Expression Patterns of SREBF1 and SREBF2 in Liver and Adipose Tissues of Two Fat-Tailed Sheep Breeds

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Research

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Abstract

Background

The fat-tailed sheep is raised largely in China and other countries due to its high adaptability to nutritionally challenging environments and disease resistance as well as fat deposition in tails. So the purpose of the study is to explore the expression pattern and regulation mechanism of sterol regulatory element binding proteins (SREBPs) in liver and adipose tissues of fat-tailed sheep bases on its role in fat metabolism and deposition with sheep grown and development.

Methods

Two representative Chinese fat-tailed sheep breeds *i.e.* Guangling Large Tailed (GLT) and Small Tailed Han (STH) were employed to evaluate the ontogenetic expressions of *SREBF1 and SREBF2* from seven different adipose tissues and liver at 4, 6, 8, 10 and 12 month by real-time PCR. Five serum indicators were detected, and the function speculation of SREBP1 and SREBP2 were evaluated by bioinformatics approaches.

Results

Serum indicators, especially total cholesterol TC and non-esterified fatty acid (NEFA) showed obvious differences and dynamical changes with the different development ages, gender factors only had a significant effect on serum NEFA concentrations. Both *SREBF1* and *SREBF2* mRNA expression in sheep were significantly affected by breed, tissue, age factors, and show a significant positive correlation (r = 0.286). Merely the expression of *SREBF1* gene in STH is significantly higher than that in GLT, but *SREBF2* gene expression is opposite. The expressions of *SREBF1/2* in liver are extremely higher than that in seven adipose tissues, the interaction of two factors between breed and month age, breed and tissue, and tissue and gender also significantly affects its expression. Subcellular locations and function prediction imply that SREBP1/2 expressions are closely related with metabolism processes in cells.

Conclusions

The mRNA expression profiling of *SREBF1* and *SREBF2* showing a breed-specific, gender-specific, and temporal and spatial expressions differences, which imply that *SREBF1/2* play a crucial role in lipid metabolism regulation during growth and development of two fat-tailed sheep. This study provides a phenotypic basis for further revealing the genetic mechanism in lipid metabolism and fat deposition that causes differences in ovine tail types, which also provides a novel insight for improving quality of meat.

Background

Domesticated sheep are economically important livestock species, and provide many daily necessaries for human consumption including meat, milk, and wool worldwide.[1] High-quality domesticated sheep breed is a valuable genetic resource for the global animal husbandry.[2] The fat-tailed sheep is widely bred in China and other countries, due to its high adaptability to different climatic conditions, disease resistance and high production in poor nutritional situations.[3] Among, the fat deposition in the tails plays a crucial role for the sheep to adapt to nutritionally challenging environments.[4] It is known that traits such as fatty acid content and lipid deposition are vital for sensory,

nutritional and technological properties of mutton.[5] Lipid metabolism is also an extremely important physiological process maintaining nutrient adjustment, homeostasis, and animal health.[6–8] Thus, exploring the mechanism of fat metabolism and deposition of fat-tailed sheep has profound meaning to improve meat quality in food and agriculture fields.

Sterol regulatory element binding proteins (SREBPs) can regulate the lipid homeostasis by regulating its target genes, which are crucial for the cholesterol and fatty acid metabolism.[9,10] The mature forms of SREBPs are transcriptionally active and trans-located into the nucleus where bind to the promoters of SREBPs target genes, most of which are involved in lipid metabolism.[11] For example, SREBP1c which is encoded by *SREBF1* mainly promotes the fatty acid synthesis by activating many genes involved in lipid metabolism in fat tissue and liver.[9] SREBP2, another member of the SREBPs nuclear transcription factor family, which is encoded by *SREBF2*, activates the target gene transcription and the gene expressions on the cholesterol biosynthesis pathway by binding to the sterol regulator and promoter/enhancer in the lipid synthetic enzyme gene.[12,13] However, the role of the transcriptional regulation of SREBPs in fat-tailed sheep is unclear.

Therefore, two representative Chinese fat-tailed sheep breeds were employed in this study. Five serum lipid metabolism indicators, including triglyceride (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC), were determined based on their body size and weight measurement traits. More importantly, the transcriptional levels and function speculation of SREBP1 and SREBP2 in the various adipose tissues and liver with the development stages were evaluated by qRT-PCR and bioinformatics approaches. All these investigations will provide a new insight for revealing the regulation function of SREBP1 and SREBP2 in fat metabolism and deposition, as well as in meat quality from the different fat-tailed sheep.

Materials And Methods

Animals and Ethical Statement

Total 80 individuals of Guangling Large Tailed sheep (GLT) and Small Tailed Han sheep (STH) with half for each breed were selected according to the genders and ages in this study. GLT, a local outstanding breed distributed in the mountain regions of northern Shanxi province, is typically of large tails for fat deposit and good mutton quality but low fecundity. STH is characterized by small fat tails but higher fecundity in the plain regions of Shandong and Hebei provinces. The disparity of fat deposition in tail between GLT and STH is obvious in different development stages. The experimental animals are fed and managed as described before.[14] The feeding, management and slaughtering were conducted according to the National (GB 13078 – 2001 and GB/T 17237 – 1998) and the Agricultural Standards (NY 5148-2002-NY 5151–2002) of the People's Republic of China. At 4, 6, 8, 10 and 12 month of age, 8 GLTs and 8 STHs of a half male/female sheep were slaughtered respectively for sample collection.

This study was carried out under the guidelines for the care and use of animals for scientific purposes. The protocol was approved by the Institutional Animal Care and Use Ethics Committee of Shanxi Agricultural University.

Body measurement

The body size and weight measurement traits of all sheep, *i.e.* body height at withers (BH), body length (BL), heart girth (HG), cannon circumference (CC), tail length (TL), tail width (TW), live weight (LW), carcass weight (CW), and absolute tail fat weight (ATW) were measured by using measuring tape and platform scale according to the standard

procedure. Relative tail fat weight (RTW) and dressing percentage (DP) were calculated as follows: RTW = ATW/CW; DP = CW/LW.

Serum and tissues collection

Four males and four females for each breed were randomly selected and slaughtered at 4, 6, 8, 10 and 12 months of age, respectively. Blood samples were collected from external jugular vein. Blood was allowed to clot, followed by centrifugation at 3000 rpm for 10 min. The serum was collected and sent to measurement of serum biochemical parameters. Adipose tissues, *i.e.* tail fat (TA), great omental fat (GO), subcutaneous fat (SC), small omentum fat (SO), perirenal fat (PR), retroperitoneal fat (RP), mesenteric fat (MT) and liver (LV), were rapidly dissected, weighed and placed in liquid nitrogen and stored at − 80 °C.

Measurement of serum biochemical parameters

Serum samples were separated from all sheep to measure the contents of key biochemical indicators related to lipid metabolism and deposition. The indicators included triglyceride (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC). The concrete operations were conducted according to the manufacturer's manual of kits (Nanjing Jiancheng Bioengineering Institute, China).

RNA Extraction and Real-Time RT-PCR

Total RNA was extracted from liver and adipose in tail, subcutaneous, great and small omental, retroperitoneal, mesenteric, perirenal tissues by Trizol (Invitrogen) following manufacturer's instructions. All of the RNA samples were treated with DNase I, followed by standard reverse transcription using SYBR® PrimeScript™ RT-PCR Kit. Real-Time qPCR was carried out using SYBR® Green PCR Master Mix (Applied Biosystems). The programs for the amplification were as following: activation of polymerase at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. The analysis of dissociation curves was always performed after 45 cycles. Primers were designed by Primer 3 Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and checked by Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). The primers are listed as followed:

SREBF1, Primer_F: GAGCTTCGTGGTTTCCAGAG; Primer_R: ATCCAGAAGCTGGTGTGTCC

SREBF2, Primer_F: TTTGAGGAGGAAGCGAAGAC; Primer_R: CCATGTGTACGTCGGAACAG

β-actin, Primer_F: GATCATTGCTCCTCCTGAGC; Primer_R: ACATCTGCTGGAAGGTGGAC

Prediction of SREBP1/2 subcellular localization and function

The subcellular locations of SREBP1/2 were predicted by the protein subcellular localization prediction tool PSORT II (http://www.genscript.com/tools/psort). The functions of these two proteins were analyzed by Prot Fun 2.2 Server (http://www.cbs.dtu.dk/services/ProtFun/) (Jensen *et al.*, 2002).

Statistical analysis

The data of body size measurements, serum biochemical parameters, and relative mRNA expressions were grouped and subjected to statistical analysis by T-test and one-way ANOVA using GraphPad Prism 8.0 software (San Diego, CA, USA). In addition, the potential interaction relationships between SREBP1/2 expression levels and other traits with breed, age, gender were analyzed by the software SPSS (version 26.0, IBM, Chicago, USA) according to the following general linear model:

$$y_{ijkl} = \mu + B_i + M_j + G_k + BM_{ij} + BG_{ik} + MG_{jk} + e_{ijkl}$$

where, y_{ijkl} is the observation for body size measurements and serum biochemical parameters, μ is the overall mean for each trait, B_i, M_j and G_k are the main effect of breed, month of age and gender, respectively, BM_{ij}, BG_{ik} and MG_{jk} are the interaction effect of breed × month of age, breed × gender and month of age × gender, respectively, and e_{ijkl} is the random error. Associations between *SREBF1/2* mRNA expression in eight tissues and slaughter and tail traits in sheep were analyzed by SPSS (version 26.0, IBM, Chicago, USA).

Results

Body size and weight in the two fat-tailed breeds

To evaluate the breed characteristics between GLT and STH, the parameters of body size and body weight were evaluated. As shown in Fig. 1. The mean body height of GLT is higher significantly than that of STH ($P < 0.01$), while the length and width of tail are less than that of GLT ($P < 0.001$). No significant differences were observed in body length, heart girth and cannon circumference (Fig. 1A). Although the live weight of STH was slightly lower compared to that of GLT ($P < 0.05$), the total carcass weights of the two breeds have no significant differences (Fig. 1B). These differences fully reflect the characteristics of these two breeds, especially in tail fat deposits.

Dynamical changes of serum lipid metabolism indicators on fat-tailed sheep

To explore the dynamic changes and lipid metabolic differences between GLT and STH, five serum biochemical parameters involved in lipid metabolism were examined between two fat-tailed breeds at different development stages of 4, 6, 8, 10, 12 months. The results showed that the average of serum TC, and NEFA at five time points are obvious different, while other parameters have no significant difference between breeds. Interestingly, only serum NEFA in ewe is significantly higher than that in rams' values (Table S1).

The changes of these indicators with the age of month are relatively complicated (Fig. 2). In STH, serum TG concentration was the highest at 4 months of age, significantly higher than other months of age, and decreased with the increase of age; TC, NEFA, HDLC, and LDLC concentrations in serum did not change significantly with age between 4 and 10 months, but there was a tendency to decrease or increase (LDLC) at the age of 12 months. Comparatively, in GLT, TG did not change significantly between 4 and 12 months with a tendency to decrease at 12 months. TC, HDLC, and LDLC show the similar changes which firstly increase significantly and then decreases from 4 to 12 months of age. The concentration of NEFA was the highest at 4 months which was significantly higher than that at 6 and 8 months, and there was a tendency to increase at 10 months and decreased again at 12 months. Those dynamical changes indicate that the lipid metabolism pattern of STH and GLT maybe different with developmental ages.

The mRNA expression profile of SREBF1 in liver and fat tissues

To determinate the role of *SREBF1* in fat metabolism regulation of fat-tailed sheep, the relative mRNA expression profile of *SREEP1* in liver and adipose tissues in two breeds with different ages were detected by real-time RT-PCR (qPCR). The result showed that the global mRNA expression was significantly different between GLT and STH ($P <$

0.05, Fig. 3A), but no significant gender difference was found (Fig. 3B, Table S2). Merely, in female, the mRNA expresses in STH higher than those in GLT ($P < 0.05$, Fig. 3C). During development age, *SREBF1* expresses relatively stable in either male or female or combination in GLT (Fig. 3D and 3E). However, the total mRNA level was the highest at 10 months of age, and significantly higher than that at 8 months of age ($P < 0.05$, Table S2). In male STH, the expression at 10-month is significantly higher than that both at 6- and 8-month ($P < 0.01$, Fig. 3E).

We also examined *SREBF1* expression levels in liver and adipose tissues, which are involved in adipogenesis and lipid metabolism. The expression in liver was extremely higher than adipose tissues including tail (TA), great omental (GO), subcutaneous (SC), small omentum (SO), perirenal (PR), retroperitoneal (RP), and mesenteric (MT) fats in both GLT and STH (Fig. 3F, G). Similar results also were observed in males of GLT and STH. The difference is that the female of GLT have not presented a tissue-specific expression, except in MT. In STH females, the expression in MT was lower than that in liver significantly ($P < 0.05$).

Correlation analysis showed that the mRNA expressions between liver and Go were extremely significantly positively correlated in GLT (Table 1, $r = 0.854$, $P < 0.01$). In STH, TA and SC, PR and GO, PR and SO presented the positive correlation ($r = 0.852$, 0.915, or 0.979, $P < 0.05$). All results reveal that *SREBF1* maybe plays a crucial role in fat metabolism regulation during growth and development of two breeds of fat-tailed sheep.

Table 1
Correlation coefficients of *SREBF1* mRNA expression in adipose tissues between two fat-tailed sheep

Tissues	Tail fat (TA)	Great omental fat (GO)	Subcutaneous fat (SC)	Small omental fat (SO)	Perirenal fat (PR)	Retroperitoneal fat (RP)	Mesenteric fat (MT)	Liver (LV)
TA	1	-0.331	0.852*	-0.068	-0.346	0.348	0.516	0.340
GO	-0.160	1	-0.279	0.516	0.915*	-0.521	-0.363	0.665
SC	0.000	0.074	1	0.300	-0.039	0.416	0.322	0.075
SO	0.138	0.422	0.132	1	0.799*	-0.046	-0.149	0.653
PR	0.376	-0.256	0.117	0.297	1	-0.259	0.070	0.435
RP	0.165	0.315	0.306	0.021	0.301	1	0.314	-0.423
MT	-0.051	0.511	0.080	0.666	0.224	0.425	1	-0.382
LV	0.223	0.854**	0.121	0.718	-0.208	0.456	0.617	1

The mRNA expression of SREBF2 **in liver and fat tissues**

Transcriptional levels of *SREBF2*, another member of *SREBFs* family, in adipose tissues and liver were further examined and shown in Fig. 4. Different from *SREBF1, SREBF2* expressed significantly higher in GLT than in STH ($P < 0.001$, Fig. 4A) and significantly higher in female than in male ($P < 0.001$, Fig. 4B), especially in female of GLT ($P < 0.001$, Fig. 4C). The age had no significant effects on the expression in GLT (Fig. 4D). However, it expressed significantly higher at 10-month-old females than in males at the same age in GLT ($P < 0.05$). In STH, it reduced to the lowest point at 8-month-old in comparison to 4-month-old ($P < 0.05$) and then went up a little. The same changing tendency was observed in female STH ($P < 0.01$, Fig. 4E).

Similar to *SREBF1*, the expression of *SREBF2* was enriched in liver than other adipose tissues. In GLT, *SREBF2* mRNA expressions in liver were significantly higher than TA ($P < 0.05$), PR ($P < 0.05$) and MT ($P < 0.01$, Fig. 4F). In STH, *SREBF2* mRNA expressions in liver were markedly higher than all the adipose tissues except GO ($P < 0.001$ or $P < 0.0001$, Fig. 4G). According to the analysis of gender, *SREBF2* mRNA expressions in liver of male GLT were significantly higher than all adipose tissues. MT of GLT is the only tissue that *SREBF2* mRNA expression differed significantly between male and female ($P < 0.001$). In female GLT, the *SREBF2* mRNA levels was significantly higher in RP than in TA ($P < 0.01$), while there were no significant differences between any other tissues (Fig. 4H, Table S3). The correlation coefficients between SC and LV, MT and LV, PR and LV, PR and SC, PR and MT, SO and MT are significant(Table 2, $P < 0.05$ or $P < 0.01$).

Table 2
Correlation coefficients of *SREBF2* mRNA expression in adipose tissues between two fat-tailed sheep

Tissues	Tail fat (TA)	Great omental fat (GO)	Subcutaneous fat (SC)	Small omental fat (SO)	Perirenal fat (PR)	Retroperitoneal fat (RP)	Mesenteric fat (MT)	Liver (LV)
TA	1	-0.163	0.058	-0.462	0.089	-0.054	0.301	-0.236
GO	0.187	1	0.424	0.680*	0.779*	-0.147	-0.163	-0.328
SC	0.149	0.218	1	0.397	0.709*	-0.327	0.064	0.066
SO	-0.093	0.148	-0.003	1	0.762*	-0.089	-0.112	0.065
PR	0.026	-0.153	0.860**	0.052	1	-0.107	-0.102	-0.703
RP	0.475	0.043	-0.060	-0.182	-0.087	1	0.464	-0.344
MT	0.209	0.145	0.568	0.697*	0.666*	0.100	1	-0.276
LV	0.107	0.158	0.672*	0.222	0.829**	0.112	0.699*	1

In STH, expression of *SREBF2* was numerically but not significantly increased in liver of male STH, but the significantly expressions variant observed between adipose tissues, such as MT and TA, GO and RP, SC and PR (Fig. 4I). In female STH, liver showed higher expression levels than any other adipose tissues, and *SREBF2* expression in MT was the lowest compared to liver and GO ($P < 0.05$). Further analysis results showed that the significantly positive correlation of *SREBF2* expressions occurred in between GO and SO, PR and GO, PR and SC, PR and SO (Table 2, $P < 0.05$). These results indicated that *SREBF2* also played a crucial role in the regulation of lipid metabolism during growth and development of the two breeds of fat-tailed sheep.

Associations between SREBP1/2 **expressions and slaughter and tail traits**

Associations between *SREBF1/2* mRNA expression in eight tissues and slaughter and tail traits in sheep were analyzed. The results demonstrated that *SREBF1* mRNA expressions in TA were significantly related to tail-type traits in GLT, such as absolute tail fat weight (ATW) and relative tail fat weight (RTW). The significant correlation also were found between LV and tail length (TL), LV and RTW, GO and TL, respectively. While in STH, significant correlation only occurred between *SREBF1* expressions in PR and body weight (BW), as well as carcass weight (CW), which was not related to tail-type traits (Table 3).

Table 3
Correlation coefficients between *SREBF1* mRNA expression in different tissues and slaughter and tail traits in sheep

Traits	Relative mRNA abundance							
	Tail fat (TA)	Great omental fat (GO)	Subcutaneous fat (SC)	Small omental fat (SO)	Perirenal fat (PR)	Retroperitoneal fat (RP)	Mesenteric fat (MT)	Liver (LV)
Guangling Large Tailed sheep(GLT)								
TL	-0.031	0.668*	0.048	0.451	-0.123	0.064	0.467	0.673*
TW	0.408	0.255	0.427	0.007	-0.017	0.335	0.285	0.432
ATW	0.651*	0.213	0.550	0.237	0.293	0.311	0.265	0.433
RTW	0.652*	0.454	0.351	0.451	0.250	0.515	0.598	0.680*
BW	0.096	-0.298	0.276	-0.079	0.111	-0.089	-0.157	-0.123
CW	0.260	-0.217	0.431	-0.160	0.114	-0.193	-0.266	-0.100
DP	0.387	-0.021	0.506	-0.192	0.139	-0.275	-0.425	-0.008
Small Tailed Han sheep(STH)								
TL	0.704	-0.335	0.445	-0.348	-0.218	0.302	0.305	-0.212
TW	0.379	0.100	0.230	0.021	0.207	0.026	-0.196	0.232
BW	0.047	0.418	0.040	0.119	0.711*	-0.169	0.135	0.637
CW	-0.005	0.376	-0.010	0.022	0.728*	-0.283	0.145	0.571
DP	-0.358	-0.446	-0.410	-0.602	-0.147	-0.629	0.018	-0.423

Note: TL: tail length; TW: tail width; ATW: absolute tail fat weight; RTW: relative tail fat weight; BW: body weight; CW: carcass weight; DP: dressing percentage. *$P < 0.05$, ** $P < 0.01$.

Relative *SREBF2* mRNA abundances in liver were significantly related negatively to tail-type traits including TL, TW, and ATW in GLT. There were also significant negatively relationships between SC and TW, as well as between GO and ATW or CW. While the only significant positively correlation to CW was *SREBF2* mRNA abundances in SC of STH (Table 4).

Table 4
Correlation coefficients between *SREBF2* mRNA expression in different tissues and slaughter and tail traits in sheep

Traits	Relative mRNA abundance							
	Tail fat (TA)	Great omental fat (GO)	Subcutaneous fat (SC)	Small omental fat (SO)	Perirenal fat (PR)	Retroperitoneal fat (RP)	Mesenteric fat (MT)	Liver (LV)
Guangling Large Tailed sheep								
TL	-0.100	0.029	-0.517	-0.006	-0.452	-0.359	-0.288	-0.694*
TW	-0.160	-0.498	-0.607*	-0.111	-0.534	-0.187	-0.266	-0.768*
ATW	-0.339	-0.611*	-0.441	-0.307	-0.253	-0.213	-0.388	-0.720*
RTW	-0.491	-0.362	-0.171	-0.239	-0.072	-0.021	-0.206	-0.549
BW	0.081	-0.459	-0.557	-0.309	-0.245	-0.215	-0.334	-0.302
CW	0.067	-0.593*	-0.507	-0.236	-0.218	-0.297	-0.429	-0.495
DP	-0.026	-0.571	-0.302	-0.391	-0.178	-0.427	-0.412	-0.588
Small Tailed Han sheep								
TL	0.484	0.486	0.295	-0.074	0.627	-0.094	0.040	-0.204
TW	0.185	0.278	0.366	0.042	0.549	0.082	0.366	0.101
BW	-0.439	0.268	0.575	0.530	0.158	-0.425	-0.053	0.473
CW	-0.385	0.234	0.663*	0.528	0.237	-0.424	-0.042	0.475
DP	0.361	-0.266	0.302	-0.136	0.080	0.214	0.070	-0.123

Note: TL: tail length; TW: tail width; ATW: absolute tail fat weight; RTW: relative tail fat weight; BW: body weight; CW: carcass weight; DP: dressing percentage. *$P < 0.05$, ** $P < 0.01$

Location and function prediction of SREBP1/2

According to SREBP1/2 expression levels in different tissues, age, and breeds, the location and function of SREBP1/2 in cell were analyzed by bioinformatics approaches. The subcellular locations results showed that SREBP1 and SREBP2 working mainly in different parts inside cells (Fig. 5A). About 78.30% of SREBP1 distributed in nucleus, and the rest in cytoplasm, vesicles of secretory system, plasma membrane, and endoplasmic reticulum. Unlike in SREBP1, only 30.40% of SREBP2 can be predicted in nucleus and 39.1% in the endoplasmic reticulum. In addition, it also distributed in vacuole, cytoplasm, and vesicles of secretory system, Golgi and mitochondria.

Functional prediction shows that both SREBP1 and SREBP2 play roles in many biological processes (Fig. 5B). SREBP1 mainly functioned in purines and pyrimidines, replication and transcription, and regulatory function, while SREBP2 mainly functioned in the process of transport and binding, purines and pyrimidines, translation and central intermediary metabolism.

Discussion

The fat-tailed sheep is raised largely in many countries due to its high adaptability to nutritionally challenging environments and disease resistance as well as fat deposition in tails.[4,15,16] The tail fat plays a crucial role during growth and development of different breed sheep.[17] Therefore, as two typical fat-tailed breeds, Guangling Large Tailed (GLT) and Small Tailed Han (STH) sheep in China were selected in this study. GLT is typically of large tails for fat deposit and good meat quality but low fecundity, and STH is characterized by small tails but higher fecundity in the plain regions. Our study firstly revealed the dynamical changes of serum lipid metabolism indicators, and the expression patterns of key genes *SREBF1* and *SREBF2* in liver and adipose tissues of GLT and STH.

Mutton is becoming attractive and alternative meats due to the low percentage of fat and good source of essential fatty acids.[5,18] It is of great importance to improve meat production traits such as live weight and carcass weight in sheep breeding.[19] In general, live and carcass weights have the same variation tendencies.[20] In this study, in spite of obvious difference in live weight between the two breeds, there was no significant difference in carcass weight. If GLT is to achieve a carcass weight similar to that of STH, it needs to be much heavier. In other words, GLT may need to consume more feed and forage than STH with comparable carcass weight. However, the feed and forage is not only converted into meat but also into tail fat, which can be derived from the result that GLT with higher live weight also has larger fat tail than STH. It is obviously not economical in sheep production.[3,21] Therefore, regulating tail fat deposition is a beneficial attempt to improve meat production performance and economic benefits.

Serum biochemical parameters are the crucial indicators for lipid metabolism which is closely correlative with production property and physiological process maintaining nutrient adjustment, homeostasis, and sheep healthy.[6–8, 22] For example, triglyceride (TG) is a fat molecule formed by the condensation of long-chain fatty acids and glycerol. Most tissues can be powered by ATP produced by the oxidative decomposition of fat.[23,24] High density lipoprotein cholesterol (HDLC) and low density lipoprotein cholesterol (LDLC) are important part of total cholesterol (TC) which affects normal lipid metabolism and resulting in metabolic disorders.[25,26] As a hydrolyzed product of triglycerides, non-esterified fatty acid (NEFA) is stored in adipose tissue and also a source of animal energy.[27] In the present study, serum indicators, especially TC and NEFA showed obvious differences and dynamical changes with the different development ages, gender factors only had a significant effect on serum NEFA concentrations. Studies have shown that TC content is positively correlated with sebum rate and liver fat rate.[28] The significantly increased serum NEFA concentration in starved cows was reported by previous study.[29] So those results implied that the fat metabolism regulation pattern in STH and GLT may be distinguishing which provide the phenotypic changes basis for revealing the genetic mechanism leading to the difference of ovine tail type.

Lipid metabolism and deposition is composed of many enzymatic reactions and often are regulated by many molecules.[9,30] In our previous studies, the roles of Lpin2/3, angiopoietin-like protein 4, miR-124-3p, and other genes and non-coding RNAs in the regulation of fat deposition from two fat-tailed sheep breeds had been discussed.[31–34] Therefore, this study mainly focuses on the crucial role of sterol regulatory element binding proteins (SREBPs) in fat-tailed sheep base on the dynamical changes of serum lipid metabolism indicators. SREBPs including SREBP-1a, SREBP-1c and SREBP2 are mainly responsible for regulating cellular lipogenesis and lipid homeostasis.[35] SREBP-1a and SREBP-1c are encoded by *SREBF1*, which involves in the synthesis of fatty acid and triglyceride, while SREBP2 is encoded by *SREBF2*, which mostly regulates the cholesterol gene expression.[36] In the present study, both *SREBF1* and *SREBF2* gene mRNA expression in sheep were significantly affected by breed, tissue, and age factors. The expression of *SREBF1* gene in STH is significantly higher than that in GLT, but *SREBF2* gene expression is opposite. Merely the expression of *SREBF1* and *SREBF2* genes in sheep showed a significant positive correlation (r = 0.286). The mRNA

expression profiling of *SREBF1* and *SREBF2* are different in the two sheep breeds, which are coincident with previous studies in other fat-tailed sheep.[37,38]

Another study indicates that there is a genetic basis for the phenotypic differences between fat-tailed and thin-tailed sheep.[39] In the comparative study of the lung cells, the expression levels of *SREBFs* on the first day of birth was significantly higher than that on the first 17.5 days of embryonic development.[40] In this study, both *SREBF1* and *SREBF2* expressions in liver and the different fat tissues at the ages of 4, 6, 8, 10 and 12 month are various. For instance, the expression of *SREBF1* in liver is extremely higher than that in seven adipose tissues, but the difference in *SREBF1* gene expression between different genders is not significant; the interaction of two factors between breed and month age, breed and tissue, and tissue and gender also significantly affects its expression. In contrast, the role of *SREBF2* gene is not exactly the same. The expression of *SREBF2* in the liver is also significantly higher than adipose tissue; the expression of female is significantly higher than that of male. Interesting, the expression of *SREBF1* gene has spatio-temporal differences, while the expression of *SREBF2* gene does not have this characteristic, but it has different roles in different genders. Those results further provide the new evidence for revealing the genetic mechanism leading to the difference of sheep tail type.

In addition, SREBPs are transcriptionally inactive when sterols are abundant, and synthesized and inserted into the endoplasmic reticulum (ER) as large precursor proteins.[41,42] The depletion of sterol can promote the release of mature SREBPs proteins and are transported from ER to the Golgi.[43] The reduction of fat mass, hepatic and plasma triglycerides was observed in SREBP1c-specific knockout mice.[44] In the present study, the results from subcellular locations and function prediction imply that SREBP1 and SREBP2 expressions are closely related with metabolism processes in cells. The differences in the expression of *SREBFs* in sheep between different breeds (different fat tail types) may be a cause of the difference in tail types between the two sheep breeds. The data from the associations between *SREBF1/2* expressions and slaughter and tail traits also further confirmed this fact. The role of transcription factor genes Srebf1 and Srebf2 in the phenotype of fat deposition and the relationship with metabolites were shown in Fig. 6.

Conclusions

Taken together, our study reveals that the serum concentrations of TC and NEFA in sheep have significant differences between breeds, and mostly indicators showed the dynamical changes with the different development ages. Importantly, the mRNA expression profiling of *SREBF1* and *SREBF2* are different in two sheep breeds, gender, and adipose tissues, showing a breed-specific, gender-specific, and temporal and spatial expressions differences. All results implied that *SREBF1/2* play a crucial role in lipid metabolism regulation during growth and development of two fat-tailed sheep, correspondingly, lipid metabolism regulation pattern in STH and GLT maybe distinguishing. This study provides a phenotypic basis for further revealing the genetic mechanism in lipid metabolism and fat deposition that causes differences in ovine tail types, which also provides a novel insight for improving quality of meat.

List Of Abbreviations

ATW Absolute tail fat weight

BH Body height

BL Body length

CC Cannon circumference

CW carcass weight

DP Dressing percentage

ER endoplasmic reticulum

GLT Guangling Large Tailed sheep

GO Great omental fat

HDLC High density lipoprotein cholestero

HG Heart girth

LDLC Low density lipoprotein cholestero

LV Liver

LW Live weight

MT Mesenteric fat

NEFA Non-esterified fatty acid

PR Perirenal fat

RP Retroperitoneal fat

RTW Relative tail fat weight

SC Subcutaneous fat

SO Small omentum fat

SREBF1 Sterol regulatory element binding factor 1

SREBF2 Sterol regulatory element binding factor 2

SREBP1 Sterol regulatory element binding protein 1

SREBP2 Sterol regulatory element binding protein 2

STH Small Tailed Han sheep

TA Tail fat

TC Total cholesterol

TG Triglyceride

TL Tail length

TW Tail width

Declarations

Ethics approval and consent to participate

The feeding, management and slaughtering of this study were conducted according to the National (GB 13078-2001 and GB/T 17237-1998) and the Agricultural Standards (NY 5148-2002-NY 5151–2002) of the People's Republic of China. This study was carried out under the guidelines for the care and use of animals for scientific purposes. The protocol was approved by the Institutional Animal Care and Use Ethics Committee of Shanxi Agricultural University.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Chen Liang detected the gene expression levels, analyzed the data, and was a major contributor in writing the manuscript. Liying Qiao and Jianhua Liu collected the tissue samples and the phenotypic data. Yongli Han and Jianhai Zhang detected the serum indicators and analyzed the data. Wenzhong Liu made a lot of helpful suggestions for this study and revised the manuscript.

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Figures

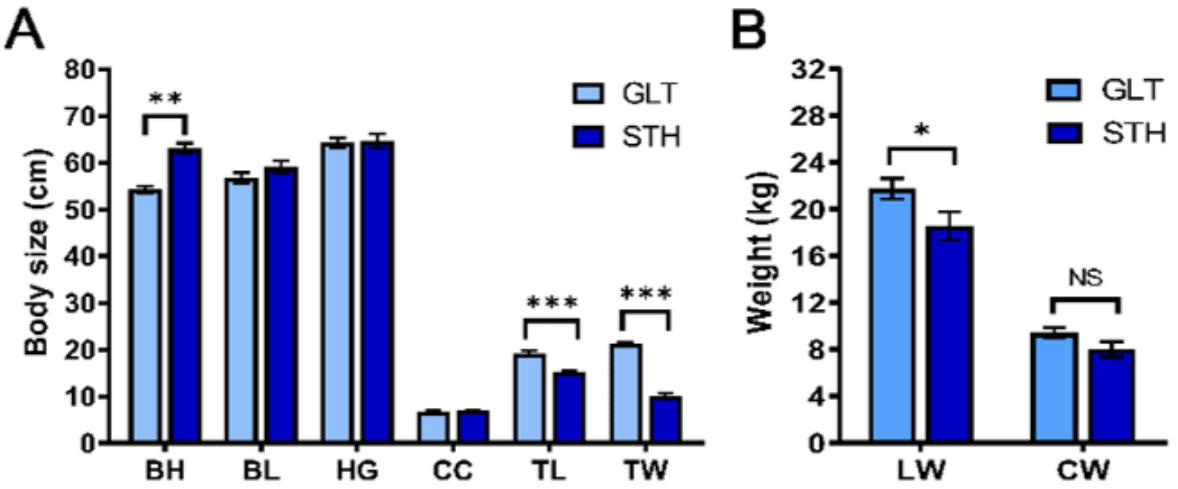

Figure 1

Characters of body size and weight in two Chinese fat-tailed sheep breed. GLT and STH indicate Guangling Large Tailed sheep and Small Tailed Han sheep respectively. (A) Comparison of body size parameters including body height (BH), body length (BL), heart girth (HG), cannon circumference (CC), tail length (TL) and width (TW) in GLT and STH, ** P<0.01; *** P<0.001. (B) Difference in live weight (LW) and carcass weight (CW) of GLH and STH. *P<0.05, NS: No significant difference.

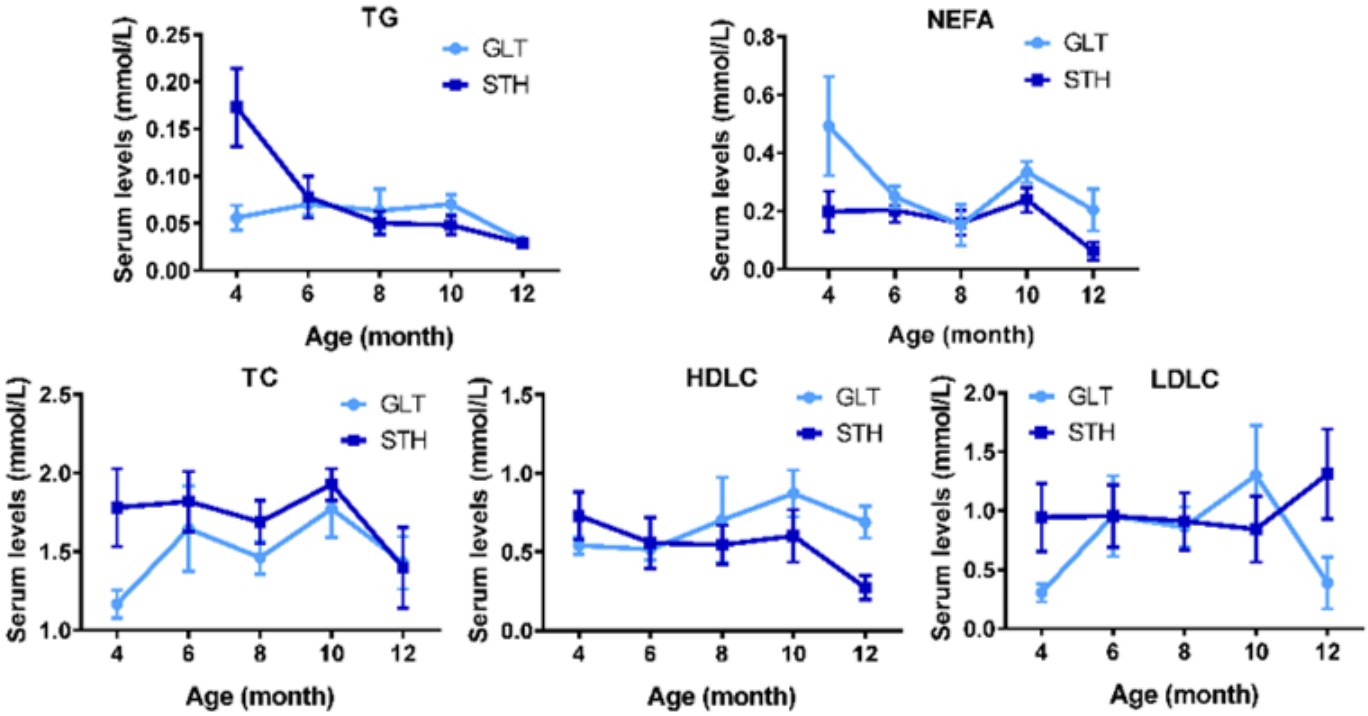

Figure 2

Dynamical changes in nine serum lipid metabolism indicators of fat-tailed sheep with age. GLT and STH indicate Guangling Large Tailed sheep and Small Tailed Han sheep respectively. TG: triglyceride; TC: total cholesterol; NEFA: non-esterified fatty acid; HDLC: high density lipoprotein cholesterol; LDLC: low density lipoprotein cholesterol.

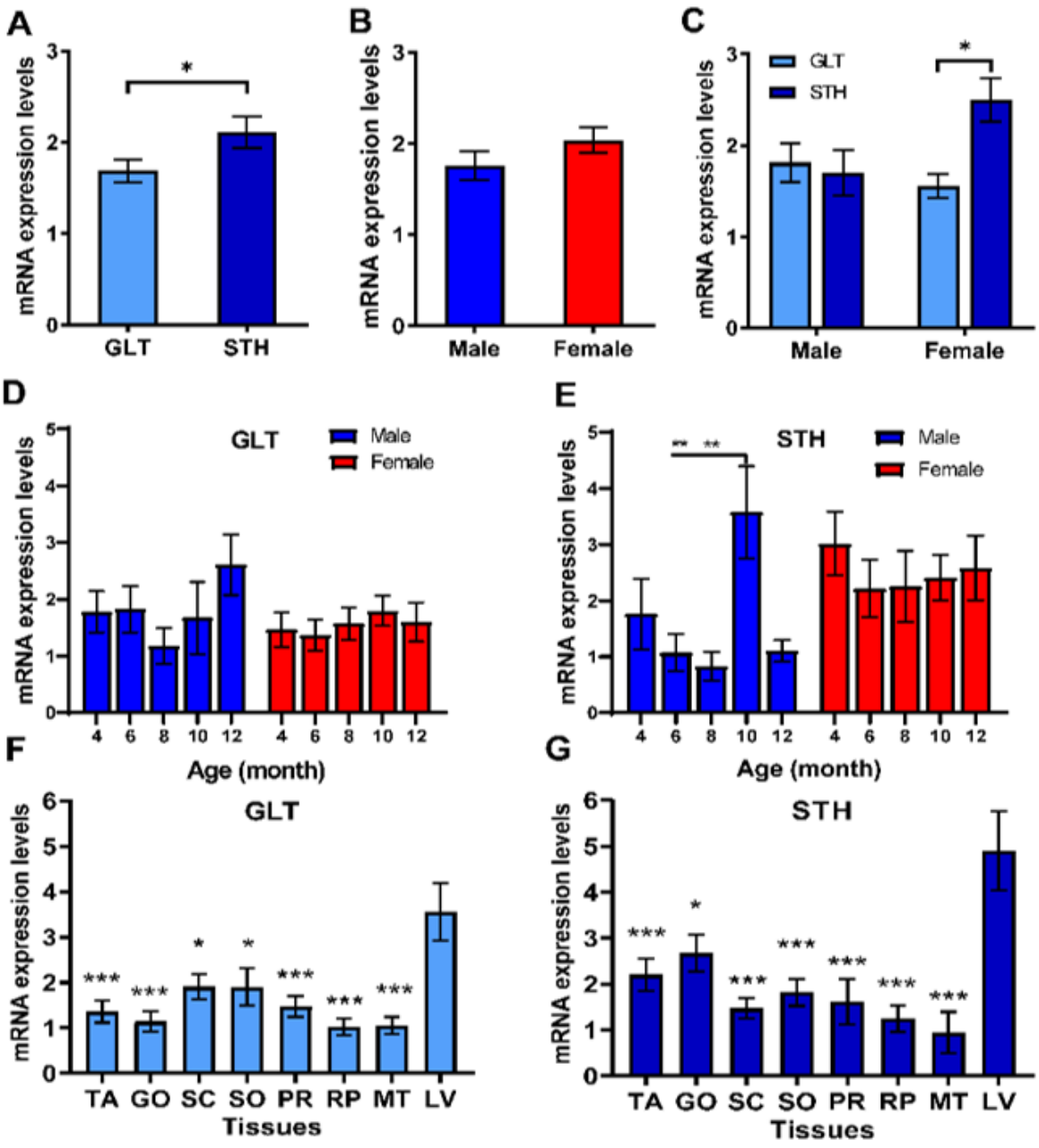

Figure 3

The mRNA expression profiles of SREBF1 in adipose tissues in two fat-tailed sheep with age by qRT-PCR. (A-C) The comparisons of global mRNA expression level of SREBF1 in all tissues between Guangling Large Tailed sheep (GLT) and Small Tailed Han sheep (STH) in terms of breed and gender. * P<0.05 indicates the significant differences between GLT and STH. (D-E) The mean expression levels of SREBF1 in tissues of GLT and STH at the different ages of 4, 6, 8, 10, 12 month, respectively. * P<0.05, ** P<0.01 indicate the significant differences among ages. (F-G) Expression abundances of SREBF1 in tail fat (TA), great omental fat (GO), subcutaneous fat (SC), small omentum fat (SO), perirenal fat (PR), retroperitoneal fat (RP), mesenteric fat (MT) and liver (LV) of GLT and STH. * P<0.05, ** P<0.01 or *** P<0.001 indicate the significant differences compared with liver tissue.

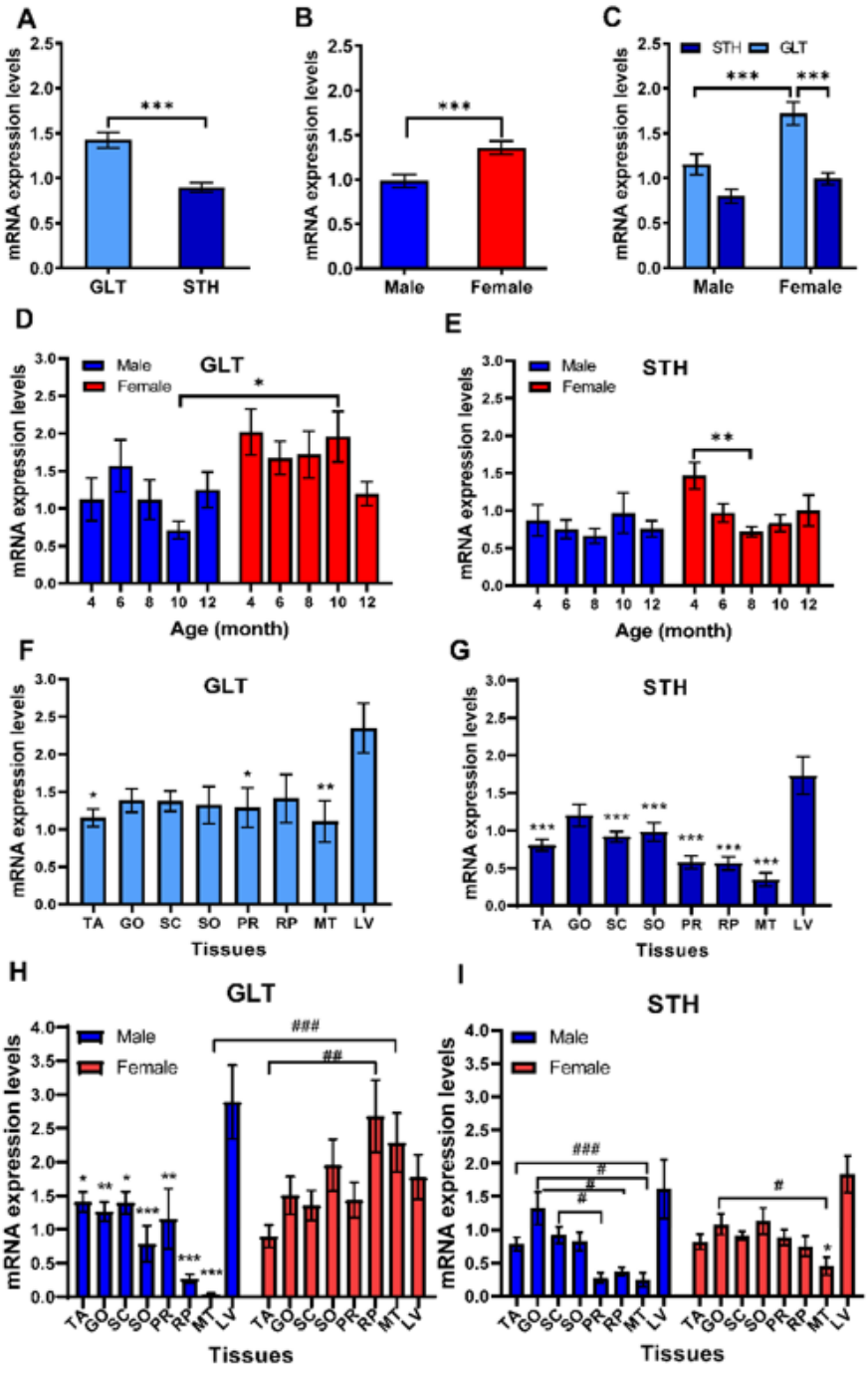

Figure 4

The mRNA expression patterns of SREBF2 in adipose tissues in two fat-tailed sheep with ages by qRT-PCR. (A-C) The comparisons of global mRNA expression level of SREBF2 in all tissues between Guangling Large Tailed (GLT) and Small Tailed Han (STH) sheep in terms of breed and gender. * P<0.05 indicates the significant differences between GLT and STH. (D-E) The mean expression levels of SREBF2 in tissues of GLT and STH at the different ages of 4, 6, 8, 10, 12 month, respectively. * P<0.05, ** P<0.01 indicate the significant differences among ages. (F-I) Expression abundances of SREBF2 in tail fat (TA), great omental fat (GO), subcutaneous fat (SC), small omentum fat (SO), perirenal fat (PR), retroperitoneal fat (RP), mesenteric fat (MT) and liver (LV) of male and female sheep. * P<0.05, ** P<0.01 or *** P<0.001 indicate the significant differences compared to the liver. # P<0.05, ## P<0.01, ### P<0.001 show the differences among adipose tissues.

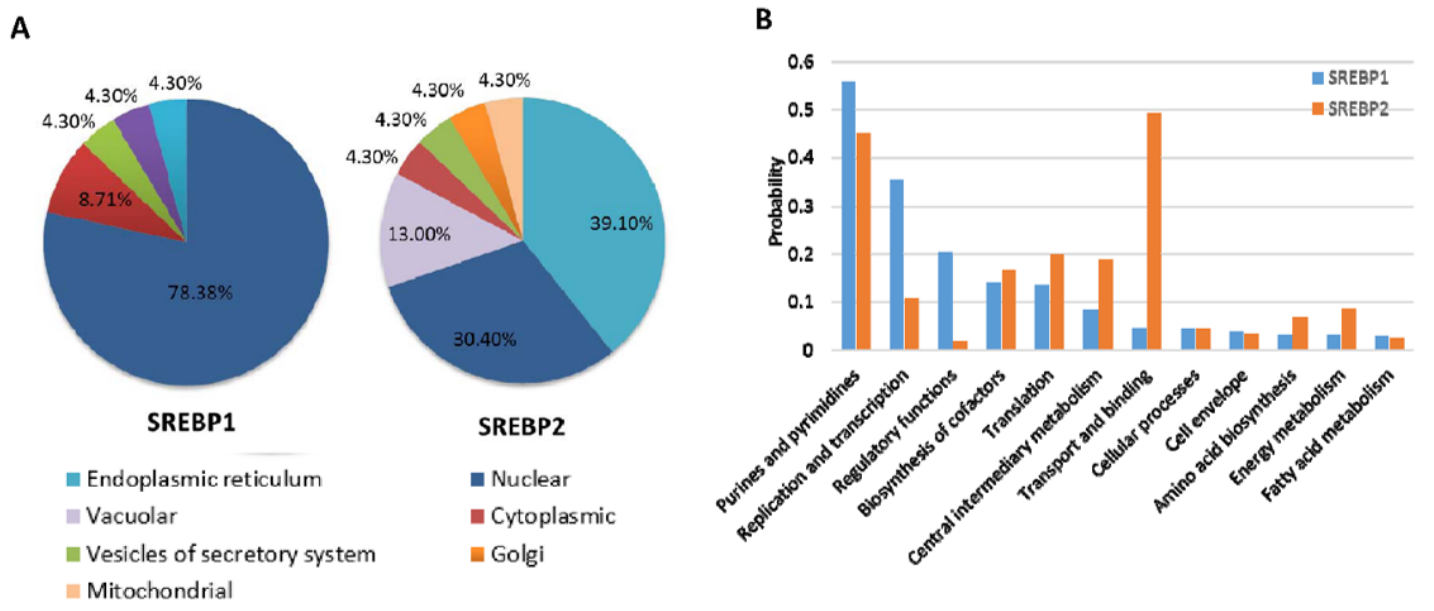

Figure 5

The subcellular location and predicted function of SREBP1/2 in sheep. (A) Subcellular localization of ovine SREBP1 and SREBP2 by contrastive analysis. (B) Prediction of functions of SREBP1 and SREBP2 in ovine.

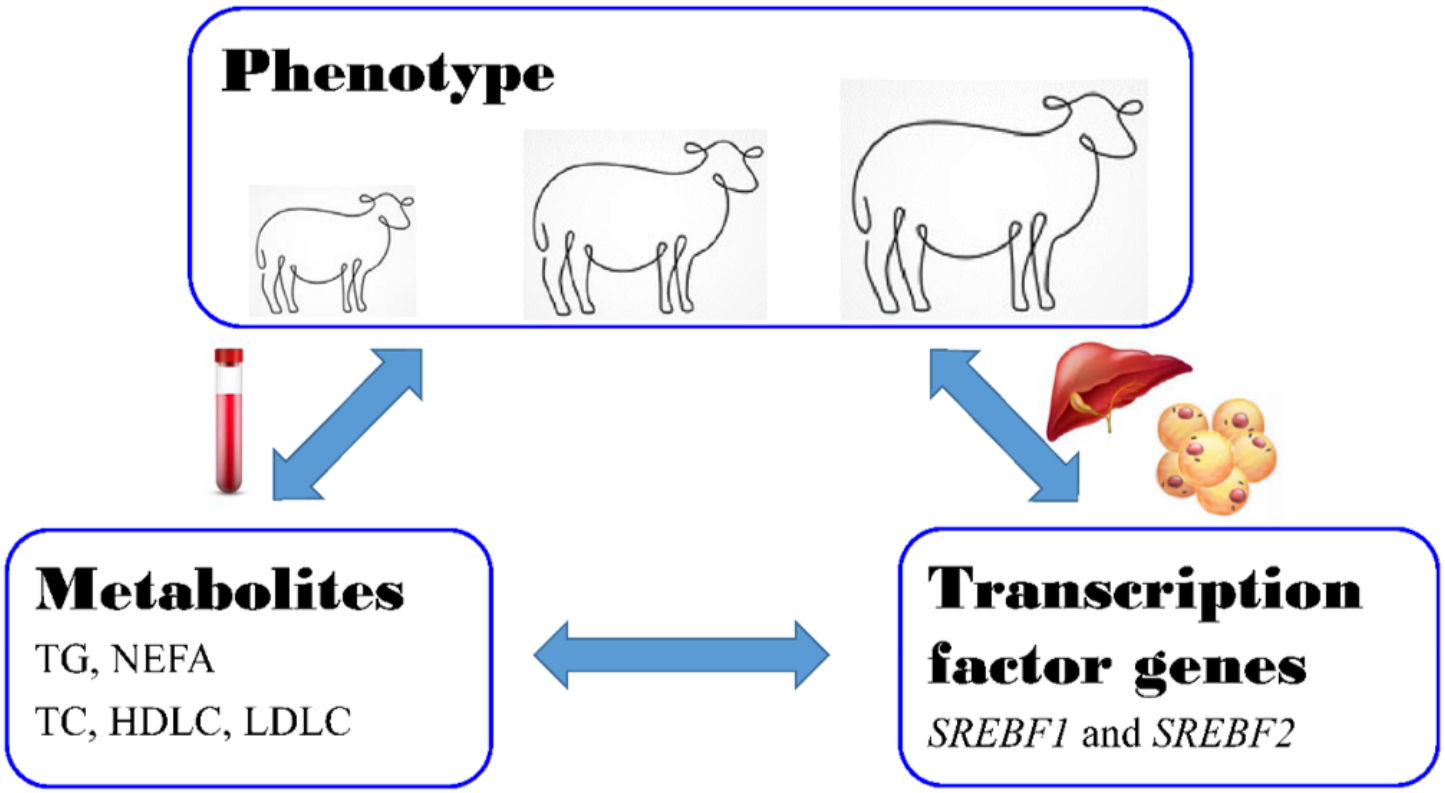

Figure 6

The main scheme of this study. The role of transcription factor genes Srebf1 and Srebf2 in the phenotype of fat deposition and the relationship with metabolites were explored in this study.

Supplementary Files

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