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DNA methylation at *LRP1* gene locus mediates the association between maternal total cholesterol changes in pregnancy and cord blood leptin levels

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Abstract

Placental lipids transfer is essential for optimal fetal development, and alterations of these mechanisms could lead to a higher risk of adverse birth outcomes. Low-density lipoprotein receptor (*LDLR*), LDL receptor-related protein 1 (*LRP1*), and scavenger receptor class B type 1 (*SCARB1*) genes are encoding lipoprotein receptors expressed in the placenta where they participate in cholesterol exchange from maternal to fetal circulation. The aim of this study was thus to investigate the association between maternal lipid changes occurring in pregnancy, placental DNA methylation (DNAm) variations at *LDLR, LRP1*, and *SCARB1* gene loci, and newborn's anthropometric profile at birth. Sixty-nine normoglycemic women were followed from the first trimester of pregnancy until delivery. Placental DNAm was quantified at 43 Cytosine-phosphate-Guanines (CpGs) at *LDLR, LRP1*, and *SCARB1* gene loci using pyrosequencing: 4 CpGs were retained for further analysis. Maternal clinical data were collected at each trimester of pregnancy. Newborns' data were collected from medical records. Statistical models included minimally newborn sex and gestational and maternal age. Maternal total cholesterol changes during pregnancy (ΔT3-T1) were correlated with DNAm variations at *LDLR* ($r = -0.32$, $p = 0.01$) and *LRP1* ($r = 0.34$, $p = 0.007$). DNAm at these loci was also correlated with newborns' cord blood triglyceride and leptin levels. Mediation analysis supports a causal relationship between maternal cholesterol changes, DNAm levels at *LRP1* locus, and cord blood leptin concentration ($p_{mediation} = 0.02$). These results suggest that *LRP1* DNAm link maternal blood cholesterol changes in pregnancy and offspring adiposity at birth, which provide support for a better follow-up of blood lipids in pregnancy.

Introduction

Metabolic adaptations to pregnancy are normal processes aiming at supporting fetal growth. These adaptations include insulin resistance and changes in blood lipid profile.[1,2] Physiological changes in maternal blood lipids during pregnancy cause a gradual increase in all circulating lipoproteins. So every women, even the ones with normal cholesterol levels before pregnancy, are experiencing temporary hyperlipidemia,[3] although normal values defined outside pregnancy might not apply. Interestingly, it was previously suggested that maternal lipid levels during pregnancy were associated with birth outcomes and might predispose the newborns to long-term health consequences.[4,5] Specifically, low maternal lipid levels may reflect an inadequate physiological response to pregnancy and have been associated with preterm delivery and lower birth weight.[6] In contrast, maternal hypercholesterolemia has been linked to poorer birth outcomes and increased cholesterol deposition in the fetal aorta that might therefore influence newborns' risk of long-term cardiovascular disease (CVD) and morbidity.[7–9]

Placenta expresses many lipoprotein receptors, including the ATP-binding cassette transporter A1 (ABCA1), low-density lipoprotein receptor (LDLR), the LDL receptor-related protein 1 (LRP1), and scavenger receptor class B type 1 (SRB1; encoding by *SCARB1* gene).[10,11] These receptors are key players for binding of lipoproteins and internalization of cholesterol efflux from maternal to fetal circulation.[11–13]

Recent studies have suggested that epigenetic modifications might explain, to some extent, the link between *in utero* environment and long-term cardiovascular disorders risk for

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newborns.[14] Epigenetics refers to DNA modifications regulating gene transcription without changes of the primary DNA sequence. DNA methylation (DNAm) is the most stable and studied epigenetic mark[15] and is generally associated with gene expression repression.[16] Importantly, the newborn epigenetic signature acquired in the womb might be persistent throughout adult life,[17,18] although this very likely not affects every gene similarly.

Changes in DNAm have been shown to be associated with *in utero* environment, including maternal lipid levels and glycemic profile during pregnancy.[19–22] Previous studies carried by our group showed that high-density lipoprotein cholesterol (HDL-C) levels during pregnancy were associated with DNAm levels at *ABCA1* and lipoprotein lipase (*LPL*) gene in the placenta.[20,23] Moreover, previous works have shown that maternal lipid levels during pregnancy were associated with changes in LDLR placental protein levels.[12]

Therefore, we hypothesized that changes in maternal blood lipids in response to pregnancy prime placental cells through changes in DNAm to optimize materno-fetal lipid transfer and fetal growth. Accordingly, this study was undertaken to assess the association between maternal lipid level changes in pregnancy, placental DNAm at *LDLR*, *LRP1*, and *SCARB1* gene loci, and anthropometric characteristics of the newborn at birth.

Materials and methods

Sample and clinical data

Sixty-nine normoglycemic women with a singleton pregnancy were selected from a cohort recruited in a founder population of French–Canadian origin from the Saguenay–Lac-Saint-Jean region of Québec (Saguenay city area, Québec, Canada). The women included in this study were aged between 18 and 40 years old, with no personal history of pregestational type 1 or 2 diabetes, polycystic ovary syndrome, or familial hypercholesterolemia. Women with a positive history of alcohol or drug abuse during the current pregnancy were also excluded. All women included in the current study were normoglycemic as defined as a 2 h-post oral glucose tolerance test (OGTT) glucose concentration < 7.8 mmol/l following a 75-g OGTT performed at 24–28 weeks' gestation according to the WHO criteria.[24]

A written informed consent was obtained for all women before their inclusion in the study, and all clinical data were de-identified. This project received the approval by the Chicoutimi Hospital Ethics Committee, in accordance with the Declaration of Helsinki.

Women were met at the ECOGENE-21 Clinical Research Center at the first, second, and third trimester of pregnancy. A registered nurse performed a complete anthropometric and metabolic profiling. Anthropometric variables and blood pressure were measured using standardized procedures.[25,26] Blood samples were obtained after a 12-h overnight fast from the antecubital vein into vacutainer tubes containing EDTA at each gestational trimester and at the time of delivery (cord blood). Plasma cholesterol, TG, HDL-C, and glucose levels were enzymatically measured using Beckman analyzers (models CX7 for lipids and DXC for glucose). LDL-C levels were calculated using the Friedewald formula.[27] Total gestational weight gain was computed by subtracting maternal weight at first trimester of pregnancy to that at the third trimester. Maternal blood lipid changes between trimesters were calculated by subtracting values at third to that at first trimester of pregnancy (ΔT3-T1 blood lipid level). ΔT3-T1 blood lipid levels represent global variation of blood lipid levels throughout pregnancy. Insulin measurements were performed using a radioimmunoassay method (Advia Centaur, Siemens). Insulin resistance levels were assessed using homeostatic model assessment – insulin resistance (HOMA-IR) [fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5] at each trimester of pregnancy.

Clinical information about newborns was collected at birth from medical files and included gestational age, sex of the newborn, and anthropometric measurements. The gestational age was calculated at the first visit from the date of the last menstrual period and was corrected afterward, as recommended, based on the date of the ultrasound scans. Birth weight *Z*-score was assessed using the 2013 Fenton growth chart.[28] Newborns' ponderal index (kg/m^3) was calculated as: newborn weight (kg)/newborn length^3 (m^3). Cord blood lipid levels were assessed as described for the mother. In addition, cord blood leptin levels, measured by ELISA as previously described (B-Bridge International) were used as a marker of adiposity as previously described.[29,30]

Placenta samples and nucleic acid extraction

Placenta samples were taken within minutes of placenta expulsion (on average < 15 min) by clinicians. Biopsies were taken near the insertion point of the umbilical cord and consisted of the chorionic plate with the chorionic villi. Biopsies were washed in PBS 1× to remove cord/maternal blood, dissected to remove conjunctive tissues, and kept in RNAlater (Qiagen, #76106) at −80°c until nucleic acid extraction. Placental DNA and RNA were purified using the All Prep DNA/RNA/Protein Mini Kit (Qiagen, #80004). The ratio of absorbance at 260–280 nm with the Ultrospec 2000 UV/Visible Spectrophotometer (Pharmacia Biotech, USA) was used to confirm the DNA purity.[30] Placenta RNA quality was assessed with Agilent 2100 Bioanalyzer RNA Nano Chips (Agilent Technologies, USA, #5067-1511) based on RNA integrity number (RIN).

DNA methylation measurement

We used the gold-standard pyrosequencing technology to determine base-specific cytosine methylation levels.[31] Pyrosequencing assays combine sodium bisulfite DNA conversion chemistry (EpiTech Bisulfite Kits, Qiagen, USA; #59104), polymerase chain reaction (PCR) amplification (PyroMark PCR Kit, Qiagen; #978703), and sequencing by synthesis assay of a target region (PyroMark Gold Q24 Reagents, Qiagen; #978802). Each pyrosequencing runs included a negative PCR control and sequencing primer control as well as a sodium bisulfite conversion control. Additionally, pyrosequencing quality control was assessed for each sample using PyroMark Q24 Analysis Software, Qiagen (v.1.0.10.134). PCR primers were selected using the PyroMark Assay Design software v2.0.1.15. The PCR and pyrosequencing primers for the amplification of the loci are described in Supplementary Table 1.

Loci within *LDLR*, *LRP1*, and *SCARB1* gene regions were selected based on a previous study reporting these regions were showing DNAm variability in the placenta.[22] Gene promoter regions and Cytosine-phosphate-Guanine (CpG) islands were also selected based on previous studies.[32,33] Overall, 43 CpG dinucleotides within *LDLR*, *LRP1*, and *SCARB1* gene loci were epigenotyped: 17 at *LDLR* gene locus (4 different regions: *LDLR*-CpGA

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Table 1. Maternal characteristics and blood lipid levels throughout pregnancy ($n = 69$)

Maternal characteristics ($n = 69$)	First trimester of pregnancy (mean ± SD)	Second trimester of pregnancy (mean ± SD)	Third trimester of pregnancy (mean ± SD)
Time of visit (weeks)	12.7 ± 1.2 ($n = 66$)	25.7 ± 0.9	36.9 ± 0.44 ($n = 65$)
Mother's age (years)	28.0 ± 3.8	–	–
BMI (kg/m^2)	23.4 ± 3.2	–	–
Fasting glycemia (mmol/l)	4.38 ± 0.34	4.29 ± 0.37	4.34 ± 0.63 ($n = 66$)
Fasting insulin (mmol/l)[a]	5.10 ± 2.52 ($n = 67$)	7.27 ± 9.86 ($n = 66$)	10.35 ± 31.61 ($n = 64$)
HOMA-IR[a]	0.99 ± 0.53 ($n = 67$)	1.38 ± 1.69	1.97 ± 11.81 ($n = 63$)
2 h-post-OGTT glucose concentration (mmol/l)	–	6.24 ± 0.75	–
Total cholesterol (mmol/l)	4.67 ± 0.83	6.29 ± 1.06[b]	6.85 ± 1.28[b,d]
LDL-cholesterol (mmol/l)	2.62 ± 0.74	3.66 ± 0.95[b]	3.87 ± 1.16[b,c]
HDL-cholesterol (mmol/l)	1.56 ± 0.32	1.78 ± 0.42[b]	1.74 ± 0.44[b]
Triglyceride (mmol/l)a	1.01 ± 0.44	1.77 ± 0.66[b]	2.76 ± 0.86[b,d]
Gravidity			
0	–	–	32 (46.4%)
1			21 (30.4%)
2			10 (14.5%)
3			5 (7.2%)
4			1 (1.4%)
Parity			
0	–	–	39 (56.5%)
1			19 (27.5%)
2			11 (15.9%)
Mode of delivery			
C-section	–	–	5 (7.2%)
Vaginal			64 (92.8%)

BM1, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment – insulin resistance; OGTT, oral glucose tolerance test; SD, standard deviation.
[a]Geometric mean is presented for this variable.
[b]Significantly different from first trimester blood lipid levels ($p < 0.001$).
[c]Significantly different from second trimester blood lipid levels ($p < 0.05$).
[d]Significantly different from second trimester blood lipid levels ($p < 0.001$).

to *LDLR*-CpGD), 9 at *LRP1* gene locus (4 different regions: *LRP1*-CpGA to *LRP1*-CpGD), and 17 at *SCARB1* gene locus (3 different regions: *SCARB1*-CpGA to *SCARB1*-CpGC) (Supplementary Table 1). When DNAm levels were found to be generally well correlated among the CpG dinucleotides from a same region ($r > 0.7$), a mean DNAm level for these CpGs was computed and used in the subsequent statistical analysis. Otherwise, the CpG dinucleotides were analyzed separately.

Table 2. Characteristics of newborns ($n = 69$)

Characteristics	Mean ± SD	Range
Sex		
Male	33 (47.8%)	–
Female	36 (52.2%)	
Gestational age (weeks)	39.4 ± 1.1	37.3–41.6
Birth weight (kg)	3.44 ± 0.43	2.00–4.31
Birth height (cm)	50.0 ± 2.0 ($n = 65$)	44.0–54.5
Birth weight *Z*-score	−0.02 ± 0.79	−2.21–1.88
Ponderal index (kg/m^3)	27.6 ± 2.6 ($n = 65$)	19.6–35.3
Placenta weight (g)	577 ± 110 ($n = 67$)	360–992
Cord blood total cholesterol (mmol/l)	1.70 ± 0.43 ($n = 61$)	0.85–2.89
Cord blood LDL-cholesterol (mmol/l)	0.86 ± 0.28 ($n = 61$)	0.39–1.90
Cord blood HDL-cholesterol (mmol/l)	0.63 ± 0.21 ($n = 61$)	0.26–1.27
Cord blood triglyceride (mmol/l)[a]	0.41 ± 0.24 ($n = 61$)	0.13–1.82
Cord blood leptin (ng/ml)	10.8 ± 8.5 ($n = 52$)	1.0–41.5

HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.
[a]Geometric mean is presented for this variable.

Relative placental mRNA level measurement

Forty-six placental RNA samples were available for mRNA quantification. They were of good quality based on a RIN > 7. Complementary DNA (cDNA) was generated from the placental total RNA using a random primer hexamer provided with the High Capacity cDNA Archive kit (ThermoFisher Scientific, USA; #4368814). Equal amounts of cDNA were run in duplicated and amplified in a 20 ul reaction containing 10 ul of 2× Universal PCR Master Mix (ThermoFisher Scientific; Life Technologies, #4366072). Primers and TaqMan probes were designed to cover exon boundaries (Life Technologies, *LDLR*: Hs01092524_m1, *LRP1*: Hs00233856_m1, and *SCARB1*: Hs00969821_m1). Each sample was calibrated to the *YWHAZ* housekeeping gene (endogenous control; *YWHAZ*: Hs00237047_m1). Relative quantification estimations were performed using an Applied Biosystem 7500 Real Time PCR System as recommended by the manufacturer. *LDLR*/*YWHAZ* C_t ratio (1/x), *LRP1*/*YWHAZ* C_t ratio (1/x), and *SCARB1*/*YWHAZ* C_t ratio (1/x) were used in the analyses.

Statistical analyses

Normal distribution of all variables was assessed using the Kolmogorov–Smirnov test. The following variables were found normally distributed after they were $\log_{10}$-transformed: TG levels, insulin levels, and HOMA-IR. Maternal blood lipid levels from each trimester of pregnancy were compared using a paired

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Table 3. Association between maternal blood lipid level changes in pregnancy and newborns' characteristics

Mothers' blood lipids levels	Cord blood total cholesterol	Cord blood LDL-cholesterol	Cord blood HDL-cholesterol	Cord blood triglyceridemia	Cord blood leptin	Birth weight *Z*-score	Ponderal index	Placental weight
ΔT3-T1 total cholesterol	***r* = 0.29** ***p* = 0.037**	***r* = 0.30** ***p* = 0.029**	*r* = 0.16 *p* = 0.245	*r* = –0.01 *p* = 0.934	***r* = –0.32** ***p* = 0.034**	*r* = –0.11 *p* = 0.376	***r* = –0.33** ***p* = 0.014**	*r* = 0.10 *p* = 0.469
ΔT3-T1 LDL-cholesterol	*r* = 0.10 *p* = 0.462	*r* = –0.01 *p* = 0.931	*r* = 0.24 *p* = 0.080	*r* = –0.08 *p* = 0.561	*r* = –0.27 *p* = 0.077	*r* = –0.06 *p* = 0.664	*r* = –0.13 *p* = 0.327	*r* = –0.03 *p* = 0.842
ΔT3-T1 HDL-cholesterol	*r* = –0.01 *p* = 0.958	*r* = 0.13 *p* = 0.356	*r* = –0.22 *p* = 0.111	*r* = 0.09 *p* = 0.539	*r* = –0.11 *p* = 0.482	*r* = –0.12 *p* = 0.371	***r* = –0.30** ***p* = 0.024**	*r* = –0.04 *p* = 0.733
ΔT3-T1 fasting triglyceride	*r* = 0.01 *p* = 0.930	*r* = –0.04 *p* = 0.792	*r* = 0.08 *p* = 0.593	*r* = 0.02 *p* = 0.864	*r* = 0.14 *p* = 0.341	*r* = –0.02 *p* = 0.865	*r* = –0.06 *p* = 0.673	*r* = –0.08 *p* = 0.574

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
p-values were obtained after consideration for maternal age, maternal IMC at first trimester of pregnancy, total gestational weight gain, gestational age, newborn's gender, and blood lipid level at third (ΔT3-T1). Statistically significant results are shown in bold.

Student's *T*-test. Partial Pearson correlations were used to investigate the association between the main variables of interest: DNAm levels, ΔT3-T1 maternal blood lipid levels, newborns' anthropometric measurements, and cord blood lipid and leptin levels. The statistical models were controlled for the following confounding factors: maternal age, maternal BMI at first trimester of pregnancy, total gestational weight gain, maternal blood lipids level at second and third trimester of pregnancy, gestational age, and newborn gender (see Figure's and Table's footnotes for details). Impacts of other confounding factors (smoking, gravity, parity, mode of delivery, and time of visits) were tested but not retained in the statistical models as results remained unchanged. Association between DNAm levels and relative mRNA levels was assessed using a partial Pearson's correlation controlling for gestational age and newborn gender. Results were considered statistically significant when *p*-values were <0.05 (two-sided).

Stepwise multivariable linear regression was used to identify predictors of the placental *LDLR* and *LRP1* DNAm level variability. The input-independent variables were maternal age, maternal BMI at first trimester of pregnancy, maternal total weight gain, ΔT3-T1 maternal total cholesterol levels, maternal total cholesterol levels at third trimester of pregnancy, gestational age, and newborn gender. Variables with a *p*-value < 0.10 were retained in the regression model.

We estimated the direct association between maternal characteristics and newborn characteristics and the mediating effect of DNAm using a nonparametric bias-corrected bootstrapping procedure as previously described.[30] This allowed us to assess whether DNAm at *LDLR* and *LRP1* gene loci mediated the association between maternal total cholesterol level changes throughout pregnancy and cord blood lipid and leptin levels. Potential confounders were considered in the statistical models: maternal age, maternal BMI at first trimester of pregnancy, maternal total weight gain, maternal total cholesterol levels at third trimester of pregnancy, gestational age, and newborn gender. Results were considered significant if *p*-values for mediation models were <0.05. Statistical analyses were performed with the IBM SPSS Statistics 24 software, USA (release 24.0.0).

Maternal lipid levels at third trimester of pregnancy were included as confounding variables in all of our statistical models in order to evaluate the independent contribution of the ΔT3-T1 maternal lipid levels to DNAm level variability and newborn characteristics. In addition, maternal blood lipid levels at third trimester are more likely representative of the lipid environment to which the placenta and newborn were exposed at birth, when the samples were collected.

Results

The characteristics of the mothers and their newborns are shown in Tables 1 and 2, respectively. At first trimester of pregnancy, women were on average 28.0 ± 3.8 years old, had a mean body mass index (BMI) of 23.4 ± 3.2 kg/m^2, fasting glucose of 4.38 ± 0.34 mmol/l, and lipid levels within the normal ranges (based on reference values in nonpregnant women) (Table 1). Maternal blood lipids tended to increase between first, second, and third trimester of pregnancy (Table 1). Babies were 47.8% male ($n = 33$), had a mean gestational age of 39.4 ± 1.1 weeks, and had a mean ponderal index of 27.6 ± 2.6 kg/m^3 (Table 2). Mean cord blood total cholesterol and leptin levels were 1.70 ± 0.43 mmol/l and 10.8 ± 8.5 ng/ml, respectively (Table 2).

We first assessed whether changes in maternal blood lipids in pregnancy were associated with newborn's physical and metabolic characteristics (Table 3). We found that higher maternal total cholesterol changes throughout pregnancy (ΔT3-T1) were correlated with higher cord blood total and low-density lipoprotein cholesterol (LDL-C) as well as lower newborns' ponderal index and cord blood leptin levels.

To determine whether the placental epigenetic signature is associated with maternal lipid profile and correlated to newborn anthropometric and metabolic profiles, we assessed DNAm levels at 43 CpG sites within *LDLR*, *LRP1*, and *SCARB1* gene loci. Only the CpG sites with DNAm levels between 10% and 90% and showing interindividual variability [standard deviation > 3%] were considered for further analysis. Among the remaining CpGs, only those correlated with their mRNA levels ($r > 0.25$ and $p < 0.1$; mean *LDLR*-CpGA, *LRP1*-CpGA2, *LRP1*-CpGC1, and *LRP1*-CpGD1; Figs. 1B, 2D, and 3C, Supplementary Figs. 1 and 2, and Supplementary Table 2) were retained for further analyses. Associations were then tested between changes in maternal blood lipids and placental DNAm levels at these four CpGs. We found that increases in total cholesterol and LDL-C levels throughout pregnancy (ΔT3-T1) were associated with lower DNAm at *LDLR*-CpGA gene locus (Fig. 1A and Supplementary Table 3). At *LRP1*-CpGA2 gene locus, increases in total cholesterol, LDL-C and HDL-C levels throughout pregnancy (ΔT3-T1) were associated with higher DNAm levels (Fig. 2A and Supplementary Table 3). All the previous associations were independent of maternal blood lipids

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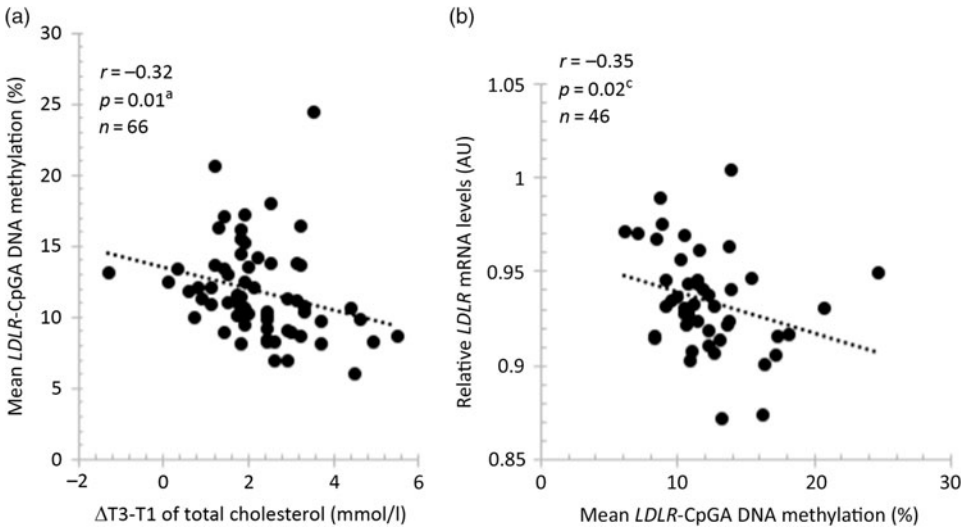

Fig. 1. Correlation between mean LDLR-CpGA DNAm levels, maternal total cholesterol level variation between third and first trimester of pregnancy (Panel A), and relative LDLR mRNA levels in placenta (Panel B). – (a) Adjusted for maternal age, maternal IMC at first trimester of pregnancy, total gestational weight gain, gestational age, and newborn's gender. (b) Adjusted for gestational age and newborn's gender.

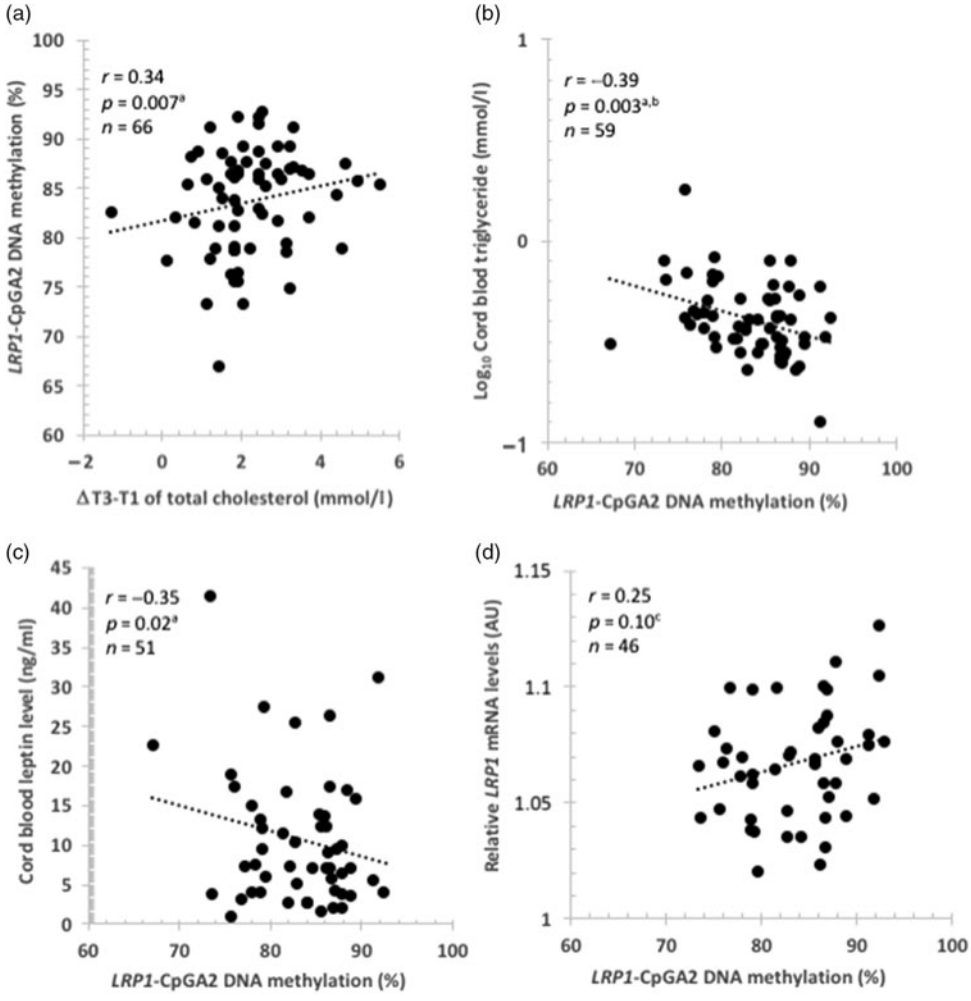

Fig. 2. Correlation between LRP1-CpGA2 DNAm levels, maternal and fetal characteristics: (A) maternal total cholesterol level variation between third and first trimester of pregnancy; (B) cord blood triglyceride levels; (C) cord blood leptin levels; and (D) relative LRP1 mRNA levels. – (a) Adjusted for maternal age, maternal IMC at first trimester of pregnancy, total gestational weight gain, gestational age, and newborn's gender. (b) Correlation coefficients (r) and p-values were computed after triglyceride levels were $\log_{10}$-transformed. (c) Adjusted for gestational age and newborn's gender.

levels at second and third trimester of pregnancy (Supplementary Table 4) and were adjusted for maternal age and BMI at first trimester, weight gain between first and third trimester of pregnancy, gestational age, and newborn gender.

We then applied stepwise multivariable linear regression analyses to assess which of the identified factors were independent predictors for DNAm levels. Predictors of placental *LDLR*-CpGA DNAm levels were changes in total cholesterol levels throughout pregnancy (ΔT3-T1), sex of the newborn, and total gestational weight gain, whereas changes in total cholesterol levels throughout pregnancy (ΔT3-T1), maternal age, and BMI at the first trimester of pregnancy predicted *LRP1*-CpGA2 DNAm levels in the placenta.

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Table 4. Multivariable linear regression analyses of *LDLR* and *LRP1* DNA methylation levels predictors in placenta

Predictors	Mean LDLR-CpGA	LRP1-CpGA2	LRP1-CpGC1	LRP1-CpGD1
ΔT3-T1 total cholesterol (mmol/l)	$\beta = -0.32$ $p = 0.007$	$\beta = 0.61$ $p = 0.004$	NS	NS
Third trimester total cholesterol (mmol/l)	NS	$\beta = -0.44$ $p = 0.030$	NS	NS
Maternal age (years)	NS	$\beta = -0.23$ $p = 0.060$	NS	NS
Gestational age (weeks)	NS	NS	NS	NS
Newborn's gender	$\beta = -0.32$ $p = 0.006$	NS	NS	NS
First trimester maternal BMI (kg/m^2)	–	$\beta = 0.26$ $p = 0.04$	–	–
Total gestational weight gain (kg)	$\beta = -0.25$ $p = 0.034$	–	NS	NS
$R^2(\%)_{ajd}$	19.1 $p = 0.001$	11.9 $p = 0.019$	–	–

BMI, body mass index; *LDLR*, Low-density lipoprotein receptor; *LRP1*, LDL receptor-related protein 1; NS, not statistically significant.
All β are standardized coefficient values.
Variables with a p-value < 0.10 were retained in the regression model.

Overall, these models explained 19.1% and 11.9% of the mean *LDLR*-CpGA and *LRP1*-CpGA2 DNAm variations, respectively (Table 4). No maternal characteristic was found to be associated with DNAm levels at *LRP1*-CpGC1 or *LRP1*-CpGD1.

Next, we assessed whether DNAm at *LDLR* and *LRP1* gene loci was associated with newborns' anthropometric characteristics and cord blood leptin and lipid profile (Supplementary Table 5). We observed that higher *LRP1*-CpGA2 DNAm levels were associated with lower cord blood triglycerides (TGs) and leptin levels (Fig. 2B, 2C). Moreover, *LRP1*-CpGC1 DNAm levels were positively correlated with cord blood total cholesterol and LDL-C levels (Fig. 3A, 3B).

Mediation analyses were finally applied to assess whether DNAm levels at *LDLR* and *LRP1* gene loci might be part of the mechanisms linking maternal and newborns' phenotypes. We found that *LRP1*-CpGA2 DNAm levels significantly mediate the association between changes in maternal total cholesterol levels during pregnancy (ΔT3-T1) and cord blood leptin levels (Fig. 4; $p_{mediation} = 0.02$). We did not detect significant mediation of DNAm at *LDLR* gene loci.

Discussion

Blood lipid levels (TG, LDL-C, and HDL-C) during pregnancy were previously associated with fetal growth and development indices suggesting that maternal dyslipidemia may be involved in fetal metabolic programming.[8,34–36] Nevertheless, these associations seemed to be mediated by several other maternal factors including the lipoprotein subtype, maternal BMI (pre- and post-conception), and/or diabetes,[8] which have been supported by a recent Mendelian randomization analysis.[37] In this study, we have decided to focus on maternal blood lipid levels changes throughout pregnancy, which have been overlooked so far. We report that rise in maternal blood lipids levels in pregnancy, in normoglycemic pregnant women, is associated with lower ponderal index and cord blood leptin levels. These associations were not observed with maternal blood lipid levels at first, second, and third trimester of pregnancy, suggesting that changes throughout the pregnancy might be more important than the absolute lipid values at the beginning, middle, or end of pregnancy.

Previous studies suggested that *in utero* environment perturbations such as maternal dyslipidemia or hyperglycemia contribute to the development of adult chronic diseases possibly through epigenetic adaptations in the placenta and offspring.[38,39] We report that rise in maternal blood lipids levels in pregnancy is associated with DNAm levels variability at *LDLR* and *LRP1* gene loci in placenta (Fig. 5). These results suggest that *LDLR* and *LRP1* epigenetic profile are sensitive to the *in utero* environment and that DNAm at these loci could explain, to some extent, the link between suboptimal maternal condition and the metabolic health programming in the newborn. In this study, we showed that DNAm variability at *LDLR* gene locus was associated with functional impact on gene expression. Although the association between *LRP1* DNAm and mRNA levels did not reach statistical significance ($p < 0.10$ but >0.05), the correlation coefficients obtained were modestly strong ($r > 0.25$) as compared to previous studies showing functional impact of DNAm on gene expression in placenta.[20,29] A significant statistical association ($p < 0,05$) between *LRP1* DNAm and mRNA levels could potentially have been reached with larger sample size. Moreover, the gene expression and DNAm studies were done on placental sample collected at delivery. Gene expression in placenta was previously showed to be highly variable according to trimesters.[40] Henceforth, the associations observed between DNAm and mRNA levels at the end of the pregnancy might be even stronger during the first or second trimester. This mechanism may indeed be important for regulation of the lipoprotein metabolism in maternal–placental fetal unit throughout the pregnancy. The functional impact of *LRP1* DNAm throughout pregnancy will need to be confirmed in larger cohort.

Using mediation analyses, we then explored the causality chain between maternal blood lipid changes, placental DNAm variations, and newborns' anthropometric and metabolic profiles (Figs. 4 and 5). We report that the associations between maternal total cholesterol changes in pregnancy and cord blood leptin levels are mediated, at least partially, by placental epigenetic adaptations at the *LRP1* gene locus. This suggests that placental DNAm responds to maternal lipid changes in pregnancy, which in turn impacts fetal growth. As we are studying complex traits, there are probably other factors contributing to interindividual variability in cord blood leptin levels, such as genetic, diet, or other environmental risk factors.[41] However, the fact that only one CpG site can explain that much of this variability is highly interesting. Moreover, even if the observed DNAm variations in our study are relatively modest, we and other groups already shown these can affect gene expression and hence could obviously have physiological consequences over a long period of time.[20,42]

Interestingly, lower placental *LRP1* mRNA levels have already been reported in pregnancies complicated by preeclampsia and characterized by a suboptimal fetal development.[10,11,43] In addition, hypercholesterolemia in pregnant rabbit has been associated with placental collagen deposition and atherosclerotic lesions that compromised placental nutrients transfer from the maternal

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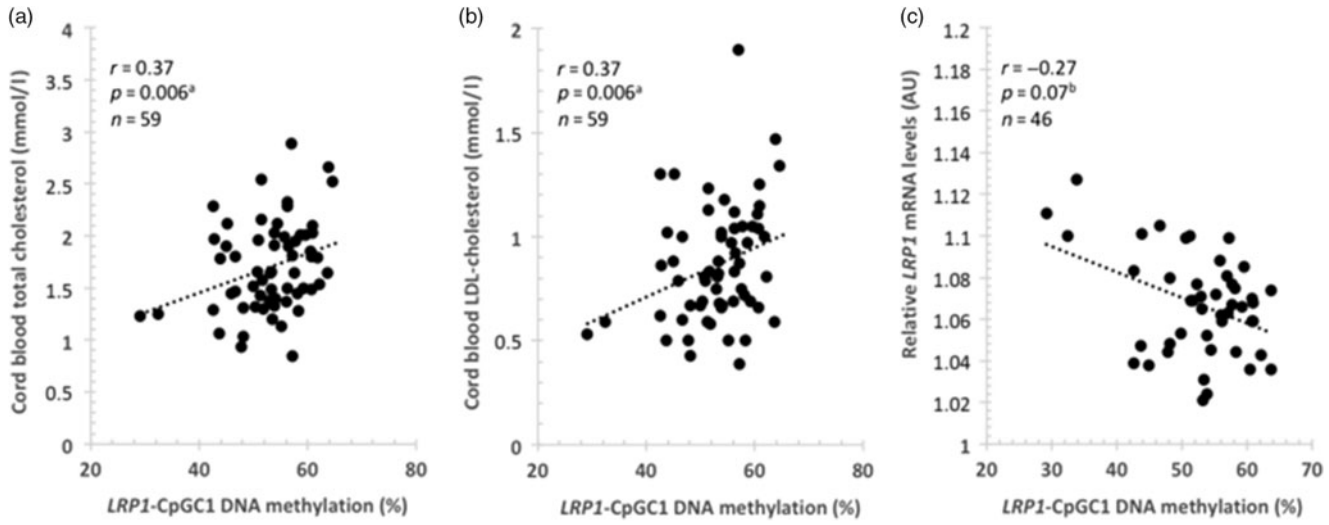

Fig. 3. Correlation between LRP1-CpGC1 DNAm levels and fetal characteristics: (A) cord blood total cholesterol levels; (B) cord blood LDL-cholesterol levels; (C) relative LRP1 mRNA levels. – (a) Adjusted for maternal age, maternal IMC at first trimester of pregnancy, total gestational weight gain, gestational age, and newborn's gender. (b) Adjusted for gestational age and newborn's gender.

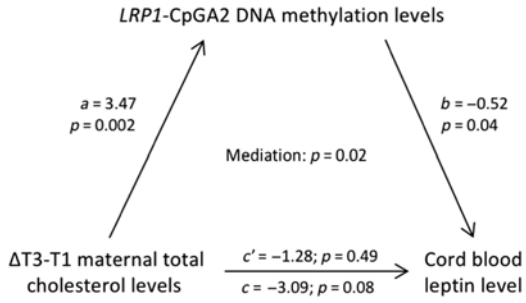

Fig. 4. Mediation analysis between maternal total cholesterol changes in pregnancy, LRP1 DNAm, and cord blood leptin levels. These results show that DNAm level variations at LRP1-CpGA2 induced by mediating the association between maternal total cholesterol changes in pregnancy and cord blood leptin levels in newborns, suggesting a causal relationship between these variables. Path a is the relationship between the ΔT3-T1 maternal total cholesterol levels and LRP1 DNAm levels. Path b is the relationship between LRP1 DNAm levels and cord blood leptin levels. Path c is the direct relationship between ΔT3-T1 maternal total cholesterol levels and cord blood leptin levels, while path c' is the indirect effect of this relationship when taking into account the LRP1 DNAm profile.

circulation to the fetus.[44] *In utero* exposure to increased maternal cholesterol levels may thus impair placental function and restrict materno-fetal nutrients transport leading to developmental restriction, possibly manifested as lower cord blood leptin levels in our study. Henceforth, we hypothesized that epigenetic modifications at *LRP1* locus in response to risen maternal blood lipids are an attempt to improve materno-placental lipids transport in functionally impaired placenta. Nevertheless, this may also have triggered a compensatory response in the newborns (increased cord blood lipids), which might increase their long-term susceptibility to CVD and adult metabolic diseases.

LRP1 encodes for a large cell surface receptor ubiquitously expressed in a variety of organs including, placenta, adipose tissue, liver, and brain.[45] It is also highly expressed in neurons: a lack of *LRP1* expression caused motor and behavioral impairment (i.e., hyperactivity, tremor, dystonia) on transgenic mice, suggesting altered neurotransmission.[46] Interestingly, *LRP1* expression in the brain could also affect body weight and adiposity through the regulation of food intake and energy expenditure: *LRP1*-KO mice showed significantly accelerated body weight gain, insulin resistance, hyperlipidemia, and an increase in plasma leptin concentration suggesting the development of leptin resistance.[47] Similarly, we report that decreased *LRP1* DNAm in placenta and corresponding mRNA levels are associated with higher cord blood leptin concentration. However, some mechanisms behind *LRP1* function are still unclear, with at least one other study reporting that the inactivation of *LRP1* in white and brown adipose tissues decreased fat storage, and had protective effects against diet-induced obesity.[48] *LRP1* inactivation also protected the mice from glucose intolerance and diabetes potentially because of their increased metabolic rate and energy expenditure, which supports that LRP1 is probably an important regulator of adipocyte energy homeostasis.[48] Interestingly, our study suggested that *LRP1* epigenetic profile in placenta is sensitive to the maternal blood lipid levels variability during the pregnancy. Previous studies showed that DNAm variability observed in a specific tissue might also be reflected in other tissues and have tissue-specific impact on gene expression regulation.[32] Moreover, recent researches suggest that newborns' methylome in different tissues, not only in placenta, might also be sensitive to suboptimal maternal condition.[49,50] Therefore, it possible that *LRP1* DNAm profile in newborns' brain or adipose tissue cells might have been affected by maternal blood lipid variability during pregnancy and could explain, to some extent, the link between suboptimal maternal condition, cord blood leptin levels, and newborns' anthropometric measurements. Nevertheless, this remains a hypothesis that will need to be investigated.

The longitudinal follow-up of our women from the first trimester of pregnancy to delivery is one of the strengths of this study. It has allowed us to accurately assess changes in maternal fasting blood lipids throughout pregnancy and its impact on the newborn methylome, anthropometric characteristics, and cord blood lipid and leptin levels. However, we cannot exclude that DNAm levels at *LDLR* and *LRP1* loci may have caused fluctuations in maternal lipemia during pregnancy because placenta samples were only analyzed at birth. The relatively small sample size ($n = 69$) and the common ethnicity of the population studied are also limiting our study, as our results may not be representative of a more diversified population. Moreover, the risk of false positive due to multiple testing cannot be excluded, as we tested a number of 4 CpG loci. Further studies are thus needed to confirm our result and to assess

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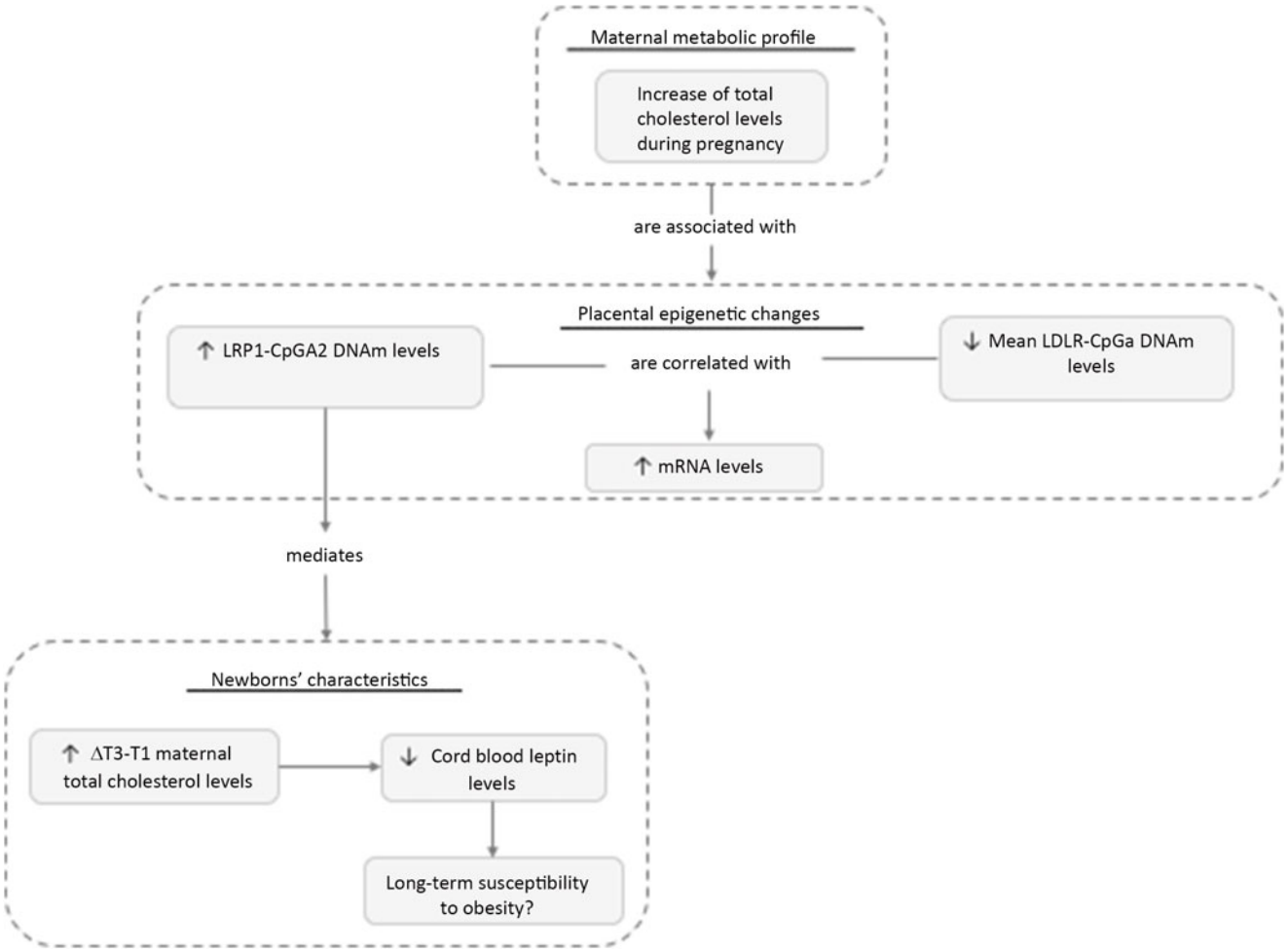

Fig. 5. Maternal metabolic variables associated with placental epigenetics changes and newborns' characteristics. Maternal blood lipid level variations throughout pregnancy were associated with placental DNAm levels changes at LDLR and LRP1 gene loci. Our results suggest that DNAm at LRP1 gene locus is causally associated with cord blood leptin levels, a biomarker of adiposity.

the functional impacts of the maternal lipid level variation on placental methylome, transcriptome, and newborns' characteristics.

Conclusion

Our study showed that maternal blood lipids levels variability in pregnancy, in normoglycemic pregnant women, is associated with placental DNAm levels changes and newborn's anthropometric measurements and blood marker of adiposity, such as cord blood leptin levels. Our findings support that placental DNAm at *LRP1* gene loci mediates the relationship between maternal blood lipid changes during pregnancy and cord blood leptin levels. This is of great interest, as we know that lipid transfer from the mother through the placenta is an important factor for adequate fetal growth and development. Moreover, these findings support that epigenetic is a crucial element in human development, although more studies are needed to fully elucidate the long-term impacts of fetal epigenetic programming. As obesity and diabetes are of great health concern these days, a better understanding of how maternal metabolic factors can influence children metabolism could be helping in the worldwide effort to prevent such epidemics.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S204017441900076X

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Authors' Contributions. AAH and SPG contributed to the study design, participated to data collection analysis/interpretation, and wrote the manuscript. EB critically reviewed the literature and wrote the manuscript. MFH, JPB, and PP participated in study design conception and revised the manuscript. DG contributed to the study design and revised the manuscript. DB participated to data collection and analysis/interpretation and revised the manuscript. LB conceived the study design, participated to data collection and analysis/interpretation, and revised the manuscript.

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Conflicts of Interest. None.

Ethical Standards. A written informed consent was obtained for all women before their inclusion in the study, and all clinical data were de-identified. This project received the approval by the Chicoutimi Hospital Ethics Committee, in accordance with the Declaration of Helsinki.

Availability of Data and Material. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Potter JM, Nestel PJ. The hyperlipidemia of pregnancy in normal and complicated pregnancies. *Am J Obstet Gynecol.* 1979; 133(2), 165–170.
2. Desoye G, Gauster M, Wadsack C. Placental transport in pregnancy pathologies. *Am J Clin Nutr.* 2011; 94(6 Suppl), 1896S–902S.
3. Bansal N, Cruickshank JK, McElduff P, Durrington PN. Cord blood lipoproteins and prenatal influences. *Curr Opin Lipidol.* 2005; 16(4), 400–408.
4. Misra VK, Trudeau S. The influence of overweight and obesity on longitudinal trends in maternal serum leptin levels during pregnancy. *Obesity (Silver Spring).* 2011; 19(2), 416–421.
5. Palinski W, Yamashita T, Freigang S, Napoli C. Developmental programming: maternal hypercholesterolemia and immunity influence susceptibility to atherosclerosis. *Nutr Reviews.* 2007; 65(12 Pt 2), S182–S187.
6. Edison RJ, Berg K, Remaley A, *et al.* Adverse birth outcome among mothers with low serum cholesterol. *Pediatrics.* 2007; 120(4), 723–733.
7. Leiva A, Fuenzalida B, Westermeier F, *et al.* Role for tetrahydrobiopterin in the fetoplacental endothelial dysfunction in maternal supraphysiological hypercholesterolemia. *Oxid Med Cell Longev.* 2015; 2015, 5346327.
8. Mudd LM, Holzman CB, Evans RW. Maternal mid-pregnancy lipids and birthweight. *Acta Obstet Gynecol Scand.* 2015; 94(8), 852–860.
9. Napoli C, D'Armiento FP, Mancini FP, *et al.* Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest.* 1997; 100(11), 2680–2690.
10. Hentschke MR, Poli-de-Figueiredo CE, da Costa BE, Kurlak LO, Williams PJ, Mistry HD. Is the atherosclerotic phenotype of preeclamptic placentas due to altered lipoprotein concentrations and placental lipoprotein receptors? Role of a small-for-gestational-age phenotype. *J Lipid Res.* 2013; 54(10), 2658–2664.
11. Wadsack C, Tabano S, Maier A, *et al.* Intrauterine growth restriction is associated with alterations in placental lipoprotein receptors and maternal lipoprotein composition. *Am J Physiol Endocrinol Metab.* 2007; 292(2), E476–E484.
12. Ethier-Chiasson M, Duchesne A, Forest JC, *et al.* Influence of maternal lipid profile on placental protein expression of LDLr and SR-BI. *Biochem Biophys Res Commun.* 2007; 359(1), 8–14.
13. Stefulj J, Panzenboeck U, Becker T, *et al.* Human endothelial cells of the placental barrier efficiently deliver cholesterol to the fetal circulation via ABCA1 and ABCG1. *Circ Res.* 2009; 104(5), 600–608.
14. Lillycrop KA, Burdge GC. Epigenetic changes in early life and future risk of obesity. *Int J Obes (Lond).* 2011; 35(1), 72–83.
15. Henikoff S, Matzke MA. Exploring and explaining epigenetic effects. *Trends Genet.* 1997; 13(8), 293–295.
16. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002; 16(1), 6–21.
17. Heijmans BT, Tobi EW, Stein AD, *et al.* Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA.* 2008; 105(44), 17046–17049.
18. Tobi EW, Lumey LH, Talens RP, *et al.* DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Human Mol Genet.* 2009; 18(21), 4046–4053.
19. Finer S, Mathews C, Lowe R, *et al.* Maternal gestational diabetes is associated with genome-wide DNA methylation variation in placenta and cord blood of exposed offspring. *Human Mol Genet.* 2015; 24(11), 3021–3029.
20. Houde AA, Guay SP, Desgagne V, *et al.* Adaptations of placental and cord blood ABCA1 DNA methylation profile to maternal metabolic status. *Epigenetics.* 2013; 8(12), 1289–1302.
21. Houde AA, Ruchat SM, Allard C, *et al.* LRP1B, BRD2 and CACNA1D: new candidate genes in fetal metabolic programming of newborns exposed to maternal hyperglycemia. *Epigenomics.* 2015; 7(7), 1111–1122.
22. Ruchat SM, Houde AA, Voisin G, *et al.* Gestational diabetes mellitus epigenetically affects genes predominantly involved in metabolic diseases. *Epigenetics.* 2013; 8(9), 935–943.
23. Houde AA, St-Pierre J, Hivert MF, *et al.* Placental lipoprotein lipase DNA methylation levels are associated with gestational diabetes mellitus and maternal and cord blood lipid profiles. *J Dev Origins Health Dis.* 2014; 5(2), 132–141.
24. Ahima RS. Digging deeper into obesity. *J Clin Invest.* 2011; 121(6), 2076–2079.
25. Norgan NG. A review of: "Anthropometric Standardization Reference Manual". Edited by T. G. LOHMAN, A. F. ROCHE and R. MARTORELL. (Champaign, IL.: Human Kinetics Books, 1988.) [Pp. vi+ 177.] £28·00. ISBN 087322 121 4. *Ergonomics.* 1988; 31(10), 1493–1494.
26. Lohman TG, Roche AF, Martorell R. *Anthropometric Standardization Reference Manual*, 1988. Champaign, IL: Human Kinetics Books.
27. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; 18(6), 499–502.
28. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* 2013; 13, 59.
29. Bouchard L, Thibault S, Guay SP, *et al.* Leptin gene epigenetic adaptation to impaired glucose metabolism during pregnancy. *Diabetes Care.* 2010; 33(11), 2436–2441.
30. Cote S, Gagne-Ouellet V, Guay SP, *et al.* PPARGC1alpha gene DNA methylation variations in human placenta mediate the link between maternal hyperglycemia and leptin levels in newborns. *Clin Epigenet.* 2016; 8, 72.
31. Marsh S. Pyrosequencing applications. *Methods Mol Biol.* 2007; 373, 15–24.
32. Guay SP, Brisson D, Lamarche B, *et al.* DNA methylation variations at CETP and LPL gene promoter loci: new molecular biomarkers associated with blood lipid profile variability. *Atherosclerosis.* 2013; 228(2), 413–420.
33. Guay SP, Brisson D, Lamarche B, Gaudet D, Bouchard L. Epipolymorphisms within lipoprotein genes contribute independently to plasma lipid levels in familial hypercholesterolemia. *Epigenetics.* 2014; 9(5), 718–729.
34. Chatzi L, Plana E, Daraki V, *et al.* Metabolic syndrome in early pregnancy and risk of preterm birth. *American J Epidemiol.* 2009; 170(7), 829–836.
35. Clausen T, Burski TK, Oyen N, Godang K, Bollerslev J, Henriksen T. Maternal anthropometric and metabolic factors in the first half of pregnancy and risk of neonatal macrosomia in term pregnancies. A prospective study. *Eur J Endocrinol.* 2005; 153(6), 887–894.
36. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Divergent metabolic and vascular phenotypes in pre-eclampsia and intrauterine growth restriction: relevance of adiposity. *J Hypertens.* 2004; 22(11). 2177–2183.
37. Tyrrell J, Richmond RC, Palmer TM, *et al.* Genetic evidence for causal relationships between maternal obesity-related traits and birth weight. *JAMA.* 2016; 315(11), 1129–1140.
38. Clausen TD, Mathiesen ER, Hansen T, *et al.* Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *J Clin Endocrinol Metab.* 2009; 94(7), 2464–2470.
39. Van der Graaf A, Vissers MN, Gaudet D, *et al.* Dyslipidemia of mothers with familial hypercholesterolemia deteriorates lipids in adults offspring. *Arteriosclerosis Thromb Vasc Biol.* 2010; 30(12), 2673–2677.
40. Sitras V, Fenton C, Paulssen R, Vartun A, Acharya G. Differences in gene expression between first and third trimester human placenta: a microarray study. *PLoS One.* 2012; 7(3), e33294.
41. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nat Rev Genet.* 2011; 12(8), 529–541.
42. Toperoff G, Aran D, Kark JD, *et al.* Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. *Human Mol Genet.* 2012; 21(2), 371–383.

https://doi.org/10.1017/S204017441900076X Published online by Cambridge University Press

43. 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; 106(11), 1221–1222.
44. Frantz E, Menezes HS, Lange KC, *et al.* The effect of maternal hypercholesterolemia on the placenta and fetal arteries in rabbits. *Acta Cir Bras.* 2012; 27(1), 7–12.
45. Hussain MM, Strickland DK, Bakillah A. The mammalian low-density lipoprotein receptor family. *Annu Rev Nutr.* 1999; 19, 141–172.
46. May P, Rohlmann A, Bock HH, *et al.* Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice. *Mol Cell Biol.* 2004; 24(20), 8872–8883.
47. Liu Q, Zhang J, Zerbinatti C, *et al.* Lipoprotein receptor LRP1 regulates leptin signaling and energy homeostasis in the adult central nervous system. *PLoS Biol.* 2011; 9(1), e1000575.
48. Hofmann SM, Zhou L, Perez-Tilve D, *et al.* Adipocyte LDL receptor-related protein-1 expression modulates postprandial lipid transport and glucose homeostasis in mice. *J Clin Invest.* 2007; 117(11), 3271–3282.
49. Cardenas A, Koestler DC, Houseman EA, *et al.* Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. *Epigenetics.* 2015; 10(6), 508–515.
50. Banik A, Kandilya D, Ramya S, Stunkel W, Chong YS, Dheen ST. Maternal factors that induced epigenetic changes contribute to neurological disorders in offspring. *Genes.* 2017; 8(6), E150.

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