Research Square

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Integrated Analysis of Serum Non-Targeted Metabolomics and Lipidomics for Exploring the Mechanism of *Lactobacillus rhamnosus* in Regulating Fat Deposition in Mice

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Abstract

Objective

This study aimed to explore the regulatory effect and potential molecular mechanism of *Lactobacillus rhamnosus* on fat deposition in mice.

Methods

Twenty weaned male C57BL/6j mice were divided into control group (CK, *n* = 10) and *Lactobacillus rhamnosus* gavage group (LA, *n* = 10). The total experimental period was 44 days, including a 14-day pre-experiment adaptation period and a 30-day formal experimental period. At the end of the trial, body weight, abdominal fat content and fecal short-chain fatty acids (SCFAs) were determined. Combined with serum non-targeted metabolomics and lipidomics, the molecular regulation mechanism of *Lactobacillus rhamnosus* on fat deposition in mice was systematically investigated.

Results

There was no significant difference in body weight between LA group and CK group, while the abdominal fat content of LA group increased significantly ($P < 0.05$). SCFA detection showed that the contents of acetic acid, propionic acid, butyric acid and isovaleric acid in LA group were extremely significantly higher than those in CK group ($P < 0.01$), while isobutyric acid and valeric acid had no significant difference (*P*>0.05). Metabolomic analysis screened a total of 2186 differential metabolites (1669 up-regulated and 517 down-regulated), which were mainly enriched in lipid metabolism-related pathways such as glycerophospholipid metabolism, α-linolenic acid metabolism, linoleic acid metabolism and arachidonic acid metabolism. Lipidomic analysis identified 185 differential lipids (132 up-regulated and 53 down-regulated). Among them, PC, PE, PS and PI were significantly up-regulated, while Cer-s and Cer-NDS were significantly down-regulated. Differential lipids were mainly enriched in glycosylphosphatidylinositol anchor biosynthesis, inositol phosphate metabolism and phosphatidylinositol signaling system pathways.

Conclusion

In conclusion, *Lactobacillus rhamnosus* can promote abdominal fat deposition in mice without affecting body weight. It may regulate fat deposition by modulating intestinal SCFAs, glycerophospholipid metabolism and phosphatidylinositol signaling pathways. This study provides experimental evidence for elucidating the interaction network between probiotics and host lipid metabolism.

Clinical trial registration

Not applicable.

Introduction

Adipose tissue is an indispensable vital component in humans and animals, which plays key physiological roles in energy storage, immune regulation and the stability of internal environmental homeostasis[1]. For livestock and poultry, fat deposition is one of the most important economic traits. It not only affects growth performance, but is also closely related to multiple meat quality indicators such as flavor, tenderness, juiciness and meat color[2]. In recent years, the important role of gut microbiota in regulating host fat accumulation has been gradually recognized.Intestinal microorganisms can decompose diet into various metabolites and signaling molecules such as short-chain fatty acids (SCFAs), bile acids (BAs) and lipopolysaccharides (LPS), thereby participating in regulating host energy utilization, lipid anabolism and immune response[3]. With the development of high-throughput sequencing technology and bioinformatics, a number of studies have confirmed that gut microbiota structure is significantly associated with lipid metabolism disorders such as obesity, diabetes and metabolic-associated fatty liver disease[4–6]. Therefore, rational intervention of imbalanced intestinal microecology is expected to improve host fat accumulation and effectively alleviate related metabolic diseases[7].

Probiotics are active microorganisms that can benefit the host when ingested at an adequate dose, serving as an important intervention means to improve host health[8]. Existing studies have confirmed that probiotics can exert positive effects on improving lipid metabolism disorders by regulating gut microbiota composition, energy metabolism and lipid metabolism[9–10]. Among them, *Lactobacillus rhamnosus*, as one of the common probiotic lactic acid bacteria, has attracted much attention in regulating host metabolism due to its good intestinal colonization ability and biological activity[11].Studies have shown that *Lactobacillus rhamnosus* can improve intestinal microenvironment, regulate the synthesis of short-chain fatty acids and related metabolic signaling pathways, thereby improving insulin resistance and reducing fat accumulation[12]. It also has positive effects on inflammatory bowel disease and cardiovascular diseases, and can prevent fatty liver and its secondary inflammation to a certain extent[13]. However, some studies have found that the effects of probiotics on improving intestinal health vary depending on dosage, strain and individual characteristics[14]. Such differences indicate that the regulation of lipid metabolism by *Lactobacillus rhamnosus* has obvious strain specificity, and its underlying molecular mechanism has not been fully clarified.

In view of the above, this study took C57BL/6J mice as animal models and intervened via intragastric administration of *Lactobacillus rhamnosus*. Phenotypic indexes such as abdominal fat content and short-chain fatty acids were determined. Combined with serum non-targeted metabolomics and broad-target lipidomics technologies, we systematically screened differential metabolites and key regulatory pathways, and clarified the metabolic mechanism of *Lactobacillus rhamnosus* regulating host fat deposition. The results provide a theoretical basis for the application of probiotics in improving human metabolic diseases, healthy breeding of livestock and poultry, and precise regulation of fat deposition.

Materials and Methods

1.1 Experimental Materials

Twenty SPF-grade weaned male C57BL/6j mice with similar body weight (12.0 ± 0.5 g) were purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The freeze-dried powder of Lactobacillus rhamnosus (ATCC7469) was provided by the College of Animal Science and Technology, Gansu Agricultural University. All experimental animals were raised in an SPF-level feeding environment with a 12 h/12 h light-dark cycle, free access to feed and drinking water, and the ambient temperature was controlled at 24 ± 2 ℃. All animal experiments were approved by the Animal Ethics Committee of the College of Animal Science and Technology, Gansu Agricultural University (GSAU-Eth-ASF-2022-010).

1.2 Experimental Methods

1.2.1 Experimental Design

Referring to the method of Chevalier, C57BL/6j mice were pretreated with antibiotics for 14 days (vancomycin 0.5 g + penicillin 1 g + neomycin 1 g + metronidazole 1 g, mixed and dissolved in 1 L pure water) to eliminate the inherent intestinal microbiota of mice. During the pretreatment period, drinking water containing mixed antibiotics was replaced every day, and mice could eat freely.

After pretreatment, mice were divided into two groups: control group (CK, n = 10) administered with normal saline by gavage, and experimental group (LA, n = 10) administered with Lactobacillus rhamnosus suspension (10^8 CFU/mL). The gavage volume was 200 μL per time, administered every other day for 30 consecutive days.

1.2.2 Bacterial Enrichment and Plate Count

The freeze-dried strain was inoculated into 5 mL LB medium (tryptone 10.0 g/L, sodium chloride 10.0 g/L, yeast extract 5.0 g/L) and mixed evenly. Then 2 mL bacterial liquid was added into 200 mL LB medium and cultured at 37 ℃ and 140 r/min for 12–48 h. When the OD_{600} value of bacterial liquid reached about 1, the bacterial liquid was diluted in gradients of 10^{-3}, 10^{-4} and 10^{-5} and spread on solid LB plates. Bacterial colonies were observed after 48 h culture and counted by a plate counter. The bacterial liquid with the concentration up to 10^8 CFU/mL was used for subsequent mouse gavage experiments(Fig. 1).

1.2.3 Sample Collection

Animals were intraperitoneally anesthetized with ready-to-use Avertin (tribromoethanol, KKL). Mice were administrated 1.25% working solution at a dosage of 200–300 μL per 10 g body weight. This anesthetic exhibits rapid onset of action and prolonged anesthesia duration with controllable side effects, superior to conventional chloral hydrate.Prior to sample collection, mice were euthanized via intraperitoneal injection of the same concentration of Avertin in compliance with international laboratory animal ethics guidelines. Complete analgesia was achieved throughout the procedure to minimize stress and suffering of experimental animals.

Mice were fasted for 12 h before slaughter. Fecal samples were collected first, subpackaged, quickly frozen in liquid nitrogen, and then stored at − 80 ℃ for subsequent SCFAs detection. All mice were anesthetized with isoflurane. Blood samples were collected from the retro-orbital venous plexus, mixed gently, placed at 4 ℃ for 3 h, and centrifuged at 3000×g for 15 min. The upper serum was separated, subpackaged and stored at − 80 ℃ for metabolomics and lipidomics analysis. Meanwhile, abdominal adipose tissues were collected, placed into 2 mL cryopreservation tubes, snap-frozen in liquid nitrogen and stored at − 80 ℃ for the determination of abdominal fat related indexes.

1.2.4 Index Determination

Abdominal fat content determination: Soxhlet extraction method was used to determine abdominal fat content. The specific procedures were as follows: the tested tissues were dried and dehydrated at 105 ℃ and ground into uniform powder; 2–5 g sample (accurate to 0.001 g) was weighed into a filter cartridge and wrapped tightly with degreased filter paper; the extraction flask was heated in a water bath at 60–70 ℃ to vaporize the extraction solvent, which was condensed and dripped into the sample tube; the solvent fully soaked the sample to gradually dissolve lipids in tissues; when the liquid level rose to the upper limit of the siphon, the solvent dissolved with lipids flowed back to the flask and circulated continuously for 6–8 h until complete extraction. After heating, most of the solvent was recovered by distillation, and the residual solvent was naturally volatilized in a ventilated environment. Finally, fat content was calculated by weighing.

SCFAs content determination: Gas chromatography (GC) was used to detect fecal SCFAs content. Briefly, 0.2 g feces was added with 1 mL double distilled water for homogenization, centrifuged at 11000×g and 4 ℃ for 10 min, and the supernatant was taken. The supernatant was mixed with 25% sodium metaphosphate solution at a volume ratio of 1:5, shaken thoroughly, and acidified in ice bath for 30 min. After centrifugation again at 11000×g and 4 ℃ for 10 min, the clear supernatant was taken and determined by gas chromatography for SCFAs content.

1.2.5 Serum Non-Targeted Metabolomics Detection

Serum non-targeted metabolomics detection was completed by Wuhan Kangsixuyuan Biotechnology Co., Ltd. Frozen samples were taken out from − 80 ℃ refrigerator, thawed and vortexed for 10 s (all subsequent operations were performed on ice). Then 50 μL sample was absorbed into a 1.5 mL EP tube, added with 300 μL 20% acetonitrile-methanol internal standard extract, fully vortexed for 3 min, and centrifuged at 12000 r/min and 4 ℃ for 10 min. A total of 200 μL supernatant was transferred to a new numbered centrifuge tube, placed at − 20 ℃ for 30 min, centrifuged again at 12000 r/min and 4 ℃ for 3 min, and 180 μL supernatant was transferred to LC injection vials for subsequent LC-MS analysis. Chromatography-mass spectrometry combined technology was adopted for sample analysis. ACQUITY Premier HSS T3 column (100 mm×2.1 mm, 1.8 μm; Waters) was used for chromatographic analysis, and mass spectrometry detection was performed in positive and negative ion modes.

Raw data were processed by XCMS software for characteristic peak identification, retention time calibration and peak alignment. Characteristic peaks with a missing rate higher than 50% in each group

were eliminated. Quality control (QC) samples were prepared by mixing extracts of all samples. The empirical cumulative distribution function (ECDF) was used to evaluate the distribution frequency of substances with coefficient of variation (CV) lower than the reference value. Screening criteria: VIP > 1, $|\log_2 FC|>1$, FDR < 0.05. Hypergeometric test was finally used for KEGG enrichment analysis of differential metabolites.

1.2.6 Lipidomics Detection

Broad-target lipidomics detection was completed by Wuhan Kangsixuyuan Biotechnology Co., Ltd. Frozen samples were thawed on ice, 20 mg sample was weighed into a labeled 2 mL centrifuge tube, grinding steel beads were added with sterile forceps, homogenized by ball mill at 30 Hz for 20 s, and centrifuged at 3000 r/min and 4 ℃ for 30 min. Then 1 mL lipid extract containing internal standard (methyl tert-butyl ether : methanol = 3:1, v/v) was added and vortexed for 15 min. Subsequently, 200 μL pure water was added, vortexed for 1 min, centrifuged at 12000 r/min and 4 ℃ for 10 min, and 200 μL supernatant was taken to a labeled centrifuge tube and concentrated to dryness. Then 200 μL lipid redissolution solvent (acetonitrile : isopropanol = 1:1, v/v) was added, vortexed for 3 min, centrifuged at 12000 r/min for 3 min, and the supernatant was taken for LC-MS/MS analysis. Thermo Accucore™ C30 chromatographic column (2.6 μm, 2.1 mm×100 mm) was used. Mass spectrometry parameters were set as follows: electrospray ionization (ESI) source, ion source temperature 500 ℃, ionization voltage + 5500 V in positive ion mode and − 4500 V in negative ion mode.

Mass spectrometry raw data were processed by Analyst 1.6.3 software. QC samples were prepared by mixing all sample extracts. ECDF was used to evaluate the distribution characteristics of substances with CV lower than the reference value. MultiQuant software was used to process raw mass spectrometry data for chromatographic peak integration and retention time correction. Screening threshold: VIP > 1, $|\log_2 FC|>1$, FDR < 0.05. Hypergeometric test was adopted for KEGG enrichment analysis of differential lipids.

1.2.7 Statistical Analysis

Excel 2019 was used for preliminary data sorting, SPSS 27.0 for statistical analysis, and Origin 2024 for plotting. Data were expressed as mean ± standard deviation. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and ns indicates no significant difference ($P > 0.05$).

Results

2.1 Determination and Analysis of Physiological Indexes in Mice

There was no significant difference in body weight between LA group and CK group. After 4 weeks of gavage, abdominal adipose tissues were collected rapidly after slaughter. The determination of abdominal fat content showed that the fat content of LA group was significantly higher than that of CK

group, indicating that gavage of Lactobacillus rhamnosus could increase abdominal fat content. Fecal SCFAs detection results showed that the contents of acetic acid, propionic acid, butyric acid and isovaleric acid in LA group were extremely significantly higher than those in CK group ($P < 0.01$), while isobutyric acid and valeric acid had no significant change ($P > 0.05$)(Fig. 2)..

2.2 Serum Non-Targeted Metabolomics Analysis

2.2.1 OPLS-DA Analysis Between Groups

To explore the overall differences of serum metabolic profiles between the two groups, orthogonal partial least squares discriminant analysis (OPLS-DA) was used to model metabolomics data. The results showed that samples of LA group and CK group were completely separated on the score plot, indicating significant differences in serum metabolism between Lactobacillus rhamnosus treated mice and control mice(Fig. 3A).

2.2.2 Identification of Serum Metabolites

Based on OPLS-DA model, with screening criteria of P < 0.01, FC > 4.0 and VIP > 2, a total of 2186 differential metabolites were identified between the two groups, including 1669 up-regulated and 517 down-regulated metabolites(Fig. 3B). Cluster heatmap showed obvious clustering separation between LA group and CK group(Fig. 3C). Compared with CK group, lipid metabolism-related metabolites such as glycerolipids, glycerophospholipids, sphingolipids and fatty acyls were significantly up-regulated in LA group, while organic acids and derivatives, carbohydrates and derivatives were significantly down-regulated. It suggested that Lactobacillus rhamnosus mainly regulated fat deposition by activating lipid metabolism-related pathways.

2.2.3 Enrichment Analysis of Differential Metabolites

KEGG enrichment analysis showed that there were 12 significantly enriched pathways between the two groups, mainly involving glycerophospholipid metabolism, α-linolenic acid metabolism, linoleic acid metabolism, arachidonic acid metabolism and other lipid metabolism-related pathways(Fig. 4).

2.3 Lipidomics Analysis

2.3.1 OPLS-DA Analysis Between Groups

OPLS-DA modeling of lipidomics data showed obvious separation between LA group and CK group, indicating significant differences in lipid metabolites between the two groups(Fig. 5A).

2.3.2 Identification of Lipid Metabolites

Under positive and negative ion modes, a total of 185 differential lipids were screened out, including 132 up-regulated and 53 down-regulated lipids(Fig. 5B). Cluster heatmap showed the distribution and clustering of differential lipid molecules between LA and CK groups. PC, PE, PS and PI were significantly

up-regulated, while Cer-s and Cer-NDS were significantly down-regulated, suggesting that Lactobacillus rhamnosus might affect fat deposition by promoting phospholipid synthesis(Fig. 5C).

2.3.3 Enrichment Analysis of Differential Lipids

KEGG enrichment analysis showed 14 significantly enriched pathways, mainly including glycosylphosphatidylinositol (GPI) anchor biosynthesis, inositol phosphate metabolism, phosphatidylinositol signaling system, glycine, serine and threonine metabolism and other lipid metabolism-related pathways(Fig. 6).

Discussion

Gut microbiota serves as a crucial regulatory factor maintaining host metabolic homeostasis, and alterations in its composition and function are closely associated with metabolic phenotypes such as fat deposition and energy metabolism imbalance[15]. As a widely applied probiotic strain, the regulatory effect and molecular mechanism of Lactobacillus rhamnosus on host lipid metabolism vary remarkably due to strain specificity and host physiological status[16]. Therefore, based on the determinations of body weight, abdominal fat content, fecal short-chain fatty acids (SCFAs), as well as metabolomics and lipidomics results, this study analyzed the changes in metabolic characteristics related to fat deposition in mice after intragastric administration of Lactobacillus rhamnosus, and further explored its potential regulatory pathways.

This study found that there was no significant difference in body weight between the LA group and the CK group, whereas the abdominal fat content was markedly increased in the LA group. The results indicated that intervention with Lactobacillus rhamnosus did not obviously alter the overall growth performance of mice, but might affect the in vivo lipid distribution pattern. In other words, the effect of this strain was mainly reflected in body composition remodeling, rather than simply causing weight gain or loss. Previous studies have demonstrated that certain probiotics can modulate adipose tissue deposition without significant changes in body weight, which may be related to adipocyte differentiation, lipid transport, and the redistribution of host energy allocation[17].

SCFAs are vital metabolites produced by gut microbial fermentation and play an indispensable role in the regulation of host lipid metabolism[18]. In the present study, the concentrations of acetic acid, propionic acid, butyric acid and isovaleric acid in feces were extremely significantly elevated in the LA group, while isobutyric acid and valeric acid showed no remarkable changes. These findings suggested that Lactobacillus rhamnosus could specifically regulate intestinal SCFA profiles in mice.

As the predominant component of SCFAs, acetic acid participates in the generation of acetyl-CoA and is involved in metabolic processes related to fatty acid synthesis[19]. Sun C et al.[20] reported that acetic acid could promote the formation of more white adipose tissue, increase the number of lipid droplets, and accelerate the differentiation rate of adipose mesenchymal stem cells in obese model mice. Propionic acid regulates systemic metabolism by inhibiting hepatic gluconeogenesis and the activities

of key enzymes involved in lipid synthesis. Butyric acid acts as the primary energy substrate for intestinal epithelial cells and can upregulate the expression of pivotal lipogenic genes, including SREBP-1C (the core regulator of de novo fatty acid synthesis) and adipocyte differentiation marker genes such as PPARγ, C/EBPα and C/EBPβ[21]. Accordingly, the increased levels of multiple SCFAs in the LA group indicated that this strain might regulate host lipid metabolism by remodeling the intestinal metabolic microenvironment.

Metabolomic analysis identified a total of 2186 differential metabolites between the two groups, among which 1669 were up-regulated and 517 were down-regulated. KEGG enrichment analysis revealed 12 significantly enriched pathways, mainly including lipid metabolism-related pathways such as glycerophospholipid metabolism, α-linolenic acid metabolism, linoleic acid metabolism, and arachidonic acid metabolism. Notably, glycerophospholipid metabolites were significantly up-regulated in the LA group. Glycerophospholipids are essential structural components of cell membranes, and their alterations generally reflect changes in membrane lipid remodeling, lipid transport, or signal transduction[22]. Combined with the enrichment results of the glycerophospholipid metabolism pathway, it was speculated that Lactobacillus rhamnosus could enhance metabolic processes associated with membrane lipid renewal and lipid synthesis, thereby modulating fat deposition. As essential fatty acids for organisms, the activation of α-linolenic acid, linoleic acid and arachidonic acid metabolic pathways can facilitate the synthesis of polyunsaturated fatty acids, and further affect adipocyte differentiation and lipid accumulation. Studies have shown that hepatic polyunsaturated fatty acids can be assembled into very low-density lipoprotein (VLDL) through derivation or direct processing, subsequently entering the blood circulation and depositing in adipose, muscle, brain and other tissues via lipoprotein lipase for utilization by the body[23]. Hence, it is inferred that Lactobacillus rhamnosus regulates fat deposition by activating these lipid metabolism-related pathways.

Lipidomic analysis revealed distinct lipid profiles between the LA group and the CK group. A total of 185 differential lipids were screened, including 132 up-regulated and 53 down-regulated lipids. These differential lipids were mainly enriched in three lipid synthesis-related pathways: glycosylphosphatidylinositol (GPI) anchor biosynthesis, inositol phosphate metabolism, and phosphatidylinositol signaling system. The key differential lipids including PC, PE, PS and PI were significantly up-regulated in the LA group. These lipids play fundamental roles in the structural constitution of cell membranes and lipoproteins, and may be closely associated with the specific abdominal fat deposition induced by Lactobacillus rhamnosus. PC, PE and PS are critical structural lipids constituting cell membranes and lipoproteins, and their metabolic changes can directly reflect the metabolic activity and functional status of adipose tissue[24]. As a storage carrier of polyunsaturated fatty acids (PUFAs), PC not only participates in the regulation of inflammatory responses[25], but also reduces serum triglyceride (TG) and total cholesterol levels to modulate blood lipid metabolism[26]. In addition, PC can activate B-cell signal transduction via PPARγ and alleviate the progression of metabolic disorders caused by abnormal lipid metabolism[27]. PE is a major component of cell membranes and is essential for maintaining cell structural integrity and selective permeability[28, 29], which may guarantee the structural stability of adipocytes under active lipid synthesis and transport, providing a structural

basis for abdominal fat deposition. PS is mainly distributed in the inner monolayer of cell membranes and participates in cell signal transduction and functional regulation[30]. Moreover, PI serves as the primary source of arachidonic acid and is an indispensable substrate for the biosynthesis of eicosanoids[31]. Therefore, metabolic variations in PI may affect the production of arachidonic acid and the biosynthesis of eicosanoids, further activate lipid metabolism signaling pathways, and modulate adipocyte differentiation.

In conclusion, intragastric administration of Lactobacillus rhamnosus could significantly increase abdominal fat deposition without affecting body weight in mice, and elevate the levels of lipogenesis-related SCFAs such as acetic acid and propionic acid. It activated lipid metabolism-related pathways including glycerophospholipid metabolism, α-linolenic acid metabolism, linoleic acid metabolism and arachidonic acid metabolism to promote lipid synthesis and adipocyte differentiation. Meanwhile, it enhanced phospholipid synthesis-related pathways, driving the transformation of lipid metabolism from energy storage to structural synthesis.

Conclusion

Lactobacillus rhamnosus up-regulates the production of short-chain fatty acids, activates glycerophospholipid metabolism, polyunsaturated fatty acid metabolism and other pathways, promotes the synthesis of PC, PE, PS, PI and other phospholipids, and further regulates lipid transport and fat deposition. This study reveals the molecular mechanism of *Lactobacillus rhamnosus* regulating fat metabolism and fat deposition in mice.

Declarations

Ethics approval and consent to participate

Not applicable. All animal experiments were approved by Gansu Agricultural University (Lanzhou, China), with the approval number GSAU-Eth-ASF-2022-010. All animal procedures were strictly performed in accordance with the guidelines formulated by the China Animal Health Commission and the Ministry of Agriculture of the People's Republic of China.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest with any financial organization regarding the materials discussed in the manuscript.

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Author Contribution

Zhu Caiye designed the research project, conceived the overall experimental scheme, drafted and revised the manuscript. Wang Yanxin and Luo Yan participated in animal feeding, sample collection and physiological index determination. Gao Bo was responsible for data sorting, statistical analysis and chart drawing. Hou Qianru participated in literature retrieval, experimental operation and manuscript polishing. Zhang Yue assisted with metabolomics and lipidomics data bioinformatics analysis. Huang Xiaoyu supervised the whole research work, provided funding support, reviewed and finalized the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

Data are available from the corresponding author on reasonable request.The metabolomics and lipidomics data supporting the findings of this study are available in the supplementary information.

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Figures

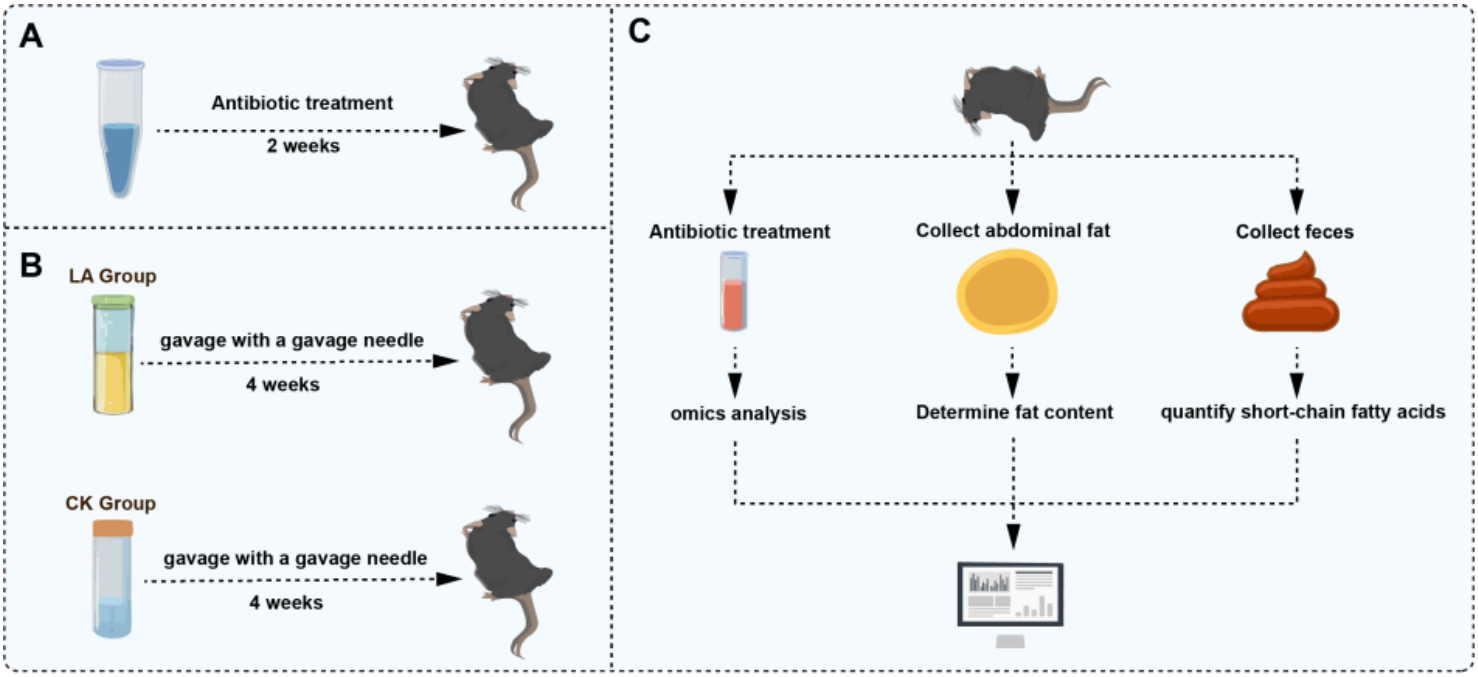


Figure 1

Schematic diagram of the mouse experimental procedure

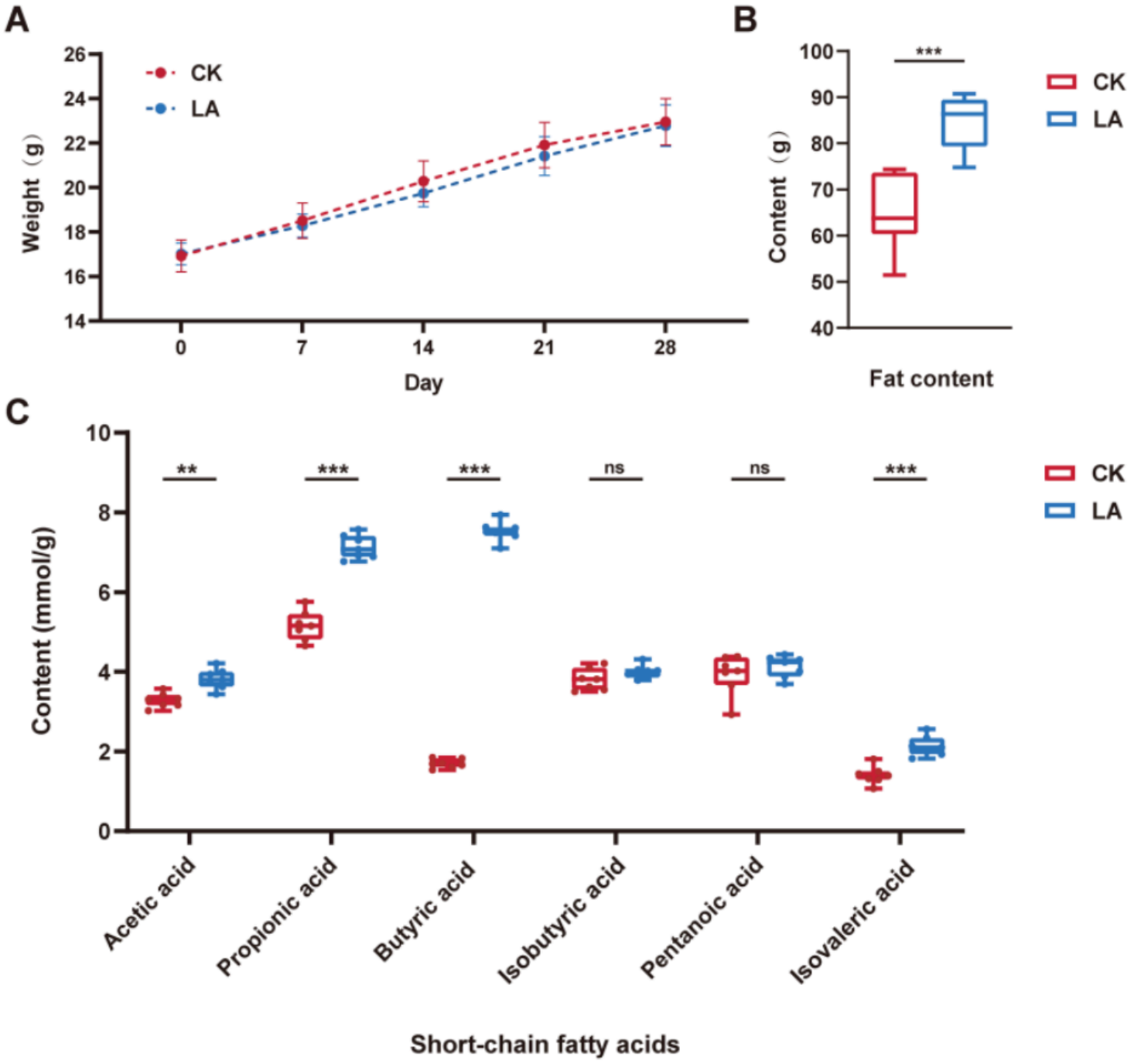


Figure 2

Determination of indexes in C57BL/6J mice administered with *Lactobacillus rhamnosus* by gavage

Note: A. Effect of *Lactobacillus rhamnosus* gavage on body weight of mice; B. Effect of *Lactobacillus rhamnosus* gavage on abdominal fat content of mice; C. Effect of *Lactobacillus rhamnosus* gavage on fecal SCFAs content of mice. *P<0.05, **P<0.01, ***P<0.001, ns means no significant difference (P>0.05).

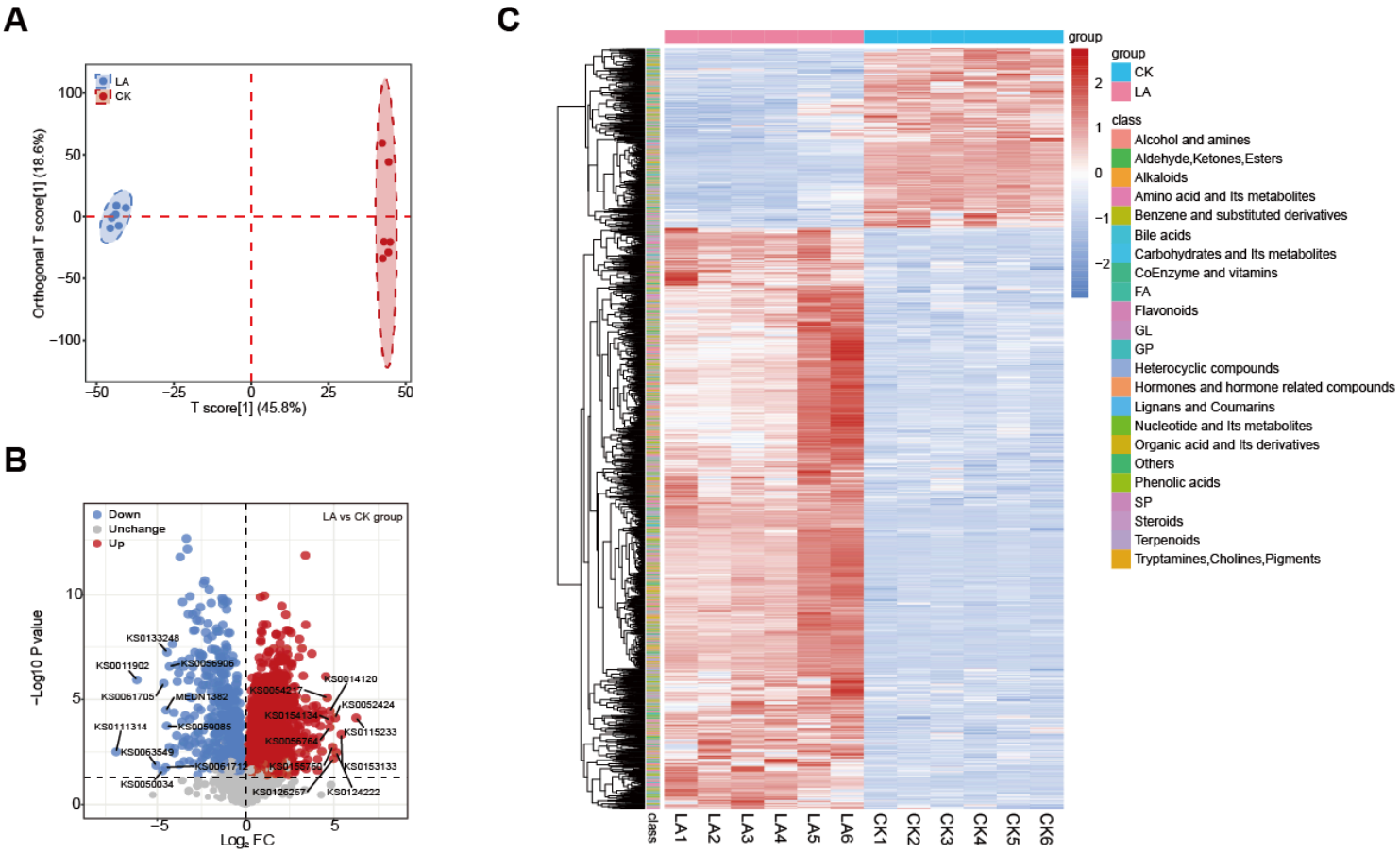


Figure 3

Serum non-targeted metabolomics profiles

A. OPLS-DA score plot; B. Volcano plot of differential metabolites; C. Cluster heatmap of differential metabolites.

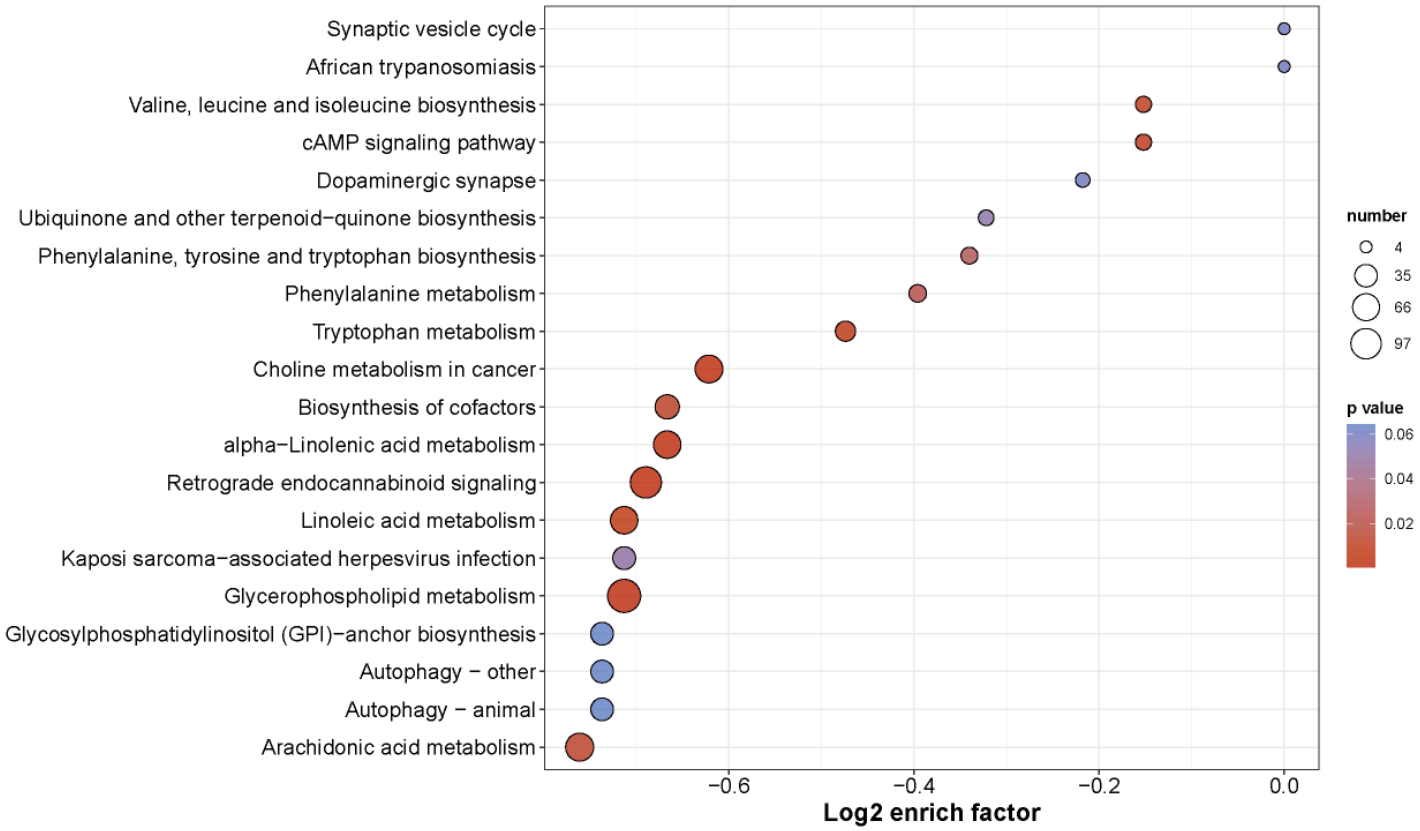


Figure 4

KEGG enrichment analysis of differential metabolites

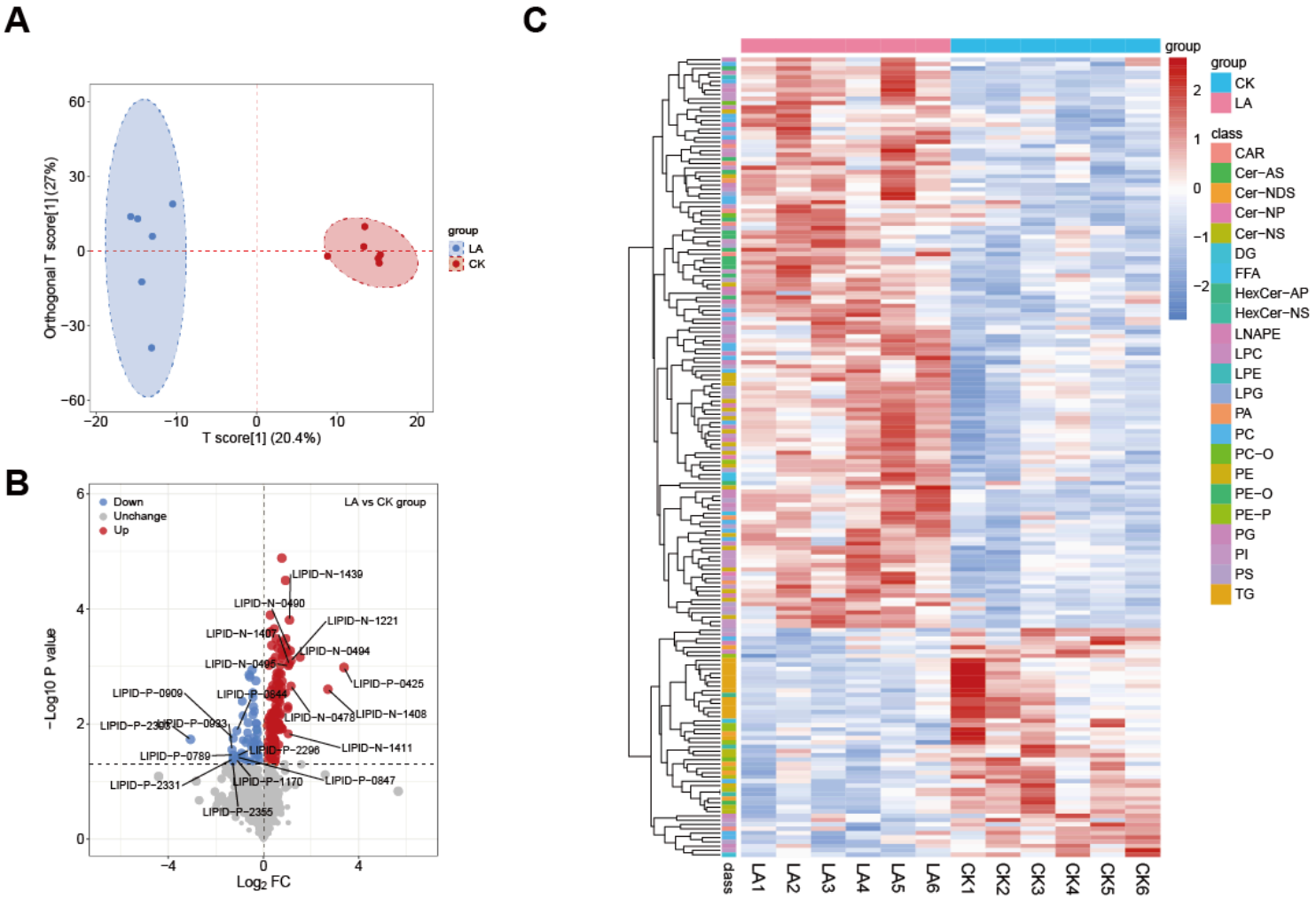


Figure 5

Lipidomics profiles

A. OPLS-DA score plot; B. Volcano plot of differential lipids; C. Cluster heatmap of differential lipids.

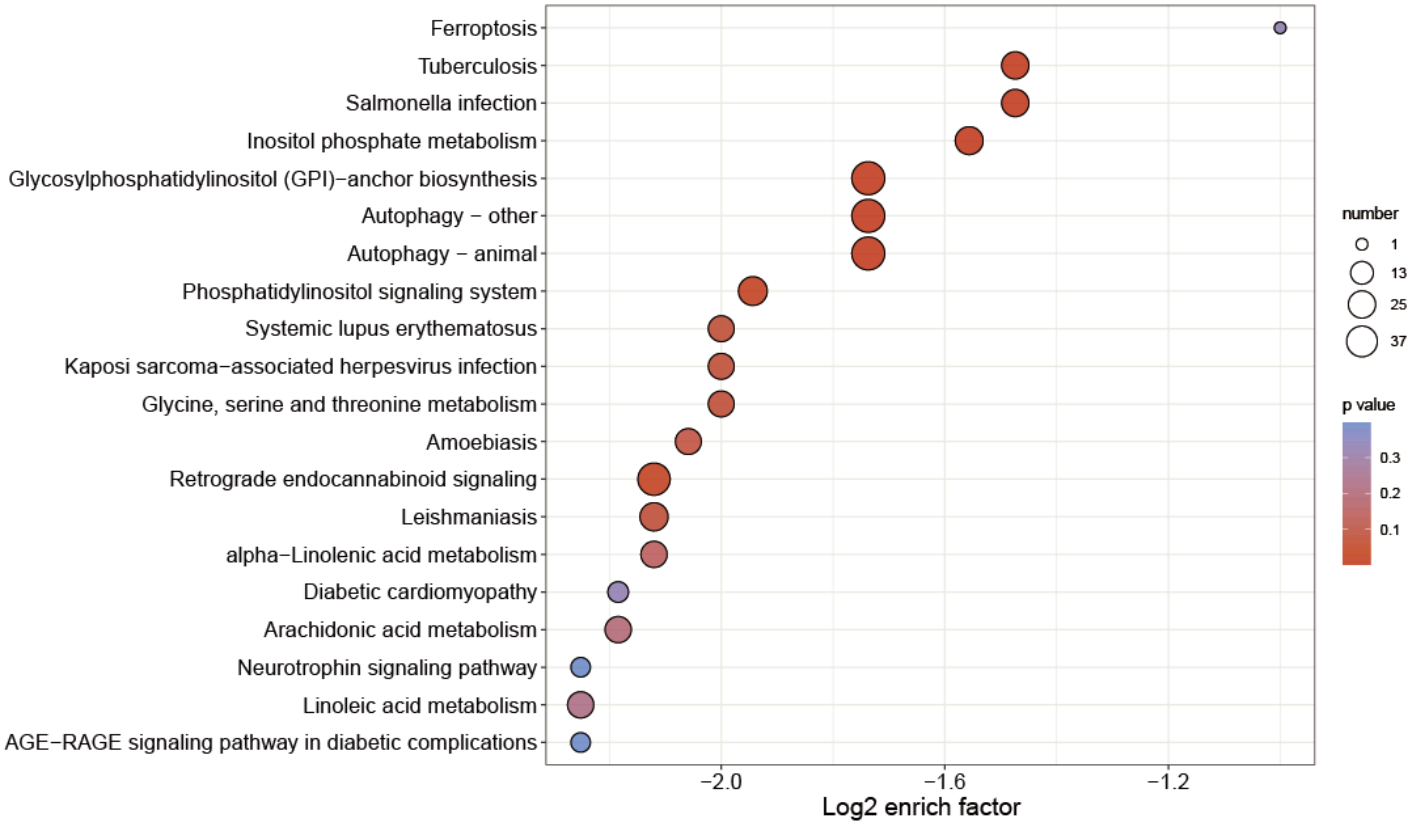


Figure 6

KEGG enrichment analysis of differential lipids