Maternal high density lipoproteins affect fetal mass and extra-embryonic fetal tissue sterol metabolism in the mouse

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Abstract **Previous studies have shown that antibodies to cubulin, a receptor on the yolk sac that binds high density lipoproteins (HDL) and cobalamin, induce fetal abnormalities. Mice with markedly low concentrations of plasma HDL-cholesterol (HDL-C) give birth to healthy pups, however. To establish whether maternal HDL-C has a role in fetal development, sterol metabolism was studied in the fetus and extra-embryonic fetal tissues in wild-type and apolipoprotein A-I-deficient mice (apoAI−/−). Maternal HDL-C content was markedly greater in apoAI+/+ mice prior to pregnancy and at 13 days into gestation. By 17 days into gestation, HDL-C content was similar between both types of mice. Fetuses from apoAI (−/− × −/−) matings were 16–25% smaller than control mice at 13 and 17 days of gestation and contained less cholesterol. The differences in size and cholesterol content were not due to a lack of cholesterol synthesis or apoA-I in the fetus. In the yolk sac and placenta, sterol synthesis rates were ~50% greater in the 13-day-old apoAI−/− mice as compared to the apoAI+/+ mice. Even though synthesis rates were greater, cholesterol concentrations were 22% lower in the yolk sac and similar in the placenta of apoAI−/− mice as compared to tissues of wild-type mice. These data suggest that a difference in maternal HDL-C concentration or composition can affect the size of the fetus and sterol metabolism of the yolk sac and placenta in the mouse.—**McConihay, J. A., A. M. Honkomp, N. A. Granholm, and L. A. Woollett. **Maternal high density lipoproteins affect fetal mass and extra-embryonic fetal tissue sterol metabolism in the mouse. *J. Lipid Res.* 2000.** 41: **424–432.**

Supplementary key words yolk sac • placenta • fetus • cholesterol • HDL • progesterone

The fetus requires cholesterol for normal development (1–3). There are two sources of cholesterol in the fetus, as in any tissue. The first source is endogenous and constitutes cholesterol synthesized within the fetus. Fetal sterol synthesis rates are elevated as compared to the adult (4–8), and may account for a significant amount of cholesterol in the fetus (4–6). The second source of fetal cholesterol, which is less well-defined, is exogenous. Exogenous cholesterol in the fetus could be derived from cholesterol synthesized in the yolk sac and placenta or from maternal lipoproteins taken up by the yolk sac and placenta that are transported to the fetus.

Both low and high density lipoproteins (LDL and HDL) are removed from the maternal circulation by the placenta and yolk sac (4, 9, 10). In the hamster, clearance rates are much greater for HDL than LDL, and HDL clearance rates are greater in the yolk sac than the placenta (9). Although the precise receptor(s) responsible for HDL clearance in these extra-embryonic fetal tissues are not yet known, several candidates exist. The scavenger receptor class B, type I (SR-BI) is present along the apical surface of the visceral endoderm of the yolk sac and along the spongiotrophoblasts of the placenta (9, 11). This receptor binds apolipoprotein A-I (apoA-I) and apoA-II, and internalizes the cholesteryl ester moiety of HDL (12–14). Cubulin (gp280), a newly described HDL receptor that binds apoA-I and internalizes the whole HDL particle, is also expressed by the endodermal cells of the yolk sac (15–18). In addition, the presence of apoE on HDL (19, 20) could result in their binding to apoE receptors expressed in the yolk sac or placenta; gp330 binds apoE-containing lipoproteins, as well as other ligands, and is present on the visceral endoderm of the yolk sac (21–23). When expression of SR-BI or gp330 is halted or activity of cubulin is blocked, fetuses die in utero and/or develop congenital malformations of the brain (15, 24–26) implying a critical role for the yolk sac and/or HDL in fetal development.

Interestingly, even though lipoprotein receptors appear to have a function in fetal development, most fetuses develop normally regardless of maternal plasma HDL-cholesterol

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; apoA-I, apolipoprotein A-I; SR-BI, scavenger receptor class B, type I; HDL-C, HDL-cholesterol; apoAI−/−, mice lacking apolipoprotein A-I; DPS, digitonin-precipitable sterol; neo, neomycin.

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(HDL-C) concentrations. One possible explanation for this apparent lack of effect is that the fetus, the yolk sac, and/or the placenta can compensate for the differences in plasma cholesterol concentrations in the dam. Thus, the purpose of the present studies was to examine sterol metabolism in fetal tissues of dams with normal versus low maternal plasma HDL-C concentrations. Consequently, the mass, cholesterol concentration, and sterol synthesis rate were examined in the fetus, the yolk sac, and the placenta of animals with extremely different HDL-C concentrations. Mice lacking apolipoprotein A-I (apoAI−/−) were the experimental animals used because their plasma cholesterol concentrations were 75–80% lower than those of normal mice due to a reduction in HDL (19, 27). To determine whether any effects found were the result of differences in maternal cholesterol concentration or in fetal genotype, heterozygote crosses (apoAI+/−) were performed and the apoAI+/+ or apoAI−/− fetal tissues within apoAI+/− dams were examined for mass and cholesterol concentration and content.

MATERIALS AND METHODS

Animals and diets

Male and non-pregnant female mice (Jackson Laboratories, Bar Harbor, ME) were housed in a temperature- and humidity-controlled room. The C56BL/6 control mice contained intact apoA-I and the experimental group consisted of C56BL/6 mice that were homozygous for the apoA-I deletion (27). Animals were subjected to 14 h of light and 10 h of darkness. When approximately 3.5 months of age, females were mated with males of the same genotype. In a majority of the matings, pairings consisted of apoAI−/− females with like males or apoAI+/+ females with like males. In one study, females that were heterozygote for the apoA-I gene were mated with like males. Females were checked daily for post-copulatory plugs at the beginning of the light cycle. The presence of a plug was marked as 0.5 days of gestation. Animals were studied at either 0, 12.75, or 16.75 days into gestation. All ages will be presented as 0, 13, and 17 days. Throughout mating and gestation, animals were fed a pelleted cereal-based diet that contained 9% lipid (Picolab Mouse Diet 20, PMI Nutrition International, Inc., Brentwood, MO). In one experiment, females were switched from the pelleted diet to a diet that consisted of ground chow (Rodent diet no. 7102, Harlan Teklad, Madison, WI) with 2% (wt/wt) added cholesterol at day 10 of gestation. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

Tissue mass and cholesterol concentration and content

Females were anesthetized and exsanguinated, and the fetus, yolk sac, and placenta were excised at days 13 and 17 into gestation. The amnion remained with the yolk sac. The placenta consisted of both maternal and fetal tissue. The tissues were saponified, and the amount of cholesterol was measured by GLC using stigmastanol as an internal standard (4, 28). Data are presented as mg cholesterol per g tissue and per tissue.

Total plasma, VLDL, LDL, and HDL cholesterol

Total plasma cholesterol concentrations from the apoAI+/+ and apoAI−/− dams were determined enzymatically (Sigma Chemical Co., St. Louis, MO). Samples were pooled and plasma lipoproteins were separated by size on FPLC using two Superose 6HR 10/30 columns in series (Amersham Pharmacia Biotech, Upsala, Sweden) (20). Cholesterol concentration in each 0.5 ml fraction from the column was determined enzymatically.

Rates of sterol synthesis in vivo

Pregnant females were injected intraperitoneally with 50 mCi of ^3H_2O (5, 29). After 60 min, dams were anesthetized and blood was collected from the vena cava. The maternal liver, fetuses, yolk sacs, and placentas were isolated. These tissues plus a sample of whole blood were saponified and digitonin-precipitable sterols (DPS) were isolated and assayed for ^3H content (4, 5, 30). The rates of synthesis are presented as nmol ^3H_2O incorporated into sterol per h per g tissue or per tissue after correction for percent water and equilibration of ^3H_2O in fetal tissues as previously described (4, 5). The amount of ^3H-DPS in blood is presented as nmol ^3H_2O incorporated into sterol per h per ml.

HDL-cholesteryl ether clearance rates

HDL was isolated from human plasma in the range of 1.070–1.210 g/ml by sequential preparative ultracentrifugation and labeled with either [1α, 2α(n)-^{3}H]cholesteryl oleyl ether or [cholesteryl-4-^{14}C]oleate as described (31, 32). The HDL was reisolated by ultracentrifugation and dialyzed overnight. The radiolabeled HDL was given as a primed-continuous infusion via a jugular catheter made of polyethylene (ID 0.28 mm, OD 0.61 mm). After 4 h, animals were injected with radiolabeled cholesteryl ester via the same catheter and killed 10 min later. Blood, the maternal liver, the yolk sac, and placenta were collected. An aliquot of the liver and plasma as well as the other tissues were saponified and sterols were extracted and assayed for their ^3H and ^{14}C content. Using the average dpm of ^3H in the plasma of each dam over the 4-h infusion and the dpm of ^{14}C at 10 min, the rates of HDL-cholesteryl ether cleared by the various tissues were calculated.

Genotype of fetal tissues

Females heterozygous for the apoA-I gene were mated with heterozygous males of the same genotype. DNA from fetal tissues was analyzed for presence of intact apoA-I gene and for presence of the neomycin (neo) gene. At 17 days into gestation, females were anesthetized, exsanguinated, and fetuses were isolated. A portion of the fetal tail was removed for genotyping with care taken to avoid contamination of the tail with maternal blood. Samples were frozen on dry ice and stored at −70°C until analyzed. The remainder of the fetus and the yolk sac was collected and cholesterol concentration was determined in each tissue as described. DNA was extracted from the frozen samples using High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's directions. The DNA samples were stored at −20°C.

The presence of the neo gene or the apoA-I gene in each tissue sample was determined by PCR in an Eppendorf Mastercycler (Eppendorf Scientific, Westbury, NY). Primers used in the detection of the gene were purchased from the University of Cincinnati DNA Core. Murine apoA-I 5′ primer (5′-GAT ATC TCG CAC CTT TAG CC-3′) and 3′ primer (5′-GAC CGC ATC CAC ATA CAC AT-3′) amplified a 380 base pair (bp) product in the intact gene. The 5′ primer (5′-AGA CAA TCG GCT GCT CTG AT-3′) and 3′ primer (5′-CTC GTC CTG CAG TTC ATT CA-3′) for neo amplified a 110-bp product. PCR reactions for apoA-I were carried out for 30 cycles. Each cycle consisted of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. Neo PCR reactions were carried out for 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. Reactions for apoA-I (50 μl) contained 0.5 μm of each primer and 1 unit of TAQ polymerase (Life Technologies, Baltimore, MD) and were performed in 1× PCR buffer (Life Technologies) modified to contain 10% glycerol, 3.0 mm $MgCl_2$, and 0.2

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mm dNTPs. Reactions for neo (50 μl) contained 0.5 μm of each primer and 1 unit of TAQ polymerase and were performed in 1× PCR buffer modified to contain 10% glycerol, 4.5 mm $MgCl_2$, and 0.2 mm dNTPs. PCR products were analyzed by electrophoresis on 10% polyacrylamide gels.

Calculations

The data are presented as mean values ± 1 SEM. The Student's unpaired *t*-test was used to compare means from data in the apoAI−/− mice to those in the apoAI+/+ mice. An asterisk above bars in the figures indicates a significant difference between means of data obtained from the apoAI−/− and apoAI+/+ tissues at the same day of gestation.

RESULTS

Similar to results in male mice lacking apoA-I (19, 27), plasma cholesterol concentrations were greater in nonpregnant female apoAI+/+ mice as compared to apoAI−/− mice (78.6 ± 1.4 mg/dl and 30.1 ± 1.4 mg/dl, respectively). The primary difference in plasma cholesterol concentrations was the lack of cholesterol carried as HDL in the apoAI−/− animals (**Fig. 1A**). As gestation progressed, maternal cholesterol concentrations decreased in the mice expressing apoA-I to 62.8 ± 2.5 and 58.5 ± 3.7 mg/dl at 13 and 17 days of gestation, respectively (Fig. 1B and C). In contrast, the plasma cholesterol concentrations increased in the apoAI−/− mice to 44.4 ± 4.7 mg/dl at 13 days and 57.6 ± 4.1 mg/dl at 17 days into gestation. When the lipoprotein profiles from these mice were evaluated, it was found that the amount of cholesterol carried in the HDL of apoAI+/+ mice decreased throughout gestation whereas it increased in apoAI−/− mice. By 17 days into gestation, HDL-C content appeared very similar in mice with or without apoA-I. Additionally, the HDL particles in apoAI−/− mice were larger than those in apoAI+/+ animals.

Prior to examination of the fetus, the ability of HDL to be cleared by the extra-embryonic fetal tissues was measured to establish the appropriateness of the mouse for the proposed studies. The murine yolk sac cleared 172 ± 22 μl of plasma of its HDL-cholesteryl ether/h per g tissue, the placenta cleared 103 ± 14 μl/h per g and the liver cleared 145 ± 21 μl/h per g at gestational day 13 (n = 4). Thus, HDL can bind and be cleared by the tissues that sequester the fetus from direct contact with the maternal circulation in the mouse, as shown previously in hamsters (9) and humans (33), making the apoAI−/− mouse an appropriate model to study the effect of maternal HDL-C concentration on fetal development.

The effect of a lack of apoA-I-containing HDL on the developing fetus was determined next. It was found that a lack of apoA-I-containing HDL during gestation resulted in a smaller fetus. The fetuses of the apoAI+/+ dams were 75 ± 2 mg and those of the apoAI−/− dams were 60 ± 2 mg at 13 days of gestation. Fetuses weighed 676 ± 20 mg and 577 ± 11 mg at 17 days into gestation for the apoAI+/+ and apoAI−/− mice, respectively. There also was approximately one less fetus per apoAI−/− dam as compared to the apoAI+/+ dam (7.7 ± 0.3 vs. 8.9 ± 0.3). The differences in fetal mass were not due to dramatic differences in maternal weights as both sets of mice weighed the same prior to gestation (21.0 ± 0.4 vs. 20.8 ± 0.2 g for apoAI+/+ and apoAI−/− mice, respectively). The smaller fetuses had slightly less cholesterol per g tissue as compared to the larger apoAI+/+ fetuses at both 13 and 17 days of gestation (**Fig. 2A**). The physiological relevance of these small differences in fetal cholesterol concentration is presently unknown. When the amount of cholesterol in the whole fetus was determined, differences became much more exaggerated. Cholesterol content was 34% greater in the apoAI+/+ mice than the apoAI−/− mice at 13 days into gestation (Fig. 2B). At 17 days, the amount of cholesterol in the whole fetus was 29% greater in the apoAI+/+ animals.

A lesser amount of cholesterol in the whole fetus can be due to a decrease in the amount of cholesterol entering the fetus or an increase in the amount of sterol being excreted. It is highly unlikely that the apoAI−/− fetus excreted greater amounts of cholesterol or bile acids. It is more likely that less cholesterol was presented to the fetus

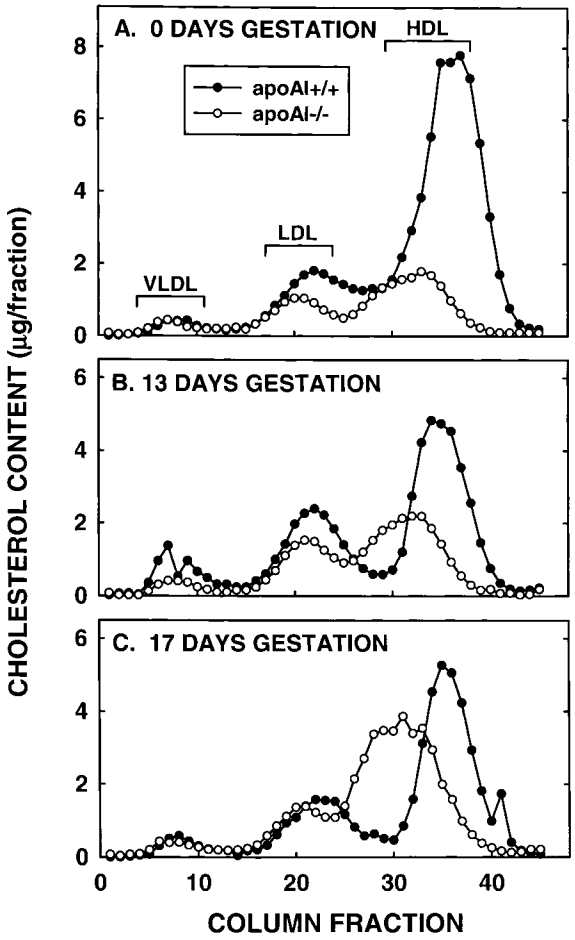

Fig. 1. Fractionation of plasma lipoproteins by Superose 6 column chromatography. Aliquots (200 μl) of pooled plasma from apoAI+/+ (closed circle) and apoAI−/− mice (open circle) at different days of gestation were applied to columns. Ages studied were 0 days of gestation (A), 13 days of gestation (B), and 17 days of gestation (C). Cholesterol was measured enzymatically in each fraction collected. VLDL, LDL, and HDL are indicated.

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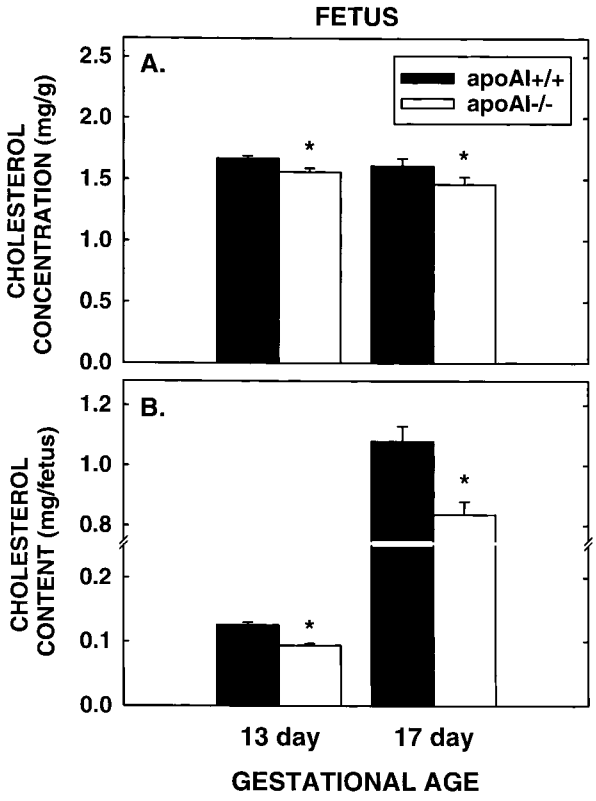

Fig. 2. Fetal cholesterol concentration and content in apoAI+/+ and apoAI−/− mice at 13 and 17 days of gestation. ApoAI+/+ fetuses were carried by apoAI+/+ dams and apoAI−/− fetuses were carried by apoAI−/− dams. Two fetuses were analyzed from each dam. Cholesterol concentration was measured in each fetus and data presented as mg cholesterol per g fetus (A) and mg cholesterol per fetus (B). Each value represents means ± 1 SEM for tissues from 14–16 dams. The asterisk indicates that the values measured in the apoAI−/− mice were significantly different from the apoAI+/+ mice at the same gestational age ($P < 0.005$).

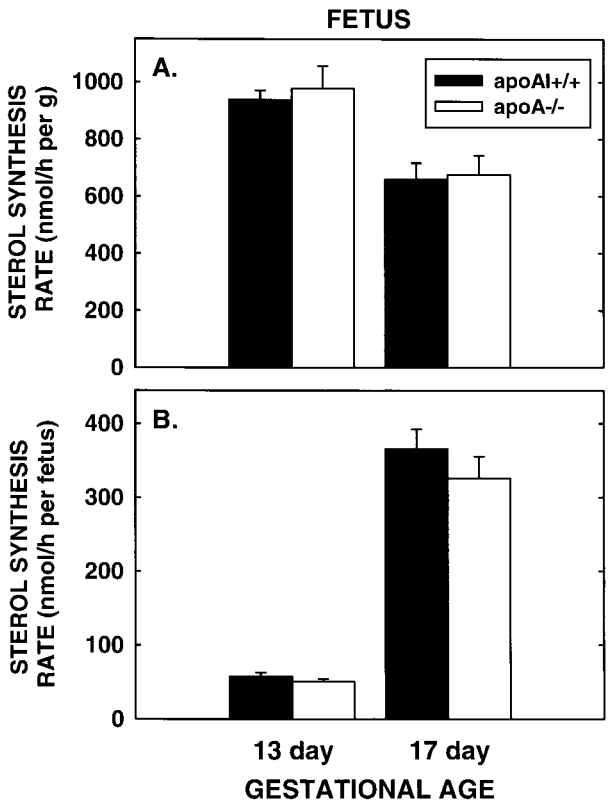

Fig. 3. Sterol synthesis rate in apoAI+/+ and apoAI−/− fetal mice at 13 and 17 days into gestation. Matings were similar to those described in Fig. 2. Two fetuses were analyzed from each dam. Sterol synthesis rates were measured in each fetus and presented as nmol ^3H_2O converted to cholesterol per h per g fetus (A) and per fetus (B). Each value represents means ± 1 SEM for tissues from 14–16 dams. Synthesis rates were similar at 13 and 17 days of gestation.

from de novo synthesis or from exogenous sources, such as the yolk sac or placenta. As a first step in the elucidation of the process responsible for the smaller fetus with less cholesterol, sterol synthesis rates were measured in the fetuses. At 13 days into gestation, synthesis rates were similar between the apoAI+/+ and apoAI−/− fetuses (824 ± 57 and 917 ± 42 nmol/h per g, respectively; **Fig. 3A**). By 17 days of gestation, synthesis rates had decreased in all fetuses to ~600 nmol/h per g. When tissue mass was taken into account, synthesis rates in the whole fetus remained similar between the two types of mice (Fig. 3B).

Even though a lack of apoA-I-containing HDL did not affect sterol synthesis rates in the fetus, it is possible that a difference in HDL-C concentration could affect sterol metabolism in the tissues that sequester the fetus. Interestingly, the yolk sac of the apoAI−/− mice synthesized ~50% more sterol than the yolk sac of the apoAI+/+ mice (**Fig. 4A**). Even though sterol synthesis rates were greater, cholesterol concentrations of the yolk sacs of the apoAI−/− mice were significantly lower compared to the apoAI+/+ mice (Fig. 4B). The decrease in yolk sac cholesterol concentration in the apoAI−/− mice was maintained through 17 days of gestation. Similar to the yolk sac, synthesis rates in the placenta at 13 days into gestation were also ~50% greater in apoAI−/− mice than the control mice (**Fig. 5A**). By 17 days of gestation, placental sterol synthesis rates were similar between the two types of mice. Unlike the yolk sac, the placenta had similar cholesterol concentrations regardless of the concentration of maternal HDL-C (Fig. 5B).

Previous studies have shown that the amount of ^{3}H-DPS, or radiolabeled sterol, in some tissues is directly proportional to the amount of ^{3}H-DPS in the blood, and the amount of ^{3}H-DPS in the blood parallels the hepatic sterol synthesis rates (5, 34). Consequently, the amount of ^{3}H-DPS in the yolk sac and placenta of the apoAI−/− mice could be the result of more ^{3}H-DPS in the blood of the apoAI−/− animals. The amount of ^{3}H-DPS in the blood of apoAI+/+ and apoAI−/− dams at 13 days of gestation was 70 ± 8 and 45 ± 7 nmol ^3H_2O converted to sterol/h per ml, respectively, and 39 ± 2 and 34 ± 2 nmol/h per ml in the apoAI+/+ and apoAI−/− dams at 17 days of gestation, respectively. Even though there was less ^{3}H-DPS in the blood of the apoAI−/− mice, it is possible that a greater proportion of the radiolabeled DPS could have been cleared by the yolk sac and placenta in these mice.

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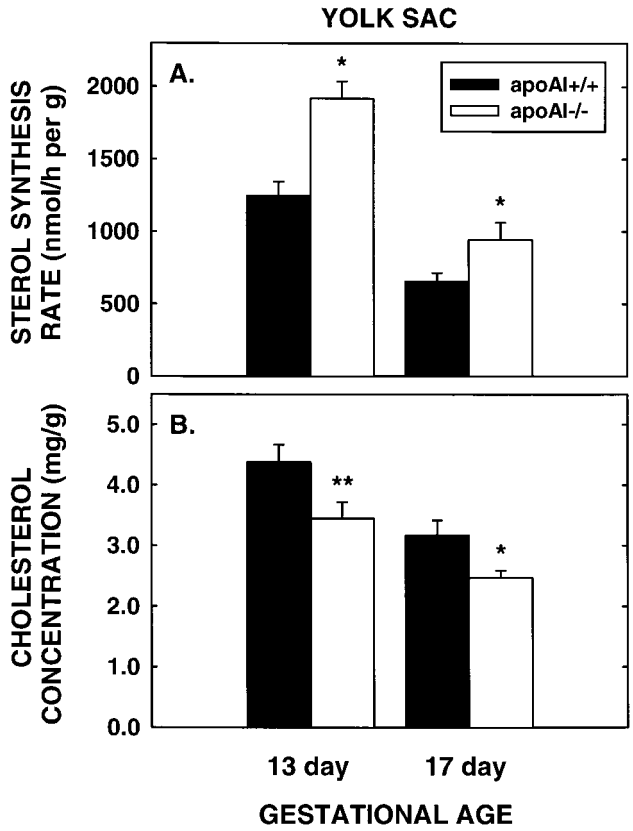

Fig. 4. Sterol synthesis rate and cholesterol concentration in the yolk sac of apoAI+/+ and apoAI−/− mice at 13 and 17 days into gestation. Matings were similar to those described in Fig. 2. Two sets of tissues were analyzed from each dam. Sterol synthesis rates were determined and presented as nmol ^3H_2O converted to sterol per h per g yolk sac (A). Cholesterol concentrations were measured and presented as mg cholesterol per g yolk sac (B). Each value represents means ± 1 SEM for tissues from 6–7 dams. The asterisk indicates that the values measured in the apoAI−/− mice were significantly different from the apoAI+/+ mice at the same gestational age ($P < 0.03$). Two asterisks indicates a strong trend for apoAI−/− mice to be significantly different from the apoAI+/+ mice at the same gestational age ($P = 0.05$).

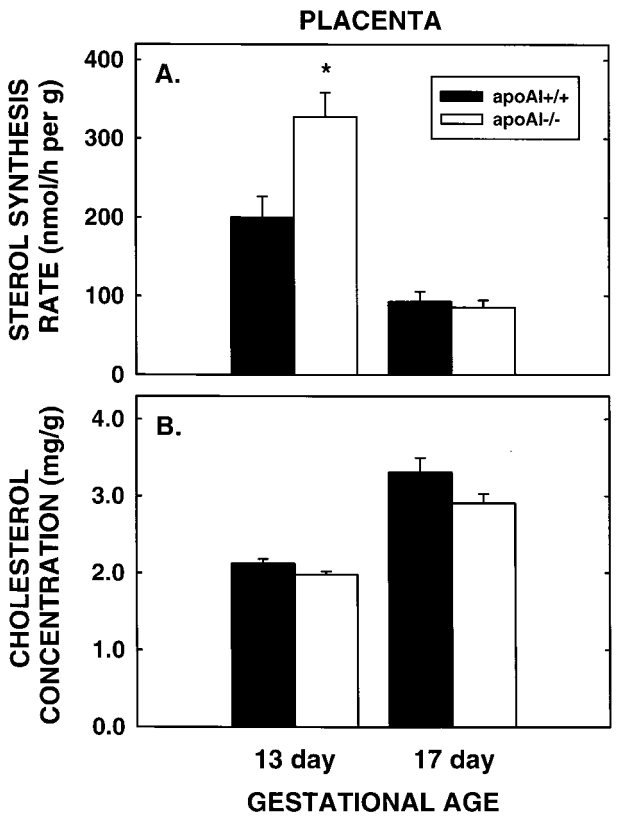

Fig. 5. Sterol synthesis rate and cholesterol concentration in the placenta of apoAI+/+ and apoAI−/− mice at 13 and 17 days into gestation. Matings were similar to those described in Fig. 2. Two sets of tissues were analyzed from each dam. Sterol synthesis rates were determined and presented as nmol ^3H_2O converted to sterol per h per g placenta (A). Cholesterol concentrations were measured and presented as mg cholesterol per g placenta (B). Each value represents means ± 1 SEM for tissues from 6–7 dams. The asterisk indicates that the values measured in the apoAI−/− mice were significantly different from the apoAI+/+ mice at the same gestational age ($P < 0.04$).

To reduce the amount of ^{3}H-DPS in the maternal blood to essentially zero and thereby negate the contribution of the maternal radiolabeled sterol to the extra-embryonic fetal tissues, dams of each genotype were fed 2% dietary cholesterol for 3 days prior to measurement of synthesis rates at a gestational age of 13. As expected, ^{3}H-DPS in the blood of the dams was much lower (~6 nmol/h per ml blood) due to suppressed hepatic sterol synthesis rates in the dam (5). More importantly, sterol synthesis rates in the yolk sac and placenta were still greater in the apoAI−/− dams (**Table 1**).

Finally, it is possible that the lower cholesterol content of the fetus and yolk sac of the apoAI−/− mice was due to a lack of apoA-I within the tissues themselves. To establish whether the primary effector of fetal size and cholesterol content was the lack of apoA-I in the fetal tissues, heterozygous females were mated with heterozygous males and the resultant apoAI+/+ and apoAI−/− fetal tissues were examined. Using this methodology, each fetus, regardless of genotype, would be exposed to the same maternal cholesterol concentration. As seen in **Fig. 6A**, both sets of fetuses weighed exactly the same (637 ± 16 mg). The fetal cholesterol content also was similar between the two genotypes of mice (Fig. 6B) due to like sizes and cholesterol concentrations (1.62 ± 0.02 mg cholesterol/g in the apoAI+/+ fetus vs. 1.56 ± 0.02 mg cholesterol/g in the apoAI−/− fetus). In addition, cholesterol concentration in the yolk sac (Fig. 4C) was similar in the apoAI+/+

TABLE 1. Sterol synthesis rates in apoAI+/+ and apoAI−/− mice fed dietary cholesterol for 3 days

Genotype of Dam	Yolk Sac	Placenta
	nmol ^3H_2O converted to sterol/h per g	
ApoAI+/+	820 ± 280	148 ± 25
ApoAI−/−	1762 ± 329	273 ± 54

All values represent means ± SEM for 3–4 sets of tissues in two dams.

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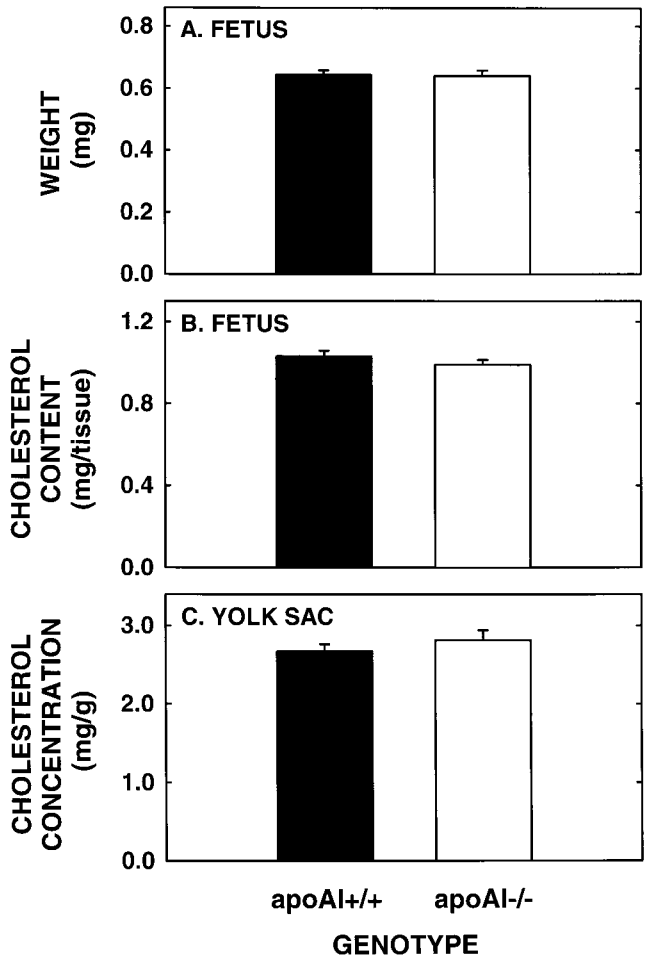

Fig. 6. Fetal weight, fetal cholesterol content and yolk sac cholesterol concentration in apoAI+/+ and apoAI−/− fetal tissues carried by heterozygous dams. Heterozygous dams were mated with heterozygous males and the resultant fetuses were genotyped as described in Material and Methods. The mass of the fetus was measured and presented as mg (A). The cholesterol content in the fetus is presented as mg cholesterol per fetus (B) and the cholesterol concentration in the yolk sac is presented as mg cholesterol per g yolk sac (C). The fetuses and yolk sacs from 10 litters yielded 22 apoAI+/+ and 20 apoAI−/− fetal units. There were no significant differences between genotypes for any of the values studied.

and apoAI−/− mice. Thus, a lack of apoA-I within fetal tissues was not responsible for the differences in fetal mass or cholesterol content nor the differences in yolk sac cholesterol concentration in the apoAI−/− fetuses carried by apoAI−/− dams.

DISCUSSION

The fetus is sequestered from direct contact with the maternal circulation. However, recent data suggest that maternal lipoproteins may be able to affect fetal metabolism via the two extra-embryonic fetal tissues that isolate the fetus. Supporting data for this concept is that, first, there are numerous lipoprotein receptors along the apical side of the visceral endoderm of the yolk sac and on the trophoblasts of the placenta (9, 11, 15, 17, 18, 22, 23). The receptors are functional in that maternal lipoproteins are taken up by the yolk sac and placenta at elevated rates as compared to other extra-hepatic tissues (5, 9). Second, the visceral endoderm and the trophoblasts have a significant amount of non-specific pinocytosis, especially the yolk sac (9, 35). Third, the yolk sac synthesizes lipoprotein particles that are secreted into the vitelline circulation and that are essential for normal growth (36, 37). To examine the impact of maternal cholesterol on the fetus in greater detail, sterol metabolism was studied in the fetus, yolk sac, and placenta of a hypocholesterolemic animal that births healthy pups, the apoAI−/− mouse.

In the present studies, fetuses carried in dams with low plasma cholesterol concentrations and no apoA-I were ~21% smaller and had a slightly lower cholesterol concentration as compared to fetuses carried by apoAI+/+ dams. As a result of the smaller mass and a lower cholesterol concentration, the apoAI−/− fetuses contained ~30% less cholesterol as compared to the wild-type fetuses. A difference in fetal size and cholesterol content would be due to either a change in fetal sterol synthesis rate or metabolism due to a lack of apoA-I within the fetus itself, or a change in sterol metabolism within the extra-embryonic fetal tissues. To begin to distinguish which process was responsible for the change in fetal mass, in vivo sterol synthesis rates were measured in both types of fetuses. Sterol synthesis rates were similar in all fetuses, regardless of genotype. Thus, the difference in size was not due to lower sterol synthesis rates in the fetuses of the apoAI−/− dams. To ascertain whether the lack of apoA-I within tissues was responsible for a change in metabolism leading to a difference in mass, heterozygote females were mated with like males to obtain fetuses that were of the apoAI+/+ or the apoAI−/− genotype. The novel aspect of these studies is that all fetuses of the apoAI+/− dams would be exposed to the same concentration of maternal lipoproteins. Interestingly, apoAI+/+ and apoAI−/− fetuses within apoAI+/− dams had similar mass and cholesterol content. Thus, the smaller fetuses were not the result of a lack of apoA-I within the fetuses themselves. Because the decrease in fetal mass was not the result of a decrease in sterol synthesis rate or a lack of fetal apoA-I, it must have resulted from a change in events occurring in the tissues that the fetus comes in direct contact with, the yolk sac and placenta.

As expected from the above discussion, sterol metabolism was affected within the yolk sac and placenta of dams lacking apoA-I. In the yolk sac at both 13 and 17 days into gestation and in the placenta at 13 days into gestation, sterol synthesis rates were ~50% greater in the tissues of the apoAI−/− mice. An increase in sterol synthesis rates is found in other tissues of apoA-I−/− mice that clear HDL at elevated rates, such as the adrenals (31, 38). An increase in tissue sterol synthesis rate is oftentimes indicative of a tissue in a negative sterol balance. A negative sterol balance results from a decrease in the amount of cholesterol entering cells, such as from less lipoprotein particles being taken up by the

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tissue, or by an increase in output. As the yolk sac and placenta take up maternal HDL at high rates, the negative sterol balance was most likely induced by a decrease in the amount of maternal-derived cholesterol presented to these tissues. The impact that the negative sterol balance has on sterol metabolism in the yolk sac or placenta will vary depending on the need of sterol for other processes within the tissues and on the degree to which sterol synthesis rates were induced.

The yolk sac has at least one role in fetal development that could be affected by a change in sterol balance. The endodermal cells of the visceral yolk sac synthesize and secrete lipoproteins. A number of intracellular components have been identified that affect lipoprotein formation and secretion by cells, including various lipids and proteins (39, 40). One of the lipid components found to affect lipoprotein formation in hepatocytes is intracellular cholesterol (39, 40). In the present studies, the yolk sacs of the apoAI−/− mice had cholesterol concentrations that were significantly lower than those in the wild-type mice. If lipoprotein secretion in the yolk sac is regulated by intracellular cholesterol levels, as in other tissues, the yolk sac of apoAI−/− mice might secrete fewer lipoproteins into the vitelline circulation than its control counterpart. Assuming this is true, the apoAI−/− fetus would receive lesser amounts of exogenous cholesterol, triglyceride and/or fat soluble vitamins to be used for energy, membranes, or as cofactors, thereby resulting in a smaller fetus. Support for this is that the cholesterol concentrations of the yolk sacs in apoAI+/− dams were similar to one another, regardless of genotype, as were fetal masses. It is presently unknown whether the cholesterol within the yolk sac-derived lipoprotein particles are those synthesized de novo, those taken up from the maternal circulation, or a combination of both.

The placenta has two possible roles in fetal development that could be affected by sterol balance. First, the placenta synthesizes steroidogenic hormones, such as progesterone (41). Using ovariectomized animals (42, 43) and animals infused with antibodies to progesterone (44), it has been shown that a lack of progesterone can delay implantation or reduce the number of embryos per dam. The decrease in progesterone that is required for these effects to occur appears to be dramatic, however. Second, the placenta recently has been shown to secrete lipid droplets into the fetal circulation (45). The lipid composition of the droplets is presently unknown. The droplets appear to be critical for development as embryos in animals devoid of the droplets are resorbed by gestational day 10 (45).

Because sterol synthesis rates were increased in the yolk sac and placenta of the apoAI −/− mice, it is not known why sterol synthesis rates were not elevated in the smaller apoAI−/− fetus, especially if the fetus is believed to receive less cholesterol from the yolk sac. One possible explanation is that fetal synthesis rates cannot be enhanced without an adequate supply of substrate. The apoAI−/− fetus could be lacking in acetyl units due to a deficiency in exogenous triglyceride-containing lipoproteins. A second possible explanation is simply that sterol synthesis rates cannot be induced in the fetus.

Based on the knowledge that the yolk sac and placenta remove HDL at relatively elevated rates (9) and that blocking HDL uptake is lethal in rats (15), one might initially assume that low maternal HDL-C concentrations, such as in the apoAI−/− mouse, would result in a severely compromised fetus. Surprisingly, the decrease in mass of fetuses in apoAI−/− versus wild-type dams was only modest. Part of the reason for the small effect was that the supporting extra-embryonic fetal tissues of apoAI−/− mice compensated for, at least partially, the decrease in the amount of HDL taken up by apoAI−/− tissues as a result of lower maternal HDL-C concentrations or abnormal HDL particles. Additional compensatory mechanisms may have existed in these tissues, such as an increase of LDL uptake (38). Another possible reason for the absence of a dramatic effect could be that the apoE/apoA-II-containing HDL of the apoAI−/− mice (19) were taken up from the maternal circulation by the yolk sac and placenta. SR-BI, cubulin, and gp330 are expressed in the mouse yolk sac and placenta (11, 14–17) and will theoretically bind the apoE/apoA-II-containing HDL. If the receptors can bind and internalize the apoE/apoA-II-containing HDL in vivo, a significant amount of lipoproteins may be internalized in the extra-embryonic tissues of the apoAI−/− mice that have high levels of HDL-C during gestation.

To summarize, these are the first studies to show a direct effect of maternal HDL-C concentration or HDL composition on fetal development. The effect could be through *1*) lower cholesterol concentrations in the yolk sac leading to fewer lipoproteins being secreted to the embryo, *2*) lower amounts of cholesterol being transported from the placenta to the fetus, *3*) lower maternal progesterone concentrations leading to delayed development, or *4*) any combination of these possibilities. These data also demonstrate for the first time the ability of the supporting fetal tissues in the rodent to compensate, at least partially, for a lack of cholesterol in the maternal circulation. It is perhaps this capacity to balance low maternal cholesterol concentrations with elevated sterol synthesis rates that allows dams to birth normal pups, regardless of plasma cholesterol levels. The role of maternal cholesterol on fetal development in the human, as in the rodent, has not been studied to a large degree partly because females with a broad range of cholesterol concentrations give birth to normal babies. The present data suggest that the apparent lack of effect of maternal plasma cholesterol concentration may be the result of the ability of supporting fetal tissues to compensate for differences in maternal cholesterol concentrations.

The authors wish to thank Raj Swain for his excellent technical assistance. These studies were supported by grants HD-34089 and DK-54504 from the National Institutes of Health. Dr. Woollett is a recipient of an Established Investigator Award from the American Heart Association.

Manuscript received 9 August 1999 and in revised form 17 December 1999.

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