Untargeted Metabolomic Characteristics of Skeletal Muscle Dysfunction in Rabbits Induced by a High Fat Diet

Huimei Fan

Sichuan Agricultural University - Chengdu Campus

YanHong Li

Sichuan Agricultural University - Chengdu Campus

Jie Wang (✉ wjie68@163.com)

Sichuan Agricultural University - Chengdu Campus

Jiahao Shao

Sichuan Agricultural University - Chengdu Campus

Tao Tang

Sichuan Agricultural University - Chengdu Campus

Tianfu Lai

Sichuan Agricultural University - Chengdu Campus

Mingchuan Gan

Sichuan Agricultural University - Chengdu Campus

Yuan Ma

Sichuan Agricultural University - Chengdu Campus

Xianbo Jia

Sichuan Agricultural University - Chengdu Campus

Songjia Lai

Sichuan Agricultural University - Chengdu Campus

Research

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Abstract

Background: Type 2 diabetes and metabolic syndrome caused by a high fat diet (HFD) have become public health problems around the world. These diseases are characterized by disrupted mitochondrial oxidation and insulin resistance in skeletal muscle, but the mechanism is not clear. Therefore, this study aims to reveal how a high-fat diet induces skeletal muscle metabolism disorder.

Methods: Sixteen weaned rabbits were randomly divided into two groups, one fed with a standard normal diet (SND) and another one fed a HFD for five weeks. Skeletal muscle tissue samples were extracted from each rabbit at the end of the 5-week trial. An untargeted metabolomics profiling was performed using ultraperformance liquid chromatography combined with mass spectrometry (UHPLC-MS/MS).

Results: The HFD significantly altered the expression levels of phospholipids, LCACs, histidine, carnosine and tetrahydrocorticosterone in skeletal muscle. Principal component analysis (PCA) and least square discriminant analysis (PLS-DA) indicated that rabbit skeletal muscle metabolism in the HFD group was significantly up-regulated compared with that of the SND group. Among the 43 skeletal muscle metabolites in the HFD group, phospholipids, LCACs, histidine, carnosine and tetrahydrocorticosterone were identified as biomarkers for skeletal muscle metabolic diseases, and may also serve as potential physiological targets for related diseases in the future.

Conclusion: The untargeted metabolomics analysis revealed that a HFD altered the rabbit skeletal muscle metabolism of phospholipids, carnitine, amino acids, and steroids. Notably, phospholipids, LCACs, histidine, carnosine and tetrahydrocorticosterone blocked the oxidative ability of mitochondria, and disturbed the oxidative ability of glucose and the fatty acid-glucose cycle in rabbit skeletal muscle.

Introduction

With the rapid development of the economy and society, great changes have taken place in the way of the human diet. It seems that the consumption of high-fat foods has become a normal part of life, which brings about many related diseases to the body. The most common is chronic metabolic disease, including obesity, hyperlipidemia, hypertension and diabetes. The basic characteristics of these chronic diseases are alterations to the TCA cycle, blocked glucose-fatty acid cycle, elevated rates of lipolysis, and impaired glucose homeostasis, making the body unable to effectively absorb and store energy, and resulting in metabolic disorders of the body [1, 2]. However, diet is considered to be the most important factor leading to metabolic disorders [3, 4].

A high-fat diet (HFD) causes obesity and metabolic diseases mainly due to the accumulation of metabolites in skeletal muscle. In addition to supporting movement and breathing, skeletal muscle is also the most important metabolic organ in the human body, which can regulate glucose homeostasis. At the end of a meal, 80% of the glucose is taken by skeletal muscles. Skeletal muscle not only plays an important role in glucose uptake, but it is also essential for ailments associated with metabolic disorders [5–8]. Over nutrition cause the deposition of metabolites in skeletal muscle, which leads to the

interruption of the mitochondrial oxidation ability of skeletal muscle. The release of insulin in the muscle of the pancreas desensitizes and inhibits absorption, leading to insulin resistance [8–10].

Moreover, there are an increasingly larger number of reports indicating that a HFD can cause skeletal muscle metabolism disorders resulting in related diseases. Studies have shown that a high-fat diet can cause blood sugar disorders and accelerate the loss of calcium in skeletal muscles, known medically as sarcopenia [2, 11]. The ability of skeletal muscle to absorb glucose decreased in mice fed a HFD, resulting in increased oxidation rate of fatty acids in skeletal muscle, leading to the accumulation of toxic metabolites and pro-inflammatory cytokines, and further aggravating insulin resistance [2]. A HFD can trigger the release of more pro-inflammatory cytokines in skeletal muscle, especially the harmful pro-inflammatory cytokine IL-6, whose overexpression leads to severe muscle atrophy and chronic, low-level skeletal muscle inflammation [2, 12, 13]. The accumulation of octanol and palmitoyl carnitine in skeletal muscle and the reduction of mitochondrial oxidative phosphorylation of glutamic acid/malic acid in mice fed with a HFD led to hyperleptinemia [2, 14]. These results suggest that a HFD can indeed lead to skeletal muscle metabolic disorders and related diseases.

In this study, we investigate whether a HFD alters the skeletal muscle metabolism physiology in rabbits. We utilize a metabolomic approach to assess the type and number of metabolites in skeletal muscle as potential therapeutic targets to elucidate blocked metabolic pathways that may contribute to HFD associated metabolic diseases in rabbits such as obesity, type 2-diabetes, and hypertension. We hypothesized that feeding rabbits with a HFD for five weeks would change rabbit skeletal muscle metabolism physiology. Thus, the aim of this study was to determine whether rabbits in a HFD group altered their skeletal muscle metabolic physiology compared to rabbits in the normal-diet group to elucidate metabolites associated with skeletal muscle metabolic disorders.

Materials And Methods

Ethics Statement

This study was approved and conducted in accordance with the ethical standards of the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, 611130, China.

Animals and feeding strategy

Sixteen weaned rabbits (35 days) with similar body weight index and health level were selected from the teaching rabbit farm of Sichuan Agricultural University and fed continuously for five weeks. Rabbits were randomly divided into two groups. One group was fed a standard normal diet (SND; n = 8), while the other received a high-fat diet (HFD; n = 8) with 10% lard added to a standard normal diet. The composition and nutrient content of the standard normal diet (SND) and the high-fat diet (HFD) were described in our previous study[15]. They were fed 120 grams per day. Water was freely available, and feed was provided

twice a day. Each rabbit was kept separately in a clean cage (600 × 600 × 500 mm) and placed in an environmentally controlled room (21–23°C, 60–75% humidity, 14-hourslight [60 lx]).

Skeletal muscle tissue collection and preparation

At the end of the 35th day of the trial, eight rabbits in each group were screened out and sacrificed by intravenous injection. 16 samples of skeletal muscle tissue were immediately collected, freeze-dried and crushed. Skeletal muscle tissue samples (100 mg) were individually grounded with liquid nitrogen and the homogenate was resuspended with prechilled 80% methanol and 0.1% formic acid by well vortex. The samples were incubated on ice for 5 min and then centrifuged at 15,000 g, 4°C for 20 min. The supernatant was diluted with LC-MS grade water to a final concentration containing 53% methanol. The samples were subsequently transferred to a fresh Eppendorf tube and centrifuged at 15000 g, 4°C for 20 min. Finally, the supernatant was injected into the Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) analysis system [16]. Equal volume samples were taken from each test sample and mixed as quality control (QC) samples to ensure the robustness of the large-scale analysis.

Metabolomic profiling

An untargeted metabolomics profiling was performed using ultraperformance liquid chromatography combined with mass spectrometry (UHPLC-MS/MS) with a Vanquish UHPLC system (ThermoFisher, Germany) coupled with an Orbitrap Q Exactive™ HF mass spectrometer (Thermo Fisher, Germany) in Novogene Co., Ltd. (Beijing, China). Samples were injected into a Hypesil Goldcolumn (100×2.1 mm, 1.9µm) using a 17-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% FA in Water) and eluent B (Methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (Methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2-100% B, 12.0 min; 100% B, 14.0 min; 100-2% B, 14.1 min; 2% B, 17 min. The Q Exactive™ HF mass spectrometer was operated in positive/negative polarity mode with a spray voltage of 3.2 kV, a capillary temperature of 320°C, a sheath gas flow rate of 40 arb, and an aux gasflow rate of 10 arb.

Statistical analysis

Statistical analyses were performed using R statistical software (R version R-3.4.3), Python (Python 2.7.6 version), and CentOS (CentOS release 6.6). When data were not normally distributed, normal transformations were attempted using the area normalization method.

A principal component analysis (PCA) was used to assess the stability of the analytical process and to visualize global metabolome differences between SND and HFD rabbit groups. To maximize class discrimination necessary for discovery of potential metabolic biomarkers between SND and HFD rabbit groups, a supervised model of partial least squares discriminant analysis (PLS-DA) was applied. The

variable importance in the projection (VIP) value was used to estimate the discriminatory power of each variable for the separation of the SND and HFD rabbit groups in the PLS-DA model, and variables with $VIP > 1$ were considered important. Furthermore, we performed a permutation test to ensure that the PLS-DA model was not over fitting.

For clustering heat maps, the data were normalized using z-scores of the intensity areas of differential metabolites and were plotted using a heatmap package in R. Pearson correlation coefficients between differential metabolites in the SND and HFD rabbit groups were analyzed in R with `cor()` and their significance tested with `Test()`. A P-value < 0.05 was considered as statistically significant. Correlation plots were drawn with R program `corrplot`.

The functions of metabolites and metabolic pathways in the SND and HFD rabbit groups were studied using the KEGG database. Metabolites with $VIP > 1$, P-value < 0.05 , and fold change ≥ 2 or $FC \leq 0.5$ were considered to be differential metabolites. Volcano plots were used to filter metabolites of interest based on metabolite values of $\log_2(\text{FoldChange})$ and $-\log_{10}(\text{P-value})$.

Results

Effect of HFD on skeletal muscle tissue metabolomics

The PCA values (Fig. 1(a) and 1(b)) and the PLS-DA results (Fig. 1(c) and 1(d)) showed that the skeletal muscle of SND and HFD rabbit groups differed from each other. The values of the model quality parameters for skeletal muscle were $R^2 = 0.84$ and $Q^2 = -0.81$ for the positive model data (Fig. 1c) and $R^2 = 0.70$ and $Q^2 = -0.82$ for the negative model data (Fig. 1d). These results showed that the PLS-DA model had a good predictive ability and there was no overfitting.

Endogenous substances with $VIP > 1$ were selected to further analyze the effects of HFD on metabolic pathways in skeletal muscle. Table 1 shows that 43 endogenous metabolites had $VIP > 1$ in skeletal muscle, and that 26 of them had higher values and 17 had lower values in HFD than in SND rabbits. The levels of these 43 metabolites were visualized in a volcano figure (Fig. 1e) and a heat map (Fig. 1f). These figures show that HFD affects the metabolism of phospholipids, carnitine, amino acids, and steroids in the skeletal muscle of rabbits, and that the HFD greatest influence occurs on amino acid and lipid metabolism.

Table 1
Potential skeletal muscle biomarkers based on UPLC-Q-TOF/MS

Name	Formula	m/z	P-value	VIP	Trend
PC (14:1e/3:0)	C25 H50 N O7 P	508.33981	0.00060252	1.96834418	↓
Hexanoylcarnitine	C13 H25 N O4	260.18542	0.001221548	2.134885168	↑
Phenylacetylglycine	C10 H11 N O3	194.08138	0.002987006	1.562157041	↓
LPC 15:1	C23 H46 N O7 P	480.30856	0.004218472	1.679235509	↓
Palmitoylcarnitine	C23 H45 N O4	400.34161	0.004662458	2.728685353	↑
5-chloro-2,8-dimethyl-4-[(3-nitro-2-pyridyl)oxy]quinoline	C16 H12 Cl N3 O3	330.05746	0.006079831	1.752888258	↓
Ala-Gln	C8 H15 N3 O4	218.11295	0.011721656	2.174643548	↑
N-Acetyl-L-carnosine	C11 H16 N4 O4	269.12411	0.012378517	1.528953387	↑
LPC 22:6	C30 H50 N O7 P	568.33807	0.016506546	1.321877932	↑
PC (14:1e/2:0)	C24 H48 N O7 P	494.32413	0.016550657	1.102232178	↓
Muscone	C16 H30 O	239.23694	0.018553432	1.998565512	↓
Propionylcarnitine	C10 H19 N O4	218.13879	0.020501301	1.659387379	↓
LPC 15:0	C23 H48 N O7 P	482.32388	0.027825481	1.548906089	↓
LPE 17:0	C22 H46 N O7 P	468.30875	0.030453786	1.236219	↓
Corticosterone	C21 H30 O4	347.22134	0.032938755	2.163429757	↓
1-Palmitoyl-Sn-Glycero-3-Phosphocholine	C24 H50 N O7 P	496.33948	0.033163212	1.248603083	↓
Tetrahydrocorticosterone	C21 H34 O4	368.27911	0.035427687	2.2470103	↑
Prolylleucine	C11 H20 N2 O3	229.15422	0.035890864	1.53343442	↓

Name	Formula	m/z	P-value	VIP	Trend
DL-Carnitine	C7 H15 N O3	162.11217	0.036302614	2.164262278	↓
L-Histidine	C6 H9 N3 O2	156.07664	0.036883235	2.296408916	↑
Carnosine	C9 H14 N4 O3	227.11374	0.038157621	1.374481323	↑
Lysops 22:5	C28 H46 N O9 P	572.29755	0.043926394	1.145501212	↑
Betaine	C5 H11 N O2	118.0863	0.046878628	1.626178977	↓

Effects of HFD on the metabolic pathways of endogenous substances in skeletal muscle

The results showed that HFD affected 13 metabolic pathways related to endogenous substances in skeletal muscle (Fig. 2). In addition, HFD affected endogenous substances related to metabolic pathways in rabbits, especially histidine and carnosine metabolism. This indicates that HFD primarily affected amino acid metabolism in skeletal muscle of rabbits after 35 days of feeding with a HFD, leading to changes in endogenous substances. A metabolite synthesis diagram is shown in Fig. 3.

Discussion

The lipids as a structural element of the cell membrane, lipids regulate metabolic homeostasis through various mechanisms, thus they are essential to maintaining homeostasis. However, lipids can also have deleterious effects on glucose metabolism and insulin sensitivity. When animals receive a HFD and obesity occurs, the body's normal metabolism slows dramatically, and adaptive mechanisms often fail to give the body appropriate feedback. The resulting influx of lipids from adipose tissue exceeds its storage capacity, leading to the accumulation of harmful lipids in skeletal muscle, which is thought to be an important factor in insulin resistance [17, 18]. Most phospholipids exist in skeletal muscle membranes and are important regulators of skeletal muscle mitochondrial respiratory function [19, 20]. Relevant studies have shown that increased phospholipids in skeletal muscle may lead to changes in mitochondrial metabolism or increase in mitochondrial membrane remodeling, making phospholipids play a role in the inflammation of skeletal muscle. Further, excess lipids have harmful effects on the body through the action of the endoplasmic reticulum. In the case of a HFD or chronic nutritional stress, lipid synthesis in the endoplasmic reticulum is disordered, which interrupts the calcium signal [19]. Lysophosphatidic acid is a kind of lipid with strong biological activity produced by the ATX enzyme. High-fat-induced changes in LPA concentrations have been associated with the development of obesity-related damage to glucose homeostasis in addition to inflammatory disease [21, 22]. Previous studies showed

that excessive endogenous LPA production in HFD mice inhibited glucose tolerance, which may lead to the deterioration of glucose tolerance [23, 24]. In this study, PC and PE (18:1) in the HFD rabbit group were significantly up-regulated, consistent with previous studies, and could be used as a potential biomarker for inflammation. However, different levels of lysophospholipids (LPC (15:1), LPC (16:1), LPC (17:1)) were significantly down-regulated compared with the SND rabbit group, contrary to previous studies. These results suggest that a HFD may not have adverse effects on LPA metabolism in rabbit skeletal muscle. In other words, not all high-fat diets have harmful effects, but they do promote skeletal muscle growth and development to some extent.

Carnitine is a type β fatty acid produced during the esterification of fatty acids. It plays a vital role in the regulation of muscle oxidative metabolism. Ninety five percent of medium-chain acylcarnitines (MCACs) and long-chain acylcarnitines (LCACs), and intermediate accumulation of fatty acid oxidation in skeletal muscle may lead to insulin resistance. There is increasing evidence that LCACs refers to the metabolic syndrome caused by excess energy [25]. Rats fed with HFD reduced LCACs activity, aggravated the circulation of glucose-fatty acid, and resulted in metabolic stiffness caused by diet [26].

Hexanoylcarnitine, propionylcarnitine and propionylcarnitine are the LCAC fatty acid derivatives of carnitine [27]. Hexanoylcarnitine was markedly increased in the HFD rabbit group than in the SND rabbit group in this study. This indicates that a LCACs increase in skeletal muscle can cause a disturbance in fatty acid-glucose circulation. This metabolism change of LCACs is helpful to improve our understanding of a potential physiological mechanism for skeletal muscle dysfunction in HFD rabbits. However, propionylcarnitine and DL-Carnitine decreased in the HFD group. High levels of propionylcarnitine and DL-Carnitine may serve as a marker of vitamin B12 deficiency, be associated with orofacial clefts, and have beneficial effects on cardiac metabolism, myocardial microvasculature function during ischemia, and coronary blood flow[28]. These two LCACs may not be harmful to the body. Although previous studies have shown that increased concentrations of these LCACs are beneficial to the body, the mechanism of the influence of low concentrations of LCACs on the body is still unclear and needs further study. Longer studies are needed to investigate if reductions of propionylcarnitine and DL-Carnitine levels could also decrease insulin sensitivity. The effect of LCACs on skeletal muscle metabolism in rabbits was described only by metabonomic methods in this paper, thus further studies on other aspects are needed.

Amino acids are closely related to the TCA cycle and glucose metabolism. Studies have shown that when dietary lipid exceeds demand, phosphorylation of nitric oxide synthase occurs in the skeletal muscle endothelium, resulting in reduced amino acid metabolism, leading to the accumulation of large amounts of amino acids in skeletal muscle, resulting in blocked synthesis and interruption of mitochondrial oxidation ability, which may cause diseases such as type 2 diabetes and insulin resistance [29]. Other studies reported that endogenous amino acids increase in skeletal muscle is related to diabetes, making amino acids a candidate biomarker for related diseases. Because of high levels of amino acids compete with glucose oxidation at the substrate level and interfere with the transcription of insulin signals, thus reducing the sensitivity of insulin signals and leading to impaired glucose oxidation [30]. Branched chain amino acids (leucine, isoleucine, and valine), aromatic amino acids (phenylalanine, tyrosine and tryptophan), and aliphatic amino acid (lysine) are generated by carboxylic acid by transamination of

intermediates dependent on glucagon and insulin secretion to regulate glucose metabolism [31, 32]. Altered levels of four amino acids were detected in rabbit skeletal muscle in this study using an untargeted metabolomics approach, namely leucine, glycine, lysine, histidine and carnosine (a histidine residue). Leucine and carnosine were significantly down-regulated whereas glycine, lysine and histidine were significantly up-regulated in skeletal muscle from the HFD group compared to the SND group. Numerous studies have shown that if the concentration of histidine is too high, a large amount of high nitrogen ammonia will be produced during the decomposition process, changing the anti-oxidation effect of carnosine, leading to accelerated lipid oxidation, and diseases related to insulin resistance. In addition to increase in the production of ammonia, high concentrations of histidine will lead to changes in the concentration of several amino acids; glutamic acid, alanine and glutamine will increase, and branched amino acids (valine, leucine and isoleucine) will decrease, leading to amino acidemia [33, 34]. In this study, histidine and carnosine levels in the HFD group were significantly up-regulated compared to the SND group, leading to significantly up-regulated glutamine levels and significantly down-regulated leucine levels, consistent with previous reports. This suggests that a HFD has specific effects on skeletal muscle metabolism in rabbits, which can be used as potential markers for metabolic diseases. However, contrary to previous research, five consecutive weeks of HFD failed to cause metabolic disorders due to high levels of lysine and glycine in rabbit skeletal muscle, which further ensured the normal operation of TCA circulation, gluconeogenesis and oxidative decomposition of glucose, and maintained the normal growth and development of rabbits. Moreover, previous studies reported that elevated plasma leucine levels were associated with insulin-resistance related diseases, but excessive histidine in skeletal muscle could lead to decreased leucine levels and insulin-resistance related diseases. These contrasting results may be an indication that leucine content in different parts of the body will have a different action mechanism, which needs further study.

Glucocorticoids containing corticosterone, cortisol, and tetrahydrocorticosterone are produced by the adrenal glands [35]. As a substance of cholesterol metabolism, glucocorticoids are an important part of the biological stress response that helps regulate body balance and glucose homeostasis. With changes in diet, bone cells can adversely affect the skeletal system by altering the activity of glucocorticoid levels causing muscle wasting, osteoporosis and insulin resistance [36]. When mice were fed a high-fat diet, lipids were deposited in skeletal muscles, resulting in increased fat decomposition, gluconeogenesis disorder, and reduced stress response. This can lead to an increase in glucocorticoids followed by metabolic syndrome and type 2 diabetes [37]. Conversely, mice fed a high-fat diet for eight weeks had similar levels of corticosterone as mice fed a low-fat diet [38]. In the present study, rabbits in the HFD group had significantly higher concentrations of tetrahydrocorticosterone than rabbits in the SND group, consistent with the above reports, indicating that tetrahydrocorticosterone could be used as a potential marker for related diseases in skeletal muscle. However, corticosterone concentration decreased significantly. Other studies showed that corticosterone, in addition to being a biological pacemaker, controls physiological processes in mammals including humans, and it can also regulate the daily circadian rhythm by adjusting the level of adrenocorticosterone controlled by the suprachiasmatic nucleus. In addition, overall corticosterone levels were higher at night than during the day because rabbits

eat 20 to 25% more at night than during the day [39]. Therefore, the results of the current study differ from those of previous studies perhaps due to the decrease of corticosterone levels in skeletal muscle caused by the unique biological characteristics of rabbits and the daytime samples taken in this trial. The level of glucocorticoids in skeletal muscle contributes to our understanding of steroid hormones, but the biological mechanism needs further research.

Conclusion

A high-fat diet caused a disturbance of the phospholipid, acylcarnitine, amino acid and steroid metabolism in skeletal muscle of rabbits. The expression levels of phospholipids were significantly up-regulated, disrupting the oxidative ability of mitochondria, and impairing glucose homeostasis, potentially leading to inflammation-related diseases; Acetylcarnitine expression was significantly up-regulated, leading to insulin resistance and impaired glucose homeostasis, which may lead to chronic metabolic diseases such as type 2 diabetes, obesity, and hypertension; The expression levels of histidine and carnosine were significantly up-regulated, leading to excessive oxidative stress, lipid oxidation, insulin resistance, and disruption of the TCA cycle, which may be associated with hyperlipidemia, aminophenemia, and type 2-diabetes; The expression level of tetrahydrocortisol increased significantly, inhibiting protein synthesis and gluconeogenesis, which may lead to metabolic syndrome and type 2 diabetes. In summary, phospholipids, LCACs, histidine, carnosine and tetrahydrocorticosterone could be used as potential biomarkers associated with chronic metabolic diseases, contributing to a better understanding of the underlying biological mechanisms, and providing a basis for the treatment and diagnosis of related diseases in the future.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included within the article.

Authors' contributions

Huimei Fan was the first author, mainly responsible for the writing of the manuscript and participating in the whole experiment process. Yanhong Li, the co-author, provided a lot of help in the experiment and the writing of the paper. Mr. Jie Wang is the corresponding author and my mentor. Give many corrections and patient guidance at the end of the manuscript. Jiahao Shao, Tao Tang, Tianfu Lai, Mingchuan Gan and Yuan Ma, Xianbo Jia and Songjia Lai also gave me a lot of help. We thank Dr. Elzo for kindly editing the manuscript and other authors help for this study. Wrote the paper: NM YJY JYL. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved and conducted in accordance with the ethical standards of the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, 611130, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Skeletal muscle metabolite score and permutation test of rabbits in normal and high-fat diet groups. a, b, PCA score graph between normal and high-fat diet groups; c, d, permutation test from PLS-DA models; e, f, volcano of metabolites with significant differences between normal and the high-fat diet groups; g is the clustering heat map of differential metabolites between normal and the high-fat diet groups.

Figure 2

Disturbed pathways in response to a high-fat diet. A. Histidine metabolism and beta-Alanine metabolism; B. ABC transporters; C. Steroid hormone biosynthesis; D. Prion diseases; E. Central carbon metabolism in cancer; F. Regulation of lipolysis in adipocytes; G. Phenylalanine metabolism; H. Glycine, serine and threonine metabolism; I. Protein digestion and absorption; J. Aldosterone synthesis and secretion; K. Aminoacyl-tRNA biosynthesis and biosynthesis of amino acids.

Figure 3

Interference and change of potential metabolic pathways in skeletal muscle of rabbits fed a high-fat diet. Different colors represent different metabolic pathways.