

# Transport of maternal LDL and HDL to the fetal membranes and placenta of the Golden Syrian hamster is mediated by receptor-dependent and receptor-independent processes

Kathleen L. Wyne\* and Laura A. Woollett<sup>1,†</sup>

Departments of Molecular Genetics\* and Internal Medicine,<sup>†</sup> University of Texas Southwestern Medical Center, Dallas, TX 75235-8887

**Abstract** Maternal lipoproteins provide nutrients to the fetus via the placenta, yolk sac, and uterine membrane plus decidua. To determine the transport processes that are responsible for the removal of lipoproteins from the maternal circulation, we measured the clearance rates of maternal LDL and HDL in vivo, as well as the tissue distribution of expression of the LDL receptor, glycoprotein 330 (gp330) and the newly described HDL receptor, SR-BI, in the placenta, yolk sac, and uterine membrane plus decidua at mid- and late-gestation of the hamster. In mid-gestation (day 10.5), LDL clearance rates of the placenta and yolk sac were similar to those in the liver ( $\approx 100 \mu\text{l/h per g}$ ) and higher than those in the decidua ( $18 \pm 3 \mu\text{l/h per g}$ ). Clearance rates for HDL-apoA-I and HDL-cholesteryl ether were similar to those of LDL in the placenta and decidua whereas rates in the yolk sac were dramatically higher ( $>1700 \mu\text{l/h per g}$ ). Additionally, albumin was cleared in the placenta and decidua at  $\approx 16 \mu\text{l/h per g}$  whereas the yolk sac cleared the protein at much higher rates ( $196 \pm 22 \mu\text{l/h per g}$ ). Low levels of LDL receptor were detected by immunoblot analysis in the placenta with trace amounts in the yolk sac. Gp330 and SR-BI were both barely detectable in the placenta but were expressed at high levels in the yolk sac. As gestation progressed to day 14.5, LDL and HDL clearance rates decreased in all three tissues; immunodetectable LDL receptor decreased in the placenta whereas the expression of gp330 and SR-BI in the placenta and yolk sac remained relatively constant. **These data suggest that the clearance of maternal lipoproteins by the placenta, yolk sac, and decidua are mediated by receptor-mediated as well as receptor-independent processes.**—Wyne, K. L., and L. A. Woollett. Transport of maternal LDL and HDL to the fetal membranes and placenta of the Golden Syrian hamster is mediated by receptor-dependent and receptor-independent processes. *J. Lipid Res.* 1998. **39**: 518–530.

**Supplementary key words** cholesterol • LDL receptor • SR-BI • gp330

Tissues that are directly involved with fetal development, including the fetus, placenta, yolk sac, and decidua, require a large amount of cholesterol for mem-

brane formation and maintenance as well as hormone synthesis. The necessary sterols can either be synthesized endogenously or acquired from circulating lipoproteins (1). Rates of sterol synthesis in the fetus, placenta, and yolk sac are greater than in other extrahepatic tissues (2–8) but cannot account for all the sterol that is accrued in these tissues in the hamster in mid- to late-gestation (7). Clearance rates for maternal low density lipoprotein (LDL) also are high in the placenta and yolk sac during this stage of gestation (6, 7) and could be a source of a significant amount of sterol. The sterols most likely support tissue growth and hormone synthesis (7, 9, 10) but may additionally be transferred to the fetus via apolipoprotein B (apoB)-containing lipoproteins (11–13).

The receptors that mediate the uptake of maternal LDL by extra-embryonic fetal tissues, such as the placenta and yolk sac, have not been clearly delineated. Several different lipoprotein receptors are expressed in the placenta, including the low density lipoprotein receptor, which is responsible for the removal of apoB-100- and apolipoprotein E (apoE)-containing lipoproteins (14), and an acetylated LDL receptor (15), which binds and internalizes negatively charged (modified) lipoproteins as in macrophages (16). Two other members of the LDL receptor gene family that are found to be expressed in the placenta (17, 18), the

Abbreviations: LDL, low density lipoprotein(s); apoB-100, apolipoprotein B-100; apoE, apolipoprotein E; VLDL, very low density lipoprotein(s); apoA-I, apolipoprotein A-I; SR-BI, scavenger receptor type BI; TCB, tyramine cellobiose; mhLDL, methylated human LDL; LDL- and HDL-C, cholesterol carried in LDL and HDL fractions, respectively; HDL, high density lipoprotein(s); gp330, glycoprotein 330; hh, hedgehog.

<sup>†</sup>To whom correspondence should be addressed at present address: Department of Pathology and Laboratory Medicine, University of Cincinnati Medical School, 231 Bethesda Avenue, Cincinnati, OH 45267.

very low density lipoprotein (VLDL) receptor and LDL receptor-related protein, also bind apoE-containing lipoproteins in vitro (19, 20). In the yolk sac, still another member of the LDL receptor gene family, glycoprotein 330 (gp330), is found in large amounts (21, 22). Gp330 binds and internalizes lipoproteins containing either apoB-100 and/or apoE in tissue culture cells (19, 23). The relative roles each of these receptors play in development of fetal tissues and membranes are not known but there may be some redundancy in receptor function as mice lacking various proteins involved in transport of lipids, such as the VLDL receptor (24) and the LDL receptor (25), are fertile.

In addition to apoB- and apoE-containing lipoproteins, high density lipoproteins (HDL) also bind to trophoblastic cells in vitro (26, 27), are cleared by the placenta in vivo (7) and stimulate production of progesterone (28). There are two different processes responsible for transport of HDL components into cells. The first is by whole particle uptake, as measured by clearance of HDL-apolipoprotein A-I (apoA-I) (29, 30). Selective uptake of the lipid component of HDL by tissues can also occur (29–32). This selective uptake is mediated by a new member of the scavenger receptor family called the scavenger receptor, class B, type I (SR-BI). Expression of the recombinant protein in cultured cells is associated with cell surface binding of HDL and transport of the lipid from the particles to the cell without any associated degradation of apoA-I or apoA-II (31). SR-BI is located in highest concentrations in tissues that selectively take up significant amounts of HDL-associated lipids from the circulation, such as the liver, adrenal glands, ovaries, and testes (30–33). Only trace amounts of SR-BI are present in the placenta of the rat and mouse (31, 32).

The present studies were undertaken to begin to examine the different pathways by which LDL and HDL are removed by the tissues that directly support fetal development. In addition, the tissue distribution of the receptors shown to remove LDL and HDL, such as the LDL receptor, gp330, and SR-BI, was determined in the placenta and yolk sac due to their relatively high clearance rates of LDL and HDL. The studies were completed in the female hamster because it is one of the few species that resembles humans in that it becomes hypercholesterolemic late in pregnancy (7, 34–36).

## METHODS

### Animals

Male and non-pregnant female Golden Syrian hamsters (*Mesocricetus auratus*) (Charles River Laboratories,

Kingston, NY), weighing 100 and 90 g, respectively, were housed in colony cages in a temperature and humidity controlled room. Animals were subjected to 12 h of darkness and 12 h of light and were fed ad libitum a ground cereal-based diet (Rodent Diet no. 8604) (Teklad Premier Laboratory Diets, Madison, WI) which had an inherent cholesterol and triacylglycerol content of 0.02% and 5% (wt/wt), respectively. After 1 week of acclimation, animals were mated (7), and the dams were studied at days 10.5 and 14.5 of gestation. Females not mated were used as non-pregnant controls. All of the animal experiments were approved by the Institutional Animal Care and Research Advisory Committee of the university.

### Determination of tissue cholesterol content

Dams were anesthetized, arterial blood was collected, and the fetus, placenta, yolk sac plus amnion, and remaining uterine membrane plus decidua were isolated. The placenta included both the maternal- and fetal-derived portions. Plasma was separated into LDL (1.020–1.063 g/ml) and HDL (>1.063 g/ml). Lipoprotein fractions and tissues were saponified, and the amount of cholesterol was measured by GLC using stigmastanol as an internal standard (37).

### Determination of native and methylated human LDL clearance and uptake rates in vivo

Plasma was isolated from blood of hamsters that had been maintained on a standard cereal-based diet and from human donors. LDL in the density range of 1.020–1.055 g/ml was isolated from both species and labeled with [<sup>125</sup>I]tyramine cellobiose (TCB) (38) or [<sup>131</sup>I] (39). The radiolabeled human LDL was extensively methylated (mhLDL) prior to use (40). The [<sup>125</sup>I]TCB-labeled LDL and mhLDL (41) were given as a primed-continuous infusion via a femoral catheter for 4 h. The infusion rates for LDL (10% of bolus) and mhLDL (3% of bolus) were determined as those that would maintain a constant dpm of <sup>125</sup>I/ $\mu$ l plasma of non-pregnant females over the 4-h infusion. Infusion rates of all lipoproteins and proteins described in this paper were determined in the same manner. All initial boluses were  $\approx$ 5,000,000 dpm in 0.5 ml. The <sup>131</sup>I ( $\approx$ 500,000 dpm in 0.5 ml) was injected via the catheter 10 min before exsanguination to correct for plasma contamination (42–44). After 4 h, animals were anesthetized, exsanguinated, and tissues were excised. Control livers from non-pregnant females, maternal livers, placentas, yolk sacs, and surrounding membranes were assayed for <sup>125</sup>I and <sup>131</sup>I. Using the average dpm of <sup>125</sup>I in the plasma of each dam over the 4-h infusion and the dpm of <sup>131</sup>I at 10 min, the rates of LDL clearance by the various tissues were calculated (42). Data are presented as  $\mu$ l of

plasma cleared of its LDL content per h per g tissue. Clearance rates were converted to uptake rates by multiplying the rate of LDL clearance by the concentration of cholesterol circulating in LDL (1.020–1.063 g/ml) (LDL-C) in the pregnant dam. The total LDL cleared by both receptor-mediated as well as receptor-independent processes was determined in hamsters infused with hamster LDL. Receptor-independent transport was measured with mhLDL. Receptor-mediated transport was the difference between total and receptor-independent clearance rates.

#### Determination of HDL-apoA-I clearance and uptake rates in vivo

HDL (1.070–1.210 g/ml) was isolated from plasma of donor hamsters fed a standard cereal-based diet. The HDL was labeled with [<sup>125</sup>I]TCB (38) or <sup>131</sup>I (39). Tissue clearance rates for HDL-apoA-I (41) were measured using a primed-continuous infusion of [<sup>125</sup>I]TCB-labeled HDL followed by an infusion of <sup>131</sup>I-HDL 10 min prior to exsanguination. Radiolabeled HDL-apoA-I was infused for 4 h at an hourly rate that was 5% of the bolus. Tissues were excised and calculations were determined as described in the previous section. Clearance rates were converted to uptake rates of HDL-C by multiplying the rate of HDL clearance by the concentration of circulating HDL-C ( $d > 1.063$  g/ml) in the pregnant dam.

#### Determination of HDL-cholesteryl ether clearance rates in vivo

Isolated hamster HDL was labeled with [ $1\alpha,2\alpha(n)$ -<sup>3</sup>H]cholesterol oleyl ether and [cholesteryl-4-<sup>14</sup>C]oleate as described previously (41). Primed-continuous infusions of the [ $1\alpha,2\alpha(n)$ -<sup>3</sup>H]oleyl ether-labeled HDL (41) were maintained for 4 h or 45 min. [<sup>3</sup>H]oleyl ether-labeled HDL was infused at an hourly rate that was 15% of the bolus. Ten min before the completion of either infusion, [<sup>14</sup>C]cholesteryl ester-labeled HDL was infused via the catheter to determine plasma contamination of tissues.

Plasma from each dam was separated into non-HDL ( $d < 1.063$  g/ml) and HDL ( $d > 1.063$  g/ml) fractions by preparative ultracentrifugation. Percentage of <sup>3</sup>H in each fraction was determined at 45 min and 4 h. In the pregnant dam,  $9.0 \pm 0.7\%$  and  $32.5 \pm 1.3\%$  of labeled cholesteryl ether was transferred to non-HDL particles ( $d < 1.063$  g/ml) after 45 min and 4 h of the infusion, respectively. Clearance rates for HDL-cholesteryl ether were determined using average dpm of <sup>3</sup>H/ $\mu$ l HDL. It should be noted that as a result of the transfer of radiolabel between lipoproteins, some of the radiolabel within tissues could be radiolabeled LDL. The amount of label removed as LDL should be minimal in that

LDL clearance rates were either equal to or less than those for HDL, and a relatively small amount of label was transferred over the entire infusion period. Regardless, all cholesteryl ether taken up by the tissues originated as HDL.

#### Determination of albumin clearance rates in vivo

Hamster albumin (Sigma Chemical Co., St. Louis, MO) was labeled with [<sup>125</sup>I]TCB (38) or <sup>131</sup>I (39). Labeled albumin (41) was infused at an hourly rate that was 2% of the bolus. The study was performed as described above.

#### Immunoblot analysis of hamster tissues

Hamster tissues were frozen in liquid N<sub>2</sub> immediately after harvesting and stored at  $-80^{\circ}\text{C}$ . Membranes were prepared as described previously (31, 32). Immunoblot analyses were performed using a polyclonal rabbit anti-bovine LDL receptor antibody (IgG-713) (45), a polyclonal rabbit anti-rat gp330 antibody (IgG-611) (46), and a polyclonal rabbit antipeptide antibody corresponding to residues 495 to 509 of human SR-BI (IgG-Q820) (47).

#### Immunocytochemical localization of SR-BI

Female hamsters that were at day 10.5 of gestation were anesthetized and the intact placenta, yolk sac, and fetus were excised and placed in fixative A (3% [wt/vol] *p*-formaldehyde, 3 mmol/L trinitro-phenol, 5 mmol/L MgCl<sub>2</sub> in 50 mmol/L Sorenson's phosphate buffer, pH 7.4) for 24 h (48). Fixed tissues were rinsed three times with PBS then processed for conventional paraffin embedding and sectioning. Deparaffinized sections were either stained with hematoxylin and eosin or immunostained after heating for 5 min  $\times$  2 in Antigen Retrieval AR-10 solution (BioGenex, San Ramon, CA) once endogenous peroxidase activity had been quenched with a 20-min immersion in 0.3% hydrogen peroxide in 20% methanol. The sections were incubated for 30 min in buffer B (0.2 mol/L NaCl, 25 mm HEPES, pH 8.0, 0.5% [wt/vol] BSA) to block nonspecific binding sites followed by an incubation for 1 h with either preimmune IgG (5  $\mu$ g/mL) or IgG-Q820 (5  $\mu$ g/mL) in buffer B. The sections were rinsed for 10 min  $\times$  2 with buffer C (0.2 mol/L NaCl, 25 mm HEPES, pH 8.0, 0.1% [wt/vol] BSA), incubated for 30 min with a biotinylated goat anti-rabbit IgG (30  $\mu$ g/ml) in buffer B, rinsed for 10 min with buffer C, rinsed in PBS and incubated with an avidin-biotinylated peroxidase complex (Vector ABC Elite Kit, Vector Laboratories, Burlingame, CA) in PBS as suggested by the manufacturer. Antibody binding was visualized with Vector VIP substrate then counterstained with Gill's hematoxylin.

### Immunocytochemical localization of human apoA-I

Femoral catheters were implanted into female hamsters that were at day 10.5 of gestation. A 0.5 ml bolus of human HDL (containing 10 mg of cholesterol) was given to pregnant dams followed by an infusion of lipoprotein at an hourly rate that was 15% of the bolus. A second set of pregnant dams was bolused and infused with sterile saline. One h later, animals were anesthetized and the intact placenta, yolk sac, and fetus were excised. Tissues were prepared and incubated as described above for the immunocytochemical localization of SR-BI except the primary antibody was either non-immune (5  $\mu\text{g}/\text{ml}$ ) (data not shown) or a monoclonal mouse anti-human apoA-I (5  $\mu\text{g}/\text{ml}$ ) (Pierce, Rockford, IL). The specificity of the anti-human apoA-I antibody was verified by immunoblot analysis of human plasma, hamster plasma, saline-infused hamster plasma, and human HDL-infused hamster plasma; apoA-I was only detected in the human plasma and human HDL-infused hamster plasma (data not shown).

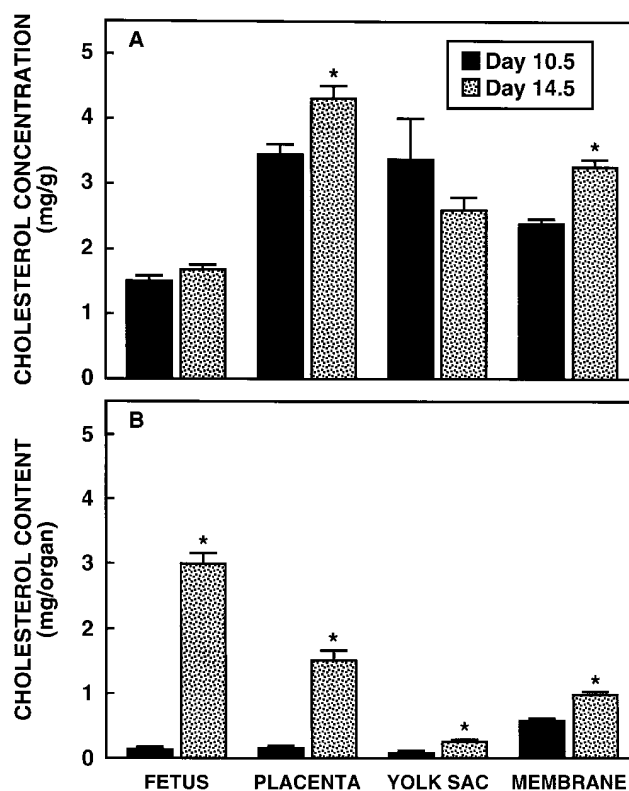
### Statistical analysis

Values are presented as means  $\pm$  SEM for livers or summed fetus, placenta, yolk sac, or decidua in each dam. Significant ( $P \leq 0.05$ ) changes between ages were determined using a Student's *t*-test.

## RESULTS

The concentration and content of cholesterol was measured in the fetus, placenta, yolk sac, and uterine membrane plus decidua at day 10.5 and day 14.5 of gestation. During these 4 days, cholesterol concentration did not change significantly in the fetus or yolk sac and increased modestly in the placenta and uterine membrane (Fig. 1A). In contrast, the sterol content of the fetus, placenta, yolk sac, and uterine membrane increased 25-, 10-, 3.6-, and 1.7-fold, respectively (Fig. 1B). The increases in cholesterol content in the fetus and yolk sac were due predominantly to an increase in tissue mass. In the placenta and uterine membrane, the increases in cholesterol content were due to an increase in both cholesterol concentration and tissue weight. The mass of the fetus, placenta, yolk sac, and uterine membrane at day 10.5 of gestation was  $0.08 \pm 0.01$ ,  $0.04 \pm 0.01$ ,  $0.02 \pm 0.001$ , and  $0.24 \pm 0.003$  mg, respectively, and increased to  $1.83 \pm 0.10$ ,  $0.39 \pm 0.04$ ,  $0.11 \pm 0.01$ , and  $0.30 \pm 0.01$  mg by day 14.5.

The clearance rates of hamster LDL at day 10.5 (Fig. 2A) and day 14.5 (Fig. 2B) of gestation were examined first. The total clearance rates of LDL were similar in

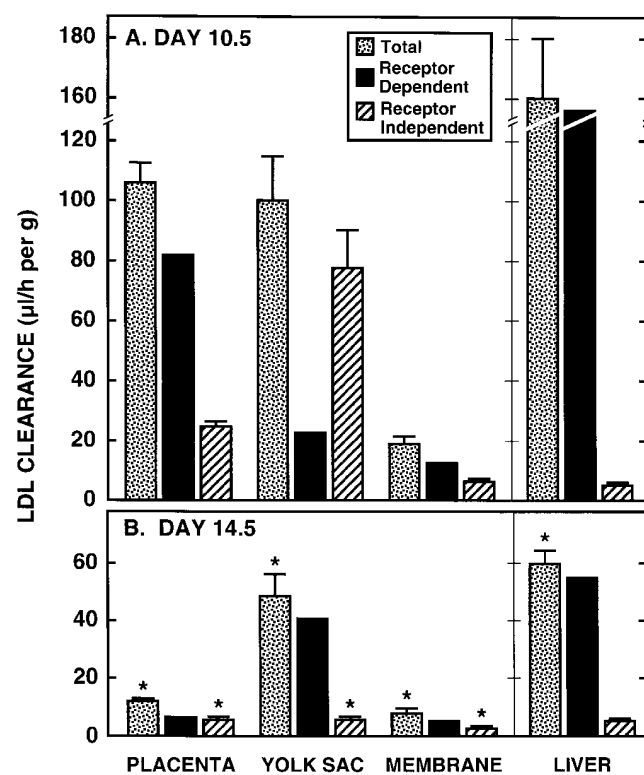


**Fig. 1.** Cholesterol concentration (A) and content (B) in fetal tissues of hamsters at day 10.5 and day 14.5 of gestation. Dams were fed a cereal-based diet throughout gestation. At the appropriate age, dams were killed and fetuses, placentas, yolk sacs plus amnions, and uterine membranes plus deciduas were removed from each dam. Placentas included the fetal plus maternal portions. Values represent means  $\pm$  1 SEM for summed fetal tissues (3–7 fetal units) in 4 dams at each gestational age. Significant differences ( $P \leq 0.05$ ) between gestational ages are shown.

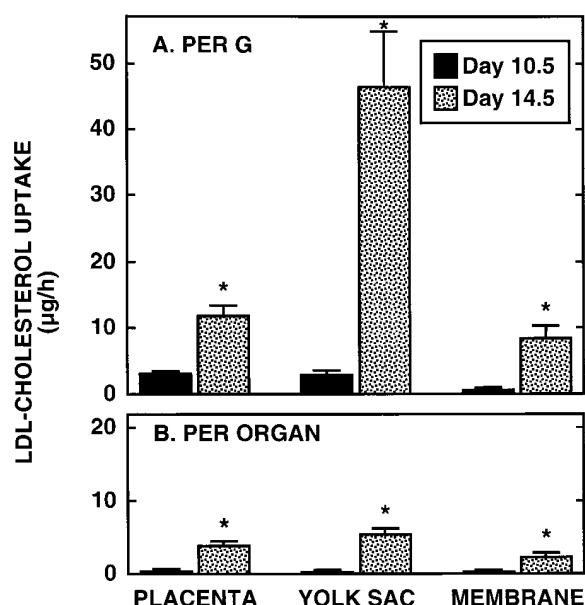
the placenta at day 10.5 and the liver of a non-pregnant female ( $86.9 \pm 8.9 \mu\text{l}/\text{h per g}$ ) (data not shown). The liver of the non-pregnant female is used for comparison because LDL clearance is increased in the liver of the pregnant hamster (Fig. 2A). In the placenta, mhLDL, an indicator of receptor-independent clearance, was cleared at 23% of the rate of homologous LDL, an indicator of total clearance, which was higher than that observed in the non-pregnant liver (6%). By day 14.5, total, receptor-mediated and receptor-independent clearance rates in the placenta decreased dramatically. Although the total LDL clearance rate in the yolk sac at day 10.5 was similar to the placenta and control liver, the proportion cleared as mhLDL was much greater (78%). As gestation progressed to day 14.5, total and mhLDL clearance rates decreased in this tissue. Similarly, total and mhLDL clearance rates in the uterine membrane decreased between day 10.5 and day 14.5 though not as dramatically as in the other two fetal tissues. Receptor-

mediated clearance in this tissue accounted for a majority ( $\approx 63\%$ ) of the total transport and decreased with the progression of gestation. Finally, clearance rates were measured in the maternal liver, a tissue that contains a significant amount of LDL receptor activity (1, 43). At day 10.5 of gestation, clearance rates were 2-fold greater in the liver of the pregnant dam as compared to the non-pregnant control. A majority of this transport was receptor-mediated (96%). As gestation progressed, total and receptor-mediated clearance decreased, whereas receptor-independent clearance remained constant.

To ascertain the actual mass of lipoprotein-cholesterol removed by each tissue ( $\mu\text{g}/\text{h}$  per g tissue or per



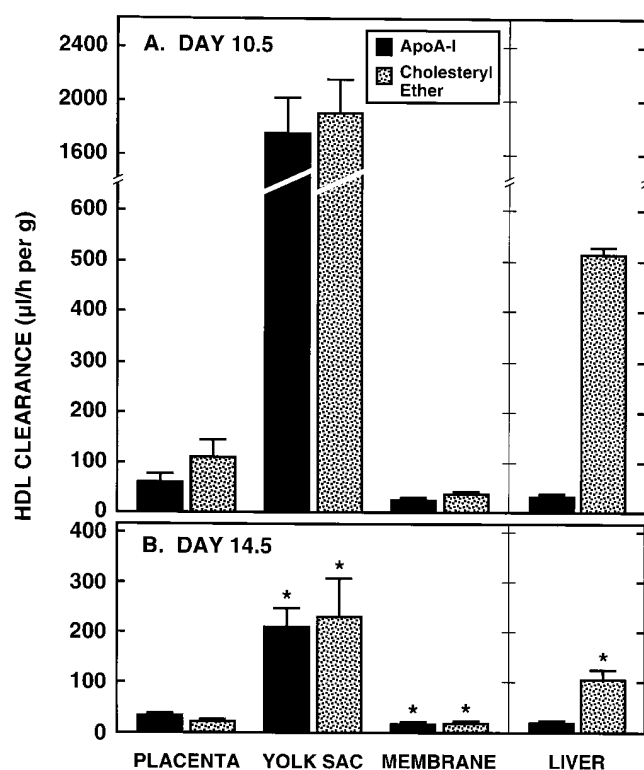
**Fig. 2.** Rates of plasma cleared of LDL in hamsters at day 10.5 (A) and day 14.5 (B) of gestation. Clearance rates were determined in vivo using a primed-continuous infusion of [ $^{125}\text{I}$ ]TCB-labeled hamster LDL to measure total LDL clearance rates and [ $^{125}\text{I}$ ]TCB-labeled methylated human LDL to determine non-receptor-mediated clearance. Receptor-mediated clearance was the difference between total and receptor-independent rates. After the infusions, dams were killed and livers, placentas, yolk sacs plus amnions, and uterine membranes plus deciduas were removed from each pregnant dam. Livers were removed from non-pregnant dams. Data are expressed as  $\mu\text{l}$  of plasma cleared of its LDL per h per g tissue. Values represent means  $\pm 1$  SEM for summed fetal tissues (2–4 fetal units) and maternal livers in 3–11 dams at each gestational age. Significant differences ( $P \leq 0.05$ ) between gestational ages are shown.



**Fig. 3.** Rates of uptake of LDL-C in fetal tissues of hamsters at day 10.5 and day 14.5 of gestation. The clearance rates for total LDL in Fig. 2 were multiplied by the LDL-C concentrations to determine uptake of LDL-C in the placenta, yolk sac and uterine membrane. Data are presented as  $\mu\text{g}$  of LDL-C taken up per h per g tissue (A) and per organ (B). Significant differences ( $P \leq 0.05$ ) between gestational ages are shown.

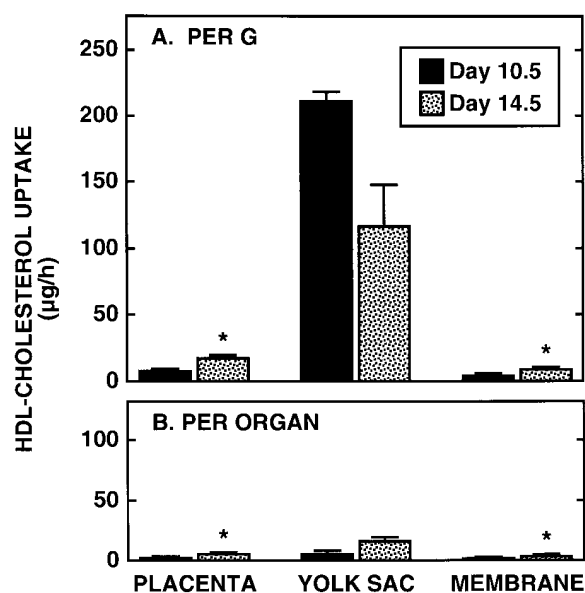
organ), the LDL clearance rates were multiplied by maternal LDL-C concentration (mg/dl). Maternal plasma LDL-C levels increased 27-fold between day 10.5 ( $3.8 \pm 0.8$  mg/dl) and day 14.5 ( $103.0 \pm 6.2$  mg/dl) of gestation. The amount of LDL-C that was taken up per g tissue increased 4-fold, 15-fold, and 14-fold in the placenta, yolk sac, and decidua, respectively, between day 10.5 and day 14.5 of gestation (Fig. 3A). When organ weight was taken into account (Fig. 3B), the increase in uptake rates during gestation was more dramatic (13-fold, 77-fold, and 24-fold in the placenta, yolk sac, and uterine membrane, respectively, between days 10.5 and 14.5). This dramatic increase in uptake in all three tissues was due to an increase in substrate (LDL-C) and tissue weight.

To determine whether HDL was taken up as a whole particle or whether the cholesteryl ester component was selectively delivered to these tissues, both the apoA-I and the cholesteryl ester moieties of HDL were labeled, and clearance rates were determined at day 10.5 (Fig. 4A) and day 14.5 (Fig. 4B) of gestation. In the control liver, which has been previously shown to selectively remove HDL-cholesteryl ester (29, 41), clearance of HDL-apoA-I was relatively low at  $6.0 \pm 0.5$   $\mu\text{l}/\text{h}$  per g whereas clearance of HDL-cholesteryl ether was much greater ( $164 \pm 17$   $\mu\text{l}/\text{h}$  per g) (data not shown). The



**Fig. 4.** Rates of plasma cleared of HDL in fetal tissues of hamsters at day 10.5 (A) and day 14.5 (B) of gestation. Clearance rates were determined in vivo using a primed-continuous infusion of [ $^{125}$ I]TCB-labeled hamster HDL-apoA-I or  $^3$ H-labeled hamster HDL-cholesteryl ether. After the infusions, dams were killed and livers, placentas, yolk sacs plus amnions, and uterine membranes plus deciduas were removed from each dam. Livers were removed from non-pregnant dams. Data are expressed as  $\mu$ l of plasma cleared of its HDL-apoA-I or HDL-cholesteryl ether per h per g tissue. Values represent means  $\pm$  1 SEM for summed fetal tissues (3 fetal units) and maternal livers in 3 dams at each gestational age. Significant differences ( $P \leq 0.05$ ) between gestational ages are shown.

clearance rates of HDL-apoA-I in the placenta at day 10.5 of gestation were 10-fold greater than those in the liver of a non-pregnant female and were not significantly different from those for HDL-cholesteryl ether. In the yolk sac, clearance of HDL-apoA-I and HDL-cholesteryl ether was extremely elevated as compared to the other tissues and the control liver. As in the placenta, clearance rates for both moieties decreased to  $\approx 210 \mu\text{l/h per g}$  at day 14.5. Clearance rates in the uterine membrane were similar for both HDL-apoA-I and HDL-cholesteryl ether at day 10.5 ( $\approx 28 \mu\text{l/h per g}$ ) and decreased by day 14.5 ( $\approx 14 \mu\text{l/h per g}$ ) of gestation. In the maternal liver, clearance rates for apoA-I remained relatively constant between days 10.5 and 14.5 of gestation whereas clearance rates for cholesteryl ether decreased 80% by day 14.5 of gestation. In all



**Fig. 5.** Rates of uptake of HDL-C in fetal tissues of hamsters at day 10.5 and day 14.5 of gestation. The clearance rates for HDL-apoA-I in Fig. 4 were multiplied by the HDL-C concentrations to determine uptake of HDL-C in the placenta, yolk sac, and uterine membrane. Data are presented as  $\mu\text{g}$  of HDL-C taken up per h per g tissue (A) and per organ (B). Significant differences ( $P \leq 0.05$ ) between gestational ages are shown.

three tissues supporting fetal development, but not the liver, clearance rates of the protein and lipid moieties of HDL were strikingly similar, raising the possibility that the whole HDL particle was internalized.

Assuming that HDL-apoA-I clearance was indicative of internalization of HDL, HDL-C uptake rates were determined by multiplying clearance rates for HDL-apoA-I by maternal plasma HDL-C concentration. The concentration of HDL-C increased 5.8-fold as gestation progressed between day 10.5 ( $9.2 \pm 0.6 \text{ mg/dl}$ ) and day 14.5 ( $53.6 \pm 0.9 \text{ mg/dl}$ ). HDL-C uptake ( $\mu\text{g/h per g}$ ) increased 2.2-fold in the placenta and 2.6-fold in the uterine membrane and did not change significantly in the yolk sac (**Fig. 5A**). When organ weight was taken into account (**Fig. 5B**), an increase in uptake rates from day 10.5 to day 14.5 was measured in the placenta ( $0.6 \pm 0.2$  to  $4.9 \pm 0.9 \mu\text{g/h per organ}$ ) and uterine membrane ( $0.6 \pm 0.1$  to  $2.5 \pm 0.4 \mu\text{g/h per organ}$ ).

To determine non-selective endocytotic activity of the various tissues, the clearance rate of radiolabeled hamster albumin was measured. Albumin was cleared by the maternal liver at the rate of  $6.0 \pm 0.7 \mu\text{l/h per g}$  at day 10.5 and did not change by day 14.5 (**Table 1**). In contrast, clearance rates were  $\approx 3$ -fold greater in the placenta and uterine membrane at day 10.5 than were rates in the liver and remained at this level throughout gestation. The rate of clearance of albumin by the yolk

TABLE 1. Clearance of albumin in various tissues of hamsters at days 10.5 and 14.5 of gestation

Gestational Age	Liver	Placenta	Yolk Sac	Membrane
days			$\mu\text{L/h per g}$	
10.5	$6.4 \pm 0.7$	$16.3 \pm 1.1$	$196.1 \pm 21.6$	$26.1 \pm 0.9$
14.5	$7.0 \pm 0.2$	$20.3 \pm 1.0^a$	$144.9 \pm 26.2$	$19.9 \pm 0.8^a$

Hamster albumin was conjugated to radiolabeled tyramine cellobiose as described in Methods. Clearance rates represent the mean  $\pm$  1 SEM for summed fetal tissues (3–4 fetal units) and maternal livers in 4 pregnant dams at each age.

<sup>a</sup>Significant differences ( $P \leq 0.05$ ) between ages are shown.

sac was strikingly high at day 10.5 and day 14.5, supporting the idea that a significant amount of endocytotic activity occurred in this tissue.

To further elucidate the mechanism responsible for the uptake of the maternal lipoproteins, the relative amounts of SR-BI (Fig. 6, upper row), the LDL recep-

tor (Fig. 6, middle row), and gp330 (Fig. 6, lower row) were evaluated by immunoblot analysis in the yolk sac and placenta using the liver as a control tissue; the samples used for the immunoblot are representative of a minimum of six separate tissue samples. SR-BI was present in the non-pregnant liver and pregnant liver in relatively equal amounts. Only trace amounts of SR-BI were present in the placenta. In contrast, the yolk sac contained high levels of immunodetectable SR-BI; the concentration of this receptor did not appear to change dramatically between day 10.5 and day 14.5 of gestation. The amount of LDL receptor in the placenta was less than that in the liver and decreased from day 10.5 to day 14.5. The large variability that occurred in the placenta was representative of the variability seen on immunoblot analyses of multiple different placental units. There was very little LDL receptor in the yolk sac at either time point. Finally, gp330 was present in the livers and placenta in trace amounts. As with SR-BI,

#### Immunoblot Analysis of SR-BI, LDLR and gp330 in the Hamster Maternal Liver, Placenta and Yolk Sac

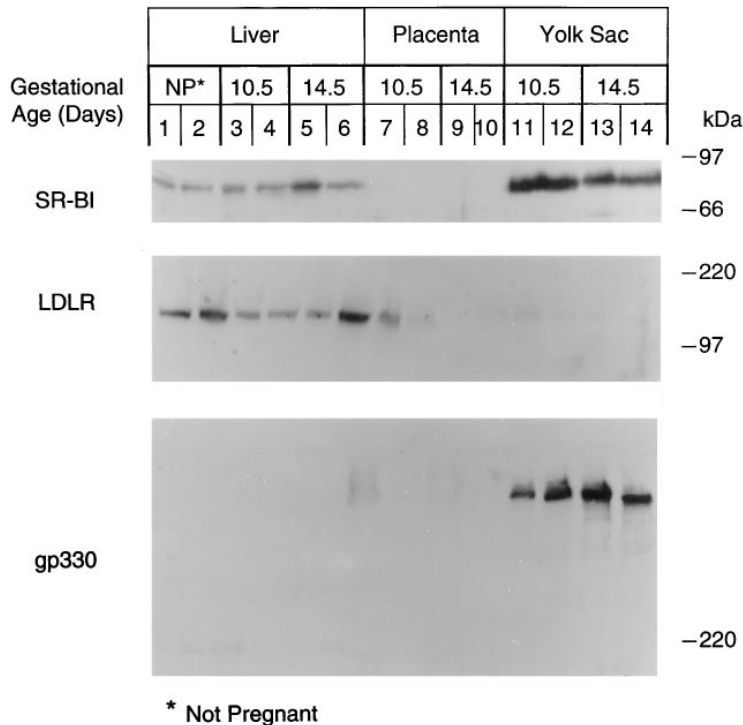
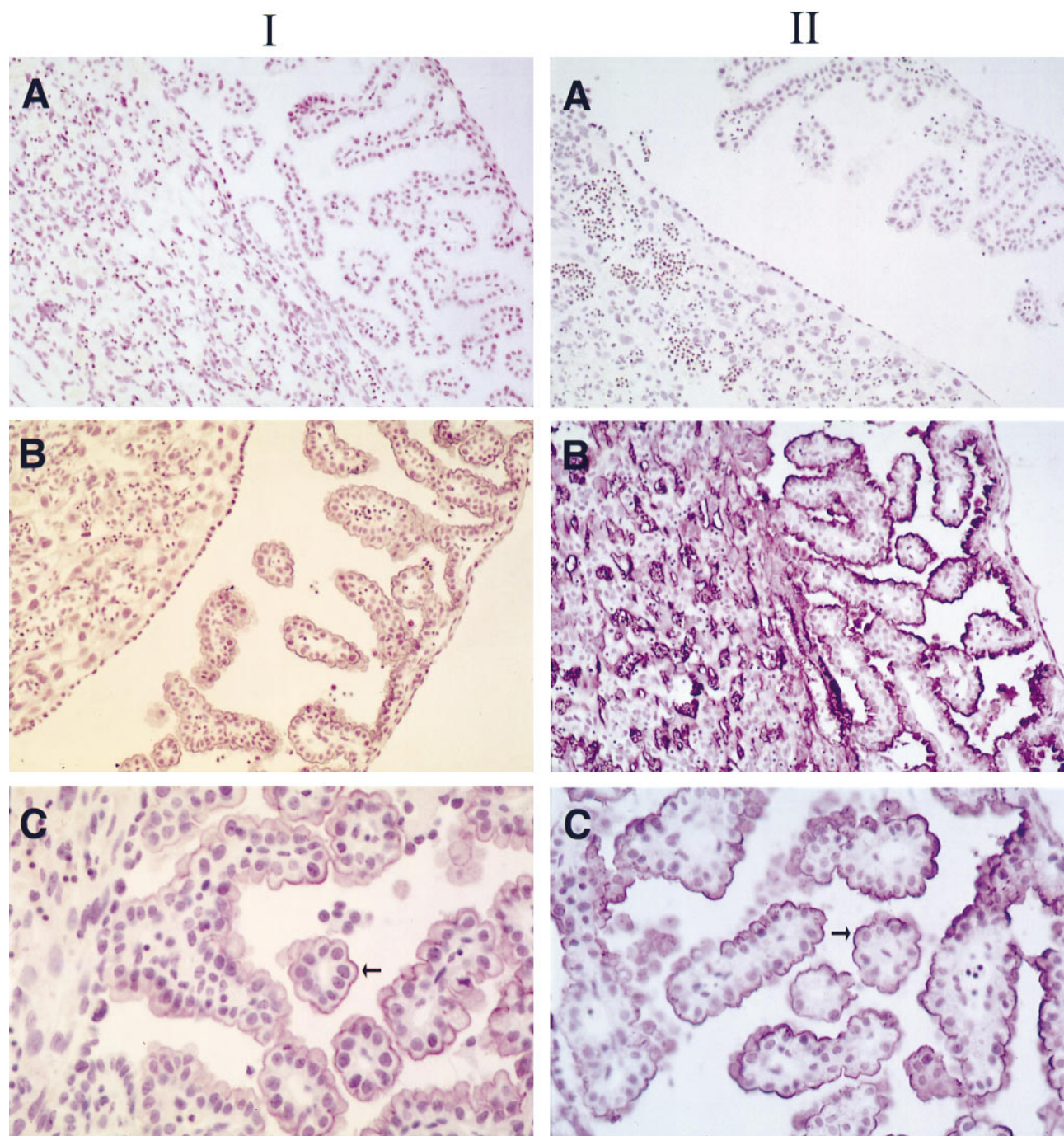


Fig. 6. Immunoblot analysis of SR-BI, the LDL receptor, and gp330 in non-pregnant livers, and livers, placentas and yolk sacs of hamsters at day 10.5 and day 14.5 of gestation. Membrane proteins from non-pregnant livers (lanes 1, 2), maternal livers at gestational days 10.5 (lanes 3, 4) and 14.5 (lanes 5, 6), placentas at gestational days 10.5 (lanes 7, 8) and 14.5 (lanes 9, 10), and yolk sacs at gestational days 10.5 (lanes 11, 12) and 14.5 (lanes 13, 14) were analyzed for SR-BI (upper row), the LDL receptor (middle row) and gp330 (lower row). Immunoblotting was performed as described in Methods. Molecular mass standards are indicated on the right in kilodaltons.



**Fig. 7.** Immunocytochemical localization of SR-BI and human apoA-I in the yolk sac and placenta of hamsters at day 10.5 of gestation. Yolk sac, placenta, and fetus were removed from the dam as a unit, immediately fixed, then processed as an intact unit. The placenta is on the left in panels A and B and the yolk sac is represented by the villi on the right of the panels. Arrows in IC and IIC depict localization of SR-BI and human apoA-I, respectively. In panel I, sections were either immunostained with preimmune IgG (A) or anti-SR-BI (B, C) as described in Methods. A  $\times 100$ , B  $\times 200$  and C  $\times 400$ . Sections in panel II were obtained from dams infused with saline (A) or human HDL (B, C) for 1 h, and were immunostained as described in Methods with anti-human apoA-I. A  $\times 100$ , B  $\times 200$  and C  $\times 400$ .

gp330 was expressed in high concentrations in the yolk sac at both 10.5 and 14.5 days.

To examine the cellular localization of SR-BI, the in-

tact yolk sac plus placenta was fixed as described in Methods and incubated with the same antibody used to detect SR-BI in the immunoblot analysis. As seen in **Fig. 7**,



panel I, SR-BI immunostaining was visualized on the cells of the yolk sac but not the placenta; even though trace amounts of SR-BI were found in the Western blot of the placenta, immunostaining of this tissue was not observed. Immunoreactive protein in the yolk sac was visualized on the apical surface of cells and was present in a fine, lacy reticular pattern that was found intracellularly but not in the nucleus. No staining was seen when sections were incubated with preimmune antibody. This pattern of staining is similar to that seen in the mouse (49) and the human (K. L. Wyne and H. H. Hobbs, unpublished data) adrenal.

To begin to ascertain the function of SR-BI in the yolk sac, human HDL was infused into dams at day 10.5 of gestation. Human HDL was used because it could be detected using antibodies specific to human apoA-I and would not cross react with hamster apoA-I. In this way, the location of exogenously administered HDL versus the apoA-I synthesized within the cells could be determined (Fig. 7, panel II). By 1 h, human apoA-I was present along the villi of the yolk sac in a pattern similar to that seen with the anti-SR-BI antibody (Fig. 7). Further studies are in progress to attempt to colocalize the endogenous HDL and SR-BI in this tissue.

## DISCUSSION

The major finding of the present studies is that lipoprotein transport by the placenta, yolk sac, and uterine membrane plus decidua is mediated potentially by a variety of different receptor-mediated as well as receptor-independent processes. Specifically, the placenta and yolk sac cleared LDL and HDL at rates significantly greater than other extrahepatic tissues, i.e., the uterine membrane plus decidua, at day 10.5 of gestation. Rates for both LDL and HDL decreased significantly by day 14.5 of gestation as maternal plasma LDL-C and HDL-C concentrations increased 27- and 5.8-fold, respectively. Clearance of both homologous LDL (total) and mhLDL (receptor-independent) occurred in all three tissues; the relative proportion of mhLDL to total clearance rates varied in each of the tissues. Finally, the whole HDL particle appeared to be internalized in all the tissues studied even though SR-BI, which has been shown to be responsible for selective transfer of cholesteryl ester (31), was expressed in high quantities in the yolk sac and was localized to surfaces of the microvilli. These studies do not rule out the possibility that cholesteryl ester is first selectively removed by SR-BI, and that uptake of the apoA-I-containing HDL occurs secondarily via a separate pathway.

The fundamental role of the placenta, yolk sac, and

uterine membrane plus decidua is to isolate the fetus from direct association with plasma constituents of the maternal circulation while simultaneously supplying the fetus with nutrients required for fetal growth. Each of these tissues has a specific function in aiding fetal development and, as such, a different requirement for cholesterol. The amount of sterol each of these tissues takes up during pregnancy-induced hypercholesterolemia is dictated by the proportion of receptor-mediated to receptor-independent transport of lipoproteins in tissues (1). If a large portion of transport is mediated by a receptor, then clearance rates will fall as substrate concentration increases and/or receptor number decreases. In contrast, if a majority of the transport is independent of a receptor, then clearance rates will remain constant unless the receptor is saturated or the function of the tissue changes. An example of these different processes is seen in the maternal liver, a tissue that contains receptor-mediated and receptor-independent transport of LDL (1, 42, 43) and HDL (30, 41). At day 10.5 of gestation, maternal plasma LDL-C and HDL-C concentrations were very low and receptor-mediated transport rates of both particles were relatively high. As lipoprotein-cholesterol concentrations increased during pregnancy, total and receptor-mediated clearance rates of LDL and clearance rates of HDL-cholesteryl ether decreased dramatically whereas clearance of mhLDL and HDL-apoA-I changed little. Thus, as demonstrated by the liver, one can now begin to elucidate the mechanisms responsible for lipoprotein clearance during gestation in the fetal tissues by examining the proportion of receptor-mediated and receptor-independent transport in these tissues.

The first layer of these three protective tissues which lies in closest proximity to the dam is the uterus. During pregnancy, the cells of this tissue fill with lipids and glycogen to form the decidua (50). The decidual cells are present not only as an anatomical barrier between the fetus and dam but also as a source of nutrients for the early developing embryo (50).

By mid-gestation (day 10.5 of the hamster), the placenta and yolk sac are functionally active and will supply a majority of nutrients to the fetus. Thus, LDL and HDL clearance rates in the membrane plus decidua at day 10.5 of gestation were no greater than those measured in other extrahepatic tissues. Clearance rates for LDL and HDL decreased in parallel with the dramatic elevation in plasma LDL-C and HDL-C concentrations which is consistent with tissues that have a majority of receptor-mediated transport. In addition to the saturation of transport sites, LDL clearance rates decreased due to a reduction in the amount of LDL receptor expressed (data not shown).

The next layer of protective tissues, which is adjacent

to the uterine membrane plus decidua, is the placenta. While the membrane plus decidua structurally separates the fetus and dam, the placenta plays a more metabolic role. Nutrients and waste products are transported between the maternal and fetal blood in the chorion villi of this tissue via a number of different types of transport processes (51, 52).

Previous studies have shown that maternal lipoproteins are one of the sources of sterol for the placenta (9, 10). As one might expect, the *in vivo* clearance rate for LDL was moderately elevated at day 10.5 of gestation as compared to other extrahepatic tissues with 77% of transport being mediated by a receptor, most likely the LDL receptor. As gestation progressed to day 14.5, the receptor-mediated clearance rate decreased. As in the uterine membrane plus decidua, this decrease was the result of a reduction in the amount of LDL receptor expressed (Fig. 6) which possibly was a result of the change in sterol balance across the tissue ( $3.5 \pm 0.2$  versus  $4.3 \pm 0.2$  mg cholesterol/g), and/or a saturation of the receptor. Interestingly, during this same time period, the rate of receptor-independent clearance decreased in the placenta. The clearance rates for albumin (Table 1) changed only slightly between day 10.5 and day 14.5 of gestation, suggesting that the decrease was not due to a change in the function of the tissue. However, as demonstrated in the maternal liver, transport that was independent of a receptor did not decrease as substrate concentration increased. It is possible that the clearance of mhLDL, which is usually indicative of receptor-independent transport in other tissues, actually was mediated by a receptor in the placenta, possibly the acetylated LDL scavenger receptor (16) which is expressed in human trophoblastic cells (15). The second source of maternal lipoproteins, HDL, was cleared at rates similar to those for LDL. As expected in a tissue lacking SR-BI, the whole HDL particle appeared to be removed by these cells. It seems likely that the high placental clearance rate for LDL, and possibly HDL (41), is primarily receptor-mediated and, as such, changes with the number of lipoprotein particles competing for transport sites and with the absolute number of transport sites.

The final layer of tissue responsible for the sequestration of the fetus from the maternal circulation is the yolk sac. In the rodent, the yolk sac is inverted and remains functional throughout gestation. Due to the absorptive properties of the microvilli that extend towards maternal tissues, the rodent yolk sac is often considered a second placenta. A separate function of this tissue is to synthesize and secrete lipoproteins into the medium of cell cultures (53) and presumably the vitelline circulation (11). Even though the human yolk sac is not inverted, it also has the capacity to synthesize various apolipoproteins (54).

In the present study, the yolk sac cleared maternal plasma constituents at relatively elevated rates as compared to other tissues, including mhLDL and albumin. Clearance rates for all maternal lipoproteins decreased as gestation progressed, including those taken up via a receptor-independent pathway. It is not possible at the present time to determine the proportion of transport that is receptor-mediated versus that which is receptor-independent in that all of the substrates of gp330 and SR-BI are not known.

It was shown in the yolk sac that both HDL-cholesteryl ether and HDL-apoA-I appeared to be cleared by the tissue at equal rates, even though SR-BI was expressed in high quantities and was localized on the surface of the villi of this tissue in the same pattern as in tissues where it is highly active (32, 49). Maternal-derived HDL was found along the apical surface of the yolk sac in this same vicinity and in a similar pattern (Fig. 7). Either the role of SR-BI is different in the yolk sac as compared to transfected CHO cells (31) and other tissues (29, 32, 41, 49), or the transport of HDL into the yolk sac is a multi-step process. Cholesteryl ester initially could be removed via SR-BI, followed by internalization of the whole HDL particle through a second process. It is possible that more HDL is removed by the yolk sac as compared to albumin and mhLDL because as the HDL is "docked" to the SR-BI on the surface of the cell, rapid endocytosis occurs, internalizing the membrane-bound particle. Interestingly, human apoA-I was not found within the yolk sac (Fig. 7). However, the lipid-containing particles taken up by the yolk sac appear to be degraded within vesicles very close to the apical surface (11) and, thus, non-degraded apolipoproteins should not be found within the cytosol. To further elucidate the transport process for HDL, and thus, the function of SR-BI in this tissue, it will be necessary to determine first, the process through which HDL is internalized using a non-degradable marker and second, saturation kinetic curves for HDL. The role of SR-BI could also be addressed by studying an animal lacking SR-BI (55).

Assuming that these clearance rates are representative of internalization of lipoprotein particles, it is possible to calculate the amount of cholesterol supplied to these tissues from the maternal circulation. Though the internalization of lipoproteins by the yolk sac has not been demonstrated *in vitro*, there are several pieces of data that when taken together suggest that the yolk sac internalizes maternal lipoproteins, as do other tissues. First, lipoprotein receptors are found on membranes of the yolk sac (21, 22) as are numerous clathrin-coated pits (56). Second, lysosomal- and phagocytic-like vesicles are located within the yolk sac near the apical surface (53, 56). Third, lipid-containing par-

ticles that are similar in size to VLDL are found in vesicles near the bases of microvilli (11). Finally, clearance rates are relatively high and thus, are most likely representative of internalization of particles. Thus, on day 10.5 of gestation, 160  $\mu\text{g}$  of LDL-C plus HDL-C was taken up by the placenta, yolk sac, and uterine membrane plus decidua. The amount of sterol taken up increased to 810  $\mu\text{g}$  per day as gestation progressed to day 14.5. Assuming that LDL-C and HDL-C uptake increased exponentially between days 10.5 and 14.5 of gestation (7),  $\approx 1.6$  mg of maternal lipoprotein-cholesterol was taken up during this 4-day period which was almost equal to that accrued by these three tissues during this same time (1.9 mg). A significant amount of sterol is synthesized simultaneously with the active removal of maternal lipoproteins (7). The excess of cholesterol could be packaged into lipoproteins within the yolk sac and transported to the fetus (11) or could be used for hormone synthesis (9, 10).

Recent studies have shown the necessity for an adequate amount of sterol in normal fetal growth, and that a lack of cholesterol in the fetus or embryo can lead to embryonic and developmental malformations. For example, individuals lacking 7-dehydrocholesterol- $\Delta^7$ -reductase, the enzyme responsible for the final step in the cholesterol biosynthetic pathway, develop the Smith-Lemli-Opitz syndrome (57, 58). The defects associated with this syndrome include mental retardation, as well as abnormal development of various organs and limbs (59). Proper fetal development is regulated in part by the hedgehog (hh) proteins (60) that require cholesterol for signaling activity; cholesterol is involved in the autoprolysis of the hh proteins and is covalently attached to the active sterol-containing amino terminal fragment (61). When the Sonic hh protein is absent in mice, holoprolencephaly occurs as does developmental abnormalities of limbs and the axial skeleton (62). A similar, though not exact, embryonic phenotype is found in mice lacking apoB (63, 64). Prior to resorption of the apoB-lacking mice, the developing embryos have low cholesterol and vitamin E concentrations (11, 63). The low concentration of these two lipids could be due to the inability of the yolk sac to synthesize and secrete lipoprotein-like particles into the vitelline circulation in the absence of apoB (11). Taken together, these findings suggest a critical role for cholesterol in proper fetal development. A significant quantity of cholesterol in the whole fetal unit is derived from the maternal circulation as shown in the present studies. To understand the importance of the various maternal lipoproteins in fetal development, the uptake of maternal-derived cholesterol by fetal tissues must now be studied in animals lacking various proteins involved in intra- and extracellular lipid transport. ■

The authors wish to thank Jeffrey Graven, Trista Prasifka, Laura Deane-Lee, and Steve Lee for their excellent technical assistance. Special thanks go to Helen Hobbs for the antibodies to the various receptors, as well as academic discussions. This research was supported by a grant-in-aid from the American Heart Association-Texas Affiliate and grants HL-09610, HL-20948, and HD-34089 from the National Institutes of Health. Dr. Wyne was supported by NIH 2 T32 DK07307-17. Dr. Woollett is a recipient of the American Heart Association Established Investigator Award.

*Manuscript received 21 July 1997 and in revised form 19 November 1997.*

## REFERENCES

- Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34**: 1637–1659.
- Carr, B. R., and E. R. Simpson. 1982. Cholesterol synthesis in human fetal tissues. *J. Clin. Endocrinol. Metab.* **55**: 447–452.
- Dietschy, J. M., T. Kita, K. E. Suckling, J. L. Goldstein, and M. S. Brown. 1983. Cholesterol synthesis in vivo and in vitro in the WHHL rabbit, an animal with defective low density lipoprotein receptors. *J. Lipid Res.* **24**: 469–480.
- Haave, N. C., and S. M. Innis. 1991. Perinatal development of hepatic cholesterol synthesis in the rat. *Biochim. Biophys. Acta.* **1085**: 35–44.
- Smith, J. L., S. R. Lear, and S. K. Erickson. 1995. Developmental expression of elements of hepatic cholesterol metabolism in the rat. *J. Lipid Res.* **36**: 641–652.
- Belknap, W. M., and J. M. Dietschy. 1988. Sterol synthesis and low density lipoprotein clearance in vivo in the pregnant rat, placenta, and fetus. Sources for tissue cholesterol during fetal development. *J. Clin. Invest.* **82**: 2077–2085.
- Woollett, L. A. 1996. Origin of cholesterol in the fetal Golden Syrian hamster: contribution of de novo sterol synthesis and maternal-derived lipoprotein cholesterol. *J. Lipid Res.* **37**: 1246–1257.
- Jurevics, H. A., F. Z. Kidwai, and P. Morell. 1997. Sources of cholesterol during development of the rat fetus and fetal organs. *J. Lipid Res.* **38**: 723–733.
- Winkel, C. A., J. M. Snyder, P. C. MacDonald, and E. R. Simpson. 1980. Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. *Endocrinology.* **106**: 1054–1060.
- Winkel, C. A., P. C. MacDonald, and E. R. Simpson. 1981. The role of receptor-mediated low-density lipoprotein uptake and degradation in the regulation of progesterone biosynthesis and cholesterol metabolism by human trophoblasts. *Placenta.* **3 (Suppl)**: 133–143.
- Farese, R. V., Jr., S. Cases, S. L. Ruland, H. J. Kayden, J. S. Wong, S. G. Young, and R. L. Hamilton. 1996. A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice. *J. Lipid Res.* **37**: 347–360.
- Lin, D. S., R. M. Pitkin, and W. E. Connor. 1977. Placental transfer of cholesterol into the human fetus. *Am. J. Obstet. Gynecol.* **128**: 735–739.

13. Connor, W. E., and D. S. Lin. 1967. Placental transfer of cholesterol-4-<sup>14</sup>C into rabbit and guinea pig fetus. *J. Lipid Res.* **8**: 558–564.
14. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science*. **232**: 34–47.
15. Alsat, E., F. Mondon, R. Rebourcet, M. Berthelie, D. Erlich, L. Cedard, and S. Goldstein. 1985. Identification of specific binding sites for acetylated low density lipoprotein in microvillous membranes from human placenta. *Mol. Cell. Endocrinol.* **41**: 229–235.
16. Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA*. **76**: 333–337.
17. Gáfvels, M. E., L. G. Paavola, C. O. Boyd, P. M. Nolan, F. Wittmaack, A. Chawla, M. A. Lazar, M. Bucan, B. Angelin, and J. F. Strauss, III. 1994. Cloning of a complementary deoxyribonucleic acid encoding the murine homolog of the very low density lipoprotein/apolipoprotein-E receptor: expression pattern and assignment of the gene to mouse chromosome 19. *Endocrinology*. **135**: 387–394.
18. Wittmaack, F. M., M. E. Gáfvels, M. Bronner, H. Matsuo, K. R. McCrae, J. E. Tomaszewski, S. L. Robinson, D. K. Strickland, and J. J. Strauss, III. 1995. Localization and regulation of the human very low density lipoprotein/apolipoprotein-E receptor: trophoblast expression predicts a role for the receptor in placental lipid transport. *Endocrinology*. **136**: 340–348.
19. Willnow, T. E., J. L. Goldstein, K. Orth, M. S. Brown, and J. Herz. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* **267**: 26172–26180.
20. Takahashi, S., Y. Kawarabayasi, T. Nakai, J. Sakai, and T. Yamamoto. 1992. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA*. **89**: 9252–9257.
21. Zheng, G., D. R. Bachinsky, I. Stamenkovic, D. K. Strickland, D. Brown, G. Andres, and R. T. McCluskey. 1994. Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/ $\alpha$ 2MR, and the receptor-associated protein (RAP). *J. Histochem. Cytochem.* **42**: 531–542.
22. Lundstrom, M., R. A. Orlando, M. S. Saedi, L. Woodward, H. Kurihara, and M. G. Farquhar. 1993. Immunocytochemical and biochemical characterization of the Heymann nephritis antigenic complex in rat L2 yolk sac cells. *Am. J. Pathol.* **143**: 1423–1435.
23. Stefansson, S., D. A. Chappell, K. M. Argraves, D. K. Strickland, and W. S. Argraves. 1995. Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B-100. *J. Biol. Chem.* **270**: 19417–19421.
24. Frykman, P. K., M. S. Brown, T. Yamamoto, J. L. Goldstein, and J. Herz. 1995. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA*. **92**: 8453–8457.
25. Ishibashi, S., M. S. Brown, J. L. Goldstein, R. D. Gerard, R. E. Hammer, and J. Herz. 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J. Clin. Invest.* **92**: 883–893.
26. Cummings, S. W., W. Hatley, E. R. Simpson, and M. Ohashi. 1982. The binding of high and low density lipoproteins to human placental membrane fractions. *J. Clin. Endocrinol. Metab.* **54**: 903–908.
27. Jorgensen, E. V., J. T. Gwynne, and S. Handwerger. 1989. High density lipoprotein<sub>3</sub> binding and biological action: high affinity binding is not necessary for stimulation of placental lactogen release from trophoblast cells. *Endocrinology*. **125**: 2915–2921.
28. Rabe, T., H. Kalbfleisch, A. Haun, and B. Runnebaum. 1983. Influence of human lipoproteins on the progesterone synthesis of human term placenta in organ culture. *Biol. Res. Preg.* **4**: 75–83.
29. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* **260**: 744–750.
30. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA*. **80**: 5435–5439.
31. Acton, S., A. Rigotti, K. T. Landschultz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*. **271**: 518–520.
32. Landschultz, K. T., R. K. Pathak, A. Rigotti, M. Krieger, and H. H. Hobbs. 1996. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98**: 984–995.
33. Andersen, J. M., and J. M. Dietschy. 1978. Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary and testes of the rat. *J. Biol. Chem.* **253**: 9024–9032.
34. Mazurkiewicz, J. C., G. F. Watts, F. G. Warburton, B. M. Slavin, C. Lowy, and E. Koukkou. 1994. Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients. *J. Clin. Pathol.* **47**: 728–731.
35. Potter, J. M., and P. J. Nestel. 1979. The hyperlipidemia of pregnancy in normal and complicated pregnancies. *Am. J. Obstet. Gynecol.* **133**: 165–170.
36. Reichen, J., G. Karlaganis, and F. Kern, Jr. 1987. Cholesterol synthesis in the perfused liver of pregnant hamsters. *J. Lipid Res.* **28**: 1046–1052.
37. Turley, S. D., M. W. Herndon, and J. M. Dietschy. 1994. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J. Lipid Res.* **35**: 328–339.
38. Pittman, R. C., T. E. Carew, C. K. Glass, S. R. Green, C. A. Taylor, Jr., and A. D. Attie. 1983. A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem. J.* **212**: 791–800.
39. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260**: 212–221.
40. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley.

1978. Role of the lysine residues of plasma lipoprotein in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253**: 9053–9062.
41. Woollett, L. A., and D. K. Spady. 1997. Kinetic parameters for HDL apoprotein A-I and cholesteryl ester transport in the hamster. *J. Clin. Invest.* **99**: 1704–1713.
42. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80**: 3499–3503.
43. Woollett, L. A., D. K. Spady, and J. M. Dietschy. 1989. Mechanisms by which saturated triacylglycerols elevate the plasma low density lipoprotein-cholesterol concentration in hamsters. Differential effects of fatty acid chain length. *J. Clin. Invest.* **84**: 119–128.
44. Woollett, L. A., D. K. Spady, and J. M. Dietschy. 1992. Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster. *J. Clin. Invest.* **89**: 1133–1141.
45. Russell, D. W., W. J. Schneider, T. Yamamoto, K. Luskey, M. S. Brown, and J. L. Goldstein. 1984. Domain map of the LDL receptor: sequence homology with the epidermal growth factor precursor. *Cell.* **37**: 577–585.
46. Willnow, T. E., J. Hilpert, S. A. Armstrong, A. Rohlmann, R. E. Hammer, D. K. Burns, and J. Herz. 1996. Defective forebrain development in mice lacking gp330/megalin. *Proc. Natl. Acad. Sci. USA.* **93**: 8460–8464.
47. Cao, G., C. K. Garcia, K. L. Wyne, R. A. Shultz, K. L. Parker, and H. H. Hobbs. 1997. Human SR-BI/CLA-1 gene structure and localization. Evidence for transcriptional control by steroidogenic factor-1. *J. Biol. Chem.* In press.
48. Wyne, K. L., R. K. Pathak, M. C. Seabra, and H. H. Hobbs. 1996. Expression of the VLDL receptor in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **16**: 407–415.
49. Rigotti, A., E. R. Edelman, P. Seifert, S. N. Iqbal, R. B. DeMattos, R. E. Temel, M. Krieger, and D. L. Williams. 1996. Regulation by adrenocorticotrophic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland. *J. Biol. Chem.* **271**: 33545–33549.
50. Langman, J. 1975. Medical Embryology. Human Development—Normal and Abnormal. The Williams & Wilkins Company, Baltimore, MD. 89–107.
51. Tropper, P. J., and R. H. Petrie. 1987. Placental exchange. In *The Human Placenta. Clinical Perspectives*. J. P. Lavery, editor. Aspen Publishers, Inc., Rockville, MD. 199–205.
52. Ahokas, R. A., and G. D. Anderson. 1987. The placenta as an organ of nutrition. In *The Human Placenta. Clinical Perspectives*. J. P. Lavery, editor. Aspen Publishers, Inc., Rockville, MD. 207–220.
53. Franke, H., D. Plonné, L. Winkler, and R. Dargel. 1992. Synthesis, secretion and immunoelectron microscopic demonstration of apolipoprotein B-containing lipoprotein particles in the visceral rat yolk sac. *Histochemistry.* **97**: 283–292.
54. Shi, W-K., B. Hopkins, S. Thompson, J. K. Heath, B. M. Luke, and C. F. Graham. 1985. Synthesis of apolipoproteins, alphafoetoprotein, albumin, and transferrin by the human foetal yolk sack and other foetal organs. *J. Embryol. Exp. Morphol.* **85**: 191–206.
55. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, and M. Krieger. 1997. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA.* **94**: 12610–12615.
56. Jollie, W. P. 1990. Development, morphology, and function of the yolk-sac placenta of laboratory rodents. *Teratology.* **41**: 361–381.
57. Irons, M., E. R. Elias, G. Salen, G. S. Tint, and A. K. Batta. 1993. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. *Lancet.* **341**: 1414.
58. Shefer, S., G. Salen, A. K. Batta, A. Honda, G. S. Tint, M. Irons, E. R. Elias, T. C. Chen, and M. F. Holick. 1995. Markedly inhibited 7-dehydrocholesterol- $\Delta^7$ -reductase activity in liver microsomes from Smith-Lemli-Opitz homozygotes. *J. Clin. Invest.* **96**: 1779–1785.
59. Smith, D. W., L. Lemli, and J. M. Opitz. 1964. A newly recognized syndrome of multiple congenital anomalies. *J. Pediatr.* **64**: 210–217.
60. Ingham, P. W. 1995. Signalling by hedgehog family proteins in *Drosophila* and vertebrate development. *Curr. Opin. Genet. Dev.* **5**: 492–498.
61. Porter, J. A., K. E. Young, and P. A. Beachy. 1996. Cholesterol modification of hedgehog signaling proteins in animal development. *Science.* **274**: 255–259.
62. Chiang, C., Y. Litingtung, E. Lee, K. E. Young, J. L. Corden, H. Westphal, and P. A. Beachy. 1996. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature.* **383**: 407–413.
63. Homanics, G. E., N. Maedo, M. G. Traber, H. J. Kayden, D. B. Dehart, and K. K. Sulik. 1995. Exencephaly and hydrocephaly in mice with targeted modification of the apolipoprotein B (*ApoB*) gene. *Teratology.* **51**: 1–10.
64. Farese, R. V., Jr., S. L. Ruland, L. M. Flynn, R. P. Stokowski, and S. G. Young. 1995. Knockout of the mouse apolipoprotein B gene results in embryonic lethality in homozygotes and protection against diet-induced hypercholesterolemia in heterozygotes. *Proc. Natl. Acad. Sci. USA.* **92**: 1774–1778.