinase, which in turn inactivates phosphofructokinase-2 and activates fructose 2,6- bisphosphatase by phosphorylation. Hence, gluconeogenesis is stimulated by a decrease in the concentration of fructose 2,6-bisphosphate, which inactivates phosphofructokinase-1 and relieves the inhibition of fructose 1,6-bisphosphatase. Xylulose 5-phosphate, an intermediate of the pentose phosphate pathway activates the protein phosphatase that dephosphorylates the bifunctional enzyme, so increasing the formation of fructose 2,6-bisphosphate and increasing the rate of glycolysis. This leads to increased flux through glycolysis and the pentose phosphate pathway and increased fatty acid synthesis.

Substrate (Futile) Cycles Allow Fine Tuning & Rapid Response

The control points in glycolysis and glycogen metabolism involve a cycle of phosphorylation and dephosphorylation catalyzed by glucokinase and glucose-6-phosphatase; phosphofructokinase-1 and fructose 1,6- bisphosphatase; pyruvate kinase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase; and glycogen synthase and phosphorylase. It would seem obvious that these opposing enzymes are regulated in such a way that when those involved in glycolysis are active, those involved in gluconeogenesis are inactive, since otherwise there would be cycling between phosphorylated and nonphosphorylated intermediates, with net hydrolysis of ATP. While this is so, in muscle both phosphofructokinase and fructose 1,6-bisphosphatase have some activity at all times, so that there is indeed some measure of (wasteful) substrate cycling. This permits the very rapid increase in the rate of glycolysis necessary for muscle contraction. At rest the rate of phosphofructokinase activity is some

10-fold higher than that of fructose 1,6-bisphosphatase; in anticipation of muscle contraction, the activity of both enzymes increases, fructose 1,6-bisphosphatase 10 times more than phosphofructokinase, maintaining the same net rate of glycolysis. At the start of muscle contraction, the activity of phosphofructokinase increases further, and that of fructose 1,6-bisphosphatase falls, so increasing the net rate of glycolysis (and hence ATP formation) as much as a 1000-fold.

THE BLOOD CONCENTRATION OF GLUCOSE IS REGULATED WITHIN NARROW LIMITS

In the postabsorptive state, the concentration of blood glucose is maintained between 4.5 and 5.5 mmol/L. After the ingestion of a carbohydrate meal, it may rise to 6.5 to 7.2 mmol/L, and in starvation, it may fall to 3.3 to 3.9 mmol/L. A sudden decrease in blood glucose (eg, in response to insulin overdose) causes convulsions, because of the
dependence of the brain on a supply of glucose. However, much lower concentrations can be tolerated if hypoglycemia develops slowly enough for adaptation to occur. The blood glucose level in birds is considerably higher (14 mmol/L) and in ruminants considerably lower (~ 2.2 mmol/L in sheep and 3.3 mmol/L in cattle). These lower normal levels appear to be associated with the fact that ruminants ferment virtually all dietary carbohydrate to short-chain fatty acids, and these largely replace glucose as the main metabolic fuel of the tissues in the fed state.

BLOOD GLUCOSE IS DERIVED FROM THE DIET, GLUCONEOGENESIS, &GLYCOGENOLYSIS

The digestible dietary carbohydrates yield glucose, galactose, and fructose

that are transported to the liver via the **hepatic portal vein**.
Galactose and fructose are readily converted to glucose in the liver .
Glucose is formed from two groups of compounds that undergo
gluconeogenesis: (1) those that involve a direct net conversion to
glucose, including most **amino acids** and **propionate** and (2) those that
are the products of the metabolism of glucose in tissues. Thus,
lactate, formed by glycolysis in skeletal muscle and erythrocytes, is
transported to the liver and kidney where it reforms glucose, which
again becomes available via the circulation for oxidation in the
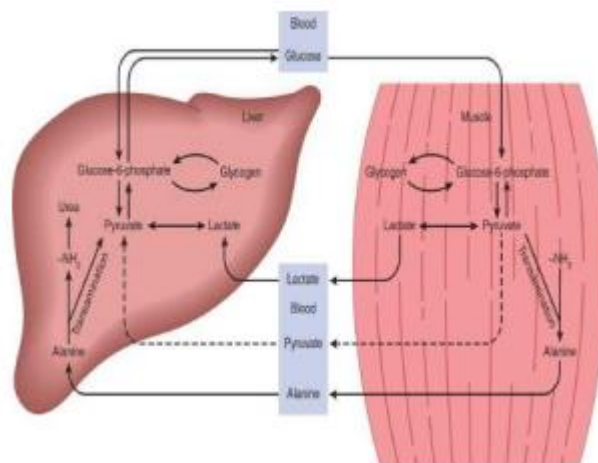tissues. This process is known as the **Cori cycle**, or the **lactic acid
cycle** (Figure 5).

FIGURE 5 The lactic acid (Cori cycle) and glucose-alanine cycles.

In the fasting state, there is a considerable output of alanine from skeletal muscle, far in excess of the amount in the muscle proteins that are being catabolized. It is formed by transamination of pyruvate produced by glycolysis of muscle glycogen, and is exported to the liver, where, after transamination back to pyruvate, it is a substrate for gluconeogenesis. This **glucose-alanine cycle** (see Figure 5) provides an indirect way of utilizing muscle glycogen to maintain blood glucose in the fasting state.

The ATP required for the hepatic synthesis of glucose from pyruvate is fromed by the oxidation of fatty acids. Glucose is also formed from liver glycogen by glycogenolysis .

Metabolic & Hormonal Mechanisms Regulate the Concentration of Blood Glucose

The maintenance of a stable blood glucose concentration is one of the most finely regulated of all homeostatic mechanisms, involving the liver, extrahepatic tissues, and several hormones. Liver cells are freely permeable to glucose in either direction (via the GLUT 2 transporter), whereas cells of extrahepatic tissues (apart from pancreatic β-islets) are relatively impermeable, and their unidirectional glucose transporters are regulated by insulin. As a result, uptake from the bloodstream is the ratelimiting step in the utilization of glucose in extrahepatic tissues. The role of various glucose transporter proteins found in cell membranes is shown in Table 2.

TABLE 2 Major Glucose Transporters

	Tissue Location	Functions
Facilitative bidirectional transporters		
GLUT 1	Brain, kidney, colon, placenta, erythrocytes	Glucose uptake
GLUT 2	Liver, pancreatic β-cell, small intestine, kidney	Rapid uptake or release of glucose
GLUT 3	Brain, kidney, placenta	Glucose uptake
GLUT 4	Heart and skeletal muscle, adipose tissue	Insulin-stimulated glucose uptake
GLUT 5	Small intestine	Absorption of fructose
Sodium-dependent unidirectional transporter		
SGLT 1	Small intestine and kidney	Active uptake of glucose against a concentration gradient

Glucokinase Is Important in Regulating Blood Glucose After a Meal

Hexokinase has a low K_m for glucose, and in the liver it is saturated and acting at a constant rate under all normal conditions. It thus acts to ensure an adequate rate of glycolysis to meet the liver's needs. Glucokinase is an allosteric enzyme with a considerably higher apparent K_m (lower affinity) for glucose, so that its activity increases with increases in the concentration of glucose in the hepatic portal vein (Figure 6). In the fasting state, glucokinase is located in the nucleus. In response to an increased intracellular concentration of glucose it migrates into the cytosol, mediated by the carbohydrate response element–binding protein (CREBP). It thus permits hepatic uptake of large amounts of glucose after a carbohydrate meal, for glycogen and fatty acid synthesis, so that while the concentration of glucose in the hepatic portal vein may reach 20 mmol/L after a meal, that leaving the liver into the

peripheral circulation does not normally exceed 8 to 9 mmol/L. Glucokinase is absent from the liver of ruminants, which have little glucose entering the portal circulation from the intestines.
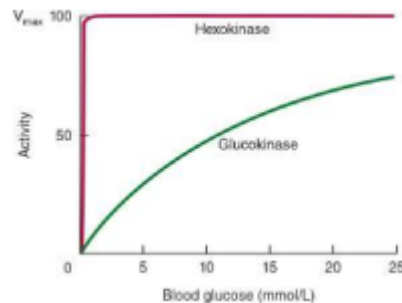
FIGURE 6 Variation in glucose phosphorylating activity of hexokinase and glucokinase with increasing blood glucose concentration.

The K m for glucose of hexokinase is 0.05 mmol/L and of glucokinase is 10 mmol/L. At normal peripheral blood glucose concentrations (4.5–5.5 mmol/L), the liver is a net producer of glucose. However, as the glucose level rises, the output of glucose ceases, and there is a net uptake.

Insulin and Glucagon Play a Central Role in Regulating Blood Glucose

In addition to the direct effects of hyperglycemia in enhancing the uptake of glucose into the liver, the hormone **insulin** plays a central role in regulating blood glucose. It is produced by the β cells of the islets of Langerhans in the pancreas in response to hyperglycemia. The β-islet cells are freely permeable to glucose via the GLUT 2 transporter, and the glucose is phosphorylated by glucokinase.

Therefore, increasing blood glucose increases metabolic flux through glycolysis, the citric acid cycle, and the generation of ATP. The increase in [ATP] inhibits ATP-sensitive K+ channels, causing depolarization of the cell membrane, which increases Ca2+ influx via voltage-sensitive Ca2+ channels, stimulating exocytosis of insulin. Thus, the concentration of insulin in the blood parallels that of the blood glucose. Other substances causing release of insulin from the pancreas include amino acids, nonesterified fatty acids, ketone bodies, glucagon, secretin, and the sulfonylurea drugs tolbutamide and glyburide. These drugs are used to stimulate insulin secretion in type 2 diabetes mellitus via the ATP-sensitive K+ channels. Epinephrine and norepinephrine block the release of insulin. Insulin acts to lower blood glucose immediately by enhancing glucose transport into adipose tissue and muscle by recruitment of glucose transporters (GLUT 4) from the interior of the cell to the plasma membrane. Although it does not affect glucose uptake into the liver directly, insulin does enhance long-term uptake as a result of its actions on the enzymes controlling glycolysis, glycogenesis, and gluconeogenesis.

Glucagon is the hormone produced by the α cells of the pancreatic islets in response to hypoglycemia. In the liver, it stimulates glycogenolysis by activating glycogen phosphorylase. Unlike epinephrine, glucagon does not have an effect on muscle phosphorylase. Glucagon also enhances gluconeogenesis from amino acids and lactate. In all these actions, glucagon acts via generation of cAMP (Table 1). Both hepatic glycogenolysis and gluconeogenesis contribute to the **hyperglycemic effect** of glucagon, whose actions oppose those of insulin. Most of the endogenous glucagon (and insulin) is cleared from the circulation by the liver (Table 3).

TABLE 3 Tissue Responses to Insulin and Glucagon

	Liver	Adipose Tissue	Muscle
Increased by insulin	Fatty acid synthesis Glycogen synthesis Protein synthesis	Glucose uptake Fatty acid synthesis	Glucose uptake Glycogen synthesis Protein synthesis
Decreased by insulin	Ketogenesis Gluconeogenesis	Lipolysis	
Increased by glucagon	Glycogenolysis Gluconeogenesis Ketogenesis	Lipolysis	

Other Hormones Affect Blood Glucose

The **anterior pituitary gland** secretes hormones that tend to elevate blood glucose and therefore antagonize the action of insulin. These are growth hormone, adrenocorticotropic hormone (ACTH), and possibly other "diabetogenic" hormones. Growth hormone secretion is stimulated by hypoglycemia; it decreases glucose uptake in muscle. Some of this effect may be indirect, since it stimulates mobilization of nonesterified fattyacids from adipose tissue, which themselves inhibit glucose utilization. The **glucocorticoids** (11-oxysteroids) are secreted by the adrenal cortex, and are also synthesized in an unregulated manner in adipose tissue. They act to increase gluconeogenesis as a result of enhanced hepatic catabolism of amino acids, due to induction of aminotransferases (and other enzymes such as tryptophan dioxygenase) and key enzymes of gluconeogenesis. In addition, glucocorticoids inhibit the utilization of glucose in extrahepatic tissues. In all these actions, glucocorticoids act in a

manner antagonistic to insulin. A number of **cytokines** secreted by macrophages infiltrating adipose tissue also have insulin antagonistic actions; together with glucocorticoids secreted by adipose tissue, this explains the insulin resistance that commonly occurs in obese people.

Epinephrine is secreted by the adrenal medulla as a result of stressful stimuli (fear, excitement, hemorrhage, hypoxia, hypoglycemia, etc.) and leads to glycogenolysis in liver and muscle owing to stimulation of phosphorylase via generation of cAMP. In muscle, glycogenolysis results in increased glycolysis, whereas in liver, it results in the release of glucose into the bloodstream.

FURTHER CLINICAL ASPECTS Glucosuria Occurs When the Renal Threshold for Glucose Is Exceeded

When the blood glucose concentration rises above about 10 mmol/L, the kidney also exerts a (passive) regulatory effect. Glucose is continuously filtered by the glomeruli, but is normally completely reabsorbed in the renal tubules by active transport. The capacity of the tubular system to reabsorb glucose is limited to a rate of about 2 mmol/min, and in hyperglycemia (as occurs in poorly controlled diabetes mellitus), the glomerular filtrate may contain more glucose than can be reabsorbed, resulting in **glucosuria** when the **renal threshold** for glucose is exceeded.

Hypoglycemia May Occur During Pregnancy & in the Neonate

During pregnancy, fetal glucose consumption increases and there is a risk of maternal, and possibly fetal, hypoglycemia, particularly if there are long intervals between meals or at night. Furthermore, premature and low-birthweight babies are more susceptible to hypoglycemia, since they have little adipose tissue to provide

nonesterified fatty acids. The enzymes of gluconeogenesis may not be fully developed at this time, and gluconeogenesis is anyway dependent on a supply of nonesterified fatty acids for ATP formation. Little glycerol, which would normally be released from adipose tissue, is available for gluconeogenesis.

The Ability to Utilize Glucose May Be Ascertained by Measuring Glucose Tolerance

Glucose tolerance is the ability to regulate the blood glucose concentration after the administration of a test dose of glucose (normally 1 g/kg body weight) (Figure 7).
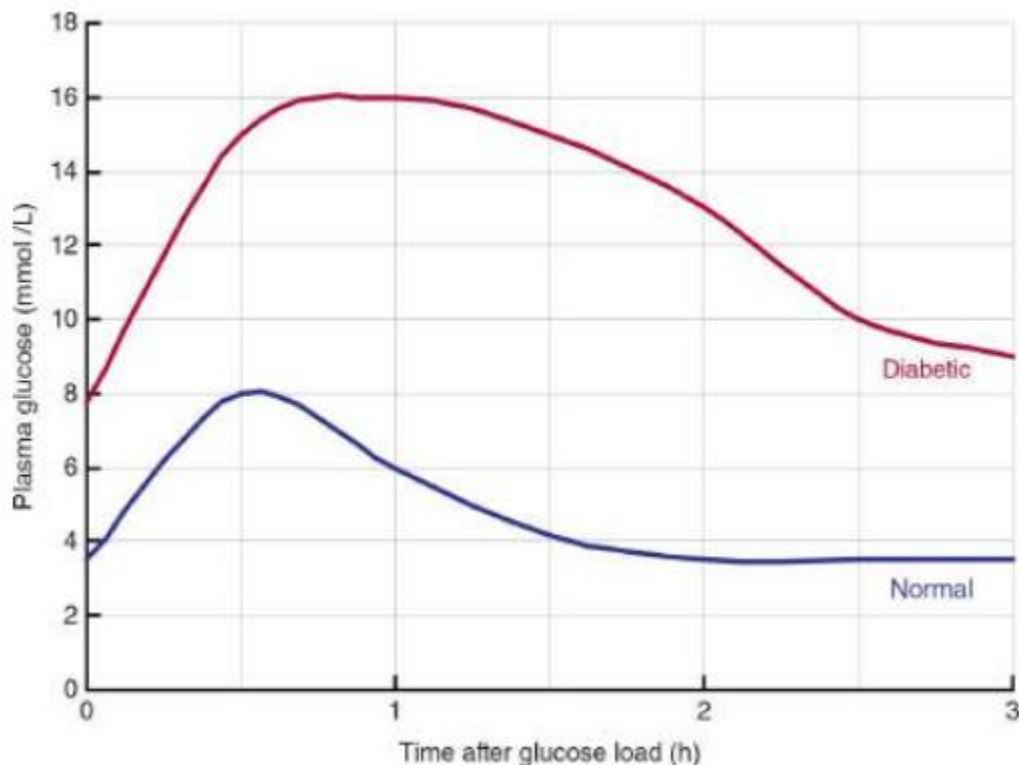
FIGURE 7 Glucose tolerance test. Blood glucose curves of a normal and a diabetic person after oral administration of 1 g of glucose/kg body weight. Note the initial raised concentration in the fasting diabetic. A criterion of normality is the return to the initial value within 2 hours.

Diabetes mellitus (type 1, or insulin-dependent diabetes mellitus [IDDM]) is characterized by impaired glucose tolerance as a result of decreased secretion of insulin because of progressive destruction of pancreatic β-islet cells. Glucose tolerance is also impaired in type 2 diabetes mellitus (noninsulin-dependent diabetes [NIDDM]) as a result of reduced sensitivity of tissues to insulin action. Insulin resistance associated with obesity (and especially abdominal obesity) leading to the development of hyperlipidemia, then atherosclerosis and coronary heart disease, as well as overt diabetes, is known as the **metabolic syndrome**. Impaired glucose tolerance also occurs in conditions where the liver is damaged, in some infections, and in response to some drugs, as well as in conditions that lead to hyperactivity of the pituitary gland or adrenal cortex because of the hormones secreted by these glands that antagonize the action of insulin. Administration of insulin (as in the treatment of diabetes mellitus) lowers the blood glucose concentration and increases its utilization and storage in the liver and muscle as glycogen. An excess of insulin may cause **hypoglycemia**, resulting in convulsions and even death unless glucose is administered promptly. Increased tolerance to glucose is observed in pituitary or adrenocortical insufficiency, attributable to a decrease in the antagonism to insulin by the

hormones normally secreted by these glands.

The Energy Cost of Gluconeogenesis Explains WhyVery Low Carbohydrate Diets Promote Weight Loss

Very low carbohydrate diets, providing only 20 g per day of carbohydrate or less (compared with a desirable intake of 100–120 g/day), but permitting unlimited consumption of fat and protein, have been promoted as an effective regime for weight loss, although such diets are counter to all advice on a prudent diet for health. Since there is a continual demand for glucose, there will be a considerable amount of gluconeogenesis from amino acids; the associated high ATP cost must then be met by oxidation of fatty acids.

SUMMARY

-----Gluconeogenesis is the process of synthesizing glucose or glycogen from noncarbohydrate precursors. It is of particular importance when carbohydrate is not available from the diet. The main substrates are amino acids, lactate, glycerol, and propionate.

-----The pathway of gluconeogenesis in the liver and kidney utilizes those reactions in glycolysis that are reversible plus four additional reactions that circumvent the irreversible nonequilibrium reactions.

-----Since glycolysis and gluconeogenesis share the same pathway but operate in opposite directions, their activities must be regulated reciprocally.

-------The liver regulates the blood glucose concentration after a meal because it contains the high K_m glucokinase that promotes increased hepatic utilization of glucose.

-----Insulin is secreted as a direct response to hyperglycemia; it stimulates the liver to store glucose as glycogen and increases uptake of glucose into extrahepatic tissues.

----Glucagon is secreted as a response to hypoglycemia and activates

both glycogenolysis and gluconeogenesis in the liver, causing release of glucose into the blood.

Glycolysis & the Oxidation of pyruvate

BIOMEDICAL IMPORTANCE

Most tissues have at least some requirement for glucose; in the brain, the requirement is substantial- even in prolonged fasting the brain can meet no more than about 20% of its energy needs from ketone bodies. Glycolysis is the main pathway of glucose (and other carbohydrate) metabolism. It occurs in the cytosol of all cells, and can function either aerobically or anaerobically, depending on the availability of oxygen and the electron transport chain (and hence of the presence of mitochondria).

Erythrocytes, which lack mitochondria, are completely reliant on glucose as their metabolic fuel, and metabolize it by anaerobic glycolysis. The ability of glycolysis to provide ATP in the absence of oxygen allows skeletal muscle to perform at very high levels of work output when oxygen supply is insufficient, and it allows tissues to survive anoxic episodes. However, heart muscle, which is adapted for aerobic performance, has relatively low glycolytic activity and poor survival under conditions of **ischemia.** Diseases in which enzymes of glycolysis (eg, pyruvate kinase) are deficient are mainly seen as **hemolytic anemias** or, if the defect affects skeletal muscle (eg, phosphofructokinase), as **fatigue.** In fast-growing cancer cells, glycolysis proceeds at a high rate, forming large amounts of pyruvate, which is reduced to lactate and exported. This produces a relatively acidic local environment in the tumor. The lactate is used for gluconeogenesis in the liver. an energy expensive

process, which is responsible for much of the **hypermetabolism** seen in **cancer cachexia**. **Lactic acidosis** results from various causes, including impaired activity of pyruvate dehydrogenase, especially in
thiamin (vitamin B1) deficiency.

GLYCOLYSIS CAN FUNCTION UNDER ANAEROBIC CONDITIONS

Early in the investigations of glycolysis, it was realized that fermentation in yeast was similar to the breakdown of glycogen in muscle. When a muscle contracts under anaerobic conditions, **glycogen disappears** and **lactate appears**. When oxygen is admitted, aerobic recovery takes place and lactate is no longer produced. If muscle contraction occurs under aerobic conditions, lactate does not accumulate and pyruvate is the end product of glycolysis. Pyruvate is oxidized further to CO_2 and water
([Figure 1]). When oxygen is in short supply, mitochondrial reoxidation of NADH formed during glycolysis is impaired, and NADH is reoxidized by reducing pyruvate to lactate, so permitting glycolysis to continue. While glycolysis can occur under anaerobic conditions, this has a price, for it limits the amount of ATP formed per mole of glucose oxidized, so that much more glucose must be metabolized under anaerobic than aerobic conditions ([Table 1]). In yeast and some other microorganisms, pyruvate formed in anaerobic glycolysis is not reduced to lactate, but is decarboxylated and reduced to ethanol.
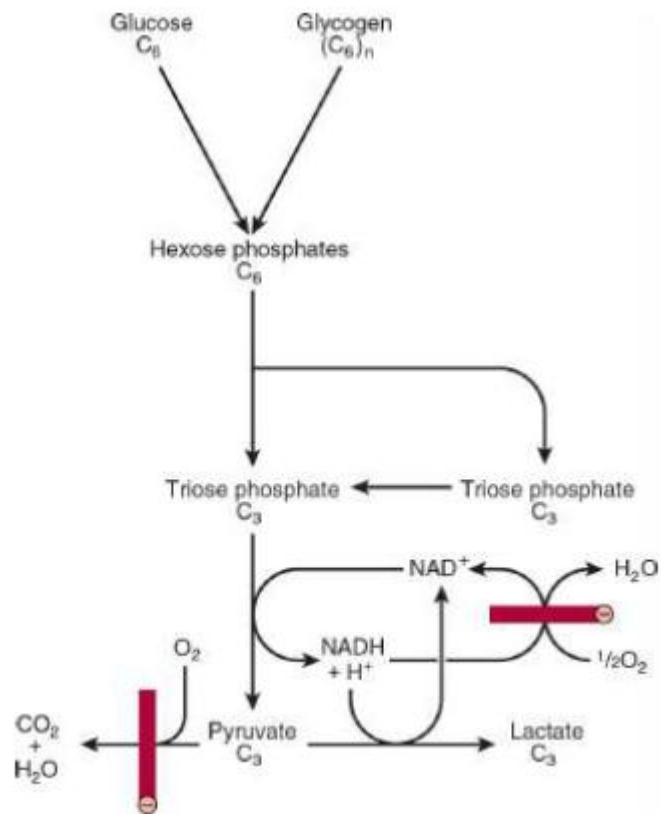
FIGURE 1 Summary of glycolysis. _, blocked under anaerobic conditions or by absence of mitochondria containing key respiratory enzymes, as in erythrocytes.

TABLE 1 ATP Formation in the Catabolism of Glucose

Pathway	Reaction Catalyzed by	Method of ATP Formation	ATP per mol of Glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	5[a]
	Phosphoglycerate kinase	Substrate-level phosphorylation	2
	Pyruvate kinase	Substrate-level phosphorylation	2
			9
	Consumption of ATP for reactions of hexokinase and phosphofructokinase		-2
			Net 7
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
	Succinate thiokinase	Substrate-level phosphorylation	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 FADH$_2$	3
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
			Net 25
	Total per mol of glucose under aerobic conditions		32
	Total per mol of glucose under anaerobic conditions		2

[a]This assumes that NADH formed in glycolysis is transported into mitochondria by the malate shuttle (see Figure 13–13). If the glycerophosphate shuttle is used, then only 1.5 ATP will be formed per mol of NADH. There is a considerable advantage in using glycogen rather than glucose for anaerobic glycolysis in muscle, since the product of glycogen phosphorylase is glucose-1-phosphate (see Figure 18–1), which is interconvertible with glucose-6-phosphate. This saves the ATP that would otherwise be used by hexokinase, increasing the net yield of ATP from 2 to 3 per glucose.

THE REACTIONS OF GLYCOLYSIS CONSTITUTE THE MAIN PATHWAY OF GLUCOSE UTILIZATION

The overall equation for glycolysis from glucose to lactate is as follows:

Glucose + 2 ADP + 2 Pi → 2 Lactate + 2 ATP + 2 H2O
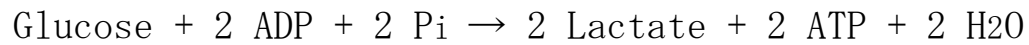
All of the enzymes of glycolysis (Figure 2) are cytosolic. Glucose enters glycolysis by phosphorylation to glucose-6-phosphate, catalyzed by **hexokinase**, using ATP as the phosphate donor. Under physiological conditions, the phosphorylation of glucose to glucose-6-phosphate can be regarded as irreversible. Hexokinase is inhibited allosterically by its product, glucose-6-phosphate.

Glycogen

Glucose-1-phosphate

Hexokinase, glucokinase

Phosphohexose isomerase

Phosphofructokinase

Glucose 6-phosphatase

Fructose bisphosphatase

Aldolase

Triose phosphate isomerase

Glucose

Glucose-6-phosphate

Fructose-6-phosphate

Fructose 1,6-bisphosphate

Dihydroxyacetone phosphate

Glyceraldehyde-3-phosphate

2 × 3 carbon sugar molecules per glucose

Glyceraldehyde-3-phosphate dehydrogenase

Pyruvate kinase

Enolase

Phosphoglyceromutase

Phosphoglycerate kinase

Pyruvate

Phosphoenolpyruvate

2-Phosphoglycerate

3-Phosphoglycerate

Bisphosphoglycerate

FIGURE 17–2 The pathway of glycolysis. ($\oplus$, —PO_3^{2-}; P_i, $HOPO_3^{2-}$; $\ominus$, inhibition.) Carbons 1–3 of fructose bisphosphate form dihydroxyacetone phosphate, and carbons 4–6 form glyceraldehyde-3-phosphate.

In tissues other than the liver (and pancreatic β-islet cells), the availability of glucose for glycolysis (or glycogen synthesis in muscle, and lipogenesis in adipose tissue, is controlled by transport into the cell, which in turn is regulated by **insulin**. Hexokinase has a high affinity (low K_m) for glucose, and in the liver, it is saturated under normal conditions, and so acts at a constant rate to provide glucose-6-phosphate to meet the liver's needs. Liver cells also contain an isoenzyme of hexokinase, **glucokinase**, which has a K_m very much higher than the normal intracellular concentration of glucose. The function of
glucokinase in the liver is to remove glucose from the hepatic portal blood following a meal, so regulating the concentration of glucose available to peripheral tissues. This provides more glucose-6-phosphate than is required for glycolysis; it is used for glycogen synthesis and lipogenesis. Glucokinase is also found in pancreatic β-islet cells, where it functions to detect high concentrations of glucose in the portal blood. As more glucose is phosphorylated by glucokinase, there is increased glycolysis, leading to increased formation of ATP. This leads to closure of an ATP-potassium channel, causing membrane depolarization and opening of a voltage-gated
calcium channel. The resultant influx of calcium ions leads to fusion of the insulin secretory granules with the cell membrane and the release of insulin. Glucose-6-phosphate is an important compound at the junction of

several metabolic pathways: glycolysis, gluconeogenesis
, the pentose phosphate pathway , glycogenesis, and
glycogenolysis. In glycolysis, it is converted to
fructose-
6-phosphate by **phosphohexose isomerase**, which involves
an aldose- ketose isomerization. This reaction is
followed by another phosphorylation catalyzed by the
enzyme **phosphofructokinase** (phosphofructokinase-1)
forming fructose 1,6-bisphosphate. The
phosphofructokinase reaction is irreversible under
physiological conditions. Phosphofructokinase is both
inducible and subject to allosteric regulation, and has
a major role in regulating the rate of glycolysis.
Fructose 1,6-bisphosphate is cleaved by
aldolase (fructose 1,6-bisphosphate aldolase) into two
triose phosphates, glyceraldehyde-3-phosphate and
dihydroxyacetone phosphate, which are interconverted by
the enzyme **phosphotriose isomerase.**
Glycolysis continues with the oxidation of
glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.
The enzyme catalyzing this oxidation, **glyceraldehyde-3-
phosphate dehydrogenase**, is NAD dependent.
Structurally, it consists of four identical
polypeptides (monomers) forming a tetramer. Four —SH
groups are present on each polypeptide, derived from
cysteine residues within the polypeptide chain. One of
the —SH groups is found at the active site of the
enzyme (Figure 3). The substrate initially combines
with this —SH group, forming a thiohemiacetal that is
oxidized to a thiol ester; the hydrogens removed in
this oxidation are transferred to NAD+. The thiol ester
then undergoes phosphorolysis; inorganic phosphate (Pi)
is added, forming 1,3- bisphosphoglycerate, and the
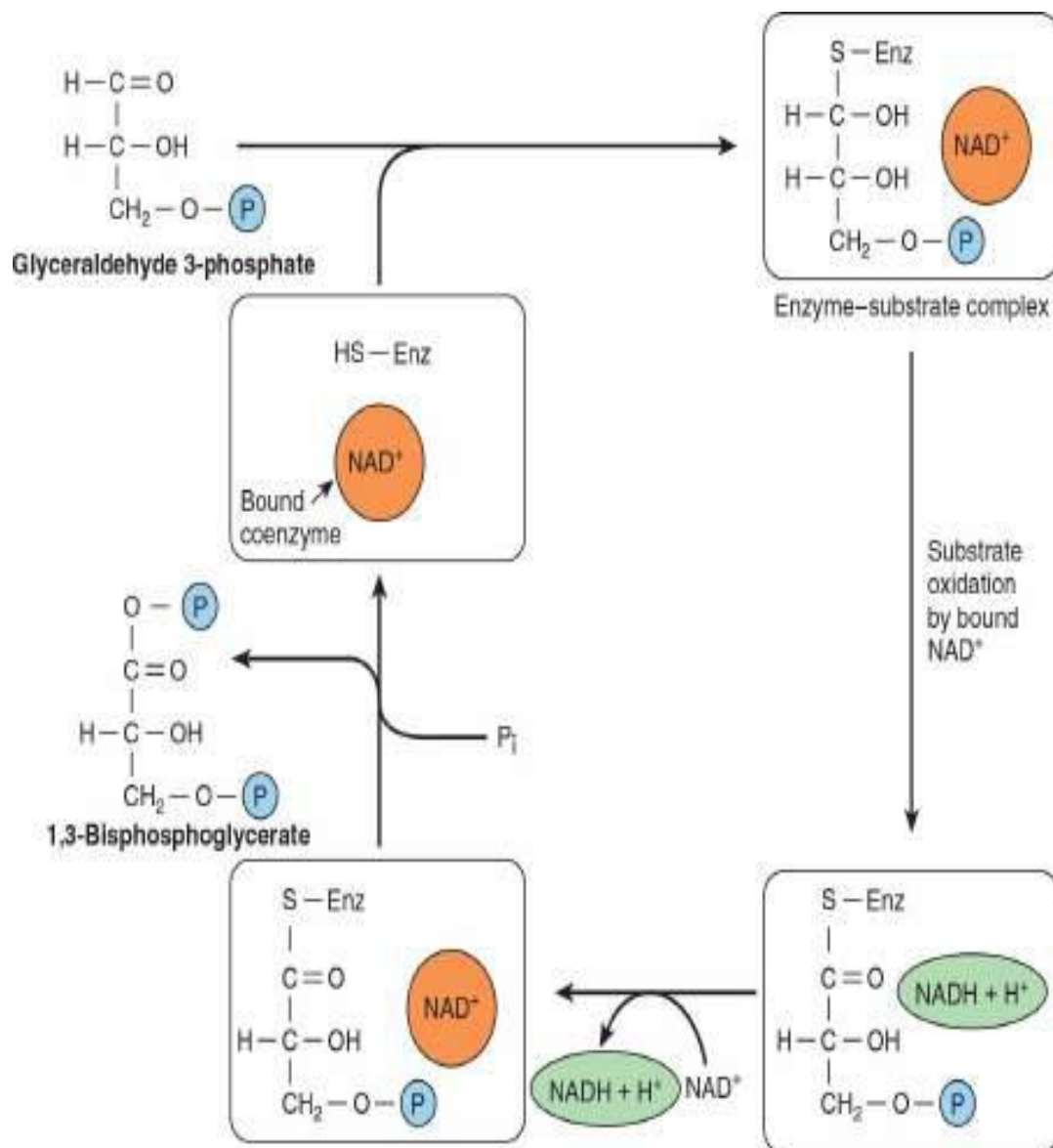free —SH group.

FIGURE 3 Mechanism of oxidation of glyceraldehyde-3-phosphate. (Enz, glyceraldehyde-3-phosphate dehydrogenase.) The enzyme is inhibited by the —SH

poison iodoacetate, which is thus able to inhibit glycolysis. The NADH produced on the enzyme is not so firmly bound to the enzyme as is NAD+. Consequently, NADH is easily
displaced by another molecule of NAD+.

In the next reaction, catalyzed by **phosphoglycerate kinase**, phosphate is transferred from 1,3 bisphosphoglycerate onto ADP, forming ATP (substrate-level phosphorylation) and 3-phosphoglycerate. Since two molecules of triose phosphate are formed per molecule of glucose metabolized, $2\times$ ATP are formed in this reaction per molecule of glucose undergoing glycolysis. The toxicity of arsenic is the result of competition of arsenate with inorganic phosphate (Pi) forming 1-arseno-3-phosphoglycerate, which undergoes spontaneous hydrolysis to 3- phosphoglycerate without forming ATP. 3-Phosphoglycerate is isomerized to 2-phosphoglycerate by **phosphoglycerate mutase**. It is likely that 2,3-bisphosphoglycerate (diphosphoglycerate, DPG) is an intermediate in this reaction. The subsequent step is catalyzed by **enolase** and involves a dehydration, forming phosphoenolpyruvate. Enolase is inhibited by **fluoride**, and when blood samples are taken for measurement of glucose,
glycolysis is inhibited by taking the sample into tubes containing fluoride. Enolase is also dependent on the presence of either Mg2+ or Mn2+ ions. The phosphate of phosphoenolpyruvate is transferred to ADP in another substrate-level phosphorylation catalyzed by **pyruvate kinase** to form $2\times$ ATP per molecule of glucose oxidized. The reaction of pyruvate kinase is essentially irreversible under physiological

conditions, partly because of the large free-energy change involved and partly because the immediate product of the enzyme-catalyzed reaction is enolpyruvate, which undergoes spontaneous isomerization to pyruvate, so that the product of the reaction is not available to undergo the reverse reaction. The availability of oxygen now determines which of the two pathways
is followed. Under **anaerobic conditions**, the NADH cannot be reoxidized through the respiratory chain, and pyruvate is reduced to lactate catalyzed by **lactate dehydrogenase**. This permits the oxidization of NADH, permitting another molecule of glucose to undergo glycolysis. Under **aerobic conditions**, pyruvate is transported into mitochondria and
undergoes oxidative decarboxylation to acetyl-CoA then oxidation to CO_2 in the citric acid cycle (see Chapter 16). The reducing equivalents from the NADH formed in glycolysis are taken up into mitochondria for oxidation via either the malate-aspartate shuttle or the glycerophosphate shuttle.

TISSUES THAT FUNCTION UNDER HYPOXIC CONDITIONS PRODUCE LACTATE

This is true of skeletal muscle, particularly the white fibers, where the rate of work output, and hence the need for ATP formation, may exceed the rate at which oxygen can be taken up and utilized. Glycolysis in erythrocytes always terminates in lactate, because the subsequent reactions
of pyruvate oxidation are mitochondrial, and erythrocytes lack mitochondria. Other tissues that normally derive much of their energy from glycolysis and produce lactate include brain, gastrointestinal

tract, renal medulla, retina, and skin. Lactate
production is also increased in septic shock, and many
cancers also produce lactate. The liver, kidneys,
and heart normally take up lactate and oxidize it, but
produce it under hypoxic conditions. When lactate
production is high, as in vigorous exercise, septic
shock,
and cancer cachexia, much is used in the liver for
gluconeogenesis, leading to an increase in metabolic
rate to provide the ATP and GTP needed. The increase in
oxygen consumption as a result of increased oxidation
of metabolic fuels to provide the ATP and GTP needed
for gluconeogenesis is seen as **oxygen debt** after
vigorous
exercise. Under some conditions, lactate may be formed
in the cytosol, but then enter the mitochondrion to be
oxidized to pyruvate for onward metabolism. This
provides a pathway for the transfer of reducing
equivalents from the cytotol into the mitochondrion for
the electron transport chain in addition to the
glycerophosphate and
malate-aspartate shuttles.

GLYCOLYSIS IS REGULATED AT THREE STEPS
INVOLVING NONEQUILIBRIUM REACTIONS

Although most of the reactions of glycolysis are freely
reversible, three are markedly exergonic and must
therefore be considered to be physiologically
irreversible. These reactions, catalyzed by **hexokinase**
(and glucokinase), **phosphofructokinase**, and **pyruvate
kinase**, are the
major sites of regulation of glycolysis.
Phosphofructokinase is significantly inhibited at
normal intracellular concentrations of ATP, this
inhibition can be rapidly relieved by 5′ AMP that is

formed as ADP begins to accumulate, signaling the need for an increased rate of glycolysis. Cells that are capable of **gluconeogenesis** (reversing the glycolytic pathway) have different enzymes that catalyze reactions to reverse these irreversible steps: glucose-6- phosphatase, fructose 1,6-bisphosphatase and, to reverse the reaction of pyruvate kinase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase. The reciprocal regulation of phosphofructokinase in glycolysis and fructose 1,6-bisphosphatase in gluconeogenesis is discussed in Chapter 19.
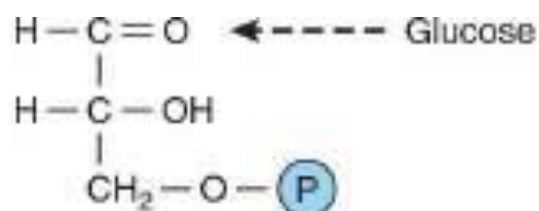
Fructose enters glycolysis by phosphorylation to fructose-1-phosphate, and bypasses the main regulatory steps, so resulting in formation of more pyruvate and acetyl-CoA than is required for ATP formation. In the liver and adipose tissue, this leads to increased lipogenesis, and a high intake of fructose may be a factor in the development of obesity.

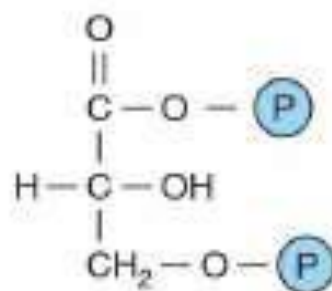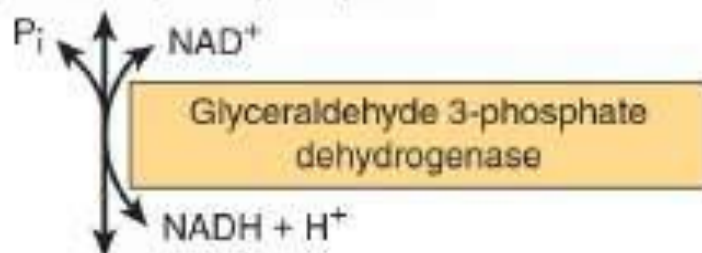## In Erythrocytes, the First Site of ATP Formation in Glycolysis May Be Bypassed

In erythrocytes, the reaction catalyzed by **phosphoglycerate kinase** may be bypassed to some extent by the reaction of **bisphosphoglycerate mutase**, which catalyzes the conversion of 1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate, followed by hydrolysis to 3-phosphoglycerate and Pi, catalyzed by **2,3-bisphosphoglycerate phosphatase** (Figure 4). This pathway involves no net yield of ATP from glycolysis, but provides 2,3- bisphosphoglycerate, which binds to hemoglobin, decreasing its affinity for oxygen, so making oxygen more readily available to tissues.
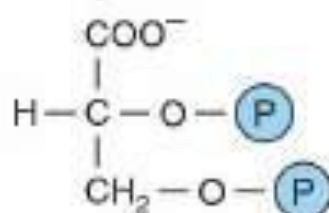
H—C=O ◄----- Glucose

H—C—OH

CH₂—O—(P)

Glyceraldehyde 3-phosphate

P_i ⤴ NAD⁺

Glyceraldehyde 3-phosphate
dehydrogenase

NADH + H⁺

O
‖
C—O—(P)

H—C—OH

CH₂—O—(P)

Bisphosphoglycerate
mutase

1,3-Bisphosphoglycerate

ADP

Phosphoglycerate
kinase

ATP

COO⁻

H—C—O—(P)

CH₂—O—(P)

2,3-Bisphosphoglycerate

P_i

COO⁻

H—C—OH

CH₂—O—(P)

2,3-Bisphosphoglycerate
phosphatase

3-Phosphoglycerate

L----▸ Pyruvate

FIGURE 4 The 2,3-bisphosphoglycerate pathway in erythrocytes.

THE OXIDATION OF PYRUVATE TO ACETYLCOA IS THE IRREVERSIBLE ROUTE FROM GLYCOLYSIS TO THE CITRIC ACID CYCLE

Pyruvate is transported into the mitochondrion by a proton symporter. It then undergoes oxidative decarboxylation to acetyl-CoA, catalyzed by a multienzyme complex that is associated with the inner mitochondrial membrane. This **pyruvate dehydrogenase complex** is analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle. Pyruvate is decarboxylated by the **pyruvate dehydrogenase** component of the enzyme complex to a hydroxyethyl derivative of the thiazole ring of enzyme-bound **thiamin diphosphate**, which in turn reacts with oxidized lipoamide, the prosthetic group of **dihydrolipoyl transacetylase**, to form acetyl lipoamide (Figure 5). In thiamin (vitamin B1 deficiency, glucose metabolism is impaired, and there is significant (and potentially life-threatening) lactic and pyruvic acidosis. Acetyl lipoamide reacts with coenzyme A to form acetyl-CoA and reduced lipoamide. The reaction is completed when the reduced lipoamide is reoxidized by a flavoprotein, **dihydrolipoyl dehydrogenase**, containing flavin adenine dinucleotide (FAD). Finally, the reduced flavoprotein is oxidized by NAD+, which in turn transfers reducing equivalents to the respiratory chain. The overall reaction is:
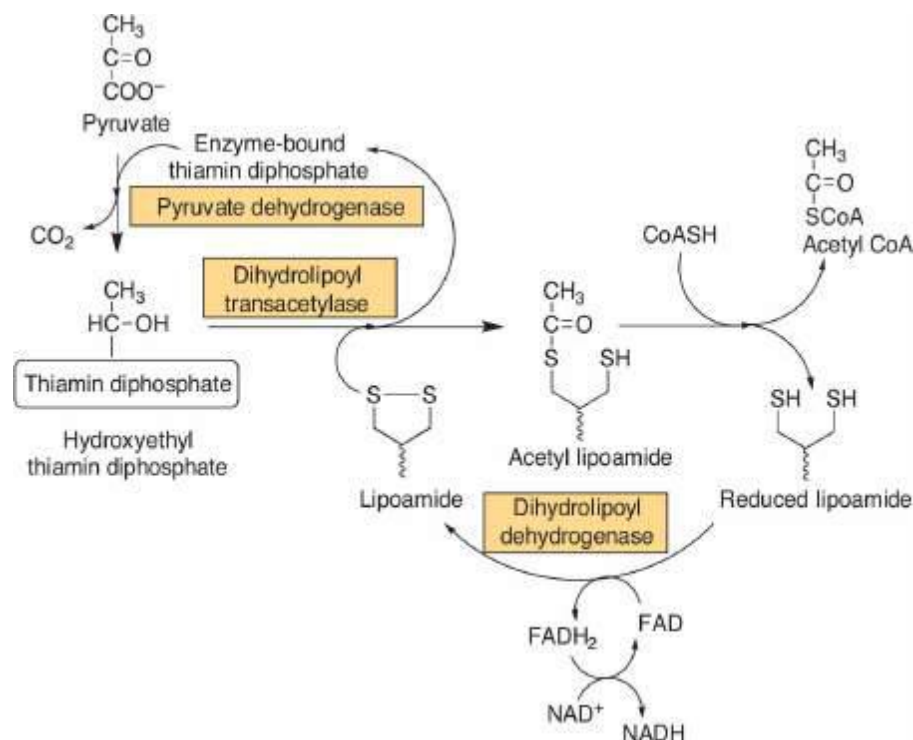
FIGURE 5 Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex.
Lipoic acid is joined by an amide link to a lysine residue of the transacetylase component of the enzyme complex. It
forms a long flexible arm, allowing the lipoic acid prosthetic group to rotate sequentially between the active sites of each of the enzymes of the complex.
(FAD, flavin adenine dinucleotide; NAD+, nicotinamide adenine dinucleotide.)
Pyruvate + NAD+ + CoA → Acetyl-CoA + NADH + H+ + CO2
The pyruvate dehydrogenase complex consists of a number of polypeptide chains of each of the three component enzymes and the intermediates do not dissociate, but are channeled from one enzyme site to the next. This

increases the rate of reaction and prevents side reactions.

Pyruvate Dehydrogenase Is Regulated by End-Product Inhibition & Covalent Modification

Pyruvate dehydrogenase is inhibited by its products, acetyl-CoA and NADH (Figure 6). It is also regulated by phosphorylation (catalyzed by a kinase) of three serine residues on the pyruvate dehydrogenase component of the multienzyme complex, resulting in decreased activity and by dephosphorylation (catalyzed by a phosphatase) that causes an increase in activity. The kinase is activated by increases in the [ATP]/[ADP], [acetyl-CoA]/[CoA], and [NADH]/[NAD+] ratios. Thus, pyruvate dehydrogenase, and therefore glycolysis, is inhibited both when there is adequate ATP (and reduced coenzymes for ATP formation)
available, and also when fatty acids are being oxidized. In fasting, when nonesterified fatty acid concentrations increase, there is a decrease in the proportion of the enzyme in the active form, leading to a sparing of carbohydrate. In adipose tissue, where glucose provides acetyl-CoA for lipogenesis, the enzyme is activated in response to insulin.
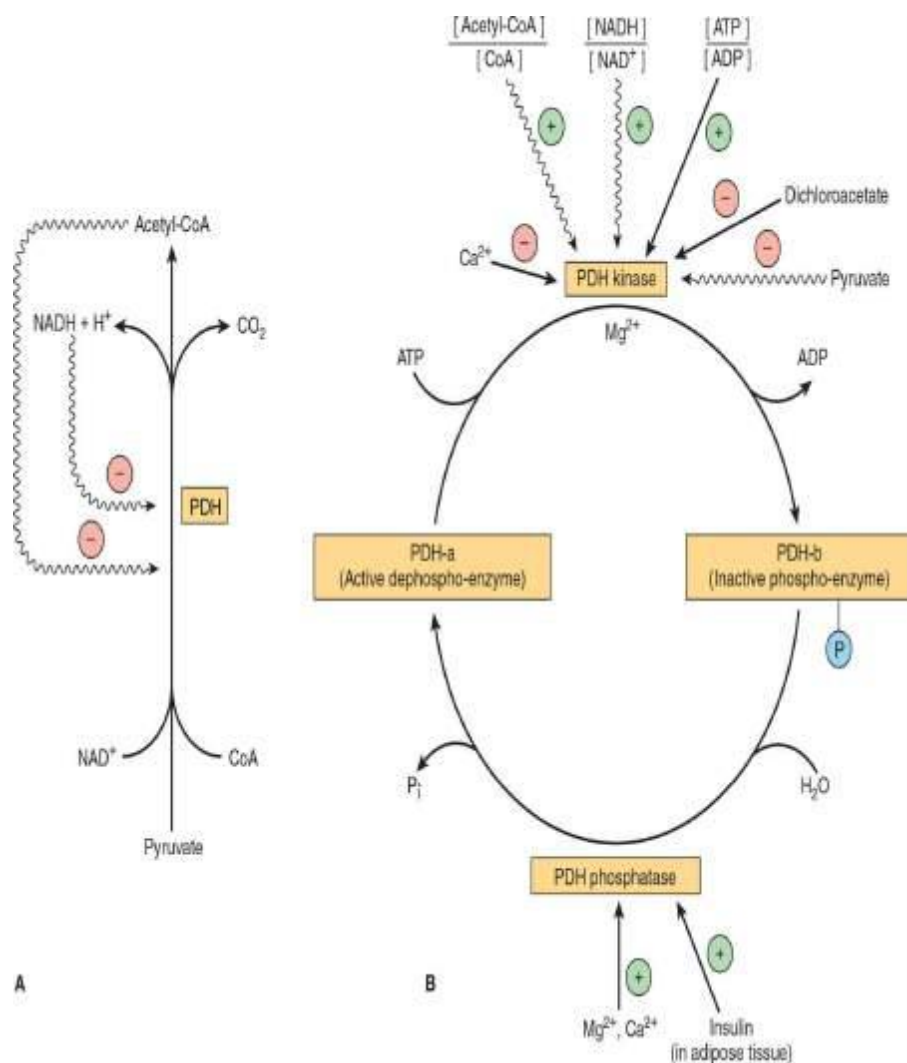
FIGURE 6 Regulation of pyruvate dehydrogenase (PDH). Arrows with wavy shafts indicate allosteric effects. (A) Regulation by end-product inhibition. (B) Regulation by interconversion of active and inactive forms.

CLINICAL ASPECTS
Inhibition of Pyruvate Metabolism Leads to Lactic Acidosis

Arsenite and mercuric ions react with the —SH groups of lipoic acid and inhibit pyruvate dehydrogenase, as does a **dietary deficiency of thiamin**, allowing pyruvate to accumulate. Many alcoholics are thiamin deficient (both

because of a poor diet and also because alcohol inhibits thiamin absorption) and may develop potentially fatal pyruvic and lactic acidosis. Patients with **inherited pyruvate dehydrogenase deficiency**, which can be the result of defects in one or more of the components of the enzyme complex, also present with lactic acidosis,
particularly after a glucose load. Because of the dependence of the brain on glucose as a fuel, these metabolic defects commonly cause neurological disturbances. Inherited aldolase A deficiency and pyruvate kinase deficiency in erythrocytes cause **hemolytic anemia**. The exercise capacity of patients with **muscle phosphofructokinase deficiency** is low, particularly if they
are on high-carbohydrate diets.

SUMMARY

--Glycolysis is the cytosolic pathway in all mammalian cells for the metabolism of glucose (or glycogen) to pyruvate and lactate.

--It can function anaerobically by regenerating oxidized NAD+ (required in the glyceraldehyde-3-phosphate dehydrogenase reaction), by reducing pyruvate to lactate.

--Lactate is the end product of glycolysis under anaerobic conditions (eg, in exercising muscle) and in erythrocytes, where there are no mitochondria to permit further oxidation of pyruvate.

--Glycolysis is regulated by three enzymes catalyzing nonequilibrium reactions: hexokinase, phosphofructokinase, and pyruvate kinase.

--In erythrocytes, the first site in glycolysis for generation of ATP may be bypassed, leading to the formation of 2,3-bisphosphoglycerate, which is

important in decreasing the affinity of hemoglobin for O2.

--Pyruvate is oxidized to acetyl-CoA by a multienzyme complex, pyruvate dehydrogenase, which is dependent on the vitamin-derived cofactor thiamin diphosphate.

--Conditions that involve an impairment of pyruvate metabolism frequently lead to lactic acidosis.

Overview of Metabolism & the Provision of Metabolic Fuel

BIOMEDICAL IMPORTANCE

Metabolism is the term used to describe the 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 fall into three categories.

(1) **Anabolic pathways** are involved in the synthesis of larger and more complex compounds from smaller precursors—for example, the synthesis of protein from amino acids and the synthesis of reserves of triacylglycerol and glycogen. Anabolic pathways are endothermic.

(2) **Catabolic pathways** are involved in the breakdown of larger molecules, commonly involving oxidative reactions; they are exothermic, producing reducing equivalents, and, mainly via the respiratory chain, ATP.

(3) **Amphibolic pathways** occur at the "crossroads" of metabolism, acting as links between anabolic and catabolic pathways, for example, the citric acid cycle.

Knowledge of normal metabolism is essential for an understanding of abnormalities that underlie disease. Normal metabolism includes adaptation to periods of fasting, starvation, and exercise, as well as pregnancy and lactation. Abnormal metabolism may result from nutritional deficiency, enzyme deficiency, abnormal secretion of hormones, or the actions of drugs and toxins. A 70-kg adult human being requires about 8 to 12 MJ (1920-2900 kcal) from metabolic fuels each day, depending on physical activity. Larger animals require less per kilogram body weight, and smaller animals more.

Growing children and animals have a proportionally higher requirement to allow for the energy cost of growth. For human beings, this energy requirement is met from carbohydrates (40-60%), lipids (mainly triacylglycerol, 30-40%), and protein (10-15%), as well as alcohol. The mix of carbohydrate, lipid, and protein being oxidized varies, depending on whether the subject is in the fed or fasting state,

and on the duration and intensity of physical work. There is a constant requirement for metabolic fuels throughout the day; average physical activity increases metabolic rate by about 40 to 50% over the basal or resting metabolic rate. However, most people consume their daily intake of metabolic fuels in two or three meals, so there is a need to form reserves of carbohydrate (glycogen in liver and muscle), lipid (triacylglycerol in adipose tissue), and labile protein stores during the period following a meal, for use during the intervening time when there is no intake of food.

If the intake of metabolic fuels is consistently greater than energy expenditure, the surplus is stored, largely as triacylglycerol in adipose tissue, leading to the development of **obesity** and its associated health hazards. By contrast, if the intake of metabolic fuels is consistently lower than energy expenditure, there are negligible reserves of fat and carbohydrate, and amino acids arising from protein turnover are used for energy-yielding metabolism rather than replacement protein synthesis, leading to **emaciation**, wasting, and, eventually, death. In the fed state, after a meal, there is an ample supply of carbohydrate, and the metabolic fuel for most tissues is glucose. In the fasting state, glucose must be spared for use by the central nervous system (which is largely dependent on glucose) and the red blood cells (which are wholly reliant on glucose). Therefore, tissues that can use fuels other than glucose do so; muscle and liver oxidize fatty acids and the liver synthesizes ketone bodies from fatty acids to export to muscle and other tissues. As glycogen reserves become depleted, amino acids arising from protein turnover are used for **gluconeogenesis.**

The formation and utilization of reserves of triacylglycerol and glycogen, and the extent to which tissues take up and oxidize glucose, are largely controlled by the hormones **insulin** and **glucagon**. In **diabetes mellitus**, there is either impaired synthesis and secretion of insulin (type I diabetes, sometimes called juvenile onset, or insulin-dependent diabetes) or impaired sensitivity of tissues to insulin action (type II diabetes, sometimes called adult-onset or noninsulin-dependent diabetes), leading to severe metabolic derangement.

 In cattle, the demands of heavy lactation can lead to ketosis, as can the demands of twin pregnancy in sheep.

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. In ruminants (and, to a lesser extent, other herbivores), dietary cellulose is fermented by symbiotic microorganisms to short-chain fatty acids (acetic, propionic, butyric), and metabolism in these animals is adapted to use these fatty acids as major substrates.

All the products of digestion are metabolized to a **common product**, **acetyl-CoA**, which is then oxidized by the **citric acid cycle** (**Figure 1**).
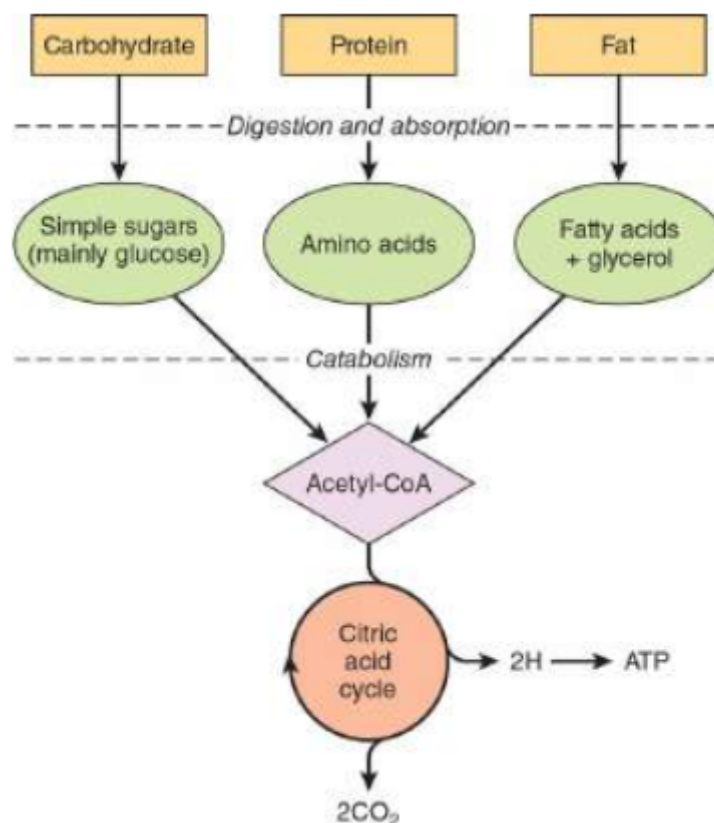

FIGURE 1 Outline of the Pathways for the Catabolism of Carbohydrate, Protein, and Fat. All these pathways lead to the

production of acetyl-CoA, which is oxidized in the citric acid cycle, ultimately yielding ATP by the process of oxidative phosphorylation.

Carbohydrate Metabolism Is Centered on the Provision & Fate of Glucose

Glucose is the major fuel of most tissues (**Figure 2**). It is metabolized to pyruvate by the pathway of **glycolysis** . Aerobic tissues metabolize pyruvate to **acetyl-CoA**, which can enter the citric acid cycle for complete oxidation to CO2 and H2O, linked to the formation of ATP in the process of **oxidative phosphorylation** (Figure 2). Glycolysis can also occur anaerobically (in the absence of oxygen) when the end product is lactate.
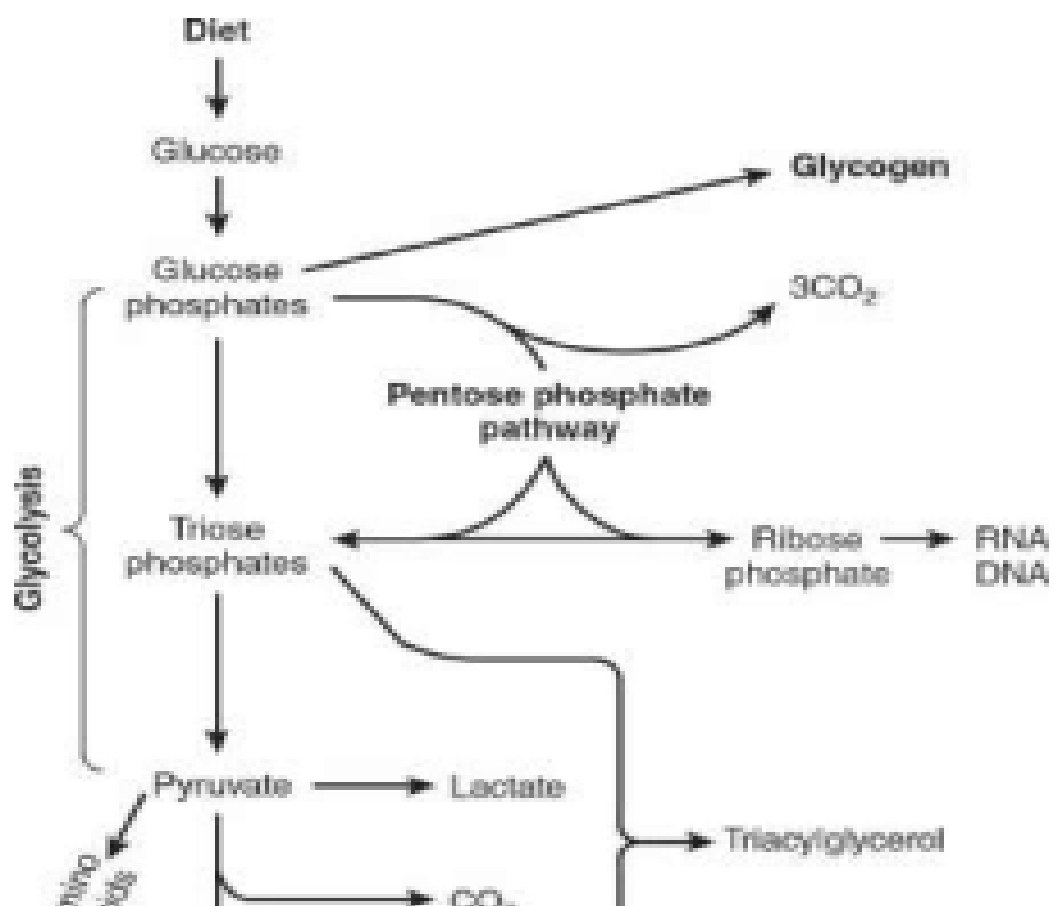
FIGURE 2 Overview of carbohydrate metabolism showing the major pathways and end products. Gluconeogenesis is not shown. Glucose and its metabolites also take part in other processes, for example, the synthesis of the storage polymer **glycogen** in skeletal muscle and liver and the **pentose phosphate pathway**, an alternative to part of the pathway of glycolysis. It is a source of reducing equivalents (NADPH) for fatty acid synthesis and the source of **ribose** for nucleotide and nucleic acid synthesis. Triose phosphate intermediates in glycolysis give rise to the **glycerol moiety** of triacylglycerols. Pyruvate and intermediates of the citric acid cycle provide the carbon skeletons for the synthesis of nonessential or dispensable **amino acids**, and acetyl-CoA is the precursor of **fatty acids** and **cholesterol** and hence of all the steroid hormones synthesized in the body. **Gluconeogenesis** is the process of synthesizing glucose from noncarbohydrate precursors such as lactate, amino acids, and glycerol.

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 (β-oxidation)** or esterified with glycerol, forming **triacylglycerol** as the body's main fuel reserve. Acetyl-CoA formed by β-oxidation of fatty acids may undergo three fates (**Figure 3**):
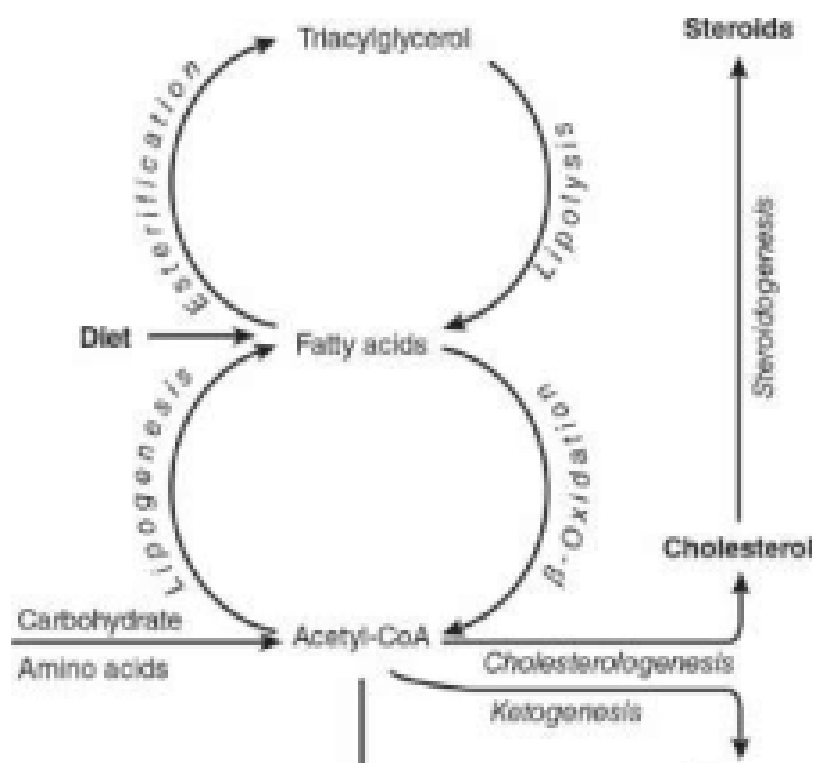
FIGURE 3 Overview of fatty acid metabolism showing the major pathways and end products.
The ketone bodies are acetoacetate, 3-hydroxybutyrate, and acetone (which is formed nonenzymically by decarboxylation of acetoacetate).
1. As with acetyl-CoA arising from glycolysis, it is **oxidized** to CO2 + H2O via the citric acid cycle.
2. It is the precursor for synthesis of **cholesterol** and other **steroids**.
3. In the liver, it is used to form the **ketone bodies**, acetoacetate, and 3- hydroxybutyrate which are important fuels in prolonged fasting and starvation.

Much of Amino Acid Metabolism Involves Transamination

The amino acids are required for protein synthesis (**Figure 4**). Some must be supplied in the diet (the **essential or indispensable amino acids**), since they cannot be synthesized in the body. The remainder are **nonessential** or **dispensable amino acids**, which are supplied in the diet, but can also be formed from metabolic intermediates by **transamination** using the amino group from other amino acids . After **deamination**, amino nitrogen is excreted as **urea**, and the **carbon skeletons that remain after transamination may**
(1) Be oxidized to CO2 via the citric acid cycle,
(2) Be used to synthesize glucose (gluconeogenesis, or
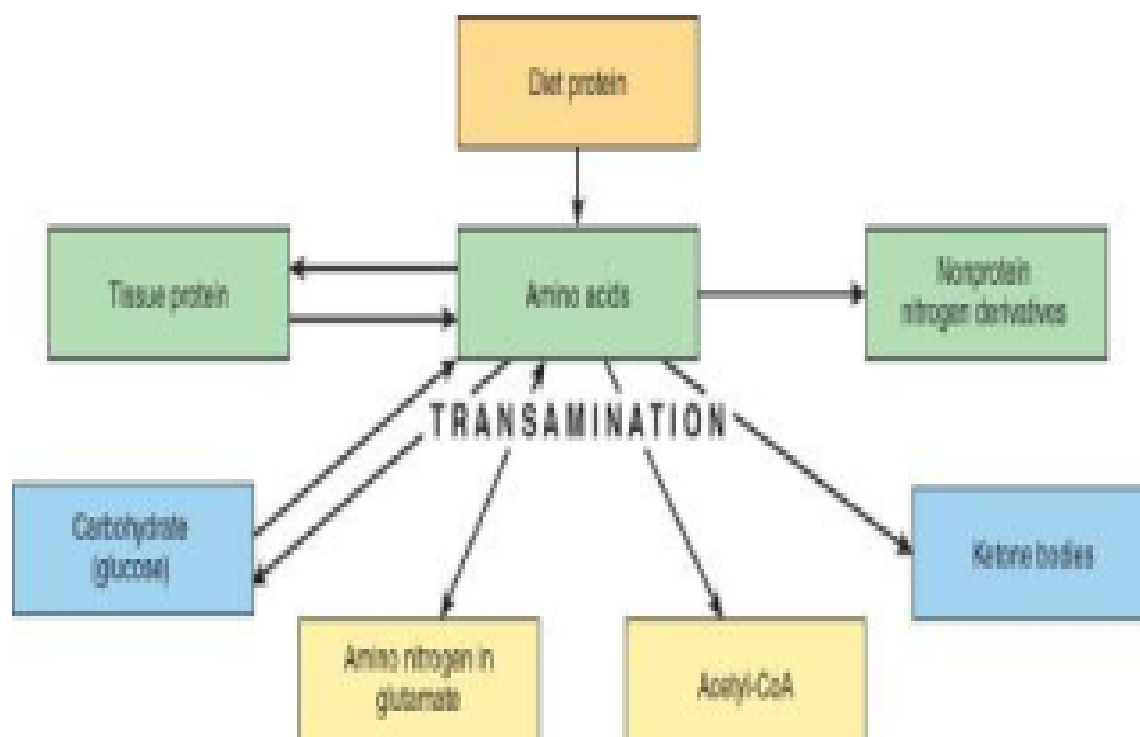 (3) Form ketone bodies or acetyl-CoA, which may be oxidized or used for synthesis of fatty acids.

FIGURE 4 Overview of amino acid metabolism showing the major pathways and end products.
Several amino acids are also the precursors of other compounds, for example, purines, pyrimidines, hormones such as epinephrine and thyroxine, and neurotransmitters.

METABOLIC PATHWAYS MAY BE STUDIED AT DIFFERENT LEVELS OF ORGANIZATION

In addition to studies in the whole organism, the location and integration of metabolic pathways is revealed by studies at two levels of organization. At the **tissue and organ level**, the nature of the substrates entering and metabolites leaving tissues and organs can be measured. 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 and **glucose** resulting from the digestion of carbohydrates are absorbed via the hepatic portal vein. The liver has the role of regulating the blood concentration of these water-soluble metabolites (**Figure 5**). In the case of glucose, this is achieved by taking up glucose in excess of immediate requirements and using it to synthesize glycogen (**glycogenesis)**, or 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 maintenance of an adequate blood concentration of glucose is essential for those tissues for which it is either the major fuel (the brain) or the only fuel (erythrocytes). The liver also **synthesizes the major plasma proteins** (eg, albumin) and **deaminates amino acids** that are in

excess of requirements, synthesizing urea, which is transported to the kidney and excreted.
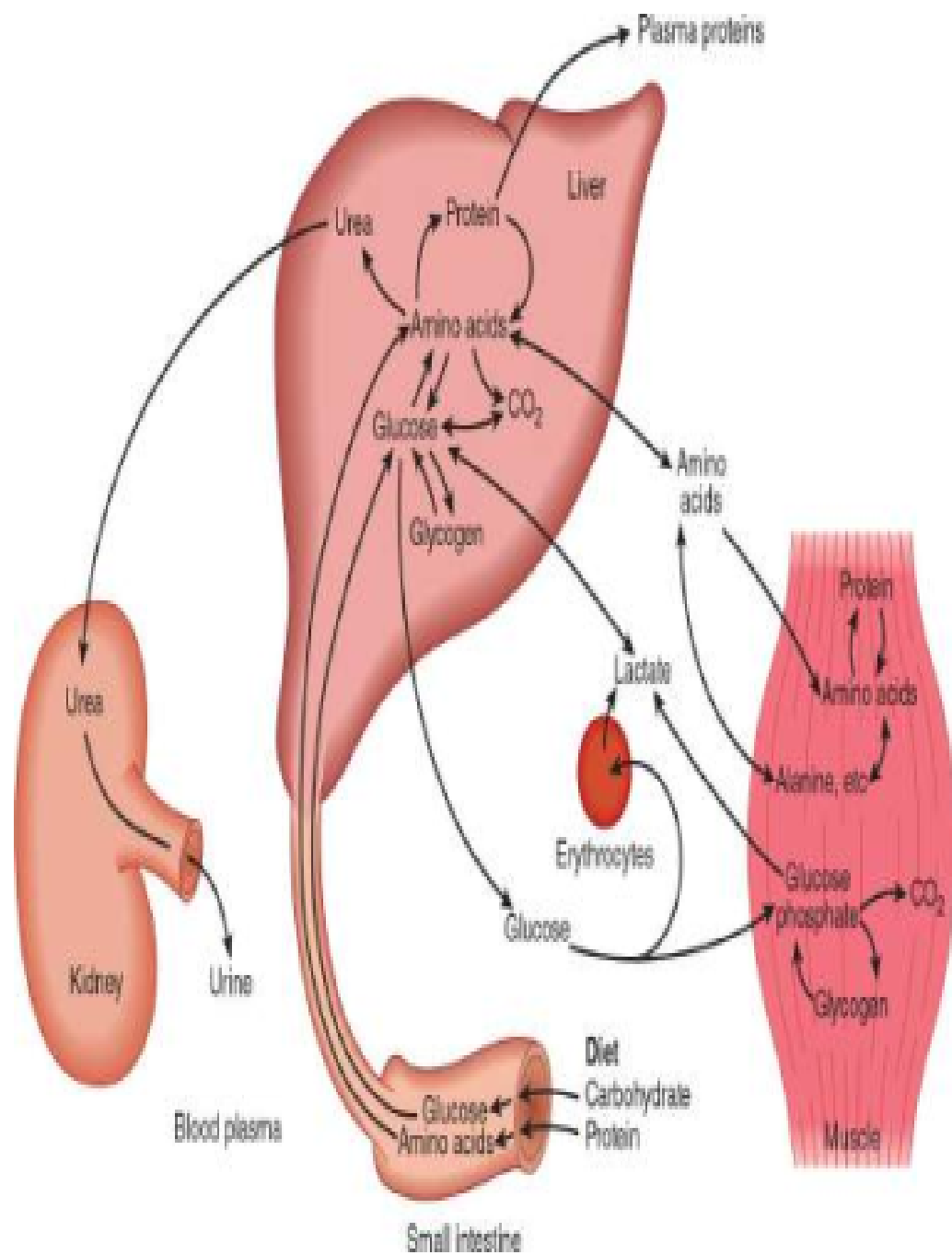
FIGURE 5 Transport and fate of major carbohydrate and amino acid substrates and metabolites. Note that there is little free glucose in muscle, since it is rapidly phosphorylated following uptake. **Skeletal muscle** utilizes glucose as a fuel, both aerobically, forming CO_2, and anaerobically, forming 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 .The main dietary **lipids** (**Figure 6**) are triacylglycerols that are hydrolyzed to monoacylglycerols and fatty acids in the gut, then reesterified 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, including vitamins A, D, E, and K. Unlike glucose and amino acids absorbed from the small intestine, 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.
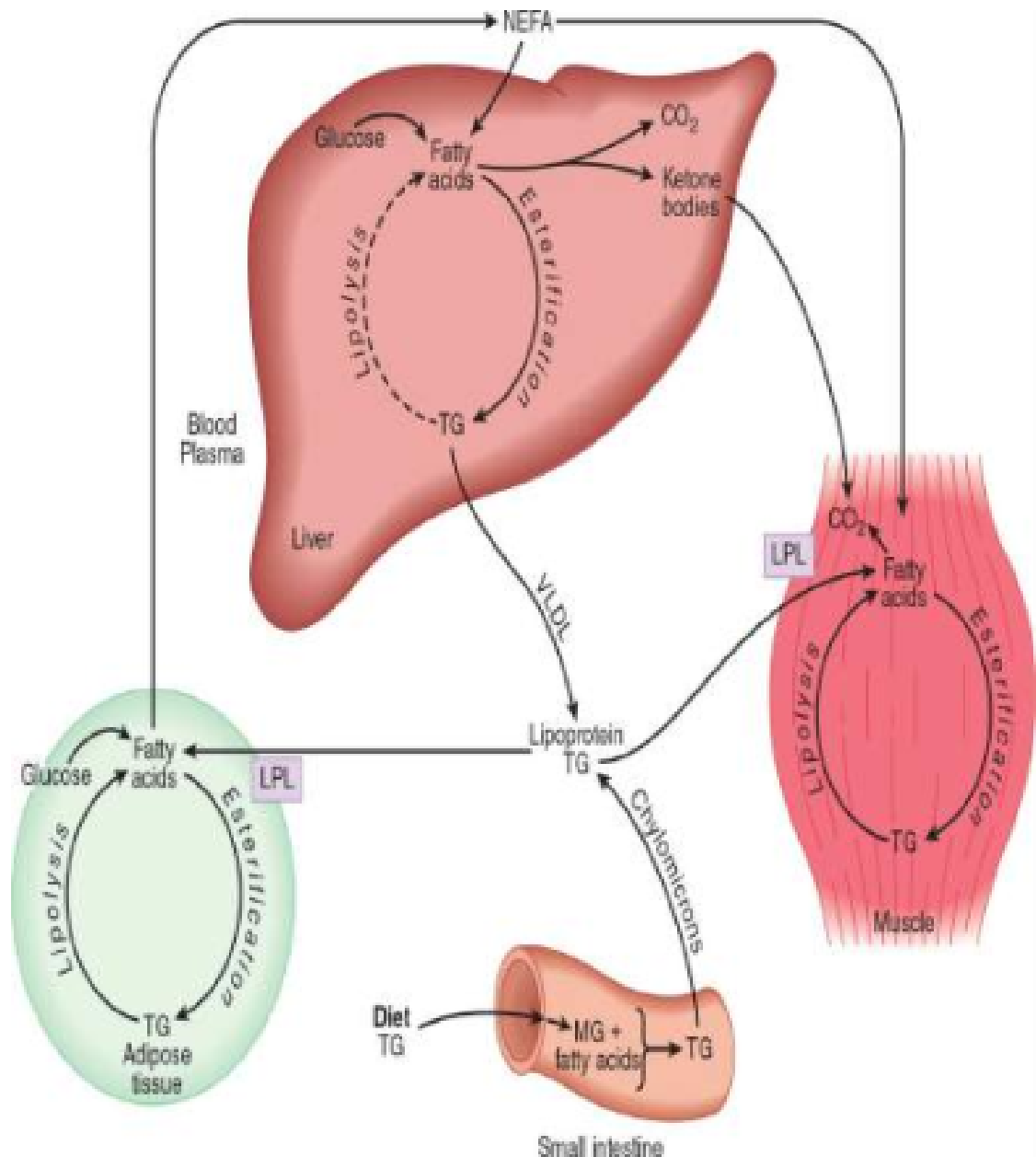
FIGURE 6 Transport and Fate of Major Lipid Substrates and Metabolites. (LPL, lipoprotein lipase; MG, monoacylglycerol; NEFA, nonesterified fatty acids; TG, triacylglycerol; VLDL, very low-density lipoprotein.)

Adipose tissue triacylglycerol is the main fuel reserve of the body. It is hydrolyzed **(lipolysis)** and glycerol and nonesterified (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, newly synthesized triacylglycerol and triacylglycerol from chylomicron remnants (see Figure3) 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 (**ketogenesis**) Ketone bodies are exported to extrahepatic tissues, where they provide 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. **Figure 7** depicts the subcellular compartmentation of metabolic pathways in a liver parenchymal cell.
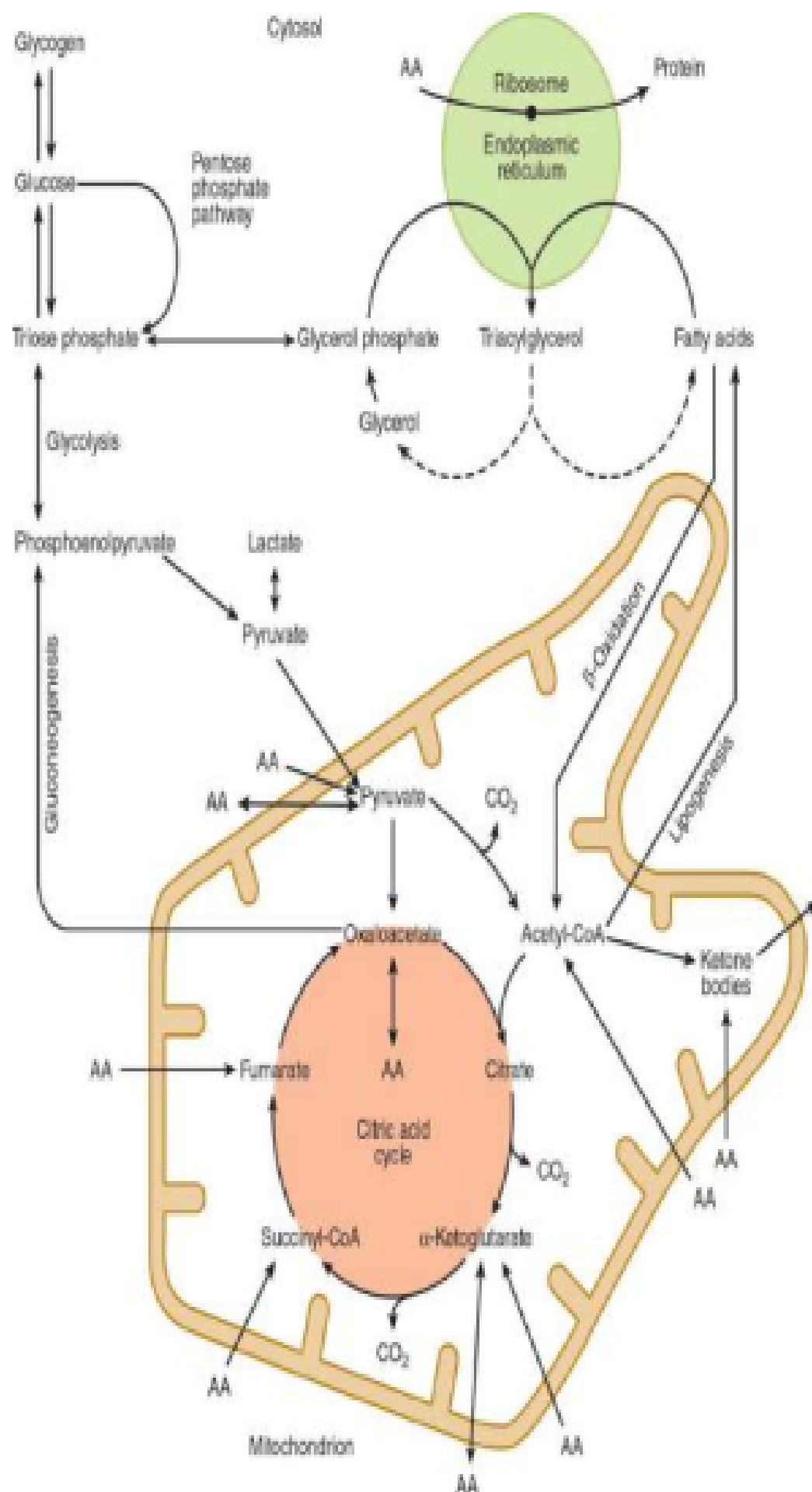
FIGURE 7 Intracellular location and overview of major metabolic pathways in a liver parenchymal cell. (AA →, metabolism of one or more essential amino acids; AA ↔, metabolism of one or more nonessential amino acids.)

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 , β-oxidation of fatty acids and ketogenesis , as well as the respiratory chain and ATP synthase .

Glycolysis , the pentose phosphate pathway, and fatty acid synthesis, all occur in the cytosol. In gluconeogenesis, substrates such as lactate and pyruvate, which are formed in the cytosol, enter the mitochondrion to yield **oxaloacetate** as a precursor for the synthesis of glucose in the cytosol.

The membranes of the **endoplasmic reticulum** contain the enzyme system for **triacylglycerol synthesis** , and the **ribosomes** are responsible for **protein synthesis** .

THE FLUX OF METABOLITES THROUGH METABOLIC PATHWAYS MUST BE REGULATED IN A CONCERTED MANNER

Regulation of the overall flux through a pathway is important to ensure an appropriate supply of the products of that pathway. It is achieved by control of one or more key reactions in the pathway, catalyzed by **regulatory enzymes**. The physicochemical factors that control the rate of an enzyme-catalyzed reaction, such as substrate concentration, are of primary importance in the control of the overall rate of a metabolic pathway .

Nonequilibrium Reactions Are Potential Control Points

In a reaction at equilibrium, the forward and reverse reactions occur at equal rates, and there is therefore no net flux in either direction.

A ↔ C ↔ D
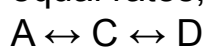
In vivo, under "steady-state" conditions, there is a net flux from left to right because there **is a continuous supply of substrate A and continuous removal of product D**. In practice, there are normally

one or more **nonequilibrium** reactions in a metabolic pathway, where the reactants are present in concentrations that are far from equilibrium. In attempting to reach equilibrium, large losses of free energy occur, making this type of reaction essentially irreversible. The enzymes catalyzing nonequilibrium reactions are usually present in low concentration and are subject to a variety of regulatory mechanisms. However, most reactions in metabolic pathways cannot be classified as equilibrium or nonequilibrium, but fall somewhere between the two extremes.

The Flux-Generating Reaction Is the First Reaction in a Pathway That Is Saturated With the Substrate

The flux-generating reaction can be identified as a nonequilibrium reaction in which the K_m of the enzyme is considerably lower than the normal concentration of substrate. The first reaction in glycolysis, catalyzed by hexokinase, is such a flux-generating step because its K_m for glucose of 0.05 mmol/L is well below the normal blood glucose concentration of 3 to 5 mmol/L. Later reactions then control the rate of flux through the pathway.

ALLOSTERIC & HORMONAL MECHANISMS ARE IMPORTANT IN THE METABOLIC CONTROL OF ENZYME-CATALYZED REACTIONS

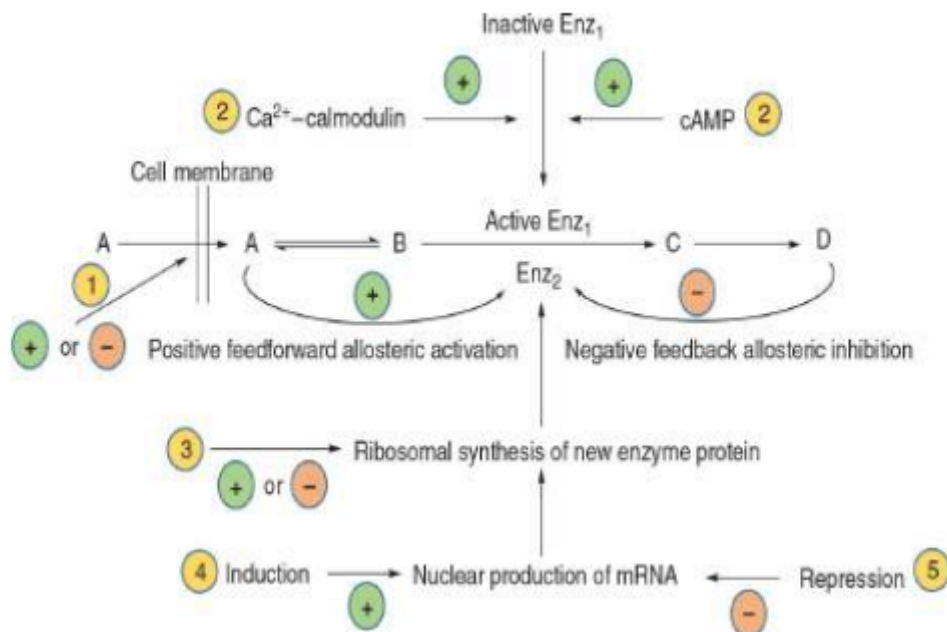In the metabolic pathway shown in **Figure 8**,

FIGURE 14–8 Mechanisms of control of an enzyme-catalyzed reaction. Circled numbers indicate possible sites of action of hormones: 1 alteration of membrane permeability; 2 conversion of an inactive to an active enzyme, usually involving phosphorylation/dephosphorylation reactions; 3 alteration of the rate translation of mRNA at the ribosomal level; 4 induction of new mRNA formation; and 5 repression of mRNA formation. 1 and 2 are rapid mechanisms of regulation, whereas, 3, 4
and 5 are slower.

$A \leftrightarrow B \rightarrow C \leftrightarrow D$

Reactions $A \leftrightarrow B$ and $C \leftrightarrow D$ are equilibrium reactions and $B \rightarrow C$ is a nonequilibrium reaction. The flux through this pathway can be regulated by the availability of substrate A. This depends on its supply from the blood, which in turn depends on either food intake or key reactions that release substrates from tissue reserves into the bloodstream, for example, glycogen phosphorylase in liver (see Figure1) and hormone-sensitive
lipase in adipose tissue (see Figure 8). It also depends on the transport of substrate A into the cell. Muscle and adipose tissue only take up glucose from the bloodstream in response to the hormone insulin.

Flux is also determined by removal of the end product D and the

availability of cosubstrates or cofactors represented by X and Y. Enzymes catalyzing nonequilibrium reactions are often allosteric proteins subject to the rapid actions of "feed-back" or "feed-forward" control by **allosteric modifiers**, in immediate response to the needs of the cell .

Frequently, the end product of a biosynthetic pathway inhibits the enzyme catalyzing the first reaction in the pathway. Other control mechanisms depend on the action of **hormones** responding to the needs of the body as a whole; they may act rapidly by altering the activity of existing enzyme molecules, or slowly by altering the rate of enzyme synthesis.

MANY METABOLIC FUELS ARE INTERCONVERTIBLE

Carbohydrate in excess of requirements for immediate energy-yielding metabolism and formation of glycogen reserves in muscle and liver can readily be used for synthesis of fatty acids, and hence triacylglycerol in both adipose tissue and liver (whence it is exported in very low-density lipoprotein). The importance of lipogenesis in human beings is unclear; in Western countries dietary fat provides 35 to 45% of energy intake, while in less-developed countries, where carbohydrate may provide 60 to 75% of energy intake, the total intake of food is so low that there is little surplus for lipogenesis anyway. A high intake of fat inhibits lipogenesis in adipose tissue and liver.

Fatty acids (and ketone bodies formed from them) cannot be used for the synthesis of glucose. The reaction of pyruvate dehydrogenase, forming acetyl-CoA, is irreversible, and for every two-carbon unit from acetyl-CoA that enters the citric acid cycle, there is a loss of two carbon atoms as carbon dioxide before oxaloacetate is reformed. This means that acetyl- CoA (and hence any substrates that yield acetyl-CoA) can never be used for gluconeogenesis. The (relatively rare) fatty acids with an odd number of carbon atoms yield propionyl-CoA as the product of the final cycle of β- oxidation, and this can be a substrate for gluconeogenesis, as can the glycerol released by lipolysis of adipose tissue triacylglycerol reserves.

Most of the amino acids in excess of requirements for protein synthesis (arising from the diet or from tissue protein turnover) yield pyruvate, or four- and five-carbon intermediates of the citric acid cycle. Pyruvate can be carboxylated to oxaloacetate, which is the primary substrate for gluconeogenesis, and the other intermediates of the cycle also result in a net increase in the formation of oxaloacetate,

which is then available for gluconeogenesis. These amino acids are classified as
glucogenic. Two amino acids (lysine and leucine) yield only acetyl-CoA on oxidation, and hence cannot be used for gluconeogenesis, and four others (phenylalanine, tyrosine, tryptophan, and isoleucine) give rise to both acetyl-CoA and intermediates that can be used for gluconeogenesis.

Those amino acids that give rise to acetyl-CoA are referred to **ketogenic**, because in prolonged fasting and starvation, much of the acetyl-CoA is used for synthesis of ketone bodies in the liver.

A SUPPLY OF METABOLIC FUELS IS PROVIDED IN BOTH THE FED & FASTING STATES

Glucose Is Always Required by the Central Nervous System and Erythrocytes

Erythrocytes lack mitochondria and hence are wholly reliant on (anaerobic) glycolysis and the pentose phosphate pathway at all times. The brain can metabolize ketone bodies to meet about 20% of its energy requirements; the remainder must be supplied by glucose. The metabolic changes that occur in the fasting state and starvation serve to preserve glucose and the body's limited glycogen reserves for use by the brain and red blood cells, and to provide alternative metabolic fuels for other tissues.

In pregnancy, the fetus requires a significant amount of glucose, as does the synthesis of lactose in lactation (**Figure 9**).
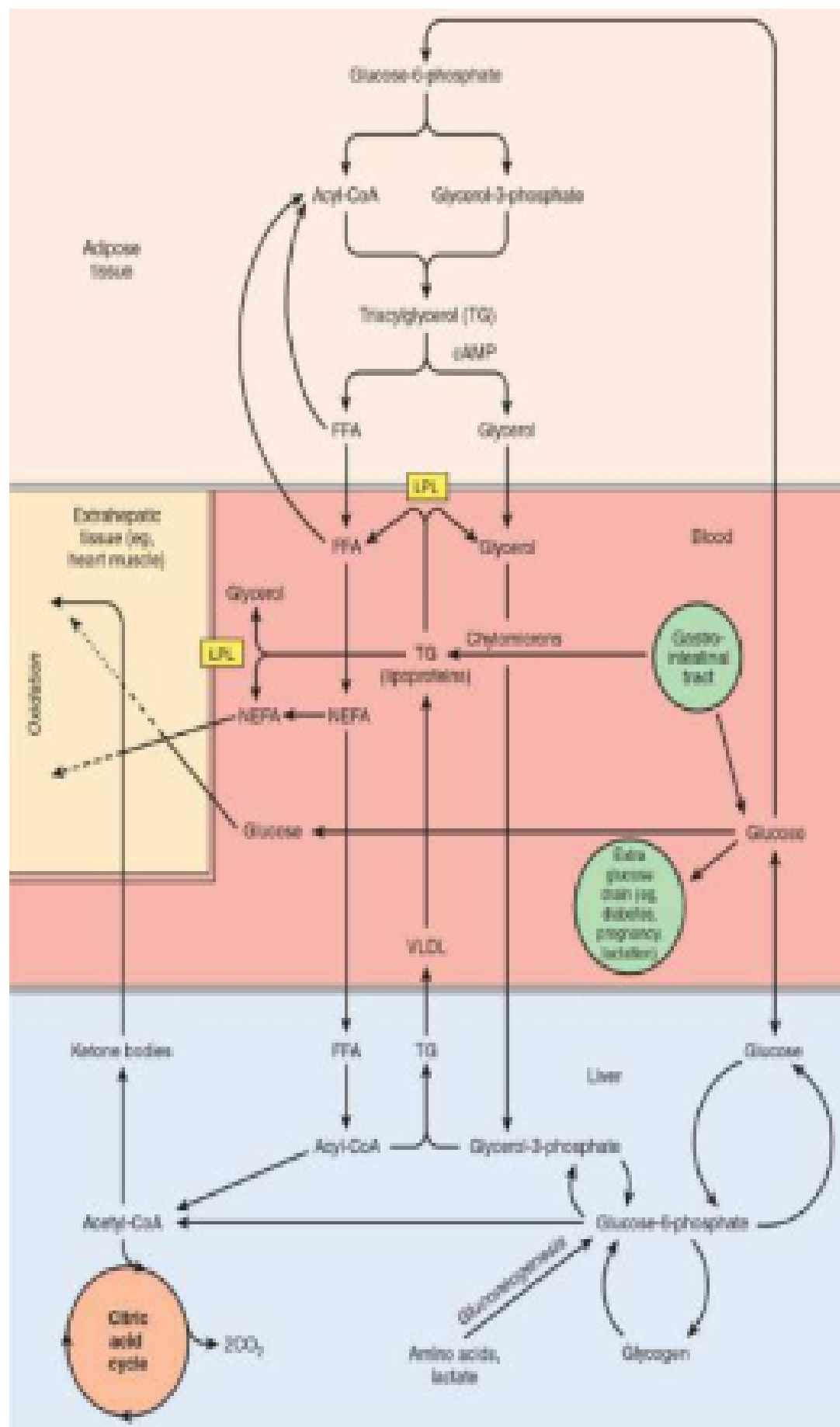
FIGURE 9 Metabolic interrelationships among adipose tissue, the liver, and extrahepatic tissues.

In tissues such as heart, metabolic fuels are oxidized in the following order of preference: ketone bodies > fatty acids > glucose. (LPL, lipoprotein lipase; NEFA, nonesterified fatty acids; VLDL, very low-density lipoproteins.)

In the Fed State, Metabolic Fuel Reserves Are Laid Down

For several hours after a meal, while the products of digestion are being absorbed, there is an abundant supply of metabolic fuels. Under these conditions, glucose is the major fuel for oxidation in most tissues; this is observed as an increase in the respiratory quotient (the ratio of carbon dioxide produced/oxygen consumed) from about 0.8 in the fasting state to near 1 (**Table 1**).

TABLE 1 Energy Yields, Oxygen Consumption, and Carbon Dioxide Production in the Oxidation of Metabolic Fuels

	Energy Yield (kJ/g)	O_2 Consumed (L/g)	CO_2 Produced (L/g)	RQ (CO_2 Produced/ O_2 Consumed)	Energy (kJ)/L O_2
Carbohydrate	16	0.829	0.829	1.00	~20
Protein	17	0.966	0.782	0.81	~20
Fat	37	2.016	1.427	0.71	~20
Alcohol	29	1.429	0.966	0.66	~20

Glucose uptake into muscle and adipose tissue is controlled by **insulin**, which is secreted by the β-islet cells of the pancreas in response to an increased concentration of glucose in the portal blood. In the fasting state, the glucose transporter of muscle and adipose tissue (GLUT-4) is in intracellular vesicles. An early response to insulin is the migration of these vesicles to the cell surface, where they fuse with the plasma membrane, exposing active glucose transporters. These insulin-sensitive tissues only

take up glucose from the bloodstream to any significant extent in the presence of the hormone. As insulin secretion falls in the fasting state, so the transporters are internalized again, reducing glucose uptake. However, in skeletal muscle, the increase in cytoplasmic calcium ion concentration in response to nerve stimulation stimulates the migration of the vesicles to the cell surface and exposure of active glucose transporters whether there is significant insulin stimulation or not.

The uptake of glucose into the liver is independent of insulin, but liver has an isoenzyme of hexokinase (glucokinase) with a high Km, so that as the concentration of glucose entering the liver increases, so does the rate of synthesis of glucose-6-phosphate. This is in excess of the liver's requirement for energy-yielding metabolism, and is used mainly for synthesis of **glycogen**. In both liver and skeletal muscle, insulin acts to stimulate glycogen synthetase and inhibit glycogen phosphorylase. Some of the additional glucose entering the liver may also be used for lipogenesis and hence triacylglycerol synthesis. In adipose tissue, insulin stimulates glucose uptake, its conversion to fatty acids, and their esterification to triacylglycerol. It inhibits intracellular lipolysis and the release of nonesterified fatty acids. The products of lipid digestion enter the circulation as **chylomicrons**, the largest of the plasma lipoproteins, which are especially rich in triacylglycerol. In adipose tissue and skeletal muscle, extracellular lipoprotein lipase is synthesized and activated in response to insulin; the resultant nonesterified fatty acids are largely taken up by the tissue and used for synthesis of triacylglycerol, while the glycerol remains in the bloodstream and is taken up by the liver and used for either gluconeogenesis and glycogen synthesis or lipogenesis. Fatty acids remaining in the bloodstream are taken up by the liver and reesterified. The lipid-depleted chylomicron remnants are cleared by the liver, and the remaining triacylglycerol is exported, together with that synthesized in the liver, in **very low-density lipoprotein**.

Under normal conditions, the rate of tissue protein catabolism is more or less constant throughout the day; it is only in **cachexia** associated with advanced cancer and other diseases that there is an increased rate of protein catabolism. There is net protein catabolism in the fasting state, when the rate of protein synthesis falls, and net protein synthesis in the fed state, when the rate of synthesis increases by 20 to 25%. The increased rate of protein synthesis in response to increased availability of amino acids and metabolic fuel is again a response to insulin. Protein synthesis is an energy expensive process;

it may account for up to 20% of resting energy expenditure after a meal, but only 9% in the fasting state.

Metabolic Fuel Reserves Are Mobilized in the Fasting State

There is a small fall in plasma glucose in the fasting state, and then little change as fasting is prolonged into starvation. Plasma nonesterified fatty acids increase in fasting, but then rise little more in starvation; as fasting is prolonged, the plasma concentration of ketone bodies (acetoacetate and 3-
hydroxybutyrate) increases markedly (**Table 2**, **Figure 10**).

TABLE 2 Plasma Concentrations of Metabolic Fuels (mmol/L) in the Fed and Fasting States

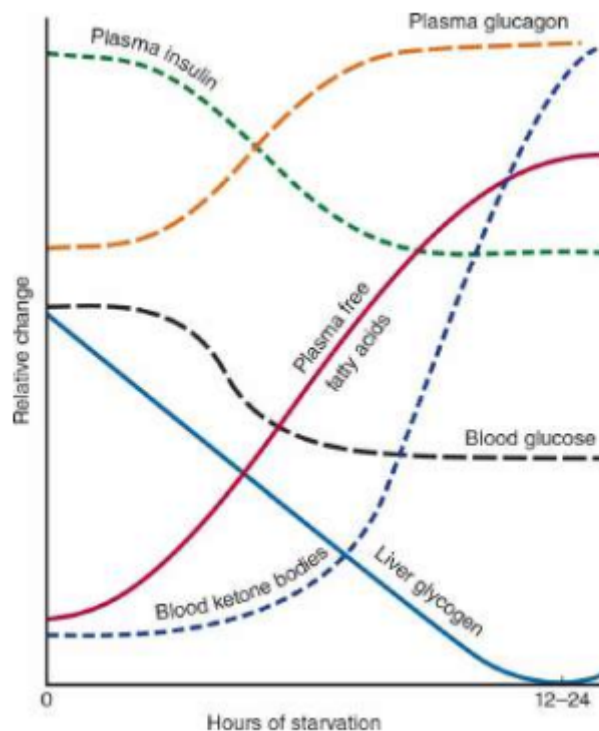|	Fed	40-h Fasting	7 Days Starvation
Glucose	5.5	3.6	3.5
Nonesterified fatty acids	0.30	1.15	1.19
Ketone bodies	Negligible	2.9	4.5

FIGURE 10 Relative changes in plasma hormones and metabolic fuels during the onset of starvation.

In the fasting state, as the concentration of glucose in the portal blood coming from the small intestine falls, insulin secretion decreases, and skeletal muscle and adipose tissue take up less glucose. The increase in secretion of **glucagon** by α-cells of the pancreas inhibits glycogen synthetase, and activates glycogen phosphorylase in the liver. The resulting glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase, and glucose is released into the bloodstream for use by the brain and erythrocytes. Muscle glycogen cannot contribute directly to plasma glucose, since muscle lacks glucose-6-phosphatase, and the primary use of muscle glycogen is to provide a source of glucose-6-phosphate for energy-yielding metabolism in the muscle itself. However, acetyl-CoA formed by oxidation of fatty acids in muscle inhibits pyruvate dehydrogenase, leading to an accumulation of pyruvate. Most of this is transaminated to alanine, at the expense of amino acids arising from breakdown of muscle protein. The alanine, and much of the keto acids resulting from this transamination are exported from muscle, and taken up by the liver, where the alanine is transaminated to yield pyruvate. The resultant amino acids are largely exported back to muscle, to provide amino groups for formation of more alanine, while the pyruvate

provides a substrate for gluconeogenesis in the liver. In adipose tissue, the decrease in insulin and increase in glucagon results in inhibition of lipogenesis, inactivation and internalization of lipoprotein lipase, and activation of intracellular hormone-sensitive lipase. This leads to release from adipose tissue of increased amounts of glycerol (which is a substrate for gluconeogenesis in the liver) and nonesterified fatty acids, which are used by liver, heart, and skeletal muscle as their preferred metabolic fuel, so sparing glucose. Although muscle preferentially takes up and metabolizes nonesterified fatty acids in the fasting state, it cannot meet all of its energy requirements by β-oxidation. By contrast, the liver has a greater capacity for β-oxidation than is required to meet its own energy needs, and as fasting becomes more prolonged, it forms more acetyl-CoA than can be oxidized. This acetyl-CoA is used to synthesize the **ketone bodies,** which are major metabolic fuels for skeletal and heart muscle and can meet up to 20% of the brain's energy needs. In prolonged starvation, glucose may represent less than 10% of whole body energy-yielding metabolism. Were there no other source of glucose, liver and muscle glycogen would be exhausted after about 18 hours fasting. As fasting becomes more prolonged, so an increasing amount of the amino acids released as a result of protein catabolism is utilized in the liver and kidneys for gluconeogenesis (**Table 3**).

TABLE 3 Summary of the Major Metabolic Features of the Principal Organs

Organ	Major Pathways	Main Substrates	Major Products Exported	Specialist Enzymes
Liver	Glycolysis, gluconeogenesis, lipogenesis, β-oxidation, citric acid cycle, ketogenesis, lipoprotein metabolism, drug metabolism, synthesis of bile salts, urea, uric acid, cholesterol, plasma proteins	Nonesterified fatty acids, glucose (in fed state), lactate, glycerol, fructose, amino acids, alcohol	Glucose, triacylglycerol in VLDL, ketone bodies, urea, uric acid, bile salts, cholesterol, plasma proteins	Glucokinase, glucose-6-phosphatase, glycerol kinase, phosphoenolpyruvate carboxykinase, fructokinase, arginase, HMG-CoA synthase, HMG-CoA lyase, alcohol dehydrogenase
Brain	Glycolysis, citric acid cycle, amino acid metabolism, neurotransmitter synthesis	Glucose, amino acids, ketone bodies in prolonged starvation	Lactate, end products of neurotransmitter metabolism	Those for synthesis and catabolism of neurotransmitters
Heart	β-Oxidation and citric acid cycle	Ketone bodies, nonesterified fatty acids, lactate, chylomicron and VLDL triacylglycerol, some glucose	—	Lipoprotein lipase, very active electron transport chain
Adipose tissue	Lipogenesis, esterification of fatty acids, lipolysis	Glucose, chylomicron, and VLDL triacylglycerol	Nonesterified fatty acids, glycerol	Lipoprotein lipase, hormone-sensitive lipase, enzymes of the

CLINICAL ASPECTS

In prolonged starvation, as adipose tissue reserves are depleted, there is a very considerable increase in the net rate of protein catabolism to provide amino acids, not only as substrates for gluconeogenesis, but also as the main metabolic fuel of all tissues. Death results when essential tissue proteins are catabolized and not replaced. In patients with **cachexia** as a result of release of **cytokines** in response to tumors and disease, there is an increase in the rate of tissue protein catabolism, as well as a considerably increased metabolic rate, so they are in a state of advanced starvation. Again, death results when essential tissue proteins are catabolized and not replaced. The high demand for glucose by the fetus, and for lactose synthesis in lactation, can lead to ketosis. This may be seen as mild ketosis with hypoglycemia in human beings; in lactating cattle and in ewes carrying a twin pregnancy, there may be very pronounced ketoacidosis and profound
hypoglycemia. In poorly controlled type 1 **diabetes mellitus**, patients may become hyperglycemic, both as a result of lack of insulin to stimulate uptake and utilization of glucose, and because in the absence of insulin to antagonize the actions of glucagon, there is increased gluconeogenesis from amino acids in the liver. At the same time, the lack of insulin to antagonize the actions of glucagon results in increased lipolysis in adipose tissue, and the resultant nonesterified fatty acids are substrates for ketogenesis in the liver.
Utilization of the ketone bodies in muscle (and other tissues) may be impaired because of the lack of oxaloacetate (all tissues have a requirement for some glucose metabolism to maintain an adequate amount of oxaloacetate for citric acid cycle activity). In uncontrolled diabetes, the ketosis may be severe enough to result in pronounced acidosis **(ketoacidosis)**; acetoacetate and 3-hydroxybutyrate are relatively strong acids. Coma results from both the acidosis and also the considerably increased osmolality of extracellular fluid (mainly as a result of the hyperglycemia, and diuresis resulting from the excretion of glucose and ketone bodies in the urine).

SUMMARY

---The products of digestion provide the tissues with the building blocks for the biosynthesis of complex molecules and also with the fuel for metabolic processes.

---Nearly all products of digestion of carbohydrate, fat, and protein are metabolized to a common metabolite, acetyl-CoA, before oxidation to CO2 in the citric acid cycle.

---Acetyl-CoA is also the precursor for synthesis of long-chain fatty acids and steroids (including cholesterol) and ketone bodies.

---Glucose provides carbon skeletons for the glycerol of triacylglycerols and nonessential amino acids.

---Water-soluble products of digestion are transported directly to the liver via the hepatic portal vein. The liver regulates the concentrations of glucose and amino acids available to other tissues. Lipids and lipid soluble products of digestion enter the bloodstream from the lymphatic system, and the liver clears the remnants after extrahepatic tissues have taken up fatty acids.

---Pathways are compartmentalized within the cell. Glycolysis, glycogenesis, glycogenolysis, the pentose phosphate pathway, and lipogenesis occur in the cytosol. The mitochondria contain the enzymes of the citric acid cycle and for β-oxidation of fatty acids, as well as the respiratory chain and ATP synthase. The membranes of the endoplasmic reticulum contain the enzymes for a number of other processes, including triacylglycerol synthesis and drug metabolism.

---Metabolic pathways are regulated by rapid mechanisms affecting the activity of existing enzymes, that is, allosteric and covalent modification (often in response to hormone action) and slow mechanisms that affect the synthesis of enzymes.

---Dietary carbohydrate and amino acids in excess of requirements can be used for fatty acid and hence triacylglycerol synthesis.

---In fasting and starvation, glucose must be provided for the brain and red blood cells; in the early fasting state, this is supplied from glycogen reserves. In order to spare glucose, muscle and other tissues do not take up glucose when insulin secretion is low; they utilize fatty acids (and later ketone bodies) as their preferred fuel.

---Adipose tissue releases nonesterified fatty acids in the fasting state. In prolonged fasting and starvation, these are used by the liver for

synthesis of ketone bodies, which are exported to provide the major fuel for muscle.

---Most amino acids, arising from the diet or from tissue protein turnover, can be used for gluconeogenesis, as can the glycerol from triacylglycerol.

---Neither fatty acids, arising from the diet or from lipolysis of adipose tissue triacylglycerol, nor ketone bodies, formed from fatty acids in the fasting state, can provide substrates for gluconeogenesis.