LIPID METABOLISM-4

Biosynthesis of Fatty Acids

- Fatty acid synthesis occurs in the liver and lactating mammary glands, primarily.
- It also occurs in adipose tissue, to a lesser extent.
- Carbohydrates and proteins obtained from the diet in excess of the body's needs can be converted to fatty acids (which are stored as triacylglycerols).

- This cytosolic process incorporates carbons from acetyl coenzyme A (CoA) into the growing fatty acid chain.
- This process needs energy (ATP) and NADPH.

- Nearly all the acetyl-CoA used in fatty acid synthesis is formed in mitochondria from pyruvate oxidation and from the catabolism of the carbon skeletons of amino acids.
- The mitochondrial inner membrane is impermeable to acetyl-CoA, so an indirect shuttle transfers acetyl group equivalents across the inner membrane.

- Intramitochondrial acetyl-CoA first reacts with oxaloacetate to form citrate, in the citric acid cycle reaction catalyzed by **citrate synthase**.
- Citrate then passes through the inner membrane on the **citrate transporter**.
- In the cytosol, citrate cleavage by **citrate lyase** regenerates acetyl-CoA and oxaloacetate in an ATP-dependent reaction.

- Oxaloacetate cannot return to the mitochondrial matrix directly, as there is no oxaloacetate transporter.
- Instead, cytosolic malate dehydrogenase reduces the oxaloacetate to malate, which can return to the mitochondrial matrix on the malate–alpha-ketoglutarate transporter in exchange for citrate. In the matrix, malate is reoxidized to oxaloacetate to complete the shuttle.

- However, most of the malate produced in the cytosol is used to generate cytosolic NADPH through the activity of malic enzyme.
- The pyruvate produced is transported to the mitochondria by the pyruvate transporter, and converted back into oxaloacetate by pyruvate carboxylase in the matrix.
- The resulting cycle results in the consumption of two ATP (by citrate lyase and pyruvate carboxylase) for every molecule of acetyl-CoA delivered to fatty acid synthesis.

- After citrate cleavage to generate acetyl-CoA, conversion of the four remaining carbons to pyruvate and CO_2 via malic enzyme generates about half the NADPH required for fatty acid synthesis.
- The pentose phosphate pathway provides the rest of the needed NADPH.

- The process of translocation of citrate from the mitochondrion to the cytosol occurs when the mitochondrial citrate concentration is high.
- This is observed when isocitrate dehydrogenase 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.

- For fatty acid synthesis to be started, malonyl-CoA is needed to be synthesized, first.
- The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by **acetyl-CoA carboxylase (ACC)**. This reaction needs HCO_3^-, biyotin and ATP to proceed.
- Carboxyl group transferred to acetyl-CoA is bound to the biotin and biotin is bound to the enzyme.

- This carboxylation is both the rate-limiting and the regulated step in fatty acid synthesis.
- The inactive form of ACC is a dimer.
- The enzyme undergoes allosteric activation by citrate, which causes dimers to polymerize, and allosteric inactivation by palmitoyl CoA (the end product of the pathway), which causes its depolymerization.

- AMP-activated protein kinase (AMPK) phosphorylates and inactivates ACC.
- AMPK itself is allosterically activated by AMP and covalently activated by phosphorylation via several kinases.
- Thus, in the presence of counterregulatory hormones, such as epinephrine and glucagon, ACC is phosphorylated and, thereby, inactivated.
- In the presence of insulin, ACC is dephosphorylated and, thereby, activated.

- The remaining series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the multifunctional, dimeric enzyme, fatty acid synthase (FAS).
- Each FAS monomer is a multicatalytic polypeptide with seven different enzymic activities plus a domain (ACP; acyl carrier protein) which carries acyl units on its terminal thiol (–SH) group during fatty acid synthesis.

- Seven enzymes present on the seven catalytic domains of the FAS sequentially catalyze the reactions of fatty acid synthesis:

1. Acetyl transacylase,
2. Malonyl transacylase,
3. Ketoacyl synthase,
4. Ketoacyl reductase,
5. Hydroxyacyl dehydratase,
6. Enoyl reductase,
7. (Palmitoyl) thioesterase

- First, a molecule of acetate is transferred from acetyl CoA to the –SH group of the ACP (AT).
- Next, this two-carbon fragment is transferred to a temporary holding site, the thiol group of a cysteine residue on the enzyme.
- The now-vacant ACP accepts a three-carbon malonate unit from malonyl CoA (MT).
- 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 (KS).

- The next three reactions convert the 3-ketoacyl group to the corresponding saturated acyl group by a pair of reductions requiring NADPH and a dehydration step.
  - The keto group is reduced to an alcohol **(KR)**.
  - A molecule of water is removed to introduce a double bond between carbons 2 and 3 (the α- and β-carbons) **(HD)**.
  - The double bond is reduced **(ER)**.

- 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.
- These seven steps are repeated, beginning with the transfer of the butyryl chain from the ACP to the Cys 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.
- Palmitoyl thioesterase cleaves the thioester bond, releasing a fully saturated molecule of **palmitate** (16:0).

- If fatty acid synthesis and oxidation were to proceed simultaneously, the two processes would constitute a futile cycle, wasting energy.
- As we noted earlier that oxidation is blocked by malonyl-CoA, which inhibits carnitine acyltransferase I.
- Thus during fatty acid synthesis, the production of the first intermediate, malonyl-CoA, shuts down oxidation at the level of a transport system in the mitochondrial inner membrane.

Sources of the NADPH required for fatty acid synthesis

- The hexose monophosphate pathway is the major supplier of NADPH 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.

Further elongation of fatty acid chains

- Although palmitate, a 16-carbon, fully saturated long-chain length fatty acid (16:0), is the primary end product of fatty acid synthase activity, it can be further elongated by the addition of two-carbon units in the smooth endoplasmic reticulum (SER).
- Elongation requires a system of separate enzymes rather than a multifunctional enzyme.
- Malonyl CoA is the two-carbon donor and NADPH supplies the electrons.

- The more active elongation system of the ER extends the 16-carbon chain of palmitoyl-CoA by two carbons, forming stearoyl-CoA (18 C).
- The brain has additional elongation capabilities, allowing it to produce the very-long-chain fatty acids (over 22 carbons) that are required for synthesis of brain lipids.

Desaturation of fatty acid chains

- Enzymes (desaturases) also present in the SER are responsible for desaturating long-chain fatty acids (that is, adding cis double bonds).
- The desaturation reactions require NADH, cytochrome b5 and its FAD-linked reductase.
- The first double bond is typically inserted between carbons 9 and 10, producing primarily 18:1(9) and small amounts of 16:1(9).

- A variety of polyunsaturated fatty acids can be made through additional desaturation combined with elongation.
- Humans have carbon 9, 6, 5 and 4 desaturases, but lack the ability to introduce double bonds from carbon 10 to the ω end of the chain. This is the basis for the nutritional **essentiality** of the polyunsaturated **linoleic and linolenic acids**.