

Is the atherosclerotic phenotype of preeclamptic placentas due to altered lipoprotein concentrations and placental lipoprotein receptors? Role of a small-for-gestational-age phenotype

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Abstract Atherosclerosis of spiral arteries in uteroplacental beds from preeclamptic women resemble those of atherosclerosis, characterized by increased plasma lipids and lipoproteins. We hypothesized that: 1) lipoprotein receptors/transporters in the placenta would be upregulated in preeclampsia, associated with increased maternal and fetal lipoprotein concentrations; and 2) expression of these would be reduced in preeclamptic placentae from women delivering small-for-gestational-age (SGA) infants. Placental biopsies and maternal and umbilical serum samples were taken from 27 normotensive and 24 preeclamptic women. Maternal/umbilical cord serum LDL, HDL, total cholesterol, and triglycerides were measured. Placental mRNA expression of lipoprotein receptors/transporters were quantified using quantitative RT-PCR. Protein localization/expression of LDL receptor-related protein 1 (LRP-1) in the preeclamptic placentae with/without SGA was measured by immunohistochemistry. Placental mRNA expression of all genes except paraoxonase-1 (*PON-1*), microsomal triglyceride transfer protein (*MTTP*), and protein disulfide isomerase family A member 2 (*PDIA2*) were observed. No differences for any lipoprotein receptors/transporters were found between groups; however, in the preeclamptic group placental *LRP-1* expression was lower in SGA delivering mothers ($n = 7$; $P = 0.036$). LRP-1 protein was localized around fetal vessels and Hofbauer cells. **This is the first detailed study of maternal/fetal lipoprotein concentrations and placental lipoprotein receptor mRNA expression in normotensive and preeclamptic pregnancies. These findings do not support a role of altered lipid metabolism in preeclampsia, but may be involved in fetal growth.**—Hentschke, M. R., C. E. Poli-de-Figueiredo, B. E. Pinheiro da Costa, L. O. Kurlak, P. J. Williams, and H. D. Mistry. **Is the atherosclerotic phenotype of preeclamptic placentas due to altered lipoprotein**

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Gestational hyperlipidemia is a common factor in pregnancy in which the maternal circulating lipid profile changes from an anabolic to a catabolic state, increasing lipids, especially triglycerides (TGs) and lipoproteins (1). From the 12th week of gestation, phospholipids, total cholesterol (TC), LDL, HDL, and TGs increase in response to estrogen stimulation and insulin resistance (2). Thus, during the first two trimesters, it is common to see maternal fat accumulation; however, in the third trimester there is enhanced lipolytic activity and decreased lipoprotein lipase (LPL) activity in the adipose tissue, consequently, fat storage declines or ceases (3). Early in gestation, the lipids are required to develop the fetal brain and central nervous system (4), to build cell membranes, and as a precursor of bile acids and steroid hormones (2). However, a maternal source of lipids is still required until term, but in lower amounts, possibly due to some fetal-derived lipids

Abbreviations: AGA, adequate-for-gestational-age; BMI, body mass index; FGR, fetal growth restriction; FGR-M, fetal growth restriction without hemodynamic changes; FGR-S, fetal growth restriction with hemodynamic changes; IQR, interquartile range; LDL-R, LDL receptor; LRP-1, LDL receptor-related protein 1; MMP, matrix metalloproteinase; MTTP, microsomal triglyceride transfer protein; PDIA2, protein disulfide isomerase family A member 2; PON-1, paraoxonase-1; SGA, small-for-gestational-age; SRB-1, scavenger receptor class B type 1; TC, total cholesterol; TG, triglyceride.

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from the lipogenic activity in the fetal liver, adrenal, and testes (2). Conversely, increased TGs during pregnancy have been shown to augment the risk of preeclampsia, preterm birth, and fetal growth restriction (FGR) (2, 5). The lipid metabolism and plasma levels are also affected by maternal factors such as body mass index (BMI), maternal weight gain, maternal nutrition, prepregnancy lipid levels, and various medical complications of pregnancy such as diabetes (6).

Complicating 2–8% of pregnancies, preeclampsia, along with the other hypertensive disorders of pregnancy, is one of the three leading causes of maternal morbidity and mortality worldwide (7). This disorder increases perinatal outcomes such as prematurity and FGR (8). Preeclampsia is generally defined as high blood pressure (systolic blood pressure of ≥ 140 mm Hg and/or diastolic blood pressure of ≥ 90 mm Hg) and proteinuria (≥ 300 mg/24 h) at or after 20 weeks gestation (9). The cause of preeclampsia remains unknown, but endothelial dysfunction, leading to compromised uteroplacental perfusion and reduced maternal-fetal transport of oxygen and nutrients is thought to be involved (10–13). Different lines of evidence indicate that abnormal lipid metabolism is involved in the pathogenesis of the disease, with acute atherosclerosis seen in preeclamptic uteroplacental beds resembling atherosclerotic lesions of coronary arteries (14). The presence of lipoprotein receptors in placental syncytiotrophoblast, specifically LDL receptor (*LDL-R*), LDL receptor-related protein 1 (*LRP-1*), and scavenger receptor class B type 1 (*SRB-1*) in third trimester placentae, have previously been shown (15). Placental expression of some of these receptors from FGR pregnancies with (FGR-S) and without (FGR-M) fetal hemodynamic changes, based on results of the Doppler velocimetry of umbilical artery and pulsatility index and from non-FGR control pregnancies have been reported. *LDL-R* mRNA levels in FGR-M were similar to controls but lower in FGR-S. In contrast, *LDL-R* protein was higher in both FGR cases than in the control group. *LRP-1* mRNA and protein levels were not altered in all FGR cases. *SRB-1* mRNA was unchanged in FGR, but protein levels were lower in FGR-S than in the other groups. The authors concluded that *LDL-R* and *SRB-1* levels are altered in FGR pregnancies and maternal plasma concentrations of LDL cholesterol are higher in the control group than in the FGR-S group (15). However, these have not previously been examined in relation to preeclampsia.

Based on the literature relating to placental and liver cholesterol transport pathways, we chose to analyze the maternal and fetal lipoprotein concentrations in combination with placental mRNA expression of *LRP-1*, *LDL-R*, *SRB-1*, ATP-binding cassette transporter A1 (*ABCA1*), paraoxonase-1 (*PON-1*), microsomal triglyceride transfer protein (*MTTP*), and protein disulfide isomerase family A, member 2 (*PDIA2*).

We therefore hypothesized that the expression of those lipoprotein receptors involved in the cholesterol pathway in the liver, are upregulated in preeclamptic placentae compared with controls as a compensatory factor. In addition, the expression of some of these receptors may be

reduced in preeclamptic placentae from women delivering small-for-gestational-age (SGA) infants versus appropriate-for-gestational-age (AGA) infants.

METHODS

Subjects and selection criteria

The study population consisted of two groups of white European women (27 normotensive, 24 with preeclampsia) (Table 1). Detailed demographics and outcome data have previously been published (16). The study was approved by the Hospital Ethics Committee of the Nottingham University Hospitals; written informed consent was obtained from each participant. Preeclampsia was stringently defined as stated in the International Society for the Study of Hypertension in Pregnancy guidelines (9). Medical and obstetric histories were obtained for each participant. The corrected birthweight percentile for each infant was computed, correcting for gestational age, gender, maternal parity, and BMI (17). SGA was defined as a centile below the 10th, and AGA as an individualized birthweight ratio between the 10th and 90th percentile (18). Table 2 provides demographic, obstetric, and pregnancy description data for the preeclamptic women delivering SGA and AGA infants.

Sample collection and measurements

Before delivery, venous blood samples were taken from mothers and immediately after placental delivery, where possible, umbilical cord venous blood was collected. Venous samples were allowed to clot prior to centrifugation at 1,400 *g* for 10 min at 4°C. Serum samples were stored at –80°C prior to analysis. The number of fetal serum samples missing was 1 in the control (*n* = 26) and 10 in the preeclamptic (*n* = 14) group. All women who took part in this study were laboring and either delivered vaginally or by emergency Caesarean section.

The lipoproteins (LDL, HDL, TC, and TG) were measured using MicroSlide technology on the Vitros Fusion 5.1 Chemistry System (New York, NY) following the manufacturer's instructions. Briefly, 200 μ l of each sample were uniformly distributed over the entire slide area that contained all the reagents for the selected assays to allow larger molecules to be broken up and penetrate into the reagent layer. All samples were analyzed in triplicate, with the inter-assay variation being less than 5% and the intra-assay variation less than 10%.

Full depth placental tissue samples were collected within 10 min of the placental delivery from halfway between the cord insertion and the periphery of the placentae, avoiding infarcts. The samples were immediately rinsed in ice-cold phosphate buffered saline, and the membranes were removed and snap-frozen in liquid nitrogen for mRNA analysis. A second sample was fixed in formalin for immunohistochemistry analysis. All samples were then stored at –80°C until analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from a known amount of placental tissue (~100 mg) using QIAzolysis reagent (Qiagen, Crawley, UK). RNA concentration and the quality of each gene were verified spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies, Labtech, Ringmer, UK); all of the samples had an A_{260}/A_{280} ratio >1.96 and were stored at –80°C. RNA (1 μ g) was then reverse transcribed using the QuantiTect Reverse Transcription kit containing a mix of random primers and oligo-dT (Qiagen) in a Primus 96 advanced gradient thermocycler (Peqlab Ltd., Fareham, UK).

TABLE 1. Clinical and biochemical data of subject groups

Parameter	NC (n = 27)	PE (n = 24)
Maternal age, years (mean ± SD)	30 ± 6.9	31 ± 6.1
Booking BMI, kg/m ² (mean ± SD)	26.2 ± 5.4	26.6 ± 5.0
Maximum systolic blood pressure outside labor, mm Hg (mean ± SD)	116 ± 4.6	156 ± 7.1*
Maximum diastolic blood pressure outside labor, mm Hg (mean ± SD)	76.0 ± 3.0	98.0 ± 4.5*
Proteinuria, g/l (median [IQR])	—	1.0 [0.3, 11.5]
Gestational age at delivery, weeks (mean ± SD)	40 ± 1.1	36.7 ± 3.8*
Birth weight, kg (median [IQR])	3.5 [3.3, 3.7]	2.9 [2.0, 3.4]*
Birthweight centile (median [IQR])	45 [23, 62]	13 [1, 82]
SGA infants [n (%)]	1 (3.7)	7 (29.2)
Preterm deliveries, ≤37 weeks gestation [n (%)]	0	14 (58.3)

NC, normotensive controls; PE, preeclampsia; IQR, interquartile range. * $P < 0.05$ between normotensive controls and women with preeclampsia.

Quantitative real-time PCR

Real-time PCR was carried out with the use of SYBR Green chemistry (2× QuantiFast SYBR Green, Qiagen) on a RotorGene 6000 (Corbett Research, Sydney, Australia) using the primers detailed in **Table 3**, following our previous protocol (19). Briefly, a pre-PCR cycle was run for 5 min at 95°C followed by 45 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 20 s. Melt-curve analysis was performed to confirm the presence of one single product and nontemplate controls run to assess contamination. Cycle threshold values were used for analysis, and abundance data were obtained by the use of quantified cDNA to generate a standard curve. Standards were quantified using densitometry and 10-fold serial dilutions (10^8 to 10^1 copies) run in parallel with the samples. Abundance data for the genes of interest were normalized to *GAPDH*, a stably expressed housekeeping gene suitable for human placental samples (20).

Immunohistochemical staining of LRP-1

LRP-1 protein expression for the 17 AGA and 7 SGA preeclamptic placentae from this cohort were analyzed by immunohistochemistry. Serial sections of placental tissue were cut (5 μm) in the same orientation from paraffin-embedded tissue blocks (Sledge Microtome, Anglia Scientific, Norwich, UK) and mounted onto SuperFrost Plus glass microscope slides (Menzel-Glaser, Braunschweig, Germany). Before use, sections were dewaxed by immersion in xylene followed by rehydration in descending concentrations of alcohol (3 min each).

Immunohistochemical staining was performed using the Dako Envision staining kits (Dako Ltd., Germany). LRP-1 rabbit polyclonal antibody (ARP58562_P050; Aviva Systems Biology, USA) was used for immunostaining of paraffin-embedded placental sections; the optimal dilution (1 in 500) was optimized. Heat-induced epitope retrieval was achieved by heating in a citrate buffer (pH 6.0) using a microwave oven for 15 min, followed by incubation for 30 min in normal rabbit serum (Sigma-Aldrich, UK) to block nonspecific binding; slides were then incubated with anti-LRP-1 overnight at 4°C. A negative control was performed for each test section by incubation with rabbit IgG. Sections were dehydrated and cleared in ascending concentrations

of alcohol and xylene before mounting in DPX (BDH, Poole, UK).

All of the slides were assessed by the same observer, blinded to pregnancy outcome. For analysis of placental sections, digital images of five randomly selected, high-power (×400 magnification) fields were captured on NIS-Elements F2.20 microscope (Nikon United Kingdom Ltd., Surrey, UK). Quantification of LRP-1 was performed as described previously (19, 21) using the Positive Pixel Algorithm of Aperio ImageScope software. This software is able to discriminate between positive- and negative-stained pixels and combines the number of positive pixels stained with the intensity of these same pixels to produce the value “positivity.” A visual check was also performed to ensure accurate discrimination of immunolabeled regions.

Statistical analysis

All tests were performed using SPSS for Windows version 19. Summary data are presented as means ± standard deviation (SD) or median and interquartile range as appropriate, the Student's *t*-test or Mann-Whitney U test were used depending on the distribution of the data, after testing using the Kolmogorov-Smirnov test. Correlations between the parameters were tested with a Spearman's rank test. The null hypothesis was rejected where $P < 0.05$.

RESULTS

Subjects

Demographic, obstetric, and pregnancy description data of the 51 participants are shown in Table 1; clinical descriptions have previously been published (16). All patients carried singleton pregnancies and the women with preeclampsia all had moderate-to-severe disease, without HELLP syndrome. The neonates from both pregnancy groups survived. The number of fetal serum samples that were missing was 1 in the control and 10 in the preeclamptic

TABLE 2. Clinical and biochemical data of the preeclamptic women delivering AGA and SGA infants

Parameter	PE AGA (n = 17)	PE SGA (n = 7)
Maternal age, years (mean ± SD)	32 ± 7.8	32 ± 2.9
Booking BMI, kg/m ² (mean ± SD)	27.2 ± 4.3	27.1 ± 6.8
Gestational age at delivery, weeks (mean ± SD)	38 ± 2.5	35 ± 5
Birth weight, kg (median [IQR])	3.2 [3, 3.5]	2 [1.6, 2.4]**
Birth weight centile (median [IQR])	47.1 [12.6, 69.4]	0.7 [0.3, 1.9]***

PE, preeclampsia; IQR, interquartile range. ** $P = 0.001$ and *** $P < 0.0001$ between PE AGA and PE SGA groups.

TABLE 3. Details the forward and reverse primer sequences and BLAST sequences

Gene	BLAST Sequence Accession Number	Primer	Length (bp)
LDL-R	NM_000527.3	5'-aggacggctacagctacc-3' 5'-ctccaggcagatgttcacg-3'	73
LRP-1	NM_002332.2	5'-gggtgcaccacctcaacat-3' 5'-agtcggtccagtcacggttc-3'	88
SRB-1	NM_005505.4	5'-catcaagcagcaggtcctta-3' 5'-cggagagatagaaggggatagg-3'	95
PON-1	NM_000446.5	5'-actatagccaagtgaagttcgagtg-3' 5'-atgagccagcaactcagctat-3'	110
ABCA1	NM_005502.2	5'-tgctcatagtcttgggactc-3' 5'-atcacctcctgtcgcatgt-3'	76
MTTP	NM_000253.2	5'-ggctggcttcacggtagc-3' 5'-gttctctcccctcgtc-3'	88
PDIA2	NM_006849.2	5'-ctccaagttctggacaacg-3' 5'-tggaccatagtgaggattg-3'	104

groups. Within the preeclamptic group, 7 women delivered SGA infants and 17 delivered AGA infants (Table 2); only 1 woman in the normotensive control group delivered an SGA infant, and thus was excluded in this analysis.

Biochemical measurements

For all maternal and umbilical serum lipoproteins, there were no significant differences ($P > 0.05$) between normotensive and preeclamptic samples. However, umbilical venous samples had significantly lower lipid and lipoprotein concentrations compared with maternal concentrations in both groups; TG (normal and preeclampsia: $P < 0.0001$), TC (normal and preeclampsia: $P < 0.001$), LDL (normal: $P = 0.003$; preeclampsia: $P = 0.01$), and HDL (normal and preeclampsia: $P = 0.001$) (Fig. 1).

Placental lipoprotein receptors, transporters, and enzyme expression

The mRNA expression of *LDL-R*, *LRP-1*, *SRB-1*, *ABCA1*, *PON-1*, *MTTP*, and *PDIA2* in placental tissue from normotensive control and women with preeclampsia are shown in Table 4. *LDL-R*, *LRP-1*, *SRB-1*, and *ABCA1* were all found to be expressed in placenta tissue, but there was no expression of *PON-1*, *MTTP*, and *PDIA2*. The placental mRNA expression of the receptors did not significantly differ between groups ($P > 0.05$ for all).

Although the numbers were small, we additionally analyzed the 10 late-onset preeclampsia women (>34 weeks gestation) separately against the control group to test for any gestational-specific differences. The same nonsignificant results were seen for all the maternal and fetal

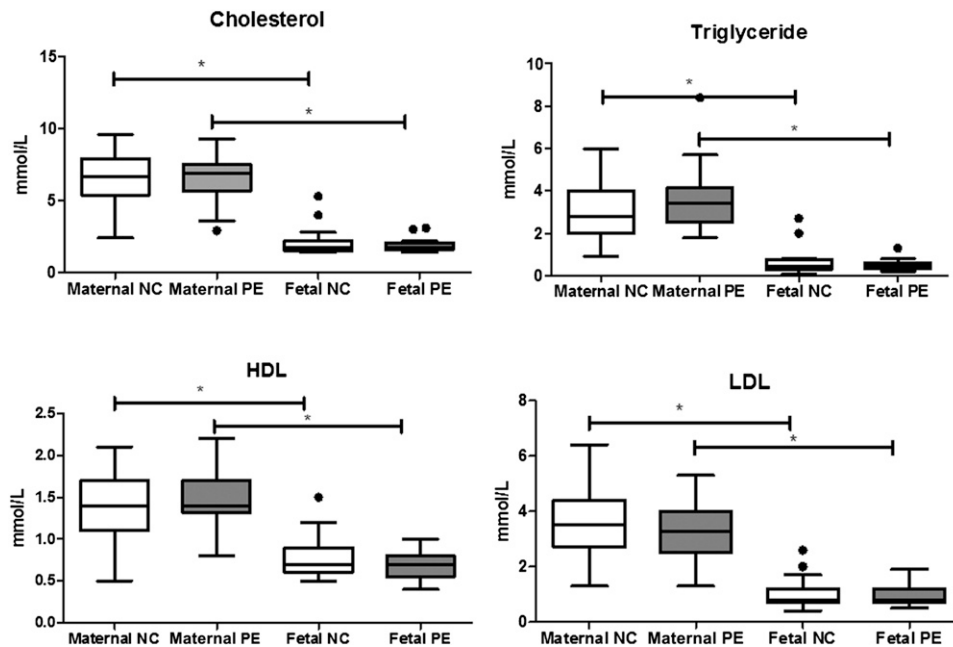


Fig. 1. Serum lipoprotein concentrations in maternal and fetal circulation, from normotensive controls and preeclampsia. The number of fetal serum samples that were missing were 1 in the control (n = 26) and 10 in the preeclamptic (n = 14). NC, normotensive control; PE, preeclampsia. Data presented as median [interquartile range]. * $P < 0.05$ between maternal and umbilical samples for all lipoproteins.

TABLE 4. Placental lipoprotein mRNA expression from normotensive controls and preeclampsia

Placenta Normalized mRNA Expression	NC (n = 27)	PE (n = 24)	P
LDL-R (median [IQR])	0.05 [0.03, 0.07]	0.06 [0.045, 0.11]	0.107
LRP-1 (median [IQR])	7.0 [5.04, 17.33]	12.3 [5.23, 23.89]	0.503
SRB-1 (median [IQR])	0.34 [0.21, 0.51]	0.32 [0.24, 0.56]	0.605
ABCA1 (median [IQR])	31.38 [20.8, 73.55]	28.8 [19.32, 82.81]	0.942

NC, normotensive control; PE, preeclampsia; IQR, interquartile range.

lipoprotein concentrations and for the placental expression of the receptors, transporters, and enzymes ($P > 0.1$ for all).

Placental lipoprotein receptor expression and SGA

In the preeclamptic group only, we found that those delivering SGA infants had significantly lower *LRP-1* expression compared with the AGA group (shown as median [IQR]) (SGA: 4.72 [3.6, 8.7]; AGA: 20.2 [13.5, 27.1]; $P = 0.036$). All the other genes did not show any differences ($P > 0.05$).

Protein expression of LRP-1 was localized mainly around fetal vessels and Hofbauer cells (Fig. 2). Visual inspection suggests lower protein expression in the preeclamptic placenta delivering SGA infants compared with the AGA group, although this was not significant when analyzed semi-quantitatively, possibly due to small sample size (median [IQR]: 0.91 [0.86, 0.95] vs. 0.87 [0.81, 0.89]; $P > 0.05$; Fig. 2).

Maternal serum, umbilical cord serum, and placental tissue

When maternal and umbilical cord serum lipoprotein concentrations were correlated with placental mRNA expression of the lipoprotein receptors, no difference between groups was found ($P > 0.05$). In addition, no associations between serum lipoproteins or receptors with birth

weights or gestational age at delivery were seen ($P > 0.05$ for both).

DISCUSSION

In the present study, we have analyzed the lipid profiles in women with preeclampsia and normotensive pregnant women. To the best of our knowledge, this is the first study that has concurrently investigated the placental, maternal, and fetal lipoprotein system comprehensively in normotensive and preeclamptic pregnancies, and associated these results with SGA infants in the third trimester of pregnancy.

Previous studies have reported that hyperlipidemia is enhanced in preeclampsia (22, 23) and has a negative impact on fetal lipid profiles (24, 25). It has been suggested that dyslipidemia may contribute to the increased oxidative stress and endothelial dysfunction, and possibly insulin resistance, which causes a compensatory increase in insulin concentration, decreased LPL activity, and increased TGs. However, factors that influence preeclampsia, such as chronic hypertension, obesity, and insulin resistance, share common features with dyslipidemia related to oxidative stress and altered vascular function. Our data showed no significant differences in maternal and fetal lipoprotein concentrations between preeclampsia and normotensive controls, which is in line with previous data on maternal levels (26). In addition, in the current study the mean BMI was 26 kg/m² in both groups (Table 1), suggesting that the abnormal lipid profile could be associated with obesity (27), and not necessarily with preeclampsia. This could also be a reason why some previous studies have reported increased lipoprotein concentrations in preeclampsia, because they may not have controlled for this. Thus, the results from this study do not suggest that changes in the lipoprotein concentrations play a direct key role in preeclampsia pathology.

All samples were collected at delivery and in the third trimester of pregnancy. Although not matched for gestation, lipoprotein concentrations are increased between the first two trimesters and last trimester, but are then stable (28); therefore these could be compared between groups even if the gestational age was not exactly the same. Furthermore, the same nonsignificant results were seen when only the term preeclamptic samples were compared with controls.

The highest mRNA expression was *ABCA1*, followed by *LRP-1*. A previous study also reported that *ABCA1* is highly expressed in human placenta (29). *LRP-1* is the direct lipoprotein receptor of intermediate density lipoprotein, a

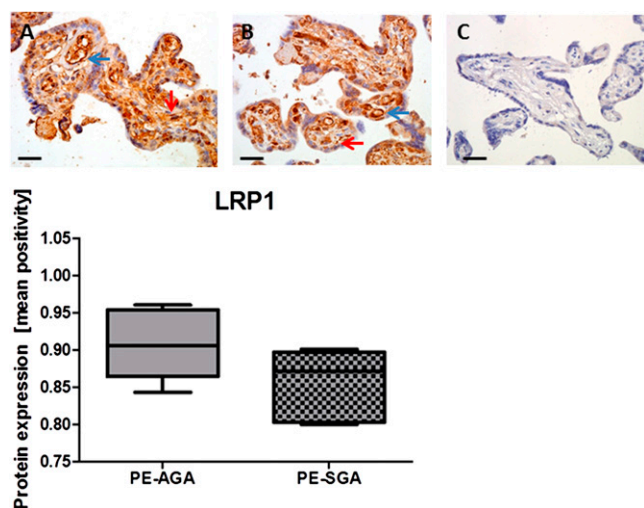


Fig. 2. LRP-1 immunostaining in preeclamptic (PE) placentae from AGA delivering mothers (n = 17) (A), SGA delivering mothers (n = 7) (B), and IgG negative control (C). In photomicrographs, positive cells appear in brown; magnification $\times 400$; scale bar = 100 μm . Protein expression was localized to Hofbauer cells (red arrows) and fetal vessels (blue arrows). In the graph, data is represented as median [interquartile range].


precursor of LDL (15). However *SRB-1* and *LDL-R*, the main cholesterol transporters (30), had very low expression in third trimester placental tissue, which may suggest that the function of *LDL-R* and *SRB-1* may not have an important role toward term, which may reflect a downregulation due to lipids derived from the fetus (2). A previous study at term concluded that *SRB-1* does not play a critical role in controlling plasma cholesterol concentration, even in pregnant women with high or low levels of cholesterol (30). Our findings are in agreement with others who have reported a marked *ABCA1* expression level in diverse placental cell types (31), and the presence of *LDL-R*, *LRP-1*, and *SRB-1* in placental tissues (15). However, in contrast to the current data, Wadsack et al. showed lower mRNA expression of *LDL-R* in FGR-S, and Stepan et al. (32) reported higher. Different criteria in selecting the study population may account for these differences (32); contrasting protein values have also been observed in several studies. In our study, we examined *LRP-1* protein localization and expression due to the differences seen in the preeclamptic plus SGA group. These data suggest lower expression in the SGA group, although this didn't reach significance, possibly due to small numbers. *LRP-1* is highly expressed in macrophages, and therefore it is unsurprising that the expression is localized to the macrophages of the placenta (Hofbauer cells).

It must be noted that cholesterol is not only transported by the receptors; experimental evidence suggests that trophoblast cells efflux cholesterol from cells like any other polarized cells (33). Three different mechanisms for cholesterol transportation from the circulation and peripheral cells to target tissues have been proposed: 1) aqueous diffusion, a protein-independent pathway based on concentration gradients; 2) the *SRB-1* mediated bidirectional transport between cells and extracellular acceptors; and 3) *ABCA1*-mediated unidirectional efflux to lipid-free apolipoproteins (34, 35). The physiological consequences of the regulation of receptors for placental function and fetal development are unclear. A yet undefined proportion of cholesterol uptake by the placenta may be released into the fetal circulation. The remaining placental cholesterol, however, is used for sterol synthesis, principally of progesterone.

When we analyzed the relationship between placental genes and SGA, we observed that *LRP-1* was significantly lower in placenta from SGA infants in the preeclamptic group. A possible mechanism for this could be through the matrix metalloproteinase (MMP) family. Membrane-type MMPs can degrade *LRP-1* into low molecular-mass fragments. Intact soluble *LRP-1* α -chain is shed into human plasma and has been identified at the blood-brain barrier following ischemia. Proteins of the MMP family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in diseases processes, such as arthritis and metastasis (36). Increased MMP-2 activity (*LRP-1* mediates its internalization) may contribute to the endothelial dysfunction that is central to the pathophysiology of preeclampsia and/or SGA.

Further analysis is necessary to understand the cholesterol pathway, especially in relation to fetal growth, in particular alterations in FGR pregnancies and gestational diabetes mellitus, which can result in extremely high birth weight infants (1). It would also be interesting to study the expression of the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, as it regulates cholesterol metabolism via degradation of *LDL-R* (37).

This is a preliminary study that contributes to the current knowledge with the need for future research. It would also be desirable to examine changes at both mRNA and protein levels of all the lipoprotein receptors, transporters, and enzymes. Another limitation of the current study is that we did not have details of exposure to antenatal steroids and other medications, surgery, or timing of the last meal, which may influence some of these alterations. Much has to be done to elucidate the placental transport in pregnancy pathologies, as the transport mechanism for lipids and lipoproteins is far from being understood.

The findings of this study do not support a direct contribution of lipid metabolism in the pathogenesis of preeclampsia. However, this is the first study that simultaneously analyzed seven lipoprotein receptors, transporters, and enzymes related to the cholesterol pathway, with the lipids and lipoprotein levels (LDL, HDL, TC, and TG) in maternal and umbilical cord serum, and in relation to fetal growth. 

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