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# Breed difference of porcine Sirtuin 1, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL)

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ABSTRACT

In the current study, we investigated the breed difference of Sirt1 gene expression in liver and pancreas between Jinhua pigs (a local fatty breed of China) and Landrace (a leaner breed). In addition, the gene expression of lipid metabolism enzymes adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) has also been examined. Results showed that the serum triglyceride (TG), and total cholesterol (TCHO) concentration of Jinhua pigs were greater than Landrace while the serum glycerol in Jinhua pigs was lower at the age of 180 d. The serum leptin concentration was also greater in Jinhua pigs. Compared with Landrace, the mRNA level and protein abundance of Sirt1 were lower both in liver and pancreas in Jinhua pigs. Consistent with Sirt1 expression, in liver and pancreas, the mRNA expression of lipolitic enzymes ATGL and HSL was always lower in Jinhua pigs than Landrace respectively. Furthermore, similar results were found in 60 d pigs. The mRNA levels of Sirt1, ATGL and HSL were decreased in 60 d Jinhua pigs comparatively. These results indicated that porcine Sirt1, as well as ATGL and HSL gene expressions are different between fatty and leaner pigs. This will provide useful information for better known of Sirt1 function in fat deposition.



## 1. Introduction

Sirtuin 1 (Sirt1), a member of sirtuin gene family, is a NAD-dependent deacetylase (Gillum et al., 2010; Nakagawa and Guarente, 2011). Sirt1 is the most studied of Sirtuins family and it has been identified to influence gluconeogenesis and fatty acid oxidation in mammals (Gillum et al., 2010; Kong et al., 2009). Under fasting, Sirt1 can elevate gluconeogenesis in liver and fatty acid oxidation in white adipose tissue (Finkel et al., 2009; Rodgers et al., 2005).

Recent studies found that genetic variation in Sirt1 was associated with visceral obesity (Peeters et al., 2008) and Sirt1 expression was lower in obese humans (Pedersen et al., 2008). Meanwhile, our previous studies found in subcutaneous adipose tissues, Sirt1 gene expression was lower in a local fatty breed of China (Jinhua) than lean pigs (Landrace) (Shan et al., 2010). Sirt1 has also been found decreased in diabetic rats compared to normal rats (Tikoo et al., 2007). Liver and pancreas are important tissues involved in the insulin signaling pathway playing critical roles in fat deposition (Yang et al., 2006). In liver, Sirt1 can promote glucose output (Rodgers et al., 2005) and cholesterol metabolism (Walker et al., 2010). And in pancreas, insulin secretion can be positively affected by Sirt1 (Finkel et al., 2009; Yang et al., 2006). However, there is no data about difference of Sirt1 expression between fatty and lean animals in liver and pancreas.

Triglycerides (TAGs) are the main form of energy storage in mammalian adipose tissue. Adipose triglyceride lipase (ATGL) is known as an important lipolitic enzyme which is

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involved in the first step of TAGs hydrolysis generating diacylglycerols (DAGs) and non-esterified fatty acids (NEFAs) (Lass et al., 2011; Zimmermann et al., 2004). And hormone sensitive lipase (HSL) is an enzyme cleaving the first and the second fatty acid (FA) from TAGs (Carmen and Victor, 2006; Zimmermann et al., 2009). Sirt1 could regulate fat metabolism by controlling ATGL and HSL transcription in 3T3 and porcine adipocytes (Chakrabarti et al., 2011; Shan et al., 2010). Furthermore, ATGL and HSL gene expression is different in subcutaneous adipose tissue between fatty pigs and lean pigs (Shan et al., 2010). But there are few reports about the expression of ATGL and HSL in liver and pancreas between fatty and lean animals.

Therefore, based on our previous studies (Shan et al., 2009a, 2010), the current study was conducted to investigate breed difference of Sirt1 in liver and pancreas between fatty and lean pigs using Jinhua and Landrace as animal models. Lipolytic enzymes ATGL and HSL gene expression were also researched.

## 2. Material and methods

### 2.1. Animals and experimental design

Exp. 1, 4 male Jinhua pigs (age of 180 d, average body weight 64.25 kg) and 4 male Landrace pigs (age of 180 d, average body weight 99 kg) were selected randomly and euthanized for serum, liver and pancreas samples. Blood samples were centrifugated at 2000 rpm for 10 min to collect serum. Exp. 2, 4 male Jinhua pigs (age of 60 d, average body weight 10.33 kg) and 4 male Landrace pigs (age of 60 d, average body weight 15.15 kg) were selected randomly and euthanized for investigating breed difference at different growing stage. The liver and pancreas samples were frozen in liquid nitrogen immediately, then stored at −80 °C until use for gene expression analysis of Sirt1, ATGL and HSL. All pigs were fed *ab libitum* with same commercial diet on the same farm. The chemical composition of diet was as following: Starting diet provided 14.46 kJ $kg^{-1}$ digestible energy (DE), 19.36% crude protein, 1.28% calcium, 0.66% phosphorus and 5.44% Lysine before 30 d after weaning; From 31 d to 80 d, the diet contained 14.78 kJ $kg^{-1}$ DE, 17.78% crude protein, 0.84% calcium, 0.66% phosphorus and 6.22% Lysine; from 81 d to 180 d, the diet contained 13.51 kJ $kg^{-1}$ DE, 17% crude protein, 0.84% calcium, 0.58% phosphorus and 5.16% Lysine.

### 2.2. Serum index

The serum index such as triglyceride (TG), total cholesterol (TCHO) and glycerol were measured with commercial RIA kits (Applygen Technologies Inc. Beijing, China) and serum leptin concentration was measured with a commercially available radio-immunoassay procedure using an RIA kit (Beijing North Institute of Biotechnology) (Shan et al., 2009a). Serum total protein (TP) was also detected using commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

### 2.3. Total RNA extraction and reverse transcription

According to our previous research (Shan et al., 2009a, 2010), total RNA was extracted from the liver and pancreas tissues using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). The purity and concentration of total RNA were determined by spectrophotometry absorbance measurements at 260 and 280 nm. Then, 2 μg of RNA was used for reverse transcription using a RevertAid™ Reverse Transcriptase kit (Fermentas International INC, CA).

### 2.4. Quantitative real-time PCR

Quantitative real-time PCR was investigated using StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA) and SYBR Premix Ex Taq Kit (Takara Biotechnology Co. Ltd., Dalian, China). Gene expression was analyzed using $2^{-\Delta\Delta CT}$ method and 18S rRNA was used as reference gene (Shan et al., 2010). The primers of porcine 18S, Sirt1, ATGL and HSL were listed in Table 1 (Shan et al., 2009b, 2010). All experiments were repeated thrice.

### 2.5. Protein extraction and western blot analysis

Total protein was isolated from liver and pancreas tissues using the protein extraction reagent (Pierce Biotechnology, Rockford, IL). Then measure the protein concentration as we described previously (Shan et al., 2010). Proteins (50–100 μg) were separated on a 5–8% SDS–polyacrylamide gels and electrophoretically transferred to PVDF (polyvinylidene fluoride) membranes (Millipore Corporation, Billerica, MA), and incubated with Sirt1 antibody (sc-19857, 1:400 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or glyceraldehyde-3-phosphate (GAPDH) antibody (sc-48166, 1:400 dilution; Santa Cruz Biotechnology). The secondary antibody (anti-mouse IgG, Santa Cruz Biotechnology) was diluted 2000-fold. All incubations were performed in Tris–HCl buffer, pH 7.5, with 0.1% Tween 20 and 5% dry milk. Immunodetection was performed by using ECL Western Blotting Substrate (Pierce Biotechnology, Rockford, IL, USA). Signal intensity was determined by BANDSCAN 4.5, and means were calculated for each breed (Shan et al., 2010).

**Table 1**
Specific primers used for real-time PCR verification of the porcine Sirt1, ATGL, HSL and 18S rRNA genes.

| Gene | Forward primer | Reverse primer | Annealing temperature (°C) | Accession number |
|---|---|---|---|---|
| Sirt1 | TGGGGTTTCTGTTTCTTGTGG | CTTGAGGATCAGGAAGGTCTGG | 60 | EU030283 |
| ATGL | TCACCAACACCAGCATCCA | GCACATCTCTCGAAGCACCA | 60 | EU357899 |
| HSL | ACCCTCGGCTGTCAACTTCTT | ACTTTCTCCTCCTTGGTGCTAATCT | 60 | FJ457623 |
| 18S rRNA | CCCACGGAATCGAGAAAGAG | TTGACGGAAGGGCACCA | 60 | AY265350 |