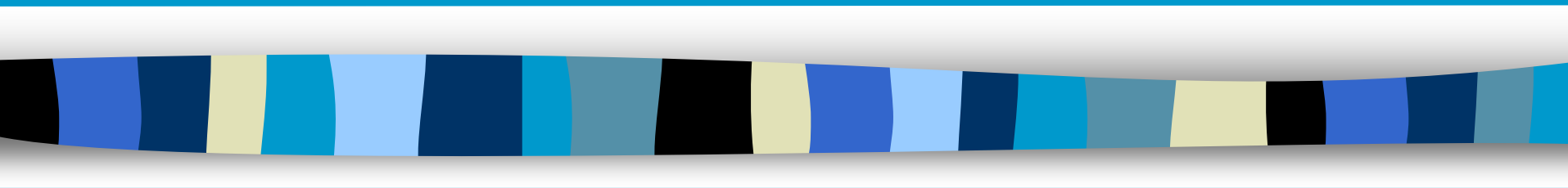
Fatty Acid Catabolism



Outline

1. Mobilization of Fats from Dietary Intake and Adipose Tissue
2. Beta-Oxidation of Fatty Acids
3. Odd-Carbon Fatty Acids
4. Unsaturated Fatty Acids
5. Other Aspects of Fatty Acid oxidation
6. Ketone Bodies

Trends in Lipid Consumption

Lipid consumption has increased

- Luxury
- Make food taste better
- Satiation (provide fullness)

Recommended	Intake	Fat
30% of calories	40% of calories - mid 1960s	13% saturated fat
	36% of calories - 1978	
	34% of calories - 1990	12% saturated fat

Composition of some known fats

Myristic acid	Coconut and palm oils, most animal and plant fats
Palmitic acid	Animal and plant fats
Stearic acid	Animal fats, some plant fats
Arachidic acid	Peanut oil
Lignoceric acid	Most natural fats, peanut oil in small amounts
Palmitoleic acid	Marine animal oils, small amounts in animal and plant fats
Oleic acid	Animal and plant fats
Linoleic acid	Corn, safflower, soybean, cottonseed, sunflower seed, and peanut oils
Linolenic acid	Linseed, soybean, and other seed oils
Arachidonic acid	Animal fats in small amounts

Food	Total lipid	Cholesterol	Saturated	Oleic	Linoleic	Linolenic
Milk	3.5	12	59	25	3	1.0
Egg	11.0	548	29	37	11	0.2
Beef -lean ground	22.0	70	50	41	3	0.7
Pork -lean	14.0	85	37	42	9-14	1.0
Chicken leg -flesh	3.5	74	27	47	22	2.0
Salmon	14.0	35	18	16	2	20.0
Whole wheat	2.0	0	21	14	55	4.0
Corn -whole	3.8	0	15	44	43	2.0
Soybeans -whole	18.0	0	13	22	54	5.0
Peanuts -butter	48.0	0	14	48	28	0.5
Coconut -fresh	38.0	0	83	5	2	0
Avocado -fresh	24.0	0	14	66	9	trace

Lipid Absorption from Lumen into Mucosal Cells

To get a lipid into the mucosal cell, it must be deesterified into free fatty acids (FFA) and monoacyl glycerols (MAG) and cholesterol. To get them out of the mucosa and into circulation, via the lymphatics, the lipids are packaged as chylomicrons. CMs are composed largely of TAGs, plus cholesterol esters and several proteins (called apoproteins).

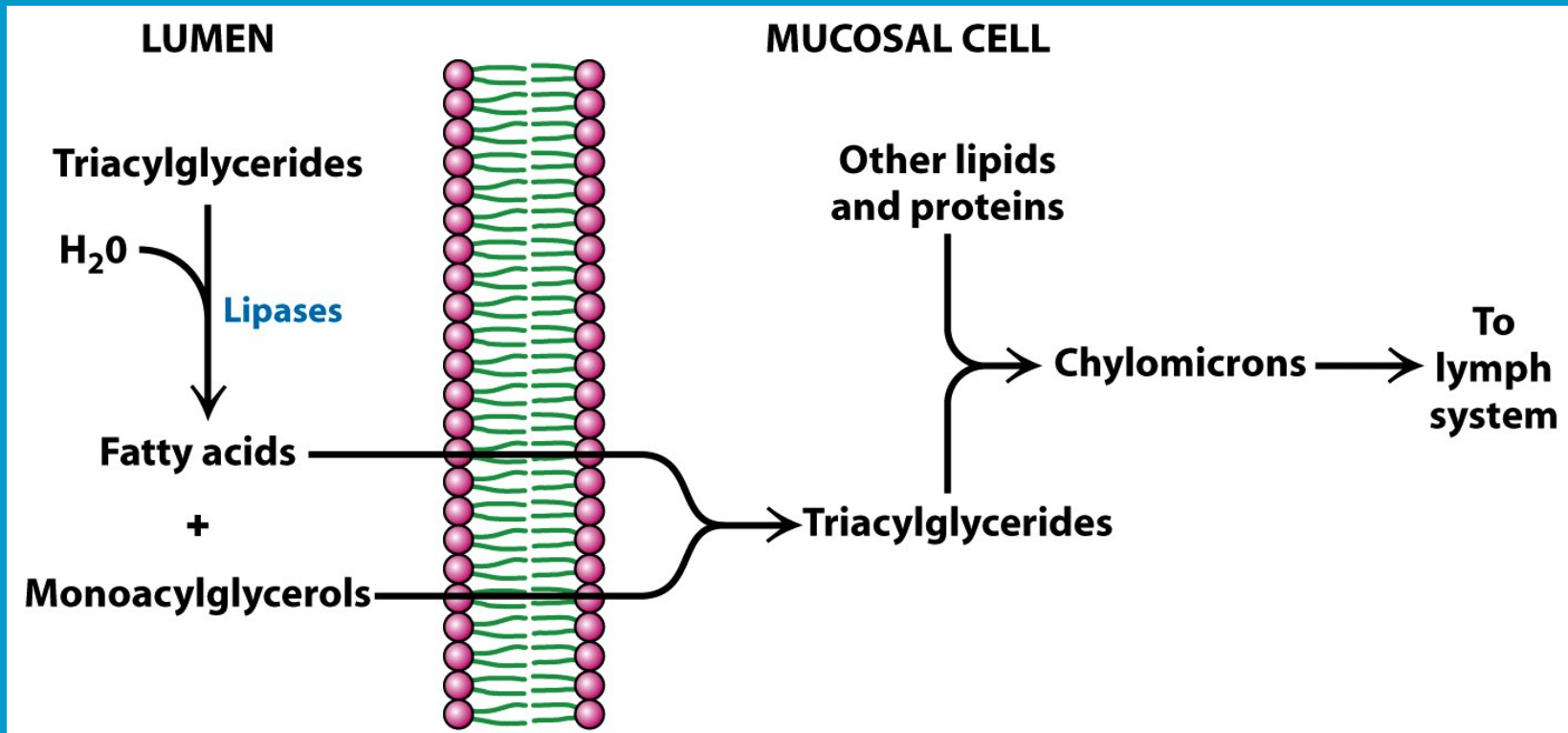


Figure 22-5
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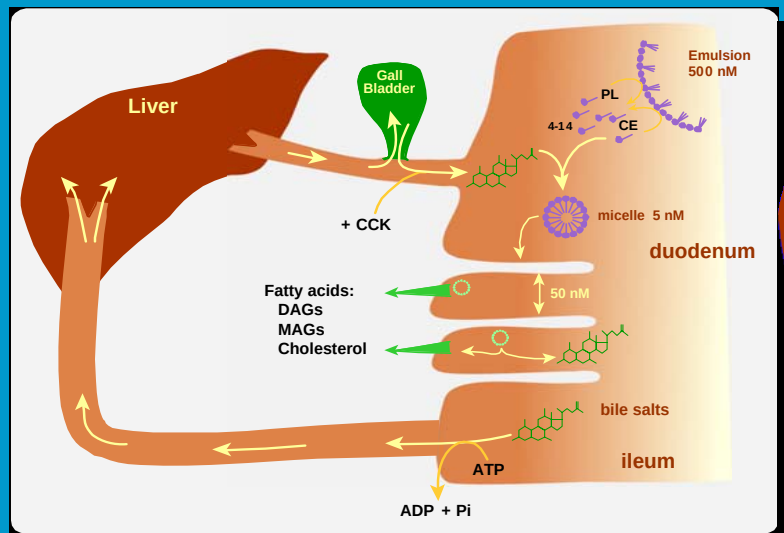
Absorption

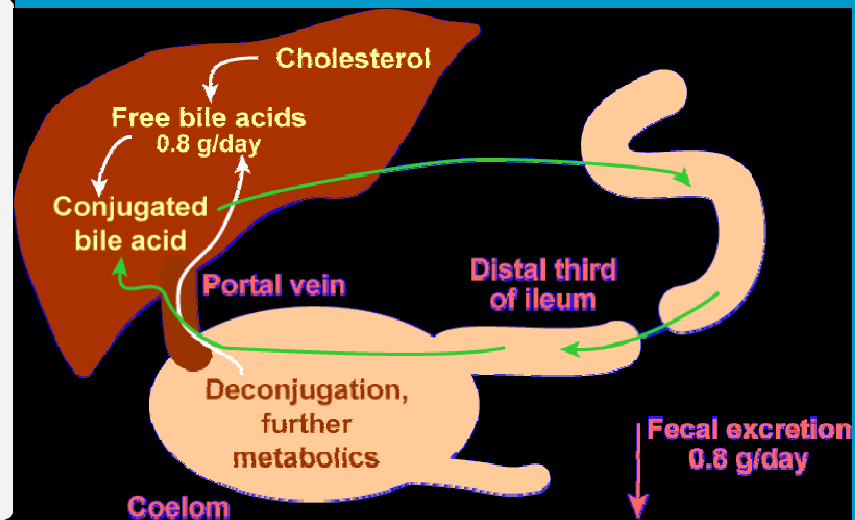
Liver: Synthesizes primary bile acids from cholesterol, sends them to storage in gall bladder. When food hits small intestine, gall bladder is stimulated to release bile acids into the sm. int. Most bile acids are derivatized with the amino acids glycine or taurine; this lowers their pKa so that they are soluble in the lower pH of the duodenum (pH 5.8 - 6.5).

Small intestine: At sufficiently high conc (**critical micellar concentration** = CMC), bile salts spontaneously aggregate to form micelles. When mixed with food, they trap lipid particles during their aggregation; this makes micelles from emulsion. The bile salt structure contains hydrophobic (rings) and hydrophilic (hydroxy group, amino acid) groups; this allows the lipid to be in contact with the aqueous environment required by degradative enzymes.

Lower small intestine, colon: Intestinal bacteria further derivatize bile acids by removing hydroxyl groups at the C-7 position. These secondary bile acids are associated with higher risks for colonic cancer (mechanism unclear).

Lipid + Bile salts + Enzymes → Bile salts FAs DAGs MAGs cholesterol → Directly absorbed into mucosal cell → Re-esterify to TAG chol ŠFA → Circulate to lymph as chylomicron





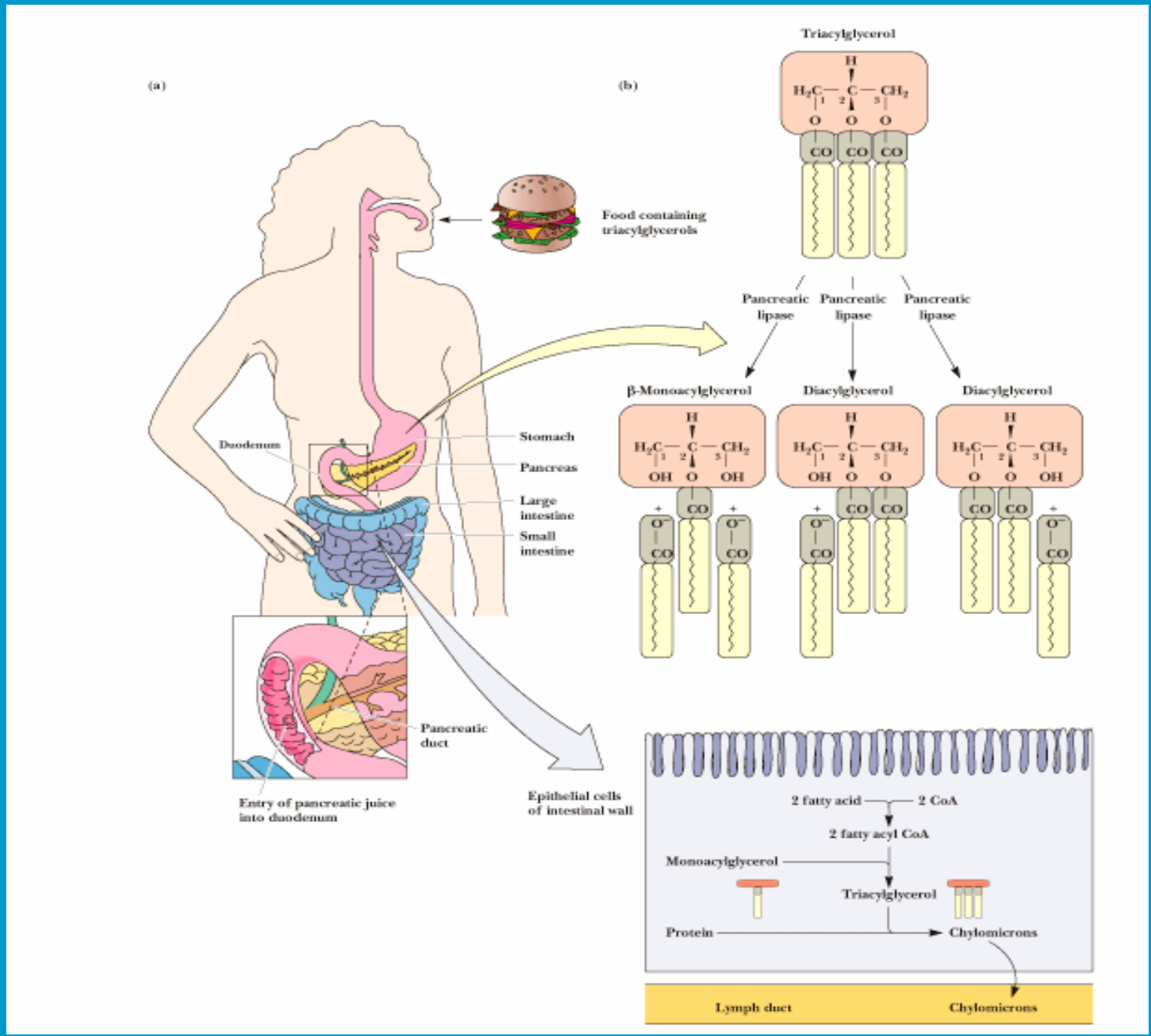

(a)
(b)
Triacylglycerol
Food containing triacylglycerols
Pancreatic lipase
Pancreatic lipase
Pancreatic lipase
β-Monoacylglycerol
Diacylglycerol
Diacylglycerol
Duodenum
Stomach
Pancreas
Large intestine
Small intestine
Pancreatic duct
Entry of pancreatic juice into duodenum
Epithelial cells of intestinal wall
2 fatty acid
2 CoA
2 fatty acyl CoA
Monoacylglycerol
Triacylglycerol
Protein
Chylomicrons
Lymph duct
Chylomicrons

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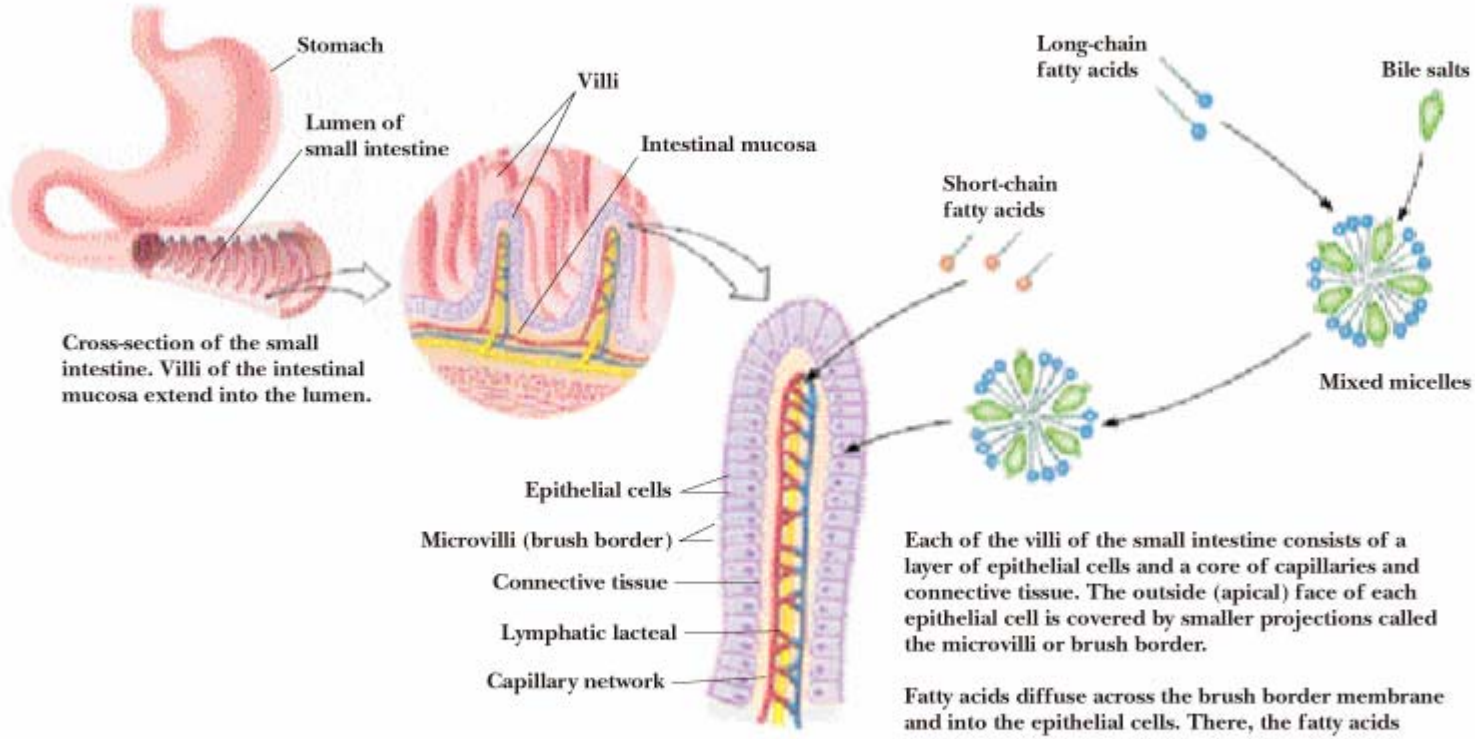
Figure 24.4



Each of the villi of the small intestine consists of a layer of epithelial cells and a core of capillaries and connective tissue. The outside (apical) face of each epithelial cell is covered by smaller projections called the microvilli or brush border.

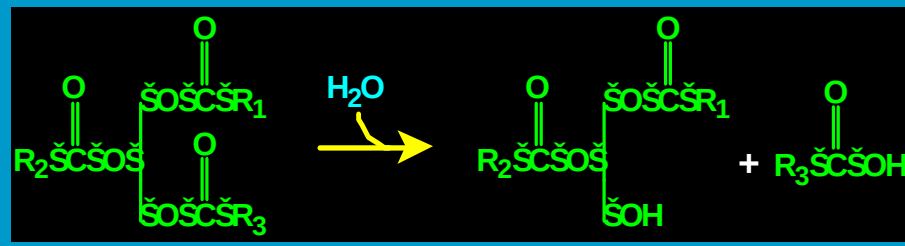
Fatty acids diffuse across the brush border membrane and into the epithelial cells. There, the fatty acids combine with glycerol to form new triacylglycerols, with aggregate with lipoproteins to form chylomicrons. Taken up by the capillaries, chylomicrons circulate to the liver and other organs.

Lipid Digestion

Mouth:

- Important largely for neonates
- Acts on milk fats - preemulsified fats

Lingual Lipase:



Stomach:

- Little to none
- Churning creates a coarse lipid emulsion
- Fat slows the release of food from the stomach

Small intestine

- Performs 90% of all lipid digestion
- Primarily performs hydrolysis and de-esterification
- Mix bile salts to convert coarse emulsion into micelles

• **Lipoprotein Lipase:** found on endothelial (vessel) walls lining tissues such as adipose and muscle. Releases FFA from TAGs in CM/VLDL for cellular uptake and usage as either energy (muscle) or storage (adipocyte). Thus insulin & glucagon differentially regulate this enzyme on muscle vs. adipose cells.

TAG → 2-MAG + FFA → cell

• **Hormone-sensitive lipase:** Only found INSIDE adipocyte. Releases FFA from adipocyte TAG stores, sends to serum. Incr by glucagon, epinephrine.

TAG → 2-MAG + FFA → serum

• **Phosphatidate Phosphatase:** in all cells, synthesizes TAG from glycerol-PO4.

α-Glycerol-3-PO4 → MAG-PO4 → 1,2-DAG → TAG

MAG-PO4 → Pi

• **Regulation of LPL Activity:**

factor	adipose	muscle
lipoprotein CII	up	up
cholesterol	down	down
starvation	up	up
excess glucose	up	down
insulin	up	down

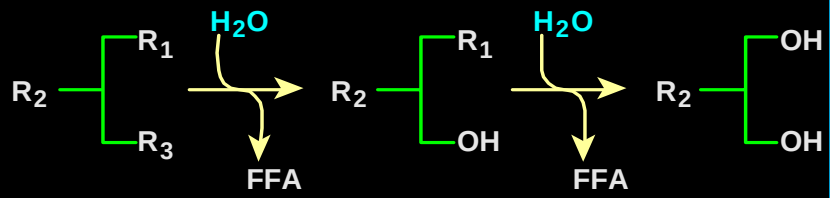
Lipid Digestion

Pancreatic Lipase

long > short, unsaturated > saturated

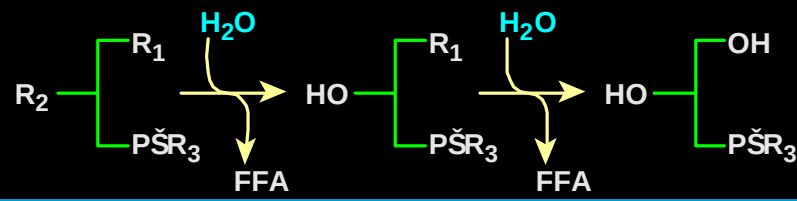
inhibited by bile salts

enhanced by Ca ++ Š Colipase



Phospholipase A2 = Lecithinase

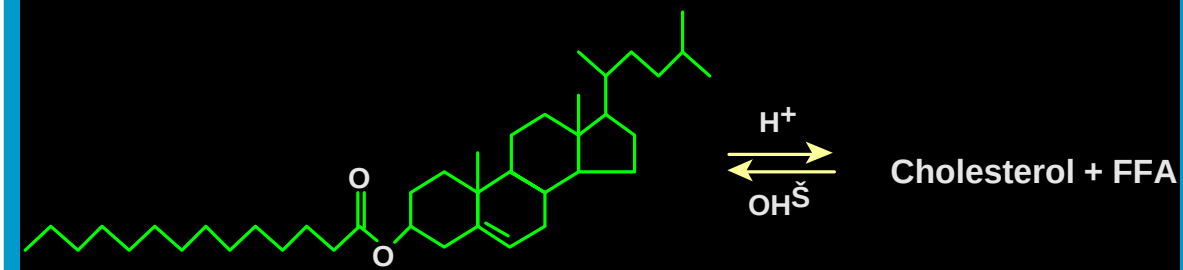
acts at C1, 2 position of PL



Cholesterol Esterase

removes FA at low pH - lumen

adds FA at higher pH - mucosa



Why Fatty Acids?

(For energy storage?)

Two reasons:

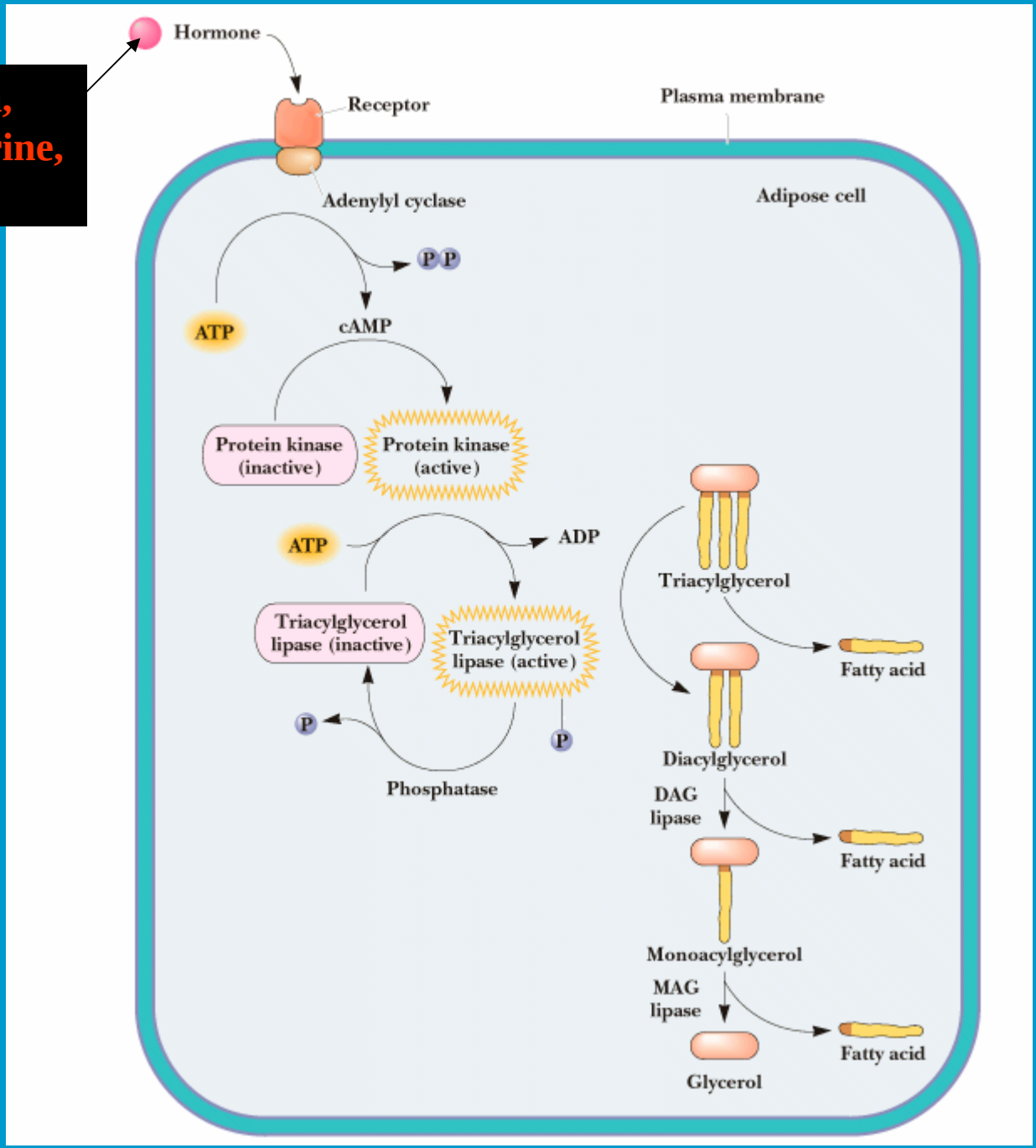
- **The carbon in fatty acids (mostly CH_2) is almost completely reduced (so its oxidation yields the most energy possible).**
- **Fatty acids are not hydrated (as mono- and polysaccharides are), so they can pack more closely in storage tissues**

Fat from Diet & Adipose Cells

Triacylglycerols either way

- **Triglycerides represent the major energy input in the modern American diet**
- **Triglycerides are also the major form of stored energy in the body**
- **Hormones (glucagon, epinephrine, ACTH) trigger the release of fatty acids from adipose tissue**

glucagon, epinephrine, ACTH



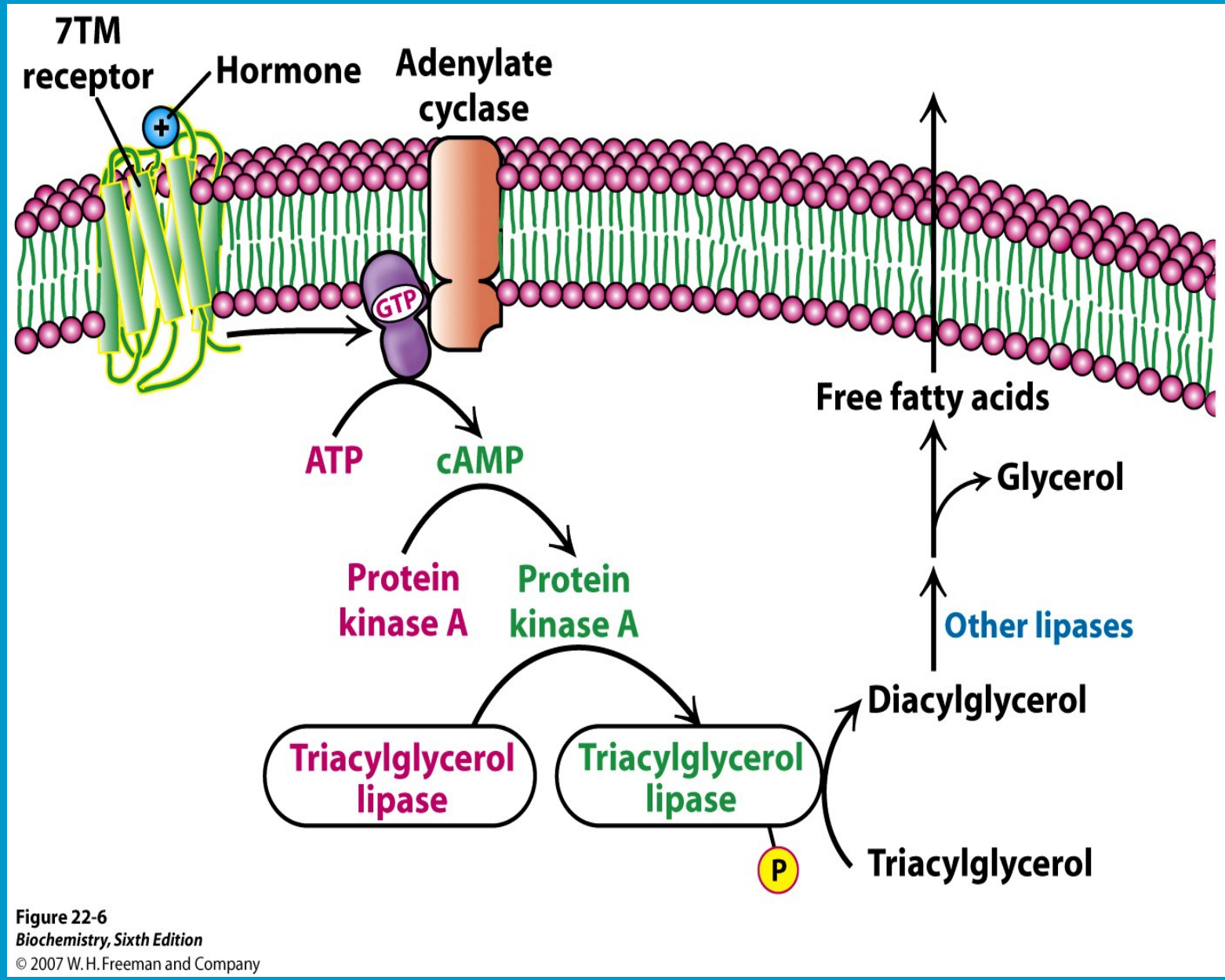


Figure 22-6
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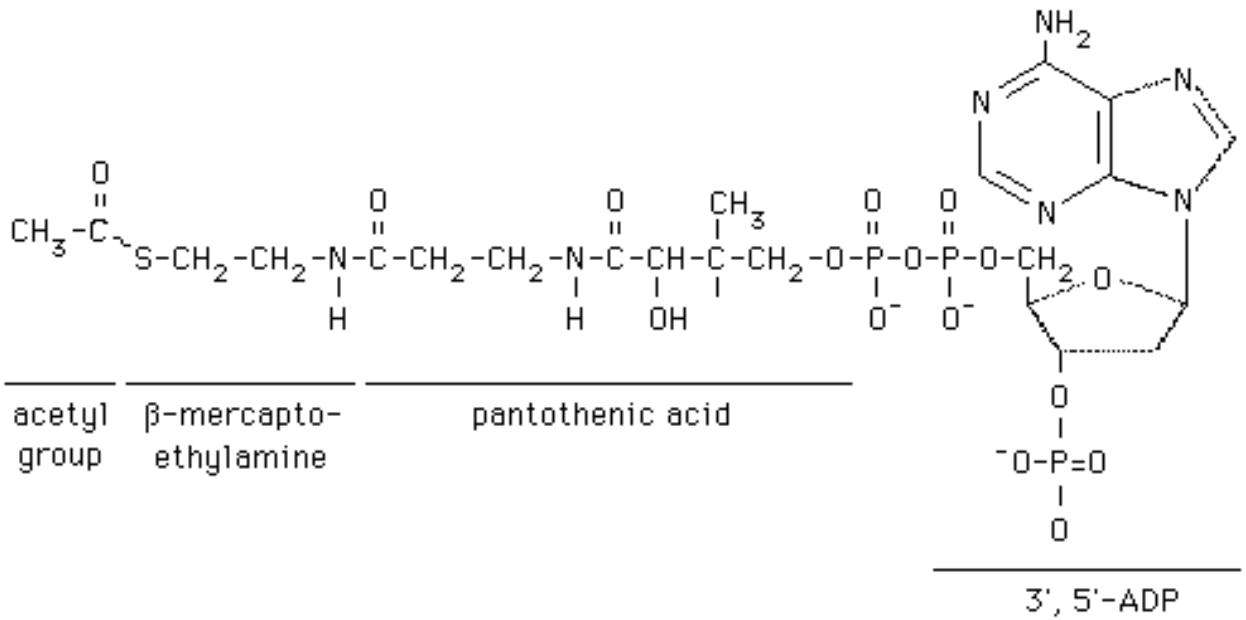
Beta Oxidation of Fatty Acids

- **Knoop showed that fatty acids must be degraded by removal of 2-C units**
- **Albert Lehninger showed that this occurred in the mitochondria**
- **F. Lynen and E. Reichart showed that the 2-C unit released is acetyl-CoA, not free acetate**
- **The process begins with oxidation of the carbon that is "beta" to the carboxyl carbon, so the process is called"beta-oxidation"**

Structure of Acetyl CoA

The structure of Acetyl CoA consists of two parts.

1. **Acetyl group**
2. **Coenzyme A**
   - **Beta-mercaptoethylamine**
   - **Pantothenic acid (not synthesized in man -- an essential nutrient)**
   - **Phosphate**
   - **3', 5'-adenosine diphosphate**



Acetyl coenzyme A, showing its constituents

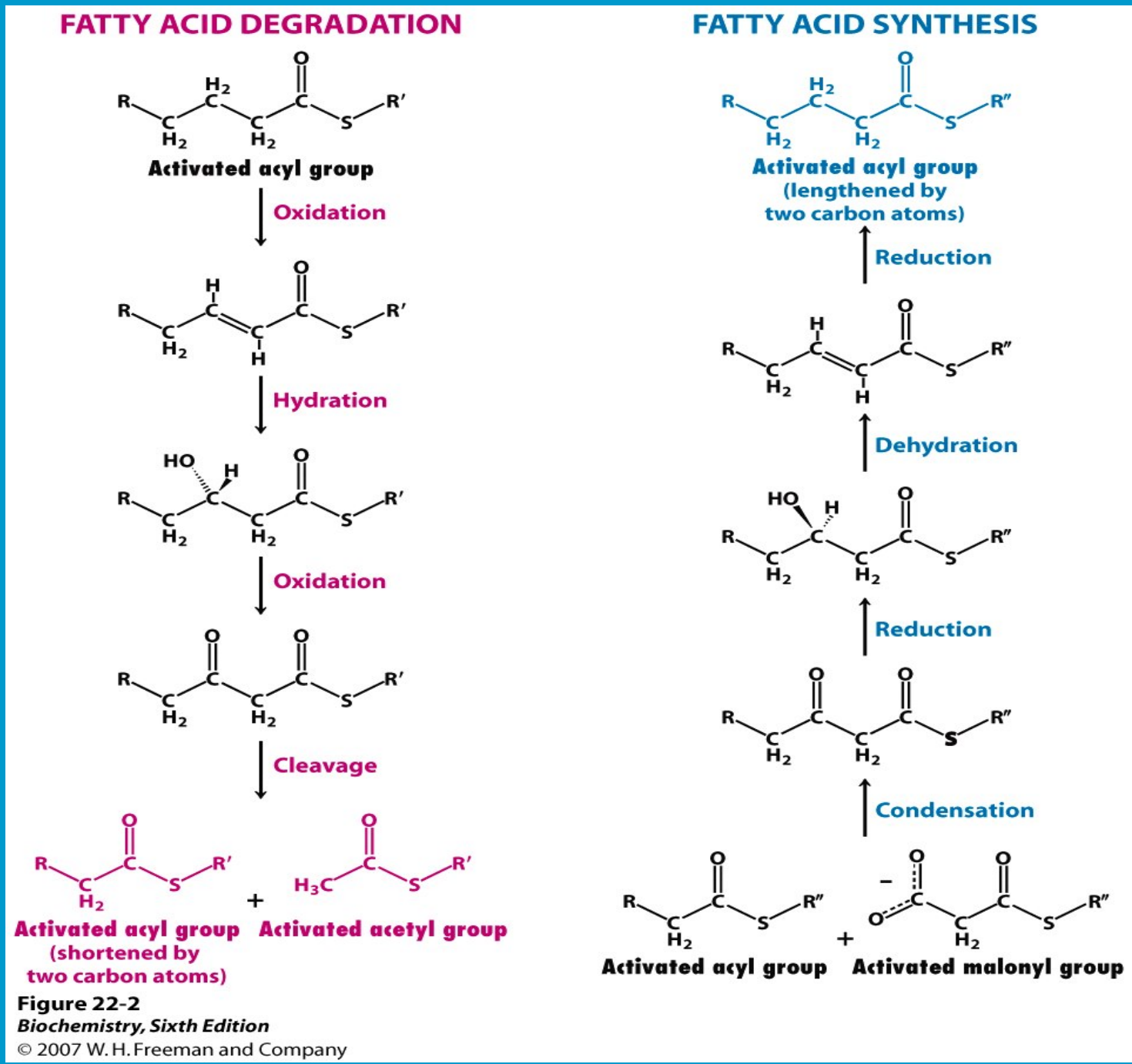


Figure 22-2
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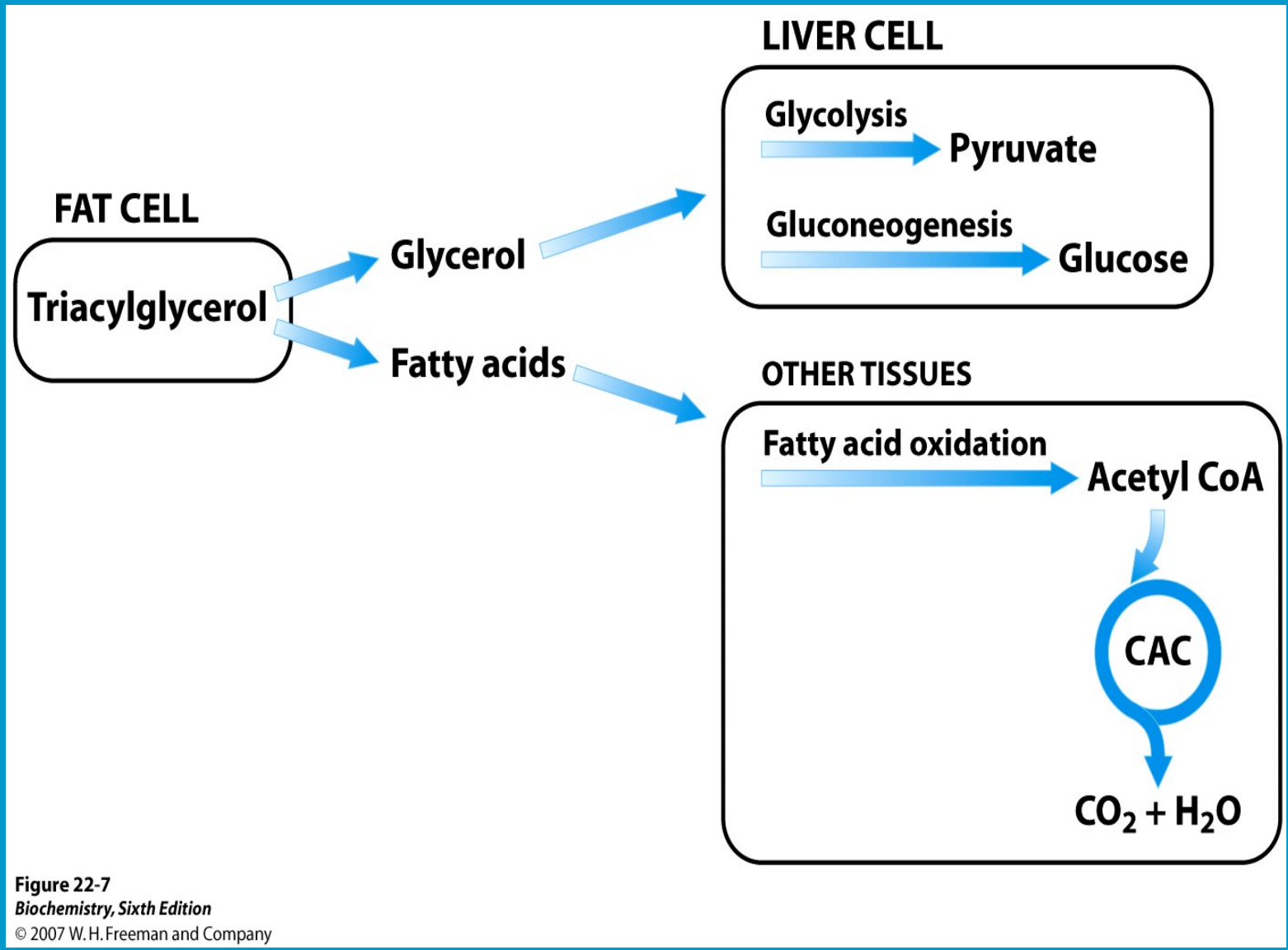

Figure 22-7
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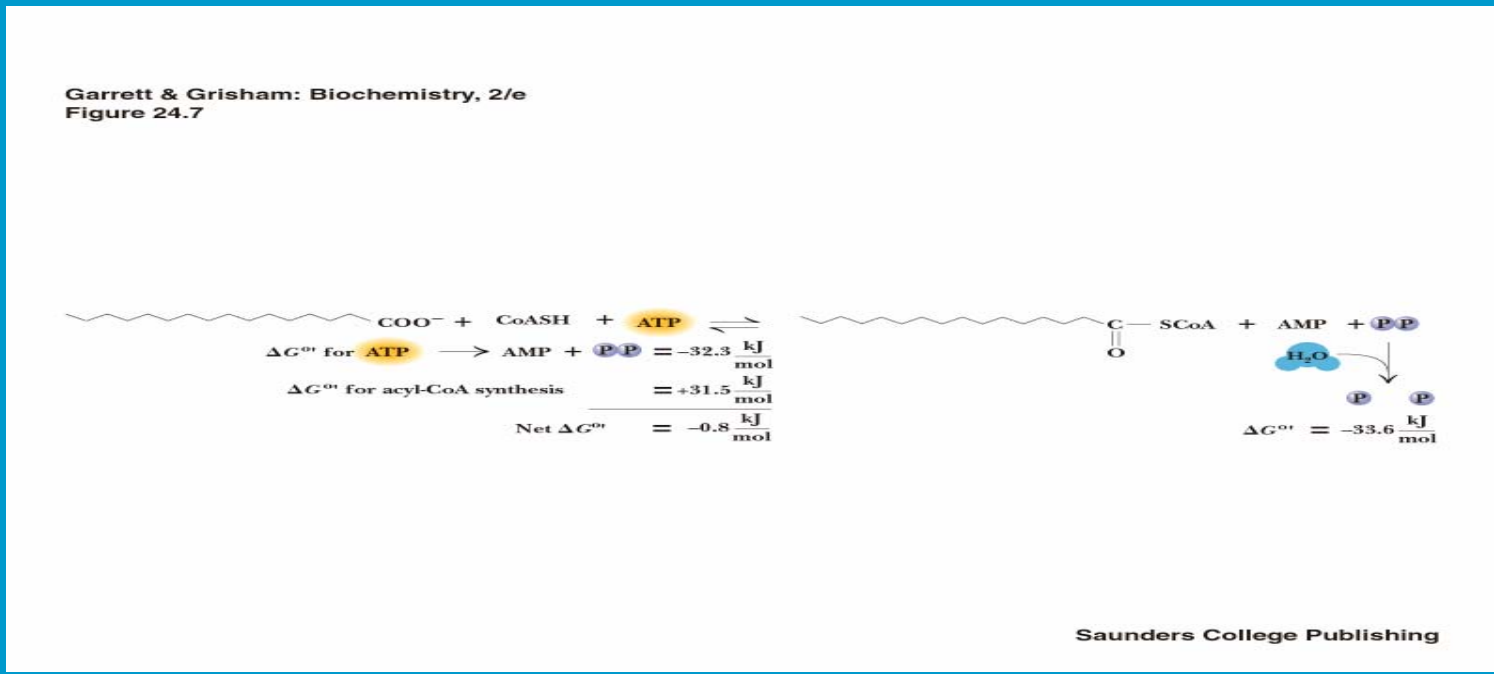
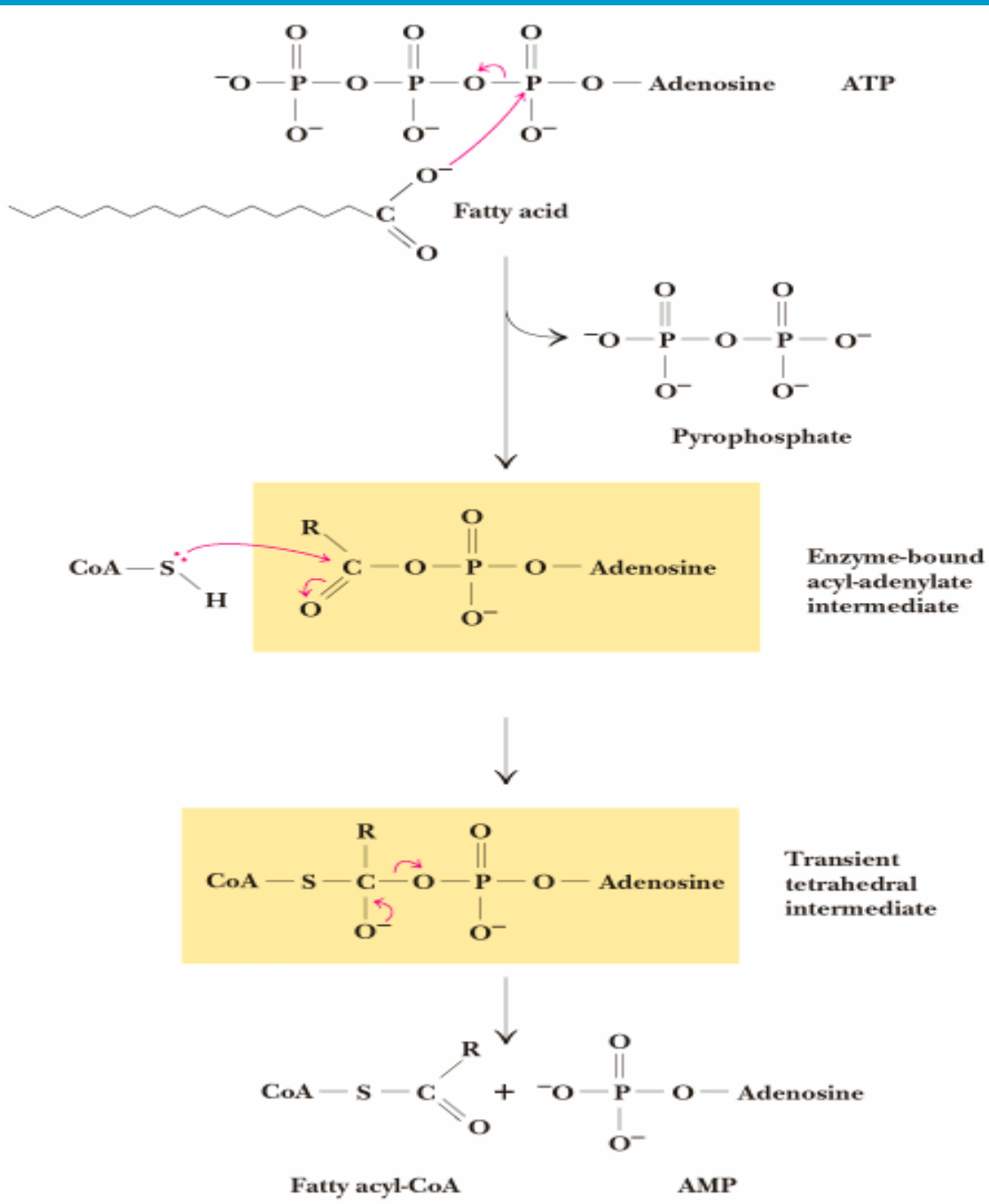
CoA activates FAs for oxidation

Acyl-CoA synthetase condenses fatty acids with CoA, with simultaneous hydrolysis of ATP to AMP and PP_i

- **Formation of a CoA ester is expensive energetically**
- **Reaction just barely breaks even with ATP hydrolysis**
- **But subsequent hydrolysis of PP_i drives the reaction strongly forward**
- **Note the acyl-adenylate intermediate in the mechanism!**

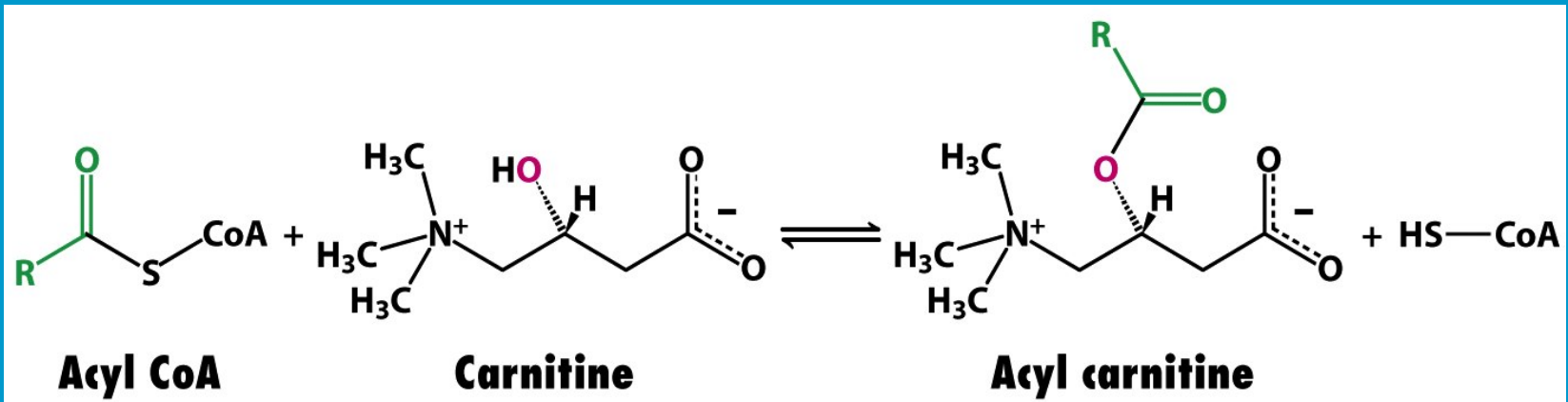



ATP
Adenosine
Fatty acid
Pyrophosphate
CoA
S
H
R
Enzyme-bound
acyl-adenylate
intermediate
Transient
tetrahedral
intermediate
Fatty acyl-CoA
AMP

Carnitine as a Carrier

Carnitine carries fatty acyl groups across the inner mitochondrial membrane

- **Short chain fatty acids are carried directly into the mitochondrial matrix**
- **Long-chain fatty acids cannot be directly transported into the matrix**
- **Long-chain FAs are converted to acyl carnitines and are then transported in the cell**
- **Acyl-CoA esters are formed inside the inner membrane in this way**



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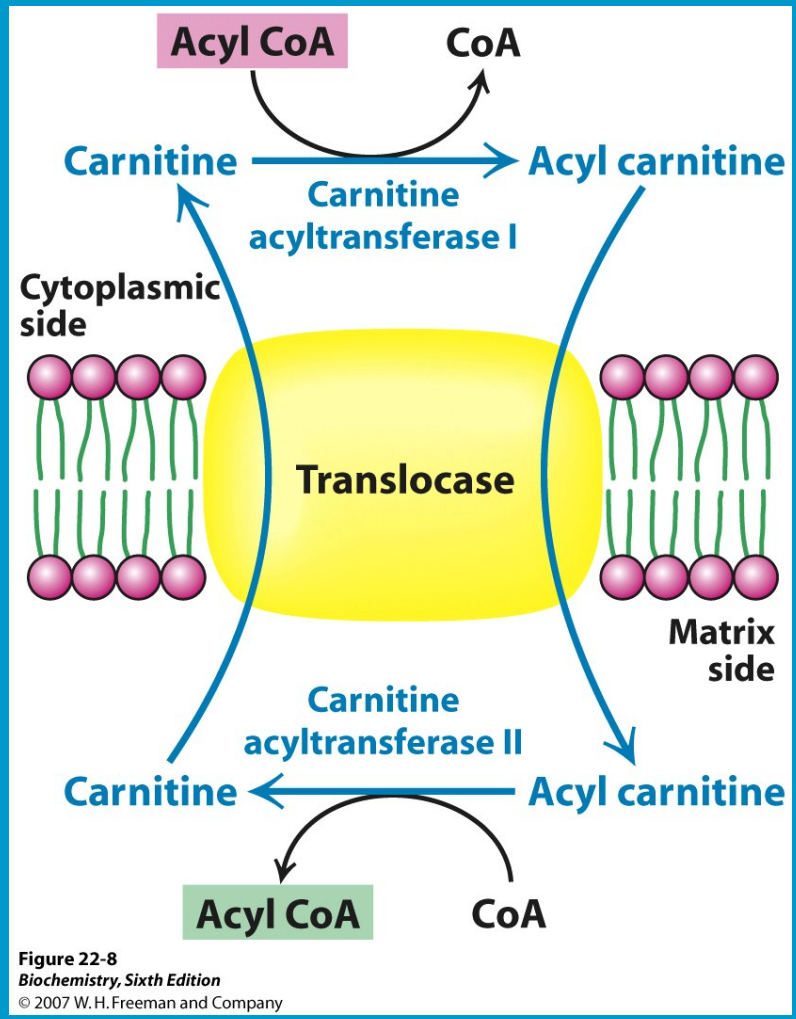

Figure 22-8
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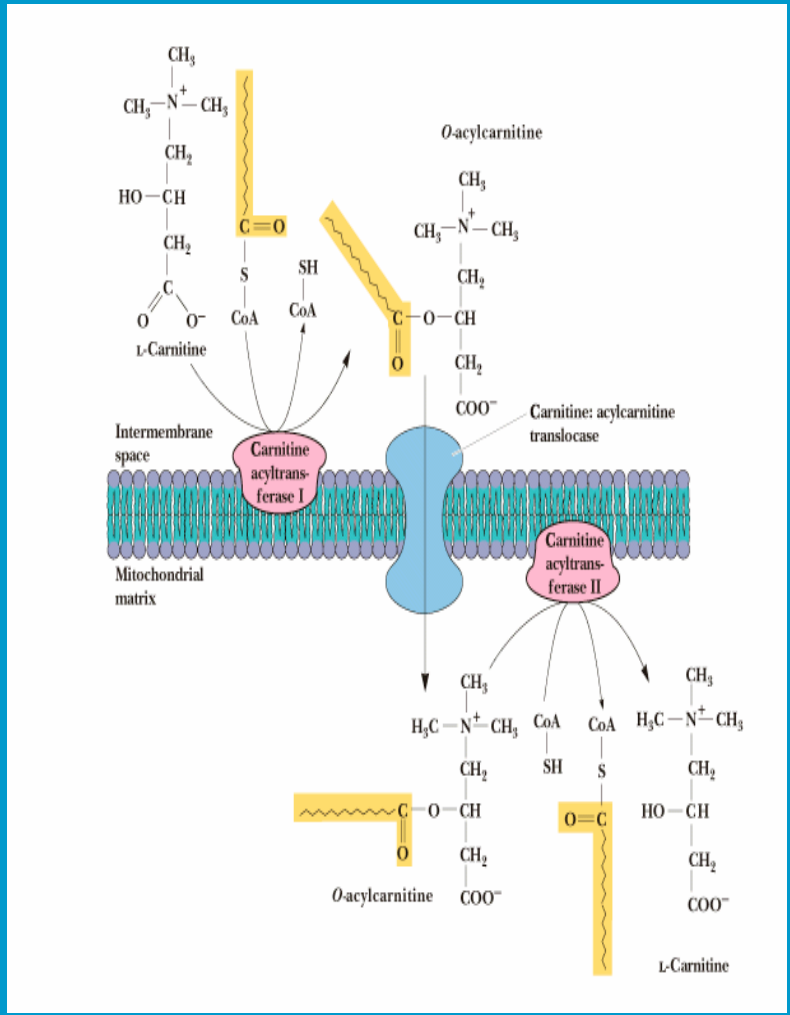
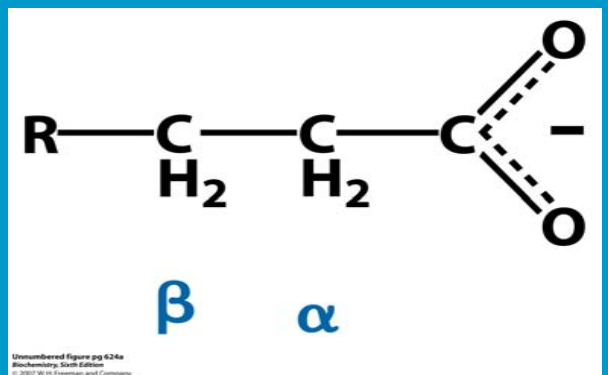


Carnitine acyltransferase 1 forms a O-acyl intermediate (which has a group transfer potential).
The eukaryotic cells maintain separate pools of CoA in the mitochondria (used in the oxidation of fatty acids, pyruvate and some amino acids) and in the cytosol (used principally in fatty acid biosynthesis)

β-Oxidation of Fatty Acids

A Repeated Sequence of 4 Reactions

- **Strategy: create a carbonyl group on the β-C**
- **First 3 reactions do that; fourth cleaves the "β-keto ester" in a reverse Claisen condensation [*CC – involves attack by a nucleophilic agent on a carbonyl carbon to yield a beta-keto acid*]**
- **Products: an acetyl-CoA and a fatty acid two carbons shorter**
- **The first three reactions are crucial and classic - we will see them again and again in other pathways**



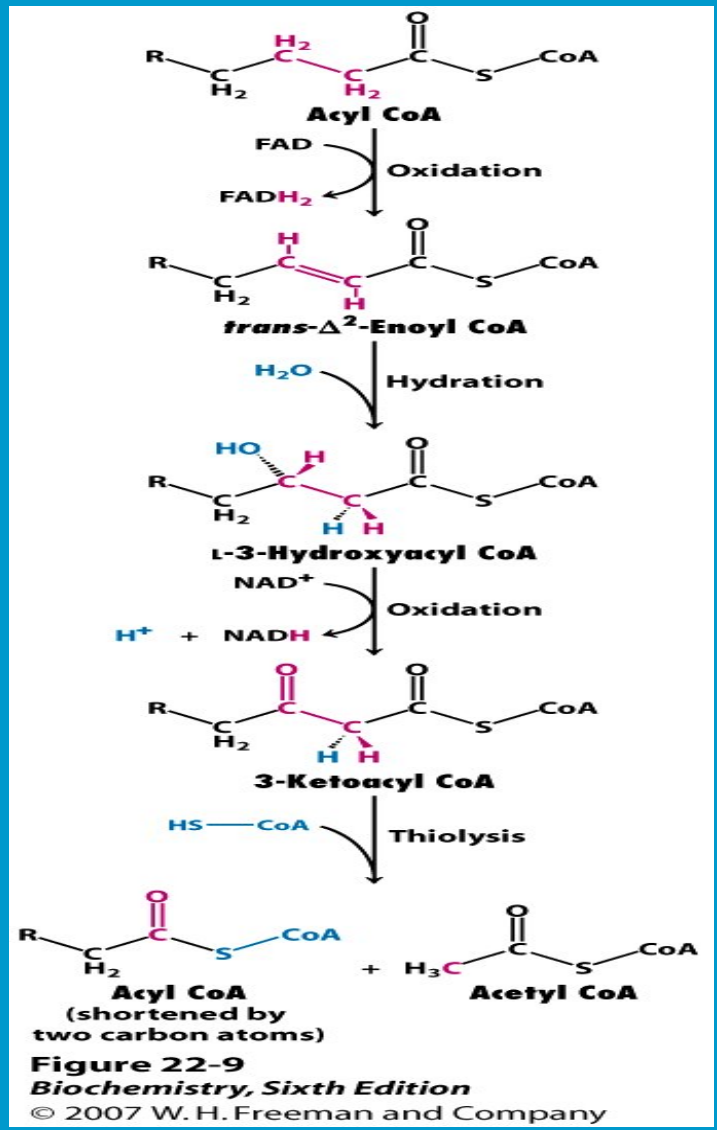


Figure 22-9
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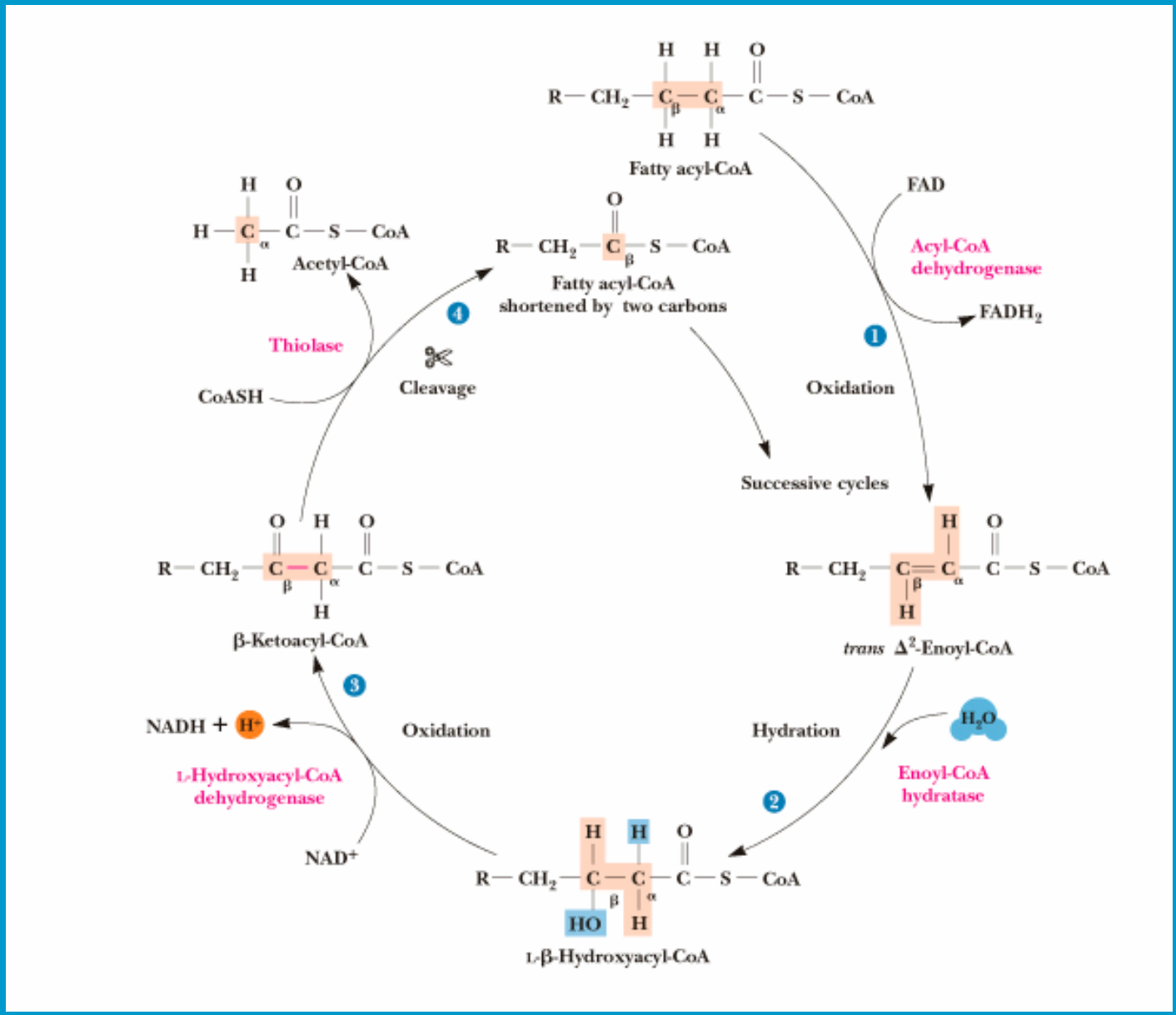
Fatty acyl-CoA
FAD
Acyl-CoA dehydrogenase
FADH2
1
Oxidation
trans Δ2-Enoyl-CoA
Hydration
H2O
Enoyl-CoA hydratase
2
L-β-Hydroxyacyl-CoA
Successive cycles
3
Oxidation
NADH + H+
L-Hydroxyacyl-CoA dehydrogenase
NAD+
β-Ketoacyl-CoA
4
Cleavage
Thiolase
CoASH
Acetyl-CoA
Fatty acyl-CoA shortened by two carbons

Acyl-CoA Dehydrogenase

Oxidation of the C_α-C_β bond

- **A family of three soluble matrix enzymes [*that differ in specificity for either long, medium or short-chain acyl-CoAs*}**
- **Mechanism involves proton abstraction, followed by double bond formation and hydride removal by FAD**
- **Electrons are passed to an electron transfer flavoprotein, and then to the electron transport chain**

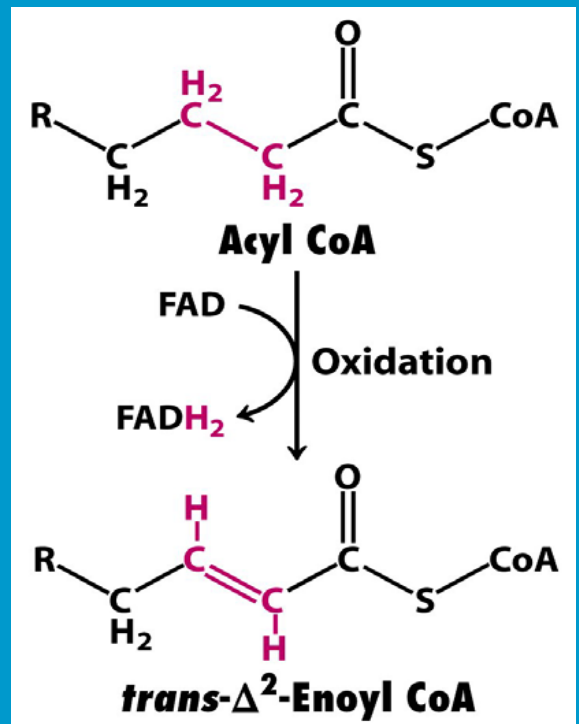

Figure 22-9 part 1
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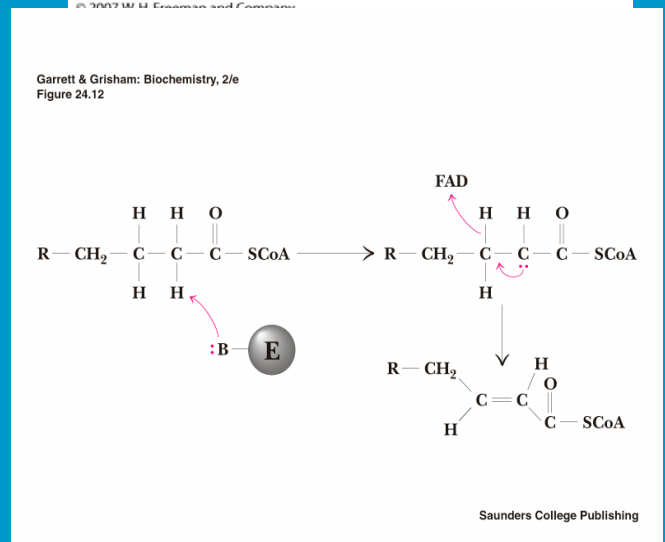

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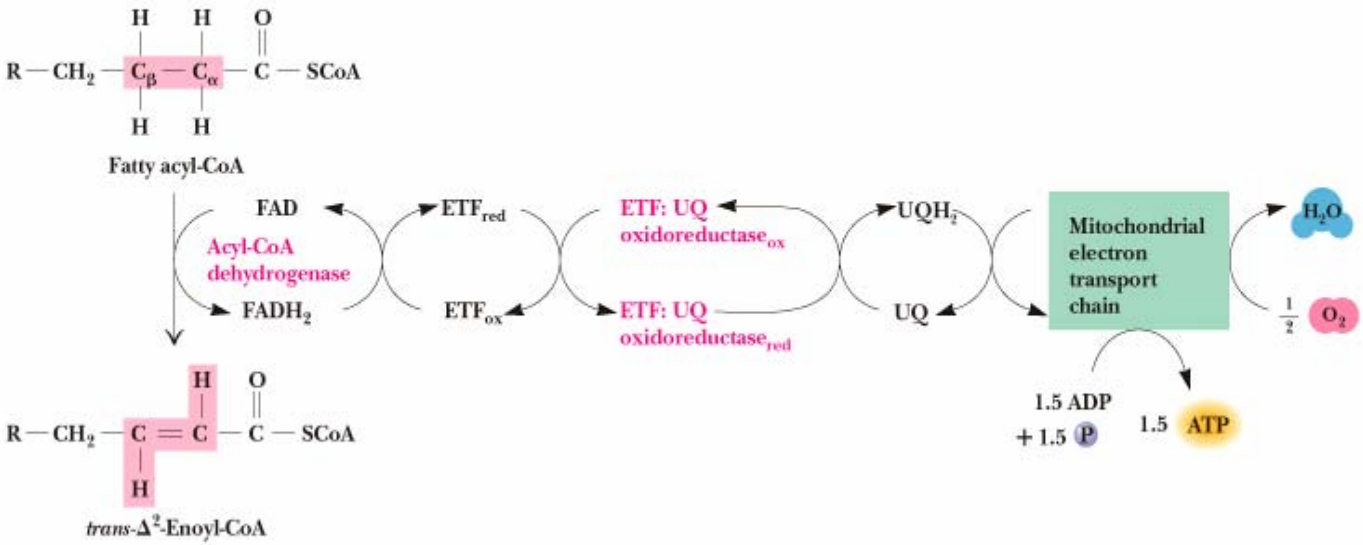
Figure 24.11



Enoyl-CoA Hydratase

Adds water across the double bond

- at least three forms of the enzyme are known
- aka crotonases
- Normal reaction converts trans-enoyl-CoA to L-β-hydroxyacyl-CoA

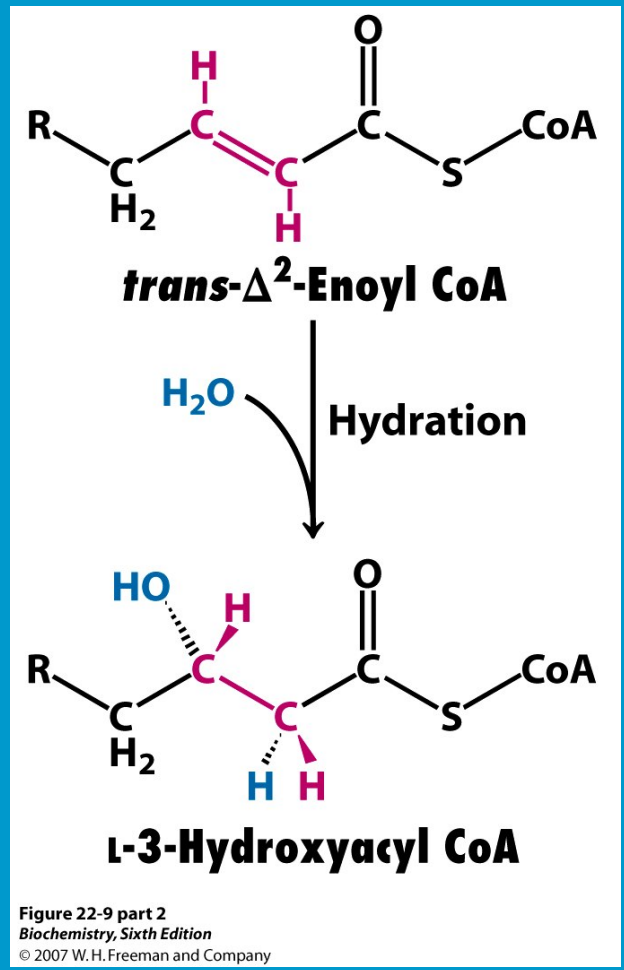


Figure 22-9 part 2
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Hydroxyacyl-CoA Dehydrogenase

Oxidizes the β-Hydroxyl Group

- This enzyme is completely specific for L-hydroxyacyl-CoA
- D-hydroxyacyl-isomers are handled differently

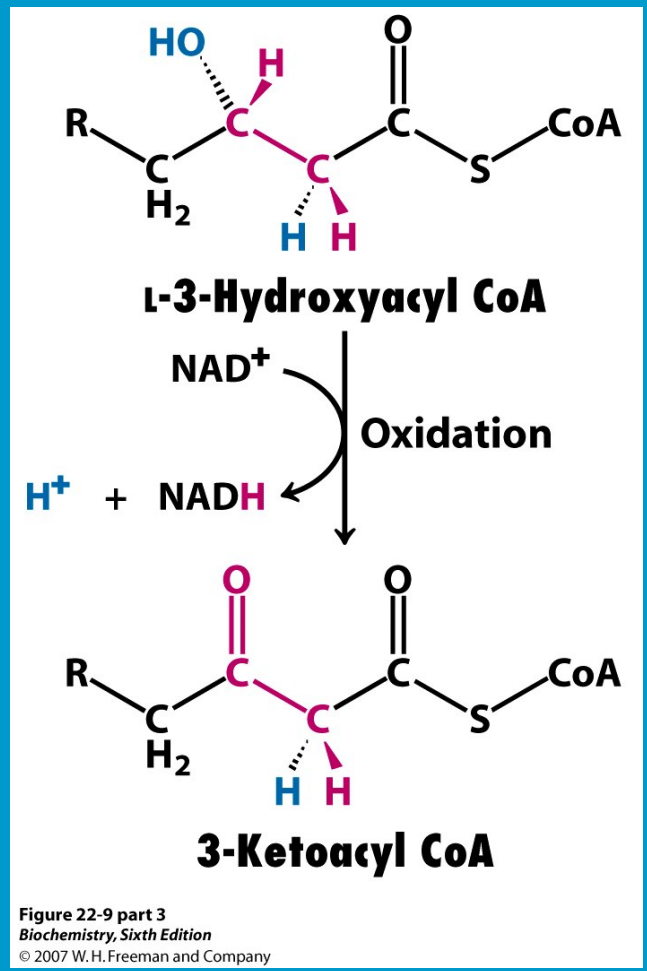

Figure 22-9 part 3
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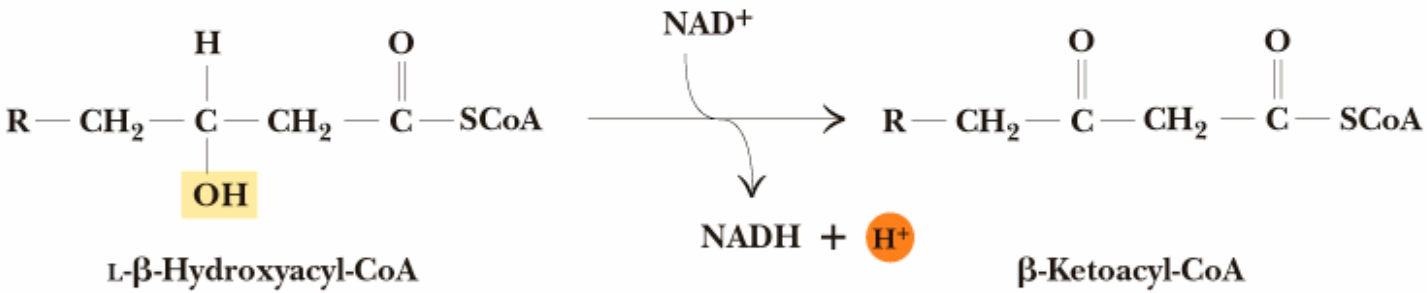

Fourth reaction: thiolase

aka β-ketothiolase

- Cysteine thiolate on enzyme attacks the β-carbonyl group
- Thiol group of a new CoA attacks the shortened chain, forming a new, shorter acyl-CoA
- This is the reverse of a Claisen condensation: attack of the enolate of acetyl-CoA on a thioester
- Even though it forms a new thioester, the reaction is favorable and drives other three

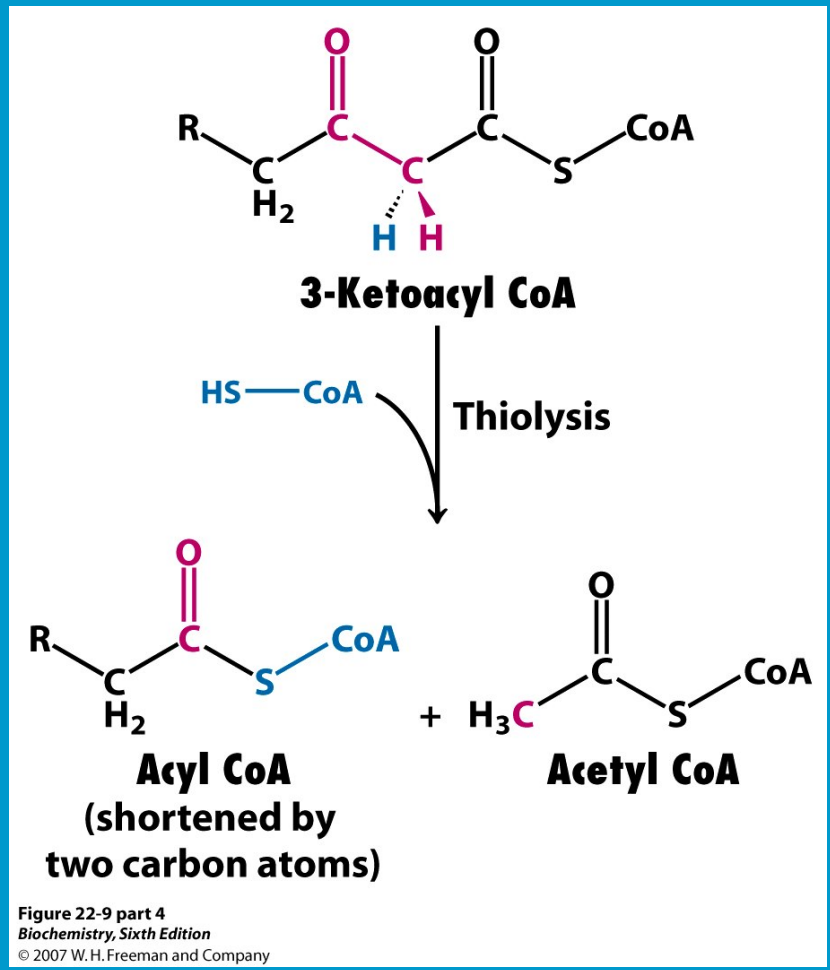

Figure 22-9 part 4
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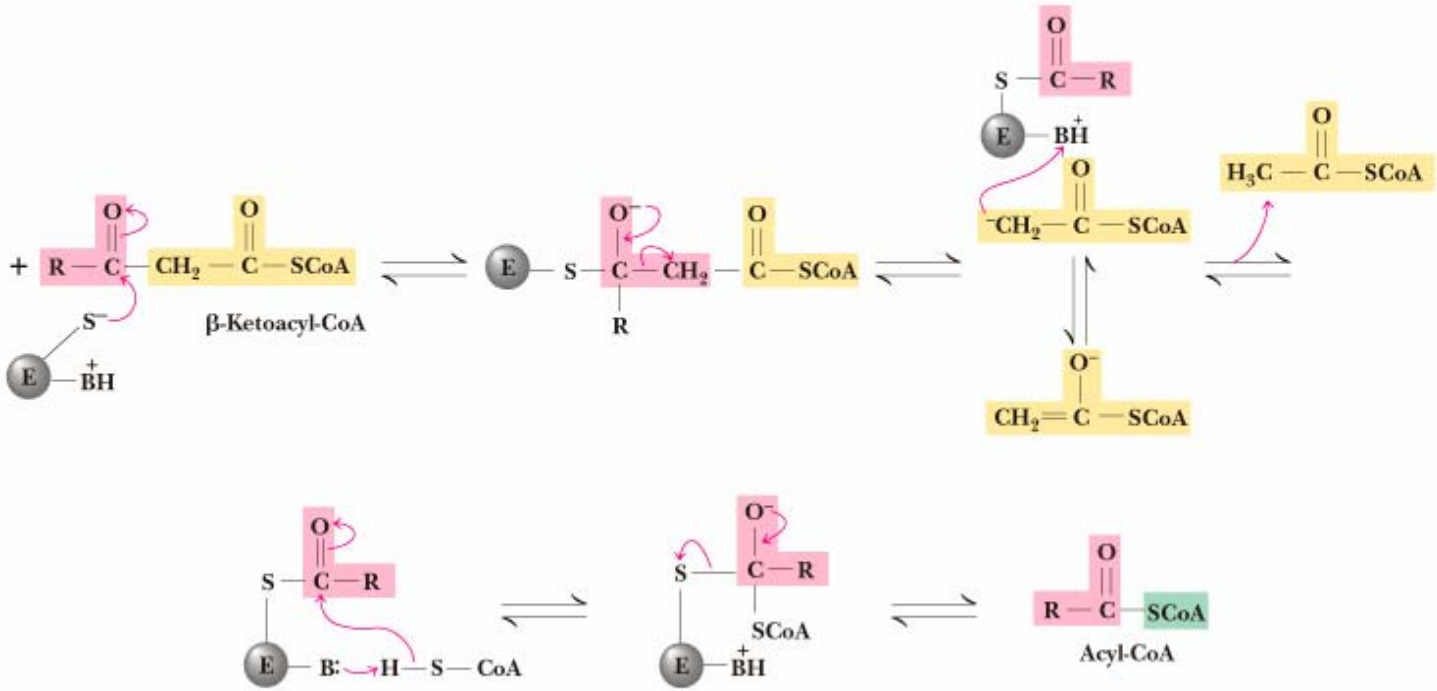

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Summary of β-Oxidation

Repetition of the cycle yields a succession of acetate units

- Thus, palmitic acid yields eight acetyl-CoAs
- Complete β-oxidation of one palmitic acid yields 106 molecules of ATP
- Large energy yield is consequence of the highly reduced state of the carbon in fatty acids
- This makes fatty acids the fuel of choice for migratory birds and many other animals

TABLE 22.1 Principal reactions in fatty acid oxidation

Step	Reaction	Enzyme
1	Fatty acid + CoA + ATP $\rightleftharpoons$ acyl CoA + AMP + PP_i	Acyl CoA synthetase [also called fatty acid thiokinase and fatty acid:CoA ligase]*
2	Carnitine + acyl CoA $\rightleftharpoons$ acyl carnitine + CoA	Carnitine acyltransferase (also called carnitine palmitoyl transferase)
3	Acyl CoA + E-FAD $\longrightarrow$ *trans*-Δ^2-enoyl CoA + E-$FADH_2$	Acyl CoA dehydrogenases (several isozymes having different chain-length specificity)
4	*trans*-Δ^2-Enoyl CoA + H_2O $\rightleftharpoons$ L-3-hydroxyacyl CoA	Enoyl CoA hydratase (also called crotonase or 3-hydroxyacyl CoA hydrolyase)
5	L-3-Hydroxyacyl CoA + NAD^+ $\rightleftharpoons$ 3-ketoacyl CoA + NADH + H^+	L-3-Hydroxyacyl CoA dehydrogenase
6	3-Ketoacyl CoA + CoA $\rightleftharpoons$ acetyl CoA + acyl CoA (shortened by C_2)	β-Ketothiolase (also called thiolase)

* An AMP-forming ligase.

Table 22-1
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Energy Yield from β-Ox of Palmitate

Palmitoyl CoA + 7 CoASH + 7 FAD + 7 NAD + 7 H_20

↓

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

FADH2 = 1.5 ATP X 7 = 10.5 ATP
NADH = 2.5 ATP X 7 = 17.5ATP
Acetyl CoA = 10 ATP X 8 = 80 ATP

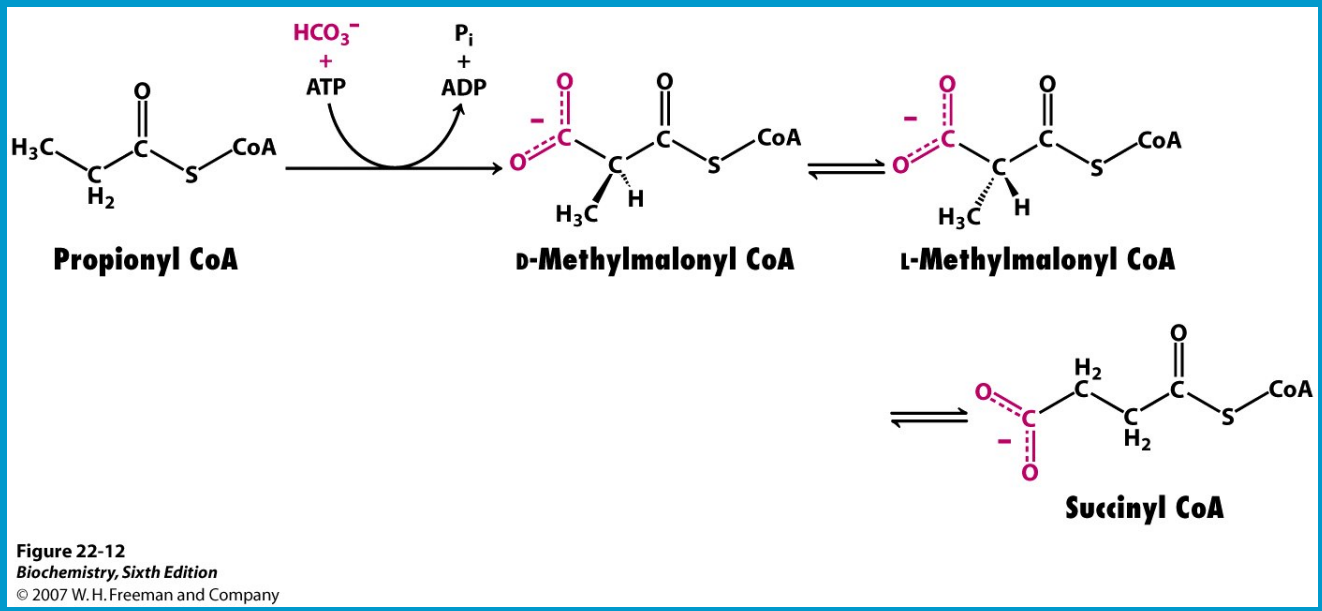
108 ATP
- 2 ATP (activation of FA)

106 ATP

Odd-Carbon Fatty Acids

β-Oxidation yields propionyl-CoA

- Odd-carbon fatty acids are metabolized normally, until the last three-C fragment - propionyl-CoA - is reached
- Three reactions convert propionyl-CoA to succinyl-CoA
- An initial carboxylation at the α-carbon of propionyl-CoA to produce D-methylmalonyl-CoA, catalyzed by a biotin-dependent enzyme, **propionyl-CoA carboxylase**.
- D-methylmalonyl-CoA is converted to the L-isomer by **methylmalonyl-CoA epimerase**.
- The third reaction, catalyzed by **methylmalonyl-CoA mutase**, involves the migration of the carbonyl-CoA group from one carbon to its neighbor. This reaction is vitamin B12-dependednt.



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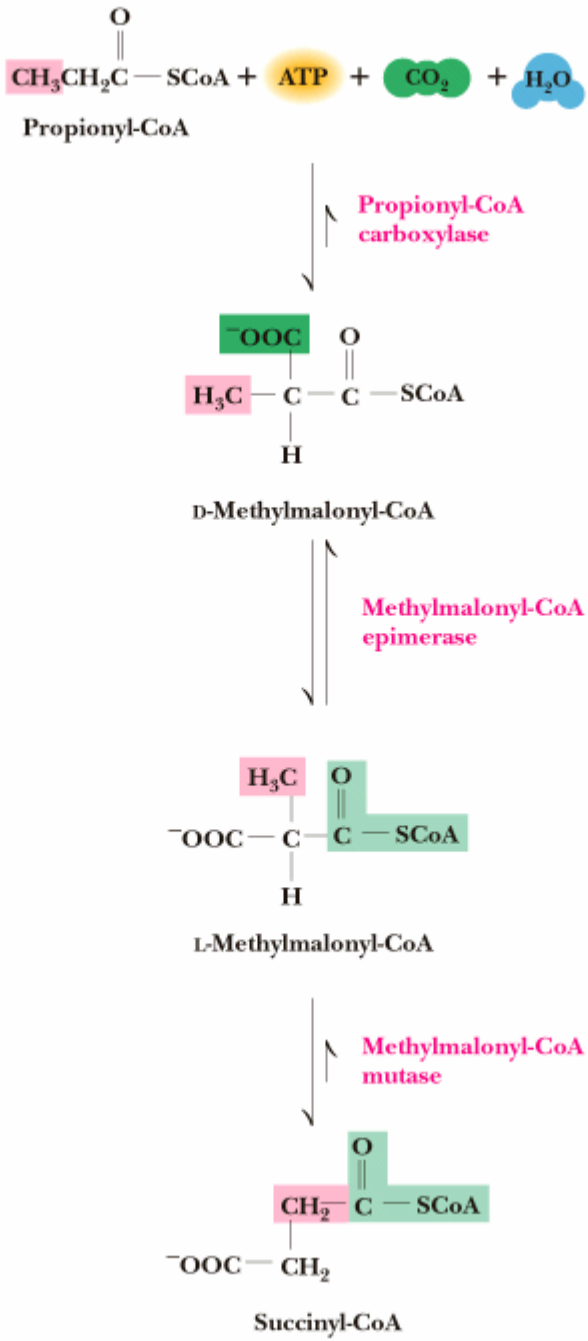
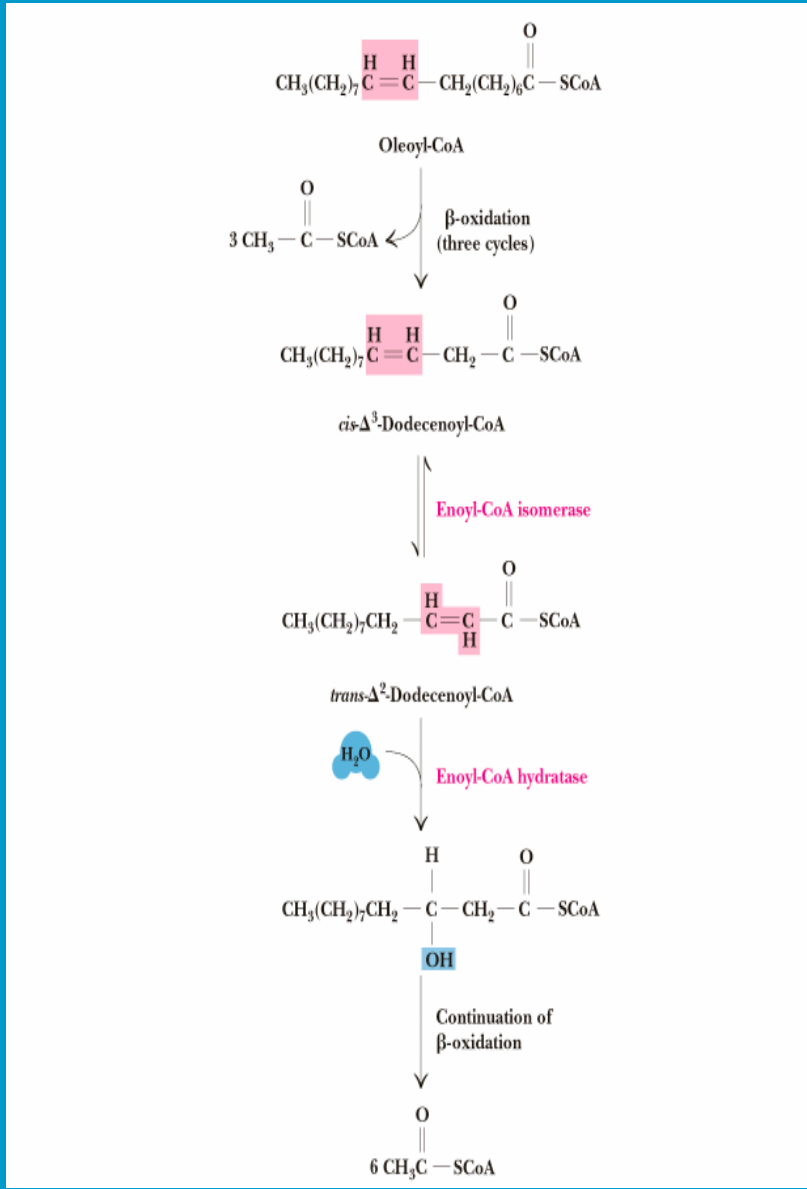


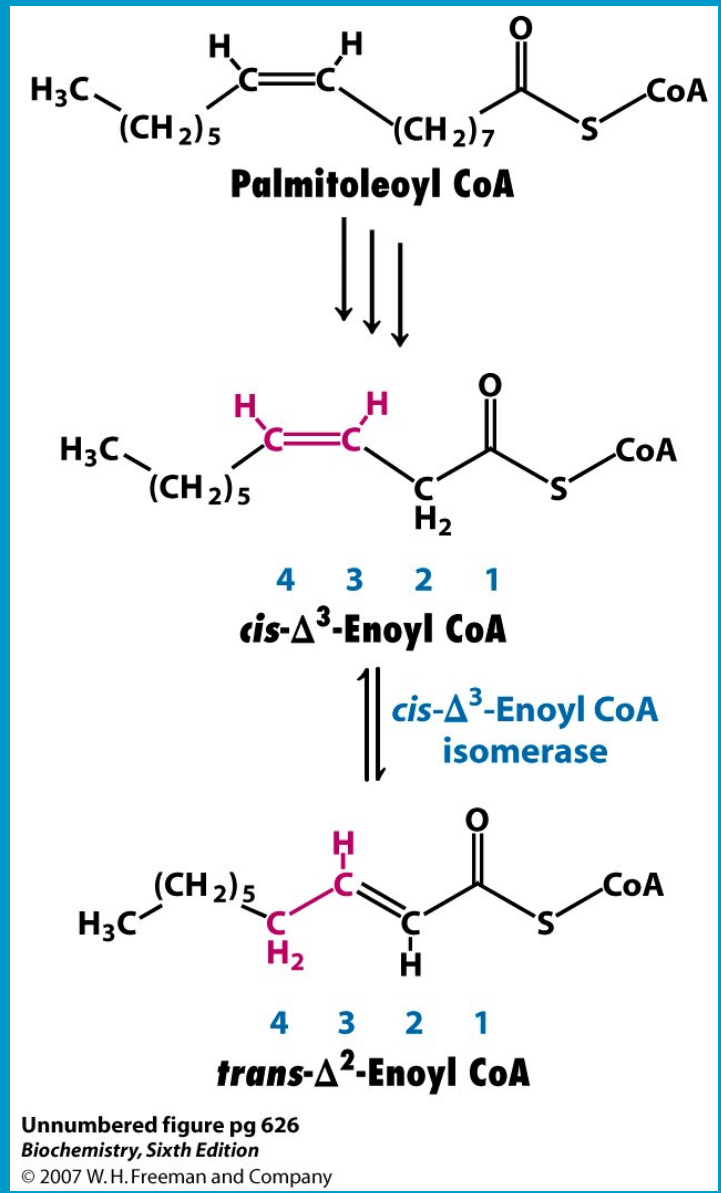
Succinyl CoA then enters the TCA cycle {succinyl CoA to malate to pyruvate to TCA cycle}

Unsaturated Fatty Acids

Consider monounsaturated fatty acids:

- Oleic acid, palmitoleic acid
- Normal β-oxidation for three cycles
- cis-Δ^3 acyl-CoA cannot be utilized by acyl-CoA dehydrogenase
- Enoyl-CoA isomerase converts this to trans- Δ^2 acyl CoA
- β-oxidation continues from this point





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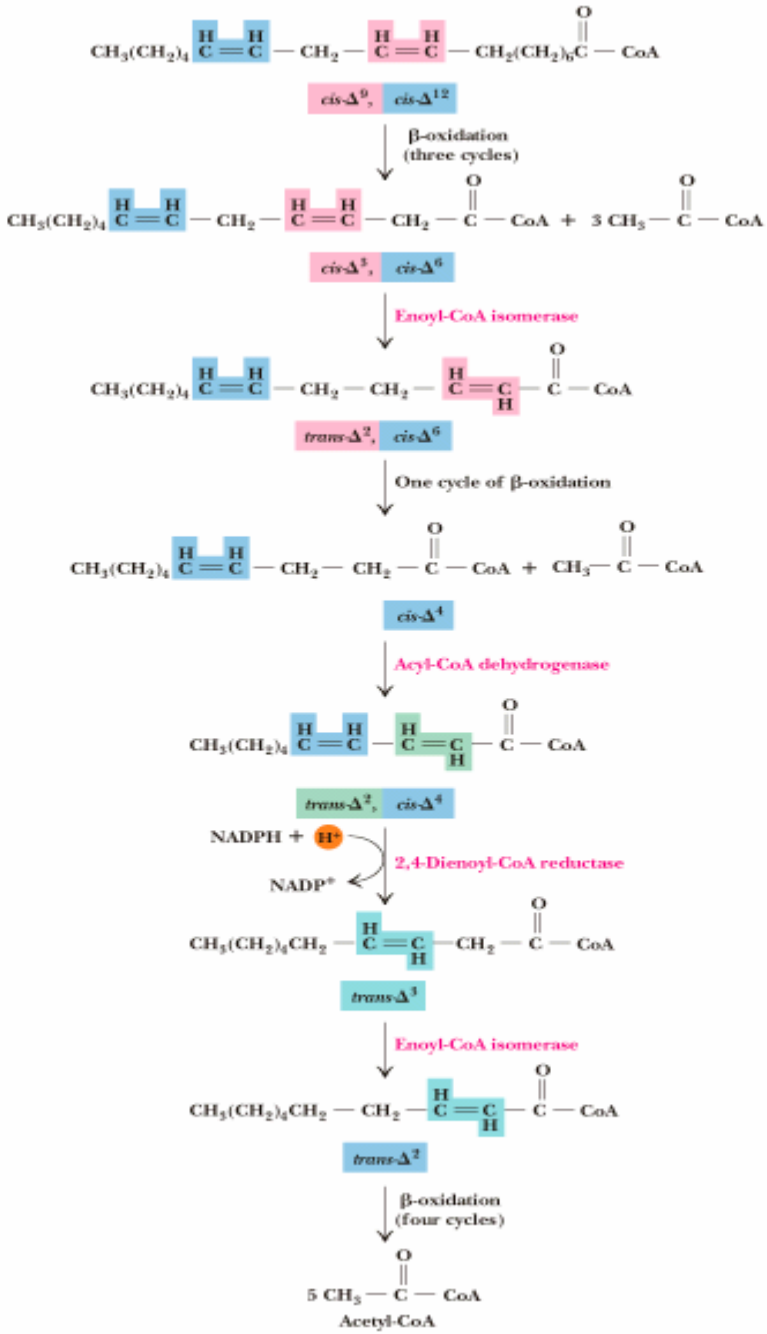
Polyunsaturated Fatty Acids

Slightly more complicated

- Same as for oleic acid, but only up to a point:
  - 3 cycles of β-oxidation
  - enoyl-CoA isomerase
  - 1 more round of β-oxidation
  - trans- Δ^2, cis- Δ^4 structure is a problem!
- 2,4-Dienoyl-CoA reductase to the rescue! – **produces a trans- Δ^3 enoyl product. This enoyl product can be converted by an enoyl-CoA isomerase to the trans- Δ^2 enoyl CoA, which then proceeds normally through the beta-oxidation pathway.**


CH3(CH2)4 — CH=CH — CH2 — CH=CH — CH2(CH2)6C — CoA
cis-Δ9, cis-Δ12
β-oxidation (three cycles)
CH3(CH2)4 — CH=CH — CH2 — CH=CH — CH2 — C — CoA + 3 CH3 — C — CoA
cis-Δ3, cis-Δ6
Enoyl-CoA isomerase
CH3(CH2)4 — CH=CH — CH2 — CH2 — CH=CH — C — CoA
trans-Δ2, cis-Δ6
One cycle of β-oxidation
CH3(CH2)4 — CH=CH — CH2 — CH2 — C — CoA + CH3 — C — CoA
cis-Δ4
Acyl-CoA dehydrogenase
CH3(CH2)4 — CH=CH — CH=CH — C — CoA
trans-Δ2, cis-Δ4
NADPH + H+
NADP+
2,4-Dienoyl-CoA reductase
CH3(CH2)4CH2 — CH=CH — CH2 — C — CoA
trans-Δ3
Enoyl-CoA isomerase
CH3(CH2)4CH2 — CH2 — CH=CH — C — CoA
trans-Δ2
β-oxidation (four cycles)
5 CH3 — C — CoA
Acetyl-CoA

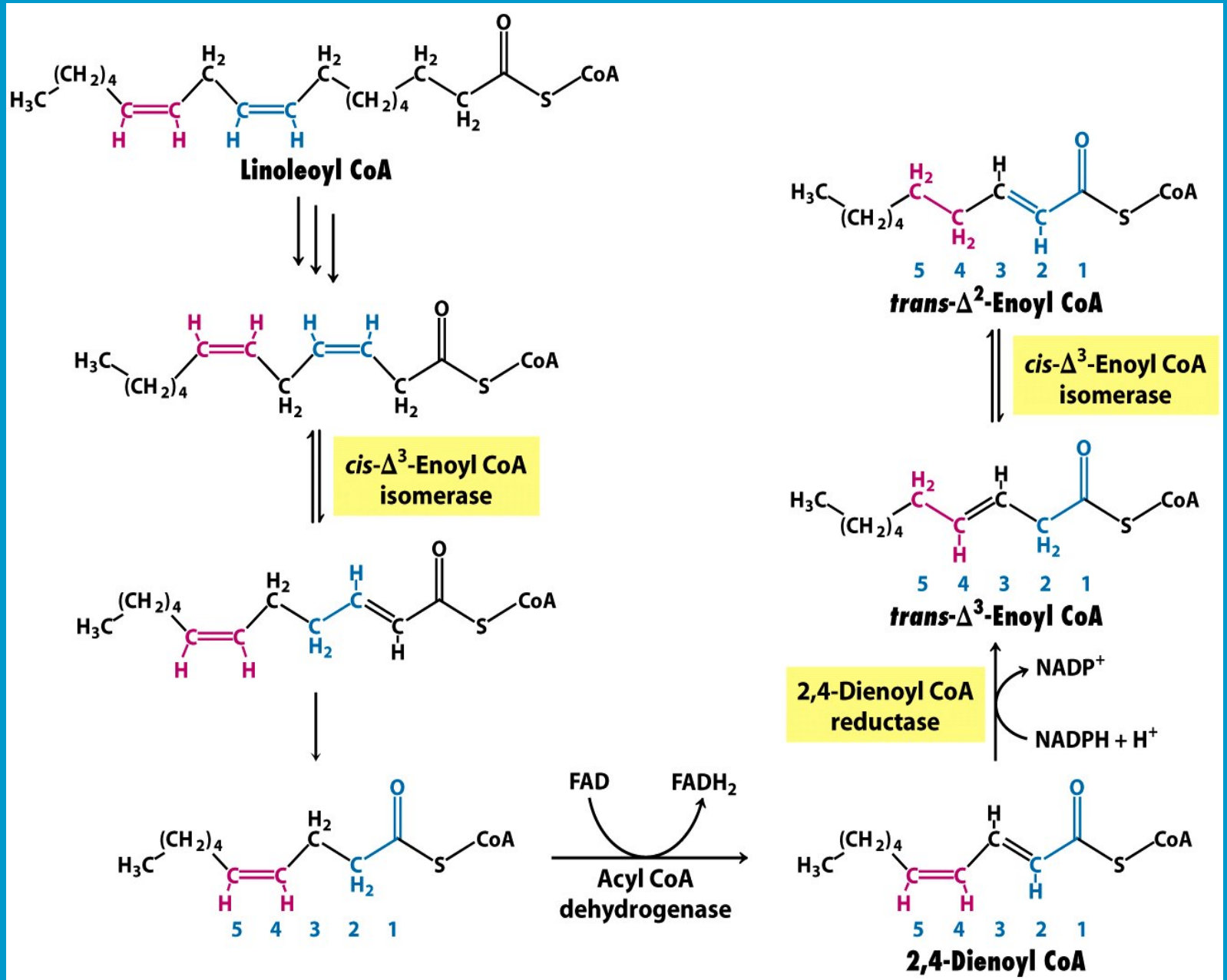


Figure 22-11
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Peroxisomal β-Oxidation

Peroxisomes - organelles that carry out flavin-dependent oxidations, regenerating oxidized flavins by reaction with O_2 to produce H_2O_2

- Similar to mitochondrial β-oxidation, but initial double bond formation is by acyl-CoA oxidase
- Electrons go to O_2 rather than e- transport
- Fewer ATPs result

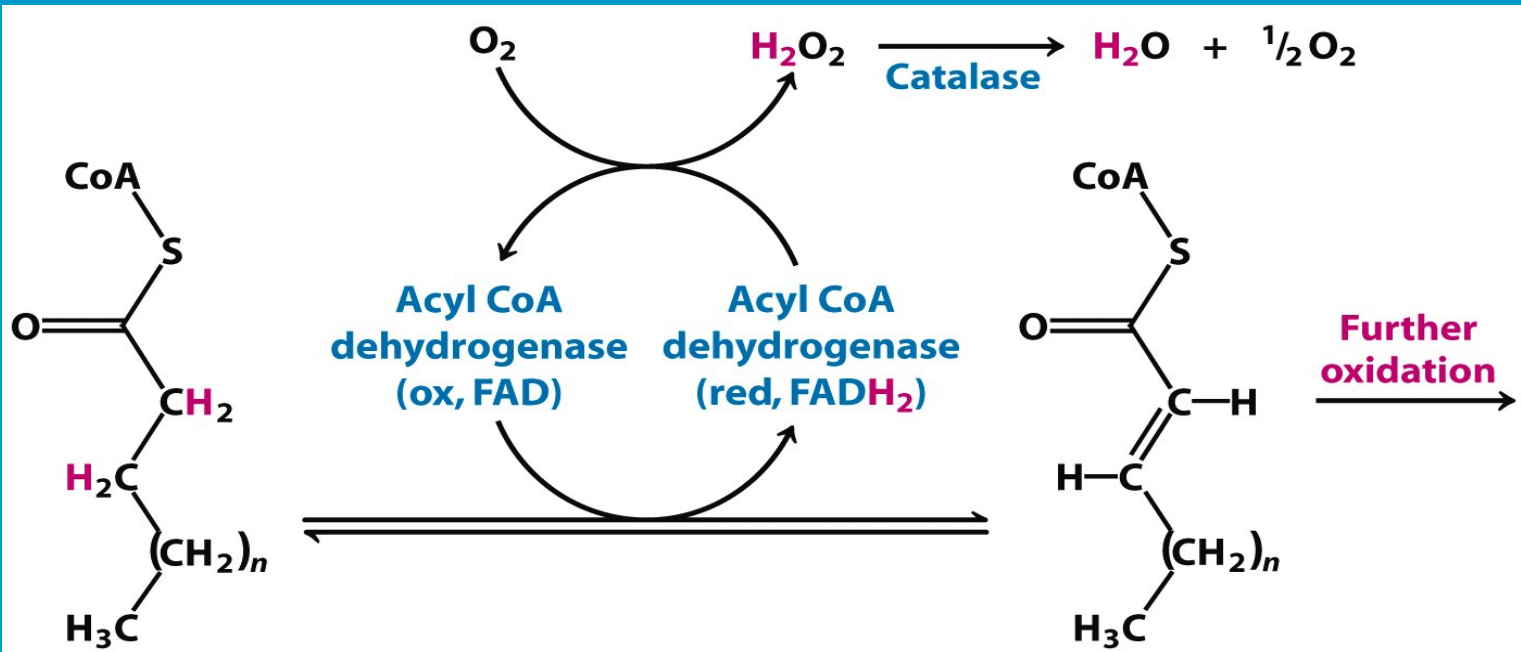

Figure 22-19
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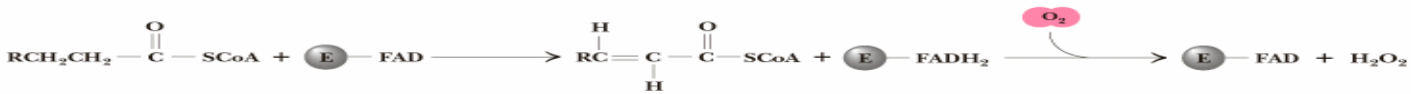
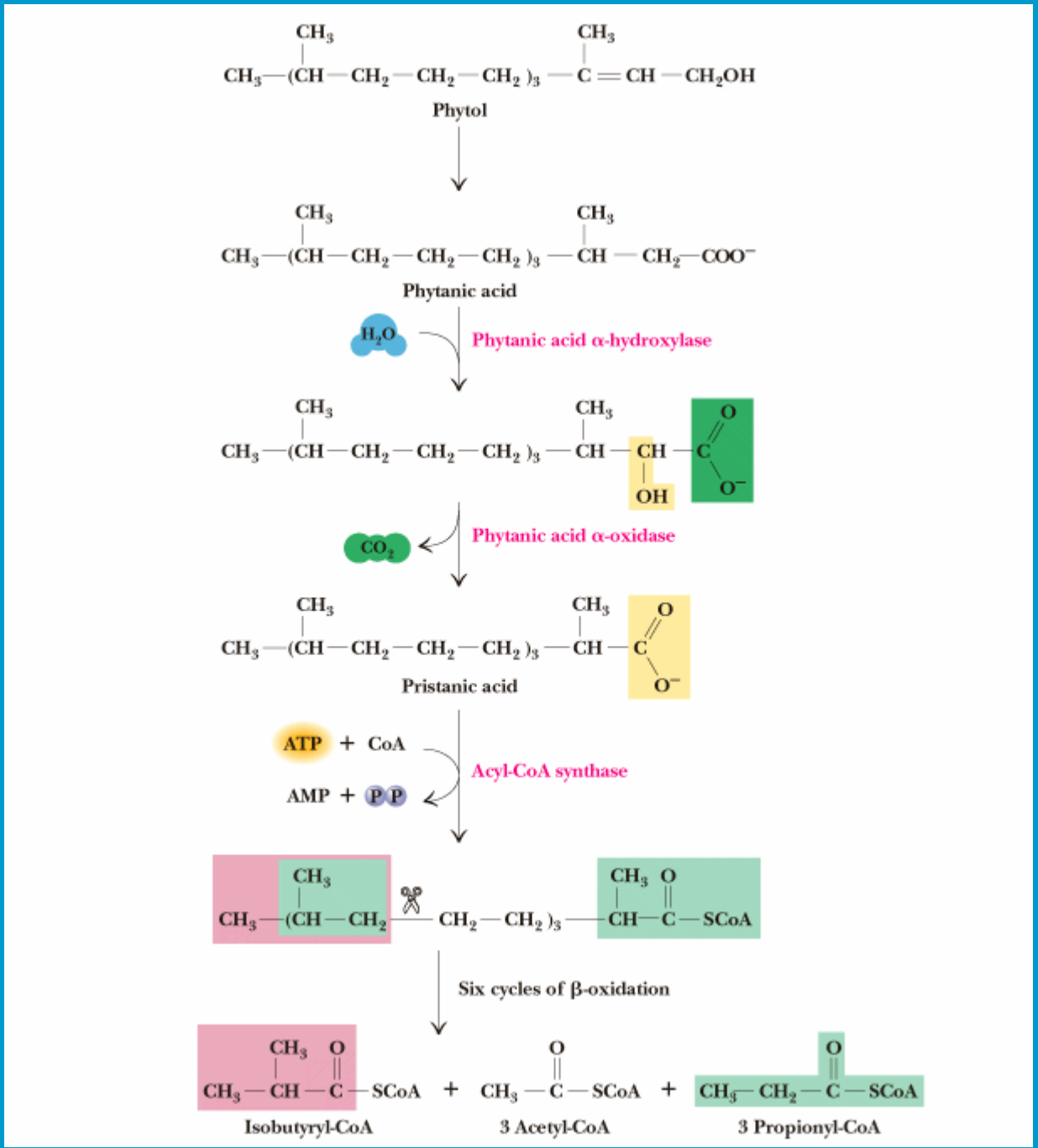
Garrett & Grisham: Biochemistry, 2/e
Figure 24.25



Branched-Chain Fatty Acids

An alternative to β-oxidation is required

- Branched chain FAs with branches at odd-number carbons are not good substrates for β-oxidation
- α-oxidation is an alternative
- Phytanic acid α-oxidase decarboxylates with oxidation at the alpha position
- β-oxidation occurs past the branch

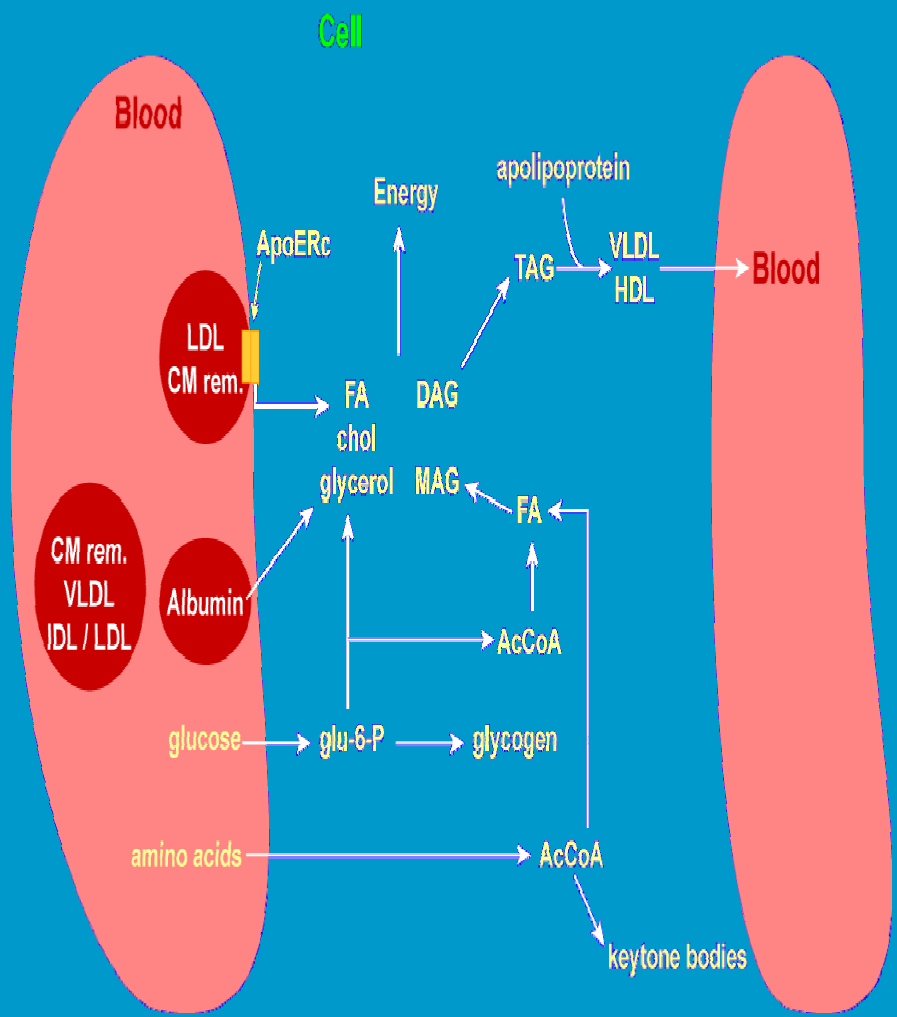

Phytol
Phytanic acid
Phytanic acid α-hydroxylase
Phytanic acid α-oxidase
Pristanic acid
ATP + CoA
AMP + PP
Acyl-CoA synthase
Six cycles of β-oxidation
Isobutyryl-CoA
3 Acetyl-CoA
3 Propionyl-CoA

Liver Clearance of Lipids

Liver is the major regulator of serum lipid levels. After a meal, it engulfs the chylomicron remnants and IDL particles, using its receptor for Apolipoprotein E. It also clears the short chain fatty acid bound with albumin. Once inside, the CM/IDL lipids are hydrolyzed to their constituent components: FFA, DAG, MAG, glycerol, and cholesterol.

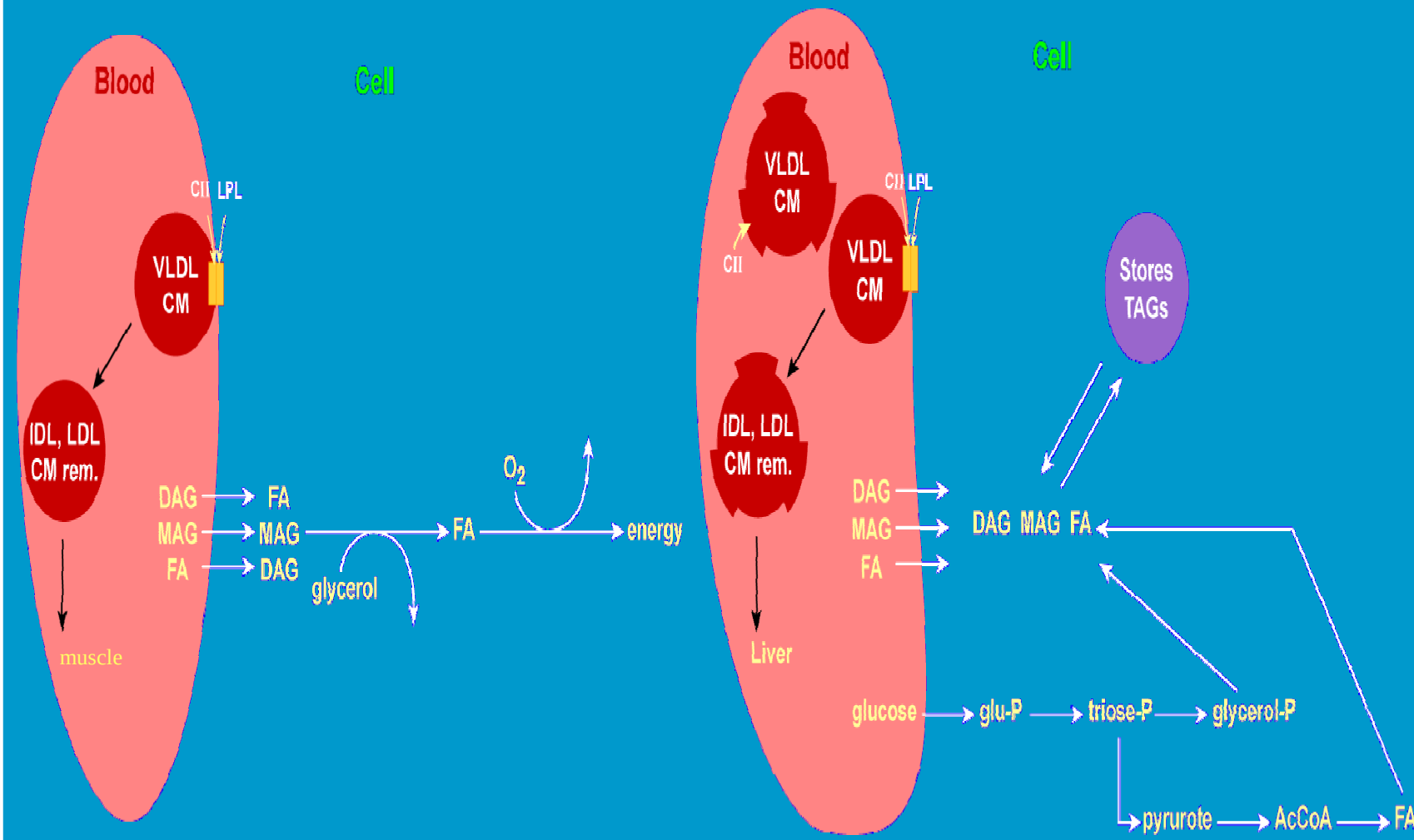
Their fate in liver is to be repackaged into new lipoprotein particles (VLDL) and sent back into circulation as the TAG/FA/Cholesterol source between meals. The glycerol backbone of TAG comes from glycerol (via glycolysis); the fatty acids come from dietary fats, and from excess amino acids and glucose (via Acetyl-CoA, the fatty acid precursor).

Liver is also the major regulator of the body's cholesterol. Liver takes up cholesterol from IDL or CM (not from LDL/HDL), and either repackages into VLDL lipoproteins or converts it into bile acids and excretes it out the gall bladder. Bile acids are the only way to remove cholesterol from the body (recall it's not oxidized for energy!)



Muscle Pathway

Adipose Pathway



Ketone Bodies

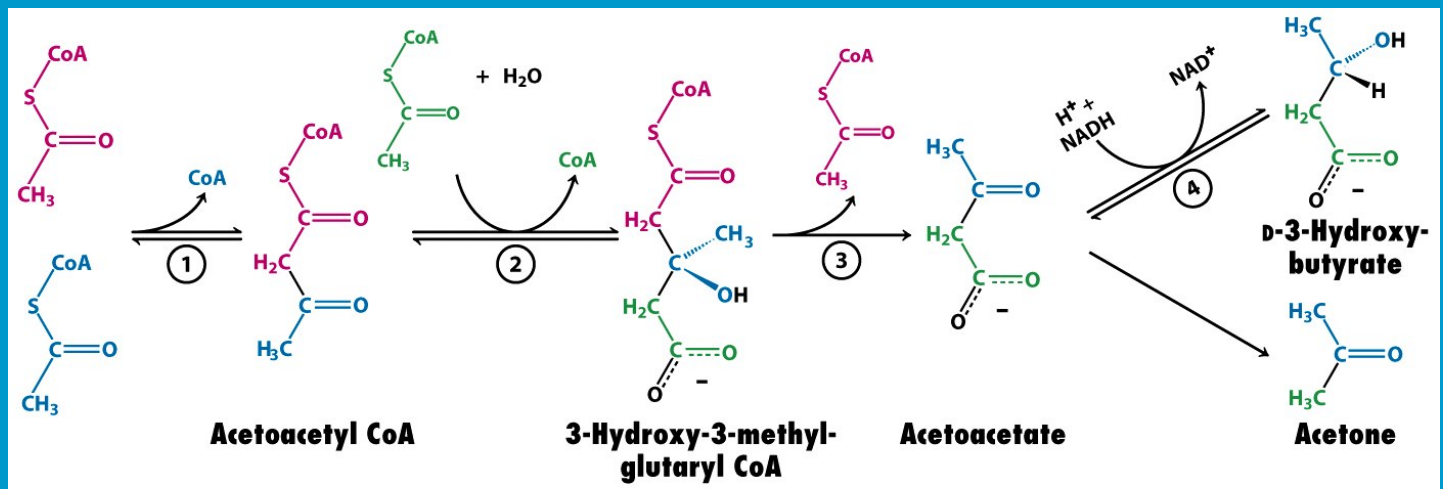
A special source of fuel and energy for certain tissues

- Some of the acetyl-CoA produced by fatty acid oxidation in liver mitochondria is converted to acetone, acetoacetate and β-hydroxybutyrate
- These are called "ketone bodies"
- Source of fuel for brain, heart and muscle
- Major energy source for brain during starvation
- They are transportable forms of fatty acids!

Ketone Bodies - II

Interesting Aspects of Their Synthesis

- Occurs only in the mitochondrial matrix
- First step - is reverse thiolase
- Second reaction makes **HMG-CoA**
- These reactions are mitochondrial analogues of the (cytosolic) first two steps of cholesterol synthesis
- Third step - HMG-CoA lyase - is similar to the reverse of citrate synthase



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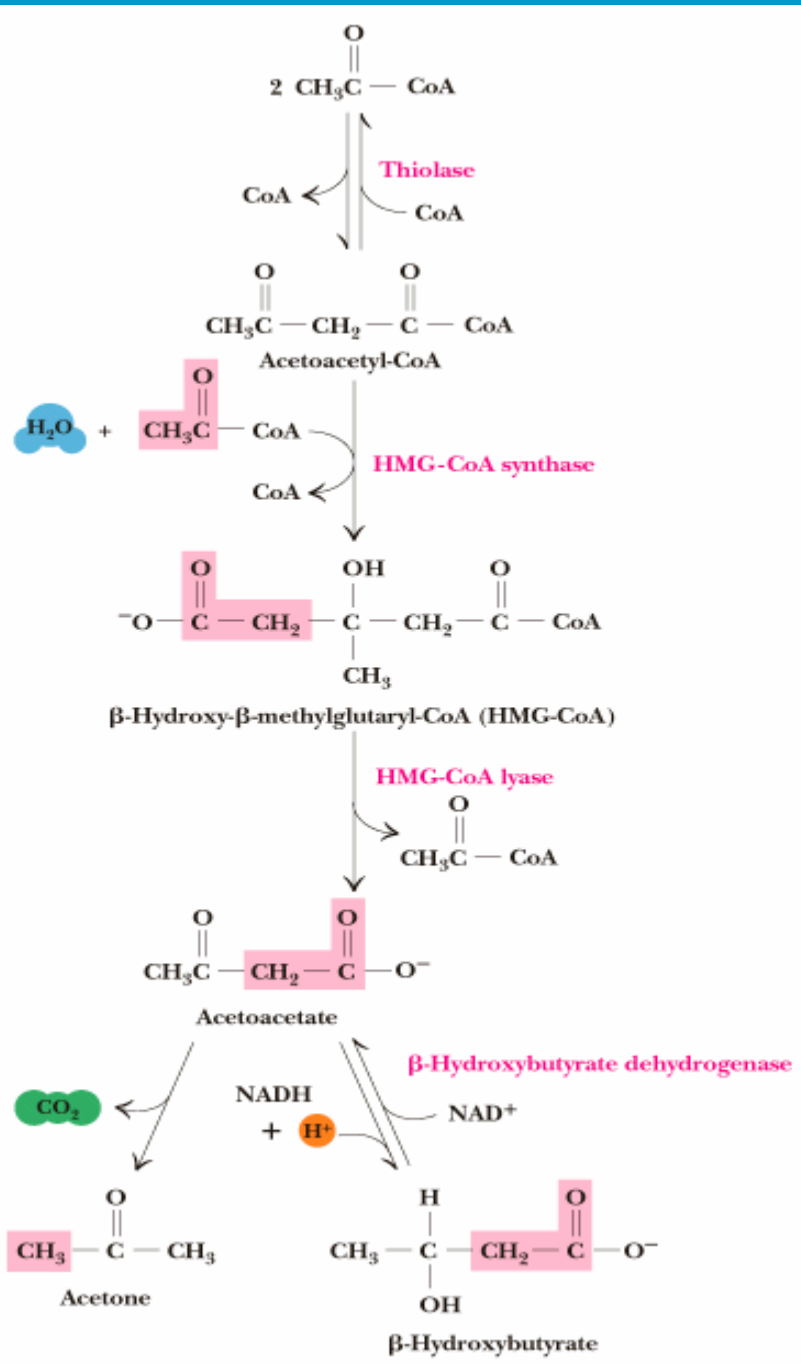
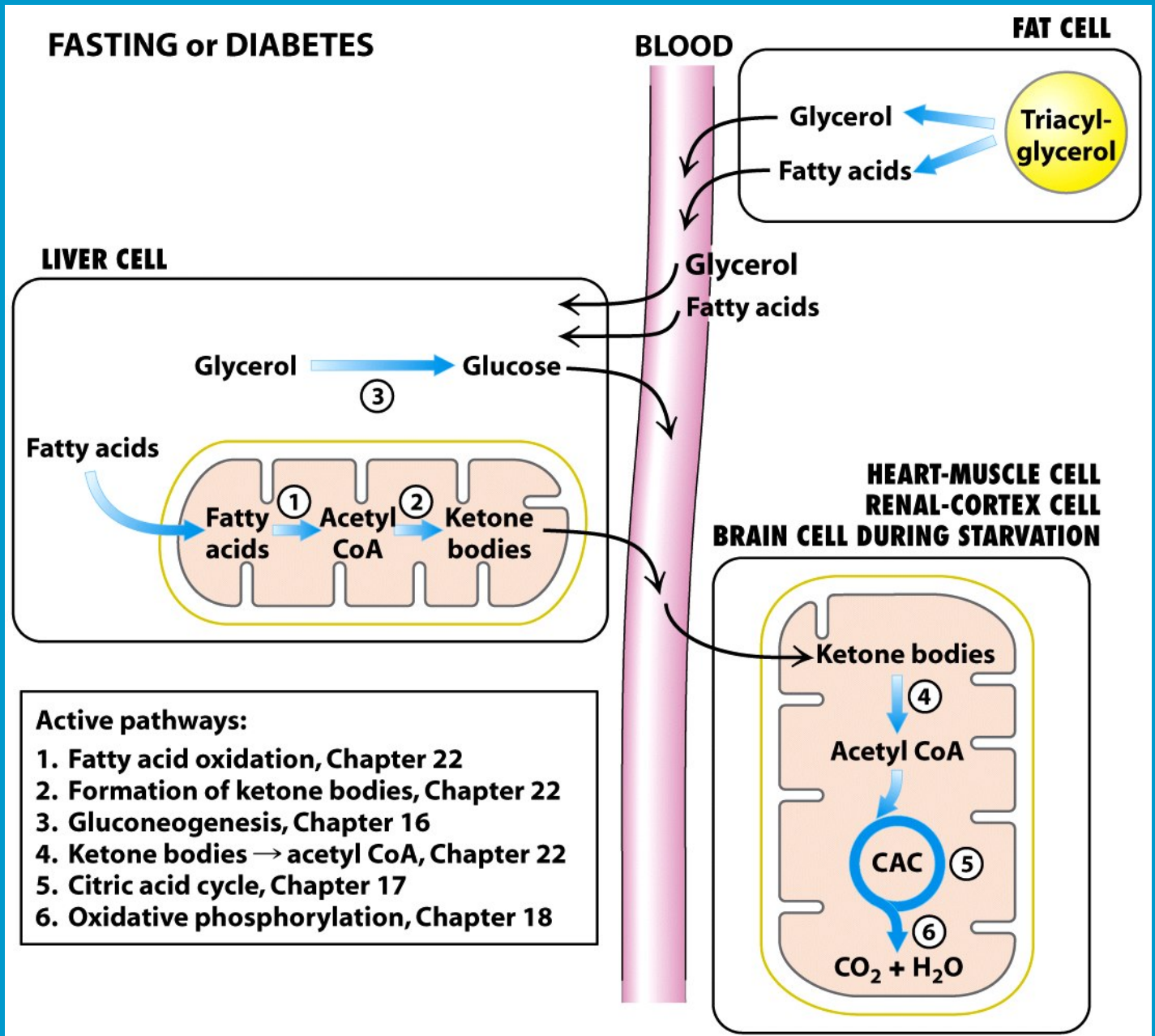

2 CH3C — CoA
Thiolase
CoA
CoA
CH3C — CH2 — C — CoA
Acetoacetyl-CoA
H2O
+
CH3C — CoA
HMG-CoA synthase
CoA
⁻O — C — CH2 — C — CH2 — C — CoA
OH
CH3
β-Hydroxy-β-methylglutaryl-CoA (HMG-CoA)
HMG-CoA lyase
CH3C — CoA
CH3C — CH2 — C — O⁻
Acetoacetate
β-Hydroxybutyrate dehydrogenase
CO2
NADH
+
H+
NAD+
CH3 — C — CH3
Acetone
CH3 — C — CH2 — C — O⁻
H
OH
β-Hydroxybutyrate

Ketone Bodies and Diabetes

"Starvation of cells in the midst of plenty"

- Glucose is abundant in blood, but uptake by cells in muscle, liver, and adipose cells is low
- Cells, metabolically starved, turn to gluconeogenesis and fat/protein catabolism
- In type I diabetics, OAA is low, due to excess gluconeogenesis, so Ac-CoA from fat/protein catabolism does not go to TCA, but rather to ketone body production
- Acetone can be detected on breath of type I diabetics



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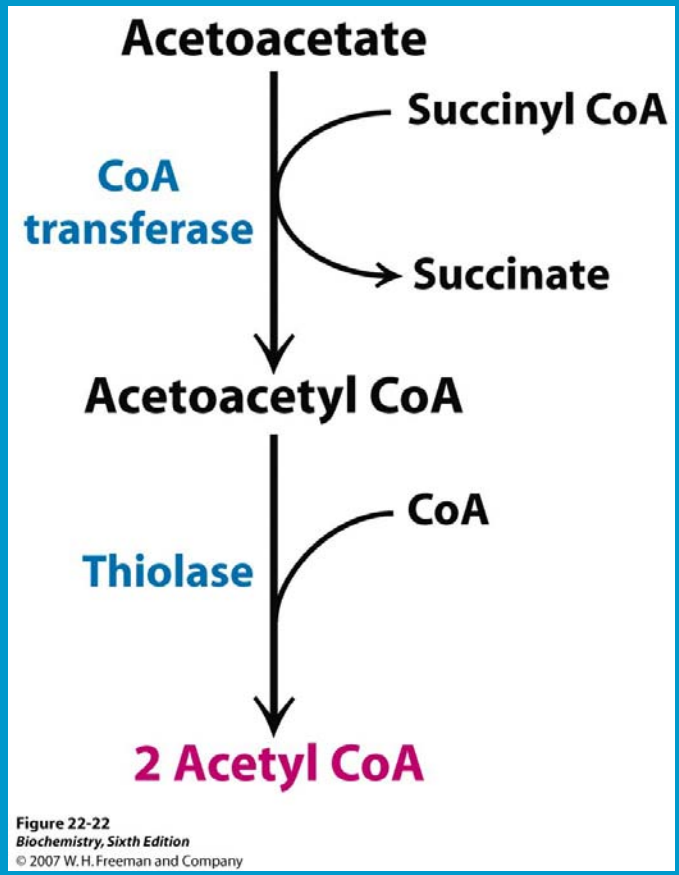
Utilization of acetoacetate as fuel



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Diabetic ketosis results in the absence of insulin

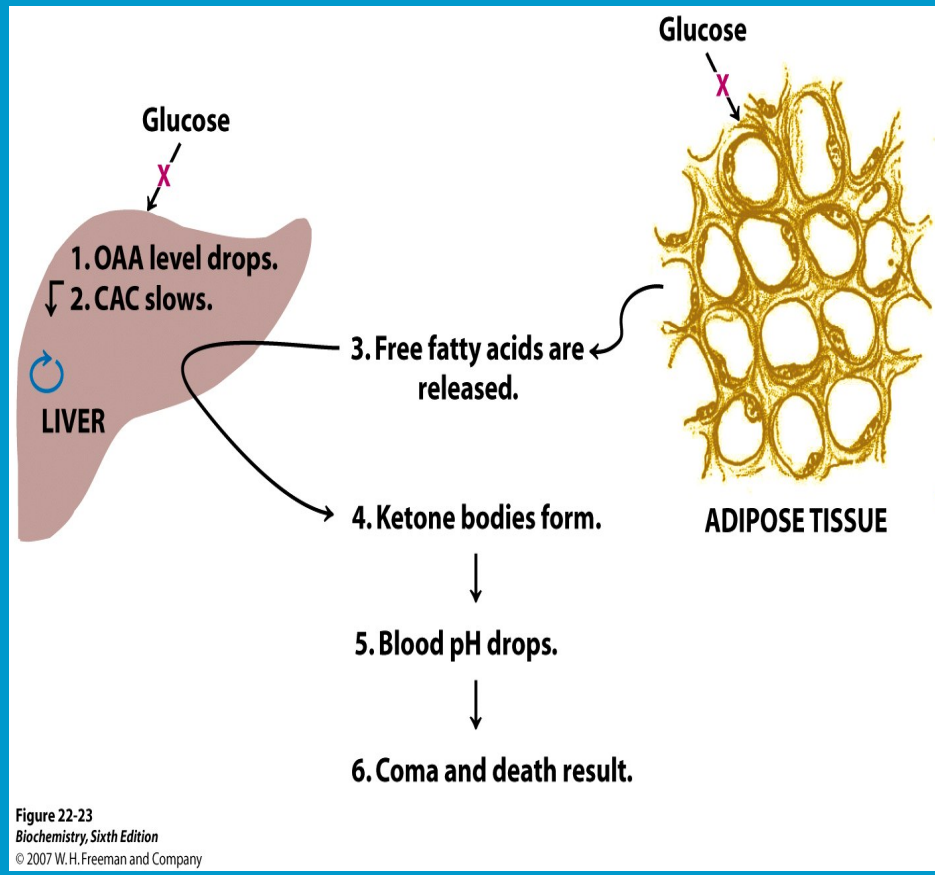

Figure 22-23
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