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Differentially Expressed Genes in Subcutaneous Fat Tissue in an Obese Pig Model Induced by a High-Fat Diet

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Abstract: This study was to develop an obese porcine model induced by a high-fat diet and determine effects of obesity on gene expression in subcutaneous fat tissue in this model. A total of 20 crossbred, castrated boars (20 kg body weight) were fed a corn-soy basal diet (Se adequate, total fat <0.82%) or the diet added with lard at 3% (20-50 kg), 5% (50-80 kg) or 7% (>80 kg) for 180 days. Results showed that pigs fed the high fat diet showed greater ($p<0.05$) body weight (146 vs. 121 kg), back fat thickness (3.5 vs. 2.3 cm) and abdominal fat content (3.5 vs. 1.9 kg), along with hyperglycemia and hyperlipidemia, compared with the control pigs. About 387 genes were found to be significantly up-regulated and 465 genes down-regulated (fold change ≥2.0) in the high-fat diet group compared to the control group. To further characterize the response of gene type to high-fat diet in subcutaneous fat tissue, 852 genes significantly altered in response to high-fat diet were classified into Gene Ontology (GO) slim terms. Genes involved in metabolic process, immune response, translation and cell cycle were significantly up-regulated ($p<0.05$). Genes involved in regulation of transcription, RNA splicing and transcription were significantly down-regulated ($p<0.05$). The results indicated that the differentially expressed genes including 26 up-regulated and five down-regulated genes were associated with significant pathways involved in organization of metabolism and primary immunodeficiency (NSFC projects 30871844, 30700585).

Key words: Obesity, pig, gene expression profile, metabolism, immune response, diet

INTRODUCTION

Obesity is generally recognized as a chronic disease defined by an overaccumulation of fat stores in adipocytes and is frequently linked with inflammation in adipose tissue and insulin resistance in peripheral tissues (Mohamed-Ali *et al*., 1998; Havel, 2002). Adipose tissue plays a critical role in metabolism, storage and release of fatty acids and the major functions of adipose tissue are to synthesize fatty acid de novo and accumulate excess energy as fat in mammals. Porcine adipose tissue has abundant mRNA for adiponectin mRNA and its receptors (Wang *et al*., 2004) and produces leptin. These results indicate that the adipose tissue in pigs can act as an endocrine tissue and express genes involved in regulating metabolism and physiological functions in other tissues. To fully understand the function of adipose tissue, an analysis of abundantly expressed genes in the tissue is needed. Currently, most of scientific literatures addressing obesity originated with rodent models but numerous disparate results between rodent models and humans (i.e., adipsin, leptin, resistin, tumor necrosis factor a and other adipokines) hindered the translation of rodent data into actionable technologies for humans (Agarwal and Garg, 2006). Compared with rodent models, pigs was an excellent model for energy metabolism and obesity in humans because it was devoid of brown fat postnatally and because of their similar metabolic features, cardiovascular systems and proportional organ sizes (Spurlock and Gabler, 2008). Although, numerous studies on obesity have been conducted, obesity induced by local grains/food was not reported. Therefore, this study was to develop an obese porcine model induced by a high-fat diet and investigate effects of obesity on the gene expression in subcutaneous fat tissue in this model.

MATERIALS AND METHODS

Animals: The protocol was approved by the Animal Care Office of Sichuan Agricultural University. A total of 20 crossbred, castrated boars (20 kg body weight, obtained from the Animal Care Office of Sichuan Agricultural University) were fed a corn-soy basal diet (Se adequate, total fat <0.82%) or the diet added with lard at 3% (20-50 kg), 5% (50-80 kg) or 7% (>80 kg) for 180 days (Table 1). Pigs were housed in individual pens with free access to diet and water. Routine immunizations were performed.

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Table 1: Ingredients and chemical composition of the control and high-fat diet (3, 5 and 7% lard)

	Control (g kg^{-1})			High-fat (g kg^{-1})		
Ingredients[1]	20-50 kg	50-80 kg	80-120 kg	20-50 kg	50-80 kg	80-120 kg
Corn	740.00	800.00	855.00	680.00	730.00	760.00
Soybean meal	193.00	141.00	90.00	225.00	160.00	110.00
Fish flour	20.00	20.00	20.00	20.00	20.00	20.00
Wheat bran	20.00	20.00	20.00	20.00	20.00	20.00
Lard	-	-	-	30.00	50.00	70.00
L-lysine	1.90	1.20	0.60	1.70	1.30	0.90
DL-methionine	-	-	-	-	0.40	0.50
L-tryptophan	-	-	-	-	0.10	-
L-threonine	0.10	-	-	-	0.30	0.30
Salt	3.00	3.00	3.00	3.00	3.00	3.00
$CaHPO_4$	4.90	0.60	-	6.00	1.40	1.40
$CaCO_3$	11.60	8.80	8.20	11.80	9.00	8.40
Trace mineral premix[2]	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin premix[3]	0.30	0.30	0.30	0.30	0.30	0.30
Chemical composition (%)						
Digestable energy (kJ kg^{-1})	3.33	3.34	3.32	3.48	3.58	3.64
Crude protein	16.24	14.47	12.80	17.08	14.72	12.83
Calcium	0.72	0.50	0.45	0.76	0.53	0.49
Phosphorus, available	0.28	0.20	0.18	0.29	0.21	0.20
Lysine	0.84	0.65	0.51	0.89	0.71	0.57
Methionine+cystine	0.49	0.45	0.42	0.51	0.49	0.46
Threonine	0.53	0.46	0.40	0.55	0.50	0.43
Tryptophan	0.16	0.14	0.11	0.17	0.15	0.12
Isoleucine	0.56	0.48	0.41	0.61	0.50	0.42

[1]Expressed on an air-dry weight basis. [2]Trace mineral premix provided per kg diet: control diet: 20-50 kg,Fe: 112 mg; Cu: 8 mg; Zn: 112 mg; Mn: 4 mg; I: 0.25 mg; Se: 0.3 mg; 50-80 kg, Fe:130 mg; Cu: 9.0 mg; Zn: 129 mg; Mn: 5.2 mg; I: 0.35 mg; Se: 0.4 mg; 80-120 kg,Fe: 123 mg; Cu: 9.3 mg; Zn: 154 mg; Mn: 6.2 mg; I: 0.42 mg; Se: 0.4 mg; High-fat diet: 20-50 kg,Fe: 117 mg; Cu: 8.4 mg; Zn: 117 mg; Mn: 4.2 mg; I: 0.26 mg; Se: 0.31 mg; 50-80 kg, Fe: 139.4 mg; Cu: 9.7 mg; Zn: 138.3 mg; Mn: 5.6 mg; I: 0.38 mg; Se: 0.43 mg; 80-120 kg, Fe: 134.86 mg; Cu: 10.2 mg; Zn: 168.8 mg; Mn: 6.8 mg; I: 0.46 mg; Se: 0.44 mg; [3]Vitamin premix provided kg^{-1} diet: retinyl acetate, 3027 mg; cholecalciferol, 22 mg; dl-a-tocopheryl acetate, 32 mg; menadione, 2 mg; thiamin, 4 mg; riboflavin, 14 mg; calcium pantothenate, 40 mg; niacin, 60 mg; pyridoxol, 6 mg; d-biotin, 0.2 mg; folacin, mg and cobalamin, 72 mg

Treatments and sampling: Body Weight (BW) and feed intake was recorded on day 1st and 180th of the trial to calculate the Average Daily Gain (ADG) and Average Daily Feed Intake (ADFI). Blood samples were taken via the anterior vena cava from overnight-fasted pigs at baseline and after 2, 4 and 6 months of treatment from individual pigs. Blood was immediately transferred to either tubes containing 0.75 USP units of sodium heparin/mL whole blood or non-heparinized tubes. Plasma samples were then centrifuged at 18,000×g for 2 min, serum samples at 2200×g for 15 min (5804R centrifuge, F45-30-11 rotor, Eppendorf) supernatants were stored at -80°C until analysis. At the end of the study, 8 pigs treatment^{-1} group were slaughtered and collected subcutaneous fat tissue. All samples were frozen in liquid nitrogen until analysis.

Plasma glucose, serum insulin and serum lipid profile: Pig (n = 8 group^{-1}) were fasted overnight for 8 h before determination of plasma glucose, serum insulin, serum lipid profile (total cholesterol, triglyceride, low density lipoprotein) and serum Nonesterified Fatty Acid (NEFA). Plasma glucose, serum insulin, total cholesterol (TC-test kit), LDL-C (LDL-C-test kit) and triglycerides (TG-test kit) were determined by an automated clinical chemistry analyzer (Roche, Model 800, Switzerland). Serum NEFA concentration was measured with commercial analysis kits obtained from the Jian Cheng Institute of Biotechnology (Nanjing, China).

Body fat profile: The backfat thickness was measured in the 5th rib (Lei *et al*., 2007) by ultrasound technology and abdominal fat was weighted.

Microarray analysis: Eight subcutaneous fat tissue samples, four derived from control diet group and four from high-fat diet group were collected in order to screen differentially expressed genes. Total RNA was isolated from frozen tissues (50 mg) using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Total RNA concentration was assessed by spectrophotometry (OD 260 nm) and purity and integrity of the RNA were determined by the absorbance ratio at 260/280 nm and visualization after agarose gel electrophoresis. An aliquot of 1 μg of total RNA was used to synthesize double-stranded cDNA prepared by T7 oligo (dT)-primed reverse transcription using a Eukaryotic Poly-A RNA Control kit (Affymetrix, Inc.); biotin-tagged cRNA was produced using the Custom MessageAmp™ II-Biotin aRNA Amplification kit (Ambion, Austin, TX,

USA.). Bio-tagged cRNA (15 μg) was fragmented to produce strands 50-200 nt in length using protocols from Affymetrix. The fragmented cRNA was hybridized to Affymetrix Bovine Genome Array containing 23,000 transcripts. Hybridization was performed at 45°C for 16 h (Affymetrix® GeneChip hybridization oven 640). The GeneChip arrays were washed and stained (streptavidin-phycoerythrin) on an Affymetrix®Fluidics station 450 (Affymetrix, Inc.) followed by scanning on an Affymetrix® GeneChip® scanner 3000.

Hybridization data were analyzed using GeneChip operating software (Gcos 1.4). Raw data from the cel files were converted to gene signal files using MAS 5.0. The expression data were loaded into GeneSpring GX 10.0 software (Agilent technologies) for data normalization and filtering. Gene detection call (present, marginal and absent) and expression levels of probesets were determined by comparing the Perfect Match (PM) and Mismatch (MM) probe-pair data also in MAS 5.0 (Wolcoxon signed rank test). In the data if a probeset had >2 present calls at least 1 age in the triplicate detection data, corresponding transcript was considered as expression in the fatt tissue transcriptome and kept for further analysis.

Normalized expression data of each transcript was subjected to log 2 transformation. An Analysis of Variance (ANOVA) test was used to identify genes of significant expression over neonatal ages in R software with age as a fixed effect. Qvalue was used to obtain False Discovery Rates (FDR). As an alternative method for the detection of differentially expressed transcripts, deriving p value for each probeset was calculated using sampling and residual shuffling permutation approach in R/MAANOVA software. False Discover Rate (FDR) was controlled by Step-down method and the FDR-adjusted permutation p values were estimated following 10000 iterations. The resulting lists of differentially expressed genes for each of both methods were compared for >1 aps. Gene Ontology (GO) and pathway analyses of these differentially expressed genes (Al-Shahrour *et al*., 2004; Alexa *et al*., 2006) were conducted using a free Web-based Molecular Annotation System 2.0 (MAS 2.0) which integrates three different open source pathway resources-KEGG, BioCarta and GenMAPP. All analysis were based on annotation information from pig databases.

Quantitative Real-Time PCR (qRT-PCR) confirmation: The same RNAs from the subcutaneous fat tissue sample were used to confirm the microarray data. Primers (Table 2) for the genes and two reference genes, *β-actin* gene (Actb) and glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) were designed using

Table 2: Primer used for target and reference genes in the Q-PCR

Genes	Accession no.	Primer pairs (5'-3' direction)
Ucp3	NM_214049	CCTCTACGACTCCGTCAAGCA
		CCAAAATCCGGGTGGTGAT
Adi	NM_214370	GCTGTTGTTGGGAGCTGTTCT
		AGGCTTCTCGGTGGTTTCCT
lepr	NM_001024587	AATCACCCGTTGGGTTCACTT
		TGGAGGCAGCGGTTTCAC
Actb	AY550069	CCCAAAGCCAACCGTGAGAA
		CCACGTACATGGCTGGGGTG
Gapdh	AF017079	CAGCAATGCCTCCTGTACCA
		CCACGATGCCGAAGTTGTC

Primer express 3.0 (Applied Biosystems). The total RNA of subcutaneous fat tissue was extracted using RNeasy lipid tissue mini kit (Qiagen no. 74804). Potential DNA contamination of the extraction was eliminated using the DNA-free kit (Ambion, catalog no. AM1906) and the RNA quality was verified by both agarose gel (1%) electrophoresis and spectrometry (A260/A280). The mRNA levels of genes were analyzed using Q-PCR (ABI 7900HT, Applied Biosystems). Briefly, RT and PCR amplifications were conducted in duplicates for both target and reference genes. Negative controls containing the template RNA and all PCR reagents but not reverse transcriptase were included to determine the RNA purity from DNA contamination. The reaction mixture (6.0 mL) contained 3.0 mL of freshly premixed 2× QuantiTect SYBR Green RT-PCR Master mix and QuantiTect RT mix (QIAGEN, catalog no. 204243), 0.4 umol L^{-1} of the primer pair and 100 ng of RNA template. The PCR consisted of 1 cycle of 48°C for 30 min, 1 cycle of 95°C for 10 min and 40 cycles of 95°C for 15 sec and 58°C for 1 min followed by the dissociation step at 95°C for 15 sec, 60°C for 20 sec and 95°C for 15 sec. To confirm the specific amplification, melt curve analysis was performed and the products were also visualized on ethidium bromide-stained 2% (wt:v) agarose gel after electrophoresis using Tris-acetate-EDTA buffer.

Relative mRNA abundances of the genes in the subcutaneous fat tissue samples were determined using the ΔCycle threshold (ΔCt) method as outlined in the protocol of Applied Biosystems. In brief, a ΔCt value was the Ct difference between the target selenoprotein gene and the reference gene ($\Delta Ct = Ct^{target} - Ct^{reference}$). Actb was used as the reference gene and its reliability was confirmed by the perfect parallelism of its Ct values in all the tissue samples. For each of the target selenoprotein genes, the ΔΔCt values of all the samples were then calculated by subtracting the average ΔCt of the liver of pigs fed of the control diet from the ΔCt of all the other tissue samples except Gpx4 and Gpx6 (the average ΔCt of the kidney of pigs fed of the control diet). The ΔΔCt values were converted to fold differences by raising 2 to the power –ΔΔCt ($2^{-\Delta\Delta Ct}$).

Statistical analysis: All results are presented as the mean±SE. Independent t-test (SPSS for Windows 10.0) was used to assess the statistical significance of differential expression levels of each gene or transcript within the eight samples (from 2 groups). Spearman correlation analysis was conducted on the results obtained from microarray and qRT-PCR technology.

RESULTS AND DISCUSSION

Body weight, hyperglycemia, hyperlipidemia and fat mass in obese pigs: After 180 days feeding as shown in Fig. 1, pigs fed the high fat diet showed greater ($p<0.05$) body weight (146 vs. 121 kg), back fat thickness (3.5 vs. 2.3 cm) and abdominal fat content (3.5 vs. 1.9 kg) than pigs fed the control diet. Blood glucose concentrations were similar between the 2 group initially (20 kg) but became 20% greater ($p<0.05$) in pigs fed the high-fat diet than pigs fed the control diet on 180 days so did of serum insulin (Table 3). Compared with the control group, the pigs fed high-fat diet showed greater ($p<0.05$) high level

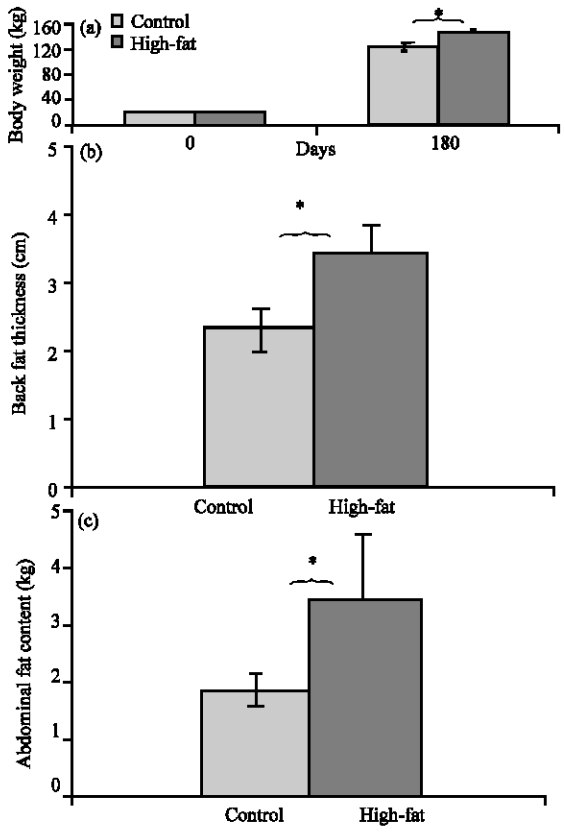

Fig. 1: Effect of high fat diet on body weight; a) back fat thickness b) abdominal content c) after 180 days feeding. Values are means±SE (n = 8 group^{-1}), $p<0.05$

in serum triglyceride (0.44 vs. 0.31), total cholesterol (4.07 vs. 2.94)) low density lipoprotein (2.13 vs. 1.33) and serum NEFA (259 vs. 101) (Table 3).

Subcutaneous fat tissue gene expression profile of obese pigs and the control: The pattern of gene expression in subcutaneous fat tissue from obese pig was analyzed by GeneChip Porcine Genome array containing 23,937 probe sets to interrogate 23,256 transcripts in pig which represents 20,201 genes. Normalized data were used to analyze the total expressed genes. About 387 genes were found to be significantly up-regulated and 465 genes down-regulated (fold change ≥2.0) in the high-fat diet group compared to the control diet group. To further characterize the response of gene type to high-fat diet in subcutaneous fat tissue, 852 genes significantly altered in response to high-fat diet were classified into Gene Ontology (GO) slim terms.

Genes involved in metabolic process, immune response, translation and cell cycle were significantly up-regulated (Table 2). Genes involved in regulation of transcription, RNA splicing and transcription were significantly down-regulated (Table 4). The results indicated that differentially expressed genes including 26 up-regulated and 5 down-regulated genes were associated with significant pathways involved in organization of metabolism and primary immunodeficiency (Table 5).

Validation of gene expression data by qRT-PCR: Three differentially expressed genes were selected to confirm their expression differences using qRT-PCR (Table 2). A t-test was conducted to compare gene expression in pigs between the control diet and high fat diet groups. The qRT-PCR data confirmed that Ucp_3 in subcutaneous fat tissue was more highly expressed in obese pigs than control.

In contrast, the opposite was true for the mRNA levels of Adi and Lepr in subcutaneous fat tissue. High-fat induced changes in gene expression closely correlated with the corresponding microarray data, although the exact fold changes differed between the two assays (Fig. 2). Obesity represents a serious threat to the health of the population of almost every country in the

Table 3: Results of plasma glucose, insulin and serum lipid profile

Serum lipid profile	Values	High fat diet	p value
Plasma glucose (mmol L^{-1})	5.17±00.43	6.21±1.20	0.05
Insulin (mmol L^{-1})	0.31±00.01	0.44±0.01	0.04
Triglycerides (mmol L^{-1})	0.31±00.01	0.44±0.01	0.02
Total cholesterol (mmol L^{-1})	2.94±00.14	4.07±0.22	0.00
LDL-cholesterol ((mmol L^{-1})	1.33±00.14	2.13±0.21	0.01
Serum nonesterified fatty acid NEFA (umol L^{-1})	101±30	259±240	0.01

Values are means±SEM; samples are taken after 180 days either diet (n = 7-8)

Table 4: Some of differentially expressed genes revealed by microarray analysis[a]

GO IDs	GO terms	Dif. gene (n)	-log P	Percentage (%)[b]
Up reg. (N = 11)				
GO:0002286	T cell activation involved in immune response	5	14.4	-
GO:0033157	Regulation of intracellular protein transport	4	13.2	-
GO:0006412	Translation	1	11.7	-
GO:0008152	Metabolic process	5	10.1	-
GO:0055085	Transmembrane transport	1	8.8	-
GO:0051017	Actin filament bundle assembly	4	8.6	-
GO:0045449	Regulation of transcription	17	8.5	21.8
GO:0006350	Transcription	17	7.7	21.8
GO:0034349	Glial cell apoptosis	2	7.5	
GO:0007049	Cell cycle	3	7.3	
GO:0006810	Transport	19	7.0	24.4
Down reg. (N = 3)				
GO:0045449	Regulation of transcription	16	7.6	48.5
GO:0008380	RNA splicing	1	7.2	
GO:0006350	Transcription	16	6.9	48.5

[a]p≤0.01, arrange by-log P from high to low

Table 5: Significant pathways involved in the differentially expressed genes[a]

Path IDs	Path terms	Dif. gene	-log P
Up reg. (N = 6)			
Path:ssc00591	Linoleic acid metabolism	5	14.0
Path:ssc00982	Drug metabolism-cytochrome P450	5	9.7
Path:ssc00980	Metabolism of xenobiotics by cytochrome P450	5	9.7
Path:ssc00830	Retinol metabolism	4	8.7
Path:ssc00590	Arachidonic acid metabolism	4	7.0
Path:ssc04115	p53 signaling pathway	3	4.4
Down reg. (N = 2)			
Path:ssc05340	Primary immunodeficiency	3	6.2
Path:ssc04614	Renin-angiotensin system	2	4.6

[a]p≤0.01, arrange by-log P from high to low

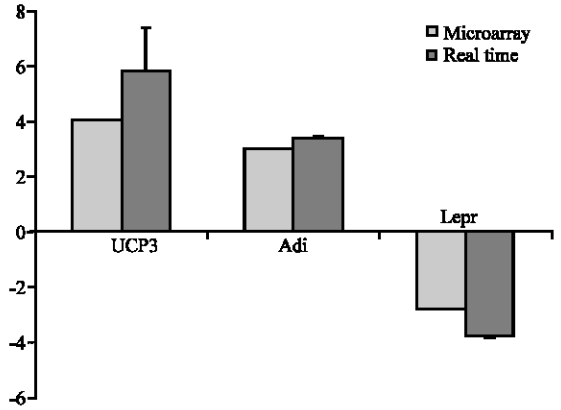

Fig. 2: mRNA expression of Ucp3, Adi and Lepr in the pig induced by high-fat diet using real-time PCR

world (Mokdad *et al*., 2003; Lei *et al*., 2007; Ye *et al*., 2004). Despite very aggressive research agendas aimed at defining the molecular basis of obesity and its comorbidities in humans, the limited availability of human samples and the small sizes of rodent samples represent one of the greatest challenges for the research of varies tissues (Spurlock and Gabler, 2008). The pig offers a greater physiological similarity, organ size likes human (Miller and Ullrey, 1987) and it easily deposits fat during growth as ideal animal model for obesity (Spurlock and Gabler, 2008). It is striking that when pig fed substantial quantities of lard and cholesterol becomes an excellent humanoid model for atherosclerosis (Gerrity *et al*., 2001) and obesity with significant visceral adipose expansion, hypertriglyceridemia and increased LDL, cholesterol (Dyson *et al*., 2006). Due to an increase in plasma FFA concentrations in normal subjects to levels comparable to those in the obese can results in the induction of oxidative stress, inflammation and subnormal vascular reactivity in addition to causing insulin resistance (Tripathy *et al*., 2003).

In the study, compared with control pigs, the pigs fed the high fat diet showed hyperglycemia, hyperlipidemia, elevated body fat accretion and serum NEFA after 180 days feeding. Apparently, the pig (high fat diet-induced) malady matches signs typical of obesity. High-fat diet triggers a complex cellular response including altering gene expression and induced obesity. To gain insight into the molecular mechanisms by which high-fat diet exerts its complex biological effects, we performed microarray analysis to assess changes in gene expression levels in response to high-fat induced obesity.

The results of microarray showed that 387 genes were up-regulated and 465 genes down-regulated in the subcutaneous fat tissue after 180 days of high-fat diet induced. To further characterize the types of genes altered, we classified 852 genes into Gene Ontology (GO) slim terms.

Gene ontology analysis revealed that high-fat diet altered genes up-regulating metabolic process, immune response, translation and cell cycle. Genes involved in regulation of transcription, RNA splicing and transcription were significant down-regulated. In the current study, genes relating to the regulation of transcription, immune response and cell cycle were found

to be significantly altered following obesity in agreement with previous reports (Dogra *et al*., 1998; Arner, 2000; Trayhurn and Beattie, 2001; Nadler *et al*., 2000; Gomez-Ambrosi *et al*., 2004; Klaus and Keijer, 2004; Weisberg *et al*., 2003).

Tissue macrophages recruited to adipose tissue in obese animals exhibit increased the expression of a broad array of genes encoding inflammatory pathway components (Xu *et al*., 2003). In the study, Cytochromes P450 (P450s) path was siginificantly up-regulated (Table 5).

Cytochromes P450 (P450s) are a multigene family of constitutively expressed and inducible enzymes involved in the oxidative metabolic activation and detoxification of many endogenous and exogenous compounds (Nebert and Dalton, 2006).The expression of these enzymes is known to be regulated by physiological, pathological, genetic, and environmental factors. White Adipose Tissue (WAT) has a very important double physiological role: through its triacylglyceride storage capacity, it is the key organ for energy homeostasis but it is also becoming increasingly apparent that WAT is a major endocrine organ (Dogra *et al*., 1998).

The adipocyte secretes many hormones and proteins that have been implicated in metabolic, neuroendocrine, immune and cardiovascular regulation. Recently, P450s were found in rat WAT and shown to be inducible through mechanisms similar to those in the liver (Nebert and Dalton, 2006).

CONCLUSION

About 387 genes were found to be significantly up-regulated and 465 genes down-regulated (fold change≥2.0) in an obese porcine model induced by a high-fat diet. In subcutaneous fat tissue, 852 genes significantly altered in response to high-fat diet were classified into Gene Ontology (GO) slim terms. Genes involved in metabolic process, immune response, translation and cell cycle were significantly up-regulated ($p<0.05$). Genes involved in regulation of transcription, RNA splicing and transcription were significantly down-regulated ($p<0.05$). The results revealed that the expression of high-fat diet influence genes involved in inflammation, metabolic diseases and cancer in adipose layer.

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REFERENCES

Agarwal, A.K. and A. Garg, 2006. Genetic disorders of adipose tissue development, differentiation and death. Annu. Rev. Genomics Hum Genet., 7: 175-199.

Al-Shahrour, F., R. Diaz-Uriarte and J. Dopazo, 2004. Fatigo: A web tool for ?nding signi?cant associations of gene ontology terms with groups of genes. Bioinformatics, 20: 578-580.

Alexa, A., J. Rahnenfuhrer and T. Lengauer, 2006. Improved scoring of functional groups from gene expression data by decorrelating go graph structure. Bioinformatics, 22: 1600-1607.

Arner, P., 2000. Hunting for human obesity genes? Look in the adipose tissue! Int. J. Obes. Relat. Metab. Disord., 24: S57-S62.

Dogra, S.C., M.L. Whitelaw and B.K. May, 1998. Transcriptional activation of cytochrome P450 genes by different classes of chemical inducers. Clin. Exp. Pharmacol. Physiol., 25: 1-9.

Dyson, M., M. Alloosh, J.P. Vuchetich, E.A. Mokelke and M. Sturek, 2006. Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atherogenic diet. Comp. Med., 56: 35-45.

Gerrity, R.G., R. Natarajan, J.L. Nadler and T. Kimsey, 2001. Diabetes-induced accelerated atherosclerosis in swine. Diabetes, 50: 1654-1665.

Gomez-Ambrosi, J., V. Catalan, A. Diez-Caballero, L.A. Martinez-Cruz and M.J. Gil *et al*., 2004. Gene expression profile of omental adipose tissue in human obesity. FASEB J., 18: 215-217.

Havel, P.J., 2002. Control of energy homeostasis and insulin action by adipocyte hormones: Leptin, acylation stimulating protein and adiponectin. Curr. Opin. Lipidol., 13: 51-59.

Klaus, S. and J. Keijer, 2004. Gene expression profiling of adipose tissue: Individual, depot-dependent and sexdependent variabilities. Nutrition, 20: 115-120.

Lei, X.G., W.H. Cheng and J.P. McClung, 2007. Metabolic regulation and function of glutathione peroxidase-1. Annu. Rev. Nutr., 27: 41-61.

Miller, E.R. and D.E. Ullrey, 1987. The pig as a model for human nutrition. Annu. Rev. Nutr., 7: 361-382.

Mohamed-Ali, V., J.H. Pinkney and S.W. Coppack, 1998. Adipose tissue as an endocrine and paracrine organ. Int. J. Obes. Relat. Metab. Disord., 22: 1145-1158.

Mokdad, A.H., E.S. Ford, B.A. Bowman, W.H. Dietz, F. Vinicor, V.S. Bales and J.S. Marks, 2003. Prevalence of obesity, diabetes and obesity-related health risk factors. J. Am. Med. Assoc., 289: 76-79.

Nadler, S.T., J.P. Stoehr, K.L. Schueler, G. Tanimoto, B.S. Yandell and A.D. Attie, 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. Proc. Natl. Acad. Sci. USA., 1997: 11371-11376.

Nebert, D.W. and T.P. Dalton, 2006. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nature Rev. Cancer, 6: 947-960.

Spurlock, M.E. and N.K. Gabler, 2008. The development of porcine models of obesity and the metabolic syndrome. J. Nutr., 138: 397-402.

Trayhurn, P. and J.H. Beattie, 2001. Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. Proc. Nutr. Soc., 60: 329-339.

Tripathy, D., P. Mohanty, S. Dhindsa, T. Syed and H. Ghanim, A. Aljada and P. Dandona, 2003. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes, 52: 2882-2887.

Wang, P.H., Y.H. Ko, B.H. Liu, H.M. Peng and M.Y. Lee *et al*., 2004. The expression of porcine adiponectin and stearoyl coenzyme a desaturase genes in differentiating adipocytes. Asian-Aust. J. Anim. Sci., 17: 588-593.

Weisberg, S.P., D. McCann, M. Desai, M. Rosenbaum, R.L. Leibe and A.W. Ferrante Jr., 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Inves., 112: 1796-1808.

Xu, H., G.T. Barnes, Q. Yang, G. Tan and D. Yang *et al*., 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest., 112: 1821-1830.

Ye, Y., Y. Shibata, C. Yun, D. Ron and T.A. Rapoport, 2004. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature, 429: 841-847.