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

Francisco Algaba-Chueca
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Elsa Maymó-Masip
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Mónica Ballesteros
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Albert Guarque
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Olga Freixes
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Núria Amigó
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Sonia Fernández-Veledo
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Joan Vendrell
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
Ana Megia (amegia.hj23.ics@gencat.cat)
Joan XXIII University Hospital in Tarragona: Hospital Universitari de Tarragona Joan XXIII
https://orcid.org/0000-0002-5101-9452

Original investigation

Keywords: birth-weight, gestational diabetes, lipoprotein profile, obesity, offspring, outcome

Posted Date: December 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-123776/v1

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 ^{1}H-NMR spectroscopy was used to profile umbilical cord serum lipoproteins. One hundred and three children were available in a two years follow-up study to evaluate associations between cord blood lipid profile and obesity.

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 in 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.(19) Subjects with a 1-hour 50-g glucose challenge test ≥ 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(20) 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(19). 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 -80ºC 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).

^{1} H-NMR spectroscopy-based cord blood lipoprotein profiling

Cord blood serum samples were analyzed using the 2D diffusion-ordered ^{1}H-NMR-based Liposcale test (Biosfer Testlab, Reus, Spain)(18). This technique has shown to be reliable with samples stored at -80º 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.^{1}H-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 ^{1}H-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 ^{1}H-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 ^{1}H-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 ^{1}H-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 ^{1}HNMR 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).

Table 1
Maternal and neonatal clinical characteristics according to gestational diabetes

	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/m^2)	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/cm^3)	2.7 ± 0.3	2.7 ± 0.3	0.633
Cb Insulin (mcUI/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 ± 3.3	7.6 ± 4.0	0.495
IDL-cholesterol (mg/dL)	4.6 ± 1.6	5.0 ± 2.0	0.291
LDL-cholesterol (mg/dL)	70.1 ± 10.1	70.6 ± 8.7	0.767
HDL-cholesterol (mg/dL)	40.9 ± 8.8	40.6 ± 8.1	0.827
VLDL-triglycerides (mg/dL)	29.4 ± 8.6	30.7 ± 9.9	0.415
IDL-triglycerides (mg/dL)	4.4 ± 1.3	4.7 ± 1.8	0.335
LDL-triglycerides (mg/dL)	4.5 ± 1.8	4.4 ± 1.9	0.925
HDL-triglycerides (mg/dL)	7.9 ± 3.7	8.6 ± 3.9	0.251
VLDL-P (nmol/L)	23.1 ± 6.3	24.0 ± 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 ± 71.7	488.0 ± 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 ± 4.2	17.4 ± 4.1	0.591
Large HDL-P (nmol/L)	0.3 ± 0.1	0.3 ± 0.1	0.107
Medium HDL-P (nmol/L)	9.7 ± 1.5	9.9 ± 1.4	0.257
Small HDL-P (nmol/L)	7.8 ± 3.8	7.1 ± 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/m^2)	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 ± 6.3^b	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 ± 279^a	3268 ± 270^c	3929 ± 251^b	< 0.001
Fat mass (%)	6.8 ± 3.6^a	11.6 ± 2.4^c	12.0 ± 2.0	< 0.001
Ponderal Index (g/cm^3)	2.5 ± 0.2^a	2.8 ± 0.3^c	2.9 ± 0.2^b	< 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 ^{1}HNMR 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)	23.20 ± 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 inversed U distribution (Fig. 1 and Tables 5 and 6).

Table 5

^{1}H-NMR assessed lipid profile across the birth-weight categories in control women.

	SGA (N = 25)	AGA (N = 25)	LGA(N = 24)	P-value
VLDL-cholesterol (mg/dL)	9.0 ± 3.8[b]	7.0 ± 2.6	5.5 ± 2.4	0.001
IDL-cholesterol (mg/dL)	5.3 ± 1.5[a]	4.1 ± 1.5	4.6 ± 1.5	0.021
LDL-cholesterol (mg/dL)	66.7 ± 12.8[b]	70.2 ± 8.1	73.6 ± 7.1	0.053
HDL-cholesterol (mg/dL)	37.5 ± 7.2[a]	44.5 ± 8.8	40.7 ± 9.2	0.018
VLDL-triglycerides (mg/dL)	34.0 ± 11.1[a,b]	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 ± 3.6[c]	6.5 ± 3.4	0.009
VLDL-P (nmol/L)	26.3 ± 7.8[a,b]	21.8 ± 4.7	21.1 ± 4.5	0.005
Large VLDL-P (nmol/L)	0.8 ± 0.3[a,b]	0.6 ± 0.3	0.6 ± 0.2	0.002
Medium VLDL-P (nmol/L)	2.2 ± 1.5[a,b]	0.9 ± 0.6	1.3 ± 0.9	< 0.001
Small VLDL-P (nmol/L)	23.3 ± 6.3[b]	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 ± 12.9[a,b]	94.2 ± 10.0	99.1 ± 11.6	< 0.001
Medium LDL-P (nmol/L)	73.8 ± 34.2[a]	48.9 ± 39.8	65.2 ± 40.4	0.045
Small LDL-P (nmol/L)	303.2 ± 54.4[a,b]	347.0 ± 38.8	341.6 ± 45.6	0.003
HDL-P (nmol/L)	16.6 ± 3.8[a]	20.0 ± 4.2[c]	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 ± 3.9[a]	9.8 ± 3.6[c]	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

^{1}H-NMR assessed lipid profile across the birth-weight categories in GDM women.

	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 R^2	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}H-NMR-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}H-NMR-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.

Funding

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness (PI 15/01562 and PI 18/00516 to AM, RTI2018-093919-B-I00 to SF-V, PI14/00228 and PI17/01503 to JV) co-financed by the European Regional Development Fund (ERDF) " A way to make Europe/Investing in your future". The Spanish Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM) (CB07708/0012) is an initiative of the Instituto de Salud Carlos III. FAC is funded by a predoctoral fellowship from AGAUR, Spain (2017FI_B_00632). SFV acknowledges the Miguel Servet tenure-track program (CP10/00438 and CPII16/00008) from the Fondo de Investigación Sanitaria, co-financed by the ERDF.

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.

Acknowledgements:

We want to particularly acknowledge all the patients and volunteers involved in this study for their collaboration. We also acknowledge the BioBank IISPV (PT17/0015/0029) integrated into the Spanish National Biobanks Network. Finally, we wish to highlight the essential contribution of the midwives and the Gynecology and Obstetrics Department of the Hospital Universitari Joan XXIII de Tarragona.

References

1. Barker DJ. The fetal and infant origins of disease. Eur J Clin Invest [Internet]. 1995 Jul [cited 2014 Sep 3];25(7):457–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7556362
2. Bao W, Srinivasan SR, Wattigney WA, Bao W, Berenson GS. Usefulness of Childhood Low-Density Lipoprotein Cholesterol Level in Predicting Adult Dyslipidemia and Other Cardiovascular Risks. Arch Intern Med [Internet]. 1996 Jun 24 [cited 2019 Aug 14];156(12):1315–20. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.1996.00440110083011
3. Cohen MS. Fetal and childhood onset of adult cardiovascular diseases. Pediatr Clin North Am [Internet]. 2004 Dec [cited 2019 Aug 14];51(6):1697–719. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15561181
4. Loughrey CM, Rimm E, Heiss G, Rifai N. Race and gender differences in cord blood lipoproteins. Atherosclerosis [Internet]. 2000 Jan 1 [cited 2019 Aug 16];148(1):57–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10580171
5. Lane DM, McConathy WJ. Factors Affecting the Lipid and Apolipoprotein Levels of Cord Sera. Pediatr Res [Internet]. 1983 Feb [cited 2019 Nov 3];17(2):83–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6828336
6. Aletayeb SMH, Dehdashtian M, Aminzadeh M, Moghaddam A-RE, Mortazavi M, Malamiri RA, et al. Correlation between umbilical cord blood lipid profile and neonatal birth weight. Pediatr Pol [Internet]. 2013 Nov 1 [cited 2019 Nov 3];88(6):521–5. Available from: https://www.sciencedirect.com/science/article/pii/S0031393913001698
7. Kumar A, Gupta A, Malhotra VK, Agarwal PS, Thirupuram S, Gaind B. Cord blood lipid levels in low birth weight newborns. Indian Pediatr [Internet]. 1989 Jun [cited 2019 Nov 3];26(6):571–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2583809
8. Koklu E, Akcakus M, Kurtoglu S, Koklu S, Yikilmaz A, Coskun A, et al. Aortic intima-media thickness and lipid profile in macrosomic newborns. Eur J Pediatr [Internet]. 2007 Feb 16 [cited 2019 Nov

3];166(4):333–8. Available from: http://link.springer.com/10.1007/s00431-006-0243-8

9. Miranda J, Simões R V., Paules C, Cañueto D, Pardo-Cea MA, García-Martín ML, et al. Metabolic profiling and targeted lipidomics reveals a disturbed lipid profile in mothers and fetuses with intrauterine growth restriction. Sci Rep [Internet]. 2018 Dec 11 [cited 2018 Dec 16];8(1):13614. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30206284
10. Kim S-M, Lee SM, Kim S-J, Kim BJ, Shin S, Kim J-R, et al. Cord and maternal sera from small neonates share dysfunctional lipoproteins with proatherogenic properties: Evidence for Barker's hypothesis. J Clin Lipidol [Internet]. 2017 Nov [cited 2018 Sep 5];11(6):1318-1328.e3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1933287417304452
11. Herrera E, Desoye G. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. Horm Mol Biol Clin Investig [Internet]. 2016 Jan 1 [cited 2018 Sep 5];26(2):109–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26351960
12. Fordyce MK, Duncan R, Chao R, Christakis M, Hsia SL, Robertson E, et al. Cord blood serum in newborns of diabetic mothers. J Chronic Dis [Internet]. 1983 Jan [cited 2019 Nov 3];36(3):263–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6826691
13. Kilby MD, Neary RH, Mackness MI, Durrington PN. Fetal and Maternal Lipoprotein Metabolism in Human Pregnancy Complicated by Type I Diabetes Mellitus [1]. J Clin Endocrinol Metab [Internet]. 1998 May 1 [cited 2019 Nov 3];83(5):1736–41. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.83.5.4783
14. Chan TC, Schwartz JJ, Garcia RE, Chin HP, Barndt R. Total serum cholesterol and plasma lipoprotein cholesterol concentrations in cord sera of newborns from hispanic mothers with gestational diabetes. Artery [Internet]. 1988 [cited 2019 Nov 3];15(4):203–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3408348
15. Sreckovic I, Birner-Gruenberger R, Besenboeck C, Miljkovic M, Stojakovic T, Scharnagl H, et al. Gestational diabetes mellitus modulates neonatal high-density lipoprotein composition and its functional heterogeneity. Biochim Biophys Acta - Mol Cell Biol Lipids [Internet]. 2014 Nov [cited 2019 Nov 3];1841(11):1619–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25130684
16. Miettinen HE, Rönö K, Koivusalo SB, Eriksson JG, Gylling H. Effect of gestational diabetes mellitus on newborn cholesterol metabolism. Atherosclerosis [Internet]. 2018 Aug [cited 2018 Sep 5];275:346–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30015298
17. Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: Application to the study of diabetic dyslipidemia. Prog Nucl Magn Reson Spectrosc [Internet]. 2013 Apr [cited 2020 Jan 27];70:1–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23540574
18. Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res [Internet]. 2015 Mar [cited 2018 Sep 5];56(3):737–46. Available from: http://www.jlr.org/lookup/doi/10.1194/jlr.D050120

19. Grupo Español de Diabetes y Embarazo (GEDE), Grupo Español de Diabetes y Embarazo. Asistencia a la gestante con diabetes. Guía de práctica clínica actualizada en 2014 | Avances en Diabetología. Av en Diabetol [Internet]. 2015 Mar 1 [cited 2015 May 11];31(2):45–59. Available from: http://www.elsevier.es/es-revista-avances-diabetologia-326-resumen-asistencia-gestante-con-diabetes-guia-90411697
20. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med [Internet]. 1998 Jul;15(7):539–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9686693
21. Catalano PM, Thomas AJ, Avallone DA, Amini SB. Anthropometric estimation of neonatal body composition. Am J Obs Gynecol. 1995;173:1176–81.
22. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weightfor- length, weight-for-height and body mass index-for-age: Methods and development [Internet]. Geneva: WHO. World Health Organization; 2006 [cited 2020 Jan 19]. Available from: https://www.who.int/childgrowth/publications/technical_report_pub/en/
23. Martinez-Perez B, Ejarque M, Gutierrez C, Nuñez-Roa C, Roche K, Vila-Bedmar R, et al. Angiopoietin-like protein 8 (ANGPTL8) in pregnancy: a brown adipose tissue–derived endocrine factor with a potential role in fetal growth. Transl Res. 2016;178.
24. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
25. Pichler G, Amigo N, Tellez-Plaza M, Pardo-Cea M, Dominguez-Lucas A, VG M, et al. LDL Particle Size and Composition and Incident Cardiovascular Disease in a South-European Population: The Hortega-Liposcale Follow-up Study. Int J Cardiol [Internet]. 2018 [cited 2020 May 24];264:172–8. Available from: https://pubmed.ncbi.nlm.nih.gov/29628276/
26. Haynes W. Benjamini–Hochberg Method. In: Encyclopedia of Systems Biology [Internet]. New York, NY: Springer New York; 2013 [cited 2020 May 31]. p. 78–78. Available from: http://link.springer.com/10.1007/978-1-4419-9863-7_1215
27. Koklu E, Kurtoglu S, Akcakus M, Koklu S, Buyukkayhan D, Gumus H, et al. Increased Aortic Intima-Media Thickness Is Related to Lipid Profile in Newborns with Intrauterine Growth Restriction. Horm Res Paediatr [Internet]. 2006 [cited 2019 Dec 16];65(6):269–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16601348
28. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care [Internet]. 2008 Sep 1 [cited 2016 Mar 13];31(9):1858–63. Available from: http://care.diabetesjournals.org/content/31/9/1858.long
29. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, Graf K, Vetter K, Abou-Dakn M, et al. Differences in the implications of maternal lipids on fetal metabolism and growth between gestational diabetes

mellitus and control pregnancies. Diabet Med [Internet]. 2011 Sep [cited 2015 Mar 7];28(9):1053–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21658120

30. Hassing HC, Surendran RP, Mooij HL, Stroes ES, Nieuwdorp M, Dallinga-Thie GM. Pathophysiology of hypertriglyceridemia. Biochim Biophys Acta - Mol Cell Biol Lipids. 2012;1821(5):826–32.
31. Chatterjee C, Sparks DL. Hepatic lipase, high density lipoproteins, and hypertriglyceridemia. Am J Pathol [Internet]. 2011 Apr [cited 2020 Aug 22];178(4):1429–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21406176
32. Gillman MW, Oakey H, Baghurst PA, Volkmer RE, Robinson JS, Crowther CA, et al. Effect of treatment of gestational diabetes mellitus on obesity in the next generation. Diabetes Care. 2010;33(5):964–8.
33. Landon MB, Rice MM, Varner MW, Casey BM, Reddy UM, Wapner RJ, et al. Mild Gestational Diabetes Mellitus and Long-Term Child Health. Diabetes Care [Internet]. 2015 Mar [cited 2017 Apr 15];38(3):445–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25414152
34. Fujita H, Okada T, Inami I, Makimoto M, Hosono S, Minato M, et al. Heterogeneity of high-density lipoprotein in cord blood and its postnatal change. Clin Chim Acta [Internet]. 2008 Mar [cited 2020 Sep 12];389(1–2):93–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18155163
35. Sreckovic I, Birner-Gruenberger R, Obrist B, Stojakovic T, Scharnagl H, Holzer M, et al. Distinct composition of human fetal HDL attenuates its anti-oxidative capacity. Biochim Biophys Acta [Internet]. 2013 Apr [cited 2020 Sep 12];1831(4):737–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23321267
36. Gugliucci A, Numaguchi M, Caccavello R, Kimura S. Small-dense low-density lipoproteins are the predominant apoB-100-containing lipoproteins in cord blood. Clin Biochem [Internet]. 2014 Apr [cited 2019 Dec 29];47(6):475–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24362269
37. Shokry E, Marchioro L, Uhl O, Bermúdez MG, García-Santos JA, Segura MT, et al. Transgenerational cycle of obesity and diabetes: investigating possible metabolic precursors in cord blood from the PREOBE study. Acta Diabetol [Internet]. 2019 Sep 6 [cited 2020 Jan 26];56(9):1073–82. Available from: http://link.springer.com/10.1007/s00592-019-01349-y
38. Simpson J, Smith AD, Fraser A, Sattar N, Callaway M, Lindsay RS, et al. Cord Blood Adipokines and Lipids and Adolescent Nonalcoholic Fatty Liver Disease. J Clin Endocrinol Metab [Internet]. 2016 Dec 1 [cited 2019 Aug 14];101(12):4661–8. Available from: https://academic.oup.com/jcem/article/101/12/4661/2765033
39. Hellmuth C, Uhl O, Standl M, Demmelmair H, Heinrich J, Koletzko B, et al. Cord Blood Metabolome Is Highly Associated with Birth Weight, but Less Predictive for Later Weight Development. Obes Facts [Internet]. 2017 [cited 2020 Jan 26];10(2):85–100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28376503
40. Stratakis N, Gielen M, Margetaki K, de Groot RHM, Apostolaki M, Chalkiadaki G, et al. Polyunsaturated fatty acid status at birth, childhood growth, and cardiometabolic risk: a pooled analysis of the MEFAB and RHEA cohorts. Eur J Clin Nutr [Internet]. 2018 May 16 [cited 2018 Sep 6]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/29765163

41. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. Br J Nutr [Internet]. 2014 Jun 14 [cited 2020 Jan 27];111(11):2024–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24521544
42. Donahue SMA, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr [Internet]. 2011 Apr [cited 2020 Jan 27];93(4):780–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21310834
43. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles M-A, Pettitt DJ. Childhood Obesity and Metabolic Imprinting. Diabetes Care. 2007;30:2287–92.
44. Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. PLoS Med [Internet]. 2019 Feb [cited 2019 Jul 14];16(2):e1002744. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30742624
45. Pathirana MM, Lassi ZS, Roberts CT, Andraweera PH. Cardiovascular risk factors in offspring exposed to gestational diabetes mellitus in utero: systematic review and meta-analysis. J Dev Orig Health Dis [Internet]. 2020 Jan 6 [cited 2020 Jun 19];1–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31902382

Figures

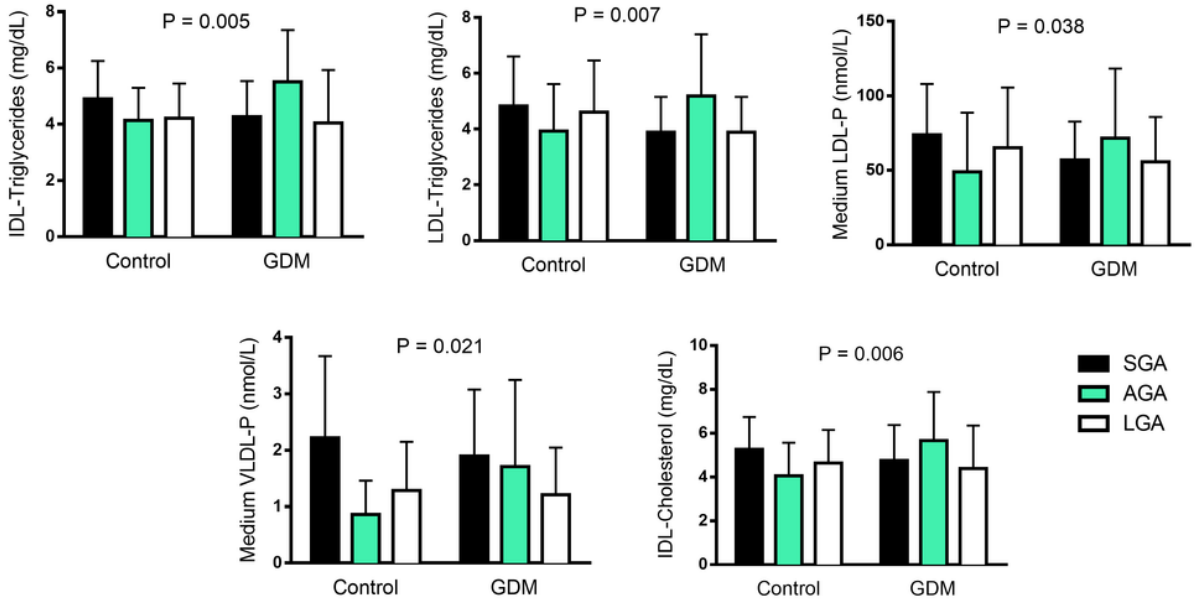

Figure 1

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.

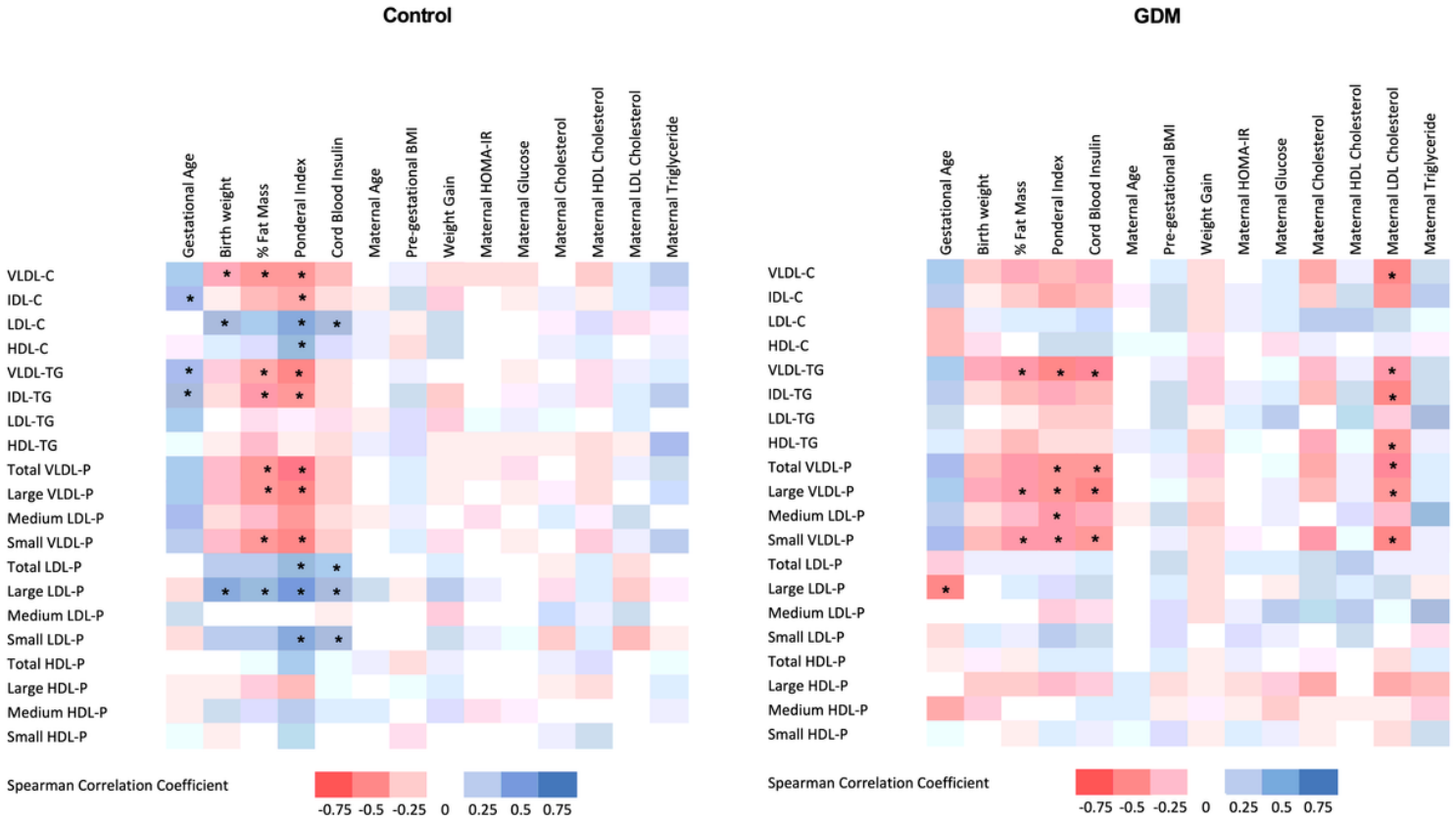

Figure 2

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.