Metabolism Of Fatty Acids, Types Of Oxidation

(Amirova M.F., abstracted from Lippincott, Richard Harvey, sixth edirion 2014)

Free Fatty Acids (FFA)

OVERVIEW Fatty acids exist "free" in the body (that is, they are unesterified) and as fatty acyl esters in more complex molecules such as triacylglycerols (TAGs). Low levels of free fatty acids (FFAs) occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting. Plasma FFAs (transported on serum albumin) are in route from their point of origin (TAG of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues). FFAs can be oxidized by many tissues, particularly liver and muscle, to provide energy and, in liver, to provide the substrate for ketone body synthesis. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids. Fatty acids attached to certain proteins enhance the ability of those proteins to associate with membranes. Fatty acids are also precursors of the hormone-like prostaglandins. Esterified fatty acids, in the form of TAGs stored in white adipose tissue (WAT), serve as the major energy reserve of the body. Alterations in fatty acid metabolism are associated with obesity and diabetes.

DE NOVO SYNTHESIS OF FATTY ACIDS

A large proportion of the fatty acids used by the body is supplied by the diet. Carbohydrates and protein obtained from the diet in excess of the body's needs for these compounds can be converted to fatty acids, which are stored as TAGs. In adult humans, fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. This cytosolic process incorporates carbons from acetyl coenzyme A (CoA) into the growing

fatty acid chain, using adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

A. **Production of cytosolic acetyl coenzyme A** The first step in de novo fatty acid synthesis is the transfer of acetate units from mitochondrial acetyl CoA to the cytosol. Mitochondrial acetyl CoA is produced by the oxidation of pyruvate and by the catabolism of certain amino acids. The CoA portion of acetyl CoA, however, cannot cross the inner mitochondrial membrane, and only the acetyl portion enters the cytosol. It does so as part of citrate produced by the condensation of acetyl CoA with oxaloacetate (OAA) by citrate synthase. [Note: The translocation of citrate to the cytosol occurs when the mitochondrial citrate concentration is high. This is observed when isocitrate dehydrogenase of the citric acid cycle is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate. Therefore, cytosolic citrate may be viewed as a high-energy signal. Because a large amount of ATP is needed for fatty acid synthesis, the increase in both ATP and citrate enhances this pathway.]

B. Carboxylation of acetyl coenzyme A to malonyl coenzyme A The energy for the carbon-to-carbon condensations in fatty acid synthesis is supplied by the process of carboxylation followed by decarboxylation of acyl groups in the cytosol. The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by acetyl CoA carboxylase (ACC), and requires CO2 and ATP. The coenzyme is the vitamin biotin, which is covalently bound to a lysyl residue of the carboxylase . ACC carboxylates the bound biotin, which transfers the activated carboxyl group to acetyl CoA.

C. Fatty acid synthesis with Fatty acid synthase (FAS) or Acyl Carrying Protein (ACP)

The series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the fatty acid synthase (FAS) or **ACP**. Each FAS monomer is a multicatalytic polypeptide with seven different enzymic domains plus a domain that covalently binds a molecule of 4 -phosphopantetheine.

[1] **An acetyl group is transferred from acetyl CoA to the –SH group of the ACP.** Domain: Acetyl CoA-ACP acetyltransacylase.

[2] Next, this two-carbon fragment is transferred to a temporary holding site, the thiol group of a cysteine residue on the enzyme.

[3] The now-vacant ACP accepts a three-carbon malonyl group from malonyl CoA. Domain: Malonyl CoA-ACP transacylase.

[4] The acetyl group on the cysteine residue condenses with the malonyl group on ACP as the CO2 originally added by acetyl CoA carboxylase is released. The result is a four-carbon unit attached to the ACP domain. The loss of free energy from the decarboxylation drives the reaction. Domain: 3-Ketoacyl-ACP synthase, also known as "condensing enzyme."

[5] The keto group is reduced to an alcohol. Domain: 3-Ketoacyl-ACP reductase.

[6] A molecule of water is removed, creating a double bond between carbons 2 and 3 (the α- and β-carbons). Domain: 3-Hydroxyacyl-ACP dehydratase.

[7] The double bond is reduced. Domain: Enoyl-ACP reductase. The result of these seven steps is production of a four-carbon compound (butyryl) whose three terminal carbons are fully saturated, and which remains attached to the ACP domain. These seven steps are repeated, beginning with the transfer of the butyryl chain from the ACP to the cysteine residue, the attachment of a molecule of malonate to the ACP, and the condensation of the two molecules

liberating CO2 . The carbonyl group at the β-carbon (carbon 3, the third carbon from the sulfur) is then reduced, dehydrated, and reduced, generating hexanoyl-ACP. This cycle of reactions is repeated five more times, each time incorporating a two-carbon unit (derived from malonyl CoA) into the growing fatty acid chain at the carboxyl end. When the fatty acid reaches a length of 16 carbons, the synthetic process is terminated with palmitoyl-S-ACP. [Note: Shorter-length fatty acids are important end products in the lactating mammary gland.

[8] Palmitoyl thioesterase, the final catalytic activity of FAS, cleaves the thioester bond, releasing a fully saturated molecule of palmitate (16:0). [Note: All the carbons in palmitic acid have passed through malonyl CoA except the two donated by the original acetyl CoA, which are found at the methyl (ω) end of the fatty acid. This underscores the rate-limiting nature of the ACC reaction.]

Major sources of the reductant NADPH2 required for fatty acid synthesis

The pentose phosphate pathway is a major supplier of NADPH, the reducatant required for fatty acid synthesis. Two NADPH are produced for each molecule of glucose that enters this pathway. The cytosolic conversion of malate to pyruvate, in which malate is oxidized and decarboxylated by cytosolic malic enzyme (NADP+-dependent malate dehydrogenase), also produces cytosolic NADPH (and CO2). [Note: Malate can arise from the reduction of OAA by cytosolic NADH-dependent malate dehydrogenase.

Storage of fatty acids as components of triacylglycerols

Mono-, di-, and triacylglycerols consist of one, two, or three molecules of fatty acid esterified to a molecule of glycerol. Fatty acids are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of "neutral fat."

1. **Synthesis of glycerol 3-phosphate**:

   Glycerol 3-phosphate is the initial acceptor of fatty acids during TAG synthesis. There are two pathways for its production. In both liver (the primary site of TAG synthesis) and adipose tissue, glycerol 3-phosphate can be produced from glucose, using first the reactions of the glycolytic pathway to produce dihydroxyacetone phosphate ([DHAP]. DHAP is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate. A second pathway found in the liver, but not in adipose tissue, uses glycerol kinase to convert free glycerol to glycerol phosphate.

TAG synthesis during fasting is low. The glucose transporter in adipocytes (GLUT-4) is insulin dependent. Thus, when plasma glucose (and, therefore, plasma insulin) levels are low, adipocytes have only a limited ability to synthesize glycerol phosphate and cannot produce TAG de novo

2. **Activation of a free fatty acid for TAG synthesis:** A fatty acid must be converted to its activated form (bound to CoA) before it can participate in metabolic processes such as TAG synthesis. This reaction, illustrated in Figure 15.6, is catalyzed by a family of fatty acyl CoA synthetases (thiokinases).
3. **Synthesis of triacylglycerol (TAG)** from glycerol 3-phosphate and fatty acyl coenzyme As: This pathway involves four reactions, shown in Figure 16.14. These include the sequential addition of two fatty acids from fatty acyl CoAs, the removal of phosphate, and the addition of the third fatty acid.

   Different fates of triacylglycerol in liver and adipose tissue

In adipose tissue, TAG is stored in a nearly anhydrous form as fat droplets in the cytosol of the cells. It serves as "depot fat," ready for mobilization when the body requires it for fuel. Little TAG is stored in healthy liver. Instead, most is exported, packaged with other lipids and apolipoproteins to form lipoprotein particles called

very-low-density lipoproteins (VLDLs). Nascent VLDLs are secreted directly into the blood where they mature and function to deliver the endogenously derived lipids to the peripheral tissues.

OXIDATION OF FATTY ACIDS

A. **Release of fatty acids from fat** The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process of lipolysis is achieved by lipases. It is initiated by adipose triglyceride lipase (ATGL), which generates a diacylglycerol that is the preferred substrate for hormone-sensitive lipase (HSL). The monoacylglycerol (MAG) product of HSL is acted upon by MAG lipase

B. H**ormone-sensitive lipase (HSL):** HSL is active when phosphorylated by PKA, a 3′ ,5′-cyclic AMP(cAMP)–dependent protein kinase. cAMP is produced in the adipocyte when catecholamines (such as epinephrine) bind to cell membrane β-adrenergic receptors and activate adenylyl cyclase. The process is similar to that of the activation of glycogen phosphorylase. In the presence of high plasma levels of insulin, HSL is dephosphorylated and inactivated.

C. Fate of glycerol: The glycerol released during TAG degradation cannot be metabolized by adipocytes because they lack glycerol kinase. Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated. The resulting glycerol 3-phosphate can be used to form TAG in the liver or can be converted to DHAP by reversal of the glycerol 3-phosphate dehydrogenase reaction. DHAP can further participate in glycolysis or gluconeogenesis.

D. **Fate of fatty acids**: The free (unesterified) fatty acids move through the cell membrane of the adipocyte and bind to plasma albumin. They are transported to the tissues, enter cells, get activated to their CoA derivatives, and are oxidized for energy in mitochondria. Regardless of their levels, plasma FFAs

cannot be used for fuel by red blood cells (RBCs), which have no mitochondria. Brain, too, does not use fatty acids for energy, but the reasons are less clear. [Note: Over 50% of the fatty acids released from adipose TAG are reesterified to glycerol 3-phosphate. Adipose tissue does not express glycerol kinase, and the phosphorylated glycerol is produced by glyceroneogenesis, an incomplete version of gluconeogenesis: pyruvate to OAA via pyruvate carboxylase and OAA to phosphoenolpyruvate (PEP) via phosphoenolpyruvate carboxykinase. The PEP is converted (by reactions common to glycolysis and gluconeogenesis) to DHAP, which is reduced to glycerol 3-phosphate. The process reduces plasma FFAs, molecules associated with insulin resistance in type 2 diabetes and obesity

β-Oxidation of fatty acids

The major pathway for catabolism of fatty acids is a mitochondrial pathway called β1oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and flavin adenine dinucleotide (FADH2).

1. ***Transport of long-chain fatty acids into mitochondria:*** After a LCFA enters a cell, it is converted in the cytosol to its CoA derivative by long-chain fatty acyl CoA synthetase (thiokinase), an enzyme of the outer mitochondrial membrane. Because β-oxidation occurs in the mitochondrial matrix, the fatty acid must be transported across the inner mitochondrial membrane that is impermeable to CoA. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is carnitine, and this rate-limiting transport process is called the “carnitine shuttle”
2. **Reactions of β-oxidation:**

The first cycle of β-oxidation consists of a sequence of four reactions involving the β-carbon (carbon 3) that results in shortening the fatty acid chain by two carbons at the carboxylate end. **The steps include**:

- an oxidation that produces FADH2,
- a hydration step,
- a second oxidation that produces NADH, and
- a thiolytic cleavage that releases a molecule of acetyl CoA.

Each step is catalyzed by enzymes with chain-length specificity. These four steps are repeated for saturated fatty acids of even-numbered carbon chains (n/2) - 1 times (where n is the number of carbons), each cycle producing one acetyl CoA plus one NADH and one FADH2. The acetyl CoA can be oxidized or used in hepatic ketogenesis (see below). The reduced coenzymes are oxidized by the electron transport chain. The final thiolytic cleavage produces two acetyl groups. [Note: Acetyl CoA is a positive allosteric effector of pyruvate carboxylase, thus linking fatty acid oxidation and gluconeogenesis.]

Energy yield from fatty acid oxidation: The energy yield from the β-oxidation pathway is high. For example, the oxidation of a molecule of palmitoyl CoA to CO2 and H2O produces 8 acetyl CoA (8x10=80 ATPs), 7 NADH, and 7 FADH2(4x7=28 ATPs), from which 80+28=108 ATP can be generated. However, activation of the fatty acid requires 2 ATP. Therefore, the net yield from palmitate is 106 ATPs

Oxidation of fatty acids with an odd number of carbons:

This process proceeds by the same reaction steps as that of fatty acids with an even number of carbons, until the final three carbons are reached. This compound, propionyl CoA, is metabolized by a three-step pathway

a. First, propionyl CoA is carboxylated, forming D-methylmalonyl coenzyme A. The enzyme ***propionyl CoA carboxylase*** has an absolute requirement for the coenzyme biotin, as do most other carboxylases.
b. Formation of L-methylmalonyl coenzyme A: Next, the D-isomer is converted to the L-form by the enzyme, methylmalonyl CoA racemase.
c. Synthesis of succinyl coenzyme A: Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the tricarboxylic acid (TCA) cycle. The enzyme methylmalonyl CoA mutase requires a coenzyme form of vitamin B12 (deoxyadenosylcobalamin). The mutase reaction is one of only two reactions in the body that require vitamin B12. [Note: In patients with vitamin B12 deficiency, both propionate and methylmalonate are excreted in the urine. Two types of heritable methylmalonic acidemia and aciduria have been described: one in which the mutase is missing or deficient, and one in which the patient is unable to convert vitamin B12 into its coenzyme form. Either type results in metabolic acidosis and neurologic manifestations.]

Oxidation of unsaturated fatty acids:

The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced. Oxidation of monounsaturated fatty acids, such as 18:1(9) (oleic acid), requires one additional enzyme, ***3,2-enoyl CoA isomerase***, which converts the 3-cis derivative obtained after three rounds of β-oxidation to the ***β*-trans derivative** required as a substrate by the enoyl CoA hydratase. Oxidation of polyunsaturated fatty acids, such as 18:2(9,12) (linoleic acid), requires an NADPH-dependent 2,4-dienoyl CoA reductase in addition to the isomerase

KETONE BODIES: AN ALTERNATE FUEL FOR CELLS

Liver mitochondria have the capacity to convert acetyl CoA derived from fatty acid oxidation into ketone bodies. The compounds categorized as ketone bodies are ***acetoacetate, 3-hydroxybutyrate*** (also called β-hydroxybutyrate), and ***acetone*** (a nonmetabolized side product). Acetoacetate and 3-hydroxybutyrate are transported in the blood to the peripheral tissues. There they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle.

Ketone bodies are important sources of energy for the peripheral tissues because

1) they are soluble in aqueous solution and, therefore, do not need to be incorporated into lipoproteins or carried by albumin as do the other lipids;

2) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and

3) they are used in proportion to their concentration in the blood by extrahepatic tissues, such as the skeletal and cardiac muscle, intestinal mucosa, and renal cortex.

Even the brain can use ketone bodies to help meet its energy needs if the blood levels rise sufficiently. Thus, ketone bodies spare glucose, which is particularly important during prolonged periods of fasting.

A. Synthesis of ketone bodies by the liver(ketogenesis)

During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced by fatty acid oxidation inhibits pyruvate dehydrogenase, and activates pyruvate carboxylase. The OAA produced is used by the liver for

gluconeogenesis rather than for the TCA cycle. Therefore, acetyl CoA is channeled into ketone body synthesis. Additionally, fatty acid oxidation decreases the NAD+ to NADH ratio, and the rise in NADH shifts OAA to malate. This also pushes acetyl CoA into ketogenesis [Note: Acetyl CoA for ketogenesis is also generated by the catabolism of ketogenic amino acids.

1. Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A: The first step, formation of acetoacetyl CoA, occurs by reversal of the thiolase reaction of fatty acid oxidation. Mitochondrial 3-hydroxy-3-methylglutaryl (HMG) CoA synthase combines a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA. HMG CoA synthase is the rate-limiting step in the synthesis of ketone bodies and is present in significant quantities only in the liver. [Note: HMG CoA is also an intermediate in cytosolic cholesterol synthesis. The two pathways are separated by location in, and conditions of, the cell.]

2. Synthesis of the ketone bodies: HMG CoA is cleaved by HMG CoA lyase to produce acetoacetate and acetyl CoA. Acetoacetate can be reduced to form 3-hydroxybutyrate with NADH as the hydrogen donor. Acetoacetate can also spontaneously decarboxylate in the blood to form acetone, a volatile, biologically nonmetabolized compound that can be released in the breath. The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the NAD+/NADH ratio. Because this ratio is low during fatty acid oxidation, 3- hydroxybutyrate synthesis is favored. [Note: The generation of free CoA during ketogenesis allows fatty acid oxidation to continue.]

Use of ketone bodies by the peripheral tissues (ketolysis)

Although the liver constantly synthesizes low levels of ketone bodies, their production becomes much more significant during fasting when

ketone bodies are needed to provide energy to the peripheral tissues. 3-Hydroxybutyrate is oxidized to acetoacetate by 3-hydroxybutyrate dehydrogenase, producing NADH. Acetoacetate is then provided with a CoA molecule taken from succinyl CoA by succinyl CoA:acetoacetate CoA transferase (thiophorase). This reaction is reversible, but the product, acetoacetyl CoA, is actively removed by its conversion to two acetyl CoAs. This pulls the reaction forward. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, RBCs), efficiently oxidize acetoacetate and 3- hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks thiophorase and, therefore, is unable to use ketone bodies as fuel.

Excessive production of ketone bodies in diabetes mellitus

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and, eventually, in the urine (ketonuria). This is seen most often in cases of uncontrolled type 1 diabetes mellitus. In diabetic individuals with severe ketosis, urinary excretion of the ketone bodies may be as high as 5,000 mg/24 hr, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dl in normal individuals). A frequent symptom of diabetic ketoacidosis (DKA) is a fruity odor on the breath, which results from increased production of acetone. An elevation of the ketone body concentration in the blood results in acidemia. Each ketone body loses a proton (H+) as it circulates in the blood, which lowers the pH. Also, in diabetic ketoacidosis, urinary loss of glucose and ketone bodies results in dehydration. Therefore, the increased number of H+ circulating in a decreased volume of plasma can cause severe acidosis (ketoacidosis).] Ketoacidosis may also be seen in cases of prolonged fasting and excessive ethanol consumption

Study Questions

1.

4-month-old child is being evaluated for fasting hypoglycemia. Laboratory tests at admission reveal low levels of ketone bodies, free carnitine, and acylcarnitines in the blood. Free fatty acid levels in the blood were elevated. Deficiency of which of the following would best explain these findings?

A. Adipose triglyceride lipase

B. Carnitine transporter

C. Carnitine palmitoyltransferase I

D. Long-chain fatty acid dehydrogenase

Correct answer = B. A defect in the carnitine transporter (primary carnitine deficiency) would result in low levels of carnitine in the blood (as a result of increased urinary loss) and low levels in the tissues. In the liver, this decreases fatty acid oxidation and ketogenesis. Consequently, blood levels of free fatty acids rise. Deficiencies of adipose triglyceride lipase would decrease fatty acid availability. Deficiency of carnitine palmitoyltransferase I would result in elevated blood carnitine. Defects in any of the enzymes of β-oxidation would result in secondary carnitine deficiency, with a rise in acylcarnitines.

2.

A teenager, concerned about his weight, attempts to maintain a fat-free diet for a period of several weeks. If his ability to synthesize various lipids were examined, he would be found to be most deficient in his ability to synthesize:

A. cholesterol.

B. glycolipids.

C. phospholipids.

D. prostaglandins.

E. triacylglycerol.

Correct answer = D. Prostaglandins are synthesized from arachidonic acid. Arachidonic acid is synthesized from linoleic acid, an essential fatty acid obtained by humans from dietary lipids. The teenager would be able to synthesize all other compounds but, presumably, in somewhat decreased amounts.