Objectives. Understand the overall pathways of sterol uptake, synthesis, transport, and utilization and their regulation; be able to explain why a deficiency of LDL receptors leads to elevated blood cholesterol. Understand the rationale for pharmacological interventions to lower cholesterol levels. Understand the overall pathways, regulation and tissue specificity of steroid hormone biosynthesis; be able to use these features of the process to explain the biochemical consequences of defects in specific synthetic enzymes.

Cholesterol structure. Cholesterol contains 27 carbon atoms arranged into four fused rings and a hydrocarbon tail.

The molecule is extremely hydrophobic. It is an essential component of cell membranes, and is the precursor for the synthesis of several important molecules. These include bile acids (needed for the digestion and absorption of dietary fat), vitamin D (needed for calcium uptake and utilization), and steroid hormones (needed for regulation of ion balance, metabolism, and sexual differentiation).

Overview of cholesterol metabolism. Cholesterol can be obtained from the diet, or can be synthesized, primarily in the liver and adrenal gland, from acetyl-CoA. Its main fates are export to peripheral tissues for membrane biogenesis and conversion to bile salts in the liver. Synthesis of steroid hormones, while essential, accounts for less than 10% of cholesterol utilization. Cholesterol is lost from the body as bile salts that are not reabsorbed, in the membranes of dead epithelial cells sloughed off from body surfaces, and as
steroid hormone derivatives excreted in the urine and feces. There is no metabolic pathway to use cholesterol for the production of energy.

**Cholesterol biosynthesis.** Cholesterol is synthesized from acetyl-CoA, with NADPH + H⁺ as a source of reducing equivalents and ATP as a source of energy, in a sequence of approximately 30 reactions. Cholesterol is not synthesized by plants or bacteria, so a diet rich in vegetable products is low in cholesterol.

Most cells in the body are capable of carrying out these reactions, but the bulk of cholesterol biosynthesis occurs in the liver. Substantial amounts are also made in the adrenal gland and gonads (where it serves as a precursor for steroid biosynthesis).
Synthesis begins in the cytosol, with the condensation of acetoacetyl-CoA and acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). This reaction is catalyzed by a cytosolic isoform of HMG-CoA synthase, distinct from the mitochondrial isoform used in ketone body synthesis. The next reaction, the conversion of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase, is the committed step of cholesterol biosynthesis. This step is also tightly regulated: both sterols (end products of the pathway) and glucagon trigger signaling cascades that cause phosphorylation and inactivation of the enzyme; insulin triggers its dephosphorylation and activation. The major form of regulation of HMG-CoA reductase, however, is at the level of transcription.

A key intermediate in cholesterol biosynthesis is farnesyl pyrophosphate, which can be covalently attached to proteins. Many proteins that are attached to the cytosolic face of the plasma membrane, including RAS GTPases, are held there via farnesyl groups.

About 20 reactions are needed to convert squalene to cholesterol; these occur in the endoplasmic reticulum.


**Transcriptional regulation of cholesterol biosynthesis.** The major factor controlling the rate of cholesterol biosynthesis and linking it to cholesterol uptake from the circulation is transcriptional regulation of HMG CoA synthase, HMG CoA reductase, and the
LDL receptor. This regulation is mediated by the transcription factor, SREBP (sterol response element binding protein). SREBP is produced as a membrane-bound protein attached to the endoplasmic reticulum of the cell via a second protein, SCAP. In cholesterol-starved cells SREBP:SCAP is transported to the Golgi apparatus where it is cleaved sequentially by two proteases (designated S1P and S2P) that release the bHLH fragment of SREBP into the cytosol. bHLH then enters the nucleus where it activates several key genes of sterol metabolism, including the HMG CoA reductase gene.

Cholesterol does not interact directly with the SCAP:SREBP complex, but rather with a third protein, INSIG. The INSIG:sterol complex traps the proteins responsible for transporting SREBP:SCAP to the Golgi apparatus and thus immobilizes SCAP:SREBP in the endoplasmic reticulum. Reduced cholesterol levels lead to dissociation of INSIG:sterol, allowing SCAP:SREBP to migrate to the Golgi complex where the signaling domain of SREBP is released by proteolysis to travel into the nucleus.

Another complication not shown in the figure is that there are three SREBP isoforms, which differ in the efficiencies with which they bind to the various SREBP-regulated genes. The different isoforms are expressed at different levels in different tissues, suggesting that regulation of sterol metabolism may be tissue-specific.

Note that all of these regulatory mechanisms operate at the level of individual cells, sensing and adjusting cholesterol levels at the single-cell level.

**Bile acids and salts.** Bile acids are cholesterol derivatives with two (commonly) or one (less commonly) additional hydroxyl groups, whose hydrocarbon tail has been shortened and oxidized to form a carboxylate group. The additional hydrophilic groups increase these molecules’ solubility in water, making them effective detergents. Conjugation of the carboxylate group with glycine or taurine further increases their solubility. These conjugates are called bile “salts”.

As indicated by the dashed arrow, a variant of these reactions may also provide a means to mobilize and remove excess cholesterol from neural tissue.
The rate of bile acid synthesis is determined largely by transcriptional regulation of 7-alpha-hydroxylase, the first enzyme in the pathway from cholesterol to bile acids. Transcription of the 7alpha-hydroxylase gene is positively regulated by SREBP, as described above. It is also positively regulated by LHR. Negative regulation by high levels of bile acids is achieved indirectly. Bile acids can form a complex with the LXR transcription factor, enabling its movement into the nucleation and transcriptional activation of the SHP gene. SHP protein in turn can form a heterodimer with LXR which, instead of acting as a transcriptional activator, acts as a transcriptional repressor for 7alpha-hydroxylase. In effect, the normal function of SHP protein is analogous to that of a dominant negative mutant protein. These regulatory events are outlined in the drawing. Note that the LXR signaling process exactly parallels the positive signaling process discussed earlier for glucocorticoid hormone receptors.

**Pharmacological interventions to manage cholesterol levels.** Normal sterol synthesis and re-utilization is controlled at multiple levels by the body to assure a sufficient supply of these essential molecules. To achieve substantial reductions of serum cholesterol, several targets are attacked simultaneously. **Statins** like “Lovastatin” are potent specific competitive inhibitors of HMG CoA reductase, and block \textit{de novo} cholesterol biosynthesis. **Resins** like cholestyramine bind bile salts in the gut, preventing their reutilization. **Ezetimbe** interferes with cholesterol uptake from the gut, possibly by modifying the activity of ABC transporters.

The resulting fall in intracellular sterol levels causes increased production of HMG CoA reductase, 7alpha hydroxylase, and LDL receptor proteins, the net effect of which is to draw cholesterol into the liver for conversion to bile acids and excretion.
**Vitamin D synthesis.** 7-dehydrocholesterol, an immediate precursor of cholesterol on the de novo biosynthetic pathway, is converted to vitamin D in a reaction driven by exposure to UV light in the skin, and enzymatic reactions that occur in the liver and kidney.

**Steroid hormones.** This diagram is an overview of the process of steroid hormone biosynthesis in the human body, highlighting a key regulated step, several enzymes useful for understanding normal tissue specificity of these events, and several key products.

In any given tissue, the amount of steroid synthesis is controlled by the availability of cholesterol in the mitochondrial matrix (determined in turn by the abundance of StAR, a cholesterol transporter protein in the inner mitochondrial membrane), and the identities of the steroids synthesized are determined by the presence or absence of specific enzymes. Although these molecules are hydrophobic, both the end products and many intermediates are transported between cells in an aqueous environment.
ates can leave the cells in which they are made and circulate as complexes either with serum albumin or with specific sterol-binding globulin proteins.

These general principles can be used to rationalize two examples of normal tissue-specific hormone synthesis, and one pathological state, congenital adrenal hyperplasia due to 21 alpha hydroxylase deficiency.

**Cortisol biosynthesis.** Cortisol is a glucocorticoid hormone, activating the transcription of genes that mediate the body’s response to stress. In the Zona reticularis and Zona fasciculata of the adrenal cortex, ACTH stimulates synthesis of StAR, leading to increased cortisol biosynthesis. Elevated cortisol levels, in turn, inhibit ACTH release, StAR protein levels fall, and cortisol synthesis is reduced as discussed in connection with amino acid metabolism and the body’s response to stress.

**Aldosterone biosynthesis.** Aldosterone, a mineralocorticoid, promotes transcription of genes in the kidney whose protein products mediate Na⁺ reuptake. These reactions occur in the Zona glomerulosa of the adrenal cortex. Here, StAR protein levels are increased in response to angiotensin II, a peptide hormone that signals reduced Na⁺ levels in the blood. StAR gene transcription is blocked in response to atrial natriuretic factor, a signal of elevated Na⁺ levels. The StAR protein has a short half-life, so transcriptional regulation effectively controls aldosterone levels under normal conditions. Although aldosterone biosynthesis and cortisol biosynthesis both require an 11 beta hydroxylation reaction, these reactions are catalyzed by two different, tissue-specific isoforms of 11 beta hydroxylase.

The B2 isoform, expressed in the Zona glomerulosa, catalyzes the conversion of 11-deoxycortisone to cortisol (as well as the following reaction) but has no activity on 11-deoxycortisol. Thus, progesterone generated in the Zona glomerulosa is converted specifically into aldosterone, while the same intermediate in the Zona reticularis and Zona fasciculata is channeled instead into cortisol production.
**Congenital Adrenal Hyperplasia.** A mutation affecting a single enzyme, 21 alpha hydroxylase, has diverse physiological effects which can be rationalized on the basis of the themes used to explain normal regulation of steroid hormone synthesis and its tissue specificity. In the adrenal cortex, loss of 21 alpha hydroxylase activity blocks the synthesis of both glucocorticoids (cortisol) and mineralocorticoids (aldosterone). To the extent that progesterone is generated, it will instead all be converted to androstenedione, which in turn can leave the adrenal cortex, bind carrier proteins in the blood, and be transported to skin or the male gonad to be converted to testosterone. Because cortisol synthesis is reduced, ACTH levels will remain high, StAR protein production in cells of the adrenal cortex will remain high, and progesterone synthesis will proceed briskly.