

## chapter

## 17

## Fatty Acid Catabolism

- 1. Energy in Triacylglycerols** On a per-carbon basis, where does the largest amount of biologically available energy in triacylglycerols reside: in the fatty acid portions or the glycerol portion? Indicate how knowledge of the chemical structure of triacylglycerols provides the answer.

**Answer** The fatty acids of triacylglycerols are hydrocarbons, with a single carboxyl group. Glycerol, on the other hand, has an —OH group on each carbon and is thus much more highly oxidized than a fatty acid. On oxidation, fatty acids therefore produce far more energy per carbon than does glycerol. Triacylglycerols have an energy of oxidation more than twice that of the same weight of carbohydrates or proteins.

- 2. Fuel Reserves in Adipose Tissue** Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients.
- (a) If 15% of the body mass of a 70.0 kg adult consists of triacylglycerols, what is the total available fuel reserve, in kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal = 4.18 kJ.
- (b) If the basal energy requirement is approximately 8,400 kJ/day (2,000 kcal/day), how long could this person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy?
- (c) What would be the weight loss in pounds per day under such starvation conditions (1 lb = 0.454 kg)?

**Answer**

- (a) Given (in the text) that the energy value of stored triacylglycerol is 38 kJ/g, the available fuel reserve is

$$\begin{aligned}(0.15)(70.0 \times 10^3 \text{ g})(38 \text{ kJ/g}) &= 4.0 \times 10^5 \text{ kJ} \\ &= 9.6 \times 10^4 \text{ kcal}\end{aligned}$$

- (b) At a rate of  $8.4 \times 10^3$  kJ/day, the fuel supply would last

$$(4.0 \times 10^5 \text{ kJ}) / (8.4 \times 10^3 \text{ kJ/day}) = 48 \text{ days}$$

- (c) If all the triacylglycerol is used over a 48-day period, this represents a rate of weight loss of

$$\frac{(0.15)(70.0 \text{ kg})}{48 \text{ days}} = 0.22 \text{ kg/day}$$

$$\text{or } (0.22 \text{ kg/day}) / (0.454 \text{ kg/lb}) = 0.48 \text{ lb/day}$$

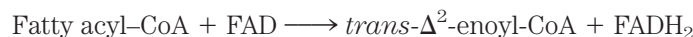
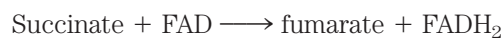
- 3. Common Reaction Steps in the Fatty Acid Oxidation Cycle and Citric Acid Cycle** Cells often use the same enzyme reaction pattern for analogous metabolic conversions. For example, the steps in the oxidation of pyruvate to acetyl-CoA and of  $\alpha$ -ketoglutarate to succinyl-CoA, although catalyzed by

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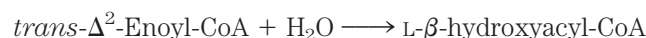
different enzymes, are very similar. The first stage of fatty acid oxidation follows a reaction sequence closely resembling a sequence in the citric acid cycle. Use equations to show the analogous reaction sequences in the two pathways.

**Answer** The first three reactions in the  $\beta$  oxidation of fatty acyl-CoA molecules are analogous to three reactions of the citric acid cycle.

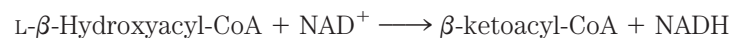
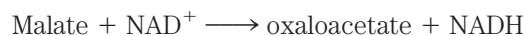
The fatty acyl-CoA dehydrogenase reaction is analogous to the succinate dehydrogenase reaction; both are FAD-requiring oxidations:



The enoyl-CoA hydratase reaction is analogous to the fumarase reaction; both add water to an olefinic bond:



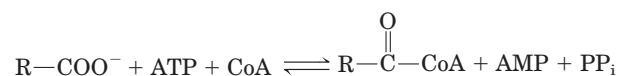
The  $\beta$ -hydroxyacyl-CoA dehydrogenase reaction is analogous to the malate dehydrogenase reaction; both are NAD-requiring and act on  $\beta$ -hydroxyacyl compounds:



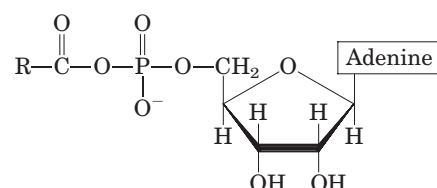
- 4.  $\beta$  Oxidation: How Many Cycles?** How many cycles of  $\beta$  oxidation are required for the complete oxidation of activated oleic acid, 18:1( $\Delta^9$ )?

**Answer** 7 cycles; the last releases 2 acetyl-CoA.

- 5. Chemistry of the Acyl-CoA Synthetase Reaction** Fatty acids are converted to their coenzyme A esters in a reversible reaction catalyzed by acyl-CoA synthetase:



- (a) The enzyme-bound intermediate in this reaction has been identified as the mixed anhydride of the fatty acid and adenosine monophosphate (AMP), acyl-AMP:



Write two equations corresponding to the two steps of the reaction catalyzed by acyl-CoA synthetase.

- (b) The acyl-CoA synthetase reaction is readily reversible, with an equilibrium constant near 1. How can this reaction be made to favor formation of fatty acyl-CoA?

**Answer** Activation of carboxyl groups by ATP could in theory be accomplished by three types of reactions: the formation of acyl-phosphate + ADP; of acyl-ADP +  $\text{P}_i$ ; or of acyl-AMP +  $\text{PP}_i$ . All these reactions are readily reversible. To create an activation reaction with a highly negative  $\Delta G'^\circ$  (effectively irreversible), the third type of reaction can be coupled to a pyrophosphatase reaction, as in the synthesis of fatty acyl-CoA molecules.

- (a)  $R\text{-COO}^- + \text{ATP} \longrightarrow \text{acyl-AMP} + \text{PP}_i$   
 $\text{Acyl-AMP} + \text{CoA} \longrightarrow \text{acyl-CoA} + \text{AMP}$
- (b) Hydrolysis of  $\text{PP}_i$  by an inorganic pyrophosphatase pulls the reaction in the direction of fatty acyl-CoA formation.

**6. Intermediates in Oleic Acid Oxidation** What is the structure of the partially oxidized fatty acyl group that is formed when oleic acid, 18:1( $\Delta^9$ ), has undergone three cycles of  $\beta$  oxidation? What are the next two steps in the continued oxidation of this intermediate?

**Answer** After three rounds of  $\beta$  oxidation, the fatty acyl-CoA has been shortened by six carbons with the removal of three acetyl-CoAs. The resulting 12-carbon intermediate is *cis*- $\Delta^3$ -dodecanoyl-CoA, with the double bond between the third and fourth carbons from the carboxyl end of the chain. Before another round of  $\beta$  oxidation can occur, that double bond must be moved from  $\Delta^3$  to  $\Delta^2$ , which is catalyzed by  $\Delta^3, \Delta^2$ -enoyl isomerase (see Fig. 17-9). Water is then added to the double bond to form the  $\beta$ -hydroxydodecanoyl-CoA derivative, which can undergo further  $\beta$  oxidation.

**7.  $\beta$  Oxidation of an Odd-Chain Fatty Acid** What are the direct products of  $\beta$  oxidation of a fully saturated, straight-chain fatty acid of 11 carbons?

**Answer** 4 acetyl-CoA and 1 propionyl-CoA

**8. Oxidation of Tritiated Palmitate** Palmitate uniformly labeled with tritium ( $^3\text{H}$ ) to a specific activity of  $2.48 \times 10^8$  counts per minute (cpm) per micromole of palmitate is added to a mitochondrial preparation that oxidizes it to acetyl-CoA. The acetyl-CoA is isolated and hydrolyzed to acetate. The specific activity of the isolated acetate is  $1.00 \times 10^7$  cpm/ $\mu\text{mol}$ . Is this result consistent with the  $\beta$ -oxidation pathway? Explain. What is the final fate of the removed tritium?

**Answer** The  $\beta$ -oxidation pathway includes two dehydrogenase enzymes that remove hydrogen (H-H) from the fatty acyl-CoA chain, first at a  $\text{—CH}_2\text{—CH}_2\text{—}$  and then at a  $\text{—CH}_2\text{—CH(OH)—}$ . The net result of the two reactions is removal of one of the two hydrogens at the point of formation of the enoyl-CoA intermediate. The two other hydrogens in the methyl group of acetyl-CoA come from water.

Palmitate contains 16 carbons, with  $(14 \times 2) + 3 = 31$  hydrogens, so each two-carbon unit contains about  $4/31$  or about  $1/8$  of the total  $^3\text{H}$  present. Thus, the counts per minute expected per acetyl-CoA, with two of the four acetyl hydrogens labeled (the other two arising from unlabeled water), is  $(2/4)(2.48 \times 10^8 \text{ cpm}/\mu\text{mol})(1/8) = 1.6 \times 10^7 \text{ cpm}/\mu\text{mol}$ , somewhat higher than observed. Exchange between  $\beta$ -ketoacyl-CoA and solvent water could cause loss of  $^3\text{H}$ .

The final fate of the tritium removed from palmitate is its appearance in water, as reduced carriers ( $\text{FADH}_2$ ,  $\text{NADH}$ ) are reoxidized by the mitochondria.

**9. Compartmentation in  $\beta$  Oxidation** Free palmitate is activated to its coenzyme A derivative (palmitoyl-CoA) in the cytosol before it can be oxidized in the mitochondrion. If palmitate and [ $^{14}\text{C}$ ]coenzyme A are added to a liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain.

**Answer** The transport of fatty acid molecules into mitochondria requires a shuttle system involving a fatty acyl-carnitine intermediate. Fatty acids are first converted to fatty acyl-CoA molecules in the cytosol (by the action of acyl-CoA synthetases) then, at the outer mitochondrial membrane, the fatty acyl group is transferred to carnitine (by the action of carnitine acyl-transferase I). After transport of fatty acyl-carnitine through the inner membrane, the fatty acyl group is transferred to mitochondrial CoA. The cytosolic and mitochondrial pools of CoA are thus kept separate, and no labeled CoA from the cytosolic pool enters the mitochondrion.

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- 10. Comparative Biochemistry: Energy-Generating Pathways in Birds** One indication of the relative importance of various ATP-producing pathways is the  $V_{\max}$  of certain enzymes of these pathways. The values of  $V_{\max}$  of several enzymes from the pectoral muscles (chest muscles used for flying) of pigeon and pheasant are listed below.

Enzyme	$V_{\max}$ ( $\mu\text{mol substrate}/\text{min}/\text{g tissue}$ )	
	Pigeon	Pheasant
Hexokinase	3.0	2.3
Glycogen phosphorylase	18.0	120.0
Phosphofructokinase-1	24.0	143.0
Citrate synthase	100.0	15.0
Triacylglycerol lipase	0.07	0.01

- (a) Discuss the relative importance of glycogen metabolism and fat metabolism in generating ATP in the pectoral muscles of these birds.
- (b) Compare oxygen consumption in the two birds.
- (c) Judging from the data in the table, which bird is the long-distance flyer? Justify your answer.
- (d) Why were these particular enzymes selected for comparison? Would the activities of triose phosphate isomerase and malate dehydrogenase be equally good bases for comparison? Explain.

**Answer**

- (a) In the pigeon, aerobic oxidation of fatty acids— $\beta$  oxidation and oxidative phosphorylation—predominates; in the pheasant, anaerobic glycolysis of glycogen predominates. Note the high citrate synthase activity in the pigeon, and the high glycogen phosphorylase and PFK-1 activities in the pheasant.
- (b) Using aerobic oxidation, pigeon muscle consumes more oxygen during flight.
- (c) The energy available per gram is higher for fat than for glycogen. In addition, anaerobic breakdown of glycogen is limited by tolerance to lactate buildup. Thus the pigeon, using predominantly the oxidative catabolism of fats, is the long-distance flyer.
- (d) The enzymes listed in the table (unlike triose phosphate isomerase and malate dehydrogenase) are the regulatory enzymes of their respective pathways and thus limit ATP production rates.

- 11. Mutant Carnitine Acyltransferase** What changes in metabolic pattern would result from a mutation in the muscle carnitine acyltransferase I in which the mutant protein has lost its affinity for malonyl-CoA but not its catalytic activity?

**Answer** Malonyl-CoA would no longer inhibit fatty acid entry into the mitochondrion and  $\beta$  oxidation, so there might be a futile cycle of simultaneous fatty acid synthesis in the cytosol and fatty acid breakdown in mitochondria. (See Fig. 17–12.)

- 12. Effect of Carnitine Deficiency** An individual developed a condition characterized by progressive muscular weakness and aching muscle cramps. The symptoms were aggravated by fasting, exercise, and a high-fat diet. The homogenate of a skeletal muscle specimen from the patient oxidized added oleate more slowly than did control homogenates, consisting of muscle specimens from healthy individuals. When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates. The patient was diagnosed as having a carnitine deficiency.
- (a) Why did added carnitine increase the rate of oleate oxidation in the patient's muscle homogenate?

- (b) Why were the patient's symptoms aggravated by fasting, exercise, and a high-fat diet?  
 (c) Suggest two possible reasons for the deficiency of muscle carnitine in this individual.

**Answer**

- (a) The carnitine-mediated transport of fatty acids into mitochondria is the rate-limiting step in  $\beta$  oxidation (see Fig. 17-6). Carnitine deficiency decreases the rate of transport of fatty acids into mitochondria and thus the rate of  $\beta$  oxidation, so addition of carnitine would increase the rate of oxidation.  
 (b) Fasting, exercise, and a high-fat diet all cause an increased need for  $\beta$  oxidation of fatty acids and thus an increased demand for carnitine shuttle activity. The symptoms of carnitine deficiency would therefore become more severe under these conditions.  
 (c) The deficiency of carnitine may result from a dietary deficiency of its precursor, lysine, or from a defect in one of the enzymes that synthesize carnitine from this precursor.

- 13. Fatty Acids as a Source of Water** Contrary to legend, camels do not store water in their humps, which actually consist of large fat deposits. How can these fat deposits serve as a source of water? Calculate the amount of water (in liters) that a camel can produce from 1.0 kg of fat. Assume for simplicity that the fat consists entirely of tripalmitoylglycerol.

**Answer** Oxidation of fatty acids produces water in significant amounts. From Equation 17-6



we know that the oxidation of 1 mol of palmitoyl-CoA produces 23 mol of water.

Tripalmitoin (glycerol plus three palmitates in ester linkage) has a molecular weight of 885, so 1 kg of tripalmitoin contains  $(1.0 \text{ kg})(1,000 \text{ g/kg})/(885 \text{ g/mol}) = 1.1 \text{ mol}$ . Complete oxidation of the three palmitoyl groups will produce

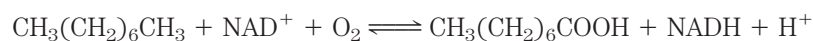
$$(1.1 \text{ mol tripalmitoin})(3 \text{ mol palmitate/mol tripalmitoin})(23 \text{ mol H}_2\text{O/mol palmitate}) = 76 \text{ mol H}_2\text{O}$$

Thus, the volume of water produced (ignoring the contribution of glycerol oxidation) is

$$(76 \text{ mol})(18 \text{ g/mol})(1 \text{ kg}/1,000 \text{ g})(1 \text{ L/kg}) = 1.4 \text{ L}$$

**Note:** in reality, this may be an overestimate. The fatty acyl groups of the triacylglycerol in the camel's fat may be less highly reduced than palmitate.

- 14. Petroleum as a Microbial Food Source** Some microorganisms of the genera *Nocardia* and *Pseudomonas* can grow in an environment where hydrocarbons are the only food source. These bacteria oxidize straight-chain aliphatic hydrocarbons, such as octane, to their corresponding carboxylic acids:



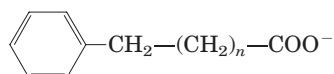
How could these bacteria be used to clean up oil spills? What would be some of the limiting factors to the efficiency of this process?

**Answer** By oxidizing hydrocarbons to their corresponding fatty acids, these microbes can obtain all their energy from  $\beta$  oxidation and oxidative phosphorylation, converting the hydrocarbons to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Theoretically, oil spills could be broken down by treatment with these microbes.

Because of the extreme hydrophobicity of hydrocarbons, close contact between substrate and bacterial enzymes might be difficult to achieve; under field conditions (e.g., an oil spill), detergents are often added to improve this contact. In addition, other nutrients, such as nitrogen or phosphorus, may be limiting for the bacterial populations, and these elements are often added to foster the growth of the hydrocarbon-oxidizers.

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- 15. Metabolism of a Straight-Chain Phenylated Fatty Acid** A crystalline metabolite was isolated from the urine of a rabbit that had been fed a straight-chain fatty acid containing a terminal phenyl group:



A 302 mg sample of the metabolite in aqueous solution was completely neutralized by 22.2 mL of 0.100 M NaOH.

- (a) What is the probable molecular weight and structure of the metabolite?  
 (b) Did the straight-chain fatty acid contain an even or an odd number of methylene ( $\text{—CH}_2\text{—}$ ) groups (i.e., is  $n$  even or odd)? Explain.

**Answer**

- (a) 22.2 mL of 0.1 M NaOH is equivalent to  $(22.2 \times 10^{-3} \text{ L})(0.100 \text{ mol/L}) = 2.22 \times 10^{-3} = 10^{-4} \text{ mol}$  of unknown metabolite (assuming that it contains only one carboxyl group) in the 302 mg sample. Thus, the  $M_r$  of the metabolite is

$$\frac{302 \times 10^{-3} \text{ g}}{2.22 \times 10^{-4} \text{ mol}} = 136$$

This is the  $M_r$  of phenylacetic acid.

- (b) Because  $\beta$  oxidation removes two-carbon units, and the end product is a two-carbon unit, the original fatty acyl chain must have had an even number of methylene groups (with the phenyl group counted as equivalent to a terminal methyl group). An odd-numbered fatty acid would have produced phenylpropionate.

- 16. Fatty Acid Oxidation in Uncontrolled Diabetes** When the acetyl-CoA produced during  $\beta$  oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies—acetone, acetoacetate, and D- $\beta$ -hydroxybutyrate. This occurs in severe, uncontrolled diabetes: because the tissues cannot use glucose, they oxidize large amounts of fatty acids instead. Although acetyl-CoA is not toxic, the mitochondrion must divert the acetyl-CoA to ketone bodies. What problem would arise if acetyl-CoA were not converted to ketone bodies? How does the diversion to ketone bodies solve the problem?

**Answer** Individuals with uncontrolled diabetes oxidize large quantities of fat because they cannot use glucose efficiently. This leads to a decrease in activity of the citric acid cycle (see Problem 17) and an increase in the pool of acetyl-CoA. If acetyl-CoA were not converted to ketone bodies, the CoA pool would become depleted. Because the mitochondrial CoA pool is small, liver mitochondria recycle CoA by condensing two acetyl-CoA molecules to form acetoacetyl-CoA + CoA (see Fig. 17–18). The acetoacetyl-CoA is converted to other ketones, and the CoA is recycled for use in the  $\beta$ -oxidation pathway and energy production.

- 17. Consequences of a High-Fat Diet with No Carbohydrates** Suppose you had to subsist on a diet of whale blubber and seal blubber, with little or no carbohydrate.

- (a) What would be the effect of carbohydrate deprivation on the utilization of fats for energy?  
 (b) If your diet were totally devoid of carbohydrate, would it be better to consume odd- or even-numbered fatty acids? Explain.

**Answer**

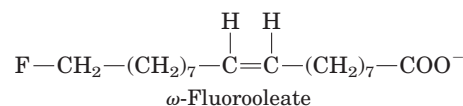
- (a) Pyruvate, formed from glucose via glycolysis, is the main source of the oxaloacetate needed to replenish citric acid cycle intermediates (see Table 16–2). In the absence of carbohydrate in the diet, the oxaloacetate level drops and the citric acid cycle slows. This increases the rate of  $\beta$  oxidation of fatty acids and leads to ketosis.

- (b) The last cycle of  $\beta$  oxidation produces two acetyl-CoA molecules from an even-numbered fatty acid, or propionyl-CoA + acetyl-CoA from an odd-numbered fatty acid. Propionyl-CoA can be converted to succinyl-CoA (see Fig. 17-11), which when converted to oxaloacetate stimulates the citric acid cycle and relieves the conditions leading to ketosis. Thus, it would be better to consume odd-numbered fatty acids.

- 18. Even- and Odd-Chain Fatty Acids in the Diet** In a laboratory experiment, two groups of rats are fed two different fatty acids as their sole source of carbon for a month. The first group gets heptanoic acid (7:0), and the second gets octanoic acid (8:0). After the experiment, a striking difference is seen between the two groups. Those in the first group are healthy and have gained weight, whereas those in the second group are weak and have lost weight as a result of losing muscle mass. What is the biochemical basis for this difference?

**Answer** The  $\beta$  oxidation of heptanoic acid (which has an odd number of carbons) produces the three-carbon intermediate propionyl-CoA, which can be converted by propionyl-CoA carboxylase to methylmalonyl-CoA, then to succinyl-CoA. This four-carbon product of fatty acid oxidation can then be converted to oxaloacetate in the citric acid cycle, and the oxaloacetate can be used for gluconeogenesis—thus providing the animal with carbohydrate as well as energy from fatty acid oxidation. Animals fed octanoic acid (with an even number of carbons) degrade it completely to acetyl-CoA by three rounds of  $\beta$  oxidation. This provides energy via the citric acid cycle but does not provide starting material for gluconeogenesis. These animals are therefore deficient in glucose, the primary fuel for the brain and an intermediate in many biosynthetic pathways.

- 19. Metabolic Consequences of Ingesting  $\omega$ -Fluorooleate** The shrub *Dichapetalum toxicarium*, native to Sierra Leone, produces  $\omega$ -fluorooleate, which is highly toxic to warm-blooded animals.



This substance has been used as an arrow poison, and powdered fruit from the plant is sometimes used as a rat poison (hence the plant's common name, ratsbane). Why is this substance so toxic? (Hint: review Chapter 16, Problem 22.)

**Answer** Oxidation of  $\omega$ -fluorooleate in the  $\beta$ -oxidation pathway forms fluoroacetyl-CoA in the last pass through the sequence. Entry of fluoroacetyl-CoA into the citric acid cycle produces fluorocitrate, a powerful inhibitor of the enzyme aconitase. As a result of this inhibition, the citric acid cycle shuts down and the flow of reducing equivalents to oxidative phosphorylation is fatally impaired.

- 20. Mutant Acetyl-CoA Carboxylase** What would be the consequences for fat metabolism of a mutation in acetyl-CoA carboxylase that replaced the Ser residue normally phosphorylated by AMPK to an Ala residue? What might happen if the same Ser were replaced by Asp? (Hint: See Fig. 17-12.)

**Answer** The Ser-to-Ala change would produce an enzyme that could not be inhibited by phosphorylation by AMPK. The first step in fatty acid synthesis would be constantly turned on, and the malonyl-CoA produced by acetyl-CoA carboxylase would inhibit entry of fatty acids into mitochondria, shutting down  $\beta$  oxidation. The Ser-to-Asp mutation would put a negatively charged Asp residue in the position occupied by  $\text{P-Ser}$  in the inhibited wild-type enzyme. This might mimic the effect of a phosphorylated Ser residue, shutting down acetyl-CoA carboxylase, inhibiting fatty acid synthesis, and stimulating  $\beta$  oxidation.

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- 21. Effect of PDE Inhibitor on Adipocytes** How would an adipocyte's response to epinephrine be affected by the addition of an inhibitor of cAMP phosphodiesterase (PDE)? (Hint: See Fig. 12–4.)

**Answer** Response to glucagon or epinephrine would be prolonged because cAMP, once formed, would persist, stimulating protein kinase A for a longer period and leading to longer-lasting mobilization of fatty acids in adipocytes.

- 22. Role of FAD as Electron Acceptor** Acyl-CoA dehydrogenase uses enzyme-bound FAD as a prosthetic group to dehydrogenate the  $\alpha$  and  $\beta$  carbons of fatty acyl-CoA. What is the advantage of using FAD as an electron acceptor rather than  $\text{NAD}^+$ ? Explain in terms of the standard reduction potentials for the Enz-FAD/ $\text{FADH}_2$  ( $E'^{\circ} = -0.219 \text{ V}$ ) and  $\text{NAD}^+/\text{NADH}$  ( $E'^{\circ} = -0.320 \text{ V}$ ) half-reactions.

**Answer** Enz-FAD, having a more positive standard reduction potential, is a better electron acceptor than  $\text{NAD}^+$ , and the reaction is driven in the direction of fatty acyl-CoA oxidation (a negative free-energy change). This more favorable free-energy change is obtained at the expense of 1 ATP; only 1.5 ATP molecules are formed per  $\text{FADH}_2$  oxidized in the respiratory chain, compared with 2.5 ATP per NADH.

- 23.  $\beta$  Oxidation of Arachidic Acid** How many turns of the fatty acid oxidation cycle are required for complete oxidation of arachidic acid (see Table 10–1) to acetyl-CoA?

**Answer** Arachidic acid is a 20-carbon saturated fatty acid. Nine cycles of the  $\beta$ -oxidation pathway are required for its oxidation, producing 10 molecules of acetyl-CoA, the last two in the ninth turn.

- 24. Fate of Labeled Propionate** If  $[3\text{-}^{14}\text{C}]$ propionate ( $^{14}\text{C}$  in the methyl group) is added to a liver homogenate,  $^{14}\text{C}$ -labeled oxaloacetate is rapidly produced. Draw a flow chart for the pathway by which propionate is transformed to oxaloacetate, and indicate the location of the  $^{14}\text{C}$  in oxaloacetate.

**Answer** Propionate is first converted to the CoA derivative. Figure 17–11 shows the three-step pathway that converts propionyl-CoA to succinyl-CoA, which can be summarized as follows. Use these descriptions to prepare your own flow diagram.

1. Propionyl-CoA carboxylase uses  $\text{CO}_2$  and ATP to form D-methylmalonyl-CoA by carboxylation at C-2 of the propionyl group.
2. Methylmalonyl-CoA epimerase shifts the CoA thioester from C-1 (of the original propionyl group) to the newly added carboxylate, making the product L-methylmalonyl-CoA.
3. Methylmalonyl-CoA mutase moves the carboxy-CoA group from C-2 to C-3 within the original propionyl unit, forming succinyl-CoA.
4. Once succinyl-CoA is formed, the citric acid cycle can convert it to oxaloacetate.

Given the stereochemistry of these reactions, the  $[^{14}\text{C}]$ -label is equilibrated at C-2 and C-3 of the oxaloacetate.

- 25. Phytanic Acid Metabolism** When phytanic acid uniformly labeled with  $^{14}\text{C}$  is fed to a mouse, radioactivity can be detected in malate, a citric acid cycle intermediate, within minutes. Draw a metabolic pathway that could account for this. Which of the carbon atoms in malate would contain  $^{14}\text{C}$  label?

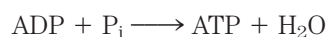
**Answer** Phytanic acid is degraded to pristanic acid by the pathway shown in Figure 17–17. Pristanic acid undergoes  $\beta$  oxidation, with each round yielding propionyl-CoA (not acetyl-CoA, as for a straight-chain fatty acid). Degradation of uniformly labeled phytanic acid produces

propionyl-CoA labeled in all three carbons of propionate. Propionyl-CoA is converted to succinyl-CoA by the series of reactions shown in Figure 17–11. The C-2 and C-3 of the succinyl moiety are labeled, and either C-1 or C-4 as well. When this succinate is converted to malate in the citric acid cycle, the malate is labeled at C-2 and C-3, and labeled half as much at C-1 and C-4.

- 26. Sources of H<sub>2</sub>O Produced in  $\beta$  Oxidation** The complete oxidation of palmitoyl-CoA to carbon dioxide and water is represented by the overall equation

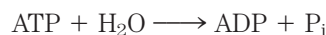


Water is also produced in the reaction



but is not included as a product in the overall equation. Why?

**Answer** ATP hydrolysis in the cell's energy-requiring reactions uses water, in the reaction



In a cell at steady state, for every mole of ATP hydrolyzed, a mole of ATP is formed by condensation of ADP + P<sub>i</sub>. There is no *net* change in [ATP] and thus no *net* production of H<sub>2</sub>O.

- 27. Biological Importance of Cobalt** In cattle, deer, sheep, and other ruminant animals, large amounts of propionate are produced in the rumen through the bacterial fermentation of ingested plant matter. Propionate is the principal source of glucose for these animals, via the route propionate → oxaloacetate → glucose. In some areas of the world, notably Australia, ruminant animals sometimes show symptoms of anemia with concomitant loss of appetite and retarded growth, resulting from an inability to transform propionate to oxaloacetate. This condition is due to a cobalt deficiency caused by very low cobalt levels in the soil and thus in plant matter. Explain.

**Answer** One of the enzymes necessary for the conversion of propionate to oxaloacetate is methylmalonyl-CoA mutase (see Fig. 17–11). This enzyme requires as an essential cofactor the cobalt-containing coenzyme B<sub>12</sub>, which is synthesized from vitamin B<sub>12</sub>. A cobalt deficiency in animals would result in coenzyme B<sub>12</sub> deficiency.

- 28. Fat Loss during Hibernation** Bears expend about  $25 \times 10^6$  J/day during periods of hibernation, which may last as long as seven months. The energy required to sustain life is obtained from fatty acid oxidation. How much weight loss (in kilograms) has occurred after seven months? How might ketosis be minimized during hibernation? (Assume the oxidation of fat yields 38 kJ/g.)

**Answer** If the catabolism of fat yields 38 kJ/g, or  $3.8 \times 10^4$  kJ/kg, and the bear expends  $25 \times 10^6$  J/day, or  $2.5 \times 10^4$  kJ/day, then the bear will lose

$$(2.5 \times 10^4 \text{ kJ/day}) / (3.8 \times 10^4 \text{ kJ/kg}) = 0.66 \text{ kg/day}$$

and in 7 months, or 210 days, will lose

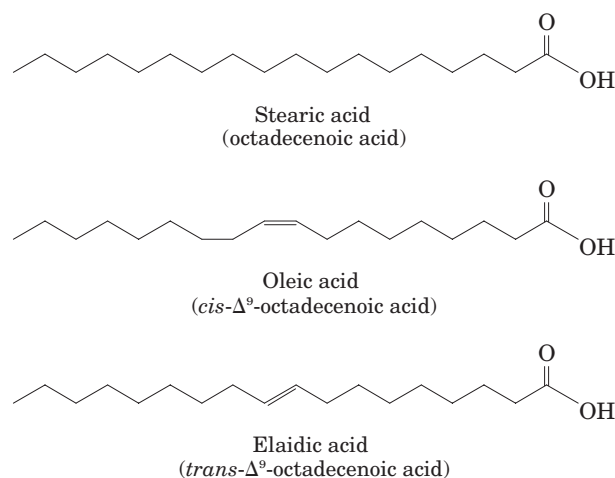
$$0.66 \text{ kg/day} \times 210 \text{ days} = 140 \text{ kg}$$

To minimize ketosis, a slow but steady degradation of nonessential proteins would provide three-, four-, and five-carbon products essential to the formation of glucose by gluconeogenesis. This would avoid the inhibition of the citric acid cycle that occurs when oxaloacetate is withdrawn from the cycle to be used for gluconeogenesis. The citric acid cycle could continue to degrade acetyl-CoA, rather than shunting it into ketone body formation.

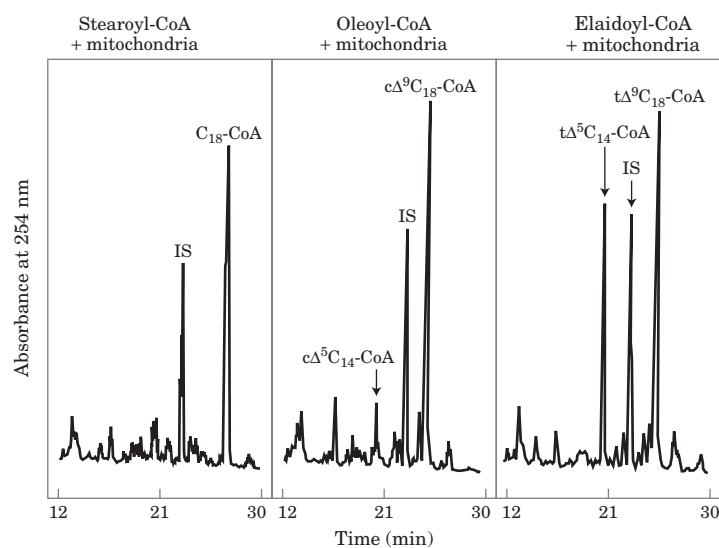
## S-208 Chapter 17 Fatty Acid Catabolism

## Data Analysis Problem

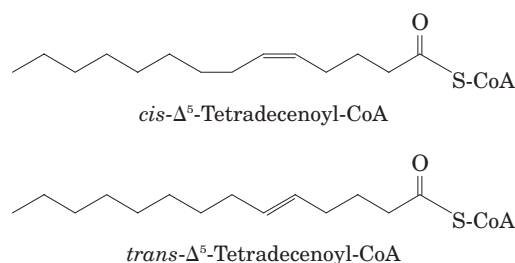
**29.  $\beta$  Oxidation of Trans Fats** Unsaturated fats with trans double bonds are commonly referred to as “trans fats.” There has been much discussion about the effects of dietary trans fats on health. In their investigations of the effects of trans fatty acid metabolism on health, Yu and colleagues (2004) showed that a model trans fatty acid was processed differently from its cis isomer. They used three related 18-carbon fatty acids to explore the difference in  $\beta$  oxidation between cis and trans isomers of the same-size fatty acid.



The researchers incubated the coenzyme A derivative of each acid with rat liver mitochondria for 5 minutes, then separated the remaining CoA derivatives in each mixture by HPLC (high-performance liquid chromatography). The results are shown below, with separate panels for the three experiments.



In the figure, IS indicates an internal standard (pentadecanoyl-CoA) added to the mixture, after the reaction, as a molecular marker. The researchers abbreviated the CoA derivatives as follows: stearoyl-CoA,  $C_{18}$ -CoA; *cis*- $\Delta^5$ -tetradecenoyl-CoA,  $c\Delta^5C_{14}$ -CoA; oleoyl-CoA,  $c\Delta^9C_{18}$ -CoA; *trans*- $\Delta^5$ -tetradecenoyl-CoA,  $t\Delta^5C_{14}$ -CoA; and elaidoyl-CoA,  $t\Delta^9C_{18}$ -CoA.



- (a) Why did Yu and colleagues need to use CoA derivatives rather than the free fatty acids in these experiments?
- (b) Why were no lower molecular weight CoA derivatives found in the reaction with stearoyl-CoA?
- (c) How many rounds of  $\beta$  oxidation would be required to convert the oleoyl-CoA and the elaidoyl-CoA to *cis*- $\Delta^5$ -tetradecenoyl-CoA and *trans*- $\Delta^5$ -tetradecenoyl-CoA, respectively?

There are two forms of the enzyme acyl-CoA dehydrogenase (see Fig. 17–8a): long-chain acyl-CoA dehydrogenase (LCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD). Yu and coworkers measured the kinetic parameters of both enzymes. They used the CoA derivatives of three fatty acids: tetradecanoyl-CoA ( $C_{14}$ -CoA), *cis*- $\Delta^5$ -tetradecenoyl-CoA ( $c\Delta^5C_{14}$ -CoA), and *trans*- $\Delta^5$ -tetradecenoyl-CoA ( $t\Delta^5C_{14}$ -CoA). The results are shown below. (See Chapter 6 for definitions of the kinetic parameters.)

	LCAD			VLCAD		
	$C_{14}$ -CoA	$c\Delta^5C_{14}$ -CoA	$t\Delta^5C_{14}$ -CoA	$C_{14}$ -CoA	$c\Delta^5C_{14}$ -CoA	$t\Delta^5C_{14}$ -CoA
$V_{max}$	3.3	3.0	2.9	1.4	0.32	0.88
$K_m$	0.41	0.40	1.6	0.57	0.44	0.97
$k_{cat}$	9.9	8.9	8.5	2.0	0.42	1.12
$k_{cat}/K_m$	24	22	5	4	1	1

- (d) For LCAD, the  $K_m$  differs dramatically for the *cis* and *trans* substrates. Provide a plausible explanation for this observation in terms of the structures of the substrate molecules. (Hint: You may want to refer to Fig. 10–2.)
- (e) The kinetic parameters of the two enzymes are relevant to the differential processing of these fatty acids *only* if the LCAD or VLCAD reaction (or both) is the rate-limiting step in the pathway. What evidence is there to support this assumption?
- (f) How do these different kinetic parameters explain the different levels of the CoA derivatives found after incubation of rat liver mitochondria with stearoyl-CoA, oleoyl-CoA, and elaidoyl-CoA (shown in the three-panel figure)?

Yu and coworkers measured the substrate specificity of rat liver mitochondrial thioesterase, which hydrolyzes acyl-CoA to CoA and free fatty acid (see Chapter 21). This enzyme was approximately twice as active with  $C_{14}$ -CoA thioesters as with  $C_{18}$ -CoA thioesters.

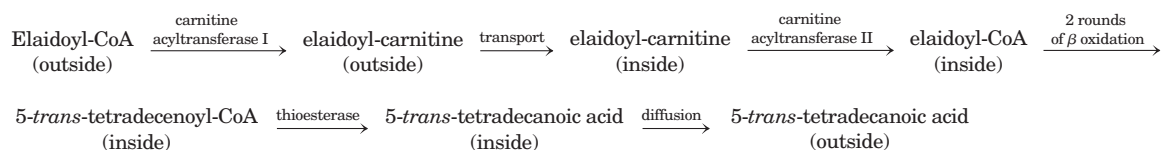
- (g) Other research has suggested that free fatty acids can pass through membranes. In their experiments, Yu and colleagues found *trans*- $\Delta^5$ -tetradecenoic acid outside mitochondria (i.e., in the medium) that had been incubated with elaidoyl-CoA. Describe the pathway that led to this extramitochondrial *trans*- $\Delta^5$ -tetradecenoic acid. Be sure to indicate where in the cell the various transformations take place, as well as the enzymes that catalyze the transformations.

## S-210 Chapter 17 Fatty Acid Catabolism

- (h) It is often said in the popular press that “trans fats are not broken down by your cells and instead accumulate in your body.” In what sense is this statement correct and in what sense is it an oversimplification?

**Answer**

- (a) Fatty acids are converted to their CoA derivatives by enzymes in the cytoplasm; the acyl-CoAs are then imported into mitochondria for oxidation. Given that the researchers were using isolated mitochondria, they had to use CoA derivatives.
- (b) Stearoyl-CoA was rapidly converted to 9 acetyl-CoA by the  $\beta$ -oxidation pathway. All intermediates reacted rapidly and none were detectable at significant levels.
- (c) Two rounds. Each round removes two carbon atoms, thus two rounds convert an 18-carbon to a 14-carbon fatty acid and 2 acetyl-CoA.
- (d) The  $K_m$  is higher for the trans isomer than for the cis, so a higher concentration of trans isomer is required for the same rate of breakdown. Roughly speaking, the trans isomer binds less well than the cis, probably because differences in shape, even though not at the target site for the enzyme, affect substrate binding to the enzyme.
- (e) The substrate for LCAD/VLCAD builds up differently, depending on the particular substrate; this is expected for the rate-limiting step in a pathway.
- (f) The kinetic parameters show that the trans isomer is a poorer substrate than the cis for LCAD, but there is little difference for VLCAD. Because it is a poorer substrate, the trans isomer accumulates to higher levels than the cis.
- (g) One possible pathway is shown below (indicating “inside” and “outside” mitochondria).



- (h) It is correct insofar as trans fats are broken down less efficiently than cis fats, and thus trans fats may “leak” out of mitochondria. It is incorrect to say that trans fats are not broken down by cells; they are broken down, but at a slower rate than cis fats.

**Reference**

Yu, W., Liang, X., Ensenauer, R., Vockley, J., Sweetman, L., & Schultz, H. (2004) Leaky  $\beta$ -oxidation of a *trans*-fatty acid. *J. Biol. Chem.* **279**, 52,160–52,167.