Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation

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ABSTRACT
Cholesterol is required for fetal development. Data obtained from recent studies in humans, rodents, and cell cultures showed that circulating maternal cholesterol can affect fetal metabolism and sterol accretion. Recent studies in our laboratory showed that the efflux of cholesterol from the basolateral side of the placental cells and the secretion of cholesterol from endodermal yolk sac cells to the fetal circulation can be regulated. The ability to manipulate the mass of maternal cholesterol that crosses to the fetus could result in a dramatic improvement in the development of fetuses that lack the ability to synthesize cholesterol, such as those with Smith-Lemli-Opitz syndrome. On the other hand, it could also accelerate the development of various age-related diseases, such as atherosclerosis.

INTRODUCTION
Cholesterol is an essential component of development. It is an integral part of cell membranes and consequently of various membrane microdomains, including lipid rafts. Cholesterol within membranes will affect the content of other lipids within membranes, specifically sphingomyelin (1–3), which could make cholesterol a fundamental mediator of metabolism through the propagation of signaling cascades (4–6). Cholesterol is also the precursor of steroid hormones such as progesterone and of metabolic mediators such as oysterol. Finally, cholesterol is essential to both the activation and propagation of hedgehog signaling; sonic hedgehog (SHH) is responsible for patterning and development of the central nervous system (7–9).

Within any given tissue, cholesterol originates from either de novo synthesis or exogenous sources. The fetal sterol synthesis rates are greater than those in other extrahepatic tissues, most likely because of the large requirement for cholesterol during this time of rapid growth (10–14). For example, an average newborn human weighs 3.2 kg. Assuming that each gram of tissue except the brain has ~1.5 mg cholesterol, the accrual of ~4.8 g cholesterol would be needed during the 9-mo growth period (15). When one includes the brain, which weighs 0.4 kg (15) and has a cholesterol concentration averaging ~7 mg/g at birth (16), the amount of cholesterol required increases to ~8 g.

An absolute requirement for fetal cholesterol was established when it was discovered that persons in whom de novo cholesterol synthesis is absent have a host of congenital birth defects (17, 18). There are 7 known defects in the cholesterol biosynthetic pathway, all of which lead to congenital malformations. Six of the defects are extremely rare and often are lethal. The seventh, and most common, defect leads to the Smith-Lemli-Opitz (SLO) syndrome, also referred to as the RSH syndrome. The defect occurs in the last step of the pathway, in which 7-dehydrocholesterol is converted to cholesterol; the enzyme that catalyzes this reaction is Δ7-7-dehydrocholesterol reductase (Δ7 reductase) (19, 20). It is interesting that, even though fetuses with null mutations in Δ7 reductase do not synthesize cholesterol, they are nonetheless born with cholesterol (albeit in very low amounts) in tissues and blood (21–23), and therefore they must have an exogenous source of cholesterol in utero. The fact that maternal plasma cholesterol concentrations are directly correlated with fetal cholesterol concentrations in rodents

1 From the University of Cincinnati Medical School, Cincinnati, OH.
2 Presented at the ASCN 44th annual meeting, Washington, DC, April 18, 2004.
3 Presentation of the Norman Kretchmer Memorial Award in Nutrition and Development supported in part by Ross Products Division, Abbott Laboratories.
4 Supported by grant no. HD34089 from the National Institutes of Health.
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Received March 23, 2005.
Accepted for publication June 21, 2005.

(24) supports the possibility that maternal plasma cholesterol concentrations are able to cross the placenta in humans. In addition, the extent of fatty streaks in the fetal aorta is correlated with maternal cholesterol concentrations in midgestation in humans (25).

The ability of the fetus to obtain maternal-derived exogenous cholesterol is complex because the maternal and fetal circulations do not come in direct contact with one another. Two tissues—the placenta and the yolk sac—separate these 2 circulations. In the human, the yolk sac develops just after implantation (day 10). By the end of the fourth week, the placenta is intact and active in that maternal-fetal exchanges occur. As gestation progresses, the yolk sac begins to regress and is essentially nonfunctional by the eighth week of gestation (26). In the rodent, although the time sequence of tissue development of the placenta and yolk sac parallels that in the human, the murine yolk sac is inverted and remains intact throughout gestation (27).

**PLACENTA**

The placenta is composed of numerous different cell types, including trophoblasts, fetal capillaries, maternal blood space that contains macrophages and red blood cells, and stroma. The actual physical barrier between the maternal and fetal circulations is made up of a layer of syncytiotrophoblasts, which are multinucleated trophoblasts. There is 1 layer of syncytialized trophoblasts in the human placenta, and there are 3 layers in the rodent.

For maternally derived cholesterol to enter the fetal circulation, cholesterol must be taken up on the maternal, or apical, side of the trophoblasts. Cholesterol is indeed taken up by the placenta and trophoblasts in the form of lipoproteins through receptor-mediated as well as receptor-independent transport (28, 29). The lipoprotein receptors expressed in the placenta or trophoblasts (or both) include the LDL, VLDL, and class A scavenger receptors; the LDL receptor–related protein (LRP); the apolipoprotein (apo) E receptor 2; megalin; cubilin; and the scavenger receptor class B type I (SR-BI) (28–37). The apo B, E receptors, those that take up VLDL and LDL, are located in clathrin-coated pits; as such, the particles are directed to the lysosomes where the cholesterol esters are hydrolyzed (38). SR-BI selectively takes up cholesteryl esters, primarily from HDL, which are hydrolyzed by neutral or acidic cholesterol esterases (39, 40). Cubilin, which also takes up HDL, acts in concert with megalin, and thus the particles can be directed to lysosomes (37). However, some ester may be hydrolyzed by other cellular enzymes (41). The maternally derived cholesterol is then available for other metabolic processes, including efflux or secretion.

Studies performed in placental explants, trophoblast cell cultures, and cultures of other polarized cells suggest that there are 3 routes by which cholesterol can exit the fetal, or basolateral, side of trophoblasts. First, the human placenta was recently shown to secrete newly formed apo B–containing lipoproteins (Figure 1, pathway A) (29). Whereas one would presume that the particles are secreted from the basolateral side and into the fetal circulation, that has not yet been shown. Second, trophoblasts secrete apo E and apo A-I from the basolateral side of the cell (Figure 1, pathway B) (43, 44), possibly complexed with cholesterol and other lipids (43, 45). Third, assuming that trophoblasts are similar to other polarized cells, trophoblasts will efflux cholesterol from cells.

Efflux occurs via 3 primary routes (46, 47). First, cholesterol can proceed down a concentration gradient to phospholipid discs or HDL via a protein-independent process (Figure 1, pathway C). Second, SR-BI can mediate the efflux of cholesterol down a concentration gradient to phospholipid discs or HDL (Figure 1, pathway D). Third, cholesterol can be effluxed to lipid-poor apolipoproteins via apolipoprotein or ATP-binding cassette transporter A1 (ABC1A) (Figure 1, pathway E). All processes theoretically could occur, because SR-BI and ABCAI are present in the placenta (48–50) and because aqueous or passive diffusion occurs in all cells (47). Because one of the goals of our laboratory is to identify the sources of the fetus’s cholesterol, we examined the mechanism of efflux of cholesterol from trophoblasts.

Cholesterol efflux was studied by using a polarized trophoblast cell line, BeWo cells. BeWo cells (subclass 30) are polarized when grown on a filter, and they transport molecules unilaterally, synthesize hormones, and express trophoblast proteins (29). In our laboratory, cells were grown to confluency and incubated with radiolabeled free cholesterol and radiolabeled cholesteryl ester as LDL (29). After 24 h, cells were washed, and acceptor was added to the bottom chamber of the filters. To initially establish the ability of trophoblasts to efflux cholesterol that originated from the apical (maternal) side from the basolateral (fetal) side, we used the most physiologic acceptor available, fetal human serum (FHS). Indeed, cholesterol that was added to the apical side of the cells was effluxed to FHS on the basolateral side (Figure 2). The amount of cholesterol effluxed to the basolateral side increased with acceptor concentration. This was the first study to show that cholesterol added to the apical side of the cells could exit the basolateral side of the cells (29).

We then incubated cells with maximal amounts of different acceptors that would distinguish the aqueous- or SR-BI-mediated transport from the ABCAI-mediated transport, those being phospholipid discs and lipid-free apo A-I, respectively (29) (Figure 3). Our group initially showed that some cholesterol was effluxed or secreted to serum-free media (SFM). It is interesting that more cholesterol was effluxed to phospholipid discs than was effluxed or secreted to SFM only. In contrast, a similar amount of cholesterol was effluxed or secreted to apo A-I and SFM. The amount of cholesterol effluxed or secreted to the media
and acceptors increased markedly when the cellular cholesterol concentration was elevated (Figure 4). We incubated cells with various metabolites known to increase ABCA1 in other cells, such as oxysterols and cAMP, and found no increase in efflux or secretion to apo A-I (29).

Thus, these data show that maternally derived cholesterol can be effluxed from the basolateral membrane by aqueous diffusion or SR-BI, assuming SR-BI is expressed in the basolateral membrane. Cholesterol also may be secreted from the basolateral side via a currently undefined process. The maternally derived sterol would most likely come from apo E- or apo B-containing lipoproteins (or both), because the receptors for these lipoproteins are most abundant in the placenta. Equally as important, efflux or secretion (or both) can be manipulated by changing cellular cholesterol concentrations. Although we cannot rule out the role of ABCA1 in the efflux of sterol from trophoblasts, it does not appear to trigger efflux from the basolateral side, at least in this cell model. These results were quite surprising because the placenta has one of the highest concentrations of ABCA1 mRNA of any tissue (50).

Assuming that the amount of cholesterol secreted or effluxed in vitro parallels that secreted or effluxed in vivo, a significant amount of cholesterol could be effluxed or secreted from the placenta to the fetus (51). For example, 5% of cellular cholesterol was effluxed or secreted to phospholipid discs in 24 h (29). Between 10 and 12 wk of gestation, ~11 g placenta is composed of placental villi, of which 30% (5.4 g) are trophoblasts (52). The knowledge that the human placenta has a cholesterol concentration of 2.5 mg/g (L Woollett, unpublished observations, 2004) would suggest that a maximum of 0.68 mg cholesterol/d could be effluxed or secreted; this would total 9.5 mg cholesterol between 10 and 12 wk of gestation. Because the fetus grows 31 g during this same period (53), it accrues a minimum of 46.5 mg cholesterol (assuming 1.5 mg cholesterol/g tissue; 54). Thus, up to ~20% of the sterol accrued by the embryo or fetus in the first trimester could be derived from maternally derived placenta cholesterol, and a possibly greater percentage could be derived from placentas with higher cholesterol concentrations.

**YOLK SAC**

The yolk sac differs from the placenta in that a definitive route of secretion of intracellular cholesterol to the fetal circulation has been known for >10 y. In the early to middle 1990s, a series of studies with yolk sac explants showed that rodent yolk sacs secreted lipoprotein particles, primarily apo B–containing particles including both VLDL and LDL (55–57). Although these particles were shown to contain newly synthesized cholesterol, the presence of maternally derived cholesterol in the nascent particles was not verified. The nascent particles were localized to the rough endoplasmic reticulum and secretory vesicles of the Golgi and were even visualized in the vitelline vessels (58); the vitelline arteries and veins make up the vasculature of the yolk sac and fuse with the fetal vessels within the embryo or fetus. In mice that are unable to synthesize apo B or microsomal triacylglycerol transfer protein (MTP), the yolk sac is devoid of these
particles and the fetuses are resorbed early in gestation, or they have various neurologic disorders including exencephalus or hydrocephalus (or both) (58–62).

The question (similar to that raised for the placenta) remains: how would cholesterol that is present in the maternal circulation be transported across the yolk sac and become incorporated into fetal tissues? First, cholesterol has to be taken up from the maternal circulation. The yolk sac expresses lipoprotein receptors for apo B,E-containing particles as well as HDL; receptors include megalin (36, 63), cubulin (64), and SR-BI (28, 30). In addition, the uptake of particles via receptor-independent processes is quite active in this tissue (27, 28). The lipoprotein cholesterol then has to be hydrolyzed to free cholesterol, packaged into new lipoproteins, and secreted into the vitelline vessels (Figure 1A). Results obtained from other lipoprotein-secreting cells, such as enterocytes and hepatocytes, make it seem likely that maternally derived cholesterol can be packaged into nascent lipoproteins and secreted from visceral endodermal cells of yolk sacs. Data from our laboratory showed that the yolk sac can indeed secrete maternally derived cholesterol, and the amount can be manipulated by changing the cholesterol concentrations in the yolk sac (24, 65).

CONCLUSIONS

Because it appears that maternal cholesterol can indeed be transported from the maternal to the fetal circulation, fetal cholesterol concentrations can theoretically change in parallel with the quantity of both endogenous and exogenous sources of sterol. Typically, fetuses have a normally high rate of sterol synthesis as compared with other extrahepatic tissues (endogenous source), regardless of maternal lipoprotein-cholesterol concentrations (exogenous source). Atypically, fetuses can have low sterol synthesis rates, as occurs with SLO syndrome, in combination with low, normal, or high maternal lipoprotein-cholesterol concentrations. Because no one has observed atypically elevated, or nonphysiologic, fetal sterol synthesis rates, this condition is not discussed here.

Normal fetal sterol synthesis rates with low, normal, or high maternal lipoprotein-cholesterol concentrations

There are several conditions, or disease states, that can lead to either low or high circulating cholesterol concentrations in the pregnant female. With respect to low plasma concentrations, women with abetalipoproteinemia or hypobetalipoproteinemia and those consuming low-cholesterol diets have extremely low sterol concentrations (endogenous source). Atypically, fetuses can have low sterol synthesis rates, as occurs with SLO syndrome, in combination with low, normal, or high maternal lipoprotein-cholesterol concentrations. Because no one has observed atypically elevated, or nonphysiologic, fetal sterol synthesis rates, this condition is not discussed here.

Low fetal sterol synthesis rates with low, normal, or high maternal lipoprotein concentrations

A change in the amount of cholesterol that crosses the placenta or the yolk sac (or both) should have the biggest effect on the development of the embryo or fetus with SLO syndrome. First, the severity of the syndrome can be correlated to maternal plasma cholesterol concentrations in most studies (21–23). Second, persons with an apo E2 allele have a more severe SLO syndrome phenotype than do those without the apo E2 allele (74); apo E2 binds defectively to the LDL receptor (75), which is abundant in the placenta (28, 76). These data suggest a critical role for the apo E–containing maternal lipoproteins in the development of fetuses with limited endogenous sources of sterol.

The number of persons who could benefit from an increase in transport of exogenous cholesterol could be significant because, even though the prevalence of SLO syndrome was shown to be 1 in 16 000–60 000 live births, depending on the geographical location (17, 77, 78), more recent studies suggested that the incidence could be much greater because the genetic prevalence of the gene is 1:30 (79). Thus, there could be from 1 in 1590 to 1 in 16 000–60 000 live births, depending on the geographical location (17, 77, 78), more recent studies suggested that the incidence could be much greater because the genetic prevalence of the gene is 1:30 (79). Thus, there could be from 1 in 1590 to 1 in 13 500 fertilized eggs with this syndrome (79).

The benefits of additional maternally derived cholesterol could vary, depending on when the extra sterol was presented to the fetus. The most dramatic benefits would occur if the exogenous cholesterol supply increased early in gestation, such as during the first trimester, because cholesterol does not cross the blood-brain barrier, at least not in the healthy person (80). The blood-brain barrier has formed by the end of the first trimester in humans (81). Additional cholesterol could affect SHH activity because cholesterol is required for the activation and propagation of the SHH signal (7, 8). SHH is first detected early in gestation when the notocord begins to form (=day 17 of gestation in humans) (82). SHH has been found to have multiple roles early in the developmental process, including roles in patterning of the
central nervous system and axon outgrowth (9); as such, even a small increase in cholesterol at these early time points could vastly improve the SLO syndrome phenotype. Second, the presentation of cholesterol before the formation of the blood-brain barrier could aid in the integrity and function of neuronal cells. Benefits of an increase in exogenous cholesterol could still occur in the second and third trimesters, however, because feeding cholesterol to children with SLO syndrome improves their functions and growth rates (83, 84). Improvements during this stage of gestation would include enhancement of basic metabolism and function due to normalized membrane integrity and cell signaling (8, 85). Study of the mutant mouse models that lack various enzymes in the cholesterol biosynthetic pathway will help delineate the role of endogenous and exogenous cholesterol in fetal metabolism and development (86–93).

**Overall summary**

It appears that the ability of maternal cholesterol to cross the placenta has both positive and negative consequences. The ability to increase the exogenous source of sterol would be beneficial for the SLO syndrome fetus that synthesizes sterol at low to null rates. In contrast, in the fetus that can synthesize sterol, although an increase in the exogenous source of sterol may or may not be detrimental to fetal development, long-term health status is likely to be affected. The effects of hypcholesterolemia and hypercholesterolemia on the metabolic process of tissues that support the fetus, the placenta, and the yolk sac have yet to be established, and those effects could markedly influence the outcome of pregnancy and long-term health issues.

I thank the staff in my laboratory during the time of these studies: Julie McConihay, Amy Honkomp, Lihang Yao, M Hayden Lichtenberg, Heather Brannan, and especially Kara Schmid. I also thank the National Institutes of Health for their support of these studies.

The author had no personal or financial conflict of interest.

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