STERSOLS 2. OXYSTEROLS AND OTHER CHOLESTEROL DERIVATIVES

STRUCTURE, OCCURRENCE AND BIOCHEMISTRY

Oxysterols are usually defined as oxygenated derivatives of cholesterol, though plant sterols can also be oxidized, and they are important as short-lived intermediates or end products in the catabolism or excretion of cholesterol. They are normally present in biological membranes and lipoproteins at trace levels only, though they can exert profound biological effects at these concentrations. However, they are always accompanied by a great excess (as much as $10^6$-fold) of cholesterol.

Because of the presence of the double bond in the 5,6-position, oxysterols can be formed rapidly by non-enzymatic oxidation (autoxidation) of cholesterol (and cholesterol esters) within tissues, when a multiplicity of different oxygenated derivatives result, but related compounds are also synthesised by specific oxygenases in cells. Once an oxygen function is introduced into cellular cholesterol, the product can act as a biologically active mediator before it is metabolized to bile acids or is degraded further, processes assisted by the fact that oxysterols are able to diffuse much more rapidly through membranes than is cholesterol itself.

1. Non-Enzymatic Oxidation of Cholesterol

There is evidence that cholesterol in a membrane environment may be attacked more readily than the polyunsaturated fatty acids by reactive oxygen species, although the opposite is true in plasma. The structures of a few of the more important oxysterols are illustrated as examples of the main types of product.
Oxysterols can vary in the type (hydroperoxy, hydroxy, keto, epoxy), number and position of the oxygenated functions introduced and in the nature of their stereochemistry. Derivatives with the A and B rings and the iso-octyl side-chain oxidized are illustrated, but compounds with oxygen groups in position 15 (D ring) are also important biologically. Usually, they are named in relation to cholesterol, rather than by the strict systematic terminology.

Oxysterols occur in tissues both in the free state and esterified with long-chain fatty acids. For example, in human atherosclerotic lesions, 80-95% of all oxysterols are esterified. Appreciable amounts of oxysterols can be present in foods, especially those rich in such as meat, eggs and dairy products, which are most probably generated non-enzymically during cooking or processing. They can be absorbed from the intestines and transported into the circulation in chylomicrons, but the extent to which dietary sources contribute to tissue levels either of total oxysterols or of individual isomers is not known.

Mechanisms of autoxidation have been studied intensively in terms of unsaturated fatty acids, and it appears that similar mechanisms operate with sterols. The first event in lipid peroxidation is an initiation reaction in which a carbon with a labile hydrogen undergoes hydrogen abstraction by reaction with a free radical, which can be a non-lipid species such as a transition metal or hydroxyl or peroxynitrile radicals, and this is followed by oxygen capture. The resulting reactive species recruits further non-oxidized lipids and starts a chain reaction termed the propagation phase. Finally, the reaction is terminated by the conversion of hydroperoxy intermediates to more stable hydroxy products by reaction with endogenous antioxidants such as tocopherols.

As an example, the reaction mechanism leading to the production of 7-oxygenated cholesterol derivatives is illustrated. In aqueous dispersions, oxidation is initiated by a radical attack forming a delocalized three-carbon allylic radical, which reacts with oxygen to produce the epimeric products 7α- and 7β-hydroperoxy-cholesterol. Subsequent enzymic and non-enzymic reactions lead to the hydroxy and keto analogues, which may be accompanied by epoxy-ene and ketodienoic secondary products.

![Reaction mechanism](image)

Reaction does not occur at the other allylic carbon 4, presumably because of steric hindrance. When cholesterol is in the solid state, reaction occurs primarily at the tertiary carbon-25, though some products oxygenated at C-20 may also be produced.

Epimeric 5,6-epoxy-cholesterol may be formed by a non-radical reaction involving the non-enzymatic interaction of a hydroperoxide with the double bond, a process that is believed to occur in macrophages especially and in low-density lipoproteins (LDL). In this instance, the initial peroxidation product is a polyunsaturated fatty acid; the hydroperoxide transfers an oxygen atom to cholesterol to produce the epoxide, and in so doing is reduced to a hydroxyl. Other non-radical oxidation processes include reaction with singlet oxygen, which is especially important in the presence of light and photosensitizers and can generate 5-hydroxy- as well as 6- and 7-hydroxy products. Cholesterol-5,6-epoxides, formed in this way, are presently of interest in relation to cancer. In addition, reaction with ozone in the lung can generate a family of distinctive oxygenated cholesterol metabolites.

Photoxidation in the retina via the action of free radicals or singlet oxygen generates unstable cholesterol hydroperoxides, which may be involved in age-related macular degeneration. For
example, these compounds can quickly be converted to highly toxic 7α- and 7β-
hydroxycholesterols and 7-ketocholesterol, depending on the status of tissue oxidases and
reductases. Three separate enzymatic pathways have developed in the eye to neutralize their
activity. 7-Ketocholesterol is a major oxysterol produced during oxidation of low-density
lipoproteins, and is one of the most abundant in plasma and atherosclerotic lesions, with a high
pro-apoptotic potential. It associates preferentially with membrane lipid raft domains.

2. Enzymatic Oxidation of Cholesterol

Within animal cells, oxidation of sterols is mainly an enzymic process that is carried out by several
enzymes that are mainly from the cytochrome P450 family of oxygenases (they have a
characteristic absorption at 450 nm). These are a disparate group of proteins that contain a single
heme group and have a similar structural fold, though the amino acid sequences can differ
appreciably. They are all mono-oxygenases. As some of them are specific to particular tissues,
there is considerable variation in oxysterol distributions between organs. For example, a primary
product is 7α-hydroxycholesterol, which is an important intermediate in the biosynthesis of bile
acids (see below) and it is produced in the liver by the action of cholesterol 7α-hydroxylase
(CYP7A1), which has a critical role in cholesterol homeostasis. The reaction is under strict
regulatory control, and any circulating 7α-hydroxycholesterol represents leakage from the liver. On
the other hand, 7β-hydroxycholesterol is produced in brain by the action of the toxic β-amyloid
peptide and its precursor on cholesterol. Whether this metabolite is involved in the pathology of
Alzheimer’s disease has yet to be determined.

An alternative pathway to bile acids starts with 27-hydroxycholesterol, which is produced by
another cytochrome P450 enzyme (CYP27A1), which introduces the hydroxyl group into the
terminal methyl carbon (C-27). While this enzyme is present in the liver, it is found in many extra-
hepatic tissues and especially the lung, which provides a steady flux of 27-oxygenated metabolites
to the liver. It is involved in some of the later stages of bile acid production (see below). In addition,
as a multifunctional mitochondrial P450 enzyme in liver, it generates both 25R,26-
hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid, which occur in small but significant
amounts in plasma.

In humans, the specific cytochrome P450 that produces 24S-hydroxycholesterol (cholest-5-ene-
3β,24-diol), cholesterol 24S-hydroxylase (CYP46A1), is located almost entirely in the smooth
endoplasmic reticulum of neurons in the brain, and even the 24S-hydroxycholesterol found in
plasma is derived from the brain. The enzyme is expressed in neurons, including those of the
hippocampus and cortex, which are important for learning and memory, and it is responsible for
most of the turnover of cholesterol in the central nervous system.

25-Hydroxycholesterol is quantitatively a relatively minor but biologically important cholesterol
metabolite. At least two cytochrome P450 enzymes, CYP27A1 and CYP3A4, catalyse the
conversion of cholesterol in vitro, as does the dioxygenase enzyme cholesterol 25-hydroxylase.
However, the relative importance of the different mechanisms of formation in vivo have still to be
elucidated. Cholesterol autoxidation does not seem to be an important contributor.

24(S),25-Epoxycholesterol is not produced by the pathways described above but is synthesised
in a shunt of the same mevalonate pathway that produces cholesterol. It may represent a measure
of newly synthesised cholesterol.

The important human pathogen, *Mycobacterium tuberculosis*, utilizes a cytochrome P450 enzyme
(CYP125) to catalyse C26/C27 hydroxylation of cholesterol as an essential early step in its
catabolism as part of the infective process.
The oxysterols formed by both autoxidation and enzymatic routes can undergo further oxidation-reduction reactions, and they can be modified by many of the enzymes involved in the metabolism of cholesterol and steroidal hormones, such as esterification and sulfation, as illustrated for 7-keto-cholesterol.

Catabolism. Because of their increased polarity relative to cholesterol, oxysterols can exit cells relatively easily. Some are converted to inert sterol esters and stored in this form, a proportion is further oxidized and converted to bile acids, and some are converted to sulfate esters (especially at the 3-hydroxyl group) or glucuronides (see below) for elimination.

3. Oxysterols – Biological Activity

In tissues in vivo, the very low oxysterol:cholesterol ratio means that oxysterols have little impact on the primary role of cholesterol in cell membrane structure and function. Indeed, it is often argued that there are few reliable measurements of cellular or subcellular oxysterol concentrations, because of the technical difficulties in the analysis of the very low concentrations of oxysterols in the presence of a vast excess of native cholesterol. Nonetheless, aside from their role as precursors of bile acids and some steroidal hormones, it is evident that oxysterols have a variety of roles in terms of maintaining cholesterol homeostasis and perhaps in signalling. Autoxidation products of cholesterol may be useful markers for oxidative stress.

While cholesterol plays a key role in its own feedback regulation, there is some evidence that oxysterols are also inhibitors of cholesterol biosynthesis, and 25-hydroxycholesterol and 24(S),25-epoxycholesterol may be especially effective, although the other side-chain oxysterols 24- and 27-hydroxycholesterol are also implicated. Several mechanisms appear to be involved, and it is suggested that they inhibit the transcription of key genes in cholesterol biosynthesis (‘sterol regulatory element binding protein’ (SREBP) transcription factors), as well as directly inhibiting or accelerating the degradation of such important enzymes in the process as HMG-CoA reductase and squalene synthase. Oxysterols could smooth out the regulation of cholesterol metabolism, preventing exaggerated responses. However, some experts in the field caution that it can be difficult to extrapolate from experiments in vitro to the situation in vivo, because of the rapidity with which cholesterol can autoxidize in experimental systems and because of the difficulty
of carrying out experiments with physiological levels of oxysterols. Claims that oxysterols are master regulators of cholesterol homeostasis in vivo are now disputed.

25-Hydroxycholesterol is also reported to have a regulatory effect on the biosynthesis of sphingomyelin, which is required with cholesterol for the formation of raft sub-domains in membranes, and together with other oxysterols to regulate the activities of some hedgehog proteins involved in embryonic development. A metabolite 7α,25-dihydroxycholesterol functions may have a role in the regulation of humoral immunity.

Oxysterols are especially important for cholesterol homeostasis in the brain, which contains 25% of the total body cholesterol, virtually all of it in unesterified form, in only about 2% of the body volume. Cholesterol is a major component of the plasma membrane especially, where it serves to control the fluidity and permeability. This membrane is produced in large amounts in brain and is the basis of the compacted myelin, which is essential for conductance of electrical stimuli and contains about 70% of the cholesterol in brain. This pool is relatively stable, but the remaining 30% is present in the membranes of neurons and glial cells of gray matter and is active metabolically. Even in the foetus and the newborn infant, all the cholesterol required for growth is produced by synthesis de novo in the brain not by transfer from the circulation across the blood-brain barrier, which consists of tightly opposed endothelial cells lining the extensive vasculature of the tissue. The fact that this pool of cholesterol in the brain is independent of circulating levels must reflect a requirement for constancy in the content of this lipid in membranes and myelin. In adults, although there is a continuous turnover of membrane, the cholesterol is efficiently re-cycled and has a remarkably high half-life (up to 5 years). The rate of cholesterol synthesis is a little greater than the actual requirement, so that net movement of cholesterol out of the central nervous system must occur.

If cholesterol itself cannot cross the blood-brain barrier, its metabolite 24(S)-hydroxycholesterol is able to do so with relative ease. When the hydroxyl group is introduced into the side chain, this oxysterol effects a local re-ordering of membrane phospholipids such that it is more favourable energetically to expel it, and this can occur at a rate that is orders of magnitude greater than that of cholesterol per se, though still only 6-7 mg per day. There is a continuous flow of this metabolite from the brain into the circulation, where it is transported by lipoprotein particles to the liver for further catabolism, i.e. it is hydroxylated in position 7 and then converted to bile acids.

Both 24(S)-hydroxycholesterol and 24(S),25-epoxycholesterol are believed to be important in regulating cholesterol homeostasis in the brain. They interact with the specific nuclear receptors involved in the expression and synthesis of proteins involved in sterol transport. 24(S)-Hydroxycholesterol also down-regulates trafficking of the amyloid precursor protein and may be a factor in preventing neurodegenerative diseases.

27-Hydroxycholesterol diffuses across the blood-brain barrier in the reverse direction from the bloodstream into the brain, where it does not accumulate but is further oxidized and then exported as steroidal acids. This flux may regulate certain key enzymes within the brain, and there are suggestions that the balance between the levels of 24- and 27-hydroxy-cholesterol may be
relevant to the generation of β-amyloid peptides. 27-Hydroxycholesterol is also an element in cholesterol elimination from macrophages and arterial endothelial cells.

Especially high levels of 24(S)-hydroxycholesterol are observed in the plasma of human infants and in patients with brain trauma, while reduced levels are found in patients with neurodegenerative diseases, including multiple sclerosis and Alzheimer’s disease (it may be protective against β-amyloid peptide, the amyloidogenic peptide found in brain in this condition). In contrast, 7β-hydroxycholesterol, produced by this protein, is pro-apoptotic, but any links with the disease are unproven.

Oxysterols appear to be important for many aspects of cholesterol turnover and transport, and there have been many reports of involvement in disease processes, especially atherosclerosis and the formation of human atherosclerotic plaques, but also cytotoxicity, necrosis, inflammation, immuno-suppression, phospholipidosis and gallstone formation. Those oxysterols formed non-enzymatically are most troublesome in this regard. For example, they are enriched in pathologic cells and tissues, such as macrophage foam cells, atherosclerotic lesions, and cataracts. They may regulate some of the metabolic effects of cholesterol, but as cautioned above, effects observed in vitro may not necessarily be of physiological importance in vivo. Similarly, it has been argued that plasma oxysterols could serve as markers of oxidative stress, but the experimental difficulties in analysis have been such that their value has been limited. Sample handling remains a problem, but the newer methods of mass spectrometry with electrospray ionization now enable direct analysis of even the reactive hydroxy-, hydroperoxy- and ozonide-containing oxysterols.

4. Cholesterol 3-sulfate

The strongly acidic sulfate ester of cholesterol occurs in all mammalian cells, but it is especially abundant in keratinized tissue, such as skin and hooves. Although present at low levels, it can be the main sulfolipid in many cell types, but especially kidney, and reproductive and nervous tissues. In many organs, it appears to be concentrated in epithelial cell walls or in plasma membranes. Cholesterol sulfate is the main circulating sterol sulfate in plasma, although it is there accompanied by dehydroepiandrosterone sulfate, the function of which is unknown. In addition, 7-ketocholesterol sulfate has been found in primate retina, while 24-hydroxycholesterol occurs in bovine brain as its sulfate ester.

![Cholesterol sulfate structure](image)

The sulfate moiety is added to sterols by a family of cytosolic sulfotransferases (SULTS), some of which are specific for particular sterols; SULT2B1b preferentially catalyses the conversion of cholesterol to cholesterol sulfate, for example. It is de-sulfated by a microsomal arylsulfatase.

Cholesterol sulfate may have a role in ensuring the integrity and adhesion of the various skin layers, while also regulating some enzyme activities. For example, it functions in keratinocyte differentiation, inducing genes that encode for key components involved in development of the barrier. Cholesterol sulfate is generated in normal epidermis, but then is desulfated in the outer epidermis as part of a ‘cholesterol sulfate cycle’ that is a powerful regulator of epidermal metabolism and barrier function. However, it is evident that the lipid may have many other functions. It may play a part in cell adhesion, differentiation and signal transduction. In addition, it
has a stabilizing role, for example in protecting erythrocytes from osmotic lysis and regulating sperm capacitation.

Sterol sulfates have been detected occasionally in lower life forms, such as the sea star, *Asterias rubrius*, and the marine diatom, *Nitzschia alba*.

5. **Cholesterol Glycosides and Other Cholesterol Derivatives**

Cholesterol is found linked covalently to specific proteins, known as the hedgehog signalling family, where it functions to anchor the protein in a membrane, but this is discussed in our web page on proteolipids. Cholesteryl glucoside and acyl cholesteryl glucoside have been found in the skin of snakes and birds. Cholesteryl glucoside occurs also in human fibroblasts, and some rat tissues, where it may act as a mediator of signal transduction in the early stages of stress. As with plant and fungal steryl glycosides, these have a sugar β-glucosidic linkage. In addition, a cholesterol-conjugate with glucuronic acid has been isolated from human liver (33 nmol/g wet tissue) and plasma, but its origin, function and metabolic fate are unknown.

Some bacterial species contain cholesterol glycosides, and four unusual glycolipids, i.e. cholesteryl-α-glucoside, cholesteryl-6'-O-acyl-α-glucoside, cholesteryl-6'-O-phosphatidyl-α-glucoside, and cholesteryl-6'-O-lysophosphatidyl-α-glucoside, occur in the pathogenic bacterium *Helicobacter pylori*, for example. The key enzyme involved in their biosynthesis is a membrane-bound, UDP-glucose-dependent cholesterol-α–glucosyltransferase. These lipids appear to support the pathogenicity of the organism. Cholesterol 6-O-acyl-β-D-galactopyranoside and its non-acylated form are significant components of membranes of the spirochete *Borrelia burgdorferi*, which is the causative agent of Lyme disease. Sterol glycosides are more common constituents of plants (see our web page on plant sterols).

6. **Vitamin D**

Vitamin D encompasses two main sterol metabolites that are essential for the regulation of calcium and phosphorus levels and thence for bone formation in animals. However, these have many other functions, including induction of cell differentiation, inhibition of cell growth, immunomodulation, and control of other hormonal systems. In addition to the well known responsibility for rickets, Vitamin D deficiency is associated with various cancers and autoimmune diseases.

Ultraviolet light mediates cleavage of 7-dehydrocholesterol with opening of the second (B) ring in the skin to produce pre-vitamin D, which rearranges spontaneously to form the secosteroid vitamin D₃ or cholecalciferol. The newly generated vitamin D₃ is transported to the liver where it is subject to 25-hydroxylation and thence to the kidney for 1α-hydroxylation to form 1α,25-dihydroxyvitamin D₃ (calcitriol), a high affinity ligand for the vitamin D receptor. Vitamin D₂ or ergocalciferol is derived from ergosterol and is obtained from plant and fungal sources in the diet.

Vitamin D₃ functions by activating a cellular receptor (vitamin D receptor or VDR), which alters the transcription rates of the specific genes responsible for the biological responses.
7. Steroidal Hormones and their Esters

These lipids cannot be discussed in depth here. In brief, in addition to the bulk sterols, animal tissues produce small amounts of vital steroidal hormones, including oestrogens and progesterone, which are made primarily in the ovary and placenta during pregnancy, and testosterone mainly in the testes (the structure of pregnenolone is illustrated below as an example). Pregnan neurosteroids are produced in the central nervous system. Conversion of cholesterol to pregnenolone in mitochondria is the rate-limiting step and involves first hydroxylation and then cleavage of the side-chain.

Steroidal esters accumulate in tissues such as the adrenal glands, which synthesise corticosteroids such as cortisol and aldosterone and are responsible for releasing hormones in response to stress and other factors. It is also apparent that fatty acyl esters of oestradiols, such as dehydroepiandrosterone, accumulate in adipose tissue in postmenopausal women. Small amounts estrogens, acylated with fatty acids at C-17 hydroxyl, are also found in the plasma lipoproteins. In each instance they appear to be biologically inert storage or transport forms of the steroid. Eventually, esterified steroids in low density lipoproteins (LDL) particles are taken up by cells via lipoprotein receptors, and they hydrolysed to release the active steroid.

There is currently great pharmaceutical interest in oleoyl-estrone, a naturally occurring hormone in humans, which was found to induce a marked loss of body-fat while preserving protein stores in animals, a desirable goal for anti-obesity drug as body protein loss is an unwanted side effect of fat loss via calorie restriction.

**Recommended Reading**


