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Body composition, serum lipid levels, and transcriptomic characterization in the adipose tissue of male pigs in response to sex hormone deficiency

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ABSTRACT

It is known that the male hypogonadism plays an important role in regulating adipose metabolism. In the present study, fifteen pairs of full male sibs were divided into a castrated group and an intact group with a paired experiment design. The pigs were slaughtered at an age of 175 days. The carcass characteristics and fat deposit of the studied animal were measured, and the hormone and serum lipid levels of the peripheral blood samples were determined, and the differentially expressed genes of the back fat between the two groups were screened with porcine genome array. Our results showed that the absence of male gonadal steroids attributed to castration significantly raised the serum lipid levels and increased fat accumulation in the pigs. A total of 225 differentially expressed genes were identified between the boars and barrows and 135 of them were upregulated. The analysis of Gene Ontology categories and KEGG pathway indicated that these differentially expressed genes were mainly involved in metabolism of lipid, carbohydrate, amino acid, xenobiotics biodegradation, and immune diseases pathways. Our results indicated that there were higher capacity of fatty acid of synthesis, enhanced uptaking capacity of fatty acids and cholesterol, inhibited lipolysis, and enhanced carbohydrate metabolism in the adipose tissue of barrows compared to boars. The findings of the present study provide new insight into the mechanisms of adipose metabolism induced by hormone deficiency.

1. Introduction

Male hypogonadism has been shown to be associated with obesity, diabetes, cardiovascular disease and metabolic syndrome (Kupelian et al., 2006; Ullah et al., 2011; Grosman et al., 2014; Naifar et al., 2015; Aoki et al., 2016; Zheng et al., 2016), and fully understanding pathways regulated by androgens is of fundamental significance to reveal the mechanism of this phenomenon. Male pigs are characterized by high testicular secretion of steroid hormones, and castration-induced sex hormone deficiency in male pigs has been shown to lead to increased body fat percentage and altered hormonal patterns (Christoffersen et al., 2010; Yao et al., 2011), which are similar to what has been found in human males with hypogonadism. Barrows have about 5% more separable fat than boars, and backfat from barrows contains less water, less protein, more lipids, and less unsaturated fatty acids than that from boars (Babol and Squires, 1995).

Overwhelming evidence has shown that mRNA levels are affected by environmental, physiological, and genetic factors (Cheung and Spielman, 2002; Sharbaf et al., 2016; Gan et al., 2016). Microarrays provide a powerful tool for exploring mRNA differences in biological specimens by measuring the expression levels of thousands of genes in a single experiment, alterations of which may lead to physiological and pathological changes. A number of studies have been conducted to analyze the complete porcine transcriptomes of different tissues and to identify important pathways using this technique (Lim et al., 2011; Hsu et al., 2012; Kiewisz et al., 2014).

White adipose tissue plays a critical role in energy homeostasis and is also a major endocrine organ that responds to nutrient, neural, and

Abbreviations: T, testosterone; IGF-I, insulin-like growth factor I; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; LPS, lipase; LP (a), lipoprotein a; GCOS, GeneChip Operating Software; NCBI, National Center for Biotechnology Information; RMA, robust multichip average; FDR, false discovery rate; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; DAG, diacylglycerol; TAGs, triacylglycerols; PAP, phosphatidic acid phosphatase; HK1, Hexokinase-1

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hormonal signals and secrets adipokines that control feeding, thermogenesis, immunity, and neuroendocrine function (Ahima and Flier, 2000; Galic et al., 2010). The main objective of this study was to investigate the transcriptomic characterization of the white adipose tissue of male pigs affected by castration-induced sex hormone deficiency, which previously has not been well defined. We contrasted the differences in transcriptional profiles between barrows and boars using an Affymetrix porcine oligonucleotide array analysis and determined the gene networks regulated by castration-induced sex hormone deficiency. The results of this study will help to expand our knowledge of fat accumulation and lipid metabolism due to hypogonadism.

2. Materials and methods

2.1. Ethics statement

All pigs were managed under normal husbandry conditions. All experimental animal protocols were approved and performed in accordance with the requirements of the Animal Care and Use Committee at China Agricultural University (approval ID 2008-017). Surgical castration was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Animals

Fifteen pairs of full male sibs from the crossing of Pietrain sire and Landrace dam (n = 30 male piglets) were selected at 7 d of age from the Zhonghe breeding farm in Beijing. All pigs were weaned at 30 d of age. At 7 d of age, both testicles of one piglet in each pair were removed by surgical castration under sodium pentobarbital anesthesia, and the another remained intact. Three diets were used during the growth phase according to live weight. The daily feed was comprised of the following: 63.0, 64.0, and 65.0% maize; 7.0, 10.0, and 14.0% wheat bran; 22.0, 15.0, and 10.0% soybean meal; and 2.0, 3.0, and 5.0% rapeseed meal; 5.0, 4.0, and 2.0% fish meal during the phases of live weight $<$ 50 kg, between 50 and 80 kg, and $>$ 80 kg, respectively. All pigs were allowed ad libitum access to feed and water until slaughter.

2.3. Body evaluation and tissue collection

Blood samples were collected from each pig at 35, 91, and 175 d of age. Serum was separated from the collected blood by centrifugation at 2000 × g for 15 min at 4 °C and stored at − 80 °C for subsequent analyses.

At 175 d of age, all pigs were killed by electric stunning and exsanguinations. Feeding machines were turned off 24 h before slaughter, and the surplus feed was accessed for 8 h, which resulted in a 16-hour fast. All pigs had unlimited access to water all the time. The weight of the left and right sides of each carcass was recorded, and the left side was then physically dissected into bone, muscle, fat, and skin, and the weight of each individual item recorded. Back-fat thickness was measured at the 1st, 3rd, 6th, and last rib as well as at the last lumbar vertebrae, and the middle layer of back fat at the 6th rib was removed immediately after slaughter and frozen in liquid nitrogen for RNA isolation.

2.4. Serum analyses

Serum concentrations of testosterone (T), insulin-like growth factor I (IGF-I), leptin, and insulin were measured using commercial ^{125}I RIA kits (Beijing North Institute of Biological Technology, Beijing, China). Serum total cholesterol (T-CHO), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), lipase (LPS), and lipoprotein a (LP (a)) levels were measured with a 7600-020 Automatic Analyzer (Hitachi, Tokyo, Japan). All reagents for the assays were purchased from Sekisui Medical Co., Ltd., Tokyo, Japan. All samples were assayed in triplicate, and the mean of each triplicate was used in the statistical analyses.

2.5. Total RNA isolation and microarray hybridization

According to the standard manufacturers' standard instructions, total RNA from adipose tissue was isolated with a Qiagen RNeasy Lipid Tissue Mini Kit, and residual DNA was removed from the RNA samples using an RNase-Free DNase set (Qiagen, Germany). The ratios of absorbance at 230 nm, 260 nm, and 280 nm were used to assess the concentration and purity of RNA using a NanoDrop spectrophotometer (NanoDrop ND-1000, USA). An optical density (OD) 260/280 ratio of 1.8 to 2 and an OD 260/230 of 1.8 or greater were accepted as good quality RNA. The integrity of total RNA was assessed by 1.2% (w/v) agarose gel electrophoresis, and samples exhibiting good RNA quality (an approximate 2:1 ratio of 28S to 18S) were used to generate labeled targets.

For small designs in which only few arrays are available for each biological condition, pooled RNA samples dramatically reduce the effect of biological variation and improve accuracy making substantive features easier to find (Kendziorski et al., 2005). All the fifteen pairs of full sibs were divided into six subgroups. An equal amount of total RNA from every 5 boars was pooled in a subgroup, and RNA from every 5 barrows in the corresponding full sibs was pooled in another subgroup. Each pooled RNA sample was hybridized to a separate array, and a total of six gene chips were used in this study resulting in three biological duplicates.

The microarray target sample processing, target hybridization, washing, staining, and scanning steps were processed according to the manufacturer's standard instructions (GeneChip Porcine Genome Array, Affymetrix, USA). Briefly, 5 μg of total RNA from each pooled RNA sample was used to synthesize cDNA using a one-cycle cDNA synthesis kit. The resulting cDNA was used to transcribe biotinylated cRNA by T7 RNA polymerase and was further fragmented and applied to the Affymetrix GeneChip Porcine Genome Array. Following hybridization at 45 °C for 16 h, the array was washed and stained with SAPE wash buffer in an Affymetrix GeneChip Fluidics Station 450. Fluorescent signals were scanned using an Affymetrix GeneChip Scanner 3000.

2.6. Array analysis

The Porcine Genome Array provides comprehensive coverage of the *Sus scrofa* transcriptome. The array contains 23,937 probe sets that interrogate approximately 23,256 transcripts from 20,201 *Sus scrofa* genes. Hybridization data were analyzed using GeneChip Operating Software (GCOS) v.1.4. The microarray results were deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus data repository (GenBank accession No. GSE45710; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45710). The scanned images were first assessed by visual inspection and then the CEL files generated by GCOS were used as the raw data for further analysis with R/BioConductor version 2.0 (Gentleman et al., 2004). Summarized expression values were computed using the robust multichip average (RMA) approach (Irizarry et al., 2003), corrected for batch effects, and utilized for clustering analysis and differentially expressed gene analysis using a moderated t-statistic. In detail, to identify genes that were differentially expressed in the microarray data (barrow and boar samples), we performed a differentially expressed gene analysis using a moderated t-statistic as implemented in the limma package to assess differential expression using linear models (Smyth, 2004). Limma simultaneously analyzes comparisons between many RNA targets in arbitrary, complicated designed experiments. Empirical Bayesian methods were used to provide stable results even when the number of arrays was small. The P-values calculated for each gene by limma were adjusted to Q-values after correcting for multiple testing using the Benjamini and Hochberg false discovery rate (FDR) strategy (Benjamini and Hochberg, 1995). We set