The pattern of development for gene expression of sterol regulatory element binding transcription factor 1 in pigs

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ABSTRACT: Sterol regulatory element-binding transcription factor 1 (*SREBF1*) has been implicated as a key regulator of adipocyte differentiation and lipid metabolism. The pattern of *SREBF1* gene expression in different growth stages and the relation with adipose deposition is studied. Fifteen female Duroc × Landrace × Yorkshire pigs in five groups, each group of three pigs at live weight 1, 30, 50, 70 and 90 kg, were used to study the developmental gene expression of *SREBF1* in the subcutaneous adipose tissue by means of semi-quantitative RT-PCR. The results showed that porcine *SREBF1* mRNA was present in a very low concentration at birth and continually increased to the highest expression at 90 kg growth stages, *SREBF1* mRNA levels increased as pigs grew and deposited fat from 1 to 90 kg live weights ($P < 0.05$). The present data indicated a close positive correlation between the levels of *SREBF1* gene expression and the fat deposition rate in pigs ($P < 0.05$).

Keywords: *SREBF1*; gene expression; fat deposition; pig

SREBF1 (sterol regulatory element-binding transcription factor 1) is a member of the basic helix-loop-helix leucine zipper (bHLH-LZ) family of transcription factors. It has also been termed sterol regulatory element-binding protein 1 (*SREBP1*). *SREBF1* is highly expressed in fat and liver, and its mRNA is induced at the early stage of adipocyte differentiation (Kim et al., 1998). *SREBF1* plays important roles in adipocyte differentiation and regulation of lipogenesis (Tontonoz et al., 1993). The overexpression of *SREBF1* in fibroblasts (Kim and Spiegelman, 1996) or in the liver of transgenic mice induces lipoprotein lipase and fatty acid synthase. *SREBF1* is involved in the control of energy metabolism in the human adipose tissue (Ribot et al., 2001) and appears to be a key link between cholesterol and triglyceride metabolism, adipogenesis as well as insulin sensitivity (Cho et al., 2004).

Efficient production of good-quality pig meat may be obtained by reducing the total fat amounts while fat depots important for meat quality are kept at optimum levels. The aim of this experiment was to determine the pattern of *SREBF1* gene expression in different growth stages and the relation with adipose deposition to supply a clue to regulate meat production quality.

MATERIAL AND METHODS

Animals

In total fifteen female Duroc × Landrace × Yorkshire pigs in five groups, each group of three animals, at live weight 1, 30, 50, 70 and 90 kg were euthanized under anaesthesia and exsanguinated after 12 h fasting and *ad libitum* access to water. The subcutaneous adipose tissues were quickly dissected and frozen in liquid nitrogen, then stored at –70°C until extraction for the total RNA. Left half-carcasses without head, legs and guts (except kidney) were weighed. Subcutaneous, ventral and mesentery adipose tissues in the left half-carcass were dissected and weighed; the fat deposition rates were calculated. All the animal experiments were done according to the guidelines of

the National Institute of Animal Health for animal experiments.

Extraction of RNA

Total RNA from porcine tissues was isolated using TRIzol Reagent (Gibco BRL) as described by the manufacturer (Sigma, USA). Extracted RNA was resuspended in 30 µl ultra-pure water. The purity and concentration of RNA were checked using a spectrophotometer at 260 and 280 nm.

cDNA cloning

The synthesis of first strand cDNA was performed using Reverse Transcription System kit (First Strand cDNA-synthesis Kit, Promega, USA) as described by the manufacturer with oligodT-primer and using approximately 1 µg of total RNA as template.

PCR

The reverse-transcribed cDNA was amplified with Taq DNA polymerase (Promega, USA) by a polymerase chain reaction (PCR) in a thermocycler (Gene Amp PCR System 9600, Pharmacia, Japan) using paired sense and antisense primers (*SREBF1*: S: 5'-AAGCGGACGGCTCACAATG-3'; A: 5'-GCTTGCGATGCCTCCAGAA-3'. ACTB: S:5'-GGAGATCGTGCGGGACAT-3'; A:5'-GTTGAAGGTGGTCTCGTGG AT-3'). The product size for *SREBF1* gene is 365 bp, for ACTB gene it is 318 bp. Primer sequences for *SREBF1* and ACTB encoding for β-actin were designed on the basis of known sequences deposited in Genebank (AY307771, NT005058). The conditions for PCR for *SREBF1* were as follows: denaturation at 94°C for 2 min, followed by 31 cycles of amplification at 94°C for 50 s, 57°C for 50 s, and 72°C for 1 min, and followed by final extension at 72°C for 10 min. The conditions for PCR for *ACTB* were as follows: denaturation at 94°C for 2 min, followed by 31 cycles of amplification at 94°C for 50 s, 53°C for 50 s, and 72°C for 1 min, and followed by final extension at 72°C for 10 minutes.

DNA sequencing

The PCR products were electrophoresed on 1% (w/v) agarose gel and selected electrophoresis bands were collected, then the gel was purified using QIAquick Gel Extraction Kit (QIAGEN). The purified amplified products were directly ligated into pGEM-T Easy Vector (Promega, USA) and transformed into *E. coli* JM109. Plasmids were then isolated and purified for DNA sequencing using Wizard Miniprep Kit (Promega, USA). The sequencing of the inserts was performed using ABI PRISM Dye Terminator Kit (Perkin Elmer) and analysed on ABI PRISM 310 Genetic Analyser (Perkin Elmer).

Sequence analysis

The BLAST sequence analysis program (http://www.ncbi.nlm.nih.gov//BLAST) was used for initial comparisons of the sequence of PCR products obtained with sequences deposited in the GeneBank. Amplified DNA was 99% homologous to the known sequence of *SREBF1* and ACTB deposited in GeneBank.

mRNA expression analysis

The expression of pig *SREBF1* mRNA was determined by semi-quantitative RT-PCR (Kousteni et al., 1999) using the housekeeping gene *ACTB* as internal control. The PCR products were electrophoresed on 1% (w/v) agarose gel. Electrophoresis band intensities of the PCR products were quantified by NIH Image Version 1.62 software (Pharmacia, Japan).

Data analysis

All the data were analyzed using the ANOVA procedure (SAS Institute, 1989) and the treatment means were compared by Duncan's multiple range test. Statistical significance was at $P < 0.05$ for all statistical tests.

RESULTS

Developmental patterns of *SREBF1* gene expression

Developmental patterns of *SREBF1* gene expression of adipose tissue in pigs with live weight of 1, 30, 50, 70 and 90 kg were evaluated using semi-quantitative RT-PCR, which allows the relative quantity of *SREBF1* mRNA levels of pigs in different growth stages. The electrophoresis results of PCR products of three pigs of each group are shown in Figure 1. *SREBF1* gene mRNA was detected in the adipose tissue of pigs with live weight of 1, 30, 50,

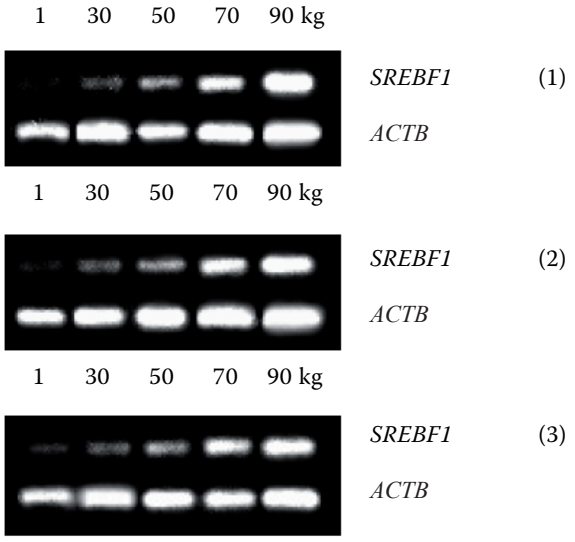

Figure 1. Electrophoresis of RT-PCR products for *SREBF1* and *ACTB* genes in the subcutaneous adipose tissue of pigs weighing 1, 30, 50, 70 and 90 kg. (1), (2), (3): The products from the first pig, the second pig and the third pig in each weight group respectively

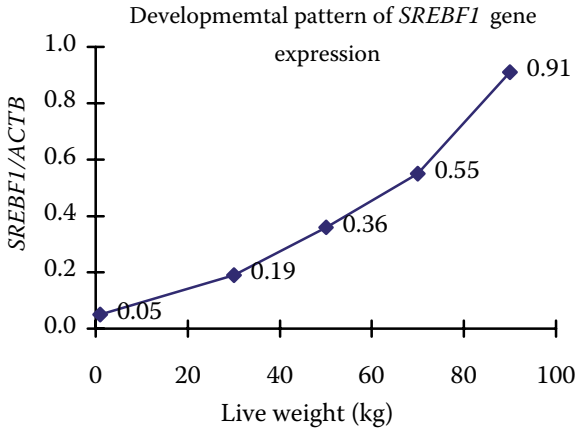

Figure 2. The pattern of *SREBF1* mRNA levels in the subcutaneous adipose tissue of pigs weighing 1, 30, 50, 70 and 90 kg. The data show the mean mRNA level of three pigs in each weight group as the ratio of the band intensity of each PCR product to the corresponding *ACTB* PCR product

70 and 90 kg. As shown in Figure 2, the *SREBF1* gene expression was weight-dependent in the porcine adipose tissue. *SREBF1* mRNA was present at a very low concentration at birth, the relative quantity of *SREBF1* mRNA (*SREBF1*/*ACTB*) steadily increased from 0.05, 0.19, 0.36, 0.55 to 0.91 as pigs grew ($P < 0.05$). The highest expression of *SREBF1* mRNA was observed in the heaviest animals (90 kg).

Developmental pattern of fat deposition

Table 1 shows that fat (subcutaneous, ventral and mesentery adipose tissues) deposition rates were 7.94%, 11.48%, 14.99%, 16.75%, 20.73%, respectively, at different growth stages of 1, 30, 50, 70, 90 kg live weight in pigs. The ratio increased significantly from 1 to 90 kg ($P < 0.01$). Table 1 also shows that the weight of subcutaneous adipose tissue, ventral adipose tissue and mesentery adipose tissue at different stages of pig growth and the weight of three adipose tissues increased significantly with live weights from 1 to 90 kg ($P < 0.01$), and the percentage of subcutaneous adipose increased with live weight.

The relation between *SREBF1* gene expression and fat deposition

Figure 2 and 3 show that the *SREBF1* mRNA levels increased as fat deposited from 1 to 90 kg growth stages ($P < 0.05$). Correlation analysis showed that there was a positive correlation between the levels of *SREBF1* gene expression and the fat deposition rate ($r = 0.89$, $P < 0.05$) from 1 to 90 kg growth stages in pigs.

Table 1. Developmental pattern of adipose deposition (mean ± SD)

Body weight (kg)	Subcutaneous adipose (kg)	Ventral adipose (kg)	Mesentery adipose (kg)	Fat deposition rate (%)
1	$0.031^{a} \pm 0.017$	$0.003^{a} \pm 0.001$	$0.028^{a} \pm 0.002$	$7.94^{a} \pm 0.75$
30	$1.236^{b} \pm 0.036$	$0.042^{a} \pm 0.002$	$0.395^{b} \pm 0.043$	$11.48^{b} \pm 0.23$
50	$3.890^{c} \pm 0.053$	$0.254^{b} \pm 0.124$	$0.605^{c} \pm 0.099$	$14.99^{c} \pm 0.43$
70	$6.661^{d} \pm 0.536$	$0.344^{c} \pm 0.068$	$1.029^{d} \pm 0.059$	$16.75^{d} \pm 1.13$
90	$11.12^{e} \pm 0.308$	$0.920^{c} \pm 0.076$	$1.798^{e} \pm 0.226$	$20.72^{e} \pm 0.57$

Means within a column with different superscripts are significantly different ($P < 0.05$)

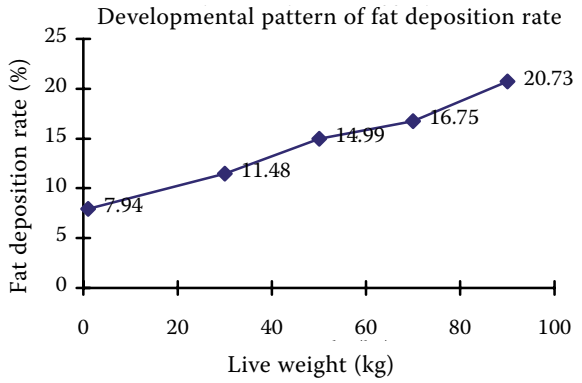

Figure 3. The pattern of fat deposition rate of pigs weighing 1, 30, 50, 70 and 90 kg. The data show the mean of fat deposition rates of three pigs in each weight group

DISCUSSION

Developmental pattern of *SREBF1* gene expression

This study found that *SREBF1* mRNA was present at birth in a very low concentration (only 5.49% of that at the stage of 90 kg live weight) while the relative quantity of *SREBF1* mRNA (*SREBF1*/*ACTB*) steadily increased to the highest value at 90 kg live weight as pigs grew. Ding et al. (1999) also found that the porcine *SREBF1* transcript was at a very low concentration at birth and continually increased during postnatal development to reach its the highest value at 28 days in the postnatal pig. Transcripts of *SREBF1* were reported to be present at substantial levels in preadipocytes of the 3T3-F442A and 10T1/2 clonal cell lines and increased many times after differentiation (Tontonoz et al., 1993). At day 0, the transcripts for porcine *SREBF1* were at the same concentration as at day 10. The transcript concentration doubled by day 2, and reached a plateau at 4–7 days of differentiation over the 9–10 days period of porcine S/V cell differentiation *in vitro* (Ding et al. 1999). In contrast, another report (Kim and Spiegelman, 1996) indicated very low *SREBF1* transcript levels at confluence in both 3T3-L1 and 3T3-F442A cells; the transcript increased markedly in both clonal cell lines at 1 day after addition of differentiation medium. Presumably, subtle differences in experimental details, including the culture conditions, led to differences in the pattern of *SREBF1* transcript expression, even when using the same cell lines. Rat S/V cells had very low concentrations of the *SREBF1* transcripts before addition of differentiation medium, with a several-fold increase after 1 day and 10-fold increase after 3 or 5 days of differentiation (Hansen et al., 1998).

Developmental pattern of fat deposition

The weight of total adipose tissue increased significantly ($P < 0.01$), the fat deposition rate increased significantly from 1, 30, 50, 70 to 90 kg growth stages in pigs ($P < 0.01$). The results indicated that the capacity of adipose deposition in pigs increased as pigs grew. Wang and Shao (1989) reported that the fat percentage increased significantly with the increase of weight in commercial lean pigs. The study of Souza et al. (2004) showed that the fat content of the carcass was significantly correlated ($P < 0.001$) with animal age.

The subcutaneous adipose tissue had the highest proportion out of the three adipose tissues in each growth stage in this study. This is in accordance with Kolstad (2001), who found that the subcutaneous fat depot was the dominating fat depot at all stages of 10, 25, 50, 85 and 105 kg live weight, containing more than a half of total body fat.

The relation between *SREBF1* gene expression and fat deposition

SREBF1, which has important roles in adipocyte differentiation, plays also a key role in lipogenesis. This study showed that there was a positive correlation between the levels of *SREBF1* gene expression and the fat deposition rate. *SREBF1* mRNA levels increased as fat deposited from 1 to 90 kg growth stages in pigs. Ribot et al. (2001) reported that the *SREBF1* mRNA levels of adipose tissue were lower in the weight-loss group than in controls by 28%.

The mechanism by which *SREBF1* regulates lipogenesis is not clear, and several reports showed partly the mechanism from different aspects. Using the adenovirus-mediated transfection of a powerful dominant negative form of *SREBF1* in rat hepatocytes, Foretz et al. (1999) demonstrated that this factor was absolutely necessary for the stimulation of gene expression of L-pyruvate kinase, fatty acid synthase, S14 (thyroid hormone-inducible hepatic protein), and acetyl coenzyme A carboxylase by glucose. These results demonstrate that *SREBF1* plays a crucial role in mediating the expression of lipogenic genes induced by glucose and insulin.

The promoters for both leptin and fatty acid synthase are transactivated by *SREBF1* (Kim et al.,

1998). A mutation in the basic domain of *SREBF1* that allows E-box binding but destroys sterol regulatory element-1 binding prevents leptin gene transactivation but has no effect on the increase in FAS promoter function. Molecular dissection of the FAS promoter shows that most if not all of this action of *SREBF1* is through an E-box motif at –64 to –59, contained with a sequence identified previously as the major insulin response element of this gene. These results indicate that *SREBF1* is a key transcription factor linking changes in the nutritional status and insulin levels to the expression of certain genes that regulate systemic energy metabolism.

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