Sterols from the Post-Lanosterol Part of Cholesterol Synthesis: Novel Signaling Players

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Introduction

Cholesterol is the major sterol in all mammalian cells and is crucial for viability of cells. It is the only lipid not dedicated to metabolism of energy and its storage. The majority of cholesterol resides in cellular membranes, where it influences the order of phospholipid chains and contributes to membrane fluidity, integrity and heterogeneity. Further roles of cholesterol also include cell cycle regulation and protein modification, as well as being the starting point for the synthesis of steroid hormones and bile acids (Fig. 1).

The synthesis of cholesterol is a housekeeping pathway and supposedly takes place in all mammalian cells. In mammals, there are more than 100 genes associated with cholesterol synthesis and synthesis regulation [1]. Cholesterol synthesis itself includes over 20 reactions, starting from acetyl coenzyme A [2]. This chapter focuses on the second part of cholesterol synthesis, from lanosterol on (Fig. 2). It is called the post-squalene or sometimes post-lanosterol portion of cholesterol synthesis since lanosterol is the first sterol intermediate in the pathway. Initially, the post-squalene pathway has been divided into the Bloch and Kandutsch–Russell branches [3]. In the Bloch branch, the final reaction is the conversion of desmosterol to cholesterol by sterol-Δ24-reductase (DHCR24); thus, all intermediates from lanosterol to desmosterol contain Δ24 double bonds. In contrast, in the Kandutsch–Russell branch, DHCR24 acts already on lanosterol; thus all intermediates from 24,25-dihydrolanosterol to 7-dehydrocholesterol contain a saturated side chain. Since DHCR24 can, in principle, metabolize any cholesterol synthesis intermediate from lanosterol on, the two branches cannot be treated separately. Study of DHCR24 substrate specificity in vitro showed 24-dehydrolathosterol as the most

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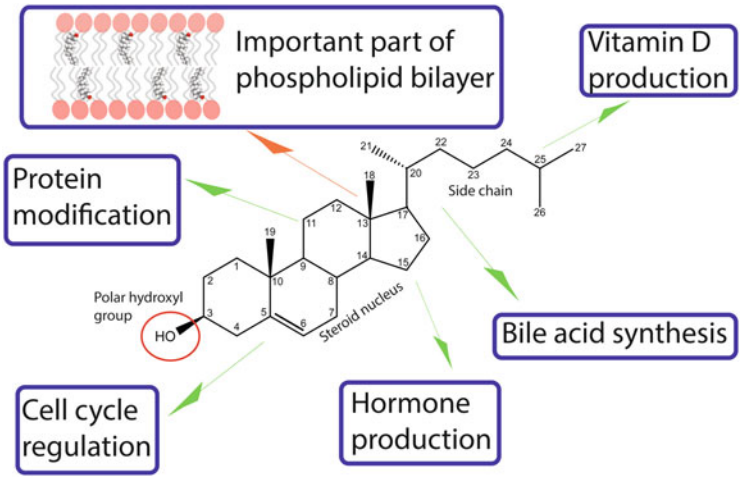


Fig. 1 The molecular structure of cholesterol with carbon atoms numbering based on IUPAC-IUB recommendations. Red ring represents the polar hydroxyl group of cholesterol molecule and the rest of molecule is nonpolar. The figure represents major roles of cholesterol in the human body. The major function of cholesterol is in the membrane as the basic part of phospholipid bilayer

reactive substrate, suggesting that cholesterol synthesis preferentially starts with the Bloch branch from lanosterol to 24-dehydrolathosterol and is then shifted to Kandutsch–Russell branch via lathosterol. In this case, 7-dehydrocholesterol presents the last intermediate before cholesterol. According to theoretical predictions, there could be many more intermediates in cholesterol synthesis [4]. The predicted number of sterol metabolites from lanosterol to cholesterol is 72, comprising a metabolic network. This has been calculated by an algorithm that applied a binary code, labelling each C atom of the sterol ring by either 1, if containing a substituents, or 0 for no substituents. However, two important facts were not considered during enzyme activity coding. First, the enzymes SC4MOL, NSDHL, and HSD17B7 remove the methyl groups at position 4 in a specific order, where methyl group at position a is removed first. After its removal, methyl group at position b rotates to position a and is removed in the following step. The coding system was designed to remove the 4b methyl group first to create molecules that have one methyl group at position 4a. Second, a single bond at position 7(8) seems to completely inhibit activity of SC5DL, as no molecules with single bond at 7 (8) and double bond at 5(6) were found in any reports. A single bond at 7(8) may influence positioning of hydrogen atoms at position 6 and thus may prevent interaction of substrate with SC5DL [4].

We will now describe the major intermediates of cholesterol synthesis, their roles as signaling molecules, and links to human diseases.

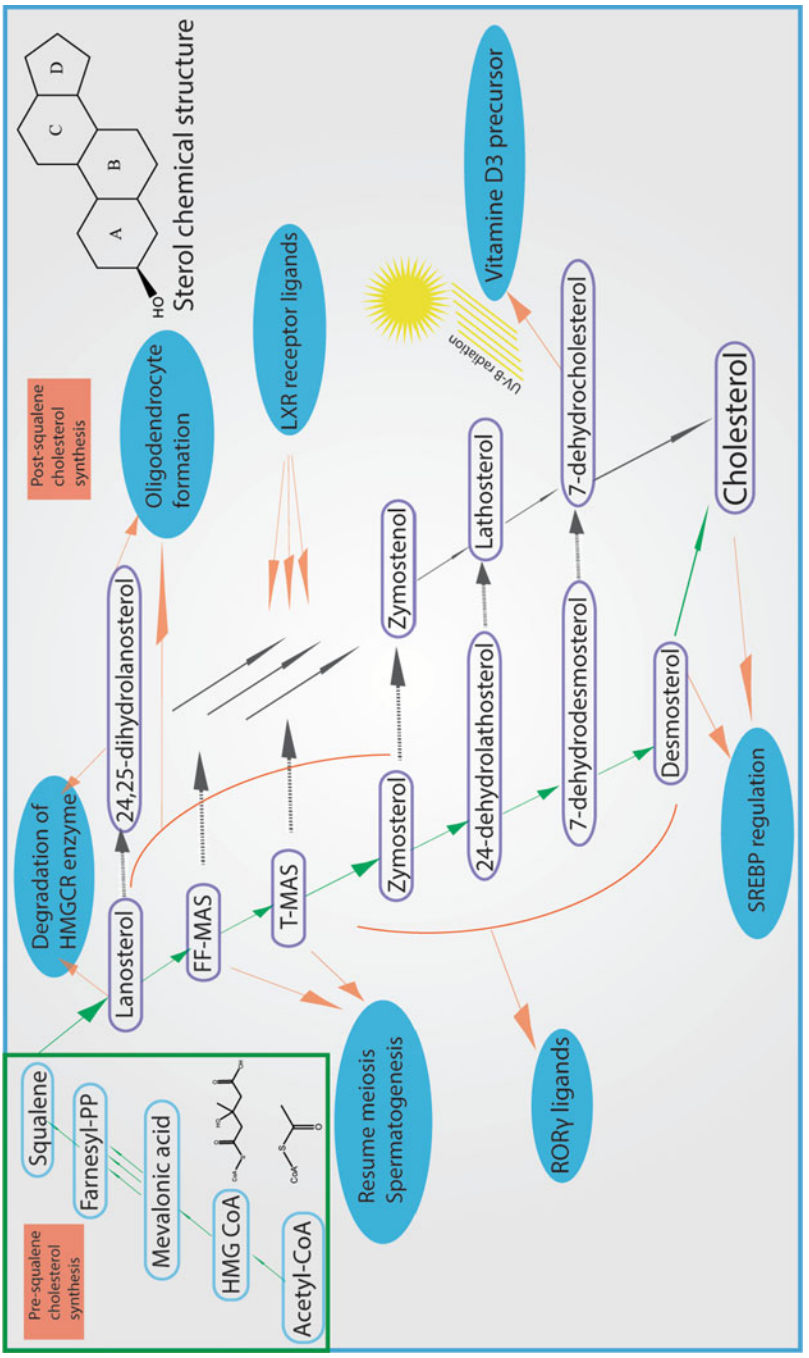


Fig. 2 Cholesterol synthesis with its intermediates. Figure represents the major cholesterol intermediates and their physiological functions in blue circles. The synthesis is from two parts, the pre-squalene in green square (not discussed in this work) and post-squalene in which lanosterol is the first molecule with sterol structure. With green arrows, the most probable synthesis pathway is shown

Sterol Intermediates from Lanosterol to Cholesterol

Lanosterol and 24,25-Dihydrolanosterol: The Branch Points of Sterol Synthesis

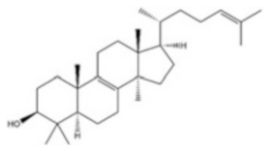
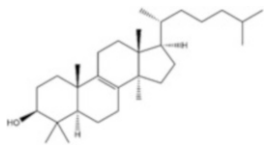
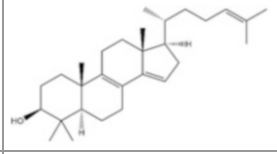
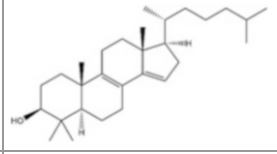
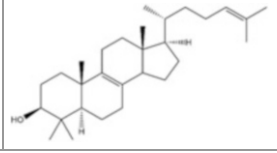
Lanosterol is the first cyclic intermediate of cholesterol synthesis pathway and the first sterol molecule in the post-squalene part of the synthesis (Sterol structures in Table 1). Lanosterol is formed from squalene by squalene epoxidase/monooxsyenase (SQLE) and lanosterol synthase (LSS) [5]. From lanosterol on the cholesterol synthesis is done by enzymes that are membrane bound in endoplasmic reticulum [6]. In the Bloch branch lanosterol is converted into FF-MAS (Follicular fluid meiosis-activating sterol) by CYP51 enzyme [7].

Addition of lanosterol efficiently stabilizes co-chaperon CHIP. CHIP acts as a molecular switch for both proteasomal and lysosomal mechanisms, which reduce aggregation of misfolded proteins, that often cause pathological conditions [8]. According to some studies, lanosterol surprisingly reverses protein aggregation in cataracts of animals. Lanosterol can dissolve precipitates and even amyloid-like fibril structures, which are the cause of cataracts in individuals. Lanosterol effectively treats cataracts in rabbit and dog lenses in vivo. Treating the cataract lenses with lanosterol was not yet successful in humans [9]. The "dissolving" ability of lanosterol seems to be partially controversial. Sterol intermediates of cholesterol synthesis, in particular desmosterol (discussed later), have been accused to cause cataracts and resulted in removal of a cholesterol lowering drug Triparanol from the market in early 1960s, as reviewed [10]. Triparanol was supposed to lower the level of cholesterol in the blood and reduce the risk of heart attacks. However, many people who took the drug went blind from an unusual form of cataract. It was suggested that accumulating desmosterol is causing the cataracts, and consequently, that all sterol intermediates toward cholesterol, from lanosterol and on, likely have harmful effects and should not accumulate in the cells. This also negatively influenced further drug development efforts to find novel hypolipidemic drugs with non-statin properties, that would inhibit enzymes in the late part of cholesterol synthesis [10].

For lanosterol, it was proven to have toxic effects in CHO-7 cells. Addition of lanosterol to cholesterol auxotrophs failed to support growth and killed the cells. Surprisingly, lanosterol killed also the wild-type cells, which underlines its toxic effect. The explanation has been that lanosterol indirectly suppressed cholesterol synthesis [11] and promoted the degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), and in this way downregulated cholesterol synthesis [12]. Alternative explanations claimed that sterols that have two methyl groups at position 4, such as lanosterol, cannot replace cholesterol in membranes, which results in defective membranes and cell death.

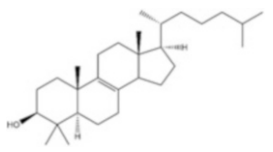
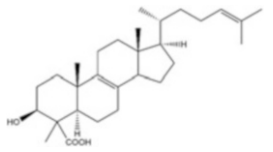
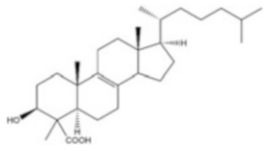
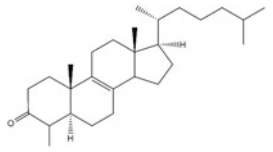
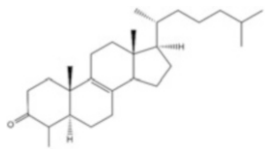
Recently Hubler et al. discovered new roles of sterols that have a double bond between C8 and C9 like lanosterol, FF-MAS, T-MAS, and zymostenol. Their results are showing that these sterols have an important role in oligodendrocytes formation.

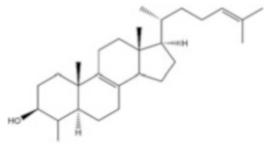
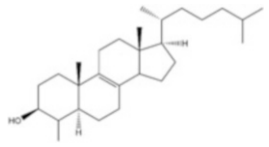
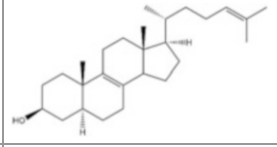
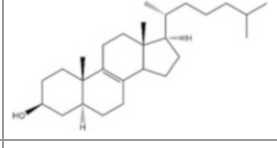
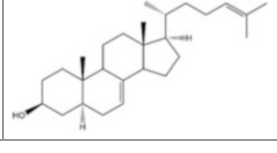
Table 1 The 19 known cholesterol intermediates, from lanosterol toward cholesterol

Trivial name	Chemical name	Formula	Structure	Molecular weight (g/mol)	Converted by
Lanosterol	4,4,14α-Trimethyl-5α-cholesta-8(9),24-dien-3β-ol	$C_{30}H_{50}O$		426.72	CYP51A1
24,25-Dihydrolanosterol	4,4,14α-Trimethyl-5α-cholest-8(9)en-3β-ol	$C_{30}H_{52}O$		428.73	CYP51A1
FF-MAS	4,4-Dimethyl-5α-cholesta-8(9),14,24-trien-3β-ol	$C_{29}H_{46}O$		410.67	TM7SF2, LBR
Dihydro-FF-MAS	4,4-Dimethyl-5α-cholesta-8(9),14-dien-3β-ol	$C_{29}H_{48}O$		412.69	TM7SF2, LBR
T-MAS	4,4-Dimethy-5α-cholesta-8(9),24-dien-3β-ol	$C_{29}H_{48}O$		412.69	SC4MOL

(continued)

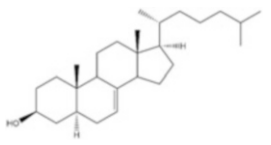
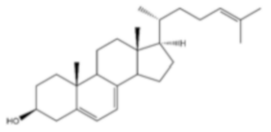
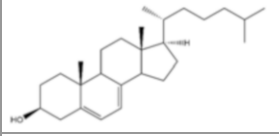
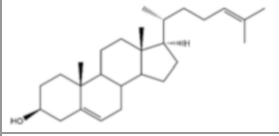
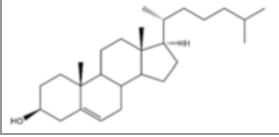
Table 1 (continued)

Trivial name	Chemical name	Formula	Structure	Molecular weight (g/mol)	Converted by
Dihydro-T-MAS	4,4-Dimethy-5α-cholest-8(9)-en-3β-ol	$C_{29}H_{50}O$		414.69	SC4MOL
4-Methyl-4-carboxyzymosterone		$C_{29}H_{46}O3$		442.34	NSDHL
4-Methyl-4-carboxy zymostenone		$C_{29}H_{48}O3$		444.36	NSDHL
4-Methyl zymosterone		$C_{28}H_{44}O$		396.34	HSD17B7
4-Methyl zymostenone		$C_{28}H_{46}O$		398.35	HSD17B7

4-Methyl zymosterol		$C_{28}H_{46}O$		398.35	SC4MOL NSDHL HSD17B7
4-Methyl zymostenol		$C_{28}H_{48}O$		400.37	SC4MOL NSDHL HSD17B7
Zymosterol	5α-Cholesta-8(9),24-dien-3β-ol	$C_{27}H_{44}O$		384.64	EBP
Zymostenol	5α-Cholest-8(9)-en-3β-ol	$C_{27}H_{46}O$		386.65	EBP
24-Dehydrolathosterol	5α-Cholesta-7,24-dien-3β-ol	$C_{27}H_{44}O$		384.64	SC5DL

(continued)

Table 1 (continued)

Trivial name	Chemical name	Formula	Structure	Molecular weight (g/mol)	Converted by
Lathosterol	5α-Cholest-7-en-3β-ol	$C_{27}H_{46}O$		386.66	SC5DL
7-Dehydrodesmosterol	5α-Cholesta-5,7,24-trien-3β-ol	$C_{27}H_{46}O$		386.66	DHCR7
7-Dehydrocholesterol	5α-Cholesta-5,7-dien-3β-ol	$C_{27}H_{44}O$		384.64	DHCR7
Desmosterol	5α-Cholesta-5,24-dien-3β-ol	$C_{27}H_{44}O$		384.64	DHCR24
Cholesterol	5α-Cholest-5-en-3β-ol	$C_{27}H_{46}O$		386.66	

Each sterol is represented with its molecule structure, chemical formula, molecular weight, and enzyme uses this sterol as substrate in the cholesterol synthesis pathway

By inhibition of enzymes in sterol synthesis, they found that 8,9-unsaturated sterols that accumulate in these cells are responsible for the promotion of oligodendrocyte formation and remyelination. The mechanism through which sterols promote oligodendrocyte formation is poorly understood. It is suspected that nuclear hormone receptors (NHR) have a role in this, but reporter assays with 20 different NHRs that are known to interact with sterols have not shown any activity in the case of oligodendrocytes. This means that the mechanism by which 8,9-unsaturated sterols promote oligodendrocytes is not yet fully understood [13].

There is no nuclear receptor identified that would be specific for lanosterol. It was shown that lanosterol can activate RORC but with a lower activity compared to some other sterols discussed below [14].

24,25-Dihydrolanosterol (DHL) can be converted from lanosterol by enzyme DHCR24 at the beginning of Kandutsch–Russell pathway of cholesterol synthesis. DHCR24 catalyzes the reduction of Δ^{24} bond in lanosterol [2]. But in most tissues lanosterol is predominantly converted to FF-MAS as proposed in the Bloch pathway. There is some evidence that 5–10% of lanosterol in the liver is converted to DHL, but not to downstream intermediates via CYP51 as proposed in Kandutsch–Russell pathway. There is possibility that some of hepatic DHL is converted to another sterol, the identity of which remains unknown [15].

DHL has a major role in posttranscriptional regulation of HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), which catalyzes the reduction of HMG CoA (3-hydroxy-3-methylglutaryl-coenzyme A) to mevalonate. This is a rate-limiting step in the entire cholesterol synthesis. DHL inhibits HMGCR by triggering degradation and ubiquitination of the enzyme. DHL causes conformational changes in HMGCR to enable interactions with the INSIG proteins. INSIG proteins recruit the E3 ubiquitin ligase gp78 and proteasome-associated protein VCP, which results in ubiquitination and degradation of HMGCR. In this way, DHL affects cholesterol synthesis and its homeostasis [16]. Because DHL is supposedly converted from lanosterol mainly in the liver, the function of cholesterol regulation by DHL through HMGCR is likely valid only for the liver [15].

Meiosis Activating Sterols FF-MAS and T-MAS

Follicular fluid meiosis-activating sterol (FF-MAS) was first identified in human ovarian follicular fluid [17]. It is a cholesterol synthesis intermediate from the Bloch pathway. It is converted from lanosterol by the previously mentioned enzyme lanosterol 14α-demethylase (CYP51). CYP51 belongs to the cytochrome P450 superfamily and converts lanosterol (and DHL) into FF-MAS together with microsomal electron transferring protein NADPH-P450 reductase [18]. The highest concentrations of FF-MAS are found in the ovary, for instance in human preovulatory follicular fluid the concentration of FF-MAS is around 1.3 μM (T-MAS concentration is half of FF-MAS) [19]. The next step in cholesterol synthesis is reduction of Δ^{14} bond in FF-MAS to form testis meiosis-activating sterol (T-MAS) that will be discussed in detail in the next paragraph. This reduction can be done by

two different enzymes encoded by two different genes, the sterol-Δ14-reductase (DHCR14) and lamin B receptor (LBR) [2]. There is evidence suggesting that mice models with DHCR14 deletion were still able to normally synthesize cholesterol [20]. On contrary, HeLa cell lines with LBR knockout were unable to effectively sustain cholesterol synthesis, despite the presence of DHCR14, which means LBR is necessary for normal cholesterol synthesis [21]. FF-MAS can also be converted by enzyme DHCR24 to form FF-MAS with saturated side chain—dihydro-FF-MAS, which is a part of Kandutsch–Russell pathway [2].

Meiosis activating sterols (MAS) were first isolated from preovulatory follicular fluid from women undergoing treatment for infertility by in vitro fertilization (FF-MAS) and from bull testicular tissue (T-MAS). MAS function as stated in name, was first associated with function of activation of oocyte meiosis [17].

Meiosis is crucial for sexual reproduction of animals and forming haploid genetically balances gametes. In mammals, oogonia enter meiosis and are transformed into oocytes. Oocytes are arrested in late prophase of the first meiotic division at diploid stage. Meiosis does not resume until the follicular unit is not mature, fully grown, and can respond to gonadotropin hormones (FSH, LH), which resume meiosis and ovulation [19]. FF-MAS has the ability to resume meiosis of oocyte [17] in the gonadotropin-dependent mechanism. FF-MAS probably works through receptor-mediated mechanism and efforts have been invested to find specific MAS receptor. Liver X receptor (LXR) is a nuclear receptor that binds FF-MAS and several oxysterols. It was a candidate for receptor-mediated mechanism of oocyte meiosis resumption, but was excluded because FF-MAS has lower affinity for LXR than oxysterols [22]. Another reason is that none of oxysterols that can bind to LXR was able to resume oocyte meiosis [19]. Data indicate that MAS signaling may work through a G-protein-coupled mechanism and may be mediated by binding to an oocyte plasma membrane-associated protein with high affinity for FF-MAS. There is a lot of evidence suggesting the role of FF-MAS in resumption of oocyte meiosis, but another set of data suggested that FF-MAS might not be a universal mediator of hormone-induced meiotic maturation. In vivo role of MAS is not clear and stays enigma [23], some experimental evidence oppose the in vivo roles of MAS. The delay in germinal vesicle breakdown after addition of MAS or AY-9944, an inhibitor of DHCR7, a later enzyme of cholesterol synthesis, was at that time the strongest evidence against the suggested role of MAS as an essential mediator of luteinizing hormone in meiosis resumption [24]. It was, on the other side, proposed that the MAPK signaling pathway is required for the MAS-like resumption of meiosis activated through one of the hormonal upstream pathways [25].

T-MAS is converted from FF-MAS with enzymes DHCR14 and LBR [2]. It was first isolated from bull testicular tissue using high-performance liquid chromatography [17]. T-MAS is the part of cholesterol synthesis pathway and is generated in all cholesterol-synthesizing cells. In most cell types T-MAS immediately converted to zymosterol [26] through several intermediates catalyzed by sterol 4,4-dimethylase enzyme complex. The first step is carboxylation of one of the 4-methyl groups by enzyme sterol-C4-methyl oxidase (SC4MOL) to form 4-methyl-4-carboxyzymosterone. 3β-hydroxy-Δ5-steroid-dehydrogenase (NSDHL)

then converts 3β-hydroxy group to 3-keto by removing one of the CO_2 and 4-methylzymosterone is formed, which is then converted to 4-methylzymosterol by enzyme 3β-keto-reductase (HSD17B7) by restoring 3β-hydroxy group. Enzymatic process is then repeated one more time and zymosterol is formed [2]. FF-MAS and T-MAS both accumulate at high concentrations in ovaries and testes, due to low expression of respective downstream enzymes [26]. In mammalian testes T-MAS concentration can be above 30 μg/g, FF-MAS in testes is present only in trace amounts [19]. The large fraction of T-MAS that is synthesized in testes is not converted to zymosterol, but to sterol that remains unidentified and is not a part of the downstream cholesterol synthesis [15]. The elevated production and accumulation of T-MAS in the testis may result from transcriptional regulation of cholesterol synthesis pathway and inhibition of the enzymes converting T-MAS into downstream cholesterol synthesis intermediates. CYP51 can escape the SREBP regulation under certain physiological conditions in the testes and can be upregulated via cAMP-dependent stimuli and cAMP-responsive element modulator (CREMτ). CREMτ is expressed only in spermatids and regulates genes associated with maturation and development of spermatids [23].

It is still not firmly established whether T-MAS is as FF-MAS associated with the resumption of meiosis. In vitro T-MAS and FF-MAS have interchangeable roles and are able to induce meiosis resumption in similar concentrations. T-MAS is more efficient in germinal vesicle breakdown assay in naked oocyte [23]. T-MAS concentration in testes is high, but its function is not clear. It may play a role in fertilization, spermatogenesis, and resumption of second meiotic division [19]. Since CYP51 that converts lanosterol to FF-MAS, unequivocally localizes to acrosomal membranes of male germ cells, it was proposed that sperm cells can synthesize MAS sterols in situ [27]. To test the role of sterols MAS in vivo, we generated a conditional male germ cell-specific knockout of *Cyp51* in the mouse. As expected, metabolic profiling revealed elevated CYP51 substrates lanosterol and 24,25-dihydrolanosterol and diminished levels of MAS, the immediate products of CYP51. To our surprise the germ cell-specific ablation of *Cyp51* did not affect testicular morphology, sperm production, or reproductive performance of the males. These results failed to show that de novo synthesis of MAS and cholesterol in male germ cells is most likely not essential for spermatogenesis [28].

Zymosterol and Zymostenol

Zymosterol is synthesized from T-MAS with removal of two methyl groups on C4 in the process involving enzymes SC4MOL, NSDHL, and HSD17B7. In the Bloch pathway zymosterol is then converted to 24-dehydrolathosterol by enzyme sterol-Δ^{8-7}-isometase (EBP), which shifts the double bond from position Δ^8 to position Δ^7. Like all cholesterol intermediates in the Bloch pathway, zymosterol can also be theoretically converted by DHCR24 to zymostenol in K–R pathway [2, 29]. But in the case of zymosterol, there is evidence that this may actually be one of the points of crossover between Bloch and the Kandutsch–Russell pathway. This was confirmed

on embryotic kidney cells HEK-293. Some data show that zymosterol is a better substrate for DHCR24 than lanosterol, which is the first intermediate in the Bloch pathway, which means that crossover between both pathways is probably downstream of lanosterol. Anyway, in most cells zymosterol is not considered to be the main substrate for DHCR24; its major physiological substrate is desmosterol on the end of Bloch pathway [15].

Zymosterol is synthesized like other sterols in rough ER. Some of the newly synthesized zymosterol in cultured human fibroblasts is rapidly (even faster than cholesterol) transferred to the plasma membrane. Then some of zymosterol is transferred back to ER, where it is converted to next sterol intermediates and finally to cholesterol. The function of this movement within the cell is unknown [30]. In studying zymosterol effect on artificial lipid monolayer, it was concluded that zymosterol possesses condensing and ordering abilities. However, the effect of zymosterol on lipid membranes is much less efficient compared to cholesterol [31].

Zymosterol is considered one of the ligands for the nuclear receptor RORγ (discussed in last chapter). Altered zymosterol concentration can through binding with RORγ [32] change the expression of genes associated with immunity, circadian rhythm, and metabolism [33]. Like other 8,9-unsaturated sterols zymosterol can also enhance the oligodendrocyte formation [13].

Zymostenol can be synthesized from zymosterol with enzyme DHCR24 [2] and is also one of the crossover points between Bloch and Kandutsch–Russell pathway of cholesterol synthesis in some tissues [15]. Zymostenol can theoretically be synthesized also from dihydro-T-MAS, an upstream cholesterol intermediate in Kandutsch–Russell pathway, through series of reactions with enzymes SC4MOL, NSDHL, and HSD17B7, like in T-MAS to zymosterol conversion [2], but this pathway is not supported with sufficient evidence. Sterols proceed down the Bloch pathway at least until zymosterol, when the demethylation of sterol nucleus is complete, and can then be converted by DHCR24 and cross into Kandutsch–Russell pathway [15].

Lathosterol and 24-Dehydrolathosterol

Lathosterol is the next cholesterol intermediate from Kandutsch–Russell pathway downstream of zymostenol. It is converted from zymostenol by enzyme EBP or from 24-dehydrolathosterol with enzyme DHCR24 [2, 29]. There is more evidence that physiologically lathosterol is synthesized from zymostenol and not from 24-dehydrolathosterol [15]. In the next step of cholesterol synthesis, lathosterol is converted to 7-dehydrocholesterol by enzyme SC5DL, which catalyzes the formation of double bond between C5 and C6.

24-Dehydrolathosterol is converted from zymosterol with changing double bond position form Δ^8 position to Δ^7 with enzyme EBP. In Bloch pathway enzyme sterol-C5-desaturase/lathosterol oxidase (SC5DL) formats double bond on C5 position and converts 24-dehydrolathosterol to 7-dehydrodesmosterol. 24-Dehydrolathosterol can also be converted by DHCR24 to lathosterol which is intermediate from Kandutsch–Russell pathway [2].

7-Dehydrocholesterol

7-Dehydrocholesterol (7-DHC) is the last intermediate in Kandutsch–Russell cholesterol synthesis pathway and it is converted from lathosterol by enzyme SC5DL. The last reaction before cholesterol is synthesized is reduction of Δ^7 double on 7-deydrocholesterol by enzyme DHCR7 [2, 34].

Apart from being precursor for cholesterol, 7-DHC is also precursor for vitamin D synthesis. Vitamin D_3 is produced in a two-step nonenzymatic process. UVB light (280–320 nm wavelength) brakes the B-sterol ring and pre-vitamin D_3 is formed, which then isomerizes to form vitamin D_3. This process happens in the skin and is dependent on UVB intensity and melanin (skin pigmentation level), which can block UVB from reaching 7-dehydrocholesterol [35].

It was shown that 7DHC can destabilize the HMGCR enzyme. In this way 7DHC can downregulate cholesterol production. This function of 7DHC was shown only in the case of 7DHC accumulation like in case of SLOS (discussed in next chapter) patients [36].

Desmosterol and 7-Dehydrodesmosterol

Desmosterol is synthesized from 7-dehydrodesmosterol by enzyme DHCR7, which removes double bond from Δ^7 position. It is the last intermediate in Bloch cholesterol synthesis pathway and is converted to cholesterol by enzyme DHCR24, which catalyzes the reduction of Δ^{24} double bond on the side chain of sterol ring [34].

Desmosterol also binds to LXR, which controls cholesterol export genes and represses inflammatory genes. Desmosterol can accumulate in macrophage foam cells and by LXR activation regulates metabolism and inflammatory response [29]. In mature testis, desmosterol can be found in high concentration (up to 12% of total sterols) and even higher in sperm where desmosterol can reach concentration up to 58% of total sterols (both data are for Rhesus monkeys). The high desmosterol level may be the consequence of lower levels of DHCR24 or its inhibition. In sperm, concentration of desmosterol is not homogenous, but desmosterol is mainly located in sperm tail. This effects the membrane fluidity and is possible to be necessary for normal motility of flagella [37].

Experiments done on J774 cells (mouse monocyte/macrophage) showed that cells substitute cholesterol with desmosterol in case of cholesterol depletion. Cells were able to survive and proliferate without cholesterol (DHCR24 mutation and medium without cholesterol). Because desmosterol and cholesterol differ only in double bond on position C24, and it is not surprising that desmosterol can substitute cholesterol in some of the processes. J774 cells with cholesterol depletion have a functional SREBP pathway and desmosterol can suppress SREBP pathway and gene expression [38]. Similarly to zymosterol, desmosterol also binds and activates the RORγ and affects the expression of genes controlled by this nuclear receptor [32].

In the Bloch pathway 7-dehydrodesmosterol is converted from 24-dehydrolathosterol by the enzyme SC5DL, which catalyzes the formation of

double bond on position C5. In the next step sterol-Δ^7-reductase (DHCR7) catalyzes the reduction of Δ^7 double bond in 7-dehydrodesmosterol to form desmosterol. In the Kandutsch–Russell pathway DHCR24 converts 7-dehydrodesmosterol to 7-dehydrocholesterol. Both desmosterol and 7-dehydrocholesterol are direct precursors of cholesterol [34].

Alternative Pathways

It is becoming clear that not all of the lanosterol and post-lanosterol intermediates are intended for cholesterol biosynthesis. One of the branches is DHL, which in liver regulates HMG-CoA reductase and is possibly converted to unknown sterol. It is known that not all of 7DHC is converted to cholesterol, but in skin also to vitamin D_3 [15]. Part of both FF-MAS and T-MAS (and dihydro-MAS forms) in testes are diverted from cholesterol synthesis and are converted to unknown sterols. A mathematical model suggested that unknown enzymes can drive the nonpolar cholesterol intermediates from the pathway. It was then experimentally confirmed that some of the enzymes from the cytochrome P450 superfamily (CYP) can catalyze non-classic reactions in cholesterol synthesis. CYP7A1, CYP11A1, CYP27A1, and CYP46A1 are enzymes for which it was shown that they can catalyze reaction with zymosterol, lathosterol, 7-DHC, desmosterol, and cholesterol. In vivo reactions of these CYP enzymes with cholesterol intermediates are not confirmed, but products could potentially have important biological activities [7].

CYP27A1 is gene encoding the mitochondrial sterol 27-hydroxylaso enzyme, which in normal physiological conditions transforms cholesterol to bile acids. By cloning CYP27A1 to *E. coli*, they found out that the enzyme is not only cholesterol specific, but can also metabolize cholesterol precursors (zymosterol, desmosterol, 7-dehydrocholesterol, and lanosterol) to form 27-hydroxy derivatives. Their function and levels in normal physiological conditions is unknown. In cells, the CYP27A1 enzyme is located on the matrix side of inner mitochondrial membrane and probably more than enzyme specificity, the mitochondrial membrane permeability for sterol molecules is crucial for the selection of the substrate [39]. CYP27A1 can theoretically convert also FF-MAS and T-MAS in the testis [7].

Accumulating Sterols Can Cause Malformations in Humans

Antley–Bixler Syndrome (ABS) Is Characterized by Accumulation of Lanosterol and 24,25-Dihydrolanosterol

Antley–Bixler syndrome (ABS) represents a group of heterogeneous disorders characterized by skeletal, cardiac, and urogenital abnormalities that have frequently been associated with mutations in fibroblast growth factor receptor 2 or cytochrome P450 oxidoreductase (*POR*) genes [40]. ABS is a rare congenital multiple malformation syndrome. Patients often have craniosynostosis and other craniofacial

anomalies, as well as other skeletal defects. Many patients also demonstrate defects in steroidogenesis and sexual development. One of the causes is mutation in a gene encoding POR. In cytochrome P450 enzymatic reactions taking place in endoplasmic reticulum, POR acts as an electron donor. This is the case also for the conversion of lanosterol to FF-MAS or 24,25-dihydrolanosterol to dihydro-FF-MAS by CYP51, which in the case of mutated POR results in accumulation of lanosterol and DHL [41].

CYP51A1 is an enzyme from the first part of post-lanosterol cholesterol synthesis and converts lanosterol in FF-MAS. To study the involvement of CYP51A1 in the ABS, the mouse model was prepared with deletion of *Cyp51* gene. Lanosterol and 24,25-dihydrolanosterol are accumulated in this knockout mice. These mice showed several ABS-like features and *Cyp51* deletion led to embryonic lethality on day 15. Lethality was caused by heart failure resulting from hypoplasia, ventricle septum, epicardial and vasculogenesis defects [42]. Some ABS patients show lower CYP51A1 activity and accumulation of lanosterol and 24,25-dihydrolanosterol substrates, suggesting that CYP51A1 is at least indirectly involved in ABS disease pathology [43].

Rare Diseases with Accumulation of 4,4′-Dimethylsterols, 4-Carboxysterols, and 14-Methylsterols

The first enzyme of T-MAS conversion to zymosterol is SC4MOL and its mutations cause a disorder called SC4MOL deficiency. It is a very rare disorder that can cause severe ichthyosiform erythroderma affecting entire body, cataracts, microcephaly, and delayed skeletal and sexual development. Disorder can be diagnosed by measuring the elevated 4,4′-dimethylsterols in the skin. There is no treatment available [44].

NSDHL is the enzyme responsible for conversion of T-MAS to zymosterol and is also associated with severe pathologies. Mutation in NSDHL causes rare disorder called CHILD syndrome, which is an X-linked and generally lethal disorder in males. CHILD syndrome causes severe skeletal defects such as aplasia of entire limb, phocomelia, limp hypoplasia, and hypodactyly. Skin is also affected and patients have inflammatory nevus with skin lesions that often follow lines of X inactivation (lines of Blaschko). Mild cognitive and heart problems are also possible [45]. The best marker for diagnosis is biochemical detection of 4-carboxysterols in affected skin flakes. Due to random X inactivation, the blood sterol profile is not always the best option [44].

Pelger–Huët anomaly and Greenberg skeletal dysplasia are two disorders associated with a defective LBR enzyme. Pelger–Huët anomaly is characterized by hypolobulation of nuclei in granulocytes and is classified as laminopathia. Greenberg skeletal dysplasia (also hydrops-ectopic calcification-moth-eaten dysplasia) is a perinatal lethal condition, with abnormal bone development and excessive fluid accumulation. Patients with Greenberg skeletal dysplasia have defect sterol metabolism. LBR is a bifunctional membrane protein located in inner nuclear

membrane. N-terminus of LBR is located in the nucleus and interacts with chromatin and intermediate filaments. Unconnected to the first function, the other function of LBR on the C-terminal end is the sterol C14 reductase activity [46]. The causes of pathological conditions associated with LBR are mutations in LBR that substantially reduce NADPH binding affinity due to changes in NADPH binding pocket site, what causes abnormal sterol metabolism [21].

Rare Diseases with Accumulation of Zymosterol and Zymostenol

Conradi–Hünermann–Happle syndrome, or X-linked dominant chondrodysplasia punctate (CDPX2, OMIM no. 302960), is a rare genetic disorder characterized by skeletal dysplasia, stippled epiphyses, cataracts, transient ichthyosis, and atrophic residua in a mosaic pattern. It is a rare disorder and in most cases lethal for males. Mutations in the gene encoding the emopamil-binding protein (EBP) have been identified as an underlying cause. Because EBP is unfunctional, the zymosterol accumulates in the tissue [40, 41]. There are over 50 different variations known in the EBP enzyme that cause missense, nonsense, frameshift, and splicing mutation in the gene. The phenotype is very variable, because of random X-inactivation, in females, linear ichthyosis and severe erythroderma at birth are common [47]. CDPX2 diagnosis can be made by detection of elevated levels of zymostenol (cholesta-8(9)-en-3b-ol) [44].

Lathosterolosis

The syndrome caused by mutation in SC5DL gene (located on chromosome 11q23.3) is called lathosterolosis (OMIM no. 607330) [41]. Because of gene mutation, enzyme is unable to convert both lathosterol and 24-dehydrolathosterol to 7-dehydrocholesterol or 7-dehydrodesmosterol. Patients with lathosterolosis have elevated levels of lathosterol in tissues; diagnosis can be made on blood, skin fibroblast, or lymphocyte samples [44]. Lathosterolosis is an extremely rare autosomal recessive disorder with few documented cases. Patients have, similar to other cholesterol synthesis disorders, axial and appendicular skeletal, craniofacial and neurological abnormalities. Also common are cataract formation, intellectual disability, neonatal microcephaly, dystrophic calcification and others [40, 41]. Treatment of one patient was successful, but with only four known patients, it is hard to conclude on treatment efficiency [48].

Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz syndrome (SLOS, OMIM no. 270400) is autosomal recessive disorder caused by different mutations in gene encoding DHCR7 enzyme, which converts 7-dehydrodesmosterol and 7-dehydrocholesterol to desmosterol or

cholesterol [44]. SLOS is the most frequent disorder from group of cholesterol synthesis defect disorders, with frequency 1:20,000–50,000. It is a multisystemic disorder, where treatment is possible only for mild cases [48]. There is high prevalence of SLOS in Northern Europe, supposedly because advantage of heterozygotes with DHCR7 mutation. This causes 7-dehydrocholesterol accumulation and more vitamin D3 production what brings advantage in areas with reduced exposure to sunlight [29]. Disorder phenotypes are extremely broad. There are patients with very mild form; on the other hand, the severely affected cases die in utero or very soon after birth. The mild cases have minor physical findings and learning problems. Most of the SLOS patients have distinctive facial appearance [41], with microcephaly, ptosis, and midface hypoplasia. Central nervous system, limb defects, urogenital and gastrointestinal anomalies are common in patients. There are many other abnormalities and defects caused by SLOS disorder. Diagnosis can be made by measuring cholesterol and its intermediate levels in plasma, amniotic fluid, or cultured skin fibroblasts, with gas chromatography combined with mass spectrometry. Cholesterol levels can be reduced or normal, 7-dehydorcholesterol and 8-dehydrocholesterol levels are elevated [44] up to 50 times. Some patients respond positively to treatment with cholesterol supplementation and they show symptoms of improvement. In addition, simvastatin therapy has been investigated, but there is no final conclusion about simvastatin efficiency [41]. Interestingly, accumulated 7-dehydorcholesterol and 8-dehydrocholesterol in SLOS patients can both be partially converted to bile acids by CYP27A1 enzyme. This unnatural bile acid synthesis pathway is happening due to the high concentration of both sterols [49].

Diseases with Accumulation of Desmosterol

Desmosterolosis (OMIM no. 602398) is disorder caused by missense mutation of DHCR24 gene (chromosome 1p32.3) that encodes the last enzyme in Bloch pathway of cholesterol synthesis, which converts desmosterol to cholesterol. DHCR24 can theoretically convert any of intermediates from lanosterol on, but its mutation causes elevation of desmosterol levels in tissue [44]. Concentration of desmosterol in patient's serum is elevated up to 120 times. Phenotype of desmosterolosis is very similar to SLOS and is associated with central nervous system and skeletal abnormalities [50].

Sterols Signaling Through Nuclear Receptors

RORγ

Nuclear hormone receptors (NHR) are transcription factors that are regulating cell processes like immune response, metabolism, reproduction, and differentiation. On the N terminus, NHR has DNA-binding domain and on the C terminus, there is

ligand-binding domain. Among the members of NHR are also retinoic acid-related receptors (RORs) that have three isoforms (α, β, γ). RORγ activation was for a longer time an enigma. Cholesterol biosynthesis intermediates and metabolites were recently proposed as ligands [14]. In a study focusing on differentiation of TH17 cells that have important role in immunity, it was shown that RORγ has a role in cell differentiation. It was found that desmosterol is an agonist and can activate the RORγ. Desmosterol is in this way very important in the control of TH17 cells differentiation [32]. RORs are also associated with circadian expression of some genes, like the core clock genes *Bmal1* and *Clock* [51] RORγ can influence the expression of some cytochromes P450 genes (*Cyp2b10*, *Cyp2b13*, *Cyp2f2*, and *Cyp4a14*) [52, 53]. In this way, altered concentration of cholesterol intermediates can change the expression of many genes and importantly affect the cell homeostasis.

Liver X Receptor LXR: A Nuclear Receptor-Binding Multiple Nonpolar Sterols

LXR receptor is probably not responsible for meiosis resumption, but FF-MAS is still its ligand though physiological relevance of this interaction is still unknown [54]. A number of post-lanosterol cholesterol intermediates can serve as activating ligands on LXR. These are DHL, T-MAS, FF-MAS, and desmosterol [29]. LXR is one of the ligand-activated transcription factors and is highly expressed in the liver, kidney gut, spleen, and adipose tissue. It forms heterodimers together with retinoic X receptor (RXR), which binds to specific DNA sequence LXR response element [54]. LXR function is to protect cells from cholesterol overload. Together with RXR they stimulate the expression of enzymes and protein transporters that are responsible for reverse cholesterol transport, cholesterol to bile acid conversion, and bile acid transport [26]. ATP-binding cassette transporter A1 is one of the cholesterol transporters whose expression is regulated through LXR/RXR heterodimers [54]. It has been reported that LXR regulates cholesterol synthesis, by downregulating the gene expression of CYP51A1 and FDFT1 (dimerization of farnesyl diphosphate) and in this way lowering the levels of cholesterol [29]. De novo fatty acid synthesis is also regulated through LXR activation, which then affects SREBP-1, acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase gene transcription [54]. LXR is also associated with repression of inflammatory genes and dopaminergic differentiation of embryonic stem cells [29].

SREBP

Sterol regulatory element-binding proteins (SREBPs) are a family of transcription factors regulating the cholesterol synthesis pathway and are activated by low sterol levels. Almost all genes that encode enzymes in cholesterol synthesis (exception is LBR located on nucleus membrane) are target of SREBPs [29]. They are regulated

by negative cholesterol feedback loop. When cholesterol levels drop below 5% of endoplasmic reticulum lipids, activation of SREBPs happens. After cholesterol levels rise above 5%, SREBPs dissociate from promotors of cholesterogenic genes. Proteins involved in this cyclic feedback regulation include the insulin-induced genes (INSIG1 and 2), the SREBP cleavage-activating protein (SCAP), site 1 and 2 proteinases (S1P and S2P) and sterol regulatory element-binding proteins SREBPs, where SREBP2 is the cholesterol-dependent transcription activator of the majority of cholesterol synthesis genes. Other signaling pathways are involved in the regulation of cholesterogenic genes in various physiological, tissue-specific, developmental, or pathophysiological conditions [10].

Conclusions

It is now evident that sterols are very active molecules and that majority have multiple roles. From lanosterol and on sterols in the cholesterol biosynthesis pathway are evidently intermediates toward cholesterol. However, we still do not understand what are concentrations and half-lives of these sterols in different tissues. Without this knowledge, it will be difficult to reveal the entire spectrum of signaling potential that these nonpolar molecules have. Meiosis activating sterols, for example, got their name due to their ability to resume meiosis in oocytes even if it was later never possible to prove the exact mechanism behind that. Even if they bind to nuclear receptor LXR, albeit with lower affinity, we do not understand how their signaling activity is performed. Discovery that the retinoic acid orphan receptor gamma binds different nonpolar sterols was a surprising fact that gave the sterol research a novel boost. While the in vitro binding capacity of RORC to zymosterol derivatives and desmosterol has been revealed in 2015 by two research groups, we still await conformation of roles in vivo. It is possible to expect that different sterols would prove to be efficient agonists of RORC depending on the tissue. It also is possible to speculate that some might be more active in regulating the circadian clock while others, for example, in promoting the immunity. It is now clear that research efforts in the area of nonpolar sterols is rejuvenated and we are awaiting novel exiting news in the near future.

References

1. Haines TH. Do sterols reduce proton and sodium leaks through lipid bilayers? Prog Lipid Res. 2001;40(4):299–324.
2. Acimovic J, Rozman D. Steroidal triterpenes of cholesterol synthesis. Molecules. 2013;18 (4):4002–17.
3. Kandutsch AA, Russell AE. Preputial gland tumor sterols. 3. A metabolic pathway from lanosterol to cholesterol. J Biol Chem. 1960;235:2256–61.
4. Belic A, Pompon D, Monostory K, Kelly D, Kelly S, Rozman D. An algorithm for rapid computational construction of metabolic networks: a cholesterol biosynthesis example. Comput Biol Med. 2013;43(5):471–80.

5. Wendt KU, Schulz GE, Corey EJ, Liu DR. Enzyme mechanisms for polycyclic triterpene formation. Angew Chem Int Ed Engl. 2000;39(16):2812–33.
6. Reinhart MP, Billheimer JT, Faust JR, Gaylor JL. Subcellular localization of the enzymes of cholesterol biosynthesis and metabolism in rat liver. J Biol Chem. 1987;262(20):9649–55.
7. Acimovic J, Goyal S, Kosir R, Golicnik M, Perse M, Belic A, et al. Cytochrome P450 metabolism of the post-lanosterol intermediates explains enigmas of cholesterol synthesis. Sci Rep. 2016;6:28462.
8. Upadhyay A, Amanullah A, Mishra R, Kumar A, Mishra A. Lanosterol suppresses the aggregation and cytotoxicity of misfolded proteins linked with neurodegenerative diseases. Mol Neurobiol. 2017;55:1169–82.
9. Zhao L, Chen XJ, Zhu J, Xi YB, Yang X, Hu LD, et al. Lanosterol reverses protein aggregation in cataracts. Nature. 2015;523(7562):607–11.
10. Rozman D, Monostory K. Perspectives of the non-statin hypolipidemic agents. Pharmacol Ther. 2010;127(1):19–40.
11. Xu F, Rychnovsky SD, Belani JD, Hobbs HH, Cohen JC, Rawson RB. Dual roles for cholesterol in mammalian cells. Proc Natl Acad Sci USA. 2005;102(41):14551–6.
12. Song BL, Javitt NB, DeBose-Boyd RA. Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. Cell Metab. 2005;1 (3):179–89.
13. Hubler Z, Allimuthu D, Bederman I, Elitt MS, Madhavan M, Allan KC, et al. Accumulation of 8,9-unsaturated sterols drives oligodendrocyte formation and remyelination. Nature. 2018;560 (7718):372–6.
14. Santori FR, Huang P, van de Pavert SA, Douglass EF Jr, Leaver DJ, Haubrich BA, et al. Identification of natural RORgamma ligands that regulate the development of lymphoid cells. Cell Metab. 2015;21(2):286–97.
15. Mitsche MA, McDonald JG, Hobbs HH, Cohen JC. Flux analysis of cholesterol biosynthesis in vivo reveals multiple tissue and cell-type specific pathways. elife. 2015;4:e07999.
16. Sato R. Sterol metabolism and SREBP activation. Arch Biochem Biophys. 2010;501 (2):177–81.
17. Byskov AG, Andersen CY, Nordholm L, Thogersen H, Guoliang X, Wassmann O, et al. Chemical structure of sterols that activate oocyte meiosis. Nature. 1995;374(6522):559–62.
18. Rozman D, Waterman MR. Lanosterol 14alpha-demethylase (CYP51) and spermatogenesis. Drug Metab Dispos. 1998;26(12):1199–201.
19. Byskov AG, Andersen CY, Leonardsen L, Baltsen M. Meiosis activating sterols (MAS) and fertility in mammals and man. J Exp Zool. 1999;285(3):237–42.
20. Bennati AM, Schiavoni G, Franken S, Piobbico D, Della Fazia MA, Caruso D, et al. Disruption of the gene encoding 3beta-hydroxysterol Delta-reductase (Tm7sf2) in mice does not impair cholesterol biosynthesis. FEBS J. 2008;275(20):5034–47.
21. Tsai PL, Zhao C, Turner E, Schlieker C. The Lamin B receptor is essential for cholesterol synthesis and perturbed by disease-causing mutations. elife. 2016;5:e16011.
22. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature. 1996;383(6602):728–31.
23. Rozman D, Cotman M, Frangez R. Lanosterol 14alpha-demethylase and MAS sterols in mammalian gametogenesis. Mol Cell Endocrinol. 2002;187(1–2):179–87.
24. Tsafriri A, Cao X, Ashkenazi H, Motola S, Popliker M, Pomerantz SH. Resumption of oocyte meiosis in mammals: on models, meiosis activating sterols, steroids and EGF-like factors. Mol Cell Endocrinol. 2005;234(1–2):37–45.
25. Motola S, Cao X, Popliker M, Tsafriri A. Involvement of mitogen-activated protein kinase (MAPK) pathway in LH- and meiosis-activating sterol (MAS)-induced maturation in rat and mouse oocytes. Mol Reprod Dev. 2008;75(10):1533–41.
26. Beltowski J, Semczuk A. Liver X receptor (LXR) and the reproductive system – a potential novel target for therapeutic intervention. Pharmacol Rep. 2010;62(1):15–27.

27. Cotman M, Jezek D, Fon Tacer K, Frangez R, Rozman D. A functional cytochrome P450 lanosterol 14 alpha-demethylase CYP51 enzyme in the acrosome: transport through the Golgi and synthesis of meiosis-activating sterols. Endocrinology. 2004;145(3):1419–26.
28. Keber R, Acimovic J, Majdic G, Motaln H, Rozman D, Horvat S. Male germ cell-specific knockout of cholesterogenic cytochrome P450 lanosterol 14alpha-demethylase (Cyp51). J Lipid Res. 2013;54(6):1653–61.
29. Sharpe LJ, Brown AJ. Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). J Biol Chem. 2013;288(26):18707–15.
30. Lange Y, Echevarria F, Steck TL. Movement of zymosterol, a precursor of cholesterol, among three membranes in human fibroblasts. J Biol Chem. 1991;266(32):21439–43.
31. Hac-Wydro K, Wydro P, Flasinski M. The comparison of zymosterol vs cholesterol membrane properties--the effect of zymosterol on lipid monolayers. Colloids Surf B Biointerfaces. 2014;123:524–32.
32. Hu X, Wang Y, Hao L-Y, Liu X, Lesch CA, Sanchez BM, et al. Sterol metabolism controls TH17 differentiation by generating endogenous RORγ agonists. Nat Chem Biol. 2015;11 (2):141–7.
33. Cook DN, Kang HS, Jetten AM. Retinoic acid-related orphan receptors (RORs): regulatory functions in immunity, development, circadian rhythm, and metabolism. Nucl Receptor Res. 2015;2:101185.
34. Luu W, Hart-Smith G, Sharpe LJ, Brown AJ. The terminal enzymes of cholesterol synthesis, DHCR24 and DHCR7, interact physically and functionally. J Lipid Res. 2015;56(4):888–97.
35. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21(3):319–29.
36. Fitzky BU, Moebius FF, Asaoka H, Waage-Baudet H, Xu L, Xu G, et al. 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. J Clin Invest. 2001;108(6):905–15.
37. Connor WE, Lin DS, Neuringer M. Biochemical markers for puberty in the monkey testis: desmosterol and docosahexaenoic acid. J Clin Endocrinol Metab. 1997;82(6):1911–6.
38. Rodriguez-Acebes S, de la Cueva P, Fernandez-Hernando C, Ferruelo AJ, Lasuncion MA, Rawson RB, et al. Desmosterol can replace cholesterol in sustaining cell proliferation and regulating the SREBP pathway in a sterol-Delta24-reductase-deficient cell line. Biochem J. 2009;420(2):305–15.
39. Pikuleva I, Javitt NB. Novel sterols synthesized via the CYP27A1 metabolic pathway. Arch Biochem Biophys. 2003;420(1):35–9.
40. Herman GE. X-linked dominant disorders of cholesterol biosynthesis in man and mouse. Biochim Biophys Acta. 2000;1529(1–3):357–73.
41. Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res. 2011;52(1):6–34.
42. Keber R, Motaln H, Wagner KD, Debeljak N, Rassoulzadegan M, Acimovic J, et al. Mouse knockout of the cholesterogenic cytochrome P450 lanosterol 14alpha-demethylase (Cyp51) resembles Antley-Bixler syndrome. J Biol Chem. 2011;286(33):29086–97.
43. Cragun DL, Trumpy SK, Shackleton CH, Kelley RI, Leslie ND, Mulrooney NP, et al. Undetectable maternal serum uE3 and postnatal abnormal sterol and steroid metabolism in Antley-Bixler syndrome. Am J Med Genet A. 2004;129a(1):1–7.
44. Kanungo S, Soares N, He M, Steiner RD. Sterol metabolism disorders and neurodevelopment-an update. Dev Disabil Res Rev. 2013;17(3):197–210.
45. Avgerinou GP, Asvesti AP, Katsambas AD, Nikolaou VA, Christofidou EC, Grzeschik KH, et al. CHILD syndrome: the NSDHL gene and its role in CHILD syndrome, a rare hereditary disorder. J Eur Acad Dermatol Venereol. 2010;24(6):733–6.
46. Turner EM, Schlieker C. Pelger-Huet anomaly and Greenberg skeletal dysplasia: LBR-associated diseases of cholesterol metabolism. Rare diseases. 2016;4(1):e1241363.

47. Braverman N, Lin P, Moebius FF, Obie C, Moser A, Glossmann H, et al. Mutations in the gene encoding 3 beta-hydroxysteroid-delta 8, delta 7-isomerase cause X-linked dominant Conradi-Hunermann syndrome. Nat Genet. 1999;22(3):291–4.
48. Corso G, Dello Russo A, Gelzo M. Liver and the defects of cholesterol and bile acids biosynthesis: rare disorders many diagnostic pitfalls. World J Gastroenterol. 2017;23 (29):5257–65.
49. Honda A, Salen G, Shefer S, Batta AK, Honda M, Xu G, et al. Bile acid synthesis in the Smith-Lemli-Opitz syndrome: effects of dehydrocholesterols on cholesterol 7alpha-hydroxylase and 27-hydroxylase activities in rat liver. J Lipid Res. 1999;40(8):1520–8.
50. Andersson HC, Kratz L, Kelley R. Desmosterolosis presenting with multiple congenital anomalies and profound developmental delay. Am J Med Genet. 2002;113(4):315–9.
51. Zhang Y, Papazyan R, Damle M, Fang B, Jager J, Feng D, et al. The hepatic circadian clock fine-tunes the lipogenic response to feeding through RORalpha/gamma. Genes Dev. 2017;31 (12):1202–11.
52. Wang C, Xie H, Song X, Ning G, Yan J, Chen X, et al. Lanosterol 14alpha-demethylase expression in the mouse ovary and its participation in cumulus-enclosed oocyte spontaneous meiotic maturation in vitro. Theriogenology. 2006;66(5):1156–64.
53. Yan J, Wang H, Liu Y, Shao C. Analysis of gene regulatory networks in the mammalian circadian rhythm. PLoS Comput Biol. 2008;4(10):e1000193.
54. Gatticchi L, Cerra B, Scarpelli P, Macchioni L, Sebastiani B, Gioiello A, et al. Selected cholesterol biosynthesis inhibitors produce accumulation of the intermediate FF-MAS that targets nucleus and activates LXRalpha in HepG2 cells. Biochim Biophys Acta. 2017;1862 (9):842–52.