Electronic Journal of Biotechnology 18 (2015) 215–220

HOSTED BY

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

mRNA transcription and protein expression of PPARγ, FAS, and HSL in different parts of the carcass between fat-tailed and thin-tailed sheep

Xiaochun Xu [a], Xuan Wei [a], Yuxin Yang [a], Wenzhi Niu [b], Qifang Kou [b], Xiaolong Wang [a], Yulin Chen [a,*]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling, People's Republic of China
[b] Tianyuan Agriculture Science and Technology Development Limited Company, Hong Sipu, Ning Xia, People's Republic of China

ARTICLE INFO

Article history:
Received 9 November 2014
Accepted 21 January 2015
Available online 2 April 2015

Keywords:
Genes
Difference
Tissues
Ovis

ABSTRACT

Background: The objective of this study was to compare the level differences of mRNA transcription and protein expression of PPARγ, FAS and HSL in different parts of the carcass in different tail-type sheep. Six Tan sheep and six Shaanbei fine-wool sheep aged 9 months were slaughtered and samples were collected from the tail adipose, subcutaneous adipose, and longissimus dorsi muscle. The levels of mRNA transcription and protein expression of the target genes in these tissues were determined by real-time quantitative PCR and western blot analyses.
Results: The results showed that PPARγ, FAS, and HSL were expressed with spatial differences in tail adipose, subcutaneous adipose and longissimus dorsi muscle of Tan sheep and Shaanbei fine-wool sheep. Differences were also observed between the two breeds. The mRNA transcription levels of these genes were somewhat consistent with their protein expression levels.
Conclusion: The present results indicated that PPARγ, FAS and HSL are correlated with fat deposition, especially for the regulating of adipose deposition in intramuscular fat, and that the mRNA expression patterns are similar to the protein expression patterns. The mechanism requires clarification in further studies.

1. Introduction

Fatty deposits have been attracting increasing attention in recent years [1,2,3,4]. Fat deposition is closely correlated with the relative protein expression levels of peroxisome proliferator-activated receptor (PPARγ), fatty acid synthase (FAS), and hormone-sensitive lipase (HSL), which are the most important transcription factors and key enzymes during adipose deposition [5,6,7]. PPARγ, a member of nuclear receptor family, is considered to be the main regulator of adipogenesis and is expressed in adipose tissue at a high level [8]. FAS is a key enzyme in fatty acid synthesis [9] and catalyzes acetyl coenzyme A, malonyl coenzyme A and nicotinamide adenine dinucleotide 2′-phosphate to synthesize fatty acids [10,11]. HSL is the rate-limiting enzyme in initiating triglyceride polymerization to form fat and influences the adipose deposition rate in mammalian tissue [12]. The fact that a knockout of HSL can significantly decrease the rates of fat hydrolysis, lipid synthesis, and adipose metabolism, suggests that HSL plays an important role in these processes [13].

The deposition efficiency of tail fat in fat-tailed sheep such as Tan sheep is higher than that in other parts of the carcass, such as subcutaneous adipose and longissimus dorsi muscle. Meanwhile, in thin-tailed sheep such as Shaanbei fine-wool sheep, the deposition efficiency of tail fat is far lower than that in other parts of the carcass. Therefore, there are likely to be remarkable differences in fat metabolism among different parts within the same breed. In addition, adipose tissue is likely to have biological effects on the "part deposition" in metabolic processes and lead to biodiversity in the animal body. Thus, to compare the distribution differences in the carcass between Tan sheep and Shaanbei fine-wool sheep is significant for theoretical research and practical applications. Many previous studies have paid attention to subcutaneous adipose [14], intramuscular adipose [15], and visceral adipose [16], while studies about tail adipose especially comparative studies of tail adipose, subcutaneous adipose and longissimus dorsi muscle, between fat-tailed and thin-tailed sheep are rare.

In this study, the levels of mRNA transcription and protein expression of PPARγ, FAS and HSL in different parts of carcass between fat-tailed Tan sheep and thin-tailed Shaanbei fine-wool sheep were determined by real-time quantitative PCR and Western blot analyses. In addition, the differences among these levels were

* Corresponding author.
E-mail address: chenyulin@nwafu.edu.cn (Y. Chen).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.03.010

compared to provide experimental data for the "part deposition" in sheep for further theoretical research.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade and of the highest purity commercially available. A PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) and SYBR Premix Ex TaqTM II were purchased from Takara Biotechnology (Dalian, China). ProteoJETTM Mammalian Cell Lysis Reagent was purchased from Fermentas Scientific Molecular Biology Corporation (Fermentas, EU). A Western Lightning ECL Kit was purchased from Perkin Elmer Corporation (Foster City, CA).

2.2. Animal treatment

Six Tan sheep from Ningxia Tianyuan Agriculture Science and Technology Development Limited Company and six Shaanbei fine-wool sheep from Shaanxi Dingbian breeding farm aged 9 months were used in this study. The animals were slaughtered according to the National Standard of China (GB 13078-2001 and GB/T 17237-1998) and Agriculture Standards of China (NY 5148-2002-NY 5151-2002). As soon as possible after slaughter, approximately 300 mg samples from tail adipose, subcutaneous adipose and longissimus dorsi muscle were extracted, packed with foil paper, placed in liquid nitrogen, and stored at -80°C until further use.

2.3. Design and synthesis of primers

Using Primer 5.0 software, primers for real-time PCR were designed based on the mRNA sequences of the target genes, PPARγ, FAS and HSL, published in NCBI (GenBank), and the β-actin gene as an internal reference. The primers were synthesized at Sangong Biotech (Shanghai, China). The sequences of the primers, annealing temperatures and the lengths of the PCR products are shown in Table 1.

2.4. Extraction and reverse transcription of RNA

Total RNA was extracted from the tail adipose, subcutaneous adipose, and longissimus dorsi muscle samples, using Trizol (TaKaRa, Tokyo, Japan), and the concentration and purity of the extracted total RNA were determined with a Maestro Nanomicro-spectrophotometer (MaestroGEN, Las Vegas, NV). Reverse transcription of the total RNA was carried out using the PrimeScript RT Reagent Kit and the products were stored at -20°C until further analysis.

2.5. Quantitative PCR

Real-time PCR was performed in a 25-ml reaction system by using SYBR Premix Ex TaqTM II. The PCR cycling conditions were 95°C for 30 s followed by 50 cycles of 95°C for 5 s, 60°C for 34 s and 72°C for 30 s. A melting curve analysis was performed at 95°C for 10 s and 60°C for 1 min, followed by a decrease in the temperature from 60°C to 95°C at a rate of 0.5°C/10 s.

2.6. Protein extraction

Total protein was isolated from the frozen tissues using ProteoJET™ Mammalian Cell Lysis Reagent which was added phenylmethanesulfonyl fluoride (PMSF) at 1:100 before use. The protein quantity was determined with the Maestro Nanomicro-spectrophotometer. The protein products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.7. Western blot analysis

Protein samples (80 μg protein) were separated by SDS-PAGE in a 12% gel using a voltage of 80 V, and then transferred to a polyvinylidene fluoride (PVDF) membrane by electroblotting. Western blotting was performed as follows: The transferred membrane was blocked with 5% bovine albumin blocking agent (BSA) for 2 h, followed by incubation with primary antibodies against PPARγ (MB0080; Bioworld Technology, Beijing, China), FAS (ab22759; Abcam, Hong Kong, China), HSL (sc-25843; Santa Cruz Biotechnology, Beijing, China) and GAPDH (bsm-0978M; Biosynthesis Biotechnology, Beijing, China) for 2 h at room temperature. The membrane was then incubated with appropriate secondary antibodies for PPARγ (CW0102; CWBIOTECH, Beijing, China), FAS (CW0105; CWBIOTECH, Beijing, China), HSL (CW0103; CWBIOTECH, Beijing, China), and GAPDH (CW0102; CWBIOTECH, Beijing, China) for 2 h at room temperature. After washing with phosphate-buffered saline (PBS), the membranes were processed for chemical luminescence with enhanced chemiluminiscent (ECL; Amersham, USA) for 3 min followed by a 1-min exposure to X-ray film. The film was developed and fixed.

2.8. Statistical analyses

The experiments were repeated three times, and the mean ± standard deviation was evaluated. Data were analyzed using SPSS software (version 10.1.0, SPSS Science, USA), and values of $P < 0.05$ were considered to indicate statistical significance. The relative expression amounts of the target genes were determined by the $2^{-\Delta\Delta CT}$ method [17,18]. Immunoblotting was analyzed by the optical density values determined by Image J software (Toronto Western Research Institute University Health Network).

Table 1
Primer sequences used for RT-PCR.

Gene	GenBank accession number	Oligos sequences	Product size (bp)	Tm (°C)
PPARγ	NM001100921.1	F: 5′-ACGGGAAAGACGACAGACAAA-3′ R: 5′-AAACTGACACCCCTGGAAGATG-3′	150	62
FAS	NM001012669.1	F: 5′-CCCAGCAGCATTATCCAGTGT-3′ R: 5′-ATTCATCCGCCATCCAGTTC-3′	87	62
HSL	NM001128154.1	F: 5′-CTTTCGCACCAGCCACAAC-3′ R: 5′-CTCGTCGCCCTCAAAGAAGA-3′	136	62
β-Actin	NM001009784.1	F: 5′-TGAACCCCAAAGCCAACC-3′ R: 5′-AGAGGCGTACAGGGACAGCA-3′	107	61

F. Forward primers; R. Reverse primers.

3. Results

3.1. Comparison of the mRNA transcription levels (MRL) of PPARγ, FAS, and HSL from fat-tailed and thin-tailed sheep

The MRL data are shown in Fig. 1 for the PPARγ gene, and the MRL differences among the tissues from either Tan sheep or Shaanbei fine-wool sheep were significant ($P < 0.05$). For Tan sheep, PPARγ MRL in tail adipose was 30.52% higher than that in longissimus dorsi muscle ($P < 0.05$), PPARγ MRL in longissimus dorsi muscle was 89.92% higher than that in subcutaneous adipose ($P < 0.05$), and PPARγ MRL in tail adipose was 1.48 times higher than that in subcutaneous adipose. For Shaanbei fine-wool sheep, PPARγ MRL in subcutaneous adipose was 79.44% higher than that in tail adipose ($P < 0.05$), and PPARγ MRL in tail adipose was 2.25 times higher than that in longissimus dorsi muscle.

For the FAS gene, the MRL differences among the tissues from either Tan sheep or Shaanbei fine-wool sheep were significant ($P < 0.05$). For Tan sheep, FAS MRL in tail fat was 54.01% higher than that in longissimus dorsi muscle ($P < 0.05$), FAS MRL in longissimus dorsi muscle was 8.76 times higher than that in subcutaneous adipose ($P < 0.05$), and FAS MRL in tail adipose was 14.04 times higher than that in subcutaneous adipose ($P < 0.05$). For Shaanbei fine-wool sheep, FAS MRL in subcutaneous adipose was 1.58 times higher than that in tail adipose ($P < 0.05$), and FAS MRL in tail adipose was 4.4 times higher than that in longissimus dorsi muscle ($P < 0.05$).

For the HSL gene, the MRL differences among these tissues from either Tan sheep or Shaanbei fine-wool sheep were significant ($P < 0.05$). For Tan sheep, HSL MRL in subcutaneous adipose was 41.07% higher than that in tail adipose ($P < 0.05$), and HSL MRL in tail adipose was 6.39 times higher than that in longissimus dorsi muscle ($P < 0.05$). For Shaanbei fine-wool sheep, HSL MRL in tail adipose was 33.15% higher than that in subcutaneous adipose ($P < 0.05$), HSL MRL in subcutaneous adipose was 2.11 times higher than that in longissimus dorsi muscle ($P < 0.05$), and HSL MRL in tail adipose was 3.14 times higher than that in longissimus dorsi muscle ($P < 0.05$).

3.2. Comparison of protein expression levels (PRL) of PPARγ, FAS, and HSL from fat-tailed and thin-tailed sheep

The PRL data are shown in Fig. 2. For PPARγ, the PRL differences among the tissues from either Tan sheep or Shaanbei fine-wool sheep were significant ($P < 0.05$). For Tan sheep, PPARγ PRL in tail adipose was 93.57% higher than that in longissimus dorsi muscle ($P < 0.05$), PPARγ PRL in longissimus dorsi muscle was 6.46% higher than that in subcutaneous adipose ($P < 0.05$), and PPARγ PRL in tail adipose was 1.06 times than that in subcutaneous adipose. For Shaanbei fine-wool sheep, PPARγ PRL in subcutaneous adipose was 1.75 times higher than that in tail adipose ($P < 0.05$), and PPARγ PRL in tail adipose was 78.81% higher than that in longissimus dorsi muscle ($P < 0.05$).

For FAS, the PRL differences in the longissimus dorsi muscle from Tan sheep and Shaanbei fine wool sheep were not significant ($P > 0.05$). For Tan sheep, FAS PRL in tail adipose was 89.97% higher than that in longissimus dorsi muscle ($P < 0.05$), and FAS PRL in longissimus dorsi muscle was 6.46% higher than that in subcutaneous adipose ($P < 0.05$). For Shaanbei fine-wool sheep, FAS PRL in subcutaneous adipose was 1.75 times higher than that in tail adipose ($P < 0.05$), and FAS PRL in tail adipose was 78.8% higher than that in longissimus dorsi muscle ($P < 0.05$).

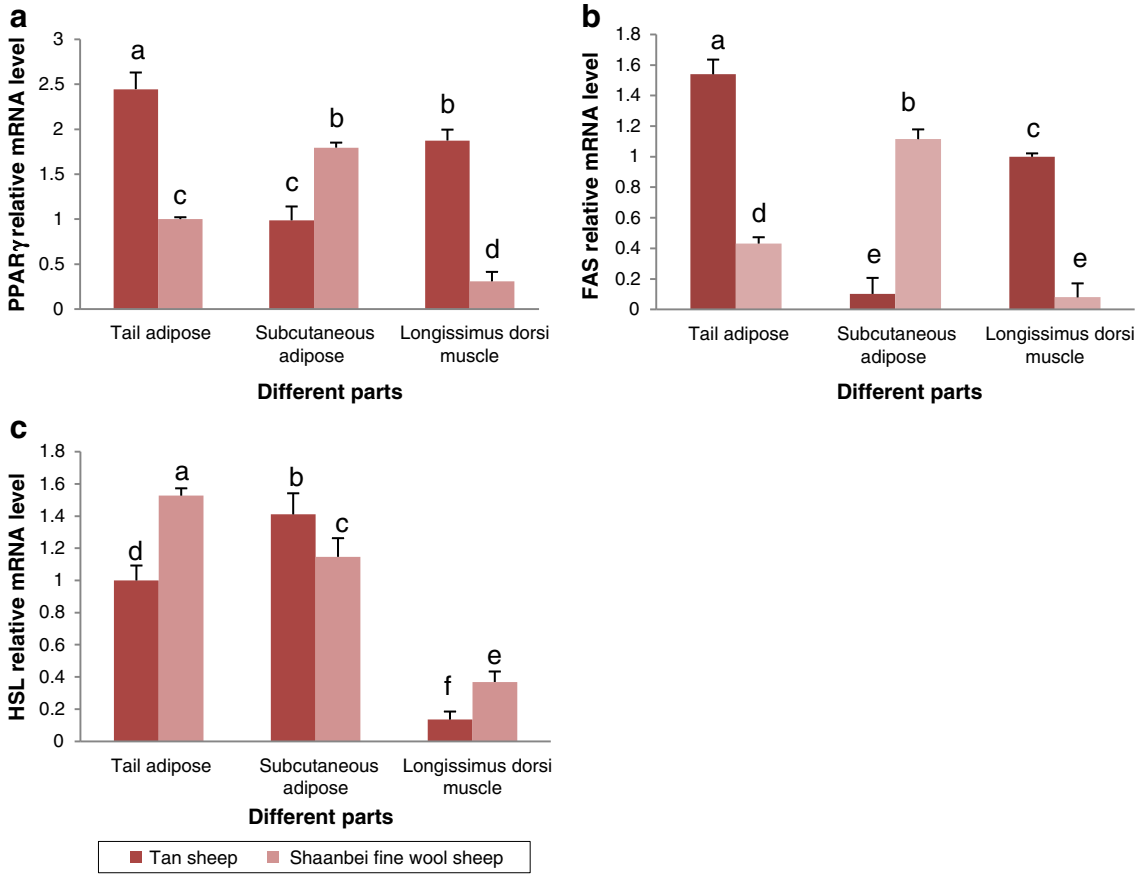

Fig. 1. PPARγ (a) FAS (b) and HSL (c) relative mRNA expression levels in tail adipose, subcutaneous adipose and longissimus dorsi muscle between Tan sheep and Shaanbei fine-wool sheep. Data are ratios of PPARγ, FAS and HSL genes' relative mRNA levels normalized to β-actin (housekeeping gene) mRNA levels. Each bar represents means ± SEM. Lowercases $P < 0.05$.

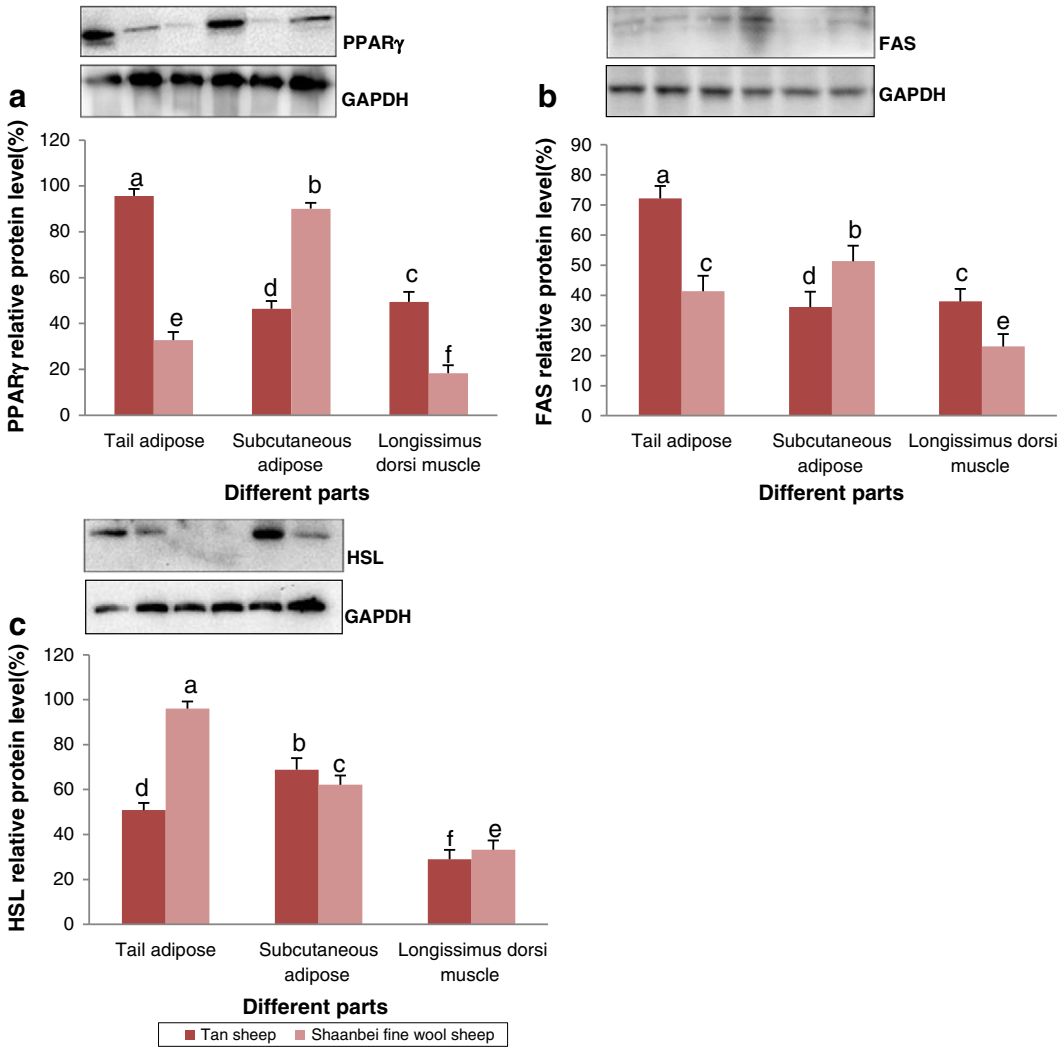

Fig. 2. (a) PPARγ, (b) FAS, and (c) HSL, relative protein expression levels in tail adipose, subcutaneous adipose and longissimus dorsi muscle between Tan sheep and Shaanbei fine-wool sheep. PPARγ, FAS, HSL, GAPDH (From left to right): subcutaneous adipose (Shaanbei fine-wool sheep), subcutaneous adipose (Tan sheep), tail adipose (Shaanbei fine-wool sheep), tail adipose (Tan sheep), longissimus dorsi muscle (Shaanbei fine-wool sheep), longissimus dorsi muscle (Tan sheep). Data are ratios of PPARγ, FAS and HSL genes' relative protein levels normalized to GAPDH (housekeeping gene) protein levels. Each bar represents means ± SEM. Lowercases $P < 0.05$.

For HSL, PRL differences among these tissues from either Tan sheep or Shaanbei fine-wool sheep were significant ($P < 0.05$). For Tan sheep, HSL PRL in subcutaneous adipose was 35.43% higher than that in tail adipose ($P < 0.05$), and HSL PRL in tail adipose was 75.21% higher than that in longissimus dorsi muscle ($P < 0.05$). For Shaanbei fine-wool sheep, HSL PRL in tail adipose was 54.57% higher than that in subcutaneous adipose ($P < 0.05$), HSL PRL in subcutaneous adipose was 86.98% higher than that in longissimus dorsi muscle ($P < 0.05$), and HSL PRL in tail adipose was 1.89 times higher than that in longissimus dorsi muscle.

4. Discussion

PPARγ, a member of nuclear hormone receptor superfamily, is a key transcription factor for differentiation energy metabolism in adipocytes, and mainly participates in adipogenesis, adipocyte differentiation, and gene expression regulation in special adipocytes [19,20]. Jones et al. [20] reported that the body fat content in PPARγ-knockout mice was significantly lower than that of control under the high-fat diet condition. Mukherjee et al. [21] documented that PPARγ was observed in the heart, liver, kidney, adipose tissue, and muscle tissue of humans, and showed a tissue-specific distribution in vivo. The present study showed that PPARγ was expressed in the tail adipose, subcutaneous adipose and longissimus dorsi muscle of Shaanbei fine-wool sheep and Tan sheep. In Tan sheep, PPARγ MRL in adipogenesis was significantly higher than that in longissimus dorsi muscle, and PPARγ MRL in longissimus dorsi muscle was significantly higher than that in subcutaneous adipose. However, in Shaanbei fine-wool sheep, PPARγ MRL in subcutaneous adipose was significantly higher than that in tail adipose, and PPARγ MRL in adipogenesis was significantly higher than that in longissimus dorsi muscle. Tan sheep belong to the long-fat-tailed sheep and have large fat deposits in the tail, meaning that PPARγ MRL in the tail is high. In comparison, Shaanbei fine-wool sheep belong to the long-thin-tailed sheep and have low fat deposits in the tail, meaning that PPARγ MRL in the tail is low. This work showed a consistent result for PPARγ MRL in tail adipose with those in Guangling large-tailed sheep and small-tailed Han sheep, which belong to the long-fat-tailed and short-fat-tailed sheep, respectively [22]. Grindflek et al. [23] reported that PPARγ MRL in the superficial adipose layer from Duroc pigs is higher than that in Landrace pigs. The differences in PPARγ MRL between different tissues from either Tan sheep or Shaanbei fine-wool sheep indicated that special parts of cultivars have PPARγ MRL differences, which possibly result from species characteristics.

The synthesis, decomposition, and reaction rate of triglycerides affect the accumulation of body fat. As a key enzyme in the process of triglycerides synthesizing and decomposing, FAS and HSL can affect

the composition of body fat. An increase in the FAS level can significantly decrease the composition of triglycerides in adipocytes [24,25]. Xiong et al. [26] reported that FAS level in adipose tissue has a significant positive correlation with the fat mass and fat percentage in pig carcass. Naduau et al. [27] documented that the fat content in muscle of rats treated with training and restricted feeding increased accompanied by increased levels of FAS protein and mRNA, and the same phenomena were observed in fasting monkey. The findings indicated that FAS expression had a somewhat positive correlation with fat composition in muscle. FAS mRNA expression level was the highest on day 0 in Kazak sheep and then declined with the growth, in the other breed the gene showed a 'decline–rise–decline–rise' expression manner as the animals grew [28]. Ding et al. [29] described that FAS expression shows species and tissue specificity. All of these previous reports suggested that the activities of enzymes related to fat metabolism in ruminants were affected by breed, age, and tissue specificity. The results of the present study showing that FAS MRL in tail adipose from Tan sheep was significantly higher than that in longissimus dorsi muscle and FAS MRL in longissimus dorsi muscle was significantly higher than that in subcutaneous adipose were consistent with the results observed in 9-months-old large-tail Tan sheep.

FAS MRL in subcutaneous adipose from 9-month-old Shaanbei fine-wool sheep was significantly higher than that in tail adipose, and FAS MRL in tail adipose was significantly higher than that in longissimus dorsi muscle. The distribution and amount of deposition of fat in the body play key roles in affecting the carcass quality and meat flavor. Regarding fat in different tissues, subcutaneous adipose mainly influences the carcass quality, while intramuscular fat is the material basis for the formation of marbling and affects the meat flavor. Many studies have demonstrated that intramuscular fat is directly involved in the formation of tenderness, juiciness, and flavor of meat [30,31].

The expression level of FAS was positively correlated with intramuscular fat and the most significant correlation was present in the longissimus dorsi muscle, demonstrating that FAS plays a positive role in intramuscular fat deposition, which is consistent with the physiological role of FAS. The data for Tan sheep in the present study showed a relatively high level of FAS MRL in longissimus dorsi muscle and a relatively low level of FAS MRL in subcutaneous adipose, suggesting that the longissimus dorsi muscle had a higher capacity for fat synthesis than the subcutaneous adipose. Therefore, the level of FAS expression in muscular tissue of Tan sheep is an important parameter for evaluating the quality of intramuscular adipose and can be used to develop a new line of Tan sheep. According to the present finding that the capacity for fat deposition in muscle tissue was higher in Tan sheep than in Shaanbei fine-wool sheep, new lines or breeds of Tan sheep could be developed to improve the meat quality of meat-and-wool sheep based on the commercial needs.

An increase of HSL expression level significantly decreases the amount of triglyceride deposition in adipocytes [32,33]. Research on pig muscular tissue by Chen et al. [34] showed that the intramuscular fat content exhibited a downward trend accompanied by an increase in the HSL expression level. In muscle tissue of HSL-deficient mice, Hansson [32] found that the expression levels of fat droplets in adipocytes were all increased, suggesting that glycogen can be utilized to counteract the low utilization of fat in HSL-deficient mice. The expression of HSL shows tissue specificity. Holm et al. [33] reported that the HSL mRNA levels are high in adipose and cholesterol-generating tissues, but low in cardiac and skeletal muscles. Qiaoyong [28] reported that HSL mRNA expression level had a similar model in two breeds, in Kazak sheep it was the highest on day 0 and in Xinjiang fine-wool sheep on day 30, then both decreased. The present study showed that the HSL expression levels were lowest in the longissimus dorsi muscle from Tan sheep and Shaanbei fine-wool sheep. In addition, the HSL expression level in subcutaneous adipose from Tan sheep was significantly higher than that in tail adipose, and the HSL expression level in tail adipose was significantly higher than that in longissimus dorsi muscle. Moreover, HSL MRL in tail adipose from 9-month-old Shaanbei fine-wool sheep was significantly higher than that in subcutaneous adipose, and HSL MRL in subcutaneous adipose was significantly higher than that in longissimus dorsi muscle.

In this study, we detected the mRNA and protein expression levels of PPARγ, FAS, and HSL in different parts of the carcass of Tan sheep and Shaanbei fine-wool sheep. Our data revealed that the PPARγ, FAS, and HSL mRNAs were detected at apparently similar levels to the corresponding proteins. In each analysis described so far, the correlations between mRNA and protein abundance or expression of a limited number of highly abundant proteins have been discussed. Berchtold et al. [35] demonstrated that the brain-derived neurotrophic factor (BDNF) protein levels closely followed the mRNA expression patterns in response to estrogen and exercise. The BDNF protein levels across all conditions were most closely correlated with the mRNA changes in the dentate gyrus. Hoggard et al. [36] reported that high levels of leptin and its receptor, both mRNA and protein, were expressed in the placenta. Future correlated large-scale mRNA and protein expression analyses will likely determine similar complex patterns of transcriptional and post-transcriptional control, as long as data clustering is based on the fact that proteins function in pathways and complexes.

5. Conclusion

PPARγ, FAS, and HSL are expressed in tail adipose, subcutaneous adipose, and longissimus dorsi muscle from Tan sheep and Shaanbei fine-wool sheep and the expression levels are affected by various factors such as spatial difference and breed. The expression levels of PPARγ and FAS in longissimus dorsi muscle of Tan sheep are higher than those in subcutaneous adipose of Tan sheep and longissimus dorsi muscle of Shaanbei fine-wool sheep, and the expression levels of HSL are in contrast to those of PPARγ and FAS. PPARγ, FAS, and HSL are closely related with fat deposition, especially in regulating deposition in intramuscular fat. The mechanism requires clarification in further studies.

Financial support

The National Special Research Fund for Non-profit Sector (Agriculture) (20130305905) and China Agriculture Research System (CARS-40-13) are acknowledged.

Acknowledgments

We are grateful to Ningxia Tan sheep breeding Centers and Shaanxi Dingbian breeding farm for their kind help and cooperation during the samples collection.

References

[1] Li C, Aldai N, Vinsky M, Dugan MER, McAllister TA. Association analyses of single nucleotide polymorphisms in bovine stearoyl-CoA desaturase and fatty acid synthase genes with fatty acid composition in commercial cross-bred beef steers. Anim Genet 2012;43:93–7. http://dx.doi.org/10.1111/j.1365-2052.2011.02217.x.

[2] Morris CA, Bottema CDK, Cullen NG, Hickey SM, Esmailizadeh AK, Siebert BD, et al. Quantitative trait loci for organ weights and adipose fat composition in Jersey and Limousin back-cross cattle finished on pasture or feedlot. Anim Genet 2010;41: 589–96. http://dx.doi.org/10.1111/j.1365-2052.2010.02058.x.

[3] Hérault F, Saez G, Robert E, Al Mohammad A, Davail S, Diot C, et al. Liver gene expression in relation to hepatic steatosis and lipid secretion in two duck species. Anim Genet 2010;41:12–20. http://dx.doi.org/10.1111/j.1365-2052.2009.01959.x.

[4] Moon JK, Kim KS, Kim JJ, Choi BH, Cho BW, Kim TH, et al. Differentially expressed transcripts in adipose tissue between Korean native pig and Yorkshire breeds. Anim Genet 2009;40:115–8. http://dx.doi.org/10.1111/j.1365-2052.2008.01798.x.

[5] Eeckhoute J, Oger F, Staels B, Lefebvre P. Coordinated regulation of PPARγ expression and activity through control of chromatin structure in adipogenesis and obesity. PPAR Res 2012. http://dx.doi.org/10.1155/2012/164140.

[6] Sankaranarayanapillai M, Zhang N, Baggerly KA, Gelovani JG. Metabolic shifts induced by fatty acid synthase inhibitor orlistat in non-small cell lung carcinoma cells provide novel pharmacodynamic biomarkers for positron emission tomography and magnetic resonance spectroscopy. Mol Imaging Biol 2013;15:136–47. http://dx.doi.org/10.1007/s11307-012-0587-6.

[7] Lee JH, Moon MH, Jeong JK, Park YG, Lee YJ, Seol JW, et al. Sulforaphane induced adipolysis via hormone sensitive lipase activation, regulated by AMPK signaling pathway. Biochem Biophys Res Commun 2012;426:492–7. http://dx.doi.org/10.1016/j.bbrc.2012.08.107.
[8] Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev 2000;14:1293–307. http://dx.doi.org/10.1101/gad.14.11.1293.
[9] Munoz G, Ovilo C, Noguera JL, Sanchez A, Rodriguez C, Silio L. Assignment of the fatty acid synthase (FASN) gene to pig chromosome 12 by physical and linkage mapping. Anim Genet 2003;34:234–5. http://dx.doi.org/10.1046/j.1365-2052.2003.00987.x.
[10] Yan XC, Wang YZ, Xu ZR. Regulation of fatty acid synthase (FAS) gene expression in animals. Acta Zoonutrimenta Sin 2002;14:1–4.
[11] Smith S, Witkowski A, Joshi AK. Structural and functional organization of the animal fatty acid synthase. Prog Lipid Res 2003;42:289–317. http://dx.doi.org/10.1016/S0163-7827(02)00067-X.
[12] Berraondo B, Martínez JA. Free fatty acids are involved in the inverse relationship between hormone-sensitive lipase (HSL) activity and expression in adipose tissue after high-fat feeding or β3-adrenergic stimulation. Obes Res 2000;8:255–61. http://dx.doi.org/10.1038/oby.2000.30.
[13] Jocken JW, Langin D, Smit E, Saris WH, Valle C, Hul GB, et al. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. J Clin Endocrinol Metab 2007;92:2292–9. http://dx.doi.org/10.1210/jc.2006-1318.
[14] Tsiplakou E, Flemetakis E, Kalloniati C, Zervas G. Differences in mRNA lipogenic gene expression in the subcutaneous adipose tissue of sheep and goats under the same dietary treatments. Small Rumin Res 2011;99:110–5. http://dx.doi.org/10.1016/j.smallrumres.2011.03.050.
[15] Yousefi AR, Kohram H, Zare Shahneh A, Nik-Khah A, Campbell AW. Comparison of the meat quality and fatty acid composition of traditional fat-tailed (Chall) and tailed (Zel) Iranian sheep breeds. Meat Sci 2012;92:417–22. http://dx.doi.org/10.1016/j.meatsci.2012.05.004.
[16] Negussie E, Rottmann OJ, Pirchner F, Rege JEO. Patterns of growth and partitioning of fat depots in tropical fat-tailed Menz and Horro sheep breeds. Meat Sci 2003;64: 491–8. http://dx.doi.org/10.1016/S0309-1740(02)00227-9.
[17] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001;25:402–8. http://dx.doi.org/10.1006/meth.2001.1262.
[18] Freeman LR, Zhang L, Dasuri K, Fernandez-Kim SO, Bruce-Keller AJ, Keller JN. Mutant amyloid precursor protein differentially alters adipose biology under obesogenic and non-obesogenic conditions. PLoS One 2012;7:e43193. http://dx.doi.org/10.1371/journal.pone.0043193.
[19] Bispham J, Gardner DS, Gnanalingham MG, Stephenson T, Symonds ME, Budge H. Maternal nutritional programming of fetal adipose tissue development: Differential effects on messenger ribonucleic acid abundance for uncoupling proteins and peroxisome proliferator-activated and prolactin receptors. Endocrinology 2005; 146:3943–9. http://dx.doi.org/10.1210/en.2005-0246.
[20] Jones JR, Barrick C, Kim KA, Lindner J, Blondeau B, Fujimoto Y, et al. Deletion of PPARγ in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. Proc Natl Acad Sci U S A 2005;102:6207–12. http://dx.doi.org/10.1073/pnas.0306743102.
[21] Mukherjee R, Jow L, Croston GE, Paterniti JR. Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonist. J Biol Chem 1997;272:8071–6. http://dx.doi.org/10.1074/jbc.272.12.8071.
[22] Lin PP, Gao ZY, Yuan YN, Wu JL, Liu BF, Zhou SS, et al. Developmental expression of PPARα and PPARγ mRNA in adipose tissues of different fat-tailed sheep. Acta Vet Zootech Sin 2012;43:1369–76.
[23] Grindflek E, Sundvold H, Klungland H, Lien S. Characterisation of porcine peroxisome proliferator-activated receptors γ1 and γ2: Detection of breed and age differences in gene expression. Biochem Biophys Res Commun 1998;249: 713–8. http://dx.doi.org/10.1006/bbrc.1998.9212.
[24] Sztalryd C, Komaromy MC, Kraemer FB. Overexpression of hormone-sensitive lipase prevents triglyceride accumulation in adipocytes. J Clin Invest 1995;95:2652–61. http://dx.doi.org/10.1172/JCI117967.
[25] Thompson MP, Cooper ST, Parry BR, Tuckey JA. Increased expression of the mRNA for hormone-sensitive lipase in adipose tissue of cancer patients. Biochim Biophys Acta 1993;1180:236–42.
[26] Xiong WZ, Yang F, Zhou AG. Study of regulation of exogenous recombinant somatotropin on fat metabolism in different cross-finishing pigs. Acta Vet Zootech Sin 2001;32:1–4.
[27] Nadeau KJ, Ehlers LB, Aguirre LE, Moore RL, Jew KN, Ortmeyer HK, et al. Exercise training and calorie restriction increase SREBP-1 expression and intramuscular triglyceride in skeletal muscle. Am J Physiol Endocrinol Metab 2006;291:E90–8. http://dx.doi.org/10.1152/ajpendo.00543.2005.
[28] Qiao Y, Huang Z, Li Q, Liu Z, Hao CL, Shi G, et al. Developmental changes of the FAS and HSL mRNA expression and their effects on the content of intramuscular fat in Kazak and Xinjiang sheep. J Genet Genomics 2007;34:909–17. http://dx.doi.org/10.1016/S1673-8527(07)60102-7.
[29] Ding ST, Schinckel AP, Weber TE, Mersmann HJ. Expression of porcine transcription factors and genes related to fatty acid metabolism in different tissues and genetic populations. J Anim Sci 2000;78:2127–34 (http://dx.doi.org/2000.7882127x).
[30] Van Laack RL, Stevens SG, Stalder KJ. The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. J Anim Sci 2001;79:392–7 (http://dx.doi.org/2001.792392x).
[31] Suzuki K, Irie M, Kadowaki H, Shibata T, Kumagai M, Nishida A. Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. J Anim Sci 2005;83:2058–65.
[32] Hansson O, Donsmark M, Ling C, Nevsten P, Danfelter M, Andersen JL, et al. Transcriptome and proteome analysis of hormone-sensitive lipase-null mice. J Lipid Res 2005;46:2614–23. http://dx.doi.org/10.1194/jlr.M500028-JLR200.
[33] Holm C, Kirchgessner TG, Svenson KL, Fredrikson G, Nilsson S, Miller CG, et al. Hormone-sensitive lipase: Sequence, expression and chromosomal localization to 19 cent-q13.3. Science 1988;241:1503–6. http://dx.doi.org/10.1126/science.3420405.
[34] Chen J, Yang XJ, Tong H, Zhao RQ. Expressions of FAS and HSL mRNA in longissimus dorsi muscle and their relation to intramuscular fat contents in pig. J Agric Biotechnol 2003;12:422–6.
[35] Berchtold NC, Kesslak JP, Pike CJ, Adlard PA, Cotman CW. Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. Eur J Neurosci 2001;14:1992–2002. http://dx.doi.org/10.1046/j.0953-816x.2001.01825.x.
[36] Hoggard N, Hunter L, Duncan JS, Williams LM, Trayhurn P, Mercer JG. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. Proc Natl Acad Sci U S A 1997;94:11073–8. http://dx.doi.org/10.1073/pnas.94.20.11073.