β-OXIDATION FUNCTION

To break down fatty acids to acetyl-CoA to provide fuel for the TCA cycle

(See Fig. 13-4.)

β-OXIDATION LOCATION

Mitochondria of all tissues

CARNITINE SHUTTLE

Transfers fatty acids form cytoplasm to mitochondria for β oxidation
Inhibited by malonyl-CoA

Long-chain fatty acids can slowly cross the mitochondrial membrane by themselves, but this is too slow to keep up with their metabolism. The carnitine shuttle provides a transport mechanism and allows control of β oxidation. Malonyl-CoA, a precursor for fatty acid synthesis, inhibits the carnitine shuttle and slows down β oxidation (Fig. 13-5).

β-OXIDATION CONNECTIONS

Fatty acyl-CoA *in*, acetyl-CoA and NADH, $FADH_2$ *out*
From triglycerides through hormone-sensitive lipase

β-OXIDATION REGULATION

Primary signals (effects on hormone-sensitive lipase):
Insulin turns *off*.
Glucagon turns *on*.
Epinephrine turns *on*.
Phosphorylation turns *on*.
Secondary signals: Malonyl-CoA inhibits carnitine acyltransferase.

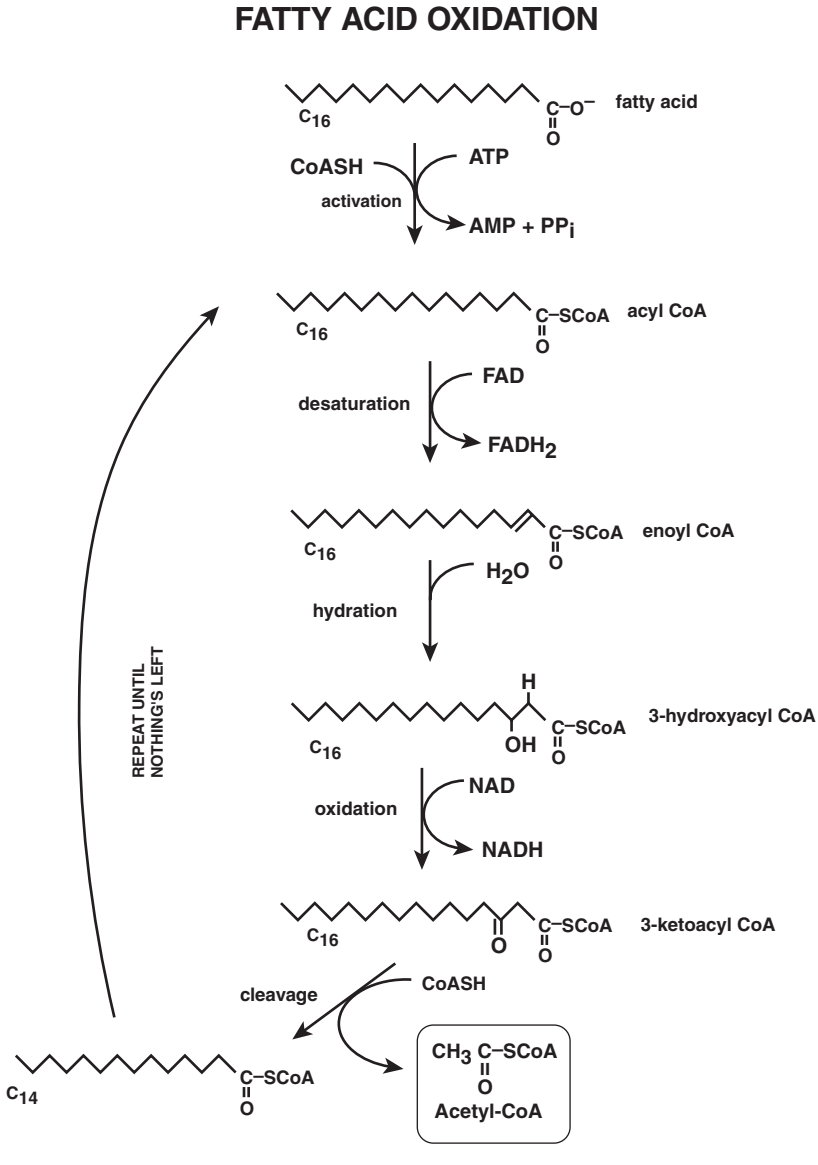

Figure 13-4 Fatty Acid Oxidation (β oxidation)

The major hormone-sensitive control point for the mobilization of fat and the β-oxidation pathway is the effect of phosphorylation on the activity of the hormone-sensitive lipase of the adipose tissue. The major direct control point for β oxidation is the inhibition of carnitine acyl-

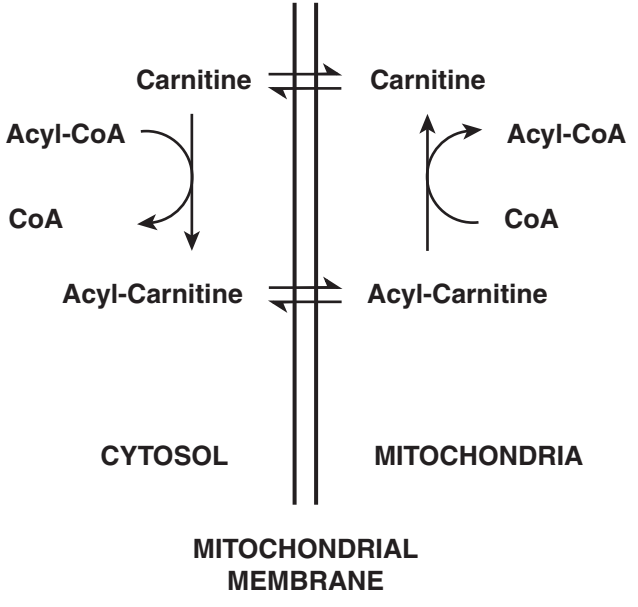

Figure 13-5
The **CARNITINE SHUTTLE** is used to transport fatty acids into the mitochondria.

transferase by malonyl-CoA. Since the malonyl-CoA required for fatty acid synthesis inhibits β oxidation, this regulation keeps the opposed pathways of fatty acid synthesis and β oxidation in check. You don't do synthesis and degradation at the same time.

β-OXIDATION ATP YIELD

C_{16} fatty acid $\longrightarrow$ $16CO_2$	129 ATP
C_{16} fatty acyl-CoA $\longrightarrow$ $16CO_2$ + CoA	131 ATP
Breakdown into steps:	
Activation of fatty acid to fatty acyl-CoA	−2 ATP
7 $FADH_2$ made from forming double bond at C-2 (7 × 2)	14 ATP
7 NADH made from oxidations during formation of 3-ketoacyl-CoA (7 × 3)	21 ATP
8 acetyl-CoA (8 × 12) through TCA cycle	96 ATP

When calculating ATP yields from β oxidation, you have to be careful to notice whether you start with the fatty acid or with the fatty acyl-

CoA. Two high-energy phosphate equivalents are required to activate fatty acids to the acyl-CoA.

$$\text{Fatty acid—}CO_2^- + \text{ATP} + \text{CoASH} \longrightarrow \text{fatty acyl—C(=O)—SCoA} + \text{AMP} + PP_i$$

All the ATP comes from oxidative phosphorylation coupled to the metabolism of acetyl-CoA by the TCA cycle. No oxygen, no β oxidation.

Each cycle of β oxidation reduces the length of the fatty acid chain by 2 carbons, produces 1 acetyl-CoA (12 ATP), 1 $FADH_2$ (2 ATP), and 1 NADH (3 ATP). Each 2-carbon unit of the fatty acid then results in the production of 17 ATPs. To figure out how much ATP a C_{20} fatty acid could make, you might remember that C_{16} fatty acid makes 129 and then add 17×2 for the additional 4 carbons ($129 + 34 = 163$). That seems like the hard way. It may be easier just to remember that each acetyl-CoA can give 12 ATPs and that each cycle generates 1 $FADH_2$ and 1 NADH. A C_{20} fatty acid would make 10 acetyl-CoA, 9 $FADH_2$, and 9 NADH and require 2 ATP equivalents for activation [$(12 \times 10) + (9 \times 2) + (9 \times 3) - 2 = 163$ ATP]. Notice that breaking a C_{20} fatty acid into 10 acetyl-CoA units requires only 9 β-oxidation cycles—the last cycle gives 2 acetyl-CoA as the product.

β-OXIDATION EQUATION

$$C_{16} \text{ fatty acid} + \text{CoA} + \text{ATP} \longrightarrow C_{16}\text{-acyl-CoA} + \text{AMP} + PP_i$$

$$C_{16}\text{-acyl-CoA} + 7NAD^+ + 7FAD \longrightarrow 8 \text{ acetyl-CoA} + 7\text{NADH} + 7FADH_2 + 7H^+$$

Each reaction of β oxidation is catalyzed by a different enzyme. Chemically, they're pretty much the same as the reverse of the individual reaction of fatty acid synthesis, with two exceptions: (1) β oxidation uses FAD for the formation of the double bond at the C-2 position, and (2) the reactions occur with the fatty acid attached to CoA rather than to the pantetheine of a multienzyme complex.

• INDIVIDUAL REACTIONS OF β OXIDATION:

Activation:

$$R—CH_2—CH_2—CO_2^- + \text{CoA} + \text{ATP} \longrightarrow R—CH_2—CH_2—C(=O)—\text{SCoA} + \text{AMP} + PP_i$$

Oxidation:

$$R—CH_2—CH_2—C(=O)—SCoA + FAD \longrightarrow R—CH=CH—C(=O)—SCoA + FADH_2$$

Hydration:

$$R—CH=CH—C(=O)—SCoA + H_2O \longrightarrow R—CH(OH)—CH_2—C(=O)—SCoA$$

Oxidation:

$$R—CH(OH)—CH_2—C(=O)—SCoA + NAD^+ \longrightarrow R—C(=O)—CH_2—C(=O)—SCoA + NADH$$

Cleavage:

$$R—C(=O)—CH_2—C(=O)—SCoA + CoA \longrightarrow R—C(=O)—SCoA + CH_3C(=O)—SCoA$$

What β oxidation actually accomplishes is the removal of a C-2 unit as acetyl-CoA from the carboxyl end of the fatty acid. This keeps happening until the fatty acid is completely converted to acetyl-CoA.

β OXIDATION OF UNSATURATED FATTY ACIDS

Double bond initially on *odd* carbon (Δ system):

- Isomerize *cis*-3 C═C to *trans*-2 C═C then proceed with normal β oxidation.
- 2 fewer ATPs per double bond

Double bond initially on *even* carbon (Δ system):

- Hydrate *cis*-2 C═C to D-3-hydroxyacyl-CoA, epimerize D-3-hydroxy to L and continue normal β oxidation.
- 2 fewer ATPs per double bond
- *Or* reduce 2-*trans*-4-*cis* C═C to 3-*trans* with NADPH; then isomerize the 3-*trans* to 2-*trans* and proceed as with normal β oxidation.
- 5 fewer ATPs per double bond

If a fatty acid already has a double bond in it, the scheme by which the fatty acid is oxidized depends on where the double bond ends up after several of the C-2 fragments have been removed by normal β oxidation. With a double bond already present, the enzyme that catalyzes the first step (insertion of the double bond at C-2) gets confused when there is already a double bond at C-2 or at C-3. The fact that the double bonds in unsaturated fatty acids are invariably *cis* also complicates life since the double bond introduced at C-2 by the desaturating enzyme of β oxidation is a *trans* double bond.

As the β-oxidation machinery chews off 2-carbon fragments, it nibbles down to one of two possible situations depending on whether the first double bond started out at an *even*- or an *odd*-numbered carbon when counting from the carboxylate end. If the double bond is on an odd-numbered carbon (as in *cis* $\Delta^9C_{18:1}$), it is metabolized slightly differently than a fatty acid in which the unsaturation is on an even-numbered carbon (as in *cis* $\Delta^{12}C_{18:1}$).

If the double bond is on an odd carbon, β oxidation removes 2-carbon fragments until it gets to the structure with a 3-*cis* double bond [R–CH═CH–CH_2–C(═O)–SCoA]. A new double bond can't be placed between C-2 and C-3 because there's already a double bond at C-3. In this situation, the activity of an isomerase simply moves the double bond from C-3 to C-2 and at the same time makes sure that the configuration is *trans*. From this point on, the metabolism is just like normal β oxidation (hydration, oxidation, cleavage). If you're counting ATPs, these unsaturated fatty acids produce 2 fewer ATPs for each double bond since there is no $FADH_2$ produced by putting in the double bond (see Fig. 13-6).

If the double bond starts out at an even carbon, β oxidation runs its normal course until the structure *cis*-2-R–CH═CH–C(═O)–SCoA is reached. The rub here is that the double bond is on an OK carbon (C-2), but it's in the wrong configuration. The double bonds in unsaturated fatty acids are invariably of the *cis* configuration, but β oxidation introduces the double bond at C-2 in the *trans* configuration. The pathway cited in most texts involves the addition of water to the 2-*cis* double bond to give the 3-hydroxy species just as in normal β oxidation, except for a mean twist. If the double bond is introduced in the *trans* configuration by β oxidation itself, hydration gives the L-3-hydroxy fatty acyl-CoA. But if the double bond is in the *cis* configuration, hydration gives the D-3-hydroxy fatty acyl-CoA. The configuration around C-3 (D vs. L) might appear trivial to you, but to the enzyme that oxidizes the C-3 (C–OH) to the carbonyl (C═O), it's night and day. The dehydrogenase won't touch the D configuration because the OH group is in the wrong place relative to the R and CoA groups. To get around this problem, there's an enzyme (an epimerase) that converts the D to the L epimer. The L epimer is then recognized by the dehydrogenase, and it's smooth sailing from there on. Since the isomerase and epimerase don't require ATP hydrolysis, ATP counting through this pathway would show that a double bond at an *even* position reduces the yield of ATP by 2 (no $FADH_2$ is formed in the first desaturation reaction).

A somewhat newer pathway, which may be the real pathway in many cells, has been discovered recently. This may or may not be described in your text. In this pathway, the fatty acyl-CoA is metabolized

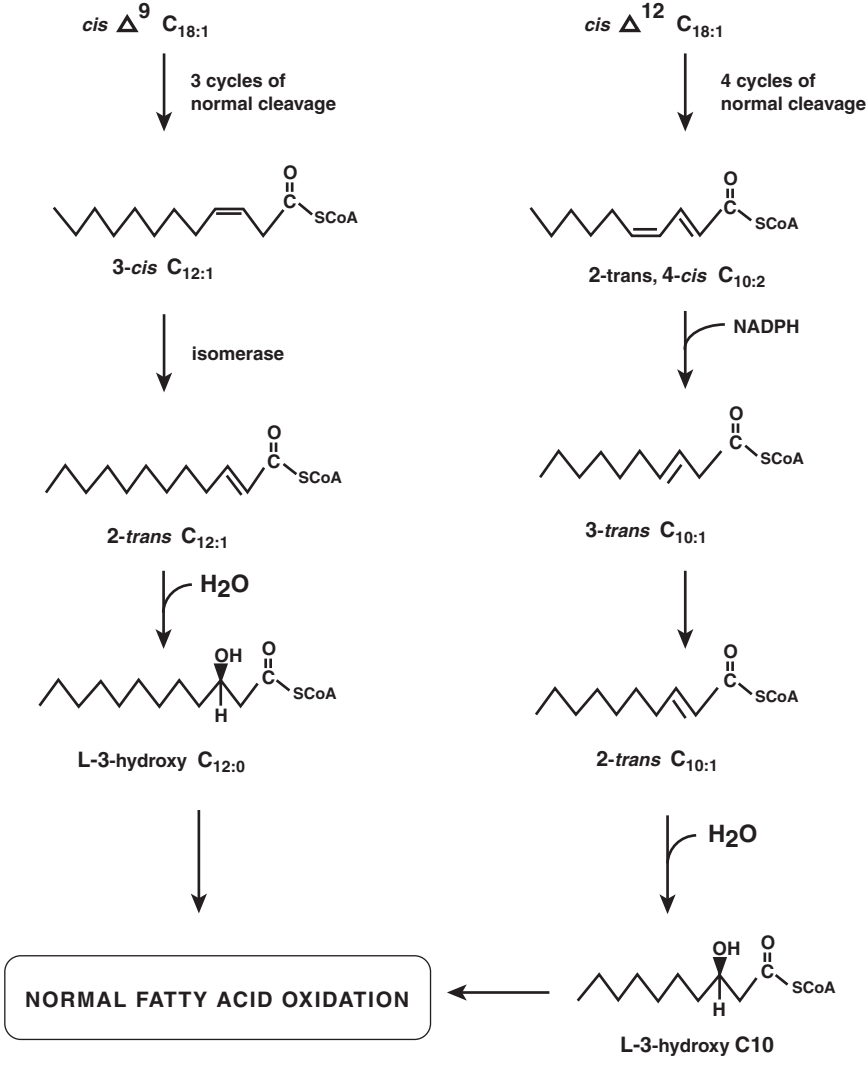

Figure 13-6 Metabolism of Unsaturated Fatty Acids
This may not be the pathway in your text. If you've not seen this mentioned, ignore it. The other (and possibly incorrect) pathway is simpler anyway.

normally, introducing a *cis* double bond at C-2 and removing 2-carbon fragments until the fatty acid contains two double bonds, 2-*trans*-4-*cis*-R–CH═CH–CH═CH–C(═O)–S–CoA. The 2-*trans* double bond is put in by the normal β oxidation, and the 4-*cis* is left from the original *cis* double bond in the unsaturated fatty acid. At this point, the 2-*trans*-4-*cis*

fatty acyl-CoA is reduced by an NADPH-dependent reductase to the *trans*-3-R–CH_2–CH═CH–CH_2–C(═O)–S–CoA. The 3-*trans* double bond is then isomerized to the 2-*trans* double bond, and β oxidation proceeds as normal. If you count ATPs by this pathway, each double bond at an even carbon would decrease the yield of ATP by 5 (assuming that the NADPH used is equivalent to NADH and would produce 3 ATPs if oxidized by the electron transport chain and that no $FADH_2$ is made in putting in the double bond).

β OXIDATION OF ODD-CHAIN-LENGTH FATTY ACIDS

Makes propionyl-CoA, which is metabolized by propionyl-CoA carboxylase (biotin) and methylmalonyl-CoA mutase (B_{12}) to give succinyl-CoA.

Since there's no good way to make a C-1 fragment during β oxidation, the metabolism of fatty acids with an odd number of carbons must finally give you a 3-carbon piece as propionyl-CoA. Odd-chain-length fatty acids aren't very abundant in nature (some shellfish and bacteria make odd-chain-length fatty acids), but they may be somewhat more common on exams. The propionyl-CoA resulting from the β oxidation of odd-chain fatty acids is metabolized by a weird pathway that is also used to metabolize the propionyl-CoA produced by the breakdown of the amino acid threonine. Two vitamins are required in this pathway, biotin and B_{12}. This is one of two places you'll see vitamin B_{12} (the other is in the metabolism of 1-carbon fragments; see Fig. 13-7).

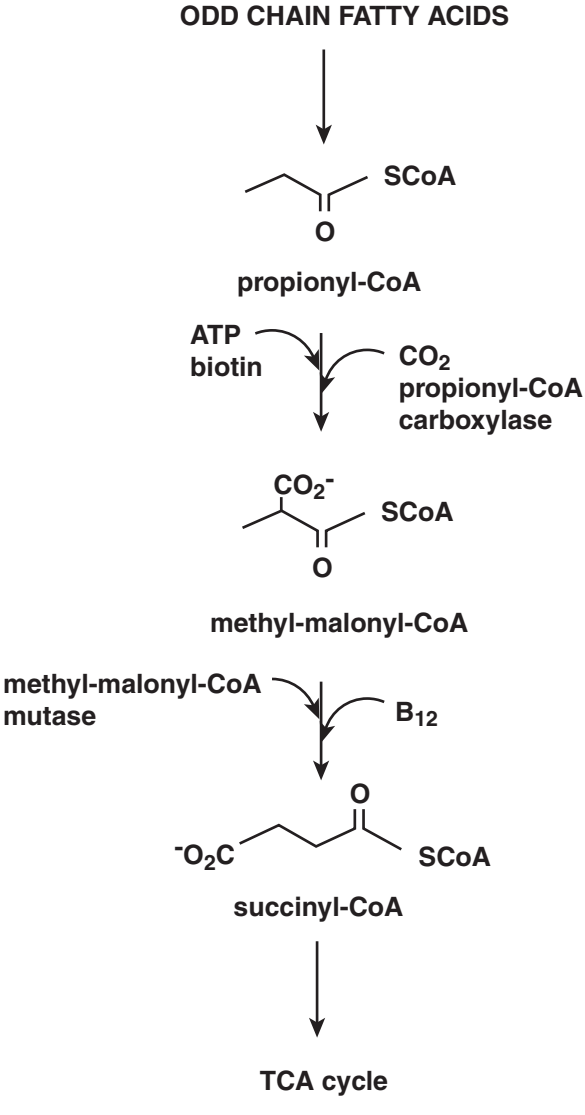

Figure 13-7
Metabolism of **ODD-CHAIN-LENGTH FATTY ACIDS** yields propionyl-CoA, which can be rearranged to succinyl-CoA and dumped into the TCA cycle.