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Multiomics reveals the ameliorating effect and underlying mechanism of polygonatum sibiricum rhizome water extract on HFD-induced mouse obesity

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Abstract

Background

Polysaccharides and ethanol extract from *Polygonatum sibiricum rhizome* were reported to improve high-fat diet (HFD)-fed mouse obesity. However, the effect and potential mechanism of *Polygonatum sibiricum rhizome* water extract (PSRwe) on HFD-induced obesity mice remains unclear. The present study was sought to comprehensively elucidate that. An obese mouse model was established by feeding HFD and PSRwe were intragastrically administered once a day for 40 days. Changes in body weight, gut microbiota and their metabolites, plasma and liver metabolomics, colonic and liver transcriptomics were explored. The compounds in PSRwe were also examined.

Results

Body weight was significantly reduced after two weeks of PSRwe administration. Meanwhile, PSRwe treatment has significantly recovered the richness and diversity of gut microbiota in HFD-caused obesity mice. Specifically, beneficial species including *Akkermansia muciniphila* and functional pathways including fatty acids biosythesis and elongation, pentose phosphate pathway, glycolysis and reductive TCA cycle were significantly enriched in PSRwe group compared with oebsity. RNA-seq revealed that the function of the up-regulated DEGs in liver of PSRwe mice were mainly concentrated on lipid and fatty acid oxidation and metabolism, while the down-regulated DEGs were mainly focused on sterols and sterol biosynthesis and metabolism. In colon, however, the function of up-regulated DEGs were primarily JAK-STAT/PI3K-Akt signaling pathway, regulation of GTPase activity, and response to cation while the down-regulated DEGs were centered on glycolysis, fructose/mannose/pyruvate metabolism, fat digestion and absorption, and PPAR signaling pathway. Metabolomics analysis indicated that betaine, an effective component from PSR water extracts, has showed higher levels in both liver and plasma of PSRwe-treated mice, indicating that it was significantly associated with obesity. In addition, correlation analysis showed that the significantly different species enriched in PSRwe group were negatively correlated with colonic DEGs related on PPAR signaling pathway, glycolysis etc. but positively correlated with JAK-STAT signaling pathway etc. Intriguingly, common metabolites in plasma and liver were negatively correlated with liver DEGs related on steroids and sterols biosynthesis and metabolism but positively correlated with fatty acid metabolism.

Conclusions

Collectively, our study demonstrated that PSRwe could significantly alleviate HFD-induced mouse obesity via either directly affect lipid metabolism through effective betaine or by changing gut microbiota and their metabolites to alter gene expression associated with fatty acid metabolism in liver and colon, suggesting PSRwe might be a promising therapeutic candidate for obesity in clinical.

Background

Polygonatum sibiricum rhizome (PSR) has the homology of medicine and dietary supplement which belongs to the Liliaceae family and is a well-known life-prolonging tonic in Chinese medicine for hundreds of years conventionally [1]. PSR has been used as an anti-aging, anti-inflammatory, anti-osteoporotic agent, as well as an immunity booster and sleep enhancer [2] because its highly diverse composition of bioactive compounds such as flavones, homoisoflavanone, alkaloids, lignins, steroid saponins, triterpenoid saponins, polysaccharides etc. PSR polysaccharides (PSP), the major pharmacology active constituent extracted from PSR, are widely applied to the therapy of cardiovascular diseases [3] and osteoporosis [4], alleviating inflammatory cytokines and promotes glucose uptake in high-glucose-/high-insulin-induced 3T3-L1 adipocytes [5], improving the palmitic acid (PA)-induced inhibition of survival, inflammation, and glucose uptake in skeletal muscle cells [5]. Intriguingly, PSP was found to play a role in high-fat diet (HFD)-induced animal obesity via regulation of lipid metabolism and inflammatory response by activating the AMPK signaling pathway [6], regulating the SIRT1-PGC1α-PPARα pathway and their downstream genes to control energy and lipid metabolisms [7], and gut-derived LPS/TLR4 pathway inhibition [8] etc. In addition, Ko et al. [9] had explored the effect and potential underlying mechanism of ethanol extract from PSR (ID1216) on HFD-induced obesity and suggested that ID1216 may be a promising therapeutic agent for improving obesity conditions through the sirtuin-1 and peroxisome proliferator-activated receptor γ coactivator-1α pathway. Collectively, PSP and PSR ethanol extract from PSR are expected to be effective measures for clinical treatment of obesity. However, PSP needs to be extracted and purified, and PSR ethanol extract is not universally available, especially for patients allergic to alcohol. Therefore, it is of great significance to explore the effect and potential underlying mechanism of water extract of PSR on obesity, which is relatively easy to obtain and of higher acceptance to the public.

The gut microbiome consists of trillions of bacteria which play an important role in human metabolism. Animal and human studies have implicated gut microbial imbalance or dysbiosis in metabolic diseases including obesity, and recent studies are beginning to unravel the involved mechanisms. Emerging evidence have supported that gut microbiota affect host metabolism by extracting energy from indigestible food intook by host and producing metabolites and cytokines. Several pathways involving gut barrier integrity, production of metabolites affecting satiety and insulin resistance, epigenetic factors, and metabolism of bile acids and subsequent changes in metabolic signaling were reported. Heiss et al [10] suggested that depletion of the gut microbiota attenuates diet-induced hypothalamic inflammation and enhances leptin sensitivity via GLP-1R-dependent mechanisms. Wu et al [11] demonstrated that intestine-specific HIF-2α ablation in mice resulted in lower lactate levels, less *Bacteroides vulgatus* and greater *Ruminococcus torques* abundance to elevate taurine-conjugated cholic acid and deoxycholic acid levels and activation of the adipose G-protein-coupled bile acid receptor, GPBAR1 (TGR5) to alter white adipose tissue thermogenesis, which deepens our understanding of how host genes regulate the microbiome and provides novel strategies for alleviating obesity. Janssen et al [12] unveiled that loss of angiopoietin-like 4 (ANGPTL4), an important regulator of triacylglycerol metabolism, in mice with diet-induced obesity uncouples visceral obesity from glucose intolerance partly via the gut microbiota, which may provide experimental data reference for explaining the mechanism of insulin secretion regulated by

gut microbiota. There is a large body of evidence demonstrating that alteration in the proportion of Bacteroidetes and Firmicutes leads to the development of obesity, but this has been recently challenged. It is likely that the influence of gut microbiome on obesity is much more complex than simply an imbalance in the proportion of these phyla of bacteria. Modulation of the gut microbiome through diet, pre- and probiotics, antibiotics, surgery, and fecal transplantation has the potential to majorly impact the obesity epidemic [13]. Thus, this study aimed to systematically elucidate the possible underlying mechanism of PSR water extract on HFD-induced mouse obesity based on the alterations of gut microbiota and their metabolites, the changes of colonic and liver gene expression, the variations of plasma and liver metabolites, as well as the complex interactions and relationships among these multiomics data.

Taken together, PSR has the ability of alleviating HFD-induced obesity, however, the role of the gut microbiome and the metabolites in colon, plasma and liver, as well as the changes of colonic and liver gene expression were unknown. Here we firstly aimed to illustrate the characteristics of the gut microbiota and their metabolites before and after PSR water extract treatment in HFD-induced obesity mice, then the blood and liver metabolites as well as liver and colonic gene expression were examined to elucidate the host genes-gut microbiome-metabolites axis in PSR water extract on HFD-induced obesity, which will provide experimental support for the usage of PSR water extract on obesity in clinic.

Results

PSRwe treatment improved HFD-induced obesity in mice

Obesity mice model was successfully constructed by HFD-fed for eight weeks. PSRwe and orlistat (a well-known irreversible inhibitor of pancreatic and gastric lipases used for orally for long-term research of obesity [14]) were intragastrically administrated to the obesity mice according to their body weight (BW) once a day for 8 weeks. All the mice were sacrificed to collect samples for multi-omics examinination after the terminal of the experiment (Figure S1A). Both PSRwe and orlistat could significantly reduce the BW of HFD-induced obese mice after two weeks of therapy, and the efficacy of PSRwe was more stable and lasting than orlistat (Figure S1B). No apparent damage for liver, lung, spleen, kidney, stomach and heart of mice during the gavage of PSRwe and orlistat were observed by H&E staining (Figure S1C). Therefore, we have explored the underlying mechanism of PSRwe in treating HFD-induced obesity based on multiomics.

PSRwe treatment altered the gut microbial composition and function in obesity

PCoA analysis based on bray-curtis distance has showed that the composition of the gut microbiota was significantly different between obesity and PSRwe group at genus level (PcoA 1 = 55.6%, Fig. 1A). Shannon and Simpson indexes were used to evaluate the alpha diversity and the results revealed that PSRwe treatment could obviously increase the species diversity and richness of the gut microbiota

compared with obesity mice (Fig. 1B). Differential analysis between PSRwe and obesity have identified 10 significant different genera (Fig. 1C), in which the relative abundance (RA) of *Akkermansia*, *Parasutterella*, *Parabacteroides*, *Klebsiella*, *Erysipelatoclostridium*, *Bacteroides*, *Alistipes*, *Ileibacterium* were significantly enriched while that of *Firmicutes* unclassified and *Mucispirillum* were obviously decreased in PSRwe treatment group compared with obesity mice. 21 significantly changed species were observed between PSRwe and obesity group (Fig. 1D). Among which, *Akkermansia muciniphila*, *Alistipes indistinctus*, *Bacteroides uniformis*, *Bacteroides stercorirosoris*, *Bacteroides intestinalis*, *Clostridium cocleatum*, *Erysipelatoclostridium ramosum*, *Ileibacterium valens*, *Klebsiella pneumoniae*, *Klebsiella variicola*, *Klebsiella quasipneumoniae*, *Lachnospiraceae bacterium* 10 − 1, *Lactobacillus reuteri*, *Lachnospiraceae bacterium* COE1, *Lactobacillus johnsonii*, *Parasutterella excrementihominis*, *Parabacteroides gordonii*, *Streptococcus acidominimus* were significantly enriched while that of *Mucispirillum schaedleri* and *Firmicutes bacterium* ASF500 were obviously decreased in the gut of PSRwe group.

Humann 3.0 was used to efficiently and accurately profile the abundance of microbial metabolic pathways. Significant changes of the functional modules were observed between groups (Figure S2A). Pathways including superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass, superpathway of glyoxylate bypass and TCA, saturated fatty acid elongation, fatty acid beta oxidation I, TCA cycle IV, gluconeogenesis III, fucose degradation, glycolysis III (from glucose), pentose phosphate pathway, oleate biosynthesis IV (anaerobic), stearate biosynthesis II (bacteria and plants), homolactic fermentation, superpathway of fucose and rhamnose degradation, NAD de novo biosynthesis I (from aspartate) were all significantly enriched in the PSRwe group, which suggested that PSRwe treatment could significantly increase the energy metabolism in obesity mice. We then focused on fatty acid metabolism and two pathways including fatty acid elongation-saturated and superpathway of fatty acid biosynthesis initiation (*E. coli*) were significantly enriched in PSRwe group (Figure S2B). Two species including *Bacteroides stercorirosoris* and *Klebsiella pneumoniae* were found to contribute to fatty acid elongation-saturated pathway. Five species including *Bacteroides caecimuris*, *Bacteroides massiliensis*, *Clostridium cocleatum*, *Erysipelatoclostridium ramosum*, *Parabacteroides goldsteinii* were observed to participate in the superpathway of fatty acid biosynthesis initiation (*E. coli*). It is worth noting that *A. muciniphila*, a promising probiotic [15], were significantly enriched in the gut of the PSRwe group (Figure S2C). We have found that *A. muciniphila* is involved in a variety of biological processes such as nucleotides biosynthesis, pyruvate fermentation, folate transformations, amino acids biosynthesis, rhamnose biosynthesis, peptidoglycan biosynthesis, and superpathway of branched chain amino acid biosynthesis.

These results suggested that PSRwe has significantly improved HFD-induced obesity in mice, as well as the gut microbial composition and function.

Colonic DEGs were correlated to the gut microbiota and their metabolites

RNA-seq was performed to depict the differentially expressed genes (DEGs) in the colon of the mice between the PSRwe and obesity group. 184 genes including *Odad4*, *Kat2b*, *Abl1*, *Mir1932*, *Cop1* etc. were up-regulated and 229 genes including *Mir702*, *Igkj1*, *Tspan11*, *Dnpep*, *Vsig10l* etc. were down-regulated in the colon of the PSRwe mice compared with the obesity group (Fig. 2A). GO annotation (Fig. 2B) has showed that the up-regulated DEGs were dynamically localized to cellular components (CC) including photoreceptor connecting cilium, condensed chromosome outer kinetochore, cytosolic small ribosomal subunit, kinetochore, complex Sin3 complex, chromosomal region, condensed chromosome kinetochore, ciliary transition zone, polysome, polysomal ribosome, and contributed to molecular function (MF) including pre-mRNA intronic binding, pre-mRNA/mRNA binding, bHLH transcription factor binding, GTPase activator activity, D1 dopamine receptor binding, GTPase regulator activity, mRNA 3-UTR binding, acetylcholine receptor activity, histone acetyltransferase activity. The up-regulated DEGs involved in biological process (BP) mainly include cellular response to cadmium ion, positive regulation of GTPase activity, cellular response to zinc ion, response to cadmium ion, cellular response to copper ion, spinal cord motor neuron differentiation, detoxification, regulation of endothelial cell proliferation. KEGG pathway analysis revealed that serotonergic/cholinergic/glutamatergic synapse, JAK-STAT signaling pathway, PI3K-Akt signaling pathway, FoxO signaling pathway, inflammatory mediator regulation of TRP channels, and antifolate resistance were the main pathways participated by the up-regulated DEGs. For the down-regulated DEGs (Fig. 2C), they were primarily localized to inclusion body, aggresome, mitochondrial inner membrane, cullin-RING ubiquitin ligase complex, organelle inner membrane, SCF ubiquitin ligase complex, ubiquitin ligase complex, mitochondrial respirasome, lytic vacuole, lysosome. The MF involved for these DEGs were mainly centered on retinol dehydrogenase activity, oxidoreductase activity/acting on the CH-OH group of donors/NAD/NADP as acceptor, aldehyde-lyase activity, androsterone dehydrogenase activity, steroid dehydrogenase activity, oxidoreductase activity/acting, protein heterodimerization activity, carbon-carbon lyase activity, GTPase activity, transferase activity/transferring pentosyl groups. The BP involved of these DEGs were principally retinoid/ diterpenoid/ terpenoid/ pyruvate/ isoprenoid metabolic process, glycolytic process, ATP generation from ADP, response to interferon-gamma, carbohydrate catabolic process, ADP metabolic process. KEGG pathway analysis has displayed that ether lipid metabolism, glycolysis/ gluconeogenesis, pentose phosphate pathway, fructose and mannose metabolism, fat digestion and absorption, PPAR signaling pathway, alpha-Linolenic acid metabolism, and apoptosis were the main pathways participated by the down-regulated DEGs. These results revealed significant differences of the colonic gene expression as well as their distinct function between PSRwe treated and untreated obesity mice.

We have also explored the gut microbial metabolites in colon and explored the association between DEGs and different metabolites. Interestingly, 165 significant different metabolites were observed between PSRwe and obesity group. Thereinto, 109 metabolites had significant high levels while 56 metabolites were obviously reduced in the gut of PSRwe treated mice compared with obesity group (Fig. 3A). The increased metabolites in the gut of PSRwe mice were mainly involved in pathways including purine/pyrimidine metabolism, cAMP signaling pathway, biosynthesis of amino acids, arginine and proline/ tyrosine/ phenylalanine/ beta-alanine metabolism, biosynthesis of cofactors, vitamin digestion

and absorption, sphingolipid signaling pathway, sphingolipid metabolism, ABC transporters, neuroactive ligand-receptor interaction, morphine addiction and caffeine metabolism, all these pathways were significantly abundant in the PSRwe group (Fugure 3B, 3C). However, the decreased metabolites in PSRwe group such as LPE, LPC, PC, Carnitine C22:2 etc., participated KEGG pathways including amino and nucleotide sugar metabolism, glucagon signaling pathway, cysteine and methionine metabolism, porphyrin and chlorophyll metabolism, all of which were obviously abundant in the obesity mice (Fig. 3B, 3D).

Complex interactions have been known to exist between the colonic gene expression and gut microbiota as well as their metabolites. To unveil that, Spear-man's rank correlation was performed for the significantly different species and DEGs, as well as the evidently different gut microbial metabolites and DEGs between the PSRwe and obesity group (Fig. 4). The down-regulated DEGs involved for the top KEGG pathways were include *Gpi1*, *Lpcat4*, *Agpat2*, *Gdpd3*, *Atf4*, *Tuba1b*, *Pla2g7*, *Aldob*, *Pla2g3*, *Acaa1b*, *Mpi*, *Mcl1*, *Fabp2*, which were positively associated with *Lachnospiraceae bacterium* 3 − 2, *Firmicutes bacterium* ASF500, and *Mucispirillum schaedleri*, which were obviously decreased in the gut of PSRwe treated mice. The up-regulated DEGs contributed to the predominant KEGG pathways contained *Chrm1*, *Homer2*, *Cyp2d10*, *Alox12*, *Il10*, *Htr2b*, *Chrnb2*, *Ep300*, *Gngt2*, *Gnas*, *Cacna1a*, *Pik3cd*, were positively associated with *Akkermansia muciniphila*, *Bacteroides stercorirosoris*, *Bacteroides uniformis*, *Alistipes indistinctus*, *Parasutterella excrementihominis*, *Parabacteroides goldsteinii* etc., which were all enriched in the gut of the PSRwe treated mice. These results indicated that the gut microbiota could regulate the gene expression in the colon wall.

To further investigate the relationship between the colonic DEGs and the gut microbial metabolites, Spearman’s rank correlation between DEGs and significantly different metabolites were performed (Figure S3). the up-regulated DEGs in PSRwe group including *Cacna1a*, *Htr2b*, *Ep300*, *Gngt2*, *Homer2*, *Chrm1*, *Alox12*, *Il10*, *Chrnb2*, *Gnas*, *Pik3cd* were significantly positively correlated with significantly different gut microbial metabolites participated in pathways including organic acid and its derivatives, nucleotide and its metabolomics, heterocyclic compounds, fatty acyl, coEnzyme and vitamins, benzene and substituted derivatives, amino acid and its metabolomics, alcohol and amines. The down-regulated DEGs including *Fabp2*, *Pla2g3*, *Acaa1b*, *Mpi*, *Aldoc*, *Atf4*, *Tuba1b*, *Pla2g7*, *Aldob*, *Mcl1*, *Gpi1*, *Olr1*, *Gdpd3*, *Ldha*, *Plin2*, *Lpcat4*, *Agpat2* were positively correlated with the gut microbial metabolites contributes to pathways including carbohydrates and its metabolites, and glycerophospholipids. The above results revealed that the colonic gene expression could affect the gut microbial metabolites.

In summary, the gut microbiota and their metabolites could regulate the colonic gene expression related to lipid metabolism, fat digestion and absorption, glycolysis, Pentose phosphate pathway, JAK-STAT signaling pathway, FoxO signaling pathway, PPAR signaling pathway to affect energy absorption and metabolism in obesity, which in return, the gene expression might affect the composition and function of the gut microbiota.

Differential analysis of the liver DEGs and metabolites

Liver is the main organ for fatty acids biosynthesis and metabolism; thus, we investigated the differences for liver gene expression and metabolites between PSRwe and obesity group.

RNA-seq was used to study the liver gene expression between PSRwe and obesity group. PcoA analysis has revealed that the liver gene expression was significantly different (PcoA 1, P = 0.00067, Fig. 5A). Differential analysis has unveiled 2791 significant DEGs in the liver between the PSRwe and obesity group. Among them, 2278 DEGs were up-regulated while 513 DEGs were down-regulated in the PSRwe group (Fig. 5B). GO annotation has revealed that the up-regulated DEGs were mainly localized to peroxisome, microbody, peroxisomal membrane, microbody membrane, lytic vacuole, lysosome, cytosolic ribosome, vacuolar membrane, autophagosome membrane, and ribosomal subunit. MF of these DEGs were primarily ATPase activity, oxidoreductase activity/acting on the CH-CH group of donors/NAD or NADP as acceptor, manganese ion binding, nucleoside-diphosphatase activity, structural constituent of ribosome, transferase activity/transferring acyl groups, carboxylic ester hydrolase activity, fatty acid binding, and monocarboxylic acid binding. The BP involved for these DEGs were principally fatty acid catabolic process, fatty acid beta – oxidation, fatty acid metabolic process, monocarboxylic acid catabolic process, lipid and cellular lipid catabolic process, lipid and fatty acid oxidation, small molecule catabolic process, sulfur compound metabolic process. KEGG pathway analysis showed that fatty acid degradation, peroxisome, fatty acid metabolism, PPAR signaling pathway, biosynthesis of unsaturated fatty acids, fatty acid elongation, lysosome, ribosome, other glycan degradation, and valine/leucine and isoleucine degradation were the main pathways contributed by the up-regulated DEGs (Fig. 5C). However, for the down-regulated DEGs, they mainly function at host cellular component, peroxisome, microbody, integral component of organelle membrane, intrinsic and integral component of endoplasmic reticulum membrane. The MF of these DEGs were mainly focused on oxidoreductase activity/acting on paired donors/ with incorporation or reduction of molecular oxygen, iron ion binding, monooxygenase activity, steroid hydroxylase activity, tetrapyrrole and heme binding, estrogen 16-alpha-hydroxylase activity, retinoic acid 4-hydroxylase activity, sulfotransferase activity, aromatase activity. The BP for down-regulated DEGs were majorly sterol/steroid metabolic process, sterol/steroid biosynthesis process, cholesterol biosynthetic and metabolic process, alcohol and secondary alcohol biosynthetic and metabolic process, and organic hydroxy compound biosynthetic process. The KEGG pathways involved for the DEGs were concentrated on chemical carcinogenesis-DNA adducts, steroid hormone and steroid biosynthesis, linoleic acid metabolism, PPAR signaling pathway, terpenoid backbone biosynthesis, retinol metabolism, antigen processing and presentation, cholesterol metabolism, metabolism of xenobiotics by cytochrome P450 (Fig. 5D). Taken together, the up-regulated DEGs were mainly focused on fatty acids biosynthesis and metabolism, and lipid metabolism while the down-regulated DEGs were centered on cholesterol, steroid, and sterol metabolism.

PcoA analysis has revealed that the liver metabolites were significantly different between the PSRwe and obesity group (Fig. 6A). Differential analysis has revealed 131 metabolites significantly enriched in the liver of PSRwe mice and 88 metabolites including carnitine C10:1, carnitine C16:2, etc., which were obviously higher in the liver of obesity mice (Fig. 6B). We have then analyzed the top abundant pathways involved by these different metabolites in PSRwe and obesity group and revealed significant differences

between them. Specifically, the top abundant enriched pathways in obesity mice include ABC transporters, purine metabolism, biosynthesis of cofactors, vitamin digestion and absorption, mineral absorption, amino and nucleotide sugar metabolism, D-glutamine and D-glutamate metabolism, inositol phosphate metabolism, starch and sucrose metabolism, fructose and mannose/galactose metabolism, carbohydrate digestion and absorption, drug metabolism-other enzymes, bile secretion, taurine and hypotaurine metabolism, insulin secretion, AMPK signaling pathway, cAMP signaling pathway, cGMP-PKG signaling pathway, FoxO signaling pathway, prolactin signaling pathway, parathyroid hormone synthesis/secretion and action, C-type lectin receptor signaling pathway, sulfur relay system, butirosin and neomycin biosynthesis, lysosome, neuroactive ligand-receptor interaction, taste transduction, olfactory transduction (Fig. 6C). Meanwhile, the top abundant pathways in PSRwe group contained pyrimidine metabolism, protein digestion and absorption, biosynthesis of amino acids (arginine, lysine), glycine-serine-threonine/ phenylalanine/ tryptophan metabolism, valine, leucine and isoleucine biosynthesis and degradation, glycerophospholipid metabolism, sphingolipid metabolism, glycosylphosphatidylinositol(GPI)-anchor biosynthesis, sphingolipid signaling pathway, ether lipid metabolism, fatty acid biosynthesis and metabolism, steroid hormone biosynthesis, carbon metabolism, 2-oxocarboxylic acid metabolism, pantothenate and CoA biosynthesis, axon regeneration, axon regeneration, and monobactam biosynthesis (Fig. 6D).

The metabolites contribute to the above KEGG pathways were showed in Figure S4 and it is easy to see that most amino acids, sugars, most of LPEs and carnitine isomers, choline were significantly higher in PSRwe group, however, cytosine, adenosine, cAMP, organic acids including ippuric acid, uric acid, maleic acid, epinephrine, sphingosine, FFA, most of LPCs etc. had higher levels in the liver of obesity mice, which were consistent with the metabolic pathways. In summary, the metabolic pathways of the obese mice were mainly focused on active transport and absorption of minerals, sugars, lipids, sterols, peptides, and proteins, hormone secretion such as insulin and parathyroid hormone, bile and taurine and hypotaurine secretion and metabolism, while that of the PSRwe treated mice were mainly focused on amino acids and lipid metabolism.

Gut microbiota affect liver gene expression and its metabolites

To further characterize the differences in lipid and fatty acids metabolism between the PSRwe and obesity groups, 12 pathways including fatty acid biosynthesis/ degradation/ elongation, glycerophospholipid metabolism, glycine, serine and threonine metabolism, peroxisome, arachidonic acid metabolism, PI3K-AKT signaling pathway, FoxO signaling pathway, Jak-STAT signaling pathway, AMPK signaling pathway, and PPAR signaling pathway were precisely analyzed and the genes involved in these pathways were analyzed in detail (Fig. 7). Interestingly, most of the genes participated in these pathways (marked as red in Fig. 7) were significantly highly expressed in liver of the PSRwe treated mice, suggesting that PSRwe treatment could evidently increase the lipid and fatty acids metabolism to alleviate the symptoms of HFD-obesity.

Gut microbiota has been reported to play an important role in obesity, thus we have explored the relationship between the significant gut microbial species and the lipid and fatty acids related DEGs in liver (Fig. 8). Interestingly, the highly expressed DEGs in liver of the obese mice including *scd1*, *scd2*, *pmvk*, *pltp*, *fabp5*, *cyp7a1*, *Foxo3*, *cyp27a1*, *fabp2*, *fabp4*, *elovl6* were positively associated with the obese mice enriched species such as *Mucispirillum schaedleri*. However, the highly expressed DEGs in liver of PSRwe treated obese mice including *stk11*, *chdh*, *acaa1b*, *pias2*, *ptgr2*, *cyp4a31*, *acadm*, *pck2*, *pla2g12a*, *egfr*, *ppt1*, *g6pc* etc. were positively correlated with species enriched in the gut of PSRwe treated mice including *Akkermansia muciniphila*, *Bifidobacterium pseudolongum*, *Lactobacillus hominis*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Klebsiella quasipneumoniae*, *Klebsiella variicola*, *Klebsiella pneumoniae*, *Bacteroides uniformis*, *Bacteroides intestinalis*, *Parabacteroides gordonii* et al.

We have subsequently analyzed the correlation between the significantly different gut microbial species and the different metabolites in liver (Figure S5). It's worth noting that 5-Aminolevulina, Phosphocholine, O-Acetylcholine, which were highly in the obese mice, were negatively correlated with the obese mice enriched species such as *Mucispirillum schaedleri*. However, the highly expressed metabolites in the PSRwe mice including betaine, choline, Threonine, L-Serine, L-Aspartate etc. were negatively associated the PSRwe enriched species such as *Akkermansia muciniphila*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus hominis*, *Parabacteroides gordonii*, *Lachnospiraceae bacterium* COE1, *Klebsiella pneumoniae*, *Parasutterella excrementihominis*.

These above results suggesting that the gut microbiota could regulate the liver gene expression related to lipid metabolism, which finally changed the liver metabolites such as betaine and choline.

Differential metabolite analysis in plasma, liver tissue and PSR water extract

The plasma metabolites in PSRwe and obesity group were significantly different (Fig. 9A). Totally 212 different metabolites were detected and 58 metabolites were significantly high while154 metabolites were obviously reduced in PSRwe group compared with the obesity mice (Fig. 9B). These different metabolites were mainly involved in pathways highly abundant in PSRwe group such as thyroid hormone signaling pathway, bile secretion, fatty acid biosynthesis, biosynthesis of unsaturated fatty acids, alpha-Linolenic acid metabolism, as well as the significantly abundant pathways in obesity such as glycine, serine and threonine metabolism, tyrosine metabolism, biosynthesis of cofactors, taurine and hypotaurine metabolism, nicotinate and nicotinamide metabolism, pyrimidine metabolism, and neuroactive ligand-receptor interaction (Fig. 9C). Venn diagram was used to illustrate the similarities, differences, and relationship of metabolites among plasma, liver and water extracts of PSR and a total of 42 common metabolites were obtained (Fig. 9D). Interestingly, among these metabolites, seven metabolites including betaine, uridine, 5-Aminoimidazole ribonucleotide, Thymidine, Cytosine, N,N-Dimethylglycine, and 5-Methyluridine were significantly enriched in liver of PSRwe-treated mice compared with obesity mice. However, betaine was the only compound that enriched in the plasma of PSRwe treated mice compared to obesity group (Fig. 9E). Betaine, can be either endogenous or exogenous, was reported to enhances

lipid metabolism and improves insulin resistance [16] through gut microbiota-drived microRNA-378a family [17] in HFD-induced obese mice. Exogenous betaine might be primarily derived from PSR water extract and was absorbed into the blood to circulate to liver to affect lipid metabolism while the endogenous betaine was mainly generated from choline under the function of *chdh* and *aldh7a1* in liver. Both choline and betaine were detected in PSR water extract. As choline can be metabolized into betaine in liver, we then analyzed the expression of *chdh* and *aldh7a1* in liver and found that *chdh* was significantly highly expressed in liver of the PSRwe mice but no significant difference for *Aldh7a1*(Fig. 9F), which indicated that *chdh* encoded the necessary enzyme or enzymes to metabolize choline into betaine in liver. Taken together, betaine was significantly higher in liver of the PSRwe mice which might due to either direct absorption from PSR water extract or choline metabolism in liver.

Discussion

Obesity is a multifactorial disease resulting in excessive accumulation of adipose tissue and various metabolic diseases [18], however, hitherto there is no highly effective treatment for obesity. In this study, we have focused on the effect of decoction prepared from the traditional Chinese medicine PSR on HFD-induced mouse obesity. We have observed that intragastric administration of PSR water extracts (PSRwe) could significantly improve the symptoms of obese mice. The gut microbiota, metabolites in colonic contents, plasma and liver, as well as gene expression in colon and liver tissues were examined to elucidate the possible mechanism of PSRwe on obesity treatment.

PSRwe treatment significantly improved HFD-induced obesity and recovered the gut microbial dysbiosis

Growing evidence has identified the gut microbiota as a potential factor in the pathophysiology of obesity for their role in protecting gastrointestinal mucosa permeability and regulating the fermentation and absorption of dietary polysaccharides to regulate fat accumulation and the resultant obesity [18]. In this study, we constructed the obese mice model by feeding a high-fat diet for eight weeks and we have found that HFD could significantly reduce the richness and diversity of the gut microbiota in mice, decreased the relative abundance of *Lactobacillus*, *Prevotella*, *Enterobacter*, *Enterococcus*, *Alistipes*, *Muribaculum*, *Corynebacterium*, *Muribaculum*, *Eubacterium*, *Turicimonas*, *Acinetobacter*, *Aerococcus*, *Staphylococcus*, but increase that of *Anaerotruncus*, *Lactococcus*, *Ruthenibacterium*, *Erysipelatoclostridium*, *Acutalibacter*. The Firmicutes/Bacteroidetes ratio was significantly greater in obesity mice. These results were consistent with the published papers [19], in which more *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Mollicutes*, *Lactobacillus* (reuteri), and less *Verrucomicrobia* (*Akkermansia muciniphila*), *Faecalibacterium* (*prausnitzii*), *Bacteroidetes*, *Methanobrevibacter smithii*, *Lactobacillus plantarum* and *paracasei* were observed.

The rhizome of Polygonatum sibiricum Redoute (PSR) has long been used to treat metabolic disorders such as diabetes-associated complications. Recently, numerous studies has demonstrated that

Polygonatum stenophyllum [20], Polygonatum sibiricum polysaccharides [21], Polygonatum sibiricum rhizome ethanol extract [22] could protect against obesity in either human individuals or HFD-fed mice. However, the effect of PSR water extract (PSRwe) on obesity remains unclear. In this study we studied the effect of PSRwe on HFD-fed obese mice and orlistat (a drug used to aid in weight loss in clinical) was used as a positive control. The results revealed that treatment with PSRwe and orlistat for 40 days had both reduced the body weight of HFD-induced obese mice, but PSRwe showed more stable and lasting effect than orlistat, suggesting that PSRwe had the potential to be applied to weight loss in clinical. In addition, PSRwe could obviously recover and even improve the richness and diversity of the gut microbial dysbiosis caused by HFD, especially in increasing the relative abundance of *Akkermansia*, *Parabacteroides*, *Bacteroides*, and *Alistipes. Akkermansia muciniphila* (*A. muciniphila*), an intestinal symbiont colonizing in the mucosal layer and is considered to be a promising candidate to improve the host metabolic functions and immune responses through producing propionate and acetate [23], was significantly enriched in the gut of PSRwe treated mice. *Parabacteroides distasonis*, has probiotic roles in human and animal health [24], was significantly higher in the PSRwe group.

Consistent with changes of the gut microbial composition, the function of the gut microbiota was also significantly altered. It's easy to found that pathways related to carbohydrate metabolism, energy metabolism, lipid and fatty acids biosynthesis and metabolism were obviously greater in the PSRwe group than obesity mice. These pathways including superpathway of glycolysis, pyruvate dehydrogenase, TCA and glyoxylate bypass, superpathway of glyoxylate bypass and TCA, incomplete reductive TCA cycle, glycolysis III (from glucose), homolactic fermentation, superpathway of fucose and rhamnose degradation, pentose phosphate pathway, NAD de novo biosynthesis I (from aspartate), fucose degradation, superpathway of fermentation, glycerol degradation to butanol, superpathway of glucose and xylose degradation, fatty acid elongation-saturated, oleate biosynthesis IV (anaerobic), stearate biosynthesis II (bacteria and plants), fatty acid beta-oxidation, stearate biosynthesis III (fungi). We have focused on the pathways and species involved in fatty acids elongation and superpathway of fatty acids biosynthesis initiation. *Bacteroides stercorirosoris* and *Klebsiella pneumoniae* were significantly enriched in PSRwe group and contributed to saturated fatty acids elongation. *Bacteroides caecimuris*, *Bacteroides massiliensis*, *Clostridium cocleatum*, *Erysipelatoclostridium ramosum*, *Parabacteroides goldsteinii* were obviously higher in the PSRwe group and contributed to the superpathway of fatty acids biosynthesis initiation. These results suggested that gut microbiota compositional changes were related to their functional alterations. Meanwhile, as *A. muciniphila* has important roles in human and animal health, we have explored pathways that *A. muciniphila* was involved and found that superpathway of branched chain amino acid biosynthesis, peptidoglycan biosynthesis, folate transformations etc were the main pathways.

The above results revealed that PSRwe exerted significant therapeutic effect on HFD-induced obesity and could improve the gut microbial homeostasis caused by obesity.

PSRwe treatment altered the colonic and liver genes expression related to fat, lipid, fatty acids, sterols and steroids metabolism

It’s of great significance to study colonic genes expression under the treatment of PSRwe since gut is involved in the direct interactions for gut microbiota and PSR water extract. Totally 413 DEGs were observed for obesity and PSRwe group, among which 229 genes were up-regulated while 184 genes were down-regulated in the PSRwe group. GO annotation and KEGG pathway analysis have showed that the function of the down-regulated DEGs were mainly focused on energy metabolism such as carbohydrate metabolism, fat digestion and absorption, and ether lipid metabolism. However, the up-regulated DEGs were primarily concentrated on JAK-STAT signaling pathway, PI3K-Akt signaling pathway, FoxO signaling pathway, inflammatory mediator regulation of TRP channels, antifolate resistance, and glutamatergic/cholinergic/serotonergic synapse. Wu et al. demonstrated that HFD-induced obesity affected the activity of the Akt/FoxO3a signaling pathway [25]. Pan et al. suggested that FoxO transcription factors play a salutary role in the protection against the diet-induced fatty liver disease [26]. Jia et al. revealed that vaccarin improves insulin sensitivity and glucose uptake in diet-induced obese mice via activation of GPR120-PI3K/AKT/GLUT4 pathway [27]. PI3K/AKT pathway damaged in various tissues of the body leads to obesity and T2D as the result of insulin resistance and therapeutic targets of the PI3K/AKT pathway were identified as treatments for obesity and T2D [28]. JAK/STAT signaling pathway is required for normal homeostasis, and, when dysregulated, contributes to the development of obesity and diabetes [29]. Obesity and the brain are linked since the brain can control the weight of the body through its neurotransmitters including dopamine, serotonin, and glutamate [30]. Interestingly, we have observed a significant increase of DEGs related to neurotransmitter producing including glutamate, acetylcholine (ACh), serotonin, which could affect host physiological functions such as emotion, sleep, pain, and endocrine secretion. In addition, obesity is often characterized by increased oxidative stress and exacerbated inflammatory outcomes accompanying infiltration of immune cells in adipocytes to inhibit insulin responses and increase the risk of cardiovascular diseases and associated features [31]. In our study, we observed that PSRwe treatment had also changed DEGs related on proliferation, apoptosis, oxidative stress resistance, and anti-inflammatory cytokines by regulating pathways such as PI3K-Akt signaling pathway and FoxO signaling pathway. In all, PSRwe treatment could reduce fat biosynthesis and absorption, increase the cellular functions and physiological events, regulate the release of neurotransmitter and cytokines to relieve obesity-induced gut inflammation and discomfort.

The exploration of liver gene expression is of great significance for understanding liver lipid metabolism. 2791 DEGs were observed between obesity and PSRwe group, in which 513 DEGs were down-regulated while 2278 DEGs were up-regulated in PSRwe group. GO annotation and KEGG pathway analysis showed that the down-regulated DEGs were mainly focused on sterols and steroids metabolism including steroid (hormone) biosynthesis, linoleic acid metabolism, cholesterol metabolism, and PPAR signaling pathway. In addition, chemical carcinogenesis-DNA adducts, terpenoid backbone biosynthesis, retinol metabolism, metabolism of xenobiotics by cytochrome P450 were also the main functions focused by down-regulated DEGs. However, the up-regulated DEGs were mainly concentrated on fatty acid elongation, oxidation, metabolism and degradation, biosynthesis of unsaturated fatty acids, PPAR signaling pathway, valine,leucine,isoleucine degradation. PPAR has three subtypes, PPARα plays a role in the clearance of circulating or cellular lipids via the regulation of gene expression involved in lipid metabolism in liver and

skeletal muscle, PPARβ is involved in lipid oxidation and cell proliferation, PPARγ promotes adipocyte differentiation to enhance blood glucose uptake (KEGG PATHWAY: map03320 (genome.jp)). The above results revealed that PSRwe treatment could increase the colon and liver gene expression related on lipid, fatty acids, sterols and steroids metabolism to relieve HFD-induced mouse obesity.

Effective components from PSR water extract played a role in improving obesity

To further explore the underlying mechanism of PSRwe on obesity, metabolomics of colon contents, plasma, and liver tissues were performed. Metabolomics of colon contents has revealed 109 significantly different metabolites between PSRwe group and obesity mice, in which 56 metabolites were low expressed while 109 metabolites were highly expressed in PSRwe group. Interestingly, carnitine and L-carnitine were found significantly lower while D-Proline betaine was obviously higher in the colon of PSRwe treated mice. It's worth noting that choline, L-carnitine, betaine, D-Proline betaine, and cocamidopropyl betaine were all examined in the PSR water extract. In plasma, 58 metabolites were obviously higher and 154 metabolites were evidently lower in the PSRwe group. Among which, Carnitine, Carnitine 2-methyl-C4, Carnitine C5:0, Carnitine C9:1-OH, Carnitine C4:0, Carnitine C6:0, Carnitine C22:2, Carnitine isoC4:0, Carnitine C3:0, Carnitine C8:0, Carnitine C5:1, Carnitine C10:0 were obviously higher while betaine and D-proline betaine were evidently lower in PSRwe group. In liver, however, 131 metabolites were significantly higher while 88 metabolites were obviously lower in the PSRwe group. Thereinto, except for carnitine C8-OH and carnitine isoC4:0, other carnitine related metabolites including DL-carnitine, carnitine C10:1 etc. and betaine were significantly higher in the PSRwe group. Interestingly, Sn-Glycero-3-Phosphocholine, Sphingosyl-phosphocholine, Methacholine, the only derivatives of choline detected in both colon and plasma, showed no significance between the PSRwe and obesity group. In addition, phosphocholine, choline and acetylcholine were also detected in liver of the PSRwe mice.

Taken together, low levels of carnitine/L-carnitine and high levels of D-proline betaine were observed in colon of the PSRwe treated mice. High levels of carnitine and low levels of betaine were detected in plasma of PSRwe mice, which might be caused either by high absorption of carnitine and low absorption of betaine by the colon or metabolic enzymes are present in plasma that metabolize betaine to carnitine. What’s more, both choline, betaine and carnitine were higher in liver of PSRwe treated mice compared with the obesity group. Choline usually serves as components of structural lipoproteins, blood and membrane lipids, and precursor of the neurotransmitter acetylcholine, meanwhile, choline is oxidized to betaine to serve as an osmoregulator [32]. Both of choline and betaine are important sources of one-carbon units especially during folate deficiency [33]. Raubenheimer et al. Found that a choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a HFD [34]. Betaine supplementation causes increase in carnitine [35]. Du et al. revealed that dietary betaine prevents obesity through gut microbiota-drived microRNA-378a family [36]. Carnitine and L-carnitine are amino acid derivatives widely known for their involvement in the transport of long-chain fatty acids into the mitochondrial matrix and protects the cell from acyl-CoA accretion [37]. Amin et al. reported that

treatment with L-carnitine improved obesity and its associated metabolic problems [38]. These results reminded us that the effective components including choline, betaine, and carnitine in PSR water extract might play important roles in the relieve of HFD-induced obesity in mice. Among which, we should especially concentrate on betaine, whose abundance was significantly higher in both liver and plasma of the PSRwe treated obesity mice compared with the non-treated ones.

Conclusion

In conclusion, our results suggested that PSRwe could significantly relieve the symptoms of HFD-induced mouse obesity either by directly affect liver lipid metabolism through effective component betaine in PSR water extract or via regulating the expression of genes related to colon fat digestion and absorption and lipid metabolism or liver fatty acid oxidation, sterols and steroids metabolism in liver by restoring the gut microbiota. These results have laid a foundation for further verification of molecular mechanism by experiments and provided a theoretical guidance for the usage of PSR water extract in clinical.

Materials and methods

Preparation of PSR water extract (PSRwe)

Wild PSR harvested from Ziwuling mountain in China was used in our study. Wild PS was cut into 1 to 2 cm pieces and soak in water for 30 min (1g PSR in 6mL water), then boil it in high heat and simmer for 40 min to get the first filtrate. Add water for a second time (1g PSR in 4mL water) and simmer for 40 min to obtain the second filtrate. The two filtrates were then mixed and concentrated at 60 ℃ to obtain the final product (750g/L) for subsequent gavage operation (Figure S6A).

Establishment of obesity model and treatment

An obese mouse model was established by feeding high-fat diet (HFD) for 8 weeks and were divided into three groups including PSR, positive and model groups. PSRwe (75 mg/10 g), orlistat (6 μg/10 g) and normal saline (0.1 mL/10 g) were gavaged to the mice every day according to their body weight. The control group were given normal diet and normal saline (0.1 mL/10 g) (Ref. to Figure S1A).

Sample collection

At week 8 (the obese mice model was successfully constructed), feces from each group were collected for metagenomics. At Week 14, the mice were sacrificed at the end of the treatment, the colon contents were collected for metagenomics and metabonomics, the colon tissues were collected for RNA sequencing, plasma was collected for metabonomics, liver tissues were collected for RNA sequencing, metabonomics and histological examination (HE staining). Lung, spleen, kidney, stomach and heart were collected for HE staining.

Shotgun metagenomic sequencing and analysis of feces and colon cotents

DNA extraction

Fecal and colonic samples were thawed on ice to perform DNA extraction by using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen) according to manufacturer's instructions. Extracts were treated with DNase-free RNase to eliminate RNA contamination. DNA quantity was determined using NanoDrop spectrophotometer, Qubit Fluorometer (with the Quant-iTTMdsDNA BR Assay Kit), and gel electrophoresis.

DNA library construction and sequencing

DNA library was constructed as previously described (A metagenome-wide association study of gut microbiota in type 2 diabetes) to perform cluster generation, template hybridization, isothermal amplification, linearization, blocking and denaturation, and hybridization of the sequencing primers. Paired-end (PE) library with insert size of 350 bp for each sample was constructed, followed by a high-throughput sequencing with PE reads of length 2 × 150 bp. High-quality reads were obtained by filtering low-quality reads with ambiguous "N" bases, adapter and human DNA contamination from the BGI-SEQ500 raw reads, and by trimming low-quality terminal bases of reads simultaneously. Over 90% of the reads remained as high-quality reads (mapped to the hg19 reference).

Taxonomic and functional profiling acquisition

Taxonomic profiles were generated from high quality reads by using Metaphlan 3.0 (- input_type fastq - ignore_viruses - nproc 6) as described in the official website (MetaPhlAn3-The Huttenhower Lab (harvard.edu)).

For functional profiling, HUMAnN 3.0 (-i input_clean_data -o output –threads 10 –memory-use maximum - -remove-temp-output) was used to efficiently and accurately profile the abundance of microbial metabolic pathways and other molecular functions from the metagenomic sequencing data (clean data) according to the official website (https://huttenhower.sph.harvard.edu/humann).

Diversity calculation

Alpha-diversity (within-sample diversity, R 4.0.3 vegan: diversity (data, index = 'richness/shannon/Simpson/InSimpson')) was calculated using the richness, Shannon index, Simpson index and Inverse Simpson index depending on the taxonomic profiles. Beta-diversity (between-sample diversity, R 4.0.3 vegan: diversity (data, index = 'bray_curtis distance')) was calculated using the Bray-Curtis distance depending on the taxonomic profiles.

Transcriptomic sequencing and analysis for colonic and liver tissue

RNA extraction and library construction

Total RNA from colonic and liver tissue were isolated using the Trizol Reagent (Invitrogen Life Technologies). The concentration, quality and integrity were determined using a NanoDrop spectrophotometer (Thermo Scientific). Only samples with RNA integrity number (RIN) ≥ 7.0 were used to generate transcriptome libraries.

mRNA-enriched transcriptome libraries were constructed in the current study. Enriched mRNA was used for transcriptome library construction, using the TruSeq RNA Library Prep Kit v2 (Illumina). The sequencing library was then sequenced on NovaSeq 6000 platform (Illumina) to obtain image files to generate raw data (FASTQ format).

Quality control

Cutadapt (v1.15) software was used to eliminate connectors and low-quality reads to obtain high quality sequences (Clean Data) for further analysis. Non-rDNA/rRNA reads were then mapped to the bovine genome (UMD 3.1) using Tophat2 (version 2.0.9) as described by Kim D et al. [39] to remove potential host DNA and RNA contaminations.

Reads mapping

The reference genome and gene annotation files were downloaded from genome website. The filtered reads were mapping to the reference genome using HISAT2 v2.0.5 (HISAT2 (daehwankimlab.github.io)).

Differential analysis of genes expression

We used HTSeq (0.9.1) statistics to compare the Read Count values on each gene as the original expression of the gene, and then used FPKM to standardize the expression. Then difference expression of genes was analyzed by DESeq (1.30.0) with screened conditions as follows: expression difference multiple |log2 (Fold Change) | > 1, significant P-value < 0.05. At the same time, we used R language Pheatmap (1.0.8) software package to perform bi-directional clustering analysis of all different genes of samples. We retained heatmap according to the expression level of the same gene in different samples and the expression patterns of different genes in the same sample with Euclidean method to calculate the distance and Complete Linkage method to cluster.

GO annotation enrichment and KEGG pathways analysis

We mapped all the genes to Terms in the Gene Ontology database and calculated the numbers of differentially enriched genes in each Term. Using top GOs to perform GO enrichment analysis on the differential genes, calculate *P*-value by hypergeometric distribution method (the standard of significant enrichment is *P*-value < 0.05), and find the GO term with significantly enriched differential genes to determine the main biological functions performed by differential genes. For the transcriptomes, ClusterProfiler (3.4.4) software was used to carry out the enrichment analysis of the KEGG pathways of differential genes, focusing on the significantly enriched pathway with $P < 0.05$.

Metabolomics test of the colon contents, plasma and liver tissues

Sample preparation and extraction

Biological samples are freeze-dried by vacuum freeze-dryer (Scientz-100F). The freeze-dried sample was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. Dissolve 50 mg of lyophilized powder with 1.2 mL 70% methanol solution, vortex 30 seconds every 30 minutes for 6 times in total. Following centrifugation at 12000 rpm for 3 min, the extracts were filtrated (SCAA-104, 0.22 μm pore size; ANPEL,Shanghai, China, http://www.anpel.com.cn/) before UPLC-MS/MS analysis.

UPLC Conditions

The sample extracts were analyzed using an UPLC-ESI-MS/MS system (UPLC, SHIMADZU Nexera X2, https://www.shimadzu.com.cn/; MS, Applied Biosystems 6500 Q TRAP, https://www.thermofisher.cn/zh/home/brands/applied-biosystems.html). The analytical conditions were as follows, UPLC: column, Agilent SB-C18 (1.8 μm, 2.1 mm * 100 mm); The mobile phase was consisted of solvent A, pure water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5% B. Within 9 min, a linear gradient to 5% A, 95% B was programmed, and a composition of 5% A, 95% B was kept for 1 min. Subsequently, a composition of 95% A, 5.0% B was adjusted within 1.1 min and kept for 2.9 min. The flow velocity was set as 0.35 mL per minute; The column oven was set to 40°C; The injection volume was 2 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

ESI-Q TRAP-MS/MS

The ESI source operation parameters were as follows: source temperature 500°C; ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); ion source gas I (GSI), gas II(GSII), curtain gas (CUR) was set at 50, 60, and 25 psi, respectively; the collision-activated dissociation (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to medium. DP (declustering potential) and CE (collision energy) for individual MRM transitions was done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

PCA analysis

Unsupervised PCA (principal component analysis) was performed by statistics function prcomp within R (www.r-project.org). The data was unit variance scaled before unsupervised PCA.

Hierarchical Cluster Analysis and Pearson Correlation Coefficients

The HCA (hierarchical cluster analysis) results of samples and metabolites were presented as heatmaps with dendrograms, while pearson correlation coefficients (PCC) between samples were caculated by the cor function in R and presented as only heatmaps. Both HCA and PCC were carried out by R package ComplexHeatmap. For HCA, normalized signal intensities of metabolites (unit variance scaling) are visualized as a color spectrum.

Differential metabolites selected

Significantly regulated metabolites between groups were determined by VIP (VIP ≥ 1) and absolute Log2FC (|Log2FC| ≥ 1.0). VIP values were extracted from OPLS-DA result, which also contain score plots and permutation plots, was generated using R package MetaboAnalystR. The data was log transform (log2) and mean centering before OPLS-DA. In order to avoid overfitting, a permutation test (200 permutations) was performed.

KEGG annotation and enrichment analysis

Identified metabolites were annotated using KEGG Compound database (http://www.kegg.jp/kegg/compound/), annotated metabolites were then mapped to KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Pathways with significantly regulated metabolites mapped to were then fed into MSEA (metabolite sets enrichment analysis), their significance was determined by hypergeometric test's p-values.

Declarations

Ethics approval and Consent to participate

The animal experimental protocol was approved by animal ethics committee of Lanzhou University Second Hospital with the approval number of D2022-135.

Consent for publication

All authors agreed to the publication of this article.

Availability of data and materials

The original multiomics datasets used and/or analyzed during the current study are publicly available at China National GeneBank DataBase (CNGBdb, https://db.cngb.org/) with an access number of

CNP0004326.

Competing interests

The authors declare no competing interests.

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Authors' contributions

X. O. and Q. W. conceived the project and the article. X. O. coordinated the whole project and prepared Figure S1-S2. Q. W. was in charge of multiomics data analysis and prepared Figure 1-10, Figure S3-S8. J. C. corrected all the figures and wrote the main manuscript text. B. L. was responsible for completing animal experiments and collecting samples. Y. Y., K. G., Z. L., H. L., D. L., X. L. assisted in animal experiments and sample collection. X. X. and Z. X. assisted in multiomics data analysis. All authors reviewed the manuscript.

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Figures

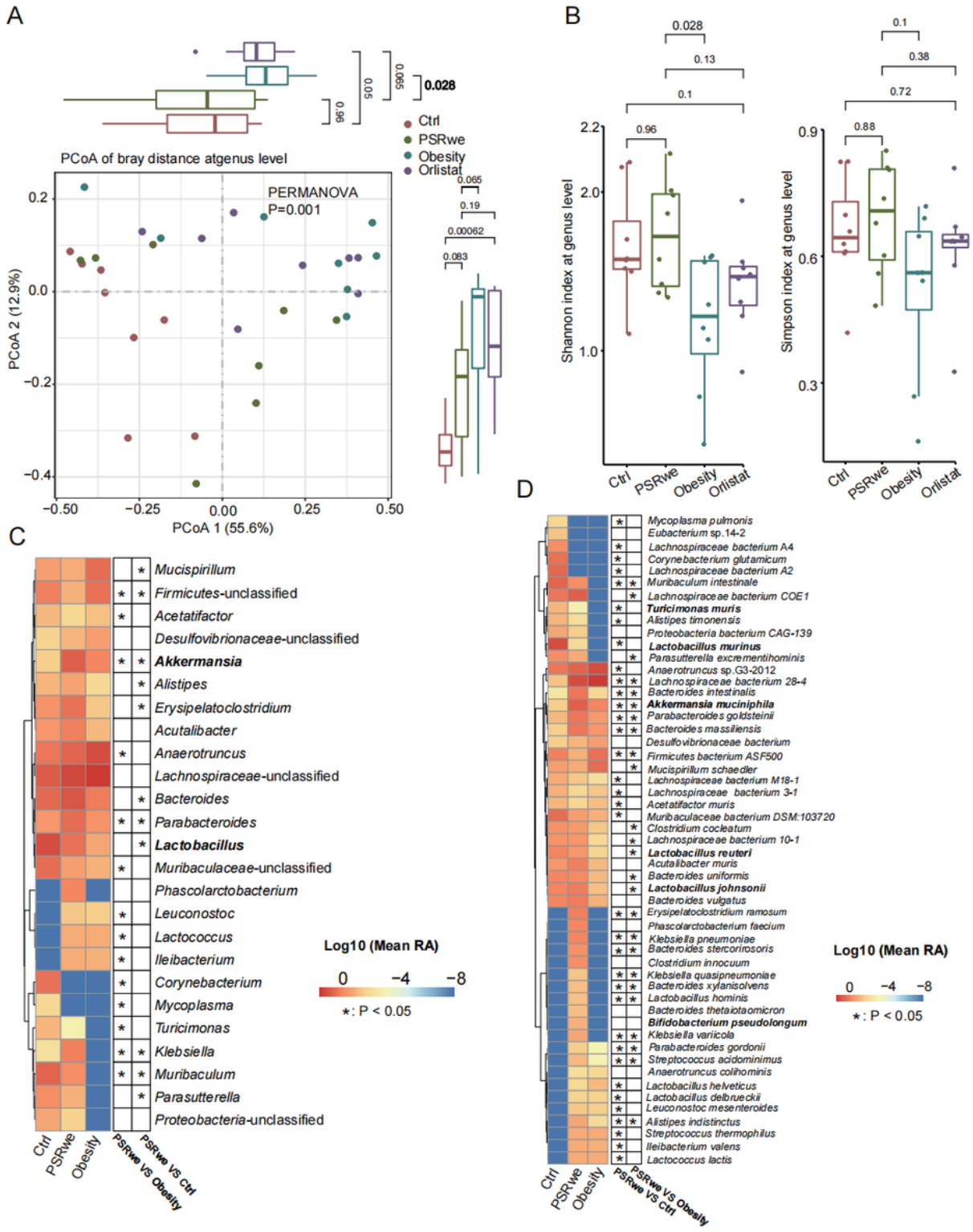


Figure 1

Changes of the gut microbial composition. (A) PCoA analysis of the gut mircobiota among groups. (B) Diversity analysis based on Shannon index and Simpson index. (C) Significantly different genera among groups. (D) Significantly different species among groups.

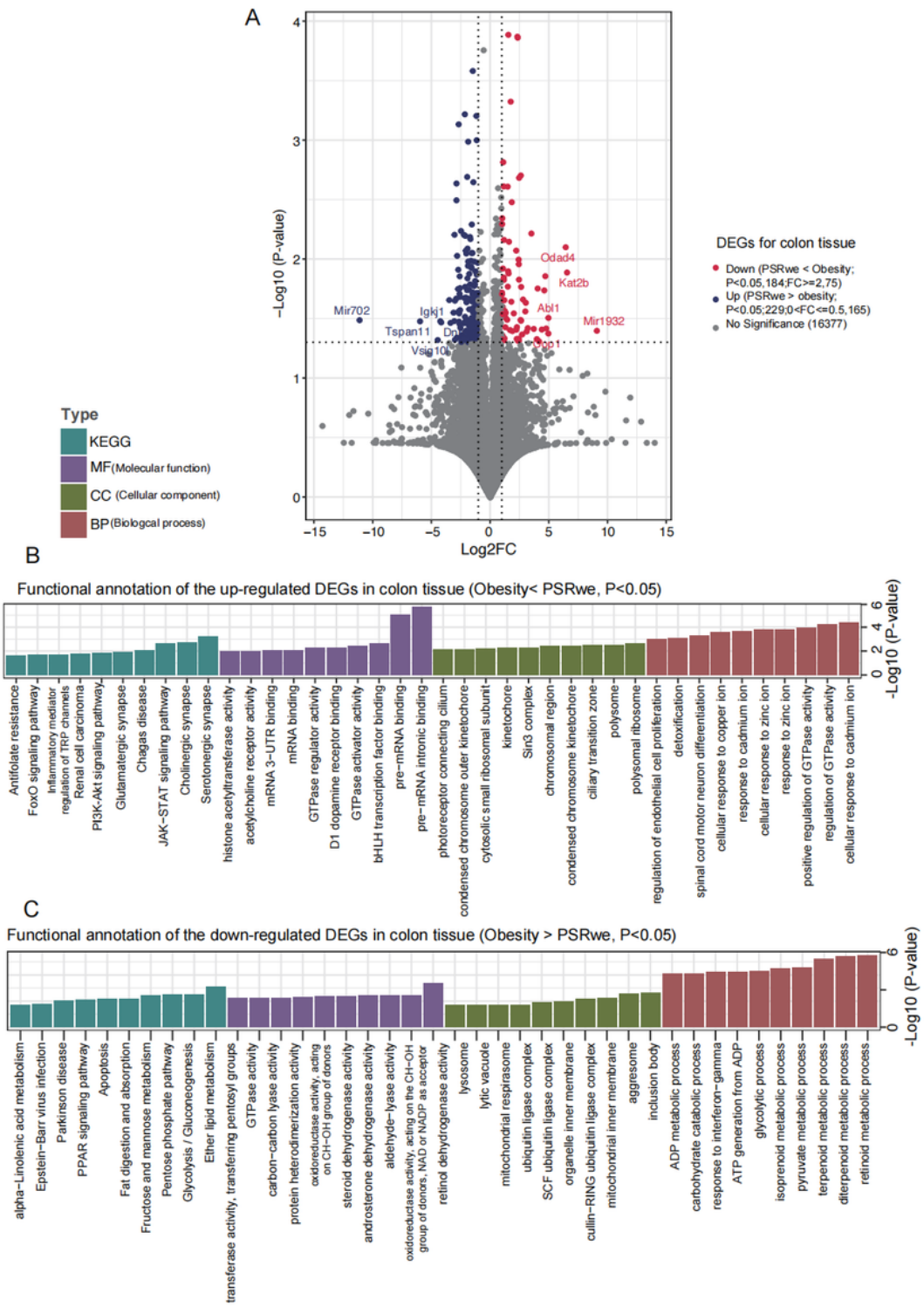


Figure 2

DEGs and their function in colonic tissues. (A) Heatmap of the DEGs between PSRwe and obesity group. GO annotation and KEGG pathway analysis of the up-regulated DEGs (B) and down-regulated DEGs (C).

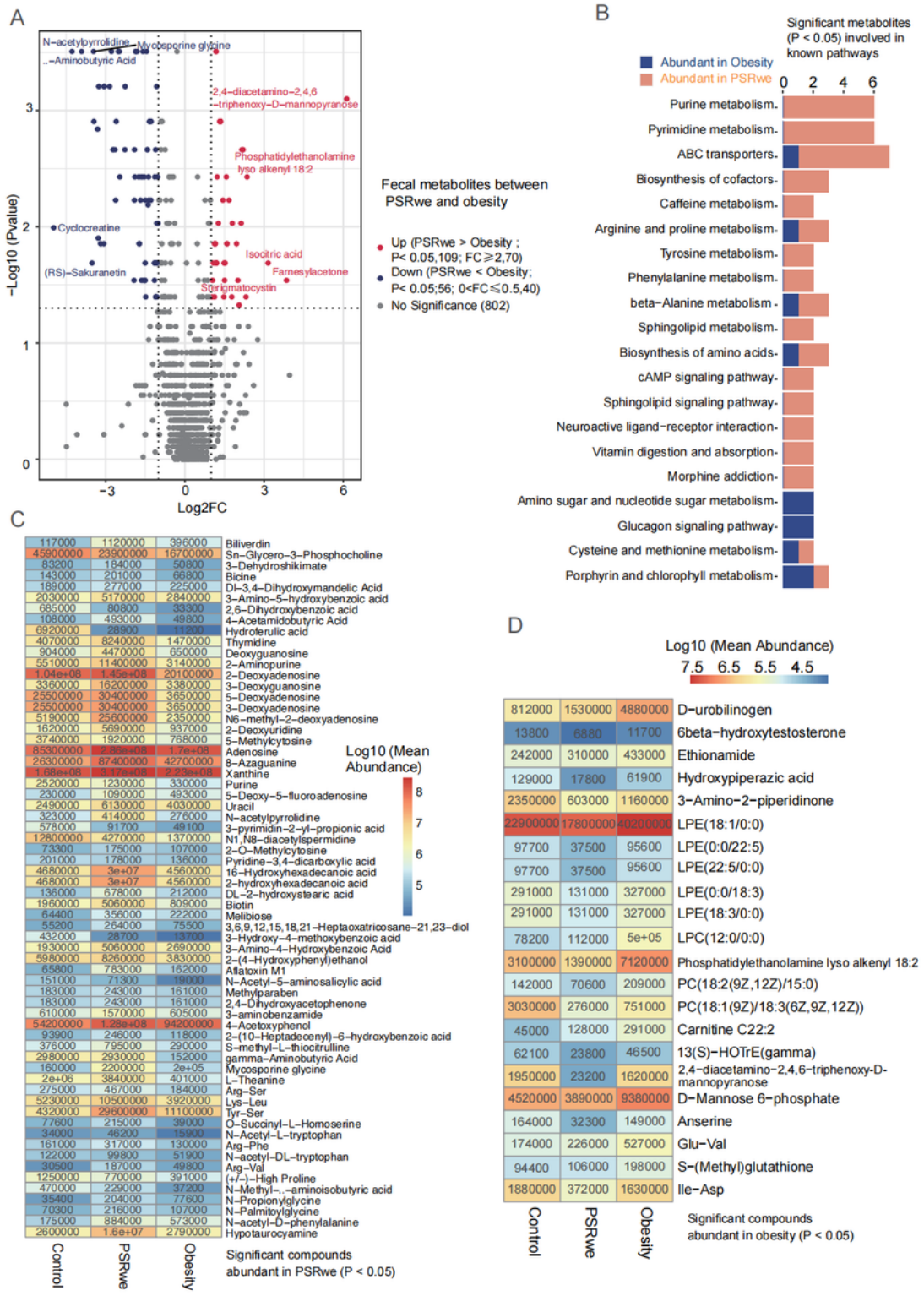


Figure 3

Differential analysis of the metabolites from colonic contents. (A) Heatmap of the metabolites between PSRwe and obesity group. (B) Pathways participated by the different metabolites. Significantly enriched metabolites in PSRwe group (C) and obesity group (D).

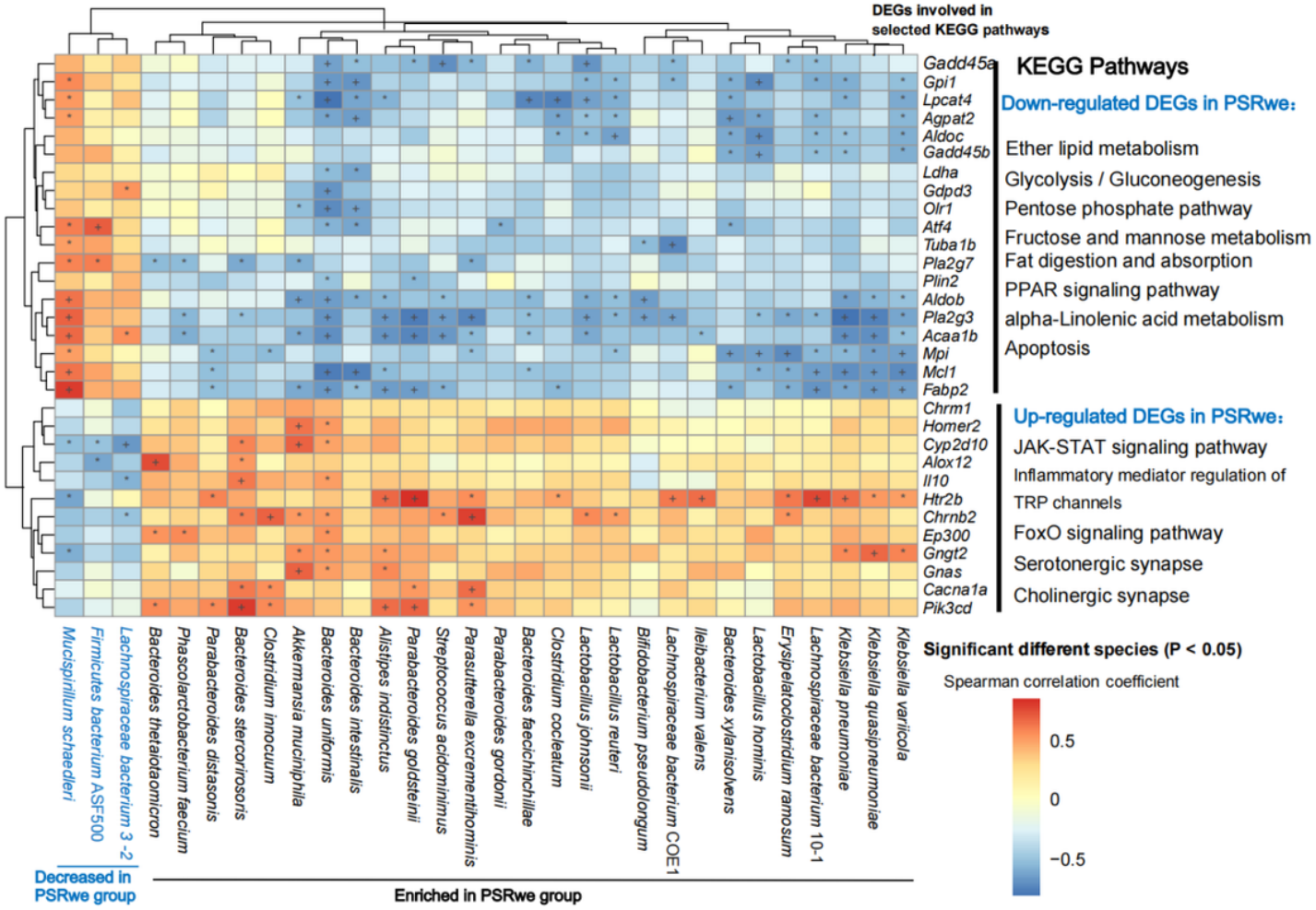


Figure 4

Correlation analysis between the different species and DEGs contribute to lipid metabolism.

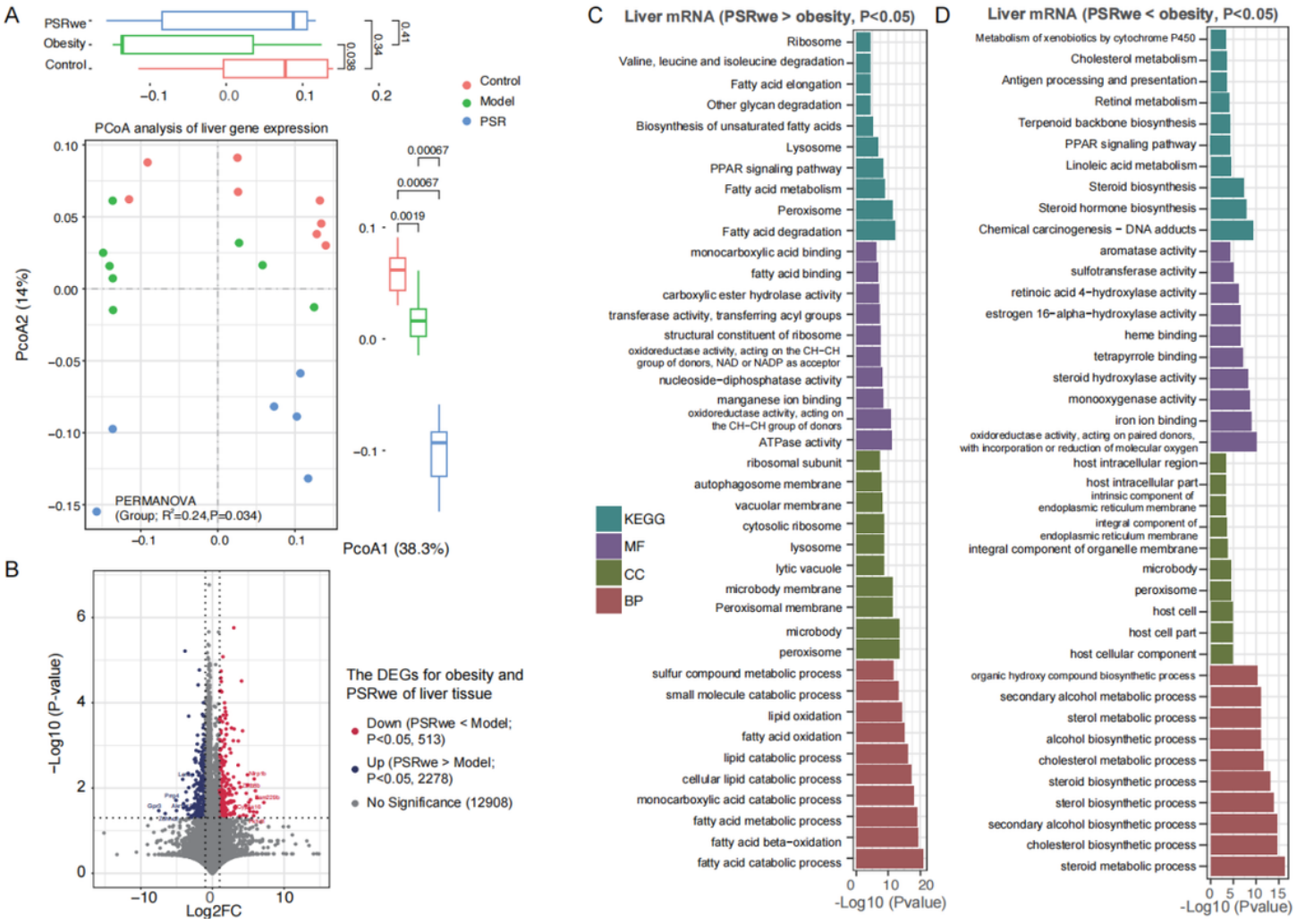


Figure 5

Liver gene expression between PSRwe and obesity group. (A) PcoA analysis of liver gene expression. (B) Differential analysis of liver DEGs. GO annotation and KEGG pathway analysis for the up-regulated DEGs (C) and down-regulated DEGs (D).

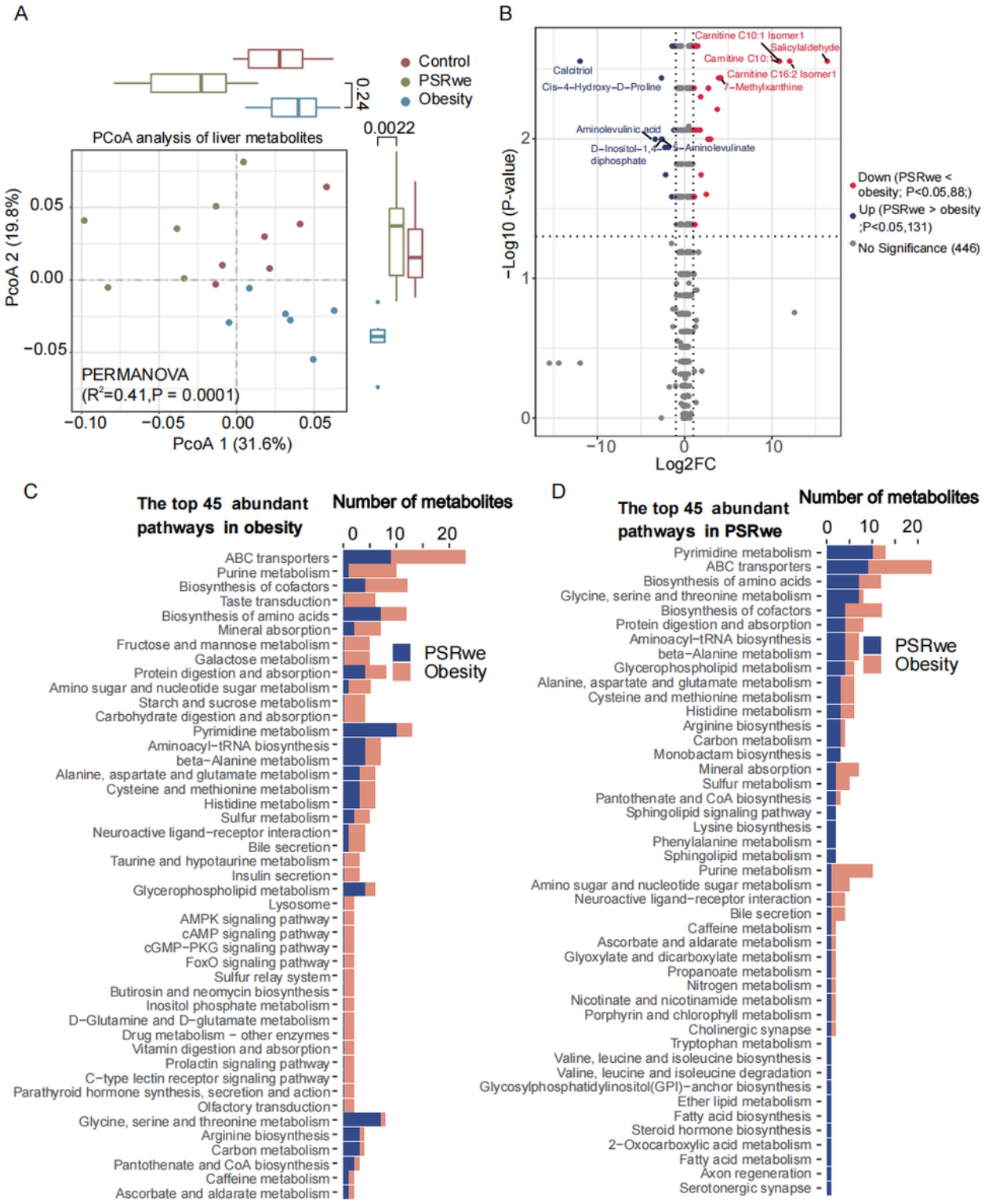


Figure 6

Liver metabolites were significantly different between PSRwe and obesity group. (A) PcoA analysis of liver metabolites. (B) Differential analysis of liver metabolites. The top 45 pathways for significantly different metabolites enriched in obesity (C) and PSRwe (D) group.

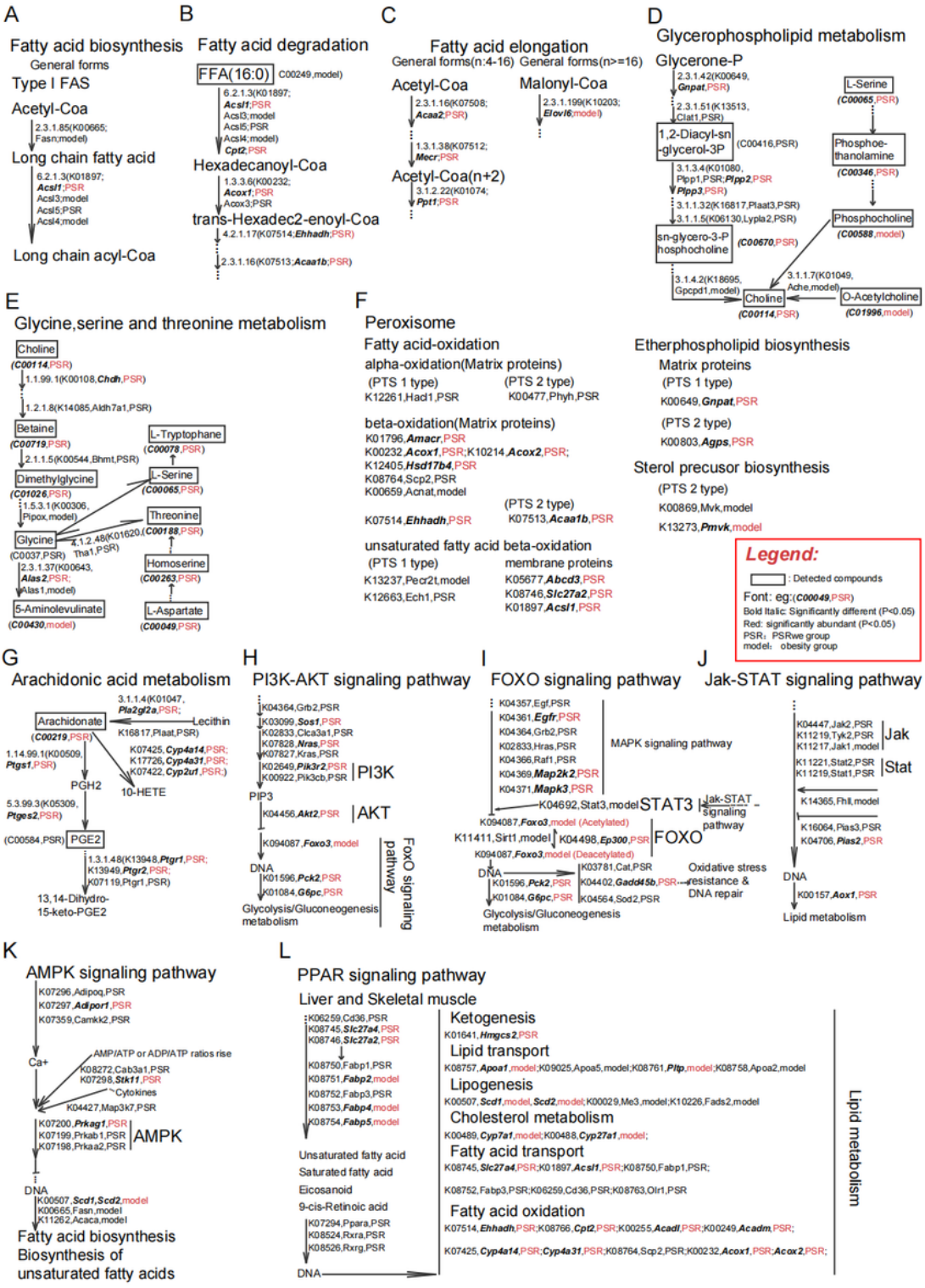


Figure 7

KEGG pathways involved for lipid metabolism.

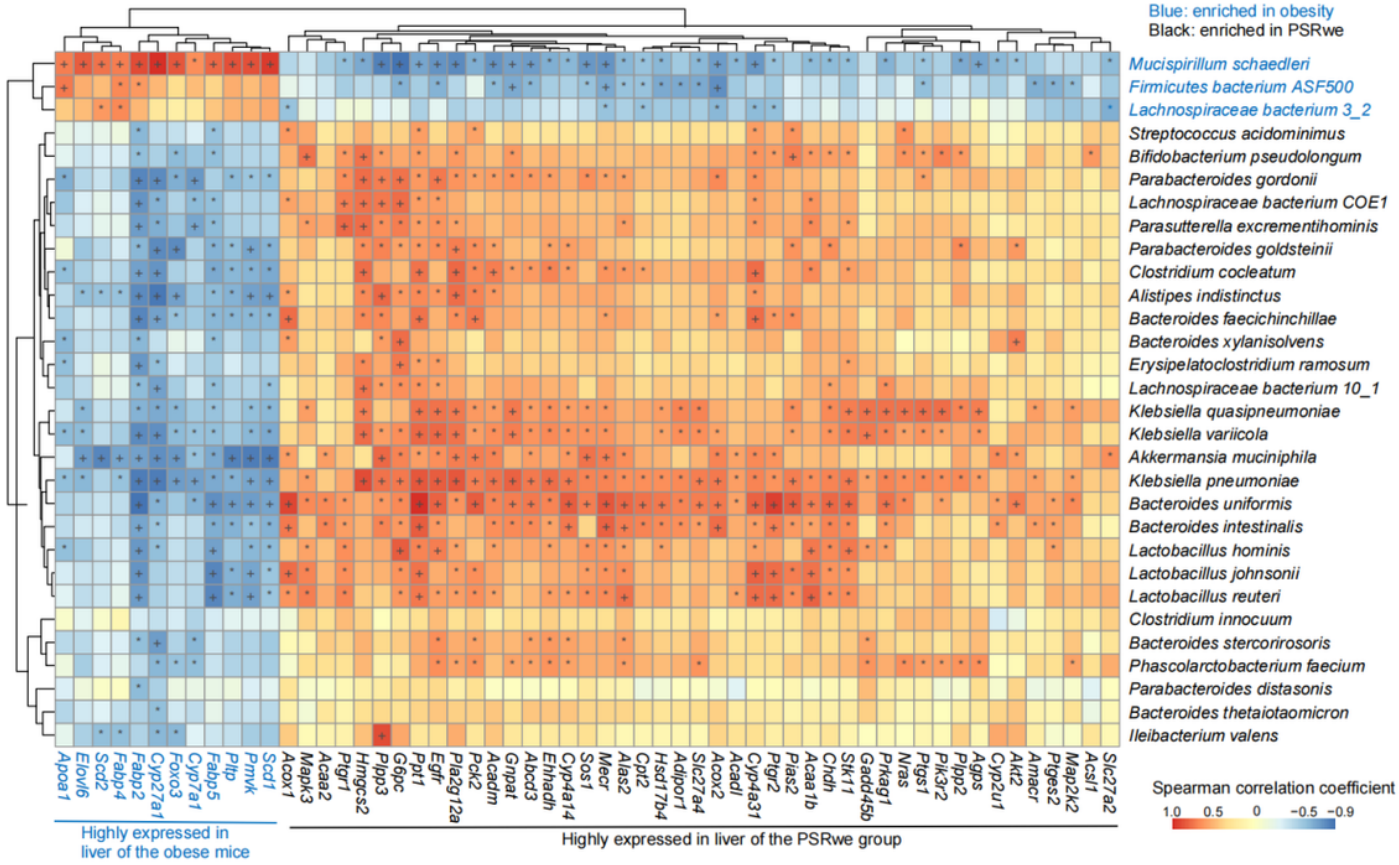


Figure 8

Correlation analysis between significantly different species and liver DEGs contribute to lipid metabolism.

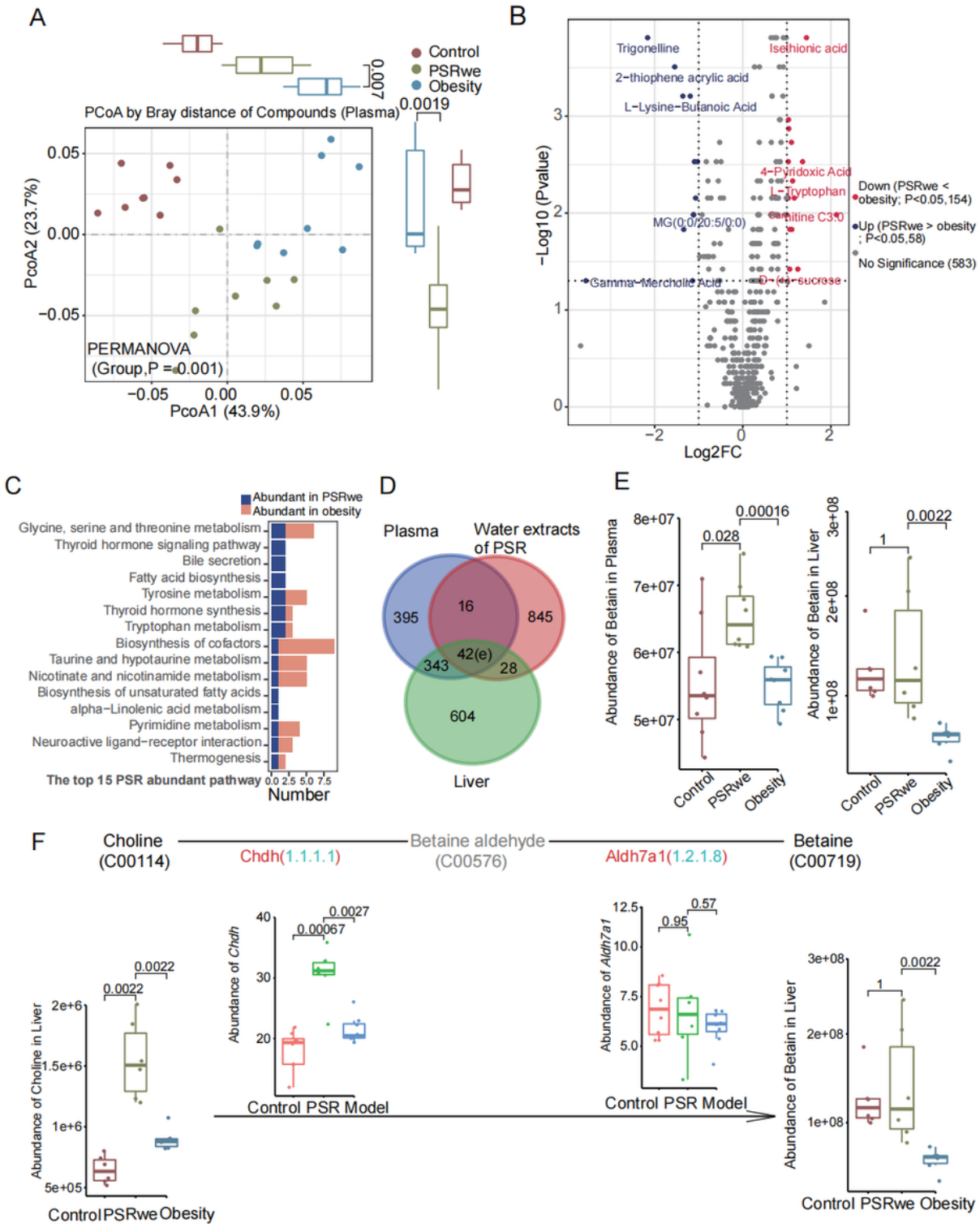


Figure 9

Discovery of effective component on obesity. (A) PcoA analysis of the metabolites in plasma. (B) Differential analysis of metabolites in plasma. (C) The top 15 pathways participated by significantly different metabolites in PSRwe group. (D) Venn diagram of plasma, liver and PSR water extracts. (E) Levels of betaine in plasma and liver. (F) Choline was metabolized to betaine by *chdh* and *aldh7a1*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplFigures.docx