Where Does Fetal and Embryonic Cholesterol Originate and What Does It Do?

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Abstract

The development of a single-celled fertilized egg, through the blastocyst stage of a ball of cells and the embryonic stage when almost all organ systems begin to develop, and finally to the fetal stage where growth and physiological maturation occurs, is a complex and multifaceted process. A change in metabolism during gestation, especially when organogenesis occurs, can lead to abnormal development and congenital defects. Although many studies have described the roles of specific proteins in development, the roles of specific lipids, such as sterols, have not been studied as intensely. Sterol's functions in development range from being a structural component of membranes to regulating the patterning of the forebrain through sonic hedgehog to regulating expression of key proteins involved in metabolic processes. This review focuses on the roles of sterols in embryonic and fetal development and metabolism. Potential sources of cholesterol for the fetus and embryo are also discussed.

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INTRODUCTION AND SCOPE

In utero development is a process that is different from any other in that cells differentiate to numerous types and proliferation abounds. Associated with increased proliferation is the requirement for membrane substrates. A key component of every membrane, and therefore one of the essential membrane substrates, is cholesterol. Cholesterol helps maintain normal membrane fluidity and lipid-rich microdomains. Because most intracellular signaling originates in specific microdomains within membranes, a change in membrane cholesterol can affect numerous metabolic processes. Cholesterol also impacts signaling through activation of the hedgehog proteins and propagation of their signal. In addition to its role as a structural component and consequently a direct or indirect activator of membrane-initiated signaling, cholesterol is the precursor of bile acids, steroid hormones, and oxysterols—all of which have been shown to affect lipid as well as glucose metabolism.

In the early 1990s, the importance of cholesterol in development became apparent when researchers discovered that fetuses that synthesize cholesterol at markedly reduced rates have numerous congenital defects. Infants with reduced cholesterol biosynthetic rates resulting from the inability to convert 7-dehydrocholesterol (7-DHC) to cholesterol have the Smith-Lemli-Opitz syndrome (SLOS; also known as RSH) (47). The defects associated with SLOS are numerous and can range from the very mild, i.e., subtle learning disorders and minor dysmorphic features, to the very severe, i.e., mental retardation and congenital abnormalities (3, 54, 93). Additionally, infants are often small for their gestational age (53). Interestingly, the outcome of the pregnancy (infant's clinical severity score) can be affected by the apoE genotype of the mother, but not that of the father, suggesting that maternal cholesterol metabolism plays a role in fetal development (131). In this review, the sources of cholesterol for the embryo (from fertilized egg to eighth week of gestation) and fetus (ninth week of gestation to birth) are discussed along with the various roles of cholesterol, including the consequences and causes of reduced cholesterol concentrations.

CHOLESTEROL METABOLISM IN STEADY STATE: SYNTHESIS AND UPTAKE

Healthy adult tissues are in steady state. In steady state, the amount of cholesterol entering cells or the body is equal to the amount leaving: input equals output. Regardless of whether a tissue is in steady state or not, cholesterol can be derived from two sources: that synthesized de novo and that taken up from the circulation (or diet).

Cholesterol biosynthesis occurs through a series of steps. The rate-limiting step for this pathway is hydroxymethylglutaryl-coenzyme A reductase (HMGR). This enzyme is highly regulated at the transcriptional, translational, and posttranslational steps (32). One of the key regulators of transcription of HMGR is the sterol regulatory element–binding protein-2 (SREBP-2). There are three forms of SREBP: SREBP-1a, SREBP-1c, and SREBP-2. Although all SREBPs are involved in the regulation of sterol synthesis rates, SREBP-2 is the strongest activator of this pathway (reviewed in 33, 42). SREBPs are synthesized as inactive, precursor forms that are localized to the endoplasmic reticulum (ER) membrane, where they bind to the SREBP cleavage–activating protein (Scap), another ER-bound protein (**Figure 1**). When cellular sterols are depleted, such as during negative sterol balance, the SREBP:Scap complex exits the ER in budding vesicles and transverses to the Golgi. Once in the Golgi, the SREBPs are cleaved to mature, activated proteins that then translocate to the nucleus, where they bind to sterol regulatory elements within the promoter regions of HMGR and other proteins. When there is an excess of cellular cholesterol, the binding of sterols to Scap induces a conformational change. The modified Scap binds to Insig-1 and -2, retaining the SREBP:Scap complex within the ER membrane. Posttranslational modifications of HMGR also impact sterol synthesis rates (33, 42). When lanosterol is replete within membranes, HMGR is ubiquitinated and subsequently degraded in proteosomes. The process involves the Insigs as well as geranylgeraniol.

Exogenous sterol is derived from the circulation in all tissues, with the possible exception of the brain (22). Cholesterol is carried in the circulation in the form of lipoproteins: very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Lipoprotein cholesterol is cleared from the plasma by receptor-mediated and receptor-independent processes. The receptor-mediated processes are saturable and the clearance rates are dependent on plasma cholesterol concentrations. The receptor-independent processes are not saturable and are independent of plasma cholesterol concentrations. For most lipoprotein receptors, especially those of the LDL receptor family, the whole lipoprotein particle is taken up by the receptors and catabolized by way of clathrin-coated pits (7, 129). However, HDL cholesteryl ester is selectively taken up by the scavenger receptor class B type I (SR-BI) (1). Cholesterol taken up through this process does not appear to be processed through the lysosomal pathway, although the SR-BI-derived cholesterol can be used for hormone synthesis and other intracellular functions if cholesterol derived from the lysosomal pathway is blocked (135). HDL can also be taken up by cubilin (36, 57), which processes the HDL by way of the lysosomal pathway (36).

CHOLESTEROL SYNTHESIS IN THE EMBRYO AND FETUS

Unlike the adult, the fetus is not in steady state and does accrue cholesterol. The rates of sterol synthesis are much greater in the fetus than in the adult (22), and can account for a significant proportion of the fetal cholesterol accrued in some species, but not all (132). Interestingly, elevated synthesis rates in the human fetal liver do not appear to decrease during gestation as they do in other animal models (22, 66, 132).

In healthy adult tissues, there is an intricate regulatory system in place such that small changes in cholesterol levels lead to marked

changes in the ability to synthesize or take up cholesterol. What about tissues not in steady state that are growing rapidly, such as tumors or fetal tissues, which must accrue a significant amount of cholesterol to maintain membrane formation? In malignant tissues, there is a blunted response to exogenous sterol such that synthesis rates, which are already elevated, do not decrease in the face of elevated sterol concentrations (109). Similarly, our laboratory has demonstrated that HMGR levels in fetal livers were not decreased to the same extent as in adult tissues with the same fold-increase in cholesterol concentrations (138). SREBP-2 appeared to be constitutively processed, as the mature levels of SREBP-2 did not change in the face of elevated cholesterol concentrations; this appears to be due either to an increase in the ratio of Scap to Insig-1 or to a decrease in intermediates of the biosynthetic pathway (reviewed in 33). Likewise, sterol synthesis rates were not fully suppressed in fetal hepatocytes treated with lipoprotein cholesterol (10). Additionally, even though hepatic sterol synthesis rates were suppressed in adult animals fed polyunsaturated fatty acids (PUFAs), sterol synthesis in PUFA-enriched fetal tissues were insensitive to PUFA-induced effects, suggesting constitutive or insensitive processing of the SREBPs into transcription factors (104).

Fetal sterol synthesis rates are not devoid of regulation, however. Carr & Simpson (11) demonstrated that hormones synthesized by the fetus and/or placenta can affect sterol synthesis rates. Estrogens, glucocorticoids, and progesterone all led to an increase in sterol synthesis rates, whereas various hormones or growth factors known to be involved in fetal growth (such as insulin) had no effect on synthesis rates.

CHOLESTEROL UPTAKE BY THE EMBRYO AND FETUS

Several lines of evidence support an exogenous source of cholesterol for the fetus and embryo. Importantly, even though fetuses and newborns with SLOS synthesize cholesterol at reduced rates (47), investigators found measurable amounts of cholesterol even in infants with the null/null genotype (69, 82). Interestingly, the severity of the SLOS phenotype was found to be affected by the apoE isoform expressed in the mothers, but not in the fathers (131), suggesting that the components in the maternal circulation that vary with apoE isoforms, such as lipoprotein concentrations or binding of lipoproteins to receptors (121, 131), could impact upon developmental processes. Napoli et al. (78) have demonstrated a direct correlation between maternal cholesterol concentrations and fetal fatty streak formation; the effects are more strongly correlated earlier in gestation. Additionally, the umbilical vein, as compared to the arteries, has greater cholesterol concentrations (112), suggesting transfer from exogenous sources to the fetus (124). Finally, some of the early studies in humans demonstrated a transfer of maternal cholesterol to the fetal circulation (reviewed in 132, 133).

To understand the possible exogenous sources of cholesterol for the embryo and fetus, one must first understand the metabolic functions and structures of tissues that nutritionally support the embryo and fetus and separate it (the embryo and fetus) from direct contact with the maternal circulation, those tissues being the yolk sac and the placenta. A brief overview of human and rodent fetal physiology is given before discussing the sources of fetal and embryonic cholesterol.

Basic Physiology of the Human Conceptus

The human zygote undergoes multiple cleavages during the first week after fertilization (reviewed in 62). In the second week, the trophoblasts that make up the outer cells of the newly formed blastocyst invade the uterine endometrium; the inner cell mass, or embryoblast, gives rise to the embryo. During this time, maternal blood and remnants of cells digested from trophoblast invasion bathe the conceptus and appear to be an excellent source of cholesterol. As development progresses, maternal blood flows into lacunae (large vacuoles)

within the uterus, forming the uteroplacental circulation and coming into direct contact with syncytiotrophoblasts. Between approximately the fourth week and the eighth week of gestation, only small amounts of maternal blood leak into the lacunae or intervillous space of the placenta, as spiral arteries are plugged early in gestation (8, 45) (**Figure 2**). The uterine glands secrete nutrients, including lipids, to the intervillous space as well (9, 38). Nutrients in the intervillous space thus contain components of maternal blood, such as cholesterol-carrying lipoproteins, and uterine gland secretions, such as lipids and presumably cholesterol. The nutrients would be taken up by syncytiotrophoblasts, exit the basolateral side, and diffuse along the stromal channels to the extracoelomic cavity. Floating within the extracoelomic cavity is the secondary yolk sac (SYC), which consists of a mesothelial layer facing outward, a mesodermal layer, and an endodermal layer facing inward (24, 50, 90). Both the endodermal and the mesothelial cell layers are covered in villi, which have extensive endocytotic absorptive properties, and take up nutrients via pinocytosis or receptor-mediated processes (134). Those nutrients can then be transported to the fetus through its network of vitelline vessels. Importantly, the SYC is thought to mediate the transport of maternally derived nutrients, possibly from the uterine gland secretions and possibly from the maternal circulation, to the embryo (24, 41, 61, 90, 106).

As development proceeds and the need for nutrition increases, a more efficient method of nutrient exchange develops. Thus, the mode of transport of maternally derived nutrition to the fetus becomes primarily hemotrophic as gestation progresses and the placenta becomes the primary route of transport (**Figure 3**). Although structurally present from about the fourth week of gestation, spiral arteries are not fully functional until about the eighth week of gestation since cytotrophoblasts appear to plug the spiral arteries until then (8, 45). Once the plugs have dispersed, the maternal blood enters the intervillous space and bathes the syncytiotrophoblasts of the chorionic villi. By this time, the extracoelemic cavity is virtually absent, the SYS has been degraded, and there is an extensive fetal vascular network at the basal membrane of the trophoblasts (49). Maternal nutrients are taken up by the syncytiotrophoblasts by receptor-mediated as well as receptor-independent processes. Once taken up, nutrients cross cells and pass through or between endothelial cells to enter the fetal circulation. Because the maternal blood within the intervillous space exchanges three to four times per minute, it is an excellent source of nutrients (i.e., cholesterol) for the developing fetus.

Basic Physiology of the Rodent Conceptus

A very popular model for human development is the rodent. The differentiation and growth of the embryos and fetuses of the rodent and the human follow the same general pattern. In rodents, the zygote implants ~5 days into gestation, which ranges from 15.5 days in length (hamster) to 21 days (rat and mouse) to 60 days (guinea pig). Most rodents, except for the guinea pig, are born quite immature compared to humans. The placentas of the rodent and the human also follow the same basic physiology. All have hemochorial placentas; i.e., maternal blood has direct contact with the trophoblasts. However, the exchange between the maternal and fetal circulations within the rodent is based on a labyrinthine matrix of blood vessels, whereas the human exchange is based on villi protruding into pools of maternal blood. Simply put, in the rodent placenta, maternal and fetal blood are circulated in vessels that are in close proximity to one another, and nutrients pass between trophoblasts separating the two vessels. In the human placenta, however, the fetal vessels are layered under a layer of syncytiotrophoblasts, which is bathed in a pool of maternal blood.

Unlike the placentas, the yolk sacs of humans and rodents are markedly different. The yolk sac in the rodent is inverted (endodermal cells face out) and is intact throughout gestation, whereas the yolk sac in the human is not

inverted (mesothelial cells face out) and is only viable in the first trimester. The rodent yolk sac is often called a secondary placenta.

Exogenous Sources of Cholesterol for the Embryo and Fetus

For maternally derived cholesterol to be taken up by the embryo and fetus, cholesterol must be taken up by trophoblasts or endodermal cells, transported across the cells, and effluxed or secreted from the basolateral side of the cells towards the fetal circulation. The transport process in the yolk sac is more defined than that in the placenta, so that tissue is discussed first.

Yolk sac. As in other tissues, the rodent yolk sac takes up maternally derived lipoprotein cholesterol through both receptor-dependent and -independent processes (133). The yolk sac expresses various lipoprotein receptors, including SR-BI, cubilin, and megalin (133). HDL appears to be taken up most readily by this tissue (134), most likely because both SR-BI and cubilin preferentially take up HDL (1, 36, 57). As demonstrated by the marked uptake of albumin as well as the presence of endocytic vesicles, extensive pinocytosis also appears to play a major role in the uptake of lipoproteins (51, 134). The exogenous cholesterol may be taken up and metabolized through the lysosomal pathway if taken up by cubilin, or it may be metabolized through intracellular esterases if taken up by SR-BI.

Entering cells is only the first step in cholesterol's movement from maternal to fetal circulations. Once in cells, the maternally derived cholesterol must have a way to exit the cells. As in the liver, the small intestine, and the heart, the rodent yolk sac synthesizes and secretes newly formed cholesterol-carrying lipoproteins from the basolateral side, or fetal-facing side, of the cell (**Figure 4**). A series of articles published in the early- to mid-1990s demonstrated that the particles secreted from rodent yolk sacs are primarily apoB-containing VLDL and LDL particles (26, 92). Regulators of lipoprotein secretion by the yolk sac are fatty acid and cholesterol availability and the ability to form apoB-containing lipoproteins (26, 68, 91, 97). Plonné et al. (91) showed that newly synthesized sterol is incorporated into the secreted lipoproteins. In very recent studies, Lichtenberg et al. (68) demonstrated that maternally derived HDL cholesterol can also be secreted from the basolateral side as lipoproteins.

What about the human condition? Because the human yolk sac cell expresses the same battery of apolipoproteins as the murine yolk sac and has the same apparent intracellular machinery to form lipoproteins (41, 61, 106), it is assumed that the human yolk sac can also synthesize and secrete lipoproteins; in fact, lipoprotein secretion by several different human yolk sac carcinoma cell lines has been demonstrated (61). Can the secretion of cholesterol be manipulated in the human, given that the yolk sac is not inverted in this species? The answer appears to be yes. The yolk sac can obtain exogenous lipids from fluid within the yolk sac cavity or within the extracoelomic cavity (52). Cholesterol and fatty acids could be present in the fluid of exocoelomic cavity from uterine gland secretions and from maternal blood that leaked around the plugged spiral arteries of the early placenta. Even if only fatty acids are present in the extracoelomic cavity and are taken up, they can drive lipoprotein formation and secretion and thereby cholesterol secretion (91). Because humans are born with abetalipoproteinemia, which is the inability to synthesize apoB-containing lipoproteins, the human yolk sac might be able to (*a*) synthesize and secrete apolipoprotein:lipid complexes since the human yolk sac does indeed synthesize apoE and apoAI (61, 106) or (*b*) transport cholesterol out of cells from an alternative route, such as efflux (see the following section) (**Figure 4**).

Placenta. Like the yolk sac, the placenta is replete with lipoprotein receptors. The lipoprotein receptors expressed in the placenta include the LDL receptor, the VLDL receptor, the class A scavenger receptor, the LDL

receptor–related protein (LRP), the apoE receptor 2, megalin, cubilin, and SR-BI (reviewed in 133). There also appears to be a component of uptake that is receptor independent (134). As with the yolk sac, the lipoprotein cholesterol can be metabolized through the lysosomal pathway (i.e., for receptors of the LDL receptor family) or by cytosolic esterases (i.e., for SR-BI).

How might the trophoblast cholesterol be secreted or effluxed from the trophoblasts? There are several mechanisms involved in the removal of cholesterol from various types of cells (**Figure 4**). In macrophages and hepatocytes, cholesterol can be effluxed to various lipid-poor acceptors through protein-mediated processes, including ABCG1, ABCA1, or SR-BI, or by way of aqueous diffusion (reviewed in 84, 137). Cholesterol can also be secreted as lipoprotein particles, as occurs in liver, intestine, heart, and yolk sac, or as apolipoprotein:lipid complexes, as occurs in macrophages (43, 46).

Several of these mechanisms have been tested in trophoblasts or placentas. First, our laboratory used a human choriocarcinoma cell line to study efflux from cells (103). We found that maternally derived LDL cholesterol does indeed exit the trophoblasts through efflux on the basolateral side to HDL and phospholipid vesicles (103). ApoAI does not appear to be an acceptor for cholesterol, indicating that ABCA1 is not involved in efflux of cholesterol even though it is expressed in trophoblasts (63). Likewise, SR-BI does not appear to be involved since this receptor is expressed on the apical side of cells, not the basolateral side where efflux occurs (123). Thus, because HDL and phospholipid vesicles are the preferred acceptors, efflux may be mediated through ABCG1 or by aqueous diffusions (56). Further support for the involvement of ABCG1 is that the amount of sterol effluxed is increased by cellular cholesterol concentration, possibly through activation of liver X receptor (LXR), which can induce ABCG1 transcription and can cause ABCG1 to redistribute to the plasma membrane (60, 126). What happens to the HDL carrying the effluxed cholesterol? Once effluxed to acceptors in the interstitial fluid or stroma, the HDL could leave the subendothelial space by transcytosis across the endothelial cells [as was shown in human umbilical vein endothelial cells by Von Eckardstein and colleagues (101)], be taken up by the endothelial cells or move through the intercellular junctions between endothelial cells (12). If taken up by the endothelium, the cholesterol would then be transported across that cell layer and effluxed or secreted into the fetal circulation.

Second, Madsen et al. (72) showed that newly synthesized apoB-containing lipoprotein particles can be secreted from placental explants, presumably (but not definitively) from the apical side. The lipoprotein particles could be taken up by the endothelial cells or could possibly move through the intercellular junctions, although pore size may be rate-limiting for transport via this route. Third, cholesterol could be secreted, not as lipoprotein particles but as apoE:cholesterol:phospholipid complexes (43, 46), as trophoblasts secrete apoE to the basolateral side of cells (99) and cholesterol is secreted in the absence of acceptors (103). Cellular cholesterol can affect both the formation of lipoprotein particles as well as secretion of apoE-containing lipid complexes as LXR is activated by cholesterol and apoE is a downstream target of LXR (103). Finally, it is tempting to speculate that cholesterol from trophoblasts can also diffuse to endothelial cells as an additional route of transport at points where vasculosyncytial membranes occur. Much remains to be done to understand how maternally derived cholesterol can enter the fetal circulation via the placenta.

Note that exogenous fetal cholesterol does not have to originate in the maternal circulation. It is possible that cholesterol synthesized by the placenta and/or yolk sac could exit cells towards the fetal circulation, just like cholesterol taken up from the maternal circulation.

Although we have some knowledge of the uptake and efflux/secretion of cholesterol by trophoblasts, we know far less about the movement of cholesterol within cells. The movement is most likely similar to the movement

of cholesterol across hepatocytes and/or enterocytes, and it most likely differs with the route by which the cholesterol is taken up and ultimately processed. For example, cholesterol taken up via receptor of the LDL receptor family or cubilin would be processed through the lysosomal pathway, thereby requiring Niemann-Pick Type C1 (NPC1) for movement (85). Other less-studied proteins that also may be involved in intracellular cholesterol transport in the placenta as in other tissues include the sterol carrier protein, Niemann-Pick C1-like 1 (NPC1L1), and ABCA2. In contrast to lysosomal processing, cholesteryl ester entering by way of SR-BI would not require NPC1, but could require cytosolic esterases and possibly other carrier proteins.

ROLES OF CHOLESTEROL IN THE EMBRYO AND FETUS

Roles of Sterol in the Embryo and Fetus

As stated above, the importance of cholesterol became apparent when Tint and coworkers (47) discovered that SLOS is the result of decreased cholesterol synthesis. As cholesterol is a part of every membrane, it could play a major role in membrane formation. In fact, cholesterol has been shown to enhance cellular proliferation (20, 27, 108). Cholesterol also maintains membrane integrity and consequently the structure and function of membrane-bound proteins (81, 102). Importantly, cholesterol is a part of lipid rafts and caveolae, or lipid microdomains (102). These domains are critical for directing the location and thereby activity of proteins into lipid-rich or -poor membrane microdomains (31). Numerous signaling processes originate in lipid microdomains (28, 110), including those related to growth (i.e., insulin signaling) (34, 87). Studies have been performed with some of the precursors of cholesterol to determine if sterol can replace cholesterol in these key functions. Huster et al. (44) showed that membrane biophysics did not change when desmosterol was present in the cell in place of, or in addition to, cholesterol. In contrast, Tulenko et al. (120) showed that cells with significant amounts of 7-DHC can form rafts, but the protein distribution within those rafts was different from that of rafts with cells containing only cholesterol. As might be expected, signaling and membrane protein activities were affected.

A few years after the biochemical defect of SLOS was discovered, Beachy and coworkers (95) discovered that a lipid moiety was covalently attached to sonic hedgehog (Shh), a protein involved in the patterning of the forebrain. Interestingly, the lipid moiety was cholesterol, and it was required for the autoproteolysis of the protein (96). Knockouts of Shh resulted in mice with cyclopia and abnormal limbs and axial skeleton (13). Three mammalian hedgehogs (Hh) are activated by cholesterol: sonic, Indian, and desert. The propagation of the Hh signal (17, 18), as well as the activation of Hh (95, 96), is cholesterol sensitive. Because the Hh play a major role in numerous essential patterning events (71), a lack of activation of Hh can have effects throughout gestation (18). Researchers have hypothesized that the affected target of the signal is Smoothened, as this protein can be found in an inactive or active state (117), possibly in response to a change in membrane composition and thus raft formation (18).

Cholesterol is also a precursor for bile acids, steroid hormones, and oxysterols. Even though there is little need to absorb significant amounts of lipids in utero, bile acids are synthesized in the fetus (105), albeit at levels lower than those in infants postpartum (40). Within the past several years, investigators have demonstrated that bile acids are key integrators of metabolism in addition to being involved in lipid absorption. They are coactivators of several nuclear receptors, including FXR, PXR, and the vitamin D receptor (73, 86, 114, 125), and they can direct effects on other signaling pathways, such as PKC (115). As might be expected, bile acids are involved in a plethora of effects concerning the maintenance of cholesterol, fatty acid, and glucose metabolism in adults (14, 64, 98). It is currently unknown how a lack of bile acids

would affect tissues that are growing rapidly, although knockouts of FXR, PXR, and the vitamin D receptor do not result in embryonic or fetal lethality (67, 107, 136). Subtle effects in knockouts have not been studied, however.

Furthermore, cholesterol is the precursor for steroid hormones, including mineralcorticoids, glucocorticoids, and sex hormones. Some of these hormones are essential for normal development of the fetus. For example, lack of estrogen, the estrogen receptors, or the androgen receptor can affect morphology of the gonads and fertility (19, 29, 100, 139). Although the lack of the mineralcorticoid receptor does not seem to impede normal development in utero (5), loss of the glucocorticoid receptor leads to neonatal mortality soon after birth due to severely retarded lung development (16). In humans, a fairly common defect in the production of adrenal steroids, due to inborn errors in adrenal steroidogenesis, can lead to congenital adrenal hyperplasia, underlying the importance of steroid hormones during development (80).

Finally, cholesterol is a precursor for oxysterols, which are activators of LXR (48, 65). As with the bile acids, LXR activation controls a multitude of processes related to lipid and glucose metabolism (reviewed in 59, 116, 118). Because LXR is expressed in the fetus (104), it is somewhat surprising that the LXRα/β knockout mouse appears to develop normally (89). However, various more subtle aspects of fetal growth have not been studied.

Roles of Cholesterol in the Extraembryonic Fetal Tissues

A brief note will be said about cholesterol in the extraembryonic fetal tissues, as changes in metabolism in the placenta and yolk sac can impact fetal development. The basic roles of cholesterol in placenta and yolk sac are similar to the roles it plays in the fetus in that cholesterol is required for membrane formation and raft integrity. Thus, because the placenta is the gateway for maternal nutrients, a change in membrane function could significantly affect the transport of a variety of compounds the fetus obtains through membrane-mediated transport, including lipids, amino acids, and glucose. Also, as mentioned in the previous section, cholesterol is the precursor for steroid hormones and oxysterols. A lack of placental steroid hormone synthesis can result in an inability to maintain pregnancy and could inhibit implantation (79). A lack of oxysterols could lead to a change in LXR activation and could thereby affect numerous basic processes involved in the maintenance of normal placental function and proliferation, as changes in LXR can affect lipid and glucose metabolism, apoptosis, and trophoblast invasion of the placenta (37, 88). Even in the absence of the LXR activation, however, mice are viable (89).

ABNORMAL STEROL SYNTHESIS IN THE EMBRYO AND FETUS

As discussed above, a lack of cholesterol synthesis has dramatic effects on development. In almost every case, congenital defects develop with reduced synthesis. Examples of defects and outcomes in mice are presented first, as embryos/fetuses of specific knockouts can be studied in utero. Various knockouts are divided into four general groupings: inhibition of sterol biosynthesis (*a*) very early in the pathway, (*b*) at the first committed intermediate to sterol biosynthesis, (*c*) between lathosterol and lanosterol, and (*d*) very late in the pathway.

Inhibition Very Early in the Sterol Biosynthetic Pathway

Inhibition of enzymes early in the sterol biosynthetic pathway leads to early embryonic lethality. ATP-citrate lyase catalyzes the formation of acetyl-CoA, a substrate for HMG-CoA synthase. Deletion of this enzyme results in embryonic lethality, with no embryos present at 8.5 dpc (4). Deletion of HMGR, which converts HMGCoA to mevalonate, results in 100% embryonic lethality (83). The lethality occurs post blastocyst formation but before implantation (83), indicating an early requirement for endogenous sterol and/or sterol biosynthetic intermediates. When SREBP-2 is deleted,

embryonic lethality occurs by 10 dpc. An interesting knockout model was recently developed wherein Insig-1 and -2 were deleted (25). As expected, sterol synthesis rates were elevated, leading to increased cholesterol concentrations. However, there was also a buildup of intermediates of the biosynthetic pathway. Even in the face of adequate cholesterol, facial malformations occurred, suggesting that the buildup of intermediates could contribute to abnormal midline facial features in individuals with inborn errors in sterol synthesis (25). Deletion of mevalonate kinase (MVK), the enzyme that converts mevalonate to phosphorylated mevalonate, is also lethal (35).

Inhibition at the First Committed Intermediate to Sterol Biosynthesis

Squalene is the first committed compound in the sterol biosynthetic pathway. Mice lacking squalene synthase are unable to synthesize squalene (119). Embryonic lethality is partial by day 9.5, whereas all embryos are resorbed by day 12.5. Embryos at 9.5 dpc have reduced growth rate and developmental immaturity, and their neural tubes fail to close.

Inhibition at Postlanosterol and Prelathosterol Steps in the Pathway

Two X-linked genes within the sterol biosynthetic pathway are also essential to development (21, 39, 70): 3β-hydroxysterol Δ^8, Δ^7-isomerase, and 3β-hydroxysterol dehydrogenase. Male hemizygotes die in utero and the heterozygous females have skin, skeletal, and eye malformations (21, 70).

Inhibition Late in the Pathway

Most fetuses with defects in sterol biosynthesis late in the pathway are viable until late in gestation or until just after birth. Pups lacking lathosterol 5-desaturase, the enzyme that catalyzes the conversion of lathosterol to 7-DHC (the second-to-last step in the sterol biosynthetic pathway), are stillborn and have craniofacial and limb-patterning defects (58). When the conversion of desmosterol to cholesterol is inhibited by knocking out Dhcr24 (3β-hydroxysterol Δ^{24}-reductase), buildup of desmosterol and reduction in cellular cholesterol ensue. Two different laboratories generated these mice and obtained different results. In 2003, Wechsler et al. (128) found that a subset of mice (32%–52%) died in utero, with the remaining surviving to adulthood. Those that survived were apparently healthy, although small in stature. In contrast, Seo and colleagues (77) generated Dhcr24$^{-/-}$ pups that died within hours of birth. The deaths were apparently the result of abnormal skin development and function; because of these deformities, the pups could not suckle. A possible difference between these two laboratories' experiments was the use of different genetic backgrounds. Pups lacking Dhcr7 (3β-hydroxysterol Δ^7-reductase), the enzyme that catalyzes the conversion of 7-DHC to cholesterol, were generated by more than one laboratory, and all had different phenotypes. All knockouts were growth retarded with neurological defects, however, and they died within the first 24 h of birth from a failure to suckle and/or hypoxia due to immature lungs (30, 122, 127). The phenotype could be partially ameliorated with nestin-driven Dhrc7 expression in brain, with survival past 24 h in 10% of the mice; however, mice still died by 17 days postpartum (140).

At least six disorders in sterol biosynthesis have been identified in humans. Possibly the biggest advance in this field occurred in 1993, when it was discovered that individuals with SLOS had low cholesterol concentrations, elevated levels of cholesterol precursors, and reduced activity of DHCR7. The other five disorders (or inborn errors), with the affected enzymes indicated in parentheses, include (*a*) mevalonic aciduria (MVK), (*b*) desmosterolosis (DHCR24), (*c*) chondrodysplasia punctata (3β-hydroxysterol Δ^8, Δ^7-isomerase), (*d*) CHILD syndrome (3β-hydroxysterol dehydrogenase), and (*e*) Greenberg dysplasia (3β-hydroxysterol Δ^{14}-reductase). Besides SLOS (DHCR7), occurrence of the other inborn errors is rare. There are several excellent,

comprehensive reviews on these disorders that describe the clinical symptoms and occurrences (55, 94), and therefore I do not discuss them here.

One might wonder why there is such a disparity of the results obtained from early versus late steps in the sterol biosynthetic pathway. Even though the endpoint of all reactions is ultimately cholesterol, early inhibition of the process results in a lack of isoprenoids as well as a lack of cholesterol. Likewise, late inhibition results in a lack of cholesterol and a buildup of different intermediates. The lack of isoprenoids occurs in reactions prior to farnesol synthesis. Isoprenoids, including geranylgeraniol and farnesol, are essential for basic cellular processes. The proteins modified by isoprenoids are numerous and varied, and include proteins of the ras, rab, and rho families; GTP-binding proteins; and G proteins (74). Farnesyl pyrophosphate is also a precursor for dolichol (74), which has been demonstrated to be essential for survival of blastocysts past implantation (76). It is understandable that embryos unable to synthesize farnesyl pyrophosphate do not develop past the implantation stage. Reactions past the formation of farnesol should not be lacking in isoprenoids, and thus defects should be due to a lack of cholesterol and/or a buildup of intermediate sterols (25). The amounts of isoprenoids in all the knockouts post squalene synthase may not be similar, depending on which intermediate is elevated. Tint and coworkers (30) have shown that HMGR activity is reduced in the Dhcr7 mice due to the ability of 7-DHC to suppress HMGR.

ABNORMAL CHOLESTEROL UPTAKE BY THE EMBRYO AND FETUS

What are the consequences of less exogenous cholesterol? Less cholesterol could be presented to the fetus due to lower maternal cholesterol concentrations, less uptake of lipoproteins, lower sterol synthesis rates in the placenta or yolk sac, less transport of sterol to the basolateral side, and/or less efflux or secretion to the fetal-facing side of trophoblasts or placental epithelial cells. This phenomenon has not been studied extensively because, until recently, it was unclear that the fetus does indeed have an exogenous source of cholesterol. In a very exciting recent study, Muenke and colleagues (23) evaluated the effect of lower maternal cholesterol concentrations on a more subtle outcome of gestation, i.e., birth weight. These researchers showed that women with lower plasma cholesterol concentrations had smaller newborns. They also demonstrated a correlation between low plasma cholesterol and microcephaly. Other studies (15, 124) have found similar results in humans, although that was not the focus of the previous studies. These studies will have far-reaching effects, as newborns with abnormal in utero growth rates, which lead to intrauterine growth–restricted infants and macrosomic infants, have an increased risk of developing age-related diseases (2, 6).

There is also an indication that less uptake of maternal lipids, and possibly cholesterol, also affects fetal growth and metabolism in the murine model. Effects thus far appear to be due to a lack of uptake by lipoprotein receptors as well as a lack of circulating maternal lipoproteins. *Lipoprotein receptors:* By comparing histology of fetuses, the total LRP2/megalin$^{-/-}$ fetus appears to be smaller than the wild-type fetus (130), suggesting a role of exogenous lipid in growth-related processes. Likewise, the embryonic lethality that occurs from a deletion of cubilin (111) is thought to be at least partly related to a lack of uptake of lipid-containing HDL. *Maternal lipoproteins:* The apoAI$^{-/-}$ mouse has very low HDL cholesterol levels. ApoAI$^{-/-}$ fetuses from crosses of apoAI$^{-/-}$ males and females are smaller than those of wild-type crosses (75), most likely due to a reduction in the amount of maternal HDL cholesterol taken up by extraembryonic fetal tissues. ApoAI$^{-/-}$ fetuses of apoAI$^{+/-}$ crosses were not smaller than their apoAI$^{+/+}$ littermates.

It seems that the effects of exogenous sterol on fetal development should be further along since a multitude of murine models exist with altered sterol metabolism. The effect of a lack of exogenous sterol on fetal development has been difficult to study in the genetically modified mouse, however, because the fetus, the placenta, and the yolk sac all have the same genotype. Thus, any changes in fetal development could be the result of a change in fetal metabolism or of a change in metabolism in the placenta or yolk sac. For example, when LRP2/megalin is deleted from placenta, yolk sac, and fetus, defective forebrain development occurs (130). Although investigators initially hypothesized that the fetal lethality was due to a lack of uptake of lipoproteins by the extraembryonic fetal tissues, more recent studies have shown that LRP2 in the fetal brain is in fact necessary for normal development (113), and perhaps the lack of lipid uptake affected only growth rates. Importantly, even though development appears normal in various murine lipoprotein receptor knockouts, a thorough study of conceptus may reveal not-yet-discovered roles of exogenous lipids in development. For example, it was thought that development of the apoAI$^{-/-}$ fetuses was normal until close examination of apoAI$^{-/-}$ fetuses of crosses of apoAI$^{-/-}$ males and females were found to be smaller than the fetuses of wild-type mice crosses (75). Researchers can begin to answer some of the current unknowns by using tissue-specific knockouts and looking for subtle effects.

SUMMARY AND PERSPECTIVE

Cholesterol is essential for development. It fulfills a number of functions ranging from being a structural entity to activating key patterning proteins to being a precursor for signaling lipids. In addition to its importance for structure, it can influence signaling because it is an integral part of all membranes and can impact lipid domain structure and protein expression. The varied roles of cholesterol in signaling and how changes can affect growth rates are unknown, however. Once the roles of cholesterol are understood, devising strategies to improve the presentation of cholesterol to the embryo or fetus could improve growth as well as other key patterning defects associated with poor outcomes. Because recent studies have shown a positive correlation between birth size and age-related diseases, including obesity, diabetes, atherosclerosis, and hypertension, understanding the roles and sources of cholesterol in the fetus can affect a significant portion of our population. These data could be used to devise strategies to enhance growth rates of small fetuses and decrease growth rates of large fetuses, thereby reducing the risk for development of age-related diseases in a large segment of the population.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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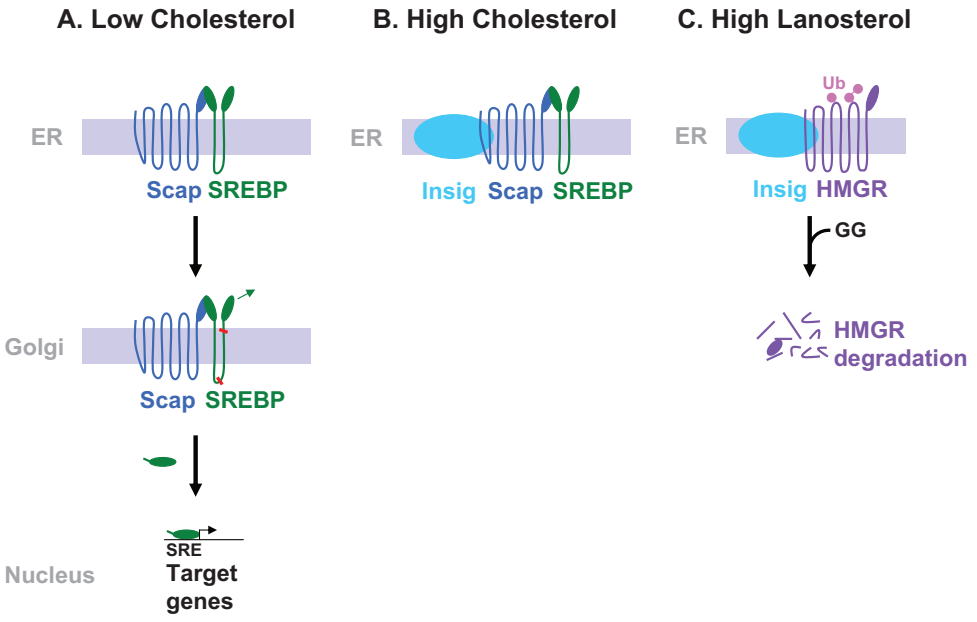

Figure 1

Processing of sterol regulatory element binding proteins (SREBPs) and hydroxymethylglutaryl-coenzyme A reductase (HMGR) regulation in the presence of low and high sterol concentrations. When cholesterol concentrations are low (*A*), Scap and SREBPs interact to form a complex, which is transported to the Golgi in vesicles that bud from the ER. In the Golgi, the SREBPs are processed to their mature forms through protease activity (as depicted by red bars). The cleaved mature SREBPs enter the nucleus and activate a number of target genes, including HMGR, by way of the sterol regulatory elements (SREs). When cholesterol levels are high (*B*), the conformation of Scap is altered, thereby allowing the protein to bind to the Insigs. When bound to the Insigs, the Scap:SREBP complex is retained in the ER, and the SREBPs are not processed to the mature forms and remain inactive. High levels of lanosterol result in the binding of HMGR to the Insigs (*C*). The Insigs are bound to another membrane protein (gp78), which is involved in the ubiquitination (Ub) of HMGR. In concert with geranylgeraniol (GG) or a GG-protein, the ubiquitinated HMGR is extracted from the ER and sent to proteosomes, where it is degraded. A more thorough description of each process can be found in an excellent review by Goldstein et al. (33).

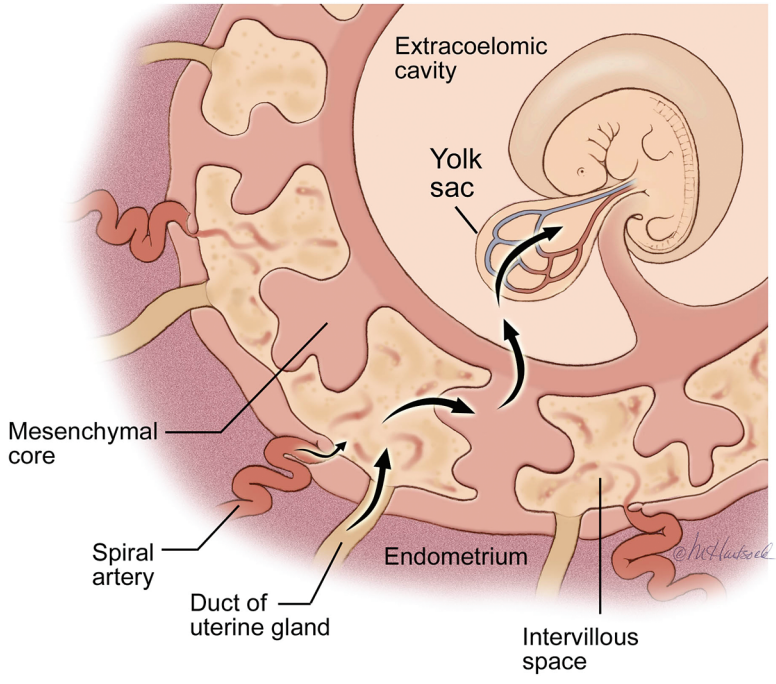

Figure 2

Early fetal/embryonic nutrition. Early in gestation, the conceptus obtains maternal nutrients from uterine gland secretions and from "'leakage" of maternal blood from spiral arteries that are plugged by cytotrophoblasts. The nutrients can pass through the cells of the mesenchymal core to the extracoelomic cavity, where they are taken up by the secondary yolk sac, incorporated into the vitelline vessels, and passed to the conceptus.

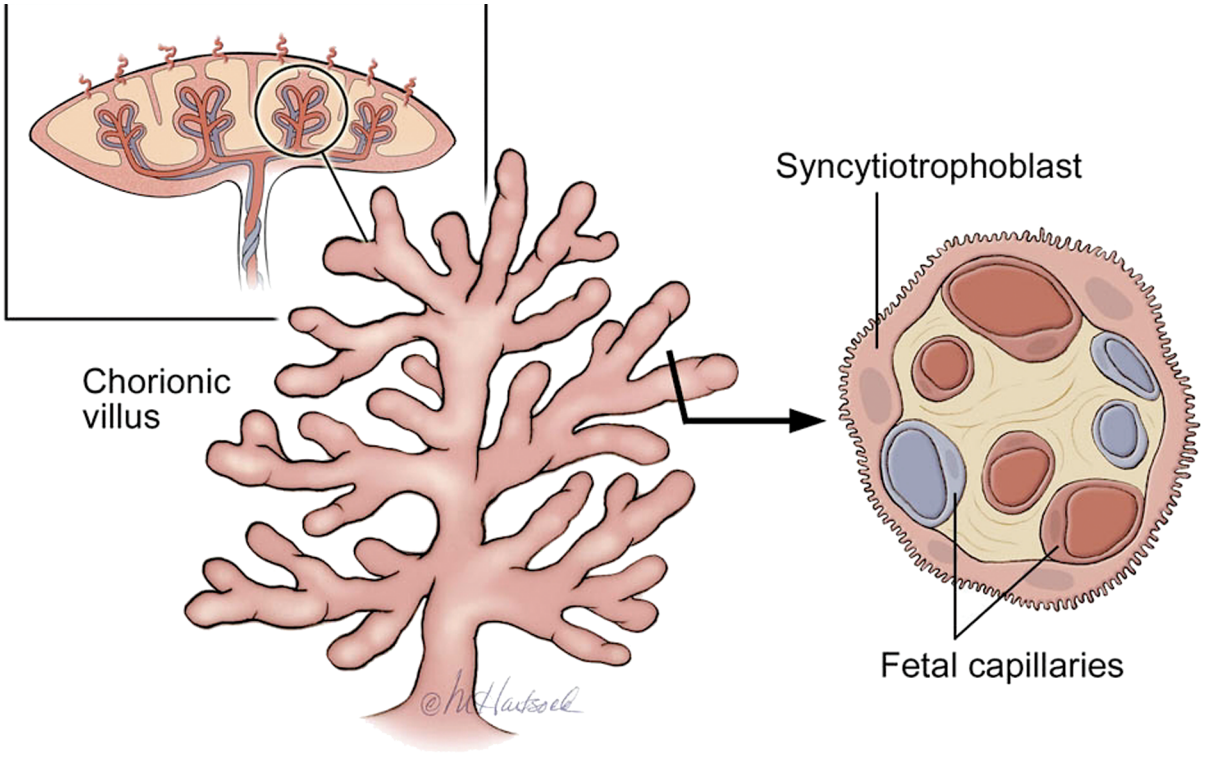

Figure 3

The chorionic villus of the placenta with the arteriocapillary-venous system within each of the cotyledons. The villus is bathed in maternal blood, which comes in contact with the syncytiotrophoblasts. Nutrients can cross these trophoblasts and enter the fetal capillaries.

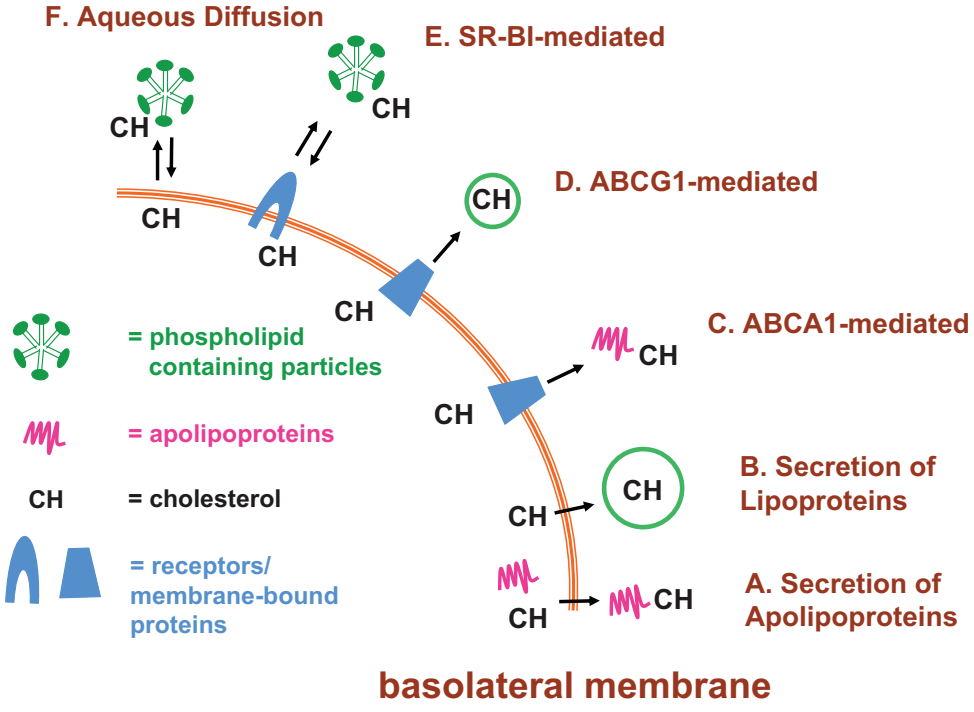

Figure 4

Secretion/efflux of cholesterol out of trophoblasts, placental endothelial cells, or visceral endodermal cells. Cholesterol can be secreted from cells complexed with apolipoprotein (*A*) or incorporated into lipoproteins as free or esterified cholesterol (*B*). Cholesterol can be effluxed by way of ABCA1 (*C*), ABCG1 (*D*), SR-BI (*E*), or by aqueous diffusion (*F*).

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Errata

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