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Influence of maternal lipid profile on placental protein expression of LDLr and SR-BI

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Abstract

Maternal hyperlipidemia is a characteristic feature during pregnancy, it has been reported that modification of the maternal lipid profile can induce disturbance during pregnancy. In this study, we evaluated the impact of maternal lipid profile on the placental protein expression of two major receptors in cholesterol metabolism, the low density lipoprotein receptor (LDLr) and the scavenger receptor type B1 (SR-B1). We demonstrate an increase in the level of maternal total circulating cholesterol leads to a significant decrease in the level of the LDLr protein expression, while the level of the SR-BI expression remains unchanged. A similar change, for LDLr, is observed in association with the maternal pre-pregnancy body mass index and weight gain. Our data suggest that the LDLr plays a role in regulating cholesterol delivered to the baby from the placenta.

Keywords: Lipid profile; Placenta; LDLr; SR-BI; Human

Maternal hyperlipidemia is one of the most consistent and striking changes to take place in lipid metabolism during late pregnancy. Normal human pregnancy is characterized by a progressive increase in the low density lipoprotein (LDL) and the very low density lipoprotein (VLDL) concentrations in the maternal circulation, as reflected by the increase of cholesterol [1]. Cholesterol is an essential component for adequate development of the fetus, being used by the placenta for the synthesis of steroid hormones [2]. Because placental *de novo* cholesterol synthesis is not sufficient to support steroid hormonal production, a major part of the hormones produced by placenta are derived from maternal plasma cholesterol [3]. Placenta express many lipoproteins receptors, including the low density lipoprotein receptor (LDLr) [4] and the scavenger receptors, such as the scavenger receptor class B type I (SR-BI) [5–7].

Among them, the LDLr is of primary importance in the binding and the internalization of the plasma-derived LDL–cholesterol and in regulating the plasma LDL concentration. The physiologic ligands of the LDLr are the LDL, which represents 65–70% of circulating plasma cholesterol in human. The LDL contain apolipoprotein B-100 (apoB-100) as primary protein component [8]. In addition to the LDLr, the LDL particles bind to other receptor such as the SR-BI [7]. The SR-BI, an 82 kDa glycoprotein, is a receptor that principally mediates the selective uptake of

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lipoproteins associated with cholesteryl esters. The SR-BI receptor is highly expressed in the human placenta cells, allowing the growing fetus to obtain a considerable portion of cholesterol from maternal lipoproteins [9].

The contribution of maternal cholesterol to the fetus under normal as well as pathological circumstances is poorly understood. Few studies suggest that maternal contribution can vary with the maternal metabolic environment during pregnancy [10,11]. Therefore, the aim of the present study is to (1) analyze the impact of changes in maternal and fetal circulating lipids on the LDLr and the SR-BI protein expression in human term placenta and (2) to evaluate if any correlation is observed with body mass index (BMI) and weight gain (WG) during pregnancy. This study suggests an important role of the LDLr in maintaining the fetal cholesterol concentration during pregnancy-related disorders/pathologies in which the lipid profile may be disturbed.

Materials and methods

The women participating in the study were recruited, before their 10th week of pregnancy, at the Service of Perinatalogy, of the Centre Hospitalier de l'Université de Montréal (CHUM), Pavilion St-Luc, Canada, from 2002 to 2006. After signing a consent form, each woman filled out an interview-administrated questionnaire, which contained general sociodemographic data, medical history, drinking, and smoking habit. To conduct this study, 74 women were selected. Women were classified in two groups according to the plasma total cholesterol concentration at term. To establish the group, we established the median of the maternal plasma total cholesterol at term (6.42 mM). Women with cholesterol concentration lower than 7 mM referred to the low cholesterol group (LC) ($n = 44$) while women with cholesterol concentration higher than 8 mM referred to the high cholesterol (HC) group ($n = 30$). Subsequently, they were reclassified into three groups according their pre-pregnancy BMI. The established normal values for BMI, according to the Health Canada (2002), were between 20 and 26 kg/m^2, while a low BMI is <20 kg/m^2, and high BMI is >26 kg/m^2. Finally, women were divided according to their WG during pregnancy. The normal values established for WG during pregnancy ranged from 11 to 18 kg. All of these women were non-smokers and did not receive any medication known to interfere with lipid metabolism. Finally, a post-natal follow-up was made to assess newborns health status as well as collecting data relative to the weight and height of the babies.

Blood and tissue samples. Blood samples were collected at each trimester and at delivery, from the mother and the cord blood. Blood samples were collected in gel Vacutainer tube (BD, Oakville, ON, Canada). The placentas, from vaginal delivery, were obtained from the collaborating hospital (CHUM, Canada), and were immediately immersed in Dulbecco's modified Eagle's medium (DMEM) (Sigma, Oakville, ON, Canada) containing a mixture of antibiotic and $NaHCO_3$. After the removal of the amnion, the chorion and the decidual layer, the placental tissue, was cut in 5 cm^2 sections and immediately frozen to liquid nitrogen and then kept at −80 °C.

Lipids determination. The plasma levels of total cholesterol (TC), LDL, high density lipoprotein (HDL), VLDL, and TG were measured using the Unicel 36 DX600 Synchron Clinical System (Beckman-Coulter, Mississauga, ON, Canada), at the Clinical Biochemistry Service of Saint-François d'Assise Hospital at Québec (Que., Canada).

Proteins extraction and Western blot analyses of the LDLr and the SR-BI. Frozen placental tissue was homogenized in ice-cold hypertonic buffer (125 mM Tris–HCl, 2 mM $CaCl_2$, 1.4% Triton X-100). The homogenate was than kept on ice for 30 min and centrifuged to collect the supernatant. Protein concentration was determined by spectrophotometric quantification using the bicinchoninic acid (BCA) reagent (Pierce, Brockville, ON, Canada). Total placental proteins (150 μg) were resolved in 8% SDS–PAGE and electroblotted to PDVF membrane (Millipore, Cambridge, ON, Canada). After incubated with a blocking solution, membranes were incubated for 90 min at room temperature, with either a human low density lipoprotein receptor (LDLr) rabbit monoclonal antibody (Fitzgerald, Concord, MA, USA), or a human scavenger receptor type BI (SR-BI) rabbit monoclonal antibody (Novus Biological, Littleton, CO, USA), or a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mouse monoclonal antibody (Chemicon International, Temecula, California, USA). Blots were probed with anti-mouse and anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies (for LDLR and SR-BI, respectively), and with a GAPDH anti-mouse-IgG (Chemicon International, Temecula, California, USA) for 90 min at room temperature. The detection was performed using the BM Chemiluminescence system (Roche, Laval, Que., Canada) and visualized by autoradiography. For semiquantitative analyses of the bands, the film was digitized and intensity of the band is doing by the Quantity One Software (Bio-Rad Laboratories, Mississauga, ON, Canada).

Statistical analyses. Data were expressed as the means ± SEM, and analyzed with the unpaired Student's t-test at $p < 0.05$ level of significance, to evaluated difference between groups. For the relationship between two variables of the same population, results are expressed as Spearman's correlation and the curve represent Pearson's linear correlation. All statistical analyses were performed using the Prism software (version 4.0.2; 2004).

Results

Population characteristics

Characteristics of mothers and newborns are presented in Table 1. In both LC and HC groups, the age of women was ±31 years old and their gestational age was about 39 weeks. No significant difference was observed in the BMI and the WG, as well as on babies' weight and height at birth and placental weight.

Maternal plasma lipids at delivery and in venous cord blood

In LC and HC groups the concentrations of TC, HDL, LDL, TG, ApoA-1, and ApoB-100 were measured at delivery in maternal and in the cord blood (Table 2). At delivery, the maternal plasma concentrations of TC, TG, and LDL–cholesterol were significantly increased into the HC group compared to LC group, corresponding to an increased level of ApoB-100, while the levels of HDL and ApoA-1 remain unchanged. No significant differences were

Table 1
Population characteristics

Maternal plasmatic cholesterol concentration	LC ($n = 44$)	HC ($n = 30$)
	<7 mM	>8 mM
Mother age (year)	30.9 ± 5.3	31.4 ± 4.2
Gestational age (week)	38.9 ± 1.5	39.2 ± 1.6
Mother BMI (kg/m^2)	23.5 ± 5.0	22.41 ± 4.36
Mother WG (kg)	14.98 ± 6.09	18.62 ± 6.81
Newborn birth weight (g)	3282 ± 648	3404 ± 462
Newborn height (cm)	51.4 ± 2	52.0 ± 5.0
Placenta weight (g)	590.2 ± 154.1	608.4 ± 142.7

Results are expressed as means ± SD where compared to LC group.

Table 2
Maternal plasma lipids at delivery and in cord blood

	LC group ($n = 44$)		HC group ($n = 30$)	
	Maternal plasma	Venous cord blood	Maternal plasma	Venous cord blood
Total cholesterol (mM)	5.74 ± 0.173	1.88 ± 0.100	$8.30 \pm 0.207^{***}$	1.67 ± 0.08
HDL (mM)	1.70 ± 0.069	0.75 ± 0.05	1.72 ± 0.111	0.72 ± 0.06
LDL (mM)	2.79 ± 0.12	0.83 ± 0.08	$4.95 \pm 0.207^{**}$	0.67 ± 0.06
Triglycerides (mM)	2.73 ± 0.170	0.63 ± 0.05	$3.65 \pm 0.230^{**}$	0.60 ± 0.07
Apo-AI (g/L)	2.05 ± 0.059	0.91 ± 0.05	2.21 ± 0.09	0.83 ± 0.04
ApoB-100 (g/L)	1.14 ± 0.04	0.30 ± 0.03	$1.64 \pm 0.05^{**}$	0.25 ± 0.03

Results are expressed as means ± SEM where $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the LC group.

observed in the cord blood samples between both groups for all parameters. Taking together, our data suggest that hypercholesterolemia at term modify the circulating lipid profile in the mother without affecting the lipid profile in newborn.

Protein expression of the LDLr and the SR-BI

As hypercholesterolemia appears to affect circulating lipid profile only on the mother side, we hypothesize that cholesterol transport in placenta could be affected. The expression of the LDLr was evaluated by Western blot in placenta. As showed in Fig. 1, the level of the LDLr expressed in placenta from HC mothers is decreased by more than 60% (1.081 ± 0.088 *vs* 0.379 ± 0.042, $^{***}p < 0.0001$) compared the LC group. At the opposite, the SR-BI protein levels are not affected (Fig. 2).

We then evaluated the impact of pre-pregnancy BMI on the modulation of the LDLr expression in term placenta. Each LC and HC groups were divided into three subgroups: low, normal, and high BMI. Of interest, our analysis reveals that the level of the LDLr protein expression in placenta is modulated by maternal pre-pregnancy BMI. In LC group (Fig. 3A, white bars), a lower maternal pre-pregnancy BMI leads to a significant increase in the expression of the LDLr compared to normal BMI (0.53 ± 0.070 *vs* 0.383 ± 0.038 $^{*}p = 0.0438$), while no such significant observation is made in the HC group (black bars). However, for HC women, a dramatic decrease of the receptor expression in the highest BMI group is observed compared to the

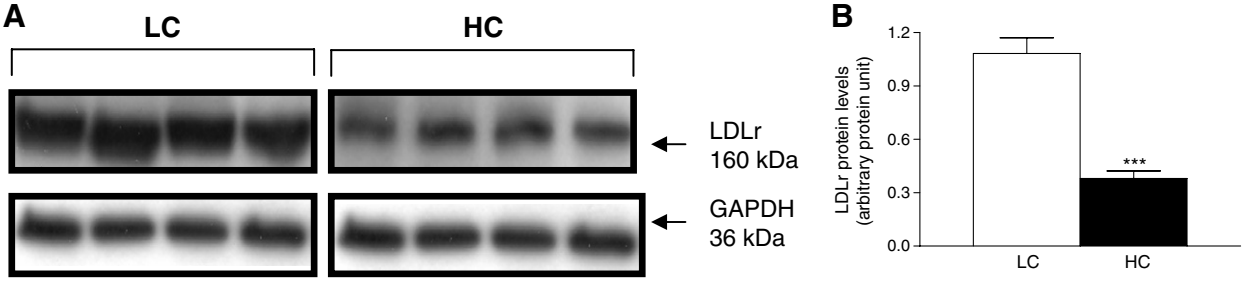

Fig. 1. Western blot analysis of the LDLr in placenta. Western blot analysis of LDLr from placenta of LC and HC groups. (A) Representative Western blot on four different placentas. (B) Relative LDLr proteins expression in placenta of LC (white bars) and HC groups (black bars). Results are expressed as a ratio of the level of the LDLr and GAPDH, measured in the same sample and the same membrane after the stripping. Results are the means ± SEM ($n = 44$ for LC and $n = 30$ for HC) $^{***}p < 0.0001$ comparing HC *vs* LC.

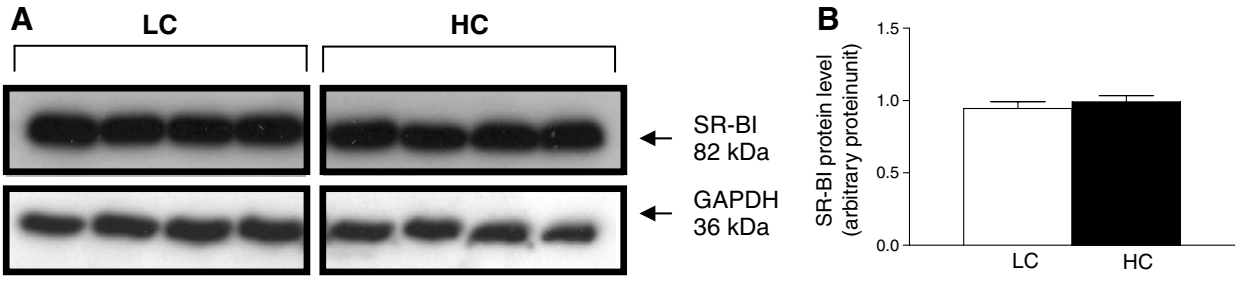

Fig. 2. Western blot analysis of the SR-BI in placenta. Western blot analysis of SR-BI from placenta of LC and HC groups. (A) Representative Western blot performed on four different placentas. (B) Relative SR-BI proteins expression in placenta of LC (white bars) and HC groups (black bars). Results are expressed as a ratio of the level of the SR-BI and GAPDH, measured in the same sample and the same membrane after the stripping. Results are the means ± SEM ($n = 44$ for LC and $n = 30$ for HC).

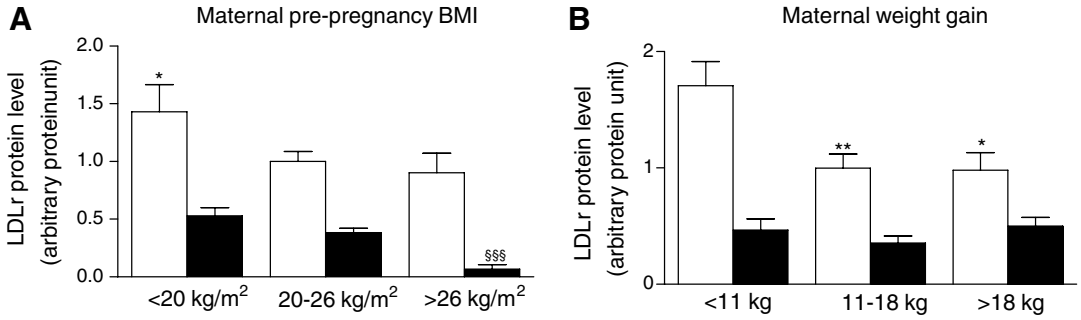

Fig. 3. Expression of the LDLr in human placenta according to the maternal pre-pregnancy BMI. Western blot analysis of LDLr from placenta of LC (white bars) and HC (black bars) group. (A) maternal BMI and (B) maternal WG. Results are expressed as a ratio of the level of the LDLr and GAPDH, measured in the same sample and the same membrane after the stripping. Results are expressed as the means ± SEM. ($n = 44$ for LC group and $n = 30$ for HC). (A) $^{*}p < 0.05$ comparing LC women with low BMI *vs* women with normal BMI, and $^{§§§}p < 0.0001$ comparing HC women with high BMI *vs* women with low and normal BMI, (B) $^{**}p < 0.001$ comparing LC women with a low WG and those with a normal WG (11–18 kg) $^{*}p < 0.05$ comparing LC women with a low WG and those with a high WG.

lowest BMI group (0.53 ± 0.070 *vs* 0.068 ± 0.039, $^{§§§}p = 0.0007$), since there is no difference for LC women. These results suggest that even if the BMI is not modified by changes in the cholesterol concentration (Table 1). In fact, the level of the LDLr expression in placenta appears to be increased when the BMI is decreased even in the absence of plasma hypercholesterolemia suggesting another regulation of the LDLr expression.

Also, we evaluate the impact of WG during pregnancy (<11 kg (low), 11–18 kg (normal), and >18 kg (high)) on the LDLr expression in the placenta of LC and HC groups. As shown in Fig. 3B, in LC group (white bars), a low WG leads to a significant increase of the LDLr expression compared to a normal and a high WG (1.708 ± 0.206 *vs* 1.000 ± 0.119, $^{**}p = 0.0033$ and 1.708 ± 0.206 *vs* 0.981 ± 0.981, $^{*}p = 0.0115$, respectively). However, in the HC group, the WG during pregnancy does not influence the expression of the LDLr in the placenta.

Correlations between the LDLr and the SR-BI proteins expressions and circulating plasma lipids at delivery

The correlation between the LDLr protein expression and many of the maternal plasma circulating lipids was evaluated by Spearman's correlation and Pearson's linear correlation curves. Negative correlation was observed between the LDLr protein expression and total maternal plasma cholesterol ($r = -0.486$) level, plasma LDL level ($r = -0.530$), and plasma apoB-100 ($r = -4340$) at term, (Fig. 4A, B, and D, respectively). No correlation was demonstrated between the LDLr protein expression and maternal plasma HDL level at term (Fig. 4C) and between the SR-BI protein expressions for all studied factors.

Discussion

In the present study, we have analyzed the lipid profile in LC and HC pregnant women, since abnormal high plasma lipid level even in fetal life has been associated with increasing risk of coronary heart diseases [12,13]. It has been reported that maternal plasma lipid status is modified during pregnancy [14,15]. In our study, we observed that in HC pregnant women, total plasma cholesterol, LDL, TG, and apoB-100 concentrations are increased compared to the levels measured in LC women. No significant difference was observed for HDL and apo-A1 levels between the two groups. In addition, we showed that modulation of maternal cholesterol concentration does not affect the fetal lipid profile measured in the venous cord blood, suggesting a modulation of the fetal metabolism or the presence of a compensatory mechanism in the placenta.

In order to verify if the placenta is able to modulate the amount of cholesterol distributed to the baby, we analyzed the expression of cholesterol transporters in HC and LC placentas. Placenta is a crucial organ for cholesterol transfer from the mother to the fetus. Cholesterol is taken up from maternal plasma LDL by the LDLr and by SR-BI [16] and next transported into cells by the ATP binding cassette transporter 1 (ABCA-1) [17]. We demonstrated in this paper that increase of the concentration of plasma cholesterol, and especially changes of the level of circulating LDL, modifies the expression of the LDLr protein in placenta, while the level of the SR-B1 protein is not affected. This suggests that in placental cells as in hepatocytes, the expression of the LDLr protein is under the control of cholesterol concentration [18].

The mechanism of cholesterol action on the regulation of the LDLr expression is complex. It appears to be mediated by different transcription factors (TFs) such as sterol regulatory element-binding proteins (SREBPs), the Sp1 YY1 and the NF-Y/CBF [19]. Both SREBP-1 and -2 are crucial molecules in the regulation of cholesterol metabolism [20] however, SREBP-2 has been preferentially implicated in the control of the LDLr gene expression [21]. It was showed that when cellular cholesterol concentrations decreased, SREBP-2 is released from the Golgi [22]. The mature SREBP-2 is subsequently translocated to the nuclei [23] where it can bind to the LDLr promoter increasing

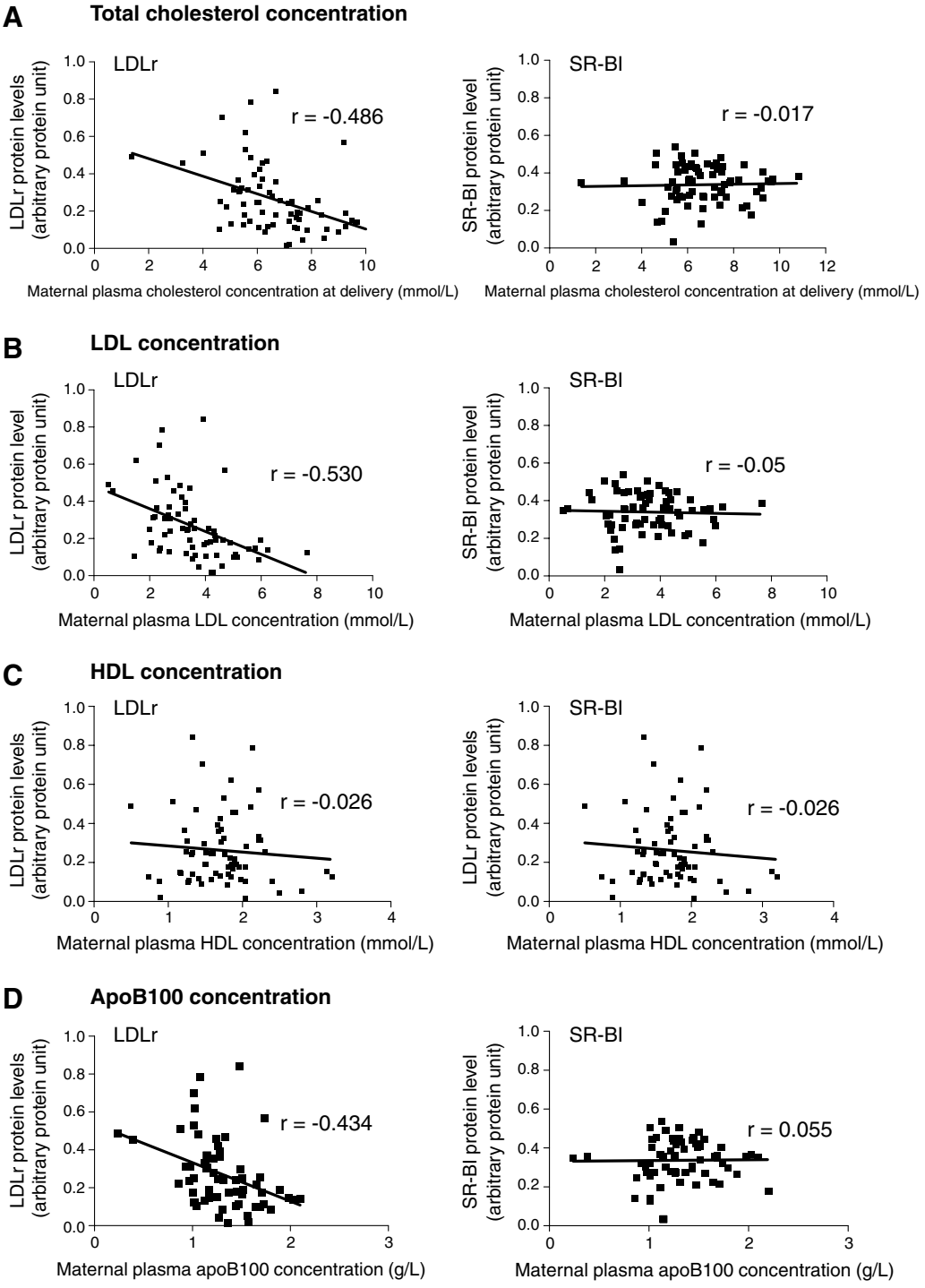

Fig. 4. Relationship of the LDLr (left panels) and the SR-BI (right panels) protein expression and different maternal plasma lipids concentration at delivery for (A) total cholesterol, (B) LDL, (C) HDL, and (D) plasma apoB-100. Results are expressed as Spearman's correlation and the curve represent Pearson's linear correlation.

gene transcription [22]. However, we did not detect any difference in the level of mature SREBP-2 expression in HC *vs* LC placentas (unpublished data). This may suggest that in placenta, cholesterol does not regulate the LDLr gene transcription *via* SREBP-2 but probably *via* other TFs such as Sp1 YY1 and NF-Y/CBF. It was also demonstrated that insulin activates the gene expression of the LDLr [24,25]. This may explain while in HC women, augmentation of the BMI increases the LDLr expression as hyperinsulinemia is often observed when obesity is associated with hypercholesterolemia [26,27].

The expression of the placental LDLr is not affected by the WG in the HC group, while in the LC group its expression is significantly increased in women with a low WG.

This increase may be attributed to a mechanism in which the LC women are able to compensate for a lower level of circulating cholesterol especially when it is associated with a low WG. It has been previously reported that a high WG during pregnancy is associated with an increase in the concentration of the serum lipoproteins correlating with fetal macrosomia [28]. However, in our study, no fetal macrosomia has been observed in the HC group despite a significant increase in the level of plasma LDL. This may be explained by the fact that in the HC group, the level of the placental LDLr expression is not affected by the WG. Taking together, our data demonstrate that WG during pregnancy may affect the expression of the LDLr but only when it is associated with a low circulating concentration of cholesterol.

Cholesterol may be transfer from the placenta to the fetal circulation by either aqueous gradient diffusion or from newly synthesized lipoproteins in placenta *via* the SR-B1 receptor [29]. A previous study suggests that SR-BI plays a major role during different stages of fetal development as in human lacking functional LDLr, fetal development and cholesterol supply to the fetus are normal compared to controls [30]. In our study, we showed that expression of placental SR-BI is not modified by changes in maternal plasma cholesterol level. In addition, no correlation was found between the placental expression level of SR-BI and the maternal plasma HDL and LDL concentrations. Taking together, our study suggests that at term, SR-BI does not play a critical role in controlling the plasma cholesterol concentration in both placenta and fetus.

In conclusion, our study shows the importance of the modulation of placental LDLr expression in the control of cholesterol concentrations in both term placenta and fetus. More precisely, we demonstrated that expression of the LDLr protein level in term placenta is inversely correlates with maternal total plasma cholesterol, plasma LDL, and plasma apoB-100 concentrations probably explaining the absence of hypercholesterolemia in the fetus. In addition, we showed that increase in maternal BMI is associated with a decrease in the LDLr expression in both HC and LC women, while a low maternal WG during pregnancy increased the LDLr expression only in LC women. Also, our study clearly demonstrates that during pregnancy, the LDLr in human term placenta plays a crucial role in the control cholesterol homeostasis in both placenta and fetus and this in response to changes in maternal lipid profile.

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