Fetal HDL/apoE: a novel regulator of gene expression in human placental endothelial cells

Manuela Augsten,1 Hubert Hackl,2 Birgit Ebner,3 Angela Chemelli,4 Otto Glatter,4 Gunther Marsche,5 Uwe Lang,1 Gernot Desoye,1 and Christian Wadsack1

1Clinic of Obstetrics and Gynecology, Medical University of Graz, Graz; 2Division of Bioinformatics, Biocenter, Innsbruck Medical University, Innsbruck; 3Center of Medical Research, Medical University of Graz; 4Department of Chemistry, Karl-Franzens University of Graz; and 5Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria

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Physiol Genomics 43: 1255–1262, 2011. First published September 27, 2011; doi:10.1152/physiolgenomics.00109.2011.—Maternal lipoproteins have been studied extensively in human pregnancies, but little is known about the role of fetal lipoproteins. The vascularized human placenta interfaces between the mother and fetus to transfer nutrients for sustaining pregnancy. Unlike that of adults, fetal high-density lipoprotein (HDL), which is in contact with placental vessels, is characterized by a high proportion of apolipoprotein E (apoE). We hypothesize this unique composition of fetal HDL affects key functions of the growing fetal tissues. The aim was to identify genes regulated by apoE-rHDL by incubating human placental endothelial cells (HPEC) with either fetal HDL or apoE-rich reconstituted HDL particles (apoE-rHDL). HPEC were exposed to 15 μg/ml fetal HDL, 15 μg/ml apoE-rHDL, or medium for 16 h, respectively. Microarray analysis determined genes regulated by fetal HDL and apoE. Characterization of HDL particles revealed a different hydrodynamic radius for apoE-rHDL (13.70 nm) compared with fetal HDL (18.11 nm). Stepwise gene clustering after microarray experiments identified 79 differentially expressed genes (P < 0.05) when cells were exposed to HDL compared with controls. Among them 16 genes were downregulated, whereas five genes were upregulated by twofold, respectively. When HPEC were incubated with apoE-rHDL 18-fold more genes (1,417, 12% of transcripts) were regulated (P < 0.05) in contrast to HDL. Thereof, 172 genes were downregulated and 376 genes upregulated (twofold). In the common subset of 38 genes regulated by both HDL particles, genes involved in cholesterol biosynthesis and cell protection prevailed. Strikingly, results suggest that HDL has the capability of regulating metallothioneins, which may have an effect on oxidative stress in HPEC.

reconstituted high-density lipoprotein particle; microarray analysis; metallothioneins; cholesterol biosynthesis

PREGNANCY AFFECTS MATERNAL metabolism of various substrates and nutrients including lipids. This is reflected by increased maternal serum concentrations of fatty acids, triglycerides, and cholesterol and by changes in lipoprotein concentrations and compositions (10). In addition to the metabolism of the mother, the fetus has gained interest in recent years. The finding of early atherosclerotic lesions in early childhood associated with maternal dyslipidemia (32) has warranted studies on neonatal lipoproteins. Thus, functional studies of neonatal lipoproteins may allow an early identification of lipoprotein metabolic abnormalities with long-term consequences. Since the fetal circulation is in contact with the placental vascular endothelium, an understanding of the impact of neonatal lipoproteins on the placental-fetal vasculature may give new insights into potential control mechanisms with which the fetus regulates lipid and cholesterol homeostasis at the feto-placental interface.

The plasma lipoprotein profile of cord blood significantly differs from adult plasma in its composition. In particular, HDL represents the main lipoprotein class in cord blood, whereas very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are present at much lower concentration than in adult plasma (3, 33). The total cholesterol concentrations in the cord blood reaches one-third of that in adults, and thereof 50–60% are partitioned to LDL and 40–50% to HDL (8, 9). Hence, cord blood HDL transports a large amount of cholesterol within the fetal circulation. Subfractionation of the fetal HDL into HDL2 and HDL3 by density gradient ultracentrifugation revealed that HDL2 represents the majority of the HDL mass (9) in cord blood, whereas in the adult plasma HDL2 is prevalent.

Although in adult lipoprotein metabolism the antiatherogenic role of HDL (12) has been well established, there is little information on the functions of fetal HDL. The majority of cholesterol in cord blood is required for the rapidly growing fetal tissues as a cell-membrane component and for sustaining cholesterol-dependent transmembrane signaling (11, 40). Furthermore, fetal cholesterol serves as precursor for progesterone synthesis and is involved in the activation of sonic hedgehog signaling, a molecule responsible for the development of the central nervous system (7).

A main feature of fetal HDL is the high proportion of apolipoprotein E (apoE) (5). ApoE is a plasma constituent of all triglyceride-rich lipoproteins such as VLDL and participates in lipoprotein metabolism through its interaction with cell surface receptors (20). It is synthesized in many tissues, including the liver, intestine, adrenal glands, kidney, testes, ovary, and brain (26).
Three major apoE isoforms designated E2, E3, and E4 are encoded by three alleles at the apoE locus. They give rise to six common phenotypes. The most common isoform, E3, is found in ~64% of the population and is considered the “neutral” Apo E genotype. It is characterized by higher plasma cholesterol levels and decreased levels of triglycerides and apoE compared with the E2 allele. Consistently, in adults elevated levels of apoE are associated with impaired clearance of remnant particles containing apoE2 (18).

It is tempting to speculate that the unique HDL composition in the fetal circulation allows fetal HDL to exert key functions for development and metabolism of fetal tissues. There is scant information, but cholesterol enriched with apoE is essential for the proliferation of brain cells and myelin formation (26), a process that is very important during early fetal development. These data suggest that the function of apoE-rich HDL in the fetal circulation is different from adult HDL with its major apolipoprotein A1 (apoA1) and may extend to functions unrelated to its lipid cargo (2).

The aim of this study was to identify HDL and apoE-regulated genes in human placental endothelial cells (HPEC), which in vivo are exposed to fetal HDL. Furthermore, we hypothesize that apoE as the predominant apolipoprotein of fetal HDL mediates important functions for fetal and placental development.

MATERIAL AND METHODS

Isolation of Endothelial Cells From the Human Placenta

Human placental tissue was obtained from healthy women after an uncomplicated pregnancy at term. The amnion was removed, and macrovascular vessels were dissected in a size of 5–7 cm for the isolation of HPEC as described previously (24). Informed consent was obtained from the mothers, and ethical approval was granted by the ethics committee of the Medical University of Graz.

Cell Culture Experiment

Primary isolated cells were cultured in endothelial basal medium (EBM; Clonetics, Cambrex, Walkersville, MD), supplemented with 5% fetal calf serum (FCS), and several growth factors (EGM-MV BulletKit, Clonetics).

For the microarray experiment five HPEC preparations, each isolated from different placentas, were used. After 48 h HPEC were washed once with 37°C 1× HBSS, and cells were treated with 15 μg/ml native fetal HDL, 15 μg/ml reconstituted HDL (rHDL), or EBM (as vehicle control), respectively. The concentration of 15 μg/ml fetal and apoE-rHDL was chosen on the basis of published results demonstrating 17.6 μg/ml apoE in cord blood HDL (9). All cell incubations were performed without medium supplements and FCS. After 16 h RNA was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Each treatment and each biological replicate were run and analyzed independently on a separate microarray. For microarray analyses 18 μg total RNA were used and RNA aliquots were retained for the qRT-PCR verification of regulated genes. RNA quality was tested with the 2100 Bioanalyzer (Agilent). Cell viability was tested by measuring lactate dehydrogenase (LDH) release from cells into the culture supernatant using the Roche/Hitachi cobas c System (Roche).

Isolation of Fetal HDL

At delivery, blood was collected from the umbilical cord and after centrifugation at 2,500 rpm for 10 min, EDTA-plasma was obtained. Due to the small amounts of collected cord plasma fetal HDL was isolated by an adapted ultracentrifugation method (37). After adjusting plasma density to 1.24 g/ml with potassium bromide, we transferred 1.7 ml plasma to a Quick-Seal Bell-top Tube (Beckman Coulter) for the TLA-100.4 rotor (Beckman Coulter). Plasma was centrifuged at 100,000 rpm for 2.5 h after the centrifugation tube was refilled with a potassium bromide solution (density 1.006 g/ml). For visualizing the HDL fraction within the tubes, DiI dye (1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate) was added in one reference tube as an indicator. HDL was reisolated as described elsewhere (36). The purity of fetal HDL was tested by lipid gel electrophoresis and further chemically characterized (27). The retention factor (Rf) values were calculated after staining the gels with Coomassie brilliant blue. Light scattering analyses of the particles allowed determination of the hydrodynamic radius of the molecules (38). The protein concentration of fetal HDL was determined by the Bradford reagent (Sigma Aldrich, St. Louis, MO).

Preparation of Discoidal Reconstituted apoE-rich HDL Particle

Discoidal rHDL containing t-α-phosphatidylcholine (Avanti Polar Lipids) and free cholesterol (Sigma Aldrich) both dissolved in chloroform and apolipoprotein E3 (apoE3, Invitrogen) was prepared as described previously (41). Briefly, 3.23 μmol of phospholipid was mixed with 13.7 nmol free cholesterol, followed by evaporation of the solvents under a stream of argon. The resulting film was solubilized under vortexing with 110 μl Na-cholate solution (10% Na-cholate in 0.2 M potassium phosphate buffer, pH 7.4) until a clear solution was obtained, followed by a drop-wise addition of 259 μl recombinant apoE3 (3.3 nmol) mixed with 300 μl potassium phosphate buffer (0.2 M) (13). Human recombinant apoE3 was solubilized in 0.7 M ammonium bicarbonate buffer. Extensive dialysis of reconstituted apoE-rich HDL (apoE-rHDL) against PBS for 12 h followed with at least six buffer changes. After dialysis apoE-rHDL was stored under argon atmosphere at 4°C. Protein of apoE-rHDL was determined by Bradford and scrutinized by lipid gel electrophoresis. The Rf values were calculated after staining the gels with Coomassie brilliant blue. The hydrodynamic radius of the particle was measured by light scattering analysis as described (38).

Gene Expression Analysis

Applied Biosystems Human Genome Survey Arrays V2.0 were used to determine the transcriptional profiles of primary HPEC incubated with either fetal HDL or apoE-rHDL. Digoxigenin (DIG)-labeled cDNA probes were generated by reverse transcription of 18 μg of total RNA using the Chemiluminescent RT-Labeling kit (Applied Biosystems) as described by the protocol. Array hybridization, chemiluminescence detection, image acquisition, and analysis used Applied Biosystems Chemiluminescence Detection Kit and Applied Biosystems 1700 Chemiluminescence Microarray Analyzer following the manufacturer’s instructions (2008).

Array processing. Each microarray was first prehybridized at 55°C for 1 h in hybridization buffer with blocking reagent (Applied Biosystems). Oligo-5T-primed, DIG-labeled cDNA targets were first fragmented mixed with internal control target and hybridized to the equilibrated microarrays in a volume of 1.5 ml at 55°C for 16 h. After hybridization, the arrays were washed with hybridization wash buffer and chemiluminescence rinse buffer. Enhanced chemiluminescence signals were generated by incubating arrays with alkaline phosphatase-conjugated anti-DIG antibody followed by incubation with chemiluminescence enhancing solution and a final addition of chemiluminescence substrate. Four images were collected for each microarray using the ABI 1700 Chemiluminescent Microarray Analyzer. Images were auto-gridded, and the chemiluminescent signals were quantified.

Data analysis. Data transformation and normalization: expression values <10 were set to 10. Data were normalized to the 50th centile (intra-array normalization), and each probe was normalized to the raw.
median expression (interarray normalization). The median interassay variability (coefficient of variation) for all three groups of hybridization control probes was ~5%. The mean normalized signals over all negative controls and assays were 0.54 ± 1.1.

Data were filtered based on the signal-to-noise ratio (signal-to-noise >3 in all samples of the respective biological replicate group) and nonchanging probes (normalized expression levels from 0.667 to 1.334 in at least three of three conditions). The remaining probes in the data set were tested for significant different expression in one of the three conditions (HDL, apoE-rHDL, control) with one-way analysis of variance. *P* values were corrected for multiple hypothesis testing based on the false discovery rate (FDR) using the Benjamini-Hochberg method (4). At an FDR cut-off of 5% 2,387 probes were considered significantly differentially expressed. These probes were then tested between two different groups (HDL vs. control or rHDL vs. control) and considered significantly differentially expressed when *P* < 0.05 (Welch’s *t*-test) and relative expression values were averaged over biological replicates. For further analysis only transcripts with current RefSeq annotation (NM_) were considered and, where stated, only probes with twofold change (log2 fold change >1 or log2 fold change <−1) were used. All data analyses were performed using GeneSpring software 7.3.1 (Agilent Technologies). Clustering and heat-map visualizations of gene expression were performed using Genesis (42).

The experiment is loaded in ArrayExpress (ArrayExpress accession, E-MEXP-3299; specified release date, 2011-07-09).

cDNA Synthesis and qRT-PCR

qRT-PCR was used to confirm microarray data of regulated genes. To this end at least 2 μg RNA was used for cDNA synthesis (Invitrogen). Standard procedures of qRT-PCR followed manufacturer’s instructions (Applied Biosystems) The following TaqMan Gene Expression Assays were used: ANGPTL4, Hs01101125_m1; MT2A, Hs01591333_g1; MT1X, Hs00745167_sH; ACAT2, Hs00255067_m1; DHCR24, MT4, Hs00745167_sH; MT2A, Hs01591333_g1; MT1X, Hs00745167_sH; MT4, Hs00255067_m1; DHCR24, Hs000181192_m1; ACAT2, Hs00255067_m1; DHCR24, Hs00207288_m1; RLP30 served as endogenous control gene.

| Table 1. Electrophoretic mobility (Rf values) and hydrodynamic radii of fetal HDL, rHDL, and apoE |
|---------------------------------------------------|---------------------------------|
| **Fetal HDL**                                      | **Hydrodynamic Radii, nm (± σ)** |
| 0.526 (± 0.010)                                    | 18.11 (± 0.53)                  |
| **rHDL**                                           |                                 |
| 0.544 (± 0.010)                                    | 13.70 (± 0.33)                  |
| **apoE**                                           |                                 |
| 0.313 (± 0.045)                                    | 100.26                         |

apoE-rHDL (0.544 ± 0.01) resulted from the different composition of both molecular assemblies. The lipid moiety of fetal HDL, which accounts for the typical spherical structure of mature HDL, contains mainly cholesteryl ester, free cholesterol, triglycerides, and phospholipids. In contrast discoidal apoE-rHDL was prepared with one single phospholipid (L-α-phosphatidylcholine) and free cholesterol. Moreover, the structural differences between native fetal HDL and apoE-rHDL are accounted for by the association with HDL of hydrophilic apolipoproteins and a variety of enzymes (6), whereas apoE-rHDL comprises only one single apoE (Fig. 1). These structural differences could also be confirmed by calculating the hydrodynamic radii (Table 1) of each sample obtained after light scattering analysis.

The lowest Rf value (0.313 ± 0.045) with the highest hydrodynamic radius (100.26) was obtained for free apoE solubilized in potassium phosphate buffer (Table 1). The very high Stokes diameter of the apoE sample suggests the formation of apoE protein aggregates without association of lipids.

**Cell Viability**

As a first step, cell viability and morphology were assessed visually by light microscopy. Adherent viable HPECs form cell monolayers with cobblestone morphology (24). After 16 h incubation with fetal HDL or apoE-rHDL cells appeared viable. To further ascertain cell viability, LDH was measured in the supernatants of HPEC after 16 h incubation with 15 μg/ml fetal HDL, apoE-rHDL, and with the vehicle control. Cellular LDH release was decreased compared with the control after incubation with both HDLs. Therefore, 15 μg/ml was chosen as a suitable concentration for cell experiments.

**Microarray Data Revealed Commonly Regulated Genes by Treating HPEC With Differently Composed HDLs**

To identify the impact of apoE on gene regulation in HPEC the results of the microarray experiment were analyzed in a stepwise manner (Fig. 2).

In the first analysis step all significantly (*P* < 0.05) regulated genes (1,496) in HPEC were summarized in a Venn diagram (Fig. 3A). Among the 11,224 regulated transcripts in the HPEC, 79 (0.65%) were differentially expressed when cells were exposed to fetal HDL compared with untreated cells. Within this group 16 genes were upregulated more than twofold and five genes downregulated more than twofold. When HPEC were treated with apoE-rHDL 1,417 genes (12%) were differentially expressed (*P* < 0.05) compared with untreated cells. Of these 172 genes were downregulated more than twofold and 376 genes were upregulated more than twofold. Among both sets of genes regulated by fetal and apoE-rHDL 38 (0.3%) were commonly regulated.

**RESULTS**

**Characterization of apoE-rHDL**

apoE-rHDL was subjected to two different methods of characterization to confirm that the isolated fractions contained solely particles with no free apoE protein. First the electrophoretic mobility and hydrodynamic radii of the particles were measured by light scattering. Fetal HDL was also thoroughly scrutinized after isolation from plasma and prior to its use in cell culture experiments. Table 1 summarizes the size distribution of apoE-rHDL compared with isolated fetal HDL and free apoE, respectively. The larger particle size of fetal HDL compared with apoE-rHDL (18.11 ± 0.53 vs. 13.7 ± 0.33 nm), as well as the lower electrophoretic mobility (Rf values of the protein staining) of fetal HDL (0.526 ± 0.01) compared with
Secondly, 1,105 transcripts with current RefSeq annotation were considered for further analyses and were classified in different groups (A–G) as summarized in the heat maps (Fig. 3B) showing each treatment separately. Within the groups C, D, and E an overlap of 27 significantly up- and/or downregulated genes in response to both treatments could be observed (Fig. 3C).

In the third step of analysis we focused on the largest common subset of genes (groups C, D, E; Fig. 3C), which are regulated by fetal HDL and apoE-rHDL. Among these two biological functions were predominant: 1) genes involved in lipid metabolism: LDLR (low density lipoprotein receptor), ACAT2 (acyetyl-Coenzyme A acetyltransferase 2, synonym: acetoacetyl Coenzyme A thiolase), DHCR24 (24-dehydrocholesterol reductase), ANGPTL4 (angiopoietin-like 4), and 2) stress response genes: metallothioneins (MT1X, MT2A, MT4).

**QRT-PCR Confirmed Regulation of Lipid Metabolism and Stress Response Genes**

The microarray data of seven genes selected on their regulation by fetal HDL and apoE-rHDL were confirmed using qRT-PCR (Table 2). The significant \((P < 0.001)\) decrease of MT2A and MT1X could be confirmed by qRT-PCR. However, the regulation of MT4 could not be validated although two different TaqMan assays were used. The qRT-PCR expression of most relevant lipid-associated genes could be verified by qRT-PCR except for LDL-R after apoE-rHDL treatment.
**DISCUSSION**

The purpose of the present study was to identify the effect of fetal HDL on the endothelium of the human placenta. This interest was sparked by the distinct composition of fetal as opposed to adult HDL leading to the hypothesis that the protein cargo of HDL may represent a fetal signal to the placenta. We have decided to use placental arterial endothelial cells (EC) as the cell type for all experiments, because the arterial vascular system represents the circulation carrying blood from the fetus to the placenta. Hence, any fetal signal to regulate its own organ placenta would primarily act upon its arterial EC.

In addition we aimed at identifying potential effects of apoE as one of the major apolipoproteins of fetal HDL. The strategy that we have chosen was to compare the effects of native fetal HDL with those of a discoidal particle composed of a cholesterol core, surrounded by phospholipids and one single apolipoprotein, apoE. This strategy was based on the assumption that a proportion of genes regulated by native fetal HDL and the apoE-rHDL particle is accounted for by apoE present in both. Thus the overlap, i.e., the commonly regulated genes, would represent the apoE effect of the native fetal HDL. The alternative approach of comparing apoE-rHDL with a particle

**Table 2. Fold change values of commonly regulated genes (groups C, D, E)**

<table>
<thead>
<tr>
<th>GenBank</th>
<th>Gene Symbol</th>
<th>apoE-rHDL Microarray</th>
<th>apoE-rHDL qRT-PCR</th>
<th>Fetal HDL Microarray</th>
<th>Fetal HDL qRT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_005952.2</td>
<td>MT1X</td>
<td>0.488</td>
<td>0.352†</td>
<td>0.332</td>
<td>0.478†</td>
</tr>
<tr>
<td>NM_005946.1</td>
<td>MT2A</td>
<td>0.517</td>
<td>0.411†</td>
<td>0.532</td>
<td>0.565†</td>
</tr>
<tr>
<td>NM_032935.1</td>
<td>MT4</td>
<td>0.405</td>
<td>n.v.</td>
<td>0.521</td>
<td>n.v.</td>
</tr>
<tr>
<td>NM_000527.2</td>
<td>LDLR</td>
<td>2.037</td>
<td>1.097m</td>
<td>0.617</td>
<td>0.548†</td>
</tr>
<tr>
<td>NM_014762.1</td>
<td>DHCR24</td>
<td>0.280</td>
<td>0.226†</td>
<td>0.442</td>
<td>0.721*</td>
</tr>
<tr>
<td>NM_005891.1</td>
<td>ACAT2</td>
<td>0.389</td>
<td>0.249†</td>
<td>0.513</td>
<td>0.472†</td>
</tr>
<tr>
<td>NM_139314.1</td>
<td>ANGPTL4</td>
<td>3.599</td>
<td>3.018†</td>
<td>2.293</td>
<td>2.337*</td>
</tr>
</tbody>
</table>

*P < 0.05, †P < 0.01; ns, not significant; n.v., not verified.
lacking apoE, i.e., made up of phospholipids and cholesterol only, was regarded too unphysiologic and, therefore, was not a suitable option.

Since the majority of apoE in the cord blood is associated with HDL, we focused on the role of apoE-HDL complex rather than on free apoE in the fetal circulation. All particles were carefully characterized (Table 1, Fig. 1). This does by no means discount the fact that mature HDL carries a wide range of proteins in addition to apoE, which all may exert transcriptional effects on target cells. Moreover, fetal HDL and apoE-rHDL differ in size, form, and composition (Fig. 1), making likely a different handling and processing by the EC including different binding, uptake, degradation, metabolism, efflux, and signaling. Some studies strongly suggest that in addition to the shape of the particles (discoidal vs. spherical) the lipid and apolipoprotein composition of HDL affects its receptor affinity (25).

The key finding of this study was that both fetal HDL and apoE-rHDL, which comprises apoE combined with only small amounts of phospholipids and cholesterol in a recombinant particle, alter and control gene expression in HPEC. The difference in effect size of fetal and apoE-rHDL on gene expression is striking. It may be related to the ability of both entities to bind to various different receptors on the cell surface due to their different lipid and protein composition. Whereas ligand binding to distinct receptors and subsequent internalization and lysosomal degradation has been well established, the potential for gene regulation via HDL-cell interaction has not been fully appreciated.

While a previous study investigated the effect of ApoA1, the main apolipoprotein of adult HDL, on human coronary aortic EC (28), the present study focused on apoE-regulated genes. Microarray analyses identified a pronounced difference in the number of regulated placental genes (79 genes and 1,417 genes) after treatment of HPEC with fetal HDL and apoE-rHDL, respectively. This range in transcriptional effects may reflect the different origin of both molecules. Fetal HDL is a natural component of cord blood occurring in vivo, whereas apoE-rHDL is an artificial molecule, mimicking a discoidal premature HDL particle.

Among all regulated genes, a small group of commonly regulated genes (38) between fetal HDL and apoE-rHDL could be identified, which may reflect an apoE-mediated effect. According to functional clustering groups of these genes participate in lipid metabolism, especially cholesterol biosynthesis, and in antioxidative effects. The genes in the latter cluster were all members of the metallothionein family.

**ApoE Regulates Genes Participating in Cholesterol Metabolism**

Cholesterol homeostasis in EC is poorly understood. Vascular EC do not accumulate cholesterol such as macrophages even though they also express a variety of receptors that allow them to recognize and respond to cholesterol and have the biochemical pathways for sterol synthesis (19). It has been suggested that EC maintain their cholesterol homeostasis by cholesterol efflux and downregulation of endogenous cholesterol biosynthesis when cholesterol is supplied (30). Therefore, it is not surprising that HPEC exposed to fetal HDL or apoE-rHDL, both particles representing an exogenous cholesterol source, lower the expression of key genes involved in cholesterol biosynthesis. ACAT2 catalyzes the initial step of cholesterol biosynthesis, i.e., the conversion of acetyl-CoA to acetoacetyl-CoA (21, 31, 43), and DHCR24 catalyzes the final step in the cholesterol biosynthesis, the conversion from desmosterol to cholesterol (46).

LDLR is an additional target of fetal HDL and showed a minor (0.6-fold change) but significant ($P = 0.02$) reduction under this treatment. In contrast apoE-rHDL was upregulated twofold in response to apoE-rHDL, but this effect could not be confirmed by qRT-PCR. The difference between fetal HDL and apoE-rHDL in cholesterol content and hence diverse extracellular cholesterol supply to HPEC may account for LDLR regulation. In addition the promoter region of LDLR contain a sterol regulatory element 1 (15, 17), making it also susceptible to cholesterol regulation. High intracellular cholesterol levels prevent their transcription (45), which might explain their reduced expression in HPEC in response to fetal HDL similar to another study (30). Furthermore, forced overexpression of apoE in mice reduces plasma cholesterol levels and prevents diet-induced hypercholesterolemia in contrast to the controls (39). These results together with our findings underline the importance of apoE as a determinant of lipoprotein levels with the capability to influence cholesterol metabolism.

The present study also indicates that the expression of genes participating in cholesterol biosynthesis is not only regulated by the amount of cholesterol but may also be affected by phospholipids or the apolipoprotein composition of the lipoprotein itself.

**ANGPTL4**, a secreted glycoprotein, is upregulated in response to fetal HDL and apoE-rHDL. This protein participates in angiogenesis exerting pro- and/or antiangiogenic functions depending on the cellular context and other factors present (14, 22). If confirmed in HPEC this would open new perspectives for regulating placental angiogenesis and warrant further investigations.

In the human placenta blood vessel formation is required for embryogenesis and placental vascularization. HDL has prosurvival and proangiogenic properties. It stimulates tube formation, EC migration and vasorelaxation (1). Therefore, the increase of ANGPTL4 in response to fetal HDL may represent a proangiogenic fetal signal. An even more pronounced increase was observed in response to apoE-rHDL and suggests that the HDL, native or apoE-rHDL, effect is mediated by apoE. Further work is needed to substantiate this attractive notion.

**ApoE Regulates Genes Contributing EC Protection**

Fetal HDL and apoE-rHDL reduced the metallothionein MT1X and MT2A expression (Fig. 3). This is a novel and unexpected finding. MTs, which encode a family of small proteins characterized by a high metal [Zn(II), Cu(II)] and toxic metal (cadmium, mercury) content that contribute to cellular protection from reactive oxygen species (ROS). These proteins are able to scavenge a wide range of ROS including superoxide, hydrogen peroxide, hydroxyl radicals, and nitric oxide (23, 35). Their antioxidative potency is threefold higher than that of glutathione (35), the most abundant antioxidant in the cytosol (44).
Within the human, term placenta MTs are secreted by maternal (decidual cells) and fetal (trophoblasts, amniotic epithelium) cells (16). Recently, we found a 35% higher MT expression in placental arterial than in venous EC (Gomes L. and Desoye G., unpublished data). This may represent a mechanism to protect the placenta from toxic effects of fetal waste products, which accumulate in the arterial branch of the vascular tree.

In the adult the protective and antioxidative effect of HDL on the vascular endothelium has been widely recognized (29). The synthesis of MTs in general is increased by various stressful conditions that mainly cause lipid peroxidation and induce the synthesis of MT isoforms (23). However, to the best of our knowledge, the regulatory effect of HDL on MT expression levels has never before been described. Although the HDL-induced downregulation of MTs at first sight seems to contradict an HDL-mediated protective effect, we interpret it as consequence of HDLs general antioxidative effect, which confers protection for the EC thus making additional protection by MTs unnecessary.

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**Disclosures**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**Author Contributions**

Author contributions: M.A., B.E., A.C., and O.G. performed experiments; M.A., H.H., B.E., A.C., and O.G. analyzed data; M.A. and H.H. edited and revised manuscript; C.W. conceived and designed research; C.W. approved final version of drafted manuscript; G.M., U.L., G.D., and C.W. edited and revised manuscript; G.M. interpreted results of experiments; M.A. and H.H. prepared figures; M.A., H.H., B.E., A.C., and O.G. analyzed data; M.A., H.H., A.C., O.G., and C.W. conceived and designed research; C.W. approved final version of manuscript.

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