Transcriptome and proteomics analysis revealed key genes in rabbit skeletal muscle after weight loss

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Keywords: Metabolism, Omics, Network, Rabbit, Skeletal muscle

Posted Date: September 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-868357/v1>

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Abstract

Skeletal muscle is one of the important organs of energy metabolism and is closely related to insulin secretion. Its metabolic characteristics have a major influence on fat distribution in various body tissues. However, rabbit skeletal muscle has lower intramuscular fat content, and its metabolic mechanism is still unclear. We investigated skeletal muscle growth and metabolic differences among rabbits using a comparative multi-omics approach after a 14-day weight loss by a restricted diet. Rabbit body weight and perirenal fat were significantly reduced after the weight loss. Transcriptomics data revealed 917 differentially expressed genes in skeletal muscle primarily enriched in the FOXO signaling, Glutathione metabolism, and AMPK signaling pathways. Proteomics data found 127 differential proteins concentrated in protein metabolism and immunoinflammatory pathways. Combined analysis demonstrated that eight genes (*ATP2A2*, *PDLIM3*, *GPX7*, *FKBP5*, *MYL3*, *COL5A2*, *UCHL1*, and *COL3A1*) were strongly correlated at the transcriptional and translational levels. These results provide a good reference for further revealing the mechanism of skeletal muscle metabolism in rabbits.

1 Introduction

Obesity has become an epidemic throughout the world causing a variety of metabolic diseases, such as type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), hypertension, and hyperlipidemia [1]. The lifestyle of a large number of people is characterized by little exercise and excessive intake of high-fat/high-energy diets that result in accumulation of fat in different body tissues and higher levels of obesity. Drug therapy, diet regulation, and exercise have been tried to help lose weight and lessen the obesity trend. However, these measures have been unable to achieve the desired levels of weight loss[2]. Carbohydrate-restricted and fat-restricted diets are regarded as more effective to lose weight compared to other strategies [3, 4]. Human diet interventions that include changes in nutritional components and calorie restrictions can alleviate inflammatory reactions and improve tissue insulin sensitivity caused by human obesity [5, 6]. Most studies have mainly focused on the process of weight loss in obese bodies, thus little is known about the process of weight loss in potentially obese normal bodies.

Skeletal muscle is a key organ for glucose metabolism and a primary target of insulin action. Its oxidative metabolic activity plays an important role in regulating intramuscular glucose and lipid metabolism influencing body fat deposition [7]. Previous studies demonstrated that fatty acid deposition in skeletal muscle of obese individuals is significantly higher than that in normal individuals, and can lead to changes in types of muscle fibers that can reduce skeletal muscle sensitivity to insulin [8]. Skeletal muscle secretes most myogenic factors regulating the energy metabolic homeostasis of tissues such as liver, adipose, and kidney through autocrine and paracrine mechanisms [9]. Thus, studying skeletal muscle growth and metabolism can improve our understanding of the metabolic unbalance associated with animal obesity. Weight loss can be regarded as a process of continuous reduction of fatty acid deposition in the body. Previous studies of weight loss have found that the oxidative capacity of skeletal muscle is reduced and more likely inclined to store fatty acids in a state of obesity [10]. The level of cellular oxidative metabolism in skeletal muscle, which has obvious differences among individuals, is

one of the important factors affecting intramuscular fatty acid deposition that can be improved by restoring the oxidative capacity and glucose tolerance of skeletal muscle after weight loss [11, 12]. Many studies confirm that various genes related to oxidative metabolism are involved in fatty acid metabolism of skeletal muscle, such as forkhead box O1 (FoxO1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), mitochondrial uncoupling protein-3 (UCP3), carnitine palmitoyltransferase (CPT-1), and adipose tissue triglyceride lipase (ATGL) [13–15]. However, the growth and metabolism of skeletal muscle is a dynamic and complex process, and these genes can only account for some of the skeletal muscle metabolic factors, but they cannot fully explain its internal mechanism.

Omics analyses are essential to obtain appropriate information about biological processes and molecular mechanisms. Studies with obese mice have found that the IL6, AMPK and PPAR signal pathways are involved in the process of gaining weight in muscle of obese mice [16]. Pig muscle fat deposition studies revealed that glycolytic enzymes such as glycerin 3-phosphate, and creatine kinase M are related to higher levels of adipogenesis and glycolysis rates in obese pigs. Further, transcriptomic analyses indicated that miRNAs and genes are involved in the regulation of the cell cycle, fatty acid biosynthesis, and actin cytoskeleton regulation. Among them, SOX2, SIRT1, KLF4, PAX6, and genes in the transforming growth factor β superfamily are involved in the regulation of the FOXO signaling pathway, stem cell pluripotency regulatory signaling pathway, and PI3K-Akt signaling pathway, which are closely related to skeletal muscle growth and development in pigs [17, 18]. Nevertheless, protein is the main component of skeletal muscle and performs important biological functions that need to be better understood to solve biological problems. Further, comparative multi-omics analyses provide needed information to understand biological processes associated with disease prediction. Comparative multi-omics analyses of mouse and human liver lipid metabolism identified PSMD9 as an important regulator of lipid metabolism [19]. However, fat deposition in skeletal muscle of domestic rabbits is less well-understood than in other domestic animals [20]. This information suggests that differences in muscle growth and metabolism among rabbits exist, and that knowledge of their specific genetic and molecular mechanisms would provide useful information to explain muscle metabolic diseases in rabbits and other species.

The objective of this study was to analyze differences in skeletal muscle growth and metabolism in young rabbits after weight loss using comparative transcriptomics and proteomics analyses. We constructed a key metabolic regulatory molecular network to explain potential molecular regulatory mechanisms for skeletal muscle growth and metabolism. Outcomes of this research are expected to deepen our understanding of rabbit skeletal muscle development, provide reliable information for improving rabbit meat quality, and contribute to solving human skeletal muscle diseases.

2 Materials And Methods

2.1 Experimental design and sample extraction

Sixteen healthy female Tianfu black rabbits of 35 to 70 days of age were selected from the teaching and research rabbit farm of the Sichuan Agriculture University and randomly divided into a control group

(CG_M: n = 8) and an experimental group (EG_M: n = 8). Rabbits from the two groups received the same feeding regimen before the age of 70 days, and they were free to eat and drink at will during this period. The specific experimental scheme can refer to our previous reference[21]. From age of 70 days to 90 days, the eight rabbits from the EG group were subjected to weight loss by restricting the amount of feed supplied to them. The amount of feed supplied to the eight EG rabbits gradually decreased from 60g/day to 20g/day during a 5-day adaptation period (71 to 75 days of age), then maintained at 20g/day for 15 days (76 to 90 days of age). Six CG_M rabbits were humanely slaughtered at 70 days of age, and six EG rabbits were sacrificed at 90 days of age. Samples from the right femoral muscle were collected for the six slaughtered rabbits in each group, rapidly frozen with liquid nitrogen, and stored at - 80 °C for subsequent analyses.

2.2 RNA isolation, library preparation, and RNA-sequencing

The total RNA of six muscle samples from the CG_M and EG_M were isolated using RNAiso Plus (Code No. 9108, TAKARA). The integrity and purity of the total RNA were evaluated using Agilent 2100 BioAnalyzer and Nanophotometer spectrophotometer, respectively. The RNA libraries were constructed using NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®. To ensure library quality, the insert size of each library was evaluated using Qubit2.0 Fluorometer and Agilent 2100 BioAnalyzer after library construction was completed. Subsequently, Illumina sequencing was carried out using RNA pools of the various libraries according to their effective concentration and target sequenced data. To ensure the quality and reliability of the data analysis, low quality reads of raw data (Qphred < = 20 bases for over 50% of the total read length) and reads with adapter and invalid bases were filtered and removed. The HISAT2 software was used to compare clean reads to those from the reference rabbit genome quickly and accurately (GCF_000003625.3). The Subread software package was utilized to remove lower quality (values lower than 10) and unpaired reads. Sequencing depth and gene length were corrected using a calculated value of Fragments Per Kilobase per Million Mapped Reads (FPKM). The DESeq2 program was used for analyzing differential gene expressions.

2.3 Detection and identification of proteins

Samples of skeletal muscle kept at -80 °C were grinded into powder under low temperature conditions, quickly transferred to a 1.5ml centrifuge tube with pre-cooled with liquid nitrogen, and an appropriate amount of protein lysis solution added (100 mM NH_4HCO_3 (pH = 8) (Sigma/5330050050), 8 M Urea and 0.2% lauryl sodium sulfate (SDS)) to fully dissolve them, followed by centrifugation at 12000g at 4°C for 15min. The supernatant from the centrifugation was placed in a clean tube for 1 h at 56°C, followed by addition of 10mM dithiothreitol (DTT, Purchased from Sigma/D9163-25G). Subsequently, a sufficient amount of iodoacetamide (IAM) was added to the tube and left in the dark for 1h at room temperature. Then, the content of the tube was mixed with 4-times volume of precooled acetone by vortexing, incubated at -20°C for at least 2 h, and centrifuged at 12000g at 4°C for about 15 min to collect the precipitate. An appropriate amount of solution buffer (0.1 M triethylammonium bicarbonate (TEAB, pH 8.5) and 6 M Urea) was added to the precipitate to dissolve it and store it at -80°C.

The total protein quality was conducted using a Bradford Protein Assay Kit (Purchased from Beyotime) according to the manufacturer's instructions. Concentration gradient ranged from 0 to 0.5ug/ul. The TMT labeling of peptides involved taking samples (120 µg) of each protein, add 100ul of a protein lysis solution, mix it with 500ul 100mM TEAB buffer well at 37°C for 4h, and then digest it overnight with 1.5 µg trypsin and CaCl₂. The solution was mixed with formic acid to achieve a pH below 3, and centrifuged at 12000g for 5 minutes. The supernatant was slowly loaded to the C18 desalting column, washed with a washing buffer (0.1% formic acid, 3% acetonitrile) three times, then eluted with an elution buffer (0.1% formic acid, 70% acetonitrile). The dry powder of the samples was redissolved by adding 100ul 0.1M TEAB buffer, and 41ul TMT labeling reagent in acetonitrile, the reaction was mixed upside down at room temperature for 2h, then 8% ammonia water was added to terminate the reaction. All labeling samples were mixed in equal volumes, desalted, and lyophilized. Subsequently, samples were fractionated using a Rigol L3000 HPLC system under UV 214 nm conditions, collected with a frequency of one a tube per minute, and finally combined into 10 fractions. All fractions were dried under vacuum conditions, and then reconstituted in a 0.1% (v/v) formic acid (FA) solution.

2.4 LC-MS/MS analysis

Shotgun proteomics analyses were performed to construct transition libraries using an EASY-nLCTM 1200 UHPLC system (Thermo Fisher) coupled with an Q Exactive HF-X mass spectrometer (Thermo Fisher) operated in a data-dependent acquisition (DDA) mode. Protein results were analyzed using the Proteome Discoverer 2.2 (PD 2.2, Thermo) search engine. Reliably identified peptides and proteins were retained and verified using false discovery rates (FDR). Peptides and proteins with FDR greater than 1% were removed. Quantified differentially expressed proteins were analyzed using t-tests with the screening criteria of $P < 0.05$ and $|\log_2(\text{FoldChange})| \geq 1$. The Interproscan software was used to conduct a functional characterization of uniquely identified proteins. The identified proteins were compared using BLAST (BLAST evalue $\leq 1e-4$) with the KEGG and COG databases, and the proteins with the highest scores selected for annotation.

2.5 Analysis of differentially expressed genes, and proteins

Differentially expressed genes (DEGs) and proteins (DEPs) were classified and analyzed for functional enrichment with the DAVID online software (<https://david.ncifcrf.gov/>) using the Gene Ontology (GO; <http://www.geneontology.org/>) and the Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.genome.jp/kegg/>) databases. The GO terms and KEGG pathways with corrected P -values < 0.05 were considered to be significantly enriched. R software programs were used to make volcano, heatmap, and correlation plots.

3 Results

3.1 RNA transcriptomic profiles of rabbit skeletal muscle tissue

There were clear anatomical differences between rabbits from the control (CG_M) and experimental groups (EG_M) after the weight loss between 70 days and 90 days of age (Fig. 1). Perirenal fat, an important component of visceral fat, significantly decreased after weight loss. The HE-stained sections of skeletal muscle showed that CG_M rabbits had higher levels of intramuscular fat than EG_M rabbits after weight loss. Thus, rabbits subjected to a restricted diet showed visible anatomical signs of weight loss. Sequencing data were subjected to quality control, namely filtering, sequencing error rate check, and GC content distribution check. After quality control, the sequencing data had values of Q20 and Q30 above 90% (Table S1). Comparison of clean reads from samples to the reference rabbit genome showed they covered over 85% of the total map (Table S2). These results indicated that sequencing results had a high degree of reliability and could be used for subsequent analyses.

3.2 Screening and functional analysis of differentially expressed genes (DEGs)

Detection of DEGs utilized the $|\log_2(\text{FoldChange})| > 1$ and $P < 0.01$ criteria. Specifically, 917 DEGs were identified in comparisons between EG_M and CG_M rabbits, 356 up-regulated and 561 down-regulated (volcano plot in Fig. 2A) and their expression levels are shown in the heatmap in Fig. 2B. The functional enrichment analysis was conducted with the DAVID software using the GO and KEGG databases. The GO functional enrichment results indicated an overrepresentation of GO terms related to the mitotic cell cycle process, supramolecular fiber organization, nuclear division in biological processes (BP), collagen-containing extracellular matrix, extracellular matrix, extracellular space in cellular component (CC), glycosaminoglycan binding, actin binding, and microtubule binding in molecular functions (MF) (Fig. 2C). This indicated that genes involved in nuclear division and muscle cell differentiation play an important role in the process of weight loss. We also observed 20 highly correlated pathways (Fig. 2D) primarily associated with ECM-receptor interaction, protein digestion and absorption, PI3K-Akt signaling, and cell cycle. Notably, the FoxO signaling, Glutathione metabolism, HIF-1 signaling, AMPK signaling, and PPAR signaling pathways were related to metabolism and may play important roles in skeletal muscle after weight loss.

3.3 Identification, and Functional enrichment analysis of differentially expressed protein (DEPs) in EG_M and CG_M

A total of 1985 unique DEPs were identified in the EG_M and CG_M rabbit (Table S1). Peptide length was largely distributed between 7 bp and 25 bp, and the cumulative proportion of identified DEPs containing unique peptides was over 95% of the total DEPs. Subsequently, we detected 127 DEPs (18 up-regulated and 109 down-regulated) in EG_M vs. CG_M rabbit comparisons with the criteria of $FC > 1.2$ or < 0.83 and $P < 0.05$ (volcano plot in Fig. 3A, and heatmap in Fig. 3B). The GO functional enrichment results (Fig. 3C) indicated that most DEPs were significantly concentrated in: 1) BP terms such as translation, cellular macromolecule biosynthetic process, cellular protein metabolic process, and protein metabolic process; 2) CC terms such as ribosome, cytoplasmic part, cytoplasm, macromolecular complex, intracellular

organelles, intracellular, and cell part; and 3) MF terms such as structural molecule activity, structural constituents of ribosomes, and RNA binding. The KEGG pathway enrichment results (Fig. 3D) indicated that ribosome, complement and coagulation cascades, and legionellosis were involved in skeletal muscle metabolism. These results indicated that weight loss can cause significant changes in protein synthesis and metabolism as well as cell structure formation in skeletal muscle.

3.6 Integrated RNA-seq and proteomics analysis of skeletal muscle from rabbits in the CG_M and EG_M

An integrated RNA-seq and proteomics analysis of skeletal muscle samples from rabbits in the CG_M and EG_M was performed to better comprehend the relationships between mRNA and proteins (Fig. 4A). There was a total of 1382 genes associated with proteins, and 8 of them (ATP2A2, PDLIM3, GPX7, FKBP5, MYL3, COL5A2, UCHL1 and COL3A1), were significantly associated with proteins. A correlation analysis between DEGs identified by the transcriptome and DEPs identified by the proteome yielded a Pearson correlation coefficient of 0.146 (Fig. 4B), indicating a low degree of association between transcriptional expression and protein translation perhaps due to the complexity of these two biological processes. Nevertheless, these DEGs and DEPs may possibly play key roles in skeletal muscle biological processes.

4 Discussion

Various restricted feeding strategies have been adopted for weight loss in recent years, among them intermittent fasting or intermittent energy fasting is one of the most accepted [22, 23]. The fasting concept relies on maintaining a minimum feed intake without specifying a specific way to lose weight [24]. For example, fasting days and limited energy intake. The metabolism of skeletal muscle of young rabbits at 70 days of age is in a relatively vigorous period, and fat deposition increases in leg muscles increases gradually after 70 days of age [25]. Hence, skeletal muscle of rabbits were studied in CG_M and EG_M rabbits from 76 days to 90 days of age to assess their metabolic characteristics during a 15-day weight loss period. Previous studies found that dietary restriction significantly reduced perirenal fat and scapular fat content in rabbits [26]. Body weight and visceral fat of rabbits were significantly reduced at the end of weight loss period, and they drank larger quantities of water during the weight loss period, thus their urine was light yellow. These results indicated that dietary restriction caused phenotypic differences in CG_M and EG_M rabbits.

Skeletal muscle is affected by a variety of factors during the process of weight loss, such as hormones, disease, sex, and age [27]. Energy-restricted diets can activate insulin signaling pathways and reduce abnormal reactions of mTORC1 and ERK1/2 signaling pathways and expression of negative growth regulatory factors in skeletal muscle of obese mice [28, 29]. Moreover, caloric restrictions can reduce skeletal muscle mass, particularly muscle fiber length and cross-sectional area, and inhibit the expression of the PGC-1 α /Expression of IGF-1/AKT1 signaling pathway [30]. We found that functional pathway enrichment of DEGs in the transcriptome was mainly related to metabolism and growth signaling pathways such as the FOXO signaling, glutathione metabolism, HIF-1 signaling, AMPK signaling, and

PPAR signaling pathways. The FOXO signaling pathway mediates insulin and insulin-like growth factors that inhibit cell metabolism, growth, differentiation, oxidative stress, and autophagy [31]. Under insufficient nutritional conditions, skeletal muscle will undergo proteolysis and amino acid degradation to maintain glycolipid metabolism in the liver. This degradation pathway is closely related to the regulatory pathway mediated by Forkhead Box (Fox) O transcription factors [32]. Conversely, the differentiation of skeletal muscle is also associated with the insulin pathway that inhibits signaling factors in the FOXO signaling pathway that mediates autophagy regulation and transcriptional protein degradation [33]. Our study also indicated that dietary restriction causes skeletal muscle loss through the insulin-mediated FOXO signaling, AMPK signaling, and PPAR signaling pathways by degradation of amino acids to provide nutrients for the liver and sustain the normal metabolic state of the organism.

Proteins are the embodiment of biological functions. The biological process from gene to protein is influenced by a variety of cell growth and metabolism regulatory factors. The proteomic analysis of skeletal muscle after weight loss indicated that the biological pathways involved in DEPs were mostly related to cellular protein metabolic processes, confirming the crucial role of protein metabolism in skeletal muscle. However, the KEGG enrichment analysis showed that these DEPs were also related to the ribosome, complement cascade, coagulation cascade, and legionellosis pathways, which are closely associated with immune pathways. The complement and coagulation cascade pathways are involved in the metabolic processes of human obesity and diabetes mellitus that influence a variety of human diseases [34]. Our results suggested that there was also an inflammatory response in rabbit skeletal muscle after weight loss, which may be caused by lower nutrient levels after dietary restriction.

Comparative omics analyses can demonstrate potential mechanisms for biological processes [35]. Consistent with previous research, a low Pearson correlation coefficient (0.146) existed between comparative genomic and proteomic analyses indicating the existence of complex regulatory mechanisms [36]. Interestingly, eight genes were involved in chronic muscle fiber formation. Collagen V [col(V)] is the main connective tissue protein, which plays an important role in the formation of muscle fibers. The majority of intramuscular collagen is located around the muscle bundle. Different types of collagen play key roles in the regulation of the homeostasis of skeletal muscle, and are involved in many biological functions including signal transduction, muscle fiber formation, and immunity [37]. Two types of COL5A3 and Collagen VI are involved in the development and metabolism of skeletal muscle, and they are also closely related to insulin resistance in obese individuals [38, 39]. The COL5A2 and COL3A1 genes in CG_M and EG_M rabbits were highly correlated with transcription and translation levels, suggesting a vital role in the regulation of muscle fiber formation. Muscle fiber composition is closely associated with insulin resistance. Two important muscle fiber types (type I and type II) exist in skeletal muscle. Type I muscle fibers are a chronic muscle fibers that have many mitochondria for oxidative metabolism and can increase their number after exercise or weight loss. Type II are a fast muscle fibers that contain few mitochondria and are mainly involved in glycolytic metabolism of skeletal muscle. The number of type I slow muscle fibers is lower than the number of type II fast muscle fibers in obese individuals [40, 41]. Thus, slow muscle fibers are important for muscle metabolic processes during obesity. The ATP2A2 protein is an ATPase in the sarcoplasmic reticulum that is closely related to Ca^{2+} transport in the

regulation of Ca²⁺ + signaling pathway. It is also a regulator of slow muscle fiber formation [42, 43]. The *PDLIM3* gene contains a NH₂-terminal PDZ domain and a COOH-terminal LIM domain, both of which can enhance the connections between alpha-actinin and actin filaments to regulate muscle differentiation [44]. Similarly, its alternative splicing form can cause muscle atrophy[45]. The Myosin light chain 3 (MYL3) and the Ubiquitin C-terminal hydrolase L1 (UCHL1) are involved in the formation of slow muscle fibers. Specifically, MYL3 is considered a biomarker protein associated with myopathy [46–48]. Notably, the transcriptome and proteome analyses in our study showed a strong correlation between gene and protein expression levels of ATP2A2, PDLIM3, MYL3, and UCHL1, which are likely involved in the regulation of the development of slow muscle fibers. The GPX7 protein is a member of the Glutathione peroxidase family and plays an important role in the antioxidant reaction of cells. It catalyzes Glutathione (GSH) to oxidized Glutathione (GSSG), reducing toxic hydrogen peroxide and lipid peroxides to non-toxic hydroxyl compounds [49] [50]. Obese patients also have lower serum GPX expression levels related to the proportion of body fat [51]. Synchaperone FKBP5 is a protein that regulates cell stress response. Its genetic variants are associated with T2D-related traits and other stress-related diseases [52]. Obese mice with the *FKBP5* knockout gene show improved glucose tolerance and enhanced insulin signaling in skeletal muscle [53]. It is highly expressed in the hypothalamus and hippocampus tissues in response to a high-fat diet [54]. Lastly, the *GPX7* and *FKBP5* genes are involved in metabolic processes associated with oxidative stress in skeletal muscle. The mechanism of insulin resistance in skeletal muscle is always associated with regulatory factors of fat metabolism [55]. Thus, we speculated that these proteins may play an important role in the regulation of cell membrane signal transduction in rabbit skeletal muscle after weight loss, and indirectly promote the oxidative metabolic capacity of muscle cells.

Conclusion

In summary, we used two omics procedures (transcriptomics and proteomics) to analyze metabolic differences between skeletal muscle tissue samples from CG_M and EG_M rabbits after a 15-day weight loss induced by a restricted diet. Dietary restriction caused significant changes in rabbit skeletal muscle growth and metabolic pathways such as insulin-mediated FOXO signaling, AMPK signaling, and PPAR signaling pathways. Further, we found significant differences in several skeletal muscle metabolic pathways including protein catabolism and trace element absorption capacity. These important metabolic differences between CG_M and EG_M rabbits after weight loss may provide new insights for rabbit and human metabolic diseases.

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the ethical standards of and approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, China (DKY-2018102005).

Consent for publication

Not applicable.

Availability of data and material

Please contact author for data requests.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the major research project fund from the Sichuan Province (Grant number 2021YFYZ0033) and the Earmarked Fund from the China Agriculture Research System (Grant number CARS-43-A-2).

Authors' contributions

Li Yanhong and Wang Jie participated in the design of the study and performed the statistical analysis. Tang Tao and Shao Jiahao carried out the investigation and project administration. Mauricio A. Elzo and Jia Xianbo participated in Writing - Review & Editing. Chen Shiyi and Lai Songjia participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Novogene Bioinformatics Technology Co.Ltd for providing valuable assistance and the members in our teams for their timely help..

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Tables

Table 1

Correlational genes of DEPs and DEGs in skeletal muscle of rabbits from the control (CG_M) and the experimental group (EG_M)

Gene ID	Description	Protein(log2FC)	Protein(Pvalue)	Tran(log2 FC)	Tran(Pvalue)
ATP2A2	SERCA-type calcium pumps	-0.29407	0.026054	-3.65421	0.000153
PDLIM3	PDZ and LIM domain 3	0.308938	0.022504	1.13341	6.62E-05
GPX7	Glutathione peroxidase-7	-0.39055	0.024828	-1.00856	0.000758
FKBP5	FK506-binding protein 51	0.354755	0.026298	3.797986	2.08E-13
MYL3	Myosin light chain 3	-0.52648	0.015801	-4.9007	0.0005
COL5A2	Collagen type V alpha 2 chain	0.382835	0.043679	-1.06688	0.015576
UCHL1	Ubiquitin carboxy-terminal hydrolase L1	0.326808	0.003951	1.867891	6.34E-09
COL3A1	Alpha 1 (III) collagen	0.451926	0.035566	-1.35329	0.005631

Figures

CG_M

EG_M

Figure 1

Perirenal fat anatomical differences. (A) and skeletal muscle tissue morphological differences (Hematoxylin-Eosin staining, B) in rabbits from the control group (CG_M) and the experimental group (EG_M).

Figure 2

Functional enrichment analysis of DEGs in the control (CG_M) and the experimental (EG_M) rabbit groups. A) DEG volcano map. The abscissa contains values of $\log_2$ (FoldChange), the ordinate contains values of $-\log_{10}P$ value, and the dashed blue lines are the thresholds of the DEG screening criteria. B) Heatmap of the expression of the DEGs in each rabbit group. Brackets in the horizontal axis represent DEG groups and brackets in the vertical axis represent number of DEG. Cluster analyses were conducted

for both horizontal and vertical data. Blue to red represents expression levels from -1.5 to 1.5. C) GO terms of the DEG enrichment diagram. The horizontal axis contains GO terms, and the vertical axis contains significance levels of GO terms, represented by $-\log_{10}P$ value. Different colors represent different functional classifications. D) KEGG pathways of the DEG enrichment diagram. The abscissa contains ratios of numbers of DEGs in CG_M vs. EG_M rabbits to total numbers of DEGs in a KEGG pathway, and the ordinate shows the KEGG pathways. Plots C) and D) show the KEGG functional enrichment map of DEGs in CG_M vs. EG_M rabbit (10 significantly enriched pathways).

Figure 3

Functional enrichment analysis of differentially expressed proteins (DEPs) in the control (CG_M) and experimental (EG_M) rabbit groups. A) Volcano plot of DEPs in the CG_M and EG_M rabbit. The horizontal axis contains $-\log_2$ FoldChange values and the vertical contains $-\log_{10}P$ values, the black dots represent non-significant DEPs, the red dots represent up-regulated DEPs, and the green dots represent

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