Sex-specific Metabolic Characterization of Serum and Liver of Mice with High-fat Diet

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Abstract

Background: Obesity exhibit sex differences is well established, but its metabolic mechanism remains unclear. Thus, investigation of metabolic pattern of male and female mice with high-fat diet (HFD) is of substantial importance for explore the potential mechanism linking gender differences in obesity.

Methods: In the present study, we analyzed the metabolic changes in serum and liver of male and female mice with high-fat diet using nuclear magnetic resonance-based metabolomic approach.

Results: Principle component analysis show that the metabolic pattern of serum and liver of male mice with HFD was significantly distinguished from the other groups. Furthermore, the accumulation of low-density lipoprotein/very low-density lipoprotein was found in the serum of male mice with HFD. Moreover, metabolomic results of liver reveal that tricarboxylic acid cycle and amino acid metabolism are increased in female mice with HFD.

Conclusion: In conclusion, our results suggest that the differences in energy and amino acid metabolism of males and females were most likely influence the predisposition to obesity.

Background

The prevalence of obesity has risen substantially in recent decades, having been considered as a significant challenge to health around the world [1]. Obesity is a complex chronic disease, caused by an imbalance between food intake and energy expenditure [2, 3], associated with inflammation reaction, oxidative stress, endothelial dysfunction and disturbances in lipid and glucose metabolism [4, 5]. Accumulating evidence indicates that obesity is a potential risk for many metabolic syndromes, including glucose intolerance, insulin resistance, hyperinsulinemia, dyslipidemia, nonalcoholic fatty liver disease, and type 2 diabetes [6–8].

It is noteworthy that there were sex differences in the susceptibility to obesity. A meta-analysis study reported that young males were more likely to be obese than young females [9]. Studies have identified that adipose tissue distributed in the abdominal or visceral region (male-pattern body fat distribution) carries a much greater risk for obesity than that distributed subcutaneously (female-pattern) [10, 11]. Nunez et al. further showed that the removal of ovarian hormones increases the susceptibility of female mice to obesity, indicating the important role of ovarian hormones play in the different sensitivity to obesity between males and females [12]. In addition, Pettersson et al. have demonstrated that female mice could maintain the anti-inflammatory environment in the intra-abdominal adipose tissue by expanding T lymphocyte populations, consequently against high-fat diet (HFD) induced obesity, whereas HFD-fed males developed hyperinsulinemia and adipose tissue inflammation [13]. Taken together, mounting evidence has demonstrated that females are more protected from obesity caused by HFD, but the reasons are not completely established. Therefore, understanding the metabolic differences between males and females may allow the discovery of better prevention and treatment strategies for obesity.

The liver is the major organ for maintaining glucose, triacylglycerol, cholesterol, and lipid metabolic homeostasis [14]. Notably, liver tissues have been often employed to elucidate the mechanisms of obesity [15]. Wang *et al*. proposed that HFD could induce fatty degeneration, hyperlipidemia, obesity, insulin resistance, and other disorders of metabolism by regulating the signaling pathways of lipid metabolism in the liver [16]. However, sex differences in liver metabolism associated with obesity have been reported less.

In the present study, we analyzed the metabolic patterns with NMR-based metabolomics in serum and liver tissues of male and female mice exposed to HFD for 12 weeks. The present study contributes to an understanding the potential metabolic mechanism of sex-specific sensitivities to obesity.

Methods

Animals and treatments

Male and female C57BL/6J mice aged 7 weeks (male, body weight: 22~26g; female, body weight: 15~19g; n=24 for each gender) were obtained from the SLAC Laboratory Animal Co. Ltd. (Shanghai, China) and housed in a specific pathogen-free (SPF) environment at the Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China) with standard conditions (room temperature = 23 ± 2℃; humidity = 45 ± 5%) and 12h/12h light-dark cycle (light on 8:00 am).

All mice had access to a standard rat chow and tap water. After adaptive feeding for one week, the mice were randomly assigned to four groups as follows: (1) M-NC (male mice, given a normal diet, n=12); (2) F-NC (female mice, given a normal diet, n=12); (3) M-HFD (male mice, given a high-fat diet, n=12); (4) F-HFD (female mice, given a high-fat diet, n=12). The normal diet contained 10 kcal% fat, 20 kcal% protein and 70 kcal% carbohydrate; the HFD contained 60 kcal% fat, 20 kcal% protein and 20 kcal% carbohydrate. Blood glucose levels were determined with a tail nick and handheld glucometer (One Touch Ultra, Lifescan, Milpitas, CA, USA). All animal experiment protocols involved were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

Oral Glucose Tolerance Test (OGTT) and Insulin Resistance Test (ITT) Assays

OGTT was conducted at 12 weeks after HFD feeding. Briefly, after a 12 h fasting, tail-vein blood was collected and measured with a glucometer at 0, 15, 30, 60, 90, 120 min after glucose treatment (orally administrated 1g/kg D-glucose, dissolved in water). ITT was carried out 3 days after OGTT to ensure recovery. Serum insulin levels were detected with an insulin ELISA kit at 0, 15, 30, 60, 90, 120 min after insulin treatment (intraperitoneally injected 0.5 IU/kg).

Sample Collection and Preparation

After 12 weeks of HFD feeding, blood samples were obtained from the retro-orbital plexus following the sacrifice of the mice, and centrifuged at 3000 rpm for 15 min at 4 °C to afford the serum, then stored at

-80 °C until analysis. In this study, 200 μL of the thawed serum sample was diluted with 250 μL of phosphate buffer (0.2 mM Na_2HPO_4/NaH_2PO_4, pH =7.4) and 50 μL D_2O. The diluted serum sample was mixed by vortex and centrifuged at 10,000 g for 15 min at 4 °C. Finally, transfer 500 μL of supernatant into a 5 mm NMR tube for NMR analysis.

Liver samples were isolated immediately after mice sacrificed, and snap-frozen in liquid nitrogen, then stored at -80 °C until analysis. The frozen liver sample was weighted into an Eppendorf tube containing ice-cold methanol (4 mL/g) and cold distilled water (0.85 mL/g), then homogenized by a handheld homogenizer (Fluko F8, Essen, German). Next, cold chloroform (2 mL/g) and cold distilled water (2 mL/g) were added into a tube and mixed using a vortex. After standing on ice for 15 min, the mixture was centrifuged at 12000 *g* for 15 min at 4℃. Lastly, transfer the supernatant into a fresh tube, lyophilized for 48 h. The lyophilized extract from the liver was reconstituted with 500 μL D_2O containing 0.6 mM sodium trimethylsilyl propionate-d_4 (TSP) and transferred into a 5 mm NMR tube for NMR analysis.

NMR-based Metabolomic Analysis

^1H NMR spectra of serum and liver samples were measured using a Bruker AVANCE III 600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany). To acquire NMR spectra of serum, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with receiver gain value fixed at 298k was used, minimizing the line-broadening effect of macromolecules such as proteins. The typical parameters were set as follows: date points = 256 k, spectral width = 12335.5 Hz, relaxation delay = 4 s, acquisition time = 2.66 s/scan. For liver spectra, a standard single-pulse sequence (ZGPR) with water signal pre-saturation was employed. The main parameters including, date points = 256 k, spectral width = 12000 Hz, relaxation delay = 6 s, acquisition time = 2.66 s/scan.

Topspin software (v2.1 pl4, Bruker Biospin, Germany) was used to manually correct the phase and baseline of the ^1H NMR spectra. The spectra of serum were referenced to the methyl peak of lactate (CH3, 1.33 ppm), while the spectra of liver extract were referenced to the TSP peak (0.00 ppm). All spectra were aligned in MATLAB (R2012a, The Mathworks Inc., Natick, MA, USA) by using “icoshift” procedure [17]. NMR metabolic signals were assigned through Chenomx NMR suite 7.0 (Chenomx Inc., Edmonton, Canada) on the basis of Human Metabolome Database. To quantify the metabolite levels of serum, the peak area was manually integrated using Topspin software. For metabolite quantification of liver tissue, each integrated peak was normalized to the total spectral area for minimizing differences, following calculate according to the peak area by reference to the internal TSP concentration, expressed as relative units (r.u.).

Data reduction and multivariate pattern recognition analysis

The SIMCA-P+ V12.0 software package (Umetrics, Umea, Sweden) was used for multivariate pattern recognition analysis of the integral values. Principal component analysis (PCA) was performed to examine overall changes in metabolic patterns between male and female mice with different diets. Partial

least squares-discriminant analysis (PLS-DA) was employed to identify the metabolic patterns between different sex or diets in mice serum, while orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed to determine differences in mice liver. In addition, R^2 (the explained variance of the model) and Q^2 (the predictive capability of the model) were calculated to assess the goodness of fit and the predictive capability of the model, respectively. In general, these two parameters approaching 1.0 indicates that the model is excellent. The significance of variables in PLS-DA was evaluated using the variable importance in the projection (VIP). When the values of VIP were greater than 2.0, these variables were considered significant. Moreover, the S-Plot generated from the OPLS-DA model was used to identify the significant metabolites that primarily contributed to class discrimination.

Statistical Analysis

The results were expressed as the means ± standard deviation (SD). All statistical analysis was performed in SPSS software (version 13.0, SPSS). The differences between two groups were evaluated by the dependent samples t-test, and pair-wise multiple comparisons were analyzed by Student's t-test with Bonferroni adjustment, with $P < 0.05$ being considered statistically significant. Pearson correlation analysis were carried out by Matlab function ("corrmartrix", r2012a). The correlation heatmap was displayed by the 'heat map' module of R software (version 3.3.1, http://www.R-project.org). In the heatmap, red indicates a positive correlation, while blue indicates a negative correlation, with a brighter color reflecting a higher correlation.

Results

Male mice are more sensitive to HFD-induced weight gain, glucose tolerance, and insulin resistance

The final body weight of male mice with 12 weeks of high-fat diet feeding was substantially higher than that of normal diet (ND)-fed male mice (41.5 ± 5.32 g versus 29.0 ± 1.5 g in male controls, $P<0.001$), while there was no significant difference in the body weight of female mice between HFD and ND (23.8 ± 1.8 g versus 22.1 ± 0.84 g in female controls，$P=0.07$) (Figure 1A). In addition, the random blood glucose of male mice increased remarkably in response to HFD, in parallel with that the female changed a little (Figure 1B). To evaluate glucose tolerance in HFD mice, the oral glucose tolerance test (OGTT) was conducted at 12 weeks. The result showed the characteristic rapid rise of blood glucose in all mice, peaking within 15-30 min, and approaching the baseline level by 120 min after glucose challenge. However, the blood glucose level of male-HFD mice remained elevated after 120 min compared to other mice, indicating that the HFD-fed male mice were more likely to develop glucose tolerance (Figure 1C). Insulin resistance test (ITT) and fasting serum insulin level results also showed that male-HFD mice were with lower insulin sensitivity and easier to develop hyperinsulinemia than female-HFD mice (Figure 1D, E). Interestingly, there was no obvious changes in food and water intake between males and females (Figure 1F, G). Collectively, these results suggested that male mice are more sensitive to HFD-induced obesity compared to female mice.

NMR spectra and pattern recognition analysis of serum and liver samples

Typical ^{1}H NMR spectra of serum sample is shown in figure 2 A. A series of metabolites were identified, involving amino acid metabolism: leucine, valine, isoleucine, alanine, glutamate, tyrosine, and phenylalanine; lipid metabolism: Low-density lipoprotein/very low-density lipoprotein (LDL/VLDL), and unsaturated fatty acid (poly-UFA); ketone body metabolism: 3-hydroxybutyrate (3-HB) and acetate; energy metabolism: lactate and creatine. To evaluate the differences in metabolic patterns of serum samples, principal component analysis (PCA) was conducted. Results based on PCA show that the male mice with HFD group (M-HFD) was clearly separated from the other three groups (figure 2B).

Figure 2C displayed the representative ^{1}H-NMR spectra in liver samples, containing many metabolites involved in amino acid metabolism (leucine, valine, isoleucine, alanine, glutamate, glutamine, glutathione, aspartate, lysine, glycine, tyrosine, phenylalanine, nicotinurate and betaine), energy metabolism (lactate, succinate, creatine, GTP and fumarate), membrane metabolism (choline and phosphocholine), ketone body metabolism (acetate and 3-hydroxybutyrate), as well as nucleic acid metabolism (uridine, inosine and AMP). Similar to the metabonomics data of serum samples, the M-HFD liver metabolic pattern was significantly distinguished from the other three groups. Moreover, the metabolic pattern of HFD-fed female mice was closer to the normal diet male (M-ND) group (Figure 2D).

Sex difference in dietetic effects on the metabolome in the serum of mice

To further explore the metabolic patterns, partial least squares-discriminate analysis (PLS-DA) and VIP statistics were conducted. The goodness of fit and the predictive capability of the model were shown in Supplementary Table1. A clear discrimination was observed between male and female mice fed with HFD along the PLS1 direction (Figure 3A). The VIP scores indicated that LDL/VLDL played a pivotal role in the separation of both groups (Figure 3B). However, there was no significant difference between males and females with ND, with the value of VIP being less than 2.0 (Figure 3C, D). Additionally, lactate is the major contributor to the separations in female mice with a ND or HFD, but not LDL/VLDL (Figure 3E, F). Interestingly, LDL/VLDL played a pivotal role in the separation of male mice with a ND and HFD (Figure 3G, H). Overall, LDL/VLDL could be the major affected metabolite in the serum of male mice with HFD.

Metabolic analysis of liver samples in male and female mice

Supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) and S-plot were subjected to identify key metabolites that contributed to metabolic pattern changes in liver. Through a pairwise comparison of OPLS-DA, it was seen that either in male or female mice, the metabolic patterns of the liver samples were obviously different between the ND and HFD (Figure 4A, C). Additionally, the S-plots suggests that the separation in male mice was ascribed to the increases of succinate, glutathione, and phosphocholine, as well as decreases in betaine and glycine (Figure 4B). For female mice, the increase of betaine, lactate, choline, phosphocholine, inosine, and the decrease of creatine contributed to the distribution of the two groups (Figure 4D). Figure 4 E and G displayed that the male group was clearly separated from the female group under ND and HFD conditions, respectively. With normal diet, betaine,

glycine, choline, alanine, and lactate are the main factors for the differences (Figure 4F). In addition, betaine, inosine, choline, lactate, and glutathione were shown through the loading of S-plot, leading to the separation of M-HFD and F-HFD (Figure 4H). The goodness of fit and the predictive capability of the OPLS-DA were displayed in Supplementary Table 2.

Male mice are more prone to HFD-induced lipid metabolism disorder

Further quantitative analysis and pathway analysis of various metabolites in mouse serum revealed that the content of LDL/VLDL increased dramatically in male mice after HFD (68.81±8.95 in M-HFD versus 9.12±2.55 in M-NC, $P<0.001$), while the female mice had no change (46.66±16.85 in F-HFD versus 46.77±17.93 in F-NC, $P=0.917$). More importantly, the LDL/VLDL level of female mice was greater than that of male mice (46.77±17.93 in F-NC versus 9.12±2.55 in M-NC, $P<0.001$) in respond to ND. However, the LDL/VLDL increased markedly in the serum of male mice with HFD feeding, even higher than that of female mice (68.81±8.95 in M-HFD versus 46.66±16.85 in F-HFD, $P<0.05$). Additionally, HFD led to the decrease of poly-UFA in the serum of male mice (17.17±1.51 in M-HFD versus 14.37±2.63 in M-NC, $P<0.05$), while almost no change in female (14.22±5.48 in F-HFD versus 14.53±4.19 in F-NC, $P=0.815$). Except 3-hydroxybutyrate and lactate, most metabolite levels decreased after HFD-feeding both in male and female mice, but there was no significant difference. The quantitative data analysis of other metabolites was shown in Supplementary Table3 and 4. Additionally, LDL/VLDL and poly-UFA are substances related to lipid metabolism. Together, our results indicate that male mice were more susceptible to HFD-induced lipid metabolism disorders than females (Figure 5).

Changes in metabolites level in liver of mice on different diet conditions

In the liver, the concentrations of metabolites such as amino acid metabolites, energy metabolites, ketone body metabolites, nucleic acid metabolites, and membrane metabolites in female mice were increased after HFD feeding (Figure 5 and Supplementary Table 5, 6). However, most metabolites levels in male mice were reduced, such as short-chain amino acid, alanine, aspartate, lysine, glycine, tyrosine, phenylalanine, lactate, creatine, uridine, inosine, acetate, choline, betaine. In addition, the levels of liver metabolites in male mice were generally higher than those in female with normal diet, and most of them had significant differences. As expected, the levels of most metabolites in male mice reduced after feeding HFD, while the levels in female mice elevated, even close to the levels of the ND-fed male mice group. These results are consistent with the similar metabolic pattern of F-HFD and M-NC group. In conclusion, the enhanced metabolic capacity could be the reason that the female mice did not increase their weight, blood glucose greatly after HFD-feeding.

Betaine could correct HFD-induced abnormal lipid metabolism

To investigate the reason that caused less accumulation of LDL/VLDL in female-HFD mice serum, we analyzed the correlation on LDL/VLDL in serum with liver metabolites. The results of the heatmap showed a significant negative correlation between LDL/VLDL and betaine, especially in mice fed with HFD (Figure 6A). Combined with the analysis of the quantitative data of betaine, it showed that the level

of betaine in male mice was higher than that in females under normal dietary conditions (24.49 ± 5.42 in M-NC versus 19.24 ± 2.26 in F-NC, $P<0.05$). Unexpectedly, after 12 weeks of HFD-feeding, the betaine level in female mice increased, while the male mice decreased (19.09 ± 2.75 in M-HFD and 23.36 ± 4.20 in F-HFD, $P<0.05$). Thus, we speculated that betaine which is highly expressed in the liver of female mice, could correct the accumulation of LDL/VLDL in serum after HFD.

Discussion

It is well-established that there were sex differences in obesity, but the mechanisms with respect to metabolism behind are not well-explored. In the present study, we found that mice exhibited sex-specific susceptibility respond to the high-fat diet. Male mice were more susceptible to weight gain, elevated blood glucose, glucose tolerance, insulin resistance, and even hyperinsulinemia induced by HFD. In addition, we examined the metabolic changes in serum and liver of male and female mice with control or high-fat diet using an NMR-based metabolomics. Our metabolomic results showed that the metabolic patterns in serum and liver of M-HFD were largely differentiated from other groups.

By metabolomics analysis of serum, we found that LDL/VLDL is the main contributor to separation of metabolic patterns in male and female mice with HFD. Low-density lipoprotein and very-low-density lipoprotein are two kinds of beta-lipoproteins, which play a main role in transporting lipids. Studies have reported that excessive intake of saturated fat and trans fatty acids is the main reason for the high level of LDL/VLDL in vivo, while plasma triglycerides and cholesterol increased as LDL/VLDL elevated [18–20]. A series of evidence showed that LDL/VLDL highly expressed in obesity, and the abnormal elevation of LDL/VLDL is closely related to the disorder of lipid metabolism [21, 22]. Results of present study showed that LDL/VLDL level was significantly increased in the serum of male mice with HFD compared with the control group, but not in female. Metabolomics analysis of mice serum revealed that the HFD treatment may promote LDL/VLDL accumulation in male, indicating that LDL/VLDL may play essential roles in the sex difference of obesity.

The metabolic analysis of liver tissue also elucidated that male mice were largely differentiated from female in response to HFD, hence we further analyzed the correlation between LDL/VLDL and liver metabolites. The heatmap clarified that LDL/VLDL and betaine were significantly negatively correlated, especially after HFD-feeding. Betaine is an obligatory intermediate in the catabolism of choline, mainly participates in the conversion of homocysteine to methionine in the human liver and kidney [23]. Previous studies have proved that supplementation of betaine can reduce lipid accumulation and improve glucose homeostasis [24, 25]. Additionally, many studies also suggested that betaine can improve insulin resistance and limit obesity in mice fed with HFD [26]. Furthermore, Wang et al. reported that the administration of betaine can reverse hepatic fat accumulation and injury in mice by improving the function of adipose tissue [27]. Pekkinen et al. also validated that betaine can relieve obesity-related metabolic disturbances [28]. Combined with the quantitative data of hepatic betaine, we speculated that female mice could resist the exogenous signal of HFD by increasing the content of hepatic betaine,

stabilizing the LDL/VLDL. By contrast, the decreased of betaine level in male mice resulted in a large accumulation of LDL/VLDL, leading to the disorder of lipid metabolism.

Energy metabolism is vital to keep normal function in vivo [29]. In our study, the results of metabolomics showed that the contents of lactate and creatine in the liver of the F-HFD group increased significantly compared with the F-NC group, whereas there was no significant change between M-NC and M-HFD group. Lactate is the key product of glycolysis in the tricarboxylic acid cycle and creatine is the key component of the creatine kinase phosphatase system, both of which are important substances involved in energy balance in vivo [30, 31]. Thus, these findings may indicate that female mice enhance energy metabolism to resist weight gain and blood glucose induced by HFD.

Amino acids metabolism also affects normal physiological function [32]. On the one hand, amino acids are substrates of various metabolic pathways, such as the TCA cycle, protein synthesis, and gluconeogenesis [33]. On the other hand, amino acids as metabolic regulators, especially glucose metabolic regulators, can stimulate the rise of insulin and glucagon [34]. A series studies conducted that obesity would lead to aberrant amino acid metabolism [35, 36]. In the present study, we verified that HFD can reduce the levels of most amino acid metabolites in male mice, including leucine, valine, isoleucine, alanine, aspartate, lysine, glycine, tyrosine, phenylalanine, and nicotinurate, whereas significantly increase the levels in female mice. These results indicated that changes in blood glucose caused by HFD in female mice were not obviously, which was related to the increase of amino acid metabolism. This might be attributable to that amino acids accelerated the cycle of tricarboxylic acid, resulting in the induction of glucose consumption, or amino acids stimulated the secretion of insulin to produce a hypoglycemic effect.

Based on the results of liver metabolomics analysis, we found that female mice exhibited a positive metabolic pattern after HFD feeding. Compared with the F-NC group, the levels of most metabolites related to energy metabolism and amino acid metabolism in the F-HFD group were significantly increased. On the contrary, the male mice in the HFD group showed a negative metabolic pattern with the reductions of amino acid and energy metabolism levels. This could be the essential reason that the female mice did not increase their body weight and blood glucose level significantly, while the males became obese.

Conclusion

In this study, we found that male mice with HFD had significant weight gain, increased blood glucose, glucose tolerance, insulin resistance, and lipid metabolism disorders compared to female mice. Moreover, the NMR-based metabolomics analysis conducted to explore the potential mechanism linking gender differences in obesity. After 12 weeks of HFD, the liver of male mice showed a negative metabolic pattern, while female mice showed a positive metabolic pattern, accelerating the tricarboxylic acid cycle by improving energy metabolism and amino acid metabolism, thus resisting the weight gain, glucose tolerance, and insulin resistance induced by HFD. In addition, female mice could correct the disorder of

lipid metabolism caused by LDL/VLDL accumulation in serum by improving the expression of betaine in the liver (Fig. 6B). Overall, our study suggesting that energy and amino acid metabolism play vital roles in the sex differences of obesity. However, further investigation of sex-specific metabolic changes in mice with longtime high-fat diet is required, as post-menopausal women with higher risk for obesity [37]. Moreover, we suggest that the metabolomics investigation of serum and liver tissue may provide a new insight into metabolic mechanisms underlying sex differences of obesity.

Declarations

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Not applicable.

Author contributions

Conception and design: Chen Li, Hongchang Gao. Methodology: Yi Zhou, Xinyi Wang, Qinbo Chen, Pengxi Deng, and Wuqiong Wang. Data analysis: Yi Zhou, Hong Zheng and Liangcai Zhao. Writing and revision: Yi Zhou, Chen Li. Supervision: Hong Zheng and Liangcai Zhao.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All study procedures were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University and were in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology.

Consent for publication

N/A

Competing Interests

The authors declare no competing interests.

Abbreviations

HFD: high-fat diet; ND: normal diet; PCA: Principal component analysis; PLS-DA: Partial least squares-discriminant analysis; OPLS-DA: orthogonal partial least squares-discriminant analysis; VIP: variable importance in the projection.

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Figures

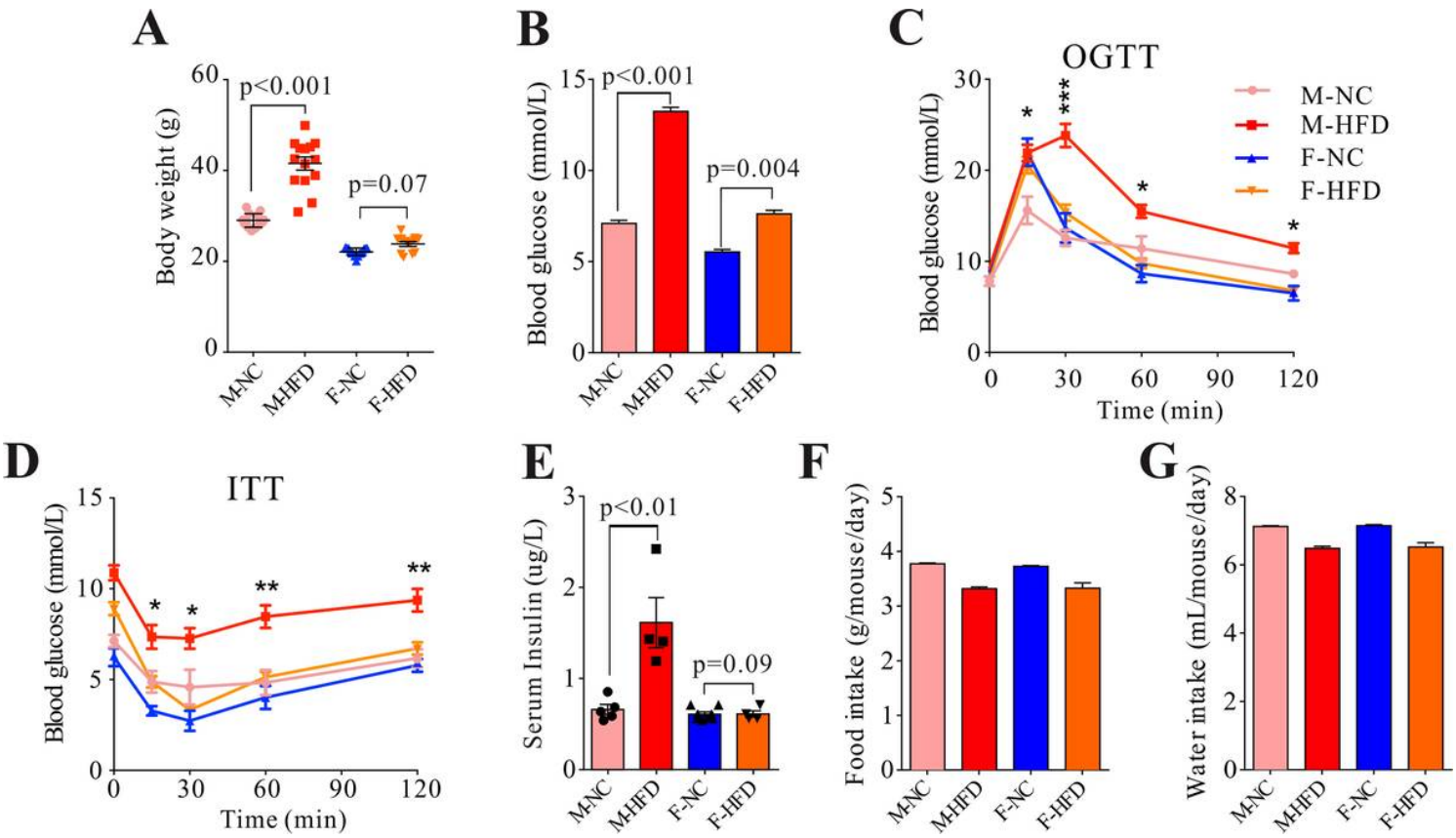

Figure 1

Effects of a high-fat diet on male and female mice. (A) body weight, (B) random blood glucose, (C) Oral Glucose Tolerance Test, (D) Insulin Resistance Test, (E) serum insulin level after 12h fasting, (F, G) food and water taken. *p<0.05, **p<0.01, ***p<0.001, n=14 each gender.

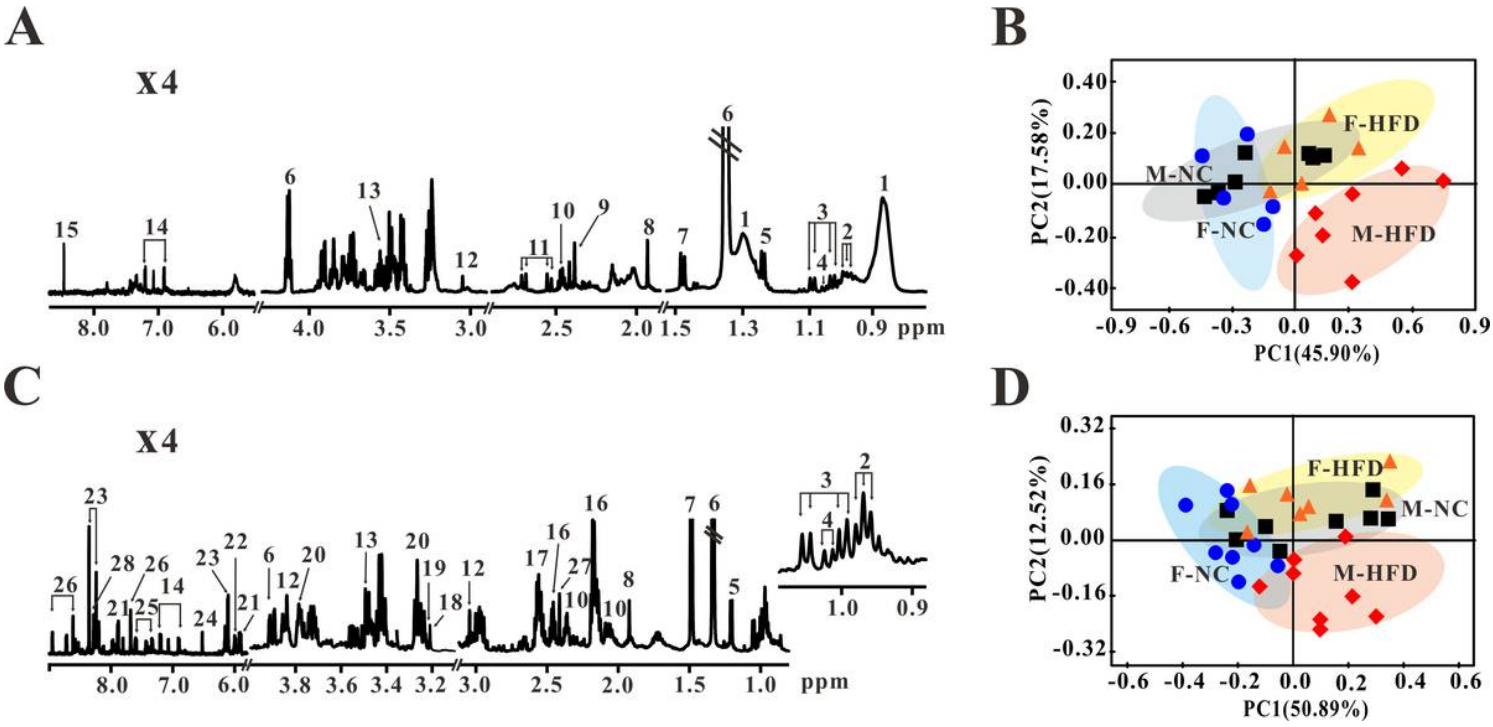

Figure 2

NMR-based serum and liver metabolomics analysis. (A) A representative 600MHz 1H NMR spectrum of mice serum. (B) A classification based on PCA was performed using the metabolomic profile in mice serum. (C) 1H NMR spectrum of mice liver. (D) A classification based on PCA was performed using the metabolomic profile in mice liver. M-NC, male mice which given a normal diet; M-HFD, male mice which given a high-fat diet; F-NC, female mice which given a normal diet; F-HFD, female mice which given a high-fat diet. Metabolite assignment: 1, LDL/VLDL; 2, leucine; 3, valine; 4, isoleucine; 5, 3-hydroxubutyrate; 6, lactate; 7, alanine; 8, acetate; 9, pyruvate; 10, glutamate; 11, citrate; 12, creatine; 13, glycine; 14, tyrosine; 15, formate; 16, glutamine; 17, glutathione; 18, choline; 19, phosphocholine; 20, betaine; 21, uridine; 22, GTP; 23, inosine; 24, fumarate; 25, phenylalanine; 26, nicotinurate; 27, succinate; 28, AMP.

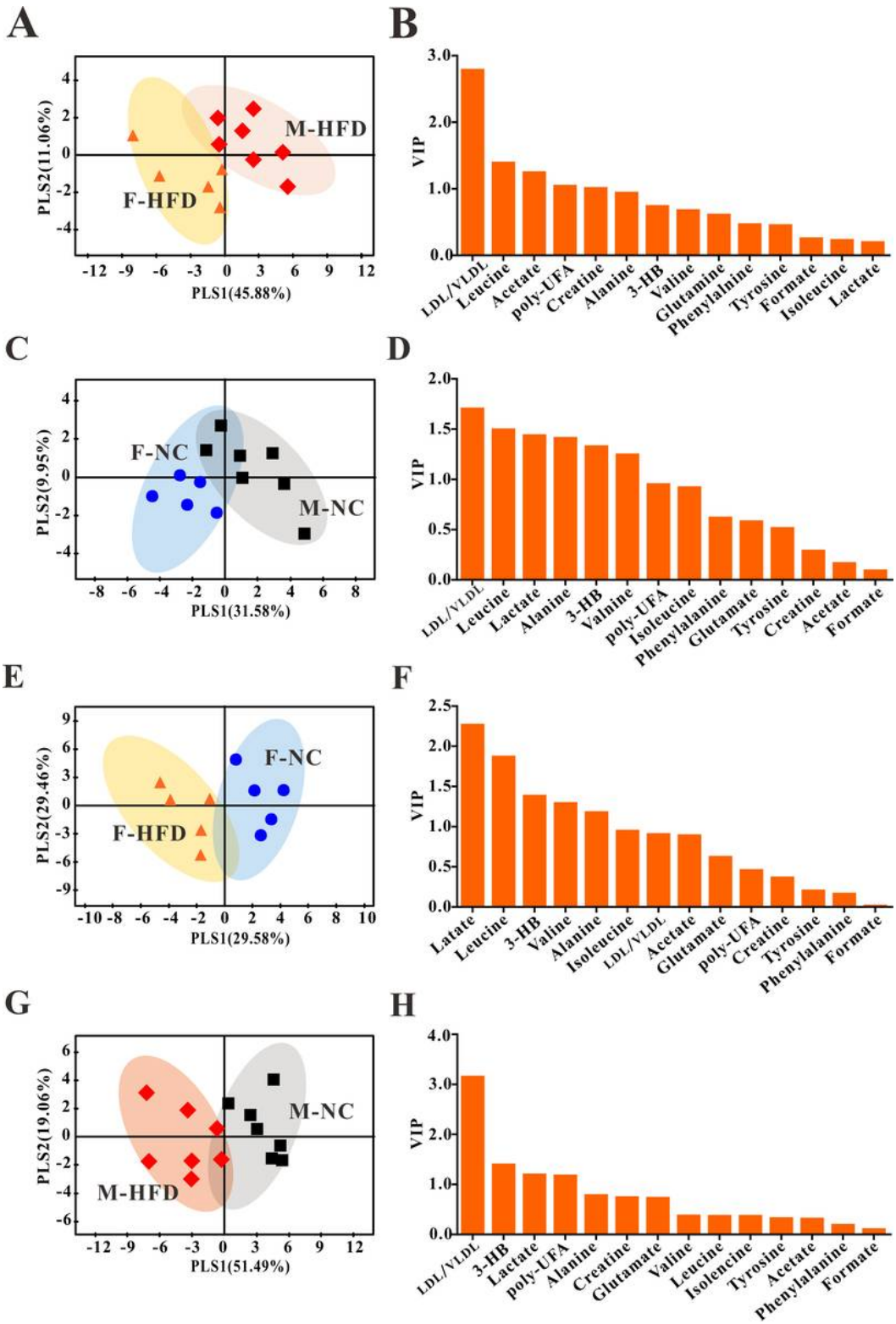

Figure 3

PLS-DA score and VIP plots obtained from NMR-based metabolomics of mice serum. Comparison of male and female mice with a high-fat diet (A, B) and a normal diet (C, D). Comparison of a normal and high-fat diet in female mice (E, F) and in male mice (G, H). It is considered as the main substance that causes differences in metabolic patterns when VIP > 2.0.

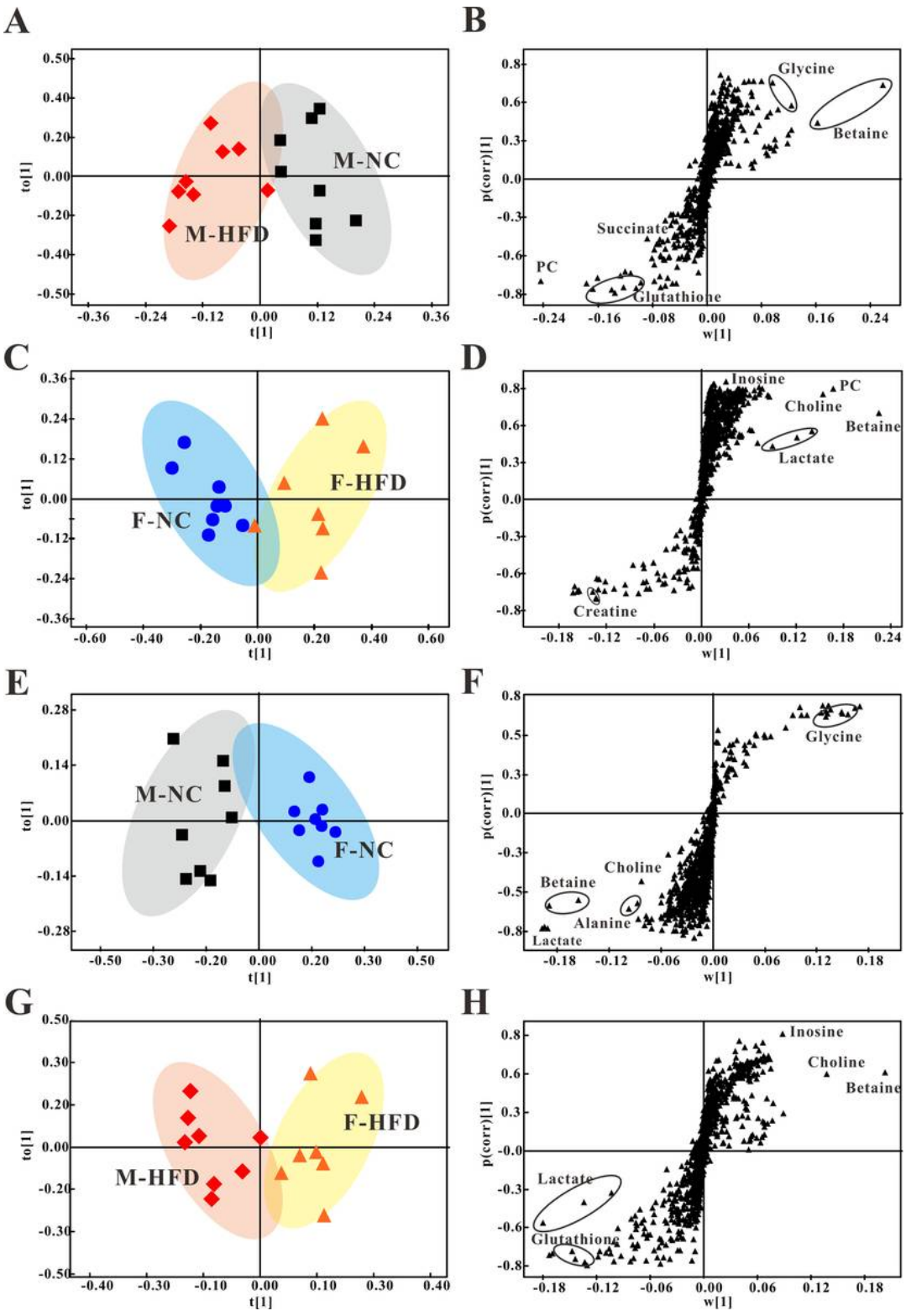

Figure 4

Pairwise comparison of OPLS-DA (left) and its corresponding S-plot (right) obtained from NMR-based metabolomics of liver samples. Comparison of a normal and high-fat diet in male mice (A, B) and in female mice (C, D). Comparison of male and female mice with a normal diet (E, F) and a high-fat diet (G, H). The substances marked in S-plot are important contributors to metabolic differences.

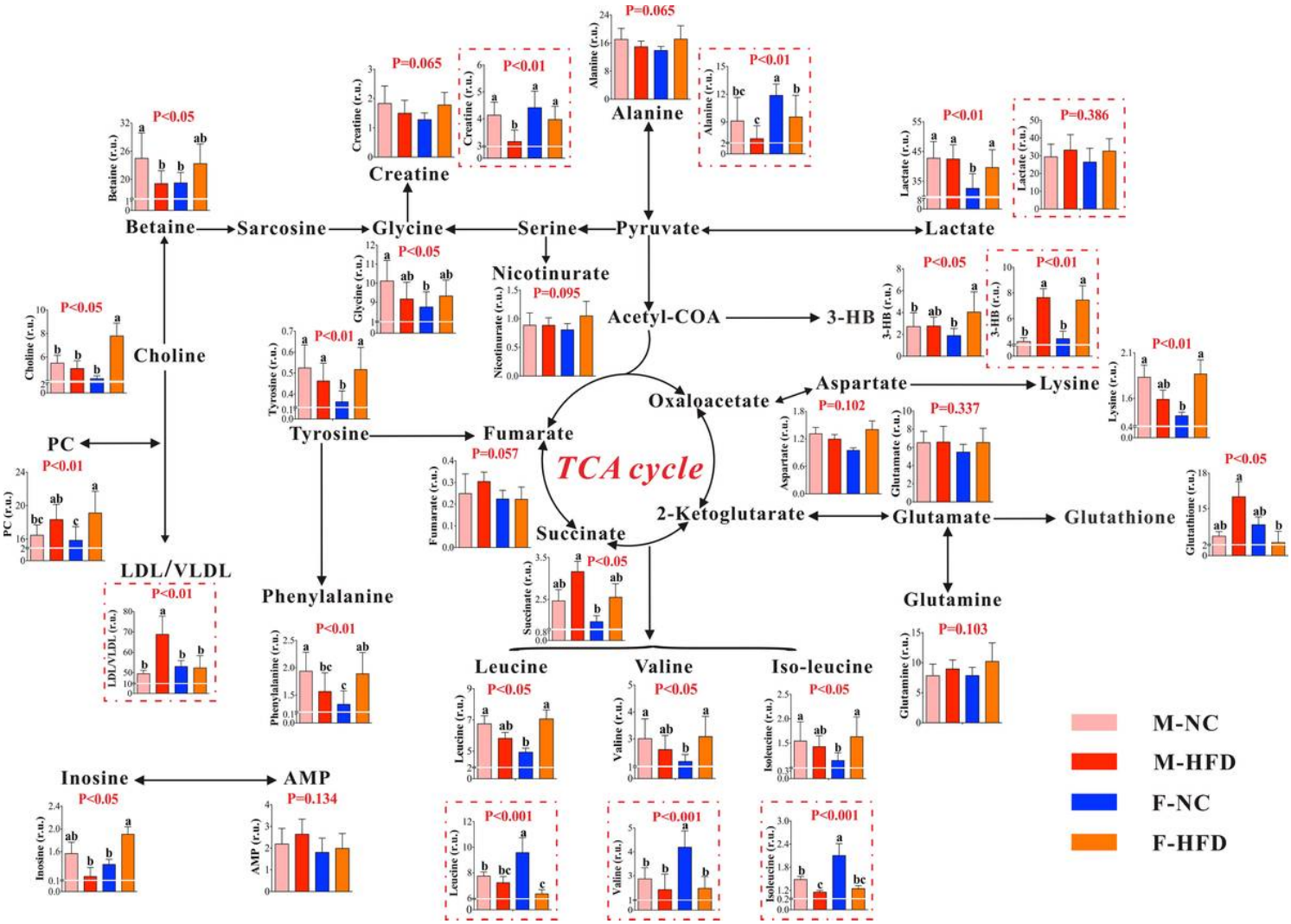

Figure 5

Metabolic changes in serum and liver samples of mice under a normal and high-fat diet. Data were expressed as mean ± SD (n = 8 for each gender); r.u., relative unit. Red outline box indicates metabolites detected from serum samples; Ohers indicates metabolites detected from liver samples. Significance was determined using Student's t-test with Bonferroni adjustment for multiple comparisons. Different letter codes indicate significant differences ($P < 0.05$).

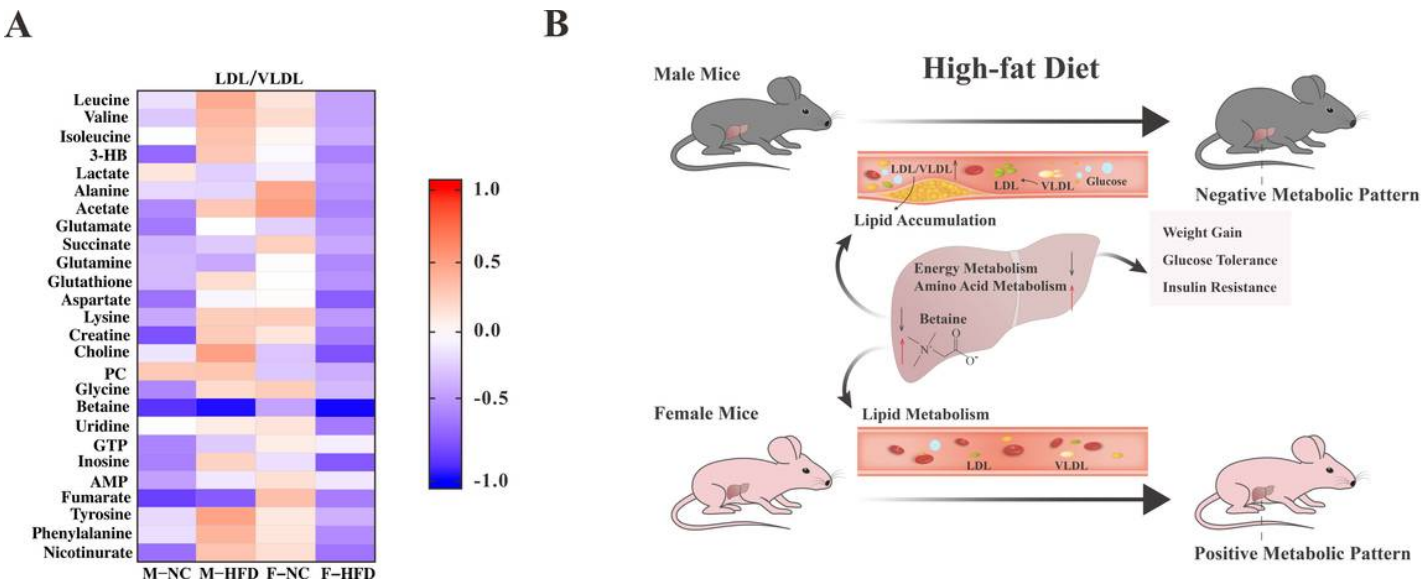

Figure 6

(A) Heatmap showing the correlation between LDL/VLDL in serum and liver metabolites in male and female mice under different dietary conditions. A darker color represents a higher correlation. (B) Female mice can resist obesity caused by HFD through improving energy metabolism and amino acid metabolism of the liver, whilst correct the lipid metabolism disorder through elevating the level of betaine in the liver.

Supplementary Files

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