Compound Tea Lowers Lipid Levels by Promoting Organic Acid Metabolism

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Abstract: The lipid-lowering effects of compound tea were investigated to determine the mechanism underlying its action and provide theoretical support for the development and application of this compound tea. Specific pathogen-free male Kunming mice were randomly divided into four groups: a model control group (NK), positive control group (YK), blank control group (CK), and compound tea treatment group (DH), with 10 mice in each group. After 30 days of intragastric administration of compound tea, the body weight, liver coefficient, liver morphology, and liver metabolism of organic acids were analyzed in mice with hyperlipidemia. The weight of mice in the NK group was significantly increased compared to that of the mice in the CK group, whereas the weight gain and liver-to-body ratio of mice in the DH group exhibited no significant difference. Fifty-two differential organic acid metabolites were detected. Compared to the CK group, b-ureaisobutyric acid and n-acetyl-aspartyl-glutamate in the DH group were significantly upregulated, whereas creatine and 14 other organic acids were significantly downregulated. In the YK group, 13 organic acids, including argininosuccinic acid, were upregulated, whereas 17 organic acids, including chlorogenic acid, were downregulated. Compound tea had a better ability to regulate blood lipids compared with the YK (positive control, Xuezhikang). This may be because compound tea can regulate the metabolism of organic acids, such as chlorogenic acid, and their associated metabolic pathways, as well as ameliorate dyslipidemia induced by a high-fat diet in mice, thereby reducing body weight and blood lipid levels.

Key words: organic acids; hyperlipidemia; compound tea; metabolomics

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基于有机酸代谢的复合茶降脂作用

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摘要：探讨复合茶的降脂作用，为茶叶的降脂机理及复合茶开发和应用提供理论支撑。SPF 级雄性 KM 小鼠随机分为模型对照组（NK）、阳性对照组（YK）、空白对照组（CK）和复合茶处理组（DH）4 组，每个组 10 只；灌胃给予复合茶 30 d 后，分析复合茶对高脂血症小鼠的体重、肝系数和肝组织形态以及肝脏有机酸代谢的影响。结果表明：与 CK 组相比，NK 组小鼠的体重极显著上升，而 DH 组的增重、肝体比均无显著差异；共检测到 52 种差异显著有机酸代谢物，与 CK 组相比，DH 组中有 B-脲基异丁酸和 N-乙酰天冬氨酰谷显著上调，肌酸等 14 种有机酸代谢物显著下调；YK 组中有精氨基琥珀酸等 13 种显著上调，绿原酸等 17 种显著下

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调。研究表明：复合茶具有较强的降脂作用和肝脏保护作用，且效果优于 YK 组（血脂康处理）；其可能原因是复合茶调节绿原酸等有机酸代谢物及其代谢途径，改善了高脂膳食诱导的小鼠血脂异常，从而达到减肥降脂作用。

关键词：有机酸；高脂血症；复合茶；代谢组

Hyperlipidemia is a disorder of lipid metabolism, which is characterized by abnormal concentrations of lipids (including high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides and total cholesterol) in plasma[1,2]. The occurrence and development of hyperlipidemia might be accompanied by the changes and dysfunction of small molecule metabolites in the body. Similarly, the changes and functional obstruction of many small molecule substances in the body should also reflect the pathogenesis of hyperlipidemia[3].The liver, an important organ for body metabolism, can secrete bile, store glycogen, and regulate the metabolism of protein, fat, and carbohydrates[4].The dynamic imbalance of lipid metabolism[5] can cause diseases such as hyperlipidemia, diabetes, coronary heart disease, and non-alcoholic fatty liver[6,7].Clinically, drugs such as statins and fibrates are increasingly being used in the treatment of hyperlipidemia, which can reduce the content of triglycerides and total cholesterol in blood lipids, but they are prone to dose-dependent, liver toxicity and other side effects[8].

Tea is rich in active ingredients such as tea polyphenols, tea polysaccharides and theanine, which has the effects of weight loss and lipid reduction[9,10]; especially related to promoting reverse cholesterol transport[11], activating fat catabolism in the liver[12], so as to achieve the effect of reducing fat and losing weight. Long-term and reasonable consumption of tea can regulate the levels of cholesterol and triglycerides in plasma and liver, and reduce the incidence of hyperlipidemia and diseases such as hyperlipidemia, obesity and hypercholesterolemia[13]. Kudingcha is rich in triterpenesaponins, which can improve the level of lysophospholipids (Lyso-GPLs) and prevent hyperlipidemia induced by a high-fat diet[14]. Flavonoids and alkaloids in lotus leaves have antioxidant, hypoglycemic, weight loss, blood lipid lowering and other effects[15]. It was reported that honeysuckle was a commonly used traditional Chinese herbal medicine. Its main components included chlorogenic acid, flavonoids and saponins, which had the functions of antipyretic, immune regulation, cholagogic and liver protection, hypoglycemic and lipid-lowering[16]. The compound tea prepared with mint leaves as the main raw materials could reduce the content of LDL-C, TC, and TG, regulated blood lipid levels, and effectively improve liver tissue lesions[17]. Tea can reverse the original metabolic syndrome, prevent fat accumulation in the liver, effectively reduce the non-alcoholic fatty liver disease and liver inflammatory reaction caused by high-fat diet. It has anti-diabetes, anti-inflammatory and antioxidant effects[18,19], and no genetic toxicity, long-term and appropriate consumption is safe and reliable[20].

Metabonomics has the characteristics of systematicness, comprehensiveness, integrity and dynamics, which has been widely used in toxicology, functionality, food nutrition science and fields closely related to human health[21]. Metabolomics technology can detect and annotate biomarkers and metabolic pathways in order to establish the relationship between endogenous metabolites before and after stimulation or interference with the physiological mechanism of the disease[22]. Hyperlipidemia is a metabolic disease with a complex mechanism. Therefore, metabolomics technology can be selected when studying the pathogenesis of hyperlipidemia[23]. Metabonomics technology was used to investigate the effect of compound tea on the metabolism of organic acids in hyperlipidemia, and to preliminarily determine the potential biomarkers and metabolic pathways of hyperlipidemia mice after treatment with tea samples, aiming to explore the lipid-lowering mechanism of compound tea and provide some reference for its development and utilization.

1 Materials and methods

1.1 Preparation of materials

The compound tea was composed of Duyun broad-leaved Kuding, Duyun ancient tree green tea, lotus leaf, Duyun original tree sweet tea, honeysuckle, and wild mint[24]. The extraction method was operated

according to the methods of ZHOU et al[25,26]. All reagents were of analytical grade and purchased from Merck (Germany).

1.2 Animal experiment

The experimental design was operated according to that of Zhou et al[25,26]. After feeding KM mice for 7 days, they were randomly divided into four treatment groups: compound tea treatment group (DH, 840 mg/kg), Xuezhikang treatment group (YK, 90 mg/kg), model control group (NK) and normal control group (CK), with 10 mice in each group. The experiment was intervened for one month, during which the physiological activity, body weight and liver coefficient of mice were observed and recorded.

1.3 Sample preparation and extraction

Tissue sample was thawed on ice. 50 mg of one sample was homogenized with 1000 μL of ice-cold methanol/water (70%, *V/V*). The cold steel balls was added to the mixture and homogenized for at 30 Hz for 3 min. After that, the mixture was whirled for 1 min, and then centrifuged with 12,000 r/min at 4 ℃ for 10 min. Finally, the collected supernatant was going to be used for LC-MS/MS analysis.

1.4 HPLC Conditions

LC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM A system, https://www.shimadzu.com/; MS, QTRAP® 6500+ System, https://sciex.com/) was used to analyze the sample extracts. The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40 ℃; flow rate, 0.4 mL/min; injection volume, 2 μL; solvent system, water (0.04% acetic acid): acetonitrile (0.04% acetic acid); gradient program, 95:5 *V/V* at 0 min, 5:95 *V/V* at 11.0 min, 5:95 *V/V* at 12.0 min, 95:5 *V/V* at 12.1 min, 95:5 *V/V* at 14.0 min.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap massspectrometer (QTRAP), QTRAP® 6500+ LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 500 ℃; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (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. Aspecific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

1.5 Data processing and analysis

R 4.0.3 software was used to perform statistical analysis on the data. All data were expressed as mean ±SD. $p<0.01$ indicated that the difference was extremely significant, $0.01<p<0.05$ indicated that the difference was significant, and $p>0.05$ mean that the difference was not significant. The mass spectrometry data were processed and analyzed quantitatively by analyst 1.6.3 software and multiple reaction monitoring (MRM) of triple quadrupole mass spectrometry, and the peaks of all metabolites were extracted and corrected. All data sets were analyzed by OPLS-DA model after processing, and the effectiveness of the model was evaluated by the values of prediction parameters R2X, R2Y and Q2, whose size directly reflected the reliability of the model.

2 Results and analysis

2.1 Effects of compound tea on body weight, liver coefficient and liver tissue morphology of mice

It can be seen from table 1, there was no significant difference in the initial weight of healthy 5-week-old SPF male KM mice among groups ($p>0.05$). After the experiment, the final body weight and weight gain of NK mice were significantly higher than those of the CK group ($p<0.01$). The final weight and weight gain, liver weight and liver body ratio of mice in DH group and YH group were significantly lower than those in NK group ($p<0.01$), while the weight gain and liver body ratio of mice in YH group and CK group were significantly different ($p<0.05$). In addition, there was no difference in

weight gain and liver body ratio between DH group and CK group, indicating that compound tea had a strong lipid-lowering effect and the effect was better than that in YK group.

Table 1 Effects of compound tea on body weight and liver coefficient of mice

Group	initial body wt/g	final body wt/g	weigt gain/g	liver wt/g	Liver wt /body wt/g
CK	40.33 ± 1.37^{Aa}	43.25 ± 1.01^{De}	2.92 ± 0.95^{De}	2.52 ± 0.06^{Cd}	5.82 ± 0.16^{Bbc}
NK	40.94 ± 1.08^{Aa}	51.50 ± 1.56^{Aa}	10.56 ± 1.75^{Aa}	3.11 ± 0.08^{Aa}	6.04 ± 0.15^{Aa}
YK	40.51 ± 1.83^{Aa}	45.16 ± 1.00^{Ccd}	4.65 ± 1.54^{CDcd}	2.55 ± 0.11^{Ccd}	5.05 ± 0.26^{Bc}
DH	40.08 ± 0.66^{Aa}	43.92 ± 1.16^{CDde}	3.86 ± 1.24^{CDde}	2.53 ± 0.13^{Cd}	5.76 ± 0.30^{Bc}

Note: values in capital letters are expressed as mean ±SD of 10 mice in each group, and different letters in the same column are at $p<0.01$; the values in lower case in the same column at $p<0.05$.

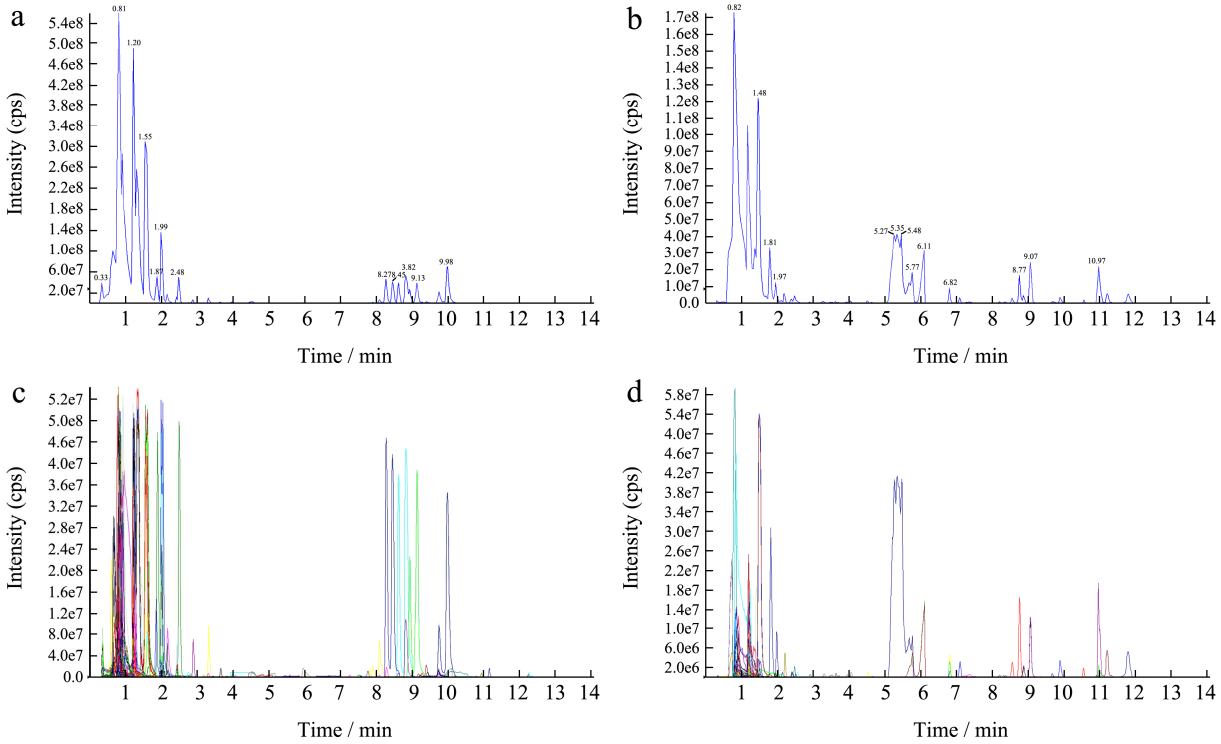


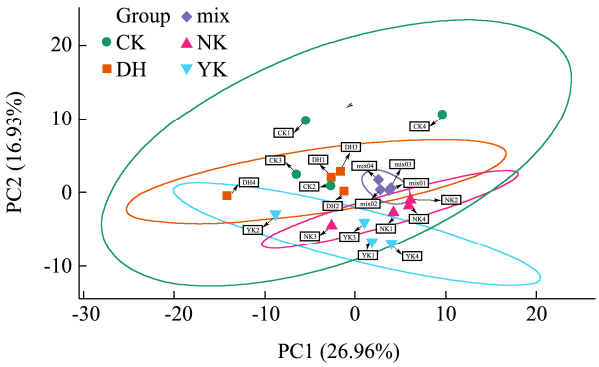
Fig.1 Total ion current diagram of mixed sample QC sample

Note: (a) and (c) are positive ion mode, (b) and (d) are negative ion mode.

2.2 Qualitative and quantitative analysis of metabolites in mice liver

The analyst 1.6.3 software was used to process the mass spectrometry data. During the analysis process, every 10 samples were inserted into the quality control (QC) samples prepared by the mixed sample extract to monitor its repeatability. The total ion current diagram (TIC) of mass spectrometry is a spectrum obtained by continuously depicting the sum of the intensities of all ions in the mass spectrum at each time point, which can accurately determine the repeatability of metabolite extraction and detection. The multi reaction monitoring (MRM) mode metabolite detection multi peak diagram showed the substances that could be detected in the sample, and the chromatographic peaks of different colors represented the detection of different metabolites (Fig.1).

2.3 Principal component analysis (PCA)



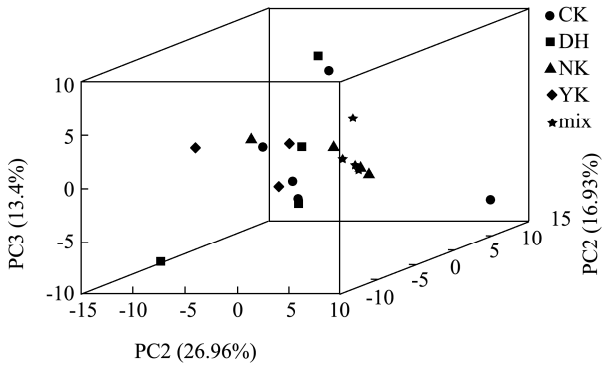


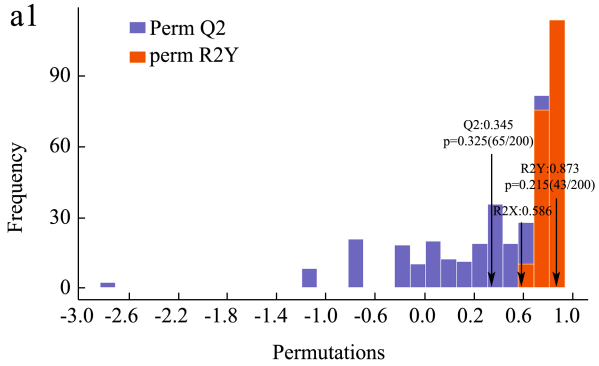
Fig.2 Principal component analysis (PCA) score chart and three-dimensional chart

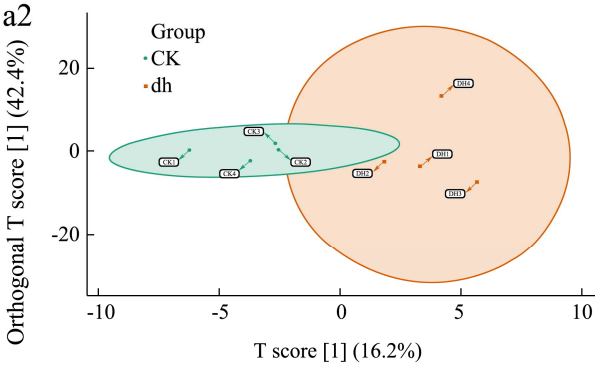
Note: The x-axis represents the first principal component and the y-axis represents the second principal component.

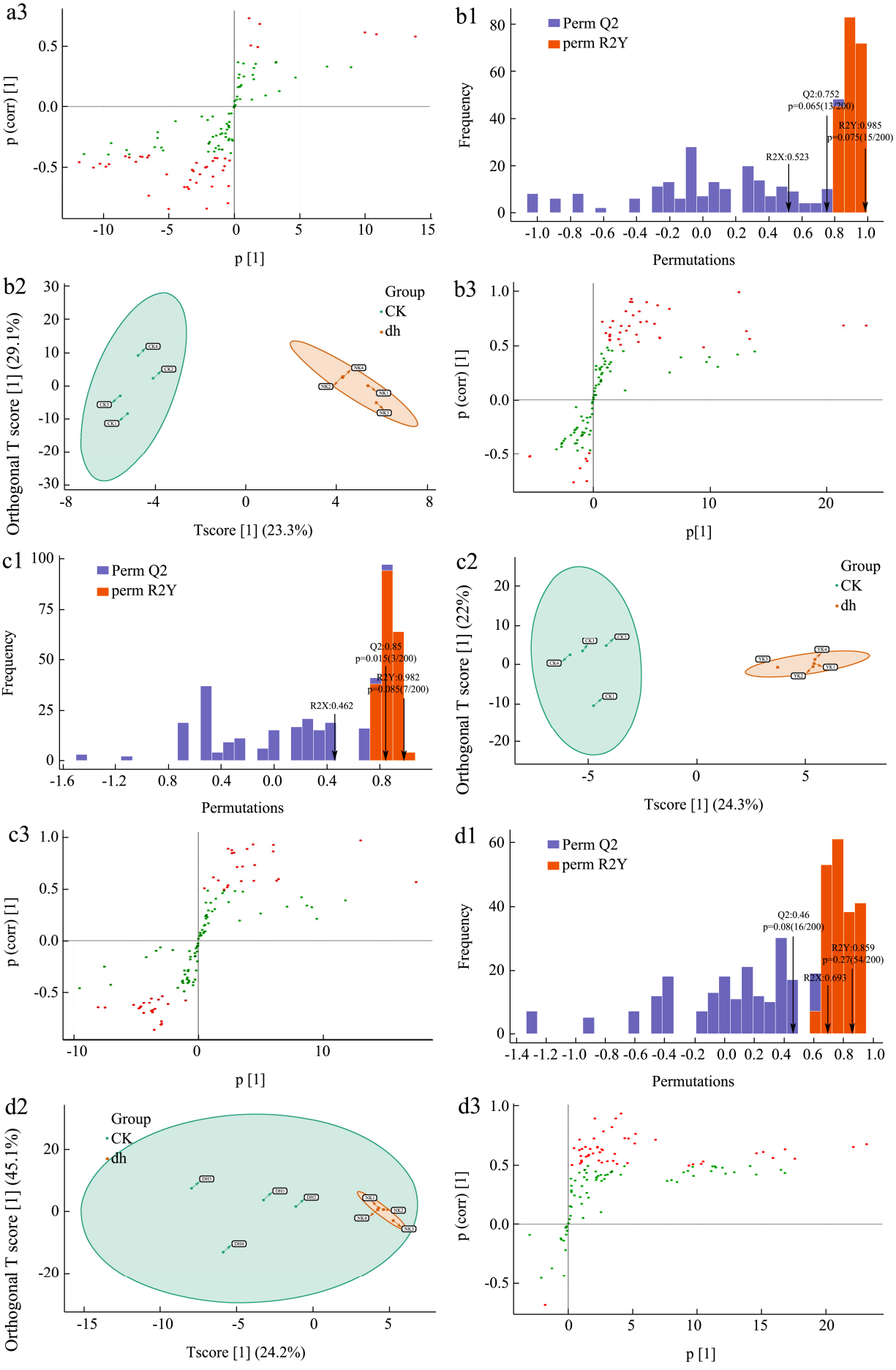
Principal component analysis (PCA) was used to preliminarily determine the overall difference in metabolites and anomalies between and within each treatment group. The intra-group PCA results showed that the more aggregated the metabolites, the smaller the difference, and the inter-group PCA results showed that the higher the overlap, the smaller the metabolite differences. PCA analysis showed that there was no significant difference between samples in each group, which had similar metabolic characteristics, and the test results were stable and repeatable (Fig.2). The sample overlap between the CK_vs_DH group was relatively highest, the metabolite difference was the smallest, and the separation trend between the metabolites was not significant. Therefore, the metabolite correlation between the two groups was very high, indicating that high-fat mice fed compound tea had no obvious effect on the metabolic characteristics of mice. The separation trend between the NK group and the CK group was obvious, suggesting that the metabolites of the NK group and the two groups of mice were significantly different. Except for individual overlap between the NK group and the DH group and the YK group (which might be caused by biological differences), the separation trend was also obvious. The results showed that there were significant differences in metabolites between compound tea treatment and positive drug treatment compared with the NK group. Among the CK_vs_YK group, the sample separation trend was larger, and the metabolism difference was significant; while between the YK_vs_DH group, the separation trend was smaller, indicating that the compound tea and the positive drug had similar effects on the metabolites of the organism.

2.4 Partial least squares-discriminant analysis (OPLS-DA)

According to OPLS-DA analysis, the X matrix information was decomposed into two types of information related and unrelated to Y, and the model was established. In order to prove its reliability, 200 verification experiments were carried out. The abscissa represented the accuracy of the model. The model was the best when $p<0.05$ of R2Y. The verification results showed that the model was meaningful and could be analyzed according to VIP Value analysis to screen for differential metabolites. Between the CK_vs_DH group (Fig.3a, Q2=0.345, p=0.325) and the NK_vs_DH group (Fig.3d, Q2=0.46, p=0.08), the Q2 score was less than 0.5, indicating that the model had some deviations. Among the CK_vs_YK group (Fig.3c, Q2=0.85, p=0.015), YK_vs_DH group (Fig.3f, Q2=0.684, p=0.03), CK_vs_NK group (Fig.3b, Q2=0.752, p=0.065) and the NK_vs_YK group (Fig.4 e, Q2=0.522, p=0.135), the Q2 scores were all greater than 0.5, which was regarded as an effective model. In addition, from the OPLS-DA score chart, it can be seen that except for the overlap of samples between the CK_vs_DH and NK_vs_DH groups, the difference in metabolites was less obvious (Fig.3 a, d), and the separation trend of samples between other groups is significant, indicating that the metabolites The difference is obvious (Fig.3 b, c, e, f).






a3
p (corr) [1]
p [1]
b1
Perm Q2
perm R2Y
Frequency
Q2:0.752
p=0.065(13/200)
R2Y:0.985
p=0.075(15/200)
R2X:0.523
Permutations
b2
Group
CK
dh
Orthogonal T score [1] (29.1%)
Tscore [1] (23.3%)
b3
p (corr) [1]
p[1]
c1
Perm Q2
perm R2Y
Frequency
Q2:0.85
p=0.015(3/200)
R2Y:0.982
p=0.085(7/200)
R2X:0.462
Permutations
c2
Group
CK
dh
Orthogonal T score [1] (22%)
Tscore [1] (24.3%)
c3
p (corr) [1]
p [1]
d1
Perm Q2
perm R2Y
Frequency
Q2:0.46
p=0.08(16/200)
R2Y:0.859
p=0.27(54/200)
R2X:0.693
Permutations
d2
Group
CK
dh
Orthogonal T score [1] (45.1%)
Tscore [1] (24.2%)
d3
p (corr) [1]
p [1]

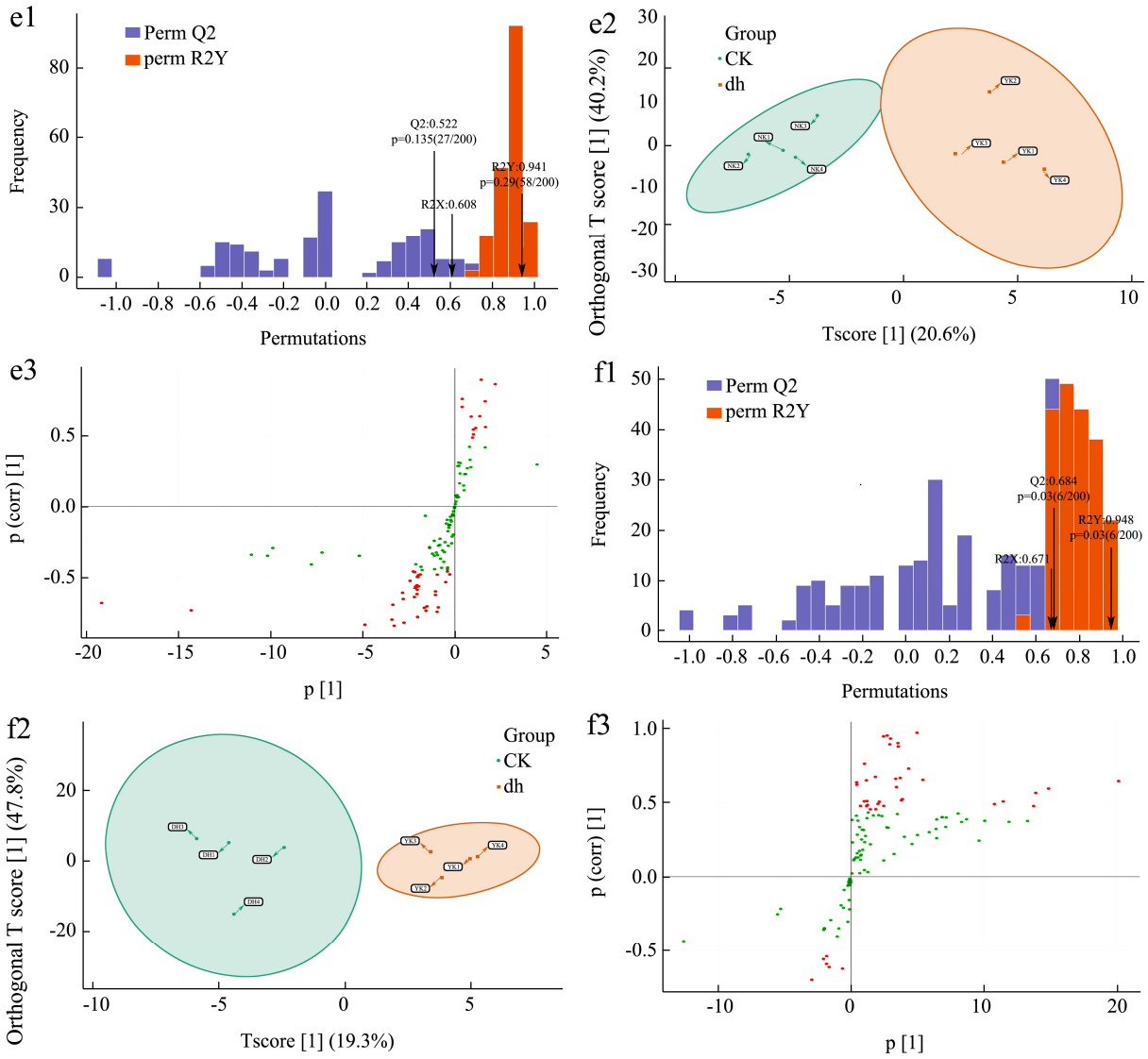


Fig.3 Score diagram, verification diagram, S-plot of OPLS-DA

Note: CK group and DH group (a), CK group and NK group (b), CK group and YK group (c), NK group and DH group (d), NK group and YK group (e), YK group and DH group (f).

2.5 Differential metabolite screening and statistical analysis

Hierarchical cluster analysis (HCA) can evaluate the characteristic differences of organic acid metabolite accumulation in high-fat mice caused by tea sample treatments, including homogeneity within treatment and variability between treatments. Metabolites with fold change ≥2 or ≤0.5 and VIP≥1 were selected as significant difference metabolites. Screening was carried out in the presence of biological duplicate samples, among which 52 significantly different metabolites were screened out (Table 2), the number of significantly different metabolites in each group and the statistical details of the number of up- and down-regulated metabolites (Table 3). The results showed that N-acetylaspartyl glutamic acid, D-hydroxypropionic acid, squintaurine, kynuric acid, xylonic acid, γ-glutamic acid-leucine, diphosphate 20 metabolites including hydroxyacetone, urocanic acid and furfural were significantly up-regulated, and creatine and chlorogenic acid were significantly down-regulated ($log_2FC<-3.7$, $log_2FC<-3.4$) in the CK_vs_NK group. In CK_vs_DH group, there were 16 differential metabolites, of which 2 were up-regulated (B-urea isobutyric acid and N-acetylaspartyl glutamate) and 14 were down regulated (2,3-dimethylbutyric acid, 2-aminoethane sulfurous acid β- hydroxyisovaleric acid, adipic acid, 2-methylglutaric acid, etc), and the most

down regulated metabolites were creatine ($log_2FC<-3.9$) and chlorogenic acid $log_2FC<-2.8$). In the CK_vs_YK group, there were a total of 30 different metabolites, and 13 were up-regulated (methylmalonic acid, aminomalonic acid, DL-glyceraldehyde-3-phosphate, N-lactoylphenylalanine, γ-glutamic acid -leucine, etc), among which the most up-regulated metabolite was DL-glyceraldehyde-3-phosphate ($log_2FC>4.6$); 17 down-regulated, represented by creatine. In the NK_ vs _DH group, 13 differential metabolites were all down-regulated, with DL-glyceraldehyde-3-phosphate ($log_2FC<-4.4$) and dihydroxyacetone phosphate ($log_2FC<-4.0$) down-regulating the most. In the NK_ vs _YK group, there were 7 different metabolites and all were down-regulated, including mandelic acid, 5-hydroxycaproic acid, N-acetylaspartyl glutamic acid, kynuric acid, sebacic acid, DL-2-aminocaprylic acid and N-acetyl-DL-valine (down-regulated the most, $log_2FC< -2.9$). In the YK_vs_DH group, there were 17 different metabolites, only 1 was up-regulated (B-ureidoisobutyric acid), and the remaining 16 were all down-regulated (D-hydroxypropionic acid, L-lactic acid, aminomalonic acid, DL-glycerin Aldehyde-3-phosphate, allyl ester, N-lactoylphenylalanine, etc), of which DL-glyceraldehyde-3-phosphate was down-regulated the most ($log_2FC<-4.7$). In addition, among all the differential metabolites screened out, the most up-regulated substances were DL-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, and the most down-regulated substances were creatine, chlorogenic acid, and DL-glyceraldehyde-3-phosphate. Dihydroxyacetone phosphate and N-acetyl-DL-valine (Fig.4).

Arginaminosuccinic acid is a derivative of succinic acid, which is an important intermediate product of TCA cycle. It is not only the main way of glucose degradation, but also the main energy source of organisms. Colle et al[27] showed that succinic acid can increase the level of glutathione (GSH) by up-regulating the activity of γ-glutamylcysteine ligase (GCL), and change energy metabolism, showing protection against mitochondrial dysfunction-derived oxidative stress effect, so as to achieve lipid-lowering, anti-inflammatory and antioxidant effects. Arginine succinate synthase (ASS) catalyzes the synthesis of arginine succinate from aspartic acid and citrulline, and plays an important role in the urea cycle, nitric oxide and arginine synthesis. Its expression is related to carbohydrate nutrients and *in vitro* oxidation stress related[28]. Chlorogenic acid (5-caffeoylquinic, CA) can inhibit the expression of p53, PGC-1, FAS and other genes, restrain glycerol-3-phosphate dehydrogenase (GPDH) activity[29], reduce liver fat accumulation[30], and relieve kidney oxidative stress, inflammation and apoptosis process of tubules[31], thereby showing significant effects of lowering blood lipids and protecting the kidneys. What's more, the phenolic hydroxyl group of the caffeic acid part of CA is very important for its lipid-lowering activity[32]. When the phenolic hydroxyl group of the caffeic acid part is destroyed, the role of regulating the transcription of adipogenic genes such as srebp1c/1a, ACC, Fas and PPAR is also eliminated. Michael et al[33] reported that chlorogenic acid and caffeic acid (CH) could promote the activity of E-NTPDase, inhibit the activities of ADA and ACE, reduce the contents of TC, TG and LDL-C ($p<0.05$), and increase the content of HDL-C. In addition, chlorogenic acid (CGA) and metformin (MET) inhibit the expression of fatty acid synthase, promote the phosphorylation of AMP activated protein kinase, reduce the content of triglycerides in the liver, and more effectively reduce inflammation and lipid accumulation[34]. Glycerol and fatty acids are generated by fat hydrolysis, in which glycerol can be produced under the action of glycerol kinase-α-phosphoric acid, glycerol-α-phosphoric acid generates dihydroxyacetone phosphate (DHAP) under the action of glycerol phosphate dehydrogenase. DHAP, an intermediate product of glucose metabolism, participates in the synthesis of phospholipid acids and fats and can be further transformed into glyceraldehyde-3-phosphate (GAP), which can generate sugar along the reverse reaction of glycolysis. Consequently, DHAP and GAP are important intermediates of lipid metabolism and glucose metabolism. Compared with the CK group, the DHAP and GAP contents of the NK group showed an upward-regulation trend, indicating that the high-fat diet affected DHAP and GAP, which in turn affected the metabolism of glucose and lipid; while the DHAP and GAP contents showed a downward-regulation trend in

NK group, suggesting that the compound tea extract can improve the glucose metabolism and fat metabolism of hyperlipidemia mice, thereby regulating blood lipids.

Studies have shown that compared with the CK group, the content of argininosuccinic acid in the YK group ($log_2FC>1.2$) was higher than that in the NK group ($log_2FC>0.8$); the chlorogenic acid metabolites of the YK group and the DH group were down-regulated, and the YK group (The down-regulation of chlorogenic acid in $log_2FC<-3.9$) was more than that in the DH group ($log_2FC<-2.8$), indicating that the DH group had a better effect on improving and regulating hyperlipidemia mice than the YK group; The increase in the content of argininosuccinic acid in the liver could be explained as the result of increased fat catabolism; suggesting that the positive drug Xuezhikang had a better effect on lowering blood lipids and could promote fat catabolism.

Table 2 Significant metabolites

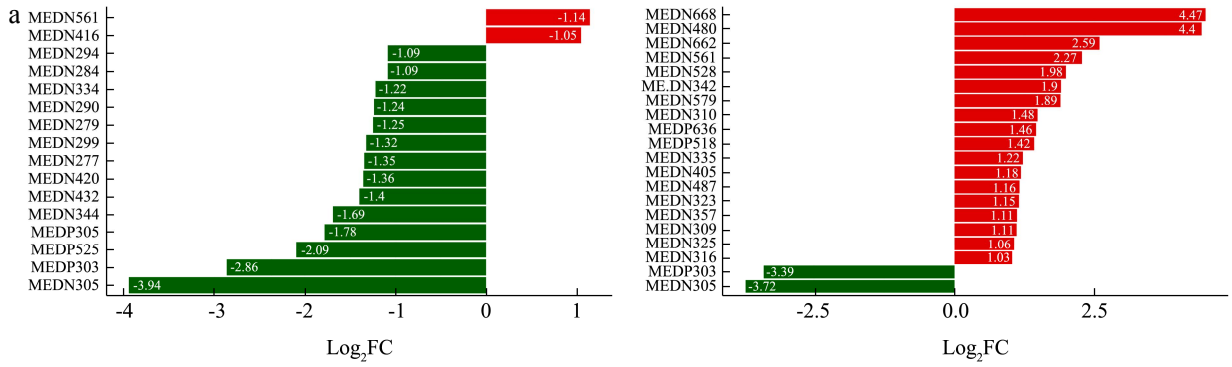
Metabolite	RT/min	CK_vs_DH	CK_vs_NK	CK_vs_YK	NK_vs_DH	NK_vs_YK	YK_vs_DH
2,3-dimethylbutanine	2.68	↓		↓			
2-aminoethanesulfinic acid	0.76	↓		↓			
2-hydroxy-4-methylvaleric acid	3.65	↓			↓		
β-hydroxyisovaleric acid	2.16	↓		↓			
N-(3-Methyl-1-oxo-2-butenoic acid) aminoacetic acid	2.89	↓			↓		
adipic acid	2.63	↓		↓			
creatine	0.78	↓	↓	↓			
mandelic acid	3.07	↓		↓		↓	
suberic acid	3.86	↓		↓			
B-ureidoisobutyric acid	1.42	↑					↑
2-methylglutaric acid	2.76	↓		↓			
5-hydroxyhexanoic acid	2.66	↓			↓	↓	
N-Acetyl Aspartyl Glutamate	1.24	↑	↑			↓	
chlorogenic acid	2.85	↓	↓	↓			
creatinine	0.71	↓		↓			
N-acetyl-L-alanine	0.78	↓		↓	↓		
D-hydroxypropionic acid	1.14		↑		↓		↓
P-hydroxyphenyl lactic acid (HPLA)	2.69		↑				
mitaurine	0.79		↑				
canine uric acid	2.90		↑			↓	
L-lactic acid	1.06		↑		↓		↓
methyl malonic acid	1.37		↑	↑			
sebacic acid	5.09		↑			↓	
xylic acid	0.80		↑				
aminomalonic acid	1.40		↑	↑			↓
DL-glyceraldehyde-3-phosphate	1.05		↑	↑	↓		↓
allyl ester	0.85		↑		↓		↓
(3-methoxy-4-hydroxyphenyl) ethylene glycol sulfate	1.74		↑	↑			
n-lactyl phenylalanine	3.98		↑	↑	↓		↓
γ-glutamic acid-leucine	2.69		↑	↑			
dihydroxyacetone phosphate	0.81		↑		↓		
urocanic acid	0.86		↑	↑			↓

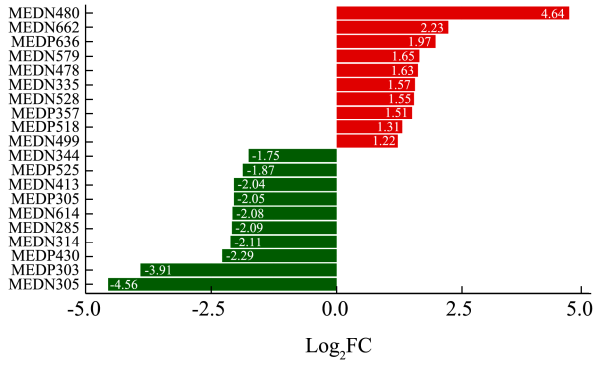
Continued table 2

Metabolite	RT/min	CK_vs_DH	CK_vs_NK	CK_vs_YK	NK_vs_DH	NK_vs_YK	YK_vs_DH
stachytine hydrochloride	0.86		↑	↑			
furfural	0.76		↑	↑	↓		↓
trans-4-(aminomethyl) cyclohexanecarboxylic acid	0.87		↑				
methyl succinic acid	2.29			↓			
glutaric acid	1.95			↓			
α-hydroxyisobutyric acid	1.61			↓			
ethyl malonic acid	2.12			↓			
argininosuccinic acid	0.90			↑			
dimethylmalonic acid	2.30			↓			↓
d-pipecolic acid	0.89			↑			
2-aminoadipate	0.80			↓			
alpha-phenylpiperidinyl-2-acetic acid	1.59			↑			↓
N-acetyl-L-histidine	0.80			↑			↓
(S)-2-hydroxybutyric acid	1.67				↓		↓
aspartame	2.84				↓		↓
DL-2-aminocaprylic acid	3.16					↓	
N-acetyl-DL-valine	2.28					↓	
4-arginine butyric acid	0.80						↓
5-aminovaleric acid	0.74						↓
guanidinoacetic acid	0.82						↓

Table 3 Statistical table of the number of differential metabolites

Differential metabolite grouping information	Number of metabolites with significant difference	Number of up-regulated metabolites	Number of down-regulated metabolites
CK_vs_NK	22	20	2
CK_vs_DH	16	2	14
CK_vs_YK	30	13	17
NK_vs_DH	13	0	13
NK_vs_YK	7	0	7
YK_vs_DH	17	1	16

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MEDP430
MEDP303
MEDN305
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2.23
1.97
1.65
1.63
1.57
1.55
1.51
1.31
1.22
-1.75
-1.87
-2.04
-2.05
-2.08
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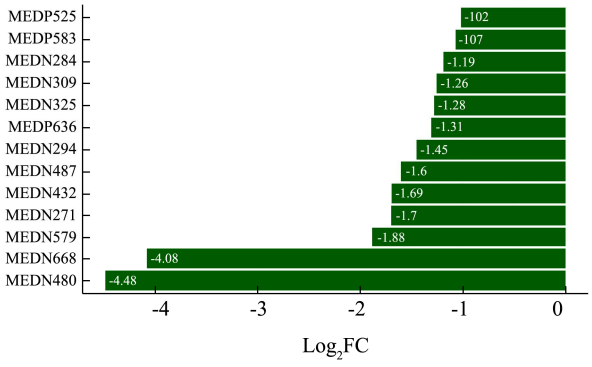
5.0
Log2FC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-102
-107
-1.19
-1.26
-1.28
-1.31
-1.45
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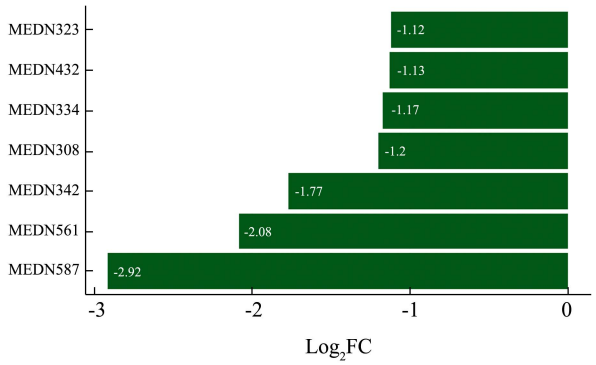
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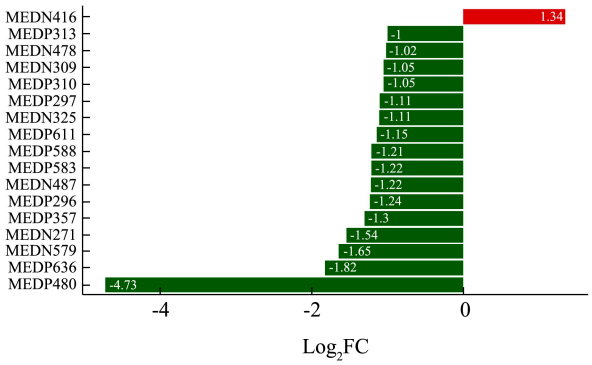
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-1.21
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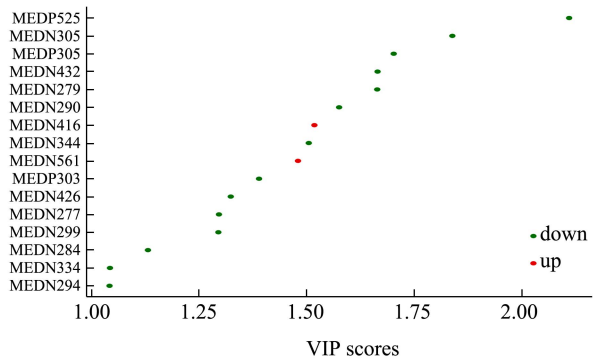
Log2FC

b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MEDN290
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MEDN561
MEDP303
MEDN426
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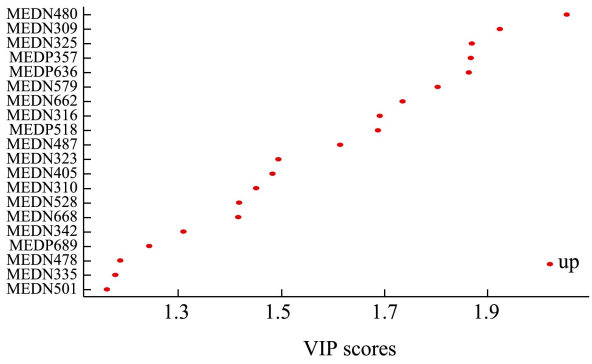
1.00
1.25
1.50
1.75
2.00
VIP scores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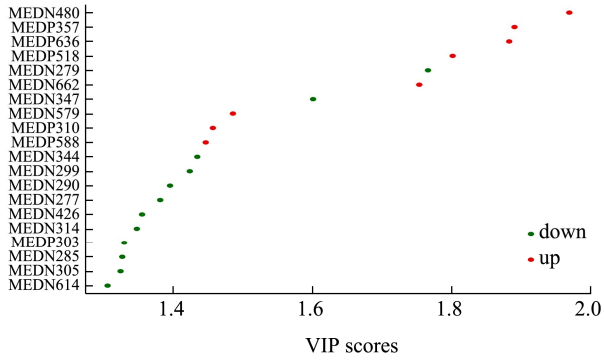
1.7
1.9
VIP scores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MEDN277
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up
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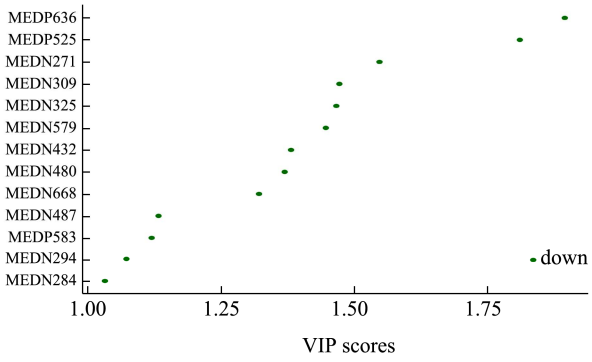
1.8
2.0
VIP scores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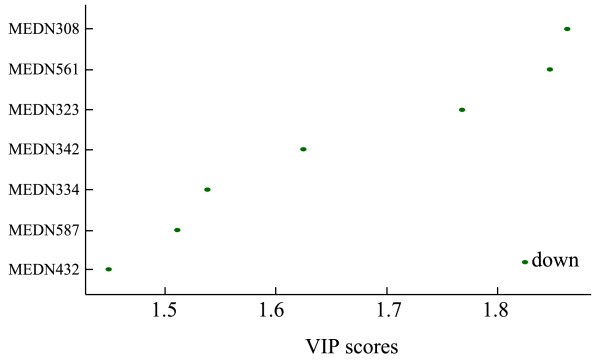
down
1.00
1.25
1.50
1.75
VIP scores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down
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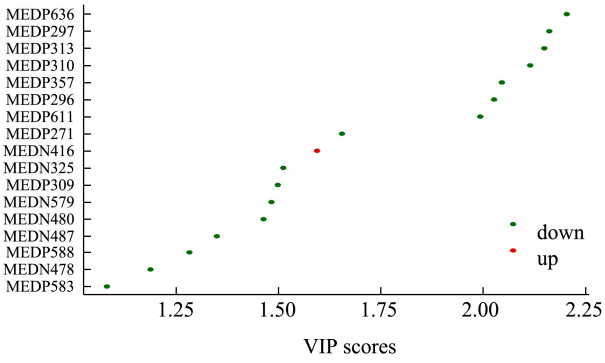
1.6
1.7
1.8
VIP scores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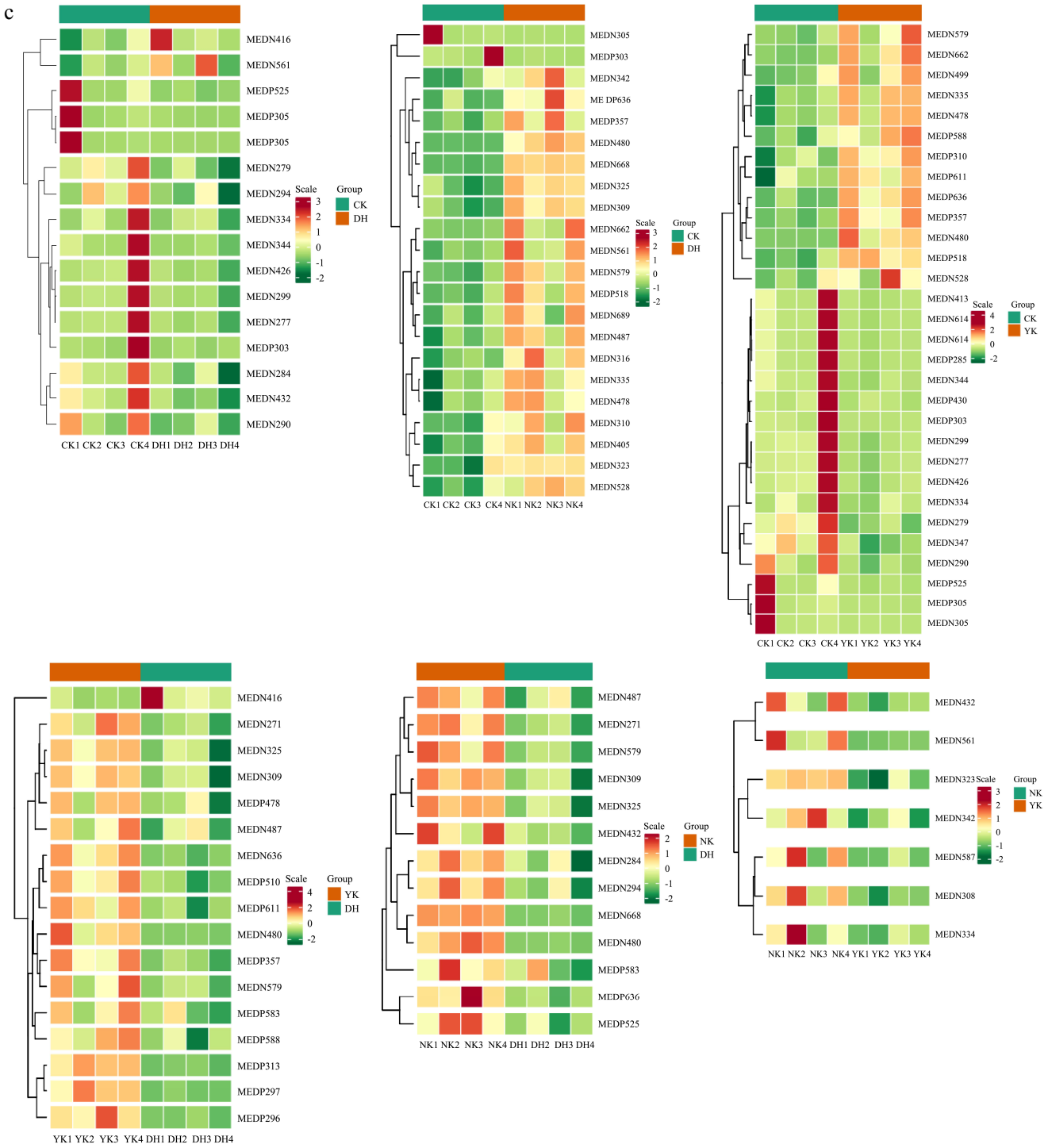


Fig.4 Bar graph, VIP value graph, cluster thermograph of different metabolites

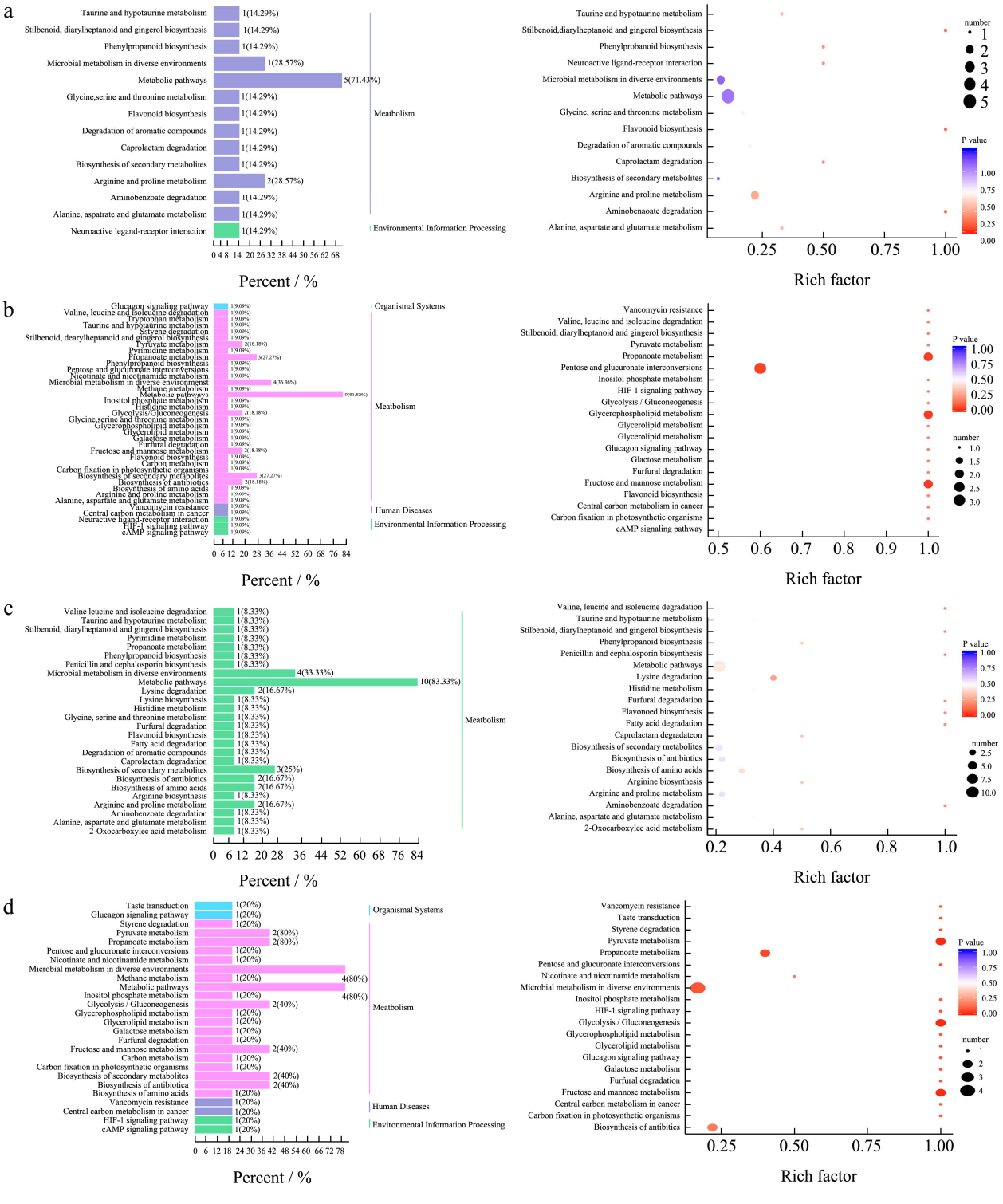
Note: Different metabolites between groups. (a) Bar graph; (b) VIP score Plot; (c) Cluster heat map.

2.6 Functional annotation and enrichment analysis of differential metabolites

According to the self-built database, the KEGG database and the UPLC-MS/MS detection platform, the multivariate statistical analysis, annotation, and classification of significant difference metabolites were carried out. The results showed the pathway types involved in each group of differential metabolites include metabolic pathway (such as serine and threonine metabolism), environmental information processing (such as microbial metabolism in different environments), tissue system (such as neuroactive ligand receptor interaction, glucagon signaling pathway), biosynthesis (such as diarylheptanes, *Ginkgo biloba* alcohol

biosynthesis) and biodegradation (such as aromatic compounds, caprolactam degradation). The comparison of each group of graphs showed that the main type of pathway was the metabolic pathway. Among them, the metabolism of arginine and proline accounts for a larger proportion in the NK_vs_YK group (Fig.5a); in other inter group comparisons, microorganisms accounted for a large proportion of metabolism in different environments.

According to the different substances and their biological interactions, the different metabolites and metabolic pathways between and within each treatment group were found, and their metabolic pathways were constructed. KEGG database and self-built database, differential metabolites can be annotated and displayed. Some results were as Fig.6.



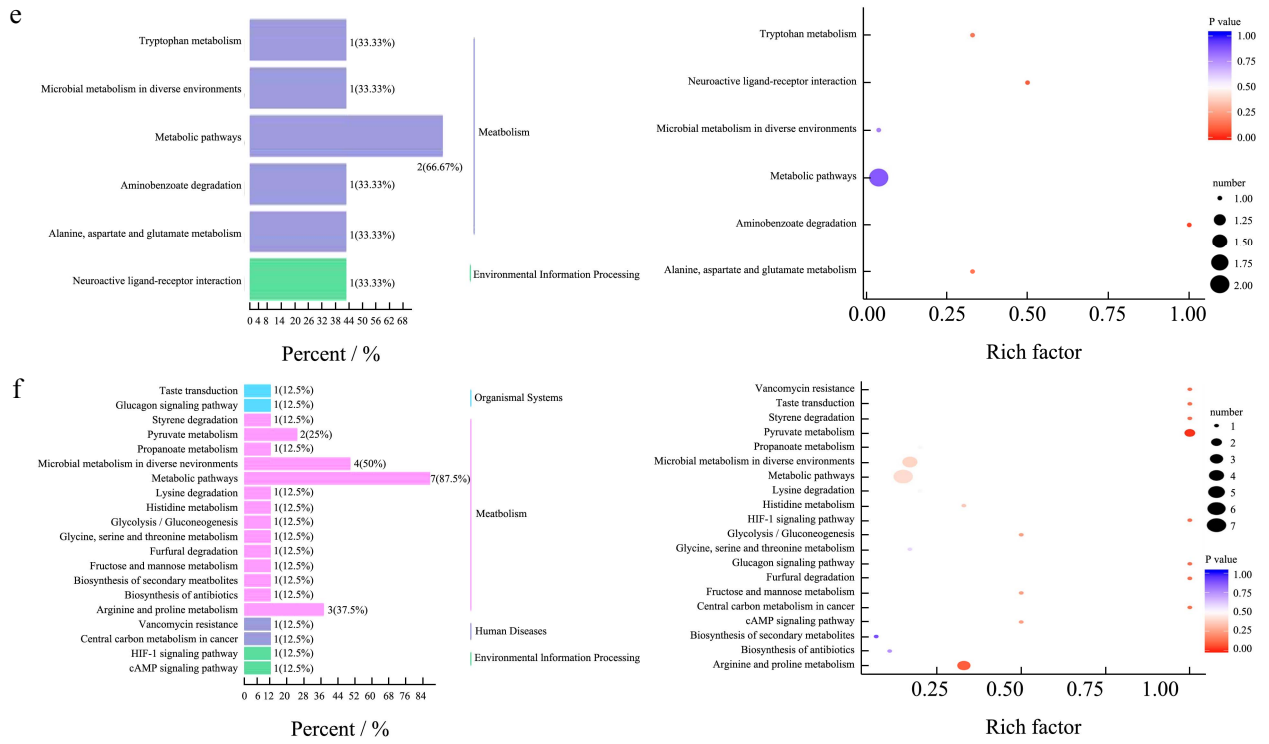


Fig.5 Classification diagram and enrichment analysis of differential metabolite KEGG

Note: CK group and DH group (a), CK group and NK group (b), CK group and YK group (c), NK group and DH group (d), NK group and YK group (e), YK group and DH group (f).

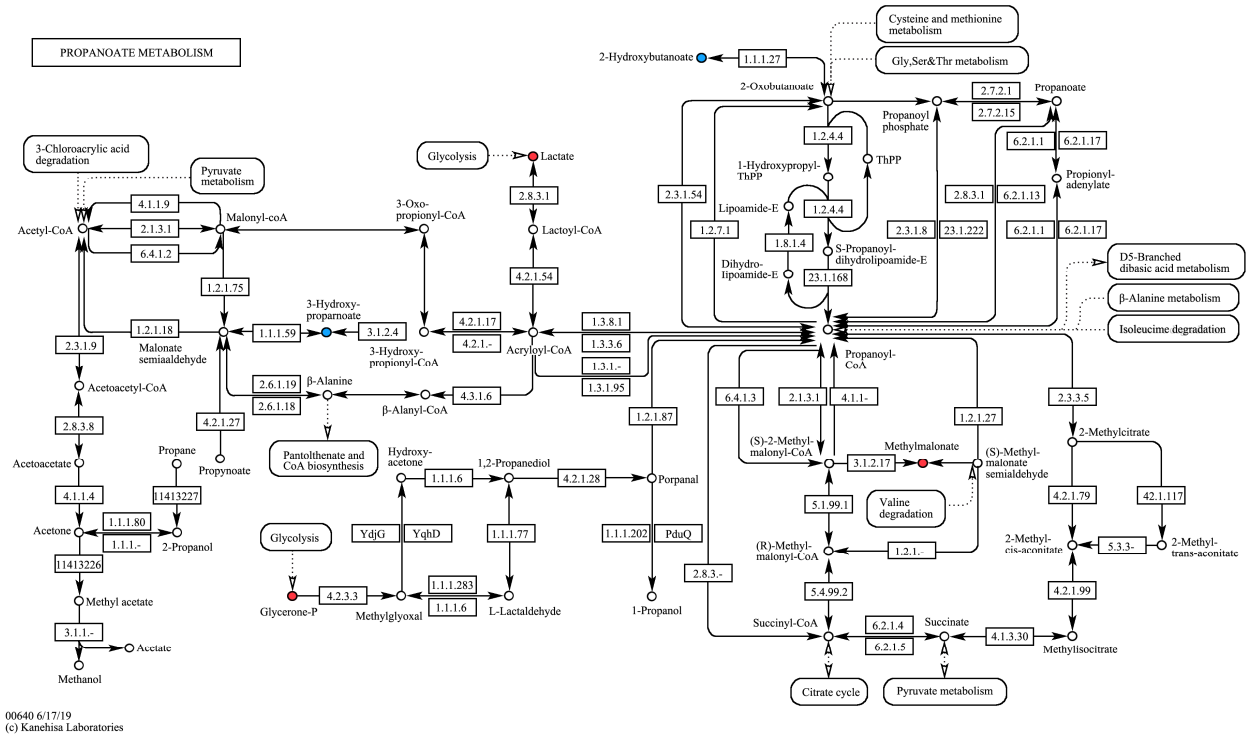


Fig.6 Pathway of differential metabolite KEGG

Note: Red indicates a significant increase, green indicates a significant decrease, and blue indicates not significant. Through the metabolic pathways, the reasons for the phenotypic differences in the research subjects can be found.

The pathways related to lipid metabolism included organic acid metabolism, lipid metabolism, glycolipid metabolism, bile acid metabolism and glycolysis[35,36], while organic acids were acidic organic compounds containing carboxyl (-COOH), common organic acids include chlorogenic acid, pyruvic acid, fumaric acid,

citric acid, maleic acid, malic acid, succinic acid, salicylic acid and other organic acids[37]. Chlorogenic acid, in particular, as an important secondary metabolite in plants, widely existed in a variety of plants, which had hypoglycemic, lipid-lowering, anti-oxidation and other pharmacological effects. It could provide new ideas and new ways for the prevention and treatment of chronic diseases such as diabetes and cardiovascular diseases caused by hyperlipidemia[38].

3 Conclusion

Methanol/chloroform mixed solvent was used to extract liver lipids for LC-MS/MS analysis. Based on MRM, MWDB and KEGG database, the organic acid metabolites of hyperlipidemia mice model fed with high-fat diet were analyzed qualitatively and quantitatively by mass spectrometry. 52 significantly different organic acid metabolites were found, the potential biomarkers and metabolic pathways of hyperlipidemia mice treated with tea were preliminarily determined. In conclusion, the compound tea extract could effectively inhibit the growth of body weight and liver weight in obese mice; it could regulate the metabolic pathways such as organic acid metabolites, glucose metabolism and fat metabolism, and improve the dyslipidemia induced by high-fat diet in mice, so as to achieve the effect of weight loss and lipid reduction. The above research results could provide some reference for the lipid-lowering and weight-loss effect of tea, and provide theoretical support for the development and utilization of the composite tea.

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