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## FETAL AND NEONATAL STEROL METABOLISM

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### ABSTRACT

Cholesterol is critical during the development of embryos, fetuses and neonates to support their growth and development. Cholesterol is a structural component of membranes in every cell, it is involved with numerous signaling events, and it is the precursor for key steroid hormones. All individuals, either *in utero* or post-partum, have two sources of cholesterol, endogenous and exogenous. In the embryo and fetus, endogenous cholesterol comes from *de novo* synthesis and exogenous sources originate in the maternal circulation; maternal cholesterol-carrying lipoproteins are taken up from the maternal circulation by the placenta or yolk sac, processed, and transported across cells to the embryo or fetus. In the neonate, endogenous cholesterol is also synthesized *de novo* whereas exogenous cholesterol is derived from the diet. Changes in maternal metabolism (diabetes or obesity) or adverse pregnancy outcomes (preterm births or preeclampsia) could lead to altered fetal sterol metabolism. In this review, we will examine fetal and neonatal cholesterol metabolism in complicated and uncomplicated pregnancies. Early identification of neonatal cholesterol abnormalities could identify infants in need of immediate treatments, mostly due to genetic disorders, and infants that could be at long-term risk of metabolic diseases.

### SOURCES OF FETAL CHOLESTEROL

A significant amount of cholesterol is accrued during gestation. A newborn that weighs  $\approx 4.5$  kg requires  $\approx 12$  g of cholesterol with concentrations ranging from  $\approx 2.2$  mg cholesterol/g liver and peripheral tissues and  $\approx 8$  mg cholesterol/g neuronal tissues [reviewed in (1,2)]. Cholesterol is not only needed to maintain structural integrity but is also required for a variety of signaling events and as precursor of steroid hormones. Signaling that depends on cholesterol is varied, and includes the presence of cholesterol in specific regions of the membranes (lipid rafts) to allow signaling proteins to aggregate and bind specific scaffold proteins and to form endosomes (3-7), the formation of unique covalent bonds between cholesterol and Hedgehog (HH) and Smoothed (8,9), and the conversion of cholesterol to active oxysterols (10,11). The fetus has two sources of cholesterol, that synthesized *de novo* and that obtained from the maternal circulation.

### Sterol Synthesis

Sterol synthesis rates are much greater in the fetus than in the adult in several species (2,12-16), including humans (2). Rates are high enough to account for a significant proportion of the fetal cholesterol in rodents (12,17-19). Synthesis rates vary between different fetal tissues and is greatest in the liver early in

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gestation. As gestation progresses, hepatic synthesis decreases to rates similar to other tissues by late in gestation (13). While the brain has the greatest cholesterol concentration, synthesis rates are not extremely elevated as cholesterol is turned over at very low rates in the brain (1).

Sterol biosynthesis is regulated at several different steps in the biosynthetic pathway primarily by the processing of transcription factor sterol regulatory element-binding protein-2 (SREBP-2) to the mature form. When cellular cholesterol concentrations are elevated in adult tissues, sterol synthesis rates and mature SREBP-2 levels are decreased (14,20). In contrast, when cellular cholesterol concentrations are elevated in the fetus, sterol synthesis rates are suppressed only marginally and mature SREBP-2 levels do not decrease (14), suggesting constitutive processing of SREBP, and higher synthesis rates in the fetus. This same lack of regulation in fetal tissues was found when fetal hepatocytes were treated with lipoprotein-cholesterol (21) and when fetuses were exposed to polyunsaturated fatty acids *in vivo* (22). Interestingly, sterol synthesis rates can be stimulated *in vitro* by hormones synthesized by the placenta, including estrogens and progesterone, possibly to ensure that essential lipids are abundantly present (23).

### Maternally-Derived Cholesterol

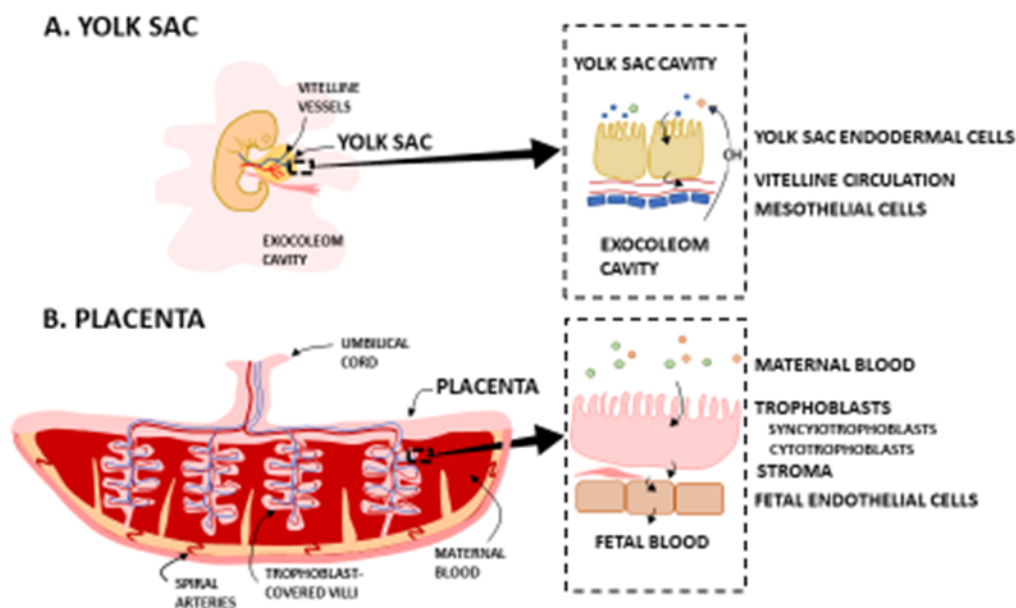
The second source of fetal cholesterol originates in the maternal circulation. Several lines of evidence support the presence of maternally-derived cholesterol in the fetal circulation. First, while there is no correlation between maternal and newborn cholesterol concentrations in term or late preterm infants, there is a direct relationship between maternal and fetal plasma cholesterol concentrations early in gestation (24). Second, correlations between maternal and fetal concentrations occur when maternal plasma

concentrations are correlated to fetal arterial and not fetal venous plasma concentrations (25). Third, fetuses of mothers with higher plasma cholesterol levels have increased intimal plaque (24). Fourth, there are significant amounts of plant sterols in the newborn circulation, 40-50% of that found in the maternal circulation (26). As these sterols are only obtained from the diet of the mother, they must cross the placental barrier. Fifth, fetuses lacking the ability to synthesize cholesterol due to a null/null mutation in one of the enzymes of the cholesterol biosynthetic pathway, such as dehydrocholesterol-7 reductase, have measurable, though low, amounts of cholesterol at birth (27,28). Finally, using a 4-vessel sampling method in pregnant women, researchers measured substantial uptake of cholesterol by the fetus with more maternal HDL-C being taken up by the fetus vs maternal LDL-C (29).

The route by which maternally-derived cholesterol is delivered to the fetus differs as the maternal-fetal interface changes during gestation (see Figure 1). Very early in gestation ( $\approx$ first 5 weeks), endocrine gland secretions containing maternally-derived cholesterol as lipid droplets bathe the blastocyst as they invade the uterine wall and are the main source of maternally-derived lipids, and overall histotrophic nutrition (30). As gestation progresses ( $\approx$ 5<sup>th</sup> to  $\approx$ 10<sup>th</sup> week of gestation), the newly formed secondary yolk sac of the embryo floats in the nutrient-rich exocoelomic cavity (31) (Fig. 1A). Nutrition at this stage is still primarily histotrophic and consists primarily of lipid-containing secretions from uterine glands and possibly some maternal lipoproteins from maternal blood which has seeped into the exocoelomic cavity (30). The human yolk sacs are not inverted, as they are in rodents, such that the highly absorptive apical side of the yolk sac faces inward (30-32), and the lipids would need to enter the yolk sac cavity via lipoprotein or endocytic receptors or other carriers (33-36). Once taken up, the cholesterol from the lipids or lipoproteins

(37) are repackaged into nascent lipoproteins (34,38,39), which are secreted into the vitelline duct artery which is integrated into the midgut of the embryo

(30,31,40). In rodents, an inability to form lipoproteins in the yolk sac is lethal (41).



**Figure 1. Scheme of the sources of cholesterol from different times of gestation. A. From about 5-10 weeks of gestation, the primary source of nutrition for the embryo/fetus is from uterine gland secretions in the form of lipid droplets (dark blue circles). There is a small amount of maternal lipoproteins (green and orange circles) that is also present in the exocoleum cavity from leakage from spiral arteries. The lipids diffuse into the yolk sac cavity and are taken up by the apical side of the yolk sac's endoderm cells via receptor- and receptor-independent mechanisms. The cholesterol is released from other components in lysosomes and repackaged into APOB-containing lipoproteins (and perhaps other lipoproteins) and secreted into the vitelline vessels which combine with the fetal circulation in the midgut. B. From 10 weeks of gestation to parturition, the fetus obtains its cholesterol from the maternal circulation in the placenta. The maternal cholesterol-carrying lipoproteins are taken up by the apical side of multi-nucleated syncytialized trophoblasts, is released from other components of the lipoproteins in lysosomes, and transported to the basolateral side of the trophoblasts. The cholesterol exits the trophoblasts to the stroma or cells within the stroma, is taken up by the fetal endothelial cells, is processed or transported to the opposite side where the cholesterol exits the cells. The routes of exit from the trophoblasts or endothelial cells are discussed in Figure 2.**

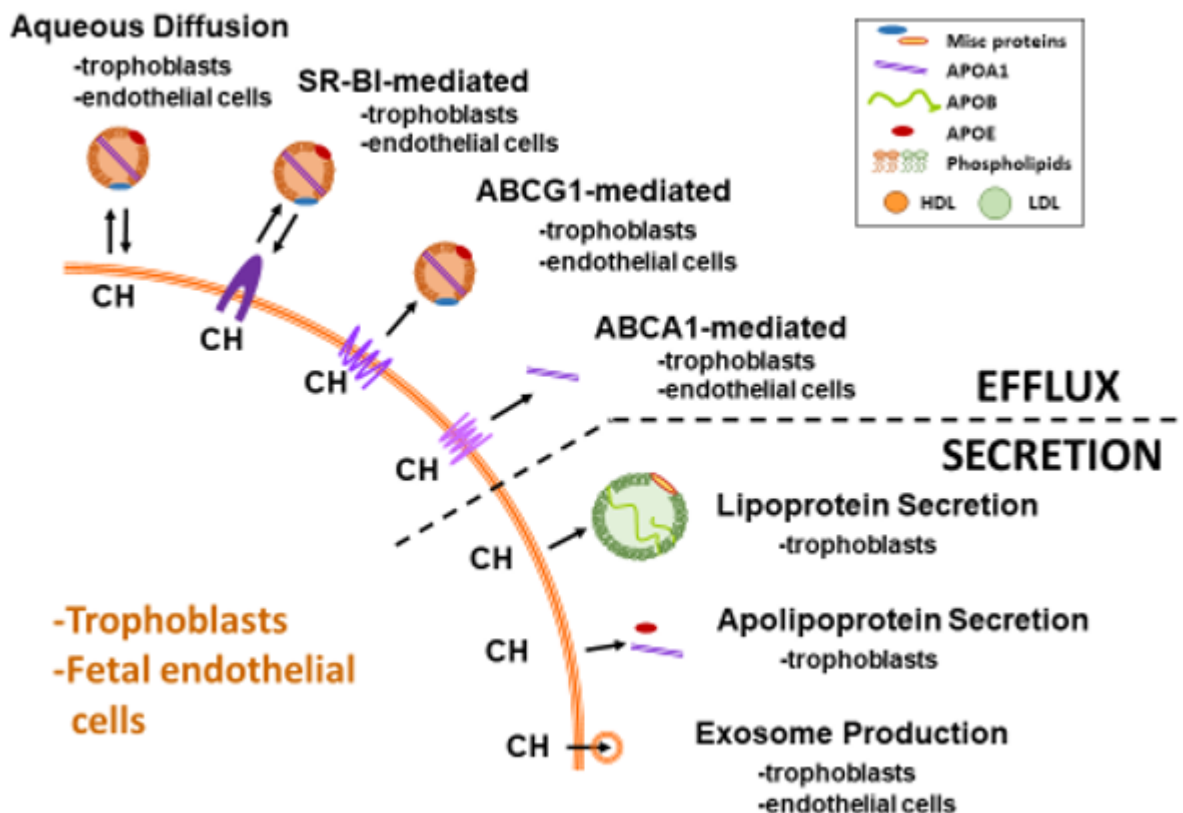
As gestation progresses to the second and third trimesters, nutrition becomes hemotrophic meaning nutrition is obtained from maternal blood. Early in gestation, the placenta does not transport nutrients as the spiral arteries that supply the placenta with maternal lipoprotein-containing blood are “plugged” by extravillous trophoblasts, blocking maternal blood from entering placental spaces that surround the trophoblasts (reviewed in (42,43)). At about 10 weeks of gestation, the “plugs” disintegrate, allowing maternal blood to enter the intervillous spaces of the placenta, directly bathing the syncytialized trophoblasts (Fig. 1B); the syncytiotrophoblasts of the placenta are polarized and take up nutrients from the maternal circulation from its apical side. The maternal blood within the intervillous space that bathes the trophoblasts exchanges 3-4 times each minute, making this an excellent source of nutrients. The maternally-derived lipoproteins are taken up via receptor-independent and receptor-dependent processes, including the LDL receptor, the VLDL receptor, the class A scavenger receptor, the LDL receptor-related protein (LRP), the APOE receptor 2, megalin, cubilin, and the scavenger receptor class B type I (SR-BI) (35,42). The sterol-containing lipoproteins are then transported to lysosomes where the sterol is released from lipoproteins via numerous lysosomal hydrolases and transported to the basolateral side of the trophoblasts by carrier and transport proteins (37). On the basolateral (fetal-facing) side of trophoblasts, the sterols exit the trophoblasts into the stroma, are subsequently taken up by and cross fetal endothelial cells, and ultimately exit these cells and enter the fetal circulation. It also is possible that the LDL or HDL are transcytosed across trophoblasts and endothelial cells as whole particles (44,45).

There are several routes by which cholesterol can exit the basolateral side of trophoblasts and fetal

endothelial cells (Figure 2), including secretion of particles and efflux of sterol to acceptors. Both processes can be regulated at several points. First, human placentas and cultured cells secrete newly synthesized APOB-containing lipoproteins (46,47) and APOA1 and APOE (48). As in other cells that secrete lipoproteins (hepatocytes and enterocytes), cellular cholesterol can drive lipoprotein-cholesterol secretion from trophoblasts (49). It is likely that other substrates would increase lipoprotein secretion from these cells. Estradiol, which is elevated during pregnancy, also increases secretion of nascent APOB-containing lipoproteins from cultured trophoblasts (47). Second, cholesterol can be effluxed from cells by either aqueous diffusion or by ATP binding cassette subfamily A member 1 (ABCA1), ABCG1, or SR-BI, all proteins which are expressed in trophoblasts (42,50). Regulation of efflux occurs at the level of cellular proteins that mediate efflux, including SR-BI, ABCA1, and ABCG1 (50-54), and by the level and type of acceptor in the circulation (55). The regulatory protein, liver X receptor (LXR), is a key mediator of ABCA1 and ABCG1 (56) (57). The levels of LXR are enhanced by oxysterols (58). Thus, changes in cellular oxysterols can enhance efflux via ABCA1/G1 (see *Adverse pregnancy outcomes*). The movement of cholesterol by SR-BI is regulated by cholesterol concentrations and phospholipids in the cells and in the accepting HDL (59). The expression of SR-BI also is regulated by a number of factors, including cellular sterol levels (60). Efflux is not only regulated by the proteins that enhance efflux, but also by the type and concentration of acceptors in endothelial spaces and circulation, with the amount of lipid-poor APOE or APOA1 and the composition of HDL being important. For example, we found that lipid-poor HDL from a fetus with the Smith-Lemli-Opitz Syndrome (SLOS) that is unable to synthesize cholesterol is a better acceptor of trophoblast cholesterol than a non-SLOS fetal HDL (61).

Likewise, when HDL was changed to a better sterol-accepting particle by the phospholipid transfer protein, efflux from endothelial cells increased (62,63). One other aspect of HDL which affects efflux is the proteome (64-66). Fetal HDL does contain more APOE than adult HDL, less cholesteryl ester transfer

protein (CETP), and equal lecithin cholesteryl acyl transferase (LCAT), a combination of proteins which support a larger particle that can efflux more cholesterol via SR-BI and ABCG1 and can't obtain cholesterol from other lipoproteins via CETP (67).



**Figure 2. Routes of exit of cholesterol from trophoblasts and fetal endothelial cells.** Cholesterol exits these cells by being effluxed out of cells to acceptors in the plasma or by being secreted. There are several routes for efflux to occur, and all proteins involved have been found in the cell types listed; aqueous diffusion to acceptors (with lipid-poor acceptors being most efficient), SR-BI mediated, ABCG1-mediated, and ABCA1-mediated. In trophoblasts, studies have shown that cells can secrete lipoproteins and apolipoproteins which will carry sterols to the circulation as they exit the cell. Finally, exosomes carry cholesterol as they exit cells.

## **FETAL LIPOPROTEIN CHOLESTEROL CONCENTRATIONS AND COMPOSITION**

The functions of lipoproteins are to transport lipids through the plasma since cholesterol, and other lipids, are lipophilic and therefore not water soluble. The two lipoproteins that carry most of the circulating cholesterol are low-density lipoprotein (LDL) and high-density lipoprotein (HDL), with lower amounts being carried as very low-density lipoproteins (VLDL). According to the National Health and Nutrition Examination Survey (NHANES), adults with an average age of  $49 \pm 18$  years have an average total cholesterol concentration of  $193 \pm 42$  mg/dl. A majority of the plasma cholesterol in adults is carried as LDL ( $115 \pm 35$  mg/dl) with HDL carrying less cholesterol ( $53 \pm 15$  mg/dl), making an average LDL-C/HDL-C ratio in adults of 2.17 (68). In contrast, plasma total cholesterol concentrations range from 51.4-96.8 mg/dl in term infants (69-80). A greater proportion of cholesterol is carried as HDL (22.1-44.9 mg/dl) versus LDL (22.0-44.9 mg/dl) in the fetus compared to the adult leading to a ratio of LDL-C/HDL-C of 0.56-1.55 in the fetus/newborn, with an average ratio of 0.99 in term infants (69,71-76,79,81).

Fetal plasma cholesterol concentrations are not constant throughout gestation, and most studies show concentrations to decrease as gestation progresses (75,82-84). The biggest decreases appear to occur with LDL-C, possibly due to increased uptake of LDL by enhanced hepatic LDL receptor activity late in gestation (85). Decreases are detected even when only term infants are compared by gestational ages, and decrease from ratios of 1.61 at 37-38 weeks of gestation to 1.27 at 41-42 weeks of gestation (83). Not all studies measure a decrease in fetal plasma cholesterol with gestational age, such as a study in Korea (86), possibly due to the location of the study

since most other studies were in resource-rich settings.

Lipoproteins (HDL, LDL, VLDL) are not comprised of just one size and type of particle, but are comprised of a spectrum of sizes (subfractions) and subspecies that carry different proteins and have different functions (64-66). This is especially true for HDL particles as over 250 distinct proteins have been associated with HDL with different combinations of protein leading to a myriad of functions (64-66,87). Not surprisingly, fetal vs adult lipoproteins differ in composition and subfraction concentrations as well as total lipoprotein-cholesterol concentrations. For example, fetal HDL particles are larger than adult HDL particles (88-90) and, small-dense LDL particles are more abundant in fetal compared to adult circulations (91). In adults, the proteins carried by HDL are involved in oxidation, inflammation, hemostasis, vitamin transport, immunity, energy balance, and lipid transport (66,92). In contrast, fetal HDL particles are enriched in proteins involved in coagulation and transport, and is lacking in proteins involved in anti-oxidative processes, such as paraoxonase I (PON1) (90). The lack of PON1 on fetal HDL suggests that these particles do not have the same anti-oxidative capacity as that found in adults (90). In addition, fetal HDL is enriched in APOE. The excess APOE could enhance the uptake of fetal HDL into fetal tissues by members of the LDL receptor family, enhance efflux of cholesterol out of endothelial cells, and affect genes related to sterol metabolism and oxidation in fetal endothelial cells (93). Unlike HDL, fetal VLDL and LDL compositions have not been studied in any detail as of yet.

## **REGULATION OF FETAL LIPOPROTEIN CHOLESTEROL CONCENTRATIONS**

In the fetus as in the adult, plasma lipoprotein-cholesterol levels are regulated by the amount of



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cholesterol entering versus that exiting the circulation. Adults in steady state have an equal amount of cholesterol entering and exiting the plasma unlike the fetus where the amount of cholesterol entering vs that exiting is not equal. The lower levels of cholesterol in fetal plasma suggests less cholesterol entering the plasma or more exiting the plasma.

### **Low-Density Lipoprotein (LDL)**

LDL-C originally enters the plasma after the liver synthesizes and secretes VLDL which is converted to LDL in the circulation. Since the liver is not functionally developed *in utero* (94,95), lipoprotein production and secretion could be low, being at least part of the cause of the low fetal LDL-C levels. The lower levels of circulating fetal cholesterol levels could also be due to an increase in uptake of lipoprotein cholesterol from the circulation by LDL receptors. Using the *in vivo* catheterized pregnant sheep model, it was found that uptake of cholesterol by tissues is greater *in utero* than in the post-partum neonatal lamb (96). This is not unexpected as fetal tissues require significant amounts of cholesterol for membrane formation and for steroid hormone synthesis (97-99).

### **High-Density Lipoprotein (HDL)**

Interestingly, HDL-C levels are relatively elevated in the fetus. Unlike VLDL and LDL, HDL is produced in the circulation and as such is not dependent upon the fetal liver for lipoprotein production. To produce HDL, cholesterol is effluxed from cells by lipid-poor APOA1 or APOE, followed by esterification of the cholesterol by lecithin cholesterol acyl transferase (LCAT), all of which are present in the fetal circulation (90).

## **ABNORMAL FETAL STEROL METABOLISM; IMPACT OF GENETIC ALTERATIONS AND ADVERSE PREGNANCY OUTCOMES**

Abnormal fetal sterol metabolism can come about by genetic alterations in the fetus and by influences of maternal factors (maternal obesity, diabetes, dyslipidemia, preeclampsia) on fetal metabolism.

### **Genetic**

Even though two sources of cholesterol exist for the fetus, a majority of fetal cholesterol is likely derived from de novo synthesis. Thus, changes in sterol synthesis could lead to unfavorable development. There are several known genetic defects in the post-squalene cholesterol biosynthetic pathway that result in altered fetal phenotypes [reviewed in (50,100-102)]; one pre-squalene defect occurs (103). Of these metabolic disorders, the most common is the SLOS. Individuals with SLOS have affected midline facial features, multiple organ and limb malformations, and intellectual disability. As sonic hedgehog (SHH) is expressed as early as 3 weeks after fertilization, and SHH is essential in a number of key developmental processes (104,105), changes in activation could have very early and significant effects. Indeed, lower SHH signaling has been associated with altered signaling that occurs in individuals with SLOS (106). Lower sterol synthesis rates in individuals with SLOS could also lead to reduced growth rates and intrauterine growth retardation (IUGR) (107). Though it was originally thought that the syndrome was due to a lack of cholesterol, the accumulation of 7-dehydrocholesterol (DHC) likely plays a role in the progression of the disease as well (108). Interestingly, a large percentage of individuals with SLOS are autistic (109) and some individuals with autism have been shown to have altered cholesterol metabolism (110) and dyslipidemia (111,112). Disruption of enzymes that occur pre-mevalonate in the sterol

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biosynthesis pathway has not been documented in live newborns, and are associated with embryonic lethality in murine deletion models [reviewed in (50)].

### **Adverse Pregnancy Outcomes**

Pregnancies complicated with diabetes, obesity, or preeclampsia often have adverse outcomes, including preterm births, altered growth rates, and in the most severe cases, stillbirths and infant mortality. The link between the altered metabolism in the pregnant females and the fetuses are often unknown but are hypothesized to be related to inflammation or oxidative stress within the placenta or fetus. Oxidative stress increases in pregnancy, and especially in pregnancies associated with diabetes, obesity, and preeclampsia (113,114). There is an increase in oxygen species during oxidative stress which are involved in the conversion of cholesterol to oxysterols (115,116).

### **Placenta and Fetal Endothelial Cells**

Oxysterols are detrimental during development as these cholesterol derivatives affect a number of signaling pathways including activation of the liver X receptor (LXR) (117) and inhibition of hedgehog signaling (118). The oxysterol-induced increase in LXR activation enhances cholesterol and oxysterol efflux from these cell types. Indeed, fetal endothelial cells of women with gestational diabetes had increases in LXR target genes, ABCA1 and ABCG1, and increased cholesterol efflux (119). Likewise, HDL-mediated cholesterol efflux and placental 27-hydroxycholesterol were increased in women with preeclampsia (120).

Studies have shown reduced HDL-C and elevated LDL-C levels in preeclamptic infants (121) and increased oxidative modifications of LDL and HDL associated with decreased PON1 activity (122). Changes in the subfraction concentrations have been

detected, as well, and infants of women with type I diabetes had greater HDL<sub>2</sub>-C and -phospholipid concentrations vs women without diabetes (123).

These changes in sterol metabolism in the placenta, endothelial cells and fetus can lead to a variety of outcomes ranging from beneficial to adverse. First, an increase in efflux due to increased placental oxysterol levels would increase fetal cholesterol and oxysterol levels, which could be deleterious to the fetus as oxysterols can inhibit hedgehog signaling (118). In contrast, this same increase in efflux from cells with increased oxysterol could reduce the sterol concentrations in the placenta, possibly protecting the cells from a build-up of oxysterols, which could be improve placental metabolism.

### **NEONATAL LIPOPROTEIN CHOLESTEROL CONCENTRATIONS AND CHOLESTEROL METABOLISM**

The three major sources of nutrition in the United States during neonatal and early infancy are human milk, cow milk-based formulas, and soy milk-based formulas. The composition of these types of diet differs in several factors that may theoretically influence cholesterol homeostasis including cholesterol content, polyunsaturated/saturated fatty acid ratio (P/S ratio), protein composition, phytoestrogen content, and the presence of hormones specific to breast milk. More recent components of milk which also can affect metabolism include miRNAs, prebiotics, and extracellular vesicles (124-126).

As with the fetus, neonates are in a rapid growth phase and require significant cholesterol for growth, energy, and normal cellular function. Infants fed human milk receive much greater quantities of cholesterol than those fed commercial formulas. Human milk contains between 10-15 mg/dl of cholesterol, cow milk-based formulas contain 1-4 mg/dl of cholesterol, and soy -



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based formulas contain no cholesterol. The soy-based formulas contain phytosterols (plant sterols), which actually inhibit cholesterol absorption (127). Not unexpectedly, breast-fed infants have higher serum cholesterol concentrations compared to formula-fed infants (128,129). In contrast to the fetus which does not suppress sterol synthesis rates, rapidly-growing neonates do suppress sterol synthesis (130-132).

### **LONG-TERM IMPACT OF ALTERED FETAL AND NEONATAL STEROL METABOLISM**

In the early 1990s, Dr. David Barker discovered that persons growing up in less affluent areas of England and Wales were at an increased risk for infant mortality and long-term ischemic heart disease compared to persons in more affluent areas (133). Specifically, the adverse long-term consequences of heart disease were related to low birthweights. This relationship was confirmed by others [reviewed in (134,135)], and was expanded to also include infants who are born large for gestational age (LGA), forming a U-shaped curve. Because of his early seminal work in this area, the “programming” of metabolism by early life environment has been coined the “Barker hypothesis” or DOHaD (Developmental Origins of Health and Disease). The importance of early nutrition has been labeled “1000 days” as that is the time from conception to the second birthday when much growth and development (and programming) (136).

It is difficult to identify any long-term effects that are specific to cholesterol as changes in cholesterol levels in the newborn or *in utero* are often associated with the oxidative stress which accompanies the adverse outcomes it is associated with. For example, some studies have shown infants of preeclamptic mothers or preterm infants to be at an increased risk of heart disease later in life (137-143). The long-term changes in metabolism that lead to programming in adulthood are likely epigenetic changes in genes controlling

metabolism [reviewed in (144)]. Indeed, the greatest epigenetic activity occurs in the first 1000 days of life (145). There are some recent treatments that are directed at changing the epigenome postnatally (not for newborns or neonates), including statins which are proposed to modify histones and various dietary regimes which can affect methylation status (146), and prenatally, including anti-oxidant compounds to reverse programming (147).

It is not only the *in utero* environment which has the potential to lead to programming of metabolic disease or heart disease, but also the type of diet fed to the newborn (148). Since breast milk and formulas vary more than just in their cholesterol content, it is almost impossible to determine if early life cholesterol due to consumption of breast milk vs soy-based formulas affects age-related development of heart disease due to so many other non-sterol-based factors. However, if one were to focus solely on cholesterol, one hypothesis would be that Infants fed cholesterol-containing human milk could be protective. Support for this is that adult men and women who were breast-fed in infancy had lower serum cholesterol concentrations (149) or higher HDL-C levels (150) compared to adults who were not fed breastfed; BMI was also lower in adults that had been breast-fed (150). Likewise, plasma total cholesterol was significantly higher in adult males that were breast fed for the shortest period of time when compared to those who were breast fed for longer times (151). In contrast, plasma cholesterol concentrations in children and baboons fed either breast milk or formula had either no difference in plasma cholesterol levels or lower plasma cholesterol levels after being fed formula (152). A review of the literature suggests that the differences in studies were due to studies using exclusive breastmilk versus those using both breast milk and formula (153), plus other outcomes could be important, including intellect and BMI (154). Future studies are needed to better characterize the long-

term effects of early cholesterol exposure on cholesterol metabolism in later childhood and adulthood.

**CLINICAL SIGNIFICANCE OF FTAL AND NEWBORN PLASMA CHOLESTEROL CONCENTRATIONS**

There are a few definitive clinical identifications that can be diagnosed with early life plasma cholesterol concentrations. As discussed earlier, plasma cholesterol levels are lower in the newborn and quite variable, making it difficult to identify infants at risk of becoming hypercholesterolemic, even newborns that are heterozygous for familial hypercholesterolemia (155). By one year of age, however, plasma levels are more stable and hypercholesterolemia becomes more obvious (155), taking into account the consumption of cholesterol-containing breastmilk at that time. This might be especially important in infants of women with

altered metabolic conditions associated with oxidative stress. The problem is that these cholesterol levels are either not routinely measured or not reported.

The plasma sterol concentrations, however, can be used to define various genetic disorders. As discussed previously, there are known defects in the post-squalene cholesterol biosynthetic pathway that result in altered fetal phenotypes [reviewed in (50,100-102)], each with unique sterol compositions depending on where the defect in the sterol biosynthetic pathway occurs. Thus, these syndromes can be identified by assaying for the specific sterols (see Table 1). This is especially useful in the milder phenotypes that might be missed, especially SLOS due to its mild form and higher prevalence. Assays for these dehydrocholesterols must be done by gas chromatography to measure non-cholesterol sterol levels, not the commonly used enzymatic assay which measures sterol levels and not type.

Table 1. Disorders of Cholesterol Biosynthesis*				
Disease	Inheritance	Gene Defect	Laboratory Findings	Phenotype
Smith-Lemli-Optiz syndrome	AR	7-dehydrocholesterol reductase gene (DHCR7)	Elevated 7-dehydrocholesterol (DHC) and 8-DHC levels	Characteristic craniofacial appearance (i.e., ptosis, small upturned nose, and micrognathia, cleft palate); microcephaly; limb anomalies (proximally placed thumbs, polydactyly, and 2–3 toe syndactyly); slow growth and poor weight gain; potential cardiac and gastrointestinal anomalies and

				intellectual disability; severity depends on mutation (null to leaky)
Sterol-4-demethylase complex	AR	SC4MOL gene defect	Elevated 4,4'-demethyl- & 4-monomethyl-sterols	Microcephaly; cataracts; slow grow; dermatitis (scaling, erythroderma)
Desmosterolosis	AR	DHCR24 Very rare with only a few patients reported.	Elevated desmosterol	Craniofacial (dysmorphic features, i.e., micro/macrocephaly, cleft palate), ambiguous genitalia; short limbs and osteosclerosis; slow growth
Lathosterolosis	AR	Lathosterol 5-desaturase (SC5D) Very rare with only a few patients reported.	Elevated lathosterol	SLOS like phenotype; craniofacial (subtle dysmorphic features, i.e., microcephaly, upturned nose); micrognathia; ptosis; cataracts; polysyndactyly or syndactyly; hypospadias
Chondrodysplasia punctata (Conradi-Hünermann syndrome; CDPX2)	X linked	Emopamil binding protein (EBP)	Elevated 8-DHC and 8(9)-cholestanol	Lethal in males Females: craniofacial (asymmetric dysmorphic features); skin (generalized congenital ichthyosis on erythrothematos base), skeletal (stippling, rhizomelic limb shortening, scoliosis); ocular (cataracts); occasional malformations (cleft palate, hearing loss)

Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD syndrome)	X linked	NADH steroid dehydrogenase-like (NSDHL) or EBP	Elevated 4-dimethyl, 4,4-dimethyl, and 4-carboxysterol intermediates (i.e., 4,4-dimethylcholesta-8, 24 dien-3 $\beta$ -ol)	Similar defects to CDPX2 but unilateral defects and no cataracts; lethal in males. Females: striking unilateral distribution of anomalies. Generalized congenital ichthyosiform erythroderma and limb deformities (right > left). Internal malformations including CNS, renal and cardiac.
Hydrops-ectopic calcification-“moth eaten” skeletal dysplasia (HEM skeletal dysplasia, Greenberg dysplasia)	AR or AD	Lamin B receptor (LBR) with DHCR14 defect	Elevated 8(9), 14-dien- 3 $\beta$ -ol and cholesta-8(9),14,24-ien-3 $\beta$ -ol	Dysmorphic facial features, hydrops fetalis, cystic hydroma, lung abnormalities, severe short-limbed dwarfism with markedly disorganized cartilaginous and bony architecture (Moth eaten appearance of long bones)
Antley-Bixler syndrome	AR	CYP51A1-associated P450 cytochrome oxidoreductase (POR) gene	elevated levels of lanosterol and dihydrolanosterol	Craniosynostosis; choanal atresia; limb abnormalities (i.e., radio humeral synostosis, and femoral bowing); ambiguous genitalia

\*The disorders listed are post-squalene. There is one defect in the pre-squalene pathway for cholesterol biosynthesis, Mevalonic Aciduria.

Though cholesterol levels are not often measured until 9-11 years of age per current recommendations for universal lipid screening per the American Academy of Pediatrics, earlier screening is appropriate if there is a

strong family history of high cholesterol or early cardiovascular events. It should be noted infants associated with pregnancies complicated with adverse outcomes are at a higher risk to develop heart disease

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later in life (137-143,156-159). Knowing this, interventions directed at improving cardiovascular risk, including maintaining a normal BMI, ideal blood pressure, ideal LDL-C etc., could be started earlier to prevent diseases from developing.

## SUMMARY

Cholesterol is essential for normal growth and development from the blastocyst through infancy. The cholesterol originates from an endogenous source (de novo synthesis) and an exogenous source (maternal lipoproteins and diet). Due to its critical role in development, sterol synthesis rates are regulated less in the fetus than neonates. If synthesis is reduced, possibly due to a genetic defect in the sterol biosynthetic pathway, abnormal development can occur. Fetal cholesterol levels can be altered,

including oxysterols, in pregnant women with various metabolic disorders, mostly those linked to oxidative stress. The consequences of these changes are unknown because even though these infants are at an increased risk to develop age-related diseases later in life (130-136, 149-152), it is not known if the long-term effects are mediated by an early exposure to cholesterol/oxysterol or other factors associated with oxidative stress. Regardless, offspring of mothers with hypercholesterolemia, preeclampsia, diabetes, obesity, or are born preterm should be monitored for future cardiovascular disease.

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