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Original Paper

Cord Blood Lysophosphatidylcholine 16:1 is Positively Associated with Birth Weight

Yong-Ping Lu[a,b,c] Christoph Reichetzeder[a] Cornelia Prehn[d] Liang-Hong Yin[c] Chen Yun[a,b] Shufei Zeng[a,b,c] Chang Chu[c] Jerzy Adamski[d,e,f] Berthold Hocher[a,g,h]

[a]Institute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke, [b]Department of Nephrology, Charité - Universitätsmedizin Berlin, Campus Mitte, Berlin, Germany, [c]Department of Nephrology, the first Affiliated Hospital of Jinan University, Guangzhou, China, [d]Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, [e]Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising-Weihenstephan, [f]German Center for Diabetes Research, München-Neuherberg, Germany, [g]Department of Histology and Embryology, Basic Medical College, Jinan University 510632 Guangzhou, [h]Key Laboratory of Study and Discovery of Small Targeted Molecules of Hunan Province, School of Medicine, Hunan Normal University, Changsha, China

Key Words
Metabolomics • Lysophosphatidylcholine • Birth Weight • DOHaD • Hypertension • Type 2 Diabetes

Abstract
Background/Aims: Impaired birth outcomes, like low birth weight, have consistently been associated with increased disease susceptibility to hypertension in later life. Alterations in the maternal or fetal metabolism might impact on fetal growth and influence birth outcomes. Discerning associations between the maternal and fetal metabolome and surrogate parameters of fetal growth could give new insight into the complex relationship between intrauterine conditions, birth outcomes, and later life disease susceptibility. ***Methods:*** Using flow injection tandem mass spectrometry, targeted metabolomics was performed in serum samples obtained from 226 mother/child pairs at delivery. Associations between neonatal birth weight and concentrations of 163 maternal and fetal metabolites were analyzed. ***Results:*** After FDR adjustment using the Benjamini-Hochberg procedure lysophosphatidylcholines (LPC) 14:0, 16:1, and 18:1 were strongly positively correlated with birth weight. In a stepwise linear regression model corrected for established confounding factors of birth weight, LPC 16:1 showed the strongest independent association with birth weight (CI: 93.63 - 168.94; $P = 6.94\times10^{-11}$). The association with birth weight was stronger than classical confounding factors such as offspring sex (CI: -258.81- -61.32; $P = 0.002$) and maternal smoking during pregnancy (CI: -298.74 - -29.51; $P = 0.017$). ***Conclusions:*** After correction for multiple testing and adjustment for potential confounders, LPC 16:1 showed a very strong and independent

Y.-P. Lu and C. Reichetzeder contributed equally to this work.

Berthold Hocher
and Liang-Hong Yin

Key Laboratory of Study and Discovery of Small Targeted Molecules of Hunan Province, School of Medicine, Hunan Normal University, Changsha, (China)
Department of Nephrology, the First Affiliated Hospital of Jinan University, Guangzhou, (China)
E-Mail hocher@uni-potsdam.de; 13725251458@126.com

association with birth weight. The underlying molecular mechanisms linking fetal LPCs with birth weight need to be addressed in future studies.

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Introduction

It is by now widely accepted, that the early life environment is not just an important factor affecting immediate birth outcomes, but is also associated with cardiovascular disease susceptibility in later life – for instance the likelihood of developing hypertension. The developmental origins of health and disease (DOHaD) hypothesis, an explanatory model for the link between early life conditions and adult disease susceptibility, has been established based on the data of epidemiological [1-4] and animal studies [5-8]. As alterations in the intrauterine environment can affect fetal growth, anthropometric measures at birth, like birth weight, birth length and head circumference are well established surrogate parameters in the investigation of developmental disease origins [9]. There is a vast amount of compelling evidence in literature demonstrating that low birth weight is associated with an increased risk for metabolic and cardiovascular disease as well as hypertension in later life [2, 10-11]. Growth and development in utero are complex processes which depend on a variety of maternal, paternal and fetal factors for an optimal outcome [12]. Due to the intricacy of involved factors, our understanding of underlying mechanisms of developmental disease origins is still limited. Recent technologic advances in high-throughput methods, like array and diverse Omics approaches have revolutionized biological research. Metabolomics may capture exposures that are notoriously challenging to quantify and improve our understanding of the link between early-life environmental factors, fetal development and disorders in later life [12-14]. Targeted metabolomics can provide detailed quantitative information on the metabolic status of an organism, adding to the better characterization of phenotypes associated with metabolic and cardiovascular sequelae over the life course [14]. Metabolic profiling was already used in characterizing maternal plasma and umbilical cord blood metabolomes in conditions such as preterm birth [15], small for gestational age (SGA) [16], low birth weight [17], very low birth weight [18, 19] and intrauterine growth retardation (IUGR) [20-22]. However, a major limitation of available studies are heterogeneous study designs, applied methodology, low sample sizes, the usage of untargeted metabolomic approaches, and the lack of replication of obtained study results [15-23].

The aim of the current study was to investigate associations between the maternal and fetal metabolome at time of birth and well established birth weight in an appropriately sized mother child cohort employing a widely used targeted metabolomic approach.

Materials and Methods

Clinic data collection

This observational study was approved by the local ethics committee and carried out at the Department of Obstetrics, Charité Universitaetsmedizin Berlin (Berlin, Germany). 226 newborns and their mothers entered the study. The majority (89.9%) were of Caucasian ethnicity – for details see also [15, 24, 25].

A structured medical history was taken. The following data were extracted into our database: age, ethnicity, weight before pregnancy, body height, gravidity, parity, hypertension and diabetes mellitus during pregnancy, smoking status before and during pregnancy, systolic and diastolic blood pressure (BP) measurements recorded during pregnancy, and the mode of delivery (normal delivery or cesarean section). As well as the newborn postnatal examination biometric data birth weight, birth length, head circumference, child sex, and Apgar scores assessed at 5 and 10 minutes were collected

Sample collection

Midwives collected maternal blood from a cubital vein in the delivery room or on the ward prior to birth. Fetal blood was collected from the umbilical cord immediately after delivery. Blood was centrifuged at 2750 g and the obtained serum samples were then stored at -80 °C until it was analyzed.

Targeted metabolomics in blood serum

Metabolite quanitification were done in the Metabolomic Platform of the Genome Analysis Center, Helmholtz Zentrum München, using FIA-ESI-MS/MS and the Absolute*IDQ*™ p150 Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). The assay allows simultaneous quantification of 163 metabolites out of 10 µL serum, and includes free carnitine, 40 acylcarnitines, 14 amino acids (13 proteinogenic + ornithine), hexoses (sum of hexoses – about 90-95 % glucose), 92 glycerophospholipids (15 lysophosphatidylcholines (lysoPC) and 77 phosphatidylcholines (PC)), and 15 sphingolipids. The method of Absolute*IDQ*™ p150 Kit has been proven to be in conformance with the EMEA-Guideline "Guideline on bioanalytical method validation (July 21st 2011") [26], which implies proof of reproducibility within a given error range. A detailed description of the sample preparation, assay procedures and nomenclature have been published previously [27-29].

Sample handling was performed by a Hamilton Microlab STAR™ robot (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a Ultravap nitrogen evaporator (Porvair Sciences, Leatherhead, U.K.), beside standard laboratory equipment. Mass spectrometric analyses were done on an API 4000 triple quadrupole system (Sciex Deutschland GmbH, Darmstadt, Germany) equipped with a 1200 Series HPLC (Agilent Technologies Deutschland GmbH, Böblingen, Germany) and a HTC PAL auto sampler (CTC Analytics, Zwingen, Switzerland) controlled by the software Analyst 1.5.1. Data evaluation for quantification of metabolite concentrations and quality assessment was performed with the Met*IDQ*™ software package, which is an integral part of the Absolute*IDQ*™ Kit. Metabolite concentrations were calculated using internal standards and reported in µM.

Statistical analysis

Data were analyzed with SPSS version 22.0. To find associations between neonatal birth weight and targeted metabolites, a three-step analysis was used: bivariate correlation analysis, *P*-values adjustment for multiple testing, and multiple linear regression analysis. In bivariate correlation analysis, B was used to estimate the strength of a correlation. To reduce false discovery rate (FDR) due to multiple testing, resulting *P*-values from bivariate correlation analysis were adjusted using the Benjamini-Hochberg (BH) procedure. The BH procedure is defined as $P_m \leq m \times q/M$ [30, 31]. M equals the total number of tested metabolites (M=163, 163 metabolites), q equals the FDR (the FDR set up at 5% in the present paper), *P*m equals the individual *P*-value's rank, and m equals the individual rank of tested metabolite. Factors known to be associated with birth weight (gestational age [32], child sex [33], maternal age [34], maternal BMI before pregnancy [35], maternal smoking during pregnancy [36, 37]) were used as confounders to calculate and adjust putative predictive metabolites in linear regression and stepwise linear regression models. For stratifying the cohort into small (SGA), appropriate (AGA), and large for gestational age (LGA) offspring, the 10th and 97th percentiles of birth weight for the gestational age were used as cut-offs defining SGA and LGA, respectively [38, 39]. A statistically significant difference was considered as $P < 0.05$.

Results

Description of the cohort

Descriptive data of the study population, which represented a regular birth cohort in regard to key characteristics such maternal age, height, BMI before pregnancy, smoking status and newborn sex, birth weight, birth length, and head circumference, are given in Table 1. For the distribution of child birth weight see Fig. 1. A.

Bivariate correlation analyses of maternal serum metabolites and child birth weight

Bivariate correlation analyses showed that four acylcarnitines (C10:1, C14:2-OH, C16:2-OH, C18:1-OH), and one sphingolipid SM (OH) C 16:1

Table 1. Detailed Descriptive Data of the mother and child (n = 226). Data are given as mean ± SE or %

Variable	Mean±SE / %
Caucasian ethnicity/other ethnicity, %	90.0/10.0
Maternal age, y	30.4±0.4
Maternal height, cm	166.3±0.5
Maternal weight before pregnancy, kg	63.2±0.6
Maternal BMI before pregnancy, kg/m²	22.3±0.3
Primigravida/primipara, %	37.4/62.6
Smoking before/during pregnancy, %	43.1/16.2
Hypertension before/during pregnancy, %	3.5/11.3
Diabetes mellitus before/during pregnancy, %	1.5/9.0
Mean weight 1st trimester kg	65.0±1.0
Mean weight 2nd trimester, kg	67.7±1.0
Mean weight 3rd trimester half , kg	75.2±0.0.9
Mean SBP in 1st trimester, mm Hg	113.1±1.0
Mean SBP in 2nd trimester, mm Hg	112.3±0.8
Mean SBP in 3rd trimester, mm Hg	113.3±0.7
Mean DBP in 1st trimester, mm Hg	68.7±0.7
Mean DBP in 2nd trimester, mm Hg	67.2±0.5
Mean DBP in 3rd trimester half, mm Hg	69.2±0.5
Gestational age at delivery, days	273.3±0.6
Child sex, male/female, %	52.2/47.8
Child birth weight, g	3372.3±30.5
Child birth length, cm	50.7±0.2
Child head circumference	34.7±0.1
Apgar score at 5 min	9.4±0.1
Apgar score at 10 min	9.6±0.1
Fetal cord blood pH	7.27±0.05

were positively correlated with birth weight (Table 2a). After p-value correction of the initially significant associations using the BH procedure (P_m > m×q/M), no more significant associations were observed.

Bivariate correlation analyses of fetal metabolites and child birth weight

Bivariate correlation analyses showed that nine LPCs (LPC 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 20:3, 20:4) were positively correlated with birth weight (Table 2b). After Benjamini-Hochberg adjustment, three LPCs (LPC 14:0, 16:1, 18:1) with P_m ≤ m×q/M remained significantly correlated with birth weight (Table 2b).

Linear regression analyses of fetal metabolites and child birth weight

Significant results obtained by FDR corrected bivariate correlation analysis were subsequently analyzed by multiple linear regression analysis, considering gestational age, child sex, maternal age, maternal BMI before pregnancy, and maternal smoking during pregnancy as confounders. LPC 14:0 (Standardized beta = 0.31, $P = 1.75\times10^{-7}$), 16:1 (Standardized beta = 0.38, $P = 6.94\times10^{-11}$), and 18:1 (Standardized beta = 0.35, $P = 7.89\times10^{-9}$) were strongly correlated with birth weight (table 3a). To investigate which of the of the three identified LPCs (LPC 14:0, 16:1 and 18:1) shows the greatest impact on birth weight, a stepwise multiple linear regression model was calculated. In this model fetal LPC 16:1 (Standardized beta = 0.39, $P = 6.94\times10^{-11}$, Table 3b model B) demonstrated the strongest correlation with birth weight.

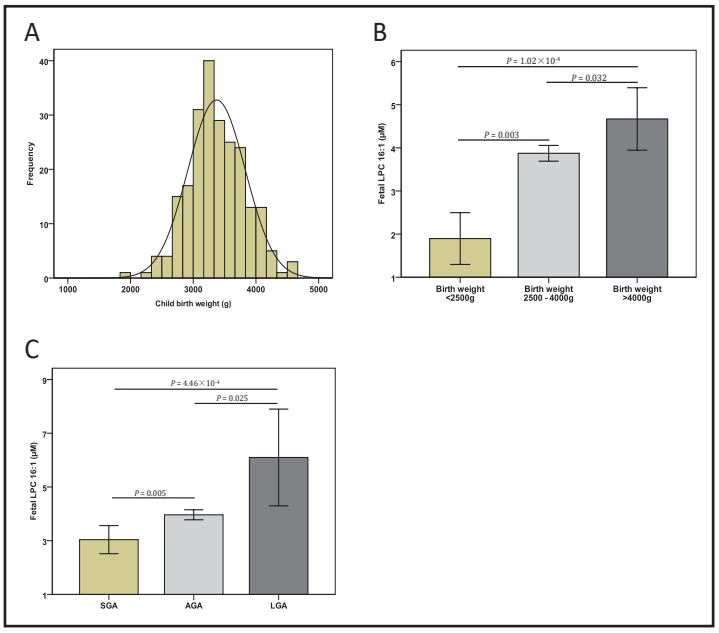

Fig. 1. Distribution of child birth weight (A), mean fetal serum LPC 16:1 in groups of child weight <2500g (n = 5), 2500-4000g (n = 199) and >4000g (n = 22) (B), and mean fetal serum LPC 16:1 in groups of SGA (n = 23), AGA (n = 198), and LGA (n = 5) (C). LPC = lysophosphatidylcholine. Data are given as mean ± SE, SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age.

Table 2. a. Correlation between maternal serum metabolites and child birth weight. (n = 226). Note: only metabolites with a p-value less than 0.05 in bivariate correlation analysis shown in the table. Cx:y = acylcarnitine, -OH = hydroxy, SM = sphingomyelin. b. Correlation between fetal serum metabolites and child birth weight. (n = 226). Note: only metabolites with a p-value less than 0.05 in bivariate correlation analysis shown in the table. LPC = lysophosphatidylcholine

a) Independent variable	Spearman Correlation	P_m value	m×q/M value
C10:1	0.144	0.031	1.23×10^{-3}
C14:2-OH	0.137	0.040	1.53×10^{-3}
C16:2-OH	0.145	0.029	9.20×10^{-4}
C18:1-OH	0.159	0.017	3.07×10^{-4}
SM (OH) C 16:1	0.156	0.019	6.13×10^{-4}
b) Independent variable	**Spearman Correlation**	**P_m value**	**m×q/M value**
LPC 14:0	0.252	2.12×10^{-4}	6.13×10^{-4}
LPC 16:0	0.223	7.42×10^{-3}	1.23×10^{-3}
LPC 16:1	0.286	2.60×10^{-5}	3.07×10^{-4}
LPC 17:0	0.200	0.003	1.53×10^{-3}
LPC 18:0	0.192	0.004	1.84×10^{-3}
LPC 18:1	0.220	8.79×10^{-4}	9.20×10^{-4}
LPC 18:2	0.139	0.037	2.76×10^{-3}
LPC 20:3	0.143	0.032	2.45×10^{-3}
LPC 20:4	0.146	0.029	2.15×10^{-3}

To rule out that the observed correlation between fetal LPC 16:1 and birth weight is an epiphenomenon mediated by hypoxic activation of phospholipase A2 and subsequent generation/accumulation of LPCs, further statistical analyses using fetal cord blood pH and APGAR scores as parameters of birth related hypoxia were performed. Fetal LPC 16:1 was not significantly correlated to fetal cord blood pH. Furthermore, quartiles of LPC 16:1 fetal serum levels were generated and fetal cord blood pH and APGAR scores at 5 min & 10 min compared within these 4 groups. There were no significant differences in fetal cord blood pH and APGAR score at 5 min & 10 min among the LPC 16:1 quartiles (For more details, see Table 4). To further confirm that fetal LPC 16:1 is independently associated with birth weight, a third linear regression model (model C) using fetal cord blood pH and APGAR score at 5 min as confounders were conducted. Also in this model, fetal LPC 16:1 (Standardized beta = 0.39, $P = 8.17\times10^{-10}$) was clearly positively correlated with birth weight (Table 3b).

To check and confirm the final result, fetal serum LPC 16:1 concentrations were compared in low (<2500g), normal (2500-4000g) and high (>4000g) birth weight groups, and in groups small (SGA), appropriate (AGA) and large for gestational age (LGA). Newborns with low birth

Table 3. a. Linear Regression models analyzing associations between fetal serum metabolites and child birth weight. (n = 226). LPC = lysophosphatidylcholine. Model A Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy, and LPC 14:0 being the independent variable and birth weight being dependent variable. Model B Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy, and LPC 16:1 being the independent variable and birth weight being dependent variable. Model C Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy, and LPC 18:1 being the independent variable and birth weight being dependent variable. 3b. Stepwise Linear Regression models analyzing associations between fetal serum metabolites and child birth weight. (n = 226). LPC = lysophosphatidylcholine. Model A Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy being the independent variable and birth weight being dependent variable. Model B Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy, and the 3 metabolites from the above table 2b ($P_m \leq m\times q/M$) being the independent variable and birth weight being dependent variable. Model C Considering gestational age, child sex, maternal age, maternal pre-pregnancy BMI, mother smoking during pregnancy, fetal cord blood pH, apgar score at 5 min and the 3 metabolites from the above table 2b ($P_m \leq m\times q/M$) being the independent variable and birth weight being dependent variable

a

Variable	Standardized Beta	t	P	95.0% Confidence interval for B
Model A (R^2 = 0.33)				
LPC 14:0	0.31	5.40	1.75×10^{-7}	213.27～458.26
Gestational age	0.42	7.32	3.62×10^{-12}	17.24～29.94
Child sex	-0.17	-2.95	0.003	-255.75～-50.81
Maternal age	0.15	2.59	0.010	2.78～20.56
Maternal pre-pregnancy BMI	0.09	1.52	0.129	-2.59～20.23
Mother smoking during pregnancy	-0.14	-2.31	0.022	-303.11～-23.90
Model B (R^2 = 0.38)				
LPC 16:1	0.39	6.87	6.94×10^{-11}	93.63～168.94
Gestational age	0.47	8.30	1.22×10^{-14}	19.99～32.44
Child sex	-0.18	-3.19	0.002	-258.81～-61.32
Maternal age	0.14	2.52	0.013	2.37～19.53
Maternal pre-pregnancy BMI	0.10	1.75	0.082	-1.26～20.77
Mother smoking during pregnancy	-0.14	-2.40	0.017	-298.74～-29.51
Model C (R^2 = 0.35)				
LPC 18:1	0.35	6.01	7.89×10^{-9}	25.38～50.13
Gestational age	0.47	8.07	5.30×10^{-14}	19.78～32.57
Child sex	-0.18	-3.15	0.002	-263.23～-60.72
Maternal age	0.16	2.79	0.006	3.62～21.10
Maternal pre-pregnancy BMI	0.09	1.62	0.108	-2.03～20.47
Mother smoking during pregnancy	-0.15	-2.53	0.012	-313.99～-38.96

b

Variable	Standardized Beta	t	P	95.0% Confidence interval for B
Model A (R^2 = 0.24)				
Gestational age	0.39	6.35	1.29×10^{-9}	14.91～28.33
Child sex	-0.13	-2.14	0.034	-225.39～-9.12
Maternal age	0.18	2.92	0.004	4.52～23.36
Maternal pre-pregnancy BMI	0.06	0.96	0.337	-6.19～17.99
Mother smoking during pregnancy	-0.15	-2.39	0.018	-328.25～-31.41
Model B (R^2 = 0.38)				
LPC 16:1	0.39	6.87	6.94×10^{-11}	93.63～168.94
Gestational age	0.47	8.30	1.22×10^{-14}	19.99～32.44
Child sex	-0.18	-3.19	0.002	-258.81～-61.32
Maternal age	0.14	2.52	0.013	2.37～19.53
Maternal pre-pregnancy BMI	0.10	1.75	0.082	-1.26～20.77
Mother smoking during pregnancy	-0.14	-2.40	0.017	-298.74～-29.51
Model C (R^2 = 0.38)				
LPC 16:1	0.39	6.49	8.17×10^{-10}	88.56～166.31
Gestational age	0.47	7.85	2.80×10^{-13}	19.51～32.59
Child sex	-0.17	-2.93	0.004	-259.87～-50.93
Maternal age	0.12	1.88	0.061	-0.42～17.93
Maternal pre-pregnancy BMI	0.08	1.32	0.190	-3.76～18.81
Mother smoking during pregnancy	-0.14	-2.41	0.017	-319.10～-32.10
Fetal cord blood pH	-0.05	-0.75	0.456	-1124.26～506.98
Apgar score at 5 min	0.16	1.82	0.070	-7.53～189.23

Table 4. Fetal cord blood pH and apgar score at 5 min & 10 min comparison according LPC 16:1 quartiles. Data are given as mean ± SE.

Variable	First quartile (n = 56)	Second quartile (n = 56)	Third quartile (n = 56)	Fourth quartile (n = 55)	P value
Fetal cord blood pH	7.25±0.01	7.25±0.01	7.29±0.01	7.27±0.01	0.072
Apgar score at 5 min	9.22±0.13	9.38±0.11	9.54±0.08	9.31±0.10	0.154
Apgar score at 10 min	9.48±0.11	9.62±0.09	9.72±0.07	9.62±0.09	0.250

weight (<2500g; n = 5) displayed significantly lower (1.90±0.21 μM) mean serum LPC 16:1 levels compared to normal (2500-4000g; n = 199; 3.85±0.09 μM) and high (>4000g; n = 22; 4.60±0.33 μM) birth weight newborns (for more details, see Fig. 1.B). Additionally, high birth weight newborns had significantly elevated serum LPC 16:1 concentrations compared to normal birth weight newborns. The same pattern of significant differences in fetal serum LPC 16:1 levels could be observed comparing SGA (n = 23; 3.04±0.25 μM), AGA (n = 198; 3.97±0.09 μM), and LGA (n = 5; 5.50±0.74 μM) newborns (for more details, see Fig. 1.C).

Discussion

The current study investigated the association between 163 maternal and fetal serum metabolites at birth and newborn birth weight in a cohort of 226 mother-newborn pairs. An initial analysis, not adjusted for confounding factors, identified associations between several maternal and fetal metabolites with birth weight. However, after FDR adjustment only fetal LPC 14:0, LPC 16:1, and LPC 18:1 were significantly associated with birth weight. Employing linear multiple regression models followed by a stepwise multiple regression model, all adjusted for confounding factors known to affect neonatal anthropometric measurements (gestational age [32], child sex [33], maternal age [34], maternal BMI before pregnancy [35], maternal smoking during pregnancy [36, 37]) revealed that out of the three LPCs, fetal serum LPC 16:1 showed the strongest independent association with birth weight. Data stratification into groups of birth weight (<2500g; 2500-4000g; >4000g;) and groups of size for gestational age (SGA; AGA; LGA) further substantiated the positive association between fetal LPC 16:1 and size at birth.

One possible explanation of the observed positive association between fetal LPC 16:1 and birth weight might be hypoxia. It is well known that hypoxia activates phospholipases that induce the generation of LPCs [40-42]. The process of giving birth is characterized by hypoxic periods of varying duration for both, the mother and the fetus. Theoretically, due to a longer duration of giving birth, larger newborns may be subjected to longer acute periods of hypoxia and thus display a stronger activation of phospholipases and higher levels of LPCs. However, in the current study we were not able to observe a significant association between data indicative of hypoxia, ie. fetal cord blood pH and APGAR scores, and fetal LPC 16:1 levels.

To the best of our knowledge, this is the second large scale study showing an independent association between fetal LPCs and birth weight. Very recently, Hellmuth et al. demonstrated in a large birth cohort a strong independent positive correlation between birth weight and several cord blood LPCs, including LPC 14.0, LPC 16:1, and LPC 18:1 [43]. Similarly, also in the current study these metabolites were independently associated with birth weight after FDR adjustment. Furthermore, also in the study by Hellmuth et al. LPC 16:1 demonstrated the strongest association with birth weight. In addition, several metabolites were negatively associated with birth weight, which might have been due to the larger cohort size of 753 fetal cord blood samples/newborns. Different from the current study, Hellmuth et al. did not use a commercially available kit for the metabolomic analyses but several methodological approaches targeting different classes of metabolites. Based on these methodological differences absolute metabolite concentrations might not be comparable between the two studies. The general tendencies of metabolite concentrations i.e. in relation to birth weight should not be affected. This underscores a putatively important role of specific LPCs in fetal growth. Based on the current state of literature, however, not much is known regarding

underlying mechanisms of the observed association. LPCs in the adult organism result from partial hydrolysis of PCs by the enzymatic action of phospholipase A2 [44], from hepatic secretion [45], and lecithin-cholesterol acyltransferase [46]. Several studies have linked LPCs with obesity, but results are contradictive, which might be due to the multifaceted incompletely understood functions of LPCs [47-49]. Higher levels of LPC species were reported in obese men [50] and also observed in an obese monozygotic twin study [51]. In a study designed to discover biomarkers that are indicative of weight change, LPC 14:0 was shown to be strongly positively associated with rapid growth and childhood obesity [52]. In contrast to these positive associations other studies showed negative associations between serum LPCs and obesity [53-57]. Overweight/obese children had decreased levels of the unsaturated LPC 18:1 [53, 54] compared with normal weight children. The comparison of adult overweight/obese subjects to their lean counterparts demonstrated increased concentrations of saturated LPCs (LPC 14:0, 18:0) and decreased concentrations of the unsaturated LPC 18:1 [55]. Also in an animal model of obesity decreased levels of the unsaturated LPCs 16:1 and 18:1 were observed [58]. Given the inhomogeneity of study results and lack of available suitable mechanistic data, it is hard to draw any firm conclusions on how LPCs might influence body weight, especially in the fetal organism. However, one mechanism that is believed to negatively affect offspring birth weight that might also be connected to LPC metabolism is fetal insulin resistance. Insulin is one of the main culprits of macrosomia in children born to diabetic mothers [59]. Contrary, attenuated fetal insulin signaling, as found in insulin resistance, has been suggested to decrease birth weight and to be a characteristic of the low birth weight phenotype [60, 61]. It was shown, that certain LPCs (among these LPC14:0) can interact with glucose metabolism independent of insulin signaling and lead to enhanced cellular glucose uptake [62]. Transcriptome analyses of human myotubes treated with LPC 16:0 and 18:1, demonstrated an increased expression of PPARδ regulated transcripts, inducing anti-diabetic and antiinflammatory effects [63]. Furthermore, it was demonstrated that LPCs (especially LPC 18:1) can enhance glucose-dependent insulin secretion in perfused rat pancreas via an orphan G-protein coupled receptor [64]. A study investigating associations between BMI, inflammation and insulin resistance demonstrated negative associations between LPC 18:1 and various adipokines and inflammatory mediators. Low levels of LPC 18:1 together with increased levels of leptin or CRP were associated with increased HOMA scores [65]. Applied to the results of the current study, altered levels of LPCs hypothetically could affect fetal growth by influencing insulin resistance and insulin secretion. Lower levels of LPCs, as found in offspring with lower birth weight, could result in increased fetal insulin resistance and decreased insulin secretion, which could negatively affect fetal growth. This hypothesis is interesting in context with previous findings from our group. In two independent previous studies we observed a negative correlation between total glycated cord blood hemoglobin and birth weight, an observation that contrasts the usual positive correlation between maternal glycemia and birth weight [66, 67]. Results of these studies indicated that lighter fetuses, when subjected to similar degrees of maternal glycemia, display an incapability of adequately lowering their blood glucose concentrations (reflected by elevated cord blood total glycated hemoglobin), in comparison to heavier fetuses. The mechanism behind these associations remained unexplored, but alterations in LPC metabolism may serve as a link in the connection between impaired fetal glucose handling and low birth weight.

The reason why LPCs 14:0, 16:1 and 18:1 were lower in the serum of lower birth weight newborns cannot be answered by our study. Several factors including maternal dietary intake, placental transfer and fetal production are possible. In the current study, levels of LPC 16:1 were about threefold higher in fetal compared to maternal serum, yet showed a barely significant positive correlation (data not shown). This finding suggests that LPC 16:1 might predominantly be generated in the fetus, but does not preclude alterations in the trans-placental transport of precursor forms. Interesting in this regard, LPC 16:0 concentrations did not differ between mother and fetus and there was a modest, highly significant correlation between fetal and maternal LPC 16:0 levels. The same pattern of absent maternal-fetal

concentration differences and presence of significant correlations could be observed for the saturated LPCs 14:0 and 18:0.

Study limitations and Outlook

One limitation of the current study is that serum metabolites were only measured at one occasion prior to birth. Furthermore, we cannot rule out if specific phenotypic and lifestyle factors which we did not account for, may have influenced the results. However, we used a very strict approach, employing BH procedure to reduce false discovery rate, followed by linear regression analysis adjusted for confounding factors known to affect neonatal anthropometric measurements. Moreover, the results of the study remain purely associative. Future studies are needed to investigate underlying mechanisms of the observed associations. Despite of the mentioned study limitations, a strength of the current study is the replication of major findings of a previous study by Hellmuth et al., [43] which is a crucial aspect of research based on high-throughput data.

In conclusion, after correction for multiple testing and adjustment for potential confounders, lysophosphatidylcholine 16:1 showed a very strong and independent association with birth weight, a surrogate parameter of intrauterine development and adult disease susceptibility. In the future, suitable preclinical studies are needed to better characterize underlying mechanisms of the observed association and to investigate if there is a mechanistic link between low birth weight, insulin resistance and alterations in LPC metabolism. Future clinical studies should include additional collection of information on possible lifestyle-related environmental factors and medication with a possible influence on the metabolic profile, as well as the measurement of serum metabolites at multiple and/or earlier occasions.

Conclusion

The aim of the current study was to investigate associations between the maternal and fetal metabolites and birth weight. There were no correlations between maternal metabolomics and birth weight. After correction for multiple testing and adjustment for potential confounders, LPC 16:1 showed a very strong and independent association with birth weight. This correlation was even stronger than classical confounding factors such as maternal smoking orr offspring sex.

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Disclosure Statement

No conflict of interest exists.

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