Acta Diabetologica (2019) 56:421–430
https://doi.org/10.1007/s00592-019-01291-z

ORIGINAL ARTICLE

# Impact of maternal BMI and gestational diabetes mellitus on maternal and cord blood metabolome: results from the PREOBE cohort study

**Engy Shokry[1] · Linda Marchioro[1] · Olaf Uhl[1] · Mercedes G. Bermúdez[2] · Jose Antonio García-Santos[2] · Mª Teresa Segura[2] · Cristina Campoy[2] · Berthold Koletzko[1]**

Received: 26 November 2018 / Accepted: 18 January 2019 / Published online: 6 February 2019
© Springer-Verlag Italia S.r.l., part of Springer Nature 2019

## Abstract

**Aims** Maternal obesity and gestational diabetes mellitus (GDM) were frequently reported to be risk factors for obesity and diabetes in offspring. Our goal was to study the impact of maternal prepregnancy BMI (pBMI) and GDM on both maternal and cord blood metabolic profiles.

**Methods** We used LC–MS/MS to measure 201 metabolites comprising phospholipids (PL), amino acids, non-esterified fatty acids (NEFA), organic acids, acyl carnitines (AC), and Krebs cycle metabolites in maternal plasma at delivery and cord plasma obtained from 325 PREOBE study participants.

**Results** Several metabolites were associated with pBMI/GDM in both maternal and cord blood ($p < 0.05$), while others were specific to either blood sources. BMI was positively associated with leucine, isoleucine, and inflammation markers in both mother and offspring, while β-hydroxybutyric acid was positively associated only in cord blood. GDM showed elevated levels of sum of hexoses, a characteristic finding in both maternal and cord blood. Uniquely in cord blood of offspring born to GDM mothers, free carnitine was significantly lower with the same tendency observed for AC, long-chain NEFA, PL, specific Krebs cycle metabolites, and β-oxidation markers.

**Conclusions** Maternal BMI and GDM are associated with maternal and cord blood metabolites supporting the hypothesis of transgenerational cycle of obesity and diabetes.

**Keywords** Gestational diabetes · Intrauterine environment · Maternal obesity · Maternal phenotypes · Metabolomics

## Introduction

Obesity and overweight have become a global epidemic [1]. In women of reproductive age, obesity poses a risk to maternal health, with consequences from gestational diabetes

Managed by Antonio Secchi.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00592-019-01291-z) contains supplementary material, which is available to authorized users.

✉ Berthold Koletzko
berthold.koletzko@med.uni-muenchen.de;
office.koletzko@med.lmu.de

Engy Shokry
engy.shokry@med.uni-muenchen.de

Linda Marchioro
linda.marchioro@med.uni-muenchen.de

Olaf Uhl
olaf.uhl@med.uni-muenchen.de

Mercedes G. Bermúdez
mgbermudez@gmail.com

Jose Antonio García-Santos
joseantonio_gsantos@outlook.es

Mª Teresa Segura
maite.segura.moreno@gmail.com

Cristina Campoy
ccampoy@ugr.es

[1] Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, LMU-Ludwig-Maximilians-Universität München, University of Munich Medical Centre, Campus Innenstadt, Lindwurmstr. 4, 80337 Munich, Germany

[2] Department of Paediatrics, School of Medicine, EURISTIKOS Excellence Centre for Paediatric Research, University of Granada, Avenida de la Investigación 11, 18016 Granada, Spain

(GDM) [2] and adverse pregnancy outcomes to later type-2 diabetes (T2D) and cardiovascular diseases [3]. It is also associated with adverse outcomes for child at birth and during neonatal, infant and later life periods [4].

GDM is defined as glucose intolerance that is first recognized during pregnancy. Risk factors have been identified (including obesity, age and family history); however, the underlying mechanisms remain an enigma. GDM might be due to a pancreatic β-cell defect similar to that in type-1 diabetes (T1D), or to a dormant, pre-existing insulin resistance (IR) clinically manifesting as GDM during the diabetogenic state of late pregnancy [5]. Women developing GDM show impaired ability to stimulate glucose disposal and to suppress both glucose production and fatty acid (FA) levels [5]. Maternal obesity and GDM, each and combined, predispose to adverse short- and long-term infant outcomes [4, 6, 7].

To provide an understanding of the connection between early metabolic programming and the increased incidence of metabolic diseases resulting from the disruption of the intrauterine environment, associations between maternal prepregnancy Body Mass Index/GDM and maternal–cord blood metabolic profiles were studied.

## Experimental section

### Participants

The study was performed on data from the PREOBE study, a prospective observational cohort study (NCT01634464). Study procedures, group classification and subject inclusion/exclusion criteria have been described by Berglund et al. [8].

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

### Sample and data collection

Maternal venous blood samples were drawn into EDTA-containing tubes at delivery. Vein umbilical cord blood samples were obtained after clamping the cord. Blood samples were processed and plasma aliquots were stored at − 80 °C until analysis. Samples for metabolomics analyses were available for 200 mothers and 124 newborns, with an overlap for 119 mother/children pairs. At recruitment, information was collected on maternal anthropometry and used to calculate pBMI [8]. Gestational weight gain (GWG) at 34 weeks and data regarding clinical outcome at delivery were collected from medical records. Biochemical analysis of serum samples was performed as previously reported [8].

Since the maternal fasting status at time of blood collection was unknown, we used a fasting blood glucose threshold (126 mg/dl) previously validated for diabetes to identify non-diabetic, non-fasting mothers. Namely, if a non-diabetic mother presents a plasma glucose value above the threshold, this indicates non-fasted status. 27 non-GDM mothers were defined as non-fasted and excluded from the analysis. Such a distinction was obviously not possible for GDM mothers [9].

### Targeted metabolomics assays

Targeted metabolomics involved analysis of 400 metabolites comprising: polar lipids [acylcarnitines (AC), diacyl-phosphatidylcholines (PCaa), acyl-alkyl-phosphatidylcholines (PCae), sphingomyelines (SM), acyl-lysophosphatidylcholines (LPCa), alkyl-lysophosphatidylcholines (LPCe)], sum of hexoses (H1), amino acids (AA), keto acids, non-esterified FA (NEFA), and Krebs cycle metabolites. NEFA analysis was performed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) as previously reported [10]. Polar lipids were analysed with flow-injection analysis tandem mass spectrometry (FIA–MS/MS) [11]. A formula CX:Y was assigned for polar lipids and NEFA where X: length of carbon chain, Y: number of double bonds, OH: indicates presence of hydroxyl group. Letters 'a' and 'e' indicate that the acyl chain is bound via an ester or ether bond to the backbone, respectively. AA analysis was done by derivatization and separation by ion-pair LC–MS/MS [12]. AA were coded according to IUPAC abbreviations.

Six plasma quality control (QC) samples per batch were consistently measured with the samples. Concentrations were calculated in µmol/l; the analytical process was controlled and post-processed by Analyst 1.6.1 and R software (R version 3.4.3).

### Metabolomics quality control (QC) and preprocessing

First, the QC for metabolomics measurements was done using a threshold of 20% and 30% for the intra- and inter-batch coefficient of variation, respectively (with allowance of max. 1 outlier measurement > 2 IQR from the next measurement). In total, 202 metabolites passed the QC. Measurements lying away than 1.5 standard deviations (SD) from the next closest measurement were removed. We corrected for batch effects by dividing metabolite concentrations by the ratio intra-batch median/inter-batch median. Then, the measurements were split into two datasets corresponding to maternal and cord blood.

Within each dataset, analytes with > 40% missing values were excluded. Sums were computed: Σ branched chain AA (BCAA), ΣLPCa, ΣPCaa, ΣPCae. Also, ratios were computed: ΣPCaa/ΣPCae, reflecting oxidative stress [13]; ΣLPCa/ΣPCaa, as a lipid biomarker of inflammation; (LPCa16:0 + LPCa18:0)/ΣPCaa as a proinflammatory biomarker [14]; (LPCa18:1 + LPCa 18:2)/ΣPCaa as

an anti-inflammatory biomarker [15]; AC ratios (AC16:0/free carnitine (Carn) and AC2:0/AC16:0) as markers of carnitine palmitoyl transferase-1 activity (CPT1) and FA β-oxidation, respectively [16]. Moreover, FA ratios were used to estimate activities of stearoyl-CoA desaturase-1 (SCD-1; 16:1/16:0, SCD-16 and 18:1/18:0, SCD-18) [17] and AA ratios Asn/Asp and Gln/Glu as indicators for anaplerosis or replenishing of Krebs cycle metabolites. Analytes, sums and ratios were log2-transformed after inspection of boxplots and quantile–quantile plots. Outliers were defined as points lying further away than 1 SD from the next measurement and were excluded.

## Statistical analysis

### Baseline characteristics

The 'overweight' and 'obese' groups were merged into one category (pBMI was considered a continuous variable). Differences between the covariates in the three groups were evaluated via Kruskal–Wallis and Fisher tests for continuous and categorical covariates, respectively.

### Associations of maternal and cord blood metabolome with BMI and GDM

The associations between metabolite concentrations in maternal/cord blood and the exposures of interest were determined using multiple linear regression models with covariates adjustment. The final model used log2 of the metabolite concentration as outcome and log2(pBMI) and GDM as independent variables, GWG at 34 weeks, maternal age, mode of delivery, and infant sex as covariates. Parity was not included in the model due to high missingness (> 50%). A sensitivity analysis including gestational age showed no differences in the effect size and was not included in the final models. For these models, median values of 157 and 111 observations were used for maternal and cord blood, respectively. Results were depicted in Manhattan plots with|log10 (*P*)|-values on *y*-axis and metabolites on *x*-axis, with the direction on *y*-axis and the magnitude indicating the sign and strength of the association, respectively. We used false discovery rate (FDR) to minimize the occurrence of false positives (type I errors), a common issue in multiple testing. FDR controls for the expected proportion of false predictions relative to the total number of predictions at the level of significance. Nevertheless, because of the exploratory nature of the analysis, we also inspected associations with uncorrected $p < 0.05$ ('trends').

# Results

Baseline characteristics of the two populations used in the analyses are summarized in Table 1A, B.

In a nutshell, our analyses of maternal and cord blood metabolites showed highly significant associations of GDM with several maternal and, to a lesser extent, cord blood metabolites, yet weaker associations with pBMI (Figs. 1, 2, 3, 4).

## Maternal prepregnancy BMI

For pBMI, none of the maternal metabolites were significant after correction for multiple testing. All BCAA, SM32:2, 34:2, PCaa38:4 and alpha-aminoadipic acid (AAA) showed positive trends with pBMI, while PCae36:1 showed a negative trend. Also, scatterplot inspection revealed positive trends for AC3:0, 4:0, 5:0, and 9:0 (Fig. 1).

In cord blood, BCAA behaved similarly except Val, while Cys showed a negative trend. Similarly, β-hydroxybutyric acid (BHBA), was positively associated with pBMI concomitant with a negative tendency for α-ketoglutaric acid. There were positive tendencies with NEFA22:4 and ΣPCaa/ΣPCae ratio with pBMI, the latter due to reduced PCae levels, while Σ(LPCa18:1 + LPCa18:2)/ΣPCaa, an anti-inflammatory biomarker, showed a negative tendency (Fig. 2).

## Maternal gestational diabetes mellitus

H1 (about 90–95% glucose, 5% other hexoses) was significantly higher in GDM in both maternal and, especially, cord blood (Figs. 3, 4). In both maternal and cord blood, Asn/Asp and Gln/Glu ratios were elevated in GDM subjects, with decrease in Asp and Glu. Another common finding is the overall decrease in the majority of phospholipids (PL). Most maternal LPCs and PCaa were decreased in GDM mothers, with strongly significant associations for LPC16:0, PCaa38:3,38:5, and some SM, especially SM32.2. PCae were not affected by GDM, thus resulting in a negative association for ΣPCaa/ΣPCae ratio.

In cord blood, LPC concentrations showed no difference with maternal GDM status, while there was a tendency for PCae and some PCaa to be decreased in GDM babies (PCae38:0 being significant after FDR correction). Interestingly, Carn was significantly lower in GDM babies with the same tendency for all AC, especially short-chain AC and acetyl carnitine. Markers for CPT1 activity and β-oxidation were higher and lower, respectively, in GDM babies. Also, an overall decrease in cord blood long-chain NEFAs and Krebs metabolites was observed in association with GDM,

**Table 1** Demographics characteristics of study participants

| | Healthy, normal weight ($n=67$) | Healthy, overweight/obese ($n=50$) | Gestational diabetes (GDM) ($n=45$) | $p$ value |
|---|---|---|---|---|
| *(A) Maternal samples* | | | | |
| Maternal age (years) | 31.00 ± 6.00 | 31.00 ± 4.75 | 34.00 ± 6.00 | 0.003*** |
| Prepregnancy BMI (kg/m$^2$) | 21.87 ± 2.66 | 28.83 ± 4.31 | 26.29 ± 8.60 | < 0.001**** |
| GWG (34 weeks) (kg) | 12.50 ± 4.47 [1] | 9.20 ± 7.90 [1] | 6.20 ± 9.60 | < 0.001**** |
| Gestational age (weeks) | 39.00 ± 1.00 | 40.00 ± 2.00 [1] | 39.00 ± 2.00 [4] | 0.067* |
| Smoking—no | 51 (85%) [7] | 41 (91%) [5] | 31 (91%) [11] | 0.623 |
| Mode of delivery—elective C-section | 10 (15%) | 17 (34%) | 20 (44%) | 0.002*** |
| Fetal sex—female | 33 (50%) | 26 (52%) | 20 (44%) | 0.764 |
| Parity—nulliparous | 12 (46%) [41] | 13 (54%) [26] | 11 (50%) [23] | 0.955 |
| Maternal glucose(mg/dl) | 76.00 ± 21.50 | 84.00 ± 30.75 | 90.00 ± 38.50 [3] | 0.001**** |
| Maternal triglycerides (mg/dl) | 213.00 ± 93.00 | 232.00 ± 94.00 | 205.00 ± 76.25 [3] | 0.257 |
| Maternal LDL cholesterol (mg/dl) | 139.00 ± 44.50 | 141.50 ± 51.50 | 115.00 ± 63.00 [4] | 0.031** |
| Maternal HDL cholesterol (mg/dl) | 69.00 ± 24.00 | 67.50 ± 20.00 | 65.00 ± 19.00 [4] | 0.032** |
| Fetal (cord) glucose (mg/dl) | 72.00 ± 23.00 [38] | 67.00 ± 28.00 [19] | 73.00 ± 23.00 [22] | 0.12 |
| Fetal (cord) triglycerides (mg/dl) | 43.00 ± 14.25 [37] | 43.00 ± 27.00 [19] | 48.50 ± 17.50 [21] | 0.867 |
| Fetal (cord) LDL cholesterol (mg/dl) | 30.50 ± 16.75 [37] | 27.00 ± 9.50 [19] | 25.00 ± 10.00 [22] | 0.212 |
| Fetal (cord) HDL cholesterol (mg/dl) | 29.00 ± 18.25 [37] | 26 ± 9.00 [19] | 27.00 ± 8.50 [22] | 0.364 |
| | Healthy, normal weight ($n=49$) | Healthy, overweight/obese ($n=40$) | GDM ($n=27$) | $p$ value |
| *(B) Cord blood samples* | | | | |
| Maternal age (years) | 31.00 ± 6.00 | 31.50 ± 4.50 | 35.00 ± 6.50 | 0.002*** |
| Prepregnancy BMI (kg/m$^2$) | 21.87 ± 2.19 | 30.11 ± 5.34 | 26.64 ± 8.40 | < 0.001**** |
| GWG (34 weeks) (kg) | 12.00 ± 3.90 | 8.10 ± 7.65 [1] | 4.70 ± 10.55 | < 0.001**** |
| Gestational age (weeks) | 39.00 ± 2.00 | 40.00 ± 2.00 [1] | 39.00 ± 2.00 [1] | 0.005*** |
| Smoking—no | 38 (84%) [4] | 32 (86%) [3] | 18 (90%) [7] | 0.932 |
| Mode of delivery—elective C-section | 6 (12%) | 16 (40%) | 11 (41%) | 0.003*** |
| Fetal sex—female | 27 (55%) | 20 (50%) | 11 (41%) | 0.497 |
| Parity—nulliparous | 11 (50%) [27] | 11 (50%) [18] | 7 (50%) [13] | 1 |
| Maternal glucose (mg/dl) | 80.00 ± 25.50 [2] | 87.00 ± 32.50 [5] | 105.00 ± 44.00 [2] | 0.026** |
| Maternal triglycerides (mg/dl) | 217.00 ± 108.50 [2] | 231.00 ± 84.00 [5] | 208.00 ± 68.00 [2] | 0.47 |
| Maternal LDL cholesterol (mg/dl) | 139.00 ± 52.50 [2] | 144.00 ± 49.50 [5] | 111.50 ± 30.00 [3] | 0.009*** |
| Maternal HDL cholesterol (mg/dl) | 68.00 ± 27.50 [2] | 64.00 ± 20.00 [5] | 59.50 ± 23.25 [3] | 0.146 |
| Fetal (cord) glucose (mg/dl) | 73.00 ± 30.00 [20] | 63.00 ± 29.00 [9] | 77.00 ± 31.00 [10] | 0.069* |
| Fetal (cord) triglycerides (mg/dl) | 42.00 ± 16.50 [19] | 45.00 ± 28.50 [9] | 47.00 ± 22.00 [9] | 0.957 |
| Fetal (cord) LDL cholesterol (mg/dl) | 28.00 ± 16.50 [19] | 29.00 ± 8.75 [10] | 27.00 ± 9.00 [10] | 0.553 |
| Fetal (cord) HDL cholesterol (mg/dl) | 29.00 ± 20.25 [19] | 25.50 ± 10.00 [10] | 25.00 ± 14.00 [10] | 0.265 |

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)

****$p < 0.001$, ***$p < 0.01$, **$p < 0.05$, *$p < 0.1$

most notably NEFA26:1, malic, and succinic acids with an elevation of 3-methyl-2-oxobutanoic acid.

## Similar and contrasting associations in GDM and obesity

In cord blood, Asn/Asp ratio was positively associated with pBMI and elevated (though not statistically significant) in GDM subjects. Ile was positively associated with pBMI and negatively with GDM.

Some maternal PCaa and SM were slightly elevated in overweight/obese and significantly reduced in GDM mothers.

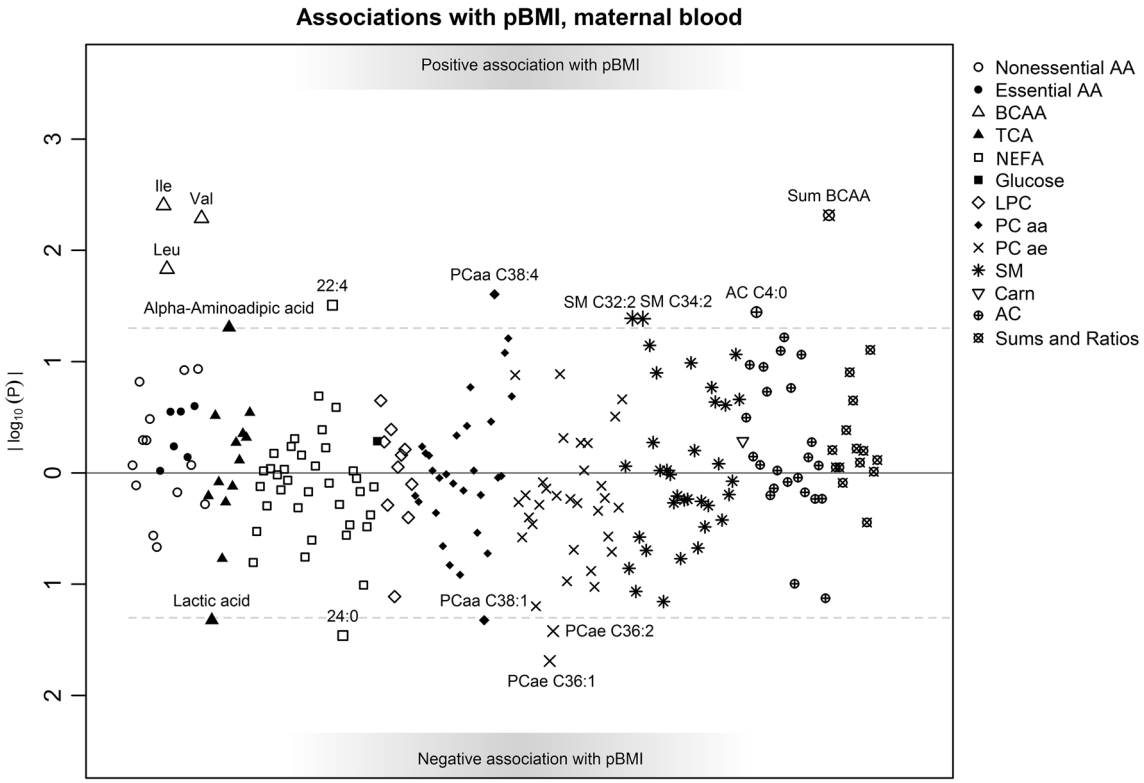


**Fig. 1** Manhattan plot showing association of metabolites in maternal plasma with maternal prepregnancy BMI (pBMI). The *y*-axis represents the log10 *p* value of pBMI; the direction (positive or negative) corresponds to the sign of the estimate for pBMI. *p* value and estimate were calculated in the linear model with the log2 metabolite concentration as dependent variable and log2 pBMI, GDM, GWG at 34 weeks, fetal sex and mode of delivery as independent variables. The dashed lines represent the uncorrected 0.05 significance level. *AA* amino acid, *AC* acylcarnitines, *BCAA* branched chain amino acid, *H1* sum of hexoses, *LPCa* acyl-lysophosphatidylcholines, *LPCe* alkyl–lysophosphatidylcholines, *NEFA* non-esterified fatty acid, *PCaa* diacyl-phosphatidylcholines, *PCae* acyl-alkyl-phosphatidylcholines, *TCA* tricarboxylic acid, *SM* sphingomyelines

## Discussion

### Influence of obesity on maternal and cord blood metabolome

In high pBMI, the most characteristic common features between maternal and cord blood were the elevated BCAA levels, which are essential AA promoting protein synthesis and turnover and glucose metabolism. In cord blood, BCAA are transplacentally transported from maternal to fetal side to be utilized for fetal growth and protein metabolism, and their transport is highly enhanced in late pregnancy to provide for the increased fetal nutrient demands [18]. This reflects other observations linking elevated BCAA and their metabolic by-products to obesity and metabolic syndrome [19]. Explanations were given showing BCAA as a consequence of obesity, others as a cause. Some authors attributed elevated BCAA levels to increased dietary protein intake, or excessive protein breakdown in skeletal muscle due to obesity-related IR [20]. Another hypothesis involves downregulation of BCAA oxidation enzymes, especially those involved in first pass BCAA metabolism, and was supported by animal and human studies [20]. There is also a speculation about role of gut microbiome in BCAA de-novo synthesis, thus contributing to their circulating levels [20].

We hypothesize that increased lipid availability associated with obesity favours FAO for satisfying energy needs. This reduces catabolism of other fuels as AA and glucose, sparing them for transfer to the fetus for fetal growth, and consequently leads to BCAA accumulation in maternal blood [21]. However, such an interpretation fails to explain the encountered elevated AC3:0 and AC5:0 levels, end products of BCAA catabolism, as well as the elevated AC4:0 and AC9:0, which were in agreement with previous reports from multiple cohorts of obese and IR subjects [19, 20].

In overweight/obese women, elevated levels of maternal hormones such as leptin, insulin, and IL-6 were discovered to play a key role in activating the mammalian target of rapamycin complex-1 (mTORC1) signalling and AA transporter activity [22], causing enhanced fetal growth. Moreover, evidence was found that placental BCAA uptake is further

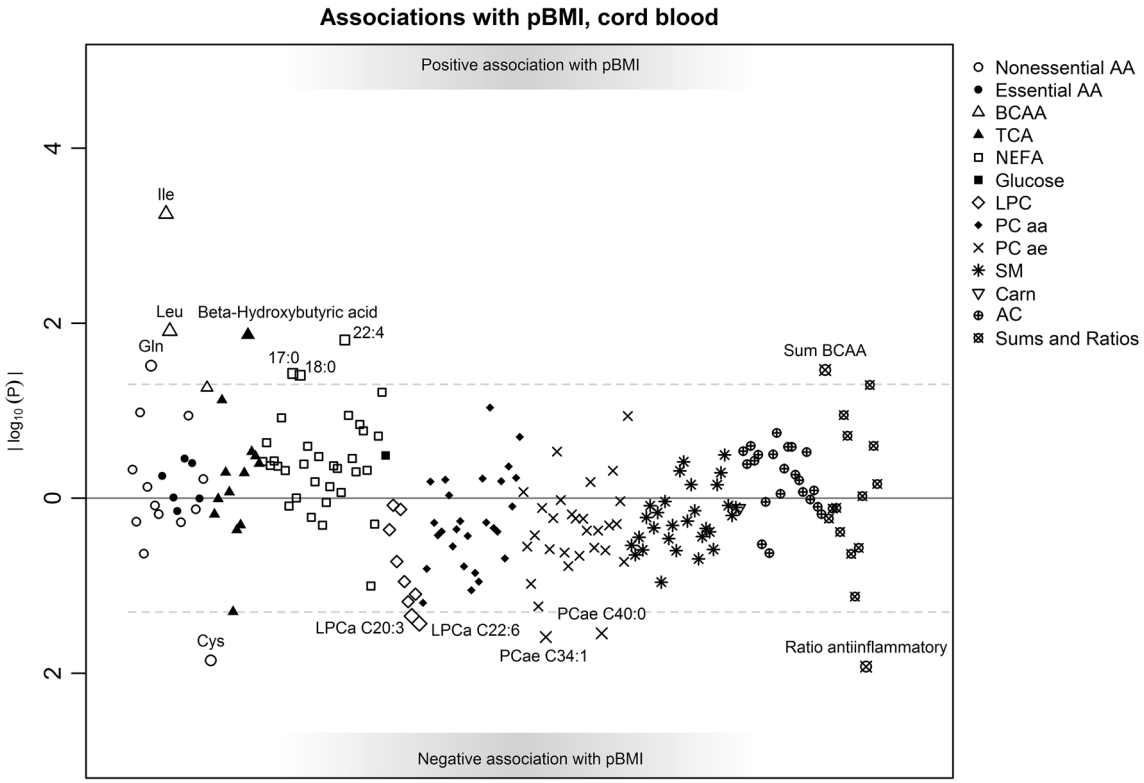


**Fig. 2** Manhattan plot showing association of cord blood metabolites with maternal pBMI. For abbreviations and explanation of the plot, see Fig. 1 legend

increased in pregnancies with enhanced maternal ketogenesis and β-oxidation rate [23].

In addition, we confirmed associations between pBMI and metabolites commonly related to inflammation, such as alpha-aminoadipic acid (AAA), LPC, and Cys. Low-grade inflammation, a common finding in obesity and diabetes, happens when excessive fat accumulation induces the release of adipokines which, in turn, lead to reactive oxygen species (ROS) production [24]. In maternal blood, mothers with high pBMI showed elevated AAA levels, whose excessive production was suggested to result from increased breakdown of Lys through oxidative stress and ROS [25]. AAA has also been identified as a potential modulator of glucose homeostasis in humans and an important factor mediating central obesity and diabetes [26]. In line with this picture, cord blood Σ(LPCa18:1 + LPCa18:2)/ΣPCaa levels, an anti-inflammatory marker, negatively associated with maternal pBMI [15]. Decreased LPCa18:1 and LPCa18:2 levels were previously associated with obesity and obesity-related factors [11, 27]. It is believed that not only the absolute concentration, but also the LPC acyl composition could be linked to inflammation, obesity, and atherogenesis, with a higher saturated-to-unsaturated LPC ratio being observed in inflammation [28]. We focused on four LPC metabolites (LPC containing 16:0, 18:0, 18:1, and 18:2 FA) and their ratios to PC because they were demonstrated to be among the most abundant serum metabolites [28]. The anti-inflammatory effect of LPC18:1 may be linked to decreased superoxide production and platelet aggregation [29] and was previously reported to be decreased among other unsaturated LPC in studies involving obese versus normal subjects [27]. Cord blood Cys was reduced suggesting that the existing oxidative stress drives Cys towards glutathione biosynthesis to fight ROS, thus enhancing its consumption and decreasing its cord blood levels [28].

Interestingly, maternal SM32:2 showed a weak positive trend with pBMI. Our group found the same tendency in another cohort of pregnant women [30] as well as a strong positive association with BMI and waist circumference in young adults [11] and children (unpublished results).

BHBA, a ketone body produced from the oxidation of fatty acids and utilized as an energy source particularly in the neonatal period and under starving conditions, was elevated only in cord blood in association with pBMI. Ketone bodies produced in maternal circulation are readily transported to the fetus as a source of energy. There are reports supporting free flow of ketone bodies from maternal to fetal circulation [31].

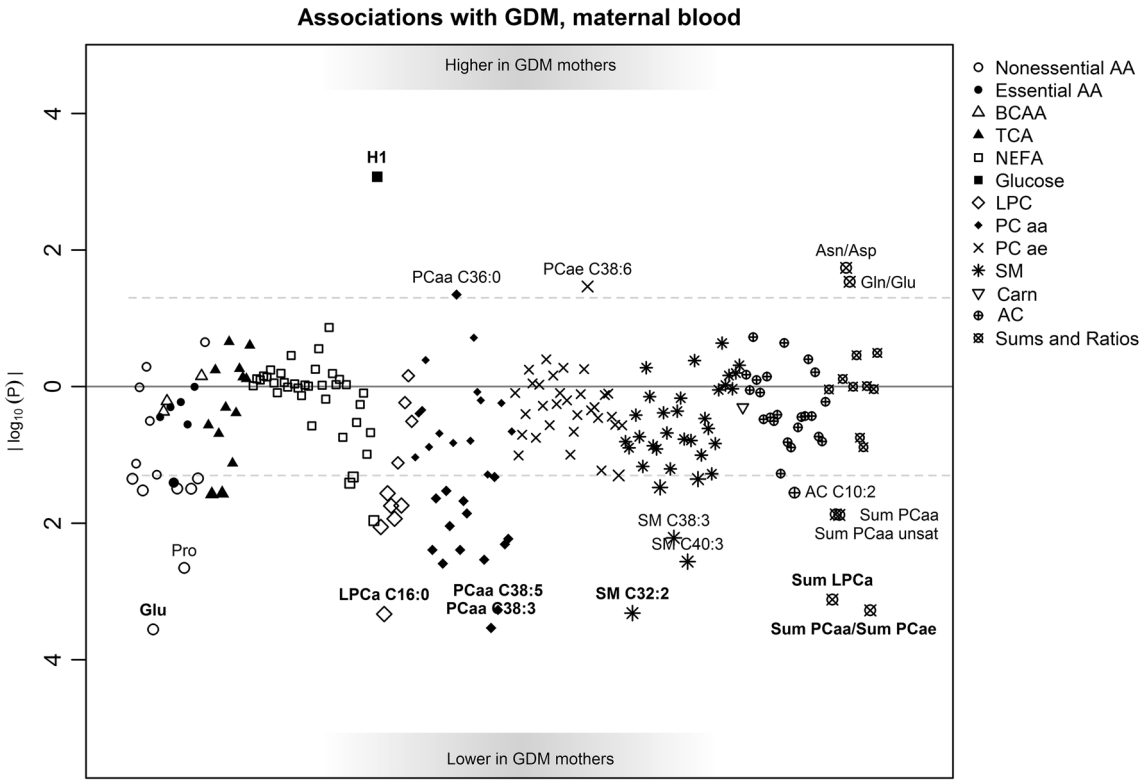


**Fig. 3** Manhattan plot showing association of metabolites in maternal plasma with gestational diabetes mellitus (GDM). The *y*-axis represents the log10 *p* value of GDM; the direction (positive or negative) corresponds to the sign of the estimate for GDM. For abbreviations and explanation of the plot, see Fig. 1 legend. Associations significant after FDR correction are marked with bold names for the metabolite

## Influence of GDM on maternal and cord blood metabolome

High pBMI and GDM share some common associations with some metabolites in maternal/cord blood which can be explained on the same basis, since obesity also presents increased IR and altered glucose tolerance, typical of GDM [8]. Trends for higher Asn/Asp levels and lower levels of some Krebs cycle metabolites were encountered in cord blood, which agrees with previous reports on these metabolites in association with IR and hyperglycaemia during pregnancy and reduced use of these metabolites for Krebs cycle replenishment [20, 32].

In GDM, we found common metabolite associations in both maternal and cord blood. Expectedly, H1 was increased reflecting maternal hyperglycaemia and enhanced transplacental transfer of glucose. Glucose is transported down its concentration gradient from maternal to fetal circulation through "GLUT1-mediated transport" or facilitated glucose transporters in basal membrane (BM) of the syncytiotrophoblast (STB) epithelium of the human term placenta [33]. Increased GLUT1 expression and activity in the BM of STB in GDM women either treated with diet alone or with insulin therapy were reported [34]. This may lead to fetal hyperglycemia and hyperinsulinemia and was proposed as a mechanism causing fetal overgrowth in GDM [34]. Another important common finding is overall trend of reduced PL levels, especially maternal LPC and PCaa. Reduced synthesis of PC and, in turn, LPC, might explain this finding being a result of reduced hepatic de-novo synthesis of PC from phosphoethanolamines (PE) and *S*-adenosylmethionine (SAM), which is catalysed by phosphatidyl–ethanolamine methyl transferase (PEMT). To our understanding, the existing oxidative stress associated with GDM drives Cys metabolism towards glutathione biosynthesis as a defensive mechanism leading to a decrease in SAM and thus decreased PC and LPC [28, 35]. Decreased synthesis of PC, a major PL in the very low-density lipoprotein (VLDL) causes fat and cholesterol accumulation in the liver (hepatic steatosis) [35, 36]. These hypotheses, however, apply only to PCaa, since PCae did not behave similarly, therefore ΣPCaa/ΣPCae ratio was decreased in GDM mothers. This could be explained by considering that ether lipids represent only a small portion of the total PL (in our study, the total PCae concentration is < 10% of PCaa), and their intracellular levels are quite low in the liver [37], so their decreased synthesis might be too small to be detected.

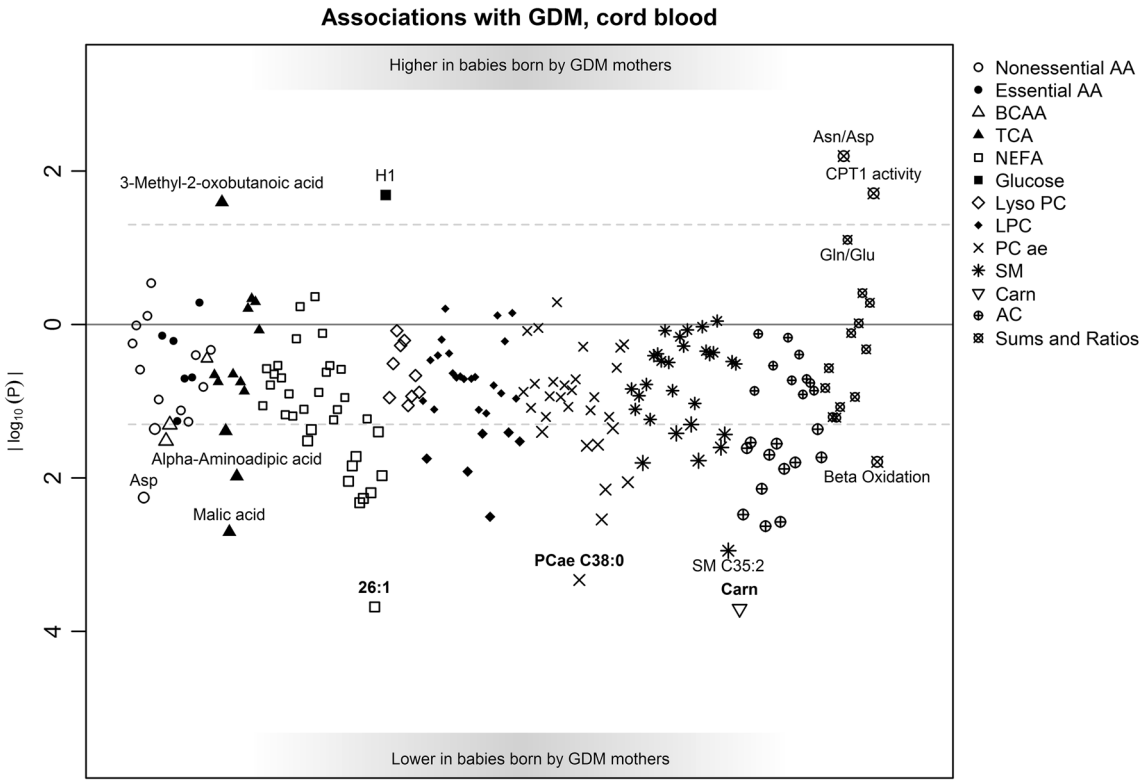


**Fig. 4** Manhattan plot showing association of metabolites in cord blood with GDM. For abbreviations and explanation of the plot, see Fig. 1 legend. Associations significant after FDR correction are marked with bold names for the metabolite

Trends for negative association with SM might be explained by either reduced synthesis [20] or increased breakdown [38]. The reduced de-novo synthesis was explained by redirection of phosphatidic acid to triglycerides (TG) rather than PL synthesis [28]. As for the increased breakdown, it was reported that GDM is associated with increased breakdown of SM resulting in ceramides, which induce inflammation and β-cell apoptosis and are negatively correlated with insulin sensitivity [28, 38].

Only in cord blood, we observed a significant decrease in Carn levels and an overall decrease in AC levels. There were different reports on Carn levels. One study reported low Carn in children with T1D [39]. In GDM pregnancies, data on Carn are scarce, but increased Carn levels in GDM mothers [40] and their offspring have been reported without clear explanation [41].This correlation of fetal and maternal Carn levels was explained due to the fetus's inability to synthetize Carn and its reliance on placental transfer [41]. Thus, one would expect that low fetal Carn would be secondary to low maternal levels. However, we found decreased fetal Carn with no differences for maternal Carn in the GDM group, along with a decrease in long-chain NEFA and fatty acid oxidation (FAO), as reflected in increased AC16:0/Carn and decreased AC2:0/AC16:0 ratios. A plausible explanation would be the reduced transport of Carn, and thus reduced initiation of FAO, as a consequence of fetal hyperglycemia. This matches the previously reported reduction in FAO, concomitant with elevated TG formation, in placentae of GDM mothers [42]. Thus, the whole picture suggests that GDM is associated with reduced placental transport of NEFAs and Carn along with incomplete or reduced FAO, which was reported in IR and diabetes [43].

Maternal blood lactic acid tended to be lower in GDM, although one might expect an elevation with increased IR, typical for GDM [44]. A plausible explanation would be the liver's ability to utilize circulating lactate for reconversion to glucose through the Cori cycle [45].

The proposed mechanisms of metabolic alterations associated with obesity and GDM are depicted in Suppl. Fig. S1. Results from linear models for all investigated metabolites are listed in Suppl. Table S2.

## Conclusion

Our study investigated the association of > 200 intermediates of energy metabolic pathways in maternal and cord blood with maternal BMI and GDM status. We observed consistent associations of BMI with BCAA as well as pronounced

association of several markers related to inflammation and β-oxidation with GDM.

**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. The data presented are part of the PhD thesis by Linda Marchioro at the Medical Faculty, LMU.

**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** All procedures followed were in accordance with the ethical standards of the bioethical Committees for clinical research of the Clinical University Hospital San Cecilio, the Mother-Infant University Hospital of Granada, and with the Helsinki Declaration of 1975, as revised in 2008.

**Informed consent** Written informed consent was obtained from all participants at the study entry.

## References

1. NCD Risk Factor Collaboration (2017) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 390(10113):2627–2642
2. Huvinen E, Eriksson JG, Stach-Lempinen B, Tiitinen A, Koivusalo SB (2018) Heterogeneity of gestational diabetes (GDM) and challenges in developing a GDM risk score. Acta Diabetol 55(12):1251–1259
3. Huvinen E, Eriksson JG, Koivusalo SB, Grotenfelt N, Tiitinen A, Stach-Lempinen B (2018) Heterogeneity of gestational diabetes (GDM) and long-term risk of diabetes and metabolic syndrome: findings from the RADIEL study follow-up. Acta Diabetol 55(12):1251–1259
4. Lowe WL Jr, Bain JR, Nodzenski M (2017) Maternal BMI and glycemia impact the fetal metabolome. Diabet Care 40:902–910
5. Kaaja R, Rönnemaa T (2008) Gestational diabetes: pathogenesis and consequences to mother and offspring. Rev Diab Stud 5(4):194–202
6. Pintaudi B, Fresa R, Dalfrà M, Dodesini AR, Vitacolonna E, Tumminia A, Sciacca L, Lencioni C, Marcone T, Lucisano G, Nicolucci A, Bonomo M, Napoli A, STRONG Study Collaborators (2018) The risk stratification of adverse neonatal outcomes in women with gestational diabetes (STRONG) study. Acta Diabetol 55(12):1261–1273
7. Leybovitz-Haleluya N, Wainstock T, Landau D, Sheiner E (2018) Maternal gestational diabetes mellitus and the risk of subsequent pediatric cardiovascular diseases of the offspring: a population-based cohort study with up to 18 years of follow up. Acta Diabetol 55(10):1037–1042
8. Berglund SK, García-Valdés L, Torres-Espinola FJ, Segura MT, Martínez-Zaldívar C, Aguilar MJ, Agil A, Lorente JA, Florido J, Padilla C, Altmäe S, Marcos A, López-Sabater MC, Campoy C, PREOBE Team (2016) Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). BMC Public Health 16:207
9. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI (2010) International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 33(3): 676–682
10. Hellmuth C, Weber M, Koletzko B, Peissner W (2012) Nonesterified fatty acid determination for functional lipidomics. Comprehensive ultrahigh performance liquid chromatography–tandem mass spectrometry quantitation, qualification, and parameter prediction. Anal Chem 84:1483–1490
11. Rauschert S, Uhl O, Koletzko B, Kirchberg F, Mori TA, Huang RC, Beilin LJ, Hellmuth C, Oddy WH (2016) Lipidomics reveals associations of phospholipids with obesity and insulin resistance in young adults. J Clin Endocrinol Metab 101:871–879
12. Harder U, Koletzko B, Peissner W (2011) Quantification of 22 plasma amino acids combining derivatization and ion-pair LC–MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 879:495–504
13. Maeba R, Hara H (2012) Serum choline plasmalogen is a reliable biomarker for atherogenic status. In: Squeri A (ed) Coronary artery disease—new insights and novel approaches. InTech, Rijeka, pp 243–260
14. Zhang W, Sun G, Aitken D, Likhodii S, Liu M, Martin G, Furey A, Randell E, Rahman P, Jones G, Zhai G (2016) Lysophosphatidylcholines to phosphatidylcholines ratio predicts advanced knee osteoarthritis. Rheumatol 55(9):1566–1574
15. Pickens CA, Vazquez AI, Daniel Jones A, Fenton JI (2017) Obesity, adipokines, and C-peptide are associated with distinct plasma and phospholipid profiles in adult males, an untargeted lipidomic approach. Sci Rep 7:6335
16. Kirchberg FF, Brandt S, Moß A, Peissner W, Koenig W, Rothenbacher D, Brenner H, Koletzko B, Hellmuth C, Wabitsch M (2017) Metabolomics reveals an entanglement of fasting leptin concentrations with fatty acid oxidation and gluconeogenesis in healthy children. PLoS One 12(8):e0183185
17. Sampath H, Ntambi JM (2008) Role of stearoyl-CoA desaturase in human metabolic disease. Future Lipidol 3:163–173
18. Myatt L, Powell T, Brown L et al (2010) Part I. nutritional regulation and requirements for pregnancy and fetal growth. In: Symonds ME, Ramsay M (eds) Maternal–fetal nutrition during pregnancy and lactation. Cambridge University Press, Cambridge, p 16
19. Butte NF, Liu Y, Zakeri IF, Mohney RP, Mehta N, Voruganti VS, Göring H, Cole SA, Comuzzie AG (2015) Global metabolomic profiling targeting childhood obesity in the Hispanic population. Am J Clin Nutr 102(2):256–267
20. Newgard CB (2012) Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell metab 15(5):606–614
21. Sandler V, Reisetter AC, Bain JR, Muehlbauer MJ, Nodzenski M, Stevens RD, Ilkayeva O, Lowe LP, Metzger BE, Newgard CB, Scholtens DM, Lowe WL Jr, HAPO Study Cooperative Research Group (2017) Association of maternal BMI and insulin resistance with the maternal metabolome and newborn outcomes. Diabetologia 60:518–530

22. Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, Jansson T, Powell TL (2013) Activation of placental mtor signaling and amino acid transporters in obese women giving birth to large babies. J Clin Endocrinol Metab 98:105–113
23. Lindsay KL, Hellmuth C, Uhl O, Buss C, Wadhwa PD, Koletzko B, Entringer S (2015) Longitudinal metabolomic profiling of amino acids and lipids across healthy pregnancy. PLoS One 10:e0145794
24. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T (2015) Oxidative stress in obesity: a critical component in human diseases. Int J Mol Sci 16:378–400
25. Zeitoun-Ghandour S, Leszczyszyn OI, Blindauer CA, Geier FM, Bundy JG, Stürzenbaum SR (2011) *C. elegans* metallothioneins: response to and defence against ROS toxicity. Mol Biosyst 7(8):2397–2406
26. Gao X, Zhang W, Yongbo W, Pedram P, Cahill F, Zhai G, Randell EW, Gulliver WP, Sun G (2016) Serum metabolic biomarkers distinguish metabolically healthy peripherally obese from unhealthy centrally obese individuals. Nutr Metab 13:33
27. Kim JY, Park JY, Kim OY, Ham BM, Kim HJ, Kwon DY, Jang Y, Lee JH (2010) Metabolic profiling of plasma in overweight/obese and lean men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). J Proteome Res 9:4368–4375
28. Dudzik D, Zorawski M, Skotnicki M, Zarzycki W, Kozlowska G, Bibik-Malinowska K, Vallejo M, García A, Barbas C, Ramos MP (2014) Metabolic fingerprint of gestational diabetes mellitus. J Proteom 103:57–71
29. Curcic S, Holzer M, Pasterk L, Knuplez E, Eichmann TO, Frank S, Zimmermann R, Schicho R, Heinemann A, Marsche G (2017) Secretory phospholipase A2 modifed HDL rapidly and potently suppresses platelet activation. Sci Rep 7:8030
30. Hellmuth C, Lindsay KL, Uhl O (2017) Association of maternal prepregnancy BMI with metabolomic profile across gestation. Int J Obes (Lond) 41(1):159–169
31. Herrera E (2002) Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 19(1):43–55
32. Scholtens DM, Muehlbauer MJ, Daya NR, Stevens RD, Dyer AR, Lowe LP, Metzger BE, Newgard CB, Bain JR, Lowe WL Jr, HAPO Study Cooperative Research Group (2014) Metabolomics reveals broad-scale metabolic perturbations in hyperglycemic mothers during pregnancy. Diabetes Care 37(1):158–166
33. Day PE, Cleal JK, Lofthouse EM, Hanson MA, Lewis RM (2013) What factors determine placental glucose transfer kinetics? Placenta 34:953–958
34. Gaither K, Quraishi AN, Illsley NP (1999) Diabetes alters the expression and activity of the human placental GLUT1 glucose transporter. J Clin Endocrinol Metab 84:695–701
35. Obeid R, Herrmann W (2009) Homocysteine and lipids: *S*-adenosyl methionine as a key intermediate. FEBS Lett 583:1215–1225
36. Li Z, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. Cell Metab 3(5):321–331
37. Braverman NE, Moser AB (2012) Review Functions of plasmalogen lipids in health and disease. Biochim Biophys Acta 1822(9):1442–1452
38. Allalou A, Nalla A, Prentice KJ (2016) A predictive metabolic signature for the transition from gestational diabetes mellitus to type 2 diabetes. Diabetes 65:2529–2539
39. Evangeliou A, Gourgiotis D, Kragianni C, Markouri M, Anogianaki N, Mamoulakis D, Maropoulos G, Tsakalidis C, Frentzayias A, Nicolaidou P (2010) Carnitine status and lactate increase in patients with type I juvenile diabetes. Minerva Pediatr 62(6):551–557
40. Pappa KI, Anagnou NP, Salamalekis E, Bikouvarakis S, Maropoulos G, Anogianaki N, Evangeliou A, Koumantakis E (2005) Gestational diabetes exhibits lack of carnitine deficiency despite relatively low carnitine levels and alterations in ketogenesis. J Matern Fetal Neonatal Med 17:63–68
41. Agakidou E, Diamanti E, Papoulidis I, Papakonstantinou E, Stergioudas I, Sarafidis K, Drossou V, Evangeliou A (2013) Effect of gestational diabetes on circulating levels of maternal and neonatal carnitine. J Diabetes Metab 4:250
42. Visiedo F, Bugatto F, Sanchez V, Cozar-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:E205–E212
43. Lackey DE, Lynch CJ, Olson KC, Mostaedi R, Ali M, Smith WH, Karpe F, Humphreys S, Bedinger DH, Dunn TN, Thomas AP, Oort PJ, Kieffer DA, Amin R, Bettaieb A, Haj FG, Permana P, Anthony TG, Adams SH (2013) Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity. Am J Physiol Endocrinol Metab 304:E1175–E1187
44. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, Young JF (2010) Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. Int J Epidemiol 39(6):1647–1655
45. Wu Y, Dong Y, Atefi M, Liu Y, Elshimali Y, Vadgama JV (2016) Lactate, a neglected factor for diabetes and cancer interaction. Mediators Inflamm 2016:6456018

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.