Acta Diabetologica (2019) 56:1073–1082
https://doi.org/10.1007/s00592-019-01349-y

ORIGINAL ARTICLE

Transgenerational cycle of obesity and diabetes: investigating possible metabolic precursors in cord blood from the PREOBE study

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Received: 8 February 2019 / Accepted: 16 April 2019 / Published online: 6 May 2019

Abstract

Aims Offspring of mothers suffering from obesity and/or gestational diabetes mellitus (GDM) were reported to be at risk of higher birth weight (BW), later obesity and diabetes. We hypothesize that infant anthropometry changes related to maternal pathological status are due to dysregulated infant metabolism.

Methods First, we inspected differences in BMI *z*-scores (*z*-BMI) between three infant groups: born to normal weight (NW; $n=49$), overweight/obese (OV/OB; $n=40$) and GDM mothers ($n=27$) at birth and 1 year. Then, we inspected associations between cord blood metabolites and 1-year Δ *z*-BMI in the three infant groups at birth and 1 year.

Results No statistically significant difference was detected in *z*-BMI between the study groups at birth; however, GDM was associated with heavier infants at 1 year. Regarding the associations between the metabolites and *z*-BMI, phospholipids, especially those containing polyunsaturated fatty acids, were the species most impacted by the maternal metabolic status, since numerous phosphatidylcholines–PUFA were positively associated with *z*-BMI in NW but negatively in OV/OB and GDM groups at birth. Conversely, the sum of lysophosphatidylcholines was only positively associated with *z*-BMI in NW at birth but of no relation in the other two groups. At 1 year, most of the associations seen at birth were reversed in NW and lost in OV/OB and GDM groups. In the NW group, PC-PUFA were found to be negatively associated with Δ *z*-BMI at 1 year in addition to some medium-chain acylcarnitines, tricarboxylic acid metabolites, Asp and Asn-to-Asp ratio. In OV/OB and GDM groups, the non-esterified fatty acid (NEFA26:0) and His correlated with Δ *z*-BMI at 1 year in negative and positive directions, respectively.

Conclusions GDM was associated with overweight in offspring at 1 year, independent of the BW with lack of evidence on existing correlation of this finding with metabolic alterations detected in cord blood metabolome. Associations were found between cord blood metabolites and infant anthropometry at birth and were influenced by maternal OB and GDM. However, an extension of the findings monitored at birth among the three groups was not detected longitudinally showing a lack of predictive power of cord blood metabolome for later development at least 1 year.

Keywords Gestational diabetes mellitus (GDM) · Obesity · Metabolomics · Cord blood · Infant anthropometry

Introduction

The increased prevalence of obesity (OB) and overweight (OW) worldwide, especially in industrialized countries, has become a recognized public health problem. In Europe, approximately 50% of the adults are OW and 16% are obese with a corresponding prevalence of about 20% among children and adolescents depending on the countries and socioeconomic groups [1, 2].

There is currently growing evidence that childhood OB is influenced by many pre- and perinatal factors, including maternal OB and gestational diabetes mellitus (GDM)

Managed by Antonio Secchi.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00592-019-01349-y) contains supplementary material, which is available to authorized users.

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believed to set stage for pathophysiological alterations during foetal development, thus increasing risk of long-term effects, which is termed as 'metabolic programming' [3–5]. GDM is hyperglycaemia first recognized during pregnancy which could attributed to pre-existing insulin resistance (IR) further manifested during pregnancy with the lack in insulin secretion or to a pancreatic β-cell defect similar to that in type-1 diabetes (T1D) [6]. Both maternal OB and GDM were repeatedly associated with short- and long-term adverse outcomes in offspring [7–9].

Previously, we have studied the impact of maternal phenotypes (OB/GDM) on maternal cord blood metabolic profiles using data from the prospective observational PREOBE study to provide an insight into metabolic programming [10]. In an extension to our findings, our objective was to investigate how these maternal phenotypes could modulate associations between the cord blood metabolome and infant anthropometry. To this end, we inspected whether associations between cord blood metabolites and offspring BMI WHO z-scores at birth and 1 year of age (1 year) are different in offspring of normal weight (NW), OW/OB and GDM mothers and underlined possible metabolic pathways behind these differences.

Methods

Participants and ethical approval

The present work was conducted using data obtained from a subpopulation within the PREOBE study (NCT01634464). Study details including subject inclusion/exclusion criteria were described elsewhere [11].

Sample and data collection

Venous cord blood samples were obtained from 116 newborns within 5 min of delivery and prior to delivery of the placenta. Samples were collected in 5-mL EDTA-coated tubes and processed, and plasma aliquots were stored at − 80 °C until analysis. Information on maternal anthropometry was collected at time of recruitment, and weight was measured additionally at 34 weeks of pregnancy. Gestational weight gain (GWG) at 34 weeks and perinatal data including gestational age (GA) at birth, infant sex, mode of delivery (standard vaginal or caesarean section), BMI and waist circumference were collected from medical records. For the offspring anthropometry, weight and length were measured at birth and at 1 year of age; then, the corresponding BMI and WHO z-scores were calculated.

Cord blood metabolomics

The analytical pipeline covered 400 metabolites including polar lipids (diacyl-phosphatidylcholines (PCaa), acyl-alkyl-phosphatidylcholines (PCae), acylcarnitines (AC), sphingomyelines (SM), acyl-lysophosphatidylcholines (LPCa), alkyl-lysophosphatidylcholines (LPCe)), sum of hexoses (H1), amino acids (AA), keto acids, non-esterified fatty acids (NEFA) and tricarboxylic acid (TCA) cycle metabolites. The formula CX:Y was assigned for polar lipids and NEFA, where X, Y and OH indicate carbon chain length, number of double bonds and presence of hydroxyl group, respectively. Letters 'a' and 'e' indicate whether the acyl chain is bound via an ester or ether bond to the backbone, respectively. AA were coded by IUPAC abbreviations.

The analysis of the metabolite classes was performed as previously described [10]. The analytical process was processed by Analyst 1.6.1 and R software. Concentrations were expressed in µmol/l.

Data analysis

Metabolomics quality control and preprocessing

Data were analysed using R statistical software. The quality control (QC) criteria allowed intra- and inter-batch coefficient of variance (CV%) of 20% and 30%, respectively, with at most one outlying QC measurement (> 2 interquartile range (IQR) from next QC). In samples, measurements lying at > 1.5 standard deviations (SD) from the next closest measurement were set to missing. Batch effect was corrected by dividing the metabolite concentrations by the intra-batch median-to-inter-batch median ratio.

Metabolites with > 20% missing values were excluded from the analysis. Sums and ratios were calculated: sum of LPC ($\sum$LPC), LPCa/PCaa, Σ(LPCa16:0 + LPCa18:0)/ΣPCaa due to the frequently reported associations of different LPC species with weight gain and OB [12], Asn/Asp and Gln/Gly as markers for TCA cycle replenishment, NEFA16:1/16:0 and NEFA18:1/18:0 as markers for SCD-1 activity, AC16:0/AC0 and AC2:0/AC16:0 as markers for carnitine palmitoyl transferase-1 activity (CPT-1) and FA beta-oxidation, respectively [13]. Concentrations were log-transformed with base 2. Boxplots and quantile–quantile plots were used to identify outliers further away than 1 SD from the next measurement.

Population demographics

General characteristics of the study participants are summarized in Table 1. Unlike the initial PREOBE study

Table 1 Baseline characteristics of the population included in the study

Parameters	NW (n = 49)	OW/OB (n = 40)	GDM (n = 27)	*p* value
Maternal nationality				
Spanish	47 (95.9%)	39 (97.5%)	25 (92.6%)	ns
Other	2 (4.1%)	1 (2.5%)	1 (3.70%)	
Smoking during pregnancy				
No	38 (84%)	32 (86%)	18 (90%)	ns
Yes	7 (16%)	5 (14%)	2 (10%)	
(missing)	4	3	7	
Baby sex				
Boy	22 (45%)	20 (50%)	16 (59%)	ns
Girl	27 (55%)	20 (50%)	11 (41%)	
Type of delivery				
Vaginal	43 (88%)	24 (60%)	16 (59%)	**0.003**
Caesarean	6 (12%)	16 (40%)	11 (41%)	
Breastfeeding				
At 3 months	21 (54%)	14 (42%)	10 (48%)	ns
Missing	10	7	6	
Maternal age	31 ± 6.000	31.500 ± 4.500	35 ± 6.500	**0.002**
Pre-pregnancy BMI	21.872 ± 2.190	30.111 ± 5.341	26.644 ± 8.400	**< 0.0001**
Gestational weight gain (GWG) at 34 weeks	12.000 ± 3.900	8.100 ± 7.650	4.700 ± 10.550	**< 0.0001**
Gestational age at birth	39 ± 2.000	40 ± 2.000	39 ± 2.000	**0.005**
Maternal glucose at delivery	80 ± 25.500 [2]	87 ± 32.500 [5]	104.500 ± 38.250 [3]	**0.045**
Neonatal glucose at birth	73 ± 30.000 [20]	63 ± 29.000 [9]	77 ± 31.000 [10]	ns
Neonatal BMI	13.000 ± 1.400 [8]	13.303 ± 1.692 [3]	13.329 ± 1.938 [2]	ns
Neonatal BMI for age_WHO_percentile	39.000 ± 40.300 [8]	47.300 ± 48.400 [3]	47.000 ± 49.450 [3]	ns
Neonatal BMI for age WHO_z-score	− 0.210 ± 1.240 [8]	− 0.070 ± 1.330 [3]	− 0.060 ± 1.490 [2]	ns
Neonatal conicity index	1.161 ± 0.091 [29]	1.202 ± 0.077 [15]	1.172 ± 0.050 [7]	ns
Neonatal length for age WHO percentile	67.600 ± 37.200 [9]	72.200 ± 34.400 [3]	52.400 ± 54.100 [4]	ns
Neonatal length for age WHO *z*-score	0.460 ± 1.070 [8]	0.590 ± 1.060 [3]	0.330 ± 1.460 [2]	ns
Neonatal waist circumference	32.000 ± 3.000 [28]	34.000 ± 3.500 [15]	33.000 ± 3.200 [6]	ns
Neonatal weight for age WHO percentile	42.500 ± 44.425 [3]	57.950 ± 45.000	69.400 ± 52.050	ns
Neonatal weight for age WHO *z*-score	− 0.190 ± 1.178 [3]	0.200 ± 1.218	0.510 ± 1.505	ns
BMI at 1 year (1 year)	16.462 ± 1.426 [12]	16.529 ± 1.264 [9]	17.294 ± 1.919 [7]	**0.027**
1-Year BMI for age WHO percentile	46.600 ± 38.500 [12]	50.150 ± 38.175 [10]	71.550 ± 46.275 [7]	**0.047**
1-Year BMI for age WHO z-score	− 0.090 ± 1.010 [12]	0.040 ± 1.135 [9]	0.575 ± 1.462 [7]	0.064
1-Year conicity index	1.114 ± 0.078 [12]	1.095 ± 0.051 [9]	1.128 ± 0.090 [7]	ns
1-Year length for age WHO percentile	56.200 ± 50.100 [12]	55.800 ± 46.300 [11]	52.900 ± 44.450 [7]	ns
1-Year length for age WHO *z*-score	0.160 ± 1.450 [12]	0.150 ± 1.405 [9]	0.070 ± 1.200 [7]	ns
1-Year waist circumference	42.500 ± 4.200 [12]	42.500 ± 4.500 [9]	44.000 ± 4.250 [7]	0.063
1-Year weight for age WHO percentile	63.600 ± 42.200 [12]	61.000 ± 53.300 [9]	67.050 ± 24.225 [7]	ns
1-Year weight for age WHO *z*-score	0.350 ± 1.140 [12]	0.280 ± 1.500 [9]	0.445 ± 0.665 [7]	ns

Values are expressed as median ± IQR, unless otherwise stated. *ns* Non-significant *p*-value

Statistically significant *p*-values are highlighted in bold

design, for this analysis we grouped together the categories 'OW' and 'OB', thus resulting in 3 groups of infants born to NW ($n = 49$), OW/OB ($n = 40$) and GDM mothers regardless of their BMI ($n = 27$). Differences between covariates in the three groups were evaluated via

Kruskal–Wallis test or Fisher's test for continuous or categorical covariates, respectively.

Associations between the cord blood metabolites and infant anthropometry

First, univariate linear regression models were calculated between birth anthropometric measures (WHO-standardized weight, length and BMI) and log2 metabolite concentrations in each group (NW, OW/OB and GDM). Additionally, sensitivity analysis was performed by including the following covariates one at a time: maternal age, mode of delivery, GWG and GA at birth.

For the same measures at 1 year of age, multivariate regression models were used with change in the anthropometry measure as outcome, log2 metabolite concentration and anthropometry at birth as linear predictors. Sensitivity analysis was conducted as explained above, with the addition of breastfeeding information up to 1 year.

Associations were considered significant if $p < 0.05$; because of the exploratory nature of the study, we did not correct for multiple testing, but inspected the scatterplots and model diagnostics to avoid artefacts. Associations that were significant in at least one of the three groups and robust for at least three confounders were visually inspected in the groups for comparison. Moreover, differences in metabolite levels between the three groups were calculated (Supplementary Table S1) and visualized using boxplots (Supplementary Fig. S2).

Results

Baseline characteristics of mother–infant pairs in the current study are reported in Table 1. Statistically significant differences in maternal age, mode of delivery, GWG and GA at birth were observed between the study groups ($p < 0.05$). Regarding BMI, although there was no significant difference in BMI *z*-scores between the study groups at birth, a trend towards higher BMI, BMI WHO percentiles and *z*-scores ($p < 0.1$) was detected at 1 year. Boxplots were plotted and showed a clear tendency for higher BMI in the GDM infant group relative to the other two groups (Fig. 1). Pairwise differences were calculated using Mann–Whitney *U*-test showing statistically significant differences in BMI *z*-scores between the GDM versus NW group ($p = 0.023$) but not in OW/OB group.

Associations of the cord blood metabolome with *z*-BMI at birth

In the NW group, a large number of phospholipids (PL) were positively correlated with *z*-BMI, including ∑LPCa, several single species, several PUFA-containing PL (PL-PUFA), plus PCae34:0, PCae40.5, SM39:1, SM42.6 and lactic acid

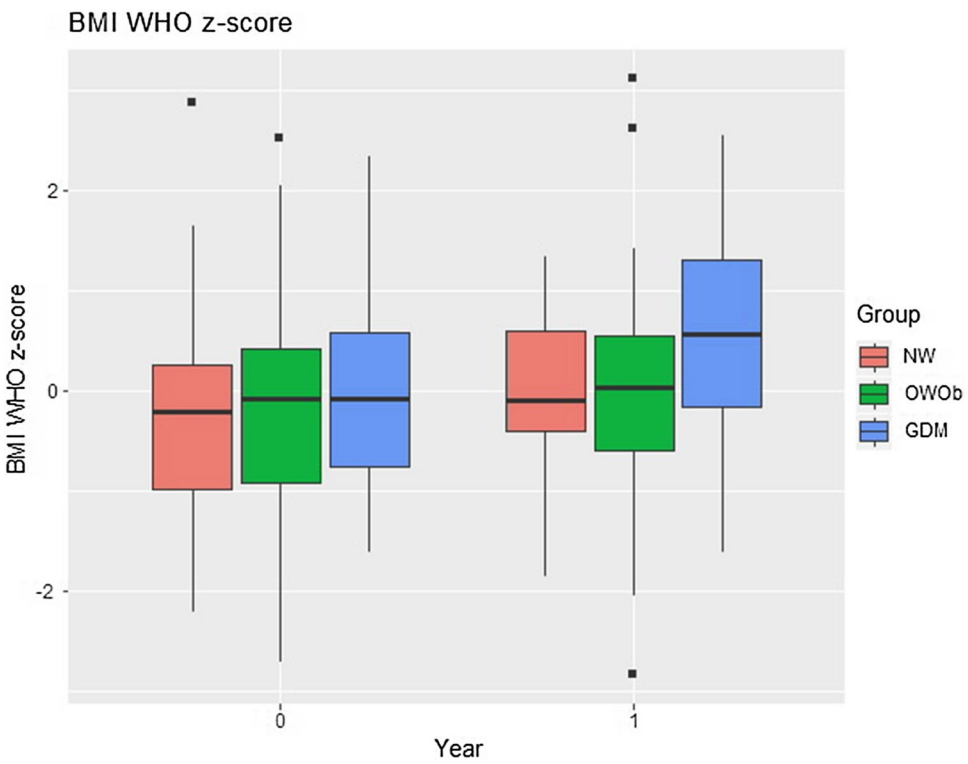

Fig. 1 Boxplots showing differences in 1-year BMI *z*-scores between infants born to normal weight (NW), overweight/obese (OW/OB) and gestational diabetes mellitus (GDM) mothers

Table 2 Significant results from the linear models for cord blood metabolites with BMI for age z-scores (z-BMI) at birth in infants born to normal weight (NW), overweight/obese (OW/OB) and gestational diabetes mellitus (GDM) mother groups

Analyte	Group	*p*-value (after FDR correction)	β	Standard error (SE)	*p*-value	Adjusted R^2
Lactic acid	NW	0.16	0.85	0.31	0.009	0.16
LPC16:0	NW	0.16	1.10	0.40	0.009	0.15
LPC18:0	NW	0.13	0.99	0.31	0.003	0.19
LPC18:1	NW	0.45	0.67	0.32	0.045	0.08
LPC20:3	NW	0.16	0.87	0.30	0.006	0.17
LPC20:4	NW	0.13	0.98	0.29	0.002	0.26
LPC22:6	NW	0.13	0.79	0.24	0.002	0.26
PCaa 36:6	NW	0.45	0.41	0.20	0.045	0.08
PCaa:38:5	NW	0.41	0.62	0.28	0.033	0.12
PCaa:38:6	NW	0.20	0.76	0.29	0.013	0.13
PCaa:40:4	NW	0.30	0.61	0.25	0.022	0.14
PCaa:40:5	NW	0.13	0.68	0.19	0.001	0.23
PCaa:40:6	NW	0.13	0.92	0.28	0.003	0.21
PCae:34:0	NW	0.45	0.59	0.29	0.047	0.10
PCae:40:5	NW	0.20	0.77	0.29	0.012	0.14
SM39:1	NW	0.45	0.73	0.35	0.044	0.08
SM42:6	NW	0.16	0.77	0.28	0.009	0.15
∑LPCa	NW	0.16	1.11	0.39	0.007	0.16
NEFA 16:2	OW/OB	0.52	− 0.64	0.22	0.008	0.21
NEFA 20:5	OW/OB	0.35	− 0.56	0.18	0.003	0.20
PCaa30:4	OW/OB	0.73	− 0.46	0.22	0.048	0.08
PCae44:5	OW/OB	0.67	− 0.86	0.36	0.026	0.16
PCae40:1	OW/OB	0.73	0.63	0.30	0.041	0.09
PCaa34:2	GDM	0.42	− 0.89	0.42	0.043	0.14
PCaa34:3	GDM	0.42	− 0.97	0.46	0.047	0.13
PCaa36:2	GDM	0.42	− 1.01	0.43	0.027	0.17
PCaa38:6	GDM	0.42	− 1.10	0.46	0.026	0.17
PCaa40:4	GDM	0.42	− 1.26	0.53	0.032	0.22
PCaa40:6	GDM	0.42	− 0.98	0.39	0.018	0.19
PCae36:1	GDM	0.42	− 1.48	0.45	0.003	0.30
PCae:C36:2	GDM	0.42	− 1.37	0.53	0.016	0.20
PCae:C36:3	GDM	0.42	− 1.06	0.39	0.013	0.22
PCae:C38:0	GDM	0.42	− 0.82	0.30	0.013	0.21
PCae:C38:2	GDM	0.42	− 1.21	0.55	0.043	0.20
PCae:C38:6	GDM	0.42	− 1.24	0.53	0.028	0.16
SM33:1	GDM	0.42	− 0.92	0.44	0.047	0.13
SM38:2	GDM	0.42	− 1.07	0.43	0.019	0.19
SM38:3	GDM	0.42	− 0.98	0.43	0.033	0.15
SM40:2	GDM	0.42	− 1.02	0.48	0.049	0.18
SM40:5	GDM	0.42	− 0.97	0.42	0.030	0.16
∑PC:aa	GDM	0.42	− 1.23	0.52	0.029	0.17
∑PC:aa:unsaturated	GDM	0.42	− 1.23	0.52	0.028	0.17

LPCa acyl-lysophosphatidylcholines; *PCaa* diacyl-phosphatidylcholines; *PCae* acyl-alkyl-phosphatidylcholines; *SM* sphingomyelins; *LPCa* acyl-lysophosphatidylcholines; *NEFA* non-esterified fatty acids

(Table 2). PCae42.2, SM44.6 and the ratio depicting CPT1 activity showed positive correlations, yet not supported by the scatterplots' inspection (Supplementary Table S3).

In the OW/OB group, fewer correlations were found, which were majorly negative (Table 2). These correlations comprised the PL-PUFA: PCaa30:4 and PCae44:5 as well as NEFA16:2 and NEFA20:5. Only PCae40:1 showed a positive correlation. Moreover, a pattern of negative correlations with BMI was detected for some medium-chain AC (AC8:0, AC8:0-OH, AC10:0, AC12:1, AC14:1) and for

NEFA14:1 and NEFA17:1 but was not confirmed by scatterplots' inspection (Supplementary Table S3).

In GDM offspring, many (mainly unsaturated) PC and SM, including ∑PCaa, were negatively correlated with *z*-BMI (Table 2). Three additional PL and Cys were negatively associated with *z*-BMI, but were not confirmed in the scatterplots (Supplementary Table S3).

Associations of the cord blood metabolome with Δ *z*-BMI at 1 year

In the NW group, we found several negative correlations between Δ *z*-BMI at 1 year and the metabolite groups: LC-PUFA and PC-PUFA, some TCA intermediates (malic and isocitric acid), as well as Asp, and medium-chain AC, even though the latter was not confirmed by the scatterplots' inspection (Supplementary Table S6). Asn-to-Asp ratio showed a positive correlation (Table 3). The LPC, strongly associated with BMI at birth, did not present significant correlations with 1-year Δ *z*-BMI; also, no trends could be seen in the plots. We additionally inspected their relationship to Δ *z*-weight and *z*-length, but no correlation was found (Supplementary Tables S7 and S8).

In the OW/OB group, only a negative correlation with NEFA26:0 could be identified; in particular, the visual inspection of LPC associations with Δ *z*-BMI showed no trend. Suspecting that the change in LPC was due to growth rather than increase with BMI, relationships between LPC, related ratios and Δ *z*-weight and Δ *z*-length were inspected. Even though not statistically significant, LPCa/PCaa and Σ(LPCa16:0 + LPCa18:0)/ΣPCaa were negatively correlated with both Δ z-weight and z-length (Supplementary Tables S7 and S8).

Also, in the GDM group, few significant correlations were found, with His being the only metabolite significantly associated with Δ z-BMI after plots' inspection (positive direction). Interestingly, the scatterplots with LOESS smoother hinted towards a parabolic relationship with LPC16:0, LPC18:0, LPC20:4 and ∑LPC, initially decreasing and then increasing with *z*-BMI (data not shown).

Significant results from the linear models for cord blood metabolites with 1-year Δ *z*-BMI are shown in Table 3. Also, results from linear models for all investigated metabolites with anthropometric measures at birth and at 1 year are listed in Supplementary Tables S3–S5 and S6–S8, respectively.

Table 3 Significant results from the linear models for cord blood metabolites with the 1-year BMI for age *z*-scores (*z*-BMI) in infants born to normal weight (NW), overweight/obese (OV/OB) and gestational diabetes mellitus (GDM) mother groups

Analyte	Group	*p*-value (FDR)	*β*	Standard error (SE)	*p*-value	Adjusted R^2
Asp	NW	0.30	− 0.25	0.10	0.017	0.65
Malic acid	NW	0.30	− 0.39	0.14	0.008	0.65
Isocitric acid	NW	0.30	− 0.93	0.33	0.009	0.64
Citric acid	NW	0.30	− 0.74	0.29	0.016	0.63
NEFA20:4	NW	0.30	− 0.42	0.16	0.011	0.63
NEFA22:4	NW	0.36	− 0.43	0.21	0.046	0.60
NEFA22:5	NW	0.32	− 0.43	0.17	0.019	0.62
NEFA22:6	NW	0.34	− 0.45	0.19	0.027	0.62
NEFA24:4	NW	0.34	− 0.47	0.20	0.026	0.62
NEFA24:5	NW	0.30	− 0.55	0.21	0.013	0.63
NEFA24:6	NW	0.36	− 0.40	0.19	0.043	0.61
PCaa36.4	NW	0.34	− 0.66	0.29	0.029	0.61
PCaa38.4	NW	0.36	− 0.67	0.31	0.043	0.60
PCaa40.4	NW	0.34	− 0.63	0.28	0.032	0.52
PCaa40.5	NW	0.36	− 0.44	0.20	0.038	0.60
PCae38.4	NW	0.30	− 0.78	0.28	0.010	0.63
PCae38.5	NW	0.34	− 0.70	0.29	0.023	0.61
PCae40.4	NW	0.30	− 0.68	0.24	0.009	0.63
SM41.1	NW	0.36	− 0.69	0.31	0.035	0.60
AC18:2	NW	0.34	− 0.44	0.19	0.031	0.59
Asn-to-Asp ratio	NW	0.30	0.36	0.12	0.004	0.68
NEFA 26:0	OW/OB	0.98	− 1.14	0.36	0.005	0.49
His	GDM	0.90	1.72	0.61	0.013	0.79

NEFA non-esterified fatty acids; *PC.aa* diacyl-phosphatidylcholines; *PC.ae* acyl-alkyl-phosphatidylcholines; *SM* sphingomyelins; *AC* acylcarnitine

Discussion

Associations of the cord blood metabolome with *z*-BMI at birth

Overall, differences were detected in the directions of associations between PL and *z*-BMI between the three groups. PL-PUFA were the most prominent species showing behavioural differences, since many of them were positively associated with *z*-BMI in the NW group but negatively in OW/OB and GDM groups. On the other hand, ∑LPC positively correlated with *z*-BMI in the NW group but showed no relation in OW/OB and GDM groups, despite significantly lower levels of several LPC species in the latter two. This implies that maternal OB/GDM suppresses these associations or alters their direction. This could be also observed in the lower metabolite levels (Supplementary Table S1). To our surprise, the three groups did not difference with regard to birth anthropometry (Table 1).

This could be explained since, in NW pregnancies, PC-PUFA and LPC are increasingly supplied from mother to foetus as a source of FA required for normal foetal growth and development [14, 15]. Conversely, in OW/OB or GDM pregnancies, it was suggested that these metabolites are not only increasingly supplied to the foetus from the maternal circulation, but even more increasingly sequestered by the active foetal metabolic tissues with higher tendency for lipid accumulation and adiposity. Thus, we hypothesize that increased accretion of unsaturated PC, especially PC-PUFA, in foetal tissues leads to lower cord blood levels of these metabolites in OW/OB and GDM groups. In other words, babies born to OW/OB mothers are 'hyper-responsive', i.e. prone to accumulate excess body fat and metabolic derangement. The hypothesis was supported by previous findings in twins and triplets, with higher total foetal mass and lower cord blood PC-PUFA, compared to singletons [16]. In general, lower levels of unsaturated PL, especially n-3 PUFA, were related to metabolic syndrome, obesity and diabetes in infants and adolescents due to their role in maintaining membranes' fluidity and preventing the molecules from packing together [17]. Moreover, a modulation in the placental FA transport and metabolism was reported in obese mothers, mediated by a variety of FA binding proteins (FABP) [18]. Alternatively, it was suggested that obesity and/or diabetes stimulates placental lipid storage pathways by upregulating expression of key proteins in the lipid esterification pathway. Thus, FA uptake by the trophoblast for esterification and storage in the placenta [19] is enacted as a defence mechanism to protect the foetus from the excess maternal lipid supply possibly associated with overweight/obesity or secondary to insulin resistance. In other words, the mother buffers the effects of extrauterine environment on the developing foetus [20, 21]. This hypothesis might provide an explanation why no difference was detected in the birth anthropometry (*z*-BMI) in the OB/GDM relative to NW groups [22].

Lower plasma LPC levels were reported in OV/OB subjects with and without T2D and were explained by changes in the metabolic status [23–27]. These changes involve increased LPC uptake by metabolically active tissues as liver, muscle or adipose tissue, however, with no evidence supporting this hypothesis [23]. It was previously suggested that increased flux of FA from the adipose tissue to the liver in obesity together with altered FA and lipoproteins metabolism causes the LPC accumulation in the liver, thus altering their secretion [28].

In line with this picture, medium-chain AC, NEFA 20:5 in the OW/OB group and Cys in the GDM group correlated negatively with z-BMI. For the former finding, we speculate that the presence of tendency for enhanced anabolism and reduced catabolic pathways in OB/OW group might initiate enhanced adiposity and reduced fatty acid oxidation, mirrored in decreased midchain AC. Medium-chain AC have been previously related to high lipolytic rates and signalling activated β-oxidation pathways [29]. For NEFA 20:5 (eicosapentanoic acid), an unesterified PUFA, we speculate that it behaves similar to PL-PUFA in this group, that is, PUFA are uptaken in esterified form and incorporated in total lipids, triacylglycerol and PL fraction of the placental and foetal tissues rather than freely circulating in cord blood [30].

Regarding Cys, it was previously related to inflammation in the intrauterine milieu, an expected finding in OB/GDM. Inflammation stimulates the release of adipokines, thus reactive oxygen species (ROS) production causing an oxidative stress [31]. This leads to decreased Cys levels, where Cys is directed towards glutathione biosynthesis to fight ROS, enhancing its consumption and decreasing its cord blood levels [32]. Unfortunately, we could not further test this hypothesis due to the lack of direct measurements for ROS species.

Associations of the cord blood metabolome with infant *z*-BMI at 1 year of age

We observed that infants of GDM mothers showed significantly higher BMI *z*-scores at 1 year than those born to NW mothers ($p < 0.05$). This finding was in line with our previous results [10], showing that although both maternal OB and GDM are associated with adverse metabolic alterations in mother and foetus, the impact of GDM is much more pronounced. Accordingly, it would not be surprising to observe a stronger impact of GDM on the infant anthropometry. These results also matched previous reports, suggesting that GDM effect on childhood growth is independent of infant

BW [33, 34], while in contrast with others claiming a relationship partially mediated by effects on BW [3, 35].

Regarding the associations of cord blood metabolites with infants 1-year Δ *z*-BMI, we did not find an extension of the associations previously monitored at birth among the three study groups. Unexpectedly, PC-PUFA that were positively associated with BW in the NW group were found to be negatively associated with Δ *z*-BMI at 1 year. In other words, 1-year higher weight gain was related to lower levels of PC-PUFA in the NW group, however, not in the OW/OB or GDM groups. This suggests that cord blood metabolome, when adjusting for the birth anthropometry, shows no predictive power for later development, at least not at 1 year. The literature about this topic is controversial: cord blood metabolome has been observed to correlate with anthropometry in children (5 years) [36] but not in adolescents (15 years) [12]. These discrepancies could be attributed to the role played by postnatal factors, such as diet quality (i.e. exposure to postnatal high-calorie or high-fat feeding, food preferences and dietary habits [37, 38]) and epigenetic modifications that might manifest at later time points. Lack of consistency of the associations between birth and 1 year further supports this hypothesis.

Another important observation is that the associations seen in two important species (PC-PUFA and medium-chain AC) with 1-year Δ *z*-BMI in the NW group are similar to those seen in the OB/OW at birth. This could mean that a baby, regardless of his/her mother's phenotype, can develop an in utero metabolism favouring later overweight/obesity not appearing at birth and needing more time to manifest.

Conversely, none of these associations were found in the OW/OB group, while LPCa-to-PCaa and Σ(LPCa 16:0 + LPCa 18:0)-to-ΣPCaa ratios were negatively associated with Δ length and weight, that is, overall growth but not risk of overweight. In GDM, we found mild indications for nonlinear relationships between LPC16:0, LPC18:0, LPC20:4 and ∑LPC with z-BMI, initially decreasing and then increasing. We speculate that the directions of associations between these metabolites and *z*-BMI might be dependent on their concentration ranges.

Advantages and limitations

Our study is one of the few investigating the independent impact of maternal BMI and GDM on associations of cord blood metabolites with infant anthropometry, using a metabolite panel of > 200 metabolites involved in energy metabolism. Nonetheless, some limitations deserve comment: primarily the limited sample number in the study groups and lack of information on socioeconomic status, smoking habits, diet and education, which impeded the investigation of potential confounding variables such as elevated maternal age or higher incidence of caesarean section in the GDM and OW/OB groups. The study investigates correlations with BMI, which do not fully capture growth. Unfortunately, body composition measures, including fat-free mass and lean mass which could be a better measure of growth in childhood, were unavailable for analysis.

Conclusion

Our results indicate that GDM is associated with overweight in offspring at 1 year independently of the birth weight, but no strong associations of 1-year anthropometry and cord blood metabolome could be found in this group. In this study, associations between the cord blood metabolome and infant anthropometry at birth in OB/GDM groups hinted to possible future offspring overweight and adiposity. However, lack of extension of these correlations to the 1-year infant anthropometry indicates poor predictive power of cord blood metabolome for later development, at least at 1 year.

Acknowledgements The authors thank the study participants, the obstetricians, paediatricians and technicians of the EURISTIKOS team and the PREOBE team at the University of Granada. We are grateful to Stephanie Winterstetter, Alexander Haag and Tina Honsowitz for their support in the analysis.

Funding This work was supported by Andalusian Ministry of Economy, Science and Innovation, PREOBE Excellence Project (Ref. P06-CTS-02341), Spanish Ministry of Economy and Competitiveness (Ref. BFU2012-40254-C03-01 and Ref. SAF2015-69265-C2-2-R), the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG 322605), European Commission research projects EarlyNutrition, FP7–FP7 KBBE-2011-1 (289346 y) and Horizon2020 DynaHEALTH (633595).

Compliance with ethical standards

Conflict of interest None of the authors reports conflicts of interest.

Ethical approval The study was approved by the Bioethical Committees for Clinical Research of the Clinical University Hospital San Cecilio, the Mother-Infant University Hospital of Granada, Spain.

Informed consent Participation was voluntary, and written informed consent was obtained from participants at study entry.

References

1. OECD (2016) Overweight and obesity among adults. In: Health at a glance: Europe 2016: state of health in the EU cycle. OECD Publishing, Paris
2. Ahrens W, Pigeot I, Pohlabeln H et al (2014) Prevalence of overweight and obesity in European children below the age of 10. Int J Obes (Lond) 38(Suppl 2):S99–S107. https://doi.org/10.1038/ijo.2014.140

3. Schaefer-Graf UM, Pawliczak J, Passow D et al (2005) Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. Diabetes Care 28(7):1745–1750
4. Catalano PM, McIntyre HD, Cruickshank JK et al (2012) The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. Diabetes Care 35(4):780–786. https://doi.org/10.2337/dc11-1790
5. Yessoufou A, Moutairou K (2011) Maternal diabetes in pregnancy: early and long-term outcomes on the offspring and the concept of "metabolic memory". Exp Diabetes Res 2011:218598. https://doi.org/10.1155/2011/218598
6. Buchanan TA, Xiang AH (2005) Gestational diabetes mellitus. J Clin Investig 115(3):485–491. https://doi.org/10.1172/JCI24531
7. Blackwell SC, Landon MB, Mele L et al (2016) Relationship between excessive gestational weight gain and neonatal adiposity in women with mild gestational diabetes mellitus. Obstet Gynecol 128(6):1325–1332. https://doi.org/10.1097/aog.0000000000001773
8. Catalano PM, Hauguel-De Mouzon S (2011) Is it time to revisit the Pedersen hypothesis in the face of the obesity epidemic? Am J Obstet Gynecol 204(6):479–487. https://doi.org/10.1016/j.ajog.2010.11.039
9. Pintaudi B, Fresa R, Dalfra M et al (2018) The risk stratification of adverse neonatal outcomes in women with gestational diabetes (STRONG) study. Acta Diabetol 55(12):1261–1273. https://doi.org/10.1007/s00592-018-1208-x
10. Shokry E, Marchioro L, Uhl O et al (2019) Impact of maternal BMI and gestational diabetes mellitus on maternal and cord blood metabolome: results from the PREOBE cohort study. Acta Diabetol 56(4):421–430. https://doi.org/10.1007/s00592-019-01291-z
11. Berglund SK, García-Valdés L, Torres-Espinola FJ et al (2016) Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). BMC Public Health 16(1):207. https://doi.org/10.1186/s12889-016-2809-3
12. Hellmuth C, Uhl O, Standl M et al (2017) Cord blood metabolome is highly associated with birth weight, but less predictive for later weight development. Obes Facts 10(2):85–100. https://doi.org/10.1159/000453001
13. Sampath H, Ntambi JM (2008) Role of stearoyl-CoA desaturase in human metabolic disease. Future Lipidol 3(2):163–173
14. Haggarty P (2002) Placental regulation of fatty acid delivery and its effect on fetal growth—a review. Placenta 23:S28–S38. https://doi.org/10.1053/plac.2002.0791
15. Prieto-Sánchez MT, Ruiz-Palacios M, Blanco-Carnero JE et al (2017) Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. Clin Nutr 36(2):513–521. https://doi.org/10.1016/j.clnu.2016.01.014
16. Zeijdner EE, Houwelingen ACV, Kester ADM, Hornstra G (1997) Essential fatty acid status in plasma phospholipids of mother and neonate after multiple pregnancy. Prostaglandins Leukot Essent Fatty Acids 56(5):395–401. https://doi.org/10.1016/S0952-3278(97)90590-1
17. Karlsson M, Mårild S, Brandberg J, Lönn L, Friberg P, Strandvik B (2006) Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. Obesity (Silver Spring) 14(11):1931–1939. https://doi.org/10.1038/oby.2006.225
18. Uhl O, Demmelmair H, Segura MT et al (2015) Effects of obesity and gestational diabetes mellitus on placental phospholipids. Diabetes Res Clin Pract 109(2):364–371. https://doi.org/10.1016/j.diabres.2015.05.032
19. Visiedo F, Bugatto F, Sánchez V, Cózar-Castellano I, Bartha JL, Perdomo G (2013) High glucose levels reduce fatty acid oxidation and increase triglyceride accumulation in human placenta. Am J Physiol Endocrinol Metab 305(2):E205–E212. https://doi.org/10.1152/ajpendo.00032.2013
20. Wells J (2011) An evolutionary perspective on the trans-generational basis of obesity. Ann Hum Biol 38:400–409. https://doi.org/10.3109/03014460.2011.580781
21. Wells J (2012) A critical appraisal of the predictive adaptive response hypothesis. Int J Epidemiol 41:229–235. https://doi.org/10.1093/ije/dyr239
22. Dubé E, Gravel A, Martin C et al (2012) Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. Biol Reprod 87(1):14. https://doi.org/10.1095/biolreprod.111.098095
23. Barber MN, Risis S, Yang C et al (2012) Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. PLoS ONE 7(7):e41456. https://doi.org/10.1371/journal.pone.0041456
24. Del Bas JM, Caimari A, Rodriguez-Naranjo MI et al (2016) Impairment of lysophospholipid metabolism in obesity: altered plasma profile and desensitization to the modulatory properties of n-3 polyunsaturated fatty acids in a randomized controlled trial. Am J Clin Nutr 104(2):266–279. https://doi.org/10.3945/ajcn.116.130872
25. Heimerl S, Fischer M, Baessler A et al (2014) Alterations of plasma lysophosphatidylcholine species in obesity and weight loss. PLoS ONE 9(10):e111348. https://doi.org/10.1371/journal.pone.0111348
26. Lehmann R, Franken H, Dammeier S et al (2013) Circulating lysophosphatidylcholines are markers of a metabolically benign nonalcoholic fatty liver. Diabetes Care 36(8):2331–2338. https://doi.org/10.2337/dc12-1760
27. Wallace M, Morris C, O'Grada CM et al (2014) Relationship between the lipidome, inflammatory markers and insulin resistance. Mol BioSyst 10(6):1586–1595. https://doi.org/10.1039/c3mb70529c
28. Fabbrini E, Sullivan S, Klein S (2010) Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology (Baltimore, MD) 51(2):679–689. https://doi.org/10.1002/hep.23280
29. Lehmann R, Zhao X, Weigert C et al (2010) Medium chain acylcarnitines dominate the metabolite pattern in humans under moderate intensity exercise and support lipid oxidation. PLoS ONE 5(7):e11519. https://doi.org/10.1371/journal.pone.0011519
30. Gazquez A, Ruiz-Palacios M, Larque E (2017) DHA supplementation during pregnancy as phospholipids or TAG produces different placental uptake but similar fetal brain accretion in neonatal piglets. Br J Nutr 118(11):981–988. https://doi.org/10.1017/s0007114517002951
31. Marseglia L, Manti S, D'Angelo G et al (2014) Oxidative stress in obesity: a critical component in human diseases. Int J Mol Sci 16(1):378–400. https://doi.org/10.3390/ijms16010378
32. Dudzik D, Zorawski M, Skotnicki M et al (2014) Metabolic fingerprint of gestational diabetes mellitus. J Proteomics 103:57–71. https://doi.org/10.1016/j.jprot.2014.03.025
33. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA (2003) Maternal gestational diabetes, birth weight, and adolescent obesity. Pediatrics 111(3):e221–e226
34. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ (2007) Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. Diabetes Care 30(9):2287–2292. https://doi.org/10.2337/dc06-2361
35. Baptiste-Roberts K, Nicholson WK, Wang N-Y, Brancati FL (2012) Gestational diabetes and subsequent growth patterns of offspring: the National Collaborative Perinatal Project. Matern Child Health J 16(1):125–132. https://doi.org/10.1007/s10995-011-0756-2
36. Cole TJ, Singhal A, Fewtrell MS, Wells JC (2016) Weight centile crossing in infancy: correlations between successive months show evidence of growth feedback and an infant-child growth transition.

Am J Clin Nutr 104(4):1101–1109. https://doi.org/10.3945/ajcn.116.139774
37. Beauchamp G, Mennella J (2009) Early flavor learning and its impact on later feeding behavior. J Pediatr Gastroenterol Nutr 48(1):S25–S30. https://doi.org/10.1097/mpg.0b013e31819774a5
38. Mennella JA (2014) Ontogeny of taste preferences: basic biology and implications for health. Am J Clin Nutr 99(3):704s–711s. https://doi.org/10.3945/ajcn.113.067694

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