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Postnatal expression pattern of PPARγ gene in pigs

Junfang JIANG[1], Yongqing JIANG[1], Zhigang ZHU[2], Zirong XU[2]

[1]Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, No.145 ShiQiao Road, Hangzhou, 310021 - CHINA

[2]Animal Science College, Zhejiang University, No.164 QiuTao Road, Hangzhou, 310029 - CHINA

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Abstract: PPARγ is a member of the peroxisome proliferator-activated receptor subfamily of nuclear hormone receptors. PPARγ has been showed to be a key regulator of adipocyte differentiation and lipid metabolism. The pattern of PPARγ gene expression in different growth stages and its relation with adipose deposition in pigs is studied. Fifteen female Duroc×Landrace×Yorkshire pigs in 5 groups with 3 pigs in each grup, at live weight of 1, 30, 50, 70, and 90 kg were used to study the developmental gene expression of PPARγ in subcutaneous adipose tissue by means of semi-quantitative RT-PCR. The results showed the PPARγ mRNA levels of porcine adipose tissue increased as pigs grew and deposited fat from 1 to 90 kg live weight ($P < 0.05$). The present data indicated a close positive correlation between the levels of PPARγ gene expression and the fat deposition rate in pigs. The experiment showed the total adipose weight increased significantly with body weight ($P < 0.05$).

Key words: PPARγ, gene expression, fat deposition, pigs

Introduction

PPARγ is a member of the peroxisome proliferator-activated receptor subfamily of nuclear hormone receptors. The receptor is specifically expressed at high levels in mammalian adipose tissue (1). PPARγ has been shown to play a key role in mediating differentiation of adipocytes and regulating fat metabolism (2). Experiments with heterozygous PPARγ mutant mice suggest that PPARγ, in addition to adipogenesis, may also have an important regulatory role in adult lipogenesis. It was observed that, on a high fat diet, mice with only 1 copy of the PPARγ gene gained less weight than wild-type mice and had significantly smaller adipose tissue fat stores (3). PPARγ plays a very important role in adipocyte differentiation, adipogenesis, and lipogenesis. Moreover, PPARγ has been shown to play pivotal roles in insulin sensitization and fatty acid metabolism (4). The results of recent studies indicate that activating PPARγ might have important anti-atherosclerotic effects (5). PPARγ is an important regulator of cardiac metabolism, PPARγ control myocardial metabolism by transcriptionally regulating genes encoding enzymes involved in fatty acid and glucose utilization (6).

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

* E-mail: jiang_junfang@sohu.com

optimum levels. In order to explore novel methods to control pig fat deposition, it may be beneficial to accumulate knowledge of the expression of regulatory factors that are involved in adipocyte differentiation during adipogenesis in pigs. The present study was designed to determine the pattern of PPARγ gene expression in different growth stages and its relation with adipose deposition in pigs to enable the improvement of meat production quality by regulating fat deposition in genes, as well as the treatment of adipose tissue disorders, such as atherosclerosis, obesity, and diabetes.

Material and methods

Animals

Total fifteen female Duroc×Landrace×Yorkshire pigs in 5 groups, with 3 pigs in each group, at live weight of 1, 30, 50, 70 and 90 kg were euthanized under anesthesia and exsanguinated after a 12 h fast and ad libitum access to water. Subcutaneous adipose tissue was quickly dissected and frozen in liquid nitrogen, then stored at -70 °C until the extraction of total RNA. Left half carcass without head, hoof, tail, guts (excepting kidney) were weighed. Subcutaneous adipose tissue, ventral adipose tissue, and mesentery adipose tissue in the left half carcass were dissected and weighed; the fat deposition rate was calculated. All the animal experiments were carried out according to the guidelines for animal experiments at the National Institute of Animal Health.

Extraction of RNA and cDNA cloning

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.

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 transcribed cDNA was amplified with Taq DNA polymerase (Promega, USA) by polymerase chain reaction (PCR) in a thermocycler (Gene Amp PCR system 9600, Pharmacia, Japan) using paired sense and antisense primers (PPARγ: S: 5'-CACAGAGTATGCCAAGAACA-3'; A: 5'-GCTATAAATATCGCCAGGTC -3'. β-actin: S: 5'-GGAGATCGTGCGGGACAT-3' A: 5'-GTTGAAGGTGGTCTCGTG GAT-3'). The product sizes of PPARγ and β-actin genes are 279 and 318 bp, respectively. Primer sequences of PPARγ and β-actin gene were designed based on known sequences deposited in Genebank (AY346131, NT005058). The conditions for PCR for PPARγ were denaturation at 94 °C for 2 min, followed by 31 cycles of amplification at 94 °C for 50 s, 51 °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 β-actin were 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 min.

DNA sequencing and sequence analysis

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

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 in the GeneBank. Amplified DNA was 0.99 homologous to the known sequence of PPARγ and β-actin deposited in GeneBank.

mRNA expression analysis

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

Data analysis

All the data were analyzed using the ANOVA procedure (7) (SAS. Institute, 1989) and the treatment means were separated by the Duncan's multiple range test. Statistical significance was at $P < 0.05$ for all statistical tests.

Results

Developmental pattern of PPARγ *gene expression*

Developmental pattern of PPARγ gene mRNA expression of adipose tissue in pigs with 1, 30, 50, 70 and 90 kg live weight were evaluated using semi-quantitative PCR analysis, which allows the relative quantity of PPARγ mRNA levels of pigs in different growth stages. The electrophoresis results of PCR products of 3 pigs of each group are shown in Figure 1. PPARγ mRNA was detected in adipose tissue of pigs with live weight of 1, 30, 50, 70 and 90 kg. As shown in Figure 2, porcine adipose tissue PPARγ gene expression was weight-dependent. PPARγ mRNA was present in low concentration at birth. The relative quantity of PPARγ mRNA levels (PPARγ/*β-actin*) steadily increased from 0.15, 0.28, 0.42, 0.72 to 1.05 as pigs grew ($P < 0.05$). The highest expression of PPARγ mRNA was observed at a later stage of 90 kg body weight.

Developmental pattern of fat deposition

Fat (subcutaneous, ventral, and mesentery adipose tissues) deposition rate was 0.08, 0.11, 0.15, 0.17, 0.21 at different growth stages of 1, 30, 50, 70, 90 kg live weight of pigs, respectively (Table). The ratio increased significantly at all stages studied ($P < 0.01$). The weight of subcutaneous, ventral, and mesentery adipose tissues and the total weight of the 3 adipose tissues increased significantly between all stages studied ($P < 0.01$), and percentage of subcutaneous adipose increased with live weight (Table).

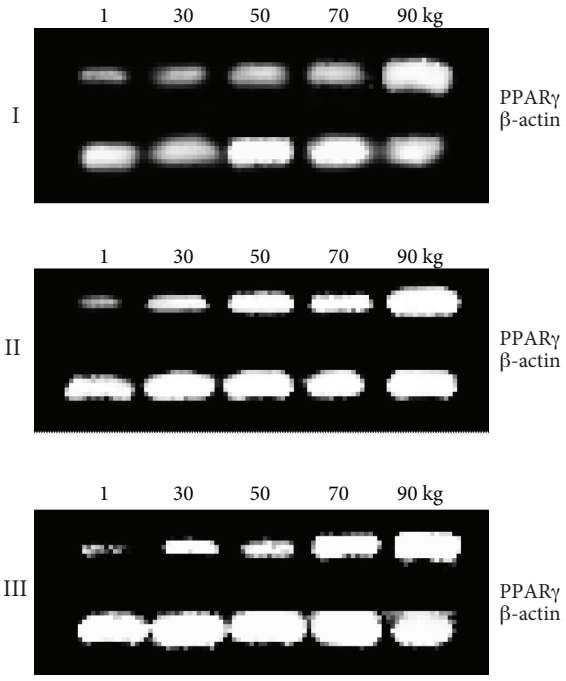

Figure 1. Electrophoresis of RT-PCR products for PPARγ and β-actin genes in the subcutaneous adipose tissue of pigs weighed 1, 30, 50, 70 and 90 kg. I, II, III: The results from the first pig, the second pig, and the third pig in each weight group, respectively.

The relation between PPARγ *gene expression and fat deposition*

PPARγ mRNA levels increased as fat deposited from 1 to 90 kg growth stages (Figures 2 and 3; $P <$

Table. Developmental adipose deposition (mean ± s.d.).

Body weight	Subcutaneous adipose (kg)	Ventral adipose (kg)	Mesentery adipose (kg)	Fat deposition rate (fat/carcass)
1 kg	$0.031^{a} \pm 0.017$	$0.003^{a} \pm 0.001$	$0.028^{a} \pm 0.002$	$0.079^{a} \pm 0.001$
30 kg	$1.236^{b} \pm 0.036$	$0.042^{a} \pm 0.002$	$0.395^{b} \pm 0.043$	$0.115^{b} \pm 0.005$
50 kg	$3.890^{c} \pm 0.053$	$0.254^{b} \pm 0.124$	$0.605^{c} \pm 0.099$	$0.149^{c} \pm 0.009$
70 kg	$6.661^{d} \pm 0.536$	$0.344^{c} \pm 0.068$	$1.029^{d} \pm 0.059$	$0.167^{d} \pm 0.015$
90 kg	$11.12^{e} \pm 0.308$	$0.920^{c} \pm 0.076$	$1.798^{e} \pm 0.226$	$0.207^{e} \pm 0.021$

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

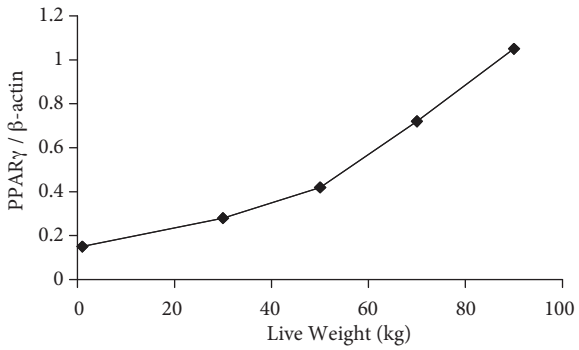

Figure 2. Developmental pattern of PPARγ gene expression. The pattern of PPARγ mRNA levels in the subcutaneous adipose tissue of pigs weighed 1, 30, 50, 70 and 90 kg. The data show the mean mRNA levels of three pigs in each weight group as the ratio of the band intensity of each PCR product to the corresponding β-actin PCR product.

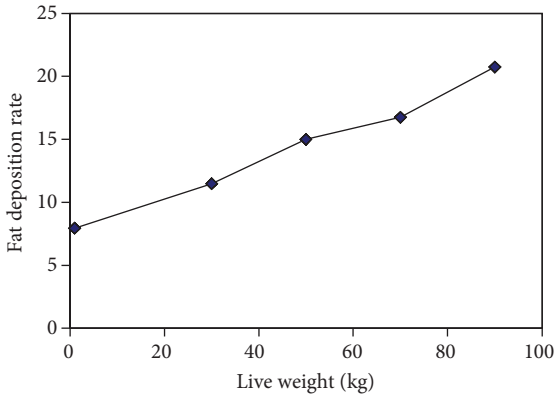

Figure 3. Developmental pattern of fat deposition rate. The pattern of fat deposition rate of pigs weighed 1, 30, 50, 70 and 90 kg. The data show the mean of fat deposition rate of three pigs in each weight group.

0.05). Correlation analysis showed that there was a positive correlation between levels of PPARγ gene expression and the fat deposition rate($r = 0.90$, $P < 0.05$) from 1 to 90 kg growth stages in pigs.

Discussion

This study showed that the relative quantity of PPARγ mRNA levels (PPARγ/*β-actin*) increased as pigs grew from 1 and 90 kg live weight. Grindflek et al. (8) also indicated that porcine PPARγ transcripts were at low concentration in subcutaneous adipose tissue at birth and were barely detectable in internal adipose tissue. The transcripts increased in both depots by 5 weeks of age and increased more by 6 months of age. Ding et al. (9) reported that PPARγ transcript was at very low concentration at birth and continually increased during postnatal development to reach its greatest value at 28 days postpartum.

The pattern of development for porcine PPARγ transcripts was different in vivo from observed in vitro: in vitro, at day 0, PPARγ transcripts of porcine S/V cell were about 0.35 of that on day 10 of cell culture; there was a gradual increase in the steady-state concentration of the PPARγ transcripts between day 0 and day 10 (9). The pattern in porcine S/V cell was somewhat different than observed in rodent-derived cells. The porcine S/V cell transcript concentration was relatively high at day 0 and gradually increased over the entire period through day 10. In 3T3-L1 and 3T3-F442A clonal cells, the PPARγ transcripts are present at very low levels in the confluent preadipocytes and increase rapidly, beginning at approximately 1 or 2 days after addition of differentiation medium (10). In rat S/V cells derived from the epididymal fat pad, the PPARγ transcripts are at a very low concentration before addition of differentiation medium; transcripts increase several-fold after 1 day and 10-fold after 3 days of differentiation (11). The regulation of adipocyte differentiation may be quite different in vitro from in vivo because of modulation of the process in vivo by paracrine and endocrine factors, blood flow, and concentration of substrates and metabolites.

The weight of total adipose tissue increased significantly and the fat deposition rate increased significantly from 1, 30, 50, 70 to 90 kg growth stages in pigs ($P < 0.01$). The results indicated the capacity of adipose deposition in pigs increased as pigs grew. Wang and Shao (12) reported that fat percentage increased significantly with the increase in the weight of commercial lean in pigs. The study of D'Souza et al. (13) showed the fat content of the carcass was significantly correlated ($P < 0.001$) with animal age. The fat percent in the carcass, shoulder, loin, belly, and ham primal cut significantly increased ($P < 0.05$) from 16 to 25 weeks of age. The backfat depth at the

P2 site significantly increased ($P < 0.001$) from 16 to 25 weeks of age. The backfat depth along the carcass midline at the fore, middle, and hind sites significantly increased ($P < 0.001$) in pigs from 16 to 25 weeks of age.

The subcutaneous adipose tissue had the highest proportion out of 3 adipose tissues in each growth stage in this study, which is in accordance with Kolstad (14) who found the subcutaneous fat depot is the dominating fat depot at all stages of 10, 25, 50, 85, and 105 kg live weight, containing more than half of total body fat.

Three PPAR isoforms (α, β, and γ) have been identified and cloned, and they differ in their tissue distribution and ligand specificity, PPARβ is ubiquitously expressed in many tissues, PPARα is found predominantly in hepatocytes, cardiomyocytes, and enterocytes, PPARγ, which was identified as an isotype of PPAR highly expressed in adipose tissue, is indeed a key transcription factor involved in terminal differentiation of adipocytes in white adipose tissue (WAT) and brown adipose tissue (BAT) (1). The results in this study showed PPARγ mRNA levels increased as pigs grew and deposited fat from 1 and 90 kg live weight growth stages. There was positive correlation between the levels of PPARγ gene expression and the fat deposition rate ($r = 0.89$, $P < 0.05$). Mice with only 1 copy of the PPARγ gene gained less weight than wild-type mice and had significantly smaller adipose tissue fat stores on a high fat diet (3). One of the most illuminating set of experiments has been carried out with chimeric mice derived from both wild-type embryonic stem cells and embryonic stem cells with a homozygous deletion of PPARγ (15). It was observed that cells lacking PPARγ were absent from white adipose tissue, but present in other tissues, leading to the important conclusion that PPARγ is required for adipogenesis. PPARγ regulates adipocyte differentiation and the expression of adipocyte-characteristic genes such as lipoprotein lipase (*LPL*), leptin, adipocyte fatty acid binding protein (*aP2*), fatty acid synthase (*FAS*), phosphoenolpyruvate carboxykinase (PEPCK), acyl-CoA synthetase, fatty acid translocase (*CD36*), and fatty acid transport protein (16), resulting in adipocyte differentiation, glyceroneogenesis, accumulation of triglycerides in these adipocytes, and activation of many of the genes involved in adipocyte metabolism, ultimately, an increase in total WAT mass (1).

The volume of lipid an individual adipocyte can accumulate is finite, whereas the capacity of adipose tissue to expand in response to overfeeding is virtually without limit. Significant expansion of adipose tissue mass, therefore, necessitates the differentiation of new adipocytes from precursor cells (17). Such differentiation can occur at any time throughout the life of an adult organism. As a lipid-activated transcription factor, PPARγ represents a molecular link between systemic lipid metabolism and adipocyte differentiation. PPARs are lipid sensors capable of adapting gene expression to integrate various lipid signals coming from intracellular signaling pathways, from inter-organ crosstalk or even from the diet (18). Given their partially overlapping yet specific expression patterns, the receptors cooperate to efficiently regulate metabolic functions (19) as well as other cellular processes, such as proliferation, differentiation or apoptosis that are essential to the fate of the tissues and organs in which they are expressed.

The characterization of PPARγ, a central regulator of adipocyte development, can open the door for novel approaches to regulate fat deposition and the treatment of disorders of adipose tissue. In the clinic, PPARγ agonists are effective in treating T2DM patients, ongoing research indicates that modulation of PPAR activity might be an effective therapy for additional maladies associated with the metabolic syndrome, including obesity (5). Administration of PPARγ activators decreases the size of atherosclerotic lesions in low-density lipoprotein receptor knockout and apolipoprotein E knockout mice, which are murine genetic models of the disease. Activation of PPARγ induces lipid efflux from macrophages, so inhibiting their transformation into lipid-laden foam cells and augmenting peripheral cholesterol transport (5).

As a ligand-dependent transcription factor, PPARγ represents an ideal target for intervention. It may be possible to limit adipocyte differentiation and fat deposition using specific antagonists of PPARγ according to the developmental pattern of PPARγ gene expression.

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