Cord blood Advanced Lipoprotein Testing shows an interaction between gestational diabetes and birth-weight: an observational study

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Abstract

Background
Abnormal lipid metabolism is observed in gestational diabetes mellitus (GDM) and in neonates with abnormal fetal growth, however, how these alterations specifically affect the umbilical cord blood lipoprotein profile is not well understood.

Objective
To assess the impact of GDM on the cord blood lipoprotein profile across birth-weight categories by using Advanced Lipoprotein Testing.

Methods
observational study involving 74 control and 62 GDM pregnant women and their offspring. Newborns were classified according to birth-weight as small (n = 39), adequate (n = 50) or large (n = 49) for gestational age (SGA, AGA and LGA, respectively). Two-dimensional diffusion-ordered 

Results
Baseline characteristics of the two groups were similar except for gestational weight gain. The size, lipid content, number and concentration of particles within their subclasses were similar between offspring born to GDM and control mothers. Using two-way analysis of variance, we observed an interaction between GDM and birth-weight categories for IDL-cholesterol content and IDL- and LDL-triglyceride content, and the number of medium VLDL and LDL particles, specifically in AGA neonates. Small LDL particles were independently associated with offspring obesity at two years.

Conclusions
In this selected cohort, GDM disturbs triglyceride and cholesterol lipoprotein content across birth-weight categories, and AGA neonates born to GDM mothers display a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to mothers with normal glucose tolerance.

Background
Fetal growth and development is a particularly vulnerable period in life greatly affected by maternal environment. Prenatal exposure to nutritional stressors has been associated with fetal programming, which can impact both metabolism and physiology and, consequently, predispose to later development of cardiovascular and metabolic diseases, including obesity.\(^{(1)}\) In this context, it has been proposed that cardiovascular disease can begin early in life\(^{(2)}\) and that atherosclerosis may originate during the fetal period.\(^{(3)}\)

Birth-weight is strongly determined by neonatal fat mass and gestational age, and fetal growth disorders can result from impaired maternal and fetal lipid metabolism. In fact, the levels and composition of cord blood lipids, apolipoproteins and lipoproteins are affected by both maternal and fetal factors.\(^{(4–6)}\) Disturbed lipid profiles at birth have been described in small and large for gestational age (SGA and LGA, respectively) neonates.\(^{(6–9)}\) When compared with adequate for gestational age (AGA) peers, SGA neonates show higher levels of triglycerides, triglyceride-enriched very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and lower levels of total cholesterol.\(^{(6, 9, 10)}\) By contrast, LGA neonates display higher LDL, HDL and total cholesterol levels than AGA neonates.\(^{(8)}\)

Diabetic pregnancies are associated with a higher incidence of fetal growth disorders and there is evidence that disturbances in maternal metabolism strongly contribute to these situations.\(^{(11)}\) Changes in cord blood lipoprotein concentrations have been reported in mothers with type 1 diabetes mellitus, including an increased cholesterol content of LDL and a decrease in HDL.\(^{(12, 13)}\) The situation appears more complex in gestational diabetes mellitus (GDM), with some studies showing no differences with normal glucose tolerant mothers and others showing lower HDL- and higher VLDL- and LDL-cholesterol concentrations.\(^{(14)}\) Additionally, qualitative changes in HDL remodeling resulting in an altered functionality have been reported in GDM neonates,\(^{(15)}\) but it does not seem to affect newborn cholesterol metabolism in both obese and well-controlled GDM mothers.\(^{(16)}\) \(^{1}\)H-nuclear magnetic resonance (\(^{1}\)H-NMR)-based analysis of lipoproteins has established that the number of LDL and HDL particles is a more powerful index of cardiovascular risk than classical cholesterol determinations, given the large variability in the amount of cholesterol per particle and in particle size.\(^{(17)}\) \(^{1}\)H-NMR-based tests have also demonstrated the incomplete conversion of VLDL into LDL in diabetes, which results in a higher prevalence of VLDL and small and dense LDL particles.\(^{(17)}\)

Given the heterogeneity of growth patterns and the inconclusive findings in the cord blood lipoprotein profile of infants from GDM mothers, a comprehensive characterization of the main lipoproteins, including the assessment of the size and number of particles, is necessary to identify possible alterations in fetal lipoprotein metabolism and their potential consequences for fetal health. In the present study, we used the Liposcale test, a novel advanced lipoprotein assessment method based on 2D diffusion-ordered \(^{1}\)H-NMR,\(^{(18)}\) in order to examine for correlations between fetal growth disorders and differences in umbilical cord blood lipoprotein profile. Additionally, we want to explore the potential association with offspring outcomes, including obesity, at two years of age.
Methods

Study subjects

All the women-child pairs included in this study belonged to a pre-birth cohort and gave birth at the Department of Obstetrics of the Hospital Universitari de Tarragona Joan XXIII between June 2010 and May 2017. GDM and control women were recruited at their first prenatal visit at the Obstetrics Department and were followed until delivery. Maternal and umbilical cord blood samples were stored in a biobank collection with the associated clinical data. In this study, the participants were selected according to the birth-weight category, and we included a similar proportion of infants born to mothers with GDM and control women who were appropriate, small or large for gestational age. They also had to fulfill the following criteria at the end of pregnancy: (1) singleton pregnancy, (2) accurate gestational age confirmed by an ultrasound examination before 20 weeks of gestation, (3) absence of fetal anomalies, (4) cord blood serum availability and (5) maternal lipid information from the third trimester exam. Neonates with major congenital anomalies, intrauterine infections or born from women with chronic and inflammatory diseases were excluded. One hundred and thirty-six neonates and mother pairs fulfilled these criteria (74 control participants and 62 participants with GDM) and were included in this study, which was performed in accordance with the tenets of the Declaration of Helsinki and whose protocol was reviewed and approved by the Hospital Universitari de Tarragona Research Ethics Board (ref: 243/2016). All participants provided informed consent before inclusion.

All mothers were screened for GDM between 24–28 weeks of pregnancy following the Spanish Diabetes and Pregnancy Group recommendations. Subjects with a 1-hour 50-g glucose challenge test $\geq$ 140 mg/dL underwent a 3-hour 100-g oral glucose tolerance test. Subjects with 2 or more values above the threshold proposed by the National Diabetes Data Group were considered to have GDM, whereas those with all values below the threshold were classified as controls.

Care for GDM was managed according to the Spanish guidelines for diagnosis and therapy of GDM. GDM women were given an individualized diet with at least 40% of carbohydrates and they were instructed to self-monitor blood glucose 6 times a day (fasting and 1-hour postprandial). Insulin therapy was recommended when fasting glucose and/or 1-hour post-prandial values were repeatedly over 95 mg/dl (mmol/L) or over 140 mg/dl (mmol/L), respectively. According to these criteria, thirty-one women were treated only with diet, and 31 women required also insulin.

Clinical and demographic data

Demographic and obstetric information on participants was collected via an interviewer-administered questionnaire, which paid particular attention to GDM risk factors. Maternal anthropometry included height, pre-pregnancy weight and weight at the end of pregnancy. Pre-pregnancy weight was self-reported and we check its concordance with the weight recorded in the first prenatal visit (before the 10th week of pregnancy). Pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) were calculated
according to the formulas: Pre-pregnancy BMI = Pre-pregnancy Weight (kg)/Height (m)² and GWG = Final weight-Pre-pregnancy weight.

Infant data included sex, gestational age, way of delivery and anthropometry. Neonatal length and weight were measured after delivery using a measuring board to the nearest 0.1 cm and a calibrated scale to the nearest 10 g. Ponderal index (PI) was calculated by the formula: PI = Birth-weight (g)/length (cm)³. Suprailiac skinfold thickness was measured within the first 48 hours of life and was used to calculate the fat mass percentage. (21) Neonates were classified according to gestational age- and sex-specific growth charts of the World Health Organization (WHO). (22) Infants were considered LGA if the birth-weight was over the 90th percentile while SGA if the birth-weight was under the 10th percentile. The remainders were considered AGA. The distribution of neonates according to birth-weight category was: 25 AGA, 25 SGA and 24 LGA in the control group and 25 AGA, 14 SGA and 23 LGA in the GDM group.

**Infant growth and child BMI**

Height and weight information from birth at two years of age was collected for 103 children. We defined obesity as a BMI ≥ 85th percentile according to age- and sex-specific BMI tables of the WHO growth standards. (22)

**Umbilical cord blood collection**

Umbilical cord blood was obtained immediately after delivery. Serum was immediately separated by centrifugation, divided into aliquots and stored at -80°C until analysis.

**Laboratory analysis**

Maternal fasting serum samples were obtained between the 33rd and 36th weeks of pregnancy to determine glucose, triglycerides, total and HDL-cholesterol in an ADVIA 2400 (Siemens AG, Munich, Germany) autoanalyzer by standard enzymatic methods (23). LDL-cholesterol was calculated using the Friedewald formula. Plasma insulin was determined by immunoassay in an ADVIA Centaur System (Siemens AG. Munich, Germany). This assay shows a cross-reactivity of 0.1% to intact human proinsulin and the primary circulating split form des 31,32 proinsulin. Insulin resistance was estimated using homeostatic model assessment of insulin resistance (HOMA)-IR, as described. (24).

**¹H-NMR spectroscopy-based cord blood lipoprotein profiling**

Cord blood serum samples were analyzed using the 2D diffusion-ordered ¹H-NMR-based Liposcale test (Biosfer Testlab, Reus, Spain) (18). This technique has shown to be reliable with samples stored at -80°C for more than a decade (25). The test provides information about size, lipid concentration (cholesterol and triglycerides) number of particles, and concentration of particles within their subclasses (large, medium and small) for the main classes of lipoproteins: VLDL, LDL, intermediate-density lipoprotein (IDL) and HDL. ¹H-NMR spectra were recorded on a Bruker Avance III 600 spectrometer (Bruker BioSpin, Rheinstetten, Germany).
Statistical analysis

Statistical significance was set at p value < 0.05. Data were analyzed with SPSS software v20.0 (IBM, Armonk, NY) and presented as percentages for categorical variables, mean (SD) for normally-distributed continuous variables, and median (interquartile range) for non-normally distributed variables. Normality of the data was tested with the Kolmogorov-Smirnov test. Non-normally distributed quantitative variables were used after log10 transformation, when required. For comparisons of proportions, differences between groups were analyzed using the chi-square test, while for comparisons of normally- and non-normally-distributed quantitative variables an unpaired t-test or Mann-Whitney U test was applied. One-way analysis of variance (ANOVA) was used to test differences among three or more groups and the two-way ANOVA was used to examine potential interactions between GDM and birth-weight categories, and the Bonferroni procedure for post hoc analyses was performed for multiple comparisons. Spearman’s rank correlation coefficients were used for the analysis of the relationships between ¹H-NMR-assessed lipoprotein profile and maternal and offspring metabolic and clinical variables. To control the false discovery rate (FDR), the Benjamini-Hochberg (B-H) procedure was used and only those values significant with the B-H correction were considered(26). Logistic regression was applied to investigate the independence of the association between ¹H-NMR-assessed large LDL and small LDL particles, offspring obesity (percentile ≥ 85th = 1), and the normal weight (percentile < 85th = 0), adjusted for potential confounders (GDM, gestational age at delivery, birth-weight, sex, pre-gestational BMI and gestational weight gain)

Results

Clinical characteristics and cord blood ¹H-NMR-based lipoprotein profile of the studied population

Clinical and metabolic characteristics of the two groups are shown in Table 1. Clinical and laboratory parameters and the ¹H-NMR-lipoprotein profile (Table 2) were similar between GDM and control groups with the exception of GWG, which was significantly lower in the GDM group. As well, GWG (7.2 ± 4.4 vs 10.1 ± 5.1 kg; p = 0.023) and fasting glucose (78 ± 8 vs 89 ± 12 mg/dL mg/dL; p < 0.001) were lower in the GDM group of women treated only with diet compared to those who needed insulin therapy. In the ¹HNMR assessed lipoprotein profile, no difference was observed between the two groups, except for a lower concentration of medium HDL-P in GDM treated with insulin compared with those treated with diet (10.3 ± 1.2 vs 9.6 ± 1.4; p = 0.026).
|                                | Control (n = 74) | GDM (n = 62) | P-value |
|--------------------------------|-----------------|--------------|---------|
| Maternal age (years)           | 32.5 ± 5.4      | 33.5 ± 4.3   | 0.257   |
| Pre-gestational BMI (kg/m²)    | 25.5 ± 5.2      | 26.6 ± 5.1   | 0.207   |
| Gestational Weight Gain (kg)   | 12.3 ± 6.2      | 8.6 ± 4.9    | <0.001  |
| Smoking, n (%)                 | 14 (18.9)       | 8 (12.9)     | 0.363   |
| M cholesterol (mg/dL)*         | 246 ± 40        | 236 ± 44     | 0.165   |
| M HDL cholesterol (mg/dL)*     | 73 ± 13         | 72 ± 15      | 0.530   |
| M LDL cholesterol (mg/dL)*     | 121 ± 54        | 124 ± 36     | 0.775   |
| M triglycerides (mg/dL)*       | 205 ± 79        | 203 ± 81     | 0.898   |
| HOMA-IR*                       | 2.0 (1.2–3.3)   | 2.9 (1.66–4.22) | 0.052 |
| Gestational age (weeks)        | 39 (38–40)      | 39 (38–40)   | 0.749   |
| Birth weight (g)               | 3259 ± 603      | 3310 ± 697   | 0.645   |
| SGA/AGA/LGA (n)                | 25/25/24        | 14/25/23     | 0.353   |
| Fat mass (%)                   | 11.7 ± 4.3      | 11.9 ± 3.8   | 0.789   |
| Ponderal Index (g/cm³)         | 2.7 ± 0.3       | 2.7 ± 0.3    | 0.633   |
| Cb Insulin (mcU/mL)            | 4.5 (2.1–8.0)   | 6.3 (2.9–12.1) | 0.058 |

Data presented: mean ± SD and median (IQR, 25–75), for parametric and nonparametric variables, respectively. Differences between variables: t-test and the Mann-Whitney U test as required. GDM: gestational diabetes mellitus; M: maternal blood, Cb: cord blood. BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL: low density lipoproteins; HDL: high-density lipoproteins, SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age * Maternal blood was obtained between the 33th and 36th week. Only 70 control and 51 GDM women were available for the analysis.
### Table 2
Cord blood $^1$H-NMR-assessed lipoprotein profile according to gestational diabetes.

|                      | Control (n = 74) | GDM (n = 62) | P-value |
|----------------------|-----------------|--------------|---------|
| VLDL-cholesterol (mg/dL) | $7.2 \pm 3.3$  | $7.6 \pm 4.0$ | 0.495   |
| IDL-cholesterol (mg/dL)     | $4.6 \pm 1.6$  | $5.0 \pm 2.0$ | 0.291   |
| LDL-cholesterol (mg/dL)     | $70.1 \pm 10.1$ | $70.6 \pm 8.7$ | 0.767   |
| HDL-cholesterol (mg/dL)     | $40.9 \pm 8.8$  | $40.6 \pm 8.1$ | 0.827   |
| VLDL-triglycerides (mg/dL)  | $29.4 \pm 8.6$ | $30.7 \pm 9.9$ | 0.415   |
| IDL-triglycerides (mg/dL)   | $4.4 \pm 1.3$  | $4.7 \pm 1.8$  | 0.335   |
| LDL-triglycerides (mg/dL)   | $4.5 \pm 1.8$  | $4.4 \pm 1.9$  | 0.925   |
| HDL-triglycerides (mg/dL)   | $7.9 \pm 3.7$  | $8.6 \pm 3.9$  | 0.251   |
| VLDL-P (nmol/L)            | $23.1 \pm 6.3$ | $24.0 \pm 7.5$ | 0.416   |
| Large VLDL-P (nmol/L)      | 0.6 (0.5–0.8)  | 0.6 (0.5–0.8)  | 0.848   |
| Medium VLDL-P (nmol/L)     | 1.1 (0.6–2.0)  | 1.1 (0.7–2.4)  | 0.471   |
| Small VLDL-P (nmol/L)      | 21.0 ± 5.1     | 21.8 ± 6.3     | 0.408   |
| LDL-P (nmol/L)             | $484.9 \pm 71.7$ | $488.0 \pm 62.0$ | 0.79    |
| Large LDL-P (nmol/L)       | 92.5 ± 13.0    | 93.1 ± 11.0    | 0.782   |
| Medium LDL-P (nmol/L)      | 61.9 ± 39.3    | 62.1 ± 36.9    | 0.973   |
| Small LDL-P (nmol/L)       | 330.4 ± 50.1   | 332.7 ± 42.1   | 0.775   |
| HDL-P (nmol/L)             | $17.8 \pm 4.2$ | $17.4 \pm 4.1$ | 0.591   |
| Large HDL-P (nmol/L)       | $0.3 \pm 0.1$  | $0.3 \pm 0.1$  | 0.107   |
| Medium HDL-P (nmol/L)      | $9.7 \pm 1.5$  | $9.9 \pm 1.4$  | 0.257   |
| Small HDL-P (nmol/L)       | $7.8 \pm 3.8$  | $7.1 \pm 3.9$  | 0.306   |

Data presented: mean ± SD and median (IQR, 25–75), for parametric and nonparametric variables, respectively. Differences between variables: t-test and the Mann-Whitney U test as required. GDM: gestational diabetes mellitus; VLDL: very low-density lipoproteins; IDL: intermediate-density lipoproteins; LDL: low density lipoproteins; HDL: high-density lipoproteins; VLDL-P: VLDL number of particles; IDL-P: IDL number of particles; LDL-P: LDL number of particles; HDL-P: HDL number of particles.

One hundred and thirty-six neonates born to GDM (N = 62) and control (N = 74) mothers were categorized into three groups based on birth-weight categories according to age- and sex-weight specific charts (Table 3). As expected, there were significant differences in birth-weight, percentage of fat mass and PI.
across groups, increasing from the SGA to the LGA group. There were also differences between birth-weight groups for GWG and cord blood insulin. To note, in the GDM group the type of intervention (only diet or diet plus insulin) was distributed similarly in the three birth-weight groups (p = 0.321)

Table 3
Maternal and neonatal clinical characteristics according to birth-weight groups.

|                        | SGA (n = 39) | AGA (n = 50) | LGA (n = 49) | P-value |
|------------------------|-------------|-------------|-------------|---------|
| Maternal age (years)   | 31.6 ± 4.6  | 33.91 ± 5.0 | 32.8 ± 5.0  | 0.102   |
| Pre-gestational BMI (kg/m²) | 25.3 ± 4.2  | 25.9 ± 5.3  | 26.7 ± 5.7  | 0.445   |
| Gestational Weight Gain (kg) | 8.6 ± 5.0   | 10.0 ± 5.3  | 12.9 ± 6.3b | 0.002   |
| Smoking, n (%)         | 11 (28.2)   | 5 (10.6)    | 6 (12)      | 0.053   |
| M cholesterol (mg/dL)* | 247 ± 36    | 235 ± 46    | 244 ± 42    | 0.395   |
| M HDL cholesterol (mg/dL)* | 73 ± 10       | 76 ± 17     | 69 ± 12     | 0.053   |
| M LDL cholesterol (mg/dL)* | 130 ± 47     | 116 ± 35    | 124 ± 57    | 0.439   |
| M triglycerides (mg/dL)* | 191 ± 70     | 204 ± 79    | 209 ± 74    | 0.552   |
| HOMA-IR*               | 1.7 (1.2–3.2)| 2.2 (1.4–4.5)| 2.4 (1.6–3.4)| 0.272   |
| Gestational age (weeks)| 39 (39–40)   | 39 (38–40)  | 39 (38–40)  | 0.599   |
| Birth weight (g)       | 2598 ± 279a  | 3268 ± 270c | 3929 ± 251b | < 0.001 |
| Fat mass (%)           | 6.8 ± 3.6a   | 11.6 ± 2.4c | 12.0 ± 2.0  | < 0.001 |
| Ponderal Index (g/cm³) | 2.5 ± 0.2a   | 2.8 ± 0.3c  | 2.9 ± 0.2b  | < 0.001 |
| Cb insulin (mcUI/mL)   | 2.5 (1.1–4.3)| 4.3 (2.2–4.7)| 8.2 (6.2–14.1)b| < 0.001 |

Data presented: mean ± SD and median (IQR, 25–75), for parametric and nonparametric variables, respectively. Analysis of variance (ANOVA) and the Bonferroni procedure for post hoc analyses; M: maternal blood; Cb: cord blood. BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL: low density lipoproteins; HDL: high-density lipoproteins, SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age; a: P < 0.05 between SGA and AGA; b: P < 0.05 between SGA and LGA; c: P < 0.05 between AGA and LGA. * Maternal blood was obtained between the 33th and 36th week. Only 70 control and 51 gestational diabetes mellitus women were included in the analysis.

In the ¹HNMR assessed cord blood lipoprotein profile, cholesterol content in VLDL, LDL and HDL lipoproteins was different between the three birth-weight groups, with the LGA and AGA groups showing the highest cholesterol content in VLDL and HDL lipoproteins, respectively, and the SGA group showing the lowest cholesterol content in LDL and HDL lipoproteins. Regarding triglyceride content, SGA showed a
higher VLDL triglyceride content than AGA and LGA, and AGA showed higher HDL triglyceride content than SGA and LGA. No differences were observed in LDL lipoproteins (Table 4).
Table 4
Cord blood $^1$H-NMR-lipoprotein content and particle profile according to birth-weight categories.

|                         | SGA (n = 39) | AGA (n = 50) | LGA (n = 49) | P-value |
|-------------------------|--------------|--------------|--------------|---------|
| VLDL-cholesterol (mg/dL)| 8.5 ± 3.6 $^b$ | 8.1 ± 3.5 $^c$ | 5.8 ± 3.3 | 0.001   |
| IDL-cholesterol (mg/dL) | 5.1 ± 1.4 | 4.8 ± 2.0 | 4.5 ± 1.7 | 0.353   |
| LDL-cholesterol (mg/dL) | 66.3 ± 11.3 $^{a,b}$ | 72.1 ± 9.5 | 72.0 ± 7.1 | 0.005   |
| HDL-cholesterol (mg/dL) | 37.3 ± 6.6 $^a$ | 44.2 ± 8.7 $^c$ | 40.1 ± 8.5 | < 0.001 |
| VLDL-triglycerides (mg/dL) | 34.1 ± 10.1 $^{a,b}$ | 29.0 ± 8.2 | 27.5 ± 8.3 | 0.002   |
| IDL-triglycerides (mg/dL) | 4.7 ± 1.3 | 4.8 ± 1.7 | 4.1 ± 1.6 | 0.092   |
| LDL-triglycerides (mg/dL) | 4.5 ± 1.7 | 4.6 ± 2.1 | 4.3 ± 1.7 | 0.797   |
| HDL-triglycerides (mg/dL) | 7.4 ± 3.6 $^a$ | 10.1 ± 3.2 $^c$ | 7.0 ± 3.8 | < 0.001 |
| VLDL-P (nmol/L) | 26.1 ± 7.1 $^b$ | 23.6 ± 6.1 | 21.2 ± 6.6 | 0.003   |
| Large VLDL-P (nmol/L) | 0.8 ± 0.3 $^b$ | 0.7 ± 0.3 | 0.6 ± 0.3 | 0.001   |
| Medium VLDL-P (nmol/L) | 2.0 (0.9–2.7) $^{a,b}$ | 0.7 (0.5–1.5) | 1.1 (0.6–1.5) | 0.001   |
| Small VLDL-P (nmol/L) | 23.2 ± 5.71 $^b$ | 21.68 ± 4.82 | 19.35 ± 5.84 | 0.005   |
| LDL-P (nmol/L) | 455.7 ± 79.7 $^b$ | 504.0 ± 62.6 | 493.8 ± 50.8 | 0.002   |
| Large LDL-P (nmol/L) | 86.2 ± 12.6 $^b$ | 94.1 ± 10.6 | 97.0 ± 10.9 | < 0.001 |
| Medium LDL-P (nmol/L) | 67.5 ± 32.1 | 59.0 ± 44.6 | 60.6 ± 35.7 | 0.555   |
| Small LDL-P (nmol/L) | 302.1 ± 48.5 | 350.9 ± 37.4 | 336.9 ± 41.3 | < 0.001 |
| HDL-P (nmol/L) | 15.8 ± 3.7 $^{a,b}$ | 19.9 ± 4.1 $^c$ | 16.7 ± 3.5 | < 0.001 |
| Large HDL-P (nmol/L) | 0.4 ± 0.1 $^a$ | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.374   |
| Medium HDL-P (nmol/L) | 9.5 ± 1.2 | 10.0 ± 1.4 | 9.9 ± 1.6 | 0.194   |
| Small HDL-P (nmol/L) | 6.0 ± 3.9 $^a$ | 9.6 ± 3.6 $^c$ | 6.5 ± 3.0 | < 0.001 |

Data presented: mean ± SD and median (IQR, 25–75) for parametric and nonparametric variables, respectively. Analysis of variance (ANOVA) and the Bonferroni procedure for post hoc analyses. VLDL: very low-density lipoproteins; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; VLDL-P: VLDL number of particles; IDL-P: IDL number of particles; LDL-P: LDL number of particles; HDL-P: HDL number of particles. SGA: small for gestational age, AGA: appropriate for gestational age; LGA: large for gestational age; $a$: P < 0.05 between SGA and AGA; $b$: P < 0.05 between SGA and LGA; $c$: P < 0.05 between AGA and LGA.
Lipoprotein particle size and number was also different across the three groups. The number of VLDL-particles (VLDL-P) was highest in the SGA group and lowest in the LGA group, which was consistent with the differences observed among particle sizes (large, medium and small VLDL-P). The number of LDL-P was lower in the SGA group than in the AGA and LGA groups, and the same distribution was observed for large and small LDL-P. The number of HDL-P was highest in the AGA group and showed an inverse U distribution when compared with the LGA and SGA groups. This phenomenon was observed specifically for small size particles (Table 4).

**GDM alters the cord blood lipoprotein profile across birth-weight categories**

While the cord blood lipoprotein profile was similar in offspring born to GDM and control mothers, we found some interactions when we assessed the effect of both birth-weight categories and GDM. AGA neonates born to GDM mothers had higher IDL-cholesterol and -triglyceride content, and LDL-triglyceride content than the SGA and LGA groups, whereas those of control mothers had lower concentrations. The same pattern was also observed with medium VLDL-P and LDL-P, which followed an inverted U distribution (Fig. 1 and Tables 5 and 6).
Table 5

|                                   | SGA (N = 25) | AGA (N = 25) | LGA(N = 24) | P-value |
|-----------------------------------|--------------|--------------|-------------|---------|
| VLDL-cholesterol (mg/dL)          | 9.0 ± 3.8b   | 7.0 ± 2.6    | 5.5 ± 2.4   | 0.001   |
| IDL-cholesterol (mg/dL)           | 5.3 ± 1.5a   | 4.1 ± 1.5    | 4.6 ± 1.5   | 0.021   |
| LDL-cholesterol (mg/dL)           | 66.7 ± 12.8b | 70.2 ± 8.1   | 73.6 ± 7.1  | 0.053   |
| HDL-cholesterol (mg/dL)           | 37.5 ± 7.2a  | 44.5 ± 8.8   | 40.7 ± 9.2  | 0.018   |
| VLDL-triglycerides (mg/dL)        | 34.0 ± 11.1ab| 26.4 ± 5.8   | 27.8 ± 5.9  | 0.003   |
| IDL-triglycerides (mg/dL)         | 4.9 ± 1.4    | 4.1 ± 1.2    | 4.2 ± 1.2   | 0.084   |
| LDL-triglycerides (mg/dL)         | 4.8 ± 1.8    | 3.9 ± 1.7    | 4.6 ± 1.9   | 0.178   |
| HDL-triglycerides (mg/dL)         | 7.5 ± 3.4    | 9.6 ± 3.6c   | 6.5 ± 3.4   | 0.009   |
| VLDL-P (nmol/L)                   | 26.3 ± 7.8ab | 21.8 ± 4.7   | 21.1 ± 4.5  | 0.005   |
| Large VLDL-P (nmol/L)             | 0.8 ± 0.3ab  | 0.6 ± 0.3    | 0.6 ± 0.2   | 0.002   |
| Medium VLDL-P (nmol/L)            | 2.2 ± 1.5ab  | 0.9 ± 0.6    | 1.3 ± 0.9   | < 0.001 |
| Small VLDL-P (nmol/L)             | 23.3 ± 6.3b  | 20.3 ± 5.1   | 19.2 ± 3.8  | 0.013   |
| LDL-P (nmol/L)                    | 461.4 ± 90.5 | 488.1 ± 57.2 | 505.9 ± 57.3| 0.09    |
| Large LDL-P (nmol/L)              | 84.4 ± 12.9ab| 94.2 ± 10.0  | 99.1 ± 11.6 | < 0.001 |
| Medium LDL-P (nmol/L)             | 73.8 ± 34.2a | 48.9 ± 39.8  | 65.2 ± 40.4 | 0.045   |
| Small LDL-P (nmol/L)              | 303.2 ± 54.4ab| 347.0 ± 38.8 | 341.6 ± 45.6| 0.003   |
| HDL-P (nmol/L)                    | 16.6 ± 3.8a  | 20.0 ± 4.2c  | 16.8 ± 3.7  | 0.004   |
| Large HDL-P (nmol/L)              | 0.3 ± 0.1    | 0.3 ± 0.1    | 0.3 ± 0.1   | 0.913   |
| Medium HDL-P (nmol/L)             | 9.1 ± 1.1    | 9.9 ± 1.3    | 10.0 ± 1.8  | 0.06    |
| Small HDL-P (nmol/L)              | 7.1 ± 3.9a   | 9.8 ± 3.6c   | 6.4 ± 3.3   | 0.004   |

Data presented: mean ± SD. Analysis of variance and the Bonferroni procedure for post hoc analyses; a. P < 0.05 between SGA and AGA; b: P < 0.05 between SGA and LGA; c. P < 0.05 between LGA and AGA; GDM: gestational diabetes mellitus; SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age; VLDL: very low-density lipoproteins; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; VLDL-P: VLDL number of particles. IDL-P: IDL number of particles; LDL-P: LDL number of particles; HDL-P: HDL number of particles;
Table 6

|                      | SGA(N = 14) | AGA(N = 25) | LGA(N = 23) | P-value |
|----------------------|-------------|-------------|-------------|---------|
| VLDL-cholesterol (mg/dL) | 7.6 ± 3.1   | 9.1 ± 3.9\(^c\) | 6.1 ± 3.9   | 0.035   |
| IDL-cholesterol (mg/dL)  | 4.8 ± 1.6   | 5.7 ± 2.2   | 4.4 ± 2.0   | 0.072   |
| LDL-cholesterol (mg/dL)  | 65.6 ± 8.8\(^a\) | 74.1 ± 9.7  | 70.2 ± 5.8  | 0.004   |
| HDL-cholesterol (mg/dL)  | 36.9 ± 5.5  | 43.8 ± 8.8  | 39.5 ± 7.9  | 0.044   |
| VLDL-triglycerides (mg/dL) | 34.3 ± 8.5  | 31.7 ± 9.6  | 27.3 ± 10.3 | 0.074   |
| IDL-triglycerides (mg/dL) | 4.3 ± 1.3   | 5.5 ± 1.8   | 4.1 ± 1.9   | 0.017   |
| LDL-triglycerides (mg/dL) | 3.9 ± 1.3   | 5.1 ± 2.2\(^c\) | 3.9 ± 1.3  | 0.035   |
| HDL-triglycerides (mg/dL) | 7.2 ± 3.9\(^a\) | 10.7 ± 2.6\(^c\) | 7.2 ± 3.9  | 0.008   |
| VLDL-P (nmol/L)          | 25.8 ± 5.9  | 25.6 ± 6.8  | 21.3 ± 8.4  | 0.076   |
| Large VLDL-P (nmol/L)    | 0.8 ± 0.3   | 0.8 ± 0.3   | 0.6 ± 0.3   | 0.060   |
| Medium VLDL-P (nmol/L)   | 1.9 ± 1.2   | 1.7 ± 1.5   | 1.2 ± 0.8   | 0.200   |
| Small VLDL-P (nmol/L)    | 23.1 ± 4.9  | 23.1 ± 5.2  | 19.5 ± 7.5  | 0.083   |
| LDL-P (nmol/L)           | 446.1 ± 59.3\(^a\) | 520.6 ± 64.9\(^c\) | 481.2 ± 40.3 | < 0.001 |
| Large LDL-P (nmol/L)     | 89.0 ± 11.9 | 94.0 ± 11.3 | 94.8 ± 9.7  | 0.134   |
| Medium LDL-P (nmol/L)    | 56.9 ± 25.8 | 71.6 ± 46.7 | 55.7 ± 30.1 | 0.241   |
| Small LDL-P (nmol/L)     | 300.2 ± 38.2\(^a,b\) | 355.0 ± 36.4  | 330.7 ± 36.4 | < 0.001 |
| HDL-P (nmol/L)           | 14.6 ± 3.4\(^a\) | 19.8 ± 4.1\(^c\) | 16.7 ± 3.4  | 0.001   |
| Large HDL-P (nmol/L)     | 0.4 ± 0.1   | 0.3 ± 0.1   | 0.3 ± 0.1   | 0.057   |
| Medium HDL-P (nmol/L)    | 10.0 ± 1.0  | 10.0 ± 1.5  | 9.8 ± 1.5   | 0.853   |
| Small HDL-P (nmol/L)     | 4.2 ± 3.3\(^a\) | 9.5 ± 3.7\(^c\) | 6.6 ± 2.8   | < 0.001 |

Data presented: mean ± SD. Analysis of variance (ANOVA) and the Bonferroni procedure for post hoc analyses; a: P < 0.05 between SGA and AGA; b: P < 0.05 between SGA and LGA; c: P < 0.05 between LGA and AGA; GDM: gestational diabetes mellitus. SGA: small for gestational age, AGA: appropriate for gestational age, LGA: large for gestational age; VLDL: very low-density lipoproteins; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; VLDL-P: VLDL number of particles. IDL-P: IDL number of particles; LDL-P: LDL number of particles; HDL-P: HDL number of particles.
Relationship of \(^{1}\)H-NMR-assessed lipoprotein profile with clinical and laboratory parameters

We next examined the relationship of \(^{1}\)H-NMR-based lipoprotein profile and maternal clinical and laboratory variables and neonatal outcomes, separately in both GDM and control groups. These results are shown in Fig. 2. Only correlations that remain significant after FDR correction are marked in the heat map with an asterisk. In the GDM group, maternal LDL-cholesterol was negatively associated with cord blood VLDL-P (total number, large and small particles), cholesterol and triglyceride content in VLDL, IDL and HDL-triglyceride content.

LDL-cholesterol concentrations were negatively associated with birth-weight in the GDM group, while no association was observed in the control group. Cord blood insulin was strongly and positively associated with small and large LDL-P and LDL-cholesterol content in the control group, while a negative relationship was observed with VLDL-P and VLDL-triglyceride content in the GDM offspring.

In both groups, neonatal adiposity was negatively correlated with cord blood VLDL-P, VLDL- and IDL-triglyceride content, and positively associated with cord blood LDL-cholesterol content and LDL-P, being these associations stronger in the control group.

Cord blood \(^{1}\)H-NMR-lipoprotein profile is associated with obesity at two years

To assess whether cord blood lipoprotein profile could be used as a biomarker for offspring outcomes, we explored its potential association with obesity at 2 years of life in a subset of participants. One hundred and three children were available in the follow-up study. No differences in sex, GDM or birth-weight categories distribution, birth-weight, maternal pre-pregnancy BMI, GWG or cord blood lipid profile were observed between the children lost to follow-up and those that remained in the study. Of the 103 children studied, 78 were normal weight and 25 were obese. Obese children were born to women with higher BMI, had higher birth-weight and were more exposed to GDM during the intrauterine life compared with normal weight children. Additionally, they showed a higher number of cord blood small (352 ±29 vs 326±51 nmol/L; P = 0.019) and large LDL-P (98±11 vs 91±13 nmol/L; P = 0.022). No other differences were observed between the two groups. To further assess the independence of these associations, we performed logistic regression analysis. We found that small LDL-P were associated with infant obesity at two years after adjusting for potential confounders (Table 7), whereas large LDL-P showed a trend (P = 0.058).
Table 7
Adjusted odds ratio for the association between large and small LDL particles with the development of obesity at two years of age.

|                    | Model R² | Exp (B) | 95% CI for exp (B) | P-value |
|--------------------|----------|---------|--------------------|---------|
| Large LDL-P* (nmol/L) | 0.234    | 1.052   | 0.998–1.109        | 0.058   |
| Small LDL-P* (nmol/L)   | 0.251    | 1.018   | 1.002–1.034        | 0.023   |

Statistical Analysis: Logistic regression analysis. *Adjusted for gestational diabetes mellitus, gestational age at delivery, birth-weight, sex, pre-gestational BMI and gestational weight gain. LDL-P: LDL particles. CI: confidence interval.

Discussion

Both GDM and abnormal growth patterns have been associated with long-term adverse outcomes on offspring and changes in the lipoprotein composition have been proposed as potential markers of cardiovascular diseases later in life. In this study, taking advantage of a thorough lipoprotein profiling by using $^1$H-NMR, we show for the first time that GDM modifies the umbilical cord blood lipoprotein profile in AGA neonates. In particular, GDM alters IDL-lipoproteins, triglyceride content in LDL, and medium-size VLDL-P and LDL-P in those children. By contrast, GDM offspring belonging to the LGA and SGA groups have a lipoprotein profile more similar to controls. Besides, we found that cord blood small LDL-P, known to be associated with atherosclerosis development, have a predictive value for later obesity in the offspring.

Both under and overnutrition in utero affects the lipoprotein profile of neonates.$^{4-7}$ SGA neonates are reported to exhibit higher cord blood triglyceride concentrations,$^{(6, 9, 27)}$ higher VLDL and IDL concentrations, and lower HDL concentrations when compared with equivalent AGA neonates.$^{(10)}$ Some of these findings have also been reported in fetal macrosomia$^{(6, 8)}$ and GDM pregnancies.$^{(28)}$ However, standard lipid profiling have failed to identify differences between offspring born to healthy pregnant and GDM mothers.$^{(29)}$ Nonetheless, a more extensive characterization of lipoproteins, as have been stated in other metabolic disorders such as diabetic dyslipidemia,$^{(17)}$ could provide more accurate information on the regulation of lipoprotein metabolism in fetal life and its potential implications for metabolism in later life. Thus, using $^1$H-NMR-based cord blood lipoprotein profiling we detected differences according to fetal growth categories in GDM women, revealing a disturbed cholesterol and triglyceride metabolism even in AGA neonates. This pattern may denote an excessive transfer of triglycerides to LDL, and the further increased cholesterol-poor LDL particles in the liver.$^{(30)}$ Furthermore, nutritional factors and dysfunctional HDL lipoproteins,$^{(31)}$ found in cord blood of infants of GDM mothers,$^{(15)}$ may induce an abnormal hepatic lipase activation also increasing IDL half-life. This scenario is similar to the dyslipidemia associated with diabetes and insulin-resistant states, where an increased generation of IDL, small and dense LDL particles, and triglyceride-enriched HDL particles is observed,$^{(30)}$ and which has been related to an increased atherogenic risk. These findings lead us to hypothesize that postnatal insulin resistance, which has been described in offspring of GDM women, may be programmed in utero and
would be present even in AGA neonates, suggesting that a good glycemic control during pregnancy is not enough to prevent long term complications, as has been previously reported.\(^{(32, 33)}\)

When analysing the lipoprotein profile according to birth-weight categories (Table 4), most of the differences observed in the whole group were replicated both, in GDM and controls separately (Tables 5 and 6). These findings may support an effect of fetal growth accretion instead of the glucose status. In this context, it must be remarked that the evidence of cord blood lipoprotein as biomarkers of later cardiovascular disease as it happens in adult life, is scarce. However, we tend to extrapolate what we observe in adult life to fetal life, despite it may have different interpretation. In fact, differences in lipoprotein composition between adults and fetuses has been described (excess apoE is found on fetal HDL particles which are large in size with absence of paroxonase I\(^{(34, 35)}\), suggesting that these particles do not have anti-oxidant capacity; small LDL poorer in lipids content\(^{(36)}\), etc.), highlighting the need to better understanding of how lipid metabolism in utero relates to lipid metabolism in adults and in turn, how these metabolic changes in fetus impacts on adult cardiovascular health.

Previous studies exploring the potential relationship between prenatal lipid metabolism and adverse metabolic outcomes in offspring have generated inconsistent results.\(^{(37–42)}\) Following other reports,\(^{(43, 44)}\) we confirm that GDM, pre-pregnancy BMI, and GWG during pregnancy are all associated with offspring obesity in early life. Furthermore, we found that small LDL-P in cord blood were associated with early obesity, even after controlling for confounding factors. These findings support the notion that disturbances in the lipoprotein metabolism at birth may have lasting effects independently of birth weight or maternal metabolic status.

There is evidence that an altered fetal lipoprotein profile is associated with aorta intima thickness in SGA and LGA neonates,\(^{(8, 27)}\) indicating a potentially increased atherosclerotic risk, already at birth. We are aware that our results cannot establish a direct link between the \(^{1}\text{H-NMR}-\text{assessed lipoprotein profile, observed in GDM-AGA newborns, with an increased atherogenic risk but, however, it offers new clues to understand the high metabolic and cardiovascular risk in the offspring of GDM pregnant women.\(^{(45)}\)\) Long-term studies are guaranteed to confirm whether cord blood \(^{1}\text{H-NMR}-\text{based lipoprotein profiling can be implemented as a useful biomarker of later metabolic diseases beyond two years of age.\}

One of the main limitations in observational studies is the inability to attribute causation between the observed associations. However, we have considered many confounding variables to mitigate bias in the analysis. Thus, the main prenatal factors were addressed, and the groups were comparable for maternal BMI and birth-weight categories. Otherwise, to reach a sufficient sample size in the three birth-weight categories, the SGA and LGA groups were overrepresented, and further population-based studies are needed to determine the role of lipoprotein composition and subfractions in the pathogenesis of metabolic diseases in offspring.

The strengths of this study include a longitudinal birth cohort with almost complete maternal data that establish a temporal relationship between the outcome and the exposure to GDM. The novelty of the
lipoprotein assessment, which allows us to identify different fetal metabolic behaviors, is also a big asset in the experimental methods.

Conclusions

GDM disturbs triglyceride and cholesterol lipoprotein concentrations across birth categories, with GDM-AGA neonates showing a profile more similar to adults with dyslipidemia and atherosclerosis than those born to normal glucose tolerant mothers. Moreover, an altered fetal lipoprotein pattern is associated with obesity development at 2 years. Overall, these findings suggest that the fetal lipoprotein profile might be an early biomarker of development of later diseases.

Abbreviations

AGA
adequate for gestational age
BMI
body mass index
FDR
false discovery rate
GDM
gestational diabetes mellitus
GWG
gestational weight gain
HDL
high-density lipoproteins
HOMA-IR
homeostatic model assessment of insulin resistance
IDL
intermediate-density lipoproteins
LDL
low-density lipoproteins
LGA
large for gestational age
NMR
nuclear magnetic resonance
-P
number of particles
PI
ponderal index
SGA
small for gestational age
VLDL
very low-density lipoproteins
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the tenets of the Declaration of Helsinki and the protocol was reviewed and approved by the Hospital Universitari de Tarragona Research Ethics Board (ref: 243/2016).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

NA is a stockowner in Biosfer Teslab and has a patent for the lipoprotein profiling described in the present manuscript. The other authors declare that they have no competing interests.

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Author’s contributions

FAC: Conceptualization, methodology, formal analysis, investigation, writing-original draft, visualization.
EMM: methodology, investigation. MB: methodology, investigation, resources, supervision. AG: methodology, investigation, resources. OF: methodology, investigation, resources. NA: methodology,
investigation, and resources. SFV: Conceptualization, resources, writing-review and editing, supervision, project administration, funding acquisition. JV: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing-original draft, writing-review and editing, visualization, supervision, project administration, funding acquisition. AM: Conceptualization, methodology, validation, resources, writing-review and editing, supervision, project administration, funding acquisition.

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Figures
Figure 1

Differences in cord blood 1H-NMR-assessed lipoprotein pattern among growth groups in GDM and control mothers. Statistical analysis: two-way analysis of variance. Data shown as mean±SD. GDM: gestational diabetes mellitus; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; IDL: intermediate-density lipoproteins; IDL-C: cholesterol content in IDL; IDL-TG: triglyceride content in IDL; LDL-TG: triglyceride content in LDL; LDL-P: LDL number of particles; VLDL-P: VLDL number of particles; AGA: appropriate for gestational age; LGA: large for gestational age, SGA: small for gestational age.

Figure 2

Heat map of the associations between cord blood 1H-NMR-assessed lipoprotein profile and maternal and neonatal clinical variables in control (left panel) and GDM (right panel) mothers. GDM: gestational diabetes mellitus; % Fat Mass: percentage of fat mass, Cb: cord blood; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; IDL: intermediate-density lipoproteins; VLDL-C: cholesterol content in VLDL; VLDL-TG: triglyceride content in VLDL; VLDL-P: VLDL number of particles; IDL-C: cholesterol content in IDL; IDL-TG: triglyceride content in IDL; LDL-C: cholesterol content in LDL; LDL-TG: triglyceride content in LDL; LDL-P: LDL number of particles; HDL-C: cholesterol content in HDL; HDL-TG: triglyceride content in HDL; HDL-P: HDL number of particles; Pre-pregnancy BMI: pre-gestational body mass index; HOMA-IR: homeostatic model assessment Insulin Resistance. Spearman Correlation coefficients. *Indicates significant associations after applying B-H procedure for FDR correction.