Is the atherosclerotic phenotype of pre-eclamptic placentas due to altered lipoprotein concentrations and placental lipoprotein receptors? – role of a small-for-gestational-age phenotype

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Running footline: Pregnancy lipoprotein molecules in pre-eclampsia

Abbreviations:

ABCA1, ATP-binding cassette transporter A1; AGA, Adequate-for-gestational-age; BMI, Body mass index; FGR, Fetal growth restriction; *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *LDL-R*, Low Density Lipoprotein Receptor; *LRP-1*, Low density lipoprotein receptor-related protein 1; *MTTP*, Microsomal triglyceride transfer protein; *PDIA2*,Protein disulfideisomerase 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, Triglycerides.

Abstract

Artherosis of spiral arteries in uteroplacental beds from pre-eclamptic women resemble those of atherosclerosis, characterised by increased plasma lipids and lipoproteins. We hypothesised 1) lipoproteins receptors/transporter in placenta would be up-regulated in pre-eclampsia, associated with increased maternal and fetal lipoprotein concentrations; 2) expression of these would be reduced in pre-eclamptic placentae from women delivering small-for-gestational-age (SGA) infants. Placental biopsies, maternal and umbilical serum samples were taken from 27 normotensive and 24 pre-eclamptic women. Maternal/umbilical cord serum LDL; HDL; total cholesterol and triglycerides were measured. Placental mRNA expression of lipoprotein receptors/transporters were quantified using qRT-PCR. Protein localisation/expression of LRP-1 in the pre-eclamptic with/without SGA was measured by immunohistochemistry. Placental mRNA expression of all genes except *PON-1*, *MTTP* and *PDIA2* were observed. No differences for any lipoprotein receptors/transporters were found between groups; however, in the pre-eclamptic group placental *LRP-1* expression was lower in SGA delivering mothers (n = 7; P=0.036). LRP-1 protein was localised 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 pre-eclamptic pregnancies. These findings do not support a role of altered lipid metabolism in pre-eclampsia, but may be involved in fetal growth.

Supplementary keywords: Hypertension; lipids; LDL cholesterol; HDL cholesterol; Triglycerides; ABCA1; LRP-1; LDL-R; SRB-1;Fetal growth.

Introduction:

Lipids and Pregnancy

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, specially triglycerides (TG) and lipoproteins (1). From the 12^{th} week of gestation, phospholipids, total cholesterol (TC), low density cholesterol (LDL), high density cholesterol (HDL) and TG increase in response to oestrogen 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 are still required until term, but in lower amounts, possibly due to some fetal derived lipids from the lipogenic activity in the fetal liver, adrenal and testes (2). Conversely, increased TG during pregnancy has been shown to have an augmented risk of pre-eclampsia, 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, pre-pregnancy lipid levels and various medical complications of pregnancy such as diabetes. (6).

Pre-eclampsia

Complicating 2–8% of pregnancies, pre-eclampsia, 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). Pre-eclampsia is generally defined as high blood pressure (systolic blood pressure $\geq$ 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 pre-eclampsia 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 atherosis seen in pre-eclamptic uteroplacental beds resembling atherosclerotic lesions of coronary arteries (14). The presence of lipoproteins receptors in placental syncytiotophoblast, specifically Low Density Lipoprotein Receptor (*LDL-R*), Low density lipoprotein receptor-related protein 1 (*LRP-1*) and Scavenger Receptor class B type 1 (*SRB-1*) in third trimester placentae have previously been shown (15). Placentae expression of some of these receptors from FGR pregnancies with (FGR-S) and without (FGR-M) fetal hemodynamic changes, based on the result of 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-I* mRNA was unchanged in FGR, but protein levels were lower in FGR-S than in the other groups. They concluded that LDL-R and SRB-I 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 pre-eclampsia.

Based on the literature relating to placental and liver cholesterol transport pathways, we chose to analyse the maternal and fetal lipoprotein concentrations in combination with placenta 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 disulfideisomerase family A, member 2 (*PDIA2*).

We therefore hypothesised that the expression of those lipoprotein receptors involved in the cholesterol pathway in the liver, are up-regulated in pre-eclamptic placentae compared to controls as a compensatory factor. In addition, the expression of some of these receptors may be reduced in pre-eclamptic 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 pre-eclampsia) (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. Pre-eclampsia 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 10^{th}, and AGA when the individualised birthweight ratio was between the 10^{th} and 90^{th} percentile (18). Table 2 provides demographic, obstetric, and pregnancy description data for the pre-eclamptic 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 1400*g* for 10 minutes 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 pre-eclamptic (n = 14) group. All women who took part in this study were labouring and either delivered vaginally or by emergency Caesarean section.

The lipoproteins (LDL, HDL, TC, TG) were measured using MicroSlide technology on the Vitros Fusion 5.1 Chemistry System (New York, USA) following the manufacturers' instructions. Briefly, 200 µl 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 analysed 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 ten minutes of the placental delivery from half way between the cord insertion and periphery of the placentae, avoiding infarcts. The samples were immediately rinsed in ice-cold phosphate buffered saline, the membranes removed, snap frozen in liquid nitrogen for mRNA analysis. A second samples 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 QIAzollysis reagent (Qiagen, Crawley, UK). RNA concentration and 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 μg) was then reverse transcribed using the QuantiTect Reverse Transcription kit containing a mix of random primers and oligodT (Qiagen, Crawley, UK) in a Primus 96 advanced gradient thermocycler (Peqlab Ltd, Fareham, UK).

Quantitative real time polymerase chain reaction (RT-PCR)

Real-time PCR was carried out with the use of SYBR Green chemistry (2x QuantiFast SYBR Green, Qiagen, Crawley, UK) 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 minutes 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 1 single product and non-template 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 normalised 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 pre-eclamptic placentae from this cohort were analysed 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 minutes 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 optimised. Heat-induced epitope retrieval was achieved by heating in a citrate buffer (pH 6.0) using a microwave oven for 15 minutes, followed by incubation for 30 minutes 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, United Kingdom).

All of the slides were assessed by the same observer, blinded to pregnancy outcome. For analysis of placental sections, digital images of 5 randomly selected, high-power (×400 magnification) fields were captured on NIS-Elements F2.20 microscope (Nikon United Kingdom Ltd, Surrey, United Kingdom). 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*-tests 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 pre-eclampsia 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 pre-eclamptic groups. Within the pre-eclamptic group, 7 women delivered SGA infants and 17 delivered AGA infants (Table 2); only one women in the normotensive control group delivered an SGA infant 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 pre-eclamptic samples. However, umbilical venous samples had significantly lower lipids and lipoproteins concentrations compared to maternal concentrations in both groups, TG (normal & pre-eclampsia: $P<0.0001$); TC (normal & pre-eclampsia: $P<0.001$); LDL (normal: $P=0.003$; pre-eclampsia: $P=0.01$) and HDL (normal & pre-eclampsia: $P=0.001$) (Figure 1).

Placental lipoprotein receptors, transporter 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 pre-eclampsia are shown in Table 3. *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 analysed the 10 late-onset pre-eclampsia women (>34 weeks' gestation) separately against the control group to test for any gestational specific differences. The same non-significant results were seen for all the maternal and fetal lipoprotein concentrations and for the placental expression of the receptors, transporters and enzymes (P > 0.1 for all).

Placental lipoprotein receptors expression and SGA

In the pre-eclamptic group only, we found that those delivering SGA infants had significantly lower *LRP-1*expression compared to AGA group (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 localised mainly around fetal vessels and Hofbauer cells (Figure 2). Visual inspection suggests lower protein expression in the pre-elcamptic placentae delivering SGA infants compared to AGA group, although was not significant when analysed 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; Figure 2).

Maternal, 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 analysed the lipid profile in women with pre-eclampsia 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 pre-eclamptic pregnancies, and associated these results with SGA infants, in the third trimester of pregnancy.

Previous studies have reported that hyperlipidemia is enhanced in pre-eclampsia (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 TG. However, factors that influence pre-eclampsia 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 pre-eclampsia 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/m^2 in both groups (Table 1), suggesting that the abnormal lipid profile could be associated with obesity (27), and not necessarily with pre-eclampsia. This could also be a reason why some previous studies have reported increased lipoprotein concentrations in pre-eclampsia, who 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 pre-eclampsia 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 2 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 non-significant results were seen when only the term pre-eclamptic samples were compared to 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 IDL, a 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 towards term, which may reflect a down-regulation 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*, *SRB-1* in placenta 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 localisation and expression due to the differences seen in the pre-eclamptic 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 expression in macrophages and therefore it is unsurprising that the expression is localised to the macrophages of the placentae (Hofbauer cells).

It must be noted that cholesterol is not only transported by the receptors but also 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 placenta may be released into the fetal circulation. The remaining placental cholesterol, however, is used for sterol synthesis, principally of progesterone.

When we analysed the relationship between placental genes and SGA, we observed that *LRP-1* was significantly lower in placentae from SGA infants in pre-eclamptic group. A possible mechanism for this could be through the matrix metalloproteinase (MMP) family. Membrane-type MMPs (MT-MMP) can degrade LRP-1 into low molecular-mass fragments. Intact soluble LRP-1 alpha chain (sLRP-1) 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 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 internalisation) may contribute to the endothelial dysfunction that is central to the pathophysiology of pre-eclampsia, 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 subtillisin/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 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 pre-eclampsia. However, this is the first study that simultaneously analysed 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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References

1. Desoye G, Gauster M, Wadsack C. Placental transport in pregnancy pathologies. Am J Clin Nutr. 2011 Dec;94(6 Suppl):1896S-902S.

2. Ghio A, Bertolotto A, Resi V, Volpe L, Di Cianni G. Triglyceride metabolism in pregnancy. Adv Clin Chem. 2011;55:133-53.

3. Vrijkotte TG, Krukziener N, Hutten BA, Vollebregt KC, van Eijsden M, Twickler MB. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: the ABCD study. J Clin Endocrinol Metab. 2012 Nov;97(11):3917-25.

4. Carr BR, Simpson ER. Cholesterol synthesis in human fetal tissues. J Clin Endocrinol Metab. 1982 Sep;55(3):447-52.

5. Pecks U, Brieger M, Schiessl B, Bauerschlag DO, Piroth D, Bruno B, et al. Maternal and fetal cord blood lipids in intrauterine growth restriction. J Perinat Med. 2012 Apr;40(3):287-96.

6. Alvarez JJ, Montelongo A, Iglesias A, Lasuncion MA, Herrera E. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. J Lipid Res. 1996 Feb;37(2):299-308.

7. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol. 2012 Feb;36(1):56-9.

8. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009 Jun;33(3):130-7.

9. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20(1):IX-XIV.

10. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol. 2000 Jul;183(1):S1-S22.

11. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, et al. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am J Pathol. 1997 Dec;151(6):1809-18.

12. Huppertz B, Peeters LL. Vascular biology in implantation and placentation. Angiogenesis. 2005;8(2):157-67.

13. Campos B, Chames M, Lantry JM, Bill JP, Eis A, Brockman D, et al. Determination of non-bilayer phospholipid arrangements and their antibodies in placentae and sera of patients with hypertensive disorders of pregnancy. Placenta. 2006 Feb-Mar;27(2-3):215-24.

14. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med. 1999 Dec;222(3):222-35.

15. Wadsack C, Tabano S, Maier A, Hiden U, Alvino G, Cozzi V, et al. Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition. Am J Physiol Endocrinol Metab. 2007 Feb;292(2):E476-84.

16. Mistry HD, Wilson V, Ramsay MM, Symonds ME, Broughton Pipkin F. Reduced selenium concentrations and glutathione peroxidase activity in preeclamptic pregnancies. Hypertension. 2008 Nov;52(5):881-8.

17. Gardosi J. Customised centile calculator. www.gestation.net; 2012 [cited 2012 March 26].

18. Cetin I, Foidart JM, Miozzo M, Raun T, Jansson T, Tsatsaris V, et al. Fetal growth restriction: a workshop report. Placenta. 2004 Sep-Oct;25(8-9):753-7.

19. Mistry HD, McCallum LA, Kurlak LO, Greenwood IA, Broughton Pipkin F, Tribe RM. Novel expression and regulation of voltage-dependent potassium channels in placentas from women with preeclampsia. Hypertension. 2011 Sep;58(3):497-504.

20. Murthi P, Fitzpatrick E, Borg AJ, Donath S, Brennecke SP, Kalionis B. GAPDH, 18S rRNA and YWHAZ are suitable endogenous reference genes for relative gene expression studies in placental tissues from human idiopathic fetal growth restriction. Placenta. 2008 Sep;29(9):798-801.

21. Williams PJ, Mistry HD, Innes BA, Bulmer JN, Broughton Pipkin F. Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human. Placenta. 2010 May;31(5):448-55.

22. Ray JG, Diamond P, Singh G, Bell CM. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. Bjog. 2006 Apr;113(4):379-86.

23. Lorentzen B. Fasting serum free fatty acids and triglycerides are increased before 20 weeks of gestation in women who later develop preeclampsia. Hypertens Pregnancy. 1994;13:103-9.

24. Belo L, Caslake M, Gaffney D, Santos-Silva A, Pereira-Leite L, Quintanilha A, et al. Changes in LDL size and HDL concentration in normal and preeclamptic pregnancies. Atherosclerosis. 2002 Jun;162(2):425-32.

25. Catarino C, Rebelo I, Belo L, Rocha-Pereira P, Rocha S, Castro EB, et al. Fetal lipoprotein changes in pre-eclampsia. Acta Obstet Gynecol Scand. 2008;87(6):628-34.

26. Emet T, Ustuner I, Guven SG, Balik G, Ural UM, Tekin YB, et al. Plasma lipids and lipoproteins during pregnancy and related pregnancy outcomes. Arch Gynecol Obstet. 2013 Feb 12.

27. Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis. 2011 Jul;217(1):3-46.

28. Herrera E, Ortega H, Alvino G, Giovannini N, Amusquivar E, Cetin I. Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. Eur J Clin Nutr. 2004 Sep;58(9):1231-8.

29. Bhattacharjee J, Ietta F, Giacomello E, Bechi N, Romagnoli R, Fava A, et al. Expression and localization of ATP binding cassette transporter A1 (ABCA1) in first trimester and term human placenta. Placenta. 2010 May;31(5):423-30.

30. Ethier-Chiasson M, Duchesne A, Forest JC, Giguere Y, Masse A, Mounier C, et al. Influence of maternal lipid profile on placental protein expression of LDLr and SR-BI. Biochem Biophys Res Commun. 2007 Jul 20;359(1):8-14.

31. Nikitina L, Wenger F, Baumann M, Surbek D, Korner M, Albrecht C. Expression and localization pattern of ABCA1 in diverse human placental primary cells and tissues. Placenta. 2011 Jun;32(6):420-30.

32. 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. 1999 Nov;106(11):1221-2.

33. Woollett LA. Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation. Am J Clin Nutr. 2005 Dec;82(6):1155-61.

34. Rothblat GH, de la Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: integration of old and new observations provides new insights. J Lipid Res. 1999 May;40(5):781-96.

35. Yancey PG, Bortnick AE, Kellner-Weibel G, de la Llera-Moya M, Phillips MC, Rothblat GH. Importance of different pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol. 2003 May 1;23(5):712-9.

36. Selvais C, D'Auria L, Tyteca D, Perrot G, Lemoine P, Troeberg L, et al. Cell cholesterol modulates metalloproteinase-dependent shedding of low-density lipoprotein receptor-related protein-1 (LRP-1) and clearance function. FASEB J. 2011 Aug;25(8):2770-81.

37. Levy E, Ouadda AB, Spahis S, Sane AT, Garofalo C, Grenier E, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. Atherosclerosis. 2013 Apr;227(2):297-306.

Figure Legends

Figure 1. Serum lipoprotein concentrations in maternal and fetal circulation, from normotensive controls and pre-eclampsia. The number of fetal serum samples that were missing were 1 in the control (n = 26) and 10 in the pre-eclamptic (n = 14). HDL: high density cholesterol; LDL: low density cholesterol; Mat NC: Maternal normal control; Fetal NC: Fetal normal control; Mat PE: Maternal pre-eclampsia; Fetal PE: Fetal pre-eclampsia. Data presented as median [IQR].*P< 0.05 between maternal and umbilical samples for all lipoproteins.

Figure 2. LRP-1 immunostaining in pre-eclamptic placentae from A) AGA (n = 17) and B) SGA (n = 7) delivering mothers and C) IgG negative control. In photomicrographs, positive cells appear in brown; magnification x400; scale bar = 100 μm. Protein expression was localised to Hofbauer cells (red arrows) and fetal vessels blue arrows). In the graph data is represented as median [IQR].

Table 1. Clinical and Biochemical data of subject groups

Parameter	**NC (27)**	**PE (24)**
Maternal age, y (mean, SD)	30 ± 6.9	31 ± 6.1
Booking body mass index, kg/m^2 (mean, SD)	26.2 ± 5.4	26.6 ± 5.0
Max. systolic blood pressure outside labour, mm Hg (mean, SD)	116 ± 4.6	156 ± 7.1*
Max. diastolic blood pressure outside labour, 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, wks (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: pre-eclampsia. *P<0.05 between normotensive controls and women with pre-eclampsia.

Table 2. Clinical and Biochemical data of the pre-eclamptic women delivery AGA and SGA infants.

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

PE: pre-eclampsia; AGA: appropriate-for-gestational-age; SGA: small-for-gestational-age. ***P*=0.001 and ****P*<0.0001 between PE AGA and PE AGA groups.

Table 3.Details the forward and reverse primers sequences and BLAST sequences

Gene	BLAST sequence Accession No.	Primer	Length (bp)
LDL-R	NM_000527.3	5’-aggacggctacagctaccc-3’ 5’- ctccaggcagatgttcacg-3’	73
LRP-1	NM_002332.2	5’-ggtgtcacccacctcaacat-3’ 5’- agtcggtccagtacacgtttc-3’	88
SRBI	NM_005505.4	5’-catcaagcagcaggtcctta-3’ 5’-cggagagatagaaggggatagg-3’	95
PON-1	NM_000446.5	5’-actatagtccaagtgaagttcgagtg-3’ 5’-atgagccagcaactcagctat-3’	110
ABCA1	NM_005502.2	5’-tgctgcatagtcttgggactc-3’ 5’-atcacctcctgtcgcatgt-3’	76
MTTP	NM_000253.2	5’-ggctggtcttcacggtagc-3’ 5’-gttctcctccccctcgtc-3’	88
PDIA2	NM_006849.2	5’-ctccaagttcctggacaacg-3’ 5’-tggaccccatagtggagttg-3’	104

LDL-R: Low-Density Lipoprotein Receptor; LRP-1: Low density lipoprotein receptor related protein 1; SRB-1: Scavenger Receptor class B type 1; PON-1: Enzyme paraoxonase-1; ABCA1: ATP-binding cassette transporter A1; MTTP: Microsomal triglyceride transfer protein; PDIA2: Protein disulfideisomerase family A, member 2

Table 3. Placental lipoprotein mRNA expression from NC and PE

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

LDL-R: Low-Density Lipoprotein Receptor; LRP-1: Low density lipoprotein receptor related protein 1; SRB-1: Scavenger Receptor class B type 1; ABCA1: ATP-binding cassette transporter A1.

Figure 1

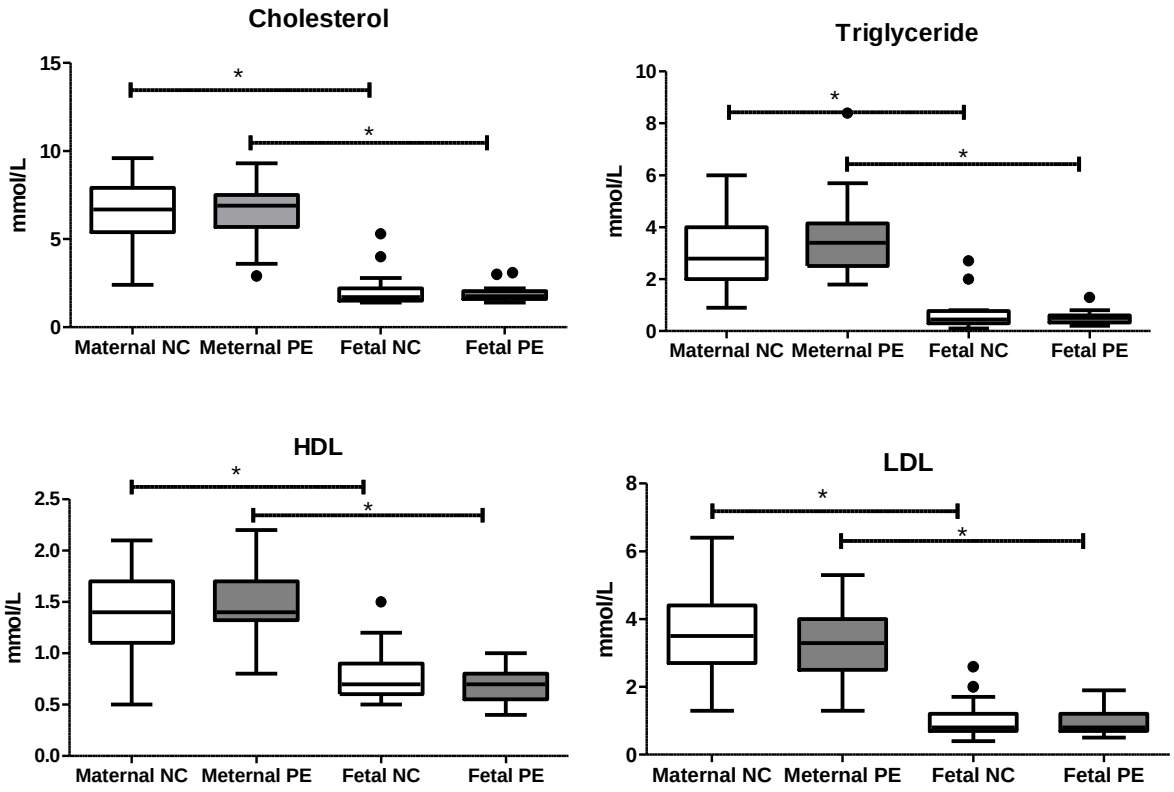
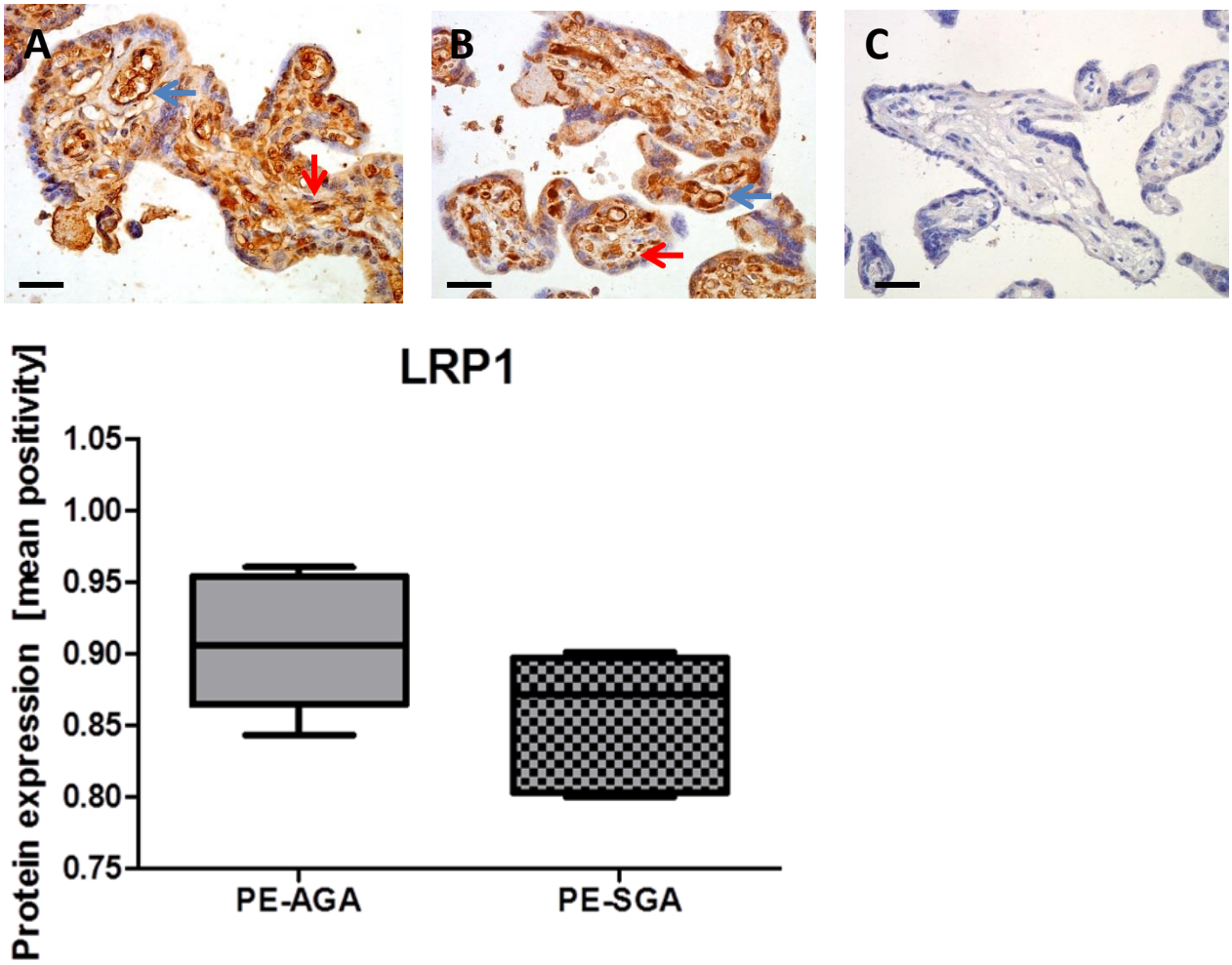

Cholesterol
mmol/L
15
10
5
0
*
*
Maternal NC
Meternal PE
Fetal NC
Fetal PE
Triglyceride
mmol/L
10
8
6
4
2
0
*
*
Maternal NC
Meternal PE
Fetal NC
Fetal PE
HDL
mmol/L
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Maternal NC
Meternal PE
Fetal NC
Fetal PE
LDL
mmol/L
8
6
4
2
0
*
*
Maternal NC
Meternal PE
Fetal NC
Fetal PE

Figure 2


A
B
C
LRP1
Protein expression [mean positivity]
1.05
1.00
0.95
0.90
0.85
0.80
0.75
PE-AGA
PE-SGA