Transport of cholesterol across a BeWo cell monolayer: implications for net transport of sterol from maternal to fetal circulation

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Abstract **The placental transport of various compounds, such as glucose and fatty acids, has been well studied. However, the transport of cholesterol, a sterol essential for proper fetal development, remains undefined in the placenta. Therefore, the purpose of these studies was to examine the transport of cholesterol across a placental monolayer and its uptake by various cholesterol acceptors. BeWo cells, which originated from a human choriocarcinoma, were grown on transwells for 3 days to form a confluent monolayer. The apical side of the cells was radiolabeled with either free cholesterol or LDL cholesteryl ester. After 24 h, the radiolabel was removed and cholesterol acceptors were added to the basolateral chamber. Cholesterol was found to be taken up by the apical surface of the placental monolayer, transported to the basolateral surface of the cell, and effluxed to fetal human serum, fetal HDL, or phospholipid vesicles, but not to apolipoprotein A-I. In addition, increasing the cellular cholesterol concentration further increased the amount of cholesterol transported to the basolateral acceptors. These are the first studies to demonstrate the movement of cholesterol across a placental cell from the maternal circulation (apical side) to the fetal circulation (basolateral side).**—Schmid, K. E., W. S. Davidson, L. Myatt, and L. A. Woollett. **Transport of cholesterol across a BeWo cell monolayer: implications for net transport of sterol from maternal to fetal circulation.** ***J. Lipid Res.*** **2003.** 44: **1909–1918.**

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Cholesterol is essential for proper development of the fetus. During gestation, the fetus grows exponentially and requires a large supply of cholesterol to support membrane formation, to synthesize hormones and bile acids (1, 2), and to ensure proper development of the central nervous system (3–5). Healthy fetuses have elevated sterol synthesis rates to meet the high demand for cholesterol (2, 6–8). Consequently, fetuses that have disruptions in cholesterol biosynthesis (9) experience abnormal development, as demonstrated by individuals with Smith-Lemli-Opitz syndrome (SLOS) (10–12). SLOS is an autosomal recessive disorder that occurs with a relatively high incidence of ~1 in 10,000 to 1 in 40,000 births (13–15). Patients with SLOS have a defect in the enzyme 3β-hydroxysterol-Δ^7-reductase causing a reduced conversion of 7-dehydrocholesterol to cholesterol. This defect results in abnormally low tissue and plasma cholesterol concentrations and causes a range of congenital birth defects from cranio-facial abnormalities to limb malformation to holoprosencephaly (13, 16). Cholesterol supplementation in children with SLOS has resulted in vast clinical improvement, including enhanced growth, rapid developmental progress, and lessening of behavioral problems (17–20). Due to these marked improvements postpartum, exogenous cholesterol may also enhance the development of the fetus and possibly lessen the severity of birth defects.

The fetus, as any tissue, has two potential sources of cholesterol. One is endogenously synthesized cholesterol within the fetus itself, and the second is an exogenous cholesterol, which is transported through or from the yolk sac and/or placenta (9, 21, 22). In humans, the yolk sac disappears early in development, but the placenta remains active and intact throughout gestation. The placenta is comprised of several cell types, with the main placental barrier formed from a layer of trophoblasts that control the passage of molecules from the maternal to the fetal circulation (23).

Although it is well accepted that the transport of various molecules, including amino acids, glucose, and fatty acids, occurs across the placenta (24, 25), the transport of cho-

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Abbreviations: ABCA1, ATP binding cassette transporter A1; FHS, fetal human serum; GLC, gas-liquid chromatography; LPDS, lipoprotein-deficient serum; LRP, low-density receptor-like protein; SFM, serum-free media; SLOS, Smith-Lemli-Opitz syndrome.

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lesterol remains unclear and undefined. Early studies that examined the in vivo transport of radiolabeled cholesterol from the maternal circulation to the fetal circulation have yielded inconsistent results. In these studies, the amounts of fetal cholesterol originating from the maternal circulation ranged anywhere from 0% to 50% (26–29). In addition, it was hypothesized that the fetus may not require exogenous sterol inasmuch as the in vivo sterol synthesis rates were sufficient in the rat fetus to account for nearly all of cholesterol accrued during gestation (7, 8). In contrast, recent studies suggest that maternal cholesterol may be a source of fetal cholesterol. First, a positive correlation exists between maternal plasma cholesterol concentration and fetal tissue cholesterol concentration (30, 31). Second, increasing maternal plasma cholesterol can reverse a drug-induced SLOS-like syndrome in rodents (32). Finally, SLOS fetuses of human and murine origin that contain two null alleles for Δ7 reductase have measurable plasma and tissue cholesterol concentrations (15, 33, 34). Either the cholesterol in these fetuses is derived from the maternal circulation and placenta, or an alternate pathway of cholesterol synthesis exists (9, 15, 20).

To begin to define the mechanism of cholesterol transport across the placenta, BeWo cells were used as a model of placental monolayer function. BeWo cells were chosen because they originated from a human choriocarcinoma (35) and have been extensively utilized to study the transport of various substances across the placenta, such as amino acids, transferrin, glucose, and fatty acids (36–40). BeWo cells grow as undifferentiated cytotrophoblasts and form confluent monolayers when grown on permeable membranes. In addition, they demonstrate polarized membrane expression of apical and basolateral protein markers and tight junction formation (38, 41). Further, BeWo cells display biochemical marker enzymes and demonstrate hormone secretion common to normal trophoblasts (35, 36). Thus, the purpose of the current studies was to examine the transport of cholesterol from the apical chamber, across the BeWo cells, to cholesterol acceptors in the basolateral chamber. Indeed, it was found that cholesterol from the apical chamber could be incorporated into the cells and effluxed to acceptors in the basolateral chamber. In addition, the efflux of cholesterol to the basolateral chamber was enhanced by increasing cellular cholesterol concentrations. This model for the maternal and fetal circulations demonstrates that fetal cholesterol (basolateral chamber) can originate from the maternal circulation (apical chamber).

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) and Trypsin-EDTA solution were purchased from Sigma (St. Louis, MO). Transwell-Clear® polyester membranes (12 mm diameter, 0.4 μm pore size) were purchased from Fisher Scientific. Heat-inactivated fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (DPBS), and the penicillin-streptomycin mixture were purchased from Gibco/Invitrogen (Grand Island, NY). [1α,2α (n)-^{3}H]cholesterol, [1α,2α (n)-^{3}H]cholesteryl oleate, and HiTrap™ heparin HP columns were obtained from Amersham Biosciences (Piscataway, NJ). L-α-Phosphatidyl choline was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All other standard chemicals or products were purchased from either Sigma or Fisher Scientific.

Cells

The BeWo cell line originated from a human choriocarcinoma (35, 42). The b30 clone was a generous gift from Dr. Kenneth Audus (University of Kansas, Lawrence, KS) with permission from Dr. Alan Schwartz (Washington University, St. Louis, MO). Cells were cultured as described previously, with few exceptions (38). Briefly, cells were maintained in DMEM (pH 7.4) with 10% FBS, 0.37% sodium bicarbonate, and 1% antibiotics. Cells were grown in T-75 flasks and typically passaged after 2–3 days in culture. Cells were harvested by exposure to trypsin-EDTA solution and seeded onto translucent polyester transwell membranes that were coated with 0.002 mg of rat tail collagen. Cells seeded at a concentration of 75,000 cells/ml typically formed monolayers by day 3 postseeding as determined by mannitol transport and microscopic examination of fixed monolayers (38). Cells remained a monolayer until day 5 postseed, then grew in multiple cell layers. All cells used in the study were between passage 25 and 50.

Human fetal serum

Blood was obtained from the umbilical vein of the placenta from term cesarean section or vaginal births as approved by the Institutional Review Board of the University of Cincinnati. Fetal human serum (FHS) was prepared by centrifugation and immediately sterile filtered. Serum was used within 2 weeks or frozen, thawed, and used within 2 months. No differences in efflux were seen with fresh versus frozen FHS. Fetal HDL was obtained from FHS by sequential preparative ultracentrifugation.

Immunoblot analysis of receptor proteins

Cells were washed and scraped in DPBS and lysed with Nonidet P-40 lysis buffer (43). Indicated amounts of protein were separated by 7.5% SDS-PAGE electrophoresis and transferred to nitrocellose. For detection of LDL receptor, the primary antibody (C7 ATCC CRL-1691) was generously provided by Dr. David Hui (University of Cincinnati, Cincinnati, OH). For detection of scavenger receptor class B type I (SR-BI), the primary antibody (400-103 Novus Biologicals, Littleton, CO) was generously provided by Dr. Philip Howles (University of Cincinnati, Cincinnati, OH). Proteins were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ).

Human LDL isolation and labeling

LDL was isolated from fresh human plasma in the density range of 1.02–1.063 g/ml by sequential preparative ultracentrifugation. LDL was labeled with [1α, 2α (n)-^{3}H]cholesteryl oleate as previously described for HDL (44, 45), except that the VLDL/LDL and liposomes were separated by column chromatography using HiTrap™ heparin HP columns. The LDL and VLDL were separated by ultracentrifugation before the LDL was dialyzed and filtered. LDL was methylated as previously described (46), and filtered.

Cholesterol acceptors

Phospholipid vesicles were prepared by solubilizing phosphatidyl choline in Tris salt buffer with sonication as described, with the exception of using phospholipid (PL) vesicles without centrifugation (47). Lipid free apolipoprotein A-I (apoA-I) was isolated from fresh human plasma as described (48).

Cell studies

Cells were grown in culture for a total of 4 days to prevent the formation of multiple layers. Cellular cholesterol was labeled either by the incorporation of free [^{3}H]cholesterol at 1 μCi/ml DMEM or by the uptake of LDL labeled with [^{3}H]cholesteryl oleate (LDL-[^{3}H]CE) at indicated concentrations.

In studies measuring cholesterol efflux with [^{3}H]cholesterol, the cells were labeled on day 2 for 24 h. On day 3, the medium was removed from the upper and lower chambers of the transwells and the cells were washed extensively with DPBS to remove all nonincorporated radioactive cholesterol. Washes were tested to ensure all radiolabeled cholesterol was removed from the chambers. Next, serum-free media (SFM) containing cholesterol acceptors at the indicated concentration were added to the lower chamber, and SFM alone was added to the upper chamber. Aliquots of media were collected at indicated time points, passed through a 0.45 μm filter, and measured by liquid scintillation. The medium was removed and the cells washed with DPBS. The cells were lysed in 1 N NaOH, and radioactivity in aliquots of cellular lysates were measured by liquid scintillation. Data are presented as the amount of [^{3}H]cholesterol in the basolateral media as a percentage of total cellular [^{3}H]cholesterol at time 0.

Uptake and transport studies were performed with LDL-[^{3}H]CE using the same protocol as above with a few exceptions. Cells were preincubated for 24 h in media containing 10% lipoprotein-deficient serum (LPDS). After 24 h, cells were labeled with varying concentrations of LDL cholesteryl ester (LDL-CE) in media containing 10% LPDS to ensure that LDL was the sole source of cholesterol for the cells. Cells were washed, acceptors added, and radiolabeled cholesterol measured as described for the [^{3}H]cholesterol experiments. Uptake data are presented as the micrograms of LDL cholesterol taken up by the cells in 24 h. Transport data are presented as the amount of [^{3}H]cholesterol in the basolateral media as a percentage of total cellular [^{3}H]cholesterol at time 0.

Cellular and media cholesterol concentration

Cellular cholesterol was extracted from cells with isopropanol and saponified. Mass cellular cholesterol was measured by gas-liquid chromatography (GLC) using stigmastanol as an internal standard (2, 49). Cellular proteins were solubilized and determined by the Markwell modification of the Lowry method (50). Data are presented as the micrograms of cholesterol per milligrams of cell protein. To measure cholesterol in the media, media from the lower chamber plus a wash of the lower chamber was collected, saponified, and extracted. Mass cholesterol was measured by GLC (2, 49). Data are presented as micrograms of cholesterol per well.

Statistics

Data are represented as the mean values ± 1 SEM. Differences between two values were tested for statistical significance ($P < 0.05$) using the two-tailed unpaired Student's *t*-test.

RESULTS

Monolayer integrity

These studies were completed using a cell line to allow one to directly study the transport of cholesterol across the placenta without the inclusion of transport across the yolk sac that may occur in the pregnant rodent in vivo (9, 51). The formation of a monolayer with tight junctions has been previously characterized in BeWo cells (37–39). Prior to the current studies, the integrity of this cell line under the conditions of these experimental manipulations was confirmed by several methods (data not shown). First, monolayer formation was confirmed by cross-sectional analysis of fixed hematoxylin and eosin Y-stained monolayers as previously described (38). Second, studies have shown that as tight junctions form between cells, the transepithelial electrical resistance increases and the permeability of mannitol, or other fluid-phase markers, decreases by about 50% (38, 52). On the basis of these results, we measured the change in mannitol flux across the monolayer on different days and determined that the flux of mannitol decreased more than 50% between days 1 and 3 of growth. Third, we confirmed that the BeWo cells transported fatty acids unidirectionally as previously described (38). Thus, the BeWo cells form monolayers with tight junctions and transport metabolites as previously described. Additionally, the confluence of the monolayer was monitored visually by daily microscopic examination to ensure there were no gaps or disruptions in the monolayer.

Model appropriateness

Previous reports have shown that placental cells are replete with lipoprotein receptors, such as the VLDL receptor, the low-density receptor-like protein (LRP), the LDL receptor, and SR-BI (53–55). To be an appropriate model, BeWo cells should express these same lipoprotein receptors. BeWo cells have been shown to express the VLDL receptor and LRP (53, 56). The presence of the LDL receptor was verified by the presence of a band migrating at ~160 kDa that correlated with bands present in the hamster liver and HepG2 cells (**Fig. 1A**). The expression of SR-BI was examined using an antibody to a portion of mouse SR-BI that shares 92% homology with the human protein. BeWo cells expressed SR-BI, which migrated as a slightly larger protein than SR-BI in the mouse liver and was correlated with a band present in another human placental cell line that has been shown to express SR-BI (Fig. 1A) (57). The presence of a larger protein was expected, because previous studies have demonstrated that human SR-BI from villous trophoblast migrates with a molecular mass slightly higher than that of rodent SR-BI (55), and the human protein sequence is 43 amino acids longer than that of the mouse.

The uptake and utilization of LDL-CE by the placenta has been well documented (45, 58, 59). As expected from lipoprotein receptor expression profiles, LDL-CE was also taken up by BeWo cells. When cells were incubated with increasing concentrations of radiolabeled LDL for 24 h, LDL-[^{3}H]CE appeared to be taken up via a process that appeared to contain both receptor-mediated and -independent components (Fig. 1B). When receptor-independent uptake LDL-CE was measured directly in cells using methylated LDL-CE, the uptake of methylated LDL was 35% that of total LDL-CE uptake (1.13 ± 0.05 μg LDL vs. 0.35 ± 0.01 μg methyl-LDL) (Fig. 1B, insert). In addition, over 90% of the cholesteryl ester that entered the cell became hydrolyzed to free cholesterol as determined by thin layer chromatography (data not shown).

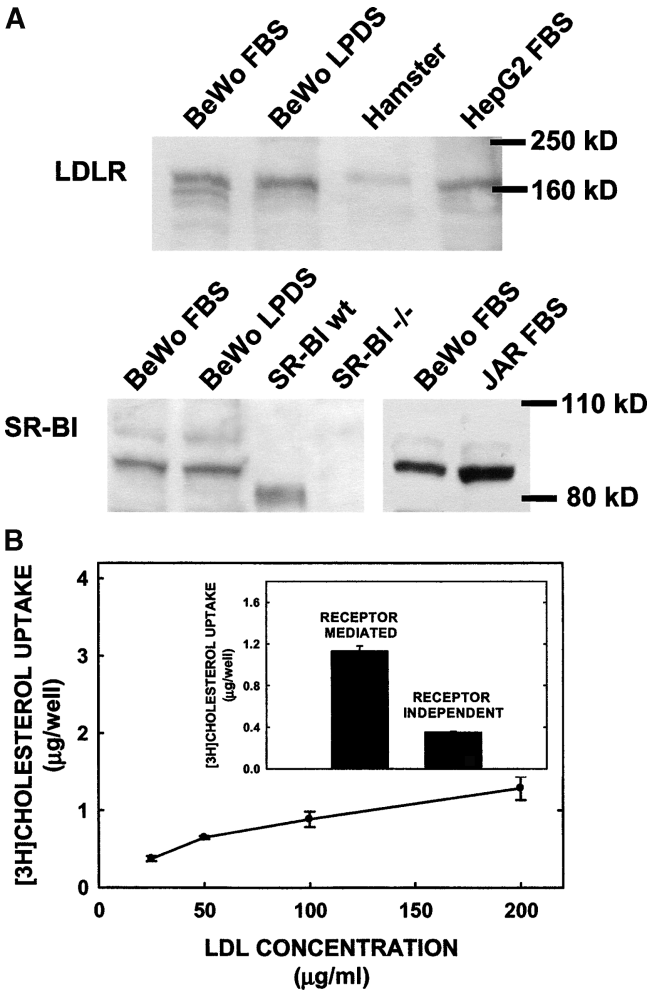

Fig. 1. Expression of lipoprotein receptors and the uptake of LDL cholesteryl ester (LDL-CE) in BeWo cells. A: Cellular or liver protein was extracted, separated by SDS-PAGE, and immunodetected with antibody to LDL receptor or scavenger receptor class B type I (SR-BI). LDL receptor is represented at the 160 kDa molecular weight marker, and SR-BI is represented slightly above the 80 kDa molecular weight marker. Blots are representative of three independent experiments. B: Cells were grown in lipoprotein-deficient serum (LPDS) for 24 h before being radiolabeled on the apical side with [^{3}H]CE-labeled LDL for 24 h. After labeling, the cells were washed, lysed, and an aliquot of lysates counted to determine [^{3}H]cholesteryl oleate (LDL-[^{3}H]CE) uptake. Insert shows uptake of [^{3}H]CE-labeled LDL versus uptake of [^{3}H]CE-labeled methylated-LDL. Each value represents the mean ± 1 SEM of triplicate samples that are representative of three independent experiments.

When the cells were incubated with LDL-CE on the basolateral surface, the amount taken up was 20% of that taken up from the apical side, implying a primarily unidirectional process (data not shown). Additionally, 50% of the basolateral-derived CE was effluxed back to the basolateral chamber.

Efflux of cholesterol from cells to FHS

Components in adult human serum are able to promote cholesterol efflux from various cell types (60–62). To determine if FHS could promote cholesterol efflux from the basolateral surface of BeWo cells, total cellular cholesterol was labeled with [^{3}H]cholesterol, and the cells were incubated with increasing concentrations of FHS in the lower chamber. The efflux of cholesterol increased between 10% and 40% FHS. The rate of efflux appeared greater up to 10% FHS and then decreased through 40% FHS (**Fig. 2A**). In order to remain in the nonsaturated portion of this curve, 10% FHS was chosen for all remaining experiments. The time course of cholesterol efflux to 10% and 0% FHS was examined over 24 h. The efflux of cholesterol to 10% FHS increased most rapidly from 2 h to 8 h, after which time the rate of increase was slower but continued until 24 h (Fig. 2B). The efflux of cholesterol to 0% FHS (control) was minimal to 24 h (Fig. 2B).

Knowing that BeWo cells take up LDL-CE, we next examined the efflux of LDL-CE. As with cells labeled with [^{3}H]cholesterol, the efflux of cellular LDL-[^{3}H]CE to 10% and 0% FHS was examined over 24 h. Similar to [^{3}H]cholesterol-labeled cells, cells labeled with LDL-[^{3}H]CE demonstrated a steady increase of efflux to 10% FHS and showed minimal cholesterol efflux to 0% FHS (**Fig. 3A**). Because fetal serum contains a significant amount of apoE-rich HDL (HDL cholesterol concentration about 35 mg/dl) (63), we examined the contribution of fetal HDL in the efflux of cholesterol to FHS. When BeWo cells were incubated with 100 µg/ml of fetal HDL protein, cholesterol efflux was greater than that effluxed to SFM ($P <$ 0.05), and slightly less than that seen to FHS (2.50 ±

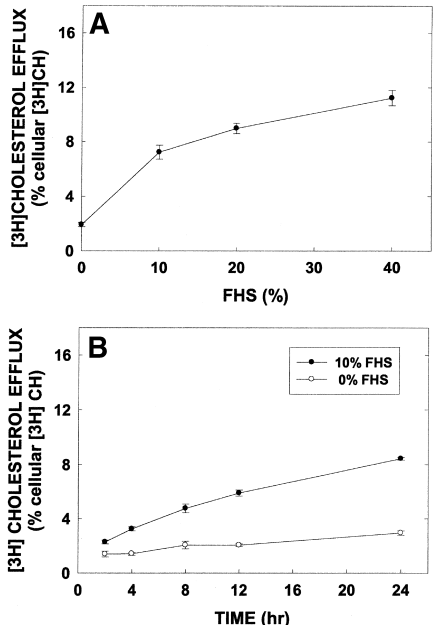

Fig. 2. Efflux of cholesterol from the basolateral surface of BeWo cells to fetal human serum (FHS). The apical side of cells were labeled with [^{3}H]cholesterol for 24 h. After labeling, the cells were washed and incubated with (A) 0–40% FHS in the basolateral chamber for 24 h or (B) 0 or 10% FHS for 2–24 h. An aliquot of the basolateral media was counted and data are represented as the amount of [^{3}H]cholesterol effluxed as a percentage of cellular [^{3}H]cholesterol at time 0. Each value represents (A) the mean ± 1 SEM of triplicate samples from three different sources of FHS or (B) the mean ± 1 SEM of triplicate samples that are representative of three independent experiments (B).

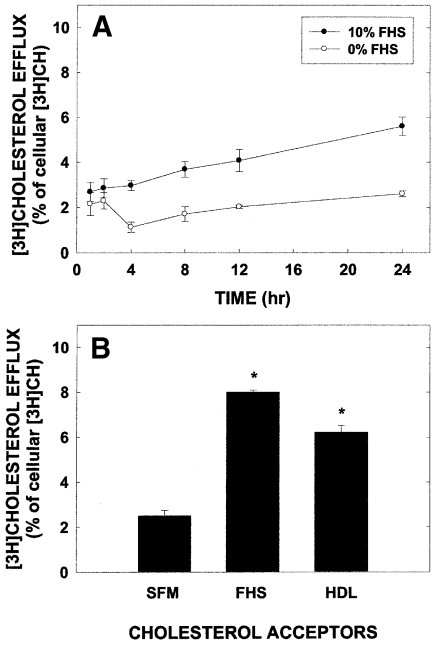

Fig. 3. Efflux of LDL-CE from the basolateral surface of BeWo cells to FHS and fetal HDL. Cells were grown in LPDS for 24 h before being radiolabeled on their apical side with [^{3}H]CE-labeled LDL for 24 h. After labeling, the cells were washed and incubated with (A) 0 or 10% FHS in the basolateral chamber for 2–24 h or (B) serum-free media (SFM), 10% FHS, or 100 μg/ml fetal HDL protein for 24 h. An aliquot of basolateral media was counted, and data are represented as the amount of [^{3}H]cholesterol effluxed as a percentage of cellular [^{3}H]cholesterol at time 0. Each value represents the mean ± 1 SEM of triplicate samples that are representative of (A) three independent experiments and (B) two independent experiments. * Significant differences ($P < 0.05$) between acceptors and SFM are shown.

0.24% vs. 6.23 ± 0.29% vs. 8.01 ± 0.08% for SFM, HDL, and FHS, respectively) (Fig. 3B). Because levels of efflux were similar, FHS was used for all following experiments.

Net efflux of cholesterol from cells to FHS and noncholesterol acceptors

Although FHS and HDL are physiological acceptors for cholesterol efflux, they contain lipoprotein cholesterol. Thus, it was necessary to differentiate net cholesterol efflux from exchange of membrane cholesterol with serum cholesterol (61, 64). To examine net cholesterol efflux, cells were incubated with 1% FHS in the basolateral chamber for 12 h, the media was collected, and cholesterol mass was measured; less FHS was used in these studies in order to detect small changes in cholesterol mass. Net movement of cholesterol was indicated by a significant increase ($P < 0.05$) of cholesterol mass in the basolateral media from 1 h to 6 h (5.84 ± 0.52 μg vs. 7.28 ± 0.21 μg), which remained constant through 12 h (**Fig. 4**).

To further examine the mechanism of net cholesterol efflux from BeWo cells, cells were incubated with either PL or lipid-poor apoA-I as acceptors. The efflux of [^{3}H]cholesterol significantly increased over SFM ($P < 0.05$) when PL were used as an acceptor (2.82 ± 0.16% vs. 5.47 ± 0.27%), but not when lipid-poor apoA-I was an acceptor (2.82 ± 0.16% vs. 2.87 ± 0.29%) (**Fig. 5**). The efflux to the basolateral chamber continued unaffected even in the presence of acceptors in the apical chamber (data not shown). No efflux to apoA-I was demonstrated from BeWo cells despite the fact that the ATP binding cassette transporter A1 (ABCA1) mRNA was detected by RT-PCR using previously described primers (65) and immunoblot analysis (400–105 anti-ABCA1 antibody, Novus Biologicals) (data not shown). In addition, cholesterol efflux to apoA-I was not manipulated when cells were treated with 10 μM cAMP, 10 μM 22-hydroxycholesterol, or the combination of 10 μM 22-hydroxycholesterol and 1 mM 9-*cis*-retinoic acid (data not shown), all of which are known to increase ABCA1 activity (66–70). The expression of ABCA1 mRNA in BeWo cells was not affected by polarization or treatment with oxysterols and 9-*cis*-retinoic acid for 24 h (data not shown).

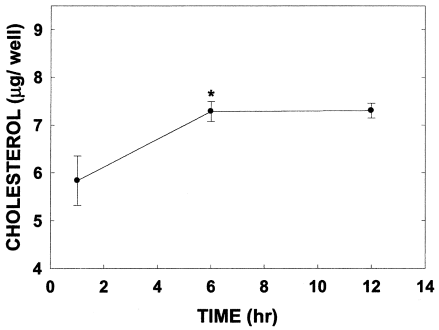

Fig. 4. Net cholesterol efflux from the basolateral surface of BeWo cells to 1% FHS. Cells were incubated with 1% FHS in the basolateral chamber. After 24 h, media from the basolateral chamber and a wash were collected and saponified, and the cholesterol content was measured by gas-liquid chromatography (GLC). Data are presented as the micrograms of cholesterol in the basolateral chamber. Each value represents the mean ± 1 SEM of six samples that are representative of two independent experiments. * Significant differences ($P < 0.05$) between consecutive time points are shown.

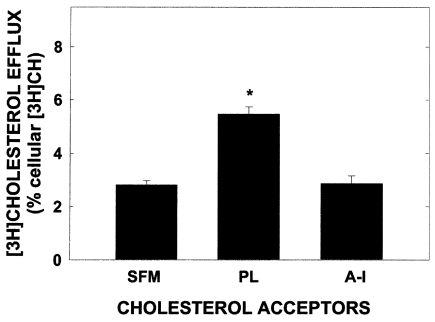

Fig. 5. Efflux of cholesterol from the basolateral surface of BeWo cells to noncholesterol-containing acceptors. The apical side of cells were labeled with [^{3}H]cholesterol for 24 h. After labeling, the cells were washed and incubated with SFM, 100 μg/ml phospholipid vesicles (PL), or 50 μg/ml apolipoprotein A-I (apoA-I) in the basolateral chamber for 24 h. An aliquot of the basolateral media was counted, and data are represented as the amount of [^{3}H]cholesterol effluxed as a percentage of cellular [^{3}H]cholesterol at time 0. Each value represents the mean ± 1 SEM of triplicate samples that are representative of three independent experiments. * Significant differences ($P < 0.05$) between acceptors and SFM are shown.

Increased cellular cholesterol concentration and cholesterol efflux

Before examining the role of cellular cholesterol concentration on cholesterol efflux, it was determined if exogenous LDL cholesterol could manipulate the cholesterol concentration of BeWo cells. Incubating the cells with increasing concentrations of LDL cholesterol produced an increase in cellular cholesterol concentration from 6.30 ± 0.21 μg to 14.00 ± 0.60 μg cholesterol/mg protein (**Fig. 6**). This increase shows a steep initial slope followed by a decrease in slope, as expected for a receptor-dependent process.

Cells were then labeled with [^{3}H]cholesterol and incubated with either 0 or 200 μg/ml LDL protein, and cholesterol efflux was examined. Increasing cellular cholesterol concentration increased the efflux of cholesterol by 4- to 5-fold to all acceptors, including SFM (**Fig. 7**). Again, the efflux of cholesterol to PL and 10% FHS was significantly greater ($P < 0.05$) than SFM (4.1 ± 0.2% vs. 12.1 ± 0.4% vs. 20.1 ± 1.6% for efflux to SFM, PL vesicles, and 10% FHS, respectively). However, the efflux of cholesterol to apoA-I was not greater than that to SFM (4.1 ± 0.2% vs. 3.7 ± 0.3%).

To confirm that the increase in cholesterol efflux from the placental cells was due to the uptake of LDL cholesterol, cells were incubated with increasing concentrations of LDL-[^{3}H]CE. As seen with free cholesterol-labeled cells, increasing cellular cholesterol concentration with exogenous LDL increased cholesterol efflux to PL (278 ± 33 dpm to 1078 ± 191 dpm) and 10% FHS (670 ± 103 dpm to 2,126 ± 206 dpm) while there was marginal increase to SFM (210 ± 26 dpm to 500 ± 47 dpm) (**Fig. 8**).

DISCUSSION

These are the first studies to show that placental cells can take up cholesterol from the apical surface of the cell and transport it to the basolateral surface of the cell for efflux to components in FHS. This efflux of cholesterol appears to be, at least, a diffusion-mediated and/or SR-BI-mediated process to phospholipids, as opposed to an apolipoprotein-mediated process (71–74). Furthermore, these studies suggest that increasing cholesterol concentrations in the placental cell can increase the amount of cholesterol available for efflux to the fetal circulation.

In accordance with previous findings in the placenta, BeWo cells behave like normal trophoblasts and serve as a good model for lipoprotein transport in the placenta. These cells take up LDL-CE via receptor-mediated and receptor-independent processes (45, 58). In addition, BeWo

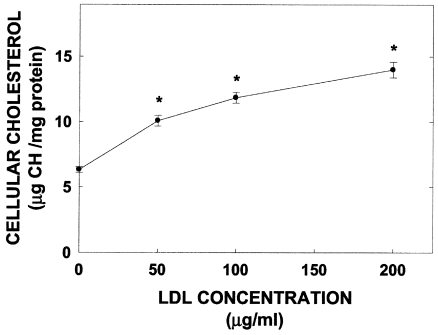

Fig. 6. Cholesterol content of BeWo cells loaded with increasing concentrations of LDL. Cells were grown in LPDS for 24 h then incubated with increasing concentrations of LDL protein in LPDS. After 24 h, the cells were washed, and lipids extracted into isopropanol, and cells kept frozen until analyzed for protein content. The lipid extract was saponified and cholesterol content measured by GLC. Data are presented as the micrograms of cholesterol per milligram of cell protein. Each value represents the mean ± 1 SEM of six samples that are representative of two independent experiments. * Significant differences ($P < 0.05$) are shown between cholesterol content of cells incubated with 0 μg/ml LDL protein and cells incubated with each of the other LDL concentrations.

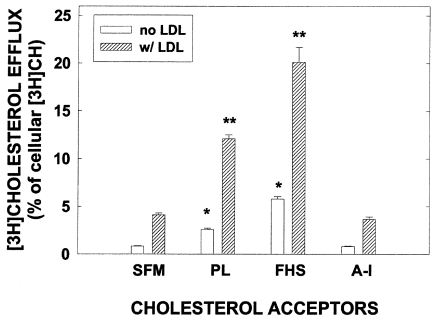

Fig. 7. Efflux of cholesterol from the basolateral surface of BeWo cells incubated with or without exogenous LDL cholesterol. The apical sides of cells were labeled with [^{3}H]cholesterol for 24 h with or without 200 μg LDL protein/ml media. After labeling, the cells were washed and incubated with either SFM, 100 μg/ml PL, 10% FHS, or 50 μg/ml apoA-I in the basolateral chamber for 24 h. An aliquot of the basolateral media was counted, and data are represented as the amount of [^{3}H]cholesterol effluxed as a percentage of cellular [^{3}H]cholesterol at time 0. Each value represents the mean ± 1 SEM of triplicate samples that are representative of three independent experiments. * Significant differences ($P < 0.05$) between acceptors and SFM with no LDL are shown. ** Significant differences ($P < 0.05$) between acceptors and SFM with LDL are shown.

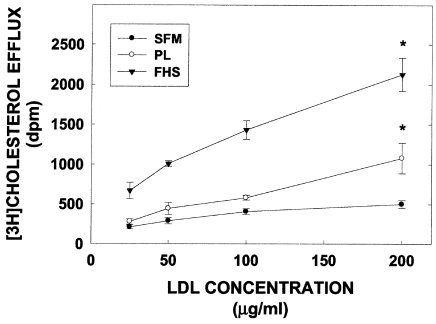

Fig. 8. Efflux of cholesterol from the basolateral surface of BeWo cells loaded with increasing concentrations of LDL. Cells were grown in LPDS for 24 h before being radiolabeled on their apical sides with LDL-[^{3}H]CE. After labeling, the cells were washed and incubated with SFM, 100 μg/ml PLs, or 10% FHS in the basolateral chamber for 24 h. An aliquot of basolateral media was counted, and data are represented as actual dpm in the basolateral chamber. Each value represents the mean ± 1 SEM of triplicate samples that are representative of three independent experiments. * Significant differences ($P < 0.05$) between acceptors and SFM are shown at the highest LDL concentration.

cells express the LDL receptor and SR-BI, as do placental cells (75, 76). However, these data are not in agreement with a recent study suggesting that BeWo cells do not express SR-BI (57). The discrepancy between studies could be due to the b30 subclone of BeWo cells or to the specificity of the different antibodies used for protein detection in the two studies.

In addition to the apical uptake of LDL-CE in BeWo cells, these studies demonstrated that LDL-derived cholesterol can be transported to the basolateral surface of the cell and effluxed to various components in FHS, including HDL. Although FHS and HDL contain cholesterol, the movement of cholesterol is not just an exchange of membrane cholesterol for lipoprotein cholesterol (61, 64), but involves a net transport of cholesterol mass from the cell to the serum. This net efflux is demonstrated by the increase of cholesterol mass in the basolateral media with 1% FHS and the movement of cholesterol to PL (71, 72, 77). These data provide a physiological pathway of cholesterol movement from the maternal to the fetal circulation.

Early studies have demonstrated that macrophages and other cells efflux cholesterol to components in whole serum (60–62). There are three proposed mechanisms for the removal of cholesterol from these cells: diffusion-mediated release, SR-BI-mediated efflux, and apolipoprotein-mediated efflux (78). During the diffusion-mediated process, cholesterol moves out of the cell membrane and down a concentration gradient to cholesterol-poor acceptors, such as phospholipid discs (73, 77, 79, 80). The rate of cholesterol efflux can be manipulated by the distribution of lipids and the enrichment of cholesterol in the membrane (79, 80). During SR-BI-mediated efflux, cholesterol is transported from the cell to a phospholipid-containing acceptor, such as HDL (81). The rate of efflux by SR-BI is increased by enrichment of the acceptors with phosphatidyl choline (82). During apolipoprotein-mediated efflux, apoA-I in the subendothelial space interacts with the cholesterol transport protein ABCA1 to facilitate the efflux of membrane cholesterol to form nascent HDL particles (74, 83–85). In certain macrophages, cholesterol efflux is increased with the up-regulation of ABCA1 protein by treatment of the cells with cAMP or 22-hydroxycholesterol and 9-*cis* retinoic acid (66–69).

The data presented here support a diffusion-mediated and/or a SR-BI-mediated release of cholesterol from BeWo cells. First, the efflux of cholesterol from the basolateral membrane was highest when phospholipid-containing particles (PL and HDL) were used as acceptors, while no apparent efflux was seen to apoA-I (86). Second, the efflux to PL was manipulated by changing the cholesterol content of the cell. There was a 4- to 5-fold increase in the amount of cholesterol effluxed from the cell when cellular cholesterol concentrations were increased. Interestingly, this increase was even seen in the presence of SFM, as indicated in Fig. 7. It is possible that the ability of BeWo cells, and the placenta, to secrete apoE into the fetal circulation (87) may facilitate the removal or secretion of cholesterol from the cell into SFM (88). We are currently investigating this possibility. Although these data support the SR-BI-mediated efflux as a possible mechanism of cholesterol, future studies are needed to determine the precise role of SR-BI in cholesterol efflux from the placenta.

ABCA1 has been shown to efflux phospholipids and cholesterol to lipid-free apolipoproteins, including apoA-I (70, 74). However, this particular efflux of cholesterol was not apparent in the BeWo cells. Despite multiple manipulations to up-regulate the expression of ABCA1, there was no increase of cholesterol efflux to exogenous apoA-I. ABCA1 is expressed on, and promotes efflux from, the basolateral surface of multiple cells (89, 90). However, it should be noted that although the mRNA expression of ABCA1 is highest in the placenta (91), the cellular location of the protein is yet to be determined. While cholesterol efflux to exogenous apoA-I was not apparent, it does not rule out the possibility that ABCA1 may mediate the efflux of cholesterol to apolipoproteins in FHS or to fetal HDL. Future investigation to determine the role of ABCA1 in the placenta will be performed.

Although the current studies were performed in vitro, the results can be applied to the in vivo situation. For example, these data suggest that fetal cholesterol concentrations can be increased via manipulation of maternal plasma cholesterol concentrations and of placental cholesterol concentrations, as previously implicated by this laboratory (31). Increasing the amount of cholesterol available for efflux could have significant implications for the developing fetus. Recent studies have shown that increasing the amount of exogenous cholesterol available to the SLOS children after birth can have a positive impact on their development and well-being (17–20). Therefore, increasing the supply of exogenous cholesterol as early as the embryonic or fetal stages may further improve the development, or lessen the severity, of possible birth defects in the SLOS fetus. While it may be of extreme benefit to increase cholesterol in fetuses with certain sterol synthesis defects, it might not be beneficial to increase exogenous cholesterol to all fetuses. Studies have demonstrated that maternal hypercholesterolemia can enhance the formation of atherosclerotic lesions in the fetus (92, 93) and possibly cause metabolic problems later in life.

The studies presented here show the merit of BeWo cells as a model for the manipulation of fetal cholesterol concentrations by an exogenous source. They demonstrate the ability of placental cells to transport maternal-derived cholesterol (from the apical chamber) across the placental cell for efflux into the fetal circulation (the basolateral chamber). Furthermore, they show that cholesterol efflux can be enhanced when the cells are exposed to increasing levels of LDL cholesterol. Understanding and dissecting this pathway may aid in the development of future in utero treatments for fetuses with defects in the cholesterol biosynthetic pathway.

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