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Original Article

Correlation between four metabolism-related genes in different adipose tissues and adipocyte morphology in Xinjiang brown cattle

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Abstract: Objective: Thirty adult healthy Xinjiang brown cattle were selected and fasted for 12 h before slaughtering. Perirenal fat, subcutaneous fat, pericardial fat and intermuscular fat were collected. Fluorescence quantitative PCR was performed to detect the expressions of leptin gene (lep), fatty acid synthetase gene (fas), lipoprotein lipase gene (lpl) and obesity-associated gene (fto). Cryosectioning technique was employed to prepare the sections of intermuscular fat, subcutaneous fat, perirenal fat and pericardial fat. Adipocyte area and adipocyte number per unit area were calculated using Motic microscopic imaging system. Correlation analysis was carried out with the expression of the four genes regulating lipid metabolism. Results showed that the expressions of the four fat metabolism genes in Xinjiang brown cattle were specific to tissues. Only fas gene did not show significant differential expression. The lep and lpl genes were strongly expressed in intermuscular fat and pericardial fat; fto gene was most strongly expressed in pericardial fat. Four genes showed different expressions in the same adipose tissues. In intermuscular fat, the expression of lep gene was obviously higher than that of other genes; in subcutaneous fat, the expression of fas gene was significantly higher than that of other genes ($P<0.05$); in perirenal fat, lpl and fto genes were significantly expressed ($P<0.05$); in pericardial fat, the expression of fto gene was considerably higher than that of other genes. Adipocyte area and adipocyte number were specific to tissues, and in the decreasing order of adipocyte area, adipose tissues were ranked as follows: perirenal fat, subcutaneous fat and pericardial fat, intermuscular fat; in the decreasing order of adipocyte number per unit area, adipose tissues were ranked as follows: intermuscular fat and subcutaneous fat, pericardial fat, perirenal fat. Except fas and fto genes, intermuscular adipocyte area was in significant negative correlation with lep gene expression ($r=-0.3656$; $P<0.05$); intermuscular adipocyte area was also correlated with lpl expression ($r=-0.51$; $P=0.0581$). Pericardial adipocyte area was significantly negatively correlated with the expressions of lep and lpl genes ($r=-0.4133$, $r=-0.47$; $P<0.05$). Perirenal adipocyte area did not show significant correlations with the expression of any of the four genes ($P>0.05$). The expression of four genes regulating lipid metabolism was not significantly correlated with the corresponding adipocyte number ($P<0.05$).

Keywords: Xinjiang brown cattle, different positions, genes regulating lipid metabolism, adipocyte area, adipocyte number per unit area

Introduction

Xinjiang is the major province of animal husbandry in China and cattle breeding industry is one of the leading industries. At the end of 2010, the total number of cattle on hand was 4.5603 million, among which Xinjiang brown cattle and the hybrids totaled over 1.80 million, accounting for 40%. As the dominant variety in cattle breeding industry of Xinjiang, Xinjiang brown cattle are third-generation dairy-beef dual-purpose breeds. Since selection and breeding are not carried out systematically for over 20 years after the breeding of Xinjiang brown cattle, the cattle breeding industry now faces the problem of unclear direction of breeding direction. There is an overall decline of genetic properties and productivity of Xinjiang brown cattle for both dairy and beef purposes. Compared with the excellent beef cattle varieties, the production performance and the meat qualities of Xinjiang brown cattle decrease. The following problems are identified: (1) For the breeding of new varieties for beef purpose, con-

ventional selection and breeding methods are used along with crossbreeding. These methods can hardly improve meat quality within a short period. The introduction of foreign varieties, especially a single variety, can lead to the changes of biodiversity of beef cattle as well as the deterioration of excellent qualities of Xinjiang brown cattle and the unique flavor of beef; (2) Xinjiang brown cattle have complex genetic background, and the physiological and biochemical processes of lipid metabolism related to meat quality are unknown. This is an important reason for the underdevelopment of molecular marker-assisted breeding of Xinjiang brown cattle, which brings an adverse impact on the beef cattle breeding industry of Xinjiang.

We detected the tissue-specific changes of the expressions of four genes related to lipid metabolism of Xinjiang brown cattle, which were leptin gene (lep), fatty acid synthetase gene (fas), lipoprotein lipase gene (lpl) and obesity-associated gene (fto). The expressions of these genes at different positions (subcutaneous fat, intermuscular fat, pericardial fat and perirenal fat) were observed. Adipocyte morphology at different positions was discussed besides the differences of expression, and a correlation analysis was carried out between tissue specificity of gene expression and adipocyte morphology. The findings provide important basis for regulating fat distribution and screening of molecular markers related to meat quality by biological techniques. This is of high importance for improving meat quality of Xinjiang brown cattle and breeding of new beef-purpose breeds.

Materials and methods

Experimental animals

According to Variety Certification Standard for Xinjiang Brown Cattle, 20 adult healthy drylot Xinjiang brown cattle showing uniform appearance characteristics, age and nutritional status were randomly selected from Yili Western Tianshan Agricultural and Livestock Development Co., Ltd. These cattle were fasted for 12 h before slaughtering.

Equipments and reagents

Equipments: Light Cycler 2.0 Instrument (Roche, USA), PCR Mycycler Thermal Cycler (Bio-Rad, USA), NanoDrop® ND-2000 Spectrophotometer (NanoDrop, USA), Leica CM3050 S cryostat (Leica, Germany), Motic BA400 Microscope (Motic China Group Co., Ltd., Xiamen); Motic Images Advanced 3.2 Software.

Reagents: TRNzol® Reagent, RNase-free, dd-H_2O, Oligo(dT)15, 2 × Taq PCR MasterMix, Ribonuclease Inhibitor, dNTP Mixture (Tiangen Biotech (Beijing) Co., Ltd); Dntp Mixture (Tiangen Biotech (Beijing) Co., Ltd); M-MLV5 × Reaction Buffer and M-MLV Reverse Transcriptase (Promega, USA); DEPC (Beijing Dingguo Changsheng Biotech Co., Ltd); TCS0803 Flat cap strip, TLS0851 Individual PCR Tubes (Bio-RID, USA); SYBR®Primix Ex TaqTM (Tli RNaseH Plus) (TaKaRa, Japan).

Method

Sample collection: Sampling for fluorescence quantitative PCR: The method by Zhang and Lu et al. was used to collect adipose tissues (intermuscular fat, perirenal fat, subcutaneous fat, pericardial fat), each for 300 g. The samples were immediately frozen in liquid nitrogen, labeled and then preserved in liquid nitrogen.

Cryosectioning: Intermuscular fat, perirenal fat, subcutaneous fat and pericardial fat were cut into small blocks and subpackaged in 3 cyrogenic tubes. The tubes were labeled, placed into gauze bags and taken back to laboratory for cryosectioning.

Total RNA extraction and concentration and purity detection

Total RNA was extracted using TRIzol reagent from adipose tissues according to the instruction. Then 2 μl of the extracted RNA was taken to determine the integrity by 1% agarose gel electrophoresis. As a result, 28 s and 18 s bands were clearly visualized, and reverse transcription was carried out.

Fluorescence quantitative PCR and product detection

PCR proceeded in CFX96 TM PCR Instrument under the following conditions: activation of fluorescence signals at 95°C for 30 s, denaturation at 95°C for 5 s, annealing at 60°C for 30 s, 39 cycles; 95°C for 10 s, 65°C for 5 s, photographing, 95°C for 5 s, plotting of dissolution curve. To ensure specificity of amplification, the plotted dissolution curve should have only one peak. Then 5 μl of RT-PCR product was sub-

Table 1. Primers of target genes

Gene	Primer	Sequence (5'-3')	Length of the fragment (bp)
gapdh	F	TCATAGACAAGATGGTGAAGGTC	163
	R	TGGGTAGAGTCATACTGGAACAT	
lep	F	AGACACCGTGAAAAGGAGA	207
	R	CACTGAATGTTTGTGGAATG	
lpl	F	AGTGCCTGCTTGTTTGTG	286
	R	TATGCCCTTTCTGTTCCT	
fas	F	TCTGGGTTCACTTGTCA	206
	R	GAGTAGCCTGGTTTCCT	
fto	F	TACTGTCCTTATTTGCTTATG	259
	R	AAAGCCCTCATTTTCCAG	

jected to 2% agarose gel electrophoresis, using D2000 DNA marker (**Table 1**).

Data processing and statistical analysis

Calculation formula of relative expression of each gene was as follows:

$$F=2^{-\left[\left(\frac{\text{Average Ct value of target gene in control group}}{\text{Average Ct value of housekeeper gene in control group}}\right)-\left(\frac{\text{Average Ct value of target gene from the samples to be detected}}{\text{Average Ct value of housekeeper gene from the samples to be detected}}\right)\right]}$$

The names of internal reference gene glyceraldehyde-3-phosphate dehydrogenase (gapdh) and various template samples were input into Excel. Relative Ct value from RT-PCR was also input. Specific samples were selected as reference according to average Ct value. Here we used mRNA content of intermuscular fat as reference. Then Ct method was used to convert the expressions of gene in other samples into relative expressions of the gene with respect to the detected sample. The calculation formula was ECt, where E is amplification efficiency with the default value of 2 for presumably ideal amplification. The adipose tissue sections were observed under the 10 × 10 microscope and the images were collected. For each sample, 3 sections were chosen and processed using Motic Images Advanced 3.2 software. Six fields of view were chosen for each section to determine adipocyte area and adipocyte number per unit area. The results were averaged and expressed as mean ± standard deviation (SE). One-way ANOVA was carried out for adipocyte morphology using Graphpad prism software; correlation analysis was performed with the expressions of genes regulating lipid metabolism.

Results

Dissolution curve

The use of 6 internal reference genes precluded an influence of primer dimmer and non-specific amplification on the results. The dissolution curve had only one non-specific peak, indicating good specificity of the designed primers and full optimization of PCR conditions.

Specific expressions of genes regulating lipid metabolism in different adipose tissues

Using intermuscular fat as control, the expressions of three genes regulating lipid metabolism in adipose tissues at different positions are shown in **Figure 1**. In subcutaneous fat and perirenal fat, lep expression was significantly lower than that in intermuscular fat ($P<0.01$); however, no difference was found in lep expression in pericardial fat and intermuscular fat ($P>0.05$). The expressions of fas in subcutaneous fat, perirenal fat and pericardial fat were not significantly different from that in intermuscular fat ($P>0.05$). The expression of fas in subcutaneous fat seemed to increase compared with that in intermuscular fat. The lpl expression in intermuscular fat was very much higher than that in subcutaneous fat ($P<0.01$); but no significant differences were detected compared with the expression in perirenal fat and pericardial fat ($P>0.05$).

Metrological results of adipocyte morphology in different adipose tissues of Xinjiang brown cattle

Adipocyte area in intermuscular fat was taken as control for comparison of peer data in **Figures 2** and **3**. Adipocyte area in intermuscular fat of Xinjiang brown cattle was significantly lower than that in perirenal fat ($P<0.01$). The differences with adipocyte area in subcutaneous fat and pericardial fat were not significant ($P>0.05$).

Number of intermuscular adipocytes per unit area was taken as control for comparison of peer data in **Figures 3** and **4**. The number of

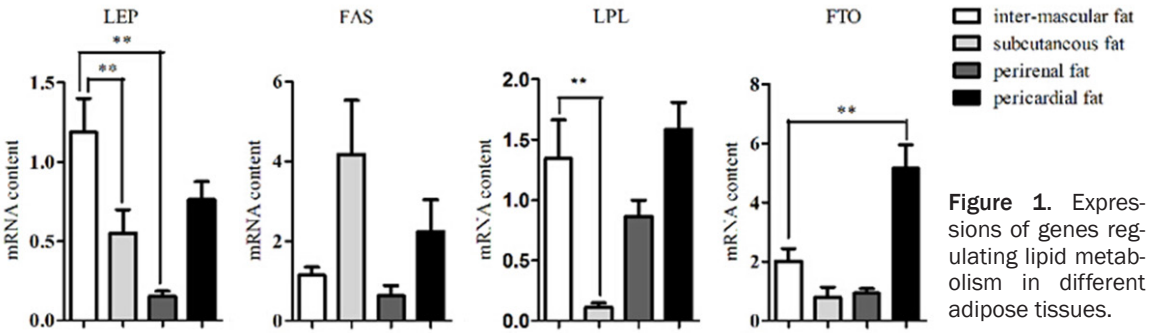

Figure 1. Expressions of genes regulating lipid metabolism in different adipose tissues.

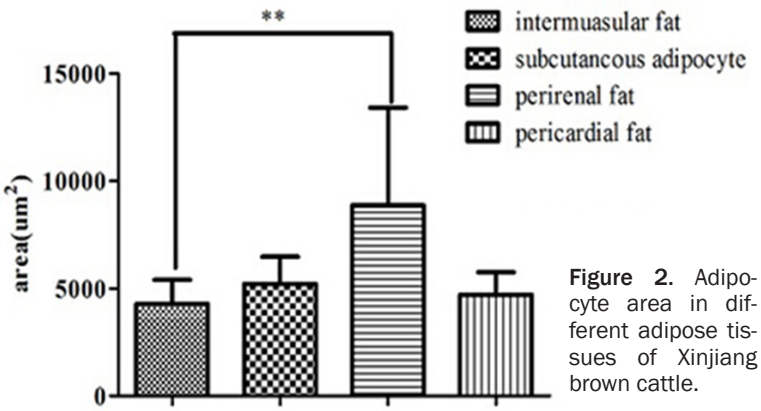

Figure 2. Adipocyte area in different adipose tissues of Xinjiang brown cattle.

intermuscular adipocytes per unit area was extremely significantly higher than that in perirenal fat and pericardial fat ($P<0.01$); the differences with number of subcutaneous adipocytes per unit were not significant ($P>0.05$).

Correlation between expressions of four genes regulating lipid metabolism and adipocyte morphology

Correlation between expressions of four genes regulating lipid metabolism and adipocyte area: As shown in **Table 2**, adipocyte area was significantly negatively correlated with lep expression (-0.3656; $P<0.05$) and correlated with lpl expression (-0.51; $P=0.0581$). Adipocyte area was not obviously correlated with fas and fto expressions ($P>0.05$). Subcutaneous adipocyte area showed no significant correlations with the expression of any of the four genes ($P>0.05$); perirenal adipocyte area showed no significant correlations with the expression of any of the four genes either ($P>0.05$); pericardial adipocyte area was significantly negatively correlated with lep expression (-0.4133; $P<0.05$) and lpl expression (-0.47; $P<0.05$); however, no significant correlations were found with the expressions of fas and fto.

Correlation between expressions of genes regulating lipid metabolism in adipose tissues and adipocyte number: As shown in **Table 3**, adipocyte number per unit area was not significantly correlated with the expressions of any of the four genes ($P>0.05$). At the other 3 positions, adipocyte number per unit area did not show significant correlations with the expressions of the four genes either ($P>0.05$).

Discussion

Expressions of the genes regulating lipid metabolism in different adipose tissues

mRNAs of many adipocyte factors are expressed specifically in different adipose tissues of Kuroge Washu, Limousin and Angus cattle. Bonnet et al. reported that lep and fas expressions in the subcutaneous fat of Limousin and Angus cattle were higher than that in intermuscular fat; lpl expression was not different in subcutaneous fat and intermuscular fat; but lpl expression in subcutaneous fat in Limousin cattle was higher than that in Angus cattle, indicating the interspecific difference [1]. Yamada et al. studied the expressions of vascular growth factors and lep gene and found that lep expressions in perirenal fat and intermuscular fat were higher than those in subcutaneous fat. In Xinjiang brown cattle, lep expression in intermuscular fat was higher than that in subcutaneous fat, which agreed with the results by Yamada et al.; however, the lep

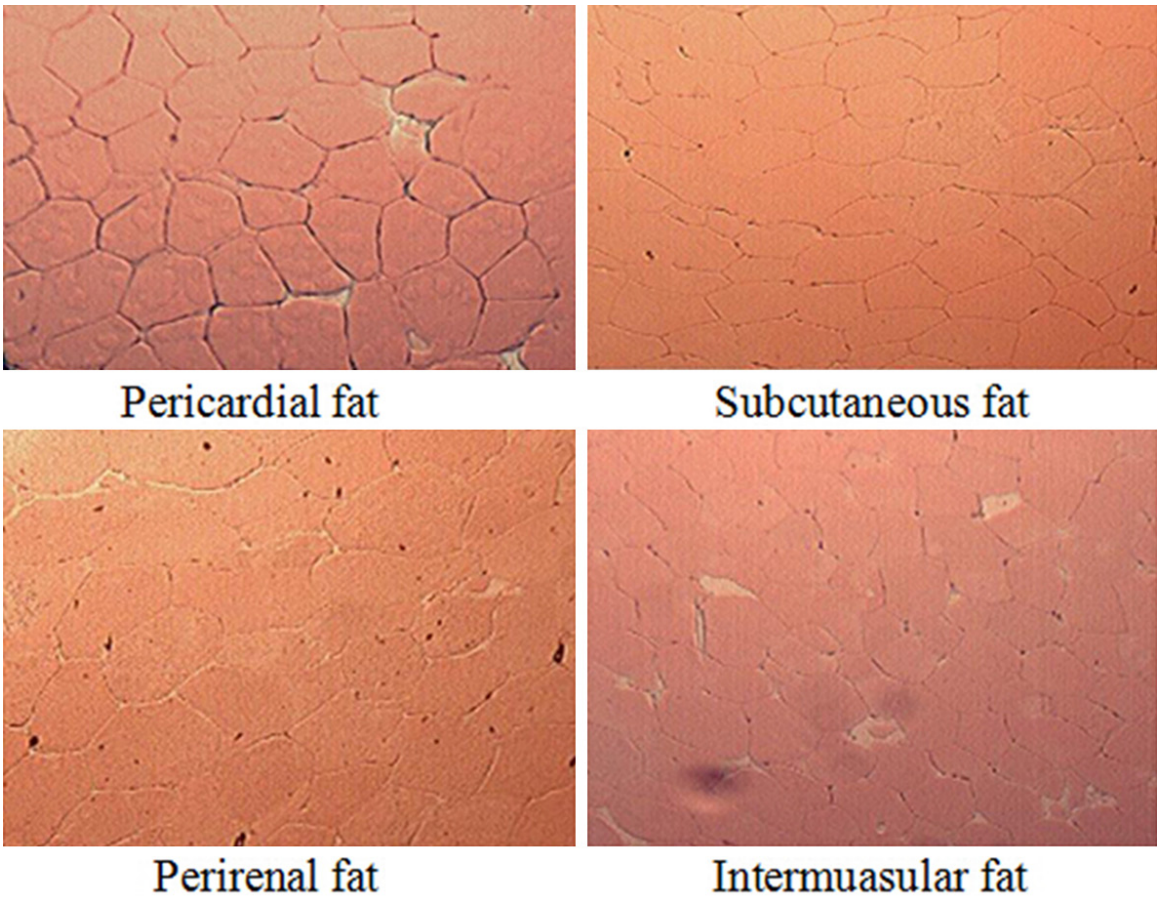

Figure 3. Adipose number per unit area in different adipose tissues of Xinjiang brown cattle.

expression in perirenal fat was not consistent in the two studies [2].

A specific protein or enzyme exhibits the same genetic potential in any type of cells within the same organism. But this potential is not necessarily fulfilled throughout the life cycle of the organism or in all cells. Gene expressions are subjected to temporal and spatial regulations, resulting in differential expressions of genes over time and in different tissues. Differential expression of lep, lpl and fto in different tissues of the same organism is one form of spatial regulation. We compared the expressions of lep, fas, lpl and fto in four different adipose tissues of Xinjiang brown cattle. All four genes were expressed in subcutaneous fat, intermuscular fat, perirenal fat and pericardial fat, though their expression intensity varied. fas gene was expressed in all these adipose tissues, and the difference was not significant ($P>0.05$). Thus fas expression was not subjected to spatial regulation; lep gene was strongly expressed in intermuscular fat and pericardial fat, but only lowly expressed in perirenal fat. This result agreed with that by Shen Liqin [3]; lpl gene was strongly expressed in intermuscular fat and pericardial fat, probably due to the intense production of triglyceride in intermuscular fat and pericardial fat; fto gene was most strongly expressed in pericardial fat. Tissue-specific expressions of the 3 genes may be attributed to differences in the rate of fat deposition at different positions as well as physiological functions, metabolic type and regulatory action.

Fatty acids are synthesized under the action of fas complex. Factor-3 signaling pathway is inhibited through lep-mediated cell factor signaling, which increases the expression of fas. The current studies on the mechanism of fat deposition are mainly concerned with a single gene and the influence on traits. But few studies are carried out over the mechanism of interactions between the genes [4]. Our results

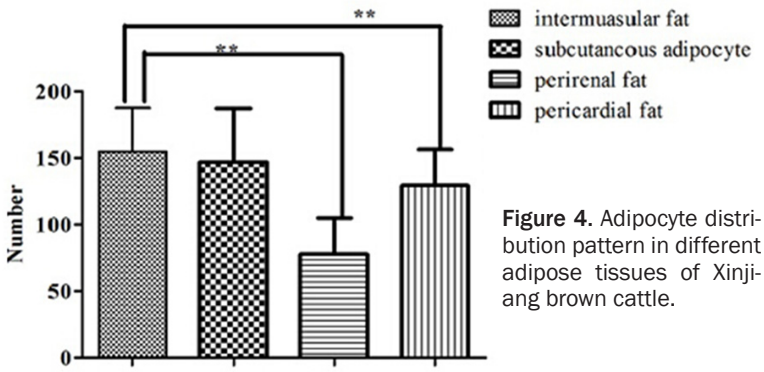

Figure 4. Adipocyte distribution pattern in different adipose tissues of Xinjiang brown cattle.

showed that lep expression in subcutaneous fat of Xinjiang brown cattle was extremely lower than fas expression. Lep expression in perirenal fat and pericardial fat was insignificantly lower than fas expression. It was indicated that downregulation of lep in vivo promoted the expressions of fatty acid synthase in white adipose tissues, leading to accelerated fatty acid synthesis and the increase of fat mass. This result was consistent with that by Nogalska et al. [5]. However, lep expression in intermuscular fat was higher than fas expression, and this result required further explanation.

Analysis of metrological results of adipocyte morphology in different adipose tissues of Xinjiang brown cattle

Studies show that the proliferation and enlargement of adipocytes are the major factors leading to fat deposition. Adipocyte proliferation mainly occurs in preadipocytes. During the late stage of development, preadipocytes and adipocytes are differentiated or die. As number of adipocytes decreases, more triglyceride is synthesized in fat and adipocytes are enlarged. It is found by Brenda that cattle adipocyte proliferation mainly occurs before the age of 11 months. After that, adipocyte number remains stable, while the adipocytes become enlarged. Zhang et al. [6] showed that adipocyte enlargement in rats occurred in parallel with body weight changes. Goldrick et al. found that fat deposition was mainly due to adipocyte enlargement in male rats. Yang et al. [7] and Hausman et al. [8] studied adipocytes in lard pigs and lean-meat pigs. Adipocytes in pigs grew by enlargement. Spalding found by studies on human obesity that adipocyte number in individuals aged over 20 years old remained constant while adipocyte enlargement was the major form of fat deposition in adults. The above studies confirm that fat deposition occurs through adipocyte enlargement. Kouba M investigated subcutaneous adipocytes in the crossbreeds of Yorkshire pigs and Duroc pigs. The thickness of adipose tissues on the back of lard pigs was larger by 1/5 compared with that in lean-meat pigs; the adipocyte size was greater by 1/5, while the number of adipocytes per unit gram of adipose tissues decreased. As shown by **Table 3**; **Figure 4**, intermuscular adipocyte area of Xinjiang brown cattle was extremely lower than perirenal adipocyte area, while the adipocyte number per unit area in intermuscular fat was extremely higher than that in perirenal fat. Adipocyte area was not significantly different in intermuscular fat, subcutaneous fat and pericardial fat. The difference in adipocyte area in intermuscular fat, subcutaneous fat and pericardial fat was not significant, and the difference in adipocyte number per unit area compared with subcutaneous fat was not significant; but the former was extremely higher than adipocyte number per unit area in pericardial fat; moreover, subcutaneous adipocyte area was extremely lower than perirenal adipocyte area. It was thus indicated that in the later stage of development of Xinjiang brown cattle, preadipocytes and adipocytes differentiated or died, leading to the decrease of adipocyte number, the increase of triglyceride in fat and enlargement of adipocytes.

Adipose tissues are the major site of energy storage in animals, and their morphology and functions are affected by various factors. The size and functions of adipocytes are closely related to adipose tissue distribution and fat deposition rate [9]. Huaser et al. [10] found that the development of intermuscular fat in pigs started later than that in other positions. In the study by Kauba et al. [11], the development of intermuscular fat and subcutaneous fat in pigs was slower, while the growth of perirenal fat was faster. In a decreasing order of adipocyte area in Xinjiang brown cattle, different adipose tissues were ranked as follows: perirenal fat > subcutaneous fat > pericardial fat > intermus-

Table 2. Correlation between expressions of four genes regulating lipid metabolism and adipocyte area

Gene	Adipocyte area							
	Intermuscular fat		Subcutaneous fat		Perirenal fat		Pericardial fat	
	r	P	r	P	r	P	r	P
lep	-0.37	<0.05	0.07	>0.05	0.12	>0.05	-0.41	<0.05
fas	0.17	>0.05	0.15	>0.05	-0.18	>0.05	0.51	>0.05
lpl	-0.51	<0.05	0.05	>0.05	0.13	>0.05	-0.47	<0.05
fto	0.34	>0.05	0.01	>0.05	-0.17	>0.05	0.5	>0.05

Table 3. Correlation between expressions of genes regulating lipid metabolism in adipose tissues and adipocyte number

Gene	Adipocyte number/mm^2							
	Intermuscular fat		Subcutaneous fat		Perirenal fat		Pericardial fat	
	r	P	r	P	r	P	r	P
lep	0.27	>0.05	-0.04	>0.05	-0.3	>0.05	0.46	>0.05
fas	0.27	>0.05	-0.14	>0.05	-0.02	>0.05	0.59	>0.05
lpl	0.27	>0.05	-0.13	>0.05	0.25	>0.05	0.13	>0.05
fto	0.48	>0.05	0.25	>0.05	-0.21	>0.05	0.09	>0.05

cular fat; in a decreasing order of adipocyte number per unit area, intermuscular fat > subcutaneous fat > pericardial fat > perirenal fat. These results may be explained by the factors of preadipocyte number, preadipocyte differentiation, blood supply, innervation and hormonal regulation.

Correlation between expressions of four genes regulating lipid metabolism and adipocyte morphology

During later stage of animal development, lpl plays important roles in adipocyte differentiation and triglyceride distribution. It is found by various researches that lpl is a key enzyme determining the adipocyte area. Hocqutte et al. studied the influence of lpl activity in cattle on intramuscular fat content, discovering that the transcriptional activity of lpl gene affected intramuscular fat content. Lpl expression serves as an important genetic marker of intramuscular fat content in cattle. Lpl is expressed in almost every tissue of animals, and the higher the expression in white adipose tissues, the easier the fat deposition will be. Lpl expression varies with tissues and positions and is significantly affected by adipocyte proliferation and differentiation. Gao et al. [12] found that lpl mRNA expression in early stage of development of Erhualian pigs was consistent with intramuscular fat growth; in the later stage, lpl expression decreased, while intramuscular fat content increased. The increase of lpl expression in early stage is due to the increase of adipocyte number along with the growth of adipose tissues. Therefore lpl is an important marker of differentiation of preadipocytes into mature adipocytes. As shown by the present study, lpl expression in pericardial fat of adult Xinjiang brown cattle was negatively correlated with adipocyte area at the corresponding position; intermuscular adipocyte area showed the trend of negative correlation with lpl expression. In subcutaneous fat and perirenal fat, lpl expression was not significantly correlated with adipocyte area, and lpl expression was not significantly correlated with adipocyte number per unit area of intermuscular fat. During the late stage of development, fat deposition in intermuscular fat and pericardial fat occurred through filling of lipid droplets in adipocytes without an obvious adipocyte proliferation in all four adipose tissues. Relative expression of genes controlling fat deposition decreased, and the relative proportion of transcripts in total RNA decreased, leading to a rapid decline on the transcript level.

Leptin is expressed by the ob gene in adipose tissues. Leptin binds to specific receptors in the hypothalamus to reduce appetite or act on

sympathetic nervous system. By this mechanism leptin facilitates fat decomposition and lowers body weight. Moreover, leptin regulates the expressions of various metabolism-related genes either directly or indirectly [13, 14]. Luo et al. investigated the influence of leptin on preadipocyte differentiation and found that 30 nmol/L and 100 nmol/L leptin significantly enhanced lpl mRNA expression while inhibited adipocyte proliferation. 150 nmol/L leptin effectively reduced lpl mRNA expression and promoted adipocyte proliferation. The same conclusions were drawn with preadipocytes in rats regardless of the concentration of leptin. In mature adipocytes, leptin directly promoted the decomposition of triglyceride, from which it is known that lep does not control adipocyte differentiation, but directly acts on adipocytes and reduces fat synthesis. In the present study, lep expression in intermuscular fat and pericardial fat of adult Xinjiang brown cattle was negatively correlated with adipocyte area at the corresponding positions. However, lep expression was neither significantly correlated with adipocyte area nor with adipocyte number in subcutaneous fat and perirenal fat. Lep can increase the activity of lpl, inhibiting the proliferation of intermuscular and pericardial adipocytes; lep acts directly on adipocytes at these positions, promoting the decomposition of triglyceride, reducing adipocyte area and number. Since the analysis was carried out only on mRNA level, we did not know whether mRNA expression of lep affected lpl protein expression and enzyme activity.

FAS is a key enzyme in fatty acid synthesis which consists of 8 functional domains. Six of eight functional domains are related to the synthesis of fatty acid chains, and the eighth functional domain provides the site for the attachment of FAS [15]. This functional domain catalyzes acetyl coenzyme A and malonyl coenzyme A to produce saturated fatty acids, typically palmitic acid and also some stearic acids and short-chain fatty acids. According to some studies, Gannan black yaks have a relatively low fatty acid content in intermuscular fat and perirenal fat and a high content of unsaturated fatty acids [16]. It is thus known that more FAS is synthesized in perirenal fat than in intermuscular fat. We found through the present study that fas expression in perirenal adipocytes in adult Xinjiang brown cattle was more correlated with adipocyte area compared with intermuscular fat. It was also found that adipocyte area in perirenal fat was significantly larger than that in intermuscular fat.

Activity of fas gene in adipose tissues plays a crucial role. Xiong's study indicates that fas expression in adipose tissues of pigs was extremely positively correlated with carcass fat content and fat percentage. Naduau et al. found that intramuscular fat content increased in rats after processing. As FAS protein level increased, fas expression showed a positive relationship with intramuscular fat deposition. Chen et al. found that fas expression was not significantly correlated with intramuscular fat content. They suggested the expression of FAS, as a rate-limiting enzyme in fat synthesis, was not the sole factor determining the production of intramuscular fat. Fas expressions in intermuscular fat, perirenal fat, subcutaneous fat and pericardial fat of Xinjiang brown cattle were weakly correlated with adipocyte area and number at the corresponding positions. Other lipid metabolism-related genes may come into play in the expression of fas expression.

ZABENA et al. found that FTO rs 9939609 was obviously correlated with morbid obesity of Spanish adults. The expression of Fto gene in subcutaneous fat showed a positive correlation with the expressions of perilipin and leptin genes. FISCHER et al. suggested that fto gene deletion in mice led to retarded growth and an obvious decrease of body weight. FREDRIKSSON et al. selected mice fasted for 48h and found that the positive regulation by the hypothalamus had a significantly positive correlation with fto expression. Niu [17] performed qRT-PCR to detect the genes related to fat synthesis and decomposition (FAS, SCD1, DGAT2, ACOX1, CPTIa). With over-expression of fto, genes related to fat synthesis (fas, scd1) were significantly upregulated; the genes related to fat decomposition (acox1, cptia) were downregulated to an insignificant degree. The mouse model for a partial loss of fto function carried one point mutation, which led to the isoleucine-to-phenylalanine mutation at codon 367 (1367F). This model did not present with a reduction of body weight and fat content until later stage of fto 1367F maturation. Other studies indicate that fto is transcriptional coactivator that regulates fat synthesis on the transcriptional level by

enhancing mutual binding of CCAAT/enhancer-binding proteins. We found that fto expression in four adipose tissues of Xinjiang brown cattle was not significantly correlated with adipocyte area and adipocyte number per unit area. The reason may be that fto gene and other lipid metabolism-related genes jointly influence obesity or that fto gene regulates fat deposition on the protein level, rather than on the mRNA level. There is one thing that cannot be denied: fto gene is involved in energy metabolism and balance. But how fto gene is involved in fat metabolism of cattle is unknown. Future direction of research will be functional identification and action mechanism of fto gene.

Conclusion

Four genes regulating lipid metabolism in Xinjiang brown cattle, namely, lep, fas, lpl and fto, were expressed to varying extent in different adipose tissues. Except fas, the expressions of other three genes showed some differences in different adipose tissues. Even the same gene was expressed differently in different adipose tissues in Xinjiang brown cattle.

Adipose area and number in Xinjiang brown cattle vary from one position to another. In a decreasing order of adipocyte area, adipose tissues were ranked as follows: perirenal fat > subcutaneous fat and pericardial fat > intermuscular fat; in a decreasing order of adipocyte number per unit area, intermuscular fat and subcutaneous fat > pericardial fat and perirenal fat. Such differences can be explained by the factors of preadipocyte number, preadipocyte differentiation, innervation, blood supply and hormonal regulation.

Expressions of four genes regulating lipid metabolism are correlated with adipocyte morphology. In pericardial fat, the expression of lpl was negatively correlated with adipocyte area, and there was the trend of negative correlation in intermuscular fat. Expression of lpl in subcutaneous fat and perirenal fat was not significantly correlated with adipocyte area and number. Fat deposition was mainly featured by filling of lipid droplets in cells without an obvious adipocyte proliferation.

Lep expression in intermuscular fat and pericardial fat was significantly negatively correlated with adipocyte area, but such correlation was not observed in subcutaneous fat and perirenal fat. However, we only determined mRNA level, and whether mRNA level of lep gene has an impact on LPL protein expression and enzyme activity requires experimental confirmation.

Fas and fto expressions in four adipose tissues were not obviously correlated with adipocyte area and number. Fto gene was involved in energy metabolism and balance. But there has been no report concerning the regulatory mechanism of fto gene in lipid metabolism of cattle. Future studies should focus on functional identification and action mechanism of fto gene.

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Disclosure of conflict of interest

None.

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