Lipid Biosynthesis

Objectives:
I. Describe how excess carbohydrate and/or amino acid consumption leads to fatty acid and triacylglycerol production.
II. What is the precursor of fatty acid biosynthesis (lipogenesis)?
   A. Where is it generated?
   B. How is it transported to the site of fatty acid biosynthesis?
   C. What other necessary precursors can be / are generated as part of the transport process?
III. Describe how the precursor is activated for the biosynthesis pathway.
IV. The Fatty Acid Biosynthesis Complex.
   A. Describe the Fatty Acid Biosynthesis Complex.
   B. Describe the six recurring reactions of fatty acid biosynthesis.
   C. What coenzymes are required for lipogenesis
   D. What is the final product of the Fatty Acid Biosynthesis Complex?
V. What other reactions are necessary for the complete biosynthesis of the fatty acids needed by the cell?
VI. Compare / Contrast β-oxidation of fatty acids and fatty acid biosynthesis.
   A. State at least three differences between lipogenesis (fatty acid synthesis) and β oxidation.
VII. Describe the control points of fatty acid biosynthesis.
   A. Allosteric control
   B. Control by Reversible Covalent Modification
   C. Hormonal control
      1. How does the control of lipogenesis integrate with the control of β-oxidation?
      2. What is the Triacylglycerol Cycle and Glyceroneogenesis?
VIII. Integrate fatty acid biosynthesis with carbohydrate metabolism.
   A. Describe the regulation of lipid and carbohydrate metabolism in relation to the liver, adipose tissue, and skeletal muscle
   B. Summarize the antagonistic effects of glucagon and insulin.
IX. Describe the synthesis of
   A. Phosphatidate.
   B. The Triacylglycerols.
   C. The Phosphoglycerides.
   D. Sphingosine / Ceramide.
X. Cholesterol Biosynthesis.
   A. In general terms, describe the synthesis of cholesterol.
   B. What is the precursor of cholesterol (cholesterologenesis/steroidalgenesis)?
      1. Where is it generated?
      2. How is it transported to the site of cholesterol biosynthesis?
   C. Describe how the initial steps of cholesterol synthesis are similar to ketone body synthesis.
   D. Describe how the initial steps of cholesterol synthesis differ from ketone body synthesis.
   E. What other isoprenes necessary for normal cellular function are synthesized by the cholesterol biosynthesis pathway?
   F. What is the control point(s) of cholesterol biosynthesis?
   G. How is cholesterol biosynthesis controlled?
XI. Ask yourself “What If Questions”; e.g., Why does an individual with prolonged excess calorie intake but low cholesterol intake have high cholesterol levels?

Background

Fatty acid biosynthesis occurs when the body is energy rich. Postprandial, ATP is generated by glycolysis and the final common pathways. NADPH is generated from glucose by the pentose phosphate pathway. The positive allosteric effects of xylulose-5-phosphate on Phosphoprotein Phosphatase 2A (this phosphatase removes phosphate from Phosphofructokinase-2, simulating the kinase activity and inhibiting the phosphatase activity) assure that the rate of pyruvate and ATP generation (glycolysis, TCA, & ET/OxPhos) matches the rate of NADPH production (Pentose Phosphate Pathway), these products are needed for biosynthesis, cell growth, and cell division. As much glucose as possible is stored as glycogen. The amino acid pool is restocked by the amino acids absorbed from the meal. These amino acids are used to synthesize proteins and other nitrogen containing compounds. Dietary lipids are transported from the gut and stored in the tissues, especially the adipose, or they are used to refurbish cell membranes. Any glucose left over as well as any amino acids in excess of those needed to restock the amino acid pool and/or for the other anabolic pathways are converted to fatty acids and stored as triacylglycerols.

Fatty Acid Biosynthesis

The majority of fatty acid biosynthesis occurs in the cytosol of the liver. The fatty acids synthesized in the liver are used for triacylglycerol, phosphoglyceride, and sphingolipid synthesis. These products are bundled in VLDL’s and transported to the other tissues. Adipose tissue also produces a reasonable amount of fatty acids. Other tissues have an extremely limited capacity for fatty acid biosynthesis, the products used primarily for membrane biosynthesis and remodeling.

The precursor for fatty acid biosynthesis is acetyl-CoA and fatty acids are assembled from this acetyl-CoA two carbons at a time. Acetyl-CoA as the precursor explains why the majority of fatty acids in nature contain an even number of carbon atoms. Acetyl-CoA for fatty acid biosynthesis comes predominantly from glucose and amino acid metabolism. Using acetyl-CoA obtained from fatty acids via β-oxidation is a futile cycle. In eukaryotes, a small amount of acetyl-CoA is generated in the cytosol by amino acid catabolism. The majority of acetyl-CoA is generated in the matrix of the mitochondria by the pyruvate dehydrogenase complex. The pyruvate to fuel this complex comes from glycolysis and amino acid catabolism. How does the acetyl-CoA generated in the mitochondria get into the cytoplasm for fatty acid biosynthesis since there is no transporter for carboxylic acids coupled to acetyl-CoA in the inner mitochondrial membrane. The acetyl-CoA gets into the cytoplasm as citrate.

Transport → The Citrate Shuttle

Citrate is transported out of the mitochondria via a specific transport protein. Once in the cytoplasm the citrate is cleaved to acetyl-CoA and oxaloacetate by the enzyme Citrate Lyase (ATP-Citrate Lyase). The acetyl-CoA formed in the cytoplasm is the precursor for the biosynthesis of fatty acids, isoprenes and cholesterol. Oxaloacetate, the other product formed in the cytosol by Citrate Lyase can not directly reenter the mitochondria; it is reduced to malate by the action of the cytosolic isoenzyme of Malate Dehydrogenase. NADH (from the glyceraldehyde-3-phosphate dehydrogenase step of glycolysis) donates the electrons for
this reduction.

Cytosolic malate has several possible fates. It can be transported into the mitochondria, converted to oxaloacetate by mitochondrial Malate Dehydrogenase, and the oxaloacetate can (re)enter the TCA cycle. An acetate can be coupled to it forming citrate, and the citrate used by the TCA cycle or used to transport additional acetate fragments into the cytoplasm. This cycle occurs when the cytoplasm has sufficient NADPH for fatty acid biosynthesis. If the cell needs additional NADPH for fatty acid biosynthesis, the malate is oxidized and decarboxylated to form pyruvate and CO₂. NADP accepts the electrons from this oxidation forming NADPH. The pyruvate generated by Malic Enzyme (re)enters the mitochondria where it can be carboxylated to form oxaloacetate or decarboxylated to form acetyl-CoA.
The reaction catalyzed by Malic Enzyme run in reverse in the mitochondria is one of the anapleurotic reactions of the TCA cycle. Mitochondrial Malic Enzyme uses NADH rather than NADPH because the mitochondria contains very low levels of the NADP/NADPH pair of cosubstrates.

Activation Step

Before the cytosolic acetyl-CoA can be used for fatty acid biosynthesis it must be activated. The acetyl-CoA is activated by the addition of CO₂ (HCO₃⁻) to the methyl group of the acetate moiety. This reaction is catalyzed by Acetyl-CoA Carboxylase, a Biotin requiring carboxylase. The activation step is the rate limiting and one of the controlling steps of fatty acid synthesis. Malonyl-CoA the product of this reaction is the activated precursor for fatty acid biosynthesis.

![Acetyl-CoA Carboxylase Reaction](attachment:image.png)

In bacteria the synthesis of fatty acids is carried out by six independent enzymes that are associated during the synthesis process with an seventh protein, ACYL CARRIER PROTEIN (ACP). Two of each subunit type assembles into an active dimer - a metabolon. The growing fatty acid is covalently linked to ACYL CARRIER PROTEIN and it acts as a swinging arm, carrying the growing fatty acid from enzyme to enzyme. The working end of ACP is a PHOSPHOPANTETHINE covalently linked to 2-Thiolethylamine, the same functional group that forms the working end of Coenzyme A. The fatty acid intermediates are coupled to this group by a thioester bond, exactly as carboxylic acids are linked to CoA-SH.

In mammals the synthesis of fatty acids is catalyzed by a single large protein containing six different enzyme activities, each in its own domain. This large protein is called the Fatty Acid Synthase Complex. A seventh domain on the mammalian Fatty Acid Synthase Complex contains...
a phosphopantetheine–2-thiolethylamine prosthetic group and functions to carry the growing fatty acid from active site to active site. This domain on the mammalian protein is called acyl carrier protein which is a misnomer since it is not an independent protein, rather the phosphopantetheine–2-thiolethylamine group is covalently linked to a serine side chain on the synthase complex. A better term would be acyl carrier domain, but acyl carrier protein is always used. A dimer is the active form of this enzyme; two of these large protein molecules loosely associate during the synthesis process. The acyl carrier protein of one enzyme subunit carries the growing fatty acid from active site to active site on the other subunit. With this arrangement, two fatty acids are synthesized simultaneously.

The six enzyme activities contained on this large protein are (ACP stands for acyl carrier protein):

1. Malonyl/Acetyl-CoA-ACP Transferase (MAT)
2. \(\beta\)-Ketoacyl-ACP Synthase (KS)
3. \(\beta\)-Ketoacyl-ACP Reductase (KR)
4. \(\beta\)-Hydroxyacyl-ACP Dehydratase (DH)
5. Enoyl-ACP Reductase (ER)
6. Thiolase (Palmitoly Thioesterase) (TE)

The Reactions

Fatty acid biosynthesis can be divided into three phases: loading, condensation, & reduction.

The loading phase consists of two reactions, both catalyzed by Malonyl/Acetyl-CoA-ACP Transferase. To start the synthesis process the Malonyl/Acetyl-CoA-ACP Transferase activity transfers the acetate group from an acetyl-CoA to the -SH group on ACP and then from the -SH group of ACP to an -SH group in the active site of the \(\beta\)-Ketoacyl-ACP Synthase activity. \(\beta\)-Ketoacyl-ACP Synthase is the “second” enzyme activity of the synthase complex as described above. In this reaction it is acting as a substrate for Malonyl/ Acetyl-CoA-ACP Transferase.

During the second loading reaction the Malonyl/Acetyl-CoA-ACP Transferase activity transfers a malonyl group from malonyl-CoA to ACP. At the end of the loading phase the ACP swinging arm is carrying a malonate group and the \(\beta\)-Ketoacyl-ACP Synthase activity is loaded with an acetate group.
The **CONDENSATION PHASE** is a single reaction, the β-Ketoacyl-ACP Synthase activity catalyzes the condensation between the acetate group covalently linked in its active site and the malonate group on ACP. The CO₂ that was added to form the malonate is released and a new bond is formed between the resulting two acetate fragments. The product formed during the first pass through the synthase complex is a four carbon β-ketoacyl group (3-ketoacyl group) covalently linked to ACP; β-ketoacyl-ACP.

Three reactions occur during the **REDUCTION PHASE**. ACP now moves the β-ketoacyl intermediate to the β-Ketoacyl-ACP Reductase site of the enzyme complex. At this site the ketone group is reduced to a β-hydroxy group; a β-hydroxyacyl-ACP. NADPH supplies the electrons for this reduction reaction.

The β-hydroxyacyl group is now moved to the β-Hydroxyacyl-ACP Dehydratase site. At this site the β-
hydroxyl group on the intermediate along with a hydrogen from the α carbon (C2) are removed as water. The intermediate is dehydrated to form an enoyl bound to ACP; an enoyl-ACP.

The enoyl intermediate is carried to the Enoyl-ACP Reductase site by ACP where the carbon-carbon double bond is reduced to form a saturated intermediate bound to ACP; an acyl-ACP. NADPH donates the electrons for this reduction. This ends the first cycle through the pathway. A four carbon saturated intermediate bound to ACP has been synthesized.
The next cycle starts at the loading phase. After the first cycle through the synthase complex and during all subsequent cycles the growing fatty acid bound to ACP is transferred to the -SH group in the active site of the β-Ketoacyl-ACP Synthase by the action of Malonyl/Acetyl-CoA-ACP Transferase (see above). This frees the ACP to accept a malonate group during the second loading reaction. Once the loading phase has been completed the condensation step catalyzed by β-Ketoacyl-ACP Synthase joins the four carbon intermediate with the malonyl group on ACP to yield a six carbon β-ketoacyl-ACP intermediate with the release of CO₂.
This intermediate is reduced by β-Ketoacyl-ACP Reductase, dehydrated by β-Hydroxyacyl-ACP Dehydratase, and reduced by Enoyl-ACP Reductase to a six carbon saturated acyl-ACP that is then passed to the -SH group in the active site of Ketoacyl-ACP Synthase. Malonyl/Acetyl-CoA-ACP Transferase loads ACP with a third malonate group and the cycle repeats - condensation, reduction, dehydration, and reduction.

The cycle repeats a total of seven times, until the 16 carbon palmitate molecule is formed. Palmitate is too large for the Malonyl/Acetyl-CoA-ACP Transferase activity to move from ACP to the active site of the Ketoacyl-ACP Synthase. Thiolase (Palmitoly Thioesterase) is the sixth enzyme activity of the complex that comes into play. Thiolase catalyzes the hydrolytic release of the palmitate from ACP into the cytoplasm.
is the final product of the Fatty Acid Synthase Complex.
Elongation and Desaturation

The fatty acids stored as triacylglycerols and the fatty acids of membrane lipids are not all palmitate. They are usually longer than 16 carbons and often unsaturated. The cell contains additional enzymes in the mitochondria and in the endoplasmic reticulum to elongate and desaturate the newly synthesized fatty acids.

Endoplasmic reticulum (ER) is the primary location for fatty acid elongation and desaturation. The ER system for fatty acid elongation employs four individual enzymes - β-Ketoacyl-CoA Synthase, β-Ketoacyl-CoA Reductase, β-Hydroxyacyl-CoA Dehydratase, and Enoyl-CoA Reductase of the fatty acid synthase enzyme and NADPH is used as the reducing agent. The major difference between elongation in the ER and the synthesis performed by the synthase enzyme is that in the ER malonyl-CoA is condensed with a fatty acyl-CoA rather than to an intermediate carried on the acyl carrier domain.

Elongation in the mitochondria employs three of the four enzymes of β-oxidation run in the reverse direction. Thiolase (Acyl-CoA Acetyltransferase) catalyzes the condensation of acetyl-CoA with a fatty acyl-CoA, L-3-Hydroxyacyl-CoA Dehydrogenase (β-Hydroxyacyl-CoA Dehydrogenase) catalyzes the first reduction using NADH as the reducing agent, and Enoyl-CoA Hydratase catalyzes the dehydration step. The last reduction step of the elongation pathway in the mitochondria employs an enzyme not from β-oxidation. An Enoyl-CoA Reductase in the mitochondria reduces the double bond in the intermediate to a single bond using NADPH.
Mammalian liver smooth ER contains four desaturase enzymes. There are Δ⁴, Δ⁵, Δ⁶, and Δ⁹ Fatty Acyl-CoA Desaturases that introduce double bonds into newly synthesized fatty acids with a wide variety of chain lengths. The Fatty Acyl-CoA Desaturases are mix-function oxidases, they oxidize two substrates and pass four electrons to O₂ to form 2 H₂O. During the desaturation reaction a pair of electrons, two hydrogen atoms, are removed from the fatty acid and a second pair of electrons are donated by NADPH. These four electrons are passed by a series of electron carriers to O₂ to form two H₂O molecules. Mammalian cells cannot introduce double bonds into fatty acids beyond position 9 (between carbon 9 & 10), which means that mammalian cells cannot synthesize linoleate (18:2Δ⁹,12) or linolenate (18:3Δ⁹,12,15). Only plants have desaturases that can introduce double bonds at the Δ¹² (Ω⁶) and Δ¹⁵ (Ω³) positions. For this reason linoleate and linolenate are essential fatty acids for mammals. Linoleate and linolenate must be obtained from plant sources in the diet.

Hormonal Control of Fatty Acid Biosynthesis

Fatty acid biosynthesis is controlled at three levels, it is controlled by hormones, it is controlled by allosteric enzymes, and it is controlled at the level of gene expression.

Insulin stimulates fatty acid biosynthesis by a mechanism that is not completely understood. Insulin stimulates the activity of ATP-Citrate Lyase. This enzyme exists in one of two forms, an inactive monomeric form and an active polymeric form. Insulin stimulates the conversion of ATP-Citrate Lyase from the inactive monomeric to the active polymeric form.

Glucagon and epinephrine inhibit fatty acid biosynthesis. In the liver cell protein kinase A, when activated by glucagon, phosphorylates Acetyl-CoA Carboxylase. Acetyl-CoA Carboxylase when phosphorylated is inhibited, significantly decreasing the rate limiting step of fatty acid biosynthesis. Acetyl-CoA Carboxylase, like ATP-Citrate Lyase exists as an active polymerized form and an inactive monomeric form. Phosphorylation of Acetyl-CoA Carboxylase by PKA stimulates the depolymerization and inactivation of this enzyme.

Summary of Hormonal Control
Carbohydrate & Lipid Metabolism

Insulin

1. Stimulates the activity of ATP-Citrate Lyase by stimulating its polymerization (mechanism unclear).
2. Activates Protein Phosphatase 2A which dephosphorylates and activates Acetyl-CoA Carboxylase.
3. Stimulates the synthesis of the Fatty Acid Synthesis Complex (Protein Kinase B phosphorylation of transcription factors).
4. Stimulates glucose uptake by mobilizing Glut4 transporters (Protein Kinase B path).
5. Stimulates Glycolysis by stimulating the synthesis of Hexokinase, Phosphofructokinase-1, Phosphofructokinase-2, and Pyruvate Kinase (Protein Kinase B phosphorylation of transcription factors).


7. Stimulates Glycogenesis by stimulating the phosphorylation of $G_M$ protein at Site 1 — Site 1 phosphorylated $G_M$ protein stimulates Protein Phosphatase 1 to remove phosphate from Glycogen Synthase rendering it active (Protein Kinase B path).

8. Site 1 phosphorylated $G_M$ protein stimulates Protein Phosphatase 1 to remove phosphate from Glycogen Phosphorylase A converting it to Phosphorylase B rendering it inactive (Protein Kinase B path).

9. Inhibits Gluconeogenesis by repressing the synthesis of Phosphoenolpyruvate Carboxykinase, Fructose-1,6-bisphosphatase, and Glucose-6-phosphatase (Protein Kinase B phosphorylation of transcription factors).

Glucagon

1. Active Protein Kinase A phosphorylates Acetyl-CoA Carboxylase causing depolymerization rendering it inactive.

2. Active Protein Kinase A phosphorylates Perilipin stimulating its movement.

3. Active Protein Kinase A phosphorylates Hormone Sensitive Lipase (Triacylglycerol Lipase) activating it.

4. In the Liver - Active Protein Kinase A phosphorylates Pyruvate Kinase rendering it inactive. PKA phosphorylates Phosphofructokinase-2 inhibiting the kinase activity and stimulating the phosphatase activity. This combination inhibits glycolysis in the liver.

5. Active Protein Kinase A phosphorylates Glycogen Synthase rendering it inactive.

6. Active Protein Kinase A phosphorylates Glycogen Phosphorylase Kinase rendering it active. Active Glycogen Phosphorylase Kinase phosphorylates Glycogen Phosphorylase rendering it active.

7. Active Glycogen Phosphorylase Kinase phosphorylates Glycogen Synthase rendering it inactive.

8. Increased cAMP concentrations brought about by Glucagon stimulates the synthesis of Phosphoenolpyruvate Carboxykinase, Fructose-1,6-bisphosphatase, and Glucose-6-phosphatase. The increased cAMP concentrations induces the synthesis of these proteins by interacting with transcription factor CREB (CYCLIC AMP RESPONSE ELEMENT BINDING PROTEIN).
Allosteric Control of Fatty Acid Metabolism

Acetyl-CoA Carboxylase is also an allosteric enzyme. It is allosterically inhibited by fatty acyl CoA’s, especially palmitoyl-CoA. The fatty acyl CoA’s could be derived from fatty acids activated for β-oxidation or they could be the final products of fatty acid biosynthesis. Either way, when the cytoplasmic concentration of fatty acids, in the form of fatty acyl CoA’s is high the rate limiting step of fatty acid biosynthesis is inhibited. Acetyl-CoA Carboxylase is also allosterically regulated by citrate. High cytoplasmic concentrations of citrate allosterically activate Acetyl-CoA Carboxylase.

Control at Gene Expression

Fatty acid biosynthesis is also controlled at the level of gene expression. When animals ingest high concentrations of certain Ω6 and Ω3 unsaturated fatty acids, the expression of many lipogenic enzymes is suppressed in the liver.

AMP-Activated Protein Kinase Regulation of Triacylglycerol Metabolism

AMP and AMP-Activated Protein Kinase (AMPK) play a role in controlling/integrating fatty acid synthesis and β-oxidation. AMP levels are a sensitive measure of the energy charge of the cell. When the energy charge of the cell is low, the AMP concentrations are high, and the AMP-Activated Protein Kinase is activated. AMPK once active phosphorylates and inactivates Acetyl-CoA Carboxylase turning off fatty acid
Glycerol-3-phosphate Acyltransferase is also phosphorylated and inactivated by AMPK stopping triacylglycerol synthesis (see below). This kinase phosphorylates and stimulates Hormone-Sensitive Lipase and Malonyl-CoA Decarboxylase. These actions increase the rate of β-oxidation by releasing more fatty acids into the circulation and by decreasing the concentration of malonyl-CoA. Decreased levels of malonyl-CoA removes the inhibition on Carnitine Acyl Transferase I so that the fatty acids that were released can be transported into the mitochondria.

The synthesis of triacylglycerols and phosphoglycerides can begin with several different metabolic intermediates and proceed by different routes depending upon the tissue. All cell types can begin with dihydroxyacetone phosphate obtained from glycolysis. All tissues contain Dihydroxyacetone phosphate Acyltransferase that catalyzes the transfer of a fatty acid from a fatty acyl-CoA to carbon 1 of dihydroxyacetone phosphate forming a 1-acyldihydroxyacetone phosphate. This enzyme is highly specific for saturated fatty acids. The 1-acyldihydroxyacetone phosphate is then reduced to 1-acylglycerol-3-phosphate by the action of Acyldihydroxyacetone phosphate Reductase.

Synthesis of Triacylglycerols and Phosphoglycerides
Alternatively, Dihydroxyacetone phosphate is reduced to glycerol-3-phosphate by the action of Glycerol-3-phosphate Dehydrogenase. NADH donates the electrons for this reduction. Liver, kidney, intestinal mucosa, and lactating mammary gland can take Glycerol and phosphorylate it by the action of Glycerol Kinase forming glycerol-3-phosphate and ADP. The enzyme Glycerol-3-phosphate Aeryltransferase (Acyltransferase I) catalyzes the transfer of a fatty acid from a fatty acyl-CoA to carbon 1 of glycerol-3-phosphate to form 1-acylglycerol-3-phosphate. This enzyme is highly specific for saturated fatty acids.

1-Acylglycerol-3-phosphate Aeryltransferase (Acyltransferase II or Lyso Phosphatidic Acid Aeryl Transferase) catalyzes the transfer of a second fatty acid from a fatty acyl-CoA to carbon 2 of the monoacylglycerol-3-phosphate forming a diacylglycerol-3-phosphate. Acyltransferase II has a high specificity for unsaturated fatty acids. The product of this series of reactions, diacylglycerol-3-phosphate, is also known as PHOSPHATIDATE.

The PHOSPHATIDATE is the precursor for the triacylglycerols and the phosphoglycerides. Synthesis of this diverse group of molecules occurs by several different routes. In some pathways phosphatidate is dephosphorylated by the action of Phosphatidate Phosphatase to form diacylglycerol. Diacylglycerol is used as a precursor for the synthesis of triacylglycerols, phosphatidylcholine, and phosphatidylethanolamine.

Triacylglycerols are synthesized from diacylglycerol by the action of Diacylglycerol Aeryltransferase (Acyltransferase III). This enzyme catalyzes the transfer a fatty acid from fatty acyl-CoA to the free hydroxyl group of diacylglycerol to form a triacylglycerol.

Before phosphatidylcholine can be synthesized the choline molecule must be activated by a two step process. First, phosphocholine is formed when a phosphate is transferred from ATP to choline by the action of Choline Kinase. The phosphocholine is then transferred to CTP by the action of CTP:Phosphocholine Cytidylyltransferase to form CDP-choline and pyrophosphate. Phosphocholine Transferase then catalyzes the transfer of the phosphocholine from CDP-choline to diacylglycerol forming CMP and phosphatidylethanolamine.

The synthesis of phosphatidylethanolamine occurs by a similar sequence of reactions. Ethanolamine is activated to phosphoethanolamine by the Ethanolamine Kinase catalyzed transfer of a PO₄⁻³ from ATP. CTP:Phosphoethanolamine Cytidylyltransferase catalyzes the transfer of phosphoethanolamine to CTP forming CDP-ethanolamine and P₂O₇⁻⁴. Phosphoethanolamine Transferase then transfers phosphoethanolamine from CDP-ethanolamine to diacylglycerol forming CMP and phosphatidylethanolamine.

Phosphatidylserine is synthesized from phosphatidylethanolamine by an exchange reaction. Base Exchange Enzyme catalyzes the reversible exchange of ethanolamine for serine. A mitochondrial enzyme, Phosphatidylserine Decarboxylase converts phosphatidylserine to phosphatidylethanolamine. No other pathway for converting serine to ethanolamine has been found in animals. Phosphatidylethanolamine can be converted to phosphatidylcholine by the addition of three methyl groups donated by S-adenosylmethionine (see amino acid metabolism section). It should be apparent from this discussion that serine is the precursor for both ethanolamine and choline.
To synthesize PHOSPHATIDYLINOSITOL the phosphatidate is activated by reaction with CTP forming CDP-diacylglycerol and pyrophosphate. CTP Phosphatidate Cytidylyltransferase catalyzes this reaction. Under the action of the enzyme Phosphatidylinositol Synthase, CDP-diacylglycerol reacts with inositol to form CMP and phosphatidylinositol.

To synthesize DIPHOSPHATIDYGLYCEROL (CARDIOLIPIN), a glycerol-3-phosphate is linked to CDP-diacylglycerol (from above) by the action of Phosphatidylglycerol-3-phosphate Synthase forming phosphatidylglycerol-3-phosphate and CMP. Phosphatidylglycerol is formed when Phosphatidylglycerol-3-phosphate Phosphatase hydrolytically removes the phosphate from phosphatidylglycerol-3-phosphate. Under the action of Cardiolipin Synthase a second molecule of CDP-diacylglycerol is coupled to phosphatidylglycerol forming diphosphatidyglycerol and CMP.

Sphingolipid Synthesis

The synthesis of SPHINGOLIPIDS starts with the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine. The enzyme 3-Ketosphinganine Synthase catalyzes this step. The ketone group on carbon three is then reduced to a hydroxyl group by the action of 3-Ketosphinganine Reductase. NADPH donates the electrons for this reduction. The product of this reaction is sphinganine. Sphinganine is converted to N-acylsphinganine by the transfer of a fatty acid from an acyl-CoA to the amino group on sphinganine. Sphinganine Acyltransferase (Ceramide Synthase) catalyzes this reaction. The N-acylsphinganine is converted to ceramide by the action of a Dihydroceramide Reductase, a DESATURASE. The Reductase is an FAD linked Mixed Function Oxidase. The enzyme takes 2 electrons from N-acylsphinganine to form the trans double bond and 2 electrons from NAD(P)H and transfers them to O2 to form two H2O.

Transfer of choline from phosphatidylcholine to ceramide results in the formation of sphingomyelin and diacylglycerol. Transfer of galactose from UDP-galactose or glucose from UDP-glucose to ceramide results in the formation of cerebrosides. The transfer of additional sugars from UDP-sugars results in the formation of globosides and gangliosides from ceramide. Very specific transferases catalyze the addition of carbohydrates to ceramide to form the cerebrosides, globosides, and gangliosides. The peroxisome is the site of sphingolipid synthesis. The mutation (lack) of any of the enzymes involved in sphingolipid synthesis, especially the glycosyltransferases, result in the abnormal accumulation of precursors. This abnormal accumulation of precursors has deleterious effects on the cell and organism.

Membrane lipids, especially the sphingolipids of the brain, are constantly undergoing renewal and remodeling. Lysosomes contain a variety of phospholipases, amidases, and glycosidases that catalyze the hydrolytic breakdown and recycling of membrane lipid components. There are certain inborn errors of metabolism in which genetic defects leads to a deficiency in a particular degradative lysosomal enzyme, especially the glycosidases that catalyze the removal of sugars from the oligosaccharide chains of globosides and gangliosides. These inborn errors in metabolism result in lysosomal storage diseases that have deleterious effects on the cell, brain, and organism.
The Triacylglycerol Cycle and Glyceroneogenesis

The balance between biosynthesis and degradation is also controlled by the **Triacylglycerol Cycle**. On average 75% of the fatty acids released from triacylglycerols in the adipose are re-esterified rather than oxidized for energy. Some of the re-esterification occurs in the adipose before the fatty acids are released into the blood and some of it occurs in the liver. The liver absorbs the fatty acids, incorporates them into triacylglycerols, the triacylglycerols into VLDL’s, and releases the VLDL’s into the blood stream. Once in the blood stream, the tissues can absorb the lipids from the VLDL’s by the usual means - ApoC-II activation of Lipoprotein lipase. Much of the triacylglycerols in the VLDL’s is reabsorbed by the adipose. Flux through the Triacylglycerol Cycle between adipose and liver is low when other fuels (glucose) are available, and increases as glucose levels drop. However, the proportion of fatty acids re-esterified remains fairly constant at 75% regardless of the metabolic conditions. This cycle reflects a balance between fatty acid use and synthesis. The function of the cycle is poorly understood. One possible function is that the fatty acids in the blood serve as an energy reserve that can be activated more rapidly than fatty acid release from the adipose.

The Triacylglycerol Cycle raises the question: Where does the glycerol come from for this cycle, especially during starvation? It comes from **Glyceroneogenesis** in the adipose, an abbreviated form of gluconeogenesis. Glyceroneogenesis starts with pyruvate, from lactate or amino acids, and ends at dihydroxyacetone phosphate, that is then reduced to glycerol-3-phosphate by the cytosolic NAD linked Glycerol-3-phosphate Dehydrogenase. Glyceroneogenesis coupled with re-esterification of free fatty acids in the adipose controls the rate of fatty acid release.

Glucocorticoids (e.g., cortisol) reciprocally regulate the levels of **PEP-Carboxykinase** in adipose and liver, and the levels of **PEP-Carboxykinase** control both gluconeogenesis and glyceroneogenesis. When stressed, glucocorticoids are released stimulating the expression of **PEP-Carboxykinase** in the liver. This increases gluconeogenesis, glyceroneogenesis, the synthesis of triacylglycerols, their packaging into VLDL’s, and their release by the liver into the blood. The glucocorticoids suppress expression of **PEP-Carboxykinase** in adipose. This results in a decrease in fatty acid re-esterification and more fatty acids are released into the blood. The net result is an increase in flux through the triacylglycerol cycle; more fatty acids released from the adipose and more triacylglycerols (VLDL’s) released by the liver. Overall, the release of the glucocorticoids result is an increase in fuel molecules (glucose via gluconeogenesis and triacylglycerols in the VLDL’s) present in the blood. When the glucocorticoids are not present, the flux through the cycle declines because there is less **PEP-Carboxykinase** in the liver and more in the adipose, and more of the fatty acids are re-esterified in the adipose before release into the blood.
CHOLESTEROL BIOSYNTHESIS can be divided into four stages.

1. Three acetate units are condensed to form a six carbon intermediate mevalonate.
2. Activated isoprene units (Δ3-Isopentyl pyrophosphate & Dimethylallyl pyrophosphate) are formed from mevalonate.
3. A combination of six 5-carbon isoprene units polymerize to form the 30 carbon linear structure of squalene.
4. Finally, squalene is cyclized to form the four ring steroid nucleus, and an additional series of changes leads to the final product cholesterol.

The cholesterol biosynthesis pathway is a branched pathway. It produces several other essential isoprenoids in addition to cholesterol. Other molecules produced from this pathway include ubiquinone (CoQ), dolichol, farnesyl, and geranylgeranyl for lipid linked proteins.

Cholesterol biosynthesis starts with acetyl-CoA derived from pyruvate or from amino acids. The first two steps of cholesterol biosynthesis are identical to the first two steps of ketone body synthesis with one difference. Cholesterol biosynthesis occurs in the cytosol, whereas ketone body biosynthesis occurs in the matrix of the mitochondria. In the first step two molecules of acetyl-CoA condense under the action of Thiolase in the cytoplasm to form acetoacetyl-CoA and CoA. In the second step a third molecule of acetyl-CoA reacts with acetoacetyl-CoA under the action of HMG-CoA Synthase to form CoA and β-hydroxy-β-methylglutaryl-CoA (3-hydroxy-3-methylglutaryl-CoA or HMG-CoA). Now the pathway diverges from the ketone body pathway.

In the third step HMG-CoA is reduced by two molecules of NADPH forming mevalonate. During the reduction CoA is released. This step is catalyzed by HMG-CoA Reductase. HMG-CoA Reductase is the rate controlling step of the pathway. It is allosterically inhibited by cholesterol. This ends stage 1.

Mevalonate is phosphorylated to mevalonate-5-phosphate by the action of Mevalonate Kinase and the mevalonate-5-phosphate is phosphorylated to mevalonate-5-pyrophosphate by the action of Phosphomevalonate Kinase. The mevalonate-5-pyrophosphate is decarboxylated to form isopentenyl pyrophosphate by the action of Pyrophosphomevalonate Decarboxylase. Some of the isopentenyl pyrophosphate is isomerized to dimethylallyl pyrophosphate by the action of Isopentyl Pyrophosphate Isomerase. Isopentenyl pyrophosphate and dimethylallyl pyrophosphate are activated isoprene units. Stage 2 is complete.

One isopentenyl pyrophosphate is joined head to tail with one dimethylallyl pyrophosphate to form the ten carbon intermediate geranyl pyrophosphate. A second isopentenyl pyrophosphate is added head to tail to the geranyl pyrophosphate to form the 15 carbon intermediate farnesyl pyrophosphate, and two farnesyl pyrophosphates then come together head to head to form the 30 carbon intermediate squalene. In a series of about 20 steps, some of which are poorly understood, the squalene is converted to cholesterol.
Control of Cholesterol Biosynthesis

Cholesterol biosynthesis is a very energy expensive pathway. Eighteen acetate fragments carried by CoA are required to synthesize 1 molecule of cholesterol, i.e., 36 carbons are required to synthesize one 27 carbon cholesterol molecule. If these 18 acetate fragments were passed through the TCA cycle and ET/OxPhos 180 ATP would be generated. When present in excess, the cell transfers the electrons from NADPH to NAD and utilizes the resulting NADH in ET/OxPhos to generate ATP. This reaction is catalyzed by Nicotinamide Nucleotide Transhydrogenase. From the 12 NADPH molecules used in cholesterol biosynthesis 30 ATP would be generated. Add to this the 12 ATP necessary to form the 6 molecules of mevalonate-5-phosphate and one can begin to see how energy expensive the process is. When excess
cholesterol is metabolized the cell gets none of this energy back, in fact it costs additional energy to convert cholesterol to the bile acids, bile salts, and bile esters for excretion. With this large energy expense of cholesterol biosynthesis, it is apparent why this pathway is so tightly controlled. Cholesterol synthesis is controlled at multiple levels all involving the enzyme HMG-CoA Reductase.

Cholesterol biosynthesis up to squalene formation occurs in the cytoplasm whereas the later stages occur in the smooth endoplasmic reticulum. The primary site / type of control of cholesterol biosynthesis is the synthesis (gene expression) / degradation of the enzyme HMG-CoA Reductase. When either LDL-cholesterol or mevalonate levels fall, the amount of HMG-CoA Reductase can rise as much as 200 fold due to an increase in enzyme synthesis and a decrease in enzyme degradation. When either LDL-cholesterol or mevalonate levels rise in the smooth endoplasmic reticulum these effects are reversed; less enzyme is synthesized and the rate of degradation is increased. A group of proteins, the Sterol Regulatory Element-Binding Protein (SREBP) are embedded in the Smooth ER membrane. Bound to SREBP is SREBP Cleavage-Activating Protein (SCAP). SCAP binds cholesterol and other sterols and when bound with cholesterol the SREBP-SCAP complex remains bound in the smooth ER membrane. When the cholesterol concentration drops, cholesterol is released from SCAP and SCAP undergoes a conformational change liberating SREBP. SREBP and the released SCAP migrates from the smooth ER membrane to the Golgi. In the Golgi SREBP undergoes two proteolytic cleavages and the cleaved amino terminal domain travels to the nucleus where it binds to gene promoter sequences, inducing gene expression. Cholesterol, via SREBP, controls the expression of about 20 of the enzymes necessary for its synthesis. When the cell has adequate to high cholesterol, the pathway for cholesterol...
synthesis is inhibited and the mevalonate present in the cell can be / is used for the synthesis of the other necessary isoprenoids.

Reversible Covalent modification: HMG-CoA Reductase is phosphorylated by AMP-activated protein kinase. Phosphorylation inhibits the activity of HMG-CoA Reductase. Protein kinase A activated by glucagon or epinephrine phosphorylates and activates Phosphoprotein Phosphatase Inhibitor 1. The activated Phosphoprotein Phosphatase Inhibitor 1 binds to Phosphoprotein Phosphatase 1 inhibiting its activity. With Phosphoprotein Phosphatase inhibited it cannot dephosphorylate and activate HMG-CoA Reductase. These coupled control mechanisms will save energy and precursors when the cell is in an energy poor state. Insulin stimulates dephosphorylation of the enzyme. The dephosphorylated form is the active form. Phosphoprotein phosphatase 1 is the enzyme primarily responsible for the removal of phosphate and activation of HMG-CoA Reductase.