



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Black tea extract ameliorates fatty liver and fatty kidney in HFD-induced obese mice

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ARTICLE INFO

Article history:

Received 1 April 2024

Received in revised form 21 April 2024

Accepted 5 September 2024

Keywords:

Black tea extract

Ectopic lipid deposition

Fatty liver

Fatty kidney

Lipidomics

ABSTRACT

Black tea is a kind of full-fermented tea with therapeutic potential for metabolic diseases. Ectopic lipid deposition (ELD) is an essential risk factor for organ injury in metabolic syndrome, especially in liver and kidney, for which effective interventions are lacking. Here, we explored whether black tea extract (BTE) improves fatty liver and fatty kidney, as well as identified the potential lipid biomarkers for ELD and lipid targets of BTE on the improvement of ELD. Transcriptome data from diet-induced obese mice were analyzed to confirm high-fat-diet feeding disturbs lipid metabolism in the liver and kidney. BTE prominently inhibited body weight gain, improved glucose metabolism, as well as reduced lipid droplet accumulation in the liver and kidney. Moreover, lipidomic profiling analyses identified 28 lipid classes in the liver while 29 in the kidney with that up-regulated glycerides and phosphatidylcholines, as well as down-regulated cardiolipin were the characteristic changes in ELD. BTE prominently reduced glycerides in ELD, thereby constituting the basis of its anti-ELD effect. Specifically, BTE displayed stronger effects on lowering cholesterol ester (CE) in fatty liver, while also affecting phospholipids and sphingolipids in fatty kidney. Ultimately, integrative analysis identified CE18:2 and triglyceride (TAG)56:4 (20:2) as the potential lipid biomarkers for BTE in improvement of ELD. BTE could be an effective food supplement for the prevention and treatment of ELD. Notably, CE18:2 and TAG56:4(20:2), as the potential lipid biomarkers, may facilitate the research and development of anti-ELD drug.

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1. Introduction

Ectopic lipid deposition (ELD) refers to the abnormal accumulation of fat in organs such as liver, kidney, pancreas and

heart. As an important risk factor for organ injury in individuals with metabolic syndrome, ELD can exacerbate various metabolic disorders including insulin resistance, abnormal glycolipid metabolism and hypertension^[1-2]. The liver and kidney are involved in the synthesis, metabolism and excretion of lipids, thus particularly susceptible to lipid deposition in various metabolic diseases, especially obesity. Non-alcoholic fatty liver disease (NAFLD), resulting from ELD in

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the liver, can progress to non-alcoholic steatohepatitis (NASH) and potentially advance to cirrhosis and liver cancer^[3]. Likewise, fatty kidney is a serious clinical consequence contributing to chronic kidney disease (CKD), due to fat storage in kidney^[4]. Instead of 2 individual organs, mounting evidence states liver-kidney crosstalk connecting NAFLD and CKD^[5-6]. Interestingly, fatty liver gains more considerable attention as a research hotspot, but fatty kidney is lack of understanding nevertheless.

Lipids are a group of biomolecules with crucial roles in living organisms, tissues and cellular processes, which could be identified, characterized, and quantified by lipidomics analysis as one of the omics approaches^[7]. Over the past decades, lipid targets have been discovered in cancer, diabetic nephropathy, gestational diabetes, etc.^[8-10]. To differentiate between NASH and steatosis, aqueous metabolites and concise “signatures” of lipids including ether-linked glycerophospholipids, 1-deoxy-ceramide species, lysophosphatidylinositols, have been identified to serve as biomarkers of disease progression in NAFLD^[11]. Regarding fatty kidney, a publication has proposed that anorexia-induced lipolysis plays a role in both hepatic and renal steatosis, necessitating further exploration^[12]. Therefore, unveiling the key lipid targets would greatly facilitate the clinical diagnosis and treatment, and thus is prerequisite for combating fatty liver, fatty kidney and associated complications.

Apart from the most popular consumed drink with 5 000-year history worldwide, tea is also a distinctive natural product possessing the multiple pharmacological activities^[13-15]. NASH, diabetes, hyperuricemia, colitis, stress and more diseases have been ameliorated by the extract, polyphenols of green tea, yellow tea or black tea^[16]. Furthermore, green tea intake dwindles aberrant hepatic lipid species of lysophospholipids (LPLs) by lipidome in high-fat diet (HFD)-induced obese (DIO) mice, which are associated with inflammation and obesity^[17]. As a fully-fermented tea undergoing post-fermentation or oxidation, black tea has been discovered to exhibit lipid-lowering, anti-cancer, prevention and treatment of cardio-cerebral diseases and metabolic diseases^[18-20]. Though the weight-lowering efficacy of black tea has been known^[16]. However, the effects on ELD are still unclear yet.

Our current findings demonstrated that black tea extract (BTE) ameliorated the metabolic syndrome, including weight loss, correcting glycolipid metabolic disorder and insulin resistance. More importantly, BTE exhibited the dramatical properties in improvement of fatty liver and fatty kidney manifested by biochemical, histological examination, especially lipidomics profiling. Not only the ELD in liver and kidney was alleviated, but the predominant alteration of glycerides, phospholipids in both organs was affirmed by lipidomic analysis. Hence, our study provided the insight into potential lipid biomarkers for clinical diagnosis of ELD, particularly prospective therapeutics of black tea as food supplement for prevention and treatment of fatty liver and fatty kidney.

2. Methods and materials

2.1 Preparation of BTE

The BTE used in the current study was produced by a patent-authorized manufacturing process^[21]. Firstly, raw tea leaves were harvested in Fuliang County of Jiangxi Province in China, and converted to black tea *via* withering, rolling, fermentation and drying.

Then, BTE powder was produced by a series of extraction procedure, including impurity removal, crushing, soaking, centrifuging, ceramic membrane filtration, reverse osmosis concentration and vacuum freeze-drying. Finally, we utilized systematic analysis and high-performance liquid chromatography (HPLC) analysis to identify 11 kinds of the characteristic components. Relevant results have been shown in our previous publication^[21].

2.2 Transcriptomics analysis

Transcriptional data of fatty liver and fatty kidney were obtained from GEO dataset (GSE159090 and GSE185466). Differential expression genes (DEGs) were identified by GEO2R online tool, using a cutoff of $P < 0.05$ and $|\log_2 \text{fold change}| > 1$. Gene enrichment analysis was performed by R software utilizing clusterProfiler package. Chord plot was plotted by <https://www.bioinformatics.com.cn> (last accessed on 20 Feb 2023).

2.3 Animal experiment

The male 6-week-old C57BL/6J mice obtained from Beijing HFK Bioscience Co., Ltd. (Beijing, China) were housed in a standard barrier system with free access to water and food. The animal procedures were approved by the Animal Care and Use Committee on the Ethics of Animal Experiments of the Institute of Materia Medica (No. 00005544). After 1-week adaptive feeding, the mice were randomly divided into 3 groups ($n = 6$). CD group were fed on chow diet (CD) and administrated 0.5% carboxymethyl cellulose sodium (CMC-Na). HFD group were fed on HFD (60 kcal% fat, Research Diets, Inc, USA) and administrated with 0.5% CMC-Na. The amount of brewed black tea each time is 2 g, and for frequent tea drinker it is 2 g twice, totaling 4 g. Thus, taking 2 g is considered a moderate amount, and 4 g is considered a high amount. Based on a yield of 37.5%, the extract of black tea is about 0.75 and 1.5 g/60 kg for humans. When converted using body surface area between humans and mice, the dose is equivalent to 150 and 300 mg/kg in mice ($0.75/60 \times 12.3 = 153.75 \text{ mg/kg}$, $1.5/60 \times 12.3 = 307.5 \text{ mg/kg}$). According to our previous study, 300 mg/kg BTE exert the most significant effect on metabolic regulation toward DIO mice^[21]. BTE group were fed on HFD and administered with 300 mg/kg BTE by oral gavage once a day. The body weight was recorded once a day while food and water intake were recorded twice a week. After 15-week administration, the mice were anesthetized to collect blood, liver, and kidney. The liver and kidney were divided into 3 parts after weighting, for lipid extraction stored in liquid nitrogen, pathological section storing in 10% buffered formalin, Oil red O staining storing in -20°C .

2.4 Blood glucose test

The blood glucose was measured in the 15th week of administration using AccuCheck glucometer (FreeStyle Optium Neo, Abbott, USA). Postprandial blood glucose (PBG) was determined without fasting, while fasting blood glucose (FBG) was determined after 4-h fasting. Glucose tolerance test (GTT) were performed after 4-h fasting with blood glucose level tested at 0, 30, 60 and 120 min after peritoneal injection of 2 g/kg glucose solution. Insulin tolerance test (ITT) was performed after 4-h fasting with blood glucose level

tested at 0, 15, 30, 60 and 90 min after peritoneal injection of 0.75 U/kg insulin solution. Area of under curve (AUC) of GTT = 15×0 min blood glucose + 30×30 min blood glucose + 45×60 min blood glucose + 30×120 min blood glucose. AUC of ITT = 7.5×0 min blood glucose + 15×15 min blood glucose + 22.5×30 min blood glucose + 30×60 min blood glucose + 15×90 min blood glucose.

2.5 Determination of blood biochemistry indexes

Blood samples were obtained for the determination of various biochemical indexes. Plasma concentrations of triglyceride (TAG), total cholesterol (CHO), alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine (Cre) were assessed using kits obtained from Biosino Bio-Technology and Science Incorporation (Beijing, China) and analyzed with an automated biochemical analyzer (TOSHIBA, Tokyo, Japan).

2.6 Plasma insulin determination

Mouse ultrasensitive insulin ELISA kit (ALPCO, USA) was utilized to measure the insulin content in the plasma according to the instructions provided by the manufacturer. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by following:

$$\text{HOMA-IR} = \frac{\text{FBG} \times \text{plasma insulin concentration}}{22.4}$$

2.7 Hematoxylin and eosin (HE) staining

After the tissues were fixed in 10% buffered formalin, the paraffin was used to embed the fixed tissues, followed by sample sectioning, and staining with.

2.8 Oil red O staining

The tissues were rapidly frozen at -20°C . Subsequently, the frozen tissues were cut into $15\ \mu\text{m}$ slices and fixed by 10% buffered formalin for 30 min. After washing, the slices were stained with Oil red O for 10–15 min protecting from light, followed by differentiate briefly in 60% ethanol and washing. Mayer's hematoxylin stain solution was used to stain nuclear. Finally, the sections were mounted in glycerin jelly.

2.9 Lipid extraction

Lipid extraction was conducted using a modified Bligh/Dyer extraction method^[22]. The extraction solvent, composed of chloroform (1:2, *V/V*) with 1% butylated hydroxytoluene (BHT), was prepared. Liver and kidney tissues were incubated at 4°C and 1 500 r/min for 1 h. Following incubation, phase separation was achieved by adding 350 μL of ice-cold Milli-Q water and 250 μL of ice-cold chloroform. Subsequently, samples were centrifuged at $16\ 260 \times g$ at 4°C for 5 min, and the lower liquid phase was transferred to a new tube. This extraction process was repeated, and the extractions were combined and dried using a SpeedVac under organic mode. The resulting lipid extract was then resuspended in 200 μL of chloroform (1:1, *V/V*).

2.10 Lipidome analysis

The Exion UPLC-QTRAP 6500 Plus (Sciex) LC-MS system was utilized for lipid analysis. Separation of polar lipids was performed using a Phenomenex Luna silica column ($150\ \text{mm} \times 2.0\ \text{mm}$, $3\ \mu\text{m}$). Mobile phase A consisted of 89.5% chloroform, 10% methanol, and 0.5% ammonium hydroxide, while mobile phase B was composed of 55% chloroform, 39% methanol, 0.5% ammonium hydroxide, and 5.5% water. The elution conditions were as follows: 0–5 min: 95% mobile phase A; 7 min: 60% mobile phase A; 7–11 min: 60% mobile phase A; 11.01 min: 30% mobile phase A; 11.01–26 min: 30% mobile phase A; and 26–31 min: 95% mobile phase A. Multiple reaction monitoring (MRM) was established to identify and quantify lipids using an internal standard. For separation of polar and neutral lipids, a Phenomenex Kinetex C_{18} column ($4.6\ \text{mm} \times 100\ \text{mm}$, $2.6\ \mu\text{m}$) was utilized with an isocratic elution of 48% chloroform, 48% methanol, and 0.1 mol/L ammonium acetate. Free CHO, alcohol, and related esters were analyzed by atmospheric pressure chemical ionization in HPLC-MS/MS. Bioinformatic analysis was performed using the OmicStudio tools at <https://www.omicstudio.cn/tool> and Bioinformatics platform.

2.11 RNA isolation and reverse transcription quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated by TRIcom Reagent (TR201, Jianshi Bio, China) and Direct-zol kit (TR205-200, Zymo, USA). A total of 1 000 ng of total RNA was used for reverse-transcription to obtain cDNA by transcription system buffer (R333, Vazyme). qPCR reaction was performed according to the protocol of SYBR qPCR Master Mix (Q712, Vazyme) and the primer sequences were listed in Table S1.

2.12 Statistical analysis

All the data were presented as mean $\pm$ SEM. Unpaired *t*-test was used to examine difference between 2 groups, while One-way ANOVA was used to examine difference between 3 groups. *P* value < 0.05 was considered as significant difference.

3. Results

3.1 HFD disturbed the genes expression of lipid metabolism in liver and kidney

Transcriptome data of fatty liver and fatty kidney were obtained from GEO datasets (GSE159090 and GSE185466). Compared with lean mice, the liver and kidney in DIO mice changed significantly at transcriptional level. Using a cutoff of $P < 0.05$ and $|\log_2 \text{fold change}| > 1$, DEGs were plotted. Specifically, total of 128 genes were found to be down-regulated, and 225 genes were up-regulated in the liver (Fig. 1A). Meanwhile, 196 genes were down-regulated while 186 genes were up-regulated in the kidney (Fig. 1B). To explore the potential effect of HFD on liver and kidney in DIO mice, enrichment analysis was performed by 353 DEGs in liver (Fig. 1C) and 382 DEGs in kidney (Fig. 1E) respectively. In liver of DIO mice, 16 terms related to biosynthesis, transport and localization of lipid were enriched, including very long-chain fatty acid metabolic process, long chain

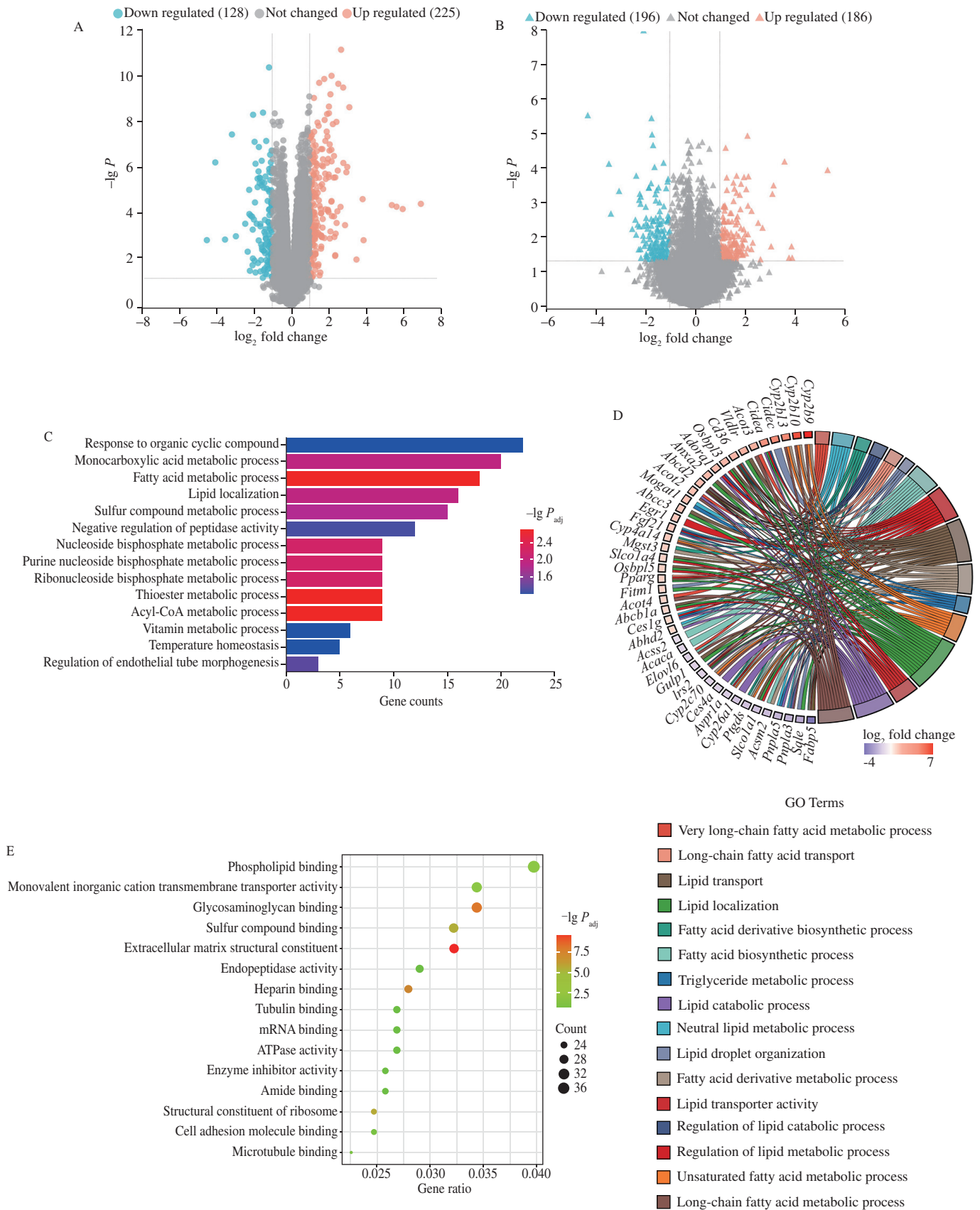


Fig. 1 HFD feeding induced alteration of lipid metabolism genes in liver and kidney. Volcano plot was utilized to present DEGs in (A) liver and (B) kidney of DIO mice compared with lean mice ($P < 0.05$, $|\log_2$ fold change $| > 1$). Gene Ontology (GO) enrichment of DEGs in (C) liver and (E) kidney was presented by histogram and bubble charts respectively. The relationship between lipid metabolism terms and DEGs in (D) liver and (F) kidney was presented by chord plot.

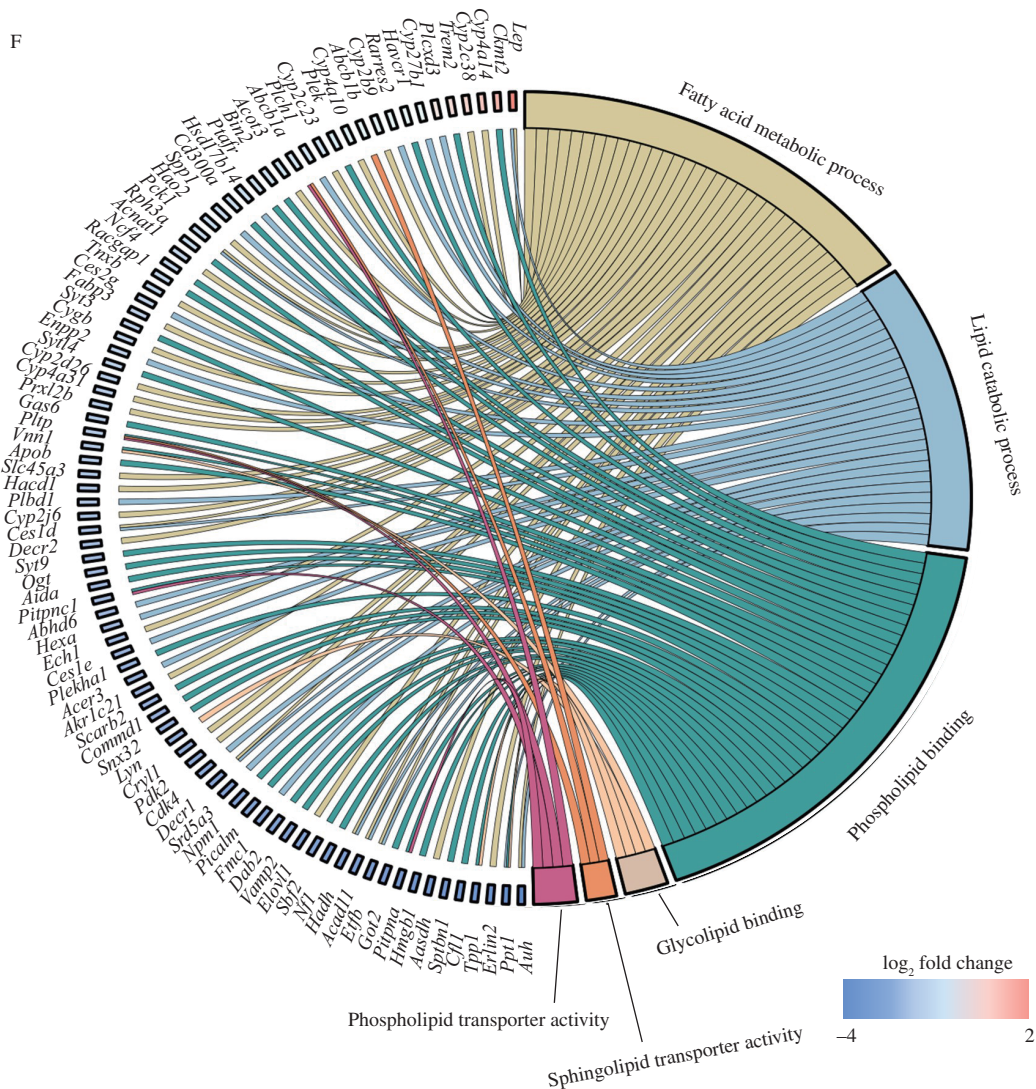


Fig. 1 (Continued)

fatty acid transport, TAG metabolic process and so on (Fig. 1D). Similarly, HFD feeding also affected lipid metabolism in kidney, including lipid catabolic process, glycolipid binding, sphingolipid transporter activity, phospholipid transporter activity, which were enriched with statistical difference as most of the down-regulated DEGs (Fig. 1F). Thence, HFD feeding disrupted fatty acid metabolism in both liver and kidney. However, it is noteworthy that the HFD affected TAG metabolism in the liver, while sphingolipid, glycolipid and phospholipid metabolism in the kidney. Taken together, the findings of transcriptomics analysis suggested that HFD feeding interfered with gene expression related to lipid metabolism in both the liver and kidney.

3.2 BTE ameliorated HFD-induced glucose and lipid metabolism disorders in DIO mice

As a popular beverage, black tea was recognized as a food supplement to regulate blood lipid. To further investigate whether black tea could improve fatty liver and fatty kidney in DIO mice,

BTE was orally administered at the beginning of HFD feeding. Since the 6th week, the body weight of HFD group has been significantly higher than CD group. Similarly, the BTE group has exhibited a significant lower body weight compared to the HFD group, starting from the 6th week (Fig. 2A). The 15-week administration of BTE significantly decreased the body weight gain by 45.5% compared with HFD group (Fig. 2B) with no effect on 24-h food and water intake (Figs. 2C and D), suggesting that BTE could protect against HFD-induced obesity without affecting appetite center in DIO mice. Given that lipid and glucose disorders, we found that TAG was decreased moderately, while plasma CHO was much higher in HFD group. BTE administration decreased plasma TAG and CHO level moderately (Figs. 2E and F). Moreover, PBG and FBG were significantly decreased in BTE group (Figs. 2G and H). Lower plasma insulin level, HOMA-IR, as well as AUC of GTT and ITT in BTE group suggested that BTE improved HFD-induced hyperglycemia and insulin resistance (Figs. 2I-N). Taken together, we preliminarily affirmed that BTE alleviated HFD-induced glucose and lipid metabolism disorders in DIO mice.

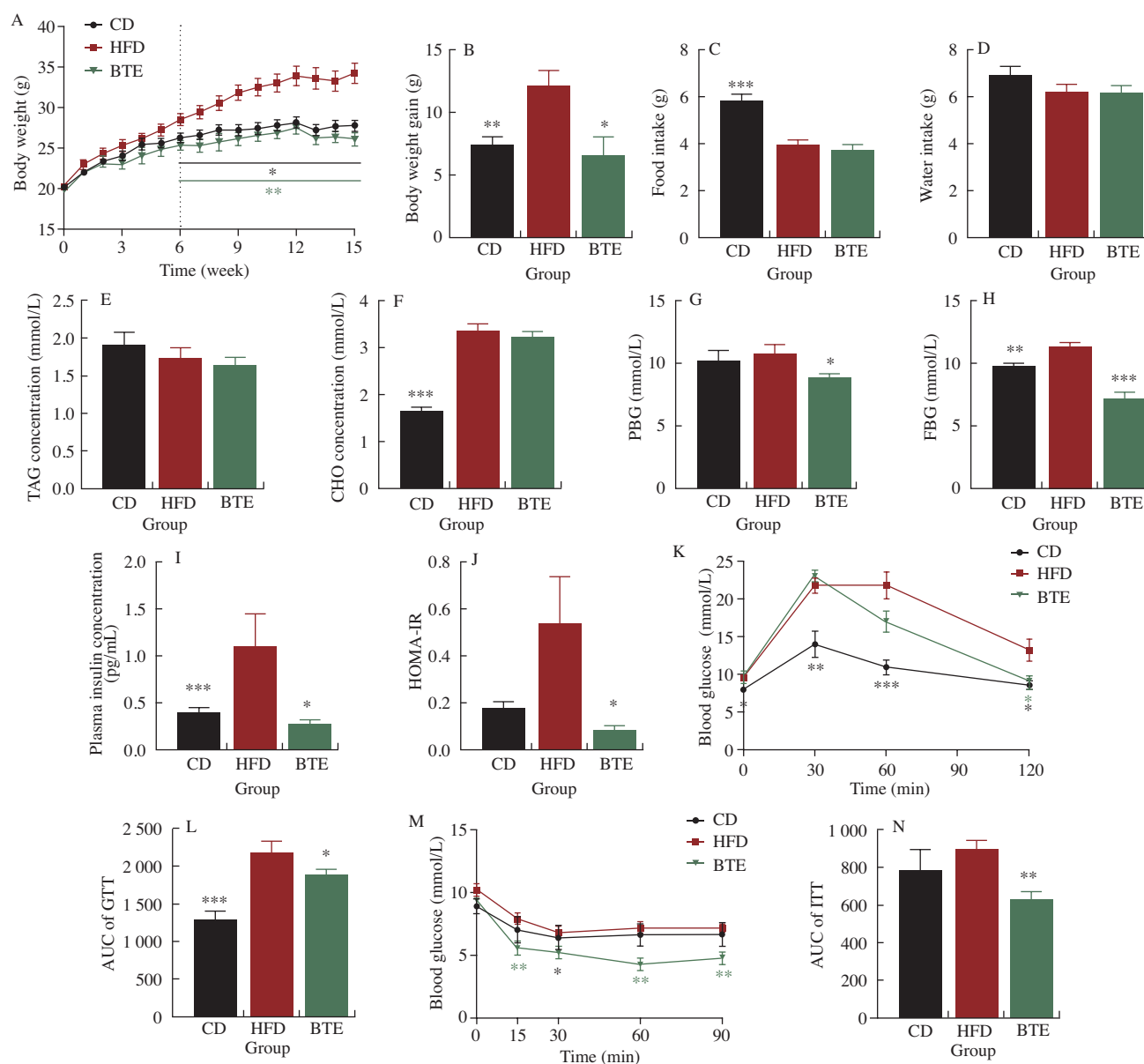


Fig. 2 BTE improved HFD-induced glucose and lipid metabolism disorder in DIO mice. The 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with BTE for 15 weeks. (A) Body weight curve during the entire experimental period and (B) body weight gain at 15th week of mice were measured. (C) The 24 h food intake and (D) 24 h water intake were recorded twice a week. Plasma (E) TAG, (F) CHO and (G) PBG were detected. (H) FBG was detected after 4-h fasting. (I) Plasma insulin level was detected by ELISA and (J) HOMA-IR was calculated. (K) GTT and (M) ITT were performed and (L, N) AUC were calculated. Data were presented as mean ± SEM, *n* = 6. Compared with HFD group, **P* < 0.05; ***P* < 0.01, ****P* < 0.001.

3.3 BTE alleviated HFD-induced lipid accumulation in liver and kidney of DIO mice

In order to examine the lipid accumulation in liver and kidney, we excised liver and kidney for further analyses. The liver (Fig. 3A) and kidney (Fig. 3C) of HFD group tended to be reddish, while BTE group reversed to dark red, close to CD group. After calculating the organ coefficient (organ weight/body weight), we found that BTE did not affect the liver coefficient (Fig. 3B) while increased the coefficient of kidney slightly compared with HFD group, but without significant difference (Fig. 3D). HE staining showed the dramatical vacuolar degeneration or steatosis in liver and renal tubular (Figs. 3E and F), consistent with Oil red O staining (Figs. 3G and H), which was alleviated

by BTE administration. To further evaluate liver and kidney function, ALT, AST, BUN and Cre concentration were detected. BTE could significantly decrease the concentrations of ALT and AST (Figs. 3I and J), but no obvious alteration was observed in BUN and Cre between BTE and HFD group (Figs. 3K and L). In summary, 15-week administration of BTE could significantly alleviate HFD-induced fatty liver and fatty kidney in DIO mice.

3.4 BTE improved lipid profiling of fatty liver and fatty kidney in DIO mice

To further investigate the potential therapeutic effect of BTE on fatty liver and fatty kidney, we utilized MRM lipidomic approach to

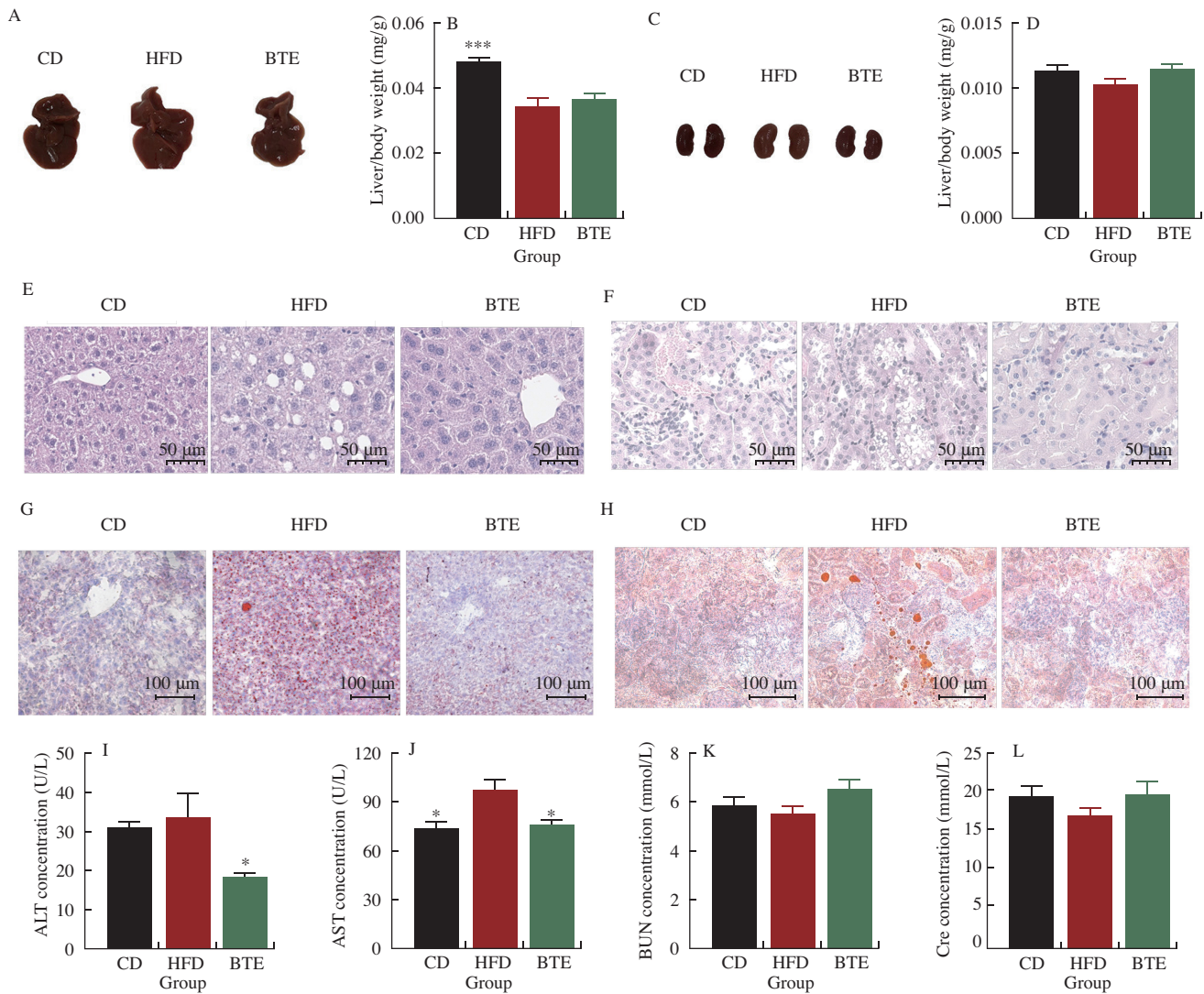


Fig. 3 BTE alleviated fatty liver and fatty kidney in DIO mice. Typical images of (A) liver and (C) kidney, (B) liver coefficient and (D) kidney coefficient. Representative image in HE staining of (E) liver and (F) kidney, as well as Oil red O staining of (G) liver and (H) kidney. Plasma (I) ALT, (J) AST, (K) Bun and (L) Cre were detected. Data were presented as mean $\pm$ SEM, $n = 6$. Compared with HFD group, $*P < 0.05$.

comprehensively characterize the effects of BTE on the content and composition of lipids in fatty liver and fatty kidney of DIO mice. Total 28 lipid classes and 459 lipid molecules were identified in the liver, while 29 lipid classes and 458 lipid molecules were identified in the kidney (Fig. 4A). TAG was the lipid class with most lipid molecules identified in both liver and kidney, including 116 kinds of TAGs in the liver and 81 kinds of TAGs in the kidney. Cardiolipin (CL) was the second most lipid molecules detected, with 59 CLs in the liver and 62 CLs in the kidney (Fig. 4A). Principal component analysis (PCA) revealed that the lipid metabolite pattern of HFD group was significantly different from CD group in both liver and kidney, while BTE group exhibited a moderate similarity to CD group (Figs. 4B, E). In the liver, 5 of top 10 lipids contributing to Dim-1 were phosphatidylethanolamines (PEs) (Fig. 4C), while CL78:14(18:2) contributed the most to Dim-2 (Fig. 4D). Similarly, 5 of top 10 lipids contributing to Dim-1 in the kidney belonged to PE (Fig. 4F), with phosphatidylcholines (PC) 40:1 and PC40:3 being the top 2 lipids and TAG being the most lipids contributing to Dim-2 (Fig. 4G). Taken together, we preliminarily found that BTE improved the lipid

metabolite pattern of fatty liver and fatty kidney towards the CD group. The analysis also revealed that glycerides and phospholipids emerged as the pivotal lipid molecules responsible for alleviating fatty liver and fatty kidney conditions following BTE therapy.

3.5 BTE remodeled the overall composition of lipid classes in fatty liver and fatty kidney

To further clarify the key lipids in the improvement of BTE on fatty liver and fatty kidney, we quantified each lipid molecule for subsequent analysis. Utilizing $P < 0.05$, $|\log_2 \text{fold change}| > 1$ as cutoff, 3D volcano was plotted to figure out the differential lipid molecules in liver (Fig. 5A) and kidney (Fig. 5B). After visualizing the abundance of lipid classes in the liver through a heatmap, we observed that most of lipid classes were significantly altered in response to HFD feeding with a decrease in the abundance of CL, PE, and lyso-phosphatidylethanolamines (LPE), as well as an increase in TAG, diacylglycerols (DAG), free fatty acids (FFA), PC, sphingosine (Sph), and cholesterol ester (CE). However, BTE treatment was

able to partially reverse the abundance of certain lipid classes in the liver, such as FFA, DAG, TAG and PC (Fig. 5C). The change in lipid classes was more pronounced in the kidney after BTE administration with decrease of most lipid classes, including DAG, ceramides (Cer), PC, lysophosphatidylcholine (LPC), etc. (Fig. 5D). As correlation network shown, CL was negatively correlated with most of the rest, while plasmalogen PC, plasmalogen PE, Sph, CHO and Monosialo-dihexosyl

gangliosides (GM3) were positively correlated with most of the rest in both liver and kidney (Figs. 5E and F). Additionally, in liver, sulfatides (SL) and PE were negatively with other lipid classes (Fig. 5E), while positively with other lipid classes in kidney (Fig. 5F). The analysis of lipid classes indicated that BTE could mitigate the composition of lipid classes, especially FFA in fatty liver, Cer and LPC in fatty kidney, as well as TAG, DAG and PC in both fatty liver and fatty kidney.

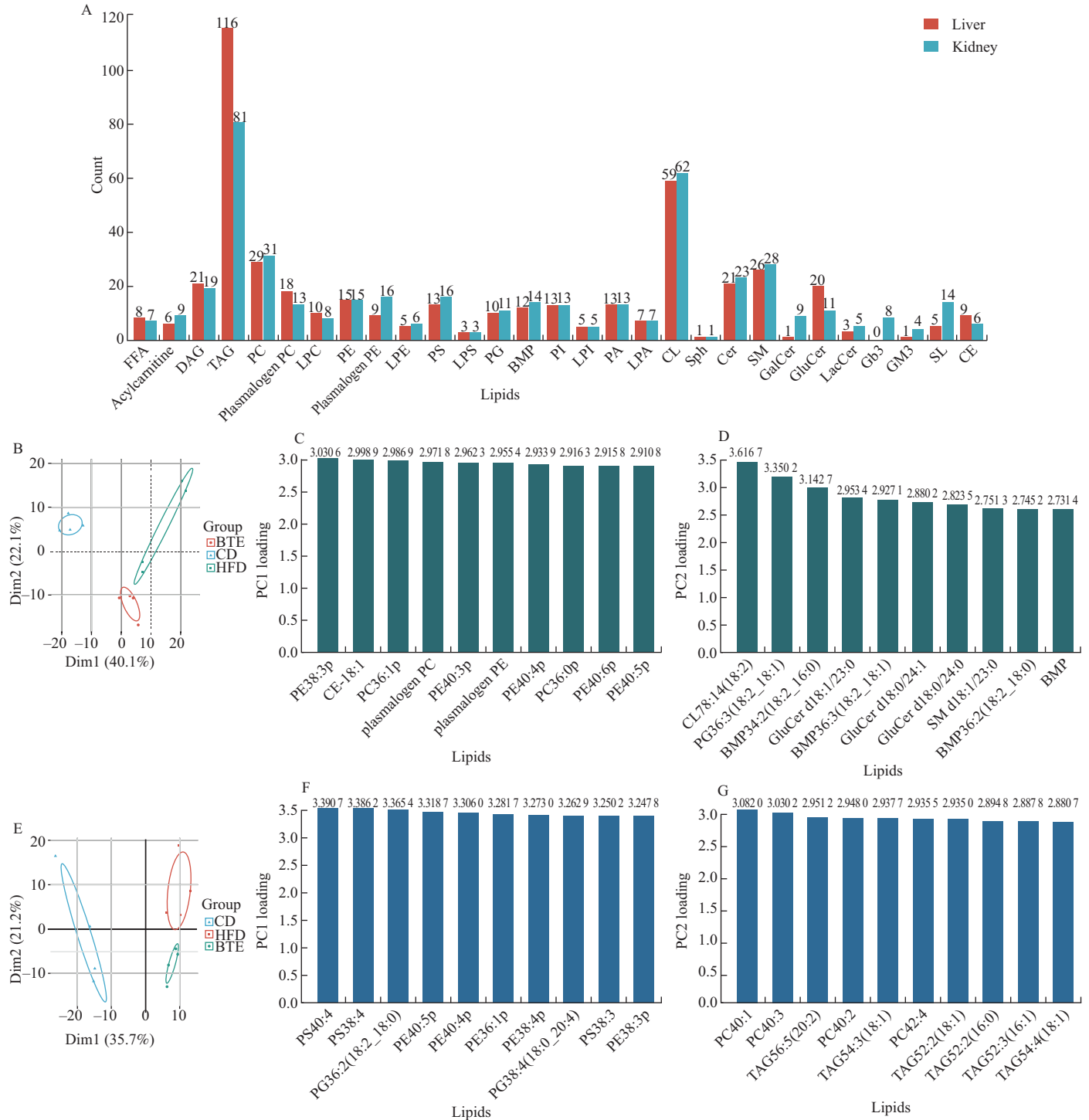


Fig. 4 Lipid profiling was altered in response to BTE administration in liver and kidney of DIO mice. 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with 300 mg/kg BTE for 15 weeks. (A) The total lipids detected by MRM in liver and kidney. PCA of the lipid composition of (B) liver and (E) kidney. The loading graphs presented each top 10 lipids with the highest contribution to Dim-1 and Dim-2 in (C, D) liver and (F, G) kidney. PG, phosphatidylglycerols; BMP, bis (monoacylglycerol) phosphate; PS, phosphatidylserines; PI, phosphatidylinositols; LPI, lyso-PI; PA, phosphatidic acid; LPA, lyso-PA; LPS, lyso-PS; SM, sphingomyelins; GalCer, galactosylceramides; GluCer, glucosylceramides; LacCer, lactosylceramides; Gb3, ceramide trihexoside.

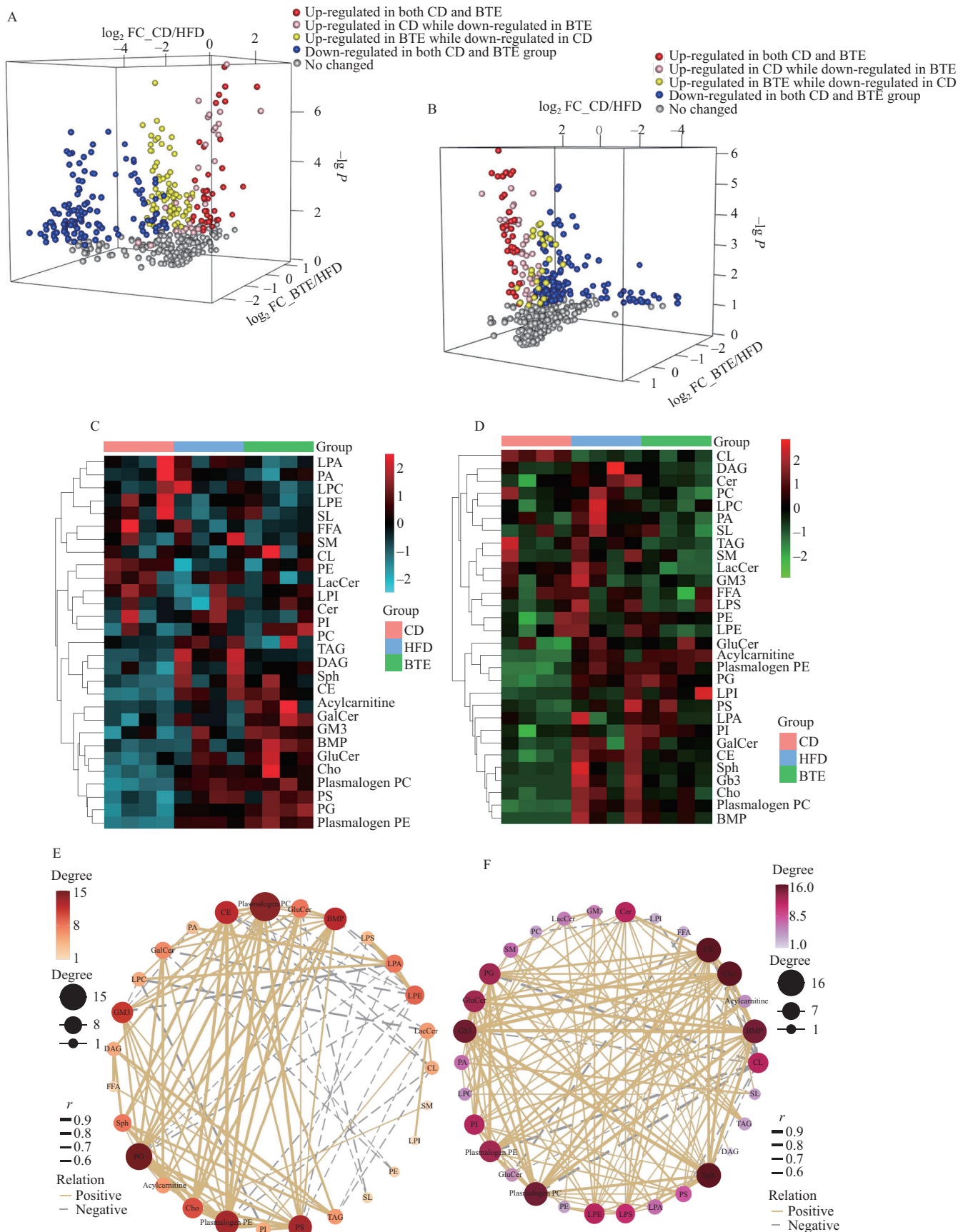


Fig. 5 BTE remodeled the overall composition of lipid classes in fatty liver and fatty kidney. The 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with 300 mg/kg BTE for 15 weeks. 3D volcano plots presented the differential lipid metabolites in (A) liver and (B) kidney ($n = 4$). FC, fold change. Heatmap visualized the abundance of lipid class in (C) liver and (D) kidney. Correlation network of the lipid classes in (E) liver and (F) kidney.

3.6 BTE reduced glyceride and CE accumulation in fatty liver and fatty kidney

DAG or TAG are formed when 2 or 3 FFA esterified with hydroxyl groups of glycerol. The liver is the central organ for glycerides synthesis, which facilitates transport and storage of FFA. Therefore, the accumulation of glycerides is a well-established characterization for NFLAD and associated with significant glomerulosclerosis and proteinuria in kidney. In the current study, we revealed that the most up-regulated lipid was TAG56:6(22:4) at 20-fold in fatty liver and TAG56:4(20:2) at 5.4-fold in fatty kidney, compared with CD group. Compared with HFD group, BTE administration reduced the abundance of most species of TAGs detected in both fatty liver (Figs. 6A and B) and fatty kidney (Fig. 6C) with TAG50:3(16:1) at 0.21-fold in liver and TAG52:3(16:1) at 0.14-fold in kidney. What's more, 14 out of 16 DAGs in liver were up-regulated in DIO mice, among which 12 DAGs were down-regulated by BTE administration (Fig. 6D). Almost all DAG species were up-regulated in the kidney of DIO mice, which were lowered by BTE (Fig. 6E). As the main substrates for glycerides synthesis, most FFAs increased in both fatty liver and fatty kidney. However, interestingly, FFA20:5 (eicosapentaenoic acid, EPA), as one of several omega-3 fatty acids, was found to decrease in both fatty liver and fatty kidney. Similar as glyceride, all FFAs were suppressed by BTE administration (Figs. 6F and G), which may contribute to the decrease in glyceride.

CHO is synthesized in the liver as the primary component of cell membranes. High levels of circulating CHO may accumulate in vascular endothelium leading to atherosclerosis. Our findings demonstrated that CHO significantly increased in both fatty liver and fatty kidney, whereas, BTE slightly reduced the concentration of CHO in fatty kidney, but without effect on fatty liver (Figs. 6H and I). CE are formed by esterification between the carboxylate group of a fatty acid and the hydroxyl group of CHO and usually positively correlated with CHO. Hence, BTE reduced most of CE detected in fatty kidney (Fig. 6K) rather than in fatty liver (Fig. 6J). Specifically, CE(18:2) was significantly decreased in fatty liver and kidney after BTE administration (Figs. 6J and K). In accordance with the alteration of glyceride, BTE down-regulated glyceride synthesis-related genes of sterol regulatory element-binding protein 1 (*Srebp1*) in fatty liver, while up-regulated glyceride lipolysis-related gene of adipose triglyceride lipase (*Atgl*) in fatty liver and fatty kidney (Fig. 6L). Interestingly, BTE did not exert significant effect on CE metabolism genes in both fatty liver and fatty kidney (Figs. 6L and M). In conclusion, BTE treatment reduced lipid deposition mainly characterized by glycerides and FFA, as well as particularly more effectively in reducing CHO and CE in the kidney as compared to the liver, thus suggesting potential benefits in the amelioration of fatty liver and fatty kidney.

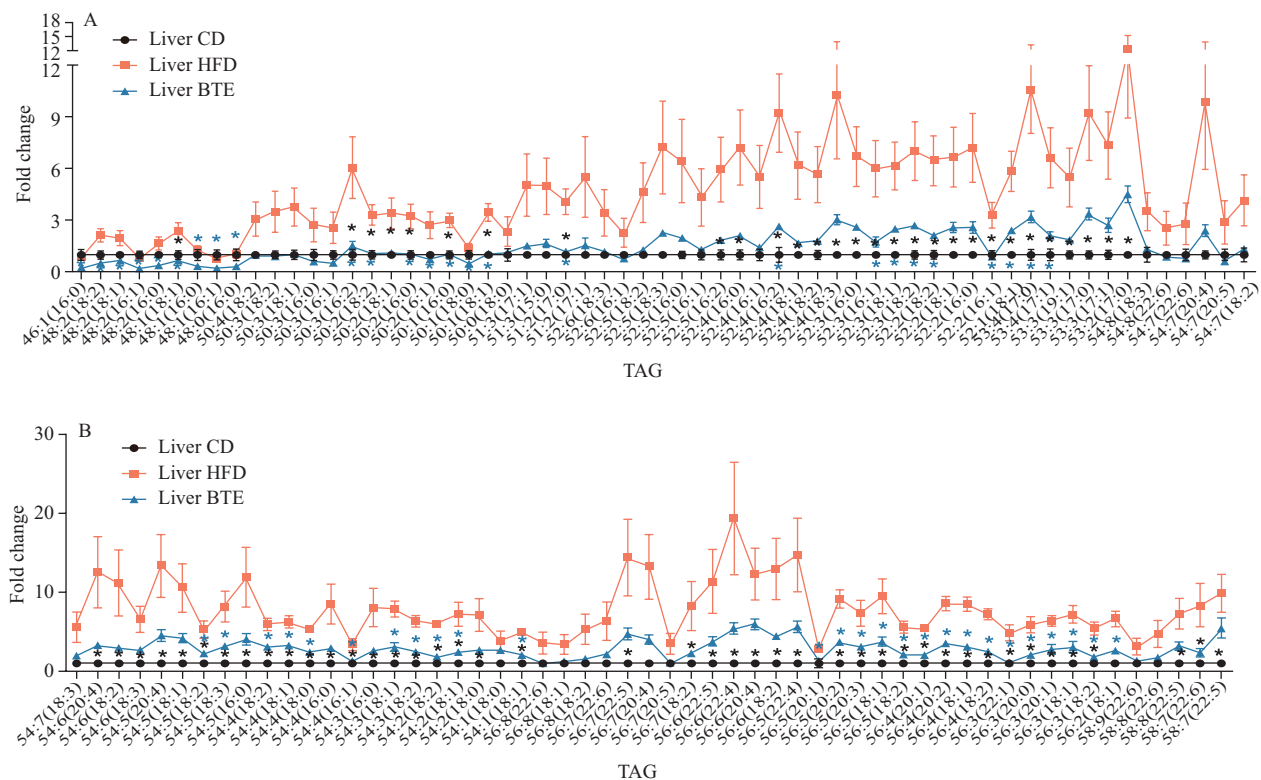


Fig. 6 BTE reduced glyceride, FFA and CE accumulation in fatty liver and fatty kidney. The 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with 300 mg/kg BTE for 15 weeks. Fold change of representative TAG concentration in (A, B) liver and (C) kidney. Fold change of representative DAG concentration in (D) liver and (E) kidney. Fold change of FFA concentration in (F) liver and (G) kidney. CHO concentration in (H) liver and (I) kidney. Fold change of CE concentration in (J) liver and (K) kidney. qPCR analysis of glyceride and CE metabolism gene expression in (L) liver and (M) kidney ($n = 6$). Data were presented as Mean $\pm$ SEM, $n = 4$ for lipidomics. Compared with HFD group, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

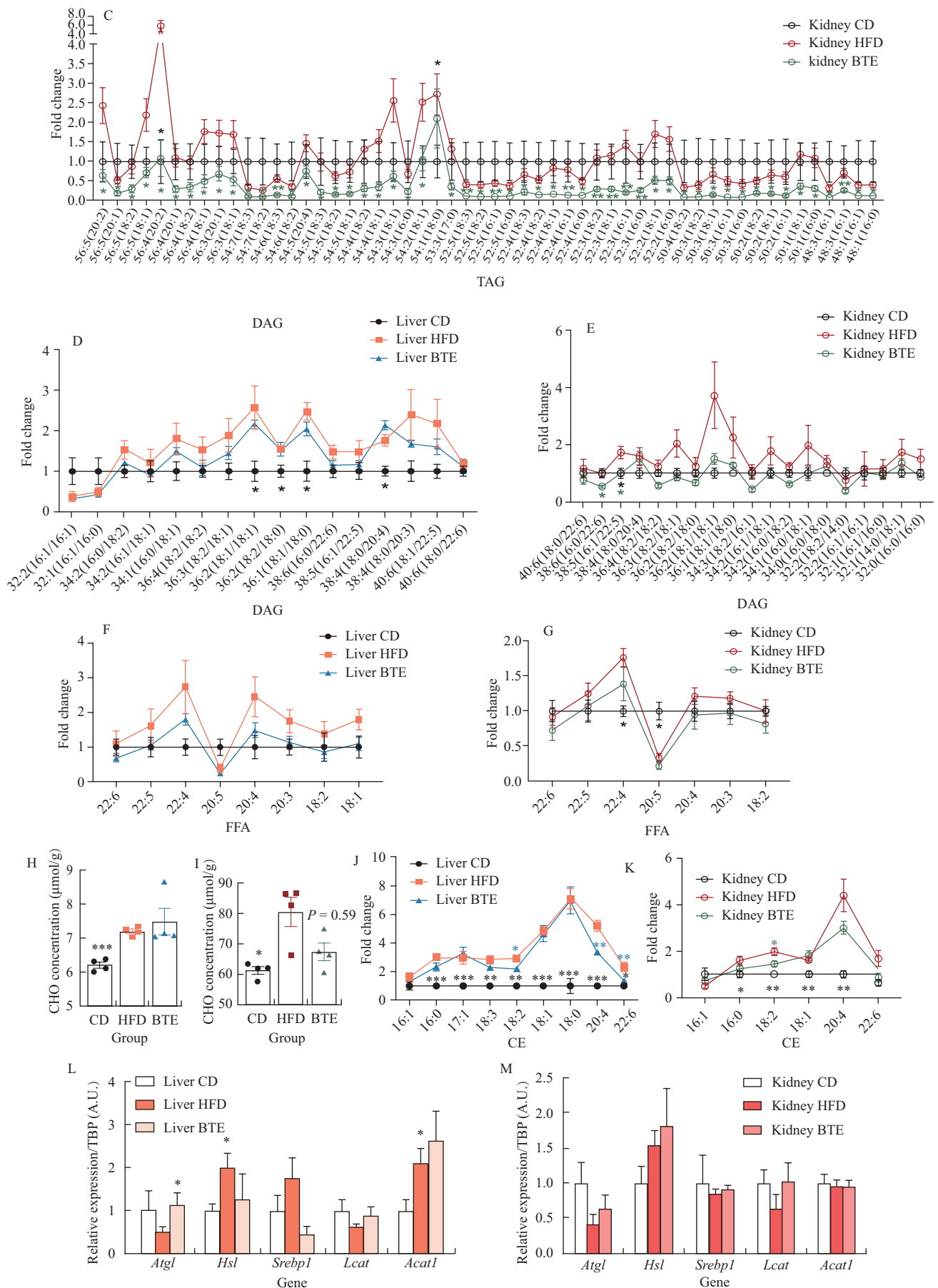


Fig. 6 (Continued)

3.7 BTE altered sphingolipid metabolism in fatty liver and fatty kidney

Sphingolipids are a class of lipids characterized by the presence of a sphingoid base backbone and a long-chain fatty acid, whose accumulation may contribute to immune cell trafficking, cytokine production and the progression of kidney disease^[23]. As described previously (Figs. 5C and D), the metabolism disorders of sphingolipid in the liver and kidney were observed after HFD feeding. In fatty liver, BTE significantly reversed the HFD-induced decrease in SM d18:1/24:0 and SM d18:1/26:1 (Fig. 7A), whereas dramatically decreased SM d18:1/16:0 and SM d18:1/20:0, as well as other SM lipids with side chains of 16–22 carbons in fatty kidney (Fig. 7B). For ceramide species, 16 out of 23 species in kidney were abnormally increased which were lowered by BTE (Fig. 7D) but without any change in fatty liver (Fig. 7C). The color density in heatmap showed that most lipids of GluCer, GalCer and LacCer were dramatically changed in fatty liver and fatty kidney (Figs. 7E and F). Specifically, LacCer d18:1/24:1, LacCer d18:1/24:0 and GluCer d18:0/25:1, GluCer d18:1/25:1, GluCer d18:0/26:1 and GluCer d18:0/26:1 were down-regulated while GluCer d18:0/17-23:0 were up-regulated with different degree in fatty liver, among which BTE reversed the changes of GluCer d18:1/25:1, GluCer d18:0/25:1, GluCer d18:0/26:1, LacCer d18:1/24:1 (Fig. 7E). In the kidney, BTE reversed the change of GalCer d18:1/24:1, LacCer d18:1/20:0 and LacCer d18:1/22:0 (Fig. 7F). SL is produced by ceramide degradation. 4 out of 5 SL lipids were reduced in fatty liver, among which SL d18:1/24:0 was increased by BTE (Fig. 7G). A total of 8 kinds of SL species were abnormally up-regulated in fatty kidney while down-regulated by BTE with significant difference in SL d18:1/20:0 (Fig. 7H).

Additionally, BTE down-regulated 7 kinds of Gb3 species which were elevated in fatty kidney (Fig. 7I), whereas Gb3 was not identified in the liver. Further investigation revealed that the expression of serine palmitoyltransferase long chain base subunit 1 (*Sptlc1*) and serine palmitoyltransferase small subunit B (*Sptssb*) mRNA was up-regulated in fatty liver following BTE administration, indicating an activation of gene expression in sphingolipid production, which was inconsistent with lipidomics change. However, no significant alterations were observed in these genes in the context of fatty kidney (Figs. 7J and K). Taken together, BTE significantly alleviated HFD feeding-induced sphingolipid metabolism disorders, including SM and Cer in both fatty liver and fatty kidney, as well as GalCer, GluCer, LacCer, SL and Gb3 in fatty kidney. The impact of BTE on sphingolipid metabolites was more substantial in fatty kidney than in fatty liver although the impact of BTE on sphingolipid-producing genes was more pronounced in fatty liver compared to fatty kidney.

3.8 BTE reduced phospholipids abundance more in fatty kidney than fatty liver.

Phospholipids are essential components of cellular and exosome membranes. As a kind of choline-containing phospholipids, decreased hepatic PC increased the risk of cardiovascular disease in NAFLD patients^[24], while high level of PC was detected in ferroptosis-activated acute renal failure^[25]. Interestingly, as shown in Figs. 8A and B, HFD feeding induced the elevation of total PC which was moderately reduced by BTE administration in both fatty liver and fatty kidney (Figs. 8A and B). BTE had a stronger regulatory effect on PC with a dramatic decrease in 10 kinds of PC in the kidney and a significant reduction in 5 kinds of PC in the liver (Figs. 8C and D). LPC has been

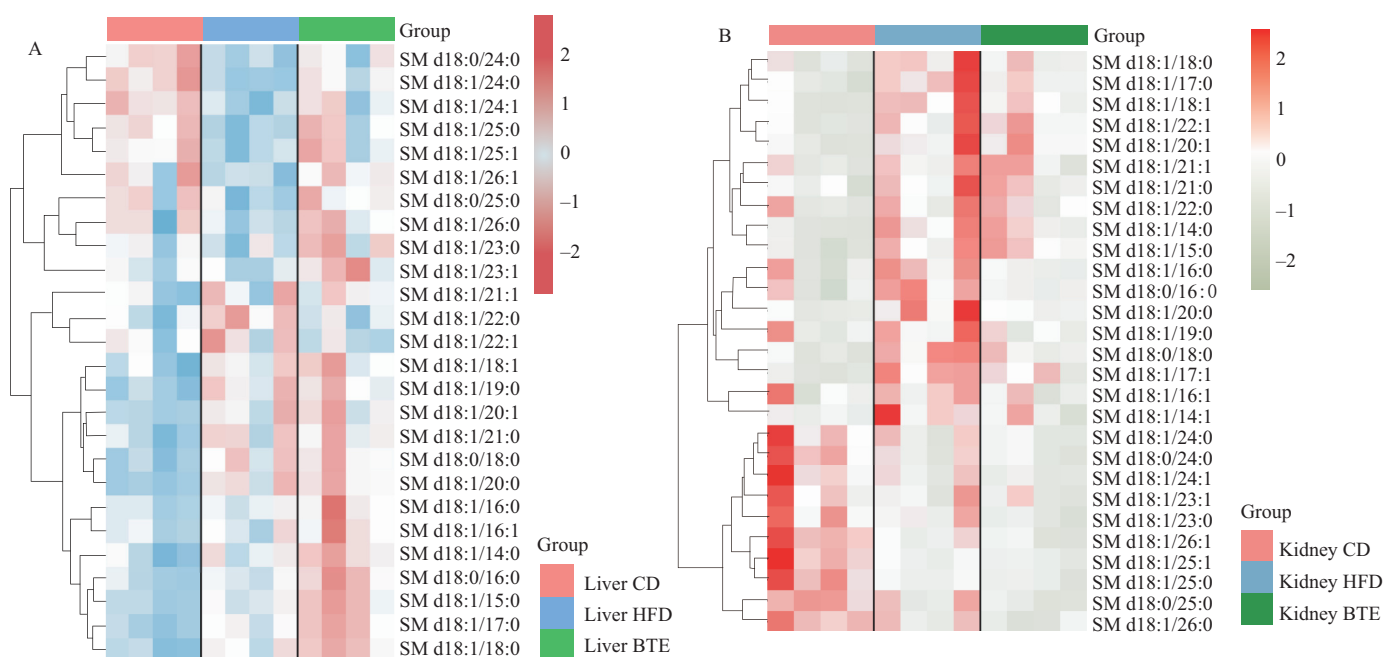


Fig. 7 BTE improved sphingolipid metabolism disorder in fatty liver and fatty kidney of DIO mice. The 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with BTE for 15 weeks. Heatmap visualized lipid molecules abundance of SM in (A) liver and (B) kidney, Cer in (C) liver and (D) kidney, LacCer, GluCer and GalCer in (E) liver and (F) kidney, Fold change of SL concentration in (G) liver and (H) kidney, as well as (I) Gb3 in kidney. qPCR analysis of sphingolipid metabolism-related gene expression in (J) liver and (K) kidney ($n = 6$). Data were presented as mean $\pm$ SEM, $n = 4$ for lipidomics. Compared with HFD group, * $P < 0.05$, ** $P < 0.01$.

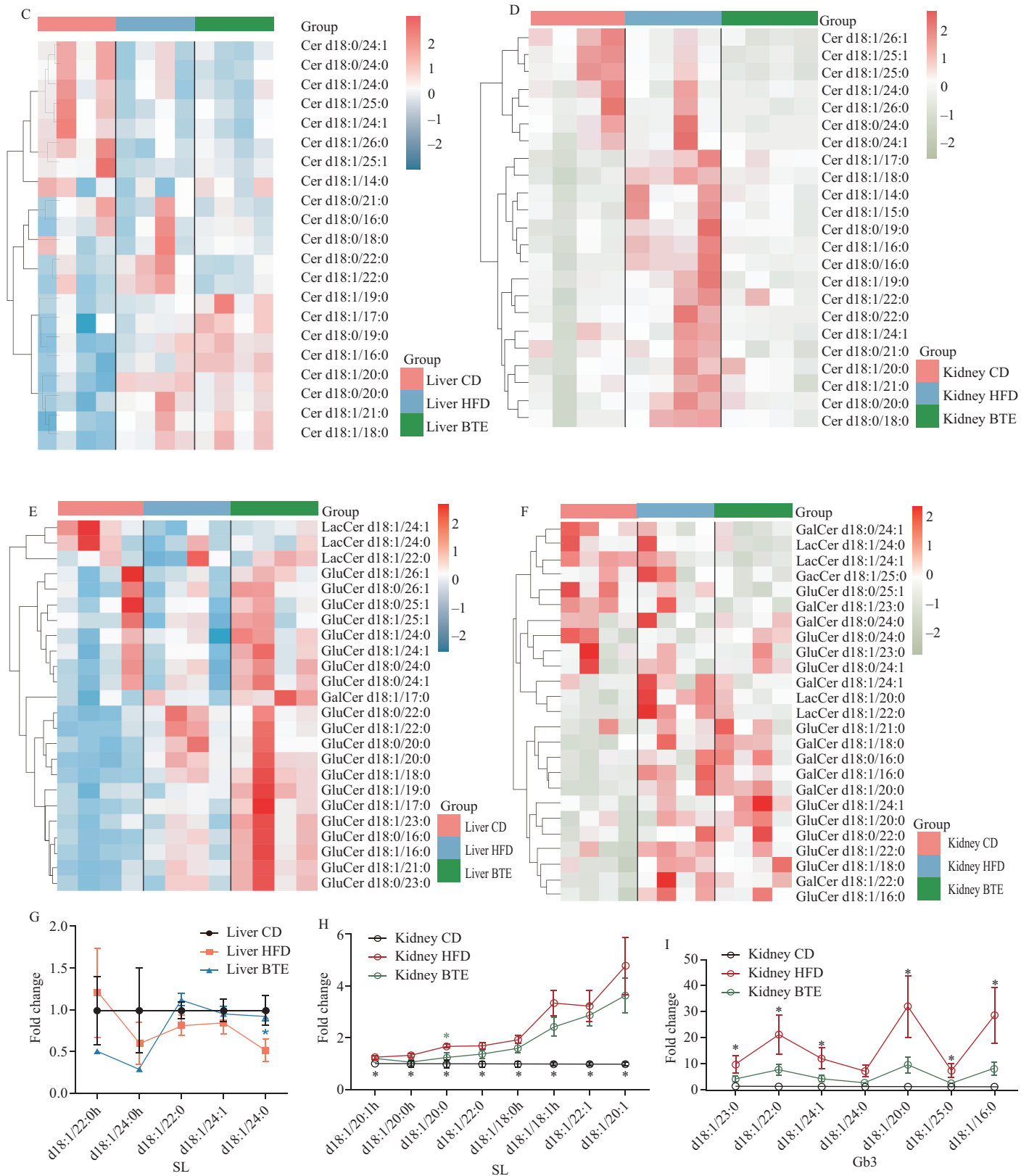


Fig. 7 (Continued)

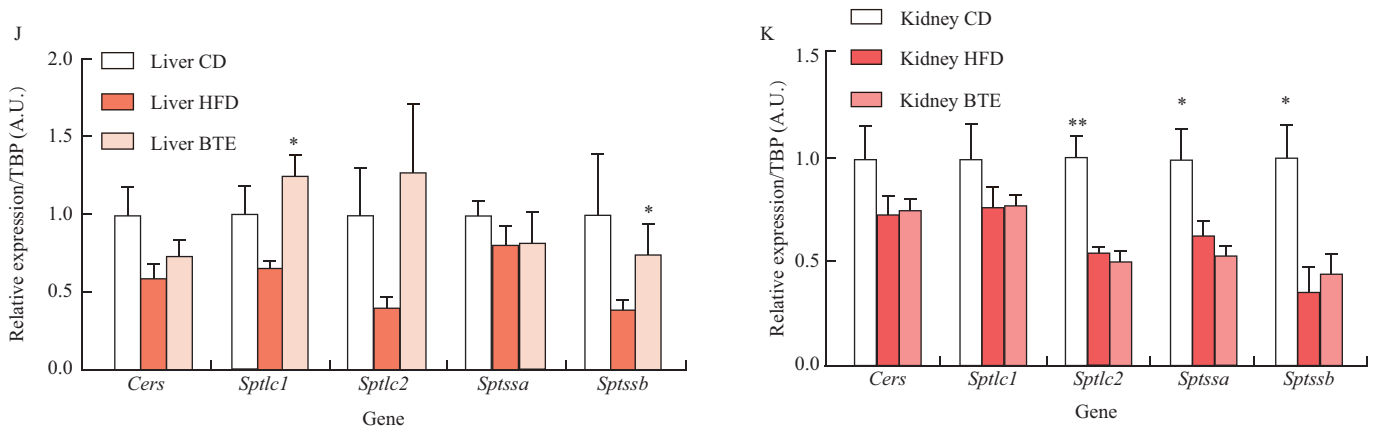


Fig.7 (Continued)

recognized as a key factor associated with CHO biosynthesis and mitochondrial integrity in liver^[26]. We observed that the abundance of LPC increased in both fatty liver and fatty kidney and BTE reversed this alteration, particularly in the fatty kidney (Figs. 8E and F). Specifically, half of LPC molecules were up-regulated in fatty liver and BTE reduced almost the LPC molecules in liver, except LPC18:3 (Fig. 8G). Meanwhile, all LPC molecules were up-regulated in fatty kidney, while BTE resulted in a decrease of almost LPC molecules, except LPC20:4 (Fig. 8H). Over-accumulated PA impaired hepatic insulin resistance and effected renal G-protein coupled receptor (GPCR) signaling pathway^[27-29]. Total PA abundance was up-regulated in fatty kidney rather than fatty liver. (Figs. 8I and J). BTE administration, although, did not alleviate the abnormal levels of PAs in fatty liver (Figs. 8I and K), decreased total level of PA class and all the PA species in fatty kidney (Figs. 8J and L).

Surprisingly, the effect of BTE on total BMP was opposite in fatty liver and fatty kidney, with a significant increase in fatty liver and a decrease in fatty kidney (Figs. 8M and N). Specifically, BMP molecules increased after HFD feeding and BTE somehow led to an increase in all BMP species in fatty liver (Fig. 8O). Conversely, in the kidney, all of 14 BMP species were detected were up-regulated in HFD group and down-regulated by BTE (Fig. 8P). We then detected the gene expression related to phospholipids metabolism and found that BTE upregulated carnitine palmitoyltransferase 1B (*Cpt1b*) gene in fatty liver and phospholipase D1 (*Pld1*) gene expression in fatty kidney (Figs. 8Q and R). Overall, the improvement of BTE on phospholipids in fatty kidney was stronger than fatty liver.

3.9 Comprehensive analysis identified the key lipids for BTE in improvement of fatty liver and fatty kidney

To figure out the specific lipid targets for BTE in improving fatty liver and fatty kidney respectively, Venn analysis was performed by the significant differential lipids in HFD group (compared with CD group) and BTE group (compared with HFD group). In liver, BTE significantly increased 6 lipids that were decreased due to HFD feeding (Fig. 9A). Notably, 3 specific species of CLs, including CL76:14(16:1), CL78:13(18:2) and CL76:13(18:2), were down-regulated by 0.28-, 0.62-, and 0.68-fold respectively in the HFD group, whereas, were elevated by 1.89-, 1.40- and 1.39-fold after BTE administration,

respectively (Fig. 9B). Furthermore, Venn analysis also revealed that BTE prominently decreased 51 lipids which were significantly up-regulated in fatty liver, predominantly including 43 TAGs, 3 CEs, 2 CLs, and 2 PCs (Figs. 9C and D). Based on these findings, it suggested that the protective effects of BTE against fatty liver may be attributed to the increase in levels of CLs, accompanied by the decrease in levels of TAGs.

However, in fatty kidney, the study revealed no overlap between the down-regulated lipids in HFD group (compared with CD group) and the up-regulated lipids observed in BTE group compared with HFD group). Despite this observation, BTE still exerted a significant reduction in the levels of 17 lipids that were significantly up-regulated in fatty kidney (Fig. 9E). Specifically, TAG56:4(20:2) was increased for 5.7-fold in fatty kidney, while BTE treatment was able to effectively counteract this increase, close to CD group. Furthermore, out of the 17 lipids were down-regulated by BTE in the context of fatty kidney, among which the majority were classified as Cer (5 lipids), with 2 classified as PE and 2 as PC. Based on these findings, these specific lipids may play a key role in mediating the beneficial effects of BTE on fatty kidney. (Figs. 9F and G).

3.10 CE18:2 and TAG56:4(20:2) were potential key lipid targets for BTE in improvement of ELD

In addition to the independent effects of BTE on the lipid profile of fatty liver and fatty kidney, it is very important to identify the lipid biomarkers which elucidated the common basis of fatty liver and fatty kidney, especially BTE exhibited the beneficial therapeutic effect on ELD. Multiple Venn analyses were employed to explore the abnormally overlapped lipids in fatty liver and fatty kidney (Fig. 10A, Table S2). As the result, the Venn plot revealed 33 down-regulated lipids and 50 up-regulated lipids in both tissues. The down-regulated lipids included 24 CLs, 3 PCs, 1 SM, 1 PS, etc. (Fig. 10B) and 50 up-regulated lipids included 12 PCs, 8 PEs, 7 PGs, 6 SMs, etc. (Fig. 10C). Therefore, the depletion of CL and abnormal accumulation of PC may be the signature of ELD. Surprisingly, PI36:1 was down-regulated in fatty liver but up-regulated in fatty kidney (Figs. S1A and B, Table S2). CL74:8(18:2), CL74:7(18:2), CL76:9(18:2) and CL76:9(20:3) were up-regulated in fatty liver but down-regulated in fatty kidney (Figs. S2A and B, Table S2).

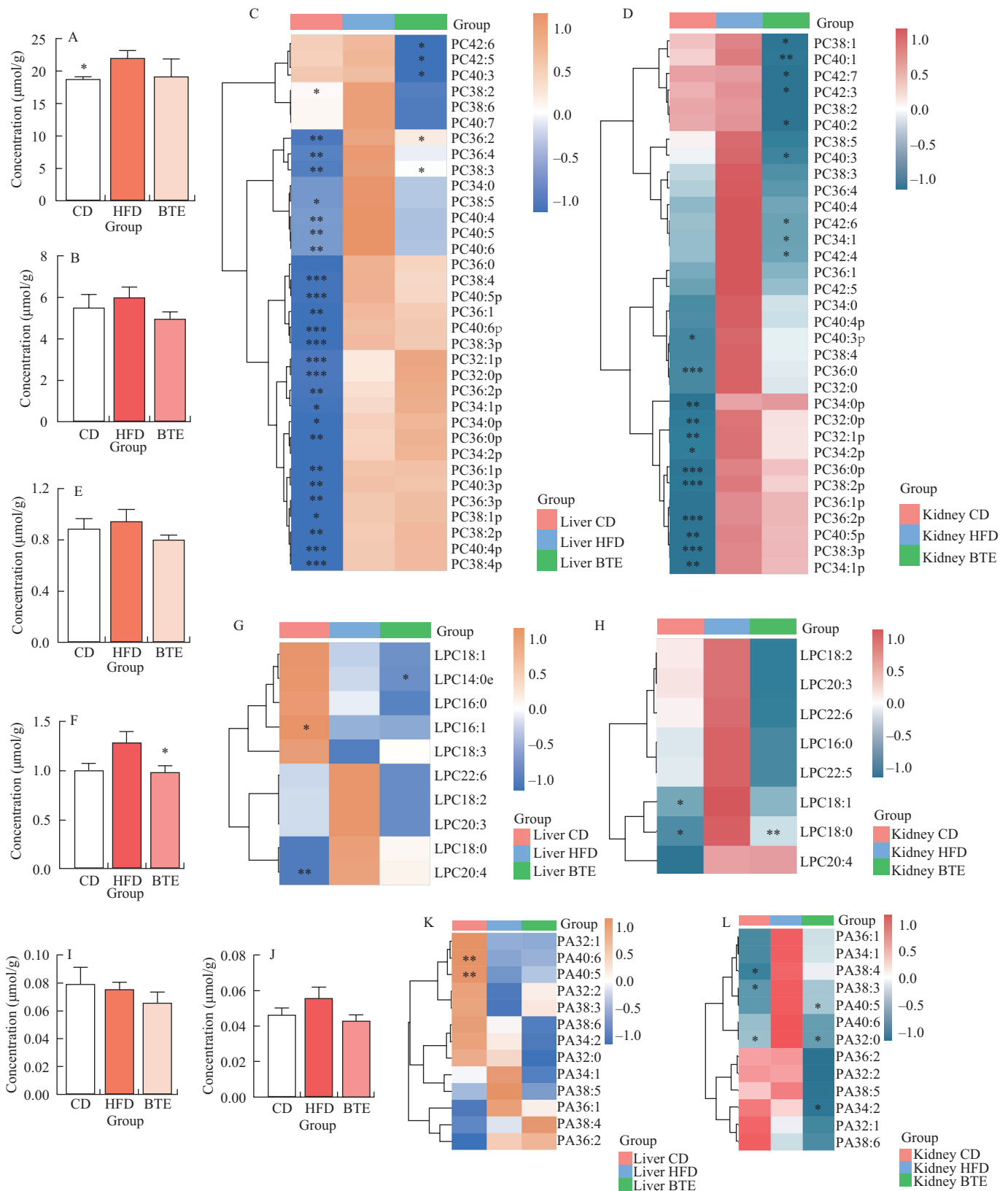
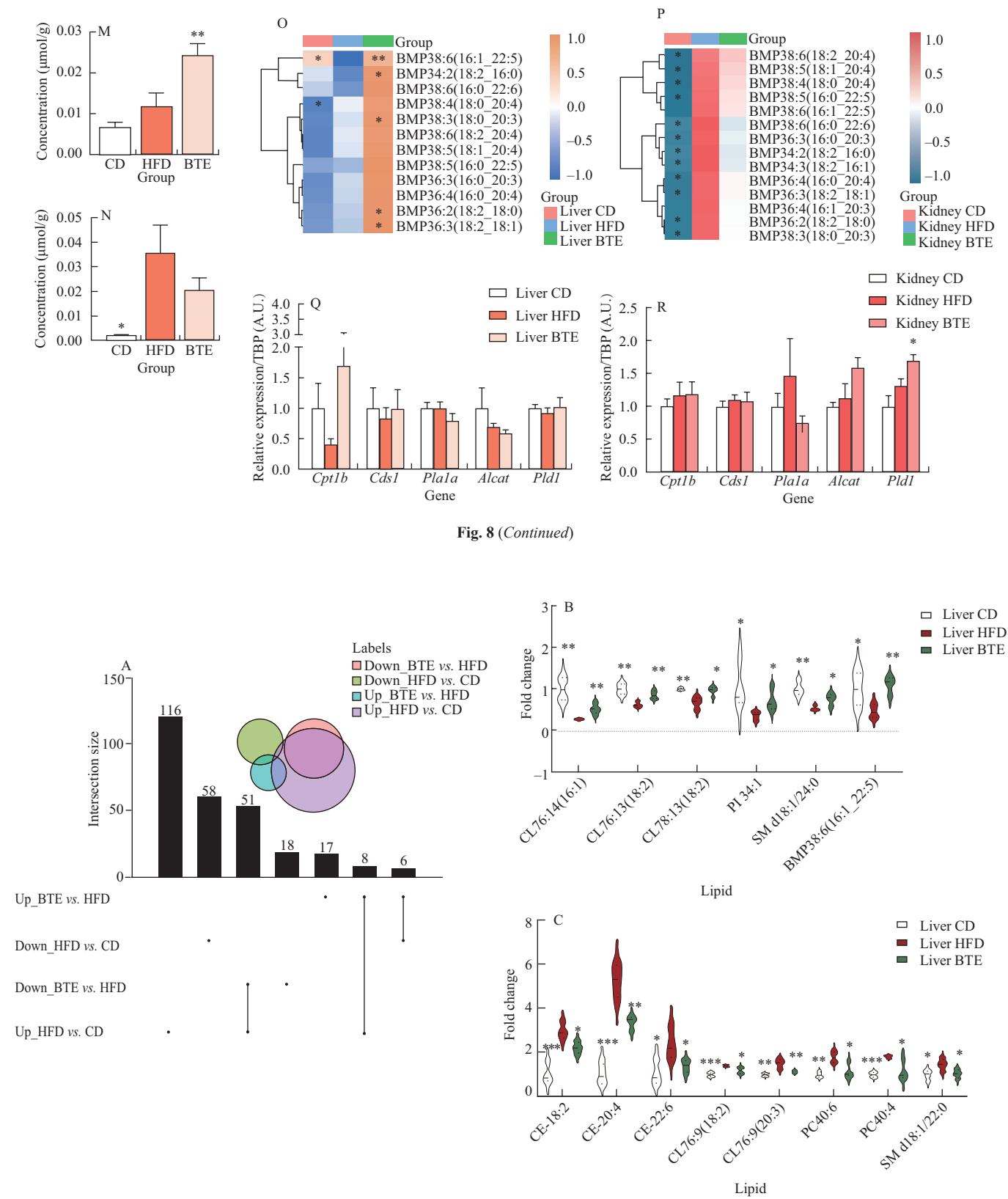


Fig. 8 Effect of BTE on phospholipid abundance in fatty liver and fatty kidney of DIO mice. The 6-week-old C57BL/6J mice were fed on CD or HFD (60 kcal% fat) and concurrently orally administered with BTE for 15 weeks. Concentration of total PC in (A) liver and (B) kidney. Heatmap visualized lipid molecules abundance of PC in (C) liver and (D) kidney. Concentration of total LPC in (E) liver and (F) kidney. Heatmap visualized lipid molecules abundance of BMP in (G) liver and (H) kidney. Concentration of total PA in (I) liver and (J) kidney. Heatmap visualized lipid molecules abundance of PA in (K) liver and (L) kidney. Concentration of total BMP in (M) liver and (N) kidney. Heatmap visualized lipid molecules abundance of BMP in (O) liver and (P) kidney. qPCR analysis of phospholipid metabolism gene expression in (Q) liver and (R) kidney ($n = 6$). Data were presented as mean $\pm$ SEM, $n = 4$ for lipidomics. Compared with HFD group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



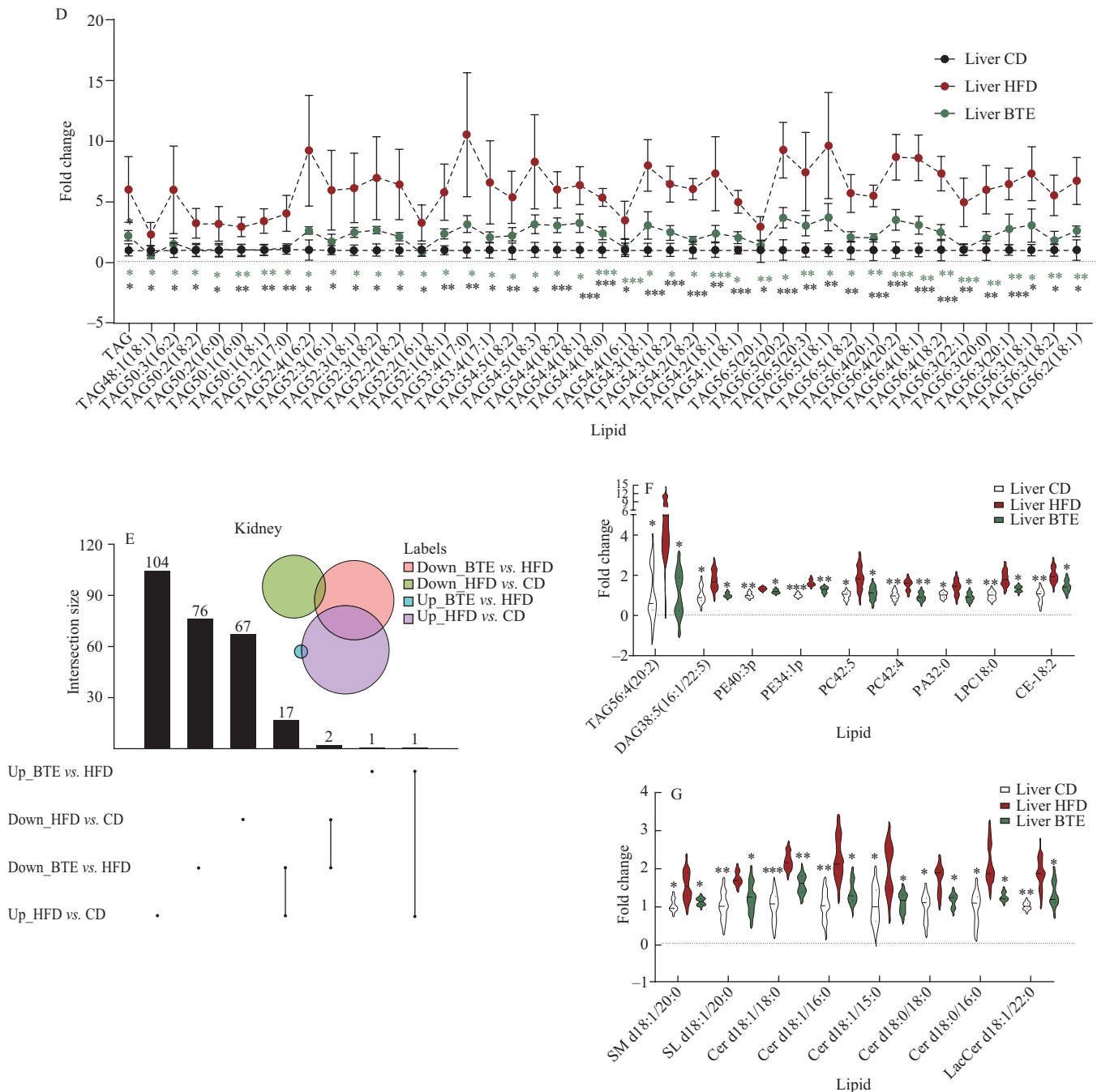


Fig. 9 (Continued)

Further multiple Venn analyses were performed in BTE group compared with HFD group (Table S3). As shown in Fig. 10D, 31 lipids were down-regulated but without any up-regulated lipids in both 2 tissues. A total of 31 lipids included 26 TAG class, 4 PC class and 1 CE class, indicating that alleviating ectopic accumulation of TAG largely contributed to the beneficial effect of BTE (Fig. 10E). Further Venn analysis was also performed in down-regulated lipids by BTE obtaining from Fig. 10C, and up-regulated lipids obtaining from Fig. 10E to identify the lipids that play a key role in the improvement effect of BTE on both fatty liver and fatty kidney. Among the lipid

molecules identified in the overlap, 2 molecules were CE18:2 and TAG56:4(20:2) (Fig. 10F). CE18:2 was up-regulated for more than 2-fold in both fatty liver and fatty kidney and decreased by 24.6% and 27.0% after BTE administration, respectively (Fig. 10G). TAG56:4(20:2) was up-regulated for more than 5-fold in both tissue and decreased by 58.9% and 81.4% after BTE administration (Fig. 10H). Taken together, BTE could reduce the lipid accumulation in both fatty liver and fatty kidney, with TAG56:4(20:2) and CE18:2 being the lipid targets for BTE in ameliorating ELD.

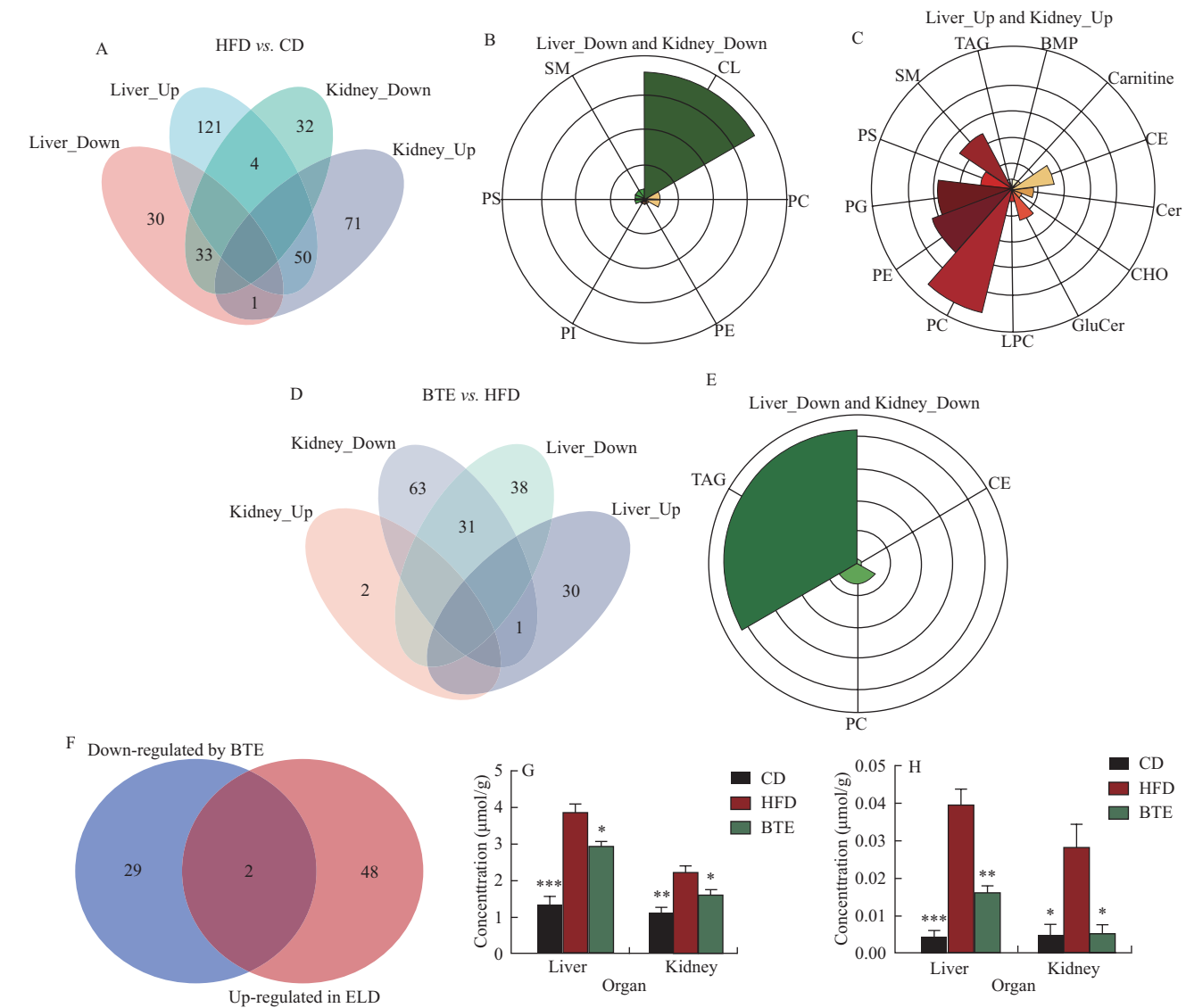


Fig. 10 Identification of lipid biomarkers for BTE in alleviating ELD. (A) Multiple Venn analysis of altered lipids in HFD group compared with CD group. (B) Down-regulated and (C) up-regulated lipids in HFD group compared with CD group, different color indicates different lipid class. (D) Multiple Venn analysis of altered lipids in BTE group compared with HFD group. (E) Down-regulated lipids in BTE group compared with HFD group, different color indicates different lipid class. (F) Venn analysis of the down-regulated lipids in BTE group with significant difference and up-regulated lipids in HFD group with significant difference, which obtained (G) CE18:2 and (H) TAG56:4(20:2) in the overlap. Data were presented as Mean $\pm$ SEM, $n = 4$. Compared with HFD group, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4. Discussion

ELD in liver, leading to NAFLD, can progress to more severe liver diseases such as hepatitis and cirrhosis. Apart from NAFLD, 14%–30% CKD is also associated with ELD^[29–30]. Increasing number of research divulged a link between fatty liver and fatty kidney to promote the progression of each other^[31]. Besides blood flow, fatty acid oxidation, inflammatory cytokine and increased lipoprotein released by fatty liver may contribute to CKD, while renin-angiotensin system dysfunction and uremic toxins induced by CKD could deteriorate fatty liver^[32–33]. Thus, comprehensive alleviation of fatty liver and fatty kidney in early obesity is impendent to prevent obese patients against NAFLD and CKD. As expected, utilizing transcriptome analysis, we affirmed that HFD could induce lipid metabolism disorder in both liver and kidney, including TAG, sphingolipid and phospholipid. Meanwhile, BTE treatment did improve lipid and glucose metabolism, as well as inhibit

body weight gain in DIO mice. More importantly, BTE alleviated lipid accumulation in liver and kidney shown by HE staining and Oil red O staining, indicating the potential therapeutic activity of BTE on fatty liver and fatty kidney.

Lipids are involved in the composition of cellular structures, intercellular information transfer, energy storage and energy supply, thereby serving as a group of essential metabolites. Lipidome is a comprehensive analysis of all lipids assisting the clinical biomarker discovery and disease diagnosis. Di-22:6-bis(monoacylglycerol) phosphate (di-22:6-BMP) has been treated as a biomarker for a form of Parkinson's disease^[34]. Acylethanolamides and LPA were considered as potential targets for treatment of cocaine addiction^[35]. Consistent with the results of transcriptional analyses, 15-week HFD feeding induced a significant alteration in both liver and kidney with most of lipid classes up-regulated, especially glycerides and phospholipids, as well as down-regulated CLs. The consistent

alteration in lipid class composition of liver and kidney induced by HFD feeding may provide the material foundation for crosstalk between fatty liver and fatty kidney.

As a prominent feature of obesity, glyceride disorder contributes to ELD. The ectopic DAG can activate protein kinase C (PKC) and inhibit insulin-PI3K-Akt signaling, thereby leading to insulin resistance^[36-37]. TAG molecules play a crucial role in the storage and transport of fatty acids, thus, over-accumulation of TAG often occurs first in the liver as the primary organ responsible for fatty acid production^[38]. High intrahepatic TAG has been revealed to be associated with CKD in obese patients^[39]. We observed up-regulated DAG in fatty liver and fatty kidney in DIO mice; meanwhile, BTE moderately reduced DAG, which was consistent with the improvement of insulin resistance. Additionally, TAG has been considered as a biomarker for predicting kidney injury in several diseases, including systemic lupus erythematosus (SLE)^[40]. In our current study, both transcriptomics and lipidomics showed a dramatic change of glyceride metabolism in fatty liver and fatty kidney, suggesting that glyceride disorders are the basic pathological change of ELD. BTE consistently reversed the change of glyceride synthesis and lipolysis-related genes, as well as reduced TAG abundance in the liver and kidney as a directly strong evidence of fatty liver and fatty kidney improvement, even though the TAG alterations in two tissues were partially distinct. HFD feeding induced significant increase of TAG up to 20-fold with the fold increasing as the increase of carbon chain's length, whereas, TAG lipids displayed inconsistent alteration with elevation or reduction in fatty kidney. The strong effect on decreasing glyceride levels in tissues may be the primary mechanism for BTE to protect against fatty liver and fatty kidney.

Sphingolipids could directly act as signaling molecules through 5 GPCRs called S1PR1-5, leading to activation and production of cytokine in fatty liver, or indirectly prompt the inflammation and fibrosis *via* the products of sphingolipid catabolism in CKD^[41-42]. The metabolomics and lipidomics analysis also indicate the association of sphingolipids levels with obesity and insulin resistant^[42]. However, the disorder of sphingolipids is less significant than expected and the effect of BTE on sphingolipids in fatty liver is limited. On the other hand, BTE exhibited the excellent effects on fatty kidney with decrease in all of sphingolipids and ceramides. Interestingly, the transcriptional regulation related to sphingolipid is different from lipidomics. Sphingolipids synthesis genes were down-regulated in fatty liver and fatty kidney, and BTE reversed them in fatty liver rather than fatty kidney, probably due to other sphingolipid synthesis pathway which remains unclear. Metabolites serve as direct indicators of an organism's physiological states. Therefore, the increased levels of sphingolipids reflect the organism's current physiological condition accurately. Recent studies suggested that sphingolipids can alter the glomerular filtration barrier by affecting the function of podocytes and ceramides were positively correlated with proteinuria and the severity of diabetic kidney disease (DKD)^[43-44]. The significant regulatory effect of BTE on sphingolipids and their derivatives in fatty kidney may lay the fundament for improving renal function.

Phospholipid is the main lipid in phospholipid bilayer of cell membrane. The hepatic PC concentration had a significantly positive correlation with liver fibrosis stage^[45]. Additionally, renal PC synthesis would be enhanced in response to kidney injury as the initiation signal of renal compensatory effect^[46]. In our current study, transcriptomics

and lipidomics revealed that HFD feeding disturbed phospholipid metabolism, which was reversed with BTE administration. Especially, BTE up-regulated *Cpt1a* gene expression in fatty liver and *Pld1* gene in fatty kidney, which has been demonstrated to alleviate microvesicular steatosis in periportal hepatocytes and ameliorate apoptosis in chronic renal toxicity^[47-48] as potential evidence of phospholipid regulation of BTE in transcriptional level. What's more, BTE down-regulated total PC in both fatty liver and fatty kidney. LPC is predominantly generated through the turnover of PC *via* the action of phospholipase A2. When acyl-CoA is present, LPC is efficiently recycled back to PC through the enzymatic activity of lysophosphatidylcholine acyltransferase (LPCAT). Although the correlations between LPC and hepatic failure are controversial^[49], in the current study, half of LPC molecules were up-regulated in fatty liver with side chain of more than 18 carbon, whereas, another half of LPC molecules were down-regulated, suggesting that the side chain of lipid molecule might contribute to upregulation or downregulation of LPC in liver induced by HFD feeding. As for kidney, LPC (16:0) and (18:0) were able to activate PPAR- δ , increase perilipin 2 and lipid droplets in DKD, which could be a promising target^[50]. Consistently, feeding HFD led to the increased levels of all LPC molecules with LPC (16:0) and (18:0) being particularly noteworthy in the kidney, revealing the underlying role of LPC in the process from fatty kidney to DKD. Importantly, BTE administration alleviated all LPC molecules accumulation in fatty kidney, which may be a potent mechanism for BTE to treat DKD.

PA was synthesized from DAG and PC *via* 2 pathways, including the phospholipase D pathway and diacylglycerol kinases pathway^[51]. However, we found that HFD feeding did not increase hepatic PA abundance, despite up-regulated the upstream DAG and PC, hinting the impaired activity of phospholipase D or diacylglycerol kinases in fatty liver. BTE reduced upstream DAG and PC, probably improved phospholipase D and diacylglycerol kinases activity, thus decreased PA concentration in fatty liver. However, the activity of phospholipase D or diacylglycerol kinases in fatty kidney might not be impaired, hence, increased DAG and PC levels due to HFD feeding led to an increase of PC, resulting in high conversion to PA in fatty kidney. On the other hand, the accumulated PA in podocytes may contribute to proteinuric kidney disease^[52]. However, BTE administration decreased PA abundance in fatty kidney, thus reduced the risk of proteinuric kidney disease.

The BMP-enriched vesicles serve as binding structures in catabolic breakdown of sphingolipids. Thus, the reductive effect of BTE on renal BMP was similar as sphingolipids, which may be the underlying mechanism of the decrease of sphingolipids by BTE. A reduction in the lysosome pool can prompt hepatic steatosis and lipotoxicity^[53]. The elevated hepatic BMP was consistent with our finding in lipid metabolism disorder. However, BTE somehow elevated BMP abundance, which conflicts with the alleviative effect of BTE on lipid metabolism and needs to be elucidated further. Taken together, the improvement effect of BTE on phospholipids in fatty kidney was stronger than fatty liver, hinting the potential effect of BTE on DKD.

As important organs in metabolism and detoxification, the liver and kidney play crucial roles in maintaining the homeostasis in the body and initially get impaired in various metabolic disease. Thus, identifying lipid targets for fatty liver and kidney can aid in treatment for ELD-related diseases. In the current study, the downregulation

of CLs and upregulation of some glyceride, PCs, PGs and PEs are the common feature of fatty liver and fatty kidney. BTE prominently reduced glyceride lipids and CE in fatty liver, whereas, BTE displayed a stronger effect on glycerides, phospholipids and sphingolipids in fatty kidney. In accordance with the previous findings, we demonstrated that among the lipids down-regulated by BTE in both fatty liver and fatty kidney, 26 lipids belonged to the TAG class, which would be the basis for BTE in alleviating fatty liver and fatty kidney. Comprehensive analysis finally revealed that CE18:2 and TAG56:4(20:2) were the lipid biomarkers for BTE in improvement of fatty liver and fatty kidney. CE and TAG are the main storage lipids in mammals. The over-accumulated TAG triggered the initiation of oxidative stress and energy compromise in liver and accelerated progression of hepatic steatosis to NASH^[54]. The persistence of TAG induced by acute kidney injury could lead to CKD, although the inflammation and fibrotic drive subside over time^[55]. The accumulation of CHO within foam cells, facilitated by CE storage, plays a crucial role not only in the development of atherosclerosis but also in inflammation. This process is also implicated in connecting various pathologies including type 2 diabetes and CKD^[56]. Thus, the suppression of abnormal accumulated CE and TAG might be the lipid target for BTE in alleviating ELD.

In summary, the current study demonstrated that glycerides metabolism disorder may be the fundament of ELD. At the same time, CL, PC, PG and PE metabolism disorders are the common feature of fatty liver and fatty kidney. Notably, BTE could alleviate HFD-induced ELD of liver and kidney revealed by lipid profiling alteration utilizing lipidomic analysis. The downregulation of glyceride, especially TAG, is the common pharmacological basis of BTE on alleviating both fatty liver and fatty kidney. The stronger effect of BTE on renal sphingolipids, phospholipid and BMP could be the lipidomics fundament for alleviating fatty kidney. Particularly, CE18:2 and TAG56:4(20:2) are potential lipid targets for BTE in improvement of ELD, which may be developed as lipid target for ELD treatment. Overall, our study provided a novel insight into comprehensively understanding the pathogenesis and therapeutical target strategy for fatty liver and fatty kidney.

Declaration of competing interest

The authors declare that they have no competing financial interests and conflicts.

Acknowledgements

This research was funded by National Natural Science Foundation of China (NSFC 82070877); the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2023-JKCS-29); the Fundamental Research Funds for the Central Universities (3332023155); CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-005).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2024.9250391>.

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