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E-mail: jzus@zju.edu.cn

A high-fat diet increases body fat mass and up-regulates expression of genes related to adipogenesis and inflammation in a genetically lean pig*#

Xue-fen YANG, Yue-qin QIU, Li WANG, Kai-guo GAO, Zong-yong JIANG†‡

Laboratory of Animal Nutrition and Feed (South China), Ministry of Agriculture / State Key Laboratory of Livestock and Poultry Breeding / Guangdong Key Laboratory of Animal Breeding and Nutrition / Guangdong Public Laboratory of Animal Breeding and Nutrition / Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

†E-mail: jiangz28@qq.com

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Abstract: Because of their physiological similarity to humans, pigs provide an excellent model for the study of obesity. This study evaluated diet-induced adiposity in genetically lean pigs and found that body weight and energy intake did not differ between controls and pigs fed the high-fat (HF) diet for three months. However, fat mass percentage, adipocyte size, concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), insulin, and leptin in plasma were significantly higher in HF pigs than in controls. The HF diet increased the expression in backfat tissue of genes responsible for cholesterol synthesis such as *Insig-1* and *Insig-2*. Lipid metabolism-related genes including sterol regulatory element binding protein 1c (*SREBP-1c*), fatty acid synthase 1 (*FASN1*), diacylglycerol *O*-acyltransferase 2 (*DGAT2*), and fatty acid binding protein 4 (*FABP4*) were significantly up-regulated in backfat tissue, while the expression of proliferator-activated receptor-α (*PPAR-α*) and carnitine palmitoyl transferase 2 (*CPT2*), both involved in fatty acid oxidation, was reduced. In liver tissue, HF feeding significantly elevated the expression of *SREBP-1c, FASN1*, *DGAT2*, and hepatocyte nuclear factor-4α (*HNF-4α*) mRNAs. Microarray analysis further showed that the HF diet had a significant effect on the expression of 576 genes. Among these, 108 genes were related to 21 pathways, with 20 genes involved in adiposity deposition and 26 related to immune response. Our results suggest that an HF diet can induce genetically lean pigs into obesity with body fat mass expansion and adipose-related inflammation.

Key words: Genetically lean pig; Diet-induced obesity; High-fat diet; Adiposity deposition; Microarray analysis; Inflammation
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‡ Corresponding author

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ORCID: Yue-qin QIU, https://orcid.org/0000-0002-6989-7026

1 Introduction

A high-fat (HF) diet is accepted as a critical factor leading to the obesity epidemic (Schrauwen and Westerterp, 2000; Bray et al., 2004). Considerable interest has been shown in using pigs as models for human obesity research because of the physiological and pathophysiological similarities between pigs and humans (Rocha and Plastow, 2006). Most pig models are minipigs that are genetically predisposed to obesity and associated disorders (Christoffersen et al., 2013; Toedebusch et al., 2014). However, little information exists regarding the relevant characteristics of a polygenic model of diet-induced obesity in pigs. Domestic pigs, especially the fast-growing lean strains that have undergone intense selection for production traits, may provide a useful alternative for the investigation of the pathogenesis of diet-induced obesity (Rocha and Plastow, 2006). In the present study, we investigated whether an HF diet could induce domestic lean pigs into obesity and thus body fat accumulation. We determined the transcriptome of subcutaneous adipose tissue and liver tissue in this polygenic model of diet-induced obesity.

2 Materials and methods

Experimental procedures for this study were approved by the Animal Experimental Committee of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China.

2.1 Animals and diets

One hundred and twelve castrated male pigs (Duroc-Landrace-Yorkshire) of similar age ((55±3) d) and body weight (BW, (18.06±0.22) kg) were randomly assigned to either a control (4% fat, 13.38 MJ/kg feed) or HF (11.4% fat, 15.55 MJ/kg feed) diet for three months. Animals undergoing each treatment were maintained in seven replicate pens, each pen housing eight pigs. The concentrations of proteins, essential amino acids, and other nutrients contained in both diets were adjusted to meet the required National Research Council (NRC; 1998) minimal levels (Table 1) for 20 to 60 kg pigs.

2.2 Experimental design and tissue sampling

The pigs were provided with unlimited access to their respective experimental diets and water. Feed intake on a per pen basis and individual BW were recorded weekly. One week before the end of the experiment, feed was withheld for 12 h before heparinized blood was collected from the anterior vena cava using vacuum tubes (with anticoagulant). The blood was then centrifuged for 10 min at 3000*g* at 4 °C, and obtained plasma was stored at −80 °C until further analysis.

At the end of the experiment, one pig with BW closest to the average BW of each pen was killed for sample collection (n=7 for both groups). Pigs were electrically stunned, exsanguinated, and processed following standard commercial procedures. The eviscerated carcass was split longitudinally through the midline, and the left side was weighed and dissected into lean mass, major white fat mass, bone, and skin.

Table 1 Composition and nutrient levels of control and high-fat diets

Group	Ingredient (%)						
	Corn	Soybean meal	Soy oil	Wheat bran	Fish meal	Premix*	Total
Control	66.10	24.00	0.00	4.50	1.40	4.00	100.00
High fat	61.45	24.00	7.55	0.00	3.00	4.00	100.00

Group	Nutrient value				
	Digestive energy (MJ/kg, measured)	Crude protein (%)	Lysine (%)	Methionine+cystine (%)	Crude fat (%)
Control	13.38	16.10	0.82	0.59	4.00
High fat	15.55	16.00	0.86	0.59	11.40

Experimental diets were self-made following commercial procedures at the feed mill located in the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China. * The following nutrients per kilogram of diet were provided: cholecalciferol 700 IU; retinol 5000 IU; α-tocopherol 25 mg; menadione 2.5 mg; thiamin 1.5 mg; riboflavin 5 mg; cyanocobalamin 0.02 mg; pantothenic acid 7.5 mg; nicotinic acid 20 mg; folic acid 0.5 mg; biotin 0.04 mg; Cu 8 mg; Fe 60 mg; Zn 60 mg; Mn 35 mg; I 0.35 mg; Se 0.3 mg

These tissues were weighed and calculated as proportions of the left half-carcass. Samples of the dorsal white adipose tissue (WAT) and longissimus muscle between the 9th and 10th ribs were obtained from the right half-carcass and immediately stored in 10% formaldehyde solution for subsequent histological analysis. Samples of the dorsal WAT and liver tissue were also snap-frozen in liquid nitrogen and stored at −80 °C, pending RNA isolation.

2.3 Tissue histology

The histological examination of the dorsal WAT and muscle samples was conducted following procedures described previously (Ma et al., 2015), and the diameter, volume, and density of adipocytes and muscle fibers were determined.

2.4 Plasma biochemical analysis

The plasma concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glucose were measured with an automatic biochemical analyzer (Beckman CX5, Beckman Coulter Inc., Brea, CA, USA) using Beckman assay kits (TC#467825, TG#445850, HDL-C#650207, LDL-C#969706, and Glucose#442640, respectively). The plasma concentrations of insulin (H203) and leptin (H174) were measured using commercial radioimmunoassay kits (NBI, Beijing, China).

2.5 Real-time PCR validation

Total RNA was isolated from dorsal adipose and liver tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA (1 μg) was synthesized into complementary DNAs (cDNAs) using a reverse transcription kit (TaKaRa, Tokyo, Japan). The synthesized cDNA was diluted (1:10, v/v) and real-time quantitative polymerase chain reaction (qPCR) amplification was determined with SYBR green I (TaKaRa) and specific primers for porcine messenger RNA (mRNA) sequences (Table 2). Conditions for qPCR were an initial denaturation at 95 °C for 180 s, followed by 40 cycles at 95 °C for 15 s and 58 °C for 30 s, with a final elongation at 72 °C for 30 s. The expression of the target genes relative to the β-actin was evaluated by the $2^{-\Delta\Delta C_T}$ method: $\Delta C_T = C_{T\ (\text{target gene})} - C_{T\ (\beta\text{-actin})}$ and $\Delta\Delta C_T = \Delta C_{T\ (\text{HF diet pigs})} - \Delta C_{T\ (\text{control pigs})}$.

2.6 Porcine microarray analysis

To explore the molecular basis for the effects of an HF diet on adiposity and obesity, cDNA microarray analysis was used to profile transcripts in dorsal WAT. GeneChip Porcine Genome Arrays (Affymetrix, Santa Clara, CA, USA), containing 23937 probe sets that interrogated approximately 23256 transcripts from twenty 201 *Sus scrofa* genes, were used. Total RNA was extracted from the dorsal WAT of individual pigs using TRIzol reagent (Invitrogen). The integrity and concentration of RNA were evaluated with Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA), and the RNA was further purified using an RNA cleanup kit (Qiagen, Dusseldorf, Germany). Equal amounts of total RNA from the seven individual pigs per treatment were pooled. Approximately 6 μg of RNA from each treatment group was amplified and labeled using the Affymetrix 3' IVT labeling kit (Lot No. 900228) and then hybridized with the GeneChip arrays for 16 h. After hybridization, the arrays were washed and stained using an Affymetrix fluidics station 450 and then scanned using an Affymetrix 3000 7G Plus scanner. The genes that met the criteria of a false discover rate value of ≤0.05 and an average fold change of at least 1.5 in either direction were considered to be significant. The GenMAPP-CS software package (http://www.genmapp.org) was used for gene ontology (GO) and pathway analyses.

2.7 Statistical analysis

Parameters including Insig-1, Insig-2, HMG-CoA reductase (HMGCR), sterol regulatory element binding protein 1c (SREBP-1c), fatty acid synthase 1 (FASN1), hepatocyte nuclear factor-4α (HNF-4α), diacylglycerol *O*-acyltransferase 1 (DGAT1), DGAT2, fatty acid binding protein 4 (FABP4), carnitine palmitoyl transferase 1 (CPT1), CPT2, proliferator-activated receptor (PPAR)-γ, and PPAR-α were performed using GraphPad Prism Version 5 (GraphPad software), and data were presented as mean±standard error of mean (SEM). Significance was evaluated using a two-tailed Student's *t*-test. A value of $P<0.05$ was considered a statistically significant difference. Data (BW at baseline, BW at the end of experiment, average daily feed intake, average daily energy intake, percentage fat mass, adipocyte diameter, adipocyte volume, adipocyte density, percentage lean mass, muscle fiber diameter, muscle fiber density, TC, TG, HDL-C,

Table 2 Primer sequences used in this study

Gene	Direction	Sequence (5'→3')	Product size (bp)	GenBank accession
DGAT1	Forward	AGGACGGACACGACGAT	287	NM_214051.1
	Reverse	GAACGCAGTCACAGCAAA		
DGAT2	Forward	TCCTGTCTTTCCTCGTGC	131	NM_001160080.1
	Reverse	ACCTTTCTTGGGCGTGT		
HNF-4α	Forward	ATCGCCACCATCGTCAA	200	NM_001044571.1
	Reverse	CCTCACCCTTTCCACTACCA		
SREBP-1c	Forward	AAGCGGACGGCTCACAA	121	NM_214157.1
	Reverse	GCAAGACGGCGGATTTATT		
FASN1	Forward	CCTGGGAAGAGTGTAAGCA	108	NM_001099930.1
	Reverse	GGAACTCGGACATAGCG		
PPAR-γ	Forward	AAGACGGGGTCCTCATCTCC	149	NM_214379.1
	Reverse	CGCCAGGTCGCTGTCATCT		
PPAR-a	Forward	TCAAGAGCCTGAGGAAACCC	153	NM_001044526.1
	Reverse	CAAATGATAGCAGCCACAAAGAG		
β-actin	Forward	CATCGTCCACCGCAAAT	210	NC_010445
	Reverse	TGTCACCTTCACCGTTCC		
FABP4	Forward	GTTACGGCTTCTTTCTC	293	NM_001002817.1
	Reverse	ATGGTGCTCTTGACTTT		
Insig-1	Forward	GGACTCCTGGACAAAACG	191	NM_001244521.1
	Reverse	GATTCGGGATTCTGGATAAA		
Insig-2	Forward	CTTTGGTTCAGACAGTTGG	225	NM_001129968.1
	Reverse	CTACGCACCGCATTACA		
HMGCR	Forward	ACAGGATGAAGTAAGGGAGA	234	NM_001122988.1
	Reverse	CGAAGTAGGTGGCGAGA		
CPT1	Forward	GAAACGGAGCAAGGGTA	182	NM_001007191.1
	Reverse	GTGGTGAACGGAAAGGA		
CPT2	Forward	CACTTGTTTGCTTTGCG	130	NM_001246243.1
	Reverse	GCTGGTGGACAGGATGTT		
CES1	Forward	AGCAGGAGGGGAAAGTG	193	NM_214246.2
	Reverse	CAGGCAGTGAACAAAGACA		
CES3	Forward	GGGACTCCTGCTTTCTGT	172	NM_001243625.1
	Reverse	TGGGCTATGCTGTAATGG		
COMT	Forward	CTCGCAGATGGTGGATG	138	NM_001195330
	Reverse	AGTTGGCAGAAGTGGGTT		
SC4MOL	Forward	GGAAAACCAGTGGAAATG	186	NM_213752.1
	Reverse	TGCCAGGTATCCTCAATC		
CCL2	Forward	GAAGAGTCACCAGCAGCAAG	215	NM_214214.1
	Reverse	TGAGGGTATTTAGGGCAAGTT		
CXCL12	Forward	TGCCCTTGCCGATTCTT	193	NM_001009580
	Reverse	GAGTGGGACTGGGTTTGTTT		

DGAT1, diacylglycerol *O*-acyltransferase 1; *DGAT2*, diacylglycerol *O*-acyltransferase 2; *HNF-4α*, hepatocyte nuclear factor-4α; *SREBP-1c*, sterol regulatory element binding protein 1c; *FASN1*, fatty acid synthase 1; *PPAR-γ*, proliferator-activated receptor-γ; *PPAR-α*, proliferator-activated receptor α; *FABP4*, fatty acid binding protein 4; *HMGCR*, HMG-CoA reductase; *CPT1*, carnitine palmitoyl transferase 1; *CPT2*, carnitine palmitoyl transferase 2; *CES1*, carboxylesterase 1; *CES3*, carboxylesterase 3; *COMT*, catechol-*O*-methyltransferase; *SC4MOL*, sterol-C4-methyl oxidase-like; *CCL2*, chemokine (C–C motif) ligand 2; *CXCL12*, chemokine (C–X–C motif) ligand 12

LDL-C, glucose, insulin, and leptin) were analyzed using the general linear model (GLM) procedure of SAS 9.2 (SAS Institute, Cary, NC, USA), and presented as mean±SEM. P value of <0.05 was considered significant.

3 Results

3.1 Growth performance

No difference was detected in BW between the two dietary groups of pigs, either at the start (baseline) or at the end of the experiment (Table 3). The average daily feed intake in the HF diet group was significantly less than that in the control diet group (P<0.05), whereas energy intake did not differ between the groups.

3.2 Body composition and histological analyses of the dorsal WAT

The lean mass, dorsal muscle fiber diameter, and density were lower in the control group than in the HF-fed pigs, although with no significant statistics (Table 4, Figs. 1c and 1d). By contrast, the percentage

Table 3 Growth performance

Group	BW at baseline (kg)	BW at the end of experiment (kg)	Average daily feed intake (kg/d)	Average daily energy intake (MJ/d)
Control (n=56)	18.48±0.59	72.45±1.72	1.90±0.04	25.41±0.59
High fat (n=54)[1]	18.03±0.34	70.40±1.40	1.55±0.03*	24.12±0.62

Data were analyzed using the GLM procedure of SAS 9.2, and data are presented as mean±SEM. [1] During the experiment, two pigs were eliminated from the high-fat diet group due to disease. * P<0.05, vs. the control group

Table 4 Body composition and dorsal white adipose tissue histological results

Group	Percentage fat mass (%)	Adipocyte diameter (μm)	Adipocyte volume ($\times10^6$ μm^3)	Adipocyte density (amount/g)
Control (n=7)	12.13±0.93	94.91±1.61	0.45±0.02	2.46±0.12
High fat (n=7)	16.64±0.18*	110.67±4.66*	0.72±0.09*	1.59±0.20*

Group	Percentage lean mass (%)	Muscle fiber diameter (μm)	Muscle fiber density (amount/mm^2)
Control (n=7)	64.71±2.41	48.99±2.24	0.90±0.10
High fat (n=7)	61.53±1.01	46.35±2.76	1.07±0.07

Data were analyzed using the GLM procedure of SAS 9.2, and data are presented as mean±SEM. * P<0.05, vs. the control group

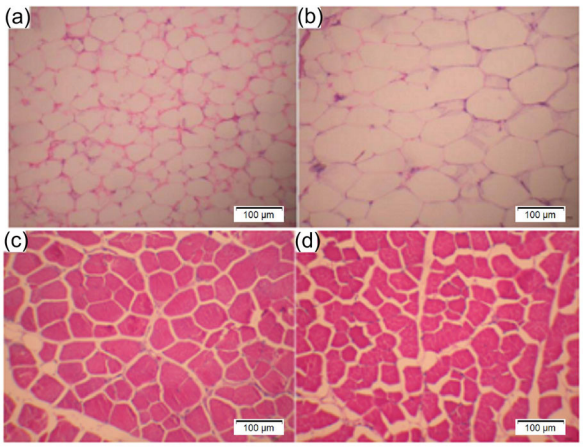

Fig. 1 Hematoxylin and eosin staining of dorsal white adipose tissue and dorsal muscle tissue
Representative images of dorsal white adipose tissue from control pigs (a) and high-fat diet-induced obese pigs (b); dorsal muscle tissue from control pigs (c) and high-fat diet-induced obese pigs (d)

of fat mass in the HF pigs was 37.2% higher than that in the control animals (P<0.05). The histological characteristics of the dorsal WAT are shown in Table 4 and Figs. 1a and 1b. The diameter (P<0.05) and volume (P<0.05) of adipocytes in the dorsal WAT were significantly increased in the HF pigs compared with the control pigs. The density of adipocytes (number per unit sectioned area) in WAT was 64.6% higher in the HF pigs than in the control pigs (P<0.05).

3.3 Increased levels of TC, TG, HDL-C, LDL-C, insulin, and leptin in HF-diet pigs

The plasma concentrations of TC, TG, HDL-C, LDL-C, and glucose were identical in the HF and control pigs at the beginning of experiment (data not shown), but after feeding for three months, the concentrations of TG, TC, HDL-C, and LDL-C were higher in HF pigs than in controls (all P<0.05, Table 5). The HF pigs also showed higher plasma concentrations of insulin and leptin (both P<0.05) than controls, but there was no difference in plasma concentration of glucose (Table 5).

3.4 Effect of HF diet on adipose expression of genes responsible for cholesterol biosynthesis and lipid metabolism in adipose and liver tissues

The relative abundances of *Insig-1* and *Insig-2* transcripts in the backfat tissue of pigs fed the HF diet increased (3.15- and 2.5-fold, respectively; Fig. 2a, P<0.05). Of the selected genes involved in lipid metabolism, relative transcript abundances of *SREBP-1c*, *FASN1*, and *DGAT2* all increased, while those of *PPAR-α* and *CPT2* decreased; the increase in *FABP4* transcripts (2.87-fold) was quite substantial. In liver tissue, HF feeding significantly elevated the expression of *SREBP-1c*, *FASN1*, *DGAT2*, and *HNF-4α* mRNA (P<0.05) and somewhat elevated mRNA expression of *HMGCR* and *Insig-1* genes, although without a statistically significant difference between

Table 5 Plasma biochemical indices and hormone levels

Group	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Glucose (mmol/L)	Insulin (μIU/ml)	Leptin (ng/ml)
Control (n=7)	2.30±0.09	0.39±0.02	0.79±0.04	1.02±0.04	6.79±0.58	9.43±0.35	1.32±0.03
High fat (n=7)	2.65±0.05*	0.48±0.02*	0.95±0.04*	1.19±0.03*	6.43±0.37	12.08±0.34*	1.91±0.13*

Data were analyzed using the GLM procedure of SAS 9.2, and data are presented as mean±SEM. * P<0.05, vs. the control group

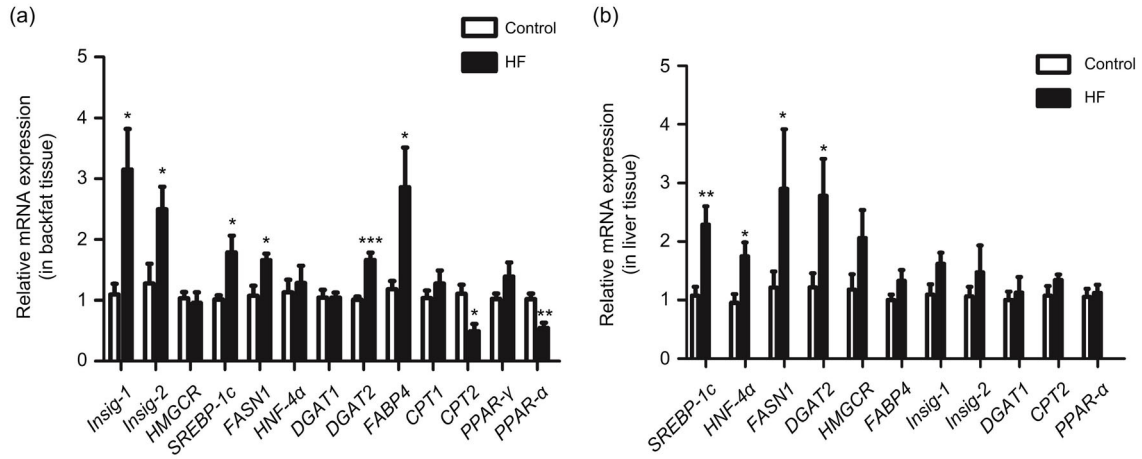

Fig. 2 Relative transcript abundance of genes involved in cholesterol synthesis and lipid metabolism in backfat tissue (a) and in liver tissue (b) of control pigs and HF-diet pigs

Data were performed using GraphPad Prism Version and expressed as mean±SEM (n=7). * P<0.05, ** P<0.01, *** P<0.001, vs. control. *HMGCR*, HMG-CoA reductase; *SREBP-1c*, sterol regulatory element binding protein 1c; *FASN1*, fatty acid synthase 1; *HNF-4α*, hepatocyte nuclear factor-4α; *DGAT1*, diacylglycerol *O*-acyltransferase 1; *DGAT2*, diacylglycerol *O*-acyltransferase 2; *FABP4*, fatty acid binding protein 4; *CPT1*, carnitine palmitoyl transferase 1; *CPT2*, carnitine palmitoyl transferase 2; *PPAR-γ*, proliferator-activated receptor-γ; *PPAR-α*, proliferator-activated receptor α; HF: high fat

the two treatment pools; the amounts of *FABP4*, *Insig-2*, *DAGT1*, *PPAR-α*, and *CPT2* in liver tissue were not affected (Fig. 2b).

3.5 Microarray analysis

A total of 576 genes differed between the two dietary pools (fold change >1.5 in either direction), of which 194 genes have detailed annotations and are accessible in the *S. scrofa* public database. Of these 194, 73 were up-regulated and 121 were down-regulated. These HF diet-responsive genes were classified using GO analysis into 15 categories of biological processes, including muscle contraction and development, ion transport and homeostasis, immune response, response to external stimulus, energy metabolism, and cell adhesion, etc. (Fig. S1).

Further analysis of the pathway maps showed that 51 up-regulated genes were significantly involved in 11 pathways (Table S1), including those associated with amyotrophic lateral sclerosis, amoebiasis, cytokine-cytokine receptor interaction, drug metabolism, insulin signaling, extracellular matrix (ECM)-receptor interaction, retinol metabolism, prion diseases, Chagas disease, the notch signaling pathway, and colorectal cancer. Of the down-regulated genes, 57 were significantly involved in the following 10 pathways (Table S2): glycolysis/gluconeogenesis, pyruvate metabolism, cardiac muscle contraction, dilated cardiomyopathy, hypertrophic cardiomyopathy, metabolic pathways, steroid hormone biosynthesis, steroid biosynthesis, propanoate metabolism, and bladder cancer. Among these 108 genes (51 up-regulated plus 57 down-regulated) that were significantly involved in various pathways, 20 genes were associated with adiposity (Table 6), including ECM remodeling (9 genes) and lipid metabolism (11 genes). Another 26 genes were involved in immune response (Table 7), including cytokine-cytokine receptor interaction (12

genes) and other immune responses (14 genes). Among these inflammation-related genes, 22 were up-regulated in pigs fed the HF diet. qPCR was further used to validate several selected gene expression results including *CES1*, *CES3*, *COMT*, *SC4MOL*, *CCL2*, and *CXCL12* obtained from microarray analysis. We observed that all data had the same direction of change by both methods, except *FASN1* (Table 8).

Table 6 Differentially expressed genes involved in adiposity in WAT from HF pigs

Pathway title	Gene ID	Description	FC (HF/control)
ECM remodeling	*COL1A2*	Collagen, type I, α2	1.96
	COL4A5	Collagen, type IV, α5	2.75
	COL5A1	Collagen, type V, α1	1.51
	COL5A3	Collagen, type V, α3	1.72
	COL6A1	Collagen, type VI, α1	1.68
	FN1	Fibronectin 1	1.63
	LAMA2	Laminin, α2	2.00
	SPP1	Secreted phosphoprotein 1/osteopontin	1.72
	LOC100513005	Thrombospondin-4-like	1.67
Lipid metabolism	*LOC100049690*	Adiponutrin	0.47
	AKR1C4	Aldo-keto reductase family 1, member C4	0.53
	CES1	Carboxylesterase 1	0.35
	CES3	Carboxylesterase 3	0.43
	COMT	Catechol-*O*-methyltransferase	0.52
	CYP19A3	Cytochrome P450 19A3	0.61
	CYP3A39	Cytochrome P450 3A39	0.53
	CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	0.53
	FASN1	Fatty acid synthase	0.54
	SC4MOL	Sterol-C4-methyl oxidase-like	0.53
	TM7SF2	Transmembrane 7 superfamily member 2	0.36

FC: fold change; HF: high fat

Table 7 Differentially expressed genes involved in adipose inflammation in WAT from HF pigs

Functional group	Gene ID	Description	FC (HF/control)
Cytokine-cytokine receptor interaction	*CCR1*	Chemokine (C–C motif) receptor 1	1.70
	CCR5	Chemokine (C–C motif) receptor 5	1.59
	CXCL12	Chemokine (C–X–C motif) ligand 12	1.71
	CXCL14	Chemokine (C–X–C motif) ligand 14	1.71
	LOC100524265	C–X–C motif chemokine 13-like	2.00
	IFNGR2	Interferon γ receptor 2	1.53
	IL18	Interleukin 18	1.70
	IL2RG	Interleukin 2 receptor, γ	2.07
	IL6	Interleukin 6	1.66
	TNF	Tumor necrosis factor	1.96
	LOC100520196	Tumor necrosis factor receptor superfamily member 3-like	1.83
	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	2.18
Other immune response	*BAX*	BCL2-associated X protein/Bax-α protein	2.87
	CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	0.48
	CASP3	Caspase 3, apoptosis-related cysteine peptidase	1.63
	CD3E	CD3e molecule, epsilon	1.83
	CCL2	Chemokine (C–C motif) ligand 2	2.15
	C-JUN	C-JUN protein/transcription factor AP-1	1.63
	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	0.50
	LOC100523020	Cytochrome c-like	1.52
	EGF	Epidermal growth factor	0.47
	FOS	FBJ murine osteosarcoma viral oncogene homolog/proto-oncogene c-Fos	1.86
	GNA14	Guanine nucleotide binding protein (G protein), α14	1.54
	LOC100153927	Similar to mitogen-activated protein kinase 1 (extracellular signal-regulated kinase 2)	0.65
	TLR4	Toll-like receptor 4	1.60
	TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	1.99

FC: fold change; HF: high fat

Table 8 qPCR validation of differentially expressed genes for microarray analysis

Gene symbol	FC (HF/control)	
	Microarray	qPCR
CES1	0.35	0.47
CES3	0.43	0.70
COMT	0.52	0.40
SC4MOL	0.53	0.68
CCL2	2.09	3.10
CXCL12	1.86	2.57
FASN1	0.54	1.55

FC: fold change; HF: high fat

4 Discussion

HF diets can lead to increased BW and body fat mass in humans and animal models (Schrauwen and Westerterp, 2000). When domestic pigs (75% Danish Landrace×25% Yorkshire; aged 3 months) were fed a high-energy diet (containing 10% (0.1 g/ml) sugar and 10% (0.1 g/ml) soy oil) ad libitum (obese group) or at 60% intake (lean group) for 5–6 months, the BW of the obese pigs was significantly higher than that of the lean pigs (Rødgaard et al., 2013). When feed intake was equivalent between the HF and control groups of minipigs, however, no difference was observed in BW (Azorín-Ortuño et al., 2012). Because feed was provided ad libitum in the present study, the daily feed intake of pigs consuming the HF diet was lower than that of pigs fed the control diet, causing no difference in energy intake or BW between the two groups (Table 3).

Body fat mass is believed to be more accurate for detecting true adiposity than the frequently used body mass index (Rocha and Plastow, 2006). In the present study, although the increased percentage of body fat mass induced by an HF diet in domestic lean pigs was not as large as that in the minipigs (Ludvigsen et al., 2015), anatomical and histological analyses showed a significantly higher percentage of body fat mass and larger adipocytes in the HF pigs compared with controls, providing further evidence for the adiposity status of these HF pigs. No significant difference was observed in the percentage of lean mass or muscle fiber size between the animals in the two groups, suggesting an independent effect of dietary fat on the expansion of fat mass.

HF diets have been widely shown to increase levels of blood lipids in animal models (Yaqoob et al., 1995; Azorín-Ortuño et al., 2012; Choi et al., 2012). Consistent with that, the present study also found that the HF diet increased plasma concentrations of TC, TG, HDL-C, and LDL-C, indicating mild hypercholesterolemia and hypertriglyceridemia in the HF pigs. Although some reports have shown significant increases in fasting serum glucose in rats subjected to long-term (5–10 months) HF diets (Chalkley et al., 2002; Choi et al., 2012), this index did not differ here, indicating that an HF diet for 3 months may not be sufficient to alter glucose homeostasis in pigs (Kim and Park, 2010; Azorín-Ortuño et al., 2012). Our result was consistent with previous studies in both swine and rat models that an HF diet leads to plasma insulin up-regulation (Guilford et al., 2017; Liu et al., 2017; Malbert et al., 2017; Yang et al., 2017), and possibly insulin resistance. The adipose-derived hormone leptin is considered to be an adiposity signal (Munzberg, 2010), and blood leptin levels are proportional to WAT stores (Gimeno and Klaman, 2005). Plasma leptin was significantly increased (45%) with fat mass expansion in the present HF pigs, confirming the increase in adiposity.

Adipose tissue is one of the most important organs for storing energy in the form of lipids and it is a major endocrine organ producing adipokines, hormones, and cytokines (Kershaw and Flier, 2004). In pigs, it was reported that adipose tissue was the central organ for fat synthesis and deposition (O'Hea and Leveille, 1969). Fat accumulation in animals depends on the levels of TG synthesis and storage and on lipid mobilization, fatty acid oxidation, and cholesterol storage (Bernlohr et al., 2002). Genetically modified animal models have highlighted that the expression of several adipogenic and lipogenic genes including *FASN1*, *DGAT*, *HNF-4α*, *SREBP-1c*, and *FABP*, plays a central role in fatty acid synthesis, TG synthesis, and lipid storage (Liu et al., 2008; Qiu et al., 2017). Consistent with these results, the present study showed that the HF diet significantly increased the relative expression of *FASN1*, *DGAT2*, *SREBP-1c*, and *FABP4* in backfat tissue and *FASN1*, *DGAT2*, *SREBP-1c*, and *HNF-4α* in the liver tissue. This study also showed that the HF diet reduced the relative expression of *CPT2* and *PPAR-α* in backfat tissue, in accordance with Li et al. (2007), who demonstrated

that these genes are related to increased fatty acid oxidation. Similarly, we found that insulin-induced genes, such as *Insig-1* and *Insig-2*, involved in cholesterol synthesis, were up-regulated by the HF diet. Coordinated regulation of pathways involving these genes in the backfat and liver tissues of HF pigs resulted in increased serum TG and cholesterol levels (15% to 23%) and increased propensity for obesity; the content of carcass fat increased by 37%.

A microarray analysis, applied to provide a transcriptional basis for changes in the WAT of HF pigs, determined that 108 genes affected by the HF diet were significantly involved in 21 pathways. Of these 108 genes, 46 (42.6%) were associated with pathways related to adiposity development or immune response.

The expression of collagen (types 1A2, 4A5, 5A1, 5A3, and 6A1) as well as fibronectin 1, laminin 2, and secreted phosphoprotein 1, was up-regulated in the WAT of HF pigs. These genes encode proteins that participate in ECM remodeling, which is indispensable during the differentiation of fibroblast-like preadipocytes into lipid-laden adipocytes (Gregoire et al., 1998). Levels of ECM proteins (type I to VI collagens, laminin, and fibronectin) are increased during adipocyte differentiation (Nakajima et al., 2002). Others also found that expression of type IV collagen is increased during this process (Weiner et al., 1989). In obese humans, adipose expression of type VI collagen (Pasarica et al., 2009) and secreted phosphoprotein 1 (encoding osteopontin protein) (Leitner et al., 2015) is up-regulated. The results obtained in our study are consistent with those reports. Some studies, however, showed decreased expression of type I collagen and fibronectin (Bortell et al., 1994) as well as of type III collagen (Weiner et al., 1989) during adipocyte development.

Abnormalities in lipid metabolism may lead to an excessive expansion of fat mass. The expression levels of numerous genes involved in lipid metabolism are reportedly up-regulated in some animal models of obesity (Kim and Park, 2010; Choi et al., 2012). Other studies reported that expression levels of cytochrome P450 (Campión et al., 2006), fatty acid transporter protein, and glycerol-3-phosphate dehydrogenase (Soukas et al., 2000) are down-regulated in obese subjects. In the present study, all the genes significantly involved in lipid metabolism were down-regulated by the HF diet. Most of these genes, including adiponutrin, sterol-C4-methyl oxidase-like, carboxylesterase (*CES1* and *CES3*), and 3α-hydroxysteroid dehydrogenase, are involved in lipolysis. Taken together, the increased expression of ECM genes along with the decreased expression of lipolysis genes probably accounts for the observed fat mass expansion in the HF pigs.

The over-expression of genes related to immune response is a key characteristic of WAT in obese subjects (Kim and Park, 2010). The excessive expansion of fat mass induces chronic inflammation in adipose tissue. Evidence shows that inflammatory pathways are activated in adipose tissue from obese subjects (Gimeno and Klaman, 2005; Choi et al., 2012). Consistent with these results, we found that 85% of the genes significantly involved in adipose inflammation were up-regulated in the HF pigs.

5 Conclusions

Obesity can be induced in genetically lean pigs by feeding an HF diet for three months with substantial expansion of body fat mass and transcriptional evidence of adipose-related inflammation. This genetically lean pig as a model for the study of diet-induced obesity may provide a useful and novel tool for research into metabolic diseases.

Contributors

Xue-fen YANG and Zong-yong JIANG conceived and designed the experiments. Yue-qin QIU, Li WANG, and Kai-guo GAO performed the experiments. Yue-qin QIU analyzed the data. Xue-fen YANG and Yue-qin QIU wrote the paper.

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Compliance with ethics guidelines

Xue-fen YANG, Yue-qin QIU, Li WANG, Kai-guo GAO, and Zong-yong JIANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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List of electronic supplementary materials

Fig. S1 Gene ontology (GO) analyses showing the significant differentially expressed genes
Table S1 Significant pathways of up-regulated genes
Table S2 Significant pathways of down-regulated genes

中文概要

题　目：高脂膳食促进瘦肉型猪的脂肪沉积及上调与脂肪生成和脂肪炎症相关基因的表达

目　的：研究高脂膳食是否会引起瘦肉型猪肥胖。

创新点：发现给瘦肉型猪饲喂高脂膳食，会引起其脂肪沉积，同时导致肥胖及脂肪炎症。

方　法：采用化学组织切片技术测量脂肪细胞和肌肉细胞的直径、体积及密度；基因芯片检测瘦肉型杜长大猪脂肪组织相关基因的变化；荧光定量聚合酶链反应（qPCR）技术检测脂肪和肝脏组织中 *Insig-1*、*Insig-2*、*HMGCR*、*SREBP-1c*、*HNF-4α*、*FASN1*、*DGAT1*、*DGAT2*、*FABP4*、*CPT1*、*CPT2*、*PPAR-γ* 及 *PPAR-α* 等基因表达水平；采用血浆生化指标仪试剂盒检测猪血浆中甘油三酯、葡萄糖、总胆固醇、高密度脂蛋白胆固醇、低密度蛋白胆固醇、胰岛素及瘦素的含量。

结　论：结果表明，高脂膳食增加瘦肉型猪的脂肪质量，增大脂肪细胞，而且增加血浆中总胆固醇、甘油三酯、高密度脂蛋白胆固醇、低密度脂蛋白胆固醇、胰岛素和瘦素的含量。高脂膳食增加背部脂肪组织中 *Insig-1*、*Insig-2*、*SREBP-1c*、*FASN1*、*DGAT2* 和 *FABP4* 等正调节脂肪生成的基因表达量，但下调 *PPAR-α* 和 *CPT2* 的表达。在肝脏组织中，高脂膳食上调 *SREBP-1c*、*FASN1*、*DGAT2* 和 *HNF-4α* 基因表达。此外，基因芯片分析的结果发现高脂膳食上调了脂肪组织中 576 个基因的表达水平，其中 20 个基因涉及到脂肪生成，26 个基因与脂肪炎症相关。综上所述，高脂膳食可以引起瘦肉型猪的脂肪沉积并导致肥胖且伴随脂肪炎症。

关键词：瘦肉型猪；膳食引起的肥胖；高脂膳食；脂肪沉积；基因芯片分析；炎症