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Oxysterols as non-genomic regulators of cholesterol homeostasis

Agata Bielska¹, Paul Schlesinger², Douglas F. Covey³, and Daniel S. Ory¹

¹Diabetic Cardiovascular Disease Center, Washington University School of Medicine, St. Louis, MO 63110, USA

²Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110, USA

³Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA

Abstract

Tight regulation of cellular and plasma cholesterol is crucial to proper cellular functioning because excess free cholesterol is toxic to cells and is associated with atherosclerosis and heart disease. Cellular cholesterol homeostasis is regulated by enzymatically formed oxygenated cholesterol derivatives termed oxysterols. Although the effects of oxysterols on transcriptional pathways are well described, the non-transcriptional mechanisms through which oxysterols acutely modulate cellular cholesterol levels are less well understood. We present emerging evidence suggesting that the membrane biophysical properties of oxysterols underlie their acute cholesterol-regulatory functions and discuss the relevance of these acute effects to cholesterol overload in physiological and pathophysiological states.

Oxysterols and cholesterol homeostasis

Cholesterol is an essential component of mammalian cellular membranes and is required for diverse cellular functions, including the maintenance of appropriate membrane permeability and fluidity, as well as the regulation of integral membrane protein function [1,2]. Maintaining narrow limits of cellular cholesterol levels is crucial because excess free cholesterol is toxic to the cell [3,4]. Cellular cholesterol homeostasis is governed by an elaborate network of transcriptional and post-translational mechanisms that are sensitive to the level of cholesterol and to oxygenated derivatives of cholesterol, termed oxysterols (Glossary). Although the effects of oxysterols on transcriptional pathways are well described [5], the non-transcriptional mechanisms through which oxysterols acutely modulate cellular cholesterol levels are less well understood. These non-transcriptional cholesterol-regulatory actions of oxysterols are essential for maintaining homeostasis in response to a cholesterol challenge. We discuss the role of oxysterols as non-genomic regulators of cholesterol homeostasis, and consider the emerging evidence that the biophysical properties of membrane oxysterols underlie these acute cholesterol-regulatory functions. We also discuss the active cholesterol hypothesis and how oxysterol activation of membrane cholesterol may

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Corresponding author: Ory, D.S. (dory@wustl.edu).

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trigger the cellular response to a rapid rise in free cholesterol. We close with a discussion on the relevance of cholesterol activation to cholesterol overload in physiological and pathophysiological states.

Types of oxysterols

The term oxysterol refers to any oxygenated form of cholesterol or cholesterol precursor. Within this group, oxysterols can be further classified based on the location of the additional oxygen group, and whether they were formed enzymatically or non-enzymatically (e.g. from reactive oxygen species during oxidative stress) (Table 1). In general, there are vast differences biologically and chemically between ring-modified oxysterols and sidechain oxysterols, both in ligand binding affinity as well as their behavior within the membrane (Table 2). Both classes of oxysterols have been reviewed extensively elsewhere [5-7], so we focus here on the biological effects of sidechain oxysterols.

Oxysterols as acute regulators of cholesterol homeostasis

In classic studies of cholesterol balance in mice, Schoenheimer and Breusch demonstrated that cholesterol feeding led to decreased cholesterol synthesis, establishing for the first time the feedback control of cholesterol homeostasis [8]. The recognition that some oxygenated cholesterol metabolites could inhibit cholesterol synthesis with even greater potency than cholesterol led Kandutsch and colleagues to propose the Oxysterol Hypothesis, which stated that oxidized forms of cholesterol – i.e. oxysterols – rather than cholesterol itself, mediate the feedback inhibition on cholesterol biosynthesis [9]. Subsequent delineation of the mechanism of feedback inhibition firmly established the crucial role for both cholesterol and oxysterols for feedback control of cholesterol homeostasis [10]. This has prompted a revised Oxysterol Hypothesis, in which endogenous oxysterols – specifically enzymatically-derived sidechain oxysterols, acting either alone or in concert with membrane cholesterol – participate at multiple steps in the control of cholesterol homeostasis [5].

A striking property of oxysterols is their ability to induce rapid changes in cellular cholesterol levels. In response to elevated free cholesterol, sidechain oxysterols are enzymatically synthesized and feedback on multiple pathways to lower the free cholesterol levels in the cell [5]. At a transcriptional level, oxysterols inhibit the processing of sterol regulatory element-binding proteins (SREBPs), transcription factors that are master regulators of cholesterol synthesis and uptake pathways [11]. Oxysterols also ligand and activate liver X receptors (LXRs), inducing cholesterol efflux and elimination pathways and limiting lipoprotein cholesterol uptake [12,13]. Concomitant inhibition of SREBP and activation of LXR pathways are essential for cell viability when challenged with a cholesterol load. However, the timescale for these transcriptional pathways to alter cholesterol levels is on the order of hours. By contrast, there is abundant evidence that oxysterols also act within minutes to lower free cholesterol acutely through non-genomic mechanisms. Sidechain oxysterols such as 25-hydroxycholesterol (25-HC) (Table 1) reduce the free cholesterol burden by increasing cholesterol esterification via the endoplasmic reticulum (ER)-resident protein acyl-CoA:cholesterol acyl transferase (ACAT) [14,15]. This permits storage of the cholesteryl esters in neutral lipid droplets, and rapidly decreases cellular free cholesterol. The role of oxysterols in promoting this esterification is twofold. First, sidechain oxysterols are potent allosteric activators of ACAT [16]. In addition to activating ACAT directly, 25-HC also promotes the movement of plasma membrane cholesterol to an ACAT-accessible pool in the ER [14,17]. ACAT activity is generally limited by the cholesterol pool available to the enzyme [18]. Thus, the oxysterol-induced expansion of this pool, which is a potent stimulator of ACAT activity, enables the cell to

adapt rapidly within minutes to excess free cholesterol, well in advance of changes in cholesterol balance mediated by SREBP and LXR target genes.

Sidechain oxysterols also rapidly modulate free cholesterol levels through regulated degradation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) [19,20]. HMGR catalyzes the rate-limiting step in cholesterol synthesis converting HMG-CoA to mevalonate, and is highly regulated at transcriptional and post-transcriptional levels via multiple sterol and non-sterol end-products of mevalonate metabolism [21,22]. At the post-transcriptional level, oxysterols such as 25-HC promote the binding of HMGR to Insig – an ER-retention protein that recruits the E3 ubiquitin ligase gp78. Upon binding to Insig, HMGR is ubiquitinated by gp78 and is ultimately degraded by the proteasome, decreasing the half-life of HMGR from ~12 h to less than 1 h [22].

Cholesterol-induced degradation of HMGR requires synthesis of endogenous oxysterols such as 27-hydroxycholesterol (27-HC) (Table 1) [19]. In studies by Lange and colleagues, primary fibroblasts from cerebrotendinous xanthomatosis (CTX) patients deficient in sterol 27-hydroxylase (CYP27A1) were challenged with free cholesterol. In contrast to control fibroblasts, in which cholesterol loading induced 27-HC synthesis, cholesterol loading of CTX cells failed either to elicit this synthetic response or to stimulate the degradation of HMGR, demonstrating that sidechain oxysterols are necessary for this rapid sterol homeostatic response.

Role of oxysterol–membrane effects in cholesterol homeostasis

Recent studies provide evidence that sidechain oxysterols can regulate cholesterol homeostatic pathways via their membrane behavior, which is distinct from the membrane effects of cholesterol. Cholesterol has a lipid ordering and condensing effect, especially in membranes composed of saturated lipids [23], causing lipid chains to pack more closely together than they would in membranes without cholesterol. Sidechain oxysterols such as 25-HC, on the other hand, exert opposing effects in membranes, displaying lipid-disordering and expansive properties [23-25] (Figure 1). The membrane-disordering effect of 25-HC probably results from the energetic penalty for inserting the sidechain hydroxyl group into the hydrophobic bilayer. Indeed, computer simulations show that cholesterol is oriented parallel to phospholipids, whereas 25-HC has a much wider range of orientations within the membrane and can be found oriented perpendicularly to the neighboring phospholipids so as to maintain hydrogen bonding in both hydroxyl groups [26].

Experiments to determine whether oxysterol membrane properties are relevant for modulating cholesterol homeostasis used oxysterol enantiomers to distinguish sterol–membrane from sterol–protein effects. These studies used the principle that enantiomers have identical membrane properties, but interact differently with chiral molecules such as proteins [25,27]. As expected, the enantiomer of 25-HC (*ent*-25-HC) did not activate the LXR pathway – which requires a stereospecific ligand-binding event. Notably, *ent*-25-HC was able both to stimulate the degradation of HMGR as well as to suppress the expression of SREBP-controlled genes to the same extent as the natural 25-HC, implying that 25-HC regulates these pathways via its membrane effects [25].

The active cholesterol hypothesis

The rapid cholesterol homeostatic effects of sidechain oxysterols are consistent with the hypothesis that a pool of chemically active cholesterol is responsible for maintenance of cellular cholesterol balance [28]. Chemical activity is a thermodynamic term that describes the effective concentration of a species in a mixture. For ideal gasses, the activity and concentration of a compound are equal and used interchangeably. In solutions, however, the

components in the mixture can interact with each other, thus lowering the effective concentration available to interact with a measurable effector molecule. For cholesterol in membranes, condensed complexes are formed between cholesterol and neighboring lipids; the chemically active cholesterol represents the fraction of cholesterol that is free from these interactions and is available to interact with other effector molecules [23,29,30]. The ability of cholesterol to interact and complex with phospholipids is well described, and the stoichiometry of these interactions depends on the lipid composition (Figure 2). Lipids with larger head-groups and saturated acyl chains are able to complex more cholesterol than corresponding lipids with smaller head-groups or unsaturated acyl chains [24,26,30-32]. Thus, the lipid composition sets the capacity of a membrane to complex cholesterol, and above that complexation threshold, any additional cholesterol possesses high chemical activity [25,28,33]. Based on these observations, it has been proposed that it is the active cholesterol that regulates intracellular cholesterol trafficking and homeostasis [28,34]. Within the context of this working model, it follows that molecules that change the membrane environment – and thus change the relative amount of active cholesterol – can acutely alter cholesterol trafficking and regulation.

An active cholesterol model is supported via multiple threshold effects, which occur when cholesterol levels rise above the physiologic capacity of the phospholipids in the membrane to complex cholesterol. For example, the kinetics of cyclodextrin (CD)-mediated cholesterol extraction can identify two separate cholesterol pools. Initial extraction is fast, with 25% of the cholesterol being extracted with a $t_{1/2}$ of ~15 sec, corresponding to the active cholesterol. The second pool of extracted cholesterol is extracted much more slowly, with a $t_{1/2}$ of ~21 min, and corresponds to complexed cholesterol [35]. As would be predicted in this model, treatment with sphingomyelinase – which hydrolyzes sphingomyelin (SM) thereby lowering the number of cholesterol-sphingomyelin complexes [36] – increases the size of the initial fast pool. A separate threshold is seen with susceptibility of membrane cholesterol to cholesterol oxidase. At lower plasma membrane cholesterol concentrations, the rate of cholesterol oxidation is slow, and remains slow as the cholesterol concentration increases. However, once the cholesterol in the plasma membrane rises above the physiological set-point of the membrane, the rate of oxidation increases eightfold, indicating that it is the active cholesterol pool that is available to the cholesterol oxidase as a substrate [23].

Another threshold effect particularly relevant to cholesterol homeostasis is the movement of plasma membrane cholesterol to the ER. The plasma membrane contains 60–80% of cellular free cholesterol, is rich in sphingolipids and saturated phospholipids, and can accommodate upwards of 50 mol% cholesterol. The ER, on the other hand, only contains about 5 mol% cholesterol [27,28]. This low level of cholesterol confers to the ER exquisite sensitivity to changes in cellular free cholesterol levels, and, accordingly, the ER serves as the nexus for multiple cholesterol homeostatic pathways. As cholesterol is added to cells, it augments the plasma membrane cholesterol pool without affecting ER cholesterol levels. Once the physiologic set-point of the plasma membrane is exceeded, however, additional cholesterol rapidly moves to the ER to be esterified by ACAT [28,37]. Stimulation of ACAT by lipoprotein treatment also only occurs once a threshold amount of cholesterol has accumulated in the cell [18]. Supporting the idea that only the active plasma membrane cholesterol is available to be trafficked to the ER, compounds that decrease cholesterol–phospholipid complexes (e.g. sphingomyelinase and membrane-intercalating compounds) cause ER cholesterol levels to rise at a lower plasma-membrane cholesterol threshold [35]. Furthermore, increasing SM content via addition of exogenous SM or ceramide (an SM precursor) increases the threshold required for acetyl-LDL (low-density lipoprotein) to stimulate ACAT activity [18].

Role of oxysterols in cholesterol activation

Within the framework of the active cholesterol model, oxysterol-induced membrane effects that disrupt cholesterol–phospholipid complexes would be predicted to increase the pool of active cholesterol rapidly (Figure 2). Indeed, many amphipathic molecules have been demonstrated to increase cholesterol activity, manifested as increased susceptibility of cholesterol to cholesterol oxidase as well as increased efflux of cholesterol to CD [38]. Similar properties have been shown for 25-HC, raising the possibility that the oxysterol utilizes the same mechanism to regulate cholesterol [39]. Cholesterol activation, therefore, offers a potential mechanism to understand how 25-HC rapidly stimulates cholesterol movement to the ER for esterification, as well as to the mitochondria to increase synthesis of 27-HC and other sidechain oxysterols [14,38].

Further evidence supporting the cholesterol-activating properties of 25-HC is provided by recent molecular dynamics simulations. Simulations incorporating both cholesterol and 25-HC within a 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) bilayer system show that 25-HC increases the availability of cholesterol within the membrane. This manifests as changes in the position and orientation of cholesterol within the membrane, as well as an increase in the solvent accessibility of cholesterol [40]. In effect, 25-HC increases the proportion of cholesterol that protrudes from the membrane at the water interface, thereby increasing the accessibility of this cholesterol pool to external acceptors.

The importance of being able to alter cholesterol activity in the membrane is highlighted by recent evidence suggesting that cholesterol-sensing transmembrane proteins respond to active cholesterol. Perfringolysin O (PFO), a cholesterol-dependent cytolysin, binds to active cholesterol in a membrane, oligomerizes, and inserts into the membrane to form pores [41–43]. Although 40 mol% cholesterol is required before PFO can bind to and perforate liposomes composed of dioleoylphosphatidylcholine (DOPC), PFO has been shown to bind to the ER at cholesterol levels just above 5 mol%, implying that the composition of the ER is such that cholesterol is active above this physiologic set-point. Strikingly, the threshold at which PFO binds to ER membranes is the same as that for the regulation of SREBP processing [44,45]. SREBP is synthesized as a precursor transmembrane protein in the ER, and binds to its escort protein SCAP (SREBP cleavage activating protein). SCAP also binds to COPII proteins, allowing the SREBP–SCAP complex to bud from the ER and transit to the Golgi. In the Golgi, SREBP is cleaved sequentially by site-1-protease and site-2-protease, releasing a mature N-terminal fragment of SREBP that can now translocate to the nucleus and activate the transcription of proteins responsible for cholesterol uptake and synthesis. Under high-cholesterol conditions, the excess cholesterol in the ER binds to SCAP, in turn enabling SCAP to bind to Insig – an ER-retention protein. Insig binding to SCAP prevents recruitment of COPII proteins, and thus prevents the transit of the SREBP–SCAP complex from the ER to the Golgi for processing [46–48]. The fact that PFO and SCAP share the same threshold for binding to cholesterol suggests that SCAP binds to the active cholesterol in the ER. This implies that regulation of active cholesterol affects cholesterol sensing as well as cholesterol trafficking.

Possible mechanisms for membrane effects at low oxysterol concentrations

Oxysterol–membrane effects observed in model membranes and computational studies entail levels of oxysterol in the membrane in the range of 30 mol% [25,26,40]. The cellular concentration of these oxysterol species is far lower than in these model systems, and is three orders of magnitude less than the cholesterol content in cells [49]. The major argument against the involvement of oxysterols in the regulation of cholesterol homeostasis pathways

via their membrane effects is that, assuming homogenous distribution of oxysterols throughout the cell, the concentration of oxysterols would be too small to alter membrane behavior and have physiological effects. However, the distribution of oxysterols within the cell remains to be determined, and it is possible that oxysterols may concentrate within specific membranes and exert local effects.

Local elevations in oxysterol concentration may occur as solvation rings near oxysterol-synthetic enzymes such as cholesterol 25-hydroxylase, which is located in the ER, or as a result of targeting these oxysterols to specific membranes. Although the precise mechanism of oxysterol transport is unknown, a group of conserved proteins known to bind oxysterols – including oxysterol binding protein (OSBP) and OSBP-related proteins (ORPs) – may play a role in the trafficking of these species (as recently reviewed in [50]). ORPs are known to target specific membranes via multiple targeting domains, including FFAT (two phenylalanines (FF) in an acidic tract) domains that target VAP (VAMP (vesicle-associated membrane protein)-associated protein) in the ER. ORPs also contain pleckstrin homology (PH) domains known to target specific phosphoinositides, allowing differential targeting of the ORPs to plasma membrane or Golgi membranes [51].

In addition to achieving high local concentrations within membranes, it is also possible that oxysterols may act within specialized membranes adapted to respond to low oxysterol concentrations. As stated above, cholesterol activity is dependent on the composition of the membrane, and saturated lipids composed of large head-groups offer more protection to cholesterol within the membrane (decreasing cholesterol activity) whereas unsaturated lipids composed of small head-groups offer less protection (increasing cholesterol activity) [30]. The extent of oxysterol-induced membrane expansion is also determined by membrane lipid composition. Support for this concept is provided by model-membrane studies in which local oxysterol concentrations of as little as 5 mol% are sufficient to expand unsaturated DOPC monolayers, whereas saturated POPC monolayers do not expand significantly [25]. Likewise, PFO and SCAP are able to sense cholesterol levels within a narrow threshold centered around 5 mol% in ER membranes, which are composed predominantly of phospholipids (85%) that have incorporated unsaturated acyl chains [25,52]. Thus, the ER appears to possess a unique membrane composition that allows for small changes in total ER cholesterol levels to be sensed. It is therefore plausible that cholesterol within such specialized membranes can be activated with submicromolar oxysterol concentrations.

Physiological relevance of rapidly adapting to large cholesterol loads

The alteration of membrane properties provides a mechanism to detect and adapt rapidly to acute changes in cellular cholesterol levels. This ability is crucial for the cell to manage large cholesterol loads, preventing accumulation of free cholesterol in the ER that leads to cellular dysfunction and death [4]. In the ER, excess cholesterol orders and increases the thickness of the ER membrane, leading to the dysfunction and inhibition of the ER calcium pump sarcoplasmic–endoplasmic reticulum calcium ATPase-2b (SERCA2b) [51]. The resulting depletion of ER calcium stores activates the unfolded protein response (UPR) and downstream expression of the death effector CHOP [3,4]. Thus, sensory mechanisms that permit the cell to respond to abrupt increases in cholesterol would prevent enrichment of ER cholesterol and possibly provide a survival advantage.

Large fluctuations in cellular cholesterol levels are routinely encountered *in vivo*, both in physiological (e.g. apoptosis) and pathophysiological (e.g. atherosclerosis) settings. Professional scavenging cells, such as macrophages, have evolved mechanisms to counter the extreme cholesterol load imposed by efferocytosis, a process whereby professional or non-professional phagocytes efficiently dispose of apoptotic cells [53,54]. Efferocytosing

cells exhibit a unique set of responses, including massive efflux of cholesterol and activation of survival pathways to prevent apoptosis [55]. Similarly, during atherogenesis rapid disposal of free cholesterol is crucial for the survival of lipid-loaded or phagocytosing macrophages, as demonstrated by the deleterious effects of administration of ACAT inhibitors before phagocytosis of apoptotic cells. Although upregulation of LXR target genes provides long-term homeostatic control, it is likely that the initial response is mediated through the membrane effects of endogenously synthesized sidechain oxysterols. Within minutes, these oxysterols facilitate the elimination of free cholesterol by stimulating the internalization of plasma membrane cholesterol and its esterification by ACAT, also providing a more soluble form of cholesterol that can be readily exported from the cell [56].

Concluding remarks

The role of sidechain oxysterols in the regulation of cholesterol homeostasis transcriptional pathways, through ligand-binding events, has been well described. Oxysterols such as 25-HC play a key role in regulating SREBP processing via binding to Insig, which in turn results in SCAP–Insig binding and the retention of the Insig–SCAP–SREBP complex in the ER [11]. Oxysterols also act as ligands for LXR, resulting in the activation of downstream cholesterol efflux pathways [12]. Less well appreciated is the central role for sidechain oxysterols in the acute response to cholesterol loads. Non-genomic regulation of cholesterol homeostasis by oxysterols provides an additional layer of control that operates on very short timescales and thus serves as a complement to the well-documented oxysterol ligand-dependent transcriptional events.

It is well established that sterols contribute to the regulation of cellular pathways via changes to membrane properties. Cholesterol content, or more specifically the effect of cholesterol on membrane thickness, is important in protein sorting among organellar membranes [57]. Similarly, changes to membrane order, thickness, or cholesterol levels have been shown to affect the function of many proteins, including EGFR, rhodopsin, and SERCA2b, among others [4,58,59]. It is also apparent that the magnitude of the change in membrane cholesterol content does not need to be large to trigger a response. Indeed, changes in cholesterol content of 1–2%, centered around the set-point of 5 mol%, are sensed by SCAP [44]. Thus, it is plausible that a regulator of cholesterol homeostasis would exploit these membrane properties to adapt quickly to increases of cellular cholesterol.

Although the active cholesterol model provides a conceptual framework for understanding the effects of oxysterols on membrane cholesterol, there are several important unresolved issues with respect to the molecular mechanisms that underpin the membrane behavior of oxysterols (Box 1). What are the precise oxysterol-membrane interactions that promote entry of cholesterol into the active pool? Perhaps oxysterols physically displace cholesterol from complexes with phospholipids, or instead act by exposing cholesterol, possibly by reducing steric shielding by phospholipid headgroups. Likewise, it is poorly understood how activation of cholesterol in the cytosolic leaflet of the plasma membrane is coupled to the machinery that traffics cholesterol to internal membranes. Further studies are also needed to reconcile the mounting evidence implicating sidechain oxysterols in modulating sterol homeostatic responses with the low intracellular concentration of oxysterols. What is the physiologically relevant site of action of oxysterols, and do they concentrate locally within specialized membranes, in which their effects on cholesterol activation are amplified? Future multidisciplinary efforts that integrate biophysical, computational and chemical biology, together with traditional cell biology approaches, will probably be required to answer these fundamental questions.

In this Opinion article we have presented the emerging evidence that sidechain oxysterols – enzymatic products of cholesterol – exert acute non-genomic cholesterol homeostatic effects by altering membrane properties, thereby allowing cells to manage large cholesterol loads rapidly and avoid triggering cytotoxic events.

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Glossary

ACAT	acyl-CoA:cholesterol acyl transferase. An ER resident protein that esterifies free cholesterol into cholesteryl esters, allowing for storage of excess cholesterol into neutral lipid droplets.
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Active cholesterol	the fraction of cholesterol that is free from complexes with neighboring lipids in the membrane, and is available to interact with cytosolic effector molecules.
CD	cyclodextrin. A family of cyclic oligosaccharides. 2-Hydroxypropyl- β -cyclodextrin, with seven sugars per ring, can bind and extract or deliver cholesterol to membranes.
COPII	a vesicle coat protein that transports proteins from the ER to the Golgi (anterograde) via vesicular budding.
CTX	cerebrotendinous xanthomatosis. Autosomal recessive disorder in which patients are deficient in sterol-27-hydroxylase (CYP27A1). CTX patients have deposition of cholestanol in the brain and other tissues, and develop progressive cerebellar ataxia and tuberous xanthomas.
DOPC	dioleoyl-phosphatidylcholine. A common membrane phospholipid with two unsaturated (oleoyl) acyl chains and a phosphatidylcholine headgroup.
Efferocytosis	a process where professional or non-professional phagocytic cells phagocytose and efficiently dispose of apoptotic cells.
25-HC	25-hydroxycholesterol. A prototypic sidechain oxysterol, with a hydroxyl group at position 25 on the sterol sidechain.
HMGR	3-hydroxy-3-methylglutaryl-CoA reductase. An ER resident enzyme that catalyzes the rate-limiting step in cholesterol synthesis, converting HMG-CoA to mevalonate.
Insig	ER-retention proteins (Insig1 or Insig2 in mammals) that binds to the SCAP-SREBP complex and retains it in the ER under high-cholesterol conditions. Insig also simultaneously binds to HMGR and the E3 ubiquitin ligase gp78, leading to the ubiquitination and subsequent degradation of HMGR under high-cholesterol conditions. Sidechain oxysterols bind to Insig to promote the Insig/SCAP and Insig/HMGR interactions.
LXR	liver X Receptor. A transcription factor that activates cholesterol efflux and elimination pathways, and limits uptake of exogenous cholesterol.
ORPs	OSBP-related proteins. A group of conserved proteins that bind oxysterols and cholesterol.
OSBP	oxysterol binding protein. A protein that binds oxysterols and cholesterol.
Oxysterols	oxygenated metabolites of cholesterol or cholesterol precursors. Oxysterols can be classified as ring-modified or sidechain oxysterols, with different activities attributed to these two groups (Tables 1,2).
PFO	perfringolysin O. A bacterial cytolysin that binds to membranes in a cholesterol-dependent manner, oligomerizes, and forms pores.
POPC	1-palmitoyl, 2-oleoyl-phosphatidylcholine. A common membrane lipid with one saturated (palmitoyl) and one unsaturated (oleoyl) acyl chain, with phosphatidylcholine as the lipid headgroup.

SCAP	SREBP cleavage activating protein. An escort protein that binds to SREBP, and to COPII proteins, allowing the SREBP–SCAP complex to bud from the ER and translocate to the Golgi.
SM	sphingomyelin, a common plasma membrane lipid, known to preferentially form complexes with cholesterol.
SREBP	sterol regulatory element-binding protein. A transcription factor that activates pathways to increase <i>de novo</i> cholesterol synthesis and uptake.

Box 1: Outstanding questions

- What are the precise oxysterol–membrane interactions that promote the entry of cholesterol into the active pool?
- How do different membrane species either augment or dampen these interactions?
- How can oxysterols modulate cholesterol homeostasis via membrane properties at low (i.e. submicromolar) intracellular concentrations?
- Where is the physiologically relevant site of action of oxysterols?
- Do oxysterols concentrate locally within specialized membranes?
- How is activation of cholesterol in the plasma membrane coupled to the machinery that traffics the cholesterol to internal membranes?

Figure 1. Model of cholesterol- and sidechain oxysterol-phospholipid interaction (a) and (c) show cholesterol in condensed packing with three phospholipids in horizontal and vertical views. (b) and (d) show similar views for 25-HC associated with three phospholipids where the alkane chains are in the more usual non-condensed conformation. This configuration promotes the membrane expansion observed upon the addition of 25-HC to phospholipid monolayers and bilayers. Increasing saturation of phospholipid acyl chains (e.g. DPPC) promotes less tilted and deeper insertion of sidechain oxysterols into membranes, thereby abrogating their membrane-expansion effects. Reproduced with permission from Gale *et al.* [25].

Figure 2. Effect of membrane composition on cholesterol capacity. In cholesterol-containing membranes, SM

(a) has a greater capacity to complex cholesterol than POPE (c) (i.e. SM membranes can complex more cholesterol before it goes into an active pool). Oxysterols disrupt the cholesterol-complexing capacity of the phospholipids (b) and (d), causing cholesterol to protrude out of the membrane (active cholesterol). The ability to disrupt lipid–cholesterol complexes is probably dependent on lipid composition, with oxysterols having a greater disordering effect in unsaturated membranes [25]. To demonstrate pictorially the effect of lipid composition on the effect of oxysterols, one oxysterol is shown as activating one molecule of cholesterol in SM (b), and one oxysterol is shown as activating two molecules of cholesterol in POPE (d). However, it is likely that much lower levels of oxysterols (i.e. <5 mol%) are sufficient for cholesterol activation, rather than the 1:1 stoichiometry shown in the model.

Table 1

Chemical structures of common sidechain and ring-modified oxysterols

Side-chain Oxysterols	Ring-modified Oxysterols
27-Hydroxycholesterol	7-Ketocholesterol
25-Hydroxycholesterol	7β-Hydroxycholesterol
24(S),25-Epoxycholesterol	7α-Hydroxycholesterol
24(S)-Hydroxycholesterol
22(R)-Hydroxycholesterol	

Table 2

Properties of sidechain and ring-modified oxysterols

Oxysterol	Side-chain Oxysterols	Ring-modified Oxysterols	References
Cholesterol-Esterification	++	+/-	5, 7, 15
HMGR Degradation	++	+/-	5, 7, 19
LXR ligand	++	–	5, 6, 12
Insig ligand	++	+/-	5, 7, 11