Metabolomic signatures for liver tissue and cecum contents in high-fat diet-induced obese mice based on UHPLC-Q-TOF/MS

Hongying Cai\textsuperscript{1,2}, Zhiguo Wen\textsuperscript{2}, Kun Meng\textsuperscript{2} and Peilong Yang\textsuperscript{1,2}\* (Correspondence: yangpeilong@caas.cn)

\textsuperscript{1} Institute of Feed Research, Chinese Academy of Agricultural Sciences, No. 12 Zhongguancun South Street, Beijing 100081, People's Republic of China
\textsuperscript{2} Full list of author information is available at the end of the article

**Abstract**

**Background**: The incidence of obesity is increasing worldwide, and it is a risk factor for diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Our previous study had demonstrated that high-fat diet induced increased weight gain, fat weight, serum cholesterol, triglyceride, and ATL levels in liver, and influenced the diversity and composition of cecal microbiota in mice. Hence, this study aimed to investigate the roles of the gut microbially derived metabolites and liver metabolites between the obese and lean mice, focusing on their association with the progression of obesity induced by high-fat diet (HFD).

**Methods**: An obesity model in mice was established with HFD for 16 weeks. Cecal contents and liver tissues metabolomics based on ultraperformance liquid chromatography-quadrupole-time-of-flight mass spectrometry and orthogonal partial least squares discriminant analyses (OPLS-DA) was performed to identify the alterations in metabolites associated with obese mice.

**Results**: Obese and lean groups were clearly discriminated from each other on OPLS-DA score plot and major metabolites contributing to the discrimination were mainly involved in glycerophospholipid metabolism, primary bile acid biosynthesis, and biosynthesis of unsaturated fatty acids pathways. HFD-induced alterations of 19 metabolites in liver and 43 metabolites in cecum contents were identified as potential biomarkers related to obesity. Specifically, chenodeoxycholic acid, taurochenodeoxycholate, and tauroursodeoxycholic acid in liver were elevated 35.94, 24.36, and 18.71-fold, respectively, PI(P-16:0/18:1(9Z)), PG(19:0/16:0), PS(P-16:0/20:2(11Z,14Z)), PI(22:1(11Z)/12:0), and PE(21:0/0:0) in cecum were enhanced 884, 640.96, 226.63, 210.10, and 45.13-fold in comparison with the lean mice. These metabolites were the most important biomarkers for discriminating between the obese and lean mice. In addition, cecum contents metabolites were strongly correlated with hepatic metabolites through gut-liver axis analysis.

**Conclusions**: HFD increased lipid profiles (i.e. glycerophospholipids, PC, PE, PI, PG, and PS) and total bile acid (primary and secondary bile acid) in liver and cecum, suggesting that they may play an important role in the progression of obesity. These metabolites can be used to better understand obesity and related disease induced by HFD. Furthermore, the level alterations of these metabolites can be used to assess the risk of obesity and the therapeutic effect of obesity management.

**Keywords**: Obesity, Metabolomics, UHPLC-Q-TOF/MS, Metabolic profiles, Biomarker, High-fat diet
the imbalance between energy intake and expenditure, which leads to an excessive accumulation of lipids. Many studies have shown that the disorder of lipid metabolism is one of the main characteristics of obesity. Lipid metabolism disorders refer to abnormal lipid profile alterations in the blood, liver, and other tissues, including hypercholesterolemia, hyperlipidemia, hyperglycaemia, and non-alcoholic fatty liver syndrome (NAFLS) [2]. At present, lipid metabolism disorders is generally considered as the risk factor leading to low quality of life and increased burden on society. Many drugs are commonly used for obesity treatment. However, some side or toxic effects have limited their clinical application [3]. Therefore, the development of a safe and effective drug for the treatment of obesity has become an international research hotspot.

Gut microbiota is now considered to be an important factor affecting human health and disease [4]. Emerging evidence suggests a strong interaction between gut microbiota and liver, known as “gut-liver axis” [5]. These interactions occur through metabolites and begin at birth [6]. This relation has been based on the evidence that the liver represents the first line defense against gut-derived antigens and approximately 70% its blood supply comes from the portal vein, the direct venous outflow of the gut [7, 8]. The abundance of bacterial metabolites may have a significant impact on human physiology and the development of disease. Metabolites derived from the gut microbiota, such as lipopolysaccharides, bile acid derivatives, amino acid derivatives, and SCFAs, are important signaling molecules that link the gut microbiota to the host [9, 10]. High-fat diet may affect the host metabolism and modulate through manipulating the gut microbiota to increase hepatic injury in animal models. Due to the close anatomical and functional associations between the liver and gut, the dysfunction of the microbial ecosystem in obesity leads to disturbances of the gut barrier, which induce the direct delivery of gut-derived gut microbes and bacterial metabolites to the liver through the portal vein and induce immune response and aggravate liver injury [11].

Deciphering signatures specific to liver alterations would be most useful for future obesity diagnostic biomarkers. Several metabolites, including fatty acids, lysophophatidylcholines (lysoPCs), and branched-amino acids (BCAAs) have been identified as potential biomarkers of obesity and related diseases through human and animal studies [12], but understanding obesity metabolism based on a few identified metabolites is limited. More identification of obesity-related metabolites is necessary for our further understanding of obesity metabolism. Although many studies have reported the relationship between the gut microbiota and liver metabolites, very little has been reported about the relationship between metabolites derived from gut and liver metabolites in dietary-induced obesity. We herein study the gut microbiologically derived metabolite signatures associated with liver metabolite alterations, focusing on their relationship with obesity progression. We specifically focus on which microbial metabolites signatures are specific to liver injury. As an emerging discipline, metabolomics provide a powerful method for the discovery of biomarkers in biological systems [13]. At present, liquid chromatography–mass spectrometry (LC–MS) has many advantages, such as sensitivity and reproducibility, and is one of the commonly used techniques in metabolomics research [14]. We previously established the obesity model in mice induced by HFD, and the results revealed that HFD led to the increased levels of TG, TC, ALT and AST in serum and liver, and lipid droplets accumulation in liver [15]. However, the mechanism of obesity and the organic dysfunction associated with obesity is not clearly understood. To explore the potential characteristic metabolites that are associated with obesity, a non-targeted metabolomics technique is performed to discover potential cecal and liver metabolites and integrative analyses through gut-liver axis are applied to find relationship between the liver specific metabolites and cecal metabolites composition. By integrating metabolomics information, we would better understand the interactions between gut and host metabolism. On this basis, combined with the changes of liver and cecum contents metabolites, the common changes of the gut-liver axis were established, which provides basic data for understanding the influence of gut-derived metabolic process of obesity and its mechanism, while also providing a rationale for metabolites-based interventions against obesity.

Materials and methods
Animals and experimental design
Eighteen female 7-week-old Kunming mice were obtained from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China), and housed in cages under a 12 h light–dark cycle. After 1-week acclimation period, the mice were randomly divided into two treatment groups (n = 9) and fed with (1) normal-chow diet, (2) high fat diet (HFD) composed of 67% (w/w) normal chow, 10% lard, 20% sucrose, 2.5% cholesterol, and 0.5% sodium cholate (Keao Xieli Feed Co., Ltd, Beijing, China) according to the previous report with slight modifications [16].

Collection of liver and cecum contents samples
After 16 weeks of experimental administration, the mice were fasted 6 h. The blood samples were collected from eighteen mice and placed at room temperature for 1 h.
Serum was obtained by centrifuged (3000g, 10 min), and stored at −80 °C for further analysis. Liver tissues were washed with ice-bold buffered saline, weighed and homogenized in saline with a homogenizer. The homogenates were then centrifuged (4 °C, 3000g, 20 min) for further analysis. The Livers and cecum contents were extracted and snap-frozen in liquid nitrogen, and then stored at −80 °C for metabolomics analysis.

Untargeted liver metabolomics analysis
The Liver tissues (60 mg) were homogenized, vortexed for 1 min and dissolved in 800 μL solvent mixture containing methanol/acetonitrile (1:1, v/v). The samples were vortexed for 1 min, and sonicated for 30 min on ice and the sonication cycle was repeated for twice, and then placed at −20 °C for 1 h, and then centrifugation at 14,000g for 20 min at 4 °C. The supernatants were collected for ultraperformance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) analysis. In parallel to the preparation of the test samples prepare a bulk quality control (QC) sample, which is made by mixing equal volumes from each of the samples. The QC samples were used to monitor the LC–MS response.

Metabolic analysis of liver samples were carried out by an Agilent 1290 Infinity LC ultra-high pressure liquid chromatograph (UHPLC) system (Agilent, Santa-Clara, CA, USA) equipped with an electrospray ionization source operating in positive and negative ion mode. For the metabolomics analysis, an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm, Waters MS Technologies, Manchester, UK) was used. The column was maintained at 25 °C and eluted at a flow rate of 300 μL/min. The mobile phase was composed of A (25 mM ammonium acetate and 25 mM ammonium hydroxide in water) and B (acetonitrile). The process of linear gradient elution was 0–1.0 min, 95% B; 1.0–8.0 min, 1–99% B; 8.0–10.0 min, 99% B; 10.0–10.1 min, 99% ~ 1% B; 10.1–12 min, 1% B. The flow rate was 500 μL/min. The injected volume was 2 μL. The QE mass spectrometer was used for its ability to acquire MS/MS spectra on information-dependent acquisition (IDA) mode and using high sensitivity modes. The settings of IDA as follows: exclude isotopes within 4 Da, candidate ions to monitor per cycle, 6. Meanwhile the QC samples were analyzed to monitor the system stability and data quality.

Untargeted cecum contents metabolomics analysis
50 mg of cecal sample was weighted to an EP tube, and 1000 μL extract solution (acetonitrile: methanol: water =2: 2: 1) with 1 g/mL internal standard was added. After 30 s vortex, the samples were homogenized at 35 Hz for 4 min and sonicated for 5 min on ice. The homogenization and sonication cycle was repeated for 3 times. Then the samples were incubated for 1 h at −40 °C and centrifuged at 12,000g for 15 min at 4 °C. The resulting supernatant was transferred to a fresh glass vial for analysis. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatants from all of the samples.

LC–MS/MS analyses were performed using an UHPLC system (1290, Agilent Technologies) with a UPLC HSS T3 column (2.1 mm ×100 mm, 1.8 μm) coupled to Q Exactive mass spectrometer (Orbitrap MS, Thermo). The mobile phase A was 0.1% formic acid in water for positive mode, and 5 mmol/L ammonium acetate in water for negative mode, and the mobile phase B was acetonitrile. The elution gradient was set as follows: 0–1.0 min, 1% B; 1.0–8.0 min, 1–99% B; 8.0–10.0 min, 99% B; 10.0–10.1 min, 99% ~ 1% B; 10.1–12 min, 1% B. The flow rate was 500 μL/min. The injected volume was 2 μL. The QE mass spectrometer was used for its ability to acquire MS/MS spectra on information-dependent acquisition (IDA) mode in the control of the acquisition software (Xcalibur 4.0.27, Thermo). In this mode, the acquisition software continuously evaluates the full scan MS spectrum. The ESI source conditions were set as following: sheath gas flow rate as 45 Arb, Aux gas flow rate as 15 Arb, capillary temperature 400 °C, full MS resolution as 70,000, MS/MS resolution as 17,500, collision energy as 20/40/60 in NCE mode, spray Voltage as 4.0 kV (positive) or −3.6 kV (negative), respectively.

Data processing
The raw UHPLC-Q-TOF/MS data were converted to the mzXML format using ProteoWizard and processed with an in-house program, which was developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. Then an in-house MS2 database (BiotreeDB) was applied in metabolite annotation. The cutoff for annotation was set at 0.3.
**Statistical analysis**

The final dataset was imported to SIMCA15.0.2 software package (Umetrics, Umeå, Sweden) for multivariate statistical analysis. Orthogonal partial least squares discriminant analyses (OPLS-DA) was performed to distinguish the overall differences in metabolic profiles between obese and lean samples and to find differential metabolites between the two groups. Then, a sevenfold cross validation was performed to calculate the value of R2 and Q2. R2 indicates how well the variation of a variable is explained and Q2 means how well a variable could be predicted. To check the robustness and predictive ability of the OPLS-DA model, 200 times permutations was further conducted. Afterward, the R2 and Q2 intercept values were obtained. Furthermore, the value of variable importance in the projection (VIP) of the first principal component in OPLS-DA analysis was obtained. It summarizes the contribution of each variable to the model. The metabolites with VIP > 1 and p < 0.05 (student t test) were considered as significantly changed metabolites. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to analyze the differential metabolites. In addition, all differentially abundant metabolites were queried against KEGG (http://www.kegg.jp/) and mapped to KEGG pathways. Enrichment analysis was performed to further explore the impact of differentially expressed metabolites and to analyze the internal relationships between differentially expressed metabolites using the Fisher’s exact test. Only functional categories and pathways with $p < 0.05$ were considered to have significant enrichment.

**Results**

**Metabolic findings in liver samples**

The hepatocytes are most exposed to gut-derived toxic factors through gut-liver axis, including bacteria and bacterial products. Examination of the metabolites composition in the liver will provide more accurate understanding about liver lipid metabolism and signaling. UHPLC-Q-TOF MS liver metabolomics analysis discovered the different metabolomics features in the obese and lean groups. As shown in Fig. 1A, B, OPLS-DA score plots displayed good separation effect between the obese and lean mice, indicating that high-fat diet induced significant systemic changes and obesity was successfully induced. The parameters of OPLS-DA models including $R^2_X=0.808$, $R^2_Y=1$, $Q^2=0.796$ for positive data, and $R^2_X=0.572$, $R^2_Y=0.993$, $Q^2=0.842$ for negative data were obtained. The permutation test was used to guard against overfitting of the OPLS-DA models. As shown

![Fig. 1](image-url)
in Fig. 1C, D, validation with 200 random permutation tests generated intercepts $R^2 = 0.999$ and $Q^2 = -0.148$ for positive data, and $R^2 = 0.944$ and $Q^2 = -0.473$ for negative data, which demonstrated that the OPLS-DA models were robust and reliable without overfitting.

The significantly differential metabolites were selected based on the criteria of an OPLS-DA model VIP > 1 and $p < 0.05$. UHPLC-Q-TOF MS metabolomics analysis discovered 19 differentially enriched metabolites between the obese and lean groups. Twenty-six of the metabolites were elevated in the obese group (fold change > 1.2) while Twenty-eight of them were decreased (fold change < 0.7).

As shown in Fig. 2, according to the KEGG reference pathways, indicators with significantly discriminative power were the “ABC transporters”, “Glycerophospholipid metabolism”, “Choline metabolism in cancer”, “Primary bile acid biosynthesis”, and “Biosynthesis of unsaturated fatty acid”, which showed significant enrichment with HFD treatment. As shown in Table 1, the levels of choline, cytidine 5′-diphosphocholine (CDP-choline), phosphorylcholine, O-phosphoethanolamine, sn-glycerol 3-phosphoethanolaminesn, and glycerophosphocholine involving in glycerophospholipid metabolism pathway, were significantly increased with fold changes of 1.38, 2.13, 2.39, 1.71, 1.94, and 2.13 respectively ($p < 0.05$), while PC (16:0/16:0) and 1-stearoyl-2-oleoyl-sn-glycerol 3-phosphocholine were significantly decreased with fold changes of 0.46 and 0.6, respectively ($p < 0.05$), which indicates high relevance to the difference between normal and high-fat diet fed mice. Compared to the lean group, HFD intervention significantly increased the amounts of oleic acid (3.89-fold), while the levels of eicosapentaenoic acid, (4Z,7Z,10Z,13Z,16Z,19Z)-4,7,10,13,16,19-docosahexaenoic acid, and alpha-linolenic acid involving in biosynthesis of unsaturated fatty acid pathway, were significantly decreased with fold changes of 0.30, 0.62, and 0.67 ($p < 0.05$), respectively.

Changes in circulating bile acids are associated with obesity and related diseases. In mice, more than 95% conjugated bile acids are taurine-conjugated bile acids, while glycine-conjugated bile acids are more abundant in human [17]. Consistent with the above report, most of bile acids in the liver were taurine-conjugated in this study. The levels of chenodeoxycholic acid (CDCA), Taurochenoxycholic acid (TCDCA) and tauroursoxycholic acid (TUDCA) in primary bile acid biosynthesis pathway were increased with the fold changes of 35.94, 24.36 and 8.71 ($p < 0.05$), respectively, while the levels of cholic acid (CA), taurocholic acid (TCA) and taurine were decreased with fold changes of 0.62, 0.35, and 0.41 ($p < 0.05$), respectively, which were potential markers related to obesity. Additionally, HFD intervention significantly increased taurodeoxycholic acid (TDCA) and taurolithocholic acid (TLCA) belonging to secondary bile acid with the fold changes of 5.88 and 4.36 ($p < 0.05$), respectively. Besides on these results, we observed that several metabolites were also significantly changed, including the levels of purines, nicotinamide, and amino acids (data not shown).

Metabolic findings in cecum contents samples
Gut-derived microbial products can reach the liver by gut-liver axis, and then have an effect on liver metabolism. In the present study, a metabolomics study of cecum contents based on UHPLC-Q-TOF/MS was conducted in mice fed with HFD. Cecum contents metabolomics presented similar results with that of liver tissue. As shown in Fig. 3A, B, OPLS-DA score plots and...
Table 1 Potential biomarkers in liver based on the UPLC-Q-TOF/MS analysis between the obese and lean mice

| Ionization mode | RT(s)  | m/z     | Metabolites                                           | Metabolic pathway                  | VIP   | Fold change | p value |
|-----------------|--------|---------|------------------------------------------------------|-----------------------------------|-------|-------------|---------|
| ESI (+)         | 788.63 | 198.0517674 | sn-Glycer 3-Phosphoethanolamines | Glycerophospholipid metabolism | 1.95  | 1.94        | 0.001   |
| ESI (+)         | 780.035 | 104.1058352 | Choline | Glycerophospholipid metabolism | 1.29  | 1.38        | 0.0009  |
| ESI (+)         | 878.204 | 489.1133277 | Gbtyidine 5’-diphosphocholine | Glycerophospholipid metabolism | 2.15  | 1.84        | 0.032   |
| ESI (+)         | 915.7575 | 142.0254256 | D-Phosphoethanolamine | Glycerophospholipid metabolism | 1.71  | 1.02        | 0.002   |
| ESI (+)         | 862.561 | 258.1094388 | Glycophorpholipid | Glycerophospholipid metabolism | 2.04  | 1.45        | 0.014   |
| ESI (+)         | 315.6605 | 756.5520135 | PC(16:0/16:0) | Glycerophospholipid metabolism | 4.63  | 0.91        | 0.013   |
| ESI (+)         | 717.05  | 242.0794821 | Phosphorycholine | Glycerophospholipid metabolism | 2.39  | 1.62        | 0.012   |
| ESI (+)         | 363.218 | 285.22  | Eicosapentaenoic acid | Biosynthesis of unsaturated fatty acids | 0.30  | 2.00        | 0.011   |
| ESI (+)         | 69.324  | 327.2320636 | (4Z,7Z,10Z,13Z,16Z,19Z)-4,7,10,13,16,19-Docosahexaenoic acid | Biosynthesis of unsaturated fatty acids | 0.62  | 12.62       | 0.0058  |
| ESI (+)         | 356.1175 | 281.2478597 | Oleic acid | Biosynthesis of unsaturated fatty acids | 0.39  | 4.70        | 0.029   |
| ESI (+)         | 73.247  | 277.2171703 | Alpha-linolenic acid | Biosynthesis of unsaturated fatty acids | 0.67  | 5.06        | 0.039   |
| ESI (+)         | 302.251 | 391.2840483 | Chenodeoxycholic acid | Primary bile acid biosynthesis | 3.94  | 6.08        | 2.9E-05 |
| ESI (+)         | 413.101 | 516.2991879 | Taurocholic acid | Primary bile acid biosynthesis | 0.35  | 1.80        | 0.034   |
| ESI (+)         | 592.106 | 126.020716 | Taurnine | Primary bile acid biosynthesis | 0.41  | 10.12       | 0.002   |
| ESI (+)         | 267.2065 | 498.2881509 | Taurochenodeoxycholic acid | Primary bile acid biosynthesis | 2.36  | 21.0        | 0.005   |
| ESI (+)         | 351.388 | 407.2790416 | Cholic acid | Primary bile acid biosynthesis | 0.62  | 2.96        | 0.035   |
| ESI (+)         | 316.661 | 517.3294166 | Taurodeoxycholic acid | – | – | - | - |
| ESI (+)         | 326.4925 | 482.3922443 | Tauroresodeoxycholic acid | – | – | - | - |
| ESI (+)         | 73.911  | 482.293108 | Taurothiolcholic acid | – | – | - | - |

ESI+ = electrospray ionization in positive ion mode; ESI– = electrospray ionization in negative ion mode; RT = retention time; VIP = variance importance for projection; PC = phosphatidylcholine.

Cluster analysis from both of positive and negative modes showed altered patterns with distinct differences in obese group versus the lean group, indicating that high-fat diet treatment induced the negative effects. The parameters of OPLS-DA models including $R^2X = 0.384$, $R^2Y = 0.992$, $Q2 = 0.92$ for positive data, and $R^2X = 0.409$, $R^2Y = 0.996$, $Q2 = 0.967$ for negative data were obtained. In addition, the OPLS-DA models validated by the permutation test with 200 random permutation tests generated intercepts $R^2 = 0.85$ and $Q2 = -0.74$ for positive data, and $R^2 = 0.78$ and $Q2 = -0.88$ for negative data (Fig. 3C, D), which demonstrated that the goodness of fit of the data.

As shown in Table 2, compared to the lean mice, it can be seen that the 43 characteristic metabolites with VIP > 1 and $p < 0.05$ belong to glycerophospholipids, bile acids, the fatty acyl group (5-acetamidovalerate, 11-dehydro-thromboxane B2, resolvin D5, alpha-dimorphic acid, 2,3-dinor-6-keto-prostaglandin F1 a, hexadecanedioc acid mono-L-carnitine ester, ethyl oleate, docosapentaenoic acid (22n-3), 9,10-epoxyoctadecanoic acid, 13-OxOODE), and glycerolipids (MG(0:0/18:3(9Z,12Z,15Z)/0:0), gingersgycerolipid A) were filtered and identified as potential biomarkers. Twenty-three of the metabolites involving in glycerophospholipid metabolism were significantly changed ($p < 0.05$), twenty of which were elevated, while three metabolites were reduced after HFD intervention. Nine phosphatidylcholines (PC) species, including PC(0:0/14:0), PC(O-16:0/2:0), PC(2:0/O-16:0)[U], PC(7:0/8:0)[U], PC(O-15:0/0:0), PC(O-16:1(11Z)/2:0), PC(O-8:0/O-8:0), PC(8:0/7:0)[U], and PC(8:0/6:0), were significantly increased 18.18, 17.86, 17.04, 9.20, 6.40, 4.15, 2.77, 1.56, and 1.52-fold, respectively, which contributed to evaluate the difference between normal and high-fat diet mice. In addition, the levels of PG(19:0/16:0), PS(P-16:0/20:2(11Z,14Z)), PI(P-16:0/18:1(9Z)), PI(22:1(11Z)/12:0), PE (21:0/0:0), and PE (13:0/0:0) were increased 640.96, 226.63, 884.00, 210.10, 45.13, and 8.17-fold with VIP > 1.5, respectively, which were the most important hepatic metabolites for discriminating between lean and obese mice. However, the levels of LysoPC(18:1(9Z)), LysoPE(15:0/0:0), and LysoPE(0:0/20:3(11Z,14Z,17Z)) were negatively affected with 0.32, 0.44, and 0.30-fold, respectively. L-palmitoylcarnitine involved in fatty acid metabolism was enhanced 16.16-fold. The levels of three metabolites involving in biosynthesis of unsaturated fatty acid,
including adrenic acid, icosenoic acid, and stearic acid were elevated 1.39, 2.31, and 1.44-fold, respectively.

Total bile acid metabolites were enhanced in comparison with the lean mice. The increased concentration of bile acid in obese mice might be associated with the disorder of liver function. As shown in Table 2, CA and TCDCA involving in primary bile acid biosynthesis were increased 4.55 and 13.71-fold, respectively. In addition, lithocholytaurine was elevated 13.57-fold, while ursodeoxycholic acid (UDCA) was decreased 0.33-fold. When compared with the normal lean mice, the results of the total metabolites belong to the fatty acyls were also increased. The KEGG results revealed that glycerophospholipid metabolism, biosynthesis of unsaturated fatty acid, and primary bile acid biosynthesis in cecum contents were highly disturbed pathways by HFD.

**Discussion**

The Metabolites produced by the gut microbiota play important roles in mediating the complex interaction between the gut microbiota and the human holobiont [9]. In our previous study, HFD intake induced the disorders of liver and serum lipid, and alterations of gut microbiota composition in obese mice model fed with HFD [15]. In this study, we investigated hepatic metabolites and cecum contents of high-fat diet-induced obese mice using UHPLC-Q-TOF MS, and their metabolic profiles were compared with that of normal lean mice by multivariate statistical analysis. We found that some hepatic and cecum contents metabolites associated with lipid metabolism and obesity-related diseases were altered by HFD intake, and these changes in metabolic profiles helped distinguish between the obese and lean mice. Based on the determination of the identified metabolites, the metabolic pathways and gut-liver axis analysis of obesity induced by a high-fat diet were proposed, which were shown in Figs. 4 and 5. Some of these metabolites have been well studied, whereas others have not.

Disorders of glycerophospholipid and fatty acid metabolism have been found to be directly related to the initiation and development of hyperlipidemia. In hepatic metabolites, compared to the lean mice, the overall levels of glycerophospholipids were increased, indicating that HFD led to lipid disorders. The levels of most glycerophospholipids were enhanced in the liver of obese mice, including choline, sn-glycerol phosphoethanolamines, CDP-choline, O-phosphoethanolamine, glycerophosphocholine, and phosphorylcholine. Excess glycerophospholipids were positively associated with fat accumulation, which agrees with the finding that an
Table 2  Potential biomarkers in cecum contents based on the UPLC-Q-TOF/MS between obese and lean mice

| Ionization mode | RT(s) | m/z       | Metabolites | Metabolic pathway                        | VIP  | Fold change | p value  |
|-----------------|-------|-----------|-------------|-----------------------------------------|------|-------------|----------|
| ESI (+)         | 366.533 | 523.381/6793 | PC(2:0/O-16:0)[U] | Glycerophospholipid metabolism    | 1.53 | 17.04       | 0.017    |
| ESI (+)         | 330.811 | 524.315/4739 | PC(O-16:0/2:0) | Glycerophospholipid metabolism    | 1.99 | 17.86       | 2.82E-05 |
| ESI (+)         | 314.942 | 495.314/3924 | PC(8:0/7:0)[U] | Glycerophospholipid metabolism    | 1.41 | 1.56        | 0.0051   |
| ESI (+)         | 368.351 | 206.190/139  | PC(O-16:1(11Z)/2:0) | Glycerophospholipid metabolism | 1.89 | 4.15        | 0.021    |
| ESI (+)         | 464.138 | 522.354/753 | PC(7:0/8:0)[U] | Glycerophospholipid metabolism    | 1.49 | 9.20        | 0.001    |
| ESI (+)         | 314.989 | 481.311/1877 | PC(8:0/6:0) | Glycerophospholipid metabolism    | 1.73 | 18.18       | 0.019    |
| ESI (+)         | 469.048 | 482.360/2632 | PC(O-8:0/O-8:0) | Glycerophospholipid metabolism | 1.05 | 1.52        | 0.040    |
| ESI (+)         | 512.919 | 467.787/1501 | PC(O-15:0/0:0) | Glycerophospholipid metabolism    | 1.38 | 6.40        | 0.0078   |
| ESI (+)         | 458.095 | 478.294/417  | LysoPC(18:1(9Z)) | Glycerophospholipid metabolism    | 1.39 | 0.32        | 0.009    |
| ESI (+)         | 421.504 | 451.298/3584 | LysoPE(16:1(9Z)/0:0) | Glycerophospholipid metabolism | 1.52 | 2.41        | 0.012    |
| ESI (+)         | 477.56  | 510.355/0402 | LysoPE(0:0/20:0) | Glycerophospholipid metabolism    | 1.58 | 1.87        | 0.00042  |
| ESI (+)         | 420.079 | 440.276/459  | LysoPE(15:0/0:0) | Glycerophospholipid metabolism    | 1.62 | 0.44        | 0.0020   |
| ESI (+)         | 399.027 | 503.358/0666 | LysoPE(0:0/20:3(11Z,14Z,17Z)) | Glycerophospholipid metabolism | 1.34 | 0.30        | 0.030    |
| ESI (+)         | 479.119 | 507.364/0197 | LysoPE(20:1(11Z)/0:0) | Glycerophospholipid metabolism    | 1.31 | 1.67        | 0.0066   |
| ESI (+)         | 451.64  | 546.354/9728 | I-(8Z,11Z,14Z-eicosatrienoyl)-sn-glycero-3-phosphocholine | Glycerophospholipid metabolism    | 1.91 | 13.14       | 0.0027   |
| ESI (+)         | 441.336 | 360.252/6856 | PG(16:0/0:0)[U] | Glycerophospholipid metabolism    | 1.59 | 4.42        | 0.022    |
| ESI (+)         | 541.67  | 765.564/852 | PG(19:0/16:0) | Glycerophospholipid metabolism    | 1.96 | 640.96      | 0.00028  |
| ESI (+)         | 506.869 | 524.429/7664 | PE(21:0/0:0) | Glycerophospholipid metabolism    | 1.90 | 45.13       | 0.0016   |
| ESI (+)         | 393.445 | 411.249/935 | PE(13:0/0:0) | Glycerophospholipid metabolism    | 1.79 | 8.17        | 0.0029   |
| ESI (+)         | 343.046 | 464.336/498 | PSP(16:0/20:2(11Z,14Z)) | Glycerophospholipid metabolism | 2.01 | 226.63      | 0.023    |
| ESI (+)         | 519.713 | 821.552/459 | PI(16:0/18:1(9Z)) | Glycerophospholipid metabolism    | 2.03 | 884.00      | 0.00014  |
| ESI (+)         | 439.284 | 837.547/032 | PI(22:1(11Z)/12:0) | Glycerophospholipid metabolism    | 2.01 | 210.10      | 1.34E-06 |
| ESI (+)         | 545.547 | 331.264/861 | Adrenic acid | Biosynthesis of unsaturated fatty acid | 1.04 | 1.39        | 0.027    |
| ESI (+)         | 560.467 | 309.271/544 | Icosenoic acid | Biosynthesis of unsaturated fatty acid | 1.35 | 2.32        | 0.00036  |
| ESI (+)         | 639.652 | 283.264/859 | Stearic acid | Biosynthesis of unsaturated fatty acid | 1.10 | 1.44        | 0.035    |
| ESI (+)         | 421.994 | 400.280/1629 | L-Palmitoylcarnitine | Fatty acid metabolism | 1.68 | 16.16       | 0.0025   |
| ESI (+)         | 392.176 | 500.285/0285 | Taurochondenoxycholic acid | Primary bile acid biosynthesis | 1.88 | 13.71       | 4.89E-06 |
| ESI (+)         | 297.733 | 407.063/3335 | Cholic acid | Primary bile acid biosynthesis | 1.41 | 4.55        | 0.0021   |
| ESI (+)         | 394.438 | 374.289/5602 | Ursodeoxycholic acid | Primary bile acid biosynthesis | 1.67 | 0.33        | 8.58E-05 |
| ESI (+)         | 363.583 | 483.322/665 | Lithocholyltaurine | – | – | – | – |
| ESI (+)         | 366.023 | 478.294/179 | 5-Acetamidovalerate | – | – | – | – |
| ESI (+)         | 424.632 | 309.180/271 | 2,3-Dinor-6-keto-prostaglandin F1 a | – | – | – | – |
| ESI (+)         | 376.480 | 430.263/936 | Hexadecanedic acid mono-L-carnitine ester | – | – | 1.87 | 9.49 | 0.00026 |
| ESI (+)         | 482.271 | 303.233/177 | Ethyl oleate | – | – | 1.56 | 4.08 | 0.0022 |
| ESI (+)         | 520.437 | 329.249/1524 | Docosapentaenoic acid (22n-3) | – | – | 1.23 | 2.07 | 0.011   |
| ESI (+)         | 453.562 | 297.107/1611 | 9,10-epoxyoctadecanoic acid | – | – | 1.22 | 3.16 | 0.018   |
| ESI (+)         | 558.79  | 700.557/389 | 13-OxoODE | – | – | 1.14 | 0.62 | 0.045   |
| ESI (+)         | 411.144 | 352.284/1858 | MG(0:0/18:3(9Z,12Z,15Z)/0:0) | – | – | 1.72 | 3.30 | 0.00039 |
| ESI (+)         | 449.367 | 676.425/8482 | Gingerglycolipid A | – | – | 1.63 | 0.034 | 0.0023  |
increased glycerophospholipid metabolites level in obese mice was positively correlated with fat accumulation [12]. Choline, like phospholipids, is an essential nutrient for the maintenance of normal liver function [18], which is absorbed mainly by the small intestine and completely metabolized in the liver [19]. The finding of the increased choline in liver was consistent with the observation that more choline is correlated with greater risks of having hepatic steatosis, NASH, and lobular inflammation [20]. A research also showed that plasma levels of free choline are positively correlated with the grade of liver steatosis and fibrosis [21]. Our results were inconsistent with a previous study in which choline-related metabolites such as choline, phosphatidylcholine, and glycerol 3-phosphate were elevated in HFD-fed mice [22]. A previous research showed that choline intake was inversely associated with the risk of nonalcoholic fatty liver disease (NAFLD) [23]. PCs are mainly synthesized through the cytidine diphosphate (CDP)-choline pathway and the PE N-methyltransferase (PEMT) pathway. However, PC (16:0/16:0) in the liver was negatively affected. This is in harmony with previous studies that reduced PC biosynthesis impairs the secretion of VLDL in the liver, thus leading to the pathogenesis of hepatic steatosis [24, 25]. The lipidomic analysis of the liver of NAFLD patients demonstrated increased TG levels and decreased PC levels [26]. Several researches also showed that PC treatment could alleviate fat accumulation [27, 28].

We found that there was a possible link between the altered microbial metabolites and liver metabolites. Most of glycerophospholipid metabolites were also enhanced in cecum contents. In addition to PC species, we also found many other glycerophospholipids, such as phosphoethanolamine (PE), phosphatidyl inositol (PI), and phosphatidylserine (PS) species, were significantly increased in the HFD group. These findings are in accordance with the notion that glycerophospholipids play several important roles in the development of NAFLD [29, 30]. Nine PCs increased, which is in accordance with a research that an enhanced PC level in plasma was positively correlated with fat accumulation [12]. In addition, excessive PCs in the cecum may be explained by PC synthesized in the liver can be secreted into bile. Biliary PC is hydrolyzed by phospholipases to lysoPC and fatty acid,
which are then absorbed through the intestinal brush border membrane [31]. On the other hand, PC produced in enterocytes is secreted into the intestinal lumen, forming part of the hydrophobic mucus layer [32]. A decrease in the PC content in the mucus layer is a characteristic of ulcerative colitis, a chronic inflammatory condition of the terminal ileum and colon [32]. These results indicated that the gut attempts to maintain phospholipid homeostasis and cellular integrity through increasing the content of PC. LysoPC resulted from the partial hydrolysis of phosphatidylcholines (PC), removing one of the fatty acid groups. Changes in LysoPCs levels are thought to be correlated with diseases with abnormal energy status, such as obesity and hyperlipidemia [33]. In contrast to the lean mice, the level of LysoPC(18:1(9Z)) was decreased after HFD intervention. This is not consistent with a study that LysoPC(18:1(9Z)) was enhanced in rats on a high-fat diet [34]. LysoPC11(18:1(9Z)), LysoPE (15:0/0:0), and LysoPE (0:0/20:3(11z, 14z, 17z)) were decreased, while lysoPE(16:1(9Z)/0:0), LysoPE(0:0/20:0), and LysoPE(20:1(11Z)/0:0) were enhanced in obese mice, which were partly matched with other reports that eleven LysoPCs and 2 lysoPEs were reduced in serum in the mice fed with HFD [12]. Furthermore, PI(P-16:0/18:1(9Z)), PI(22:1(11Z)/12:0), PE(21:0/0:0), PE(13:0/0:0), and PS(P-16:0/20:2(11Z,14Z)) were significantly enhanced after HFD intake. A previous report showed that the levels of most of PC, PE, PI, and PS species were significantly decreased in liver of NAFLD rats [35]. PE(21:0/0:0) and PE(13:0/0:0) are two kinds of glycerophosphoethanolamines. Phosphoethanolamine (PE) is a class of phospholipids found in biological membranes. Together with PC, PS, PI, PE represents the backbone of most biological membranes. Phosphoethanolamines in food break down to form phosphoethanolamine-linked amadori products, which act as a part of the Maillard reaction [36]. These products accelerate membrane lipid peroxidation and cause oxidative stress on cells exposed to them [37]. Phosphatidylserine (PS) and phosphatidylglycerol (PG) are endogenous phospholipids with assumed anti-inflammatory potential. PG is a glycerophospholipid, generally considered to be a precursor to the synthesis of cardiolipin (CL), a mitochondrial signature phospholipid that is necessary for dynamic mitochondrial function [38]. Lack of PG in mammalian cells results in CL deficiency, mitochondrial dysfunction, and

![Fig. 5 Bile acids metabolism pathway disturbed in the obese mice induced by a high-fat diet based on the metabolites of liver and cecum contents analysis. Metabolites names in blue or red indicated they were found in liver or cecum contents, respectively, and the metabolites in orange were found in both. The increased trend was indicated by ↑ and decreased trend was indicated by ↓. CA cholic acid, CDCA chenodeoxycholic acid, TCA taurocholic acid, TCDCA taurochenodeoxycholic acid, TDCA taurodeoxycholic acid, TLCA taurolithocholic acid, UDCA ursodeoxycholic acid.](https://example.com/five.png)
decreased adenosine triphosphate production [39]. Interestingly, PS (P-16:0/20:2(11Z, 14Z)), PG(19:0/16:0), and PG(16:0/06:0) [U] were significantly increased 226.63, 640.96, and 4.42-fold, respectively, which were important biomarkers in cecum. These results can be explained by that the gut attempts to maintain phospholipid homeostasis by increasing the content of PS and PG. These findings may indicate that the specific functions of different glycerophospholipids still require further clarification. The relationship between the differential hepatic and cecum contents metabolites in glycerophospholipid metabolism summarized shown in Fig. 4. Further studies are required to correlate obesity with glycerophospholipids. Consistently, pathway analysis results showed that glycerophospholipid metabolism was closely involved in hepatic and cecal metabolites metabolism.

There exists highly efficient bile acid preservation and recycling system within the body, which is termed the enterohepatic circulation. Bile acids, which are biosynthesized by the catabolism of cholesterol in the liver, are involved in regulating lipid absorption and metabolism [40, 41], and helping maintain the homeostasis of glucose, cholesterol, and triglyceride in the liver. In addition, bile acids, acting as a detergent for cellular membranes, can directly affect gut microbiota by causing membrane disruption through their amphipathic properties. The deconjugation of bile salt hydrolase (BSH) produced by gut microbiota is a prerequisite for the downstream modifications by 7-alpha-dehydroxylase to produce DCA and LCA, or by 7-alpha-hydroxysteroid dehydrogenase (HSDH) to produce UDCA. Gut microbiota convert primary bile acids to secondary bile acids, which has an important effect on bile acid signaling [42]. The relationship between the differential hepatic and cecum contents metabolites in bile acid pathway is summarized in Fig. 5. In humans, primary bile acids are CA and CDCA, which are converted by gut microbiota to secondary bile acids, such as LCA, DCA, and UDCA. Secondary bile acids have been reported to affect lipid metabolism, such as energy expenditure, insulin sensitivity, and cholesterol synthesis through farnesoid X receptor (FXR) and takeda-G-protein-receptor-5 (TGR5) pathways [43–45]. FXR signaling activation inhibits lipogenesis and promotes fatty acid oxidation and also affects cholesterol transportation [46, 47]. Bile acids differ in their potency to activate FXR and TGR5. In the present work, the results demonstrated that the concentrations of total bile acid were elevated in the liver and cecum, which is in accordance with the finding that obesity is associated with enhanced bile acid synthesis and impaired transport [48]. Increased bile acids production in patients are also associated with NAFLD, diabetes and metabolic syndrome [49–51]. Mouzaki et al. concluded that bile acid production in the livers of NAFLD was enhanced, based on the findings that faecal total bile acid and faecal primary bile acid levels in patients with NAFLD were increased [52]. This result was also supported by an epidemiological study which demonstrated that a low-fat, high-carbohydrate, and high-fiber diet consumption reduced fecal concentrations of secondary bile acid [53].

A few changes to primary and secondary metabolites in both liver and cecum contents between the lean and obese mice confirmed that lipids primarily drove this metabolic pathology. Cholic acid and chenodeoxycholic acid, known as primary bile acids, are synthesized from cholesterol in the liver and enter the small intestine where they are conjugated to glycine and taurine. Cholic acid (CA), the major bile acid, was significantly decreased 0.62-fold in the liver, which is inconsistent with the study that CA was increased 68-fold after high fat, high cholesterol, and cholate diet in mice [54]. The result was in harmony with the finding that liver level of cholic acid was significantly decreased in patients with NASH [55]. CA is a signaling molecule that activates the FXR-FGF15 pathway [56]. However, CA in cecum showed opposite results and increased 4.55-fold. High level of CA in cecum may be due to the absorption of cholate present in the high-fat diet, but it may also generated by the conversion of cholesterol to CA as a mechanism for excreting excessive cholesterol through gut-liver axis. Elevated chenodeoxycholic acid (CDCA) in the liver, which has been identified as effective ligands for FXR [41], was consistent with the report by Aranha et al. [57]. Taurochenodesoxycholic acid (TCDCA) was enhanced 24.36 and 13.71-fold in liver and cecum, respectively, is formed by conjugation of chenodeoxycholic acid (CDCA) with taurine in liver. The increased concentration of TCDCA in obese mice might be associated the disorder of liver function. The previous report indicated that TCDCA was enhanced in serum of liver cirrhosis and hepatocellular carcinoma patients [58]. Lithocholic acid (LCA) was reported to a hepatotoxic microbial metabolite [59]. Accordingly, taurolithocholic acid was elevated 4.36-fold in the liver, and lithocholytaurine was enhanced 13.57-fold in the cecum. The UDCA in the gut or the activation of the intestinal FXR pathway increases the expression of genes related to intestinal barrier function, thus benefiting the treatment of obesity and liver disease [60, 61]. Besides, UDCA was reported to improve hepatic steatosis and enhance insulin sensitivity by inducing the excretion of hepatic lipids, inhibiting hepatic long-chain FFA uptake, restoring gut mucosal integrity, and suppressing the miR-34a/SIRT1/p53 pathway in obese mice [61–63]. Our research consistently found that UDCA was significant reduced in obese mice. Thus, the change in bile acid metabolism induced by HFD plays multiple roles in regulating lipid
metabolites in obese mice. However, more studies are required to clarify the role of specific bile acid in bile acid pool as a potential mechanism in obesity and metabolic disorders.

Furthermore, the level of oleic acid was also affected by HFD. Oleic acid is a monounsaturated fatty acid, which was enhanced 3.89-fold in the liver of obese mice. This result is consistent with the finding that oleic acid was significantly enhanced in serum of liver cirrhosis and hepatocellular carcinoma patients [58]. Alpha-linolenic acid (ALA) is an essential fatty acid, which was significantly lowered by HFD in liver. The result is in harmony with the research that alpha-linolenic acid-enriched butterm attenuated high fat diet-induced insulin resistance and inflammation [63]. Accordingly, the levels of adrenic acid, icosenoic acid, and stearic acid involving in biosynthesis of unsaturated fatty acid in the cecum were significantly enhanced. The data is consistent with the report that higher proportion of stearic acid in serum phospholipids [64]. These results indicated that HFD intake had regulating effect on these metabolites involved in fatty acid metabolisms.

Most studies used male mice to construct high-fat diet-induced obesity model [12, 65, 66]. In the study, we used female mice in order to discover different outcomes in males based on their different hormonal profile. The results shared some similarities with previous studies that compared to the lean mice, a high-fat increased lipid profiles in the liver [12], and total bile acids (BAs) were enhanced in fecal metabolites in obese male mice [66]. However, the result was not in consistent with the finding that glycerol 3-phosphate and choline were significantly reduced [22], and CA was significantly elevated in the liver of male mice [54]. The specific mechanism that causes the different results in female and male mice needs further investigation. We proposed metabolic pathways associated with high-fat diet-induced obesity based on the metabolites we found in this study (Figs. 4, 5). However, the causal relationship between these identified biomarkers and the exact underlying mechanisms of the metabolites changes in obesity are still unclear. Furthermore, a large number of markers were detected by UHPLC-Q-TOF/MS but remain unidentified at present. Despite these limitations, the present study showed a cluster of obesity-associated alterations in metabolites, the proposed pathway will presumably facilitate our understanding of obesity and associated diseases symptoms.

Conclusions
The UPLC-Q-TOF MS-based liver and cecum contents studies have been performed to reveal the complex interactions between liver and gut of obese mice induced by a high-fat diet and provided important metabolic information for obesity and related diseases. A high-fat diet increased lipid profiles (i.e. glycerophospholipids, PC, PE, PI, PG, and PS) and total bile acid (primary and secondary bile acid) in liver and cecum, indicating that HFD regulated glycerophospholipid metabolism, primary bile acid synthesis, and fatty metabolism pathways. This study provides evidence regarding the important regulatory functions of metabolites produced by gut commensal bacteria as well as insights into the interaction between the microbial products and host. The differences in these metabolic profiles between the obese and lean mice may provide a better understanding of the metabolic changes of obesity, which could be used for future clinical diagnosis and treatment.
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