EXPRESSION OF PERILIPIN AND GLUCOCORTICOIDS RELATIVE REGULATING GENES IN ADIPOSE TISSUE OF PIGS WITH DIFFERENT HALOTHANE GENOTYPES

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***Abstract*—This study was aimed to investigate expression of perilipin in adipose tissue of pigs and its regulation by action of glucocorticoids. The subcutaneous and kidney adipose tissue was taken from pigs with different halothane genotypes (Hal^{NN}, homeozygous wild type and Hal^{nn}, homozygous mutant) followed by determining the expression of perilipin and other related regulating genes with relative quantitative real time Real-timePCR. The results showed that there were no significantly difference between two halothane genotype pigs in body weight, back fat thickness and its index, but Hal^{nn} pigs exhibited lower tendency in kidney fat weight and its index (P =0.09 and P =0.01, respectively) than that in Hal^{NN} pigs. Two-way variance analysis results showed perilipin mRNA expression was significantly higher (P < 0.05), but lower GR mRNA expression in adipose tissue of Hal^{nn} pigs than that in Hal^{NN} pigs (P < 0.05). There were no significant differences between two halothane genotype pigs in 11β-HSD1, FAS and HSL mRNA expression. Between subcutaneous and kidney fat depots, significant differences were found for 11β-HSD1, FAS and HSL mRNA expression but not GR and perilipin. These results suggest that perilipin mRNA expression exhibits significant halothane genotype difference, and this difference may be related to GR, meanwhile difference may be exist for fat metabolism between subcutaneous fat and kidney fat.**

***Index Terms*—Pigs; perilipin; subcutaneous fat; halothane genotype**

I. INTRODUCTION

Most of triacylglycerols (TG) are deposited in lipid droplets of adipocytes. Investigations over the past decade revealed lipid droplets as regulated organelles with surprising complexity. They are coated by specific proteins, belonging to the so-called PAT family named after the three initially identified members perilipin (PLIN1), adipocyte differentiation-related protein (ADRP), and tail-interacting protein of 47 kDa (TIP47) (Tansey et al., 2003; Bickel et al., 2009). The best characterized family member is perilipin which has been shown to play a crucial role both in lipid storage and in lipolysis (Moore et al., 2005; Miyoshi et al., 2008). At basal state, perilipin surrounds the lipid droplet to block the access of intracellular lipases to the lipids. However, once phosphorylated, it triggers a massive remodeling of the lipid droplets by increasing the surface area available to lipases and it assists the hormone sensitive lipase (HSL) in gaining access to lipid substrates (Greenberg et al., 1991). Although perilipin is thoroughly investigated in rats, there are only few reports in pigs (Li et al., 2008; Tao et al., 2008).

Glucocorticoids(GC) has direct effect on the lipid metabolism, and this effect demonstrated distinct response at diversity adipose depot(Wu et al., 2003). Glucocorticoids receptor and 11β-HSD1 mediated the GC action. Previous studies showed the plasma cortisol level exist significantly difference between Hal^{NN} and Hal^{nn} pigs (Weaver et al., 2000) . Whether this difference will lead to the adipose deposition difference and this alteration is related to the perilipin, it is still unclear. So in the present study different halothane genotypes (Hal^{NN}, homeozygous wild type and Hal^{nn}, homozygous mutant) were employed to investigate expression of perilipin in adipose tissue of pigs and its possibly regulation by action of glucocorticoids.

II. MATERIALS AND METHODS

A.Animals and sample collection

Prepubertal growing boars (12 EHL and 6 PIE) at the body weight of 60 kg were sacrificed via i.v. injection with an overdose of 3% sodium pentobarbitone. Kidney fat and Subcutaneous fat samples were taken immediately and rapidly frozen in liquid nitrogen, then stored at −80 ◦C until analysis.The experiment was undertaken following the guidelines of regional Animal Ethics Committee.

B.RNA extraction and mRNA quantification

Total RNA was extracted from homogenized adipose tissuesusing TRIzol Total RNA Kit (Invitrogen Life Technologies, USA) and subsequently purified with the RNase-Free DNase Set (Promega, USA) according to the manufacturer's instructions. Total RNA concentration was then quantified by measuring the absorbance at 260 nm with a photometer (Eppendorf, Germany). Ratios of absorption (260/280nm) of all preparations were between 1.9 and 2.1.

Two μg of total RNA were reversely transcribed in a final volume of 25 μl with M-MLV reverse transcriptase (Promega, USA) and random hexamer primers (SunShine, China). Reverse transcription was performed in a Thermal Cycler PTC0200 (Bio-Rad, USA).

Real-time PCR was performed in Mx3000P (Stratagene, USA) with specific primers. All samples were normalized with the house-keeping gene 18S rRNA. The primer for perilipin (F:5'-gcctgactttgctggatgg-3' and R:5'-cttggtgctggtgtaggtcttct-3'), ADRP(F:5'-acatggcatccgttgctgtt-3' and R:5'-ggcgtaagtgttggcaatgg-3'), GR (F:5'-ccaaactctgccttgtgtgttc-3' and R:5'-tgtgctgtccttccactgct-3'), 11β-HSD1 (F:5'-ccatgctgaagcagagcaac-3' and R:5'-aagaacccgtccagagcaaa-3'), HSL (F: 5'-accctcggctgtcaacttctt-3' and R:5'-tcctccttggtgctaatctcgt-3'), FAS(F:5'-gtcctgctgaagcctaactc-3' and R:5'-tccttggaaccgtctgtg-3'), 18S (F: 5'-cccacggaatcgagaaagag-3' and R: 5'-ttgacggaagggcacca-3') were designed and synthesized by Takara Biotechnology (China). Mock-reverse-transcription and no template controls (NTC) were used to monitor possible contaminations with genomic DNA. Pooled samples made by mixing equal quantities of cDNA from all samples were used for optimizing the PCR conditions and generating standard curves for each target gene. The specificity of the reactions was checked by melting curve analyses for each gene.The 2-ΔΔCt method was used to analyze the real-time PCR data (Livak and Schmittgen, 2001).

C.Statistical analysis

The results were expressed as mean ± s.e. Data were analysed using the general linear model procedure (Statistical Packages for the Social Sciences, 2001) and the model included the halothane genotype, adipose tissue. And after analysis, it has been confirmed that there were no interation between these two factors in all indexes. Differences were considered significant when $P < 0.05$.

III. RESULTS AND DISCUSSION

There were no significantly difference between two halothane genotype pigs in body weight, back fat thickness and its index, but Hal^{nn} pigs exhibited lower tendency in kidney fat weight and its index (P =0.09 and P =0.01, respectively) than that in Hal^{NN} pigs (Table 2). Perilipin is the main lipid coated protein. Kern et al. (2004) reports that the perilipin mRNA expression is significantly related to the body fat in human. And perilipin levels are significantly decreased in transgenic mice over expressing leptin accompanied by decreased basal lipolysis (Ke et al., 2003). In the present study, perilipin mRNA expression was significantly higher ($P < 0.05$) in adipose tissue of Hal^{nn} pigs than that in Hal^{NN} pigs. And GR mRNA expression showed significanlty difference in adipose tissue between Hal^{nn} and Hal^{NN} pigs ($P < 0.05$). There were no significant differences between two halothane genotype pigs in 11β-HSD1, FAS and HSL mRNA expression. Between subcutaneous and kidney fat depots, significant differences were found for 11β-HSD1, FAS and HSL mRNA expression but not GR and perilipin.

Table 2 Body mass and index of back fat thickness and kidney fat in Hal^{NN} and Hal^{nn} pigs

Genotypes	Body mass	Back fat thickness	Back fat index	Kidney fat weight	Kidney fat index
Hal^{NN}	62.03± 1.82	0.61 ± 0.08	0.010 ± 0.001	0.31 ± 0.04	0.51± 0.07
Hal^{nn}	59.37 ± 2.17	0.47 ± 0.09	0.008 ± 0.001	0.18 ± 0.04	0.31 ± 0.07

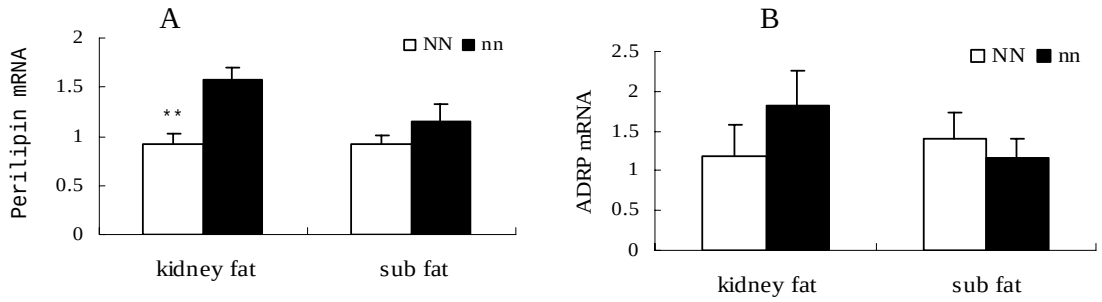

Fig. 1 Expression of perilipin (A) and ADRP(B) mRNA in adipose of Hal^{NN} and Hal^{nn} pigs

** indicates difference in the same depot position of different halothane genotype is extremely significant ($P < 0.01$).

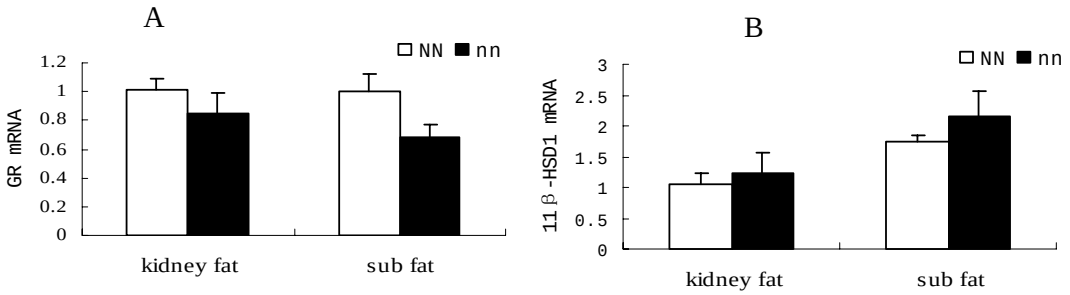

Fig. 2 Expression of GR（A）and 11β-HSD1(B) mRNA in adipose of Hal^{NN} and Hal^{nn} pigs

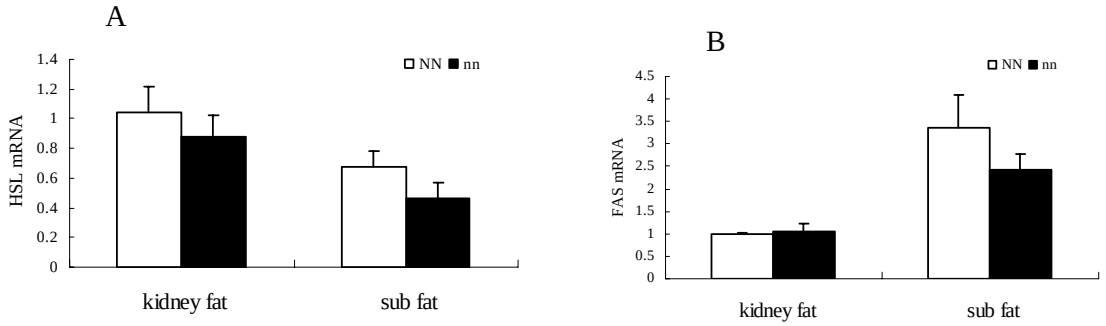

Fig. 1 Expression of FAS and HSL mRNA in adipose of Hal^{NN} and Hal^{nn} pigs

IV. CONCLUSION

These results suggest that perilipin mRNA expression exhibits significant halothane genotype difference, and this difference may be related to GR, meanwhile difference may be exist for fat metabolism between subcutaneous fat and kidney fat.

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