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MATERNAL-FETAL MEDICINE

Investigation of the impact of birth by cesarean section on fetal and maternal metabolism

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Abstract

Purpose Elective cesarean section (CS) was related to long-term adverse health effects in the offspring, but little is known about underlying mechanisms. Our study investigates the metabolic changes in both maternal and cord blood associated with CS in comparison to vaginal delivery (VD) to explore potential causal pathways.

Methods Samples obtained from PREOBE study participants were subjected to LC–MS/MS-targeted metabolomics comprising > 200 metabolites.

Results Elective CS showed an impact on both maternal and cord blood metabolomes. In maternal blood, the CS group showed lower levels of phospholipids (PL), principally ether-linked phosphatidylcholines (aaPC), pyruvic acid, branched chain keto-acids (BCKA), and other gluconeogenic substrates, but since the CS group showed different HDL levels in comparison to the VD group, we could not exclude contribution of the latter in the findings. In cord blood, the most remarkable finding in the CS group was the high levels of Cys; conversely, the lower levels of non-esterified fatty acids (NEFA), some tricarboxylic acid (TCA) cycle metabolites, gluconeogenic substrates, markers of β-oxidation, and the sum of hexoses were lower in CS-born babies in addition to tendentially lower levels of PL.

Conclusions We speculate that lower levels of maternal and fetal corticosteroids in CS, due to less stressful condition, cause metabolic perturbations at birth initiating future negative health outcomes. This further supports the early programming hypothesis.

Keywords Mode of delivery · Metabolomics · Cord blood · Metabolic adaptation · Stress hormones

Introduction

The concept of critical periods in fetal development is now well-established suggesting that early life events during certain phases of development could result in permanent

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changes and thus major impacts on later health outcomes [1]. 'Metabolic imprinting' triggered by altered intrauterine environment is considered to increase the risk of obesity and diabetes in children [2]. The mode of delivery may also have a lasting impact [1]. Cesarean section (CS) has potentially deleterious effects on both the mother and the child. In mothers, it may increase the risk of infection, thrombosis, hospital stay, and other complications [3]. In children, epidemiological studies related CS delivery to increased risk for several chronic diseases such as diabetes, obesity, allergies, asthma, and autism [4–6].

Several mechanisms have been suggested to explain the effects of CS on the development of long-term child outcomes [7]. Different microbiota colonization patterns in infants due to the absence of the initial inoculation of maternal vaginal and fecal microbiota and resulting in the use of antibiotics may play a role [8–10]. Another hypothesis is a lack of the biological stimuli concomitant with VD, which initiate metabolic changes to allow the newborn to adapt to extra-uterine life. In CS, altered responses may occur resulting in different metabolism, especially in the liver [11].

Hormonal changes are considered an integral part of normal labor necessary to support both physical and emotional changes [12]. After normal VD, fetal and infant stress hormones (predominantly adrenaline and cortisol) promote glycogenolysis and gluconeogenesis, thus maintaining plasma glucose concentrations after the discontinued glucose supply from the mother [13–15]. Conversely, lower levels of stress hormones in elective CS were related to altered insulin secretion and glucose metabolism after birth, particularly in the liver [12].

In this study, we hypothesize that the mode of delivery (VD/elective CS) has an effect on the metabolomics profile in the maternal and cord blood, which could mediate possible long-term health effects. We test this question in the PREOBE cohort using metabolomic analysis as a sensitive tool to detect the changes in metabolites in association with the mode of delivery.

Methods

Study subjects and sample collection

Pregnant women were recruited between 2008 and 2012 at the Clinical University Hospital San Cecilio, the Mother-Infant University Hospital of Granada, Spain, and their peripheral health centers, within the observational PREOBE cohort (NCT01634464) [16]. Specifics concerning the recruitment and the clinical data collection procedures have been described previously [17]. Maternal venous blood samples were drawn into EDTA-containing tubes at delivery and cord blood samples were obtained after clamping the cord. The collected samples were processed, and plasma aliquots were stored at − 80 °C and shipped on dry ice to the laboratory of the Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-Universität Munich (LMU) where the metabolomic analysis was carried out.

Since there was no information available on the fasting status of the mothers at the time point of blood collection, we used a previously validated fasting blood glucose threshold (126 mg/dl) for diabetes to identify non-fasting, non-diabetic mothers [18]. In other words, if a non-diabetic mother presents a plasma glucose level above this threshold, this indicates non-fasted status. Accordingly, non-GDM mothers defined as non-fasted were excluded from the analysis. Nevertheless, this distinction was not possible for GDM mothers due to the absence of a threshold for fasting glucose levels in diabetes.

Mode of delivery was retrieved from medical records. For the scope of this analysis, we only included data from mother/child pairs with either standard vaginal delivery (VD) or elective CS, thus disregarding study participants with emergency CS, induction of labor, or without information about the mode of delivery. In total, 162 maternal samples and 116 cord blood samples were considered in this analysis. No information was available on the duration of labor.

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, and the Research Bioethical Committee of the University of Granada. Every participant provided her oral and written informed consent at study entry.

Metabolomic analysis

Targeted metabolomics using a MS-based platform was used to measure 400 metabolites: polar lipids (acylcarnitines (AC), phospholipids (PL) including diacyl-phosphatidylcholines (daPC), acyl-alkyl-phosphatidylcholines (aaPC), sphingomyelins (SM), acyl-lysophosphatidylcholines (LPCa), alkyl-lysophosphatidylcholines (LPCe)), sum of hexoses (H1), amino acids (AA), non-esterified fatty acids (NEFA), keto-acids and tricarboxylic acid (TCA) cycle metabolites. Concentrations were calculated in µmol/l. Throughout the manuscript, a formula CX:Y was assigned for polar lipids and NEFA where X: the number of carbon units, Y: the number of double bonds. OH is added when a hydroxyl group is present in the molecule. Letters 'a' and 'e' were added to indicate whether the acyl chain is bound via an ester or ether

bond to the backbone, respectively. IUPAC abbreviations were used for AA.

TCA and NEFA analyses were done using liquid chromatography with tandem mass spectrometry without derivatization, and AA after derivatization, as previously reported [19, 20]. Polar lipids (including acylcarnitines) were measured using flow-injection analysis tandem mass spectrometry [21]. To ensure high quality of the data, standard biological quality control (QC) samples are regularly analyzed (6 samples/batch) together with the plasma and cord blood samples. The analytical process was controlled and post-processed by Analyst 1.6.1 and R software (R Project for Statistical Computing, https://www.r-project.org/, version 3.4.3).

Quality control and data preprocessing

Data analyses were performed using the R statistical software version 3.4.3. The intra- and inter-batch coefficient of variation (CV%) values of metabolites in the QC samples (6 samples/batch, 1 measurement > 2 IQR from the next measurement, defined as outlier) were set at a threshold of 20% and 30%, respectively, as accepted limits in assessment of repeatability in the metabolomics datasets. Metabolomics measurements > 1.5 standard deviations (sd) from the next closest measurement were set to missing. Boxplots were depicted for outlier visual inspection and removal, if appropriate. After applying the QC criteria, 202 metabolites were selected for further statistical analysis. The batch effect was corrected by dividing the metabolite concentrations by the ratio intra-batch median/inter-batch median. Measurements were then split in two datasets corresponding to maternal and cord blood measurements, respectively.

Metabolites with > 40% missing values were excluded from the datasets. Sums of some metabolite classes were computed including daPC, aaPC, LPCa, and BCAA (using analytes with ≥ 80% available measurements). Likewise, ratios between some species were computed including: (a) acyl carnitine ratios (AC16:0/AC0 or AC16:0/free carnitine (Carn) and AC2:0/AC16:0) as markers of carnitine palmitoyl transferase-1 activity (CPT1) and fatty acid β-oxidation, respectively [22]; (b) FA ratios as an estimate for stearoyl-CoA desaturase 1 (SCD-1; 16:1/16:0, SCD-16 and 18:1/18:0, SCD-18) [23]; (c) AA ratios, Asn/Asp and Gln/Glu, as indicator for the anaplerotic reactions or Krebs cycle replenishing; (d) LPC ratios including: ΣLPCa/ΣdaPC, as a lipid biomarker of inflammation [24]; (LPCa 16:0 + LPCa 18:0)/ΣPC aa as a pro-inflammatory biomarker [25]; (LPCa 18:1 + LPCa 18:2)/ΣdaPC as an anti-inflammatory biomarker [26]; and (e) ΣdaPC/ΣaaPC, reflecting oxidative stress [27, 28]. Logarithmic transformation (log base 2) was applied for all analytes, sums, and ratios after inspection of boxplots and quantile–quantile plots. Points > 1sd from the next measurement were defined as outliers and were excluded.

Statistical analysis

The impact of the CS delivery on the metabolite concentrations in maternal/cord blood was determined using multiple linear regression models with log2 of the metabolite concentration as outcome and mode of delivery (CS, y/n) as the independent variable. Covariate adjustment was done by adding pBMI, GDM status (y/n), GWG at 34 weeks, birth weight, and maternal age to the model. Preliminary analyses including gestational age, infant sex, and mode of anesthesia showed no significant associations with the metabolites, therefore, they were not included in the final model. The final model used was log2 of the metabolite concentration = mode of delivery (CS; y/n) + pBMI + GDM status (y/n) + GWG + birth weight + maternal age. For maternal blood only, we additionally adjusted the models for maternal HDL concentrations at delivery, since they were different in the two groups; variance inflation factors (VIFs) for GDM and HDL were calculated in all models including both covariates. Additionally, we run the models using only data from non-GDM subjects. Significant associations were additionally visually inspected via diagnostic and partial residual plots [29]. p values were corrected for multiple testing via false discovery rate (FDR) at alpha level 0.05, however, we also inspected interesting associations with uncorrected p value < 0.05 ('trends'). The results are summarized in Manhattan plots.

Results

Baseline characteristics of the populations used in the study (maternal and cord blood), subdivided into the two groups of interest (VD versus CS), are reported in Table 1. Continuous covariates were compared via Kruskal test, categorical via Chi square test. In general, elective CS was more opted for by high BMI and/or GDM mothers with heavier babies. Glucose was lower in the cord blood of CS babies. In addition, mothers who underwent CS had lower HDL cholesterol throughout pregnancy and, even more markedly, at birth.

In maternal blood, pyruvic, isocitric and 3-methyl-2-oxobutanoic acid were lower in CS mothers, with the same trend evident for two more branched chain keto-acids (BCKA) (3-methyl-2-oxovaleric acid and 4-methyl-2-oxovaleric acid) at p value < 0.05 (FDR corrected) and other gluconeogenic substrates such as two AA (Pro, Trp), lactic acid, and a TCA metabolite (malic acid) at p value < 0.05 (uncorrected). The same picture was observed for several phospholipids, with many metabolites being significantly lower in the CS group even after correction for multiple

Table 1 Baseline characteristics of the populations used in the study including maternal and cord blood samples

	Maternal blood				Cord blood			
	VD ($n=115$)	CS ($n=47$)	p value		VD ($n=83$)	CS ($n=33$)	p value	
Maternal age (years)	31 ± 5.00	33 ± 5.50	0.052		32 ± 5.50	33 ± 7.00	0.086	
Pre-pregnancy BMI (kg/m^2)	24.13 ± 7.16	26.77 ± 7.86	0.008	**	23.88 ± 6.61	27.94 ± 8.13	< 0.0001	***
GWG (34 weeks) (kg)	10.55 ± 7.15 [1]	10.70 ± 9.85 [1]	0.897	ns	10.00 ± 7.35 [1]	10.50 ± 9.30	0.824	ns
Gestational age (weeks)	39.50 ± 1.00 [1]	40 ± 1.00 [4]	0.42	ns	39 ± 1.00 [1]	40 ± 1.25 [1]	0.22	ns
Birth weight	3280 ± 545.00	3510 ± 605.00	0.04	*	3250 ± 560.00	3560 ± 576.00	0.004	**
Nationality—Spanish	107 (94%) [1]	43 (93%) [1]	1	ns	79 (95%)	29 (91%) [1]	0.396	ns
Gestational diabetes—yes	25 (22%)	20 (43%)	0.011	*	16 (19%)	11 (33%)	0.143	ns
Smoking—no	89 (88%) [14]	34 (89%) [9]	1	ns	65 (87%) [8]	23 (85%) [6]	1	ns
Child sex—female	60 (53%) [1]	19 (40%)	0.17	ns	43 (52%)	15 (45%)	0.681	ns
Maternal glucose (delivery) (mg/dl)	82.50 ± 29.50 [1]	78.50 ± 24.50 [3]	0.452	ns	86 ± 30.50 [4]	84 ± 39.50 [6]	0.41	ns
Maternal triglycerides (delivery) (mg/dl)	213 ± 93.00	237 ± 75.50 [3]	0.084		215 ± 85.50 [3]	236 ± 90.00 [6]	0.129	ns
Maternal LDL cholesterol (delivery) (mg/dl)	139.50 ± 48.75 [1]	117.00 ± 64.75 [3]	0.026	*	139.00 ± 49.00 [4]	110.00 ± 60.50 [6]	0.005	**
Maternal HDL cholesterol (delivery) (mg/dl)	69.50 ± 20.75 [1]	57 ± 17.00 [3]	< 0.0001	***	69 ± 22.50 [4]	51 ± 13.00 [6]	< 0.0001	***
Maternal HDL cholesterol (24 weeks) (mg/dl)	79 ± 23.00 [26]	64 ± 15.75 [17]	0.001	***				
Maternal HDL cholesterol (34 weeks) (mg/dl)	68 ± 15.50 [4]	61 ± 16.00 [8]	0.014	*				
Cord glucose (mg/dl)	75 ± 23.75 [52]	63 ± 30.00 [26]	0.064		76 ± 23.75 [25]	54 ± 31.50 [14]	0.004	**
Cord triglycerides (mg/dl)	44 ± 23.00 [51]	44 ± 26.25 [25]	0.383	ns	45 ± 24.00 [24]	41.50 ± 30.50 [13]	0.243	ns
Cord LDL cholesterol (mg/dl)	28.00 ± 12.75 [52]	26.00 ± 12.75 [25]	0.421	ns	28.50 ± 10.75 [25]	28.00 ± 11.00 [14]	0.864	ns
Cord HDL cholesterol (mg/dl)	27 ± 10.75 [52]	24.50 ± 16.00 [25]	0.521	ns	27 ± 14.75 [25]	27 ± 19.50 [14]	0.727	ns

Values are expressed in 'median ± interquartile range' or 'absolute number (percentage)'. Numbers in square brackets indicate the numbers of missing observations. p values refer to Kruskal–Wallis test (for continuous covariates) or Chi square test (for categorical covariates)

CS cesarean section, *VD* vaginal delivery

***$p<0.001$, **$p<0.01$, *$p<0.05$, •$p<0.1$, *ns* non-significant

testing. However, the total sum of the PC only showed a trend for lower levels in the CS group. No metabolites showed remarkably elevated levels in CS rather than VD (Fig. 1). Due to the differences in maternal HDL levels in the two groups, we further adjusted the models for maternal HDL; as VIFs were below 1.5, no multicollinearity was identified in the models. After this adjustment, only pyruvic acid remained significant, however, the observed trends were still visible for the remaining metabolites. A list of the significant metabolites before and after adjustment for maternal HDL levels is shown in Table 2a, b. In the models including non-GDM mothers only, most metabolites lost significance after adjustment for multiple testing (possibly due to the smaller sample sizes, which was of 112 observations instead of 153), but trends still remained at uncorrected 0.05 level.

In cord blood, Cys stands out as the most remarkable finding, being highly elevated in CS babies; LPC 22:6 and the sum of aaPC (especially aaPC 32:2) were also slightly elevated in the CS group. On the other hand, the majority of NEFA, as well as citric acid (a TCA metabolite), pyruvic and lactic acid were strongly reduced in CS (Fig. 2). Trends for lower levels were also identified for three amino acids (Ala, Asn, Orn, and Pro), isocitric acid, the AC2:0/AC16:0 ratio, a marker for fatty acid β-oxidation, and as expected, the sum of hexoses, though all not significant after correction for multiple testing (Table 3). Similarly, as in maternal blood, the results obtained including data from non-GDM mothers only (84 subjects) were in line with the ones including the whole population, even though most associations were not significance after correction for multiple testing. A list of the significant metabolites in cord blood is shown in Table 3. In addition, results from the linear models for all investigated maternal and cord blood metabolites are listed in Additional files (Tables S1 and S2).

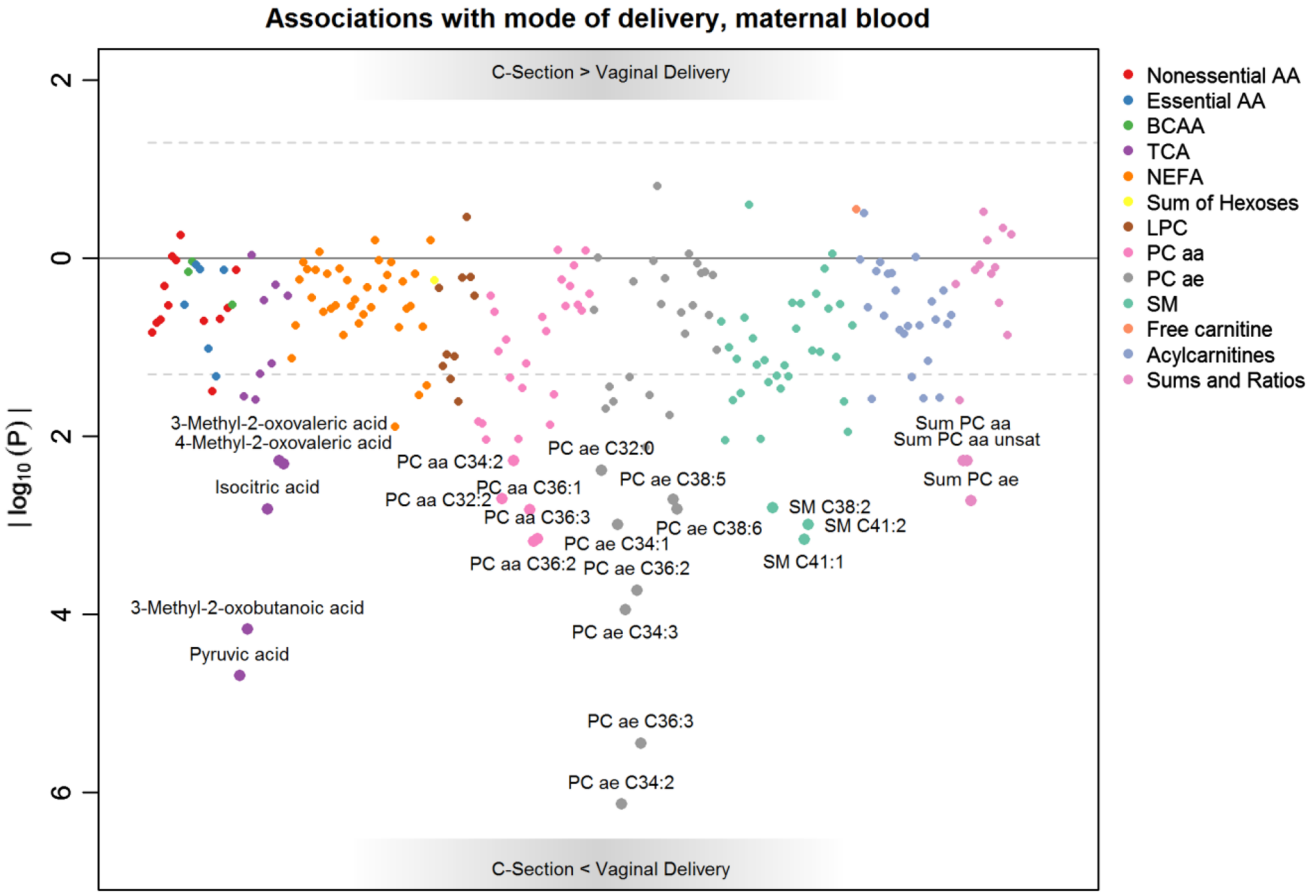

Fig. 1 Manhattan plot depicting the association of metabolites in maternal plasma with mode of delivery. The *y* axis represents the log10 *p* value of the association with mode of delivery; the direction (positive or negative) corresponds to the sign of the estimate for the association. *p* value and estimate were calculated in the linear model with the log2 metabolite concentration as dependent variable and log2 mode of delivery (CS versus VD) + pBMI + GDM status (y/n) + GWG + maternal age + birthweight as independent variables. The dashed lines represent the uncorrected 0.05 significance level. Labeled metabolites are significant at $p < 0.05$ after FDR correction. *DaPC* diacyl-phosphatidylcholine, *aaPC* acyl-alkyl-phosphatidylcholine, *SM* sphingomyelin

Discussion

To our knowledge, this study is the first to investigate the metabolic changes associated with CS delivery in both mothers and infants in humans since the majority of the studies in this regard were performed in animal models with controlled experimental settings and not in humans. CS showed remarkable effects on both the maternal and the cord blood metabolites.

In mothers, stress hormones (glucocorticoids), secreted prior to or during labor, may play a major role in observed differences. Such hormones are induced by the stress of labor and placental positive feedback in response to their circulating levels [30, 31]. Previous reports confirmed higher circulating levels of cortisol in VD in comparison to CS deliveries [32–34]. A study involving the measurement of maternal cortisol levels during spontaneous VD versus CS found that there was a significant difference in the maternal cortisol levels in VD versus CS; the highest levels of cortisol were detected at the second stage of labor in VD (i.e., when the uterine cervix is fully dilated) and were almost 1.7 times those detected during the preoperative stage in elective CS [35]. This confirms that spontaneous VD generates higher maternal stress than elective CS, creating a state of physiological hypercortisolism as a physiological response to stress (physiological and/or psychological) [36]. Other reports suggest that the passage of the fetus through the birth canal in VD increases the fetal stress, which reflected in the immediate postnatal glucocorticoid synthesis [37].

Glucocorticoids were previously reported to play a role in enhancing the synthesis of PL [38] as well in the reduction of their breakdown via inhibition of phospholipase A2 [39, 40]. The literature about the relationship between cortisol concentrations and hepatic PL synthesis is scarce and non-conclusive; however, the available data suggest that glucocorticoid receptors respond to the proportional change

Table 2 Significant results from the linear models for maternal blood including metabolite concentration as dependent variable and mode of delivery (cesarean section vs. vaginal delivery) as independent variable. Models were adjusted for log2 (pre-pregnancy BMI), GDM, GWG at 34 weeks, maternal age and birth weight

Analyte	Analyte group	*n*	Beta	*p* value	*p* value (FDR)
(A) Before adjustment for maternal HDL levels at delivery					
Pyruvic acid	TCA	160	− 0.515	**< 0.001**	**0.001**
Lactic acid	TCA	159	− 0.252	**0.028**	0.131
3-Methyl-2-oxobutanoic acid	BCKA	160	− 0.300	**< 0.001**	**0.004**
Isocitric acid	TCA	158	− 0.332	**0.002**	**0.023**
3-Methyl-2-oxovaleric acid	BCKA	160	− 0.253	**0.005**	**0.048**
4-Methyl-2-oxovaleric acid	BCKA	160	− 0.256	**0.005**	**0.048**
daPC 32:2	daPC	158	− 0.291	**0.002**	**0.024**
daPC 34:2	daPC	157	− 0.167	**0.005**	**0.048**
daPC 36:1	daPC	158	− 0.234	**0.001**	**0.023**
daPC 36:2	daPC	158	− 0.201	**0.001**	**0.017**
daPC 36:3	daPC	158	− 0.215	**0.001**	**0.017**
aaPC 32:0	aaPC	158	− 0.174	**0.004**	**0.047**
aaPC 34:1	aaPC	157	− 0.201	**0.001**	**0.020**
aaPC 34:2	aaPC	158	− 0.357	**< 0.001**	**< 0.001**
aaPC 34:3	aaPC	158	− 0.292	**< 0.001**	**0.005**
aaPC 36:2	aaPC	158	− 0.228	**< 0.001**	**0.007**
aaPC 36:3	aaPC	158	− 0.314	**< 0.001**	**< 0.001**
aaPC 38:5	aaPC	158	− 0.201	**0.002**	**0.024**
aaPC 38:6	aaPC	157	− 0.228	**0.002**	**0.023**
SM 38:2	SM	158	− 0.199	**0.002**	**0.023**
SM 41:1	SM	158	− 0.205	**0.001**	**0.017**
SM 41:2	SM	123	− 0.264	**0.001**	**0.020**
Sum daPC	Sums and Ratios	156	− 0.153	**0.005**	**0.048**
Sum daPC unsaturated	Sums and Ratios	156	− 0.154	**0.005**	**0.048**
Sum aaPC	Sums and Ratios	134	− 0.193	**0.002**	**0.024**
(B) After adjustment for maternal HDL levels at delivery					
Ala	Nonessential AA	148	− 0.167	**0.044**	0.404
Pro	Nonessential AA	155	− 0.180	**0.037**	0.404
Pyruvic acid	TCA	156	− 0.567	**< 0.001**	**0.003**
3-Methyl-2-oxobutanoic acid	BCKA	156	− 0.297	**< 0.001**	**0.026**
Malic acid	TCA	156	− 0.253	**0.050**	0.418
Alpha-Ketoglutaric acid	TCA	155	− 0.273	**0.015**	0.235
Isocitric acid	TCA	154	− 0.340	**0.003**	0.116
3-Methyl-2-oxovaleric acid	BCKA	156	− 0.240	**0.014**	0.229
4-Methyl-2-oxovaleric acid	BCKA	156	− 0.244	**0.013**	0.229
daPC 36:1	daPC	154	− 0.152	**0.045**	0.404
daPC 36:2	daPC	154	− 0.131	**0.032**	0.404
daPC 36:3	daPC	154	− 0.132	**0.042**	0.404
aaPC 32:0	aaPC	154	− 0.132	**0.041**	0.404
aaPC 34:1	aaPC	153	− 0.142	**0.026**	0.369
aaPC 34:2	aaPC	154	− 0.251	**< 0.001**	**0.031**
aaPC 34:3	aaPC	154	− 0.189	**0.012**	0.229
aaPC 36:2	aaPC	154	− 0.155	**0.014**	0.229
aaPC 36:3	aaPC	154	− 0.219	**0.001**	0.054
SM 36:0	SM	81	0.300	**0.010**	0.229
SM 38:2	SM	154	− 0.135	**0.041**	0.404
SM 41:1	SM	154	− 0.161	**0.012**	0.229
SM 41:2	SM	119	− 0.223	**0.010**	0.229
AC4:0	AC	156	− 0.373	**0.007**	0.229
AC8:1	AC	156	− 0.318	**0.033**	0.404

Beta > 0 indicates higher values of the analyte in the cesarean section group. Significant *p* values are in bold

AA amino acid, *AC* acylcarnitines, *BCKA* branched chain keto-acid, *FDR* false discovery rate, *daPC* diacyl-phosphatidylcholines, *aaPC* acyl-alkyl-phosphatidylcholines, *SM* sphingomyelin, *TCA* tricarboxylic acid

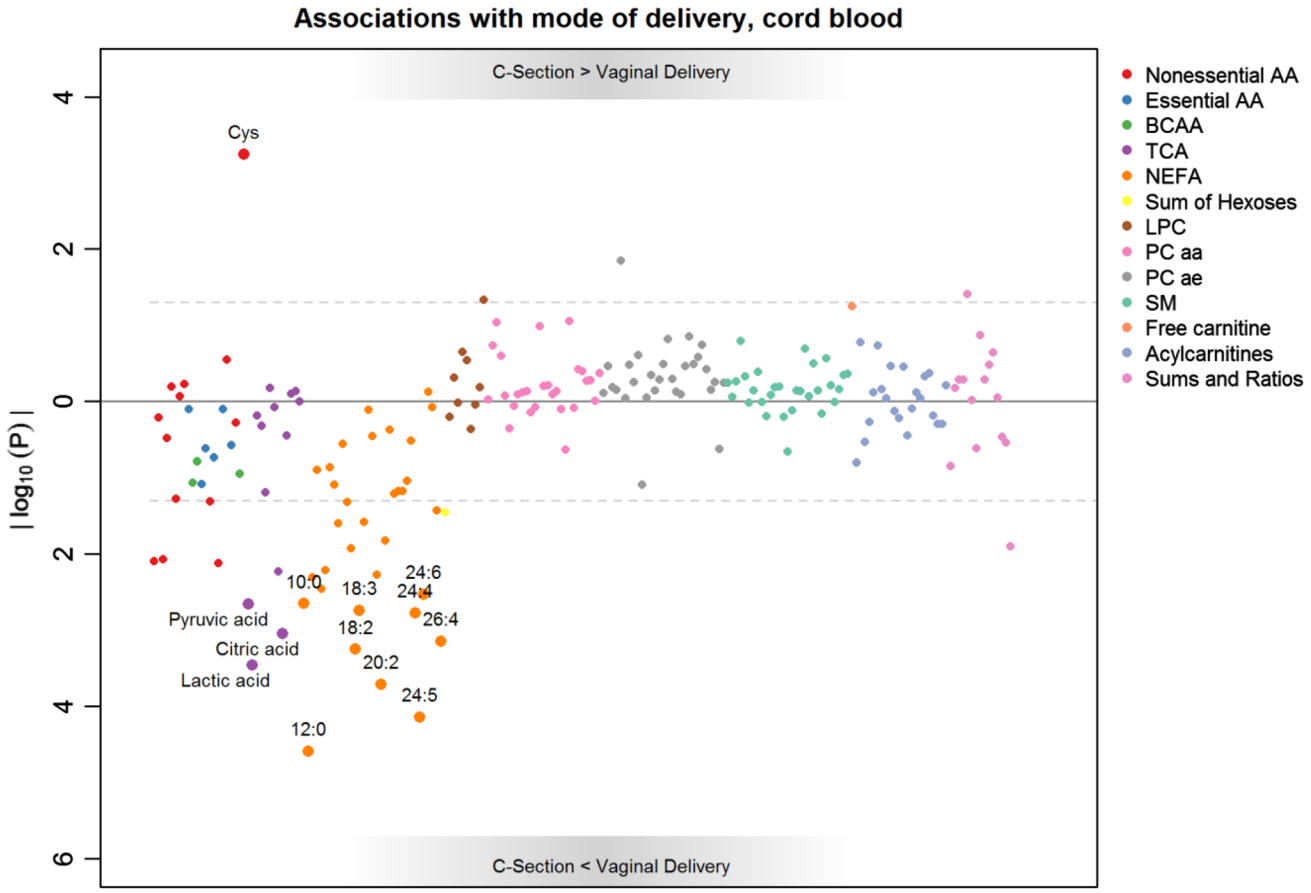

Fig. 2 Manhattan plot depicting the associations of metabolites in cord blood with mode of delivery. For explanation of the plot, see Fig. 1 legend

in glucocorticoid concentration rather than in the absolute amounts thereof [37, 41]. Therefore, the great change in cortisol during labor might enhance large hepatic PL synthesis.

In elective CS delivery, the absence of the biological stimuli present in normal labor leads to lower levels of stress hormones relative to VD [32–34], and, therefore, to the observed pattern for relatively reduced maternal PL in CS mothers [11, 40]. However, the population under study showed strong differences in the HDL profiles throughout pregnancy (Table 1), and adjusting for HDL largely reduced, but did not eliminate, the observed associations. Therefore, we suspect that the observed differences in the PL levels are to attribute to a combination of both factors, especially considering that the differences in the HDL levels were more pronounced at delivery than at earlier time points. These different levels might be attributed to the highest incidence of GDM and elevated BMI in the CS group [42, 43]. In diabetic pregnancy, increased insulin resistance may account for hypertriglyceridemia, increased VLDL production, and increased HDL catabolism leading to low plasma HDL concentration. Different explanations were provided, one of which is the reduction in the activity of lipoprotein lipase (LPL) enzyme, which could impair the maturation of HDL particles. Another explanation is the combination of CETP-mediated triglyceride enrichment of HDL coupled with the elevated hepatic lipase activity associated with insulin resistance that enhances the remodeling of HDL in the circulation with an increased HDL catabolism [44]. In addition, previous reports demonstrated a significant reduction in the activity of phospholipid transfer protein (PLTP) in association with maternal GDM [45]. PLTP is a protein that is responsible for the transfer of phospholipids from triglyceride-rich lipoproteins to high-density lipoprotein (HDL), therefore, a reduction in its activity may be associated with a decrease in HDL cholesterol levels.

The lack of exposure to glucocorticoids in CS might also explain the lower levels of pyruvic, isocitric acid and some BCKA. In CS, the hormonal inhibition of the oxidative decarboxylation of pyruvic acid (that is, its conversion to acetyl-CoA) due to glucocorticoids is compromised, thus explaining the encountered lower levels of pyruvic acid [46]. Glucocorticoids also enhance gluconeogenesis and gluconeogenic substrate supply through increased muscle protein breakdown, amino acid utilization, and

Table 3 Significant results from the linear models for cord blood including metabolite concentration as dependent variable and mode of delivery (cesarean section vs. vaginal delivery) as independent variable

Analyte	Analyte group	*n*	Beta	*p* value	*p* value (FDR)
Ala	Nonessential AA	89	− 0.285	**0.008**	0.081
Asn	Nonessential AA	114	− 0.234	**0.009**	0.082
Orn	Nonessential AA	112	− 0.150	**0.050**	0.303
Pro	Nonessential AA	112	− 0.200	**0.008**	0.081
Cys	Nonessential AA	88	0.662	**0.001**	**0.019**
Pyruvic acid	TCA	115	− 0.477	**0.002**	**0.038**
Lactic acid	TCA	104	− 0.435	**< 0.001**	**0.017**
Isocitric acid	TCA	113	− 0.243	**0.006**	0.069
Citric acid	TCA	113	− 0.328	**0.001**	**0.023**
10:0	NEFA	105	− 0.629	**0.002**	**0.038**
12:0	NEFA	104	− 1.117	**< 0.001**	**0.005**
14:0	NEFA	111	− 0.486	**0.005**	0.067
15:0	NEFA	106	− 0.883	**0.003**	0.050
16:0	NEFA	111	− 0.447	**0.006**	0.069
17:0	NEFA	109	− 0.324	**0.025**	0.196
18:0	NEFA	109	− 0.494	**0.049**	0.303
18:1	NEFA	111	− 0.463	**0.012**	0.108
18:2	NEFA	110	− 0.644	**0.001**	**0.019**
18:3	NEFA	101	− 1.011	**0.002**	**0.036**
18:4	NEFA	87	− 0.949	**0.027**	0.198
20:1	NEFA	97	− 0.991	**0.005**	0.068
20:2	NEFA	105	− 0.987	**< 0.001**	**0.013**
20:3	NEFA	110	− 0.620	**0.015**	0.122
24:4	NEFA	110	− 0.474	**0.002**	**0.036**
24:5	NEFA	110	− 0.563	**< 0.001**	**0.007**
24:6	NEFA	111	− 0.585	**0.003**	**0.046**
26:2	NEFA	111	− 0.230	**0.037**	0.257
26:4	NEFA	109	− 0.362	**0.001**	**0.020**
H1	Sum of hexoses	114	− 0.210	**0.036**	0.256
LPC 22:6	LPC	85	0.280	**0.046**	0.298
aaPC 32:2	aaPC	98	0.346	**0.014**	0.117
Sum aaPC	Sums and ratios	96	0.199	**0.038**	0.257
Ratio beta oxidation	Sums and ratios	110	− 0.367	**0.013**	0.110

Models were adjusted for log2 (pre-pregnancy BMI), GDM, GWG at 34 weeks, maternal age and birth weight

Beta > 0 indicates higher values of the analyte in the cesarean section group. Significant *p* values are in bold

AA amino acid, *AC* acylcarnitines, *BCKA* branched chain keto-acid, *FDR* false discovery rate, *H1* sum of hexoses, *LPC a* acyl- lysophosphatidylcholines, *NEFA* non-esterified fatty acid, *daPC* diacyl-phosphatidylcholines, *aaPC* acyl-alkyl-phosphatidylcholines, *ratio beta oxidation AC2:0/AC16:0*, *SM*: sphingomyelin, *TCA* tricarboxylic acid

BCAA oxidation thus elevated circulating glucocorticoids are related to elevated BCKA levels [15, 46]. In conditions where glucocorticoid release is reduced as in CS, an associated decrease in pyruvic acid and BCKA may occur. The observed differences in these metabolites could be regarded as an indirect effect of CS delivery resulting from less hormonal stimulation, primarily stress hormones, which in turn leads to these abnormal patterns of maternal metabolism at delivery.

In cord blood, we found alterations of the metabolome with CS delivery, especially with metabolites involved in liver metabolism, which may lead to altered metabolic responses as previously demonstrated in animal models [11, 34]. In these models, the lower levels of the stress hormones following elective CS were related to altered insulin function, glucose metabolism after birth, and lipid metabolism, which were attributed to the failure to activate gluconeogenesis, responsible for this chain of events [11]. In standard

vaginal delivery, fetal hypoglycemia rapidly develops after birth as a result of the cessation of the mother to fetus nutrient supply [15]. As a compensatory mechanism, a significant decrease in circulating plasma insulin occurs along with an increase in both plasma glucagon concentration and the number of glucagon receptors which results in a low insulin/glucagon ratio. Thus, the metabolism is shifted towards the release of energy stores through glycogenolysis, lipolysis, and gluconeogenesis from AA and glycerol [34]. Neonatal gluconeogenesis is further maintained by β-oxidation, a prerequisite for glucose synthesis by glycerol phosphate dehydrogenase (GPD) reaction which is triggered by elevated levels of stress hormones [15, 47]. Enhanced lipid mobilization from the adipose tissue under the effect of elevated stress hormones results in increasing the levels of circulating NEFA and triacylglycerols (TAG) which stimulates β-oxidation as a response [48, 49]. Accordingly, due to the absence of the hormonal stimulus in babies born by CS, gluconeogenesis is diminished leading to a tendency for increased lipid accumulation and reduced lipolysis which is not encountered in those born by VD. In fact, these reported results match the overall picture in our results on the cord blood which shows reduced levels of NEFA, fatty acid β-oxidation marker, TCA metabolites, substrates for gluconeogenesis (pyruvate and lactate) and sum of hexoses, providing further evidence for the underlying mechanisms of obesity risk in infants born by CS.

Another important and highly significant finding was elevated Cys in the cord blood of CS babies. High levels of Cys were linked to increased production of hydrogen sulfide (H2S) in the myometrium [50] which decreases the intensity of spontaneous contractions. That is, low levels of Cys in VD are likely to stimulate uterine contractions.

Cord blood aaPC levels tended to be slightly higher (though not significantly) in CS babies despite the observed large reduction of aaPC in the mothers. We found this surprising, considering that the fetus and the mother are subject to the same hormonal stimuli. However, plasma PC do not originate only from the liver. Lung is another organ where synthesis, transient accumulation/storage, and secretion of PC is a major function not only as a pulmonary surfactant but also to be used in membrane homeostasis, organ growth, and lipoprotein homeostasis/HDL trafficking via ABC-A1 transporter [51, 52]. Earlier reports demonstrated that the absolute amounts of lung PC normally synthesized in vivo exceed surfactant requirements by at least an order of magnitude, thus the excess PC, not destined for alveolar secretion, are returned to the circulation and presumably redirected to the liver via HDL [51]. Accordingly, PC plasma levels measure only equilibrium, and not metabolism and trafficking. We speculate that these higher levels of cord blood PC could be ascribed to an adaptation mechanism to provide for the fetal needs of PL in the early extra-uterine life. In VD, there is sufficient time for this adaptation, but in CS the hormonal stimulus is absent and thus accelerated transfer of maternal PL to the fetus might occur even at the expense of maternal PL, thus further contributing to the reduced levels of maternal PL. Alternatively, the accelerated transport of PL might be itself due to lower maternal PL levels, as this event has already been observed in starvation in animal models [53]. Anyways, there is a paucity of literature about the theme, so more studies are needed to investigate these hypotheses.

Observational studies examined the main maternal and child outcomes associated with CS deliveries. In mothers, CS was reported to negatively impact future pregnancies. It was recognized as an important risk factor for preterm birth in following pregnancies [54] causing problems as placenta previa, bleeding, abnormal fetal positions, and rupture of membranes. Conversely, CS was assumed to protect the postpartum sexual function relative to VD, since it avoids genital tract trauma; however, the reports were conflicting between positive and no association [55, 56]. CS was also reported to decrease the risk of urinary incontinence and pelvic organ prolapse with no evidence of long-term effects on the maternal metabolism [3, 47]. In the offspring, it was linked to increased future risk of obesity, asthma, among other chronic diseases [4–6, 57]. According to our results, we detected alteration in both the maternal and the cord blood metabolites in the CS relative to VD group. In CS babies, a tendency was detected for increased lipid accumulation, reduced lipolysis, and altered glucose metabolism, indicating altered hepatic metabolism, which if persistent, might contribute to future obesity and metabolic syndrome in the offspring [58, 59].

Limitations

The study presented few limitations that deserve to be mentioned. The small sample size was limited by the number of samples available for secondary metabolomic analysis and by the information about mode of delivery. An additional limitation was the lack of more detailed information on fasting status, socioeconomic level, diet, and education. These limitations can be overcome in future studies targeting mode of delivery and its related effects on both short- and long-term metabolic and health outcomes on the maternal and the offspring side.

Conclusions

The samples collected within the PREOBE study provided the unique opportunity to study the effect of elective CS on a wide range of metabolites in maternal and cord blood at the time of delivery. The impact of CS was strongly evident

in the CS babies even after adjustment for all confounders. These results not only support previous findings from observational studies reporting both short- and long-term health effects of CS on infant metabolism, but also elucidate mechanisms in the child which, on the long run, might have a negative impact and lead to the development of obesity, asthma, or other chronic diseases [4–6, 53].

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Author contributions MGB, JAG, and MTS coordinated and conducted the data and sample collection. OU performed the metabolic analysis. LM conducted the statistical analyses. ES discussed the results and wrote the paper. CC and BK are the principal investigators, responsible for the design and coordination of the research. BK and CC are also the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data, accuracy of the data analysis, and the final content of the manuscript. All the authors reviewed, edited and approved the manuscript.

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Compliance with ethical standards

Conflict of interest None of the authors report 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.

Adherence to EQUATOR network guidelines The authors declare compliance with the EQUATOR (Enhancing the QUAlity and Transparency of Research) network guidelines available at: https://equator-network.org.

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

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