Maternal and Fetal Metabonomic Alterations in Maternal Lipopolysaccharide Exposure-induced Male Offspring Glucose Metabolism Disorders

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Research

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Abstract

Maternal lipopolysaccharide(LPS)exposure during pregnancy induced metabolic abnormalities in male offspring, but the underlying mechanisms are still unclear. The aims of this study were to elucidate the underlying etiologies by characterizing the metabolic alterations in maternal serum and male fetal liver. Pregnant mice were intraperitoneally injected with LPS (50ug/kg/d) from gestational period (GD 15 to GD 17). In the GD18, maternal serum and male fetal liver were collected. The metabolic profiles were analyzed using liquid Chromatograph Mass Spectrometer (LC-MS) techniques. After LPS exposure, glycerophospholipids containing saturated fatty acids were up-regulated, and glycerophospholipids containing polyunsaturated fatty acids were down-regulated in both pregnant mice and male offspring. In addition, we observed that LPS-exposed dams also had increased saturated fatty acids levels and decreased polyunsaturated fatty acids levels. Because these abnormal glycerophospholipids and fatty acid metabolism have been identified as possibly associated with the risk of type 2 diabetes, our study has therefore identified two pathways (glycerophospholipids and fatty acid metabolism) that potentially underlie LPS induced fetal metabolic disease.

1. Introduction

The developmental origins of health and disease (DOHaD) theory has raised that in the early stages of life, especially during intrauterine life, exposure to unfavorable factors (malnutrition, hormone, smoking, endocrine-disrupting chemical, etc.) could increased the risk of chronic non-communicable diseases such as T2D, metabolic syndrome and cardiovascular diseases in adulthood[1]. Therefore, it is crucial for us to elucidate the internal mechanism of environmental disturbances of metabolic alteration of mothers and fetus.

Lipopolysaccharide (LPS), a cell wall component characteristic of gram-negative bacteria, is widely present in the environment and digestive tracts of humans and animals. Periodontal disease, intrauterine infection, respiratory infection and bacterial vaginitis can cause exposure to LPS during pregnancy[2–4]. Moreover, several studies have shown that obesity during pregnancy can also lead to systemic low-grade inflammatory conditions and the production of LPS, that is metabolic endotoxemia[5, 6]. These results suggest that exposure to LPS is an adverse exposure that cannot be ignored during pregnancy. Our previous studies have found that low-dose (50μg/kg/d) LPS exposure in the third trimester of pregnancy can lead to the occurrence of glucose metabolism disorders in adult male offspring[7], but has no effect on glucose metabolism and metabolic phenotypes in adult female offspring[8]. However, the intrauterine mechanism and early metabolic changes of glucose metabolism disorders in male offspring caused by low-dose LPS exposure during pregnancy are still unclear, and the effects of LPS on maternal metabolism are not clear.

Metabonomics is a method of quantitative analysis of all metabolites in the body of living things and searching for the relative relationship between metabolites and physiological and pathological changes[9]. Abnormal metabolites can reflect metabolic changes in the early stages of the disease[10]. During pregnancy, maternal blood is continuously exchanged with the fetus through the placenta to provide nutrients needed for growth and development. The metabolic changes of maternal blood reflect the biochemical dynamics during pregnancy, which makes it an extremely valuable material for fetal health diagnosis. In addition, liver is a key metabolic organ in the body. It has been pointed out that abnormal liver metabolism in early life may be the basis of metabolic diseases in adulthood[11]. Therefore, In this study, we analyze alterations in the male fetal liver metabolites to identify metabolic pathways associated with glucose metabolism disorders caused by maternal LPS exposure during pregnancy. In addition, the effects of LPS exposure on maternal serum metabolome were determined by analyzing metabolic changes during maternal pregnancy after LPS exposure.

2. Materials And Methods

2.1 Reagents and chemicals

LPS (serotype 0127: B8) was purchased from Sigma Chemical Co., Ltd. L-2-chlorophenylalanine was from Shanghai Hengchuang Bio-technology Co., Ltd. (Shanghai, China). Water, methanol, acetonitrile, formic acid were purchased from CNW Technologies GmbH (Düsseldorf, Germany).

2.2 Animal information

The ICR mice (8–10 weeks old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River. The humidity of the experimental animal house was maintained at 45–55% and the temperature was maintained at 20–25 °C. After 1 week of adaptation, all mice mated naturally at 9:00 p.m. at 2:4 male to female ratio. At 7:00 the next morning, the female mice were checked and the presence of a vaginal plug was designated as gestational day 0 (GD0). Thirty pregnant mice were divided randomly into two groups. In the LPS group, pregnant mice were intraperitoneally (i.p.) injected with LPS (50 ug/kg) daily from GD15 to GD17. The dose of 50 μg/kg LPS is based on prior laboratory experience[7]. In the control group, pregnant mice were i.p. injected with saline daily from GD15 to GD17. All pregnant mice in each group were sacrificed in GD18 and collected serum of pregnant mice and male fetal liver. The sex of the fetus is determined by the appearance of the gonads. Male fetal livers were weighed, rapidly frozen, and stored in -80℃ liquid nitrogen until analyzed. Because we have previously reported glucose metabolism disorders in male offspring due to LPS exposure during pregnancy[7], only included male fetal liver in the metabolomics analysis.

2.3 Sample Preparation

Maternal serum

Samples stored at -80 ℃ were thawed at room temperature. 100μL of sample was added to a 1.5 mL Eppendorf tube with 10 μL of 2-chloro-l-phenylalanine (0.3 mg/mL) dissolved in methanol as internal standard, and the tube was vortexed for 10 s. Subsequently, 300 μL of ice-cold mixture of methanol and acetonitrile (2/1, v/v) was added, and the mixtures were vortexed for 1 min, ultrasonicated at ambient temperature (25 ℃ to 28 ℃) for 10 min, stored at -20 ℃ for 30 min. The extract was centrifuged at 13000 rpm, 4 ℃ for 15 min. The supernatants (200 μL) from each tube were collected using crystal syringes, filtered through 0.22 μm microfilters and transferred to LC vials. The vials were stored at -80 °C until LC-MS analysis.

Fetal liver

30 mg accurately weighed sample was transferred to a 1.5-mL Eppendorf tube. Two small steel balls were added to the tube. 20 μL internal standard (2-chloro-l-phenylalanine in methanol, 0.3 mg/mL) and 400 μL extraction solvent with methanol /water (4/1, v/v) were added to each sample. Samples were stored at -80 ℃ for 2 min and then grinded at 60 HZ for 2 min, ultrasonicated at ambient temperature (25 ℃ to 28 ℃) for 10 min, stored at -20 ℃ for 30 min. The extract was centrifuged at 13000 rpm, 4 ℃ for 15 min. The supernatants (200 μL) from each tube were collected using crystal syringes, filtered through 0.22 μm microfilters and transferred to LC vials. The vials were stored at -80 °C until LC-MS analysis

2.4 UHPLC–Q-TOF analysis

An ACQUITY UHPLC system (Waters Corporation, Milford, USA) coupled with an AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, MA) was used to analyze the metabolic profiling in both ESI positive and ESI negative ion modes. An ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) were employed in both positive and negative modes. The binary gradient elution system consisted of (A) water (containing 0.1% formic acid, v/v) and (B) acetonitrile (containing 0.1% formic acid, v/v) and separation was achieved using the following gradient: 0 min, 5% B; 2 min, 20% B; 4 min, 60% B; 11 min, 100% B; 13 min, 100% B; 13.5 min, 5% B and 14.5 min, 5%B The flow rate was 0.4 mL/min and column temperature was 45 ℃. All the samples were kept at 4℃ during the analysis. The injection volume was 5 μL. Data acquisition was performed in full scan mode (m/z ranges from 70 to 1000) combined with IDA mode. Parameters of mass spectrometry were as follows: Ion source temperature, 550 °C (+) and 550 °C (-); ion spray voltage, 5500 V (+) and 4500 V

(-); curtain gas of 35 PSI; declustering potential, 100 V (+) and − 100 V (-); collision energy, 10 eV (+) and − 10 eV (-); and interface heater temperature, 550 °C (+) and 600 °C (-). For IDA analysis, range of m/z was set as 25–1000, the collision energy was 30 eV. The QCs were injected at regular intervals (every 10 samples) throughout the analytical run to provide a set of data from which repeatability can be assessed.

2.5 Data processing and statistical analysis

The acquired LC-MS raw data were analyzed by the progqenesis QI software (Waters Corporation, Milford, USA) using the following parameters. Precusor tolerance was set 5 ppm, fragment tolerance was set 10 ppm, and retention time (RT) tolerance was set 0.02 min. Internal standard detection parameters were deselected for peak RT alignment, isotopic peaks were excluded for analysis, and noise elimination level was set at 10.00, minimum intensity was set to 15% of base peak intensity. The Excel file was obtained with three dimension data sets including m/z, peak RT and peak intensities, and RT–m/z pairs were used as the identifier for each ion. The resulting matrix was further reduced by removing any peaks with missing value (ion intensity = 0) in more than 60% samples. The internal standard was used for data QC (reproducibility). The positive and negative data were combined to get a combine data which was imported into SIMCA software package (version 14.0, Umetrics, Umeå, Sweden). Principle component analysis (PCA) and (orthogonal) partial least-squares-discriminant analysis (OPLS-DA) were carried out to visualize the metabolic alterations among experimental groups, after mean centering (Ctr) and Pareto variance (Par) scaling, respectively. PCA represents each point in the multi-dimensional space through feature parameters.The distance between samples of the same category is less than the distance between samples of other categories.Based on this, it is used as the basis for discriminating classification and OPLS-DA can find the variables most relevant to the factors of grouping, this method is mainly for the comparison between two samples. The Hotelling's T2 region, shown as an ellipse in score plots of the models, defines the 95% confidence interval of the modeled variation. Variable importance in the projection (VIP) ranks the overall contribution of each variable to the OPLS-DA model, and those variables with VIP > 1 are considered relevant for group discrimination. Fold change (FC) is the ratio of the average expression level of a certain metabolite in the two groups of samples, FC > 1 means the expression level of the metabolite is up-regulated, and FC < 1 means the expression level of the metabolite is down-regulated. In this study, the default 7-round cross-validation was applied with 1/seventh of the samples being excluded from the mathematical model in each round, in order to guard against overfitting.

2.6 Identification of differential metabolites

The differential metabolites were selected on the basis of the combination of a statistically significant threshold of variable influence on projection (VIP) values obtained from the OPLS- DA model and p values from a two-tailed Student's t test on the normalized peak areas, where metabolites with VIP values larger than 1.0 and p values less than 0.05 were considered as differential metabolites.

3. Results

3.1 Discriminant Analysis

Principal Component Analysis (PCA) was performed to give an overview of distribution between samples. In the score plot, each point on the scores plot represents an individual sample. The PCA score plots (Figure. 1A,C) demonstrated obvious separation between control and LPS groups. To get insight into the types of metabolites responsible for the maximum separation between two groups, orthogonal projection to latent structure with discriminant analysis were performed. In the OPLS-DA model score plots (Figure. 1B,D), there were significantly separated and aggregated between control and LPS groups, indicating that LPS exposure interfered with normal metabolism of pregnant mice and liver metabolism of male fetus.

3. 2 Lps Exposure Significantly Altered Maternal Serum Metabolome

The identified metabolites were classified based on their biochemical types. According to the metabolites list, a total of 38 metabolites in the maternal serum were altered after LPS exposure, mainly reflected in glycerophospholipids and fatty acid metabolism (Table 1). Among them, 32 metabolites were up-regulated and 6 were down-regulated. Interestingly, up-regulated glycerophospholipids are mainly containing saturated fatty acid and down-regulated glycerophospholipids are containing polyunsaturated fatty acid (Table 2). Furthermore, in fatty acids, LPS induced the elevation of stearic acid, oleic acid, palmitic acid, 8,1,10-eicosatrienoic acid and 3-dehydroxycarnitine, and induced a decrease of docosahexaenoic acid (DHA) (Table 3).

Table 1
Summary of differential metabolites identified in maternal serum.

	Lipid				**Peptides**	**Carbohydrates**	**Total**
	Glycerophospholipids	**Fatty acids**	**Glycerolipids**	**Hydroxysteroids**			
Up-regulation	22	5	1	1	1	2	32
Down-regulation	2	1	1	1	1	0	6

Note: Up-regulation: LPS group showed an upward trend compared with the control group; Down-regulation: LPS group showed a downward trend compared with the control group.

Table 2
Qualitative identification results of differential glycerophospholipids in maternal serum.

No.	Metabolites	m/z	Formula	VIP	FC(LPS/CON)	Trend	*P*-value
1	PC (17:0/2:0)	532.3406	$C_{27}H_{54}NO_8P$	1.69	1.51	↗	0.003
2	PC (O-14:0/2:0)	494.3253	$C_{24}H_{50}NO_7P$	3.15	1.15	↗	0.002
3	LysoPC (O-15:0)	466.3297	$C_{23}H_{50}NO_6P$	1.72	1.25	↗	□0.001
4	LysoPC (P-17:0)	492.3458	$C_{25}H_{52}NO_6P$	1.71	1.54	↗	□0.001
5	LysoPC (P-19:1)	520.3753	$C_{27}H_{54}NO_6P$	1.47	2.15	↗	□0.00
6	LysoPC (17:1)	506.3252	$C_{25}H_{50}NO_7P$	5.56	1.15	↗	0.042
7	LysoPC (19:0)	536.3719	$C_{27}H_{56}NO_7P$	3.11	2.07	↗	□0.001
8	LysoPC (19:1)	534.3562	$C_{27}H_{54}NO_7P$	1.39	1.41	↗	0.001
9	LysoPC (18:1)	522.3554	$C_{26}H_{52}NO_7P$	5.90	1.26	↗	0.008
10	LysoPC (15:0)	480.3095	$C_{23}H_{48}NO_7P$	3.02	1.07	↗	0.021
11	LysoPC (18:0)	522.3566	$C_{26}H_{54}NO_7P$	11.82	1.19	↗	0.002
12	LysoPC (20:0)	552.4016	$C_{28}H_{58}NO_7P$	2.67	2.09	↗	□0.001
13	LysoPC (20:1)	550.3859	$C_{28}H_{56}NO_7P$	1.28	1.43	↗	□0.001
14	LysoPA(18:0e)	447.2867	$C_{21}H_{45}O_6P$	1.28	1.34	↗	□0.001
15	LysoPC (22:0)	580.4328	$C_{30}H_{62}NO_7P$	1.78	1.67	↗	0.008
16	LysoPC (O-18:0)	510.3911	$C_{26}H_{56}NO_6P$	1.34	1.53	↗	□0.001
17	PS (O-16:0/13:0)	702.4684	$C_{35}H_{70}NO_9P$	1.10	1.56	↗	0.003
18	PS (P-16:0/14:1)	690.4696	$C_{36}H_{68}NO_9P$	2.74	1.71	↗	0.028
19	PS (P-18:0/14:1)	718.5010	$C_{38}H_{72}NO_9P$	5.89	1.65	↗	0.027
20	LysoPS (P-20:0)	502.3287	$C_{26}H_{52}NO_8P$	4.39	1.29	↗	0.024
21	LysoPE (20:0)	508.3409	$C_{25}H_{52}NO_7P$	6.45	1.21	↗	0.003
22	PE (10:0/10:0)	504.3094	$C_{25}H_{50}NO_8P$	6.41	1.15	↗	0.039
23	PA (18:4/22:4)	745.4775	$C_{43}H_{69}O_8P$	1.34	0.66	↘	0.005
24	PA (20:4/22:6)	786.5037	$C_{45}H_{69}O_8P$	2.31	0.53	↘	0.002

Note:PC: phosphatidylcholine; LysoPC: lysophosphatidylcholine; PE: phosphatidylethanolamine; LysoPE: lysophosphatidylethanolamine; PS: phosphatidylserine; LysoPS: lysophosphatidylserine; PA: phosphatidic acids; VIP: variable important in projection; FC: fold Change;m/z: mass charge ratio; ↗: LPS group showed an upward trend compared with the control group; ↘: LPS group showed a downward trend compared with the control group.

Table 3
Qualitative identification results of differential fatty acids in maternal serum.

Metabolites	VIP	FC(LPS/CON)	Trend	*P*-value
Stearic acid	3.10	1.22	↗	0.000
Oleic acid	2.12	1.19	↗	0.002
Palmitic acid	1.85	1.10	↗	0.001
8,11,14-Eicosatrienoic acid	1.58	2.55	↗	0.014
3-Dehydroxycarnitine	4.60	1.19	↗	0.004
DHA	1.56	0.70	↘	0.001

Note: DHA: docosahexaenoic acid; VIP: variable important in projection; FC: fold Change; ↗: LPS group showed an upward trend compared with the control group; ↘: LPS group showed a downward trend compared with the control group.

3.3 LPS exposure significantly altered male fetal liver metabolome

As shown in Table 4, a total of 75 metabolites were changed in male fetal liver due to maternal LPS exposure during intrauterine, including glycerophospholipids, fatty acids, amino acids/peptides, nucleic acids and flavonoids. The metabolic pathway enrichment of male fetal liver demonstrated that LPS exposure mainly affects glycerophospholipid metabolism, unsaturated fatty acid anabolism, linoleic acid metabolism, and alpha-linolenic acid metabolism pathway in the male fetal liver (Fig. 2).

Table 4
Summary of differential metabolites identified in male fetal liver.

	Lipid		Carbohydrates	Amino acid/peptide	Nucleosides	Flavonoids	Total
	Glycerophospholipids	Fatty acids					
Up-regulation	21	4	7	6	0	1	39
Down-regulation	10	5	10	6	3	2	36

Note: Up-regulation: LPS group showed an upward trend compared with the control group; Down-regulation: LPS group showed a downward trend compared with the control group.

A large number of population studies have shown that changes in phospholipids, fatty acids, amino acids, hexoses, and acylcarnitine are closely related to the risk of T2D[12–31]. In the extensive reading of related literature, review, it was found that no metabolites related to the risk of T2D were found in the abnormal amino acid, nucleic acids and flavonoids metabolism caused by LPS exposure. However, abnormal fatty acid and glycerophosphate metabolism may be associated with the risk of T2D.

As shown in Table 5, a total of 31 glycerophospholipids were changed in the male fetal liver during intrauterine after meternal LPS exposed. Interestingly, similar to the changes in the maternal, glycerophospholipids containing saturated fatty acids, such as PC (O-1:0/16:0) and PC (O-14:0/2:0), were up-regulated. Moreover, the glycerophospholipid containing polyunsaturated fatty acid, such as LysoPC (17:2) and LysoPI (20:5) were down-regulated (Table 5). In fatty acids, a total of 9 fatty acids were changed. Compared with the control group, saturated fatty acids and acylcarnitine were up-regulated in the LPS group, while polyunsaturated fatty acids were down-regulated (Table 6).

Table 5
Qualitative identification results of differential glycerophospholipids in male fetal liver.

No.	Metabolites	m/z	Formula	VIP	FC(LPS/CON)	Trend	*P*-value
1	PC (O-1:0/16:0)	490.3319	$C_{25}H_{52}NO_7P$	2.37	2.05	↗	0.005
2	PC (O-14:0/2:0)	991.6746	$C_{24}H_{50}NO_7P$	1.89	1.27	↗	0.023
3	PC (O-6:0/O-6:0)	390.2763	$C_{20}H_{44}NO_6P$	1.12	1.64	↗	0.037
4	LysoPC (18:0)	522.3585	$C_{26}H_{54}NO_7P$	1.09	1.29	↗	0.014
5	LysoPC (19:0)	536.3743	$C_{27}H_{56}NO_7P$	1.57	1.27	↗	0.031
6	LysoPC (O-14:1)	450.3004	$C_{22}H_{46}NO_6P$	1.26	2.02	↗	0.002
7	LysoPC (O-15:0)	466.3323	$C_{23}H_{50}NO_6P$	9.84	1.41	↗	0.045
8	LysoPC (O-17:0)	494.3638	$C_{25}H_{54}NO_6P$	6.31	1.58	↗	0.022
9	LysoPC (P-15:0)	464.3164	$C_{23}H_{48}NO_6P$	2.60	1.86	↗	0.013
10	LysoPC (P-18:0)	508.3765	$C_{26}H_{54}NO_6P$	10.03	1.49	↗	0.032
11	LysoPC (P-19:1)	518.3635	$C_{27}H_{54}NO_6P$	1.30	2.02	↗	0.005
12	PE(14:1/15:0)	612.4361	$C_{34}H_{66}NO_8P$	1.34	2.84	↗	0.031
13	LysoPE (P-16:0e)	436.2849	$C_{21}H_{44}NO_6P$	9.13	1.71	↗	0.018
14	LysoPE (O-16:0)	438.2996	$C_{21}H_{46}NO_6P$	4.29	1.66	↗	0.029
15	LysoPE (O-18:1)	464.3165	$C_{23}H_{48}NO_6P$	9.52	1.82	↗	0.005
16	LysoPE (18:0)	462.3012	$C_{23}H_{48}NO_7P$	7.01	1.65	↗	0.025
17	LysoPE (18:1)	460.2848	$C_{23}H_{46}NO_7P$	1.43	2.14	↗	0.006
18	PS(14:1/14:1)	714.3739	$C_{34}H_{62}NO_{10}P$	1.03	1.75	↗	0.025
19	LysoPA(22:1)	510.3553	$C_{25}H_{49}O_7P$	1.21	1.24	↗	0.030
20	LysoPG(18:0)	511.3065	$C_{24}H_{49}O_9P$	3.24	1.23	↗	0.017
21	PA(O-18:0/15:0)	649.5184	$C_{36}H_{73}O_7P$	2.34	1.18	↗	0.020
22	LysoPC (17:2)	504.3114	$C_{25}H_{48}NO_7P$	5.59	0.85	↘	0.027
23	LysoPE (20:5)	498.2640	$C_{25}H_{42}NO_7P$	1.41	0.75	↘	0.009
24	LysoPE (20:4)	500.2798	$C_{25}H_{44}NO_7P$	4.15	0.82	↘	0.004
25	LysoPE (22:5)	528.3082	$C_{27}H_{46}NO_7P$	1.23	0.76	↘	0.030

Note:PC: phosphatidylcholine; LysoPC: lysophosphatidylcholine; PE: phosphatidylethanolamine; LysoPE: lysophosphatidylethanolamine; PS: phosphatidylserine; LysoPS: lysophosphatidylserine; PA: phosphatidic acids; LysoPA: lysophosphatidic acids; LysoPI: lysophosphatidylinositol; VIP: variable important in projection; FC: fold Change;m/z: mass charge ratio; ↗: LPS group showed an upward trend compared with the control group; ↘: LPS group showed a downward trend compared with the control group.

No.	Metabolites	m/z	Formula	VIP	FC(LPS/CON)	Trend	*P*-value
26	LysoPS (20:3)	548.2958	$C_{26}H_{46}NO_9P$	1.32	0.64	↘	0.001
27	LysoPS (22:2)	542.3233	$C_{28}H_{52}NO_9P$	1.50	0.66	↘	0.001
28	LysoPI (20:2)	623.3224	$C_{29}H_{53}O_{12}P$	1.03	0.71	↘	0.049
29	LysoPI (20:5)	617.2753	$C_{29}H_{47}O_{12}P$	3.12	0.49	↘	0.001
30	LysoPI (20:4)	643.2845	$C_{29}H_{49}O_{12}P$	1.99	0.74	↘	0.015
31	LysoPA(18:4)	411.1920	$C_{21}H_{35}O_7P$	1.03	0.47	↘	0.020

Note:PC: phosphatidylcholine; LysoPC: lysophosphatidylcholine; PE: phosphatidylethanolamine; LysoPE: lysophosphatidylethanolamine; PS: phosphatidylserine; LysoPS: lysophosphatidylserine; PA: phosphatidic acids; LysoPA: lysophosphatidic acids; LysoPI: lysophosphatidylinositol; VIP: variable important in projection; FC: fold Change;m/z: mass charge ratio; ↗: LPS group showed an upward trend compared with the control group; ↘: LPS group showed a downward trend compared with the control group.

Table 6
Qualitative identification results of differential fatty acids in male fetal liver.

Metabolites	VIP	FC(LPS/CON)	Trend	*P*-value
Stearic acid	1.24	1.25	↗	0.011
Cervonyl carnitine	1.54	1.53	↗	0.006
Isobutyryl-L-carnitine	1.06	2.46	↗	0.000
Stearoylcarnitine	2.37	1.41	↗	0.017
EPA	1.47	0.44	↘	0.004
DHA	1.25	0.52	↘	0.006
5,6-Epoxy-8,11,14-eicosatrienoic acid	1.31	0.76	↘	0.004
Alpha-Linolenic acid	1.35	0.86	↘	0.032
Linoleic acid	3.49	0.86	↘	0.029

Note: DHA: docosahexenoic acid; EPA: eicosapentaenoic acid; VIP: variable important in projection; FC: fold Change; ↗: LPS group showed an upward trend compared with the control group; ↘: LPS group showed a downward trend compared with the control group.

4. Discussion

LPS exposure during pregnancy is one of the adverse exposure factors in pregnancy that cannot be ignored. At present, no studies have focused on the effect of LPS exposure during pregnancy on maternal and offspring metabolism from the perspective of metabolomics. A model of low-dose LPS exposure during pregnancy causing glucose metabolism disorders in adult male offspring has been reported[7]. Metabolomics was used to analyze the metabolic changes of maternal serum and male fetal liver after LPS exposure during pregnancy. The results of this study showed that low-dose LPS exposure during pregnancy had a similar tendency to interfere with the glycerophospholipids metabolism and fatty acid metabolism of maternal and male offspring.

4.1 Effects of LPS exposure during pregnancy on maternal metabolites

Some researchers have suggested that inflammatory mediators activate the innate immune system to cause lipid remodeling of glycerolipids, glycerophospholipids, and prenols in majority of mammalian[32]. Most of previous studies have analyzed the effect of inflammation on the glycerophospholipids metabolism in RAW264.7 cells in mice[33–36]. Dennis et al. characterized lipidomic (glycerides, glycerophospholipids, sphingolipids, and fatty acids) responses of murine macrophage to inflammatory by LC/MS[32]. It reported that levels of most unsaturated free fatty acids were reduced at longer time points, whereas most free saturated fatty acids increased after treated with LPS. Furthermore, in the glycerophospholipids and glycerolipids, this trend was especially noticeable for the saturated and monounsaturated species in that the 32:0, 34:0, 34:1, 36:0, and 36:1 phosphatidic acid species increased by severalfold in response to the stimulus, whereas certain polyunsaturated species such as 38:4 phosphatidylinositol showed decreases of up to 50%[32]. She et al. identified 16 altered glycerophospholipids in LPS-treated RAW264.7 cells. Among the 16 glycerophospholipids, significant decreases in the levels of lysoPC (20:4), PC (36:4), PC (34:4), PC (35:4), PC (36:5), and PC (40:5) species were accompanied by relative increases in the levels of lysoPE (20:3), lysoPE (22:1), lysoPC (19:3), lysoPC (P–18:0), PC (O–32:3), PC (O–30:0), PC(O–32:0)species in the LPS-treated[33]. And other studies have similar results[36] [34]. These studies have suggested that LPS exposure leads to upregulation of glycerophospholipids containing saturated or monounsaturated fatty acids, and downregulation of glycerophospholipids containing polyunsaturated fatty acids. However, changes in glycerophospholipids caused by inflammation involve complex mechanisms and further research is needed.

In the present study, we found that fatty acid metabolism in maternal serum was significantly changed after LPS exposure, stearic acid, palmitic acid, oleic acid up-regulated, and 3-dehydroxycarnitine were up-regulated, while polyunsaturated fatty acid DHA was down-regulated. Stearic acid and palmitic acid are saturated fatty acids, oleic acid is a monounsaturated fatty acid, and DHA is an omega–3 polyunsaturated fatty acid. 3-dehydroxycarnitine is an acylcarnitine. 8,11,14-Eicosatrienoic acid, is an omega–9 polyunsaturated fatty acid. Dennis et al. reported that levels of most unsaturated free fatty acids were reduced at longer time points, whereas most free saturated fatty acids increased after treated with LPS [32]. These polyunsaturated fatty acids included linolenic acid, α-linolenic acid, DHA, stearidonic acid and 5,8,11,14,17-eicosapentaenoic acid, and saturated fatty acids included lauric acid (12:0), myristic acid (14:0), palmitic acid, stearic acid and oleic acid etc[32]. Another study also suggested that palmitic acid, palmitoleic acid, stearic acid, and oleic acid increased under LPS stimulation[33]. Presently, few studies have investigated the effects of inflammation on fatty acid metabolism, and more studies have focused on the pro-inflammatory or anti-inflammatory effects of fatty acids. Therefore, it is unclear whether LPS leads to saturated fatty acids or omega–9 polyunsaturated fatty acids, as well as to the down-regulation of polyunsaturated fatty acids[37–39]. Furthermore, it is controversial that whether monounsaturated fatty acids have anti-inflammatory effects[40]. Accordingly, these results suggests that inflammation may interact with fatty acids. Further research and verification are needed in the future.

4.2 Effects of LPS exposure during pregnancy on male offspring metabolome

Fatty acids (FAs) are one of the most essential substances for fetal intrauterine growth. They are involved in many energy and metabolic processes, including cell membrane, retina and nervous system growth. Fatty acid deficiency and maternal-fetal metabolic disorders can lead to fetal malnutrition and premature delivery. Importantly, it may cause metabolic and cardiovascular diseases in adulthood[41]. As the most important metabolic organ of human beings, the liver is also an important site of fatty acid metabolism. This study found that LPS exposure during pregnancy caused changes in liver glycerophospholipids and fatty acid metabolism in male fetal liver, which may be a risk factor for the development of glucose metabolism disorders in adulthood. It is now clear that fatty acids influence a range of other diseases, including metabolic diseases such as type 2 diabetes, inflammatory diseases, and cancer. Studies have suggested that saturated fatty acids, especially even saturated fatty acids (lauric acid, myristic acid, palmitic acid, and stearic acid) increase total cholesterol and low-density lipoprotein cholesterol concentrations, as well as increase body inflammation and insulin resistance. Therefore, exposure to high levels of saturated fatty acids is associated with a higher risk of coronary heart

disease, cardiovascular disease, and T2D[40]. Linoleic acid (18:2ω−6) is an omega−6 polyunsaturated fatty acid that lowers blood cholesterol and low-density lipoprotein cholesterol[43, 44]. Omega−3 polyunsaturated fatty acids, mainly including α-linolenic acid (18:3ω−3), DHA (22:6ω−3), EPA (20:5ω−3), docosapentaenoic acid (DPA, 22:5ω−3) has a wide range of biological effects, involving membranes, signal transduction, gene expression and lipid mediators, plays an important role in controlling inflammation, reducing blood triglyceride and adipocyte differentiation [45–47]. Therefore, omega−3 polyunsaturated fatty acids play an important role in reducing the risk of diabetes[48]. At the same time, EPA and DHA are essential for fetal growth and development. DHA plays a central role in the structure and function of the eye and brain [40]. Epoxy eicosatrienoic acid is a metabolite of arachidonic acid and has a variety of biological functions, and it is closely related to islet cell function, insulin resistance, blood glucose homeostasis and progression of T2D[49–51]. In this study, we found that stearic acid was up-regulated, while the omega−3 polyunsaturated fatty acids DHA, EPA, α-linolenic acid and epoxy eicosatrienoic acid were down- regulated. This suggests that intrauterine LPS exposure leads to an increase in risk factors for T2D and protective factors decrease, which may be one of the causes of disorders of glucose metabolism in adulthood. Stearic acid, alpha-linolenic acid, DHA, EPA, and epoxyeicosatrienoic acid may be early markers of glucose metabolism disorders in adult male offspring of intrauterine LPS exposure. At the same time, intrauterine LPS exposure led to a significant decrease in DHA, which is essential for fetal growth and development. This suggests that intrauterine LPS exposure may have adverse effects on the normal growth and development of the fetus and may affect the normal development of the eye or brain. In addition, the study also found that ceramide carnitine, isobutyryl-L-carnitine and stearyl carnitine were significantly up-regulated. Ceramide carnitine, isobutyryl-L-carnitine and stearyl carnitine belong to the acylcarnitine. Acetylcarnitine is produced by mitochondrial matrix enzymes from carnitine and acetyl-CoA, which are products of fatty acid beta oxidation and glucose oxidation. Forty-five acylcarnitines were analyzed in the IRAS study and found to be associated with higher concentrations of carnitine insulin resistance[31]. Rui et al found that acylcarnitine, especially acetylcarnitine C2, is a marker for diabetic patients[52]. These studies suggest that upregulation of acylcarnitine may be associated with the risk of developing diabetes. Based on this, we hypothesized that abnormal up-regulation of ceramide carnitine, isobutyryl-L-carnitine and stearyl carnitine may be an early metabolic imprint of glucose metabolism disorder in adult male offspring caused by intrauterine LPS exposure.

Recently, more studies have focused on the relationship between glycerophospholipids and T2D. Maria A et al. investigated the proportions of fatty acids in plasma phospholipids, as predictors for the worsening of T2D [53]. In the glycerophospholipids fraction, saturated fatty acids, such as myristic acid (14:0), stearic acid (18:0) and palmitic acid (16:0) ratio were associated with decreased insulin sensitivity. In addition, glycerophospholipids with a high DHA ratio are associated with increased insulin sensitivity[53]. For insulin secretion, linoleic acid (18:2n−6) is associated with increased insulin secretion in glycerophospholipids[53]. Wang et al. evaluated the relationship between phospholipids of different fatty acids and the incidence of diabetes by following 2909 people. It was found that after adjusting for factors such as age, gender, baseline body mass index, waist-to-hip ratio, alcohol intake and parental diabetes history, The incidence of diabetes is positively correlated with the ratio of cholesterol in blood and saturated fatty acids in glycerophospholipids. In cholesterol esters, the incidence of diabetes is positively correlated with the ratio of palmitic acid (16:0), palmitoleic acid (16:1n−7) and gamma-linolenic acid (20:3n−6), with linoleic acid (The ratio of 18:2n−6) is negative. In glycerophospholipids, the incidence of diabetes is positively correlated with the ratio of palmitic acid (16:0) and stearic acid (18:0) [54]. Other studies have found similar results[55–57]. In summary, the relationship between glycerophospholipids and the onset of diabetes is related to the type and proportion of fatty acids contained therein, probably because glycerophospholipids are a reservoir of free fatty acids. In this study, it was found that saturated fatty acid chains, especially glycophospholipids containing (16:0) and (18:0) chains, were up-regulated. This result suggests that abnormal changes in glycerophospholipids are another possible mechanism of glucose metabolism disorders in adult male offspring of intrauterine LPS. At the same time, these results suggest that the metabolic changes of maternal and progeny in the model of LPS exposure to male progeny glucose metabolism are mainly related to the composition and proportion of fatty acids. Therefore, we should pay attention to the changes of fatty acids during pregnancy.

Studies have found that many adverse exposures during pregnancy may lead to an increased risk of glucose metabolism in adult offspring. One study have found that bile acids and tryptophan metabolism are novel pathways involved in metabolic abnormalities in bisphenol A -exposed pregnant mice and male offspring [11]. In this study, we find that maternal serum and male fetal liver showed similar changes in glycerophospholipid and fatty acid metabolism. That indicates the abnormal glycerophospholipids and fatty acid metabolism may be the mechanism of glucose metabolism disorder in male offspring.

Despite these findings, our studies have some limitations. First, this study only characterize the alterations of maternal and male offspring metabolome after LPS exposure, but we did not explore the underlying mechanism of these changes. Second, this study did not detect the metabolites in amniotic fluid and could not explain the relationship between maternal and offspring metabolic changes. Although the detailed exploration of glycerophospholipids and fatty acid metabolism pathways is beyond the scope of this study, future analysis, including studies on the input and/or transport of fetal fatty acids, will be potentially important in elucidating the mechanism of LPS-exposured changes in glycerophospholipids and fatty acid metabolism.

5. Conclusion

In summary, we utilized LC-MS to demonstrate that maternal LPS exposure caused the changes of glycophospholipids and fatty acid metabolism in maternal serum and male fetal liver. Interestingly, the metabolic changes in the pregnant mice and male offspring are similar. Up-regulated glycerophospholipids are mainly containing saturated fatty acid and down-regulated glycerophospholipids are containing polyunsaturated fatty acid. Furthermore, in fatty acids, saturated fatty acid were up-regulated and polyunsaturated fatty acid were down-regulated. Abnormal changes in glycerophospholipids and fatty acid metabolism may be intrauterine metabolic imprinting of glucose metabolism disorders in male offspring.

Declarations

Ethics approval and consent to participate

This protocol has received ethics approval from the Ethics Committee of Anhui Medical University and the ethical approval number is LLSC20150350.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QQ, LH and MZ conceived and designed experiments. QQ and LH performed experiments. QQ performed statistical analysis. QQ and LH performed chemical analysis. QQ and LH wrote manuscript. MZ provided technical and editorial assistance.

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Competing interest

The authors declare they have no conflicts of interest that could have influenced the work reported in this paper.

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Figures

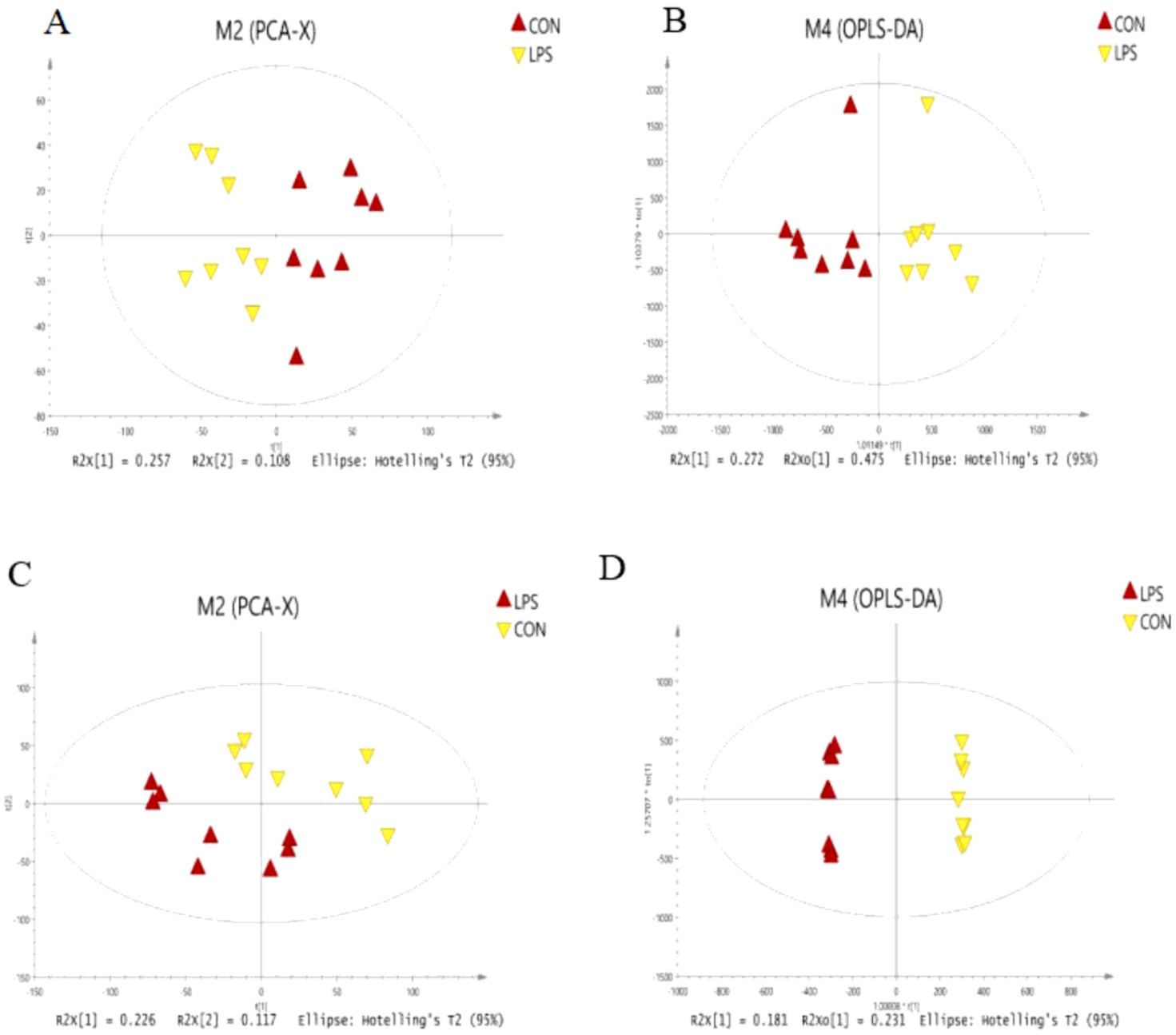

Figure 1

Discrimination analysis of maternal serum and fetal liver metabolome in LPS group and CON group. (A) Discriminant analysis of maternal serum metabolome in two groups in PCA mode; (B) Discriminant analysis of maternal serum metabolome in two groups in OPLS-DA mode; (C) Discriminant analysis of male fetal liver in two groups in PCA mode; (D) Discriminant analysis of male fetal liver in two groups in OPLS-DA mode.

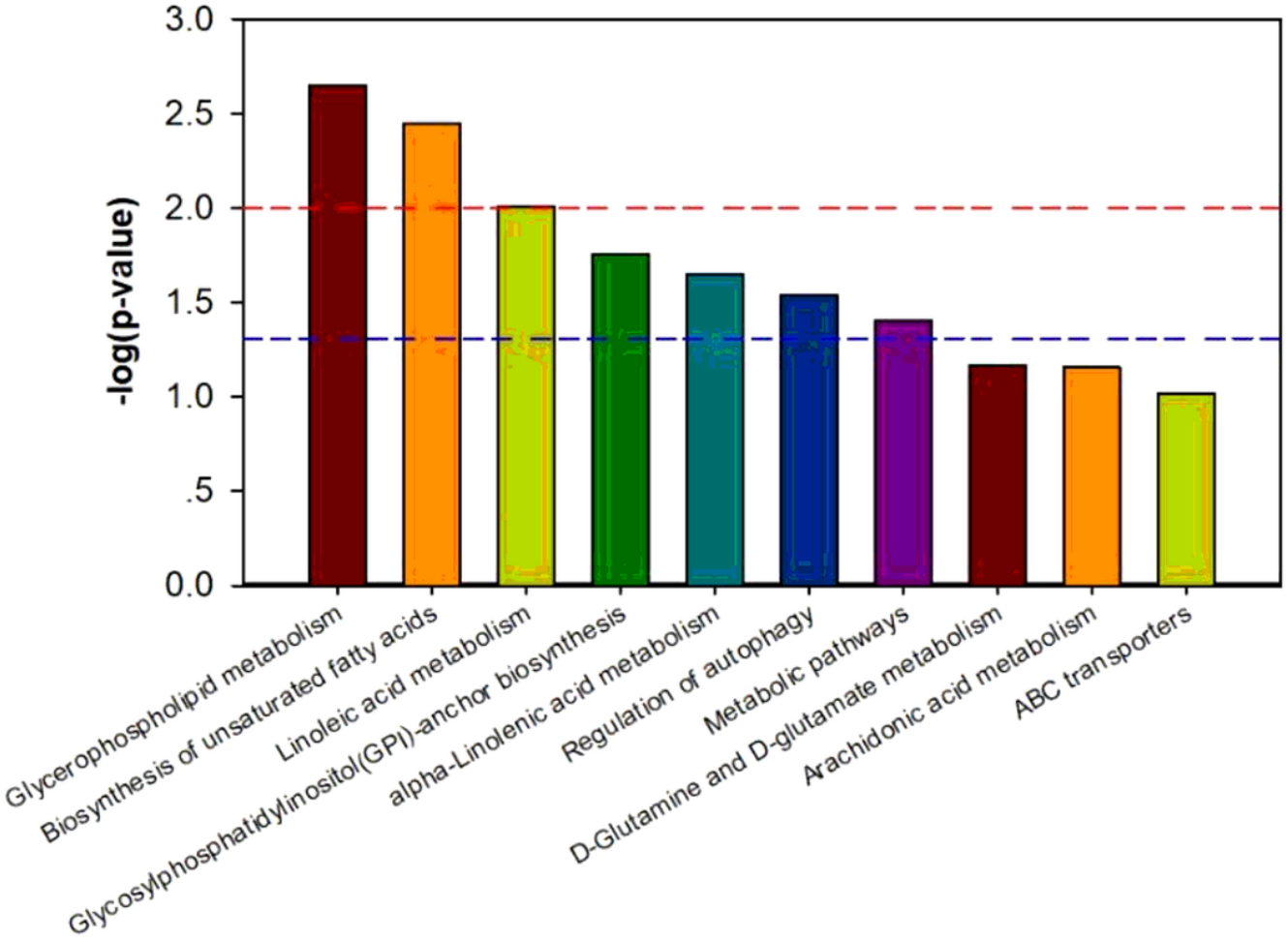

Figure 2

Metabolic pathway enrichment of male fetal liver in LPS group and CON group. The red line indicates p-value is 0.01, the blue line indicates p-value is 0.05. When the top of the column is higher than the blue line or the red line, the signal pathway represented by the column is significant.