Progress in Lipid Research 96 (2024) 101304

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review Article

How active cholesterol coordinates cell cholesterol homeostasis: Test of a hypothesis

Yvonne Lange [a,*,1], Theodore L. Steck [b,1]

[a] Department of Pathology, Rush University Medical Center, Chicago, IL 60612, United States of America
[b] Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, United States of America

ARTICLE INFO

Keywords:
Active
Cholesterol
Enterocyte
Feedback
Homeostasis
Model
Regulation

ABSTRACT

How do cells coordinate the diverse elements that regulate their cholesterol homeostasis? Our model postulates that membrane cholesterol forms simple complexes with bilayer phospholipids. The phospholipids in the plasma membrane are of high affinity; consequently, they are fully complexed with the sterol. This sets the resting level of plasma membrane cholesterol. Cholesterol in excess of the stoichiometric equivalence point of these complexes has high chemical activity; we refer to it as *active cholesterol*. It equilibrates with the low affinity phospholipids in the intracellular membranes where it serves as a negative feedback signal to a manifold of regulatory proteins that rein in ongoing cholesterol accretion. We tested the model with a review of the literature regarding fourteen homeostatic proteins in enterocytes. It provided strong albeit indirect support for the following hypothesis. Active cholesterol inhibits cholesterol uptake and biosynthesis by suppressing both the expression and the activity of the gene products activated by SREBP-2; namely, HMGCR, LDLR and NPC1L1. It also reduces free cell cholesterol by serving as the substrate for its esterification by ACAT and for the synthesis of side-chain oxysterols, 27-hydroxycholesterol in particular. The oxysterols drive cholesterol depletion by promoting the destruction of HMGCR and stimulating sterol esterification as well as the activation of LXR. The latter fosters the expression of multiple homeostatic proteins, including four transporters for which active cholesterol is the likely substrate. By nulling active cholesterol, the manifold maintains the cellular sterol at its physiologic set point.

1. Introduction

Cholesterol is a major and essential component of the lipid bilayer of animal cell plasma membranes and figures prominently in human health and disease [1–3]. An elaborate manifold of regulatory proteins manages its abundance [1,4–9]. Cells keep their cholesterol in balance by specifying how much they need, sensing how much they have and adjusting what they have to what they need. (The terms *sterol* and *cholesterol* are used interchangeably.) A simple model has been proposed to describe the mechanism of cell cholesterol homeostasis [10]. It postulates that the cholesterol in the plasma membrane is held in tight complexes with its polar lipids (at least the glycerophospholipids and sphingomyelin) up to stoichiometric equivalence. This establishes the physiologic set point of the cholesterol in the plasma membrane. Excess cholesterol is uncomplexed and has a high chemical activity [11,12]. This *active cholesterol* equilibrates with the intracellular membranes (endomembranes) and provides a negative feedback signal to the regulatory proteins in the ER and mitochondria. They respond by inhibiting the accretion of the sterol and promoting its depletion, thereby restoring the plasma membrane to its resting level.

The basic features of the active cholesterol model are well supported experimentally [10,13]. However, it remains to be shown that active

Abbreviations: ABCA1, ATP binding cassette transporter A1; ABCG1, ATP binding cassette transporter G1; ABCG5/G8, ATP binding cassette transporter G5/G8; ACAT, acyl-coenzyme A:cholesterol acyltransferase; CH, cholesterol; CM, chylomicron; ER, endoplasmic reticulum; GramD1b, GRAM domain containing 1B; 27HC, 27-hydroxycholesterol; HDL, high density lipoproteins; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IDOL, inducible degrader of the low density lipoprotein receptor; Insig, insulin-induced gene; LDLR, low density lipoprotein receptor; LXR, liver X receptor; NPC1L1, Niemann-Pick C1-like 1 protein; PFO, perfringolysin O; PL, phospholipid; Scap, sterol regulatory element-binding protein cleavage-activating protein; SR-BI, scavenger receptor class B, type I; SREBP-2, sterol regulatory element-binding protein-2..

* Corresponding author.
E-mail address: Yvonne_Lange@rush.edu (Y. Lange).
[1] Both authors contributed fully to every aspect of the manuscript.

https://doi.org/10.1016/j.plipres.2024.101304
Received 27 June 2024; Received in revised form 23 October 2024; Accepted 1 November 2024
Available online 2 November 2024

cholesterol coordinates the diverse functions that manage cholesterol homeostasis. That is, are the activities of these proteins governed by this unitary signal? We address this question by reviewing relevant literature on enterocytes, the absorptive cells that line the lumen of the intestine. Enterocytes utilize the same sterol regulatory mechanisms as other cells but also manage the body's cholesterol load. That is, they make the final call as to whether sterol molecules are retained or excreted. To this end, they integrate inputs from the diet, the bloodstream and the liver with their own sterol synthesis [6,14–17]. The secretion of cholesterol from the bloodstream to the gut is referred to as transintestinal cholesterol excretion or TICE [18–20].

Here, we consider fourteen proteins that regulate enterocyte sterol (see Fig. 1). The premise being tested is that the operative feedback signal is not cell cholesterol itself, as is often assumed, but a particular small and dynamic fraction thereof: active cholesterol. The relevant literature is supportive, but much of it is indirect. Therefore, our review is often speculative. Nevertheless, the data affirm the hypothesis. Furthermore, what is proposed for the fourteen proteins in enterocytes is applicable to other proteins that regulate their cholesterol as well as to cells generally.

2. The disposition of cellular cholesterol

Plasma membranes hold about 90 % of the cholesterol and sphingomyelin and about half of the glycerophospholipids in cells like fibroblasts [10,21]. The remaining sterol and phospholipids are distributed unequally among the intracellular organelles. Cholesterol circulates throughout the cell with a characteristic time of about an hour and appears to be at diffusional equilibrium among the membranes [10,13,22,23]. A variety of soluble and intermembrane transporters mediate this flux, mostly independent of vesicular traffic [13,24–28].

Almost all the cholesterol in cell membranes is complexed with their phospholipids, and this is a primary determinant of its intracellular distribution [10,11,13]. The different phospholipids have characteristic affinity constants and stoichiometries. Complexes of cholesterol with various disaturated phosphatidylcholines as well as sphingomyelin have apparent cholesterol:phospholipid stoichiometries of CH:PL ~ 1 mol/mol [29,30]. This is (1 mol CH)/(1 mol CH + 1 mol PL); hence, a mole fraction of 0.5 or 50 mol%. At least some unsaturated phospholipids, for example dioleoylphosphatidylcholine, appear to have CH:PL=~ 0.5; i.e., 33 mol% or a mole fraction of 0.33. Resting plasma membranes generally have CH:PL ~0.7 mol/mol; i.e., ~41 mol%. This is consistent with roughly equal amounts of phospholipids with CH:PL ~ 1.0 and 0.5 mol/mol [10]. Enterocyte brush border membranes have a CH:PL value of ~0.50–0.65 mol/mol; i.e., ~33–40 mol% [31–33]. It therefore seems that phospholipids with a stoichiometry of CH:PL 0.5 predominate in these cells. Plasma membrane cholesterol serves multiple structural roles: condensing the bilayer and modulating its elasticity, viscosity, compressibility, impermeability, thickness and lateral phase behavior [13].

The phospholipids in plasma membranes are particularly avid for cholesterol; consequently, they are normally fully complexed at their stoichiometric equivalence point (Fig. 2A) [10,11,13,34]. In sharp contrast, the intracellular membranes of a variety of cell types contain relatively little cholesterol; that is, CH:PL < 0.1 mol/mol or < 10 mol% [10,35–38]. These endomembranes are not saturated with cholesterol, since loading cells can expand their intracellular sterol content several fold [34]. It thus appears that endomembrane phospholipids have weak affinity for cholesterol (Fig. 2B) [10,11,13]. It has been estimated that the dissociation equilibrium of avid plasma membrane complexes leaves only a few percent of its cholesterol uncomplexed while perhaps 10 % of the sterol in endomembranes is dissociated [10,29,39].

The complexation of sterols with phospholipids reduces their chemical activity by orders of magnitude [29]. Consequently, the titration of membrane phospholipids with cholesterol produces a J curve in cholesterol chemical activity (dashed red line in Fig. 2A) [10,13,40]. Ligands for sterols must compete with the membrane phospholipids for their complexed cholesterol. As a result, the binding isotherms even of monomeric proteins are perforce sigmoidal [41,42]. Ligands with low affinity compete poorly with the phospholipids and only associate well with the uncomplexed sterol as it becomes available at the equivalence point (dashed blue line inFig. 2A). Two such weak ligands are

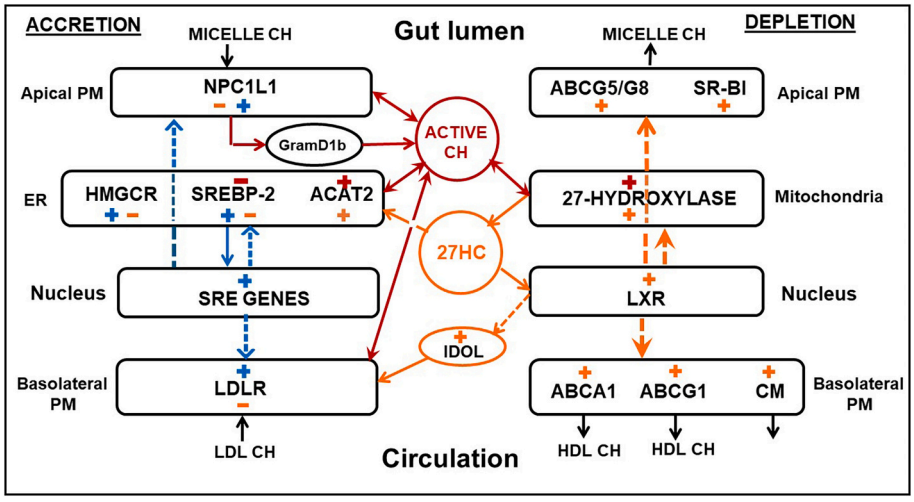

Fig. 1. How active cholesterol (CH) regulates cholesterol homeostasis: a hypothesis. This is a summary of the regulatory manifold described in the text. Plus signs signify their stimulation, minus signs signify their inhibition, solid arrows represent their activities and dashed arrows indicate their expression. The functions in the left column generally mediate cholesterol accretion; those in the right column mediate its depletion. The transcription of sterol regulatory element (SRE)-containing genes is driven by activated SREBP-2. This leads to the expression of HMGCR, LDLR, NPC1L1 and additional SREBP-2 (dashed blue arrows). The excess cholesterol acquired from intestinal micelles, circulating LDL and biosynthesis is chemically active (shown in red) and distributes among the cell membranes (double headed arrows). Active plasma membrane cholesterol is conveyed to the ER by GramdD1b and presumably by other transporters. There, it reduces the abundance of the free sterol by cueing the sequestration of SREBP-2 and promoting its own esterification by ACAT2. Active cholesterol is also the substrate for mitochondrial 27-hydroxylase. Its product, 27HC, mediates the degradation of HMGCR and the activation of LXR. The latter drives the expression of mitochondrial 27-hydroxylase, IDOL, the CM apolipoprotein B-48, and four plasma membrane transporters (dashed orange arrows). Excess cholesterol is secreted to the circulation by these transporters as well as via CM. Not shown: 27HC inhibits the expression of NPC1L1 and activated LXR inhibits the destruction of ABCA1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

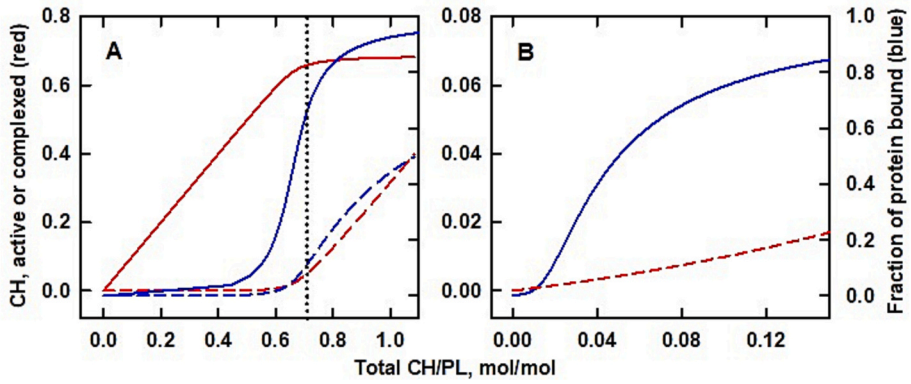

Fig. 2. Association of sterol-binding proteins with active and extractable cholesterol. Panel A. Simulated titration curves illustrate the formation of complexes of cholesterol (CH) with high affinity plasma membrane phospholipids (PL) (solid red line) up to their stoichiometric equivalence point of CH:PL = 0.71 mol/mol phospholipid (i.e., 40 mol% cholesterol; vertical line). The dashed red line is the uncomplexed (chemically active) cholesterol. Ligands with relatively weak affinity (e.g., cholesterol oxidase, methyl-β-cyclodextrin and GramD1b) associate well only with the uncomplexed sterol (dashed blue line). More avid proteins (e.g., PFO and some oligomeric plasma membrane proteins) competitively extract the sterol from its phospholipid complexes and therefore bind it below the equivalence point (solid blue line). Panel B. Some regulatory proteins are avid oligomers that bind cholesterol cooperatively (e.g., ACAT and Scap). They extract the sterol from its weak ER complexes well below its stoichiometric equivalence with the phospholipids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cholesterol oxidase and β-cyclodextrins [11,13,30,43]. Similarly, the sterol-binding domain of the cholesterol import protein, GramD1b, associates weakly with plasma membrane cholesterol in resting cells but binds strongly to the uncomplexed excess [44,45]. This characteristic befits a homeostatic function: removing the sterol that exceeds the physiologic set point of the plasma membrane (see Section 4.2).

Avid proteins, certain integral plasma membrane proteins among them, can competitively extract cholesterol from its phospholipid complexes below their stoichiometric equivalence point (Fig. 2A and B) [41,42]. Toxins like PFO are also avid; they can associate with unenriched cells so as to permeabilize them [41,46,47]. Thresholds for the binding of PFO mutants vary widely well below the stoichiometric equivalence point of the sterol [48,49]. Whether a protein extracts complexed cholesterol competitively or binds only the active excess can be difficult to discern. Thus, the membrane sterol associated with toxins and GramD1b has simply been called *accessible cholesterol* [9,28,47,50–53]. Four forms of cell cholesterol are therefore relevant to its homeostasis: complexed, uncomplexed (the active excess), extractable (that competitively removed from its complexes by a ligand) and esterified.

3. Cholesterol homeostasis

Fig. 1 applies our model to enterocytes. The left side of the figure shows the accretion of cholesterol, driven by SREBP-2 through the expression of SRE gene products (see Section 5.1). SREBP-2 also promotes its own elaboration autocatalytically in a feed-forward fashion [54,55]. The cholesterol in the cell would increase indefinitely were it not for feedback responses that inhibit and reverse its accumulation. These responses are managed by the manifold of homeostatic proteins depicted in the right side of the Figure. The hypothesis being tested is that the activity of these proteins is coordinated by excess (uncomplexed, active) plasma membrane cholesterol and its oxysterol derivatives (center of the figure).

We propose that homeostasis works like this: The regulatory manifold keeps the avid plasma membrane phospholipids replete with cholesterol at their stoichiometric equivalence point. Excess plasma membrane cholesterol spills down its chemical activity gradient to the intracellular membranes. There, it serves as a feedback signal to the regulatory elements [10,12,13]. The influx of active cholesterol can enlarge the small sterol pools in the endomembranes several-fold on a time scale of minutes [23,34]. Conversely, the cholesterol in the ER falls rapidly when that in the plasma membrane drops below its resting level [56]. The activities of ER effectors like SREBP-2, ACAT and HMGCR respond homeostatically to the local cholesterol level [12,13,38,56]. In a like manner, incrementing plasma membrane cholesterol above its physiologic set point stimulates the synthesis of the feedback signal, 27HC, in mitochondria (see Section 4.1) [57]. In that study, excess cholesterol moved from the plasma membrane to the mitochondria where it was converted to 27HC which promoted the proteolytic inactivation of HMGCR in the ER with a half-time of 8.5 min overall.

The activity of a homeostatic protein varies with its fractional saturation with cholesterol. This is determined by the cholesterol affinity of the protein, that of the phospholipids with which it competes and the local membrane concentration of the sterol [10,42]. At least some of the homeostatic proteins are high-affinity oligomers that bind the sterol well below stoichiometric equivalence of the sterol with the phospholipids, as simulated in Fig. 2B [42]. Accordingly, the thresholds for the activity of these proteins do not reflect the emergence of active cholesterol in the endomembranes but, rather, their extraction of the sterol from its complexes. This appears to be the case for ACAT tetramers, the activity of which rises sigmoidally at a bilayer cholesterol concentration of ~10 mol% (see Section 4.3) [58]. This is far below the presumed stoichiometric equivalence point for the egg phosphatidylcholine vesicles used in that membrane reconstitution study [29]. Similarly, the isotherm for the inactivation of SREBP-2 was shown to be acutely sigmoidal at an ER cholesterol concentration of ~6 mol% (see Section 5.1) [38,59]. This ER threshold lies well below the stoichiometric equivalence point of common phospholipids [29,30]. The implication that ER phospholipids have a low sterol affinity is supported by the finding that the association of PFO with isolated ER membranes has a threshold of ~6 mol% cholesterol while its binding to the cholesterol associated with avid phospholipids has thresholds of 30–45 mol% [47,59]. It therefore appears that the low threshold for SREBP-2 mobilization reflects both the weak cholesterol affinity of ER phospholipids and the cooperative binding of the sterol by the avid protein that sequesters SREBP-2; namely, Scap tetramers [10,60].

Plasma membrane cholesterol can fall below its stoichiometric equivalence point due to cell growth or cholesterol export. Cholesterol will then flow from the intracellular membranes to the plasma membrane [13,24,56]. Presumably, transporters are able to extract cholesterol from its phospholipid complexes in the intracellular membranes far below their stoichiometric equivalence point, as simulated in Fig. 2B. The extent of transfer is independent of the abundance, affinity and

velocity of the transporters; rather, it reflects the equilibration of active cholesterol down its chemical activity gradient between the endomembranes and the plasma membrane.

The homeostatic manifold uses active and extractable cholesterol differently. Active cholesterol is that delivered from the plasma membrane to the endomembranes as a homeostatic signal. Extractable cholesterol is the regulator of homeostatic activities in the endomembranes; it is also the substrate for cholesterol flux to the cell surface. Homeostatic responses are sharpened by two thresholds in tandem. One is the stoichiometric equivalence point of the plasma membrane phospholipids beyond which active cholesterol moves to the endomembranes (Fig. 2A). The other threshold is the sigmoidal dependence of the activities of the regulatory proteins on the extractable cholesterol in the endomembranes (Fig. 2B). This dual feedback system maintains the cholesterol in the plasma membrane replete at its physiologic set point while that in the intracellular membranes is variable and kept far below stoichiometric equivalence with the phospholipids by the homeostatic effectors. Maintaining a low level of endomembrane sterol makes feedback responses sensitive to small fluctuations in the plasma membrane at its threshold.

We now review evidence bearing on the hypothesis that cholesterol homeostasis in enterocytes is coordinated by active cholesterol.

4. Active cholesterol regulates homeostatic proteins

4.1. 27-hydroxylase

Side-chain oxysterols are cholesterol derivatives, some of which serve as homeostatic feedback signals [61–63]. Here, we focus on 27HC because it has been shown to be synthesized from active cholesterol [40,57]. As charted in Fig. 1 and discussed below, 27HC directly regulates the activity of at least three homeostatic ER effectors: HMGCR, ACAT and SREBP-2. Importantly, oxysterols also activate LXR and thus drive the expression of several proteins mediating cholesterol depletion (see Section 5.2) [9,64,65]. One of these is the steroidogenic acute regulatory (StAR) protein that facilitates the mitochondrial production of oxysterols [66]. This is a feed-forward mechanism that intensifies the activation of LXR. Furthermore, 25-hydroxycholesterol (from the ER) and 27HC (from mitochondria) stimulate the nuclear expression of their respective hydroxylases through LXR; these pathways are also positive feedback loops that boost LXR activity [62,67]. In addition, oxysterols inhibit the expression of NPC1L1 in enterocytes, thereby curtailing the uptake of intestinal cholesterol (see Section 6.2) [68]. Finally, oxysterols exit cells rapidly, thus adding to the elimination of excess sterol [69].

4.2. GramD1b

This shuttle protein (also called Aster-B) transports cholesterol from the plasma membrane to the ER at their contact sites in many cells, including enterocytes [28,51–53]. It is regulated by active cholesterol in two ways. First, active cholesterol is its transport substrate [45,70]. Second, the expression of GramD1b is promoted by LXR which is activated by the side-chain oxysterols synthesized from active cholesterol (see Sections 4.1 and 5.2) [71]. GramD1b might also facilitate reverse cholesterol transport down its chemical activity gradient from ER to plasma membrane but this has not been reported [56].

4.3. ACAT2

ACATs (also known as SOATs) catalyze the esterification of cholesterol in the ER. ACAT1 is abundant in most tissues while ACAT2 is expressed preferentially by enterocytes [7]. The catalytic activity of ACAT is acutely stimulated by the inward flux of excess plasma membrane cholesterol [40,56]. This is because the active sterol and its oxysterol products serve both as substrates and as allosteric activators of the enzyme [58,72,73]. The esterified cholesterol product is stored in lipid droplets or incorporated into chylomicrons for secretion to the circulation (see Section 7.3). Cholesterol is liberated from its esters by cytoplasmic (neutral) cholesterol esterase. Degradation of the esterase is promoted by oxysterols [74]. This helps to keep the concentration of unesterified sterol low, another negative feedback mechanism responding to excess cholesterol.

4.4. HMGCR

Enterocytes not only receive cholesterol from the diet, the circulation and, through bile, from the liver but they also synthesize it as needed [75]. Excess (active) cholesterol down-regulates the activity of HMGCR, the rate-limiting enzyme for sterol production in the ER. This inhibition is implemented at both the transcriptional and the post-translational level [76]. The expression of HMGCR is held in check by the combined effect on SREBP-2 of active cholesterol and its side-chain oxysterol products (see Sections 4.1 and 5.1). The enzyme is also rapidly inactivated in a hypersharp response to active cholesterol [57,77]. This feedback function requires 27HC, at least in cultured human fibroblasts [40,61]. The pathway is as follows: active cholesterol is the substrate for the synthesis of 27HC which activates Insig which binds HMGCR which promotes its conjugation with ubiquitin which stimulates its proteosomal degradation [9,76]. Squalene monooxygenase is another rate-controlling enzyme in the sterol biosynthetic pathway [8]. It is regulated by cholesterol at multiple levels, just like HMGCR; however, the agency of active cholesterol remains to be examined.

4.5. IDOL

The function of IDOL is to facilitate the destruction of LDLRs so as to curtail the ingestion of LDL cholesterol from the circulation (see Section 6.1) [78,79]. Consistent with our paradigm, the expression of IDOL is promoted by the oxysterol-mediated activation of LXR; hence, by active cholesterol (see Section 5.2) [80].

5. Active cholesterol regulates gene expression

5.1. SREBP-2

Several nuclear receptors are involved in the management of cell cholesterol [81]. SREBP-2 activates the expression of a variety of sterol regulatory element (SRE)-containing genes that encode proteins catalyzing the biosynthesis and uptake of cholesterol [7,60,82]. These include NPC1L1, LDLR and HMGCR. SREBP-2 is mobilized, cleaved and delivered to the nucleus when the concentration of the sterol in the ER falls below a resting threshold of ~6 mol% [38,59]. Importantly, SREBP-2 also stimulates its own expression autocatalytically [54,55]. This would lead to the inexorable accumulation of cell cholesterol were it not that feedback from excess cholesterol supervenes. In particular, active cholesterol and its side-chain oxysterol derivatives inhibit SREBP-2 activation by binding the regulatory proteins, Scap and Insig [60,83,84]. In addition, the oxysterols produced from active cholesterol protect Insig against proteolytic turnover and this too promotes the sequestration of SREBP-2 [60,85].

5.2. LXR

LXRα and LXRβ form heterodimers with the retinoid X receptor, RXR [86]. When activated by 27HC or other oxysterols, the LXR dimer stimulates the expression of a battery of proteins that serve to reduce excess cell cholesterol [7,9,63,87,88]. These are described in Sections 4.5 and 7 and depicted in Fig. 1. (For a different view, see Saito et al. [84].) As mentioned in Section 4.1, activated LXR also promotes the expression of GramD1b, StAR and the sterol-25 and sterol-27 hydroxylases [62,66,67,71]. These are feed-forward mechanisms that amplify LXR activity. Active LXR also down-regulates the expression of NPC1L1,

thereby reducing cholesterol ingestion [68]. Finally, oxysterol-bound LXRβ directly stabilizes ABCA1 against proteolytic clearance, an additional feedback mechanism that facilitates the depletion of excess cell cholesterol (see Section 7.1) [89].

6. Active cholesterol regulates cholesterol uptake

Cholesterol is so water-insoluble that it is only found in association with "carriers": mostly phospholipid bilayers but also low and high density lipoproteins, micelles of bile salts and sterol-binding proteins. Cholesterol molecules are distributed among these compartments through various mechanisms [25]. (a) Sterols spontaneously desorb from donors and diffuse to acceptors through the aqueous medium [19,90,91]. Their distribution reflects the sterol affinities of the donor and acceptor compartments. Typically, equilibrium is attained with a half-time of hours. (b) In contrast, cholesterol molecules are rapidly transferred between donors and acceptors during their transient collision. Presumably, sterol molecules with a high chemical activity bob in and out of membranes and are captured in a two-step "activation-collision" mechanism [92]. (c) Various transporters convey active cholesterol down its chemical activity gradient through the cytoplasm, across bilayer barriers and between organelles via intermembrane contact sites [13,24–28,93]. Enterocytes use GramD1b, NPC1L1 and SR-BI in this way (see Sections 4.2, 6.2 and 7.5) [7,53]. (d) Sterol transport can be facilitated directly or indirectly through its coupling to metabolic energy as in the case of the transport ATPases (see Sections 7.1, 7.2 and 7.4) [94,95]. The energy could be used thermodynamically to drive the sterol against its chemical gradient or kinetically to lower the activation energy barrier that slows its passive flux. Pumps that extract cholesterol from its complexes might lack an end point–if the process went too far, it could put cell cholesterol out of balance and undermine the material properties of the plasma membrane. In contrast, using metabolic energy to lower the kinetic barrier for the transfer of the active sterol down its chemical gradient would promote the removal of just the excess.

6.1. LDLR

LDL carries cholesterol through the bloodstream to the cells of the body [96]. It is taken up by endocytosis via the LDLR and delivered to late endosomes and lysosomes for digestion. The liberated sterol has high chemical activity and is transported downhill from the digestive compartments to the plasma membrane and organelles where it serves as a feedback signal [97,98]. As with other proteins mediating sterol accretion, the expression of the LDLR is promoted by SREBP-2 and is curtailed by active cholesterol (see Section 5.1). A related homeostatic mechanism is the proteolysis of the LDLR cued by oxysterols through the LXR-dependent expression of IDOL (see Section 4.5).

6.2. NPC1L1

Cholesterol is carried through the intestine in mixed micelles of bile salts, phospholipids and lipid digestion products. NPC1L1 transports the sterol from the lumen into the apical plasma membranes of enterocytes [99–102]. The protein closely resembles its lysosomal homolog, NPC1 [99,103,104]. In particular, the N-terminal domain of NPC1L1 spans the glycocalyx that protects the brush border surface and conveys sterol molecules through the surrounding unstirred water layer. A tunnel in the transporter presumably delivers the sterol to the inner leaflet of the plasma membrane bilayer, making it available to intracellular transporters. NPC1L1 cannot avoid taking up sitosterol and other plant sterols, a toxic cargo that must then be removed by ABCG5/G8 (see Section 7.4) [105,106].

NPC1L1 does not pump cholesterol. Rather, it reduces the barrier to diffusion between donor micelles in the bile and the plasma membrane [107,108]. Because the sterol is conveyed down its chemical gradient, its uptake is favored when the micelles have a high proportion of (low affinity) bile salts relative to (high affinity) phospholipids [105,109,110]. But how does the cell take up micellar cholesterol when its plasma membrane phospholipids are fully complexed? Presumably, the incoming sterol is active and immediately becomes available to GramD1b and to cytosolic transporters (see Section 4.2) [99]. The excess sterol is moved to the undersaturated intracellular membranes and thence, as physiologically mandated, to the blood stream via chylomicrons and HDL (see Sections 7.1–7.3).

NPC1L1 activity is curtailed by active cholesterol. The excess cholesterol both inhibits the nuclear expression of NPC1L1 via LXR and sequesters its transcription factor, SREBP-2, in the ER (see Sections 5.1 and 5.2) [68,82]. In addition, excess cholesterol drives the redistribution of plasma membrane NPC1L1 to the endocytic recycling compartment, ERC [111,112]. It has been suggested that a reversible vesicle internalization cycle is the mechanism for cholesterol uptake [7,102]. A plausible alternative is that endocytosis is regulatory: it sequesters NPC1L1 once the plasma membrane sterol is replete [113]. This is why the inhibitor, ezetimibe, blocks NPC1L1 internalization [113]. That is, by inhibiting cholesterol uptake, this agent reduces active plasma membrane cholesterol, the putative stimulus for the endocytosis of the protein. A cholesterol concentration dependence J curve as in Fig. 2A could test the hypothesis that active cholesterol controls the cellular disposition of NPC1L1.

7. Active cholesterol regulates cholesterol secretion

7.1. ABCA1

The mechanism by which ABCA1 uses ATP hydrolysis to transport cholesterol is uncertain and debated [95,114,115]. We favor the view that ABCA1 exports cholesterol through a multi-step process [116]. First, the hydrolysis of ATP drives the perturbation of the plasma membrane bilayer, mobilizing both its cholesterol and its phospholipids [117]. Then, the "activated" phospholipids move down their chemical activity gradient to the apoA1 bound to ABCA1. This creates a phospholipid bilayer acceptor compartment on the nascent HDL that is avid for cholesterol. The removal of phospholipids from plasma membranes renders their cholesterol active; i.e., susceptible to cholesterol oxidase, methyl-β-cyclodextrin extraction and PFO binding. Finally, the active plasma membrane sterol follows the phospholipids passively to the nascent HDL [116].

We suggest that active cholesterol is involved in this process at no less than three levels. One is through the expression of ABCA1, elicited by LXR in response to oxysterols derived from active cholesterol (see Section 5.2). Another is the ability of active cholesterol to remove inhibitory LXR from the transporter [89]. Third, it is active plasma membrane cholesterol that is transferred to nascent HDL. This supposition follows from a homeostatic mandate: only excess sterol should be removed from the cell.

7.2. ABCG1

ABCG1 transfers plasma membrane cholesterol to the nascent HDL particles generated by ABCA1, furthering their maturation [116]. Its activity is not specific: a variety of other sterol acceptors suffice in vitro. The mechanism underlying the action of ABCG1 is not well understood [95,115]. The protein is a membrane-spanning homodimeric ATPase with an apparent cholesterol-binding cavity [118]. Like ABCA1, ABCG1 apparently activates cholesterol in that it renders the plasma membrane sterol susceptible to cholesterol oxidase [116,119]. The activated sterol would then move passively to the bilayer compartment in the nascent HDL or to another acceptor. That active cholesterol suppresses the proteasomal degradation of both ABCA1 and ABCG1 is another facet of its function as a negative feedback regulator [120].

7.3. Chylomicrons

Excess plasma membrane cholesterol is esterified by the ACAT2 (see Section 4.3). The esters are stored along with triglycerides in cytoplasmic droplets or packaged in CM particles [7,16,121]. The latter are secreted by exocytosis from the basolateral membrane of the enterocytes to the lymph and thence to the systemic circulation. The expression of apolipoprotein B-48, a CM surface protein, is dependent on LXR; thus, it too depends on active cholesterol (see Section 5.2) [122].

7.4. ABCG5/G8

Heterodimers of ABCG5 and ABCG8 in the apical brush borders of enterocytes and hepatocytes secrete sterol molecules into the intestine [123–126]. The expression of the transporter is turned on by LXR; ergo, by active cholesterol (see Section 5.2). Active cholesterol also stimulates the ATPase activity of the transporter, presumably as its substrate [127,128]. The transport mechanism is unclear [95]. Like other ABC transporters, ABCG5/G8 has a hydrophobic cavity or tunnel thought to bring sterols from the inner to the outer leaflet of the plasma membrane bilayer [128]. ATP hydrolysis might pump cholesterol to the bile directly. However, extraction of cholesterol from its complexes would reduce the level of plasma membrane cholesterol below its optimum. Furthermore, it would invite a futile cycle through the return of the secreted sterol to the depleted membrane. We prefer the hypothesis that it is the excess and not the complexed plasma membrane sterol that is the substrate for transport. It would have a higher chemical activity than that in bile micelles and therefore flow downhill to the acceptors in the gut. In this view, ATP hydrolysis would serve to lower the activation energy for the facilitated diffusion of the uncomplexed excess as suggested above for the other transporters [107,108,129].

Plant sterols such as sitosterol abound in the diet, and NPC1L1 unavoidably imports these xenosterols together with cholesterol (see Section 6.2) [62,105,123,126]. Unfortunately, such xenosterols can be toxic, causing a condition called sitosterolemia [106]. ABCG5/G8 counters this hazard by transporting them back to the gut lumen [95].

7.5. SR-BI

The SR-BI channel mediates the bidirectional transfer of cholesterol, its esters, phospholipids and other lipids between extracellular partners and the plasma membrane [130–132]. SR-BI is located predominately in the apical brush border of the enterocyte [133]. (This contrasts with the hepatocyte where the protein is located in the basolateral membrane in order to import cholesterol from circulating HDL.) Active cholesterol drives the expression of the transporter through LXR activation via oxysterol production (see Section 5.2) [6,132]. Furthermore, the uncomplexed sterol is the presumed substrate for SR-BI [91,134,135]. Micelles rich in bile acids have relatively weak cholesterol affinity and so their excess cholesterol can be transported by SR-BI down its chemical activity gradient into the plasma membrane. This extra plasma membrane sterol is active: it is susceptible to attack by cholesterol oxidase, extraction by **β**-cyclodextrin and esterification by ACAT in the ER [136–138]. Cholesterol will also flow the other way – from cells to micelles – when the plasma membrane sterol has a sufficiently high chemical activity. This is the case when the sterol is in stoichiometric excess of the phospholipids [91,134]. In this way, SR-BI dissipates cholesterol activity gradients between cells and their exchange partners.

As is the case for NPC1L1 (Section 6.2), active cholesterol also manages the cellular disposition of the SR-BI. In a study of cultured hepatocytes, the transporter was shown to be associated with the basolateral membrane when the cells were deprived of cholesterol [139]. This location enables SR-BI to take up HDL cholesterol from the circulation. Cholesterol enrichment redistributed the transporter to the apical plasma membrane, enabling the hepatocytes to secrete the excess sterol to the bile. In similar experiments on isolated intestinal mucosa, feeding lipid mixtures rich in cholesterol to the explants prompted internalization of the SR-BI from the enterocyte brush borders, presumably to prevent their overload [140]. It remains to be seen whether these translocations are controlled by active cholesterol.

8. Concluding comments and suggestions

Various mathematical models have addressed the mechanism of cholesterol homeostasis [141–144]. They mostly have assumed that cell cholesterol is held at a steady state level by multiple opposed kinetic processes. Other studies have considered accessible cholesterol as a homeostatic signal but have not specified what determines the set point for the sterol [9,47,52]. Here, we have evaluated a stringent equilibrium model [10]. It has three postulates: (a) Cholesterol is maintained homeostatically at stoichiometric equivalence with the plasma membrane phospholipids. (b) Cholesterol exceeding the complexation capacity of these phospholipids is chemically active (dashed red line in Fig. 2A). (c) By equilibrating with the endomembranes, active cholesterol regulates a manifold of homeostatic proteins that keep the plasma membrane sterol at its physiologic set point. This homeostatic mechanism does not sense cholesterol deficits directly. Rather, it is the absence of active cholesterol that relieves the suppression of its accretion and halts its depletion. While the model is general, it does not consider all features of cholesterol management such as those in specialized cells like the liver and steroidogenic tissues.

Is the cholesterol in plasma membranes actually set at stoichiometric equivalence with their phospholipids, as stipulated by the model [10,12,13]? The question cannot be answered directly at present because the sterol stoichiometries of the plasma membrane phospholipids have not been well enough determined. However, we have reasoned as follows: The thresholds of the J curves for the cholesterol oxidase digestion of the sterol in vesicles of various phospholipids appear to correspond to their stoichiometric equivalence points (as depicted by the dashed blue curve in Fig. 2A.) [29]. Importantly, the threshold for the cholesterol oxidase digestion of plasma membrane cholesterol matches its physiologic set point [77,145]. It follows that plasma membranes are maintained at the stoichiometric equivalence point of their phospholipids. This premise makes the strong prediction that the resting level of plasma membrane cholesterol is indifferent to the characteristics of the regulatory proteins: their affinities, velocities, concentrations and fractional saturations.

The model predicts that the activity of each element in the homeostatic manifold responds acutely to modest variations in plasma membrane cholesterol, as illustrated by the dashed blue J curve in Fig. 2A. This has been shown for the synthesis of 27HC and for the activities of GramD1b, ACAT and HMGCR [13,40,45,56,77]. However, the generality of the hypothesis needs testing on the other regulatory proteins and on other cells. J curves can be constructed by varying the cholesterol in intact cells with methyl-β-cyclodextrin loaded with graded amounts of cholesterol [12,13,146]. There are also spot tests. Treating cells with phospholipase C or sphingomyelinase C frees the plasma membrane cholesterol from its complexes, thereby activating the sterol by shifting its J curve to the left [13]. Cholesterol also can be activated with a variety of membrane intercalating amphiphiles that competitively displace it from its phospholipid complexes [39,77,147]. Introducing lysophosphatides and alkylphospholipids into bilayers has the opposite effect: these intercalators form complexes with cholesterol, thereby reducing its chemical activity [13,40,148]. Some assays for the availability of plasma membrane cholesterol in intact cells utilize its susceptibility to digestion by cholesterol oxidase, its extraction by methyl-β-cyclodextrin and its binding by tagged cytolysins [13]. Homeostatic responses can be tracked by the processing of SREBP-2, the esterification of the sterol and the catalytic activity of HMGCR [12].

The goal of this review was to examine the hypothesis that active cholesterol coordinates cellular cholesterol homeostasis. As a test case, we considered fourteen proteins in the homeostatic manifold of the

enterocyte. The model appears to be robust; however, it needs more direct testing, as suggested above.

Dedication

This study is dedicated to the memory of Donald M. Small–inspiration, mentor and friend.

CRediT authorship contribution statement

Yvonne Lange: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Theodore L. Steck:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization.

Acknowledgments

We are grateful to Andrew J. Brown (UNSW Sydney, Australia) and Peter Tontonoz (UCLA) for their valuable feedback on the manuscript. We also wish to thank the reviewers for their astute and helpful comments. This work was supported by a grant for emeriti academic activities from the University of Chicago.

References

[1] Duan YJ, Gong K, Xu SW, Zhang F, Meng XS, Han JH. Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics. Signal Transduct Target Ther 2022;7(1):29.
[2] Guo JR, Chen SL, Zhang Y, Liu JX, Jiang LY, Hu LD, et al. Cholesterol metabolism: physiological regulation and diseases. MedComm 2024;5(2):24.
[3] Shin KC, Moussa HYA, Park Y. Cholesterol imbalance and neurotransmission defects in neurodegeneration. Exp Mol Med 2024;6.
[4] Goedeke L, Fernández-Hernando C. Regulation of cholesterol homeostasis. Cell Mol Life Sci 2012;69(6):915–30.
[5] Howe V, Sharpe LJ, Alexopoulos SJ, Kunze SV, Chua NK, Li D, et al. Cholesterol homeostasis: how do cells sense sterol excess? Chem Phys Lipids 2016;199:170–8.
[6] Yu XH, Zhang DW, Zheng XL, Tang CK. Cholesterol transport system: an integrated cholesterol transport model involved in atherosclerosis. Prog Lipid Res 2019;73:65–91.
[7] Luo J, Yang HY, Song BL. Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol 2020;21(4):225–45.
[8] Chua NK, Coates HW, Brown AJ. Squalene monooxygenase: a journey to the heart of cholesterol synthesis. Prog Lipid Res 2020;79:101033.
[9] Griffiths WJ, Wang Y. Sterols, oxysterols, and accessible cholesterol: Signalling for homeostasis, in immunity and during development. Front Physiol 2021;12.
[10] Steck TL, Tabei SMA, Lange Y. A basic model for cell cholesterol homeostasis. Traffic 2021;22(12):471–81.
[11] Lange Y, Steck TL. Cholesterol homeostasis and the escape tendency (activity) of plasma membrane cholesterol. Prog Lipid Res 2008;47(5):319–32.
[12] Steck TL, Lange Y. Cell cholesterol homeostasis: mediation by active cholesterol. Trends Cell Biol 2010;20(11):680–7.
[13] Lange Y, Steck TL. Active cholesterol 20 years on. Traffic 2020;21(11):662–74.
[14] Chuang JC, Lopez AM, Turley SD. Quantitation of the rates of hepatic and intestinal cholesterol synthesis in lysosomal acid lipase-deficient mice before and during treatment with ezetimibe. Biochem Pharmacol 2017;135:116–25.
[15] Afonso MS, Machado RM, Lavrador MS, Quintao ECR, Moore KJ, Lottenberg AM. Molecular pathways underlying cholesterol homeostasis. Nutrients 2018;10(6):18.
[16] Ko CW, Qu J, Black DD, Tso P. Regulation of intestinal lipid metabolism: current concepts and relevance to disease. Nat Rev Gastroenterol Hepatol 2020;17(3):169–83.
[17] Stellaard F. From dietary cholesterol to blood cholesterol, physiological lipid fluxes, and cholesterol homeostasis. Nutrients 2022;14(8):14.
[18] Grefhorst A, Verkade HJ, Groen AK. The TICE pathway: mechanisms and lipid-lowering therapies. Methodist Debakey Cardiovasc J 2019;15(1):70–6.
[19] Nakano T, Inoue I, Murakoshi T. A newly integrated model for intestinal cholesterol absorption and efflux reappraises how plant sterol intake reduces circulating cholesterol levels. Nutrients 2019;11(2):18.
[20] Xu HM, Xin YY, Wang JX, Liu ZX, Cao YT, Li WG, et al. The TICE pathway: mechanisms and potential clinical applications. Curr Atheroscler Rep 2023;25(10):653–62.
[21] Lange Y, Swaisgood MH, Ramos BV, Steck TL. Plasma membranes contain half the phospholipid and 90% of the cholesterol and sphingomyelin in cultured human fibroblasts. J Biol Chem 1989;264(7):3786–93.
[22] Lange Y, Ye J, Steck TL. Activation mobilizes the cholesterol in the late endosomes-lysosomes of Niemann pick type C cells. PloS One 2012;7(1):e30051.
[23] Infante RE, Radhakrishnan A. Continuous transport of a small fraction of plasma membrane cholesterol to endoplasmic reticulum regulates total cellular cholesterol. Elife 2017;6:23.
[24] Baumann NA, Sullivan DP, Ohvo-Rekila H, Simonot C, Pottekat A, Klaassen Z, et al. Transport of newly synthesized sterol to the sterol-enriched plasma membrane occurs via nonvesicular equilibration. Biochemistry 2005;44(15):5816–26.
[25] Wüstner D, Solanko K. How cholesterol interacts with proteins and lipids during its intracellular transport. Biochim Biophys Acta (BBA) - Biomembr 2015;1848(9):1908–26.
[26] Soffientini U, Graham A. Intracellular cholesterol transport proteins: roles in health and disease. Clin Sci 2016;130(21):1843–59.
[27] Litvinov DY, Savushkin EV, Dergunov AD. Intracellular and plasma membrane events in cholesterol transport and homeostasis. J Lipids 2018;2018:22.
[28] Kennelly JP, Tontonoz P. Cholesterol transport to the endoplasmic reticulum. Cold Spring Harb Perspect Biol 2023;15(2):17.
[29] Lange Y, Tabei SM, Ye J, Steck TL. Stability and stoichiometry of bilayer phospholipid-cholesterol complexes: relationship to cellular sterol distribution and homeostasis. Biochemistry 2013;52(40):6950–9.
[30] Litz JP, Thakkar N, Portet T, Keller SL. Depletion with Cyclodextrin reveals two populations of cholesterol in model lipid membranes. Biophys J 2016;110(3):635–45.
[31] Christon R, Meslin JC, Thevenoux J, Linard A, Leger CL, Delpal S. Effects of a low dietary linoleic acid level on intestinal morphology and enterocyte brush border membrane lipid composition. Reprod Nutr Dev 1991;31(6):691–701.
[32] Schulthess G, Compassi S, Boffelli D, Werder M, Weber FE, Hauser H. A comparative study of sterol absorption in different small-intestinal brush border membrane models. J Lipid Res 1996;37(11):2405–19.
[33] Prabhu R, Balasubramanian KA. Simple method of preparation of rat colonocyte apical membranes using polyethylene glycol precipitation. J Gastroenterol Hepatol 2003;18(7):809–14.
[34] Lange Y, Ye J, Steck TL. Essentially all excess fibroblast cholesterol moves from plasma membranes to intracellular compartments. PloS One 2014;9(7):e98482.
[35] Dallman PR, Dallner G, Bergstrand A. L. Ernster heterogeneous distribution of enzymes in submicrosomal membrane fragments. J Cell Biol 1969;41(2):357–77.
[36] Colbeau A, Nachbaur J, Vignais PM. Enzymac characterization and lipid composition of rat liver subcellular membranes. Biochim Biophys Acta (BBA) - Biomembr 1971;249(2):462–92.
[37] Mrak RE, Fleischer S. Lipid composition of sarcoplasmic reticulum from mice with muscular dystrophy. Muscle Nerve 1982;5(6):439–46.
[38] Radhakrishnan A, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. Cell Metab 2008;8(6):512–21.
[39] Lange Y, Ye J, Duban ME, Steck TL. Activation of membrane cholesterol by 63 amphipaths. Biochemistry 2009;48(36):8505–15.
[40] Lange Y, Ory DS, Ye J, Lanier MH, Hsu FF, Steck TL. Effectors of rapid homeostatic responses of endoplasmic reticulum cholesterol and 3-hydroxy-3-methylglutaryl-CoA reductase. J Biol Chem 2008;283(3):1445–55.
[41] Lange Y, Tabei SMA, Steck TL. A basic model for the association of ligands with membrane cholesterol: application to cytolysin binding. J Lipid Res 2023;64(4):100344.
[42] Steck TL, Ali Tabei SM, Lange Y. Estimating the cholesterol affinity of integral membrane proteins from experimental data. Biochemistry 2024;63(1):19–26.
[43] Ayuyan AG, Cohen FS. The chemical potential of plasma membrane cholesterol: implications for cell biology. Biophys J 2018;114(4):904–18.
[44] Naito T, Ercan B, Krshnan L, Triebl A, Koh DHZ, Wei FY, et al. Movement of accessible plasma membrane cholesterol by the GRAMD1 lipid transfer protein complex. Elife 2019;8.
[45] Koh DHZ, Naito T, Na M, Yeap YJ, Rozario P, Zhong FL, et al. Visualization of accessible cholesterol using a GRAM domain-based biosensor. Nat Commun 2023;14(1):6773.
[46] Ohno-Iwashita Y, Shimada Y, Waheed AA, Hayashi M, Inomata M, Nakamura M, et al. Perfringolysin O, a cholesterol-binding cytolysin, as a probe for lipid rafts. Anaerobe 2004;10(2):125–34.
[47] Das A, Brown MS, Anderson DD, Goldstein JL, Radhakrishnan A. Three pools of plasma membrane cholesterol and their relation to cholesterol homeostasis. Elife 2014;3:1–16. e02882.
[48] Johnson BB, Brena M, Anguita J, Heuck AP. Mechanistic insights into the cholesterol-dependent binding of Perfringolysin O-based probes and cell membranes. Sci Rep 2017;7(1):13793.
[49] Liu SL, Sheng R, Jung JH, Wang L, Stec E, O'Connor MJ, et al. Orthogonal lipid sensors identify transbilayer asymmetry of plasma membrane cholesterol. Nat Chem Biol 2017;13(3):268–74.
[50] Radhakrishnan A, Rohatgi R, Siebold C. Cholesterol access in cellular membranes controls hedgehog signaling. Nat Chem Biol 2020;16(12):1303–13.
[51] Naito T, Saheki Y. GRAMD1-mediated accessible cholesterol sensing and transport. Biochim Biophys Acta Mol Cell Biol Lipids 2021;1866(8):158957–64.
[52] Xiao X, Kennelly JP, Ferrari A, Clifford BL, Whang E, Gao YJ, et al. Hepatic nonvesicular cholesterol transport is critical for systemic lipid homeostasis. Nat Metab 2023;5(1):165-+.
[53] Ferrari A, Whang E, Xiao X, Kennelly JP, Romartinez-Alonso B, Mack JJ, et al. Aster-dependent nonvesicular transport facilitates dietary cholesterol uptake. Science 2023;382(6671):eadf0966.
[54] Sato R, Inoue J, Kawabe Y, Kodama T, Takano T, Maeda M. Sterol-dependent transcriptional regulation of sterol regulatory element-binding Protein-2*. J Biol Chem 1996;271(43):26461–4.

[55] Amemiya-Kudo M, Shimano H, Hasty AH, Yahagi N, Yoshikawa T, Matsuzaka T, et al. Transcriptional activities of nuclear SREBP-1a, −1c, and −2 to different target promoters of lipogenic and cholesterogenic genes. J Lipid Res 2002;43(8):1220–35.
[56] Lange Y, Ye J, Rigney M, Steck TL. Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. J Lipid Res 1999;40(12):2264–70.
[57] Lange Y, Steck TL, Ye J, Lanier MH, Molugu V, Ory D. Regulation of fibroblast mitochondrial 27-hydroxycholesterol production by active plasma membrane cholesterol. J Lipid Res 2009;50(9):1881–8.
[58] Chang CC, Lee CY, Chang ET, Cruz JC, Levesque MC, Chang TY. Recombinant acyl-CoA:cholesterol acyltransferase-1 (ACAT-1) purified to essential homogeneity utilizes cholesterol in mixed micelles or in vesicles in a highly cooperative manner. J Biol Chem 1998;273(52). 35132–41.
[59] Sokolov A, Radhakrishnan A. Accessibility of cholesterol in endoplasmic reticulum membranes and activation of SREBP-2 switch abruptly at a common cholesterol threshold. J Biol Chem 2010;285(38):29480–90.
[60] Brown MS, Radhakrishnan A, Goldstein JL. Retrospective on cholesterol homeostasis: the central role of Scap. Annu Rev Biochem 2018;87:783–807.
[61] Axelson M, Larsson O. Low density lipoprotein (LDL) cholesterol is converted to 27-hydroxycholesterol in human fibroblasts. Evidence that 27-hydroxycholesterol can be an important intracellular mediator between LDL and the suppression of cholesterol production. J Biol Chem 1995;270(25):15102–10.
[62] Brauner R, Johannes C, Ploessl F, Bracher F, Lorenz RL. Phytosterols reduce cholesterol absorption by inhibition of 27-hydroxycholesterol generation, liver X receptor alpha activation, and expression of the basolateral sterol exporter ATP-binding cassette A1 in Caco-2 enterocytes. J Nutr 2012;142(6):981–9.
[63] Odnoshivkina UG, Kuznetsova EA, Petrov AM. 25-hydroxycholesterol as a signaling molecule of the nervous system. Biochem.-Moscow 2022;87(6):524–37.
[64] Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: from cholesterol metabolites to key mediators. Prog Lipid Res 2016;64:152–69.
[65] Brown AJ, Sharpe LJ, Rogers MJ. Oxysterols: from physiological tuners to pharmacological opportunities. Br J Pharmacol 2021;178(16):3089–103.
[66] Cummins CL, Volle DH, Zhang Y, McDonald JG, Sion B, Lefrançois-Martinez AM, et al. Liver X receptors regulate adrenal cholesterol balance. J Clin Invest 2006;116(7):1902–12.
[67] Liu Y, Wei Z, Ma X, Yang X, Chen Y, Sun L, et al. 25-hydroxycholesterol activates the expression of cholesterol 25-hydroxylase in an LXR-dependent mechanism. J Lipid Res 2018;59:jlr.M080440.
[68] Duval C, Touche V, Tailleux A, Fruchart JC, Fievet C, Clavey V, et al. Niemann-pick C1 like 1 gene expression is down-regulated by LXR activators in the intestine. Biochem Biophys Res Commun 2006;340(4):1259–63.
[69] Meaney S, Bodin K, Diczfalusy U, Björkhem I. On the rate of translocation in vitro and kinetics in vivo of the major oxysterols in human circulation. J Lipid Res 2002;43(12):2130–5.
[70] Ercan B, Naito T, Koh DHZ, Dharmawan D, Saheki Y. Molecular basis of accessible plasma membrane cholesterol recognition by the GRAM domain of GRAMD1b. EMBO J 2021;40(6):e106524.
[71] Sandhu J, Li S, Fairall L, Pfisterer SG, Gurnett JE, Xiao X, et al. Aster proteins facilitate nonvesicular plasma membrane to ER cholesterol transport in mammalian cells. Cell 2018;175(2):514–529.e20.
[72] Rogers MA, Liu J, Song B-L, Li B-L, Chang CCY, Chang T-Y. Acyl-CoA:cholesterol acyltransferases (ACATs/SOATs): Enzymes with multiple sterols as substrates and as activators. J Steroid Biochem Mol Biol 2015;151(0):102–7.
[73] Long T, Liu Y, Li XC. Molecular structures of human ACAT2 disclose mechanism for selective inhibition. Structure 2021;29(12). 1410-+.
[74] Miura S, Nagura H, Sawamura F, Tomita I, Kawai E, Mochizuki N, et al. Sterol-mediated regulation of hormone-sensitive lipase in 3T3-L1 adipocytes. Lipids 2003;38(7):743–50.
[75] McFarlane MR, Liang G, Engelking LJ. Insig proteins mediate feedback inhibition of cholesterol synthesis in the intestine. J Biol Chem 2014;289(4):2148–56.
[76] Faulkner RA, Yang Y, Tsien J, Qin T, DeBose-Boyd RA. Direct binding to sterols accelerates endoplasmic reticulum-associated degradation of HMG CoA reductase. Proc Natl Acad Sci 2024;121(7):e2318822121.
[77] Lange Y, Ye J, Steck TL. How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. Proc Natl Acad Sci U S A 2004;101(32):11664–7.
[78] Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 2009;325(5936):100–4.
[79] Scotti E, Calamai M, Goulbourne CN, Zhang L, Hong C, Lin RR, et al. IDOL stimulates clathrin-independent endocytosis and multivesicular body-mediated lysosomal degradation of the low-density lipoprotein receptor. Mol Cell Biol 2013;33(8):1503–14.
[80] van Loon NM, van Wouw SAE, Ottenhoff R, Nelson JK, Kingma J, Scheij S, et al. Regulation of intestinal LDLR by the LXR-IDOL axis. Atherosclerosis 2020;315:1–9.
[81] Li Z, Zheng D, Zhang T, Ruan S, Li N, Yu Y, et al. The roles of nuclear receptors in cholesterol metabolism and reverse cholesterol transport in nonalcoholic fatty liver disease. Hepatol Communicat 2024;8(1):e0343.
[82] Alrefai WA, Annaba F, Sarwar Z, Dwivedi A, Saksena S, Singla A, et al. Modulation of human Niemann-pick C1-like 1 gene expression by sterol: role of sterol regulatory element binding protein 2. American J Physiolo-Gastrointest Liver Physiol 2007;292(1):G369–76.
[83] Lee AG. The role of cholesterol binding in the control of cholesterol by the Scap–Insig system. Eur Biophys J 2022;51(4):385–99.
[84] Saito H, Tachiura W, Nishimura M, Shimizu M, Sato R, Yamauchi Y. Hydroxylation site-specific and production-dependent effects of endogenous oxysterols on cholesterol homeostasis: implications for SREBP-2 and LXR. J Biol Chem 2023;299(1):15.
[85] Lee JN, Song B, DeBose-Boyd RA, Ye J. Sterol-regulated degradation of Insig-1 mediated by the membrane-bound ubiquitin ligase gp78*. J Biol Chem 2006;281(51):39308–15.
[86] Moschetta A. Nuclear receptors and cholesterol metabolism in the intestine. Atherosclerosis Suppl 2015;17:9–11.
[87] Wang B, Tontonoz P. Liver X receptors in lipid signalling and membrane homeostasis. Nat Rev Endocrinol 2018;14(8):452–63.
[88] Russo-Savage L, Schulman IG. Liver X receptors and liver physiology. Biochim et Biophys Acta (BBA) - Mol Basis Diseas 2021;1867(6):166121.
[89] Hozoji-Inada M, Munehira Y, Nagao K, Kioka N, Ueda K. Liver X receptor β (LXRβ) interacts directly with ATP-binding cassette A1 (ABCA1) to promote high density lipoprotein formation during acute cholesterol accumulation*. J Biol Chem 2011;286(22):20117–24.
[90] Rothblat GH, de la Llera-Moya M, Favari E, Yancey PG, Kellner-Weibel G. Cellular cholesterol flux studies: methodological considerations. Atherosclerosis 2002;163(1):1–8.
[91] Phillips MC. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem 2014;289(35):24020–9.
[92] Steck TL, Ye J, Lange Y. Probing red cell membrane cholesterol movement with cyclodextrin. Biophys J 2002;83(4):2118–25.
[93] Wustner D. Steady state analysis of influx and transbilayer distribution of ergosterol in the yeast plasma membrane. Theor Biol Med Model 2019;16(1):13.
[94] Robichaud S, Ouimet M. Quantifying cellular cholesterol efflux. Methods Mol Biol 1951;2019:111–33.
[95] Plummer AM, Culbertson AT, Liao M. The ABCs of sterol transport. Annu Rev Physiol 2021;83:153–81.
[96] Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol 2009;29(4):431–8.
[97] Luo J, Jiang L, Yang H, Song BL. Routes and mechanisms of post-endosomal cholesterol trafficking: a story that never ends. Traffic 2017;18(4):209–17.
[98] Trinh MN, Brown MS, Goldstein JL, Han J, Vale G, McDonald JG, et al. Last step in the path of LDL cholesterol from lysosome to plasma membrane to ER is governed by phosphatidylserine. Proc Natl Acad Sci 2020;117:18521–9. 202010682.
[99] Huang CS, Yu X, Fordstrom P, Choi K, Chung BC, Roh SH, et al. Cryo-EM structures of NPC1L1 reveal mechanisms of cholesterol transport and ezetimibe inhibition. Sci Adv 2020;6(25):eabb1989.
[100] Hu MQ, Yang F, Huang YW, You X, Liu DS, Sun S, et al. Structural insights into the mechanism of human NPC1L1-mediated cholesterol uptake. Sci Adv 2021;7(29):15.
[101] Long T, Liu Y, Qin Y, DeBose-Boyd RA, Li XC. Structures of dimeric human NPC1L1 provide insight into mechanisms for cholesterol absorption. Sci Adv 2021;7(34):9.
[102] Ticho AL, Calzadilla N, Malhotra P, Lee H, Anbazhagan AN, Saksena S, et al. NPC1L1-dependent transport of 27-alkyne cholesterol in intestinal epithelial cells. Am J Physiol Cell Physiol 2021;320(5):C916–25.
[103] Winkler MBL, Kidmose RT, Szomek M, Thaysen K, Rawson S, Muench SP, et al. Structural insight into eukaryotic sterol transport through Niemann-pick type C proteins. Cell 2019;179(2):485–497 e18.
[104] Saha P, Shumate JL, Caldwell JG, Elghobashi-Meinhardt N, Lu A, Zhang LC, et al. Inter-domain dynamics drive cholesterol transport by NPC1 and NPC1L1 proteins. Elife 2020;9.
[105] Yamanashi Y, Takada T, Suzuki H. Niemann-pick C1-like 1 overexpression facilitates ezetimibe-sensitive cholesterol and beta-sitosterol uptake in CaCo-2 cells. J Pharmacol Exp Ther 2007;320(2):559–64.
[106] Othman RA, Myrie SB, Jones PJH. Non-cholesterol sterols and cholesterol metabolism in sitosterolemia. Atherosclerosis 2013;231(2):291–9.
[107] Eckhardt ERM, Wang DQH, Donovan JM, Carey MC. Dietary sphingomyelin suppresses intestinal cholesterol absorption by decreasing thermodynamic activity of cholesterol monomers. Gastroenterology 2002;122(4):948–56.
[108] Petruzzelli M, Groen AK, van Erpecum KJ, Vrins C, van der Velde AE, Portincasa P, et al. Micellar lipid composition profoundly affects LXR-dependent cholesterol transport across CaCo2 cells. FEBS Lett 2009;583(8):1274–80.
[109] Cohn JS, Kamili A, Wat E, Chung RW, Tandy S. Dietary phospholipids and intestinal cholesterol absorption. Nutrients 2010;2(2):116–27.
[110] Yang F, Chen GX, Ma MH, Qiu N, Zhu LJ, Li J. Egg-yolk sphingomyelin and phosphatidylcholine attenuate cholesterol absorption in Caco-2 cells. Lipids 2018;53(2):217–33.
[111] Yu L, Bharadwaj S, Brown JM, Ma Y, Du W, Davis MA, et al. Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. J Biol Chem 2006;281(10):6616–24.
[112] Xie C, Zhou ZS, Li N, Bian Y, Wang YJ, Wang LJ, et al. Ezetimibe blocks the internalization of NPC1L1 and cholesterol in mouse small intestine. J Lipid Res 2012;53(10):2092–101.
[113] Johnson TA, Pfeffer SR. Ezetimibe-sensitive cholesterol uptake by NPC1L1 protein does not require endocytosis. Mol Biol Cell 2016;27(11):1845–52.
[114] Qian H, Zhao X, Cao P, Lei J, Yan N, Gong X. Structure of the human lipid exporter ABCA1. Cell 2017;169(7):1228–1239 e10.
[115] Yu XH, Tang CK. ABCA1, ABCG1, and cholesterol homeostasis. In: Zheng L, editor. Hdl metabolism and diseases. Singapore: Springer-Verlag Singapore Pte Ltd; 2022. p. 95–107.

[116] Steck TL, Lange Y. Is reverse cholesterol transport regulated by active cholesterol? J Lipid Res 2023;64(6):1–10. 100385.
[117] Phillips MC. Is ABCA1 a lipid transfer protein? J Lipid Res 2018;59(5):749–63.
[118] Skarda L, Kowal J, Locher KP. Structure of the human cholesterol transporter ABCG1. J Mol Biol 2021;433(21):13.
[119] Vaughan AM, Oram JF. ABCA1 and ABCG1 or ABCG4 act sequentially to remove cellular cholesterol and generate cholesterol-rich HDL. J Lipid Res 2006;47(11):2433–43.
[120] Hsieh V, Kim M-J, Gelissen IC, Brown AJ, Sandoval C, Hallab JC, et al. Cellular cholesterol regulates ubiquitination and degradation of the cholesterol export proteins ABCA1 and ABCG1*. J Biol Chem 2014;289(11):7524–36.
[121] Demignot S, Beilstein F, Morel E. Triglyceride-rich lipoproteins and cytosolic lipid droplets in enterocytes: key players in intestinal physiology and metabolic disorders. Biochimie 2014;96:48–55.
[122] Ushio M, Nishio Y, Sekine O, Nagai Y, Maeno Y, Ugi S, et al. Ezetimibe prevents hepatic steatosis induced by a high-fat but not a high-fructose diet. Am J Physiol-Endocrinol Metab 2013;305(2):E293–304.
[123] Patel SB, Graf GA, Temel RE. Thematic review series: lipid transfer proteins ABCG5 and ABCG8: more than a defense against xenosterols. J Lipid Res 2018;59(7):1103–13.
[124] Zein AA, Kaur R, Hussein TOK, Graf GA, Lee JY. ABCG5/G8: a structural view to pathophysiology of the hepatobiliary cholesterol secretion. Biochem Soc Trans 2019;47:1259–68.
[125] Wang HH, Liu M, Portincasa P, Wang DQH. Recent advances in the critical role of the sterol efflux transporters ABCG5/G8 in health and disease. In: Jiang XC, editor. Lipid transfer in lipoprotein metabolism and cardiovascular disease. Singapore: Springer-Verlag Singapore Pte Ltd; 2020. p. 105–36.
[126] Bydlowski SP, Levy D. Association of ABCG5 and ABCG8 transporters with Sitosterolemia. In: Lizard G, editor. Implication of oxysterols and Phytosterols in aging and human diseases. Cham: Springer International Publishing Ag; 2024. p. 31–42.
[127] Johnson BJH, Lee JY, Pickert A, Urbatsch IL. Bile acids stimulate ATP hydrolysis in the purified cholesterol transporter ABCG5/G8. Biochemistry 2010;49(16):3403–11.
[128] Sun Y, Wang J, Long T, Qi X, Donnelly L, Elghobashi-Meinhardt N, et al. Molecular basis of cholesterol efflux via ABCG subfamily transporters. Proc Natl Acad Sci 2021;118(34):e2110483118.
[129] Vrins C, Vink E, Vandenberghe KE, Frijters R, Seppen J, Groen AK. The sterol transporting heterodimer ABCG5/ABCG8 requires bile salts to mediate cholesterol efflux. FEBS Lett 2007;581(24):4616–20.
[130] Linton MF, Tao H, Linton EF, Yancey PG. SR-BI: a multifunctional receptor in cholesterol homeostasis and atherosclerosis. Trends Endocrinol Metab 2017;28(6):461–72.
[131] Morel E, Ghezzal S, Lucchi G, Truntzer C, de Banos JPP, Simon-Plas F, et al. Cholesterol trafficking and raft-like membrane domain composition mediate scavenger receptor class B type 1-dependent lipid sensing in intestinal epithelial cells. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863(2):199–211.
[132] Shen WJ, Azhar S, Kraemer FB. SR-B1: a unique multifunctional receptor for cholesterol influx and efflux. Annu Rev Physiol 2018;80:95–116.
[133] Lobo MV, Huerta L, Ruiz-Velasco N, Teixeiro E, de la Cueva P, Celdran A, et al. Localization of the lipid receptors CD36 and CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors mediating the intestinal absorption of dietary lipids. J Histochem Cytochem 2001;49(10):1253–60.
[134] Cai L, Eckhardt ER, Shi W, Zhao Z, Nasser M, de Villiers WJ, et al. Scavenger receptor class B type I reduces cholesterol absorption in cultured enterocyte CaCo-2 cells. J Lipid Res 2004;45(2):253–62.
[135] Fuentes M, Santander N, Cortes V. Insulin increases cholesterol uptake, lipid droplet content, and apolipoprotein B secretion in CaCo-2 cells by upregulating SR-BI via a PI3K, AKT, and mTOR-dependent pathway. J Cell Biochem 2018;120(2):1550–9.
[136] Kellner-Weibel G, de la Llera-Moya M, Connelly MA, Stoudt G, Christian AE, Haynes MP, et al. Expression of scavenger receptor BI in COS-7 cells alters cholesterol content and distribution. Biochemistry 2000;39(1):221–9.
[137] de La Llera-Moya M, Connelly MA, Drazul D, Klein SM, Favari E, Yancey PG, et al. Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL. J Lipid Res 2001;42(12):1969–78.
[138] Huang ZH, Gu D, Lange Y, Mazzone T. Expression of scavenger receptor BI facilitates sterol movement between the plasma membrane and the endoplasmic reticulum in macrophages. Biochemistry 2003;42(13):3949–55.
[139] Harder CJ, Meng A, Rippstein P, McBride HM, McPherson R. SR-BI undergoes cholesterol-stimulated transcytosis to the bile canaliculus in polarized WIF-B cells. J Biol Chem 2007;282(2):1445–55.
[140] Hansen GH, Niels-Christiansen LL, Immerdal L, Danielsen EM. Scavenger receptor class B type I (SR-BI) in pig enterocytes: trafficking from the brush border to lipid droplets during fat absorption. Gut 2003;52(10):1424–31.
[141] Paalvast Y, Kuivenhoven JA, Groen AK. Evaluating computational models of cholesterol metabolism. Biochim Biophys Acta 2015;1851(10):1360–76.
[142] Pool F, Currie R, Sweby PK, Salazar JD, Tindall MJ. A mathematical model of the mevalonate cholesterol biosynthesis pathway. J Theor Biol 2018;443:157–76.
[143] Zhang FY, Macshane B, Searcy R, Huang ZY. Mathematical models for cholesterol metabolism and transport. Processes 2022;10(1):27.
[144] Scheepers R, Araujo RP. Robust homeostasis of cellular cholesterol is a consequence of endogenous antithetic integral control. Front Cell Dev Biol 2023;11:1244297.
[145] Lange Y, Cutler HB, Steck TL. The effect of cholesterol and other intercalated amphipaths on the contour and stability of the isolated red cell membrane. J Biol Chem 1980;255(19):9331–7.
[146] Zidovetzki R, Levitan I. Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies. Biochim Biophys Acta 2007;1768(6):1311–24.
[147] Lange Y, Ye J, Steck TL. Activation of membrane cholesterol by displacement from phospholipids. J Biol Chem 2005;280(43):36126–31.
[148] Rios-Marco P, Marco C, Cueto FJ, Carrasco MP, Jimenez-Lopez JM. Pleiotropic effects of antitumour alkylphospholipids on cholesterol transport and metabolism. Exp Cell Res 2016;340(1):81–90.