Biosynthesis of Ketones & Cholesterols, Regulation of Lipid Metabolism-2015

Some people aren’t just missing the odd screw. The whole freakin’ toolbox is gone.
The major site of acetoacetate and 3-hydroxybutyrate production is the liver. They are preferred substrates for myocardiocytes and renal cortex rather than glucose. The blood carries the metabolites for energy metabolism; triacylglycerides (in the form of chylomicrons and VLDL’s), F.A. associated with albumin, ketones, amino acids, lactate and glucose. The α and β cells of the pancreas respond to the presence or absence of glucose in blood.
Transfer of acetyl-CoA from mitochondrion to cytosol via the tricarboxylate transport system. This pathway not only moves acetyl-CoA from the mito to the the cytosol but also produces the reducing power needed for the pathway, NADPH. This produces a cycling of pyruvate from mito to cytosol to form OAA besides acetyl-CoA.
After the synthesis in the endoplasmic reticulum, the LDL receptor matures in Golgi complex then migrates to the cell cell surface, where it clusters in coated pits. After internalization of LDL, multiple vesicles fuse to form endosome. Proton pumping in the endosome membrane causes the pH to drop, which in turn cause LDL to dissociate from the receptor. The LDL apolipoprotein is degraded in lysosomes. The LDL receptor remains in a vesicle, which returns to the plasma membrane to start the cycle anew.
The input to fatty acid synthesis is acetyl-CoA, which is carboxylated to malonyl-CoA. The ATP-dependent carboxylation provides energy input. The CO₂ is lost later during condensation with the growing fatty acid. The spontaneous decarboxylation drives the condensation. **Acetyl-CoA Carboxylase** catalyzes the 2-step reaction by which acetyl-CoA is carboxylated to form malonyl-CoA. As with other carboxylation reactions (e.g., pyruvate carboxylase), the enzyme prosthetic group is **biotin**. ATP-dependent carboxylation of the biotin, carried out at one active site (1), is followed by transfer of the carboxyl group to acetyl-CoA at a second active site (2). The overall reaction, which is **spontaneous**, may be summarized as: HCO₃⁻ + ATP + acetyl-CoA + ADP + Pi + malonyl-CoA.
Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of HMG-CoA. Unlike the HMG-CoA formed during ketone body synthesis in the mitochondrial matrix, the other isoenzyme HMG-CoA synthase, this form is synthesized in the cytoplasm for cholesterol synthesis. However, the pathway and the necessary enzymes are the same as those in the mitochondria. Two moles of acetyl-CoA are condensed in a reversal of the thiolase reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to HMG-CoA by the action of HMG-CoA synthase.

HMG-CoA is converted to mevalonate by HMG-CoA reductase, HMGR (this enzyme is bound in the ER) for cholesterol synthesis. HMGR absolutely requires NADPH as a cofactor and two moles of NADPH are consumed during the conversion of HMG-CoA to mevalonate. The reaction catalyzed by HMGR is the rate-limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls.
1. 3-ketothiolase, 2. HMG-CoA synthase, 3. HMG-CoA cleavage enzyme, & 4. 3-hydroxybutyrate \( dh \). The ratio of acetoacetate to hydroxybutyrate depends on the NADH/NAD in mitochondria.

Ketone bodies are produced mainly in the mitochondria of liver cells, and synthesis can occur in response to unavailability of blood glucose. This is caused by low glucose levels in the blood, after exhaustion of cellular carbohydrate stores, such as glycogen or, synthesis of ketones can occur due to excessively high levels of blood glucose that are unable to be stored as glycogen in liver and muscle.
Ketogenesis
During high rates of fatty acid oxidation, primarily in the liver mitochondria, large amounts of acetyl-CoA are generated. These exceed the capacity of the TCA cycle, and one result is the synthesis of ketone bodies, or **ketogenesis**. The ketone bodies are acetoacetate, β-hydroxybutyrate, and acetone. The formation of acetoacetyl-CoA occurs by condensation of two moles of acetyl-CoA through a reversal of the thiolase catalyzed reaction of fat oxidation. Acetoacetyl-CoA and an additional acetyl-CoA are converted to HMG-CoA by HMG-CoA synthase, an enzyme found in large amounts only in the liver. HMG-CoA in the mitochondria is converted to acetoacetate by the action of HMG-CoA lyase. Acetoacetate can undergo **spontaneous decarboxylation to acetone**, or be enzymatically converted to β-hydroxybutyrate through the action of β-hydroxybutyrate dehydrogenase. **When the level of glycogen in the liver is high the production of β-hydroxybutyrate increases.** When carbohydrate utilization is low or deficient, the level of OAA will also be low, resulting in a reduced flux through the TCA cycle. This in turn leads to increased release of ketone bodies from the liver for use as fuel by other tissues. In early stages of starvation, when the last remnants of fat are oxidized, heart and skeletal muscle will consume primarily ketone bodies to preserve glucose for use by the brain. Acetoacetate and β-hydroxybutyrate, in particular, also serve as major substrates for the biosynthesis of neonatal cerebral lipids. Ketone bodies are utilized by extrahepatic tissues through the conversion of β-hydroxybutyrate to acetoacetate and of acetoacetate to acetoacetyl-CoA. The first step involves the reversal of the β-hydroxybutyrate dehydrogenase reaction, and the second involves the action of ketoacyl-CoA transferase; **Acetoacetate + Succinyl-CoA ⇌ Acetoacetyl-CoA + succinate**

The latter enzyme is present in all tissues except the liver. Importantly, its absence allows the liver to produce ketone bodies but not to utilize them. This ensures that extrahepatic tissues have access to ketone bodies as a fuel source during prolonged fasting and starvation.
Regulation of Ketogenesis

The fate of the products of fatty acid metabolism is determined by an individual's physiological status. Ketogenesis takes place primarily in the liver and may be affected by several factors:

1. Control in the release of free fatty acids from adipose tissue directly affects the level of ketogenesis in the liver. This is, of course, substrate-level regulation.

2. Once fats enter the liver, they have two distinct fates. They may be activated to acyl-CoA's and oxidized, or esterified to glycerol in the production of triacylglycerols. If the liver has sufficient supplies of glycerol-3-phosphate, most of the fats will be turned to the production of triacylglycerols.

3. The generation of acetyl-CoA by oxidation of fats can be completely oxidized in the TCA cycle. Therefore, if the demand for ATP is high the fate of acetyl-CoA is likely to be further oxidation to CO₂.

4. The level of fat oxidation is regulated hormonally through phosphorylation of Aetyl-CoA Carboxylase, which may activate it (in response to glucagon) or inhibit it (in the case of insulin).
Pathway of cholesterol biosynthesis. Synthesis begins with the transport of acetyl-CoA from the mitochondrion to the cytosol. The rate limiting step occurs at the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, catalyzed step. The phosphorylation reactions are required to solubilize the isoprenoid intermediates in the pathway. Intermediates in the pathway are used for the synthesis of prenylated proteins, dolichol, coenzyme Q and the side chain of heme a.
Origin of the carbon atoms of cholesterol. This can be deduced from tracer experiments with acetate labeled in the methyl carbon (black) or the carboxyl carbon (red). The individual rings in the fused-ring system are designated A through D.
Formation of mevalonate from acetyl-CoA. The origin of C-1 and C-2 of mevalonate from acetyl-CoA is shown in pink. Mevalonate is then converted to 3-isopentenyl pyrophosphate in three reactions that require ATP. Mevalonate is decarboxylated to isopentenyl pyrophosphate, which is a key metabolite for various biological reactions. Three molecules of isopentenyl pyrophosphate condense to form farnesyl pyrophosphate through the action of geranyl transferase. Two molecules of farnesyl pyrophosphate then condense to form squalene by the action of squalene synthase in the ER. Oxidosqualene cyclase then cyclizes squalene to form lanosterol. Finally, lanosterol is converted to cholesterol through a 19-step process.
Conversion of mevalonate to activated isoprene units. Six of these activated units combine to form. The leaving groups of 3-phospho-5-pyrophosphomevalonate are shaded pink. The bracketed intermediate is hypothetical.
Figure 26-10
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Figure 26-11
Biochemistry, Sixth Edition
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Lanosterol

19 steps

HCOOH + 2 CO₂

Cholesterol

Figure 26-12
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Regulation of HMGR by covalent modification. HMGR is most active in the dephosphorylated state. Phosphorylation is catalyzed by AMP-activated protein kinase, AMPK, (HMGR kinase), an enzyme whose activity is also regulated by phosphorylation. Phosphorylation of AMPK is catalyzed by AMPK kinase (AMPKK). Hormones such as glucagon and epinephrine negatively affect cholesterol biosynthesis by increasing the activity of the inhibitor of phosphoprotein phosphatase inhibitor-1, PPI-1. Conversely, insulin stimulates the removal of phosphates and, thereby, activates HMGR activity. Additional regulation of HMGR occurs through an inhibition of its' activity as well as of its' synthesis by elevation in intracellular cholesterol levels. This latter phenomenon involves the transcription factor SREBP.
Pathway for sterol-accelerated degradation of HMG CoA reductase. Accumulation of 25-hydroxycholesterol, lanosterol, or 24,25-dihydrolanosterol in ER membranes triggers binding of the reductase to Insigs. A subset of Insigs is associated with the membrane-anchored ubiquitin ligase, gp78, which binds the E2 Ubc7 and VCP, an ATPase that plays a role in extraction of ubiquitinated proteins from ER membranes. Through the action of gp78 and Ubc7, the reductase becomes ubiquitinated, which triggers its extraction from the membrane by VCP, and subsequent delivery to proteasomes for degradation. The post-ubiquitination step is postulated to be enhanced by geranylgeraniol through an undefined mechanism that may involve a geranylgeranylated protein, such as one of the Rab proteins.
Regulation of cholesterol formation balances synthesis with dietary uptake. Glucagon promotes phosphorylation (inactivation) of HMG-CoA reductase; insulin promotes dephosphorylation (activation). X represents unidentified metabolites of cholesterol that stimulate proteolysis of HMG-CoA reductase.

Acyl-CoA cholesterol acyltransferase, or simply ACAT) is an intracellular protein located in the ER that forms cholesteryl esters from cholesterol.
The released SREBP enters the cell nucleus where it functions as a transcription factor to activate genes for enzymes of the cholesterol synthesis pathway. Its lifetime in the nucleus is brief, because SREBP is ubiquitinated and degraded. This activation can increase the HMG-CoA reductase level 200 fold as well as the number of LDL receptor. The serum LDL conc. is dependent upon the rate of IDL removal from the blood by the liver. This is dependent on the number of LDL receptors on the liver.
The continual alteration of the intracellular sterol content occurs through the regulation of key sterol synthetic enzymes as well as by altering the levels of cell-surface LDL receptors. As cells need more sterol they will induce their synthesis and uptake, conversely when the need declines synthesis and uptake are decreased. Regulation of these events is brought about primarily by sterol-regulated transcription of key rate limiting enzymes and by the regulated degradation of HMGR. Activation of transcriptional control occurs through the regulated cleavage of the membrane-bound transcription factor sterol regulated element binding protein, SREBP. As discussed above, degradation of HMGR is controlled by the ubiquitin-mediated pathway for proteolysis. Sterol control of transcription affects more than 30 genes involved in the biosynthesis of cholesterol, triacylglycerols, phospholipids and fatty acids. Transcriptional control requires the presence of an octamer sequence in the gene termed the sterol regulatory element, SRE-1. It has been shown that SREBP is the transcription factor that binds to SRE-1 elements. It turns out that there are 2 distinct SREBP gene, SREBP-1 and SREBP-2. In addition, the SREBP-1 gene encodes 2 proteins, SREBP-1a and SREBP-1c as a consequence of alternative exon usage. All 3 proteins are proteolytically regulated by sterols. Full-length SREBP’s have several domains and are embedded in the membrane of the endoplasmic reticulum (ER). The N-terminal domain contains a transcription factor motif of the basic helix-loop-helix (bHLH) type that is exposed to the cytoplasmic side of the ER. There are 2 transmembrane spanning domains followed by a large C-terminal domain also exposed to the cytosolic side. When sterols are scarce, cleavage of the full-length SREBP takes place with the result being that the N-terminal bHLH motif is released into the cytosol. The bHLH domain then migrates to the nucleus to direct transcription. Conversely, when sterols are abundant, cleavage of SREBP is inhibited. To control the level of SREBP-mediated transcription, the soluble bHLH domain is itself subject to rapid proteolysis.
The cleavage of SREBP is carried out by 2 distinct enzymes, one of which is regulated by sterols. The regulated cleavage occurs in the luminal loop between the 2 transmembrane domains. This cleavage is catalyzed by site-1 protease, S1P. High sterol content blocks the activity of S1P. The second cleavage, catalyzed by site-2 protease, S2P, occurs in the first transmembrane span, leading to release of active SREBP. In order for S2P to act on SREBP, site-1 must already have been cleaved. Additional studies on sterol-regulated gene expression demonstrated that cleavage of SREBP by S1P is controlled by the level and action of an additional protein termed, SREBP cleavage-activating protein, SCAP. SCAP is a large protein also found in the ER membrane and contains at least 8 transmembrane spans. The C-terminal portion, which extends into the cytosol, has been shown to interact with the C-terminal domain of SREBP. This C-terminal region of SCAP contains 4 motifs called WD40 repeats. The WD40 repeats are required for interaction of SCAP with SREBP. The N-terminus of SCAP, including membrane spans 2-6, resembles HMGR which itself is subject to sterol-stimulated degradation. This shared motif is called the sterol sensing domain, SSD. Several proteins whose functions involve sterols also contain the SSD. These include patched, an important development regulating receptor whose ligand, hedgehog, is modified by attachment of cholesterol and the Neimann Pick C1 (NPC1) protein which is involved in cholesterol transport in the secretory pathway. The function of SCAP is to positively stimulate S1P-mediated cleavage of SREBP. The function of sterols is to inhibit this positive action of SCAP. The activity of SCAP involves movement from the ER to the Golgi and back. Because the C-terminus of SCAP interacts with SREBP, movement of SCAP takes SREBP along for the ride. When sterols are low, SCAP and SREBP move to the Golgi. This transit is required for SREBP cleavage as S1P is Golgi-localized. When sterols are high, movement of SCAP is halted. Thus, the overall effect of sterols is to regulate the ability of SCAP to present SREBP to S1P.
Synthesis of the 2 primary bile acids, cholic acid and chenodeoxycholic acid. The reaction catalyzed by the $7\alpha$-hydroxylase is the rate limiting step in bile acid synthesis. Conversion of $7\alpha$-hydroxycholesterol to the bile acids requires several steps not shown in detail in this image. Only the relevant co-factors needed for the synthesis steps are shown.
As surfactants or detergents, bile acids are potentially toxic to cells, and their concentrations are tightly regulated. They function as a signaling molecule in the liver and the intestines by activating a nuclear hormone receptor, FXR, also known by its gene name NR1H4. Activation of FXR in the liver inhibits synthesis of bile acids, and is one mechanism of feedback control when bile acid levels are too high. FXR activation by bile acids during absorption in the intestine increases transcription and synthesis of FGF19, which will then inhibit bile acid synthesis in the liver. Emerging evidence associates FXR activation with alterations in triglyceride metabolism, glucose metabolism, and liver growth.
Within the liver the carboxyl group of primary and secondary bile acids is conjugated via an amide bond to either glycine or taurine before their being re-secreted into the bile canaliculi. These conjugation reactions yield glycoconjugates and tauroconjugates, respectively. The bile canaliculi join with the bile ductules, which then form the bile ducts. Bile acids are carried from the liver through these ducts to the gallbladder, where they are stored for future use. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids. In the gut the glycine and taurine residues are removed and the bile acids are either excreted (only a small percentage) or reabsorbed by the gut and returned to the liver. This process of secretion from the liver to the gallbladder, to the intestines and finally re-absorption is termed the enterohepatic circulation.
Cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. Three different mechanisms can form these: autoxidation, secondary oxidation to lipid peroxidation, and cholesterol-metabolizing enzyme oxidation. A great interest in oxysterols arose when they were shown to exert inhibitory actions on cholesterol biosynthesis.[43] This finding became known as the “oxysterol hypothesis”. Additional roles for oxysterols in human physiology include their: participation in bile acid biosynthesis, function as transport forms of cholesterol, and regulation of gene transcription.

Cholesterol is oxidized by the liver into a variety of bile acids. These, in turn, are conjugated with glycine, taurine, glucuronic acid, or sulfate. A mixture of conjugated and nonconjugated bile acids, along with cholesterol itself, is excreted from the liver into the bile. Approximately 95% of the bile acids are reabsorbed from the intestines, and the remainder are lost in the feces. The excretion and reabsorption of bile acids forms the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallises and is the major constituent of most gallstones. Although, lecithin and bilirubin gallstones also occur, but less frequently. Every day, up to 1 g of cholesterol enters the colon. This cholesterol originates from the diet, bile, and desquamated intestinal cells, and can be metabolized by the colonic bacteria. Cholesterol is converted mainly into coprostanol, a nonabsorbable sterol that is excreted in the feces.