BIOC 462b – Dr. Tischler
SYNTHESIS OF FATTY ACIDS, TRIACYLGLYCEROL, PHOSPHOLIPIDS

Text: pages 343-351; 805-817; 820-831
Sample Problems: see Exam 4 sample problems on exam page of course web site
Recommended Problems to Solve: 21-2; 21-3; 21-7

Objectives:

1. Outline the process by which acetyl groups are shuttled from the mitochondria to the cytoplasm for lipogenesis and how this also contributes NADPH for lipogenesis.

2. Describe the general mechanism of the fatty acid synthase reaction

3. Discuss the regulation of lipogenesis, especially of acetyl CoA carboxylase through polymerization-depolymerization and covalent modification, and consider how this regulation applies to physiological conditions such as consumption of carbohydrates, intake of a high fat diet and during food deprivation.

4. Outline the sources of glycerol-3-phosphate for formation of triacylglycerols and phospholipids including the role of glyceroneogenesis in the process.

GENERAL STRUCTURES OF LIPIDS (Fig. 1 – for review purposes only)

Fatty acids lacking any double bonds are termed saturated. The most common mammalian saturated fatty acid is palmitic acid (Fig. 1), which contains 16 carbons. This is the primary form in which fats are stored as triacylglycerol. Once double bonds are introduced into the hydrocarbon chain, then the fatty acids become either monounsaturated (e.g., palmitoleic acid) or polyunsaturated (e.g., linoleic acid and \( \alpha \)-linolenic acid). The coding for the fatty acids is illustrated in figure 1 with \( \Delta \) referring to the presence of one or more double bonds and the superscript indicating at which carbon(s) the double bonds commence. From a nutritional standpoint there is much conjecture about the importance of omega-3 and omega-6 fatty acids in the diet. The term omega-3 refers to a polyunsaturated fatty acid that, counting from the methyl end of the molecule, has a double bond in the 3\textsuperscript{rd} carbon position (e.g., \( \alpha \)-linolenic acid) whereas an omega-6 fatty acid has this first double bond, from the methyl end, at the 6\textsuperscript{th} carbon position (linoleic acid). These latter fatty acids are important precursors for the eventual synthesis of eicosanoid hormones discussed in the next lecture. Typically unsaturated fatty acids are incorporated into phospholipids, on the middle carbon of the glycerol backbone, rather than stored as triacylglycerol. While omega-3 and omega-6 fatty acids offer health benefits, the opposite is true for trans fatty acids in which the double bonds are in a ‘trans’ rather than a ‘cis’ configuration. The nature of the headgroup on the phospholipid can confer various functions, as discussed later in the notes. Sphingolipids are a unique group of membrane lipids that like phospholipids have a polar head group and two nonpolar tails. However, unlike phospholipids they lack a glycerol backbone and instead contain a sphingosine backbone. The polar head group of sphingolipids may have a sugar instead of a phosphate and are termed glycolipids. The simplest sphingolipid is ceramide, containing a hydrogen atom in the R (head group) position. The other sphingolipids build on this core molecule. Sphingomyelin, found mostly in membranes of erythrocytes and neurons, contains phosphocholine as its head group. Consequently, sphingomyelin is also classified as a phospholipid. In myelin, the most common fatty acids in sphingomyelin have very long chains (e.g., 24 carbons) whereas in grey matter the principal fatty acids are 16 or 18 carbons in length.
saturated fatty acid: \( \text{CH}_3-(\text{CH}_2)_{14}-\text{COOH} \)  palmitic acid

unsaturated fatty acid: \( \text{CH}_3-(\text{CH}_2)_{7}-\text{CH}=(\text{CH}_2)_{7}-\text{COOH} \)  palmitoleic acid – 16:1 (\( \Delta^9 \))

polyunsaturated fatty acid: \( \text{CH}_3-(\text{CH}_2)_{4}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_{7}-\text{COOH} \)
linoleic acid – 18:2 (\( \Delta^9,12 \)) (omega-6)

\( \text{CH}_3-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_{7}-\text{COOH} \)
\( \alpha \)-linolenic acid – 18:2 (\( \Delta^9,12,15 \)) (omega-3)

CH\(_2\)-OOC-Fatty acid\(_1\)  \( \rightarrow \)  CH\(_2\)-OOC-Fatty acid\(_1\)  (saturated)

Fatty acid\(_2\)-COO-CH  \( \rightarrow \)  Fatty acid\(_2\)-COO-CH  (unsaturated)

\( \text{CH}_2\)-OOC- Fatty acid\(_3\)  \( \rightarrow \)  CH\(_2\)-OPO\(_3\)-X  (head group)

**Figure 1.** General structures of fatty acids, triacylglycerol, phospholipids and sphingolipids.

**FATTY ACID SYNTHESIS (LIPOGENESIS) AND ACTIVATION OF FATTY ACIDS:**

*Generation and transport of carbons for lipogenesis: (Fig. 2)*

The overall balance of carbon cycling and NADPH production for lipogenesis is depicted in Fig. 2 and can be expressed as follows.

\[
\text{Acetyl CoA}_{\text{mitochondria}} + 2 \text{ ATP} + \text{NADH} + \text{NADP}^+ \rightarrow \\
\text{Acetyl CoA}_{\text{cytoplasm}} + 2 \text{ ADP} + 2 \text{ P}_i + \text{NAD}^+ + \text{NADPH}
\]

This equation simply states that carbons from acetyl CoA in the mitochondria end up in the cytoplasm for lipogenesis at the cost of 2 ATP and with the transfer of reducing equivalents (electrons) from NADH to NADPH. The two ATP are consumed in the pyruvate carboxylase and citrate lyase reactions.

Technically, any compound metabolized to acetyl CoA can serve as a precursor for fat synthesis. In adipose tissue, glucose is the most important, though dietary leucine may contribute as well. In liver, glucose and certain amino acids (those whose catabolism generate acetyl CoA, except leucine) are precursors. Although acetyl CoA is required for fatty acid synthesis, it is produced in the mitochondria from the metabolism of pyruvate via pyruvate dehydrogenase (PDH) as discussed in an earlier lecture.
pyruvate + NAD + coenzyme A (CoA) → acetyl CoA + CO₂ + NADH  

**pyruvate dehydrogenase**

Because the pool of coenzyme A in the mitochondria remains separate from the pool in the cytoplasm, acetyl CoA is not transported directly across the membrane. Instead citrate carries these carbons from the mitochondria to the cytoplasm. This is a similar event as occurs for cholesterol biosynthesis, to be discussed in the next lecture.

**Figure 2.** Export of acetyl CoA as citrate for fatty acid biosynthesis, generation of NADPH and pathway of lipogenesis.

Pyruvate is produced from glucose via glycolysis (see earlier lecture) and then transported into the mitochondrial matrix via **pyruvate translocase**. In the mitochondrial matrix, pyruvate is converted either to acetyl CoA (Fig. 2, PDH) or to oxaloacetate (via pyruvate carboxylase; Fig. 2, PC) in equal amounts.

\[
\text{pyruvate + CO}_2 + \text{ATP} \rightarrow \text{oxaloacetate + ADP + P}_i
\]

**pyruvate carboxylase**

The opposite regulation of PDH and PC permits this to occur. While PDH is inhibited by excess amounts of acetyl CoA, PC is activated by acetyl CoA. The products of these reactions combine to form citrate in the citric acid cycle via **citrate synthase** (CS).

Acetyl CoA + oxaloacetate → citrate

**citrate synthase**
The high concentration of citrate favors its transport out of the mitochondrial matrix via the \textit{tricarboxylate translocase}, driven by the concentration gradient of citrate (Fig. 2). In the cytoplasm citrate is cleaved by \textbf{citrate lyase (CL)} producing acetyl CoA, for lipogenesis, and oxaloacetate as products. The reaction requires energy obtained by hydrolysis of ATP.

\[
\text{Citrate} + \text{ATP} + \text{coenzyme A} \rightarrow \text{acetyl CoA} + \text{oxaloacetate} + \text{ADP} + \text{P}_i
\]

\textit{citrate lyase}

To recycle carrier carbons, oxaloacetate is reduced to malate via a cytoplasmic \textbf{malate dehydrogenase (MDH)} with NADH providing the reducing power.

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{malate} + \text{NAD}^+
\]

\textit{malate dehydrogenase}

Malate is then oxidized by NADP\(^+\) in a reaction catalyzed by \textbf{malic enzyme (ME)} that generates NADPH for lipogenesis.

\[
\text{Malate} + \text{NADP}^+ \rightarrow \text{Pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+
\]

\textit{malic enzyme}

The pyruvate product enters the mitochondria again to be available for synthesis of oxaloacetate. Thus oxaloacetate from the citric acid cycle is never consumed in this overall process of carbon transport.

\textit{Key enzymes in lipogenesis:}

\[
\text{acetyl CoA} + \text{HCO}_3^- + \text{ATP}^+ \rightarrow \text{malonyl CoA} + \text{ADP}^3^- + \text{Pi}^2^- \quad \textit{acetyl CoA carboxylase}
\]

\[
\text{acetyl CoA} + 7 \text{malonyl CoA} + 14 \text{NADPH} + 14 \text{H}^+ \rightarrow \text{palmitate} + 7 \text{CO}_2 + 8 \text{CoA} + 14 \text{NADP}^+ \quad \textit{fatty acid synthase}
\]

\textbf{Acetyl CoA carboxylase} is the committed step for fat biosynthesis. This enzyme is highly regulated (see below). The purpose of the reaction is to produce malonyl CoA that is responsible for providing two of its three carbons for elongating the fatty acid chain being synthesized by fatty acid synthase. While in animals this enzyme is a single polypeptide chain, in bacteria it contains three subunits.

\textbf{Fatty acid synthase} is a homodimeric enzyme consisting of multiple enzyme activities. In animals it is located in the cytoplasm whereas in plants it is found in the chloroplasts as these are the site of NADPH formation during photosynthesis.
Figure 3. General mechanism for the fatty acid synthase reaction. Steps 1 through 4 are the first cycle that generates a 4-carbon intermediate. The next 6 cycles repeat steps 5 through 8 with palmitate released as the final product (step 9). CE = condensing enzyme; ACP = acyl carrier protein.

ACP = acyl carrier protein
CE = condensing enzyme
Two components of the fatty acid synthase complex are the condensing enzyme (CE) also known as the β-ketoacyl ACP synthase and the acyl carrier protein (ACP) both of which contain free sulfhydryl groups (-SH) (Fig. 3). The SH group on ACP is derived from phosphopantetheine; thus ACP substitutes for CoA. ACP and the condensing enzyme are adjacent placing the two –SH groups in close proximity. The initial sequence of the reaction results in attachment of the acetyl group from acetyl CoA to the CE as a primer and ultimately the last two carbons of the hydrocarbon tail. The malonyl group from malonyl CoA attaches to ACP (Fig. 3, step 1). The acetyl group from the CE then condenses (catalyzed by β-ketoacyl ACP synthase) with the malonyl residue on ACP causing release of CO₂ and leaving a 4-carbon intermediate covalently bound to ACP (step 2). Finally in a series of three reactions that involve a reduction using NADPH (catalyzed by β-ketoacyl ACP reductase), a dehydration step (catalyzed by β-ketoacyl ACP dehydratase) and a second reduction reaction using NADPH (catalyzed by enoyl ACP reductase), a 4-carbon fatty acid is formed (step 3). This 4-carbon unit is then transferred to the CE site (step 4). An additional two-carbon unit is added to the acyl chain, by attachment of another molecule of malonyl CoA to the vacated site on ACP (step 5). Because the complex exists as a homodimer, two fatty acid chains can be synthesized concurrently in the complex. The process continues through six cycles that include condensation of the growing acyl chain from CE with the new malonyl group on ACP (step 6), multiple reduction of this product to form a new acyl chain that is two carbons longer (step 7) and the transfer of this acyl chain back to the CE site (step 8). Steps 6 through 8 continue until palmitate is formed and is released from the ACP site to the CE site (step 9). The final removal of palmitate, from the CE site, is catalyzed by thioesterase I, which cleaves off long chain fatty acids with 16 or more carbons. A total of seven cycles are required to form palmitate with the oxidation of 14 molecules of NADPH (2 per cycle). Note that the product of lipogenesis is the free fatty acid NOT the fatty acyl CoA form.

Sources of NADPH for fatty acid synthase:

- **malic enzyme**: Malate + NADP⁺ ---> Pyruvate + CO₂ + NADPH
- **pentose phosphate pathway**: Glucose-6-P + 2 NADP⁺ ---> Ribulose-5-P + 2 NADPH + CO₂

Malic enzyme in the cytoplasm is a source of NADPH and is active during lipogenesis, as noted above. To produce palmitate, eight molecules of acetyl CoA are required to generate the 16 carbons. These acetyl CoA molecules require the cleavage of 8 molecules of citrate, so that flux through malic enzyme will produce 8 molecules of NADPH, about 50% of the requirement for palmitate synthesis. NADPH also is generated in the pentose phosphate pathway from glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as discussed previously by Dr. Tsao.

Activation and further processing of fatty acids:

The primary product of the synthase complex is palmitic acid, NOT palmitoyl CoA. The coenzyme A derivative of palmitic acid is formed by a separate enzyme, acyl CoA synthetase. This enzyme is general for all long chain fatty acids. Note that two high-energy bonds are consumed for activation of each fatty acid since the pyrophosphate product (PP_i) is hydrolyzed.

palmitate + ATP + CoA ➔ palmitoyl CoA + AMP + PP_i

Palmitoyl CoA can undergo chain elongation and desaturation to produce long-chain unsaturated fatty acids. Elongation is a microsomal process (endoplasmic reticulum), requiring malonyl CoA and two NADPH in a process similar to that occurring on fatty acid synthase. Desaturation is also a microsomal process. Reducing power is derived from NADH or NADPH and molecular oxygen is required as well.
Immediate oxidation of newly synthesized fatty acyl CoA is prevented by the inhibitory effect of malonyl CoA on carnitine palmitoyl transferase I to prevent formation of the palmitoyl carnitine derivatives needed for transport into mitochondria.

**Regulation of lipogenesis:**

**Polymerization-depolymerization of acetyl CoA carboxylase:** Acetyl CoA carboxylase is fully active in its polymerized state, which contains 3 or more subunits (Fig. 4, [1]) compared to its monomer state [2]. The monomer retains a low activity, but only when it is not phosphorylated (see below). The polymerized state only exists in a non-phosphorylated form since phosphorylation causes depolymerization and inactivation [3]. Citrate is an allosteric activator whose binding causes a conformational change in monomers that facilitate their aggregation and hence activation of the enzyme ([4]). As you learned above (Fig. 2), citrate is transported to the cytoplasm during lipogenesis. Hence this effect of citrate on acetyl carboxylase is logical. In contrast during other conditions such as a high fat diet or when lipolysis is ongoing, palmitoyl CoA is abundant and inhibits lipogenesis. It does so by allosterically binding to the carboxylase causing the polymer to destabilize and dissociate to the individual monomers [5]. This latter effect represents "feedback" control as a signal for abundant levels of long-chain fatty acids.

**Covalent modification (phosphorylation-dephosphorylation):** When the concentration of glucose is low in the blood, its uptake and processing both by liver and fat cells is greatly diminished. Consequently, glucose becomes unavailable as a precursor for fatty acid synthesis and lipogenesis decreases. Glucagon causes inactivation of acetyl-CoA carboxylase by initiating a sequence of events that leads to phosphorylation of the enzyme. This sequence includes activation of cyclic AMP-dependent protein kinase A which either directly or via another kinase causes phosphorylation of the carboxylase and hence inactivation (Fig. 4, [6]).

Additionally, AMP-dependent protein kinase (AMPK) phosphorylates acetyl-CoA carboxylase [7]. Under conditions of low energy (low [ATP]/[AMP]) fat synthesis is undesirable because it is an energy consuming process. This low energy ratio leads to activation of AMP protein kinase by phosphorylation of this enzyme. A high ratio of [ATP]/[AMP] favors the inactive form of AMPK.

In the fed state when glucose is in excess, lipogenesis is favored largely through the effects of insulin, a hormone whose function is to promote fuel synthesis and storage. Insulin acts at several levels. 1) Insulin causes activation of citrate lyase to increase the availability of substrate (acyl CoA) for the acetyl CoA carboxylase (not shown). 2) Insulin activates protein phosphatase that catalyzes the direct dephosphorylation of acetyl CoA carboxylase so that along with binding of citrate allows for polymerization. 3) Protein phosphatase also dephosphorylates AMPK so that it is inactivated and unable to rephosphorylate the carboxylase. 4) Insulin activates phosphodiesterase that catalyzes the degradation of cyclic AMP so that protein kinase A becomes inactive (not shown). Thus these coordinated events work in concert to promote lipogenesis.
Figure 4. Regulation of acetyl CoA carboxylase

**TRIACYLGLYCEROL FORMATION:**

Palmitoyl CoA is incorporated into acylglycerols via esterification. Phosphatidic acid, the intermediate common to synthesis of triacylglycerol and phospholipid, is formed from glycerol-3-phosphate (Fig. 5). Glycerol-3-phosphate is produced from phosphorylation of glycerol via glycerol kinase, or from dihydroxyacetone phosphate (DHAP) derived from glycolysis. Adipose cells lack glycerol kinase, so that DHAP is the only source of carbon backbones in this tissue. However adipose cells also can produce DHAP from pyruvate via glyceroneogenesis(Fig. 6). Phosphatidic acid contains two fatty acyl side-chains. Triacylglycerol is formed by removal of the phosphate side group from the backbone followed by addition of a third fatty acyl group (Fig. 5). Phospholipids are formed by addition of polar head groups to the phosphate moiety of phosphatidic acid.
Figure 5. Formation of phosphatidic acid from glycerol-3-phosphate or dihydroxyacetone phosphate, and its conversion to triacylglycerol.

Figure 6. Glyceroneogenesis from pyruvate. The 5 steps not shown are identical to those in gluconeogenesis and include in sequence: enolase, phosphoglycerate mutase, phosphoglycerate kinase (using ATP), glyceraldehydes-3-phosphate dehydrogenase (using NADH) and triose phosphate isomerase (glyceraldehyde-3-P to DHAP).

PHOSPHOLIPIDS:
(see text figures 21-25 to 21-29 if you have a burning desire to see the chemistry of these pathways)

The most prevalent class of lipids in membranes is the phospholipids. Most phospholipids are similar to triacylglycerols in that their backbone is derived from glycerol. Phospholipids include: 1) phosphatidic acid, 2) phosphatidylcholine, 3) phosphatidylethanolamine, 4) phosphatidylinositol, 5) phosphatidylserine, 6) plasmalogens, and 7) sphingomyelin. Except for sphingomyelin, all other phospholipids are phosphoglycerides. Though sphingomyelin contains phosphate and therefore is classified as a phospholipid, its backbone is sphingosine rather than glycerol (see Figure 1). Phosphoglycerides contain
two fatty acyl chains esterified to the middle and terminal carbons of glycerol (see Fig. 1). The third carbon of glycerol is esterified to a phosphate group, which is esterified to a head group on the other side, forming a phosphodiester. In the absence of a head group, the phospholipid is called phospha
tid acid (see Figure 5).

Metabolism of phosphatidylethanolamine and phosphatidylserine

The pathway for synthesis of phosphatidylethanolamine occurs in the endoplasmic reticulum by condensation between diacylglycerol and ethanolamine. Phosphatidylserine is formed by the exchange of serine for ethanolamine on phosphatidyl-ethanolamine in a reversible reaction. Phosphatidyl-ethanolamine is one of the two most common phospholipids found in bilayer membranes. Along with phosphatidylserine it is primarily found in the inner leaflet of membranes.

Metabolism of lecithin (phosphatidylcholine)

Lecithin is synthesized primarily by the addition of choline to diacylglycerol. The second reaction in this sequence, phosphocholine cytidylyltransferase (PCCT), is the rate-limiting reaction for the formation of phosphatidylcholine and is regulated using a novel mechanism. PCCT is stored in the cytoplasm where it is inactive but on binding to the endoplasmic reticulum membrane becomes activated. An additional pathway for formation of phosphatidylcholine from phosphatidylethanolamine includes three successive methylations in liver and developing brain (during myelination). The source of the methyl groups is S-adenosylmethionine (SAM), a methyl carrier produced from methionine. Lecithin is the most abundant phospholipid found in cell membranes. It contains a large portion of the body's pool of choline, which is important in nerve transmission as a precursor to acetylcholine.

Dipalmitoyl lecithin, as its name suggests, bears two palmitic acid (16 carbons) molecules on carbons 1 and 2 of the glycerol backbone. This phospholipid is a major component (>80%) of lung surfactant. Surfactant, produced by epithelial cells in the lungs, is essential for stopping adherence of the fluid layers of alveoli in the lungs by breaking the surface tension. For obvious reasons, the fetus does not require surfactant until near the end of gestation. When an infant is born prematurely, the epithelial cells do not produce sufficient amounts of lecithin. The deficiency of lecithin in preterm newborns leads to respiratory distress syndrome (RDS).

Formation of phosphatidylinositol and cardiolipin via CDP-diacylglycerol:

In the formation of phosphatidylinositol, phosphatidic acid is activated by formation of CDP-diacylglycerol (CDP-DAG). CDP-DAG then reacts with inositol to yield phosphatidyl-inositol. Phosphatidylinositol-4,5-bisphosphate (PIP₂) is produced from phosphatidylinositol by further phosphorylation. In response to hormone signals, PIP₂ is cleaved by phospholipase C to yield diacylglycerol and inositol trisphosphate, two important second messengers in signal transduction. Additionally, phosphatidylinositol serves as an anchor for glycoproteins to the plasma membrane.

CDP-DAG also is needed for the synthesis of cardiolipin, a phospholipid found exclusively in mitochondrial membranes. Cardiolipin is essential for the functioning of cytochrome oxidase and the phosphate transporter in the mitochondrial inner membrane, thereby facilitating oxidative phosphorylation.
SPHINGOLIPID METABOLISM: (informational; not to be tested)

Sphingolipids can contain carbohydrates instead of phosphocholine and are classified as cerebrosides, globosides and gangliosides. These glycolipids are especially found in nervous tissue and are positioned on the outside of the plasma membrane to provide cell surface carbohydrates. The carbohydrates of membrane glycolipids, along with glycoproteins, are important in cell-cell recognition (antigens) and to protect lipids and proteins from lipases and proteases.

Cerebrosides

Cerebrosides, the simplest glycolipid, contain a single hexose (galactose or glucose). Galactocerebrosides (galactosylceramides) are mostly found in the brain. Glucocerebrosides (glucosylceramides) are located mostly in extraneural tissues, though they usually are found as an intermediate in the synthesis or degradation of globosides or gangliosides.

Globosides and gangliosides

Globosides and gangliosides constitute the more complex glycolipids, as both groups contain polysaccharide chains. The sugar residues include glucose, galactose, N-acetylgalactosamine and fucose. The notable difference between the two groups is that gangliosides also contain terminal sialic acid residues that are lacking on globosides. Gangliosides are in high concentration in ganglions of the CNS (hence their terminology). Gangliosides are identified by unique codes (e.g., G₃_M1) with subscripts of M, D, T, or Q indicating the number of sialic acid residues (1 to 4, respectively). The number in the subscript codes for a specific carbohydrate sequence. For instance subscript 3 refers to a galactose-glucose-attachment. One function of gangliosides is serving as cell surface receptors. For instance G₃_M1 in human intestinal mucosal cells is a receptor for cholera toxin, which is a protein produced by Vibrio cholerae.