Effect of maternal hypercholesterolemia on fetal sterol metabolism in the Golden Syrian hamster

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Abstract **The fetus obtains a significant amount of cholesterol from de novo synthesis. Studies have suggested that maternal cholesterol may also contribute to the cholesterol accrued in the fetus. Thus, the present studies were completed to determine whether diet-induced maternal hypercholesterolemia would affect fetal sterol metabolism. To accomplish this, maternal plasma cholesterol concentrations were increased sequentially by feeding hamsters 0.0%, 0.12%, 0.5%, and 2.0% cholesterol. At 11 days into a gestational period of 15.5 days, cholesterol concentrations and sterol synthesis rates were measured in the three fetal tissues: the placenta, yolk sac, and fetus. In the placenta and yolk sac, the cholesterol concentration increased significantly when dams were fed as little as 0.12% cholesterol ($P < 0.0167$), and sterol synthesis rates decreased in dams fed at least 0.5% or 2% cholesterol, respectively ($P < 0.0167$). In the fetus, changes in fetal cholesterol concentration and sterol synthesis rates occurred only when dams were fed at least 0.5% cholesterol, which corresponded to a greater than 2-fold increase in maternal plasma cholesterol concentrations. When the cholesterol concentration in the fetal tissues in each animal was plotted as a function of maternal plasma cholesterol concentration, a linear relationship was found ($P < 0.001$). These studies demonstrate that sterol homeostasis in fetal tissues, including the fetus, is affected by maternal plasma cholesterol concentration in a gradient fashion and that sterol metabolism in the fetus is dependent on sterol homeostasis in the yolk sac and/or placenta.**—McConihay, J. A., P. S. Horn, and L. A. Woollett. **Effect of maternal hypercholesterolemia on fetal sterol metabolism in the Golden Syrian hamster.** ***J. Lipid Res.*** **2001.** 42: **1111–1119.**

Supplementary key words fetus • embryo • placenta • fetal membranes • yolk sac • cholesterol

The Smith-Lemli-Opitz syndrome (SLOS) is a relatively common, autosomal recessive malformation syndrome that develops in utero (1). The clinical features associated with this syndrome are craniofacial abnormalities, limb malformations, and various neurological disorders (2, 3). Patients with SLOS have a reduction in plasma and tissue cholesterol concentrations due to a lack of 3β-hydroxysteroid-Δ^7-reductase activity (4). Rodents will develop an SLOS-like syndrome in utero by feeding drugs that inhibit 3β-hydroxysteroid-Δ^7-reductase to the pregnant dam (5–7). The development of the drug-induced craniofacial and limb defects can be reversed in the fetal rodents by increasing maternal plasma cholesterol concentrations early in gestation (5–8). These results suggest that the embryo can acquire cholesterol from exogenous sources, such as the maternal circulation. Consequently, maternal plasma lipoprotein cholesterol could play a nutritive role in embryonic and/or fetal development.

The developing conceptus has different nutritional sources that vary with the stage of development. Initially, the nutritional requirement of the blastocyst is minimal and can be met by intrauterine components. As the blastocyst continues to grow and divide, the nutritional requirements increase. To meet these requirements, the blastocyst becomes implanted within the uterine wall and two extraembryonic fetal tissues begin to form.

The yolk sac is the first of the two extraembryonic fetal tissues formed during gestation [reviewed in refs. (9–12)]. Both the rodent and human endodermal cells of the yolk sac are polarized with an apical and a basolateral side. The rodent yolk sac is inverted and the human yolk sac is not. In the rodent, numerous exogenous constituents are taken up on the apical side of the cells, transported to the basolateral side, and secreted into the vitelline vessels of the embryo. In the human, the same processes are thought to occur because the morphology of the tissue and the expression of proteins are similar to those in the rodent. Shortly after the formation of the yolk sac, the chorionic and allantoic mesoderms fuse to establish the placenta in both rodents and humans. Even though there are numerous types of placentas, the rodent and human discoid placentas are both chorioallantoic. The human syncytiotro-

Abbreviations: ABC-A1, ATP-binding cassette transporter 1; DPS, digitonin-precipitable sterols; HDL-C, LDL-C, and VLDL-C, cholesterol carried primarily as high, low, and very low density lipoprotein, respectively; Shh, Sonic hedgehog; SLOS, Smith-Lemli-Opitz syndrome; SR-BI, scavenger receptor class B type I.

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phoblasts and the outer layer of the rodent trophoblasts have numerous villi that are bathed in maternal blood and tissue secretions. A variety of constituents in the maternal fluids are taken up by the syncytiotrophoblasts and trophoblasts. The nutrients cross to the basolateral side of these cells and are secreted into the vessels of the umbilical cord.

Despite the fact that lipoprotein-derived maternal cholesterol is taken up by the yolk sac and/or placenta (10, 13–17), the transport of maternal cholesterol across the two extraembryonic fetal tissues and the subsequent secretion into the umbilical vein or vitelline vessels is not well defined. In addition, past studies that have attempted to examine this process have yielded extremely diverse results (18–23). In the studies presented here, the ability of maternal cholesterol to be transferred to the fetus was determined by measuring sterol concentrations and synthesis rates in the fetal tissues of dams with varying degrees of diet-induced hypercholesterolemia. We found that the fetal cholesterol concentration increased and that the sterol synthesis rate decreased in response to an elevation in maternal plasma cholesterol concentration. Sterol balance in the yolk sac and placenta also was affected by maternal cholesterol concentration. The changes in fetal sterol metabolism appeared to be dependent on the sterol balance in the yolk sac and/or placenta, which was in turn dependent on the extent of maternal hypercholesterolemia.

MATERIALS AND METHODS

Animals and diets

Male and nonpregnant female Golden Syrian hamsters (*Mesocricetus auratus*) (Charles River Laboratories, Kingston, NJ), weighing 100 and 90 g respectively, were housed in a temperature- and humidity-controlled room. Animals were subjected to a cycle of 14 h of darkness and 10 h of light, and were fed ad libitum pelleted cereal-based diet (rodent diet no. 7102; Harlan Teklad, Madison, WI). After acclimatization to the light cycle, two different sets of studies were completed. In the first set of studies, females were divided randomly into four groups. One group of animals was placed on ground chow, which had an inherent cholesterol content of 0.02%. This was the control diet. The three remaining groups were fed ground chow plus increasing amounts of cholesterol. Cholesterol (ICN Biomedicals, Aurora, OH) was added to the chow at 0.0%, 0.12%, 0.5%, and 2.0% (w/w). After 3 weeks of consuming the diets, designated females were mated. Animals were considered pregnant if they entered lordosis and copulated when placed in the same cage as a male. This was day 0 of gestation. Once mated, the females were housed separately until they were studied on day 6 or 11 of gestation. Mating was continued each day until all designated females were pregnant. The remaining females were studied in the nonpregnant state (0 days of gestation) after being fed one of the diets for 3 weeks. In the second set of studies, pregnant females were divided into two groups. One group of females was fed the control diet throughout gestation. The second group of females was fed the control diet until day 10 of gestation, at which time they were switched to the diet containing 2% cholesterol. Both sets of females were studied on gestational day 11. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

Plasma and tissue cholesterol concentrations

Dams were anesthetized and exsanguinated via the descending aorta. The maternal liver and kidney, and the fetal yolk sac, fetal and maternal placenta, and fetus were isolated. Tissues were saponified and the cholesterol concentration was measured by gas-liquid chromatography (GLC), using stigmastanol as an internal standard (24).

Plasma cholesterol concentration was determined enzymatically (Boehringer Mannheim, Indianapolis, IN). Lipoprotein cholesterol concentration was determined by simultaneously separating lipoproteins at densities of 1.020 and 1.063 g/ml by ultracentrifugation. Samples were saponified and cholesterol concentration was determined by GLC (24).

Sterol synthesis rates in vivo

Pregnant females were injected intraperitoneally with 50 mCi of ^3H_2O (14). After 60 min, dams were anesthetized and blood was collected from the descending aorta. The maternal liver was isolated as were fetuses, yolk sacs, and placentas. Two sets of fetal tissues were taken from each dam. Tissues as well as a sample of whole blood were saponified and digitonin-precipitable sterols (DPS) were isolated and assayed for ^3H content (14, 25). The rates of synthesis are presented as nanomoles of ^3H_2O incorporated into sterol per hour per gram of tissue or per tissue after correction for percent water and equilibration of ^3H_2O in the fetus (14, 26).

In a second set of studies, dams were injected with ^3H_2O and anesthetized at 15, 30, and 60 min after injection. The maternal plasma and liver and several fetuses were isolated. The percent water in the fetuses and the ^3H content was determined. The ^3H content in the maternal liver was measured simultaneously.

Calculations

The data are presented as mean values ± 1 SEM. To determine whether data could be analyzed by analysis of variance, the variability of the data was examined using a Bartlett's test for equality (27). The Bartlett's test revealed that the variability of the data was not similar between animals of the different dietary groups. Thus, significant differences were determined between data from the control group of animals and data from each group of animals fed a different level of dietary cholesterol, using a two-sample *t*-test in which equality of variances is not assumed. Because multiple *t*-tests were used, significance was not assumed to have occurred unless the *P* value obtained was less than the Bonferroni correction of the *P* value (27). To determine the Bonferroni correction, the *P* value needed to achieve significance was divided by the number of *t*-tests conducted for each measurement. Thus, a *P* value of <0.0167 (0.05/3) would be required in order to be judged statistically significant. Plasma cholesterol concentration was also analyzed by age to determine whether measurements at 6 or 11 days into gestation were different from measurements in nonpregnant dams fed similar diets. Because two tests were run for this comparison, the *P* value necessary for significance would be <0.025 (0.05/2). For the study in which animals were fed cholesterol for 0 or 24 h, significance occurred at $P < 0.05$. In addition, individual data points for fetal, yolk sac, and placental cholesterol concentrations were plotted as a function of maternal cholesterol concentration. Curves were generated for each set of data with the assumption that the line was not required to pass through the origin. The best-fit line was determined by testing for goodness of fit using both first- and second-order kinetics (SigmaStat; SPSS, Chicago, IL).

RESULTS

Hamsters were used in these studies because first, circulating LDL-cholesterol concentration increases in both hamsters and humans fed modest levels of cholesterol (28, 29) and second, because the hamster and human are both hypocholesterolemic in early to mid gestation and hypercholesterolemic in late gestation (26, 30, 31). Sterol metabolism was examined in hamsters at 6 and 11 days of gestation to correspond to early to mid gestation in humans when both the yolk sac and placenta are viable tissues and when the major patterning of tissues and organs occurs [reviewed in refs. (9) and (32)].

Effect of dietary cholesterol on sterol metabolism in the dam

Hamsters were fed 0.0%, 0.12%, 0.5%, and 2.0% cholesterol for 3 weeks to manipulate plasma cholesterol concentration. Maternal plasma cholesterol levels at 0 days of gestation varied from 128 to 547 mg/dl in response to the diets (**Fig. 1**). The addition of 0.12% cholesterol to the diet did not affect maternal plasma cholesterol concentrations significantly, whereas the addition of 0.5% or 2.0% cholesterol to the diet resulted in increases in plasma cholesterol concentration. The change in the circulating cholesterol levels in the cholesterol-fed dams was the result of an increase in cholesterol being carried as low and very low density lipoproteins (LDL- and VLDL-C) (**Table 1**; $P < 0.0167$). As gestation progressed to day 6, plasma cholesterol concentrations decreased in all animals (Fig. 1) ($P < 0.025$). Plasma and lipoprotein cholesterol concentrations were greater in dams fed 2.0% cholesterol as compared with the controls (Fig. 1 and Table 1) ($P < 0.0167$). By 11 days of gestation, maternal plasma cholesterol concentrations remained lower than those in the nonpregnant animals (Fig. 1) ($P < 0.025$). As with the other ages, animals at 11 days of gestation fed 0.5% and 2.0% cholesterol had greater plasma and lipoprotein cholesterol concentrations (Fig. 1 and Table 1) ($P < 0.0167$). Unlike the plasma cholesterol concentration, which varied dramatically with the amount of cholesterol consumed, kidney cholesterol concentration differed by only 10% in dams fed 0% or 2% cholesterol (2.25 ± 0.04 and 2.54 ± 0.04 mg/g in dams fed 0% or 2% cholesterol, respectively).

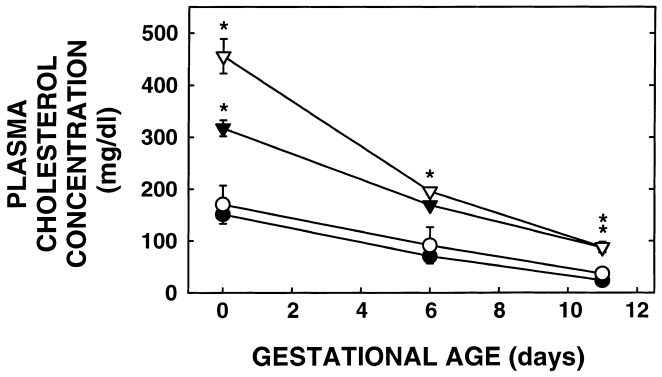

Fig. 1. Plasma cholesterol concentrations of cholesterol-fed dams at 0, 6, and 11 days of gestation. Female hamsters were placed on one of four diets containing 0.0% (closed circles), 0.12% (open circles), 0.5% (closed triangles), or 2% (open triangles) added cholesterol (wt/wt) for 3 weeks before mating and during gestation. Animals not mated (0 days into gestation) were fed the diet for 3 weeks. Maternal plasma cholesterol concentrations were measured enzymatically in animals on the three different days of gestation. The results represent means ± 1 SEM of data obtained in five to nine dams. Asterisks demonstrate significant differences ($P < 0.0167$) from the control-fed animals at each day of gestation. Differences between gestation days are described in Results.

TABLE 1. Plasma VLDL, LDL, and HDL cholesterol concentrations in cholesterol-fed hamsters at 0, 6, and 11 days into gestation

Lipoprotein Fraction	Diet 0% CH	0.12% CH	0.5% CH	2.0% CH
	mg cholesterol/100 ml			
0 days				
VLDL	21 ± 6	21 ± 2	103 ± 36	192 ± 28*
LDL	31 ± 4	52 ± 8	107 ± 6*	137 ± 18*
HDL	96 ± 4	97 ± 8	146 ± 19	129 ± 11*
6 days				
VLDL	11 ± 1	14 ± 1	27 ± 7	47 ± 7*
LDL	17 ± 5	24 ± 5	39 ± 11	42 ± 5*
HDL	45 ± 4	54 ± 6	87 ± 9*	106 ± 3*
11 days				
VLDL	5 ± 1	14 ± 4	28 ± 5*	23 ± 4*
LDL	5 ± 1	11 ± 2	25 ± 2*	22 ± 4*
HDL	13 ± 4	16 ± 8	37 ± 7*	26 ± 1*

Hamsters were fed diets containing different levels of cholesterol (CH) for 3 weeks before and during gestation. Asterisks demonstrate significant differences from the animals fed 0% cholesterol. All values represent means ± SEM for three to five animals.

Effect of dietary cholesterol on sterol metabolism in the fetal tissues

Once it was established that maternal plasma cholesterol concentration could be manipulated throughout gestation, the impact of maternal hypercholesterolemia on sterol homeostasis in the yolk sac and placenta was determined. In the control-fed dam, the cholesterol concentration in the yolk sac was 2.61 ± 0.15 mg/g (**Fig. 2A**). Cholesterol concentration in this tissue increased 56% when as little as 0.12% cholesterol was added to the diet ($P < 0.0167$). Diets consisting of 0.5% and 2.0% cholesterol also resulted in increases in tissue cholesterol concentrations as compared with tissues of control-fed hamsters ($P < 0.0167$). When the yolk sac cholesterol concentration was correlated to maternal plasma cholesterol concentration, a positive slope with a significant correlation was found (**Fig. 3A**). Dietary cholesterol had no effect on the mass of the yolk sac of animals fed cholesterol as compared with the control-fed animals; the masses of the yolk sacs were 22 ± 2, 23 ± 4, 19 ± 1, and 20 ± 1 mg in dams fed 0.0%, 0.12%, 0.5%, and 2% cholesterol, respectively. Similar to that in the yolk sac, the placental cholesterol concentration of the control-fed dam was 2.49 ± 0.07 mg/g (Fig. 2B). When dams were fed 0.12% cholesterol, placental cholesterol concentration increased 15% ($P < 0.0167$). Increases in placental cholesterol concentrations were also measured when 0.5% and 2.0% cholesterol was added to the diet ($P < 0.0167$) such that the correla-

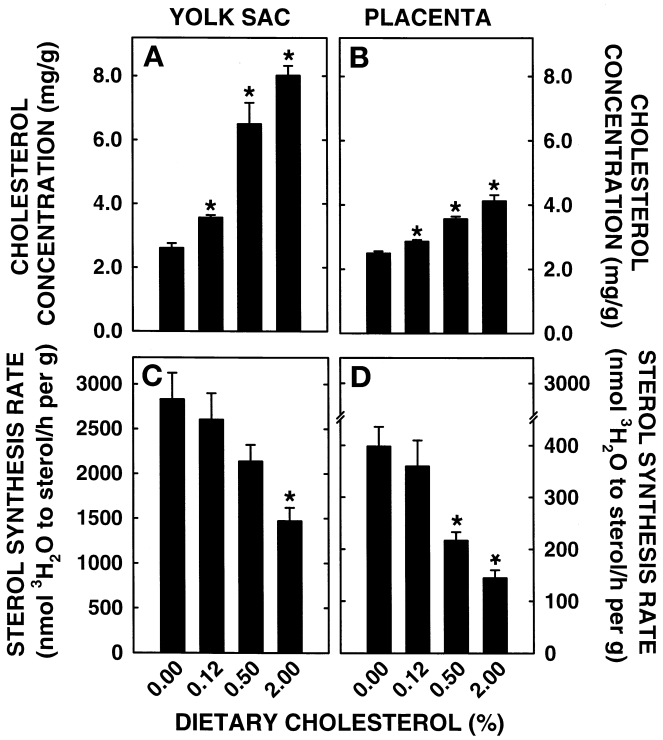

Fig. 2. Cholesterol concentrations and sterol synthesis rates in the yolk sac and placenta of cholesterol-fed dams at 11 days into gestation. Dams were fed 0.0%, 0.12%, 0.5%, and 2% cholesterol for 3 weeks before and during gestation. Cholesterol concentrations in the yolk sac (A) and placenta (B) are presented as milligrams of cholesterol per gram of tissue. Two of each of the tissues were pooled for one sample. Two sets of tissues were obtained from each dam. The results represent means ± 1 SEM of data obtained in 6 to 16 dams. Concentration of [^{3}H]DPS represents the sterol synthesis rates. Data are presented as nanomoles of ^3H_2O converted to sterol per hour per gram in the yolk sac (C) and placenta (D). Two of each of the tissues were pooled for one sample. Two sets of tissues were obtained from each dam. The results represent means ± 1 SEM of data obtained in six to nine dams. Asterisks demonstrate significant differences ($P < 0.0167$) from the control-fed animals.

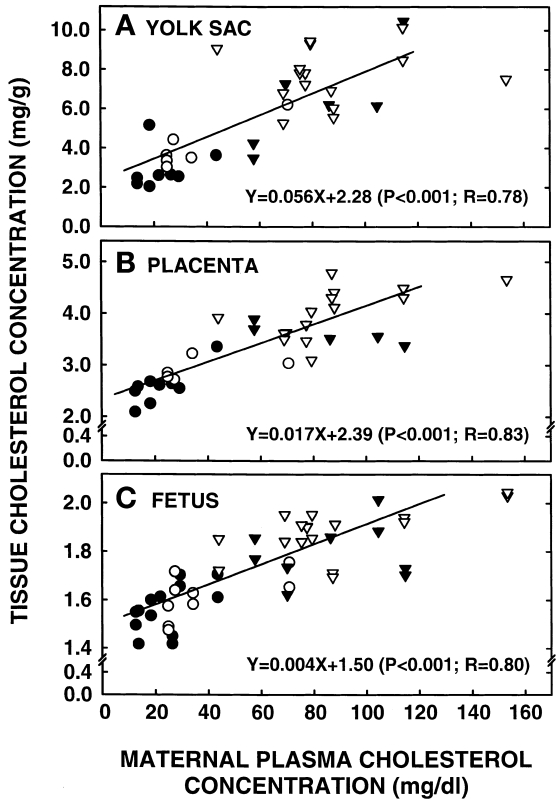

Fig. 3. Yolk sac, placental, and fetal cholesterol concentrations plotted as a function of maternal plasma cholesterol concentrations at 11 days into gestation. Dams were fed 0.0% (closed circles), 0.12% (open circles), 0.5% (closed triangles), and 2% (open triangles) cholesterol for 3 weeks before and during gestation. The cholesterol concentration of each yolk sac (A), placenta (B), or fetus (C) presented in Figs. 2 and 4 were replotted as a function of the plasma cholesterol concentration for each dam presented in Fig. 1. Lines generated from data best fit to first-order kinetics in all three tissues ($P < 0.001$). The equations for the yolk sac and placenta were y = 0.056x + 2.28 ($R = 0.78$) and y = 0.017x + 2.39 ($R = 0.83$), respectively. The equation for the fetus was y = 0.004x + 1.50 ($R = 0.80$). The slopes were all significantly greater than zero ($P < 0.001$) and varied dramatically between the three tissues.

tion of placental to maternal cholesterol concentrations yielded a line with a positive slope and significant correlation (Fig. 3B). Despite the absence of significant difference in the placental mass of females fed any diet, there was a trend ($P = 0.02$) for placental mass in dams fed 2% cholesterol to be less than mass in the control animals (92 ± 4, 83 ± 7, 91 ± 5, and 75 ± 5 mg in animals fed 0.0%, 0.12%, 0.5%, and 2% added cholesterol, respectively).

To further examine sterol balance across the extraembryonic fetal tissues, sterol synthesis rates were determined in these same tissues. The sterol synthesis rate in the yolk sac was relatively high as compared with other tissues (2,831 ± 297 nmol/h per g) (Fig. 2C). The addition of 0.12% or 0.5% cholesterol to the diet of the dam had no effect on sterol synthesis rate in the yolk sac. Only when 2.0% dietary cholesterol was fed did sterol synthesis rates in the yolk sac decrease significantly as compared with control-fed dams ($P < 0.0167$). In the placenta, the sterol synthesis rate was 398 ± 38 nmol/h per g in the control-fed animal (Fig. 2D). Placental sterol synthesis rates were less in dams fed 0.5% and 2% dietary cholesterol as compared with rates in control animals ($P < 0.0167$).

Because maternal hypercholesterolemia did affect sterol homeostasis in the extraembryonic fetal tissues, we examined sterol metabolism in the fetus itself. The fetus in a control-fed dam had a cholesterol concentration of 1.58 ± 0.02 mg/g (**Fig. 4A**). Fetal cholesterol concentration did not change when dams were fed 0.12% cholesterol. However, cholesterol concentrations in fetuses of dams fed 0.5% and 2% added cholesterol were 17% and 20% greater ($P < 0.0167$), respectively, than concentrations in fetuses in the control-fed dams. As in the yolk sac and placenta, fetal cholesterol concentrations correlated significantly with maternal plasma cholesterol concentrations (Fig. 3C). The size of the fetus (91 ± 3, 97 ± 8, 92 ± 4, and 87 ± 4 mg in dams fed the diets containing 0.0%, 0.12%, 0.5%, and 2% added cholesterol, respectively) and the number of fetuses per dam (13 ± 1, 12 ± 1, 13 ± 1, and 15 ± 1 fetuses

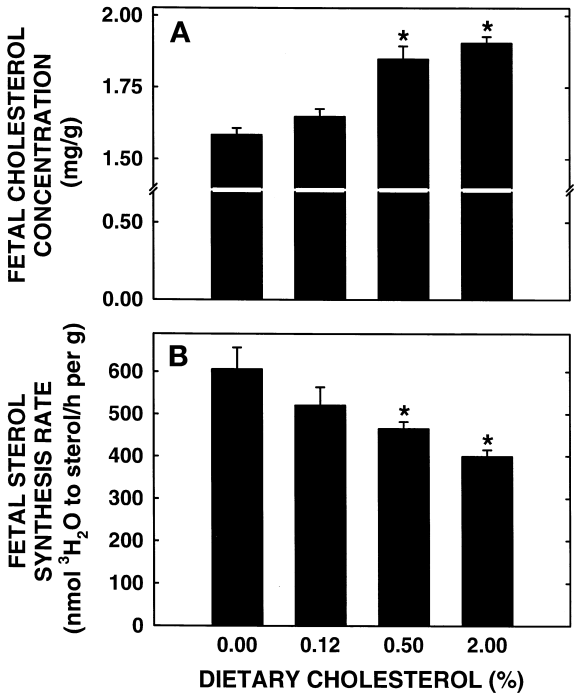

Fig. 4. Cholesterol concentrations and sterol synthesis rates in fetuses of cholesterol-fed dams at 11 days of gestation. Dams were fed 0.0%, 0.12%, 0.5%, and 2% cholesterol for 3 weeks before and during gestation. Cholesterol concentrations are presented as milligrams of cholesterol per gram fetus (A). Concentrations of [^{3}H]DPS represent sterol synthesis rates. Data are presented as nanomoles of ^3H_2O converted to sterol per hour per gram tissue (B). Two fetuses were pooled and presented as one sample. Two sets of tissues were used from each dam. The results represent means ± 1 SEM of data obtained in six to nine dams. Asterisks demonstrate significant differences ($P < 0.0167$) from the control-fed animals.

in dams fed diets containing 0.0%, 0.12%, 0.5%, and 2% added cholesterol, respectively) were not affected by dietary cholesterol. As shown previously (14, 26), sterol synthesis rates in fetuses fed no added cholesterol were relatively high (605 ± 50 nmol/h per g) as compared with other peripheral tissues (29). Sterol synthesis rates decreased significantly by 23% and 29% ($P < 0.0167$) in fetuses of dams fed 0.5% and 2% added cholesterol, respectively (Fig. 4B). Differences in synthesis rates were not due to an effect of diet on percent water in the fetuses or on the rate of transfer of ^3H_2O from the dam to the fetuses (data not shown).

In the present studies, sterol synthesis rates were determined by the amount of [^{3}H]DPS in the tissues. Previous studies have shown that the amount of [^{3}H]DPS in some tissues is representative of the amount of [^{3}H]DPS in blood and does not represent de novo sterol synthesis rates within the tissue of interest (33); the amount of [^{3}H]DPS in blood is directly related to hepatic sterol synthesis rates (33). Because the dams in this present study were fed different levels of cholesterol, their hepatic sterol synthesis rates varied dramatically. Livers of dams fed the diets containing 0.0%, 0.12%, 0.5%, and 2.0% cholesterol had synthesis rates of 630 ± 137, 180 ± 38, 23 ± 1, and 19 ± 5 nmol/h per g, respectively. As expected, the amount of [^{3}H]DPS in the blood also varied and was 27.0 ± 11.1, 5.9 ± 3.9, 1.5 ± 0.3, and 1.5 ± 0.3 nmol/h per ml in dams fed 0.0%, 0.12%, 0.5%, and 2.0% added cholesterol, respectively.

To verify that the differences in sterol synthesis rates of the fetal tissues were truly a reflection of metabolism within the tissues themselves and not the result of a change in maternal hepatic sterol synthesis rates, dams were fed dietary cholesterol for 0 or 24 h before the study. Maternal hepatic sterol synthesis rates were suppressed dramatically ($P < 0.05$) in animals fed 2% cholesterol for 24 h (**Fig. 5A**). In contrast, sterol synthesis rates in the fetus (Fig. 5B), yolk sac (Fig. 5C), and placenta (Fig. 5D) were similar in tissues of dams fed cholesterol for 0 or 24 h. These data indicate that the differences in sterol synthesis rates of fetal tissues in dams fed cholesterol throughout gestation are due to a change in metabolism within the fetal tissues themselves, and are not an artifact of changes in sterol synthesis in maternal tissues.

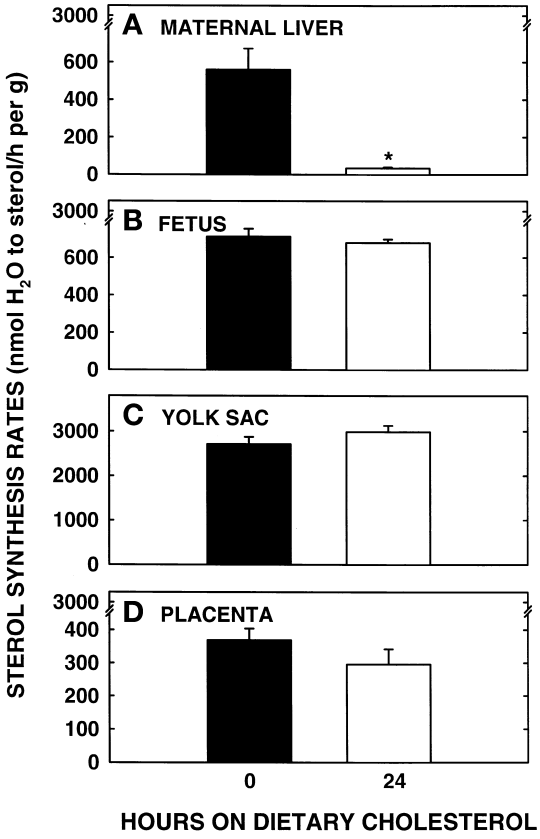

Fig. 5. Sterol synthesis rates in the maternal liver, fetus, yolk sac, and placenta of dams fed the control diet throughout gestation or of dams fed the control diet for the first 10 days of gestation and 2% cholesterol for 24 h before the study. Concentration of [^{3}H]DPS represents sterol synthesis rates. Data are presented as nanomoles of ^3H_2O converted to sterol per hour per gram maternal liver (A), fetus (B), yolk sac (C), and placenta (D). Two fetuses, yolk sacs, or placentas were pooled and represent one sample. Two sets of tissues were used from each dam. The results represent means ± 1 SEM of data obtained in three dams. The asterisk demonstrates a significant difference ($P < 0.05$) from the animals fed no cholesterol.

DISCUSSION

The primary finding of the present studies was that circulating maternal cholesterol does affect fetal cholesterol metabolism. Modest changes in maternal plasma cholesterol concentration, such as those found in dams fed 0.12% cholesterol, had no impact on fetal cholesterol concentration. In contrast, when the circulating maternal cholesterol levels were elevated 2-fold or greater, fetal sterol concentration increased significantly. Consequently, fetal cholesterol was positively correlated with maternal plasma cholesterol concentration. Because sterol synthesis rates within fetuses with high cholesterol concentrations were slightly suppressed, the increase in fetal cholesterol concentration must have resulted from more exogenous sterol being presented to the fetus.

Two-step model for accretion of maternal-derived cholesterol by the fetus

Maternal cholesterol can affect the fetus via at least two different mechanisms. *1*) Maternal cholesterol can be taken up by the yolk sac and/or placenta, processed by these two tissues, and secreted into the vessels leading to the fetus. This process represents direct secretion of the maternal lipid into the fetal circulation; *2*) maternal cholesterol can be taken up by the yolk sac and/or placenta, alter sterol metabolism within these two extraembryonic fetal tissues, and enhance secretion of sterol to the vessels leading to the fetus. This process represents an indirect effect of the maternal cholesterol on secretion of lipids to the fetus. It is possible that both of these mechanisms exist simultaneously.

The first step in the accretion of exogenous cholesterol by the fetus, via either mechanism, is an uptake of maternal lipoproteins by the yolk sac and placenta. These extraembryonic fetal tissues take up maternal lipoproteins at relatively elevated rates as compared with other extrahepatic tissues (13–17, 29), through both receptor-mediated and receptor-independent transport. In fact, these high clearance rates may be the reason for the pregnancy-induced hypocholesterolemia. The yolk sac and placenta are unique tissues in that lipoproteins appear to be taken up at significant rates by receptor-independent processes (13), even in the presence of numerous lipoprotein receptors, including the LDL receptor (13, 34), scavenger receptor, class B, type I (SR-BI) (13, 35–37), the VLDL receptor (38), the apoE receptor 2 (39), the acetylated LDL receptor (17), megalin (40, 41), and cubulin (gp280) (42–44). Because intracellular sterol balance does not appear to regulate expression of several of these receptors, such as megalin (45), or receptor-independent transport, at least in adult tissues, the extraembryonic fetal tissues may be able to take up circulating maternal cholesterol via unregulated processes. In support of this concept, the yolk sac, which has the largest percentage of receptor-independent transport, had a greater increase in cholesterol concentration than did the placenta. In addition, the placental and yolk sac cholesterol concentrations increased to a much greater extent than did cholesterol concentrations in extrahepatic tissues that have a lower rate of unregulated transport, such as the kidney.

The second step of the two proposed mechanisms is either that the maternal-derived cholesterol that is taken up by the cells is transported to the basolateral side and secreted into the vitelline vessel or umbilical vein, or that the maternal-derived cholesterol that is taken up by the cells affects sterol metabolism within the cells, thereby affecting cholesterol secretion to the fetal vessels. Data obtained here and presented previously (46) suggest that at least one of these proposed mechanisms exist.

In the yolk sac, maternal cholesterol is potentially transported to the basolateral side of the cells by being incorporated into nascent endodermal cell lipoproteins (47–49); other cells that synthesize lipoproteins, such as hepatocytes and enterocytes, incorporate exogenous sterol into newly formed lipoproteins (50–52). The lipoproteins that are formed in the yolk sac are essential to normal murine fetal development because the lack of lipoprotein synthesis leads to embryonic resorption (48, 53–55). The component(s) that are responsible for this lethality are presently unknown, but may include cholesterol because the cholesterol concentration of the resorbed fetuses in some of the studies is less than the concentration in normal fetuses (48). Various factors can affect the lipoprotein formation in these cells, such as exogenous fatty acids (56) and age (56, 57). Triglyceride and cholesterol concentration may also affect lipoprotein formation, assuming the regulation of lipoprotein formation in these cells is similar to that in hepatocytes (50, 51). Thus, an excess of maternal-derived cholesterol in the yolk sac, as seen in dams with high plasma cholesterol concentrations, could either be incorporated into the lipoproteins themselves and/or could stimulate lipoprotein secretion as a result of a change in tissue sterol concentration, resulting in an increase in the amount of sterol secreted to the vitelline vessels and the fetus.

Unlike the endodermal cells of the yolk sac, the effects of maternal-derived cholesterol on sterol metabolism and/or transport of cholesterol within trophoblasts are not presently defined. However, observations have allowed speculation about the processes responsible for transport of sterol across trophoblasts. First, lipid droplets, possibly including cholesterol, were found to be secreted into the umbilical vein of fetal mice (58). A component of these lipid droplets is essential to fetal development because a lack of droplet formation leads to embryonic lethality (58). Second, the placenta has high mRNA levels for the cholesterol transporter ATP-binding cassette transporter 1 (ABC-A1) (59–62). This transporter may aid in the efflux of cholesterol from trophoblasts to various acceptors in the fetal circulation. The expression of ABC-A1 is critical for fetal development and a loss of its activity will lead to fetal death in utero (63).

Several data obtained from these hypercholesterolemic pregnant hamsters support this two-step model for accretion of exogenous sterol by the fetus. First, a 13–50% increase in maternal plasma cholesterol concentration in dams fed 0.12% cholesterol results in significant increases in cholesterol concentrations in the yolk sac and placenta,

whereas the fetal cholesterol concentration remains unchanged with these marginal changes in maternal cholesterol levels. Fetal cholesterol concentrations change only when cholesterol levels are significantly elevated in the yolk sac and placenta. Second, the slope of the line describing the relationship between tissue cholesterol concentration and maternal plasma cholesterol concentration is much greater for the yolk sac and placenta as compared with the fetus. Thus, cholesterol concentrations increase initially in the responsive yolk sac and placenta, followed by changes in the less responsive fetus.

Effect of maternal-derived cholesterol on fetal sterol metabolism

The exogenous cholesterol accrued by the fetal tissues is not inert but appears to suppress sterol synthesis rates. These results are to be expected because the sterol regulatory binding proteins (SREBP) have been shown to be expressed in fetal tissues (64, 65), and the amount of SREBP appears to be greatest in tissues that are rapidly dividing (64); the SREBP are the primary regulators of sterol biosynthesis (66). Interestingly, sterol synthesis rates decreased simultaneously or subsequent to an increase in cholesterol concentration in the fetal tissues. These results are different from those obtained in the adult liver. In the liver, the tissue cholesterol concentration does not increase until synthesis rates have reached a nadir (67). The lack of responsiveness in the fetal tissues may be necessary to sustain the high synthesis rates required to maintain normal development.

CONCLUSION

In summary, the fetus, placenta, and yolk sac can accrue significant amounts of cholesterol from exogenous sources in early to midgestation of the hamster. Similarly, plasma cholesterol concentrations of the human fetus also increase in parallel with maternal plasma cholesterol concentrations in early to midgestation (46). The fact that maternal cholesterol appears to cross into the fetus could be used to manipulate the outcome of certain pregnancies. The pregnancies that may be most affected by exogenous sterol are those in which normal forebrain development is altered in early to midgestation, such as that which occurs in SLOS fetuses.

Cholesterol has varied roles in forebrain development, ranging from serving as a membrane component to an activator and propagator of the signal for Sonic hedgehog (Shh), one of the signaling molecules that is essential for normal patterning of the forebrain [reviewed in ref. (68)]. A lack of Shh activity has been implicated in SLOS fetuses because they have a higher occurrence of holoprosencephaly, which is an abnormal forebrain, than the general population (69). Because the severity of the congenital defects in SLOS patients appears to be correlated to tissue cholesterol concentration (70), the ability to increase fetal cholesterol early in development, as we have demonstrated is possible here, may help reduce the number and severity of anomalies found in these individuals through a variety of mechanisms.

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