GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Higher expression of acyl-CoA dehydrogenase genes in adipose tissues of obese compared to lean pig breeds

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Genet. Mol. Res. 13 (1): 1684-1689 (2014)
Received September 8, 2013
Accepted November 8, 2013
Published January 22, 2014
DOI http://dx.doi.org/10.4238/2014.January.22.5

ABSTRACT. The balance between biosynthesis and oxidation of fatty acids determines adipose deposition in mammals. Obese and lean pigs show obvious differences in total adipose mass and therefore offer an attractive model for comparative studies. We found that obese Rongchang pigs, when compared with lean Landrace pigs, exhibited significantly higher mRNA levels for five genes encoding acyl-CoA dehydrogenases involved in mitochondrial fatty-acid β-oxidation in eight different adipose tissues. These changes in gene expression were positively correlated with adipocyte volume in the eight adipose tissues. Based on these results, we hypothesize that acyl-CoA dehydrogenase genes participate in the regulation of fat mass in pigs.

Key words: Fat deposition; Acyl-CoA dehydrogenases; Fatty acid oxidation; Obese and lean pigs

INTRODUCTION

Adipose tissues play an essential role in energy homeostasis and constitute the largest energy reserve, in the form of triglycerides, in the body of animals (Walewski et al., 2010). A competition between biosynthesis and oxidation of fatty acids determines the distinct metabolic characteristics of adipose tissues. Oxidative degradation of fatty acids, especially by β-oxidation in mitochondria, regulates adipose tissue distribution and total adipose mass, and is the main energy source for homeostasis (Houten and Wanders, 2010; Wang et al., 2013). Mitochondrial fatty acid β-oxidation consists of four reaction steps, i.e., dehydrogenation, hydration, further dehydrogenation and thiolysis (Wanders et al., 2010). The initial dehydrogenation, the main rate-limiting step, is catalyzed by enzymes of the acyl-CoA dehydrogenase family (Goetzman, 2009; Atsuzawa et al., 2010).

The pig is an excellent model for biomedical research on energy metabolism and obesity. Commercial pig varieties, which are relatively inbred, have undergone strong genetic selection for production of lean meat or, in some cases, adipose tissue. This has led to remarkable phenotypic changes, making these breeds perfect models for comparative studies. Adipose tissue distribution and total adipose mass are determinant factors for pork yield and flavor (Yang et al., 2010). Therefore, a better understanding of metabolic processes in the main adipose depots could provide strategies for rational alteration of adipose tissues.

In the present study, we examined eight types of adipose tissue from lean Landrace pigs and obese Rongchang pigs, and measured differences in adipocyte volume and in the expression of five genes encoding acyl-CoA dehydrogenases that target fatty acids of different length.

MATERIAL AND METHODS

Animals and tissue collection

Nine females for each of the Landrace (a leaner, Western breed) and Rongchang (a fatty, Chinese breed) pig breeds were used in this study. The pigs were sacrificed at a commercial slaughterhouse at 210 days old, when the pigs reach peak commercial value. Eight adipose tissues [abdominal subcutaneous adipose (ASA), greater omentum (GOM), intramuscular adipose (IAD), inner layer of backfat (ILB), mesenteric adipose (MAD), pericardial adipose (PAD), retroperitoneal adipose (RAD) and upper layer of backfat (ULB)] were rapidly and manually dissected from each cleaved pig. All samples were immediately submerged in RNA*later* (Qiagen, Germany) for RNA preservation.

Measurement of the adipocyte volume

All adipose tissues were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sliced at a thickness of 6 μm and stained with hematoxylin and eosin (H&E). The mean diameter of 100 adipocyte cells was determined for each sample in randomly selected fields using the Image Pro-Plus 6.0 software (Media-Cybernetics, USA) as previously reported (Li et al., 2008).

RNA isolation, cDNA synthesis, and quantitative RT-PCR (q-PCR)

Total RNA was extracted from adipose tissues using TRIzol reagent (Invitrogen,

Carlsbad, CA, USA). Total RNA was reverse transcribed to cDNA using the oligo (dT) and random 6-mer primers provided in the PrimeScript RT Master Mix kit (TaKaRa, Dalian, China), following manufacturer instructions. The q-PCR analysis was performed in the iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) using the SYBR PrimeScript RT-PCR kit (TaKaRa, Dalian, China). Five target genes (i.e., *ACADSB*, *ACADS*, *ACADM*, *ACADL*, and *ACADVL*) were normalized to the three internal control genes (*ACTB*, *TBP*, and *TOP2B*) (Erkens et al., 2006) (Table 1). All experiments contained a negative control and all q-PCR reactions were performed in triplicate.

Table 1. Information on the primers.

Gene symbol	Sequences of primers (5′→3′)	Amplicon length (bp)	Ensembl or GenBank No.
ACADSB	F: GGCAAATGTAGACCCTG R: TGTCCAATCTGTCCCAC	183	ENSSSCG00000010723
ACADS	F: CCGTGGAAAGAGCGAAAT R: CCAAACTACTCCTCCCGAAC	143	ENSSSCG00000009916
ACADM	F: CCCCTTATTATTGGTGG R: GCTTTGGTCTTTATCCC	134	ENSSSCG00000003776
ACADL	F: GTAAGAACAAATGCCAAGA R: CAGCCACTACAATCACAAC	103	ENSSSCG00000016156
ACADVL	F: AGAGCGTTGACGTTCCC R: GCTGGCAGGCATTTGAC	105	ENSSSCT00000019531
*ACTB**	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	114	ENSSSCG00000007585
*TBP**	F: GATGGACGTTCGGTTTAGG R: AGCAGCACAGTACGAGCAA	124	ENSSSCT00000029370
*TOP2B**	F: AACTGGATGATGCTAATGATGCT R: TGGAAAAACTCCGTATCTGTCTC	137	AF222921

**β* actin (*ACTB*), TATA box binding protein (*TBP*), and topoisomerase II *β* (*TOP2B*) are the housekeeping genes.

Statistical analysis

Normalization factors (NFs) of three stably expressed internal control genes were calculated using geNorm tool (Vandesompele et al., 2002). The relative mRNA levels of five target genes were normalized with dividing the raw expression data of target gene by NF (Erkens et al., 2006). All statistical analysis was conducted with the SigmaPlot 12.0 software (Systat, San Jose, CA, USA). Data are reported as the means ± SD.

RESULT AND DISCUSSION

Adipocytes of Rongchang pigs were more voluminous than those of Landrace pigs in the eight adipose tissues (Student *t*-test, $P < 0.01$) (Figure 1). This result was consistent with the known history of the two breeds, because Landrace pigs and Rongchang pigs have been selected for reduced and increased adipose tissue, respectively.

We measured mRNA levels for five genes encoding mitochondrial acyl-CoA dehydrogenases that target fatty acids of different length: short/branched chain (*ACADSB*), short-chain (*ACADS*), medium-chain (*ACADM*), long-chain (*ACADL*), and very long-chain (*ACADVL*) acyl-CoA dehydrogenases. In almost all the adipose tissues tested, mRNA levels for the five acyl-CoA dehydrogenase genes were significantly higher in Rongchang pigs than in the Landrace breed (Student *t*-test, $P < 0.01$) (Figure 2).

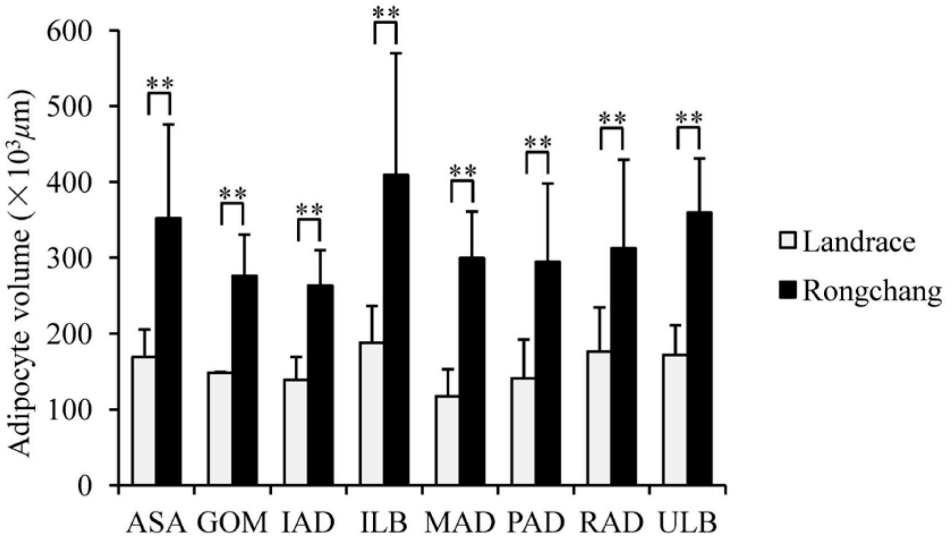

Figure 1. Adipocyte volume in Landrace and Rongchang pigs. **Statistically significant differences at the 1% level (Student's *t*-test).

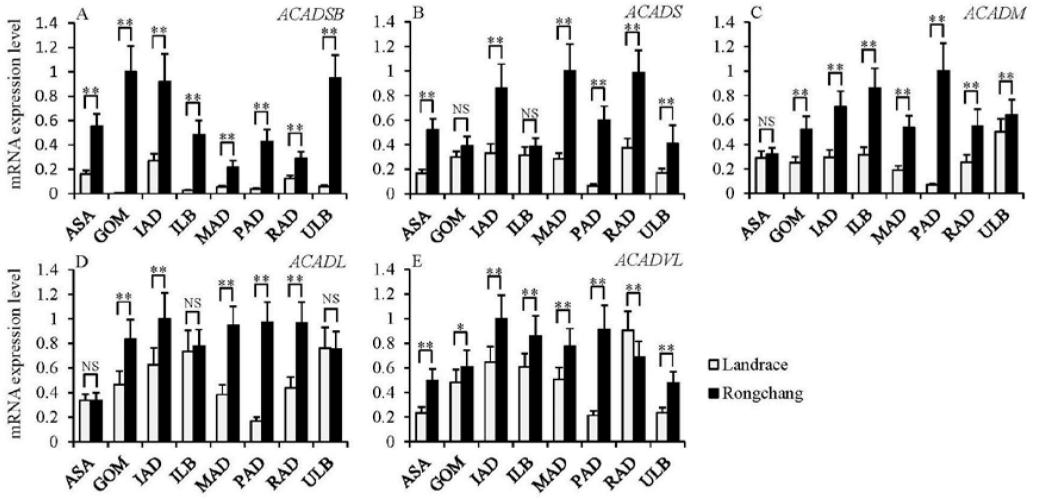

Figure 2. Levels of mRNAs for five acyl-CoA dehydrogenase genes in adipose tissues of Landrace and Rongchang pigs. *ACADSB* (**A**), *ACADS* (**B**), *ACADM* (**C**), *ACADL* (**D**), and *ACADVL* (**E**). *,**Statistically significant differences at the 5 and 1% levels, respectively (Student's *t*-test).

The *ACADSB*-encoded enzyme catalyzes the first dehydrogenation reaction of valproic acid, a fatty acid that affects branched-chain amino acid metabolism (Luís et al., 2011). Dysfunction of *ACADSB* may induce lipid oxidative damage in rats (Knebel et al., 2012). *ACADS* is mainly responsible for mitochondrial β-oxidation of C4-C6 short-chain fatty acids

(Kruger et al., 2012), and is more highly expressed in skeletal muscle of obese Korean pigs than in that of Landrace pigs (Kim et al., 2010). *ACADM*, which encodes an enzyme that can oxidize C6-C12 fatty acids (Tucci et al., 2012), is upregulated in the liver with high fat content, and its expression is positively correlated with fat content of human liver (Greco et al., 2008). *ACADL* is mainly involved in the initial oxidation step of long-chain (C10-C16) fatty acids (Tucci et al., 2012). *ACADVL*, which encodes an enzyme that can oxidize C14-C20 fatty acids (Tucci et al., 2012; Wang et al., 2013), is highly expressed in adipose tissue of high-fat diet-induced obese rats (Qiu et al., 2010; Yang et al., 2010).

In addition, we found a positive correlation between adipocyte volume and mRNA levels of the five acyl-CoA dehydrogenase genes in the adipose tissues of both pig breeds (Figure 3).

In summary, our results suggest that the genes encoding acyl-CoA dehydrogenases, which play important roles in the metabolism of fatty acids present in food, are involved in the regulation of pig fat mass.

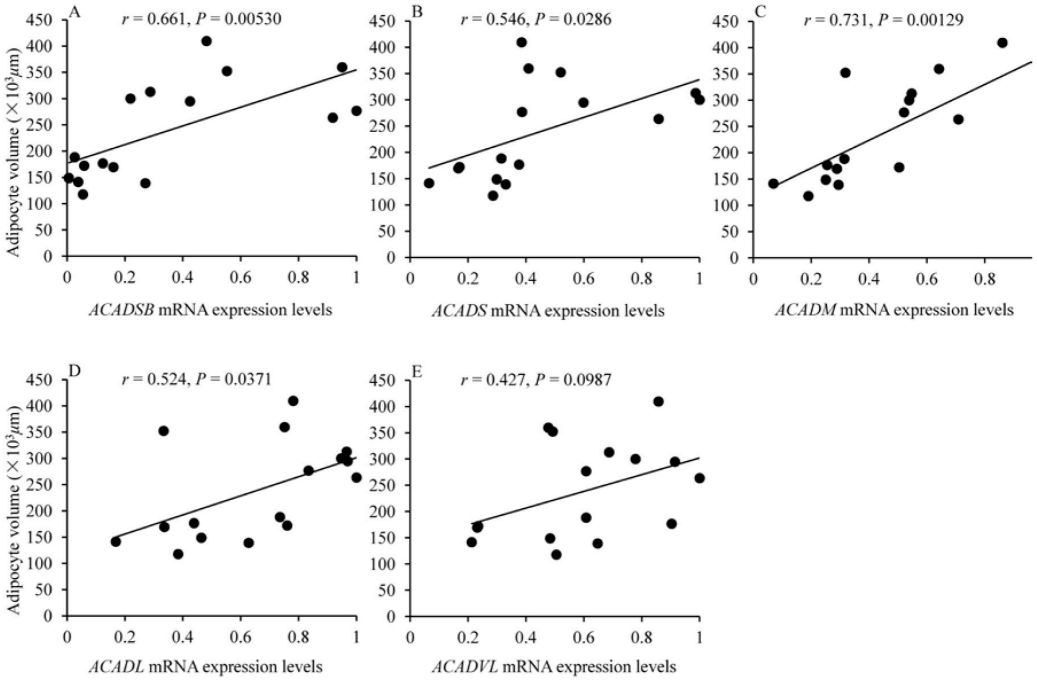

Figure 3. Pearson's correlation (N = 16) between adipocyte volume and mRNA levels for *ACADSB* (**A**), *ACADS* (**B**), *ACADM* (**C**), *ACADL* (**D**), and *ACADVL* (**E**), in eight adipose tissues of Landrace and Rongchang pigs.

ACKNOWLEDGMENTS

Research supported by the Specialized Research Fund of Ministry of Agriculture of China (#NYCYTX-009), the National Special Foundation for Transgenic Species of China (#2011ZX08006-003) (grants to X.W. Li), and the National High Technology Research and Development Program of China (''8634'' Program) (#2013AA102502) (grants to M.Z. Li).

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