CHAPTER 16
The Citric Acid Cycle

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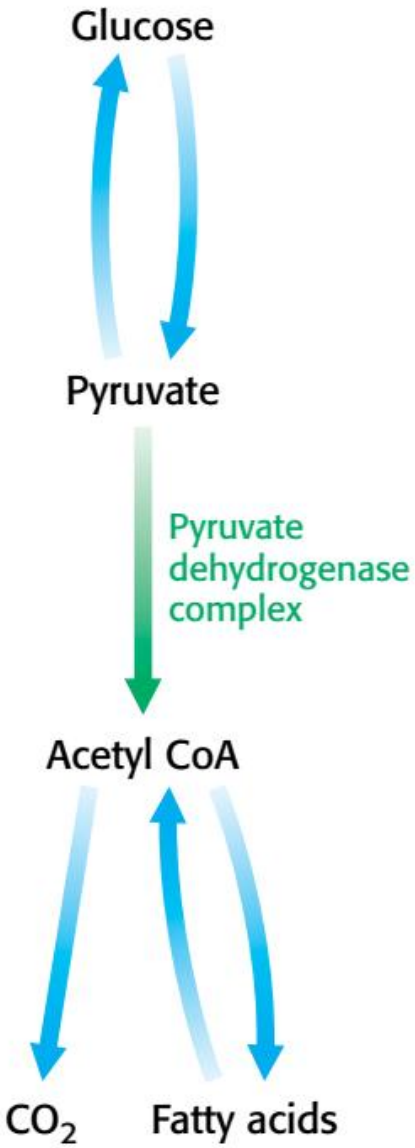
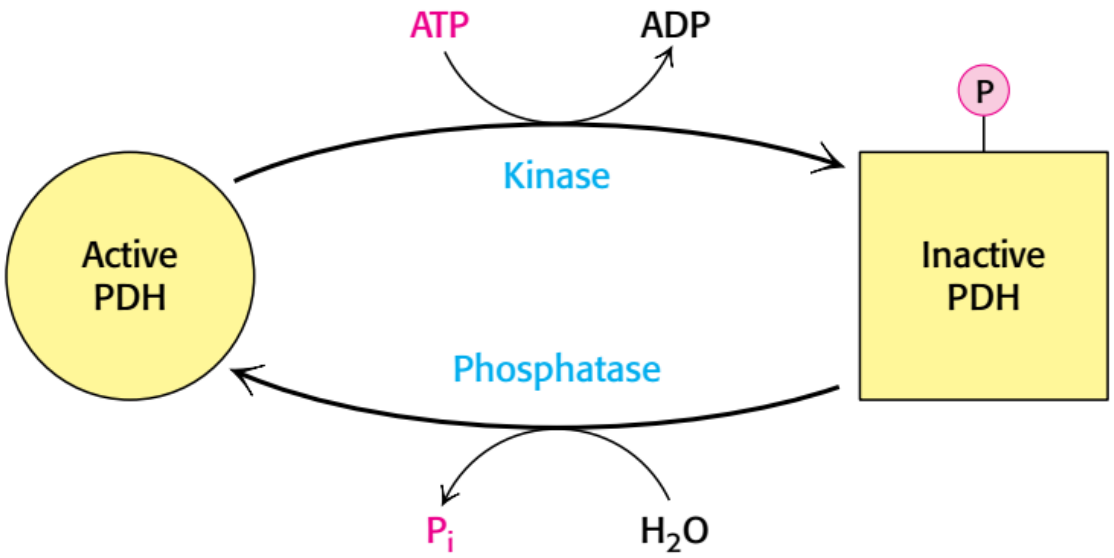
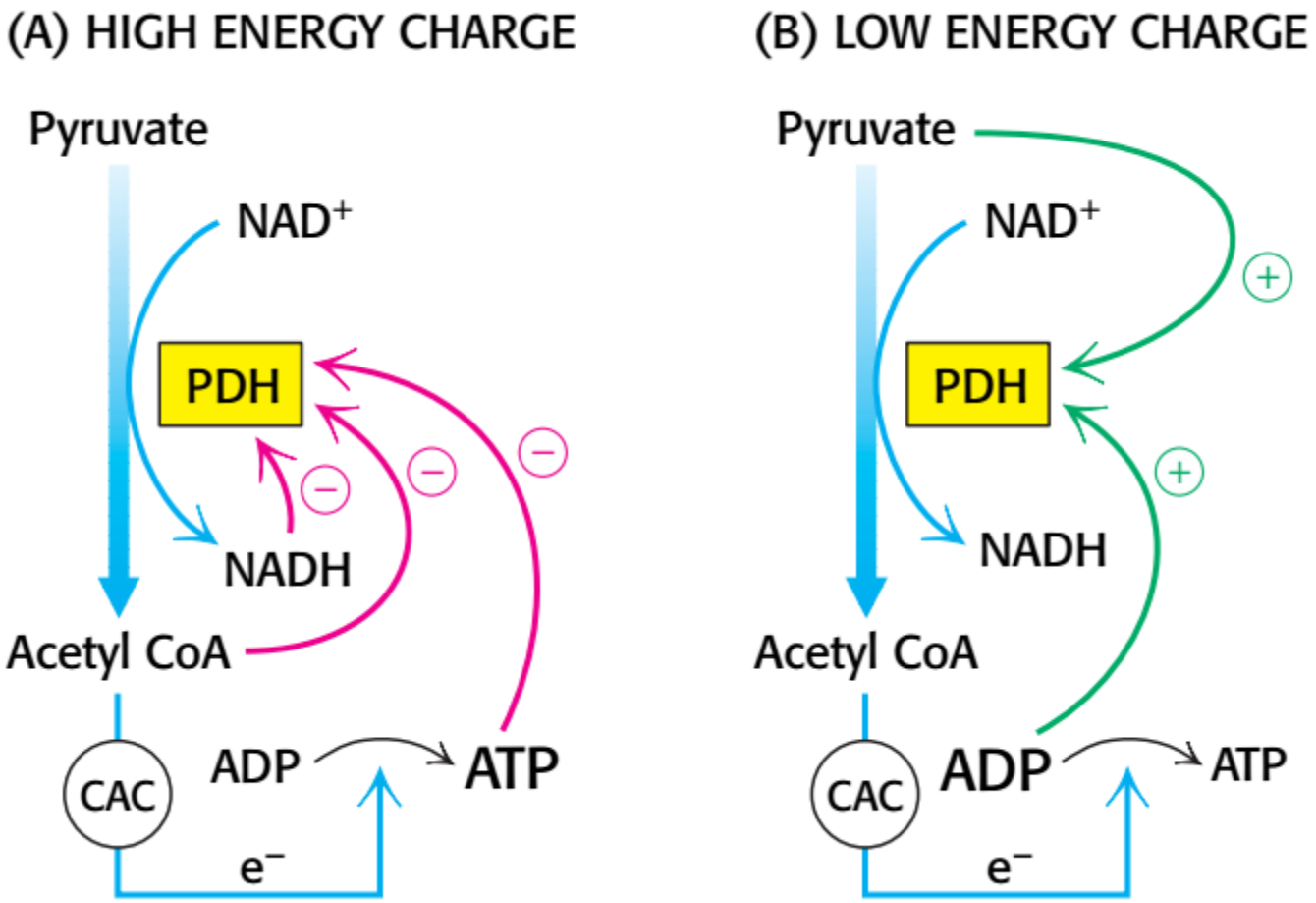
16.3 Regulation of the Citric Acid Cycle

- **PDH is regulated by allosteric and covalent mechanisms**





- **PDH is regulated by allosteric and covalent mechanisms**



- **The citric acid cycle is controlled at several points**

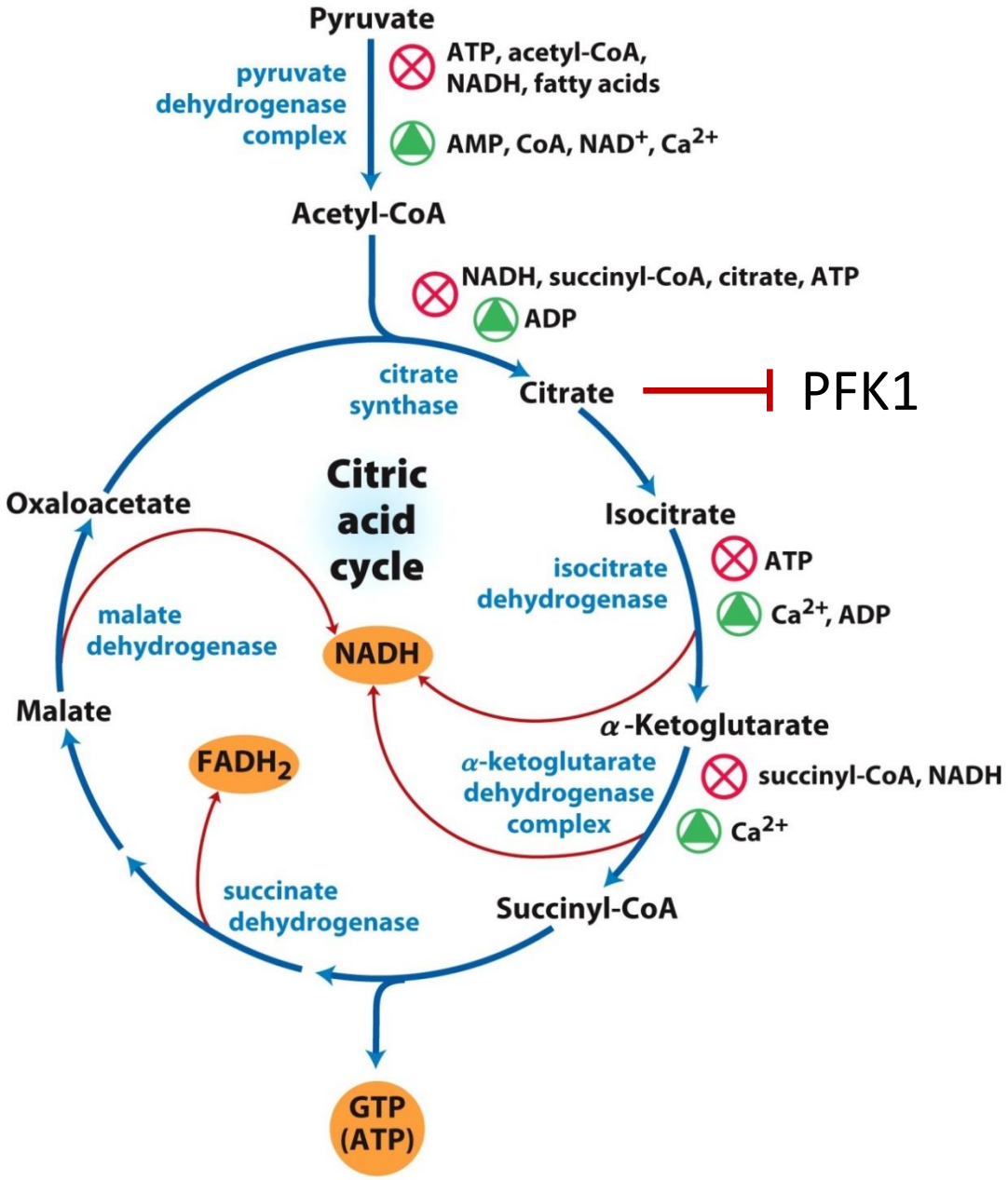
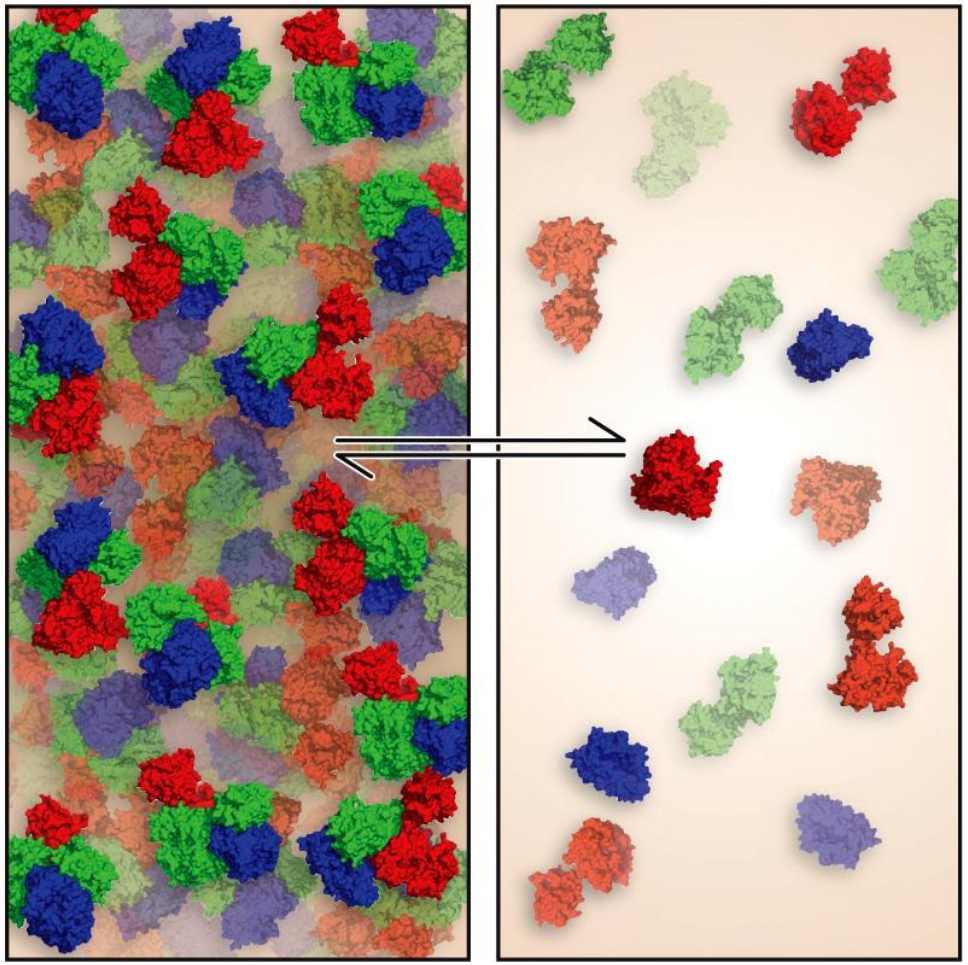


Figure 16-19

• Substrate channeling in citric acid cycle



In the cytosol, high concentrations of enzymes 1, 2, and 3 favor their association.

In extract of broken cells, dilution by buffer reduces the concentrations of enzymes 1, 2, and 3, favoring their dissociation.

Figure 16-20

Substrate channeling through multienzyme complexes may occur in the citric acid cycle

metabolon

• Some mutations in enzymes of the CAC lead to cancer

- Loss-of-function
  - Fumarase
  - Succinate dehydrogenase
- Gain-of-function
  - Isocitrate dehydrogenase 1/2

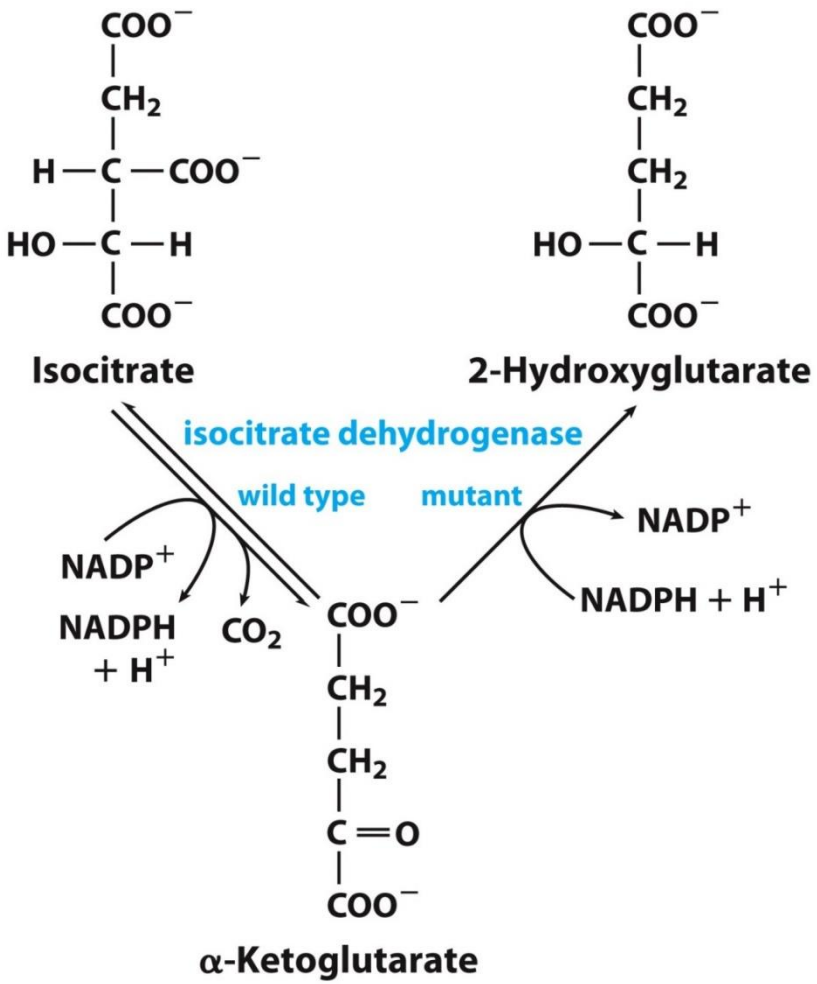
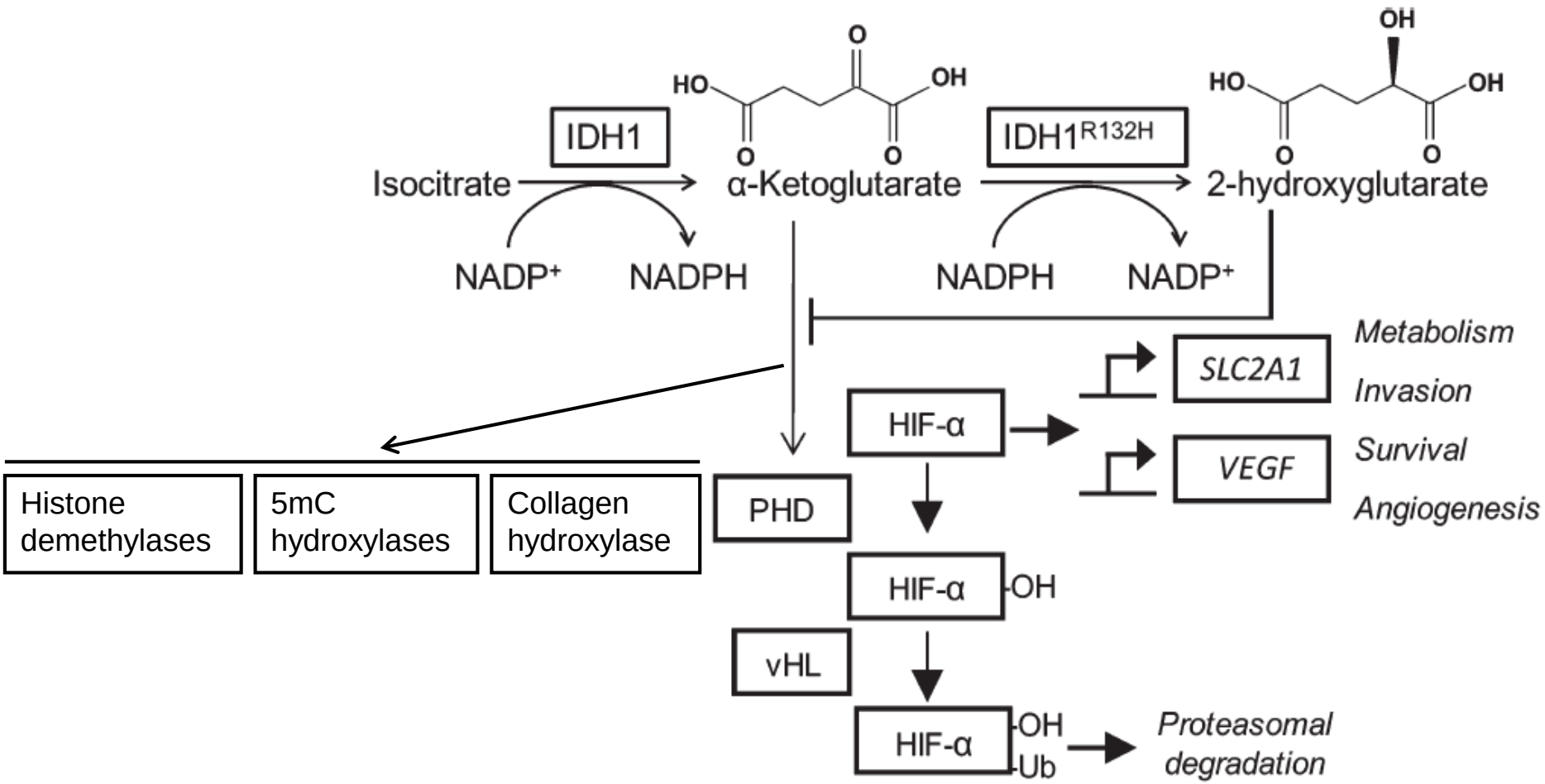


Figure 16-21

• IDH1 is mutated in many diffuse gliomas



PHD: HIF prolyl hydroxylase; vHL: von Hippel Lindau protein (E3); VEGF: vascular endothelial growth factor; SLC2A: solute carrier family 2 member 1

• Summary 16.3

- The overall rate of the citric acid cycle is controlled by the rate of conversion of pyruvate to acetyl-CoA and by the flux through citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. These fluxes are largely determined by the concentrations of substrates and products: the end products ATP and NADH are inhibitory, and the substrates NAD^+ and ADP are stimulatory.
- The production of acetyl-CoA for the citric acid cycle by the PDH complex is inhibited allosterically by metabolites that signal a sufficiency of metabolic energy (ATP, acetyl-CoA, NADH, and fatty acids) and stimulated by metabolites that indicate a reduced energy supply (AMP, NAD^+ , CoA).
- Complexes of consecutive enzymes in a pathway allow substrate channeling between them.

16.4 The Glyoxylate Cycle

- **Vertebrates cannot convert acetyl-CoA into glucose**

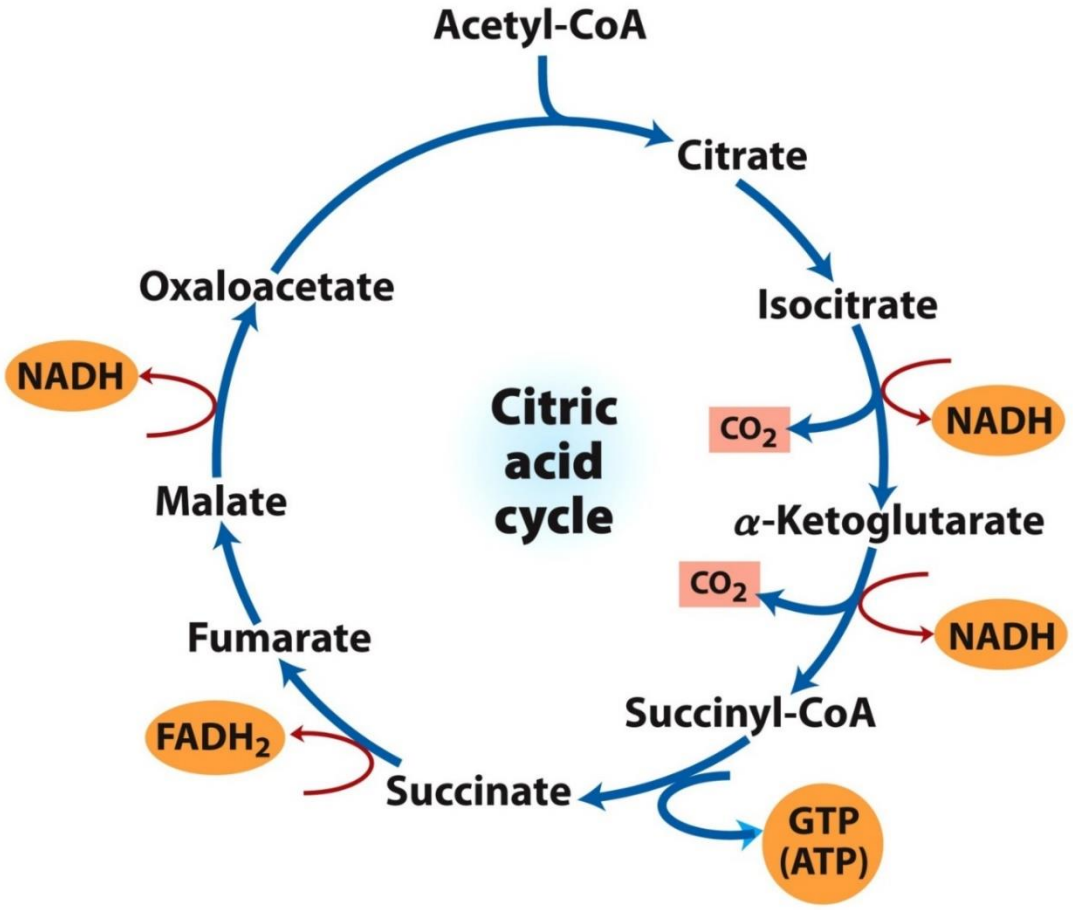
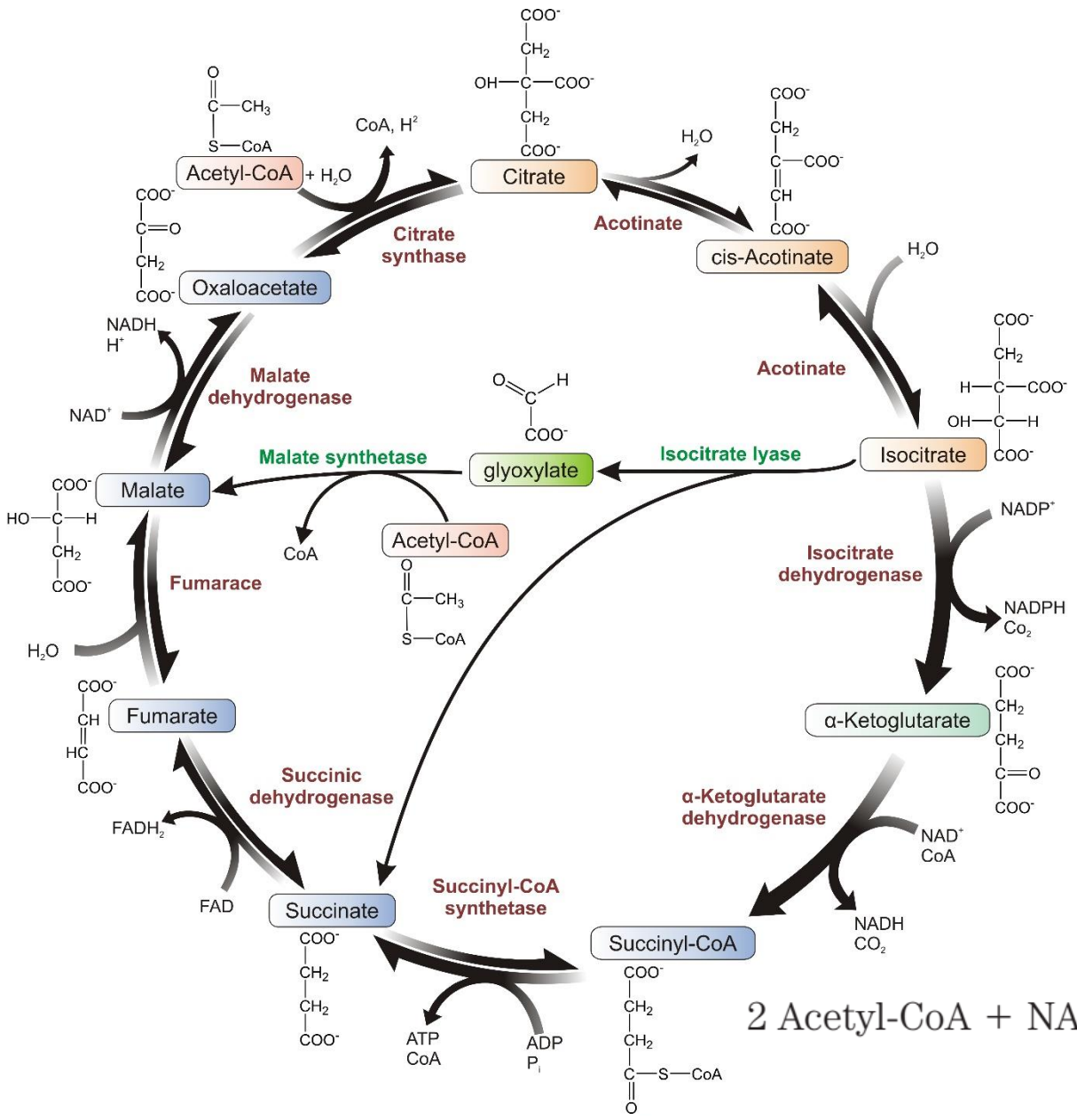


Figure 16-14

• The glyoxylate cycle



Vertebrates lack **isocitrate lyase** and **malate synthase**

$$2\ \text{Acetyl-CoA} + \text{NAD}^+ + 2\text{H}_2\text{O} \longrightarrow \text{succinate} + 2\text{CoA} + \text{NADH} + \text{H}^+$$

• Glyoxysome

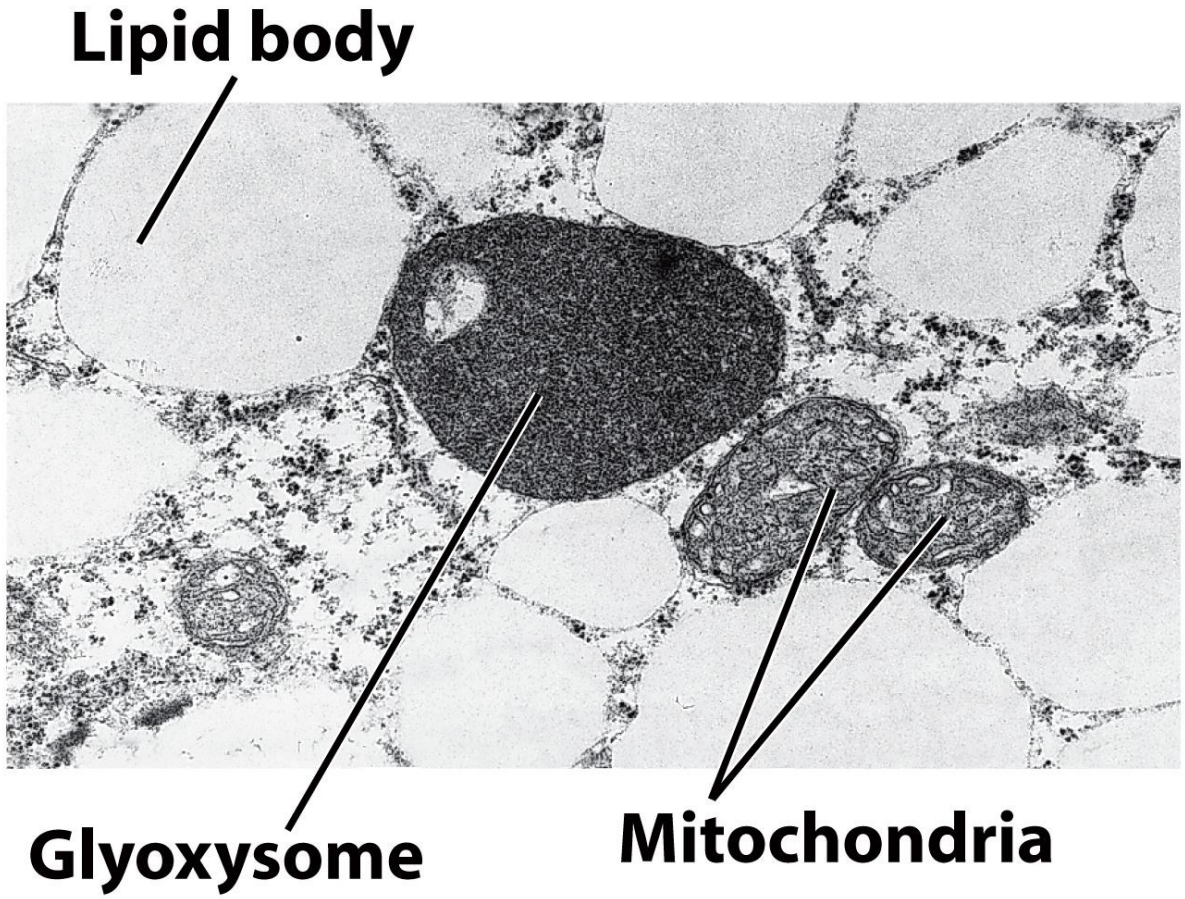
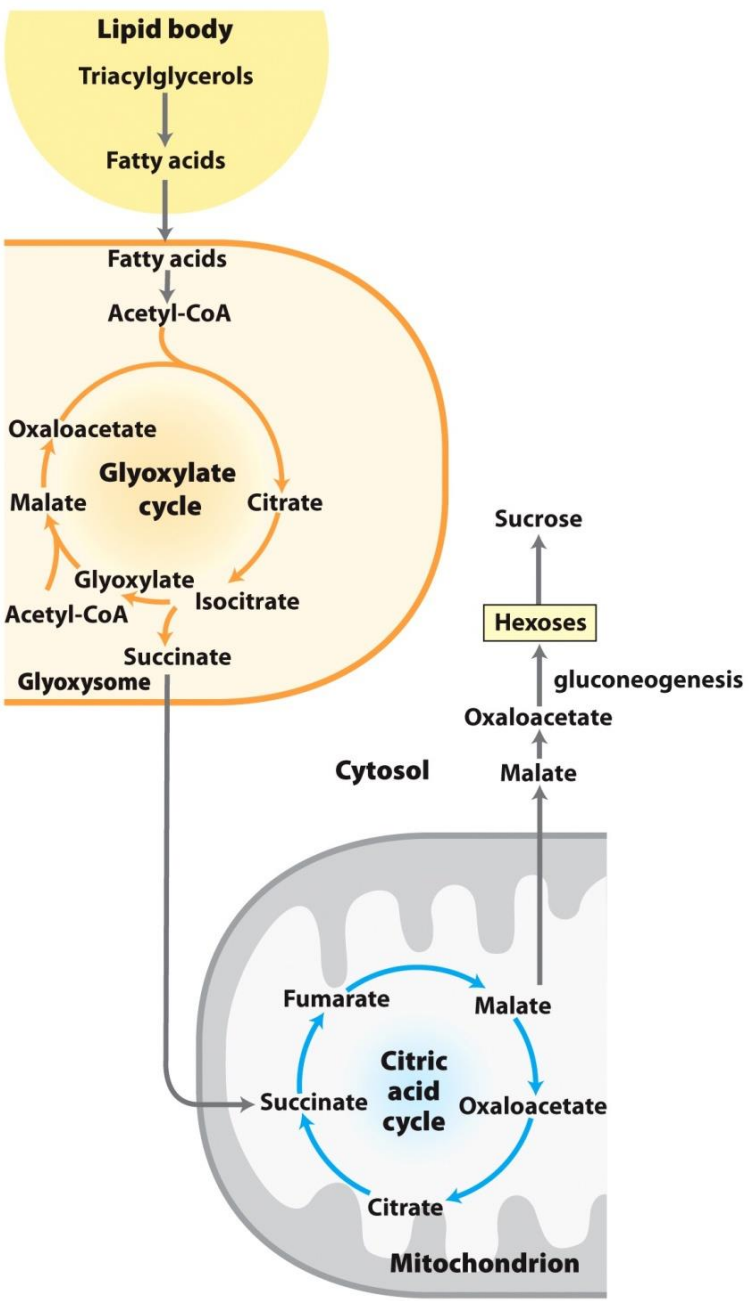


Figure 16-23

Electron micrograph of a germinating cucumber seed, showing a glyoxysome, mitochondria, and surrounding lipid bodies

- **Relationship between the glyoxylate and citric acid cycles**



- **Citric acid and glyoxylate cycle are coordinately regulated**

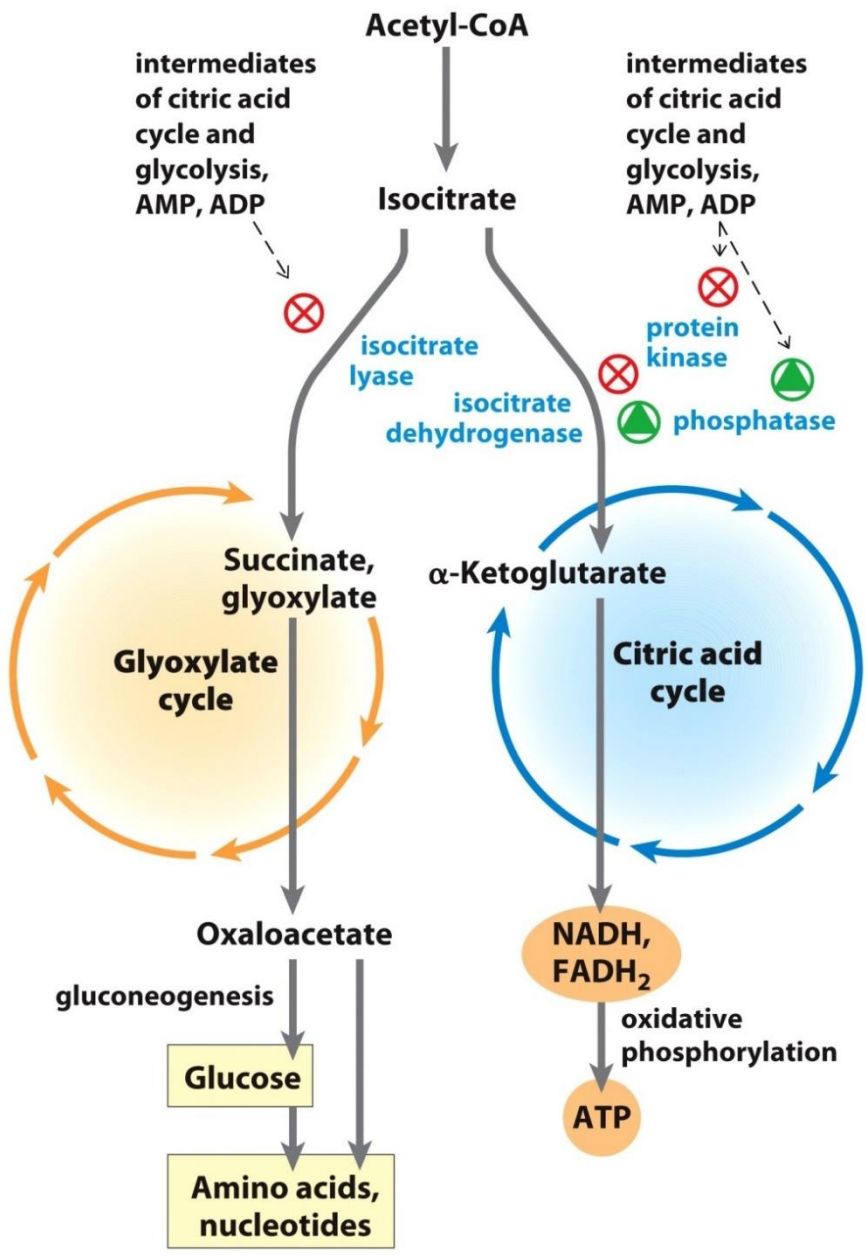


Figure 16-25

• Summary 16.4

- The glyoxylate cycle is active in the germinating seeds of some plants and in certain microorganisms that can live on acetate as the sole carbon source. In plants, the pathway takes place in glyoxysomes in seedlings. It involves several citric acid cycle enzymes and two additional enzymes: isocitrate lyase and malate synthase.
- In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the *net* formation of succinate, oxaloacetate, and other cycle intermediates from acetyl-CoA. Oxaloacetate thus formed can be used to synthesize glucose via gluconeogenesis.

• Summary 16.4

- Vertebrates lack the glyoxylate cycle and **cannot** synthesize glucose from acetate or the fatty acids that give rise to acetyl-CoA.

- The partitioning of isocitrate between the citric acid cycle and the glyoxylate cycle is controlled at the level of isocitrate dehydrogenase, which is regulated by reversible phosphorylation.

CHAPTER 17
Fatty Acid Catabolism

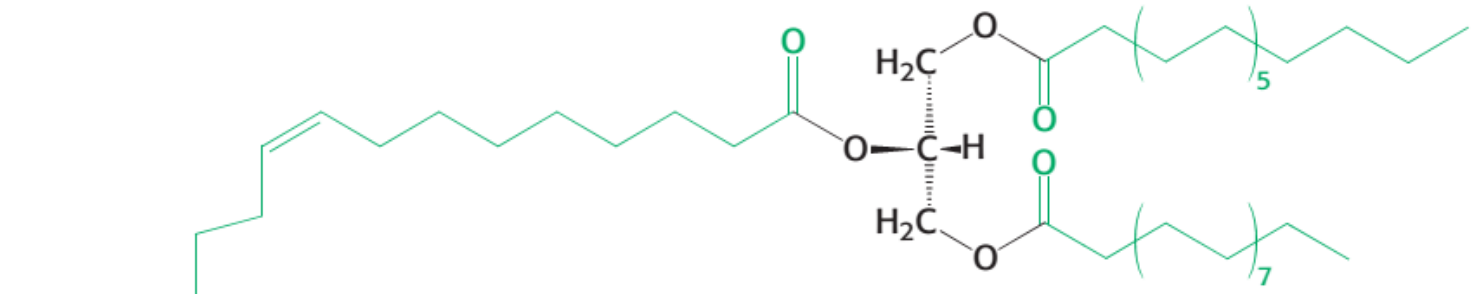
p667

• Physiological roles of fatty acids

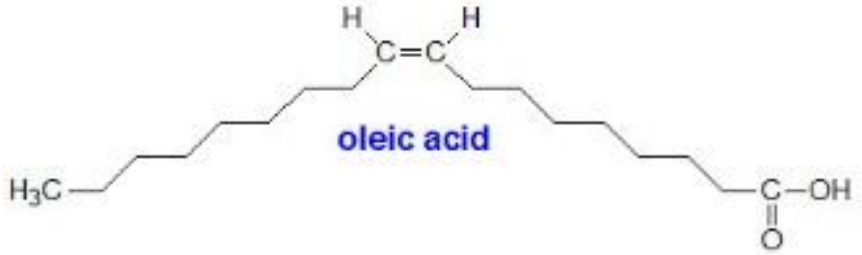
- Fatty acids are fuel molecules. They are stored as triacylglycerols (also called neutral fats or triglycerides).
- Fatty acids are building blocks of phospholipids and glycolipids.
- Many proteins are modified by the covalent attachment of fatty acids, which targets the proteins to membrane locations.
- Fatty acid derivatives serve as hormones and intracellular messengers.

• Triacylglycerols (TAG)

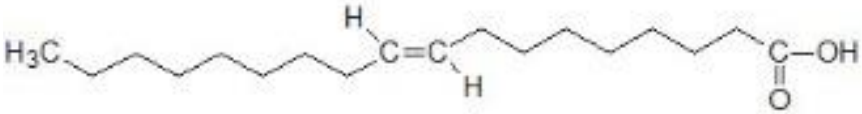
- Triacylglycerides are composed of a glycerol backbone to which 3 fatty acids are esterified.



A triacylglycerol



oleic acid



elaidic acid

• Advantages to storing energy in triacylglycerols

1. Fatty acids, the long alkyl chains of TAG, are highly reduced structure with an energy of complete oxidation (~38 kJ/g) more than twice that for the same weight of carbohydrate or protein. (high free energy yield)

2. TAGs are insoluble (unsolvated), and thus do not raise the osmolarity of the cytosol.

3. TAGs are chemically inert, without the risk of undesired chemical reactions with other cellular constituents.

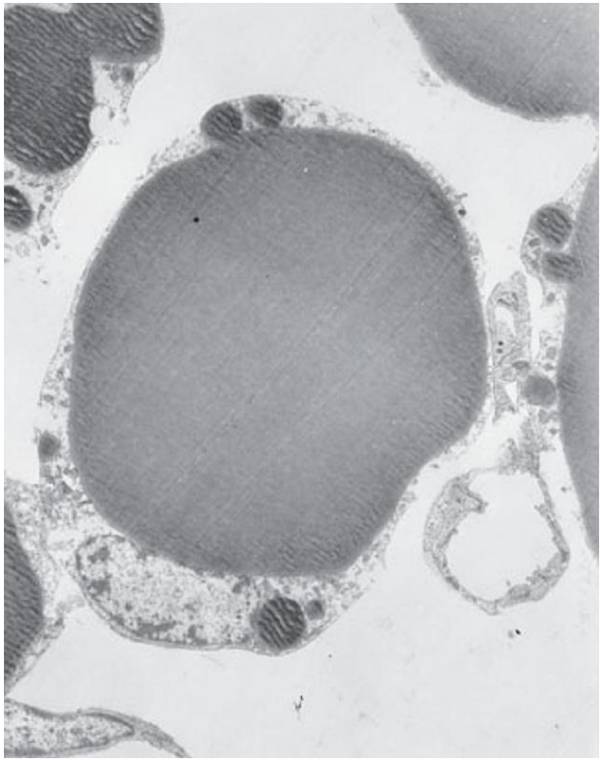
• Triacylglycerols Are a Major Form of Stored Energy

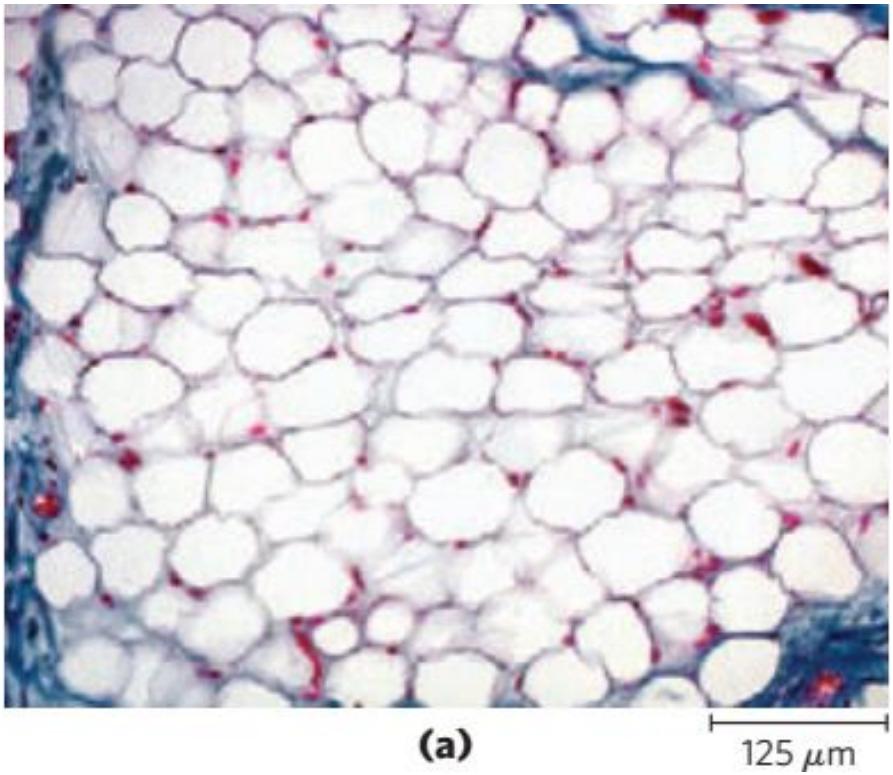
Stored metabolic fuel in a 70-kg person

Constituent	Energy (kJ/g dry weight)	Dry Weight (g)	Available Energy (KJ)
Fat (adipos tissue)	37	15,000	555,000
Protein (muscle)	17	6,000	102,000
Glycogen (muscle)	16	120	1,920
Glycogen (liver)	16	70	1,120
Glucose (extracellular fluid)	16	20	320
Total			660,360

More fat is stored than protein and carbohydrate. Fat accounts for approximately **83%** of available energy.

• Triacylglycerols Are a Major Form of Stored Energy

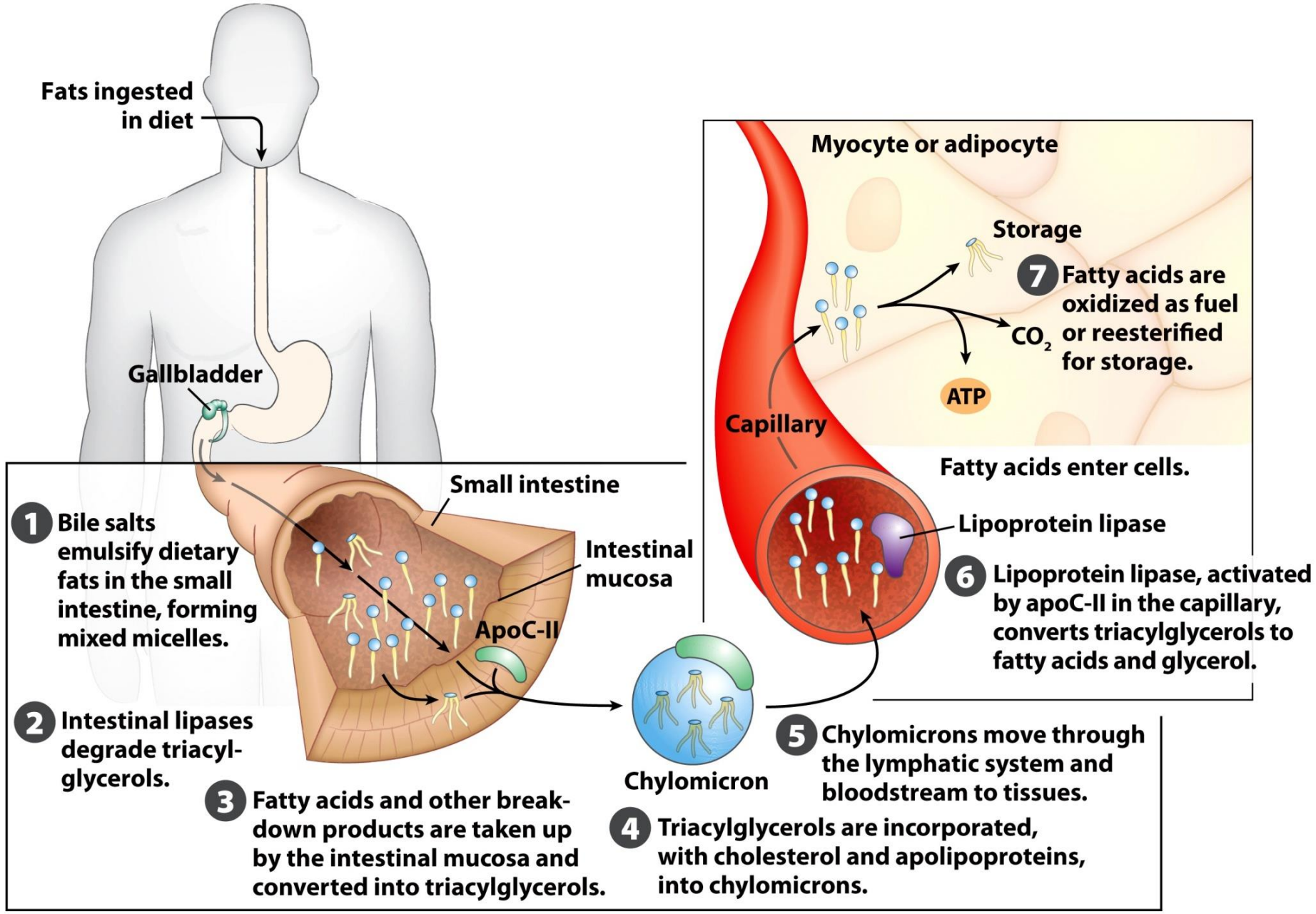




- In mammals, the major site of triacylglycerol accumulation is the cytoplasm of adipocytes.

17.1 Digestion, Mobilization and Transport of Fats

• Processing of dietary lipids in vertebrates



- **Molecular structure of a chylomicron**

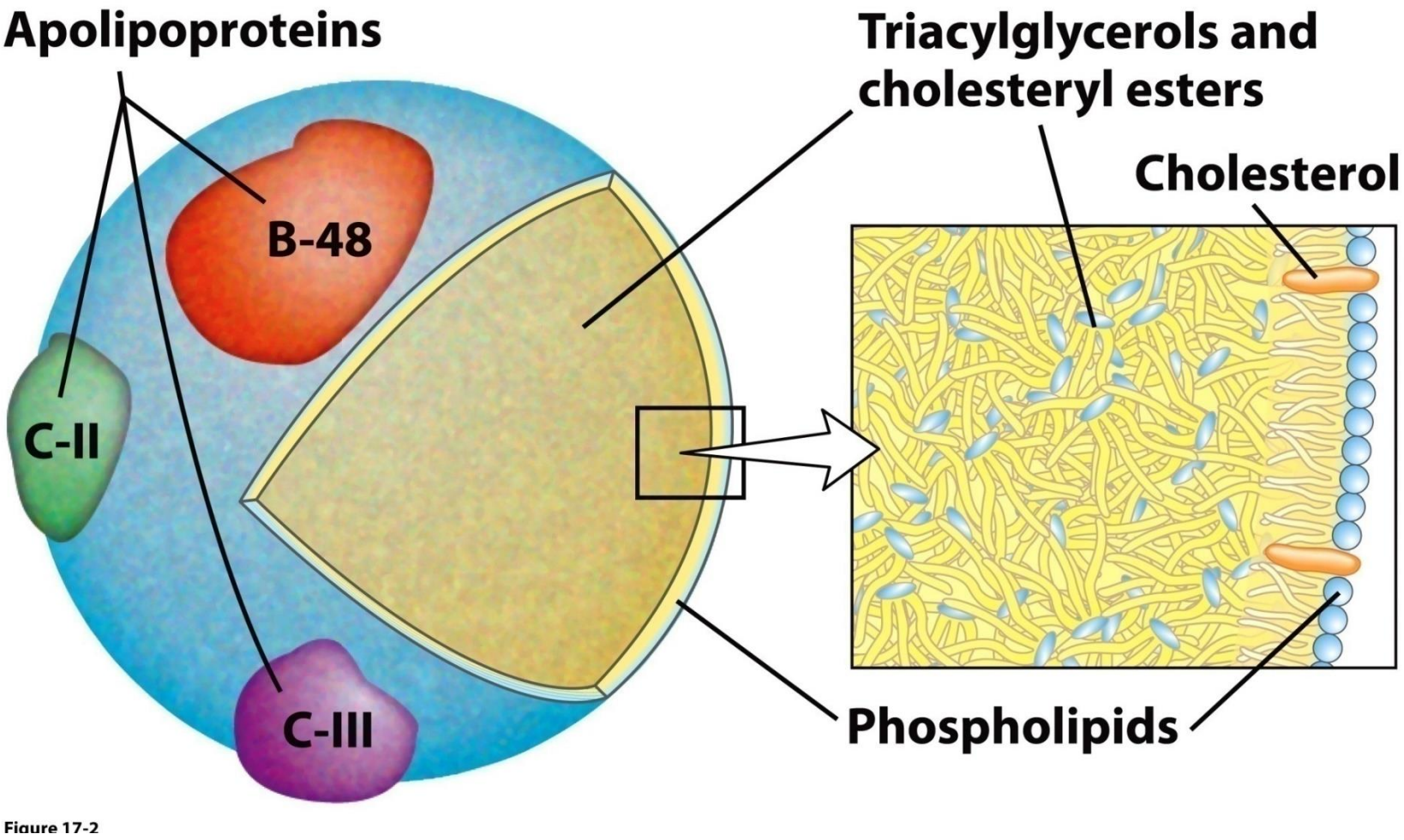
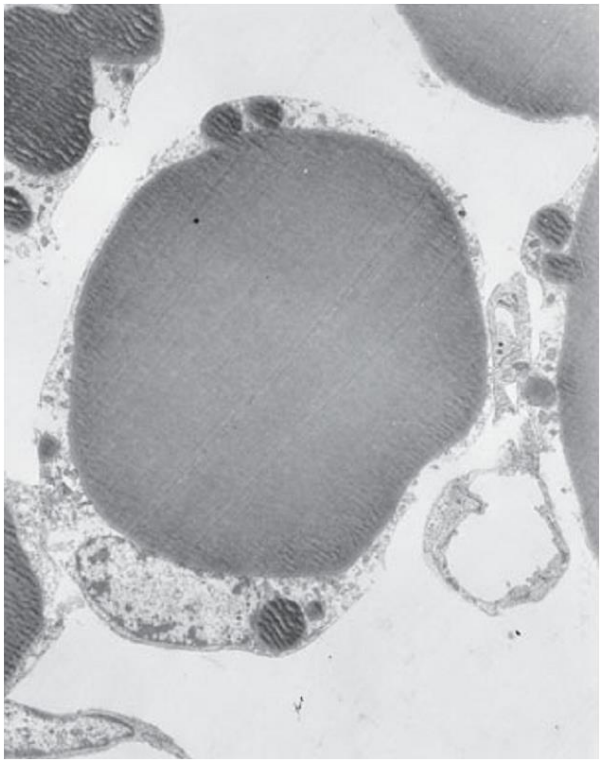
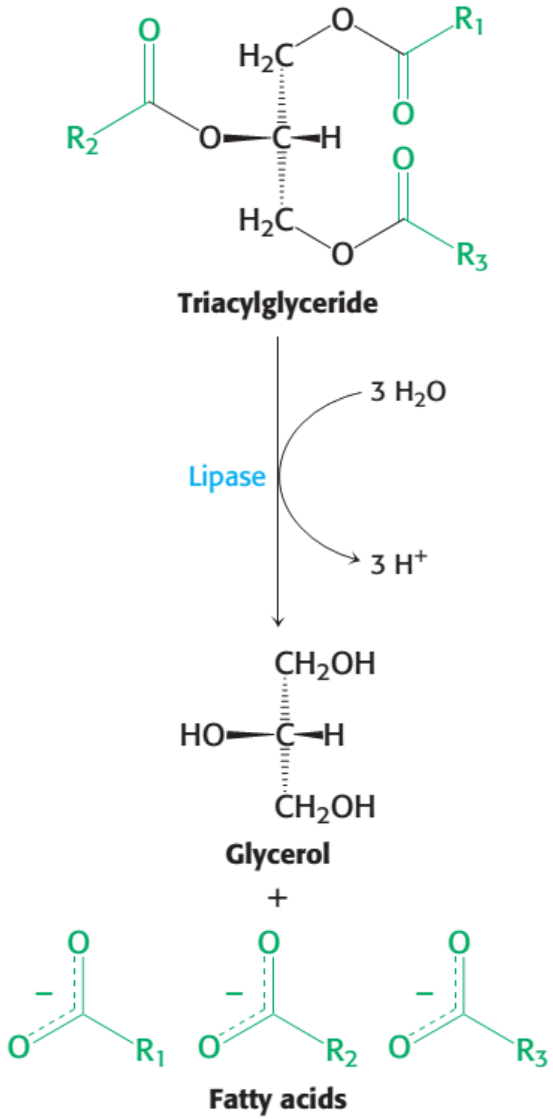


Figure 17-2

• Mobilization of Fat Stores

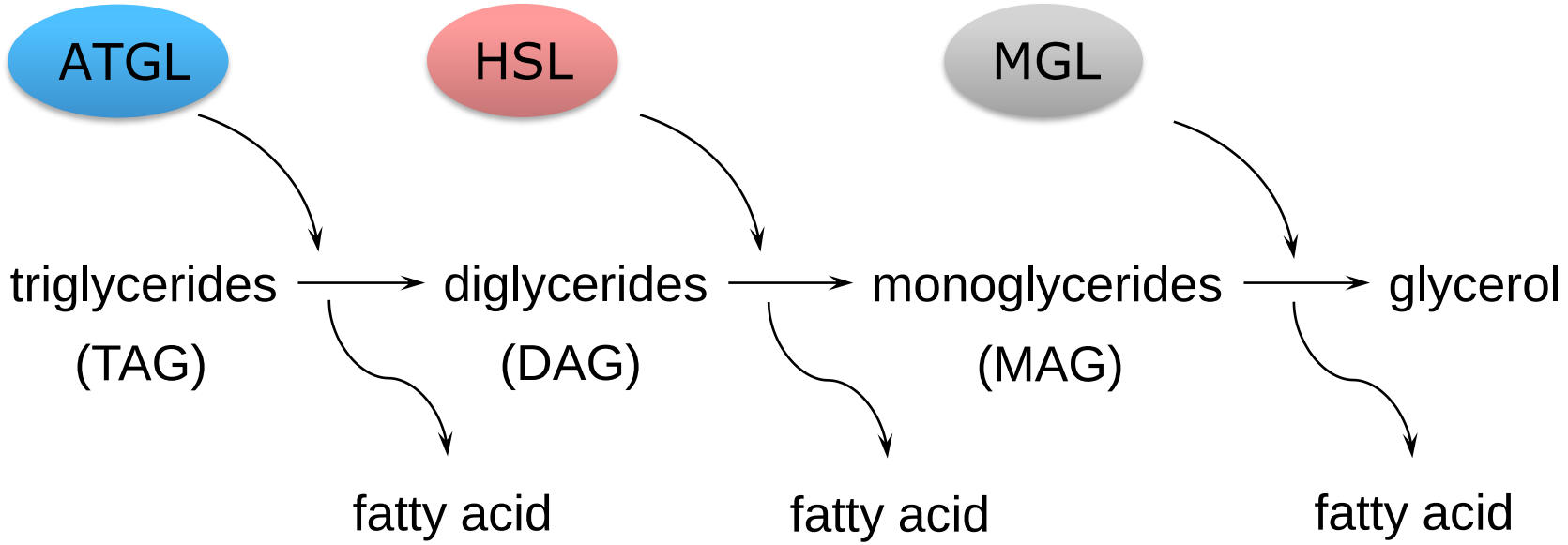




Lipolysis

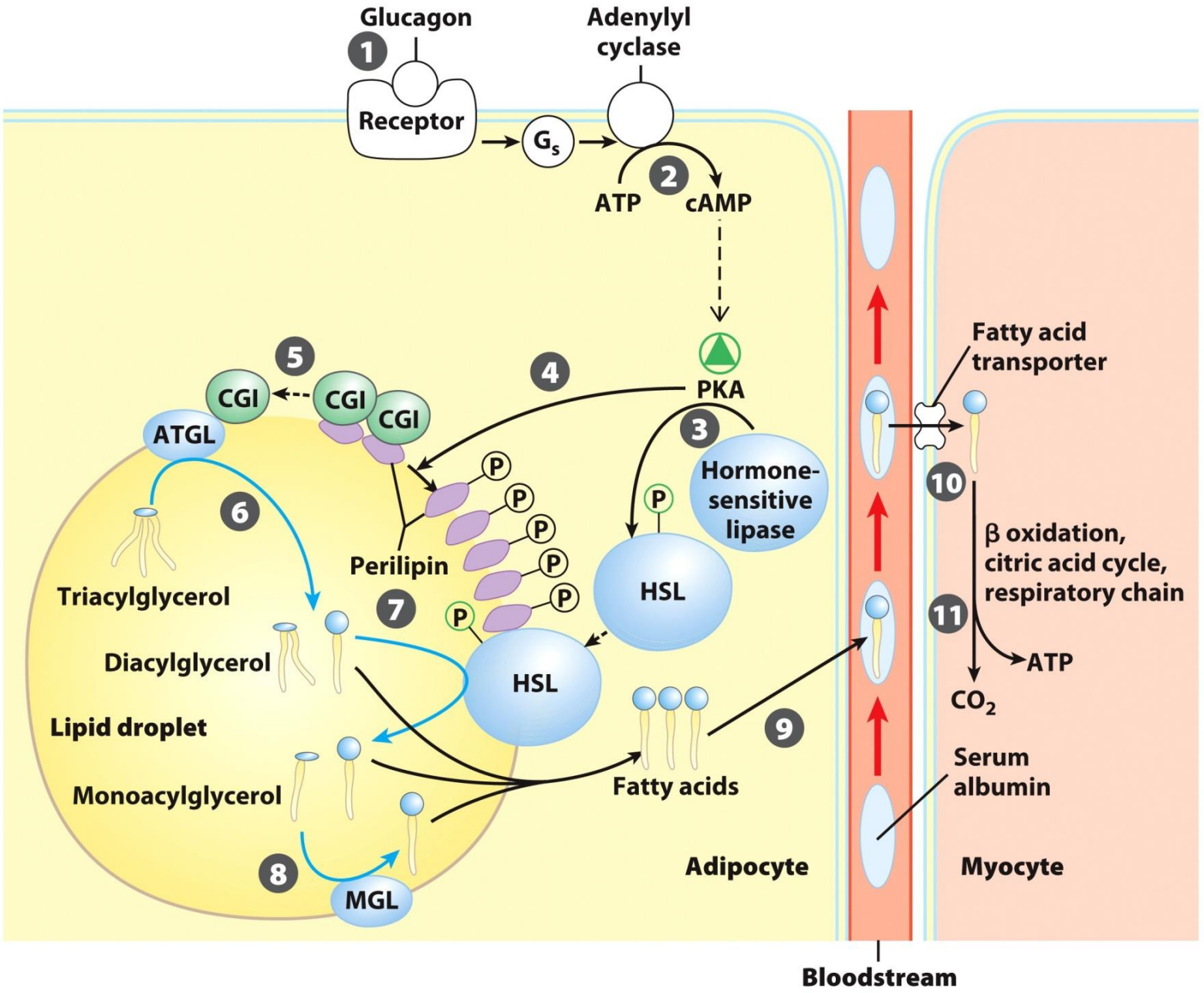
• Mobilization of Fat Stores

- The release of metabolic energy, in the form of fatty acids, is controlled by a complex series of interrelated cascades that result in the activation of triglyceride hydrolysis.

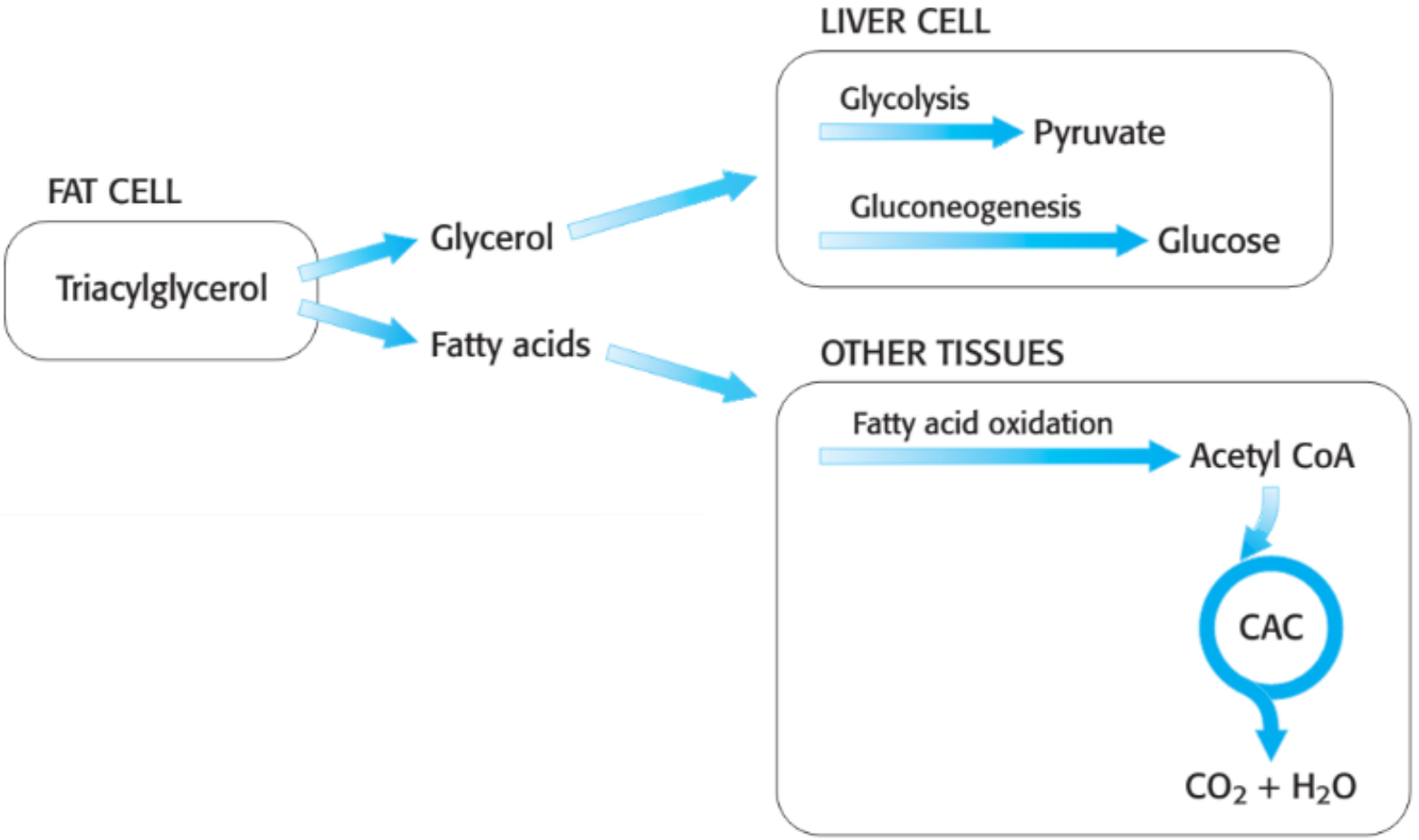


ATGL: adipose triglyceride lipase,
HSL: hormone-sensitive lipase,
MGL: monoacylglycerol lipase.

• Hormone-regulated mobilization of fat stores



- **Lipolysis generates fatty acids and glycerol**



- **Entry of glycerol into the glycolytic pathway**

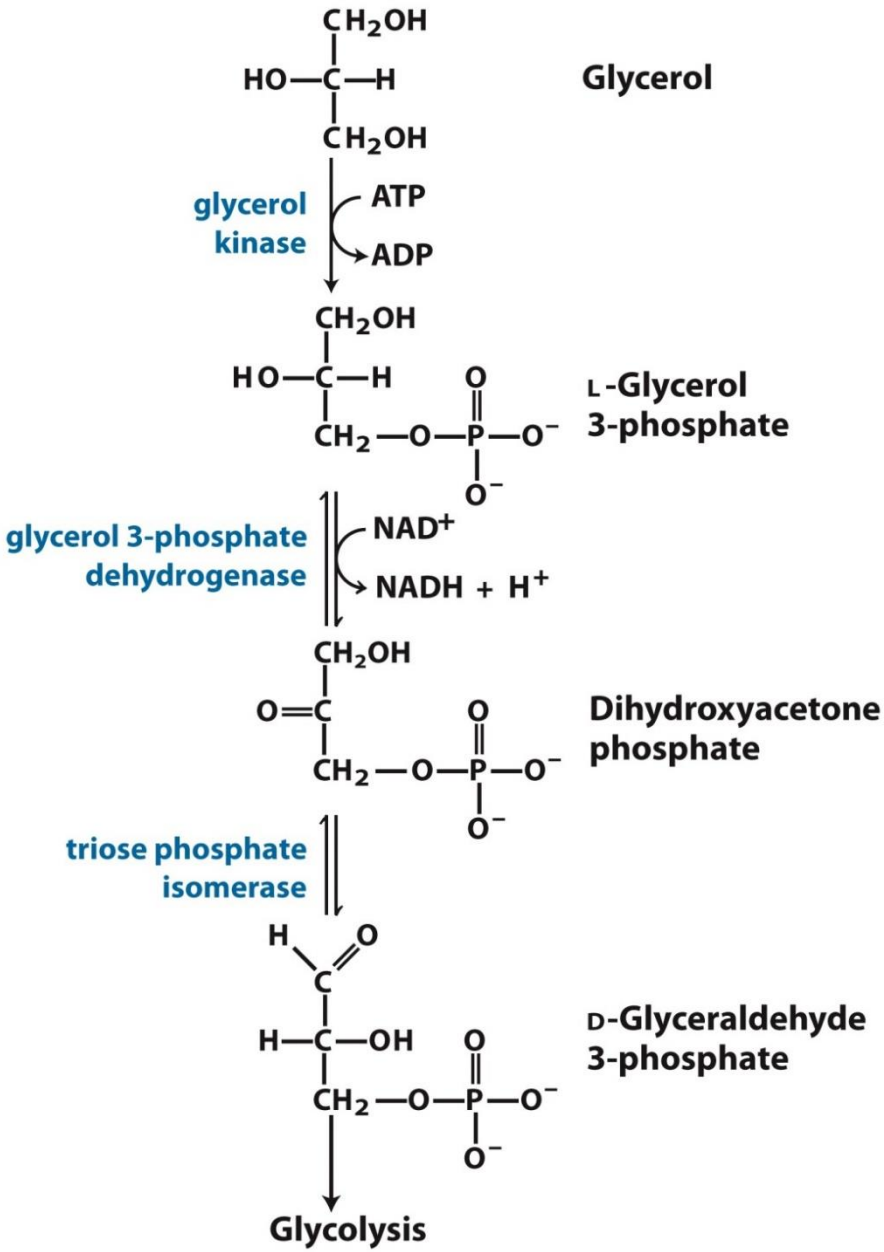
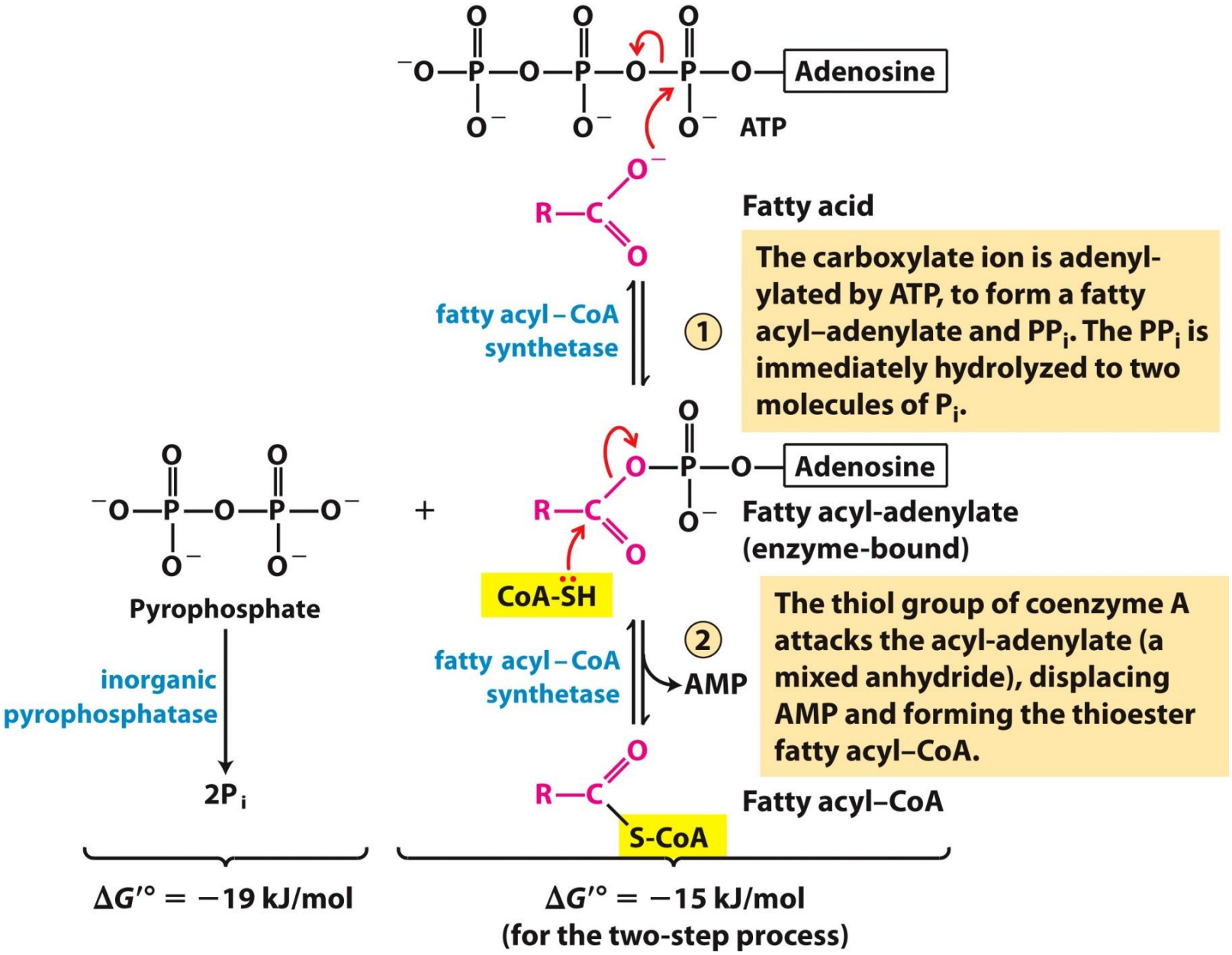


Figure 17-4

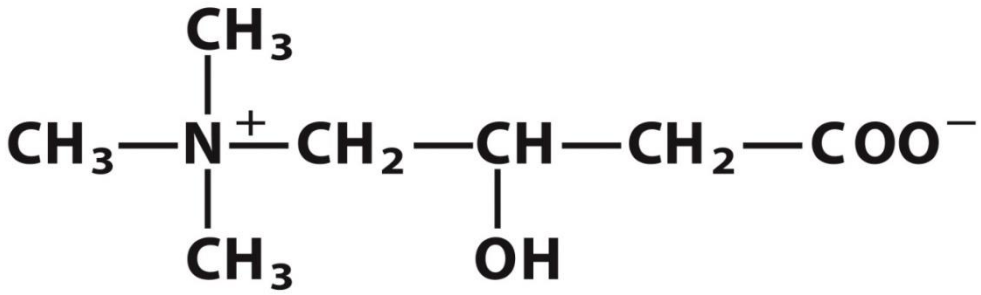
• Fatty acid oxidation in mitochondria or peroxisomes

- Oxidation of fatty acids occurs in the mitochondria and the peroxisomes.
  - Short- and medium-chain fatty acids (SCFAs and MCFAs, respectively), are oxidized exclusively in the mitochondria.
  - Long-chain fatty acids (LCFAs: 12–16 carbons long) are oxidized in both the mitochondria and the peroxisomes with the peroxisomes exhibiting preference for 14-carbon and longer LCFAs.
  - Very-long-chain fatty acids (VLCFAs: C17–C26) are preferentially oxidized in the peroxisomes.

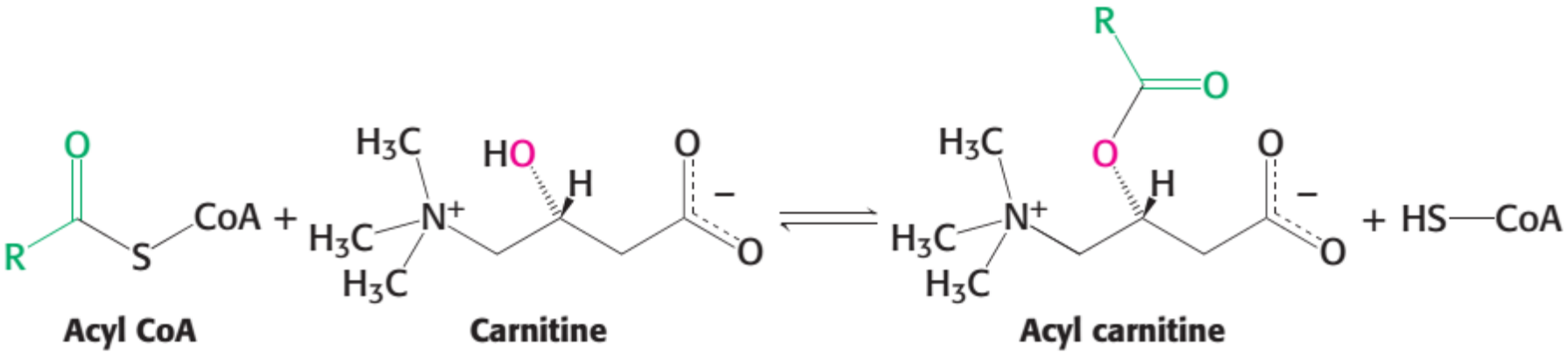
- **Fatty acids must be activated before being oxidized**



• Carnitine carries activated FA into mitochondria



Carnitine



Acyl CoA **Carnitine** **Acyl carnitine**

• Carnitine carries activated FA into mitochondria

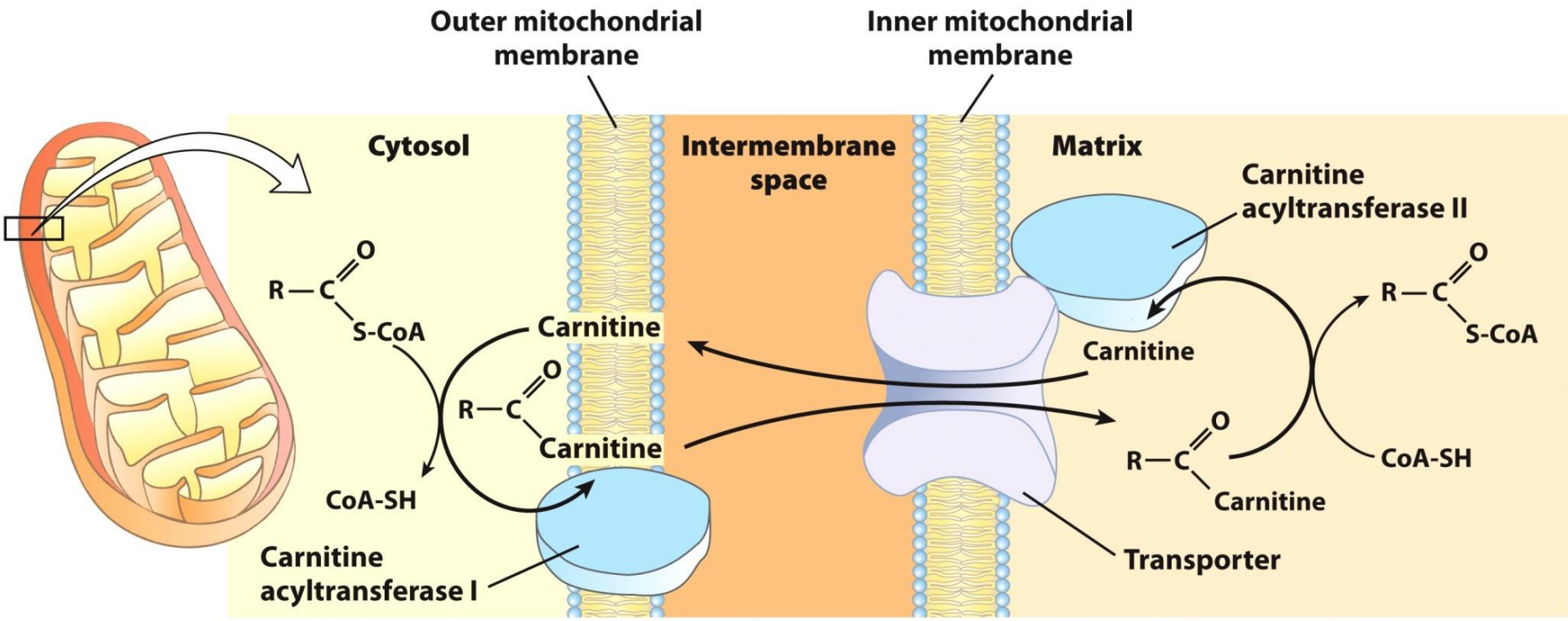


Figure 17-6
Lehninger Principles of Biochemistry, Sixth Edition

• Summary 17.1

- The fatty acids of triacylglycerols furnish a large fraction of the oxidative energy in animals. Dietary triacylglycerols are emulsified in the small intestine by bile salts, hydrolyzed by intestinal lipases, absorbed by intestinal epithelial cells, reconverted into triacylglycerols, then formed into chylomicrons by combination with specific apolipoproteins.
- Chylomicrons deliver triacylglycerols to tissues, where lipoprotein lipase releases free fatty acids for entry into cells. Triacylglycerols stored in adipose tissue are mobilized by a hormonesensitive triacylglycerol lipase. The released fatty acids bind to serum albumin and are carried in the blood to the heart, skeletal muscle, and other tissues that use fatty acids for fuel.
- Once inside cells, fatty acids are activated at the outer mitochondrial membrane by conversion to fatty acyl–CoA thioesters. Fatty acyl–CoA that is to be oxidized enters mitochondria in three steps, via the carnitine shuttle.

17.2 Oxidation of Fatty

• Stages of fatty acid oxidation

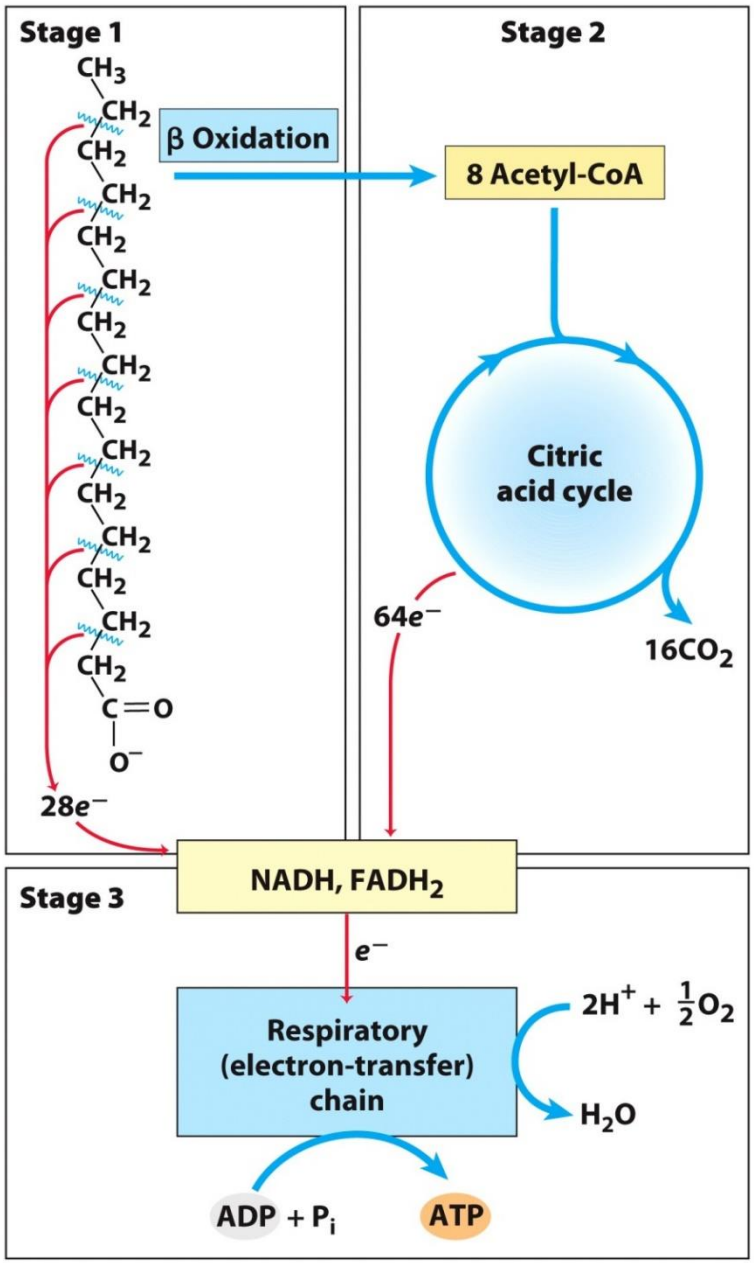


Figure 17-7

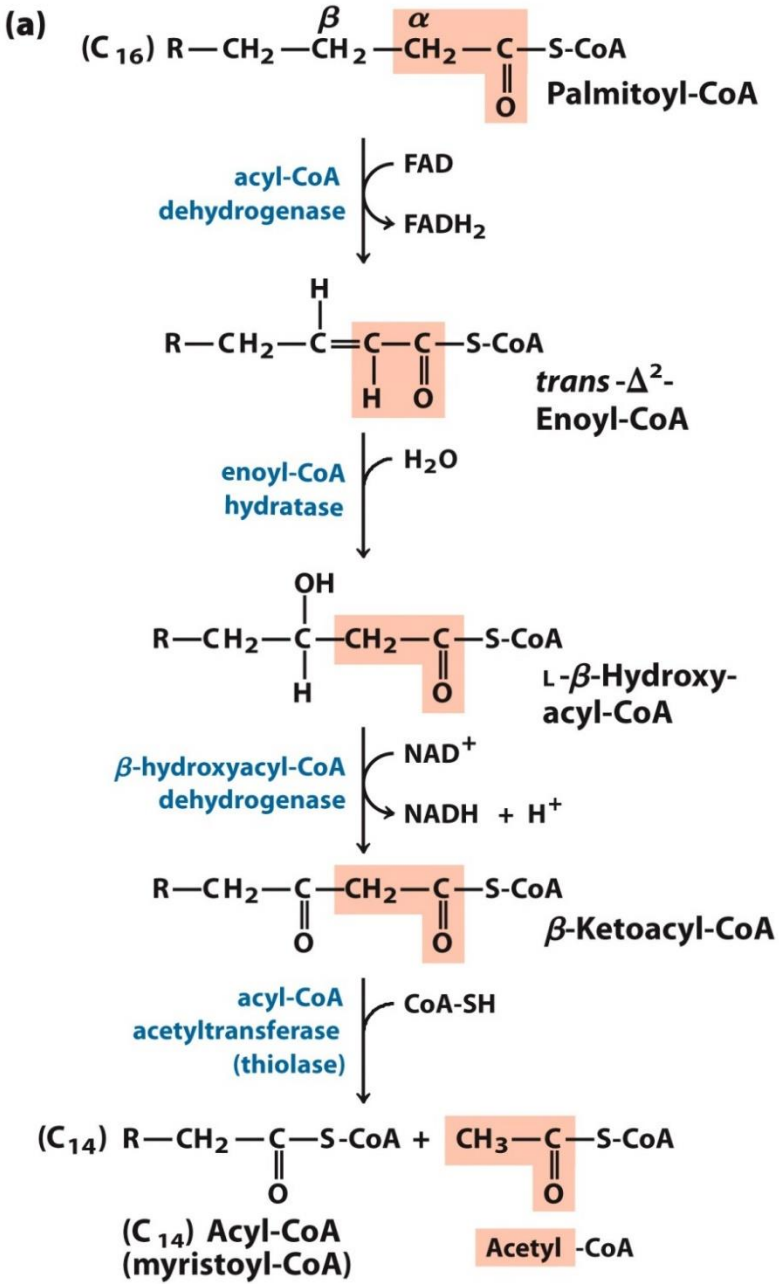
• The β-oxidation pathway

1. Oxidation

2. Hydration

3. Oxidation

4. Thiolysis



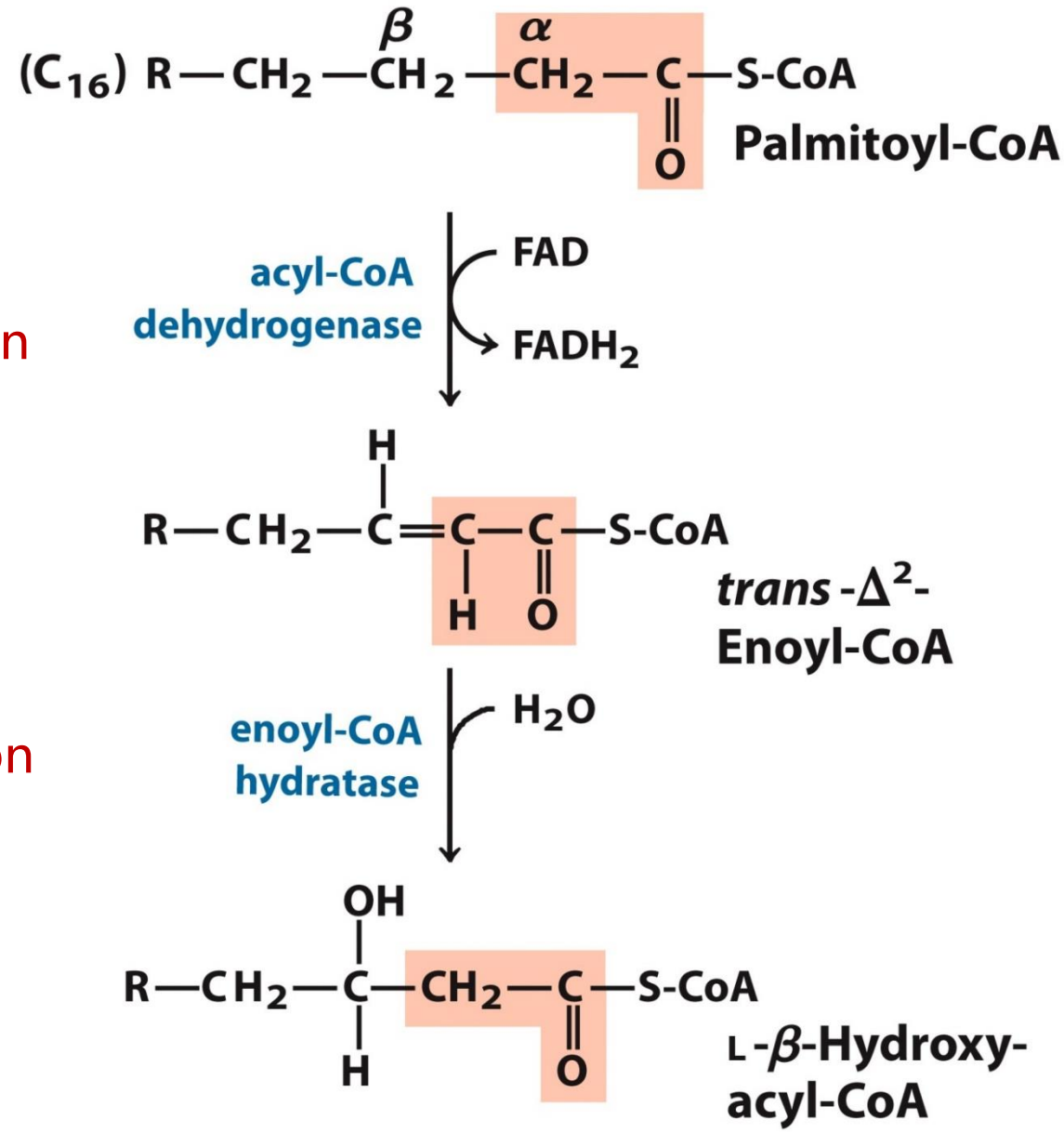
酯酰辅酶A

烯酰辅酶A

β-羟酰辅酶A

β-酮酰辅酶A

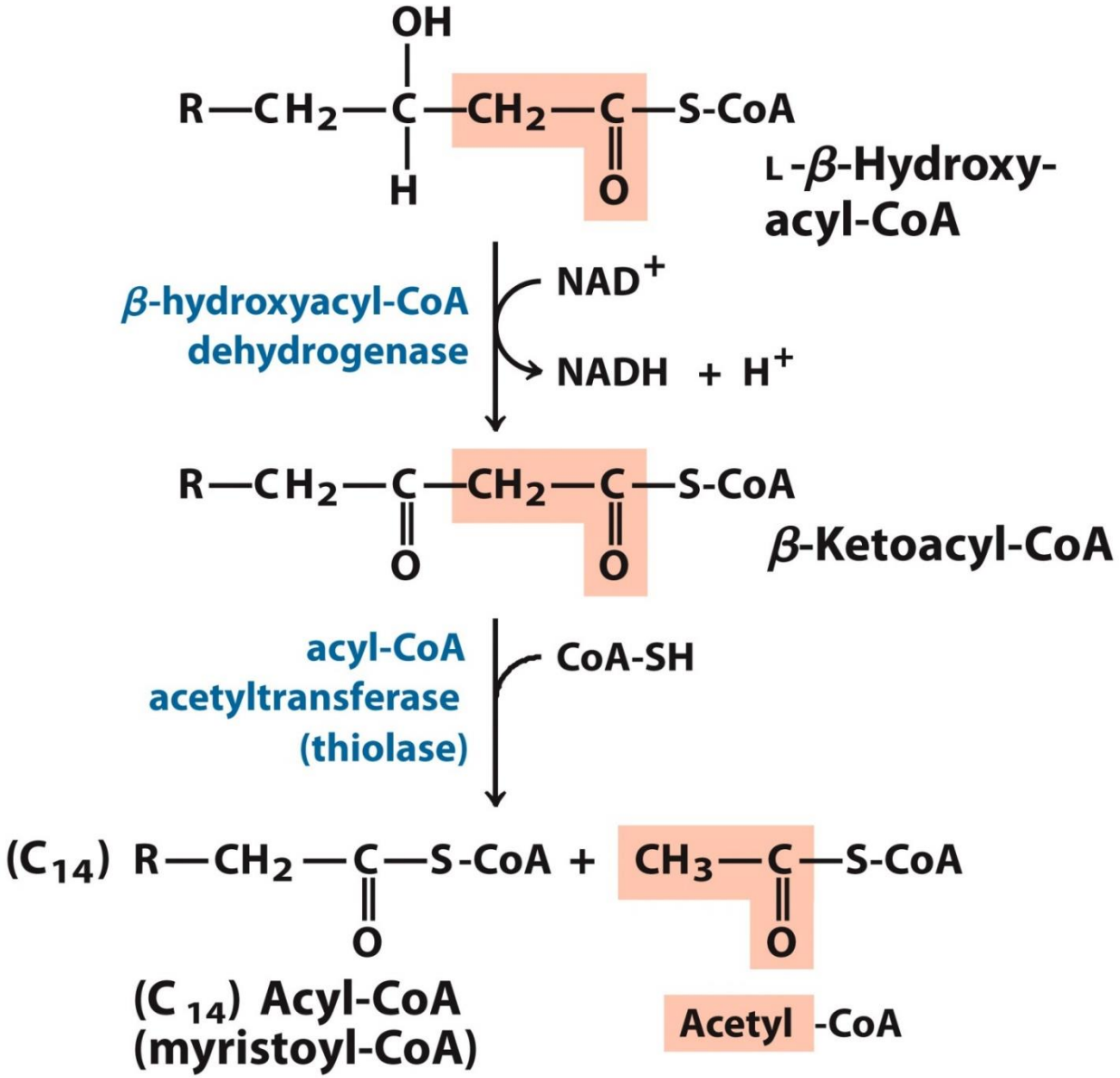
• The β-oxidation pathway



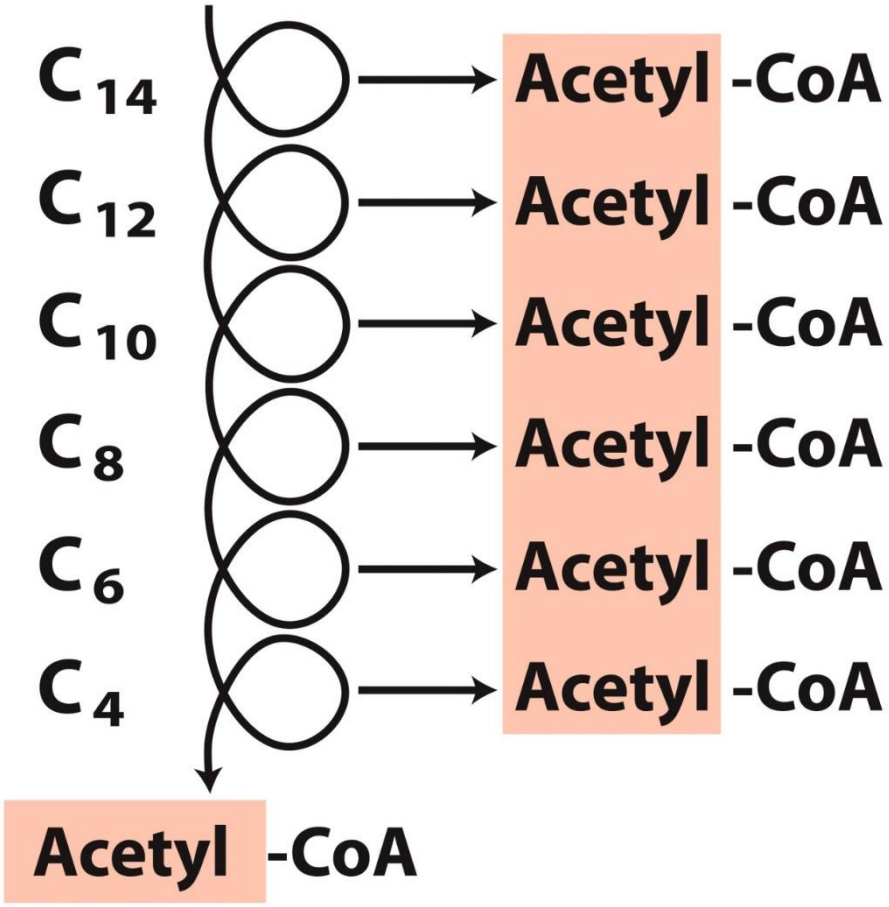
• The β-oxidation pathway

3. Oxidation

4. Thiolysis

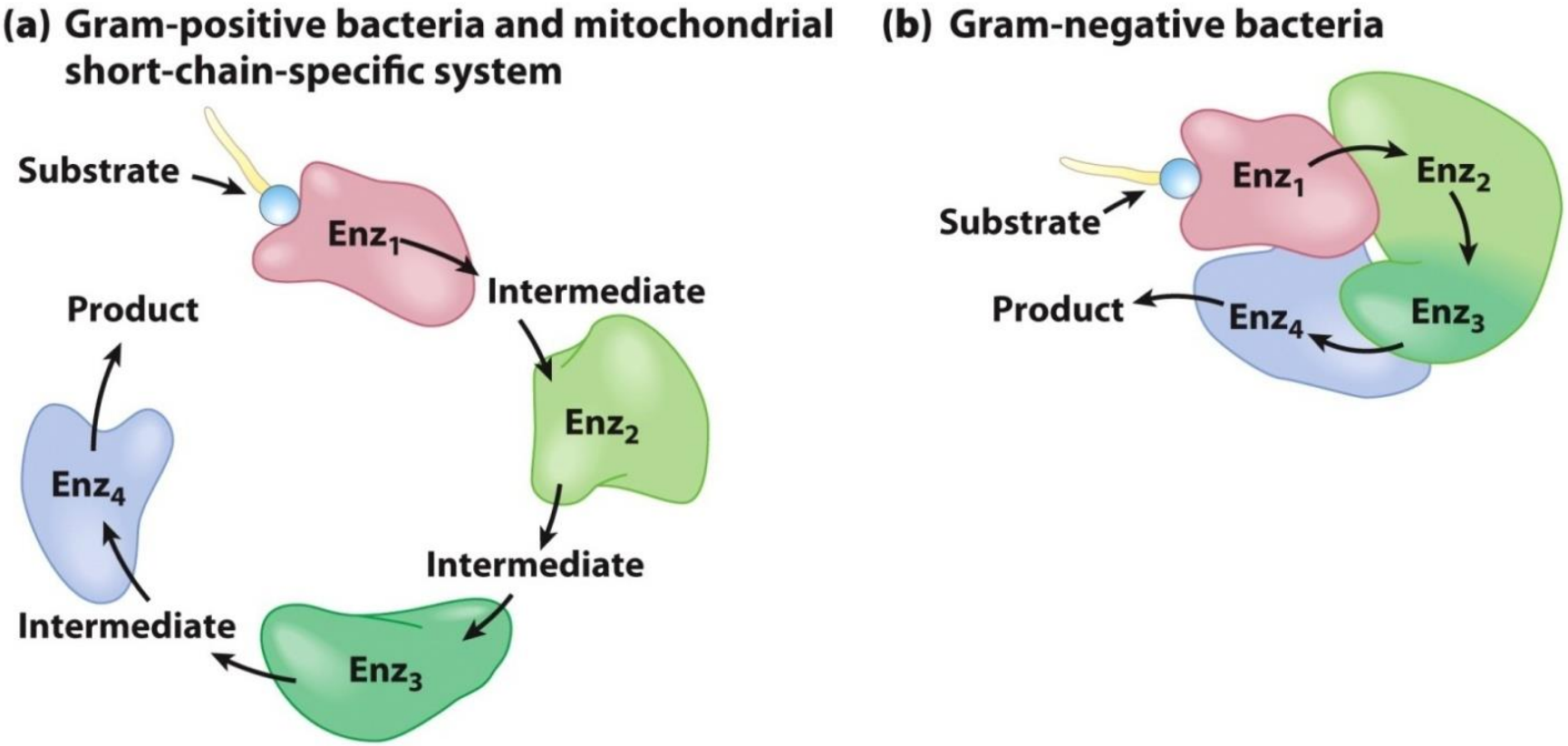


• The β-oxidation pathway

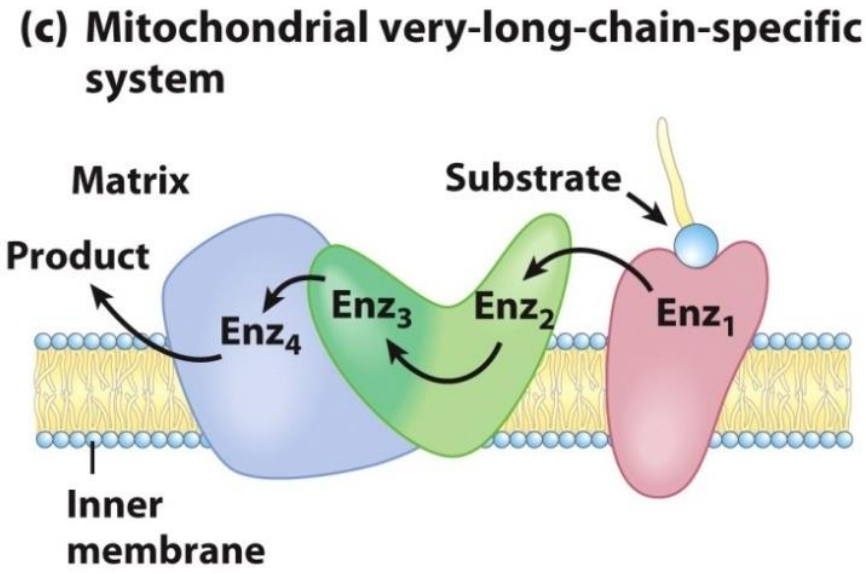


Palmitoyl-CoA + 7CoA + 7FAD + 7NAD^+ + 7H_2O ⟶
8acetyl-CoA + 7$FADH_2$ + 7NADH + 7H^+

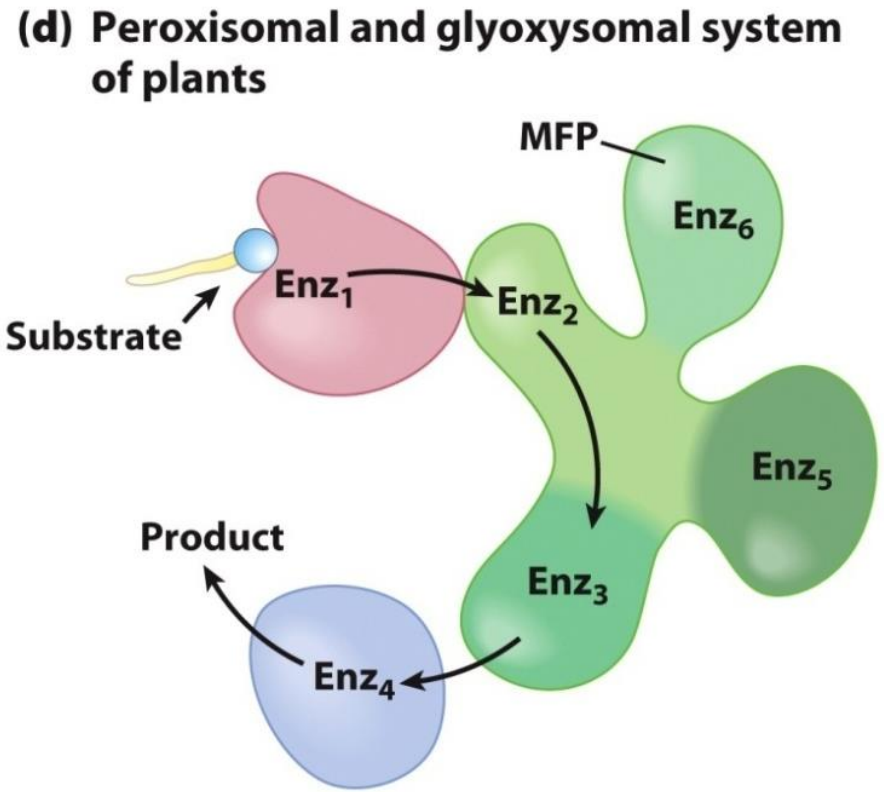
• Divergent evolution of β oxidation enzymes



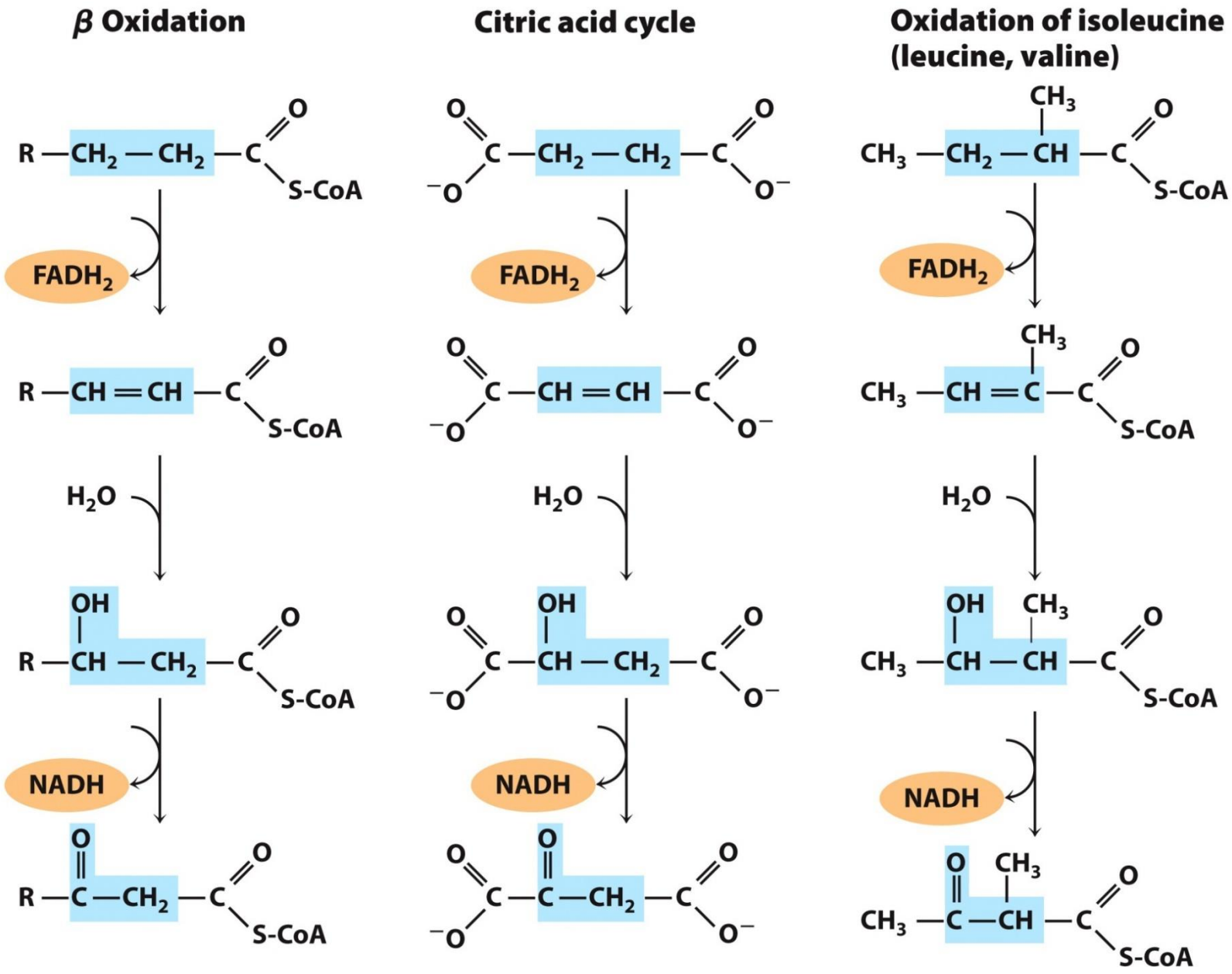
• Divergent evolution of β oxidation enzymes



Trifunctional protein (TFP)



- **Introduction of a carbonyl on the β carbon**



• Fatty acid is a superior energy source

- **The complete oxidation of palmitate yields 106 ATP.**
  - Palmitoyl CoA + 7 FAD + 7 NAD^+ +7 Co A + 7 H_2O →

    7 $FADH_2$ + 7 NADH + 7 H^+ + 8 acetyl CoA

(1 $FADH_2$ → 1.5ATP; NADH → 2.5ATP; Acetyl CoA → 10ATP)

7×1.5 + 7×2.5 +8×10 = 108

108-2 =106 ATP (6.6 ATP per Carbon atom)

TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

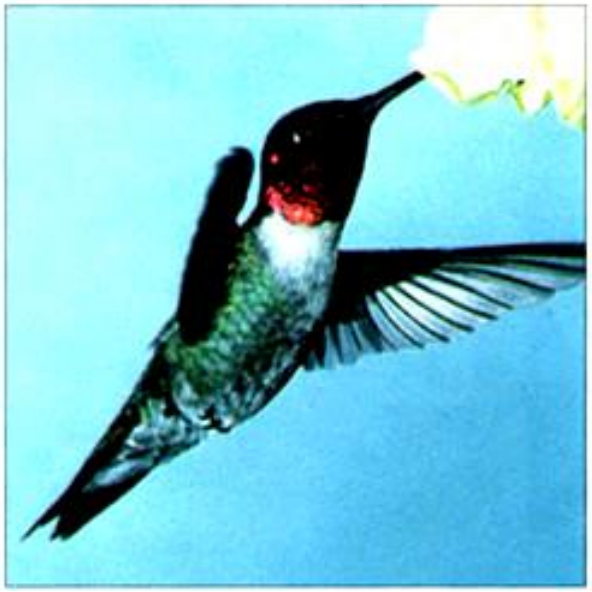
*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 526).

- **Fatty acid is a superior energy source**

Box 17-1 figure 1
Lehninger Principles of Biochemistry, Sixth Edition

- **Fatty acid is a superior energy source**

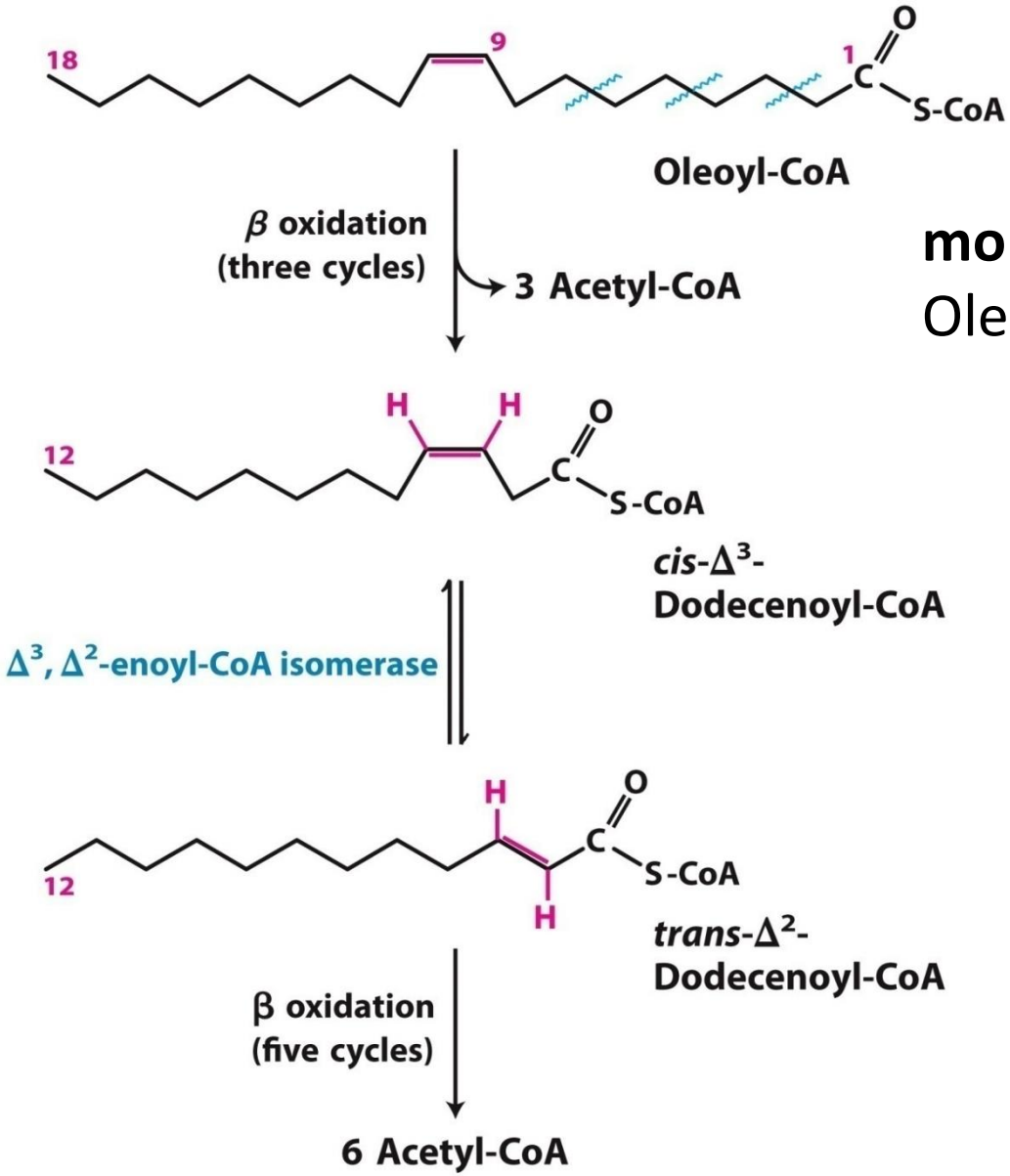


3300 Km!

Ruby-throated hummingbird: 红(玉)喉北蜂鸟　　**Golden plover:** 美洲金鸻


Golden plover
Ruby-throated hummingbird
AK
HI
YT
NT
NU
Canada
Hudson Bay
Gulf of Alaska
BC
AB
SK
MB
ON
QC
NL
WA
OR
CA
ID
NV
MT
WY
UT
AZ
CO
NM
ND
SD
NE
KS
OK
TX
MN
IA
MO
AR
LA
WI
IL
MS
MI
IN
KY
TN
AL
OH
WV
GA
FL
SC
NC
VA
PA
NY
MD
DE
NJ
CT
RI
MA
VT
NH
ME
NB
NS
North Pacific Ocean
North Pacific Ocean
North Atlantic Ocean
Gulf of Mexico
México
Cuba
PR
Guatemala
Nicaragua
Venezuela

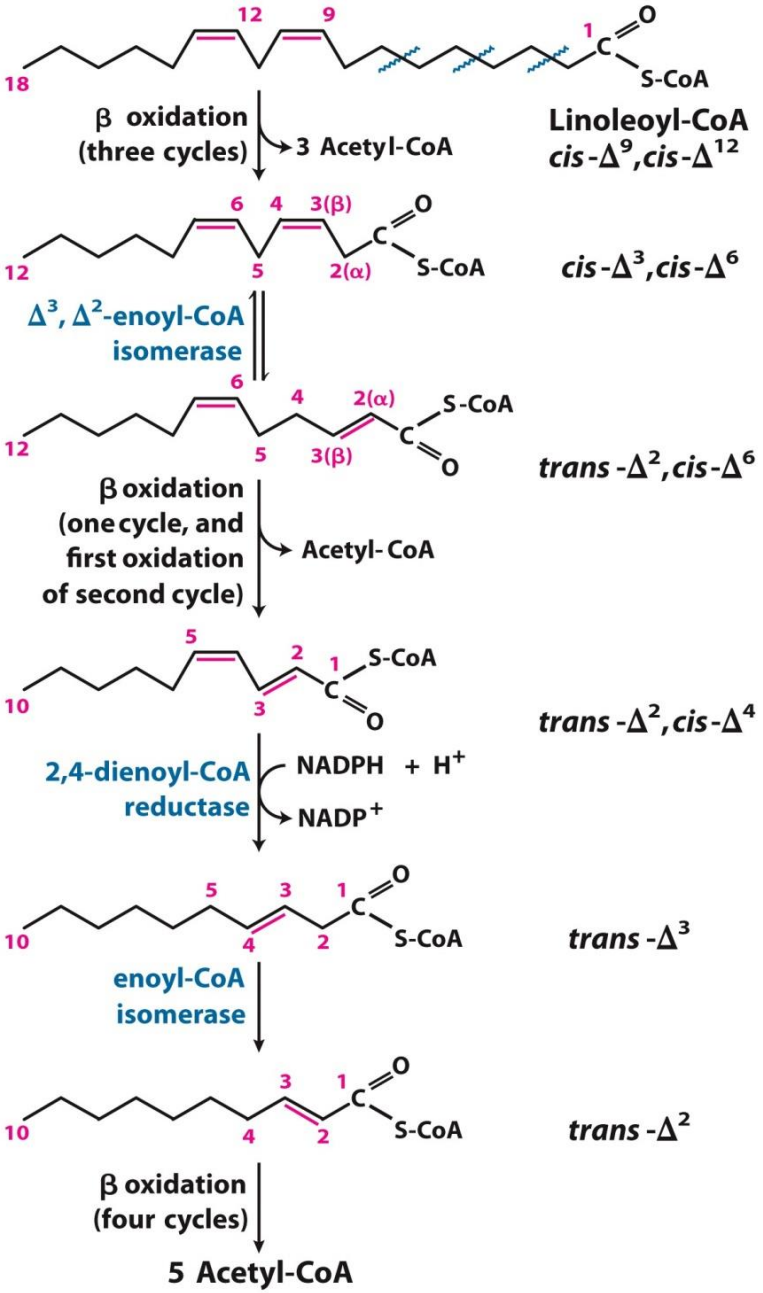
• Oxidation of unsaturated fatty acids



monounsaturated fatty acid
Oleic acid

Figure 17-10

• Oxidation of unsaturated fatty acids



polyunsaturated fatty acid
linoleic acid

• Oxidation of unsaturated fatty acids

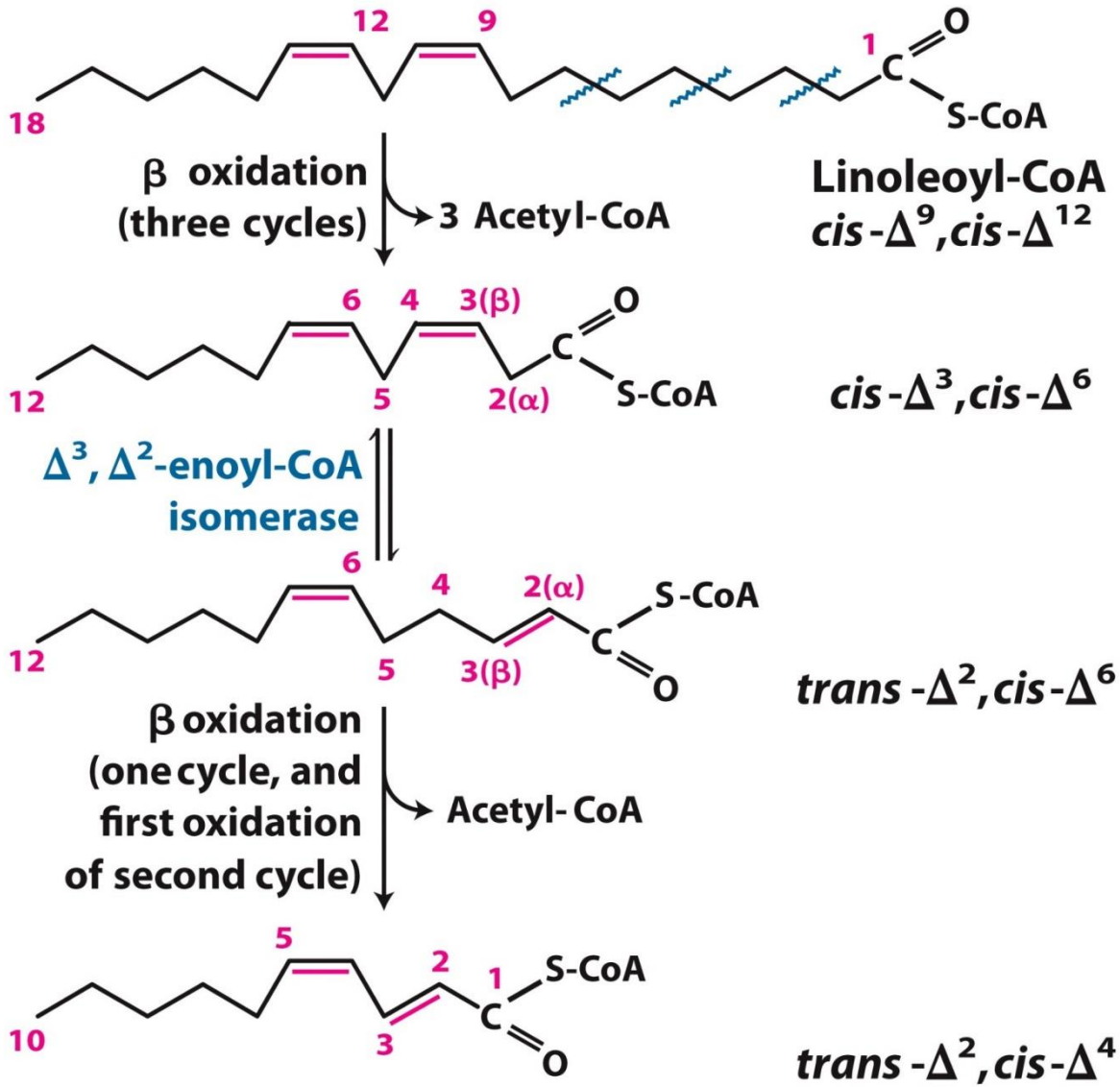


Figure 17-11 part 1
Lehninger Principles of Biochemistry, Sixth Edition

• Oxidation of unsaturated fatty acids

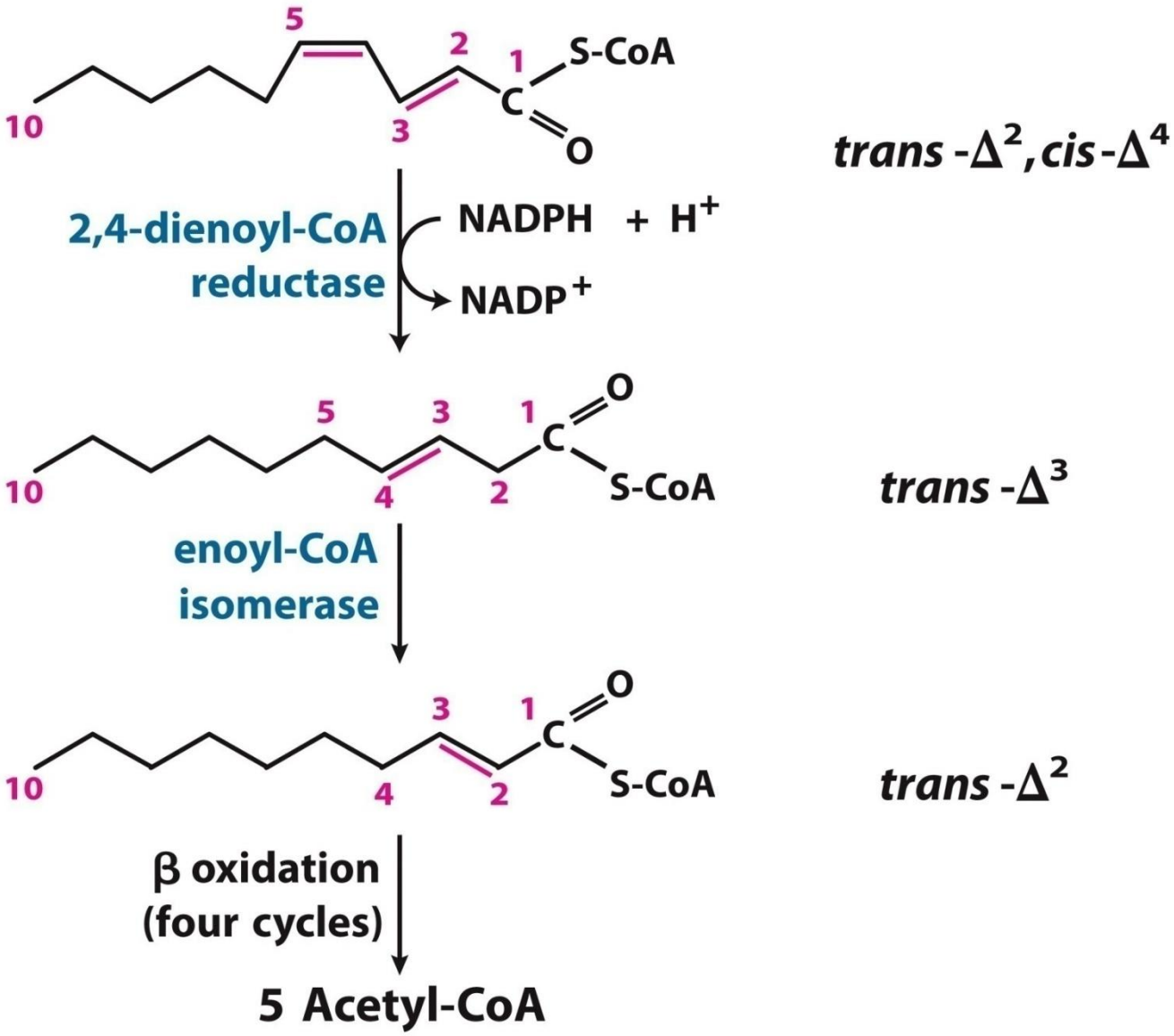
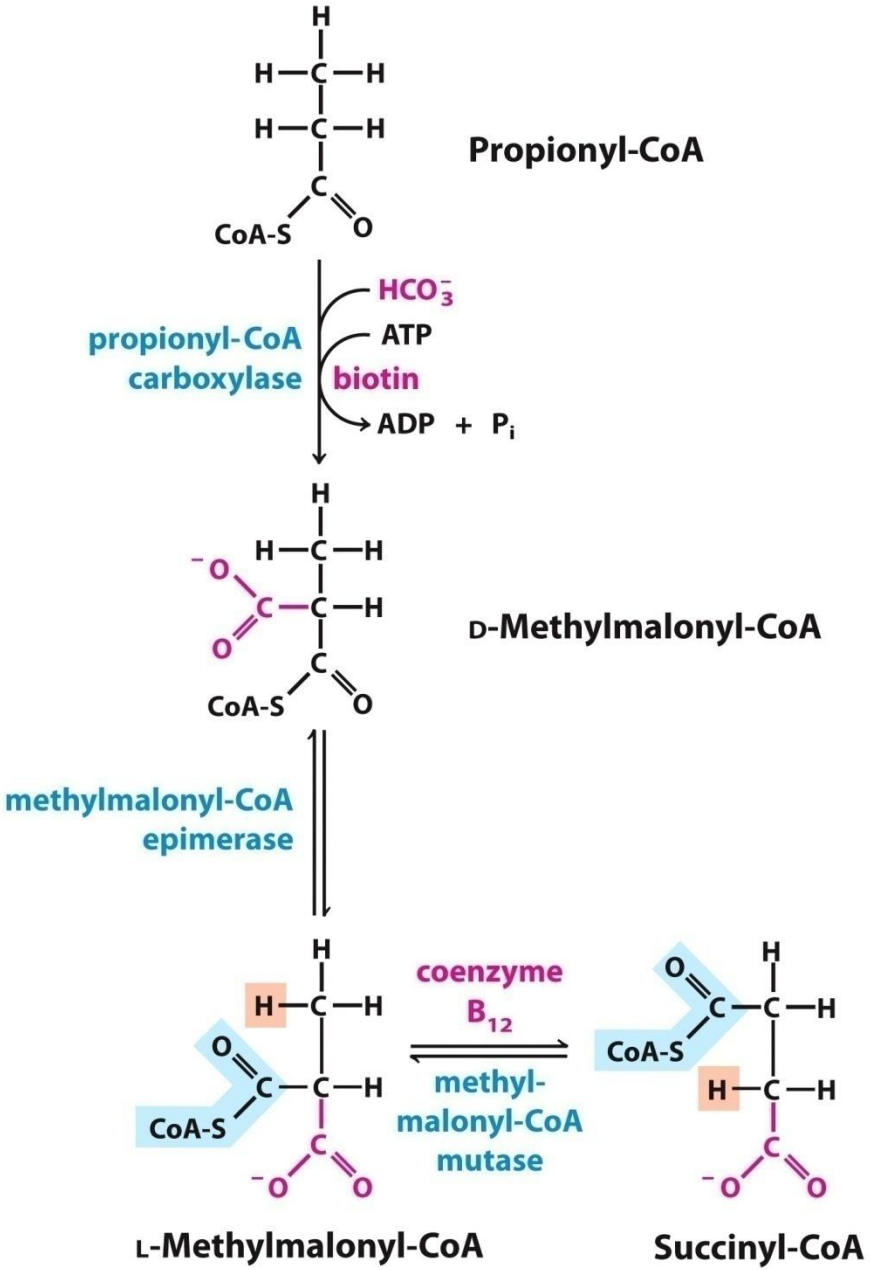
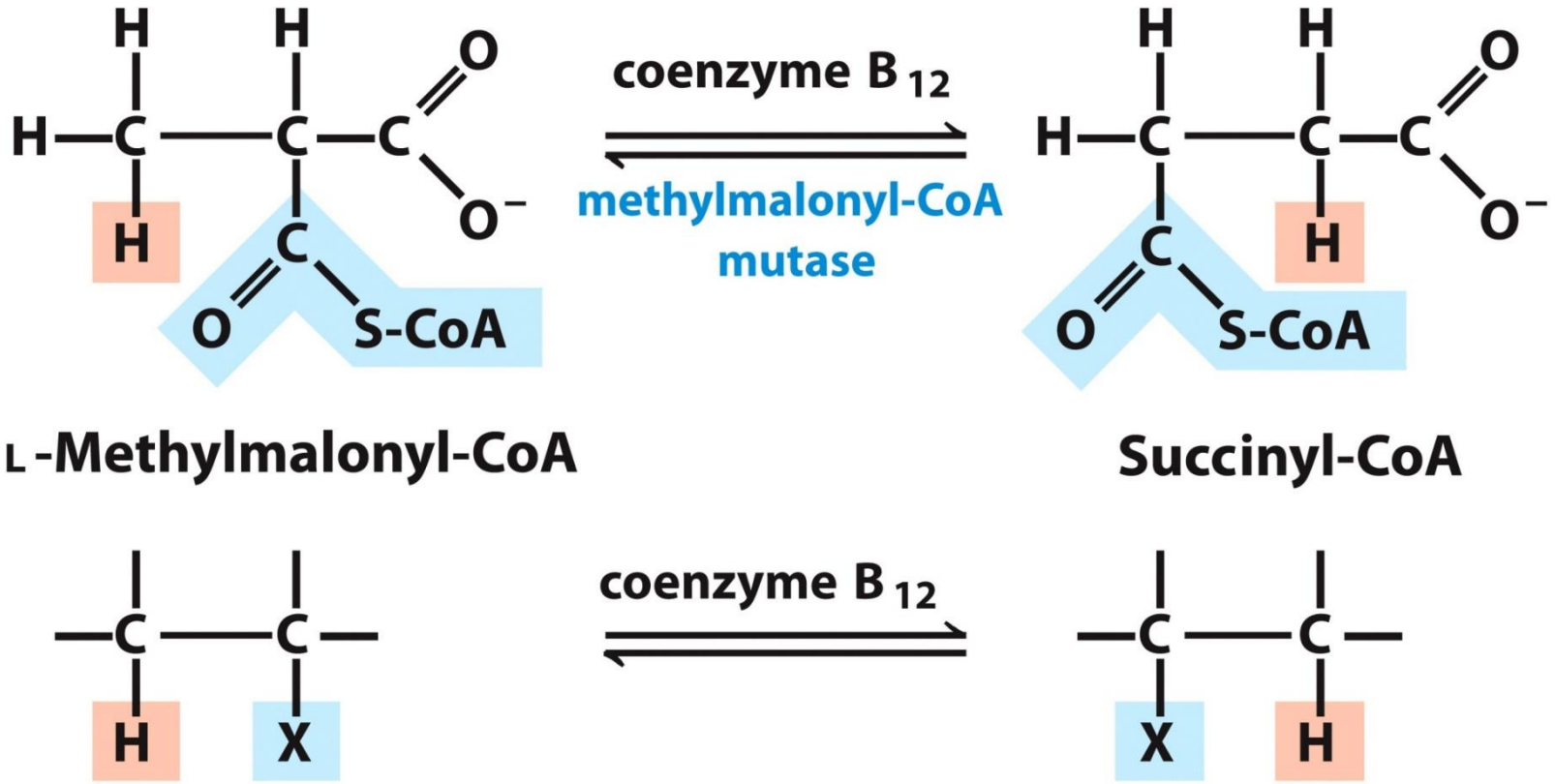


Figure 17-11 part 2
Lehninger Principles of Biochemistry, Sixth Edition

• Oxidation of odd-number fatty acids



- **Coenzyme B12**



Box 17-2 figure 1
Lehninger Principles of Biochemistry, Sixth Edition

• Regulation of Fatty Acid Oxidation

- ACC (acetyl CoA carboxylase)
- CPT1 (carnitine palmitoyl/acyl transferase I)

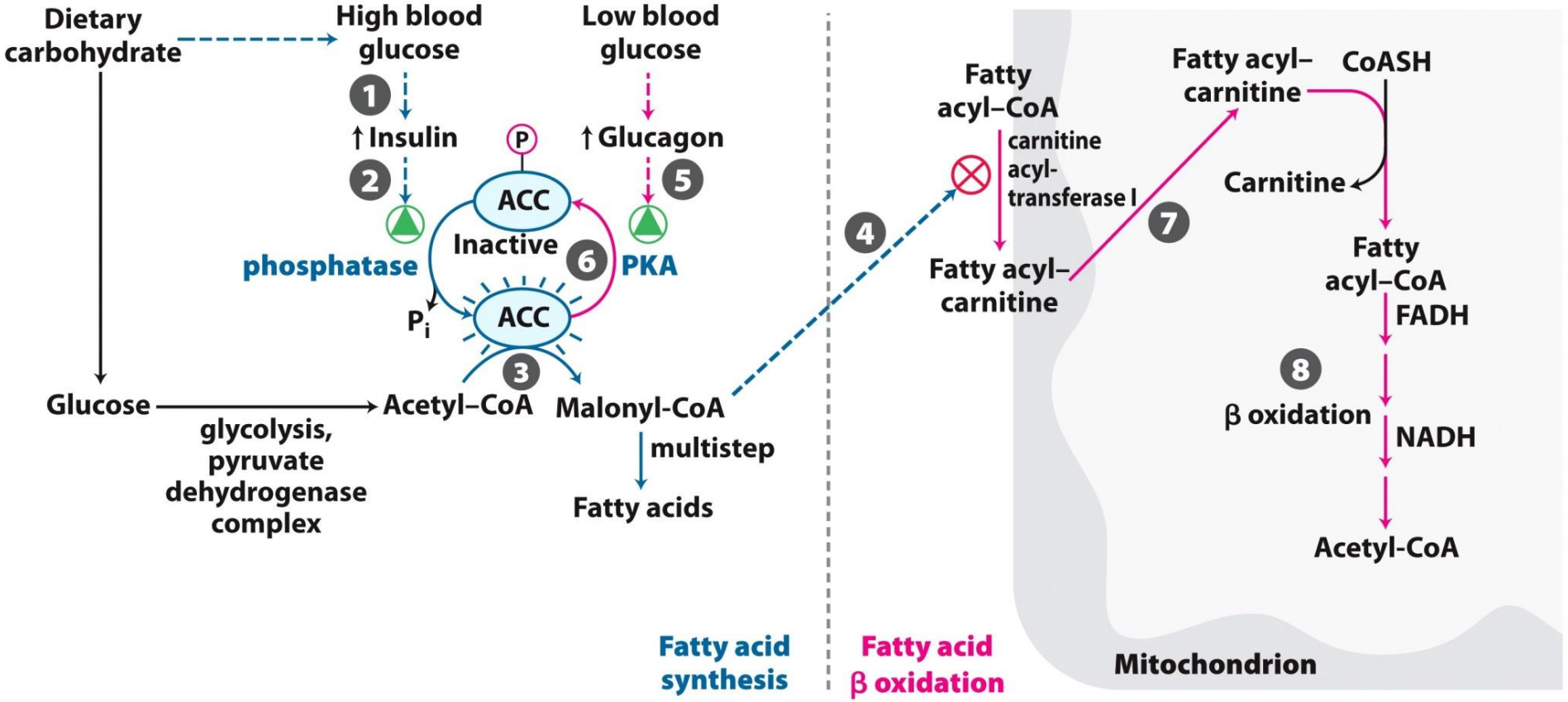
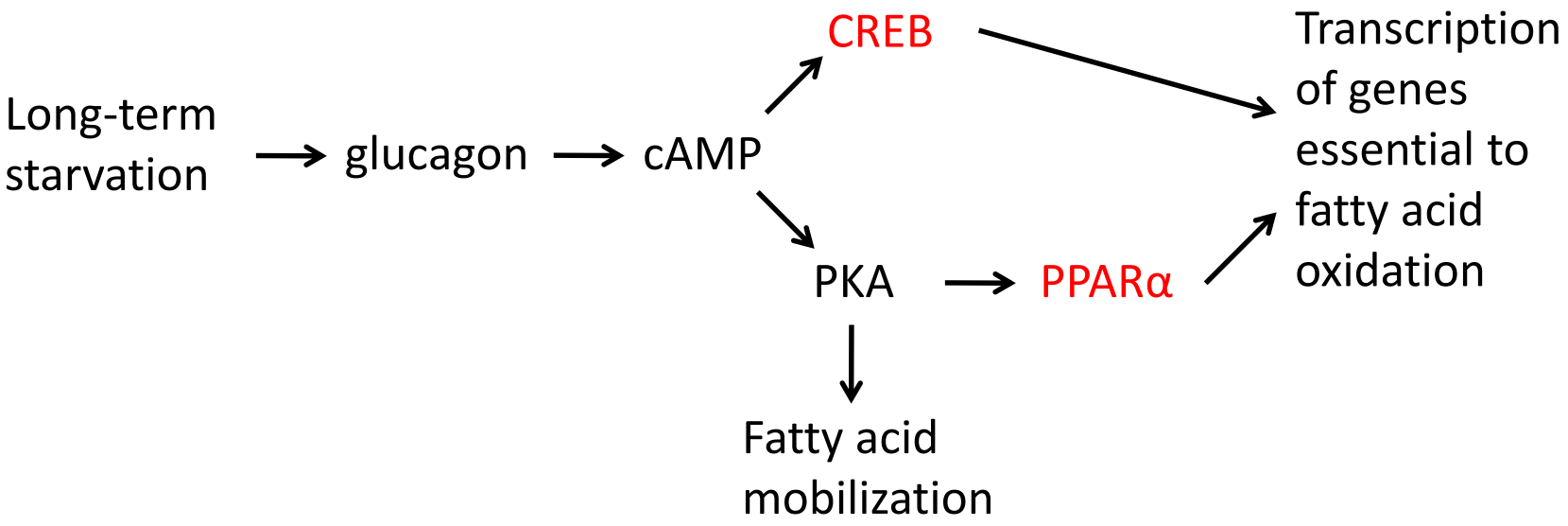


Figure 17-13

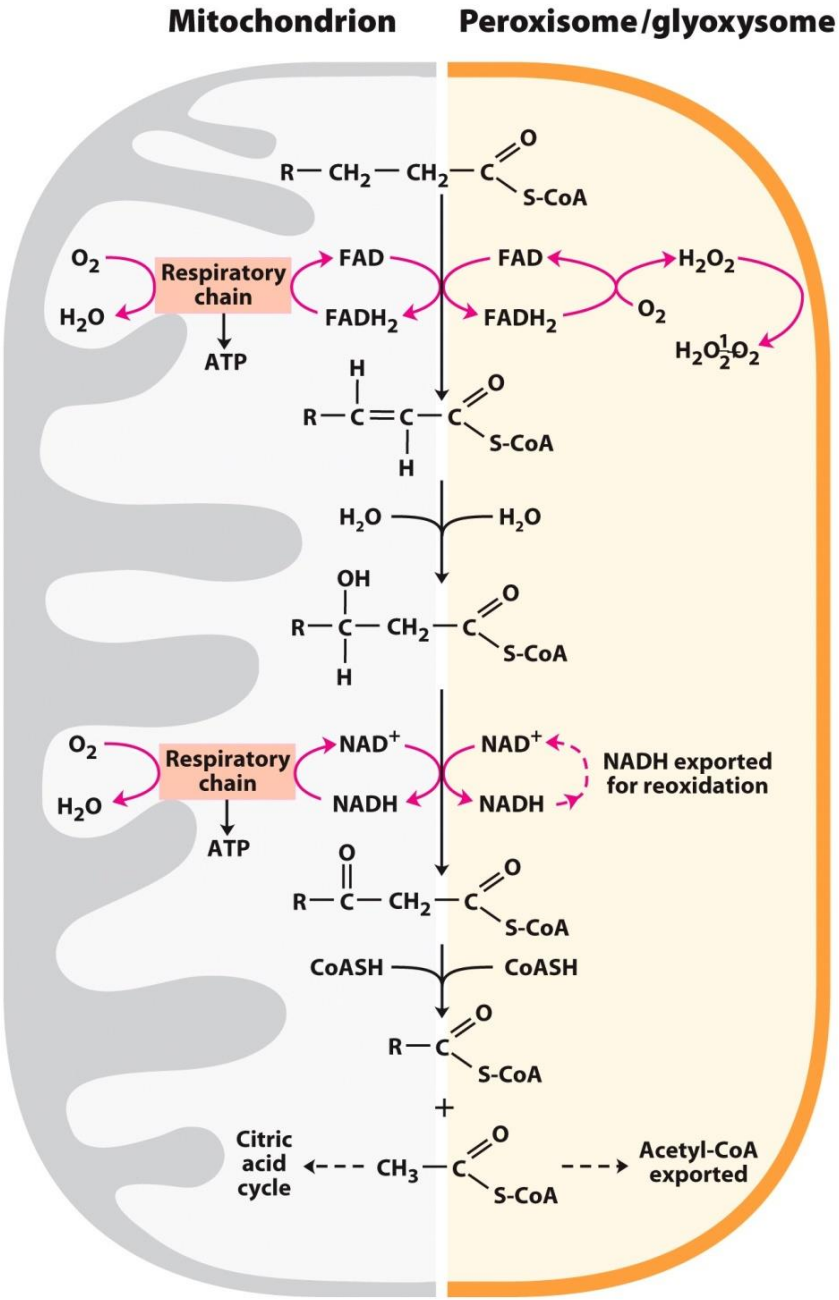
• Regulation of Fatty Acid Oxidation

Transcription factors turn on the synthesis of proteins for lipid catabolism



CREB: cAMP-response element binding protein,
PPAR: Peroxisome proliferator-activated receptor

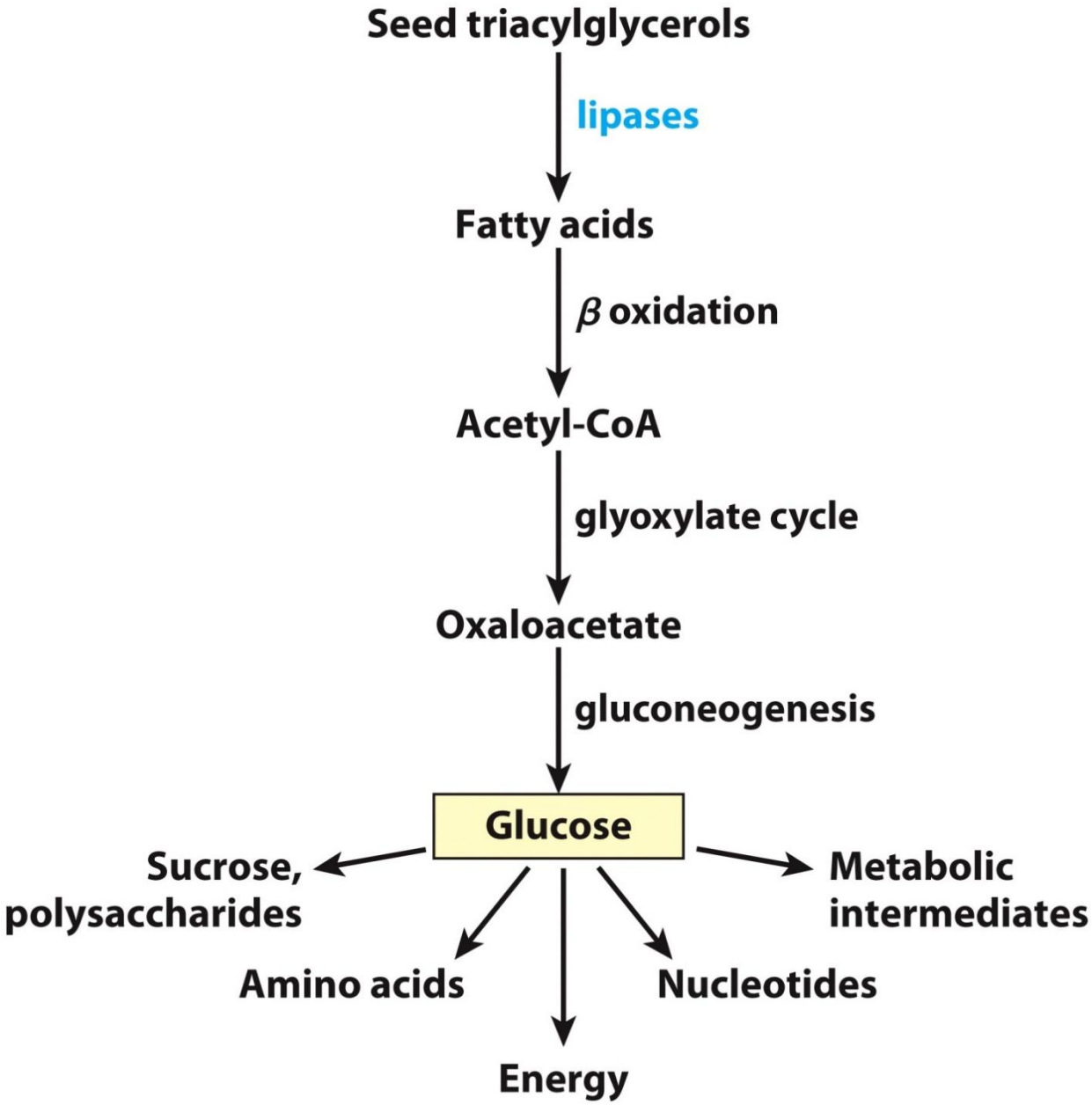
• Peroxisomes also carry out β oxidation



- The first oxidation step is catalyzed by acyl-CoA oxidases which is coupled to the reduction of O_2 to H_2O_2 by catalase.

- Octanoyl-coenzyme A is the endpoint of β-oxidation in peroxisomes.

- **Glyoxysomes use acetyl-CoA for biosynthesis**



- The ω oxidation of fatty acids in the ER

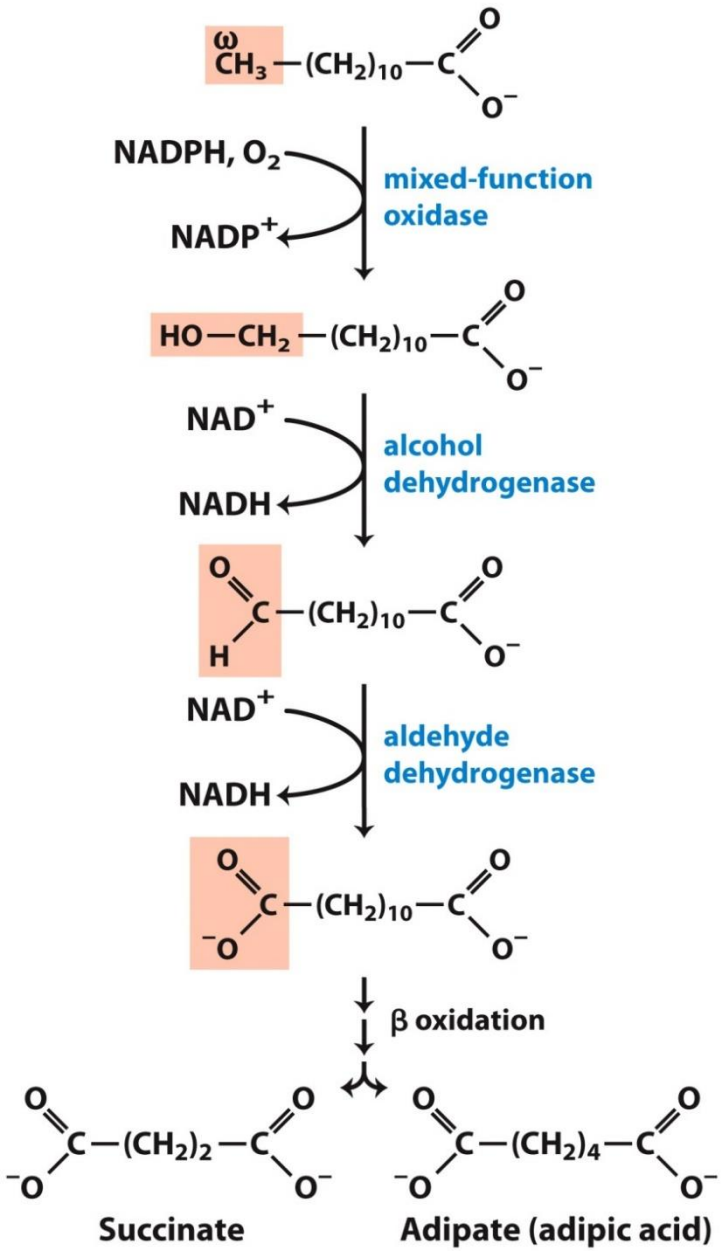


Figure 17-17

- **The α oxidation of phytanic acid in the peroxisomes**

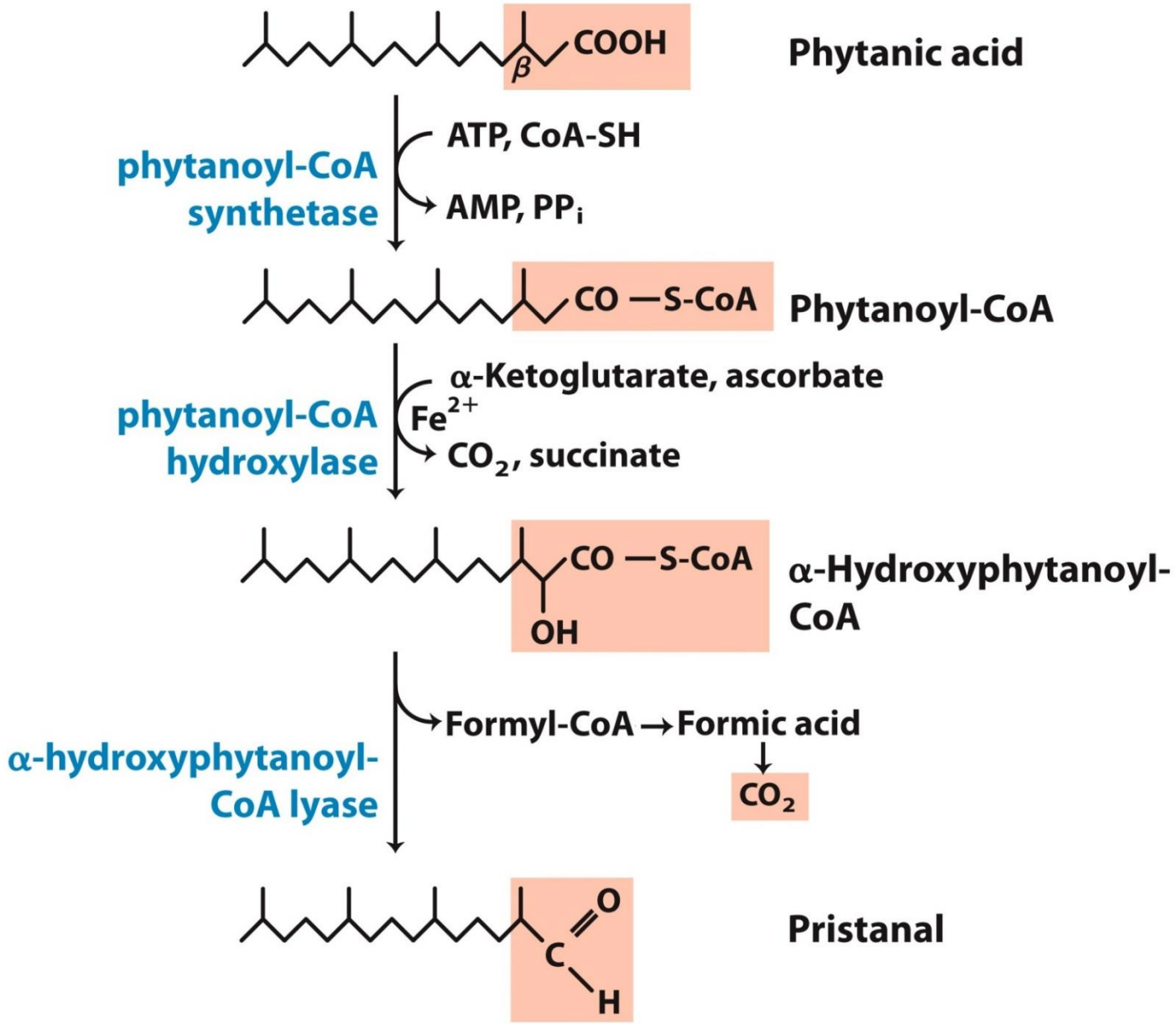
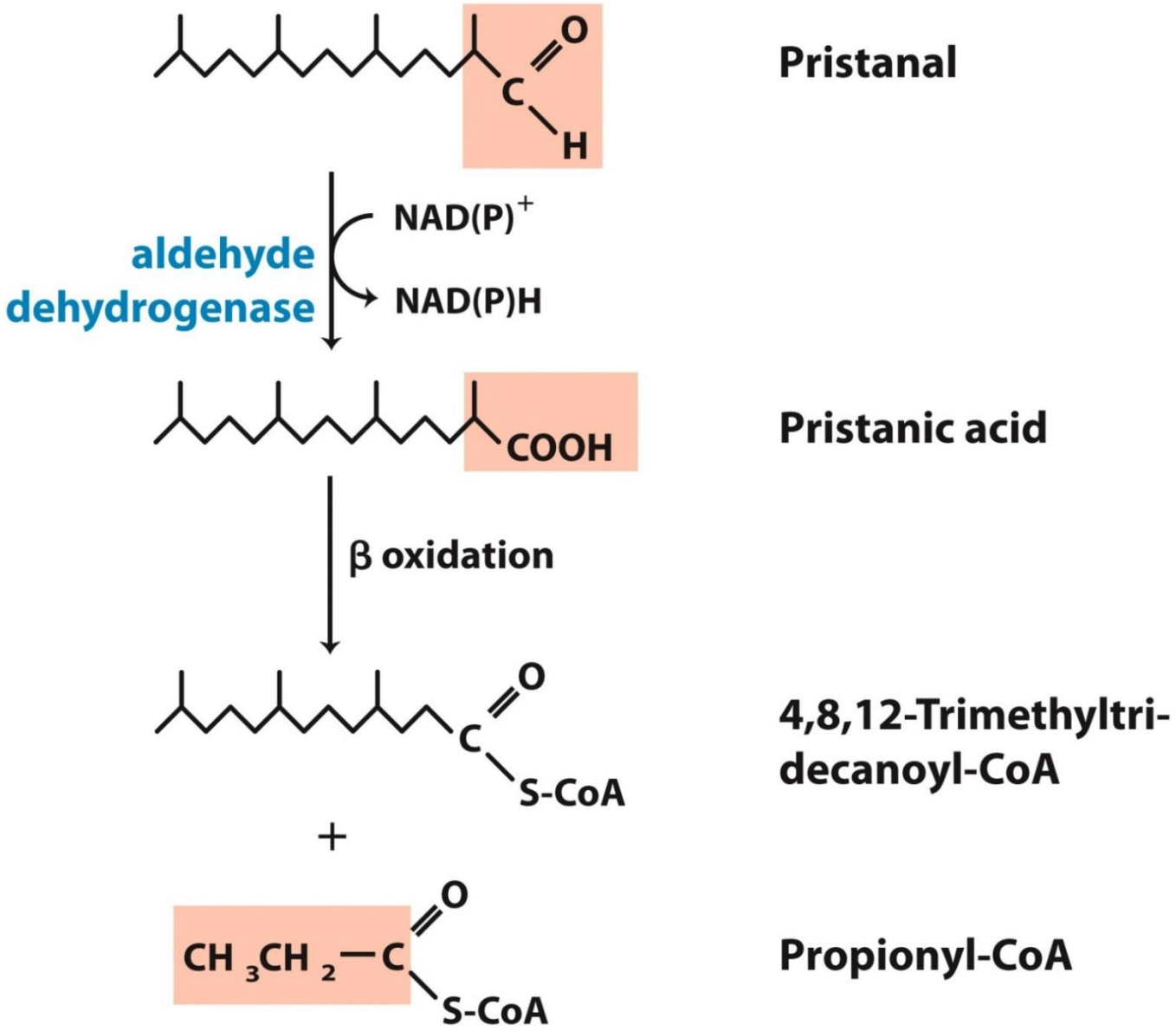
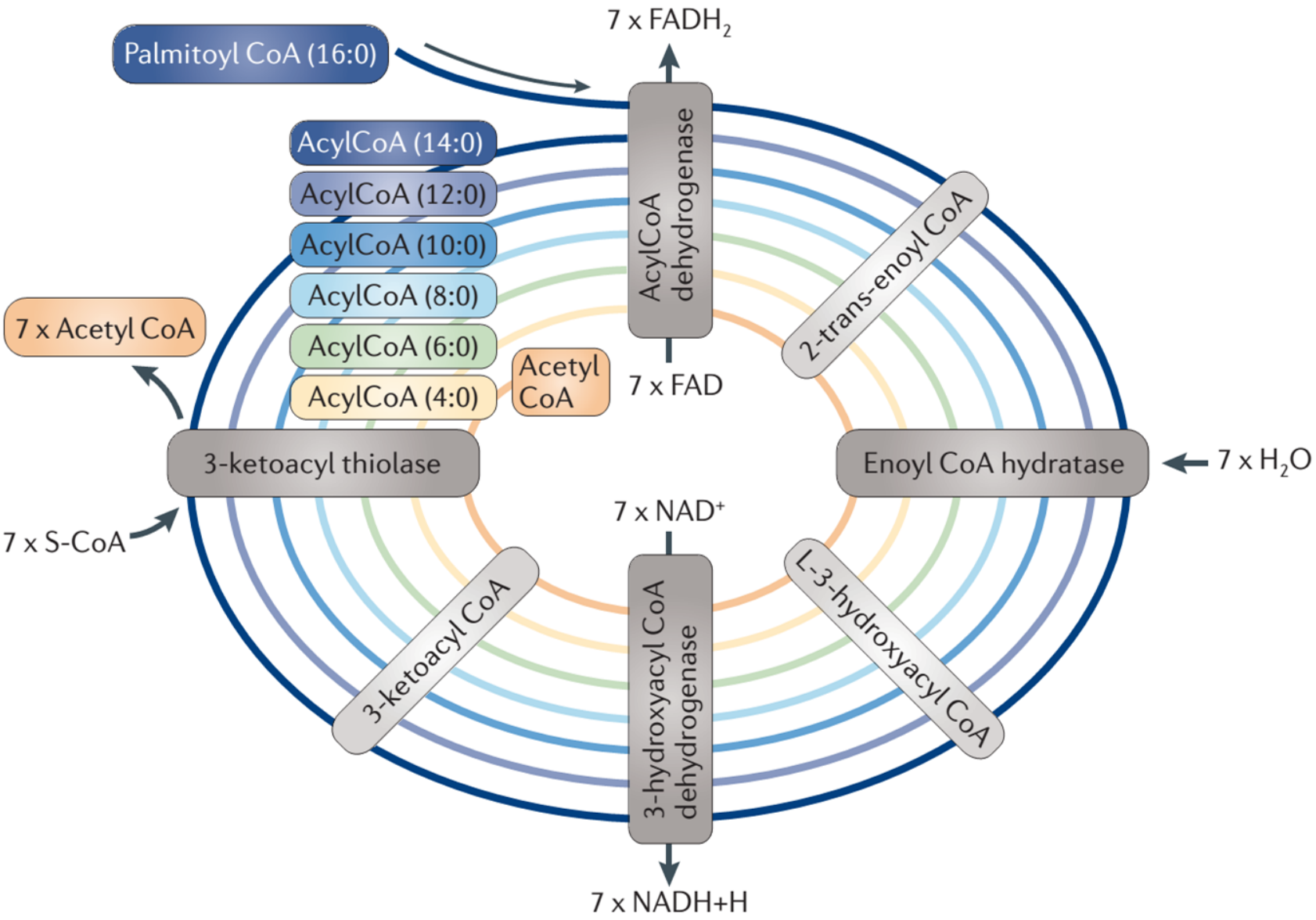


Figure 17-18 part 1

- The α oxidation of phytanic acid in the peroxisomes



• Summary 17.2


Palmitoyl CoA (16:0)
7 x FADH2
AcylCoA dehydrogenase
AcylCoA (14:0)
AcylCoA (12:0)
AcylCoA (10:0)
AcylCoA (8:0)
AcylCoA (6:0)
AcylCoA (4:0)
Acetyl CoA
7 x FAD
2-trans-enoyl CoA
7 x Acetyl CoA
3-ketoacyl thiolase
Enoyl CoA hydratase
7 x H2O
7 x S-CoA
7 x NAD+
3-ketoacyl CoA
3-hydroxyacyl CoA dehydrogenase
L-3-hydroxyacyl CoA
7 x NADH+H

• Summary 17.2

- Malonyl-CoA, an early intermediate of fatty acid synthesis, inhibits carnitine acyltransferase I, preventing fatty acid entry into mitochondria. This blocks fatty acid breakdown while synthesis is occurring.

- Oxidation of unsaturated fatty acids requires two additional enzymes: enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase.

- Odd-number fatty acids are oxidized by the β-oxidation pathway to yield acetyl-CoA and a molecule of propionyl-CoA. This is carboxylated to methylmalonyl-CoA, which is isomerized to succinyl-CoA in a reaction catalyzed by methylmalonyl-CoA mutase, an enzyme requiring coenzyme B12.

• Summary 17.2

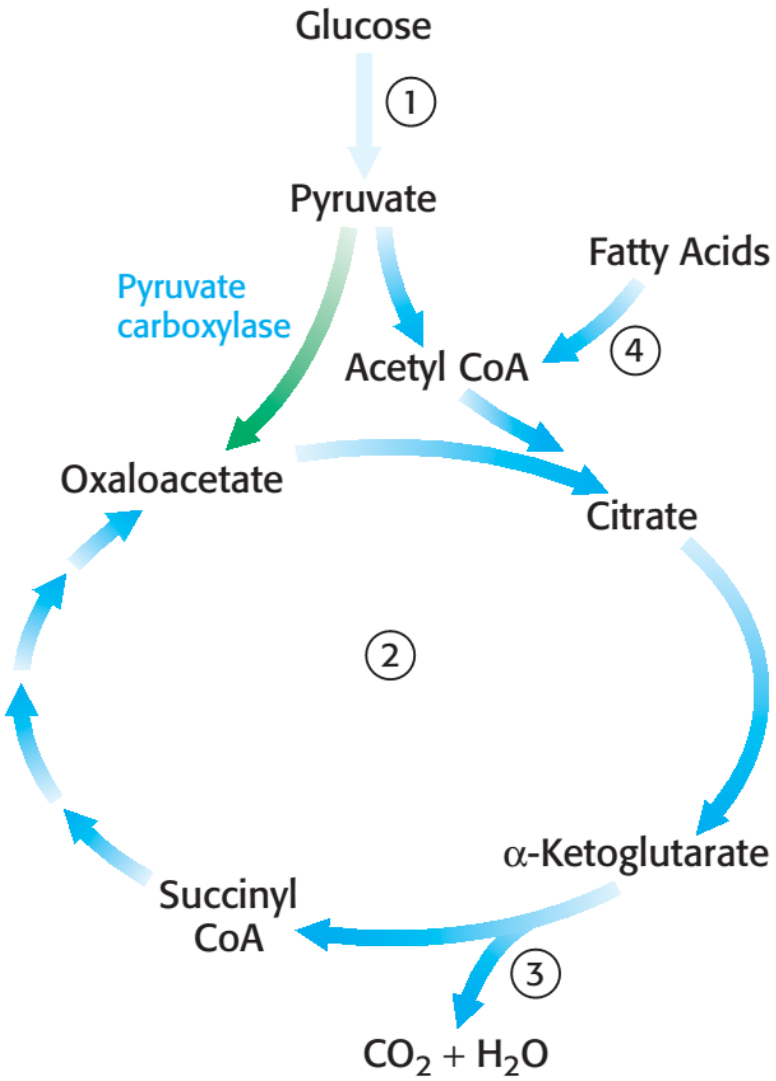
- Peroxisomes of plants and animals, and glyoxysomes of plants, carry out β oxidation in four steps similar to those of the mitochondrial pathway in animals. The first oxidation step, however, transfers electrons directly to O_2, generating H_2O_2.

- The reactions of ω oxidation, occurring in the endoplasmic reticulum, produce dicarboxylic fatty acyl intermediates, which can undergo β oxidation at either end to yield short dicarboxylic acids such as succinate.

- The reactions of α oxidation degrade branched fatty acids such as phytanic acid.

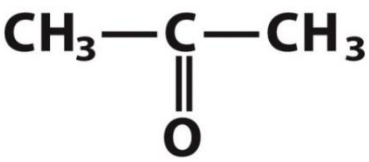
17.3 Ketone Bodies

• "Fats burn in the flame of carbohydrates"

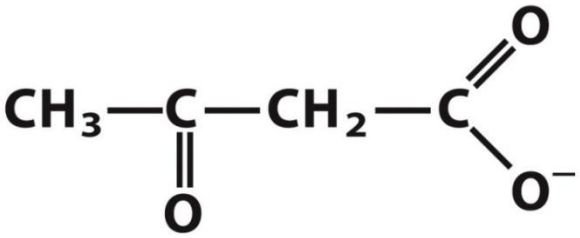


- The acetyl CoA formed in fatty acid oxidation enters the citric acid cycle only if fat and carbohydrate degradation are appropriately balanced.

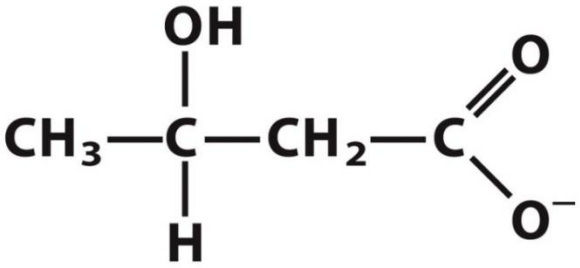
- **Ketone Body**



Acetone



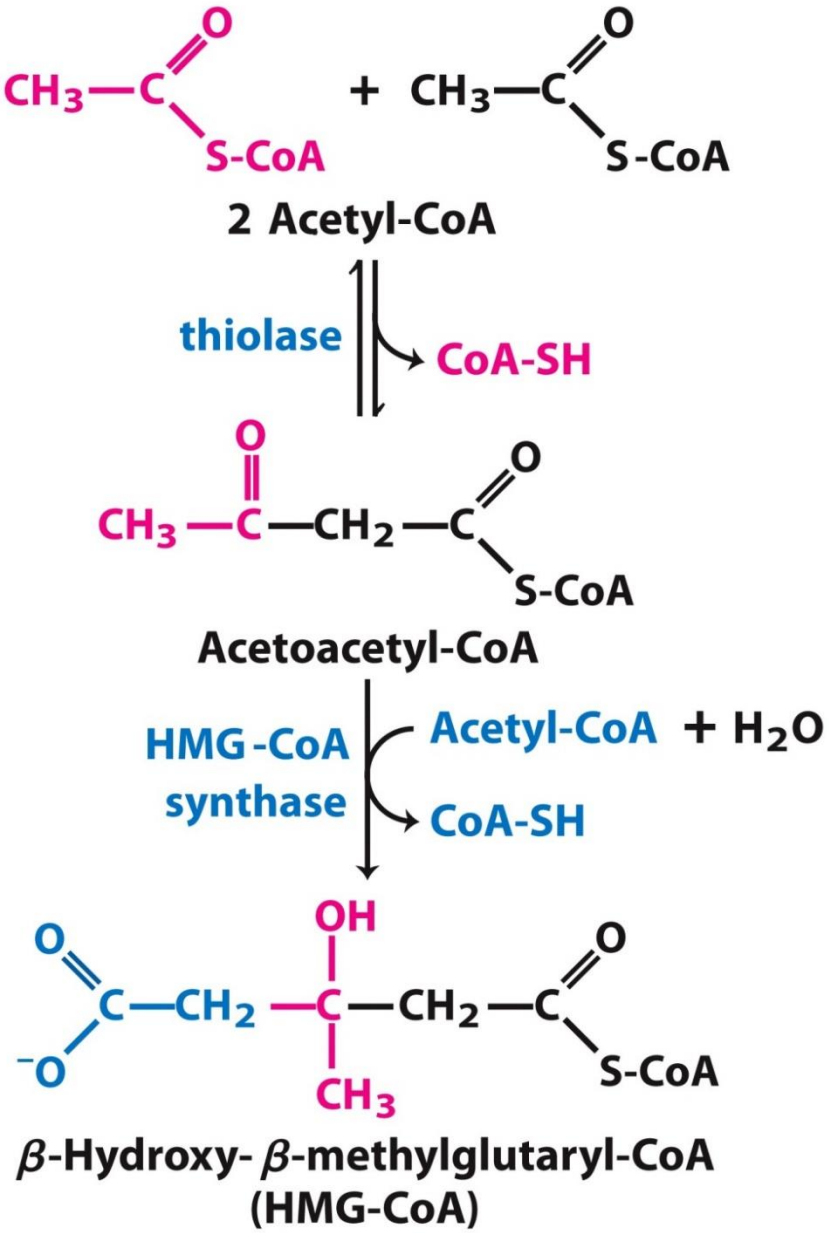
Acetoacetate



D-β-Hydroxybutyrate

Unnumbered 17 p686

• Ketogenesis



• Ketogenesis

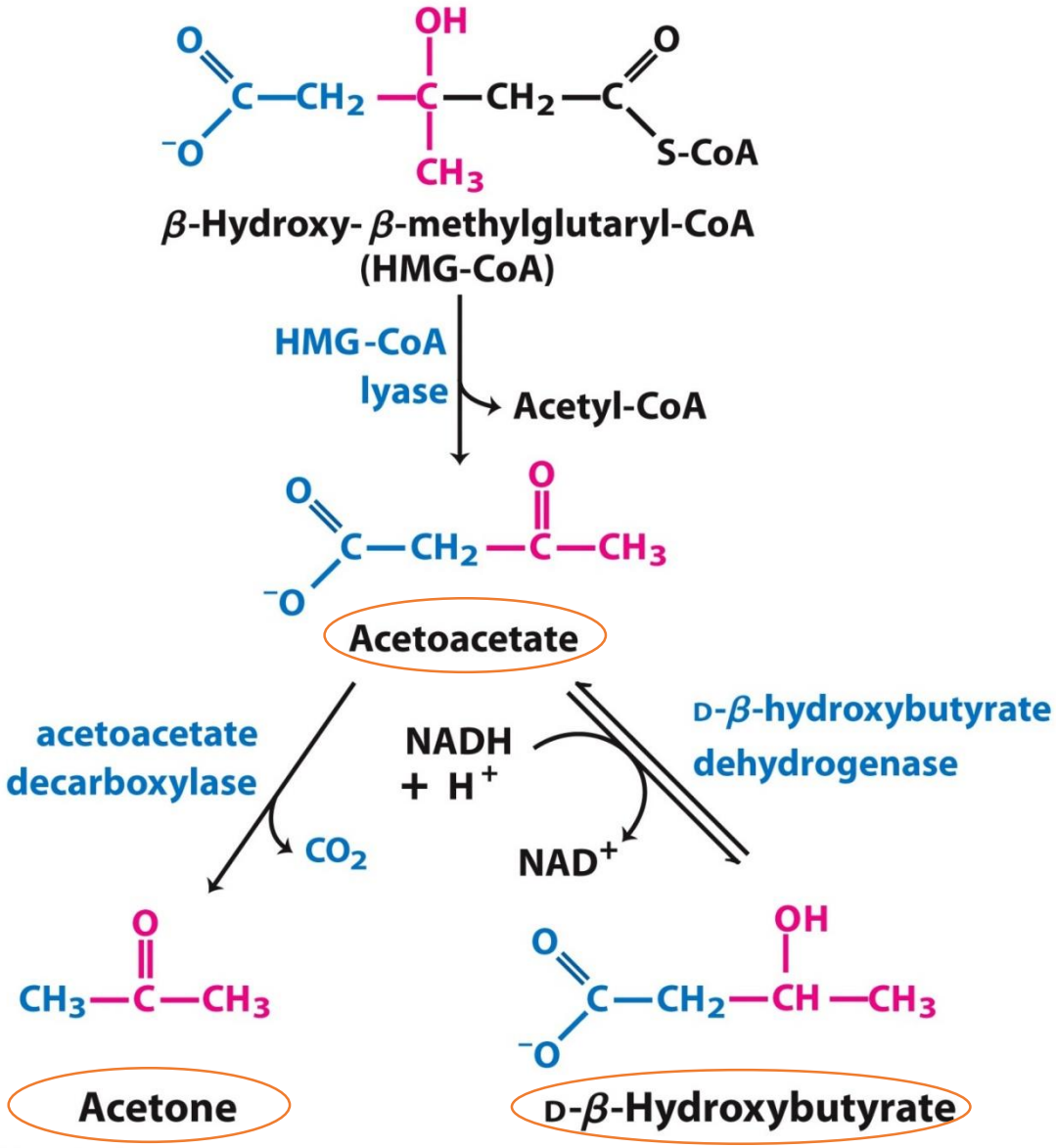


Figure 17-19 part 2
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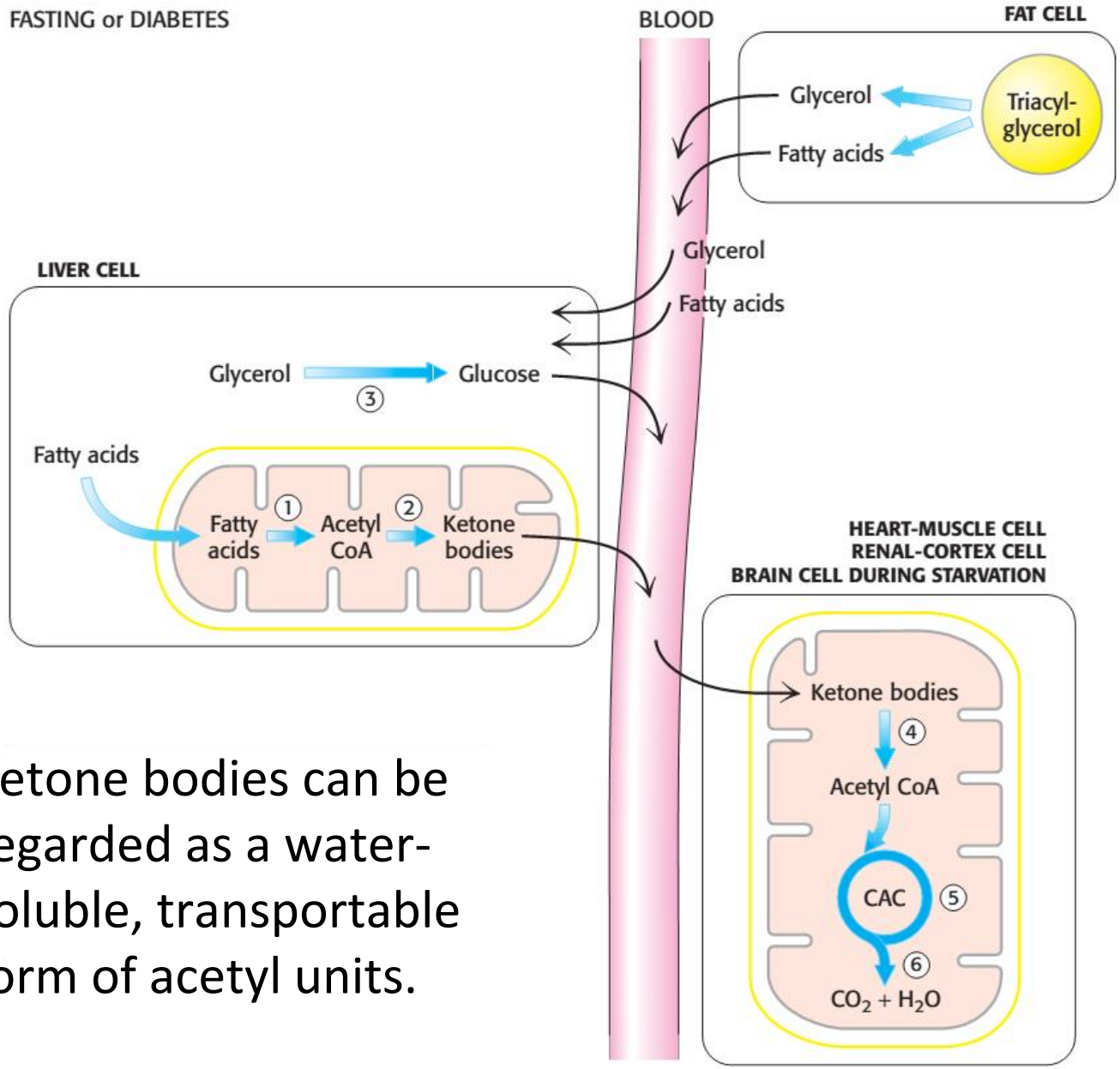
• Ketone bodies are important fuel



- Ketone bodies can be regarded as a water-soluble, transportable form of acetyl units.

• Ketone bodies are important fuel

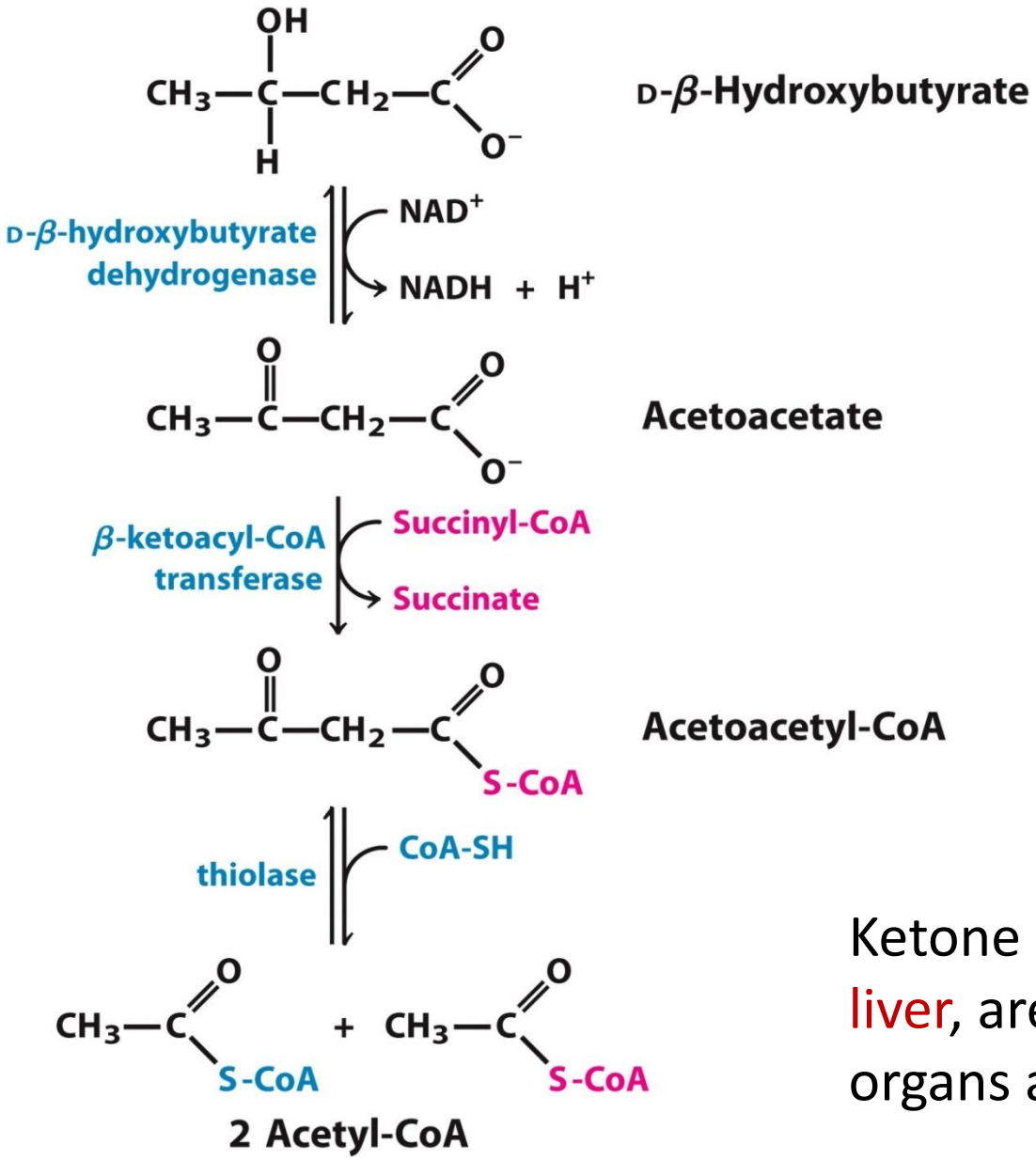


Figure 17-20

Ketone bodies, formed in the liver, are exported to other organs as fuel

- **Ketone bodies are overproduced during starvation**

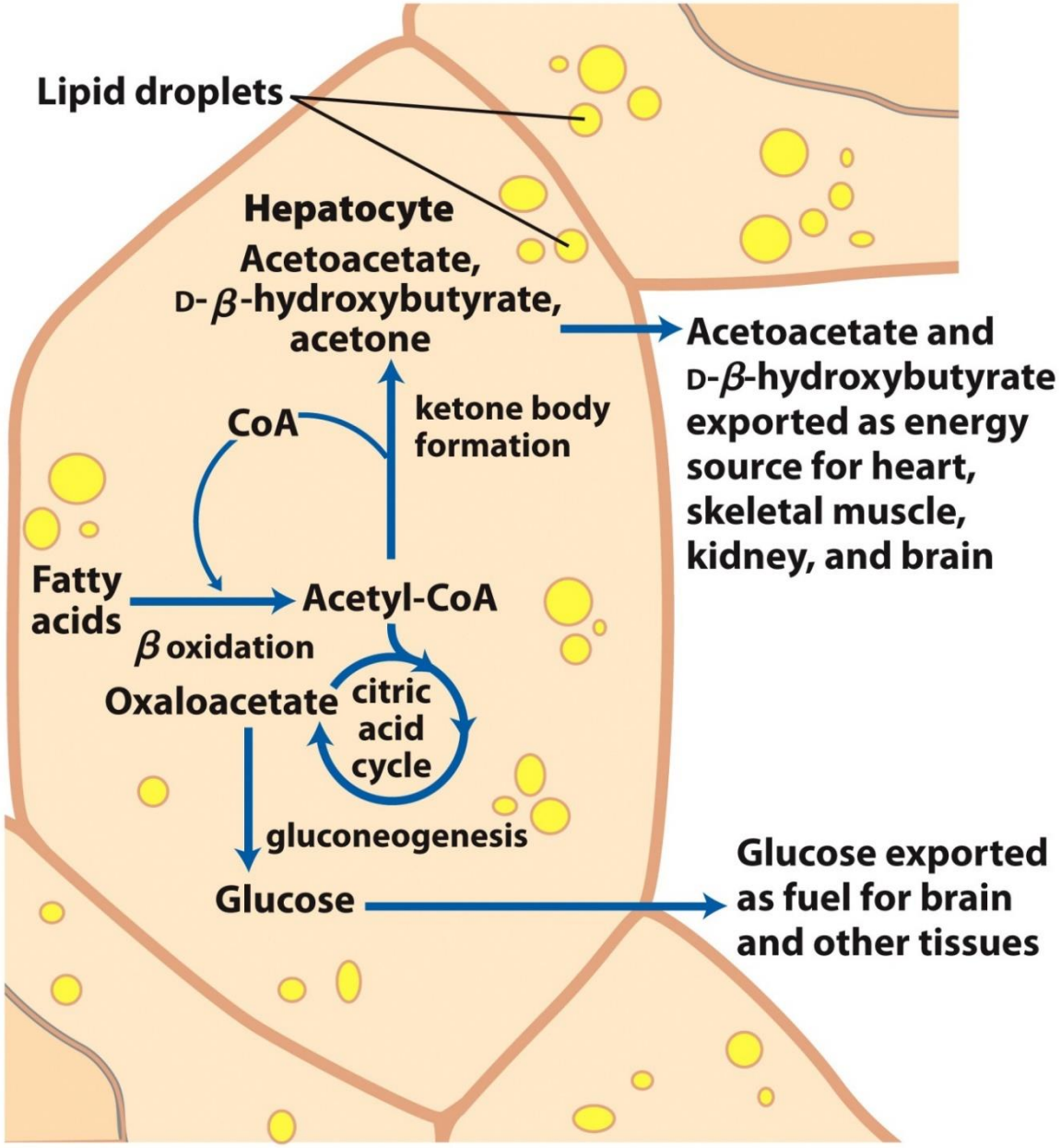


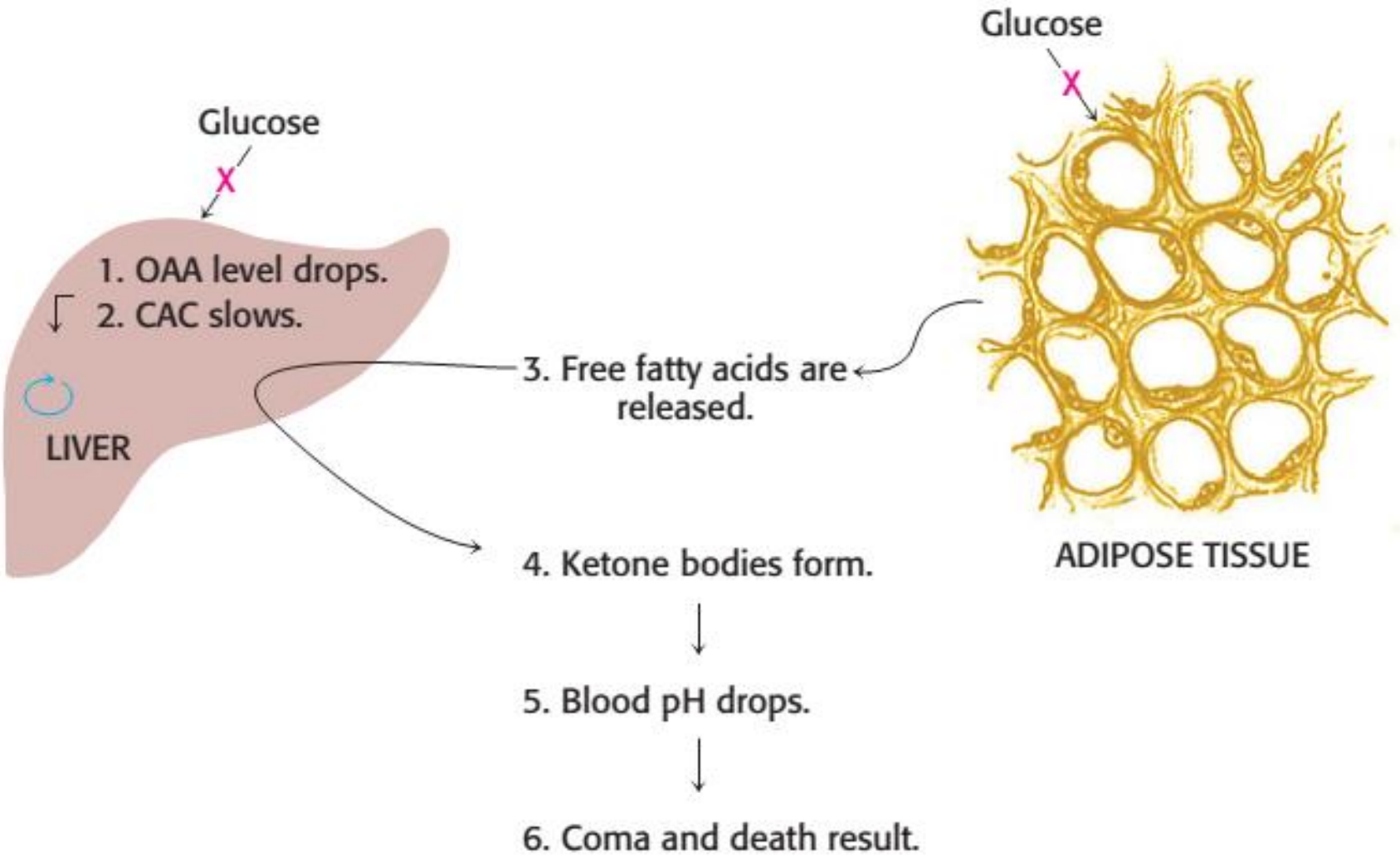
Figure 17-21

• Diabetic ketoacidosis (DKA)

Ketone bodies and diabetes mellitus

	Urinary excretion (mg/24 h)	Blood concentration (mg/100 mL)
Normal	≤125	<3
Extreme ketosis (untreated diabetes)	5,000	90

• Diabetic ketoacidosis (DKA)



• Summary 17.3

- The ketone bodies (acetone, acetoacetate, and β-hydroxybutyrate) are formed in the liver. The latter two compounds serve as fuel molecules in extrahepatic tissues, through oxidation to acetylCoA and entry into the citric acid cycle.

- Overproduction of ketone bodies in uncontrolled diabetes or severely reduced calorie intake can lead to acidosis or ketoacidosis.

The limitation of biosynthesis in humans have important consequences

The bulk of the available stored fuel in humans is fat, however, many peripheral tissues obtain their energy from breakdown of glucose, most notable is the brain, which requires 100~150g of glucose per day. Human cannot convert stored fat to either glucose or glucogenic amino acids. Moreover, humans can synthesize only 8~10 of 20 amino acids de novo from glucogenic precursor. These metabolic limitations have several consequences:

1. The human diet must include glucogenic fuel to supply peripheral tissues with glucose.
2. During even the normal overnight fast, some muscle protein is broken down to supply precursors for gluconeogenesis.
3. Resynthesis of the degraded protein, which normally occurs when the fast is broken, requires that the diet contain the 10 essential amino acids.
4. During more prolonged fasting, the daily demand (about 75g) on protein reserves for gluconeogenesis quickly becomes intolerable. Under these conditions the brain switches to utilization of ketone bodies as its principal energy source, thereby sparing muscle protein.

5. Whereas adults can tolerate prolonged fasting resulting in loss of up to ¼ of normal body weight without harm, children can not, because normal growth requires continued protein synthesis. Protein deficiency in children leads to stunted growth and the pathological condition known as *kwashiorkor*, which is characterized by apathy, edema, and low levels of many key enzymes, and is one of the most widespread children afflictions in the world.