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Integrated multi-omics analysis reveals component differences and their regulatory mechanisms of adipose tissue as lard raw material between Bamei and Large White pigs

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ABSTRACT

Bamei pigs, an indigenous Chinese breed, yield meat with a delectable flavor and boast higher carcass fat content compared to commercial breeds, making them a rich food source for humans. However, the differences in lipid and nutrient components between the adipose tissue of Bamei pigs and commercial pigs are still unclear. The study employed UPLC-MS/MS to quantify the composition of lipids and metabolites in the backfat of both Bamei and Large White pigs. A total of 428 lipids and 193 metabolites were significantly different between the 2 groups. Specifically, Bamei pig backfat exhibited altered levels of various lipids and metabolites that may potentially contribute to nutritional and flavor differences, including unsaturated triglycerides, free fatty acids, medium-chain triglycerides, essential amino acids, vitamins and antioxidants, while maintaining reduced cholesterol levels. Furthermore, we delved into the molecular mechanisms underlying these nutritional differences by analyzing significantly different 431 mRNAs and 865 proteins and integrating the regulatory network of protein-metabolite-lipid pathway. Importantly, in the pyruvate metabolic pathway of Bamei pigs, the bioprocess of lactate production was inhibited but the acetyl-CoA production was activated, suggesting the possibility that energy allocation favors the biogenesis of lipid precursors. These findings may contribute to guiding industrial food producers in enhancing the quality of lard at the genetic and molecular levels.

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1. Introduction

Pigs are the most consumed livestock species globally, accounting for approximately 36% of the meat production worldwide[1]. Due to its appealing fat content and texture, pork is widely consumed in many parts of the world, especially throughout the Americas, Central Europe, East Asia, and Southeast Asia. Autochthonous pig breeds are those that have evolved in a particular geographic region over many centuries[2], such as the Berkshire and Laiwu, are known for their high levels of fat content and marbling in carcass[3-4], which makes them a popular choice for pork products like bacon and sausage[5-6]. Commercial pig breeds, such as the Duroc and Large White (LW) pigs, are known for their fast growth rate and high carcass lean ratio, usually used to make lean pork products, such as ham and pork chops[7-8]. Differences in economic traits of different pig breeds not only satisfy diverse consumer favors, but also allow them to be excellent research materials for analyzing the molecular mechanisms that regulate the traits.

Adipocytes are the primary cells that store and release lipids and are responsible for energy balance and lipid metabolism in the organism.

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The composition and distribution of lipids in pig depends on a variety of factors, including breed, age, sex, nutrition and environmental conditions[9-10]. The fat content can influence the meat quality and food product production of the carcass. For example, intramuscular fat (also known as marbling) is highly desired in pork because it contributes to the tenderness, juiciness and flavor of the meat[3,11]. Lard, derived from pig fat tissue, is a flavorful cooking ingredient with a high smoke point, making it ideal for high-temperature frying and baking[12]. Compared to plant oils, higher levels of saturated fat may increase the risk of disease[13-14], leading some people to avoid or limit the consumption of animal fats. However, animal fats contain a variety of essential nutrients that are important for human health, including free fatty acid (FFA), cholesterol, fat-soluble vitamins, proteins and trace minerals, etc.[15-16]. A balanced intake of lipids from both animal and plant sources is the key to keeping our health[17-18]. Therefore, understanding the nutritional composition of adipose tissue and the molecular mechanisms of high fat-producing capacity for autochthonous pig breeds is crucial for improving the flavor of pork and the nutritional value of lard products.

Bamei (BM) pig, a Chinese indigenous breed, is characterized by its fatty phenotype, which includes thick backfat, high fat content, and superior meat quality[19-20]. LW pig is a lean-type commercial pig breed, which is famous for high leanness, high feed utilization and well motherhood. Currently, differences in lipids and metabolites are unclear in these 2 types of pig adipose tissue. Therefore, we used these 2 pig breeds as models and collected adipose tissues, combined with metabolomics, lipidomics, transcriptomics, and proteomics to comprehensively characterize and quantify lipids, metabolites, mRNAs, and proteins. A comprehensive understanding of the lipid and nutrient characteristics, as well as the regulatory networks governing their synthesis in pig fat tissues is crucial for improving lard to meet the increasing health demands in our diet.

2. Materials and methods

2.1 Animals and samples preparation

All research involving animals was conducted according to Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in March 2017), and approved by the Institutional Animal Care and Use Committee of Northwest A&F University (NWAFU No. 20230569). In this study, 2 kinds of pigs were used: BM (Ensembl Accession: GCA_001700235.1) and LW (world famous improved pig, Ensembl Accession: GCA_001700135.1). The pigs were all feed under standard temperature, standard humidity and standard ventilation conditions at the farm of Northwest A&F University. All pigs were fed normal diets after weaning according to the requirements specified in the Standard for Pig Feeding (NY/T 65−2004) issued by the Ministry of Agriculture and Rural Affairs of the People's Republic of China. When the pigs reached slaughter age, 5 normal and similar weight pigs were randomly selected from each group for sample collection. All pigs were humanely sacrificed as needed to reduce suffering and were not fed the night before they were sacrificed. Adipose tissues (ATs) were rapidly and manually separated from each carcass, immediately flash-frozen in liquid nitrogen, and stored at −80 °C.

2.2 Lipid extraction and lipidomic assay

The porcine backfat were used for lipid extraction and lipidomic analysis. Lipids extraction were performed as previously reported by Matyash et al.[21]. The supernatant of the extracted lipid complex solution was collected, and were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD, SCIEX, Framingham, MA, USA; MS, QTRAP® System, SCIEX, Framingham, MA, USA), for the specific process performed as Xuan et al.[22]. Raw data were matched with the Lipidmaps and annotated based on the Lipid Structure Database (LMSD: http://www.lipidmaps.org/), and HMDB (http://www.hmdb.ca). The differential lipid molecules (DLMs) between BM and LW were identified according to the criteria of variable importance in projection (VIP) ≥ 1, fold change (FC) > 1.5, and P value < 0.05.

2.3 Untargeted metabolomics profiling

Samples were removed from –80 °C, weighed an appropriate amount of tissue samples into a liquid nitrogen pre-cooled mortar, and fully ground to powder with liquid nitrogen. Metabolites were solubilized with liquid chromatography mobile phase A and separated using a ultra-performance liquid chromatography system (Waters, ACQUITY UPLC, Milford, MA, USA). The mass spectrometry data were extracted, aligned, and corrected for retention time using MetaboScape 2022, and the primary and secondary mass errors were controlled within 20×10^{-6} to ensure the accuracy of the identification results, and the structural and annotation information of the metabolites were obtained through the comparison of the spectra of NIST, HMDB, its own databases, and the integration of public databases. According to the quantitative information of metabolites obtained by database matching, the data were screened and combined with statistical algorithms to fill in missing values and correct the data, and for the samples with multiple repetitions, the corrected expression was used to calculate the metabolite difference FC between the 2 groups, meanwhile, combined with the P value of univariate t-test analysis, to obtain the difference significant metabolites (P value < 0.05 and FC > 1.5).

2.4 Transcriptome profiling

RNA-seq services were provided by Huada Co., Ltd. (DNBSEQ-T7, Shenzhen, China) according the standard procedures[23]. Transcriptome sequence data was aligned to the pig reference genome (Sscrofa11.1). Differential genes were compared between groups by normalizing and screening the number of read counts of transcripts using the R package DESeq2[24]. Genes with −lg (Q value) > 20 and $\log_2$ FC > 1.5 or < –1.5, were assigned as significantly differentially expressed.

2.5 Proteome profiling

Adipose tissues samples for proteome were analyzed with 4D-FastDIA quantitative proteomics. 4D proteomics is based on the traditional 3D, retention time + mass-to-charge ratio (m/z) + ionic strength (intensity) of the 3 dimensions to add a 4th dimension (the separation of ionic drip) and based on deep neural network library search strategy to achieve high reliability and high resolution extraction of peptide information[25]. The proteomic assays were

performed at Jingjie Bio (Bruker, timsTOF Pro2, Hangzhou, China). Based on the raw files obtained from the mass spectrometry assay, the protein database (UniProt, Sus_scrofa_9823_PR_20230316.fasta, 46 179 sequences) was searched using the DIA-NN (v 1.8) search engine with the enzymatic cut method set to Trypsin/P and the maximum number of missed cuts set to 1. Carbamidomethyl on Cys was specified as fixed modification. Deep learning was used to algorithm was used to construct a theoretical spectral library, and an inverse library was added to calculate and control the FDR < 1% caused by random matching, and the identified protein needed to contain at least one unique peptide. Proteins with a corrected P value < 0.05 and $\log_2$ FC > 0.58 or < –0.58 were assigned as significantly differentially expressed.

2.6. Pathway enrichment analysis

We performed pathway enrichment analysis for differential mRNA and proteins using the online tool g:Profiler (https://biit.cs.ut.ee/gprofiler/gost). The g:Profiler tool allowed us to perform statistical analysis and calculate enrichment scores to determine the significance of pathway enrichment. g:Profiler uses multiple testing correction by default and applies tailor-made algorithm g:SCS for reducing significance scores (user threshold 0.05). Once the pathway enrichment analysis was completed, we saved the results as a csv file and employed the ggplot2 package (version 3.4.0) in R to visualize the enriched pathways.

2.7 Transcription factor and target mRNA identification

Firstly, we identified differentially expressed proteins associated with fat deposition from results of combined transcription-proteome analysis. Subsequently, we proceeded with the g:Profiler tool to identify the transcription factors in the fat deposition related pathways. To further validate the regulatory role of these identified transcription factors, we queried the ChIP-Atlas database to identify the mRNA targets that are potentially regulated by the identified fat deposition-related transcription factors. The cell type used in the database was adipocyte with a target gene range of TSS ±5 kb and a binding score of ≥ 500. Finally, we used the pheatmap package (version 1.0.12) in R to visualize the mRNA expression levels of the identified target genes.

2.8 Multi-omics pathway mapping analysis

To conduct multi-omics pathway mapping analysis, we utilized the "pathview" package in R. The pathway view was laid out using the Graphviz engine for pathway maps (parameter: kegg.native = F), and the input data were the FC of protein expression difference and integrated lipids and metabolites abundance ($\log_2$ FC). The Pathview package first downloads the pathway map from the KEGG website and then integrates the input data to render the pathway map (https://www.bioconductor.org/packages/release/bioc/html/pathview.html).

2.9 Phenome wide association analysis using the FarmGTEx dataset

The identified key protein symbols associated with fat deposition BM were entered into PigBiobank of FarmGTEx's database (http://piggtex.farmgtex.org/), and we obtained the results of a phenome wide association analysis that included genes significantly associated with traits, the study symbol, and P value. The ggplot2 package for R software was used to plot P value bubble plots of the protein association with the trait.

2.10 Wet rendering of lard

After impurities were removed, the backfat of the pig was ground in a meat grinder. Fat and water were added at a mass ratio of 5:1, and the mixture was rendered at 100 °C and 0.02 MPa for 30 min. The lard was allowed to cool naturally in a sealed container and then stored at 4 °C.

2.11 Electronic nose analysis

The odor of the headspace gas profiles were analysed using the E-nose system PEN3-Plus (E-nose system, PEN3-Plus, Airsense Analytics GmbH, Schwerin, Germany). In a 50 mL sample vial, 1 g lard or adipose tissue were volatilized by incubation at 50 ℃ for 30 min. The response characteristics of each PEN3 system sensor are as follows: W1C (aromatic benzene compounds), W5S (nitrogen oxides), W3C (aromatic amine compounds), W6S (hydrides), W5C (aromatic short chain alkanes), W1S (methyl compounds), W1W (inorganic sulfides), W2S (alcohols, aldehydes and ketones), W2W (aromatic organic sulfides), W3S (long-chain alkanes).

2.12 Biochemical analysis

Activities of pyruvate dehydrogenase and lactate dehydrogenase, as well as the contents of pyruvate, lactate, FFA, and total cholesterol, were measured using kits (BC0385, BC0685 Solarbio, Beijing, China) according to the standard methods provided in the instruction manual.

2.13 Statistical analysis

All statistical analyses were performed in "R" (version 4.3.0) software. Data represent means ± pooled SEMs. The significance of differences was determined using the unpaired Student's t-test as indicated, and differences with $P < 0.05$ were considered as statistically significant.

3. Results

3.1 Lipid profiles of subcutaneous fat in BM and LW

Appearance, backfat, and carcass of BM are shown in Table S1, Fig. S1, where backfat thickness and carcass fat ratio of BM at the age of slaughter are higher than that of LW. The lipid profiles of adipose tissue from the 2 pig breeds were different (Figs. 1A and B). Among the 428 differential lipids, we found the following lipid categories in descending order: glycerolipids (57.5%), glycerophospholipids (26.9%), sphingolipids (11.7%), FFA (3.5%) and prenol lipids (0.7%) (Fig. 1C). Among the glycerides identified, most of the significant differences were triglycerides

with a fatty acyl chain length of more than 15 (Figs. 1D, E and S1). Compared with LW, a greater proportion of polyunsaturated lipids than saturated or monounsaturated lipids were found in the significantly up-regulated glycerides and glycerophospholipids of BM, whereas the opposite proportions were observed in the significantly down-regulated phosphatidylethanolamine and glycerides (Fig 1F). Interestingly, all FFA contents identified were up-regulated and all acylcarnitines were down-regulated (Figs. 1G and H). In addition, the content of mostly ceramides and sphingolipids was also significantly downregulated (Fig. S2).

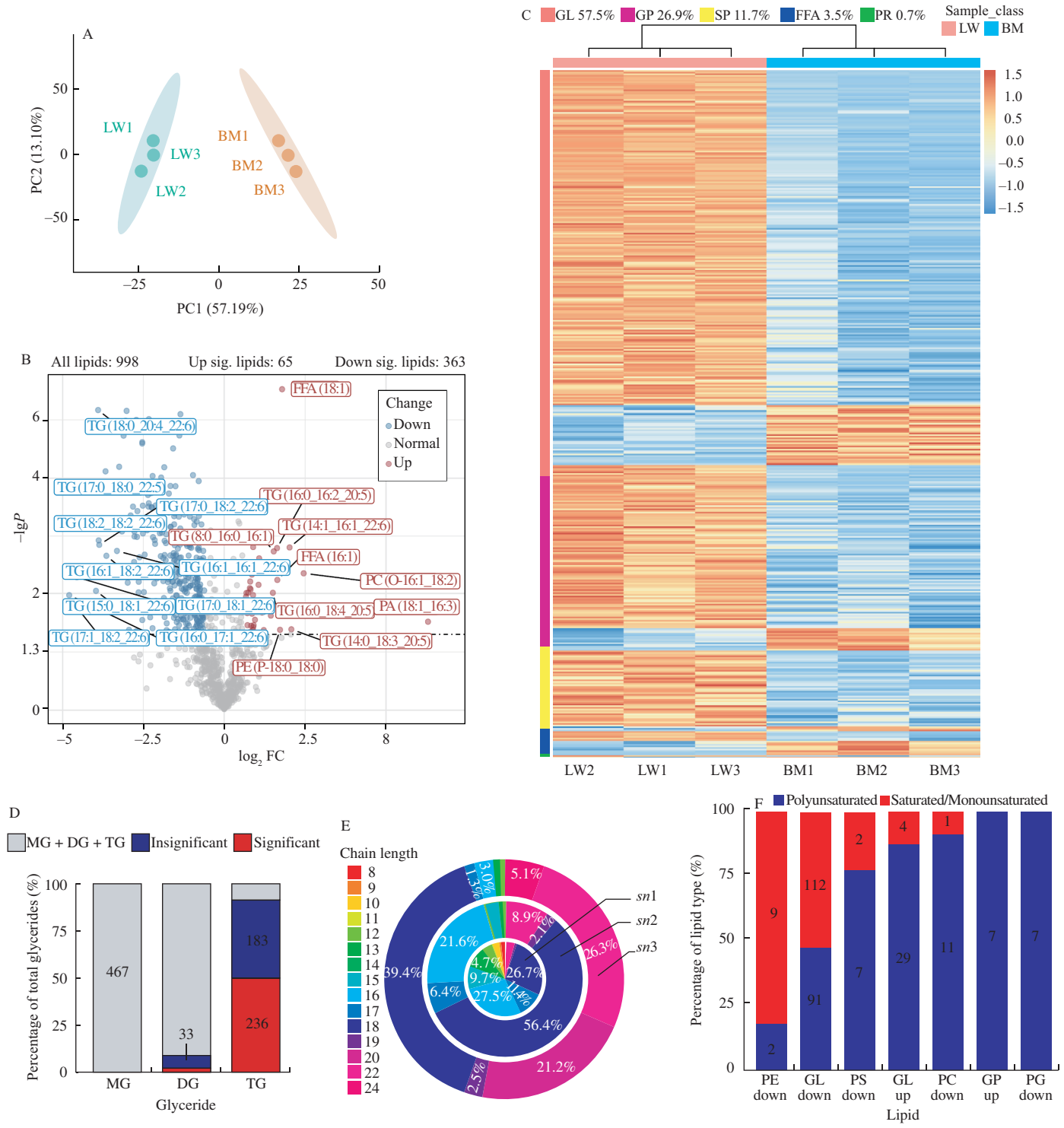


Fig. 1 Lipid profiles of subcutaneous fat in BM and LW. (A) PCA results of all identified lipids in pig backfat. (B) Volcano plots of all lipids detected from backfat, data were shown as BM/LW, P value < 0.05, $|\log_2 FC| \geq 0.58$ highlighted in red and blue. PA, phosphatidylic acid, PC, phosphatidylcholine, TG, triglyceride, PE, phosphatidylethanolamine. (C) Clustering heat map of 428 significantly different lipids. GL, glycerolipids; GP, glycerophospholipids; SP, sphingolipids; PR, prenol lipids. (D) Histogram of TG, diglycerides (DG) and monoglycerides (MG) as a percentage of all glycerides. (E) Ring pie chart of chain length percentage of triglycerides with significant differences in content. (F) Analysis of the degree of unsaturation of different categories of lipids, fatty acid chains with a number of unsaturated bonds ≥ 2 were considered polyunsaturated. (G-H) Heat map of FFA and acylcarnitine with significant differences in content.

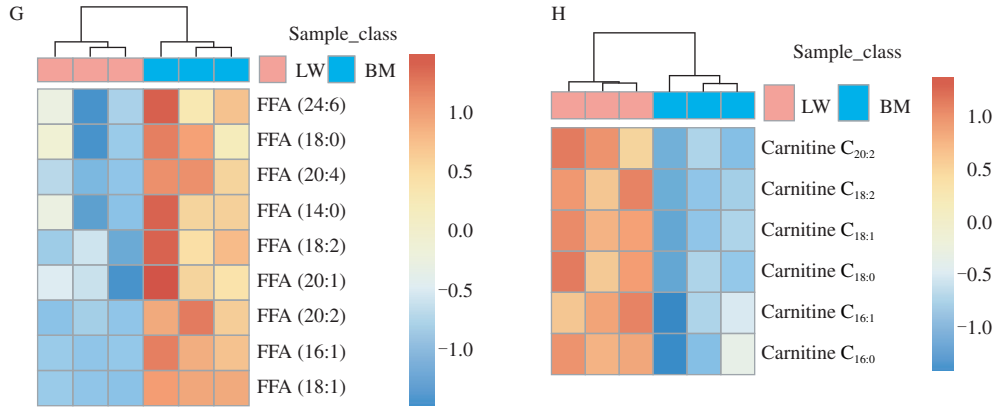


Fig. 1 (*Continued*)

3.2 *Metabolic profiles of subcutaneous fat in BM and LW*

The metabolite profiles of adipose tissue from the 2 pig breeds were different (Figs. 2A and B). Compared to LW, the metabolites that increased were mainly lipids, vitamins, and cofactors, whereas the metabolites that decreased primarily were nucleic acids (Fig. 2C). We further analyzed the content of nutrients in adipose tissue. In comparison to LW, BM had less cholesterol and cholesterol esters (Fig. 2D). More importantly, some essential amino acids, glutathione, vitamin D and some B vitamins were higher in BM (Table 1, Fig. 2E). Cholesterol synthesis and metabolism in the animal organism primarily occurs in the liver, and in adipose tissue, channel proteins on adipocyte membranes control cholesterol uptake and excretion[26].

Table1
Relative amino acid content of adipose tissue in BM and LW pigs.

Amino acid	FC	*P* value
Glutamine	4.08	2.34×10^{-2}
Leucine	2.17	3.83×10^{-2}
Cysteine	1.98	2.63×10^{-3}
Valine	1.46	2.62×10^{-2}
Argnine	1.44	3.35×10^{-2}
Isoleucine	1.34	2.67×10^{-1}
Threonine	1.05	7.27×10^{-1}
Tryptophane	0.79	4.53×10^{-1}
Alanine	0.59	1.75×10^{-1}
Methionine	0.27	5.12×10^{-4}

Note: FC = Relative content of BM/Relative content of LW.

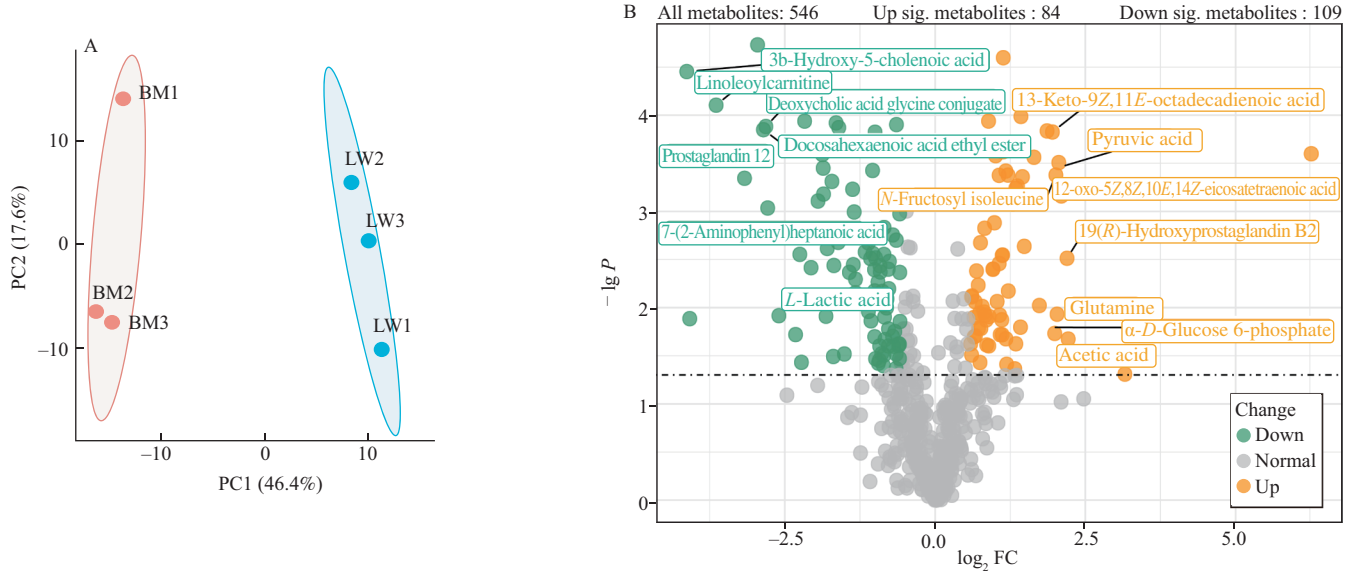


Fig. 2 Metabolic profiles of subcutaneous fat in BM and LW. (A) PCA results of all identified metabolites in pig backfat. (B) Volcano plots of all metabolites detected from backfat, data were shown as BM/LW, *P* value < 0.05, $|\log_2 FC| \geq 0$ highlighted in red and blue. (C) Histogram of the percentage of the significant difference metabolites belonging to categories. (D-E) Heat map of cholesterol and nutritional factors with significant differences in content.

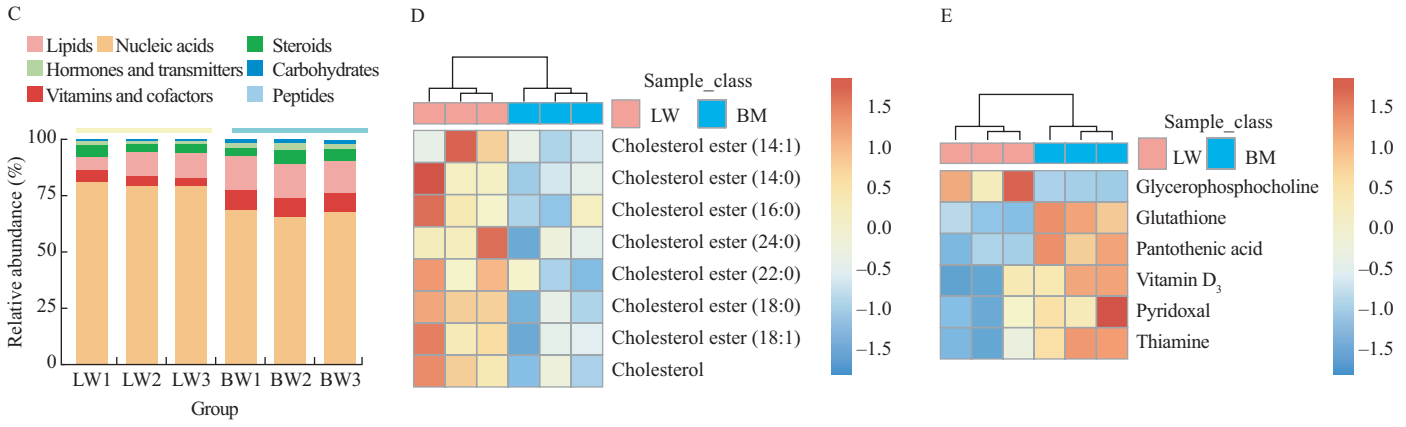


Fig. 2 (*Continued*)

3.3 Identification and comparison of differentially expression genes and proteins

Principle component analysis (PCA) and volcano plot analysis of all identified mRNAs showed a significant difference between BM and LW (Figs. 3A and B). The results of KEGG enrichment analysis of the top 30 showed that differential mRNAs were enriched and mostly upregulated in the lipid and atherosclerosis, arginine and proline metabolism, cholesterol metabolism, and cytokine-cytokine receptor interaction pathways (Figs. 3C and S4). GO enrichment analysis revealed that the differential mRNAs are mainly related to biological processes such as muscle contraction and serine biosynthesis, and are localized in extracellular matrix and muscle structures, performed molecular functions such as NADPH oxidoreductase activity, intracellular chloride channel activity, and low-density lipoprotein binding (Fig. 3D, Table S2). Proteins that secrete metabolites and act as enzymes for biochemical reactions play an important role in adipogenesis and degradation. Therefore, we identified the differential proteins between BM and LW. PCA and volcano plot analysis of all identified proteins showed a significant difference between BM and LW (Figs. 4A and B). The significantly different proteins were enriched in the pathways of valine, leucine and isoleucine degradation, fatty acid degradation, pyruvate metabolism, glycolysis/gluconeogenesis, PPAR signaling, biosynthesis of amino acids and oxidative phosphorylation. (Figs. 4C and S4). GO enrichment analysis showed that the significantly different proteins were mainly enriched in the biological processes of acetyl-CoA biosynthesis, fatty acid β-oxidation, and tricarboxylic acid metabolism, localized in the collagen trimer and pyruvate dehydrogenase complex, and performed the molecular functions of the pyruvate dehydrogenase and coenzyme A carboxylase activities (Fig. 4D). In BM, the protein expression levels of fatty acid desaturation-related enzymes, SCD1 and FADS2, were significantly up-regulated (Fig. 4E). The lipolysis key rate-limiting enzymes and the enzymes that convert carnitine to acylcarnitine, CPT1A and CPT1B, were down-regulated, whereas the expression of the carnitine acyltransferase, CPT2, and the enzyme that regulates the acyl-CoA/CoA ratio, CRAT, were up-regulated (Fig. 4F). The expression of APOE, a protein associated with cholesterol uptake in adipocytes, was significantly decreased in the adipose tissue of the BM pig compared with that of the LW pig, whereas the proteins associated with cholesterol excretion, ABCA1 and APOA1, were significantly up-regulated (Fig. 4G). We also detected changes of the expression levels of proteins related to glutathione and vitamin metabolism (Figs. 4H-K).

3.4 A combined transcriptome and proteome analysis reveals the regulation of fat deposition by transcription factors

In BM and LW adipose tissues, the expression patterns of differential mRNAs and proteins were generally isotropic (Fig. 5A). The GO pathway enrichment analysis revealed that genes associated with fat deposition pathways in BM pigs were significantly up-regulated both mRNA and protein levels, or exhibited no change in mRNA levels but significant up-regulation in protein levels (Figs. 5B and S5). Next, we predicted the transcription factors that regulate mRNAs in the pathway associated with fat deposition, and we found that the protein expression of most of these transcription factors were downregulated in BM, including *RELA*, *CEBPB*, *BRD4* and *NR3C1*, etc. (Figs. 5C and S5). It is interesting to note that, in the BM group, although the expression levels of transcription factors are reduced, the expression levels of their target mRNAs are mostly elevated (Figs. 5D and S5). In addition, we mapped differentially expressed genes/proteins (P value < 0.05) to the KEGG pathway and performed correlation analysis, found that the signaling pathway associated with adipogenesis or degradation was most relevant when protein was significantly up/down regulated but mRNA unchanged (Fig. S5). These results suggests that protein differences may have a greater impact on fat deposition than mRNA.

3.5 Multiomics KEGG pathway mapping analysis and validation

We mapped the identified differential proteins, metabolites, and lipids to the KEGG pathway to systematically analyze the molecular mechanisms underlying the differences in fat deposition capacity between BM and LW. Of the total 54 significantly enriched pathways, the glycolysis, TCA cycle, fatty acid biosynthesis, and pyruvate metabolism pathways were enriched in the highest number of biomolecules (Fig. S6). In the pyruvate metabolic pathway, compared to the LW, in the BM, the pathway for the production of lactate from pyruvate was inhibited and the pathway for the production of acetyl-CoA from pyruvate was activated. We further investigated the upstream signaling pathways of pyruvate metabolism, glycolysis and the TCA cycle, and found that the expression of most of the metabolic enzymes and metabolites in these 2 pathways were upregulated in BM. This means that in BM pigs, feed energy was used more for the production of precursors for fat synthesis. In addition, in BM, the

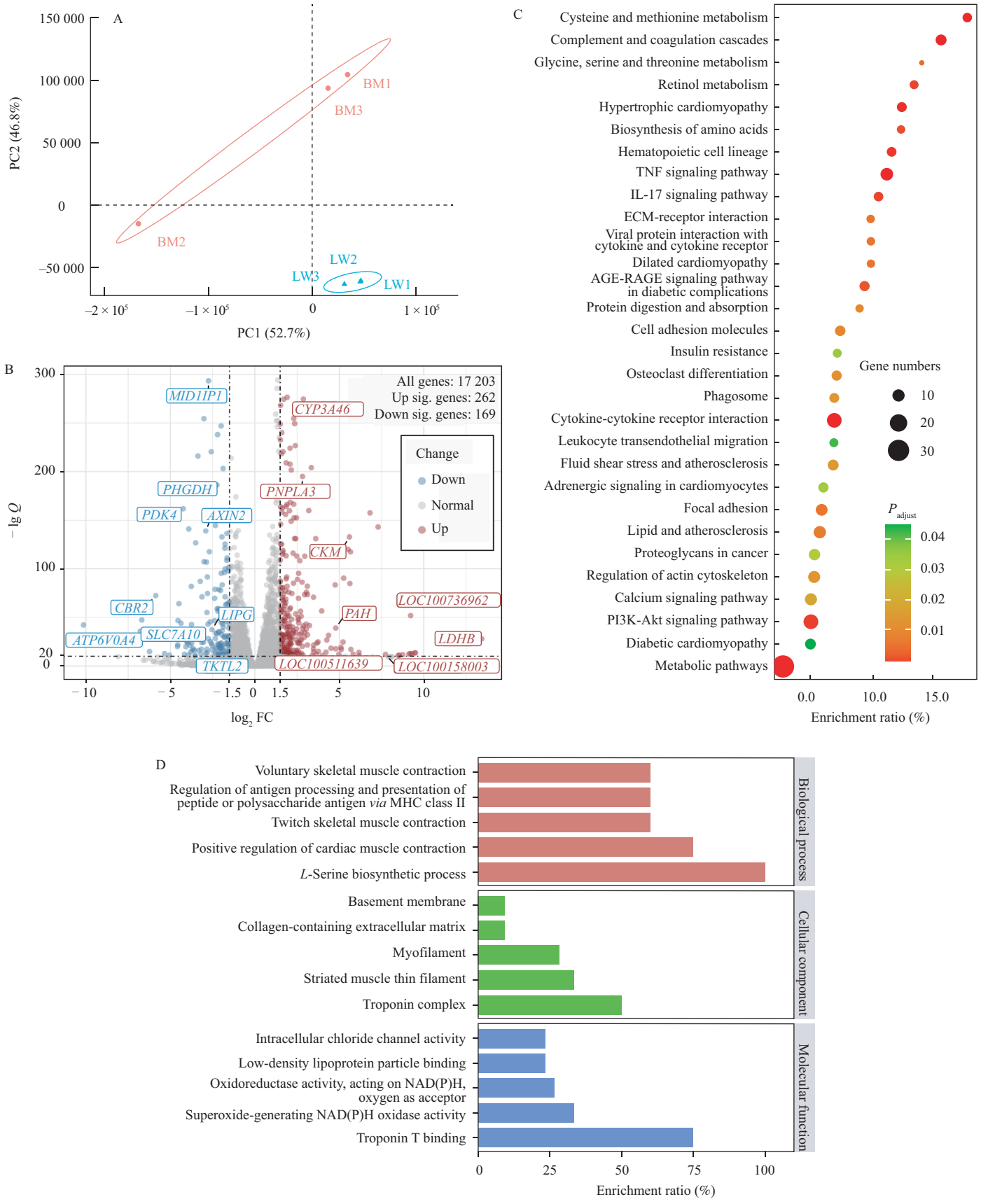


Fig. 3 mRNA profiles of backfat in BM and LW. (A) PCA results of all mRNAs in pig backfat. (B) Volcano plots of all mRNAs detected from backfat, data were shown as BM/LW, Q-values < 1×10^{-20}, $|\log_2 FC| \geq 1.5$ highlighted in red and blue. (C) Top 30 terms of significant differential mRNAs enriched in the KEGG pathway. (D) GO analysis of significantly different mRNAs.

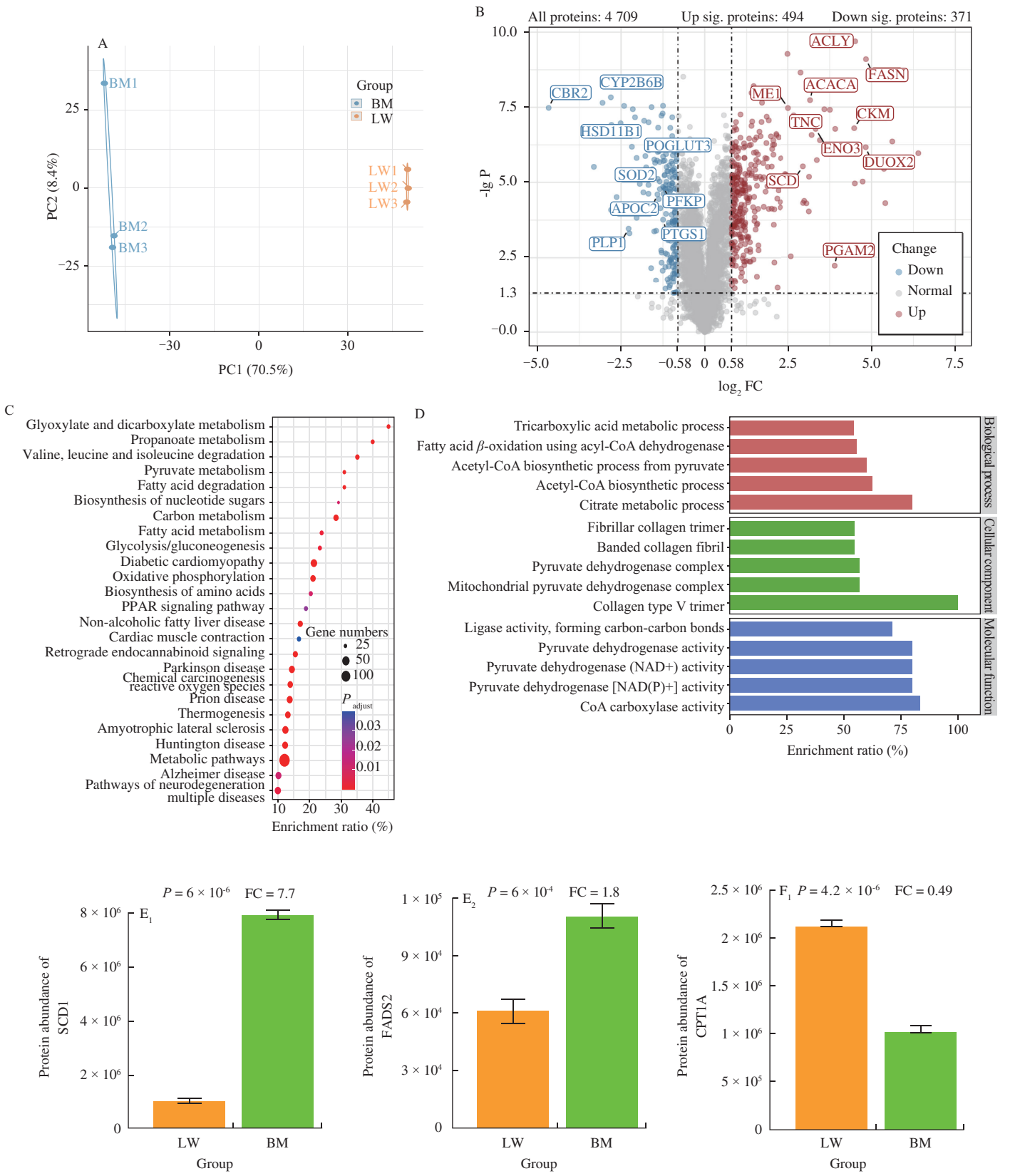
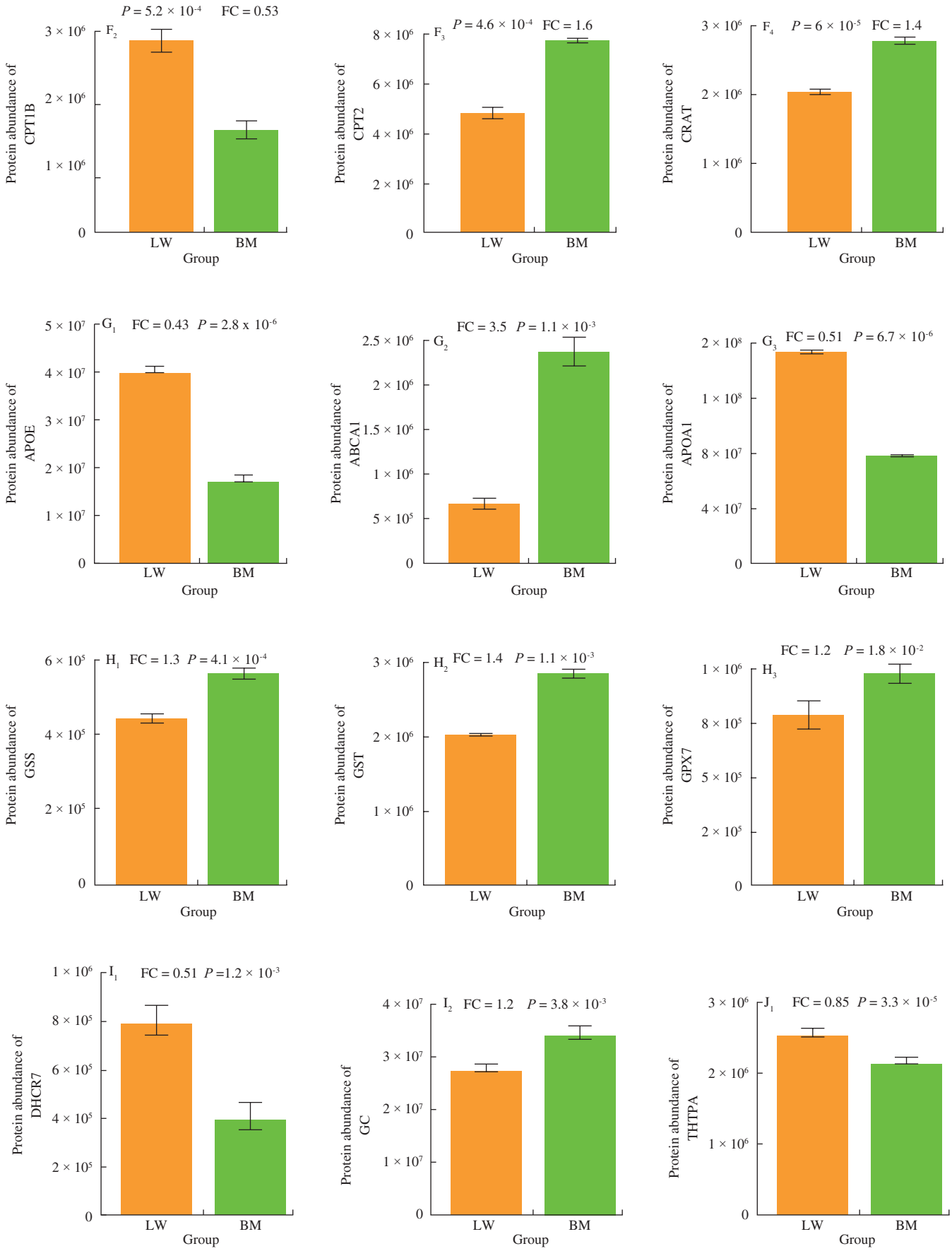


Fig. 4 Protein profiles of backfat in BM and LW. (A) PCA results of all proteins in pig backfat. (B) Volcano plots of all proteins detected from backfat, data were shown as BM/LW, P value < 0.05, |$\log_2$ FC| ≥ 0.58 highlighted in red and blue. (C) Top 25 terms of significant differential mRNAs enriched in the KEGG pathway. (D) GO analysis of significantly different proteins. Protein expression levels of fatty acid desaturation-related enzymes (E_1) SCD1 and (E_2) FADS2; key lipolysis rate-limiting enzymes (F_1) CPT1A, (F_2) CPT1B, (F_3) CPT2, (F_4) CRAT; absorption and elimination of cholesterol-related enzymes (G_1) APOE, (G_2) ABCA1 and (G_3) APOA1. Expression levels of proteins related to glutathione metabolism (H_1) GSS, (H_2) GST, (H_3) GPX7; vitamin metabolism, vitamin D ((I_2) DHCR7, (I_1) GC), vitamin B1/B5 ((J_1) THTPA, (J_2) PANK1), vitamin B_6 ((K_1) PDXP, (K_2) PDXK).



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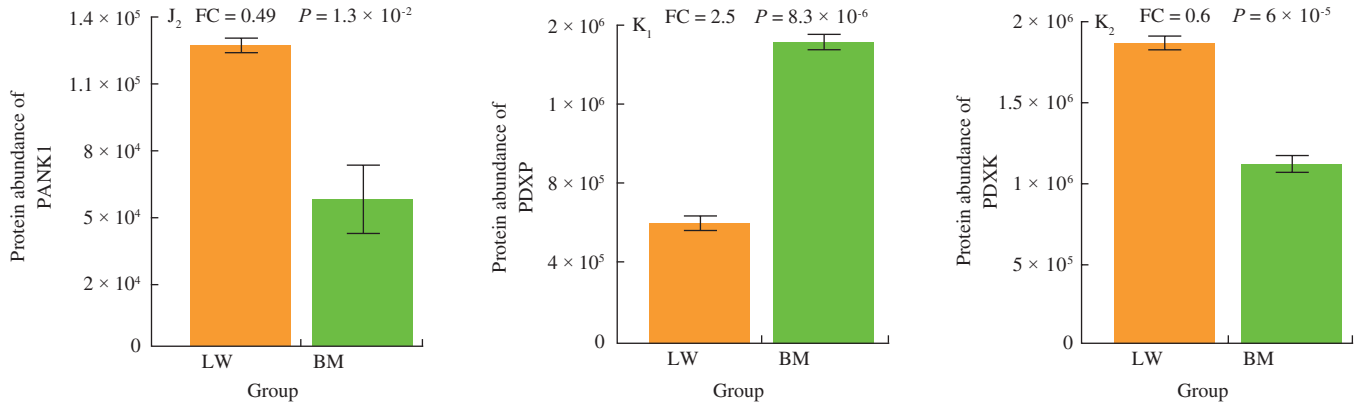

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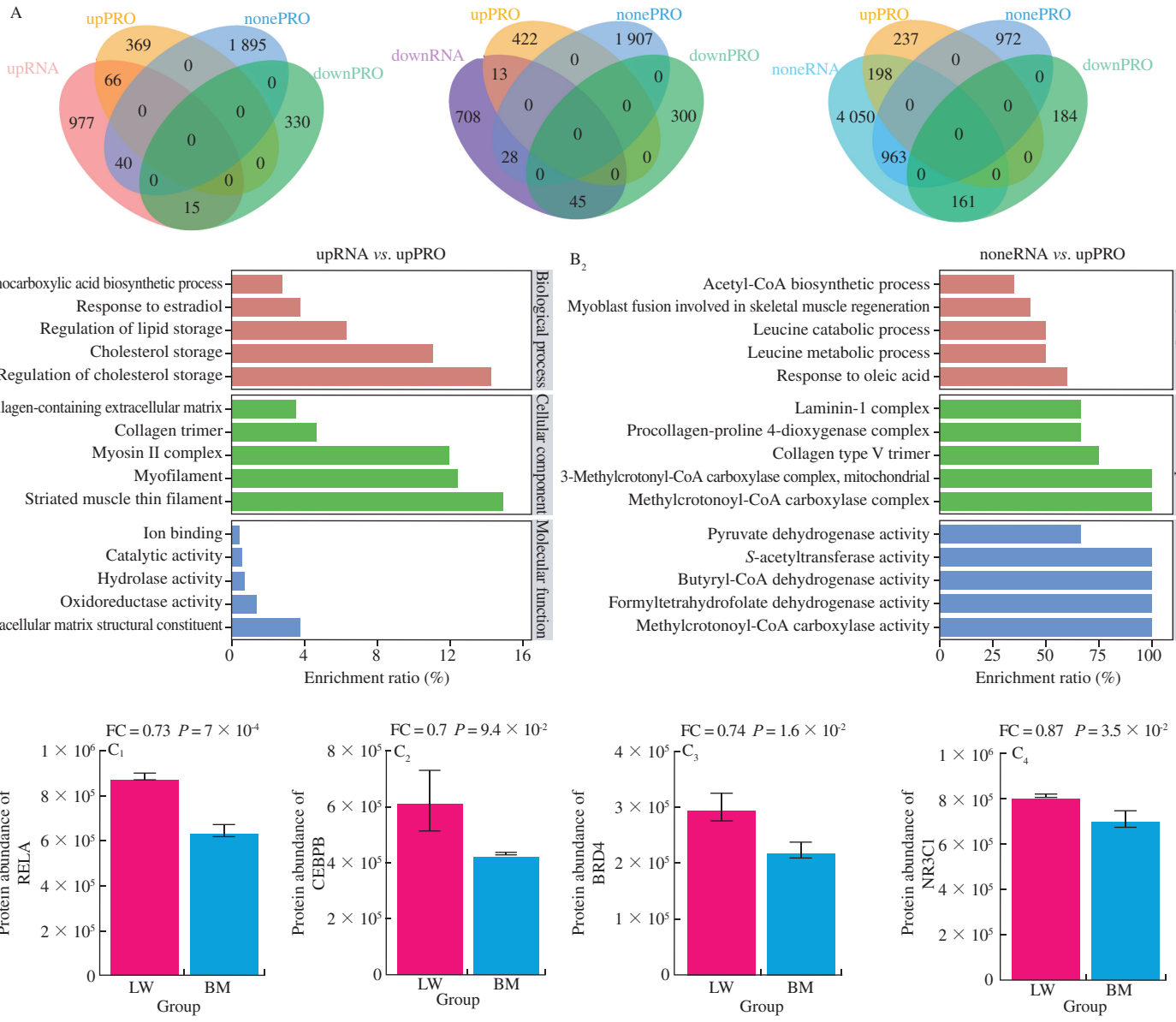


Fig. 5 A combined transcriptome and proteome analysis reveals the regulation of fat deposition by transcription factors. (A) Number of differences in mRNAs and proteins at different levels of regulation. upPRO, orange, protein significantly upregulated in BM; upRNA, red, RNA significantly upregulated in BM; downPRO, green, protein significantly downregulated in BM; downRNA, purple, RNA significantly downregulated in BM; nonePRO, dark blue, protein with no significant change between BM and LW; noneRNA, light blue, RNA with no significant change between BM and LW. (B) GO analysis of mRNAs and proteins at different levels of regulation. (C) Protein expression levels of fat deposition-related transcription factors. (D) Expression levels of mRNAs regulated by transcription factors.

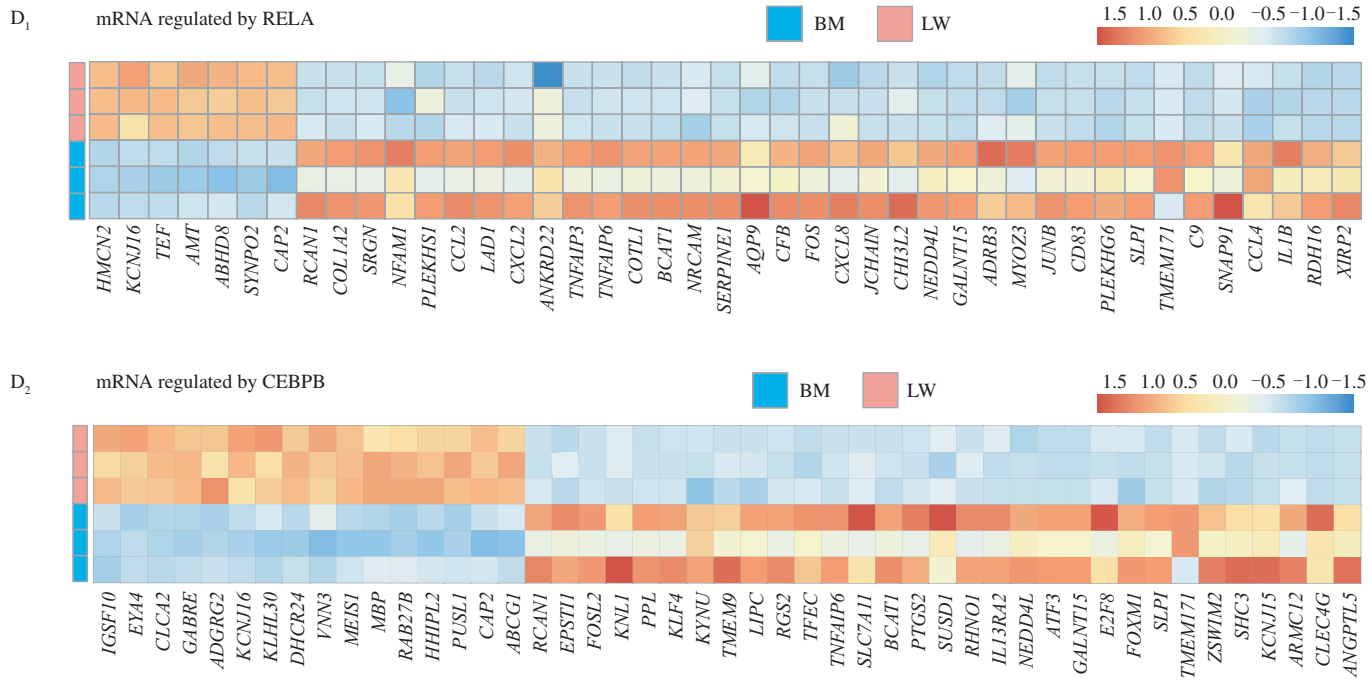


Fig. 5 (*Continued*)

fatty acid production pathway was activated, whereas the fatty acid catabolic pathway was inhibited, and in particular, the expression of CPT1A, a key rate-limiting enzyme for lipolysis, was significantly down-regulated (Fig. 6A). In the protein-lipid-metabolite integrated KEGG pathway network, we identified *ACACA*, *ACADVL*, *ACAT2*, *ACOX1*, *ACSS2*, *CRAT*, *DLAT*, and *LDHA* as key genes contributing to a significant difference in fat deposition capacity between BM and LW. And in four large sample size datasets (> 10 000 pigs), We verified that these genes were significantly associated with pig back fat thickness by phenome wide association analysis (Fig. 6B). Finally, we transformed pig fat into lard by wet process, quantitatively measured the key components content and enzyme activity in adipose tissue and lard, the results were consistent with the omics approach (Figs. 6C-E). Odor analysis results by electronic nose show that, BM lard contains more nitrogen oxides, aromatic inorganic sulfur, and organic sulfur compounds than LW lard. Relative to BM's adipose tissue, BM's lard exhibits lower levels of methyl compounds (Fig. 6F).

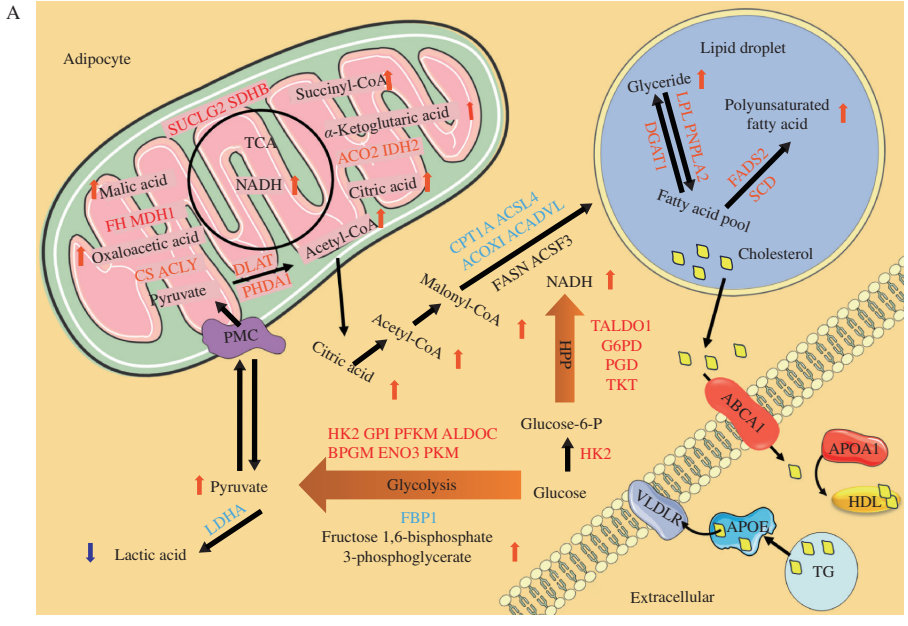
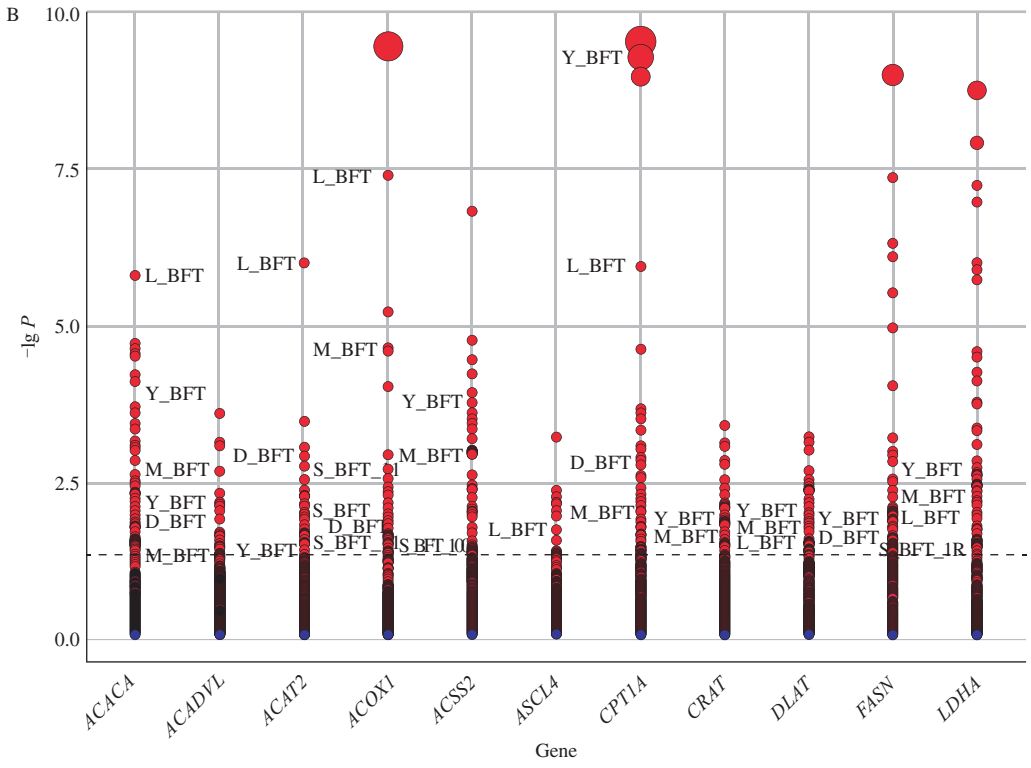


Fig. 6 Protein-lipid-metabolite integrated KEGG pathway network and Biochemical analysis. (A) Protein-lipid-metabolite integrated KEGG pathway network integrating glycolysis, TCA cycle, pyruvate metabolism, fatty acid biosynthesis, fatty acid degradation, and pentose phosphate pathways. Significantly altered proteins/metabolites and their associated bioprocesses are plotted, and detailed pathway diagrams are placed in the Supplementary Material. (B) The point plot shows the association between gene and complex trait in gene-based association analysis. D: Duroc; Y: Yorkshire; L: Landrace; M: including D, Y, L groups, Chinese autochthonous pigs, and hybrid groups between pig breeds. (C) Lard from BM and LW Pigs. (D) Quantitative results of activities of pyruvate dehydrogenase (DLAT) and lactate dehydrogenase (LDHA). (E) Quantitative results of important components in adipose tissue and lard. (F) Odor analysis results using electronic nose. *$P < 0.05$. Different lowercase letters mean significant difference ($P < 0.05$).



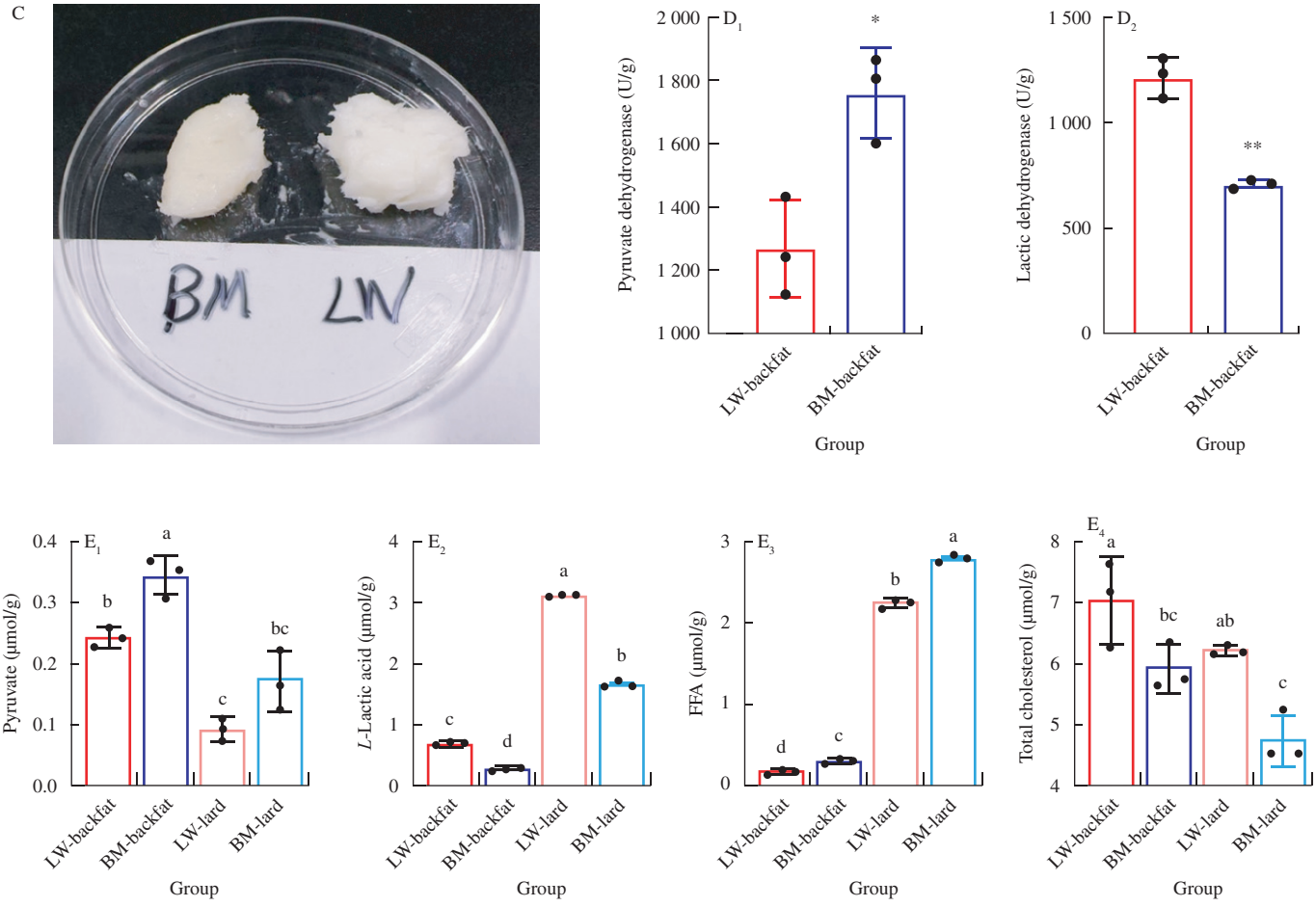


Fig. 6 (*Continued*)

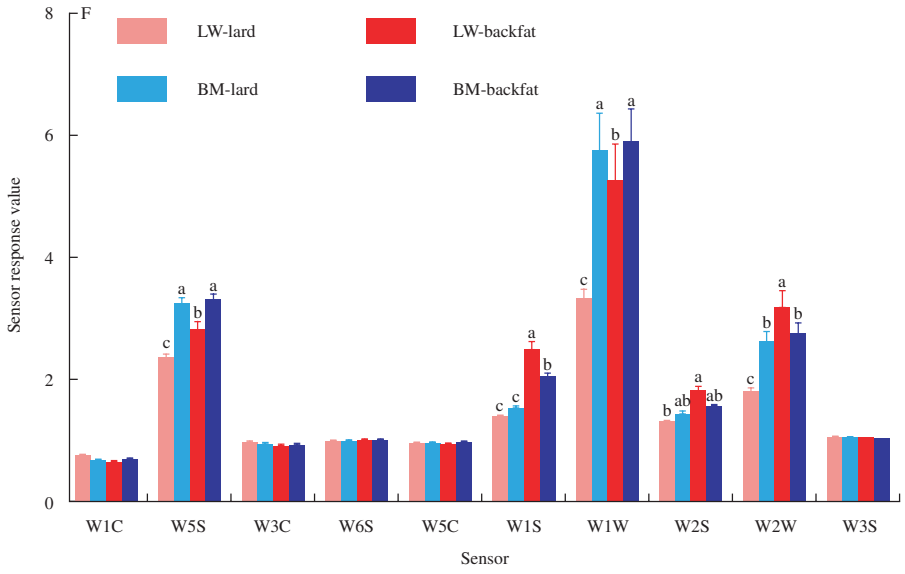


Fig. 6 (*Continued*)

4. Discussion

Lard, derived from pig fat, is a versatile cooking oil that adds a distinctive flavor and texture to dishes. Previous studies have shown that consuming lard is not good for health compared to plant oils[27-28], but lard contains some nutrients and lipids that the body needs[29]. Therefore, a balanced intake of plant and animal oils is the key to maintaining good health. In this study, we systematically analyzed the differences and molecular mechanism for these differences in nutrient composition and flavor substances of adipose tissue between fat-type BM and lean-type LW.

Studies showed that the content of saturated fatty acids and cholesterol in animals are higher compared to plants. High intake of saturated fats and cholesterol has been associated with an increased risk of cardiovascular and obesity diseases[13]. In this study, 7 cholesterol esters and 203 saturated/monounsaturated lipids were significantly lower in BM pigs than in LW pigs, whereas 36 polyunsaturated lipids were significantly higher. In addition to lipids, we detected a significant increase in vitamins D, E and B in the backfat of BM pigs. Vitamin B is a water-soluble vitamin that participates in the metabolism of sugar, protein and fat in the body as a coenzyme[30]. Pig fat tissue is notably rich in essential vitamins, particularly vitamin D and vitamin E. These fat-soluble vitamins play crucial roles in maintaining overall health. The fat-soluble nature of vitamins D and E means they are absorbed more efficiently when consumed with dietary fats. lard, is a natural source of vitamin D. This vitamin is essential for the regulation of calcium and phosphorus in the body, promoting bone health. Adequate vitamin D intake is also associated with a reduced risk of various chronic diseases[31-32]. Vitamin E is a fat-soluble antioxidant with significant levels in porcine adipose tissue. While lard is not a complete source of protein, it does contain some essential amino acids. These are the building blocks of proteins and are vital for various physiological functions in the body. Our untargeted metabolomic results indicate that the backfat of the BM contains more essential amino acids, including leucine and valine, compared to the LW. Valine and leucine are important human sports nutritional supplements that are absorbed and transported directly into muscle tissue to provide energy or to build muscle through conversion to protein[33-34]. Glutathione, an antioxidant, helps protect cells from damage caused by free radicals and contribute to overall health[35]. The main function of the glutathione S-transferase (GST) is to catalyze the combination of the electrophilic groups of certain endogenous or exogenous hazardous substances with the sulfhydryl groups of reduced glutathione to form more non-toxic derivatives[36]. In this study, glutathione content, protein expression of GSS, GST and GPX7 were significantly increased in BM pigs. These results suggests that consuming lard derived from BM fat tissue might be more beneficial for human health.

Fatty acids in lard play a role in the flavor development of cooked dishes and baked goods[37]. The length of fatty acid chains significantly influences the flavor profile of fats. Short-chain FFAs often contribute to a tangy or sour taste, while medium-chain FFAs can impart a smoother, milder flavor. Long-chain FFAs, on the other hand, may have a more complex and pronounced taste[38]. In this study, we found significant differences in the content of FFA between BM and LW pigs, all of which were long-chain and up-regulated in BM. The degradation of lipids during cooking also contributes to the generation of aromatic compounds. FFAs, in particular, are known for their role in creating appealing aromas in cooked foods[39]. The oxidation of unsaturated fatty acids produces hydroperoxides, which then further decompose into odor-active volatile secondary products[40]. During the heating process, the increased breakdown of unsaturated lipids may contribute to BM lard imparting a stronger aroma to food. Amino acids in addition to being nutrients also provide flavor to foods; for example, glycine and alanine are sweet, arginine leucine and isoleucine have a bitter taste, and aspartic acid has a sour taste[41-43]. Meanwhile, amino acids mainly are involved in Meladic reactions with reducing sugars during cooking[44], leading to browning and formation of flavor compounds, or are involved in Strecker reactions with α-dicarbonyl compounds, producing aromatic compounds, aldehydes. In our study cysteine was significantly increased in BM pigs. Cysteine is used as an additive in

baked products for meat and bread because it produces meaty flavor compounds through a Meladic reaction that imparts an attractive aroma to the food. In terms of odor components, BM lard contained more nitrogen oxides, aromatic inorganic sulfur, and organic sulfur compounds compared to LW lard. BM lard exhibited lower levels of methyl compounds relative to BM adipose tissue. However, the relationship between these compound compositions and odor requires further investigation. These results suggest that cooking with lard from the adipose tissue of the BM pig possibly confers a more enticing aroma to the food.

Considering that fat deposit is a complex trait, a single metabolomics analysis may be limited. We performed high-throughput untargeted integrated pathway analyses including mRNAs, proteins, lipids, and metabolites to find potential biomarkers that are highly correlated with fat traits. We found the central role of pyruvate metabolism in contributing to the differences in fat deposition capacity between BM and LW pigs. In glycolysis, glucose is converted to pyruvate. Pyruvate is used to synthesize fatty acids through the production of acetyl-CoA a by enzymes such as PDHA1 and DLAT[45]. These fatty acids can be esterified to triglycerides, leading to lipid deposition in adipose tissue. Aerobic metabolism of glucose is more efficient for energy production compared to anaerobic metabolism, the complete oxidation of one molecule of glucose in aerobic respiration produces a much higher yield of ATP and NADPH[46], which furthermore are used to synthesize fat. In this study, the levels of some key enzymes and metabolites were significantly up-regulated in BM, including the biological process of glucose to pyruvate, the TCA cycle that leads to the complete oxidation of glucose, and the first phase of the pentose phosphate pathway. These processes provided high levels of acetyl-CoA, ATP, and NADH for fat synthesis. In pyruvate-to-fatty acid biogenesis, the fatty acid synthesis pathway was enhanced and the fatty acid degradation pathway was inhibited. In addition, by analyzing high-throughput protein and metabolic profiling data, we uncovered differential cholesterol absorption and elimination between adipocytes of BM and LW. In our study, we found decreased protein expression of APOE and increased protein expression of ABCA1 and APOA1 in BM pigs. This may contribute to cholesterol elimination from adipocytes, resulting in lower content of cholesterol and cholesteryl esters in the BM pigs.

5. Conclusion

In the current study we found that the adipose tissue of the BM had better lipid deposition capacity and nutritional value. The category, chain length and degree of unsaturation of triglycerides, the composition of glycerophospholipids, sphingolipids, acylcarnitines and FFA were significantly differ between the 2 pig breeds. The higher levels of polyunsaturated fatty acids, valine, and leucine, along with vitamins D and B, antioxidant vitamin E, and glutathione, underscore the nutritional value of BM backfat as lard. Meanwhile, higher levels of long-chain fatty acids and cysteine may confer a rich full flavor to the BM lard. Mechanistically, we systematically analyzed the changes in mRNAs, key enzymes, and metabolites responsible for these differences. Importantly, using multi-omics pathway mapping, we found that in the pyruvate metabolic pathway of BM pigs, the bioprocess of lactate production was inhibited but the acetyl-CoA production was activated, suggesting the possibility that energy allocation favors the biogenesis of lipid precursors. The current study could contribute to a better understanding of the lipid differences of BM and LW pigs and also provide a basis for improvement of lard quality.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250355.

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