

Christian Wadsack, Silvia Tabano, Alexandra Maier, Ursula Hiden, Gioia Alvino, Veronica Cozzi, Manfred Hüttinger, Wolfgang J. Schneider, Uwe Lang, Irene Cetin and Gernot Desoye

Am J Physiol Endocrinol Metab 292:476-484, 2007. First published Sep 26, 2006;
doi:10.1152/ajpendo.00547.2005

You might find this additional information useful...

This article cites 43 articles, 21 of which you can access free at:

<http://ajpendo.physiology.org/cgi/content/full/292/2/E476#BIBL>

This article has been cited by 1 other HighWire hosted article:

Adverse Birth Outcome Among Mothers With Low Serum Cholesterol

R. J. Edison, K. Berg, A. Remaley, R. Kelley, C. Rotimi, R. E. Stevenson and M. Muenke
Pediatrics, October 1, 2007; 120 (4): 723-733.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajpendo.physiology.org/cgi/content/full/292/2/E476>

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:

<http://www.the-aps.org/publications/ajpendo>

This information is current as of January 10, 2008 .

Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition

Christian Wadsack,^{1*} Silvia Tabano,^{1,2*} Alexandra Maier,¹ Ursula Hiden,¹ Gioia Alvino,² Veronica Cozzi,² Manfred Hüttinger,³ Wolfgang J. Schneider,⁴ Uwe Lang,¹ Irene Cetin,^{2*} and Gernot Desoye^{1*}

¹Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria; ²Clinic of Obstetrics and Gynecology, University of Milano, Milan, Italy; ³Center of Physiology and Pathophysiology; and ⁴Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University of Vienna, Vienna, Austria

Submitted 9 November 2005; accepted in final form 5 September 2006

Wadsack C, Tabano S, Maier A, Hiden U, Alvino G, Cozzi V, Hüttinger M, Schneider WJ, Lang U, Cetin I, Desoye G. Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition. *Am J Physiol Endocrinol Metab* 292: E476–E484, 2007. First published September 26, 2006; doi:10.1152/ajpendo.00547.2005.—Among other factors, fetal growth requires maternal supply of cholesterol. Cellular cholesterol uptake is mainly mediated by the LDL receptor (LDL-R) and the scavenger receptor family. We hypothesized that expression levels of key receptors of these families were regulated differently in placentas from IUGR pregnancies with varying degrees of severity. Third-trimester placentas from IUGR pregnancies with (IUGR-S) and without (IUGR-M) fetal hemodynamic changes and from control (AGA) pregnancies were studied. LDL-R, LDL-R-related protein (LRP-1), and scavenger receptor class B type I (SR-BI) mRNA and protein levels were measured. Cholesterol concentration and composition of lipoproteins were analyzed enzymatically and by lipid electrophoresis, respectively, in maternal and umbilical cord blood. LDL-R mRNA levels in IUGR-M were similar to AGA but lower ($P < 0.05$) in IUGR-S. In contrast, LDL-R protein was twofold (IUGR-M) and 1.8-fold (IUGR-S) higher ($P < 0.05$) than in the AGA group. LRP-1 mRNA and protein levels were not altered in the IUGR cases. SR-BI mRNA was unchanged in IUGR, but protein levels were lower ($P < 0.05$) in IUGR-S than in the other groups. Maternal plasma concentrations of LDL cholesterol were higher ($P < 0.05$) in the AGA group (188.5 ± 23.6 mg/dl) than in the IUGR-S group (154.2 ± 26.1). Electrophoretic mobility of the LDL fraction in maternal plasma demonstrated significant changes in migration toward higher values (AGA 0.95 ± 0.06 , IUGR-M 1.12 ± 0.11 , $P < 0.001$; IUGR-S 1.28 ± 0.20 , $P = 0.002$). We conclude that LDL-R and SR-BI levels are altered in IUGR pregnancies. These differences were associated with changes in LDL, but not HDL, mobility and cholesterol concentration in maternal circulation.

pregnancy; placenta; lipids; fetal growth

CHOLESTEROL HAS MULTIPLE BIOLOGICAL ROLES that include its functioning as a structural membrane component, precursor for steroid synthesis, and activator of various cellular processes (42). Extensive steroid hormone synthesis in the placenta and the rapid growth and development of the fetus make pregnancy a condition of high cholesterol demand in the fetoplacental unit (23). In humans, both placental tissue (39) and fetal organs (5) have the capacity for de novo cholesterol synthesis. However, the high cholesterol demand in the fetal tissues may not

be fully satisfied by endogenous means. A significantly higher cholesterol concentration in the umbilical vein compared with the arteries (33) suggests transfer from maternal or placental sources to the fetus. In the third trimester of gestation, maternally-derived cholesterol reportedly contributes ~22–40% to the fetal cholesterol pool (14, 24). However, this remains controversial (25). The molecular mechanisms accounting for the uptake of maternal cholesterol into the placenta, mainly as lipoprotein-associated cholesterol (42), and for subsequent transfer, if any, into the fetal circulation, are as yet poorly understood.

Cellular uptake of maternal low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) is mainly mediated by two receptor families, LDL receptor (LDL-R) and the scavenger receptor family. In the human placenta, binding sites for LDL (16), VLDL, (41) and HDL (1) have been identified on the microvillous membrane of the syncytiotrophoblast. LDL-R-related protein (LRP-1) is a multiligand cell-surface receptor expressed in trophoblast during gestation (8) that binds apolipoprotein E-containing particles (4), such as VLDL. Because of the profound increase in circulating maternal VLDL with advanced gestation (40), syncytiotrophoblast LRP-1 may play a role in acquiring cholesterol for metabolic needs of both placenta and fetus (11, 36).

Scavenger receptors bind modified LDL as well as HDL (27). Recently, a developmental change in placental expression of the scavenger receptor class B type I (SR-BI), a high-affinity receptor for HDL, has been described with decreasing protein expression from the first to the third trimester (38).

Intrauterine growth restriction (IUGR) is a condition often associated with a decreased supply of nutrients and/or oxygen to the growing conceptus. Due to placental failure, an adequate provision of the fetus with oxygen and nutrients may result (21). Alterations in expression levels of placental lipoprotein receptors could be associated with changes in their uptake or efflux functions and might contribute to altered lipid levels in the fetal circulation in pregnancies characterized by IUGR, hence, contributing to poor fetal growth (6).

We hypothesized that the expression levels of lipoprotein receptors involved in cholesterol uptake and metabolism may be downregulated in placentas from IUGR pregnancies. Furthermore, the degree of downregulation might correlate with the severity of the pathology. Lipoprotein particles are heterogeneous in size, density, electric charge, and chemical com-

* C. Wadsack and S. Tabano contributed equally to this work as first authors; I. Cetin and G. Desoye contributed equally to this work as last authors.

Address for reprint requests and other correspondence: C. Wadsack, Clinic of Obstetrics and Gynecology Medical University of Graz, Auenbruggerplatz 14, A-8036 Graz, Austria (e-mail: christian.wadsack@meduni-graz.at).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

position. These heterogeneities, which are due to environmental factors, such as the fatty acid content of the diet (22), may also modulate fetal development. Therefore, in this study we investigated placental expression of receptors for the major lipoproteins involved in cholesterol uptake as well as maternal and fetal lipoprotein composition in IUGR pregnancies. We have related the observed changes to the severity of the pathology as represented by hemodynamic changes in the IUGR fetuses.

SUBJECTS AND METHODS

Subjects. Placental and blood samples were obtained from IUGR and appropriate-for-gestational-age (AGA) pregnancies. Gestational age was calculated from the last menstrual period and confirmed by an ultrasonographic examination performed before 20 wk of gestation. Babies with morphological malformations at birth and/or chromosomal abnormalities were excluded from the study. Mothers were excluded if they had obstetrical complications (hypertension, diabetes mellitus, or gestational diabetes) or factors predisposing to IUGR. Control pregnancies (AGA) were women who gave birth to healthy-term neonates with a birth weight between the 10th and 90th percentile according to Italian standards for birth weight and gestational age (19).

IUGR was defined on the basis of ultrasound measurements of the abdominal circumference with values below the 10th percentile of reference values, together with a percentile reduction >40% compared with the previous measurement of abdominal circumference and birth weight below the 10th percentile according to Italian standards for birth weight and gestational age (19). Intrauterine growth-restricted fetuses were divided into two groups according to Doppler velocimetry of the umbilical artery: IUGR-M was composed of fetuses with normal pulsatility index (PI); IUGR-S had abnormal PI (20). All women delivered by elective caesarean section.

At delivery placentas were trimmed free of membranes, and three pieces were excised such that they contained tissue from both the fetal and maternal side. After being washed in physiological saline containing EDTA to eliminate excess blood, they were stored at -80°C within 30 min.

Informed consent was obtained from the patients, and the ethics committee of the Medical Faculty, University of Milan, granted ethical approval.

Blood samples. Blood samples were collected at the time of delivery from a maternal brachial vein after a 12-h overnight fast as well as from umbilical artery and vein in tubes containing 1.0 mg/ml Na_2EDTA . Plasma was harvested by immediate centrifugation (4,000 rpm) in a bench centrifuge at 4°C for 10 min and stored at -80°C until electrophoretic analysis. Aliquots of plasma were used immedi-

ately for measurements of plasma LDL- (LDL-C) and HDL-cholesterol (HDL-C) concentration using commercially available enzymatic reagents for quantitative cholesterol determination (Wako Chemicals, Neuss, Germany).

Materials. Primers for LDL-R, LRP-1, and the ribosomal protein L30 were selected using the software Primer 3 that is available online at <http://www-genome.wi.mit.edu>. For LDL-R, they were forward primer (F): 5'-TGG CAT CAC CCT AGA TCT CC-3', reverse primer (R): 5'-GGT GGT CCT CTC ACA CCA GT-3', amplifying a 323-bp fragment; for LRP-1, they were F: 5'-ACC TGC CAG ATC CAG AGC TA-3''27', R: 5'-CTC TGG CTG AGG TGG AAG TC-3', amplifying a 270-bp fragment; for SR-BI, they were F: 5'-AAA TCC GGA GCC AAG TAG GT-3', R: 5'-CCA GAA CAG GCA GAG TAG TGG-3', amplifying a 231-bp fragment; for L30, they were F: 5'-CCT AAG GCA GGA AGA TGG TG-3', R: 5'-CAG TCT GTT CTG GCA TGC TT-3', amplifying a 351-bp fragment.

In a microarray analysis (Affymetrix U133A) of isolated first-trimester and term trophoblasts and placental endothelial cells (12), the ribosomal protein L30 was most stably expressed among various housekeeping genes and, hence, was used here in RT-PCR. All primer pairs were chosen to span exons to avoid amplification of traces of potentially contaminating DNA.

Rabbit antiserum against a synthetic peptide, corresponding to the linker region between the ligand binding repeats 4 and 5 of the human LDL-R (CRGLYVFQGDSSPC), was used. CRGLYVFQGDSSP and RGLYVFQGDSSPC were coupled to keyhole limpet hemocyanin via the respective cysteines, and a 1:1 mixture (250 μg of each peptide) was injected three times on days 0, 21, and 35. The antiserum was obtained on day 42. The LRP-1 antiserum was raised in rabbits against an intracellular peptide of LRP-1 (amino acid 4532 to 4544). Antibodies for SR-BI (rabbit, polyclonal) and β -actin (mouse, monoclonal) were purchased from Abcam (Novus Biologicals, Cambridge, UK). The secondary antibody used for SR-BI and β -actin detection, respectively, was goat anti-rabbit or anti-mouse HRP conjugate (Bio-Rad, Hercules, CA).

RNA extraction and one-step RT-PCR. After extraction [carried out with the phenol and guanidine thiocyanate method (7)] and quantification, 500 ng of total RNA were reverse transcribed, and the gene-specific cDNA was amplified in the same reaction using the One-Step RT-PCR kit (Qiagen, Vienna, Austria). PCR products were separated on a 3% agarose gel, and the resulting bands were scanned for optical density using the Alpha EaseFC software (AlphaInnotech, version 3.2.3). Since different amplification conditions had to be applied for targets (LDL-R and LRP-1) and housekeeping gene (L30), reactions were performed in separate tubes. After reverse transcription at 50°C for 30 min and hot-start *Taq* polymerase activation at 95°C for 15 min, the cycle parameters were as follows: LDL-R: 29 cycles of amplification at 94°C for 30 s, 58°C for 60 s, and 72°C for 60 s;

Table 1. Clinical features of the study subjects

Clinical Data	AGA (18 cases)	IUGR-M (8 cases)	IUGR-S (12 cases)	P Value	
				IUGR-M vs. AGA	IUGR-S vs. AGA
Mother					
Age, yr	33 (22–41)	35 (30–45)	33 (23–40)	NS	NS
Weeks of gestation, wk + days	39+2 (38–40+6)	37 (33+2–38+4)	33+4 (27–38+5)	0.00014	0.00001
BMI at the beginning of pregnancy*	21.9 (18.2–27.5)	20.2 (16.2–26.2)	24.1 (18.9–37.1)	NS	NS
Conceptus					
Fetal weight, g	3,284 (2,880–3,900)	1,750 (1,370–2,430)	1,380 (620–2,280)	0.00000001	0.00000001
Placental weight, g	498 (370–700)	263 (175–412)	259 (139–456)	0.000001	0.0000003
Fetal ponderal index†	26.3 (23.8–31.6)	24.7 (23.5–26.5)	14.8 (12.6–17.0)	NS	0.000004

Values are given as median (range). AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; NS, not significant; BMI, body mass index ($P > 0.05$). The IUGR group was classified into 2 subgroups (IUGR-M, IUGR-S) according to pulsatility index (PI) obtained by ultrasound measurements. *BMI = weight (kg)/height (m^2). †Ponderal index = $\text{kg}/\text{length}^3 (\text{m}^3)$.

Table 2. Fetal characteristics of AGA and IUGR groups

	Umbilical Vein									
	PI	Po ₂ , mmHg	Pco ₂ , mmHg	Glucose, mM	Lactate, mM	pH	O ₂ sat, %	Hb, g/dl	O ₂ content, mM	
AGA (18 cases)	<i>n</i> = 18 cases	28.0 (15.2–51.2)	42.9 (37.9–53.6)	3.8 (3.3–4.5)	1.8 (1.2–2.6)	7.4 (7.3–7.4)	53.1 (34.0–80.1)	14.7 (13.3–18.0)	4.6 (2.9–6.9)	
IUGR-M (8 cases)	<i>n</i> = 8 cases	23.7 (19.4–31.0)	41.5 (33.8–51.2)	3.7 (3.0–4.4)	1.7 (1.4–2.4)	7.4 (7.3–7.4)	52.0 (42.0–57.0)	15.6 (12.7–17.7)	4.8 (3.3–5.9)	
IUGR-S (12 cases)	<i>A</i> = 12 cases	21.1 (15.0–36.8)	48.8 (42.6–61.2)	3.6 (3.1–4.3)	2.5 (1.3–4.1)	7.3 (7.0–7.4)	40.8 (15.0–79.5)	16.3 (13.5–18.6)	4.0 (1.3–6.4)	
<i>P</i> values vs. AGA and IUGR-M		NS	NS	NS	NS	NS	NS	NS	NS	
<i>P</i> values vs. AGA and IUGR-S		0.04	0.03	NS	NS	0.04	NS	0.01	NS	

Values are given as mean (range); *P* > 0.05. O₂ sat, oxygen saturation; *n*, normal; *A*, abnormal. O₂ content was calculated as O₂ content = Hb (g/l) × O₂ sat. × 0.005982 (20).

LRP-1: 30 cycles of amplification at 94°C for 30 s, 57°C for 60 s, and 72°C for 60 s; SR-BI: 30 cycles of amplification at 94°C for 30 s, 59°C for 60 s, and 72°C for 60 s; L30: 28 cycles of amplification at 94°C for 30 s, 58°C for 40 s, and 72°C for 60 s. Each experiment was performed in duplicate.

Protein extraction and Western blot analysis. Proteins used in Western blot experiments were extracted from tissues by a standard method. Aliquots of protein (30 µg) quantified by the method of Lowry et al. (15) were loaded onto and separated on either 10 (LDL-R, LRP-1) or 4–12% (SR-BI) PAGE Precast Duramide gels (Cambrex Biosciences Rockland, Rockland, ME) under reducing conditions. They were subsequently transferred to a nitrocellulose membrane (Amersham Biosciences, Little Chalfont, UK) by blotting for 1.5 h at a constant voltage of 25 V. The nitrocellulose filters were incubated overnight with the primary antibody, washed, and incubated with the secondary peroxidase-labeled IgG for 1.5 h. Proteins were detected on autoradiography film using an enhanced chemiluminescence detection kit (Amersham Biosciences). After treatment with Restore Western blot stripping buffer (Pierce), filters were reincubated with β-actin antibody to correct for loading. The bands on the films were densitometrically scanned as described above.

Lipoprotein electrophoresis. Electrophoretic mobility was determined on 0.5% agarose gels, as described by Sparks and Phillips (32). Samples (6 µl) loaded onto the gel were electrophoresed at 25 mA for 90 min using a 0.06-M barbital buffer (pH 8.6; Merck). The gels were then fixed in 0.5% 5-sulfosalicylic acid dihydrate solution (Roth) for 30 min and washed for 10 min in distilled water. They were dried overnight at room temperature and stained with 1% Sudan Black B (Fluka) in 60% ethanol (Merck). Migration distance was measured from the loading point to the front of the stained band. Relative electrophoretic mobility (Rf) was calculated as the ratio of migration distance of α- or β-band relative to 5 µl of control LDL (0.7 mg protein/ml) or HDL₃ (1.0 mg protein/ml) both isolated by sequential ultracentrifugation (31) from an age-matched healthy human pregnant female donor.

Data analysis. An AGA “control sample” was included in each RT-PCR and Western blot experiment to make possible the comparison of results obtained on different gels and blots. It represents an internal standard prepared from a pool of three fresh AGA term placentas. The coefficient of variation between the intensities of bands obtained by PCR and immunoblotting for the internal standard was 21 and 28%, 17 and 18%, and 12 and 16% for LDL-R, LRP, and SR-BI, respectively. In RT-PCR experiments the expression values for each sample were calculated as the ratio between LDL-R, LRP-1, or SR-BI and L30 intensity of the band normalized to the same ratio of the AGA control sample. Similarly, the amount of LDL-R, LRP-1, and SR-BI protein from Western blot experiments was normalized to the β-actin level for each sample and then normalized to the AGA control sample.

All statistical analyses were performed using the SigmaStat for Windows (version 2.03) statistical software package (Access Softtek, San Rafael, CA). After testing for normal distribution, ANOVA between the groups and Tukey’s post hoc test were used. Linear correlation analyses (Pearson product moment correlation) were used to test for association between maternal, fetal, and all lipoprotein variables as well as for testing of gestational age-dependent changes in mRNA and protein levels. Significances were accepted with *P* < 0.05. Unless stated otherwise, data are presented as means ± SD.

RESULTS

Characteristics of the study subjects. Table 1 summarizes demographic and obstetrical characteristics of subjects with normally grown and growth-restricted fetuses. As expected, IUGR pregnancies had significantly lower fetal and placental weights than AGA; IUGR-S fetuses had lower fetal ponderal

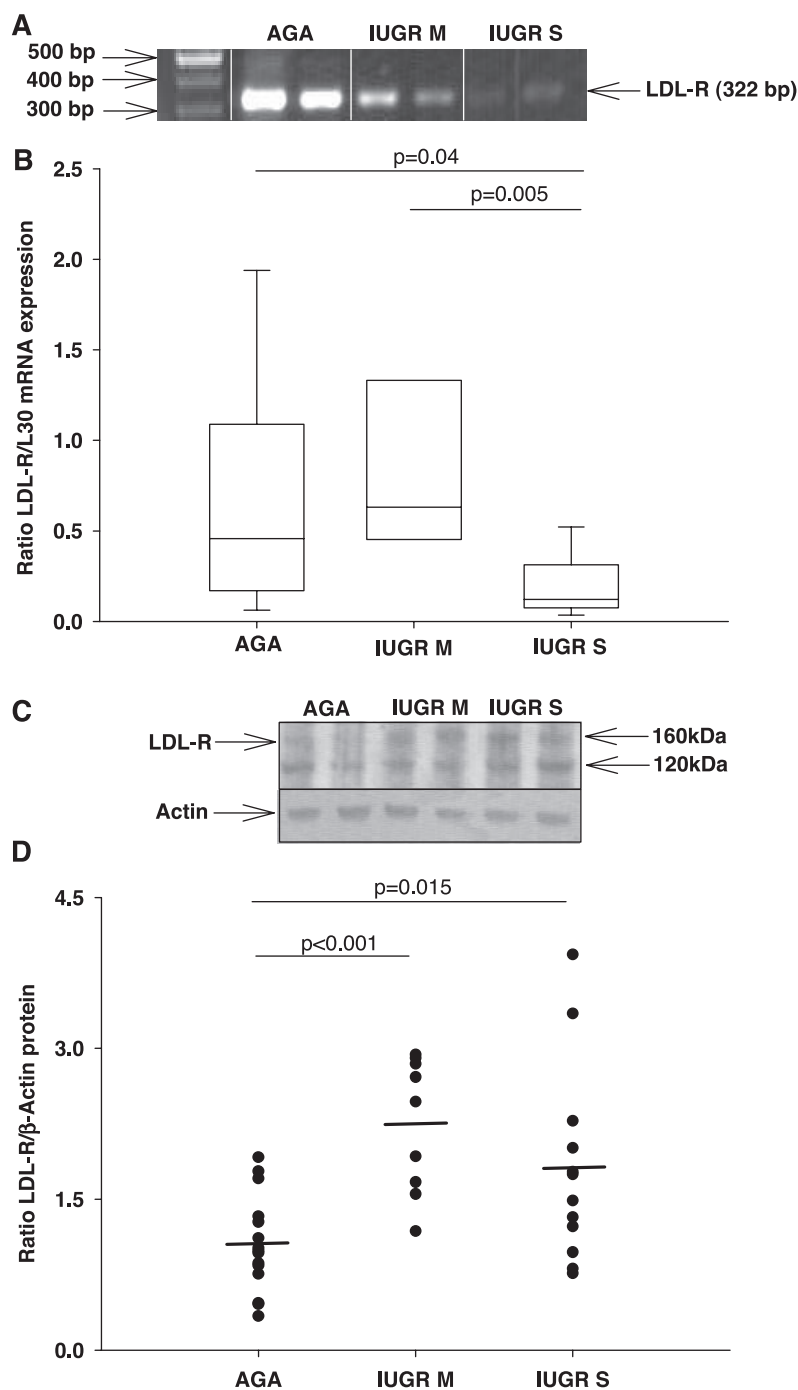


Fig. 1. *A*: semiquantitative RT-PCR for LDL receptor (LDL-R) in the various groups produced a single band of 322 bp in size. Individual data were normalized to both L30 mRNA and to an internal control included on each blot. *B*: the median value (horizontal line) in the box plot of intrauterine growth restriction IUGR-S ($n = 9$) is decreased vs. IUGR-M ($n = 5$) and appropriate for gestational age (AGA; $n = 11$). *C*: Western blot analysis of tissue samples identified 2 LDL-R bands with apparent molecular masses of 160 (mature form) and 120 kDa (precursor), with the 120-kDa protein being more prominent. *D*: mean LDL-R expression (horizontal line) was significantly increased in IUGR-M and IUGR-S compared with the AGA group. Individual data were normalized to β -actin and to the internal control in each blot.

indexes (Table 1). No significant differences were observed between IUGR-M and IUGR-S in these variables. Specific oxygenation values were different in IUGR-S compared with AGA; P_{O_2} values were significantly lower, whereas P_{CO_2} and Hb values were significantly higher in IUGR-S; moreover, lactate concentrations tended to increase in IUGR-S (Table 2).

LDL-R and LRP-1. RT-PCR for LDL-R mRNA resulted in a single product of 322 bp (Fig. 1A). Transcriptional expression levels in IUGR-M [0.83 ± 0.50 arbitrary units (AU)] were similar to those of the AGA group (0.81 ± 0.82 AU), whereas mRNA levels were lower ($P < 0.05$) in IUGR-S (0.19 ± 0.18 AU) than in IUGR-M and AGA (Fig. 1B) by 75%. Western

blot analysis of tissue samples identified two LDL-R bands at apparent molecular masses of 160 and 120 kDa. The 120-kDa protein band was more prominent and is in accordance with analyses of immunoprecipitates of the LDL-R from normal fibroblasts, which demonstrate that the receptor is detected first as a precursor of apparent 120 kDa (Fig. 1C). The precursor is converted to a mature form of apparent 160 kDa (9). Protein levels were higher in the IUGR-M (2.24 ± 0.67 AU, $P < 0.001$) and IUGR-S (1.81 ± 0.54 AU, $P < 0.05$) groups than in the AGA group (1.05 ± 0.47) (Fig. 1D). No significant relationship was observed between LDL-R expression and gestational age (data not shown).

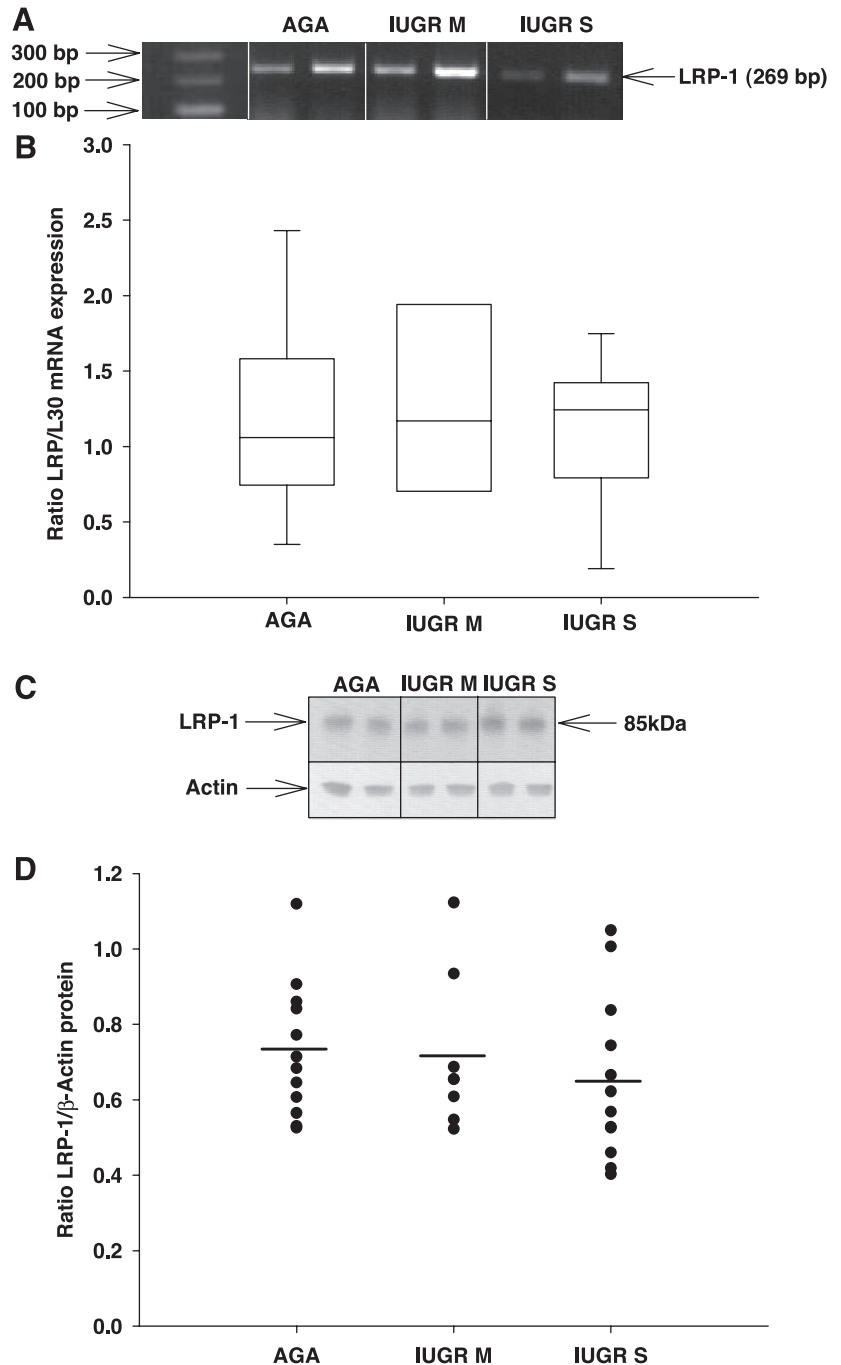


Fig. 2. *A*: semiquantitative RT-PCR for LDL-R-related protein (LRP-1) in the various groups produced a single band of 269 bp in size. Individual data were normalized to both L30 mRNA and to an internal control. *B*: the median values (horizontal line) in the box plots do not differ significantly between the groups (AGA $n = 18$, IUGR-M $n = 8$, IUGR-S $n = 12$). *C*: representative immunoblot of LRP-1 revealed 1 strong band at 85 kDa in each group, representing the transmembrane β -subunit of the protein. Individual data were normalized to β -actin and to the internal control in each blot. *D*: mean values (horizontal line) in the IUGR groups showed no expression differences compared with the control group.

RT-PCR for LRP-1 resulted in a single product of correct size at 269 bp (Fig. 2A). Similar mRNA levels were found in both IUGR-M (1.28 ± 0.24 AU) and IUGR-S (1.10 ± 0.14 AU) compared with the AGA group (1.39 ± 0.26 AU) (Fig. 2B). Moreover, a considerable variability on transcriptional levels was observed in the AGA group. Unaltered placental LRP-1 mRNA in the various groups was paralleled at the protein level (Fig. 2C). LRP-1 protein expression in the AGA, IUGR-M, and IUGR-S groups was 0.73 ± 0.12 , 0.72 ± 0.20 , and 0.65 ± 0.21 , respectively (Fig. 2D). No significant relationship was observed between LRP-1 expression and gestational age (data not shown).

SR-BI. SR-BI mRNA was detected by RT-PCR in tissue samples of all groups with a product of 231 bp in length (Fig. 3A). No quantitative differences were found between the IUGR-M (0.86 ± 0.12) and IUGR-S (0.81 ± 0.16) compared with AGA group (0.94 ± 0.35) (Fig. 3B). The antibody used to probe Western blot experiments revealed an 82-kDa SR-BI band in placentas, in agreement with published values for isolated trophoblasts (38) and other tissues (27) (Fig. 3C). Expression levels in IUGR-M (0.79 ± 0.25 AU) resembled those of the AGA group (0.94 ± 0.40 AU), but those of IUGR-S (0.53 ± 0.20 AU) were lower ($P < 0.05$) than in IUGR-M and AGA by about 50% (Fig. 3D). The expression

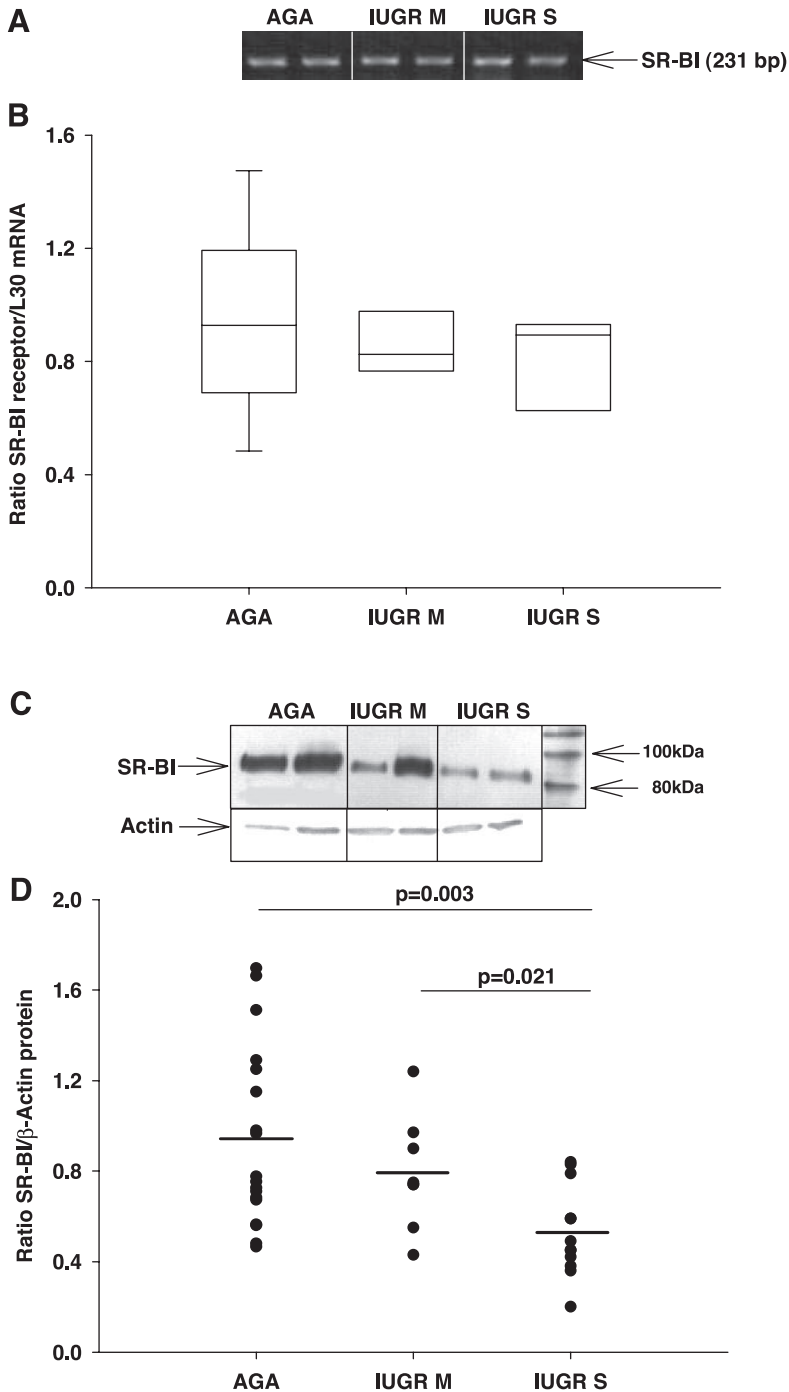


Fig. 3. A: identification of scavenger receptor class B type I (SR-BI) mRNA by RT-PCR revealed a single band of 231 bp in size. B: box plots of SR-BI mRNA levels revealed no significant differences among the groups. C: representative Western blot for AGA and IUGR groups of different severity showed a strong band at 82 kDa representing SR-BI. D: individual data were normalized to β -actin and to the internal control in each blot. The mean value (horizontal line) of IUGR-S ($n = 12$) and IUGR-M ($n = 8$) is significantly decreased vs. AGA ($n = 18$).

levels of SR-BI did not correlate with any other maternal or fetal clinical parameter either in the separate subgroups or in the total population (AGA + IUGR; data not shown). Expression of SR-BI was not related to gestational age (data not shown).

Lipoprotein electrophoresis and lipoprotein cholesterol. In the AGA group ($n = 11$) the relative electrophoretic mobilities (Rf values) in maternal plasma were 0.95 ± 0.06 [determined as the sample to LDL migration distance ratio (β -band)] and 1.03 ± 0.06 [determined as the sample to HDL₃ migration distance ratio (α -band)], respectively. They were similar to the values in the arterial (β -band: 0.96 ± 0.11 , α -band: $1.04 \pm$

0.04) and venous (β -band: 0.98 ± 0.09 , α -band: 1.16 ± 0.01) umbilical cord plasma (data not shown). In maternal plasma the β -band showed a significant change in mobility toward higher values in IUGR-S ($n = 9$), as reflected by higher Rf values [IUGR-M ($n = 8$) 1.12 ± 0.11 , $P < 0.001$; IUGR-S 1.28 ± 0.20 , $P = 0.002$] (Fig. 4). Rf values in the umbilical cord of this sample collection did not differ among the groups.

Maternal plasma concentrations of LDL-C were highest in the AGA group (188.5 ± 23.6 mg/dl) compared with the IUGR-S group (154.2 ± 26.1 mg/dl) ($P = 0.049$) and the IUGR-M group (176.6 ± 19.1 mg/dl). No difference could be observed in the arterial and venous cord plasma either for the

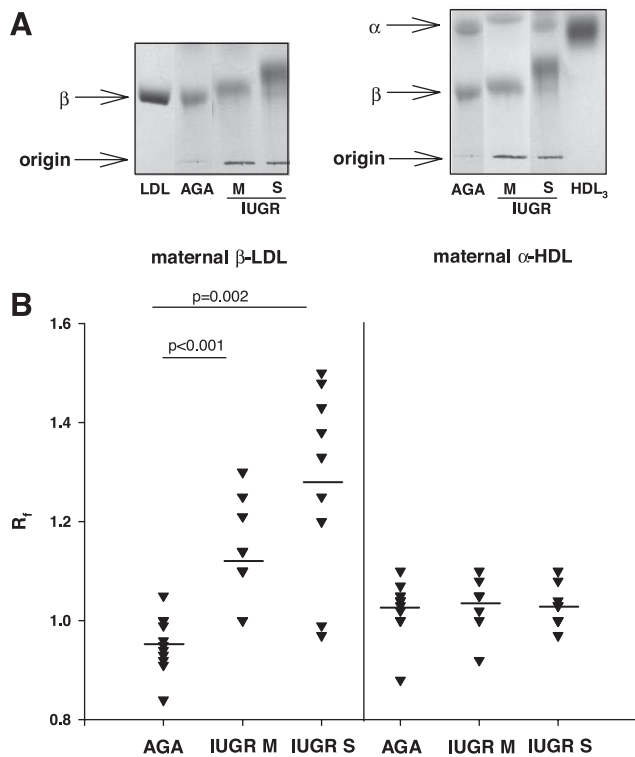


Fig. 4. Lipid electrophoresis of maternal plasma in AGA ($n = 18$), IUGR-M ($n = 8$), and IUGR-S ($n = 12$) subjects. **A:** origin and migration positions of α -migrating (HDL) and β -migrating (LDL) lipoproteins are indicated and native LDL and HDL₃ included as controls. The AGA plasma lipoproteins showed typical α - and β -migrating LDL and HDL, respectively. In the IUGR-M group, the β -fraction showed a slightly higher migrating LDL than native control LDL, whereas no differences in the α -migrating fraction were found. In the IUGR-S group, the β -lipoprotein mobility was increased compared with native control LDL, but no differences could be observed in the α -fraction. Two representative experiments with 1 plasma of each group. **B:** Rf values (mean = horizontal line) of maternal LDL and HDL in the various groups calculated as the distance ratios of LDL or HDL bands, respectively, to control lipoprotein band.

separate or for the combined (arterial: 48.4 ± 7.1 mg/dl; venous: 43.4 ± 5.3 mg/dl) study groups. When the maternal plasma concentrations of all three populations were considered together, the only significant correlation was found between β -Rf values and LDL-C ($r = -0.58$, $P = 0.0039$), which indicates changes in the lipid-to-protein ratio in LDL particles on the maternal side.

The maternal Rf values of the α -bands in both IUGR groups (IUGR-M 1.03 ± 0.06 and IUGR-S 1.03 ± 0.04) were similar to those of the AGA group (1.03 ± 0.05 ; Fig. 4). Electrophoresis of cord blood samples showed similar Rf values for the α - and β -bands in both IUGR groups and no difference in the maternal and cord blood values of the AGA group (data not shown). HDL-C concentrations in maternal (AGA + IUGR groups: 56.0 ± 3.8 mg/dl) as well as fetal (AGA + IUGR groups: 24.3 ± 3.3 mg/dl) plasma did not differ between the groups (data not shown).

DISCUSSION

The presence of lipoprotein receptors in the placenta indicates the potential for fetal tissues to take up cholesterol from maternal lipoproteins. The present study demonstrates an al-

tered expression of distinct lipoprotein receptors in IUGR compared with AGA placentas. In particular, this was found for LDL-R and SR-BI, two key receptors for the placental uptake of cholesterol from maternal LDL and/or HDL. Changes in lipoprotein receptors are not a general phenomenon associated with IUGR, since LRP-1 expression levels are not affected in the same IUGR patients. Because of a reciprocal relationship in LDL-R and LRP-1 levels (11), LRP-1 was included in the study to identify any compensatory changes for the alterations in LDL-R. However, absence of the predicted changes suggests that the mechanism resulting in the reciprocal changes in LDL-R and LRP-1 is not operative in the IUGR placenta.

Contrary to our findings, increased LDL-R mRNA levels have previously been reported in IUGR compared with AGA term and preterm placentas (35). Different criteria in selecting the study populations may account for these differences. In both studies IUGR was diagnosed in utero by biometric ultrasound, but the full range of IUGR severity as reflected by hemodynamic changes in the umbilical circulation was included only in the present study. These different findings underline the influence of the severity and stage of the IUGR condition. To our knowledge, this is the first study investigating differences in the expression of placental receptors between AGA and IUGR pregnancies classified on the basis of IUGR severity. A further difference in study design was the analysis of placental tissue obtained exclusively from elective caesarean sections, which in terms of gene expression may be more representative of the condition of the placenta during pregnancy than samples taken at the end of pregnancy after vaginal delivery, which is associated with higher fetal distress. It is pertinent that fetal cord blood lipids in uncomplicated pregnancies were significantly affected by mode of delivery (28, 45), which may have marked effects on placental function.

The discordant changes in LDL-R mRNA and protein conform to the complex mode of regulation of LDL-R protein at the level of synthesis, posttranslational processing, and degradation and may suggest a concomitant downregulation of PCSK9. This proteinase is critical for the degradation and, hence, turnover of LDL-R protein (3).

In contrast to LDL-R, SR-BI protein levels were profoundly reduced in the severe IUGR group (Fig. 3). One potential bias of our study is that IUGR pregnancies were delivered at an earlier gestational age than controls, because it is very rare to obtain placental samples from normal pregnancies delivered preterm for indications other than fetal. However, we did not observe any relationship between translational levels of LDL-R and SR-BI and gestational age. Moreover, inferences can be made from developmental changes in SR-BI protein expression levels, which decrease with gestational age (38). This would predict higher levels in earlier periods compared with the end of gestation. Therefore, it appears unlikely that the gestational age differences account for the differences in SR-BI expression levels. Similar inferences cannot be made for LDL-R because of conflicting studies reporting an increased (18), decreased (43), or unchanged (35) LDL-R expression on transcriptional as well as translational levels during gestation.

Not only are the receptor protein expression levels different in IUGR, but the composition and concentration of some maternal lipoproteins also differ. Several techniques have been employed to separate the plasma lipoproteins, including ultra-

centrifugation and electrophoresis. Electrophoretic mobility on agarose gels of small amounts of maternal and fetal plasma can be used as a proxy measure for the lipid-to-protein ratio in lipoproteins (32). An increase in the Rf values of the β -spots reflecting their electrophoretic mobility was positively correlated with the severity of IUGR. The unchanged Rf values of the particles in the arterial and venous cord plasma were in line with their comparable concentrations of LDL- and HDL-C. This finding of absent or only small umbilical venous-arterial differences in cholesterol concentrations varies from other studies (33) and may reflect too small subject collectives.

The electrophoretic mobility of the β -lipoproteins, which are mainly LDL particles, depends on particle size, electric charge, density, and chemical composition (17) and may be modified by diet (2) and altered in pathological conditions such as atherosclerosis (34), diabetes (44), or hypercholesterolemia (37). Plasma apolipoprotein (26), lipoprotein, and fatty acid concentrations between normal and IUGR pregnancies (6, 28) differ in maternal sera, and this may lead to the changes in lipoprotein composition. In IUGR, LDL particles were significantly enriched in cholesteryl ester and depleted in free cholesterol (30). These observations are in line with an increase in the Rf values of the β -spots in association with a significant decrease in LDL-C concentrations found here. This reflects a modified composition in the hydrophobic core of the LDL particle. In the present study, we have confirmed decreased cholesterol levels in fetal compared with maternal blood (28) and significantly lower LDL-C concentrations in IUGR (30). The changes are specific for LDL since, in contrast, Rf values of HDL particles and maternal as well as fetal HDL-C were unaltered. Therefore, maternal LDL appears to be more susceptible than HDL to influences by the IUGR pathology.

The physiological consequences of the regulation of receptors for placental function and fetal development are unclear. A yet undefined proportion of cholesterol taken up by the placenta may be released into the fetal circulation. The remaining placental cholesterol, however, is used for sterol synthesis, foremost of progesterone (13, 39). Provided that cholesterol taken up by LDL-R and SR-BI contribute to the same intracellular cholesterol pool, the LDL-R upregulation may represent a mechanism to compensate for the reduced cholesterol uptake mediated by SR-BI. However, this may be insufficient, since IUGR in general is accompanied by reduced maternal progesterone concentrations (29).

An association of placental receptor expression levels with fetal or placental weight could not be observed here; however, in IUGR, abnormal lipoprotein metabolism may be a factor underlying poor fetal growth (26). Maternal LDL levels, i.e., both LDL protein and cholesterol, which normally increase by ~60% during uncomplicated pregnancies (10), fail to rise appropriately in pregnancies complicated by IUGR. As a result, despite increased translational levels of the receptor in the placenta, cholesterol provision for steroid synthesis and supply to the fetus may be insufficient (30).

In conclusion, this study provides evidence for specific changes in receptors involved in placental uptake of maternal cholesterol-rich lipoproteins. These differences are evident in IUGR associated with changes in the fetal circulation, suggestive of placental failure (21). The differential changes in LDL-R and SR-BI in IUGR warrant further investigation into

placental lipid uptake and sequestration in healthy and pathological pregnancy.

ACKNOWLEDGMENTS

We are indebted to Dr. Peter Dodds, Imperial College at Wye, for his expert criticism and careful reading of the manuscript. The expert technical assistance of Birgit Hirschmugl, Graz, is gratefully acknowledged.

GRANTS

The studies reported herein have been carried out with financial support from the Commission of the European Communities within the RTD program "Quality of Life and Living Resources" and Grant QLK1-2001-00138 "Influence of Dietary Fatty Acids on the Pathophysiology of Intrauterine Foetal Growth and Neonatal Development." It does not necessarily reflect the Commission's views and in no way anticipates its future policy in this area. Further financial support came from the Jubilee Fund, Austrian National Bank, Vienna Grants 11165 (to C. Wadsack), 10896 (to U. Hiden), and 10053 (to G. Desoye).

REFERENCES

1. **Alsat E, Malassine A.** High density lipoprotein interaction with human placenta: biochemical and ultrastructural characterization of binding to microvillous receptor and lack of internalization. *Mol Cell Endocrinol* 77: 97-108, 1991.
2. **Archer WR, Lamarche B, St-Pierre AC, Mauger JF, Deriaz O, Landry N, Corneau L, Despres JP, Bergeron J, Couture P, Bergeron N.** High carbohydrate and high monounsaturated fatty acid diets similarly affect LDL electrophoretic characteristics in men who are losing weight. *J Nutr* 133: 3124-3129, 2003.
3. **Attie AD, Seidah NG.** Dual regulation of the LDL receptor—some clarity and new questions. *Cell Metab* 1: 290-292, 2005.
4. **Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK.** The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341: 162-164, 1989.
5. **Carr BR, Simpson ER.** Cholesterol synthesis in human fetal tissues. *J Clin Endocrinol Metab* 55: 447-452, 1982.
6. **Cetin I, Giovannini N, Alvino G, Agostoni C, Riva E, Giovannini M, Pardi G.** Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatr Res* 52: 750-755, 2002.
7. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156-159, 1987.
8. **Coukos G, Gafvels ME, Wisel S, Ruelaz EA, Strickland DK, Strauss JF 3rd, Coutifaris C.** Expression of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein and the 39-kd receptor-associated protein in human trophoblasts. *Am J Pathol* 144: 383-392, 1994.
9. **Cummings RD, Kornfeld S, Schneider WJ, Hobgood KK, Tolleshaug H, Brown MS, Goldstein JL.** Biosynthesis of N- and O-linked oligosaccharides of the low density lipoprotein receptor. *J Biol Chem* 258: 15261-15273, 1983.
10. **Desoye G, Schweditsch MO, Pfeiffer KP, Zechner R, Kostner GM.** Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum. *J Clin Endocrinol Metab* 64: 704-712, 1987.
11. **Gafvels ME, Coukos G, Sayegh R, Coutifaris C, Strickland DK, Strauss JF 3rd.** Regulated expression of the trophoblast alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Differentiation and cAMP modulate protein and mRNA levels. *J Biol Chem* 267: 21230-21234, 1992.
12. **Hiden U, Maier A, Bilban M, Ghaffari-Tabrizi N, Wadsack C, Lang I, Dohr G, Desoye G.** Insulin control of placental gene expression shifts from mother to foetus over the course of pregnancy. *Diabetologia* 49: 123-131, 2006.
13. **Lasuncion MA, Bonet B, Knopp RH.** Mechanism of the HDL2 stimulation of progesterone secretion in cultured placental trophoblast. *J Lipid Res* 32: 1073-1087, 1991.
14. **Lin DS, Pitkin RM, Connor WE.** Placental transfer of cholesterol into the human fetus. *Am J Obstet Gynecol* 128: 735-739, 1977.
15. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275, 1951.
16. **Malassine A, Besse C, Roche A, Alsat E, Rebouret R, Mondon F, Cedard L.** Ultrastructural visualization of the internalization of low

- density lipoprotein by human placental cells. *Histochemistry* 87: 457–464, 1987.
17. **McNamara JR, Small DM, Li Z, Schaefer EJ.** Differences in LDL subspecies involve alterations in lipid composition and conformational changes in apolipoprotein B. *J Lipid Res* 37: 1924–1935, 1996.
 18. **Murata M, Kodama H, Goto K, Hirano H, Tanaka T.** Decreased very-low-density lipoprotein and low-density lipoprotein receptor messenger ribonucleic acid expression in placentas from preeclamptic pregnancies. *Am J Obstet Gynecol* 175: 1551–1556, 1996.
 19. **Parazzini F, Cortinovic I, Bortolus R, Fedele L.** [Standards of birth weight in Italy]. *Ann Ostet Ginecol Med Perinat* 112: 203–246, 1991.
 20. **Pardi G, Cetin I, Marconi AM, Lanfranchi A, Bozzetti P, Ferrazzi E, Buscaglia M, Battaglia FC.** Diagnostic value of blood sampling in fetuses with growth retardation. *N Engl J Med* 328: 692–696, 1993.
 21. **Pardi G, Marconi AM, Cetin I.** Placental-fetal interrelationship in IUGR fetuses—a review. *Placenta* 23, Suppl A: S136–S141, 2002.
 22. **Pedersen A, Baumstark MW, Marckmann P, Gylling H, Sandstrom B.** An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. *J Lipid Res* 41: 1901–1911, 2000.
 23. **Pepe GJ, Albrecht ED.** Actions of placental and fetal adrenal steroid hormones in primate pregnancy. *Endocr Rev* 16: 608–648, 1995.
 24. **Pitkin RM, Connor WE, Lin DS.** Cholesterol metabolism and placental transfer in the pregnant Rhesus monkey. *J Clin Invest* 51: 2584–2592, 1972.
 25. **Plotz EJ, Kabara JJ, Davis ME, LeRoy GV, Gould RG.** Studies on the synthesis of cholesterol in the brain of the human fetus. *Am J Obstet Gynecol* 101: 534–538, 1968.
 26. **Radunovic N, Kuczynski E, Rosen T, Dukanac J, Petkovic S, Lockwood CJ.** Plasma apolipoprotein A-I and B concentrations in growth-retarded fetuses: a link between low birth weight and adult atherosclerosis. *J Clin Endocrinol Metab* 85: 85–88, 2000.
 27. **Rigotti A, Trigatti B, Babitt J, Penman M, Xu S, Krieger M.** Scavenger receptor BI—a cell surface receptor for high density lipoprotein. *Curr Opin Lipidol* 8: 181–188, 1997.
 28. **Rodie VA, Caslake MJ, Stewart F, Sattar N, Ramsay JE, Greer IA, Freeman DJ.** Fetal cord plasma lipoprotein status in uncomplicated human pregnancies and in pregnancies complicated by pre-eclampsia and intrauterine growth restriction. *Atherosclerosis* 176: 181–187, 2004.
 29. **Salas SP, Marshall G, Gutierrez BL, Rosso P.** Time course of maternal plasma volume and hormonal changes in women with preeclampsia or fetal growth restriction. *Hypertension* 47: 203–208, 2006.
 30. **Sattar N, Greer IA, Galloway PJ, Packard CJ, Shepherd J, Kelly T, Mathers A.** Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth restriction. *J Clin Endocrinol Metab* 84: 128–130, 1999.
 31. **Sattler W, Mohr D, Stocker R.** Rapid isolation of lipoproteins and assessment of their peroxidation by high-performance liquid chromatography postcolumn chemiluminescence. *Methods Enzymol* 233: 469–489, 1994.
 32. **Sparks DL, Phillips MC.** Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis. *J Lipid Res* 33: 123–130, 1992.
 33. **Spellacy WN, Ashbacher LV, Harris GK, Buhi WC.** Total cholesterol content in maternal and umbilical vessels in term pregnancies. *Obstet Gynecol* 44: 661–665, 1974.
 34. **St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Bernard PM, Despres JP, Lamarche B.** Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. *Circulation* 104: 2295–2299, 2001.
 35. **Stepan H, Faber R, Walther T.** Expression of low density lipoprotein receptor messenger ribonucleic acid in placentas from pregnancies with intrauterine growth retardation. *Br J Obstet Gynaecol* 106: 1221–1222, 1999.
 36. **Umans L, Overbergh L, Serneels L, Tesseur I, Van Leuven F.** Analysis of expression of genes involved in apolipoprotein E-based lipoprotein metabolism in pregnant mice deficient in the receptor-associated protein, the low density lipoprotein receptor, or apolipoprotein E. *Biol Reprod* 61: 1216–1225, 1999.
 37. **Vedie B, Jeunemaitre X, Megnien JL, Myara I, Trebeden H, Simon A, Moatti N.** Charge heterogeneity of LDL in asymptomatic hypercholesterolemic men is related to lipid parameters and variations in the ApoB and CIII genes. *Arterioscler Thromb Vasc Biol* 18: 1780–1789, 1998.
 38. **Wadsack C, Hammer A, Levak-Frank S, Desoye G, Kozarsky KF, Hirschmugl B, Sattler W, Malle E.** Selective cholesteryl ester uptake from high density lipoprotein by human first trimester and term villous trophoblast cells. *Placenta* 24: 131–143, 2003.
 39. **Winkel CA, Snyder JM, MacDonald PC, Simpson ER.** Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. *Endocrinology* 106: 1054–1060, 1980.
 40. **Winkler K, Wetzka B, Hoffmann MM, Friedrich I, Kinner M, Baumstark MW, Wieland H, Marz W, Zahradnik HP.** Low density lipoprotein (LDL) subfractions during pregnancy: accumulation of buoyant LDL with advancing gestation. *J Clin Endocrinol Metab* 85: 4543–4550, 2000.
 41. **Wittmaack FM, Gafvels ME, Bronner M, Matsuo H, McCrae KR, Tomaszewski JE, Robinson SL, Strickland DK, Strauss JF 3rd.** 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, 1995.
 42. **Woollett LA.** The origins and roles of cholesterol and fatty acids in the fetus. *Curr Opin Lipidol* 12: 305–312, 2001.
 43. **Wyne KL, Woollett LA.** 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* 39: 518–530, 1998.
 44. **Yano M, Inoue M, Maehata E, Shiba T, Yamakado M, Hirabayashi Y, Taniyama M, Suzuki S.** Increased electronegative charge of serum low-density lipoprotein in patients with diabetes mellitus. *Clin Chim Acta* 340: 93–98, 2004.
 45. **Yoshimitsu N, Douchi T, Yamasaki H, Nagata Y, Andoh T, Hatano H.** Differences in umbilical cord serum lipid levels with mode of delivery. *Br J Obstet Gynaecol* 106: 144–147, 1999.