Chapter 13

Fatty Acid Metabolism

This chapter examines the students' ability to integrate their knowledge of fatty acid metabolism with clinical problems and carbohydrate metabolism.

QUESTIONS

Select the single best answer.

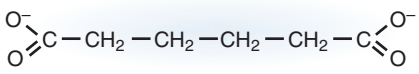
1 You prescribe ibuprofen to help reduce your patient's inflammation. Which of the following pathways is blocked as an anti-inflammatory mechanism of action of nonsteroidal anti-inflammatory drugs?
(A) Prostaglandin synthesis
(B) Thromboxane synthesis
(C) Leukotriene synthesis
(D) All eicosanoid synthesis
(E) Arachidonic acid release from the membrane

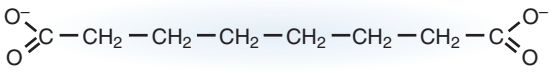
2 You have an asthmatic patient who is already on an inhaled steroid and albuterol, but is still having difficulty. You add montelukast to her regimen. Montelukast (Singulair) specifically blocks the product of which of the following metabolic pathways?
(A) Cyclooxygenase
(B) Lipoxygenase
(C) P450
(D) Cori cycle
(E) TCA cycle

3 Coconut palm tress cannot survive growing outdoors in Kansas. Which of the following is the best explanation for this finding?
(A) Coconut/palm oil is a saturated fat
(B) Coconut/palm oil is a monounsaturated fat
(C) Coconut/palm oil is a polyunsaturated fat
(D) Kansas soil is not sandy enough to support growth
(E) Kansas soil is too rocky to support growth

4 An inactivating mutation in the ETF:CoQ oxidoreductase will lead to an initial inhibition of which of the following enzymes in fatty acid oxidation?
(A) Carnitine acyltransferase I
(B) Carnitine acyltransferase II
(C) Acyl-CoA dehydrogenase
(D) Enoyl-CoA dehydrogenase
(E) β-keto thiolase

5 A 3-month-old child had her first ear infection and was feeding poorly due to the ear pain. One morning the parents found the child in a nonresponsive state and rushed her to the emergency department. A blood glucose level was 45 mg/dL, and upon receiving intravenous glucose the child became responsive. Further blood analysis displayed the absence of ketone bodies, normal levels of acyl-carnitine, and the presence of the following unusual carboxylic acids shown below. The enzymatic defect in this child is most likely in which of the following enzymes?





(A) Fatty acyl-CoA synthetase
(B) Carnitine translocase
(C) Carnitine acyltransferase I
(D) Carnitine acyltransferase II
(E) Medium chain acyl-CoA dehydrogenase

6 Regarding the child described in question 5, why were fasting blood glucose levels so low?
(A) Acyl-carnitine inhibition of gluconeogenesis
(B) Dicarboxylic acid inhibition of gluconeogenesis
(C) Insufficient energy for gluconeogenesis
(D) Dicarboxylic acid inhibition of glycogen phosphorylase
(E) Reduction of red blood cell production of lactate for gluconeogenesis

7 A 6-month-old child presents to the physician in a hypotonic state. The child has previously had a number of hypoglycemic episodes, at which times blood glucose

levels were between 25 and 50 mg/dl. Blood work shows normal levels of ketone bodies (not elevated) during hypoglycemic episodes. Carnitine levels in the blood were, however, below normal. Free fatty acid levels were elevated in the blood, however acyl-carnitine levels were normal. Dicarboxylic acid levels were non-detectable in the blood. A liver biopsy shows elevated levels of triglyceride. A likely enzymatic defect is which of the following?
(A) Carnitine acyltransferase I
(B) Carnitine acyltransferase II
(C) Medium chain acyl-CoA dehydrogenase
(D) Hormone sensitive lipase
(E) Carnitine transporter

8 Carnitine deficiency can occur in a number of ways. Secondary carnitine deficiency can be distinguished from primary carnitine deficiency by measuring which of the following in the blood?
(A) Fatty acids
(B) Acyl-carnitine
(C) Lactic acid
(D) Glucose
(E) Ketone bodies

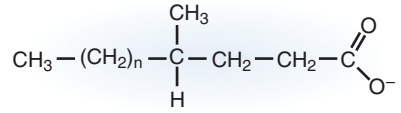
9 Which one of the following fatty acids will generate the largest amount of ATP upon complete oxidation to carbon dioxide and water?
(A) C16:0
(B) cisΔ9 C16:1
(C) cisΔ9 C18:1
(D) cisΔ6 C18:1
(E) cisΔ9, Δ12 C18:2

10 An individual contains an inactivating mutation in a particular muscle protein, which leads to weight loss due to unregulated muscle fatty acid oxidation. Such an inactivated protein could be which of the following?
(A) Malonyl-CoA decarboxylase
(B) Carnitine acyl transferase I
(C) Carnitine acyl transferase II
(D) Medium chain acyl-CoA dehydrogenase
(E) Acetyl-CoA carboxylase 2

11 The net energy yield obtained (moles of ATP per mole of substrate oxidized) when acetoacetate is utilized by the nervous system as an alternative energy source is which of the following? Consider that acetoacetate must be oxidized to four molecules of carbon dioxide during the reaction sequence.
(A) 17
(B) 18
(C) 19
(D) 20
(E) 21

12 A mouse model has been generated as an in vivo system for studying fatty acid synthesis. An inactivating mutation was created which led to the cessation of fatty acid synthesis and death to the mice. This mutation is most likely in which of the following proteins?
(A) Carnitine acyl transferase I
(B) Carnitine acyl transferase II
(C) Citrate translocase
(D) Glucose-6-phosphate dehydrogenase
(E) Medium chain acyl-CoA dehydrogenase

13 α-oxidation would be required for the complete oxidation of which of the following fatty acids?

A


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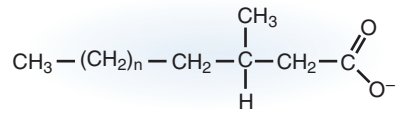

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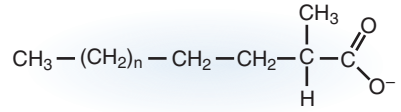

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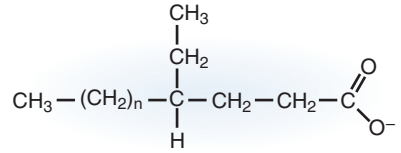

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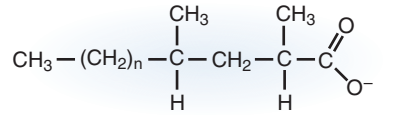


14 A 2-month-old infant with failure to thrive displays hepatomegaly, high levels of iron and copper in the blood, and vision problems. This child has difficulty in carrying out which of the following types of reactions?
(A) Oxidation of very long chain fatty acids
(B) Synthesis of unsaturated fatty acids
(C) Oxidation of acetyl-CoA
(D) Oxidation of glucose
(E) Synthesis of triacylgycerol

15 A 55-year-old man had been advised by his physician to take 81 mg of aspirin per day to reduce the risk of

blood clots leading to a heart attack. The rationale for this treatment is which of the following?
(A) To reduce prostaglandin synthesis
(B) To reduce leukotriene synthesis
(C) To reduce thromboxane synthesis
(D) To increase prostacyclin synthesis
(E) To increase Lipoxin synthesis

16 You are examining a patient who exhibits fasting hypoglycemia and need to decide between a carnitine deficiency and a carnitine acyltransferase 2 deficiency as the possible cause. You order a blood test to specifically examine the levels of which one of the following?
(A) Glucose
(B) Ketone bodies
(C) Insulin
(D) Acyl-carnitine
(E) Carnitine

17 Inhibitors specific for cyclooxygenase 2 (COX-2) were deemed more efficacious for certain conditions than inhibitors which blocked both COX-1 and COX-2 activities. This is due to which of the following?
(A) Inhibiting COX-1 increased the frequency of heart attacks
(B) Inhibiting COX-2 did not alter prostaglandin production
(C) COX-2 is specifically induced during inflammation
(D) Specifically inhibiting COX-2 reduces the rate of heart attacks
(E) COX-1 is inducible and only expressed during wound repair, while COX-2 is expressed constitutively

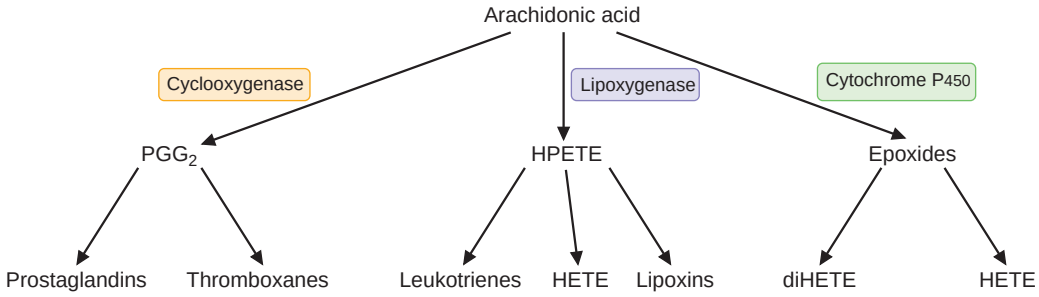
18 An individual with a biotinidase deficiency was shown to produce fatty acids at a greatly reduced rate (in the absence of supplements) as compared to someone who did not have the deficiency. This is due to which of the following?
(A) Low activity of citrate lyase
(B) Reduced activity of malic enzyme
(C) Reduced activity of acetyl transacylase
(D) Defective acyl carrier protein
(E) Reduced ability to form malonyl-CoA

19 Liver fatty acid oxidation leads to an enhancement of gluconeogenesis via which of the following?
(A) Generation of precursors for glucose synthesis
(B) Activation of pyruvate carboxylase
(C) Activation of phosphoenolpyruvate carboxykinase
(D) Inhibition of pyruvate kinase
(E) Inhibition of PFK-2

20 A 35-year-old man in New York city, originally from Jamaica, purchased an illegally imported fruit from a street vendor and, within 4 h of eating the fruit, began vomiting severely. When brought to the emergency department the man was severely dehydrated and exhibited several seizures. The toxic effects of the fruit were interfering with which of the following?
(A) Fatty acid release from the adipocyte
(B) Fatty acid entry into the liver cell
(C) Fatty acid activation
(D) Fatty acid transport into the mitochondria
(E) Oxidative phosphorylation

ANSWERS

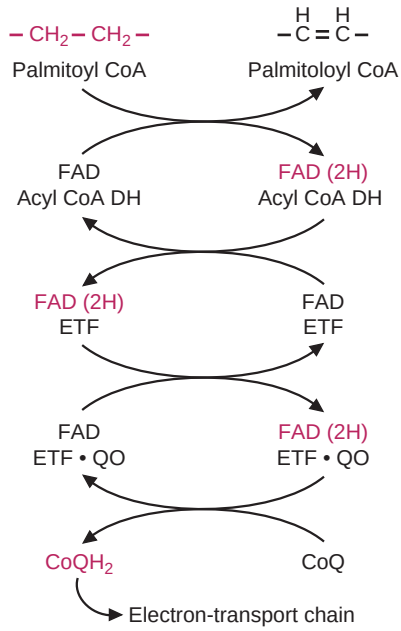
1 **The answer is A: Prostaglandin synthesis.** Eicosanoids are potent regulators of cellular function. They are derived from arachidonic acid and are metabolized by three pathways: the cyclooxygenase pathway (prostaglandins and thromboxanes), lipoxygenase pathway (leukotrienes), and the cytochrome P450 pathway (epoxides) (see the figure below). Nonsteroidal anti-inflammatory drugs (NSAIDs) do not block arachidonic acid release from the membrane (which would block all eicosanoid synthesis); however, they do interfere with the cyclooxygenase pathway. Prostaglandins affect inflammation, thromboxanes affect formation of blood clots, and leukotrienes affect bronchoconstriction and bronchodilatation. NSAIDs block prostaglandins as one of their anti-inflammatory mechanisms. Thus, while NSAIDS will block both prostaglandin and thromboxane synthesis, it is the blockage of prostaglandin synthesis which will block the inflammatory symptoms.



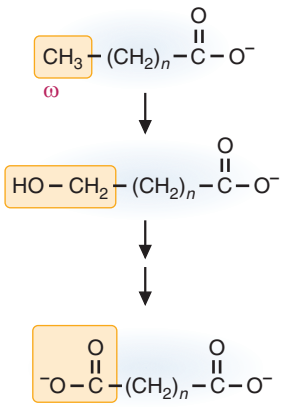
2 **The answer is B: Lipoxygenase.** Montelukast is a leukotriene blocker. Leukotrienes are formed through the lipoxygenase pathway and affect bronchoconstriction and allergy pathways (see the figure in answer to question 1). The cyclooxygenase pathway produces prostaglandins and thromboxanes. The P450 pathway produces epoxides. The Cori cycle is related to gluconeogenesis (lactate transfer from the muscle to the liver), while the TCA cycle is utilized to oxidize acetyl-CoA to CO_2 and H_2O.

3 **The answer is A: Coconut/palm oil is a saturated fat.** Saturated fats do not liquefy until a much higher temperature than that at which monounsaturated or polyunsaturated fats do (the melting temperature for saturated fats is greater than that for unsaturated fats). Conversely, saturated fats are solids at a higher temperature than unsaturated fats and cannot exist in a liquid form at a lower temperature. Since the oil of a plant is its "lifeblood," at a lower temperature, a saturated oil would solidify and the plant would die. Saturated oil plants cannot survive in a temperate climate (Kansas) and need a tropical climate of warm temperatures all year round. Only polyunsaturated oil plants can survive in a temperate climate (corn, flax, wheat, and canola). Monounsaturated oils need a warmer climate, but not as warm as the tropics (olive, peanut). Knowing where a plant grows gives a large clue as to whether the oil will be saturated, monounsaturated, or polyunsaturated. The difference in oil content between plants appears to be an evolutionary process. Kansas soil is very rich and supports growth of most plants.

4 **The answer is C: acyl-CoA dehydrogenase.** The acyl-CoA dehydrogenases catalyze the first step of the fatty acid oxidation spiral in that these enzymes create a carbon–carbon double bond between carbons 2 and 3 of the fatty acyl-CoA, generating an FADH2 in the process. The FADH2 then donates its electrons to the electron transfer flavoprotein (ETF), which then transfers the electrons to coenzyme Q (via the ETF:CoQ oxidoreductase). A lack of the oxidoreductase activity will lead to an accumulation of mitochondrial FADH2, depleting FAD levels, and reducing the activity of the acyl-CoA dehydrogenases. The lack of FAD does not directly inhibit the β-ketothiolase or enoyl-CoA dehydrogenase steps, nor does it affect the activity of the carnitine acyltransferases. The figure below shows the normal transport of electrons from FADH2 to coenzyme Q when the FADH2 is generated by the acyl-CoA dehydrogenases.



5 **The answer is E: Medium chain acyl-CoA dehydrogenase.** The child has MCAD (medium-chain acyl-CoA dehydrogenase) deficiency, an inability to completely oxidize fatty acids to carbon dioxide and water. With an MCAD deficiency, gluconeogenesis is impaired due to a lack of energy from fatty acid oxidation, and an inability to fully activate pyruvate carboxylase, as acetyl-CoA activates pyruvate carboxylase, and acetyl-CoA production from fatty acid oxidation is greatly reduced. In an attempt to generate more energy, medium-chain fatty acids are oxidized at the ω ends to generate the dicarboxylic acids seen in the question (see the figure below for an overview of ω oxidation). The finding of such metabolites (dicarboxylic acids) in the blood is diagnostic for MCAD deficiency. If there were mutations in any aspect of carnitine metabolism, there would be no oxidation of fatty acids (the fatty acids would not be able to enter the mitochondria), and the dicarboxylic acids (which are byproducts of fatty acid metabolism) would not be observed. Similarly, a mutation in the fatty acyl-CoA synthetase (the activating enzyme, converting a free fatty acid to an acyl-CoA) would also result in a lack of fatty acid oxidation, as fatty acids are not able to enter the mitochondria in their free (nonactivated) form.

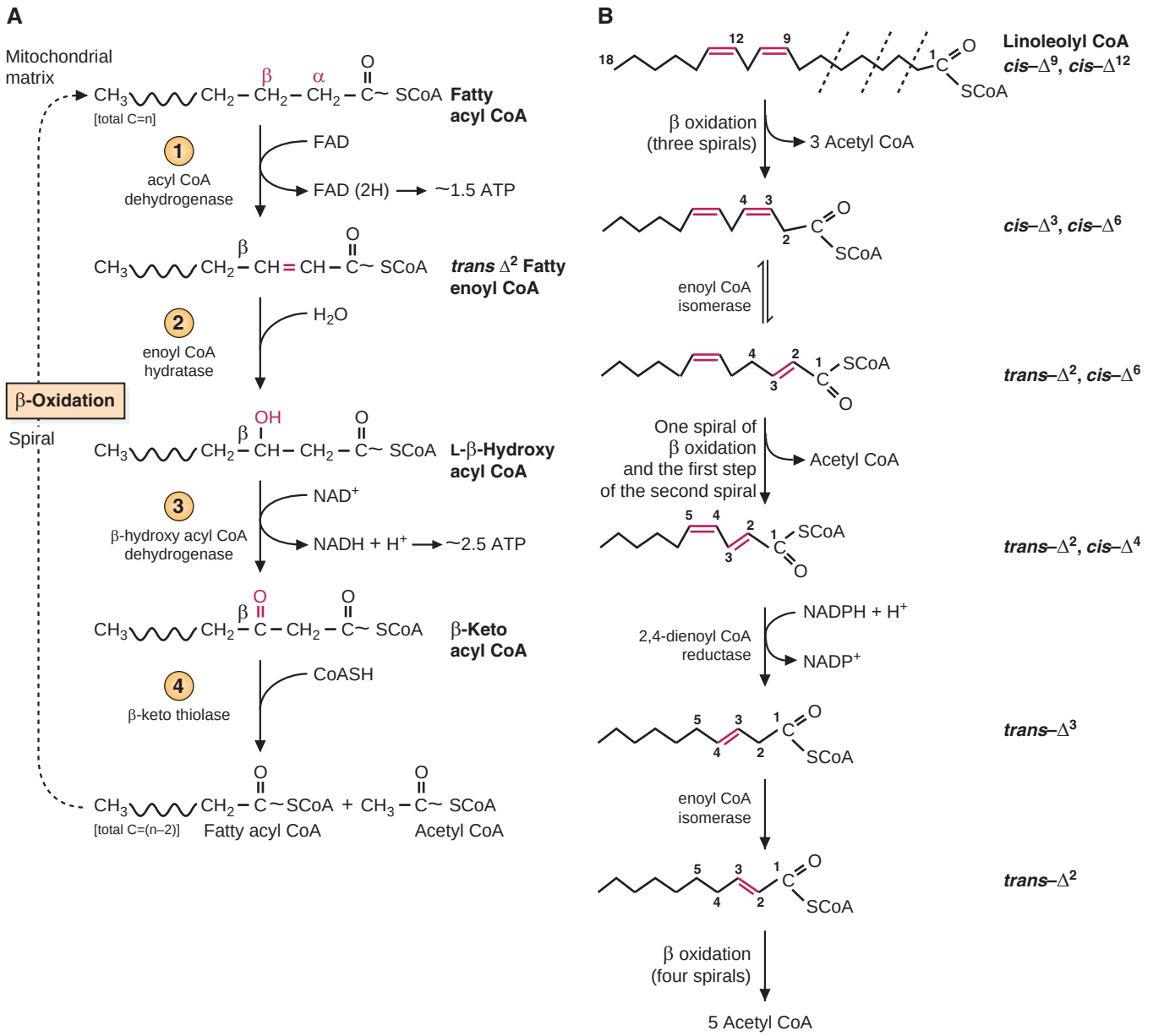


6 **The answer is C: Insufficient energy for gluconeogenesis.** Defects in fatty acid oxidation deprive the liver of energy when fatty acids are the major energy source (such as during exercise, or a fast). Because of this, there is insufficient energy to synthesize glucose from gluconeogenic precursors (it requires 6 moles of ATP to convert 2 moles of pyruvate to 1 mole of glucose). Acyl-carnitines and dicarboxylic acids have no effect on the enzymes of gluconeogenesis, nor do they hinder the ability of the red blood cell to utilize glucose through the glycolytic pathway. Additionally, acetyl-CoA levels are low due to the lack of complete fatty acid oxidation and pyruvate carboxylase, a key gluconeogenic enzyme, is not fully activated. This also contributes to the reduced gluconeogenesis observed in patients with MCAD defective.

7 **The answer is E: Carnitine transporter.** The child has a mutation in the enzyme which transports carnitine into liver and muscle cells, leading to a primary carnitine deficiency. The carnitine stays in the blood and is eventually lost in the urine (the same carnitine transporter is required to recover the carnitine from the urine in the kidney). Since the liver is carnitine deficient, ketone body production is minimal at all times, even during a fast (thus, the lack of baseline ketone bodies in the circulation under these conditions). Fatty acids will rise in circulation, as they cannot be stored in the cells as acyl-CoA. The liver shows evidence of triglyceride formation as the acyl-CoA cannot be degraded, and acyl-CoA accumulates within the cytoplasm, leading to triglyceride formation. A defect in carnitine acyl transferase 1 would lead to elevated levels of carnitine in the circulation. A defect in carnitine acyltransferase II would lead to elevated levels of acyl-carnitine in the circulation (since the acyl group cannot be removed from the carnitine). The lack of circulating dicarboxylic acids indicates that the defect is not in MCAD (medium-chain acyl-CoA dehydrogenase). A defect in hormone sensitive lipase would show a decrease in free fatty acid levels, rather than the increase observed in the patient.

8 **The answer is B: acyl-carnitine.** Primary carnitine deficiency is a lack of carnitine within the cell (such as a mutation in the carnitine transporter); secondary carnitine deficiency occurs when the carnitine is sequestered in the form of acyl-carnitine (the carnitine cannot be removed from the acyl group, such as a defect in carnitine acyl transferase 2). Thus, elevated levels of acyl-carnitine would be expected in a secondary carnitine deficiency, but not in a primary carnitine deficiency. In both types of carnitine deficiencies, fatty acid oxidation is significantly reduced, so the levels of ketone bodies, glucose, lactate, and fatty acids would be similar under both conditions.

9 **The answer is C: cisΔ9 C18:1.** An 18-carbon fatty acid will generate an additional acetyl-CoA, one NADH, and one FADH2 as compared to a 16-carbon fatty acid. Thus, the addition of two carbons will add 14 additional ATP to the overall energy yield (10 ATP per acetyl-CoA, 2.5 for NADH, and 1.5 for FADH2). An unsaturation at an odd carbon position will require the use of an isomerase during oxidation, and this will result in the loss of generation of 1 FADH2; an unsaturation at an even carbon position will require the use of the 2,4 dienoyl-CoA reductase, and this will result in the loss of generation of 1 NADPH. Thus, an unsaturation at an odd position results in the loss of 1.5 ATP, while an unsaturation at an even position results in the loss of 2.5 ATP. Thus, in comparing two 18-carbon fatty acids, one with an unsaturation at position 9, and the other at position 6, the fatty acid with the double bond at position 9 will

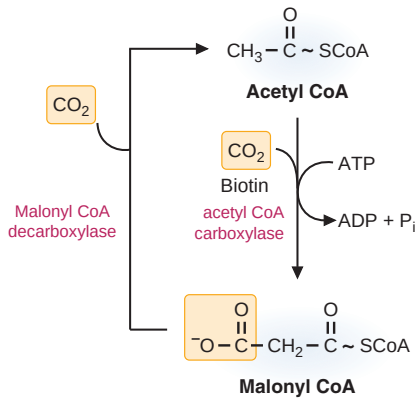


Answer 9: Panel A: The steps of β-oxidation. The four steps are repeated until an even-chain fatty acid is completely converted to acetyl-CoA. The FAD(2H) and NADH are reoxidized by the electron-transport chain, producing ATP. **Panel B**: The additional reactions required for the oxidation of unsaturated fatty acids. The two new enzymes required are the enoyl-CoA isomerase and the 2,4 dienoyl-CoA reductase.

yield one more ATP than the fatty acid with the unsaturation at position 6. An overview of the fatty acid oxidation spiral is shown above, along with the reactions required for the oxidation of unsaturated fatty acids.

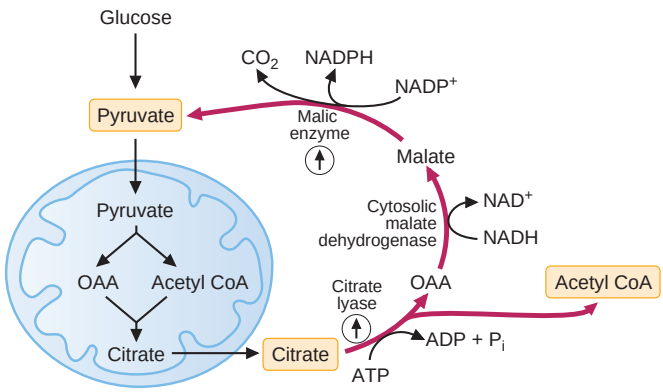
10 **The answer is E: acetyl-CoA carboxylase 2.** An inactivating mutation in acetyl-CoA carboxylase would lead to an inability to produce malonyl-CoA, which regulates fatty acid oxidation through an inhibition of carnitine acyl transferase 1. As malonyl-CoA levels increase, fatty acid oxidation is reduced, and as the levels decrease, fatty acid oxidation will increase. If malonyl-CoA decarboxylase were inactivated, malonyl-CoA levels would remain elevated, and fatty acid oxidation would be inhibited. Inactivating mutations in either carnitine acyltransferase 1 or 2 would lead to an inability to oxidize fatty acids, as they would not enter the mitochondria. A defect in medium-chain acyl-CoA dehydrogenase (MCAD) would also result in reduced fatty acid oxidation, as the initial step of the oxidation spiral would be inhibited once the fatty

acid had been reduced to about 10 carbons in length. The reactions catalyzed by malonyl-CoA decarboxylase and acetyl-CoA carboxylase are shown below.



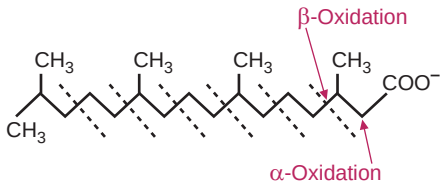
11 **The answer is C: 19.** Acetoacetate will react with succinyl-CoA to produce acetoacetyl-CoA and succinate (this costs 1 GTP, as the succinate thiokinase step is skipped). The acetoacetyl-CoA is converted to two acetyl-CoA, each of which can generate 10 ATP when completely oxidized (each acetyl-CoA generates 1 GTP, 3 NADH, and 1 FADH2). The sum, then, is 20 minus the 1 lost in the CoA transferase step, for a net yield of 19 ATP.

12 **The answer is C: Citrate translocase.** Citrate translocase is required for citrate to exit the mitochondria and enter the cytoplasm in order to deliver acetyl-CoA for fatty acid biosynthesis (see the figure below). Acetyl-CoA, which is produced exclusively in the mitochondria, has no direct path through the inner mitochondrial membrane. However, under conditions conducive to fatty acid biosynthesis (high energy levels, and allosteric inhibition of the TCA cycle), citrate will accumulate and leave the mitochondria (see the figure below). Once in the cytoplasm, citrate lyase will cleave the citrate to produce acetyl-CoA and oxaloacetate. The oxaloacetate is recycled to pyruvate, producing NADPH in the process, which is also required for fatty acid biosynthesis. A defect in either carnitine acyl transferase will not affect fatty acid biosynthesis, as those enzymes are required to transport the fatty acid into the mitochondria for its oxidation. A lack of glucose-6-phosphate dehydrogenase will not interfere with fatty acid synthesis, as malic enzyme can provide sufficient NADPH for the pathway. MCAD is involved in fatty acid oxidation and does not affect fatty acid synthesis.



Citrate leaving the mitochondria and delivering acetyl-CoA to the cytoplasm for fatty acid synthesis.

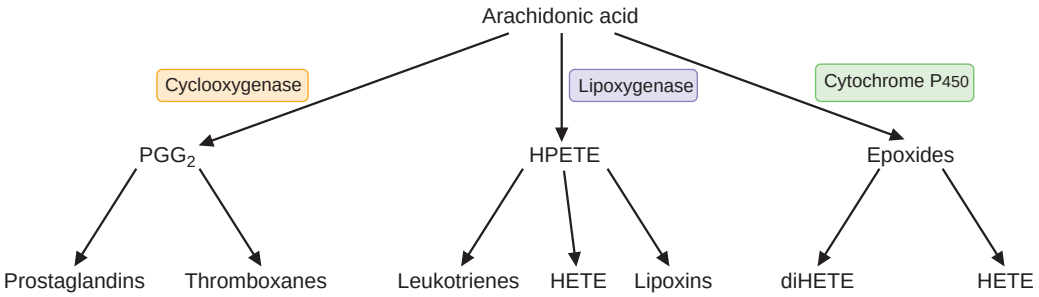
13 **The answer is B.** α-oxidation leads to the oxidation of the α-carbon of a branched chain fatty acid to generate an α-keto acid, which undergoes oxidative decarboxylation. This reorients the methyl groups on the branched chain fatty acid such that they are on the α-carbon, rather than the β-carbon. In this manner, the methyl groups do not interfere with the β-oxidation spiral (if the methyl group were on the β-carbon, a carbonyl group would be unable to form on that carbon, which would block further oxidation of the fatty acid). Answer choices A, C, D, and E are eliminated as requiring α-oxidation because, after one round of normal β-oxidation, the methyl group (or butyl group) will be on the α-carbon and would not interfere with the β-oxidation spiral. An overview of α-oxidation is shown below.



The figure depicts the oxidation of phytanic acid. A peroxisomal α-hydroxylase oxidizes the α-carbon, and its subsequent oxidation to a carboxyl group releases the carboxyl group as carbon dioxide. Subsequent spirals of peroxisomal β-oxidation alternately release propionyl and acetyl-CoA.

14 **The answer is A: Oxidation of very long chain fatty acids.** The child has Zellweger's syndrome, an absence of peroxisomal enzyme activity. Of the pathways listed as answers, only the oxidation of very long chain fatty acids is a peroxisomal function. Fatty acid synthesis occurs in the cytoplasm. Acetyl-CoA oxidation takes place in the mitochondria. Glucose oxidation is a combination of glycolysis (cytoplasm) and the TCA cycle (mitochondria). Triglyceride synthesis occurs in the cytoplasm.

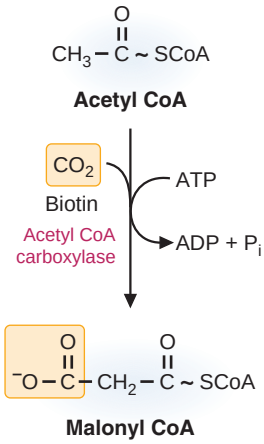
15 **The answer is C: To reduce thromboxane synthesis.** Thromboxane A2 release from platelets is an essential element of forming blood clots, and aspirin will block prostaglandin, prostacyclin, and thromboxane synthesis. It is the thromboxane inhibition which reduces the risk of blood clots. Leukotrienes and lipoxins require the enzyme lipoxygenase, which is not inhibited by aspirin. These pathways are outlined below.



16 **The answer is D: acyl-carnitine.** With a carnitine deficiency, fatty acids cannot be added to carnitine, and acyl-carnitine would not be synthesized. With a carnitine acyl-transferase 2 deficiency, the fatty acids are added to carnitine, but the acyl-carnitine cannot release the acyl group within the mitochondria. This will lead to an accumulation of acylcarnitine, which will lead to an accumulation in the circulation. The end result of either deficiency is a lack of fatty acid oxidation, such that ketone body levels would be minimal under both conditions, and blood glucose levels would also be similar in either condition. Insulin release is not affected by either deficiency, and carnitine levels, normally low, would not be significantly modified in either deficiency.

17 **The answer is C: COX-2 is specifically induced during inflammation.** COX-2 is induced during inflammatory conditions, while COX-1 is constitutively expressed. Thus, when an injury occurs, and an immune response is mounted at the site of injury, COX-2 is induced in those cells to produce second messengers that play a role in mediating the pain response. Specifically inhibiting the COX-2 isozyme will block the production of those second messengers, without affecting the normal function of COX-1. Inhibiting COX-1 may reduce the frequency of heart attacks, and inhibiting COX-2 will block prostaglandin production via the cylco-oxygenase. Recent data suggests that certain drugs that specifically block COX-2 have unwanted side effects, such as an increase in heart attacks.

18 **The answer is E: Reduced ability to form malonyl-CoA.** Biotinidase is required to remove covalently-bound biotin from proteins, which is how most of the biotin in our diet is received. In the absence of biotinidase, individuals can become functionally biotin-deficient, due to the lack of free biotin in the body (as compared to being covalently bound to proteins). The formation of malonyl-CoA, via acetyl-CoA carboxylase, requires biotin as a required cofactor (see the figure below). Citrate lyase, malic enzyme, acetyl transacylase (an activity of fatty acid synthase) and acyl carrier protein (another component of fatty acid synthase) do not require biotin for their activity.



19 **The answer is B: Activation of pyruvate carboxylase.** Fatty acid oxidation increases the levels of acetyl-CoA within the mitochondrial matrix, and acetyl-CoA is a potent activator of pyruvate carboxylase, a key gluconeogenic enzyme (it will convert pyruvate to oxaloacetate, a necessary first step to bypass the irreversible pyruvate kinase reaction). Acetyl-CoA cannot be used to synthesize net glucose, so it is not an effective precursor of glucose production. Acetyl-CoA does not activate PEP carboxykinase (that enzyme is transcriptionally controlled), nor does it affect pyruvate kinase (a cytoplasmic enzyme). PFK-2 is not regulated by acetyl-CoA (phosphorylation by protein kinase A is the key regulator effect for PFK-2 in the liver).

20 The answer is D: Fatty acid transport into the mitochondria. The man had eaten the unripe fruit of the ackee tree (from Jamaica). The unripened fruit contains the toxin hypoglycin A, which will interfere with carnitine's ability to transport acyl-carnitine groups across the inner mitochondrial membrane. This leads to a complete shutdown of fatty acid oxidation in all tissues in the affected individual, leading to severe hypoglycemia. Hypoglycin has no effect on fatty acid release from the adipocyte, or fatty acid entry into liver cells. Fatty acid oxidation is not directly inhibited, nor does this toxin directly inhibit the complexes of the electron transport chain and the proton-translocating ATPase.