Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;27:327-340

Accepted: February 10, 2011

Gene Expression Profiling of Adipose Tissues in Obesity Susceptible and Resistant Rats under a High Fat Diet

Jeong In Joo and Jong Won Yun

Department of Biotechnology, Daegu University, Kyungsan

Key Words
Brown adipose tissue • Gene profiling • Microarray • Obesity prone • Obesity resistant • White adipose tissue

Abstract
Different responses to a high fat diet (HFD) can occur even within a group of animals with the same genetic background, such as obesity-prone (OP) or obesity-resistant (OR) phenotypes, on the same feeding. To explain these phenotypes, we performed an analysis of gene expression differences in brown (BAT) and white adipose tissue (WAT) of OP and OR rats. Microarray analysis of transcripts revealed that 91 and 53 genes showed significant differences in expression between the BAT and WAT gene, respectively. Surprisingly, a majority of these genes were significantly down-regulated in adipose tissues in response to HFD feeding. K-means clustering of the expression levels of these genes identified 4 distinct groupings of genes with significant expression levels. Only a limited number of genes were significantly regulated in adipose tissues in response to HFD feeding, whereas expression levels of a large number of genes differed significantly between OP and OR rat. Our observations support that distinct discrepancies exist in gene-expression regulations in adipose tissues, and that alteration likely resulted from significant differences in genes encoding metabolic enzymes. To the best of our knowledge, this study provided the first direct comparison of gene-expression changes between OP and OR rats.

Introduction

Obesity is a major risk factor for metabolic syndromes; thus, weight reduction is an important component of therapy for metabolic disorder patients. Increased adiposity in these individuals results from an upsurge in both adipocyte number and size of individual fat cells [1]. This is because the adipocyte plays an important role in management of dietary energy excess, releasing signaling molecules known as adipokines that regulate oxidation of metabolic fuels in several organs, including the liver, skeletal muscle, heart, and pancreas [2], preventing accumulation of lipids in non-adipose tissues, avoiding lipotoxicity [3, 4]. The role of adipose tissue in etiology of obesity and its pathophysiologic consequences is increasingly recognized [5-7]. Adipose tissue and adipocyte-secreted factors influence diverse

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/11/0274-0327$38.00/0

Accessible online at:
www.karger.com/cpb

Jong Won Yun
Department of Biotechnology, Daegu University
Kyungsan, Kyungbuk 712-714 (Korea)
Tel. +82-53-850-6556, Fax +82-53-850-6559
E-Mail jwyun@daegu.ac.kr

processes, including appetite and energy homeostesis, lipid metabolism, insulin sensitivity, and inflammation [8, 9].

Propensity to overeat and become obese when eating a high-fat diet (HFD) varies widely among individuals, both across and within species or strains [10-13]. It is recognized that genetic factors play a role in this variation; however, the pathways responsible for these phenotypic differences are still largely unknown [13].

Rodents have been used extensively in many studies of obesity, particularly the high fat diet (HFD)-induced obese rodent model, which provides an efficient system for development of the obese phenotype. However, experiments have shown different responses to HFD, even within a group of animals with the same genetic background. For example, Sprague-Dawley (SD) or Wistar rats on the same HFD may manifest either obesity-prone (OP) or obesity-resistant (OR) phenotypes [14, 15].

Information regarding factors for determination of phenotypic differences between OP and OR, particular in adipose tissues is insufficient. Compared with OR mice, adipocytes from OP mice have demonstrated greater sensitivity to insulin due to greater uptake of glucose than OR, thereby increasing lipogenesis [16]. Defective oxygen consumption in brown adipose tissue (BAT) also affects obesity development. BAT of obese rats had a diminished respiratory rate, compared with lean rats, due to an inability of mitochondria to utilize free fatty acids for production of enhanced oxygen consumption [17].

Oligonucleotide or cDNA microarrays have dramatically changed the study of the pattern of gene expression by enabling simultaneous analysis of thousands of genes in a single experiment, thereby providing a comprehensive assessment of expression levels, representing a powerful strategy for research in the obesity field [18-23]. To date, only one transcriptomic profiling study of OP and OR rats under an HFD has been performed using liver tissue by Xu et al. [24]. They revealed that altered metabolic pathways in OP rats may involve increased activity of the sympathetic nervous system and Krebs cycle, increased production of ketone bodies, and an adaptive regulatory process for storage of excessive lipids in liver through a reverse cholesterol transport process.

Clearly, most cases of obesity are polygenic and represent interaction between multiple genes and the environment, wherein diet is a major component. For this reason, gene expression profiling may be helpful in the search for key player genes determining OP and OR phenotype. We hypothesized that differences in gene expression in adipocytes is likely to contribute to this response. We therefore performed gene profiling analysis in BAT and WAT of OP and OR rats using oligonucleotide microarrays to assess the causes of metabolic change and to determine which genes are involved in this response.

Materials and Methods

Animals and Breeding Conditions

Five-week-old male Sprague-Dawley (SD) rats weighing 130-150g were purchased from Daehan Experimental Animals (Seoul, Korea) and were maintained in the animal facility at the Department of Biotechnology, Daegu University. All rats were provided with water and standard chow *ad libitum* for 1 week prior to any experimental procedures in order to allow them to acclimatize to their new surroundings. Rats were raised in separate cages in order to exclude diverse effects among rats. They were randomly divided into two groups, with 8 rats fed a low fat diet (10% calories from fat) and 37 rats fed an HFD (40% calories from fat). Dietary compositions used in this study were presented in Table 1. Their weights and food intakes were taken every alternative day for 56 days and they were subdivided into OP (n=6) and OR rats (n=6) according to the highest and lowest body weight gainers, respectively. These experiments were approved by the Committee for Laboratory Animal Care and Use of Daegu University (Permission No.2009-006). All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

RNA preparation

According to the manufacturer's instructions, total RNA was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, USA), and purified using RNeasy columns (Qiagen, Valencia, USA). After processing with DNase digestion, clean-up procedures, RNA samples were quantified, aliquotted, and stored at -80°C until use. For quality control, purity of the total RNA was determined by the ratio of absorbance readings at 260 and 280 nm, the ratio of which fell within the range of 1.8-2.0. Integrity of RNA was analyzed by denaturing agarose gel electrophoresis, with the 28S rRNA band appearing approximately twice as intense as the 18S rRNA band and analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA).

Labeling and purification

According to the manufacturers' protocol, total RNA was amplified and purified using the Ambion Illumina RNA amplification kit (Ambion, Austin, USA) to yield biotinylated cRNA. Briefly, 550 ng of total RNA was reverse-transcribed to cDNA using a T7 oligo (dT) primer. Second-strand cDNA was synthesized, *in vitro* transcribed, and labeled with biotin-NTP. Following purification, cRNA was quantified using the ND-1000 spectrophotometer (NanoDrop, Wilmington, USA).

	LFD	HFD
Composition by weight, g/kg		
Casein	200	200
Cornstarch	150	155
Sucrose	500	50
Dextrose	0	132
Cellulose	50	50
Soybean oil	0	25
Corn oil	50	0
Lard	0	175
Mineral mix	35	35
Vitamin mix	10	10
TBHQ	0	0.014
DL-Methionin	3	0
L-cystine	0	3
Choline bitartrate	2	2.5
Composition by calories, %		
Protein	21	20
Carbohydrate	68	35
Fat	12	45
Total	3902 kcal/kg	4776 kcal/kg

Table 1. Dietary compositions of low fat diet (LFD) and high fat diet (HFD)

Hybridization and data export

According to the manufacturers' instructions, 750 ng of labeled cRNA samples were hybridized to each RatRef-12 expression beadchip for 16-18 h at 58°C (Illumina Inc., San Diego, USA). Following the bead array manual, detection of an array signal was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK). According to the manufacturer's instructions, arrays were scanned with an Illumina bead array Reader confocal scanner. Array data export processing and analysis was performed using Illumina BeadStudio v3.1.3 (Gene Expression Module v3.3.8).

Raw data preparation and statistical analysis

The quality of hybridization and overall chip performance were monitored by visual inspection of both internal quality control checks and the raw scanned data. Raw data were extracted using software provided by the manufacturer (Illumina GenomeStudio v2009.2 (Gene Expression Module v1.5.4). Array data were filtered by detection p-value<0.05 (similar to signal to noise) in at least 50% of samples. We applied a filtering criterion for data analysis; higher signal value was required in order to obtain a detection p-value=0.05. Selected gene signal value was transformed by logarithm and normalized by a quantile method. Statistical significance of the expression data was determined using ANOVA and the TukeyHSD test, in which the null hypothesis was that no difference exists between mean of groups in the data. False discovery rate (FDR) was controlled by adjustment of p value using the Benjamini-Hochberg algorithm. Gene-ontology analysis for a significant probe list was performed using PANTHER (http://www.pantherdb.org/panther/ontologies.jsp), using text files containing the Gene ID list and accession number of Illumina probe ID. Gene Set Enrichment Analysis (GSEA) was performed in order to determine whether an a priori defined set of genes showed differential patterns in both biological processes and molecular function states. One-tail Fisher Exact was adopted for measurement of gene-enrichment in annotation terms. Hierarchical and K-means clustering was performed using complete linkage with a Euclidian metric. GenomeStudio v2009.2 software was used for quantification and image analysis of mRNA data. R scripts were used for all other analytical processes.

Quantitative real-time RT-PCR analysis

Total RNA was isolated from adipose tissue samples with TRIZOL (QIAGEN Inc., Valencia, USA), and was then cleaned with ribonuclease-free deoxyribonuclease and the RNeasyMini kit (QIAGEN). Samples were then processed according to the manufacturer's instructions. Quality of the RNA was analyzed on a 1% (w/v) agarose gel and the concentration by a Qubit quantitation system (Invitrogen, Eugene, USA). Briefly, reverse transcription of 2 μg RNA was carried out using the iScript cDNA synthesis kit, according to the supplier's protocol. According to the manufacturer's protocol, quantitative real-time PCR was performed using the Applied Stratagene mx 3000p QPCR System (San Diego, USA). Oligonucleotide sequences were as follows: 5'-GGT CTC GCT CCT GGA AAG A-3' and 5'-GTA TGA CTC CAC TCA CGG CAA-3' primer for Gapdh, 5'-TGA GGA GGA CCG CAT TTA TC-3' and 5'-GAA GCT TCC TTC GTG ACC AG-3' primer for Acaca, 5'-AAG CTA GGA GCC TTC CTT CTC TT-3' and 5'-TTG TAG CAC AGA CGT TAC TGC AT-3' primer for Wdnm1, 5'-AGG GAC AGC AAA GAG GAT AAT G-3' and 5'-TTG GGC TTA GAG AAC AGA GAG G-3' primer for Rrad, 5'-CCA TCT TGG CGT CTG ATC TT-3' and 5'-TGG CGA GGA TAA CCA ATT TC-3' primer for Rpl30, 5'-AGC AGC CTC ATT TCT GCA CT-3' and 5'-CCC CAG AAG GAG GTG TGT AA-3' primer for Sox18, 5'-CCA GAC CTC AGC AAA CAC AA-3' and 5'-GGG TCA AAC AGG TCC TTG AA-3' primer for Ck, 5'-CAC CAG CTG AAA CCC TGA AT-3' and 5'-AGG AAT ATG CGT GCC ATA GG-3' primer for Nnat, 5'-CTG ACA AGA CGG ACA TGT GG-3' and 5'-GAA GTC TTT GGC CTC GTC TG-3' primer for Mylk2, 5'-GGA TAC AGA AAG CCG AAC CA-3' and 5'-GGA TAC AGA AAG CCA GAA CCA-3' primer for Acsl5, 5'-TAT CAC CAA CTG GGA CGA CA-3' and 5'-CCG GAG GCA TAG AGA GAC AG-3' primer for Acta1.

Statistical analysis

All experimental results were compared by one-way analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) program, and the data were expressed as the mean ± S.E.M. Group means were considered significantly different at $p<0.05$, as determined by the technique of protective least-significant difference (LSD) when ANOVA indicated an overall significant treatment effect.

Results

Effect of HFD on OP and OR phenotypes

Rats were randomly divided into two groups, with 8 rats fed a chow diet and 37 rats fed an HFD, and the latter was subdivided into OP (n=6) and OR rats (n=6) according to their body weight gain. Changes in body weight between individuals during the experimental period

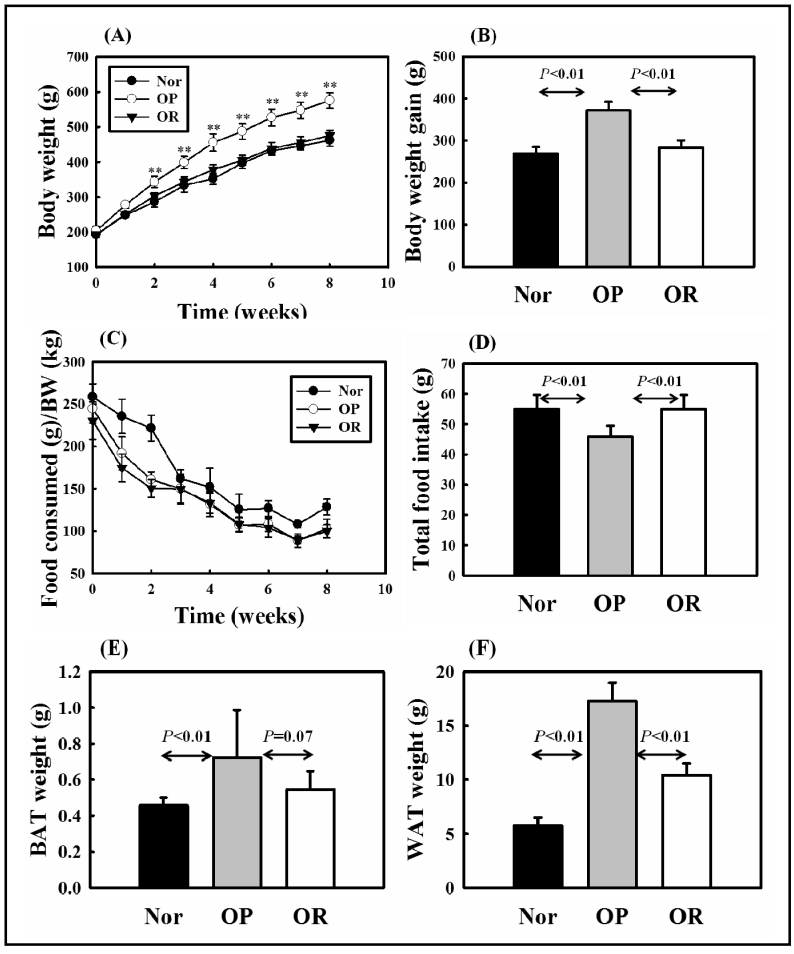


Fig. 1. HFD induced OP and OR phenotypes. (A) Body weight profiles, (B) average body weight gain, (C) food consumption profiles, (D) average food consumption, (E) average weight of epididymal WAT per body weight, (F) average weight of interscapular BAT per body weight did not differ significantly among the experimental groups of normal, OP, and OR rats (n=6 in each group). *p<0.01, **p<0.001 for significance. Error bars represent means ± S.E.M.

are shown in Fig. 1A. Body weights of rats were much the same at the beginning of this study; however, after 8 weeks, OR rats weighed 475.6 ± 15 g and OP rats weighed 575.5 ± 21 g (p<0.001, Fig. 1A). The average body weight gain of OR rats was 282.8 ± 18 g, while that of OP rats was 371.8 ± 20 g (p<0.001, Fig. 1B). Therefore, total body weight of OP rats was higher by an average of approximately 25%, compared with OR rats (Fig. 1B). Food consumption profiles did not differ significantly between the OP and OR groups. However, of particular interest, total food intake of OP rats was significantly lower (p<0.01) than that of OR rats (Figs. 1C and D). Moreover, weights of epididymal fat of WAT of OR rats per body weight were significantly lower (p<0.01) than those of OP rats (Fig. 1E). However, unexpectedly, weights of interscapular fat of BAT of OR rats were slightly lower (not significant, p=0.07) than those of OP rats (Fig. 1F).

Microarray data analysis

Oligonucleotide microarray analyses were conducted in order to determine any changes in the gene expression profile of adipose tissues between OP and OR rats in response to HFD. Expression profiling was performed for BAT and WAT from normal, OP, and OR rats (n=3). In each group the gene expression profiles of adipose tissues from normal, OP, and OR rats were generated using the Illumina RatRef-12 Bead Chip, which includes 21,290 probe sets representing >11,360 known genes and 6,856 expressed sequence tags with unknown functions. In this study, the PANTHER classification system (http://www.pantherdb.org) was used for classification of genes according to their functions [25]. In brief, gene lists can be uploaded to the size and analyzed relative to molecular functions, biological processes, and pathways.

The majority of genes showing significant differences between OP and OR rats were genes encoding proteins/enzymes of carbohydrate and lipid metabolism, cell structure and motility, developmental processes, differentiation, and cell signaling (Tables 2 and 3). This screening process led to identification of 91 and 53 genes that were found to have significant changes in expression of the BAT and WAT genes, respectively, as depicted in the dendrograms (Figs. 2 and 3). Furthermore, we compared gene expression profiles by the SAM two-class unpaired test method with criteria of two fold

Table 2. Differentially expressed genes (2≥fold change) in brown adipose tissue (BAT) of normal (NOR), obesity-prone (OP), and obesity resistant (OR). [a] Differentially expressed genes with a fold change = 2 between NOR and OP, as well as NOR and OR groups, are represented, where the OR/OP is the average fold change using the SAM two class unpaired test method. Genes showing differential expression of greater than 2-fold are shown in bold. [b] For clusters, see Figs. 2 and 4.

Accession No.	Description	Fold change[a]			Cluster[b]
		OP/NOR	OR/NOR	OR/OP	
Carbohydrate metabolism					
NM_031715.1	phosphofructokinase	**-2.10**	-1.19	**2.76**	4
NM_172091 .1	glucagon receptor	**-2.04**	-1.71	1.19	4
NM_017328.1	phosphoglycerate mutase 2	**-3.63**	-1.60	**2.27**	4
XM_342002.3	PREDICTED: muscle glycogen phosphorylase	**-5.11**	-1.55	**3.28**	4
NM_012949.1	enolase 3, β	**-5.20**	-1.47	**3.53**	4
NM_053297.1	pyruvate kinase	**-2.73**	-1.47	1.85	4
NM_022407.3	aldehyde dehydrogenase 1 family, member A1	**-2.17**	1.05	**2.27**	4
Cell structure					
XM_575617.2	PREDICTED: similar to leiomodin 3	**-2.21**	-1.42	1.55	4
NM_012935.2	crystallin, α B	**-2.65**	-1.59	1.66	4
XM_214499.4	PREDICTED: actinin α 2	**-2.34**	-1.36	1.72	4
XM_001069915.1	PREDICTED: integrin beta 1 binding protein 2	**-2.10**	-1.58	1.33	4
XM_001054854.1	PREDICTED: tropomodulin 4	**-3.56**	-1.37	**2.59**	4
XM_214563.4	PREDICTED: myotilin	**-6.26**	-1.35	**4.64**	4
NM_133424.1	actinin α 3	**-9.04**	-1.53	**5.90**	4
NM_019212.2	actin, α 1	**-9.55**	-1.31	**7.30**	4
NM_013044.2	tropomodulin 1	**-2.80**	-1.39	**2.02**	4
NM_022531.1	Desmin	**-2.84**	-1.40	**2.03**	4
Cell mobility					
XM_001054975.1	PREDICTED: myomesin 1 (skelemin) 185kDa	**-2.32**	-1.46	1.58	4
XM_217637.4	PRE DICTED: nebulin -related anchoring protein	**-2.59**	-1.61	1.61	4
XM_340818.2	PREDICTED: myosin, heavy polypeptide 4	**-2.60**	-1.53	1.70	4
XM_240481.4	PREDICTED: myomesin 2	**-2.08**	-1.38	1.51	4
NM_176079.1	myogenic differentiation 1	**-2.11**	-1.60	1.32	4
NM_020104. 1	fast myosin alkali light chain	**-4.03**	-1.38	**2.92**	4
NM_053650.1	PDZ and LIM domain 3	**-3.32**	-1.36	**2.44**	4
XM_001056398.1	PREDICTED: nebulin	**-3.41**	-1.54	**2.22**	4
NM_021666.2	Triadin	**-3.56**	-1.59	**2.25**	4
NM_00 534075.1	tropomyosin 1, α	**-7.02**	-1.50	**4.66**	4
NM_031532.1	troponin T type 3	**-8.49**	-1.50	**5.67**	4
Signal transduction					
XM_001065955.1	PREDICTED: titin	**-2.31**	-1.15	**2.01**	4
NM_00 537351.1	troponin C type 2	**-6.09**	-1.31	**4.66**	4
NM_181369.2	histidine rich calcium binding protein	**-2.57**	-1.46	1.76	4
NM_019 232.1	serum/glucocorticoid regulated kinase	**-2.00**	-1.41	1.42	4
NM_012530.1	creatine kinase	**-9.84**	-1.40	**7.05**	4
XM_001078539.1	PREDICTED: ryanodine receptor 1	**-4.08**	-1.47	**2.77**	4
NM_175844.2	actin -binding Rho activating protein	**-4.08**	-1.40	**2.92**	4
XM_00106 5492.1	PREDICTED: myeloid leukemia factor 1	**-4.54**	-1.74	**2.62**	4
XM_001056439.1	PREDICTED: SH3 and cysteine rich domain 3	**-3.48**	-1.45	**2.39**	4
NM_057191.1	kelch repeat and BTB (POZ) domain containing 10	**-2.74**	-1.46	1.88	4
NM_031688.1	synuclein, γ	**2.20**	1.53	-1.44	3
NM_031596.1	squamous cell carcinoma antigen recognized by T cells	-1.35	**-2.25**	-1.67	4
Transport proteins					
NM_012919.2	calcium channel, voltage -dependent, α2/δ subunit 1	**-2.27**	-1.50	1.51	4
NM_001008880.1	sodium channel, type IV, β	**-2.38**	-1.45	1.64	4
NM_030834.1	solute carrier family 16, member 3	**-2.53**	-1.58	1.60	4
NM_019255.1	calcium channel, voltage-dependent, γ subunit 1	**-2.01**	-1.31	1.54	4
NM_021588.2	Myoglobin	**-3.82**	-1.68	**2.27**	4
XM_001072039.1	PREDICTED: taxilin β	**-3.93**	-1.65	**2.38**	4
NM_012812.1	cytochrome c oxidase, subunit VIa, polypeptide 2	**-4.81**	-1.64	**2.93**	4

(continued next page)

or higher between the three groups of BAT and WAT. To investigate changes in genes in response to an HFD and between OP and OR rats, we displayed four clusters of dendrogram data for BAT and WAT based on the total of 91 and 53 genes, respectively (Fig. 4). A limited number of genes (Egr1, RT1-A3 in BAT and Cyp2e1, Serpina12 in WAT) were included in clusters 1 and 2 (HFD-responsive genes), whereas a large number of genes were included in clusters 3 and 4. This result demonstrated that gene alterations were more remarkable between OP and OR rats, rather than HDF response (Figs. 2, 3, and 4). Using the complementary approach, we were able to observe genes at a glance showing differential expressions of 2-fold or higher between OP and OR rats. Scatter plot analysis demonstrated differences in global expression between OP and OR rats (Fig. 6). It is noteworthy that a greater number of genes of BAT showed dramatically higher differential expression with higher magnitude, compared with WAT.

Accession No.	Description	Fold change[a]			Cluster[b]
		OP/NOR	OR/NOR	OR/OP	
Nucleoside, nucleotide and nucleic acid metabolism					
NM_138876.1	adenosine monophosphate deaminase 1	**-4.51**	-1.43	**3.15**	4
XM_217334.2	PREDICTED: apolipoprotein B editing complex 2	**-2.51**	-1.54	1.63	4
NM_001009489.1	2 ' -5 ' oligoadenylate synthetase 1K	-1.36	**-2.11**	-1.55	4
Developmental process					
XM_343196.3	PREDICTED: myosin binding protein C, slow type	**-5.27**	-1.62	**3.26**	4
NM_022499.1	Parvalbumin	**-5.95**	-1.70	**3.51**	4
NM_012605.1	myosin light chain, phosphorylatable, fast skeletal muscle	**-9.07**	-1.54	**5.89**	4
NM_017185.1	troponin I type 2	**-6.39**	-1.39	**4.06**	4
NM_181687.1	Neuronatin, transcript variant 1	**3.40**	1.19	**-2.39**	3
NM_053601.1	Neuronatin, transcript variant 2	**2.43**	1.42	**-2.04**	3
XM_001077547.1	PREDICTED: similar to Myosin heavy chain, skeletal muscle, adult 2	**-2.29**	-1.06	**2.16**	4
XM_001065955.1	PREDICTED: titin	**-7.37**	-1.56	**4.73**	4
Transcription factor					
NM_012551.1	early growth response 1	**-2.34**	**-1.24**	2.17	1
XM_001056023.1	PREDICTED: zinc finger, MYND domain containing 17	**-2.23**	-1.34	1.66	4
XM_216172.3	PREDICTED: SET and MYND domain containing 1	**-2.51**	-1.49	1.69	4
XM_227134.4	PREDICTED: similar to Nocturnin	**-2.01**	-1.34	1.50	4
NM_019328.3	Nuclear receptor subfamily 4, group A, member 2	**-2.10**	-1.76	1.20	1
NM_145669.2	four and a half LIM domains 1	**-2.16**	-1.63	1.32	4
NM_031628.1	Nuclear receptor subfamily 4, group A, member 3	**-2.17**	-1.98	1.10	4
XM_001056311.1	PREDICTED:four and a half LIM domains 3	**-2.40**	-1.69	1.42	4
XM_001076609.1	PREDICTED: bol, boule-like	**-3.01**	-1.42	**2.12**	4
Protein metabolism					
NM_138887.1	heat shock protein, α -crystallin-related, B6	**-2.11**	-1.50	1.40	4
XM_001066621.1	PREDICTED: ubiquitin specific protease 13	**-2.12**	-1.46	1.45	4
NM_001013217.1	tripartite motif-containing 54	**-3.07**	-1.44	**2.13**	4
NM_022699.2	ribosomal protein L30	**2.82**	-1.20	**-3.40**	3
NM_031531.1	serine (or cysteine) peptidase inhibitor	**2.08**	-1.01	**-2.11**	3
NM_012660.2	eukaryotic translation elongation factor 1 α 2	**-5.27**	-1.61	**3.28**	4
NM_057209.1	myosin light chain kinase 2	**-3.32**	-1.45	**2.29**	4
Immunity					
NM_001008830.1	RT1 class I, A3	**2.09**	**2.13**	1.02	2
NM_001008827.1	RT1 class Ia, locus A1	**2.04**	1.98	-1.03	3
Miscellaneous					
XM_579587.1	PREDICTED: hypothetical gene supported by NM_053959	**-2.23**	-1.54	1.45	4
NM_080399.1	DNA-damage-inducible transcript 4-like	**-2.28**	-1.64	1.39	4
XM_001078936.1	PREDICTED: dehydrogenase/reductase (SDR family) member 7C	**-4.57**	-1.60	**2.86**	4
NM_001012080.1	hemochromatosis type 2	**-5.65**	-1.45	**3.89**	4
XM_579736.1	PREDICTED: hypothetical gene supported by NM_201989	**-3.22**	-1.60	**2.02**	4
NM_172223.2	peroxisomal membrane protein 4	**-2.15**	-1.24	1.74	4
XM_001055627.1	PREDICTED: similar to CG10671-like	**-2.17**	-1.36	1.60	4
NM_139230.1	Nexilin	**-2.36**	-1.42	1.67	4
XM_001067257.1	PREDICTED: ankyrin repeat and SOCS box-containing protein 11	**-2.39**	-1.38	1.73	4
XM_237094.4	PREDICTED: ankyrin repeat domain 23	**-2.41**	-1.39	1.74	4
XM_001064588.1	PREDICTED: similar to myozenin 1	**-4.97**	-1.56	3.19	4
NM_201562.2	reticulon 2	**-3.31**	-1.50	2.21	4
XM_573265.2	PREDICTED: similar to putative SH3 BGR protein	**-3.48**	-1.48	2.35	4
NM_001011984.1	ankyrin repeat and SOCS box-containing 2	**-2.76**	-1.43	1.93	4
NM_012634.1	phosphoribosyl pyrophosphate synthetase 2	**4.17**	1.02	-4.08	3

Differential gene expression between OP and OR rats

In this study, we noted a significant alteration in expression of genes in adipose tissue, particularly in BAT between OP and OR rats. From an overview of the gene expression patterns in OP and OR groups, nearly 91 and 53 differential genes in BAT and WAT samples were found, and the dendrograms of those genes are shown in Figs. 2 and 3. Details on the differential genes are listed in Tables 2 and 3.

Large decreases (fold changes≥2) in gene expression in BAT of OP rats were observed in genes coding for carbohydrate metabolism (7 genes), cell structure (10 genes), cell mobility (11 genes), signal transduction (11 genes), transport protein encoding genes (7 genes), developmental process (6 genes), transcription factor (9 genes), and protein metabolism (5 genes) (indicated in bold type in Table 2, also see Fig. 5). Conversely, several sets of genes demonstrated increased expression levels in BAT of OP rats. Of these, genes

Table 3. Differentially expressed genes (2≥fold change) in white adipose tissue (WAT) of normal (NOR), obesity-prone (OP), and obesity resistant (OR) rats [a]Differentially expressed genes with a fold change = 2 between NOR and OP as well as NOR and OR group are represented, where the OR/OP is the average fold change using SAM two class unpaired test method. Genes showing differential expression of more than 2-fold were shown in bold. [b] For cluster, see Figs. 3 and 4.

Accession No.	Description	Fold change[a]			Cluster[b]
		OP/NOR	OR/NOR	OR/OP	
Protein metabolism					
NM_138825.1	serine (or cysteine) peptidase inhibitor	**3.36**	**3.57**	1.06	2
NM_053372.1	secretory leukocyte peptidase inhibitor	**2.95**	**4.68**	1.59	3
NM_022699.2	ribosomal protein L30	1.05	**-9.32**	**-9.74**	**3**
XM_001074100.1	PREDICTED: camello-like 3	**-2.12**	-1.07	1.99	4
NM_012619.1	phenylalanine hydroxylase	**2.46**	1.72	-1.43	3
Carbohydrate metabolism					
NM_022407.3	aldehyde dehydrogenase 1 family, member A1	**-2.97**	-1.57	1.88	4
Lipid metabolism					
NM_024390.2	hydroxyprostaglandin dehydrogenase 15	**2.41**	**2.50**	1.04	2
NM_022193.1	acetyl-coenzyme A carboxylase α	**-2.39**	-1.84	1.30	4
NM_017127.1	choline kinase α	-1.26	**-2.24**	-1.78	3
NM_031558.1	steroidogenic acute regulatory protein	-1.68	**-3.39**	**-2.02**	**3**
NM_053580.2	solute carrier family 27 (fatty acid transporter), member 1	-1.70	**-2.10**	-1.24	4
NM_001003706.1	WDNM1 homolog	**-6.46**	**-2.56**	**2.52**	**4**
NM_031543.1	cytochrome P450, family 2, subfamily e, polypeptide 1	**-5.83**	**-1.09**	**2.04**	**1**
Immunity					
NM_001007612.1	chemokine ligand 7	**3.32**	**2.38**	-1.40	3
NM_212466.2	complement factor B	**-2.19**	-1.34	1.64	4
NM_053669.1	SH2B adaptor protein 2	**-2.41**	-1.61	1.49	4
NM_182952.2	chemokine ligand 11	1.75	**2.63**	1.51	3
NM_013092.1	chymase 1, mast cell	**-2.02**	1.62	**3.27**	**4**
NM_001008513.1	chemokine ligand 21b (serine)	**-2.52**	**-3.31**	-1.31	4
Signal transduction					
XM_340809.3	PREDICTED: RAS, dexamethasone-induced 1	1.02	**-2.18**	**-2.22**	**3**
XM_001080316.1	PREDICTED: sialic acid binding Ig-like lectin 10	**2.76**	1.29	**-2.15**	**3**
NM_053338.1	Ras-related associated with diabetes	**2.59**	1.13	**-2.29**	**3**
NM_031349.2	apelin receptor	**2.17**	1.45	-1.50	3
NM_013108.1	adrenergic, β-3-, receptor	-1.46	**-2.45**	-1.67	
NM_001014071.1	ERBB receptor feedback inhibitor 1	-1.50	1.54	**2.30**	
NM_013122.1	insulin-like growth factor binding protein 2	**-6.78**	**-2.94**	**2.30**	**4**
NM_021838.2	nitric oxide synthase 3	-1.39	1.51	**2.10**	
NM_080698.1	fibromodulin	**-2.51**	-1.77	1.42	4
NM_031658.1	mesothelin	**-10.00**	**-3.11**	**3.22**	**4**
Cell structure					
NM_199498.1	keratin 19	**-3.53**	-1.63	**2.16**	**4**
NM_012935.2	crystallin, alpha B	**2.84**	**2.23**	-1.27	3
Transcription factor					
XM_001055978.1	PREDICTED: B-cell leukemia/lymphoma 6	1.25	**2.16**	1.73	4
NM_001004210.1	X-box binding protein 1	-1.29	**-2.12**	-1.65	4
NM_001024781.1	SRY (sex determining region Y)-box 18	-1.83	1.34	**2.44**	**4**
NM_00533691.1	interferon regulatory factor 7	1.20	**2.05**	1.71	4
NM_053727.2	nuclear factor, interleukin 3 regulated	-1.46	**-2.23**	-1.52	4
XM_001067431.1	PREDICTED: interferon regulatory factor 6	**-2.07**	**-2.12**	-1.03	1
Developmental process					
NM_053687.1	schlafen 3	1.10	**2.03**	1.84	4
NM_080394.2	reelin	**-2.34**	**-2.94**	-1.25	4
Transporter					
NM_019230.1	solute carrier family 22	**-2.31**	**-2.34**	-1.01	1
NM_001013080.1	chloride intracellular channel 3	**-2.30**	-1.52	1.52	4
NM_001013185.1	chaperone, ABC1 activity of bc1 complex homolog	**-2.00**	-1.68	1.19	4
NM_012792.1	flavin containing monooxygenase 1	**-2.06**	-1.60	1.28	4
Miscellaneous					
XM_579967.1	PREDICTED: LOC499428	**-2.20**	-1.58	1.40	4
XM_236574.4	PREDICTED: similar to RIKEN cDNA 1300017J02	**-2.24**	-1.42	1.57	4
NM_080906.1	DNA-damage-inducible transcript 4	-1.88	1.12	**2.11**	**4**
NM_031655.1	latexin	-1.32	1.60	**-2.12**	**4**
NM_172223.2	peroxisomal membrane protein 4	**-2.20**	-1.20	1.84	4
NM_012634.1	phosphoribosyl pyrophosphate synthetase 2	**3.50**	1.26	**-2.78**	**3**
XM_001055146.1	PREDICTED: sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2	**-2.38**	-1.72	**1.39**	**4**
NM_017195.1	growth associated protein 43	**-1.37**	1.15	**2.08**	**4**
XM_214902.4	PREDICTED: similar to suprabasal-specific protein suprabasin	**-2.44**	-1.15	**2.13**	**4**
XM_341056.2	PREDICTED: similar to uroplakin IIIb	**-3.91**	-1.88	**2.08**	**4**

encoding ribosomal protein L30 and serine peptidase inhibitor showed opposite expression patterns (up-regulated in OP rats, but down-regulated in OR rats). However, overall gene expression patterns in WAT were

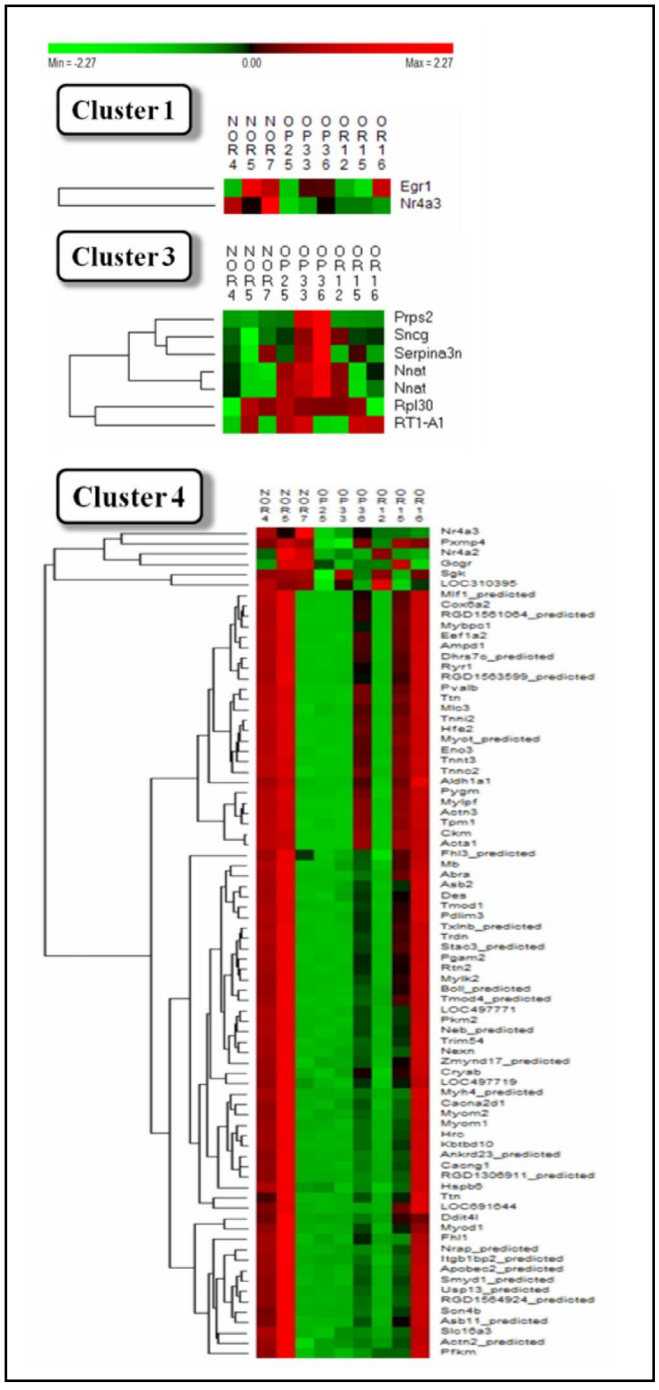


Fig. 2. Integrative clustering analysis. Expression profiles of the 91 differentially expressed genes in BAT of the OP and OR groups. A green to red color palette is used to represent expression levels, relative to the mean expression level of the control group, with green representing down-expression and red representing up-expression. Black indicates missed or excluded data.

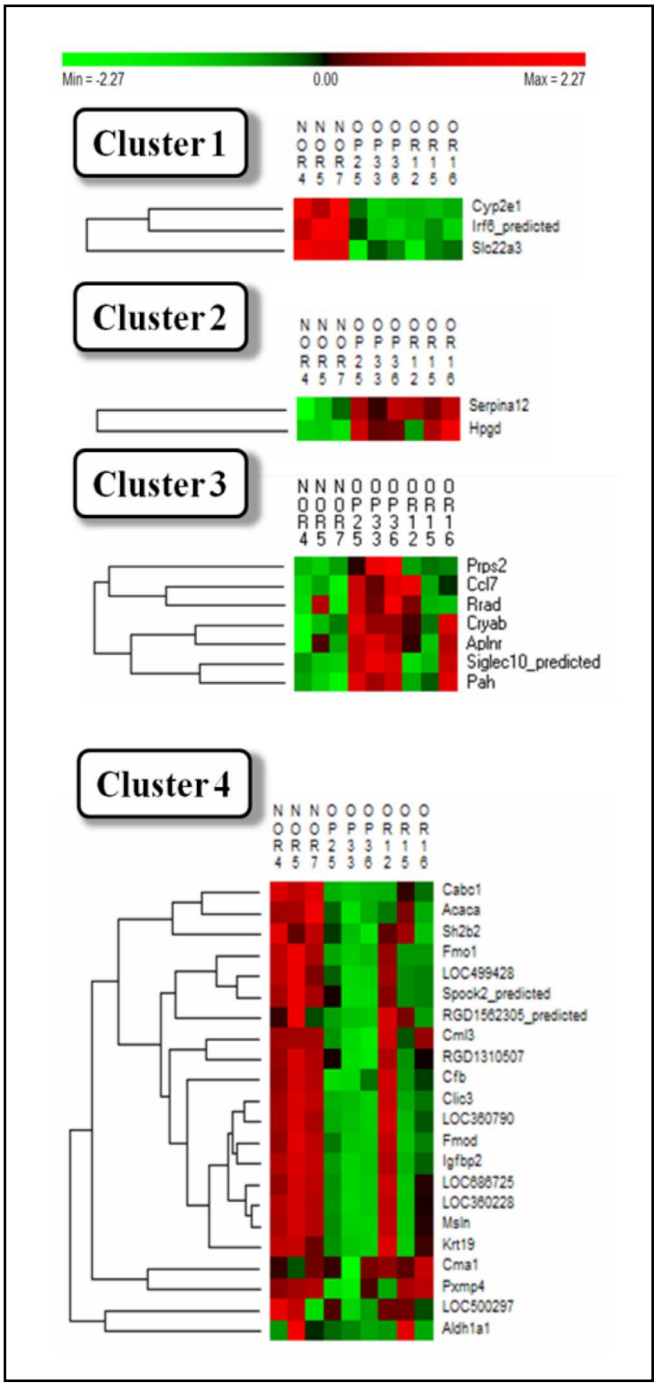


Fig. 3. Integrative clustering analysis. Expression profiles of the 53 differentially expressed genes in WAT of the OP and OR groups. A green to red color palette is used to represent expression levels, relative to its mean expression level of the control group, with green representing down-expression and red representing up-expression. Black indicates missed or excluded data.

quite different, compared with BAT. Among 53 differentially expressed genes (2≥fold changes in either OP or OR rats compared with normal rats), 45 genes were down-regulated; however, 35 genes were up-regulated in OP rats. Eight genes exhibited opposite expression patterns between OP and OR rats in response

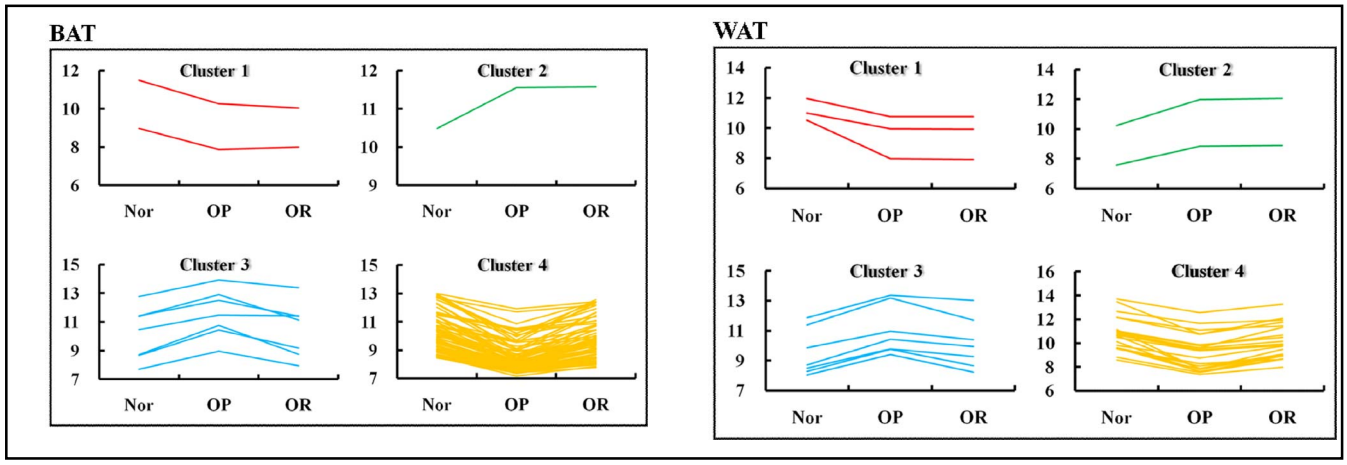
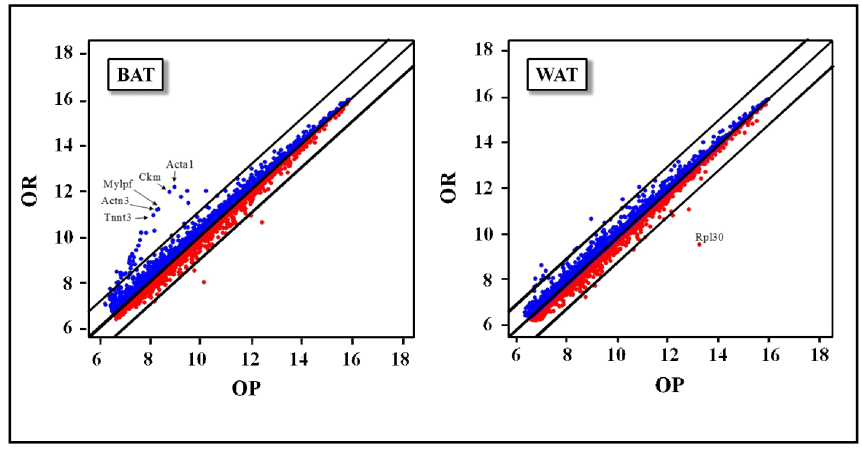


Fig. 4. K-means clustering display of significantly changed genes in BAT and WAT of normal, OP, and OR rats. The color scale shows the logarithm of the fold change of each gene relative to the average expression in the first category. Y-axis represents mean expression values of each gene in the normal, OP, and OR groups.

Fig. 5. Scatter plots comparing global gene expression profiles between OP and OR groups. Expression data for each type of adipose tissue of OP was plotted against those of the OR group. Upper and the lower diagonal lines show the two-fold regression lines. Transcript expression levels are on the $\log_2$ scale. Blue and red dots stand for up- and down-regulated genes in each adipose tissue, respectively. Five genes in BAT and one gene in WAT were indicated to stress their distinct differential expression between the two groups. For gene symbols, see the abbreviations section.



to HFD feeding.

K-means clustering of the absolute expression levels of these genes identified 4 distinct groups of genes with significant expression levels. Only a limited number of genes were significantly down- or up-regulated in both types of adipose tissue in response to HFD feeding (normal vs. OP and normal vs. OR), whereas expression levels of a large number of genes differed significantly between OP and OR rat adipose tissues (cluster 3 and 4 in Figs. 2, 3, and 4).

Validation of microarray analysis by real-time RT-PCR analysis

We selected several genes, including some important genes that were differentially expressed between OP and OR rats in order to validate their changes by quantitative reverse transcription RT-PCR. To address this issue, expression patterns of some genes, including Acta1, Mylk2, and Nnat in BAT, as well as Rrad, Sox18, and Wdnm in WAT, were further confirmed by real-time RT PCR (Fig. 7). As a result, expression patterns of all tested genes by PCR analysis were in line with those of microarray analysis.

Discussion

Rats vary in their propensity to become obese when eating a high-fat diet; however, factors that make some rats susceptible and others resistant to diet-induced obesity are unclear. Numerous studies have described various phenotypic consequences between rodent models with HFD-induced obesity and their normal weight counterparts. For example, compared with OR rats, OP rats have several deficits in central nervous system function, including prominent deficits in noradrenergic function [26]. OR rats had a significantly lower increase in plasma norepinephrine than did OP rats. Levin et al.

Fig. 6. Distribution of differentially expressed genes (2≥fold changes) from microarrays according to their molecular functions or biological process in BAT and WAT of OP and OR rats.

pointed out that plasma NE levels after glucose load in OR rats were lower than those of OP rats and that there was a positive correlation between NE levels and subsequent weight gain [26]. OP rats have a diminished rate of norepinephrine (NE) clearance from the plasma, whereas OR rats require less sympathetic activation due to increased sensitivity to NE. These results suggest that predisposal to becoming OR on an HFD can dampen sympathetic activation after a glucose load, possibly due to heightened end-organ responsiveness to NE.

A number of genes are known to determine phenotypic distinction. Several genes are proven to be involved in stimulation while others prevent adiposity through gene knock-out or transgenic studies. The former includes kruffel-like factor 15 (klf15) and kruffell-like factor 4 (klf4) [27, 28], while the latter includes the early growth response gene 1 (egr1) [29]. Expression patterns of these genes were in line with our microarray data (data not shown).

In comparison with other tissues, fewer studies have been performed using adipose tissues for identification of phenotypic differences between OP and OR rodent models. Lopez et al. [30] reported that expression levels of a number of genes in epididymal fat involved in lipid metabolism were significantly increased, whereas redox and stress protein-coding genes showed down-regulated expression in diet-induced obese Wistar rats, compared with control rats. They also reported an important finding: if HFD feeding is prolonged, expression of many of the initial genes, probably aimed at consumption of the energy surplus and prevention of excessive fat deposition, is not maintained, resulting in development of adaptation to increased lipid storage [30]. Based on these observations, we hypothesized that OR rats have developed a different adaptive ability to store excessive lipids in adipose tissues through several metabolic pathways, including β-oxidation.

Comparison of the levels of gene expression in adipose tissue of rats fed a normal diet and an HFD established that HFD feeding has a global effect on the phenotype of adipose tissue between OP and OR rats at the molecular level. Identity of genes that are differentially expressed between adipose tissue of rats fed a normal and an HFD also revealed a number of previously unknown aspects of the response of adipose tissue to HFD feeding.

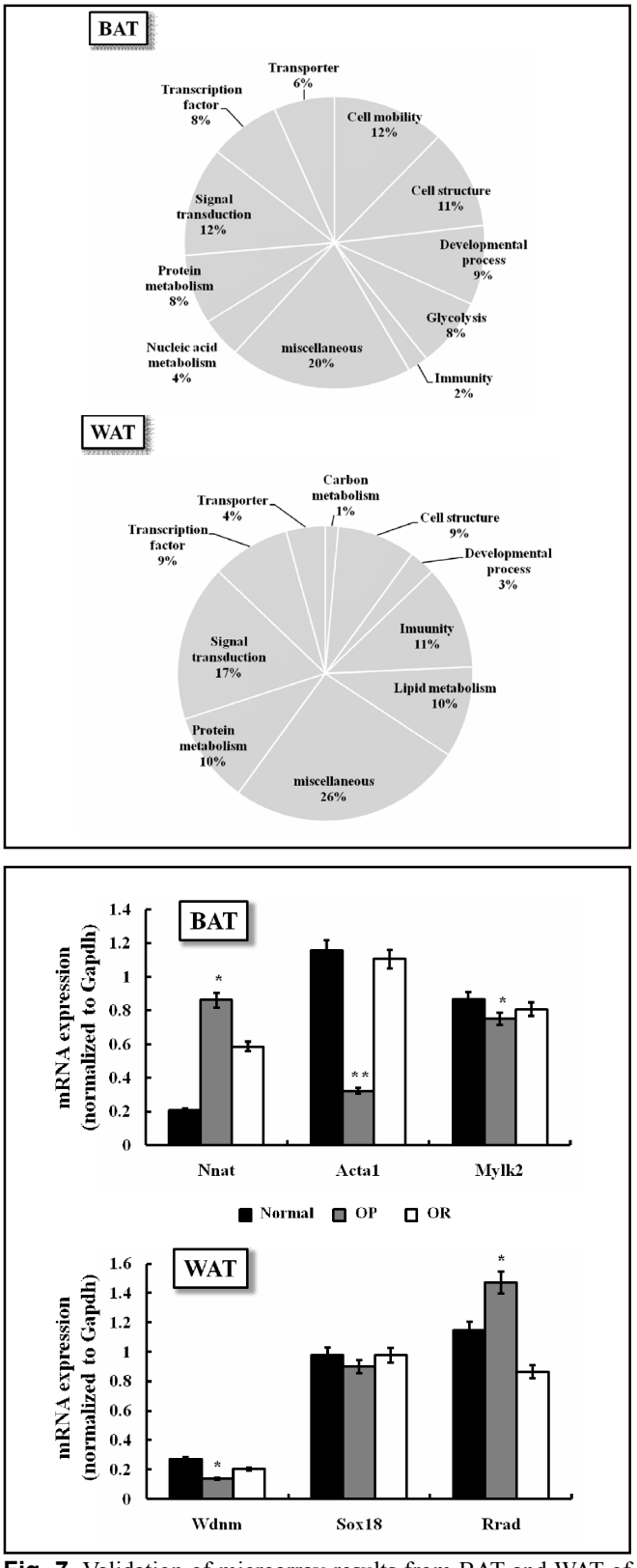


Fig. 7. Validation of microarray results from BAT and WAT of the experimental groups by real-time RT PCR analysis. Results are expressed as fold changes normalized to GAPDH gene expression and represent the mean ± SEM of triplicate independent experiments. *p<0.05, **p<0.001 for significance.

Accession No.	Description	Fold change[a)]		
		OP/NOR	OR/NOR	OR/OP
Lipid metabolism in BAT				
Fatty acid oxidation				
NM_130739.1	acyl-CoA synthetase long-chain family member 6 (Acsl6)	-1.48	-1.26	1.18
NM_031543.1	cytochrome P450, family 2, subfamily e, polypeptide 1 (Cyp2e1)	**-1.52**	-1.21	1.25
XM_230773.4	acyl-CoA synthetase short-chain family member 2 (predicted)	**-1.53**	**-1.5**	1.02
NM_053607.1	acyl-CoA synthetase long-chain family member 5 (Acsl5)	**-1.58**	-1.29	1.23
NM_053580.2	solute carrier family 27 (fatty acid transporter), member 1 (Slc27a1)	**-1.73**	**-1.61**	1.08
NM_012540.2	cytochrome P450, family 1, subfamily a, polypeptide 1 (Cyp1a1)	**-1.85**	-1.02	**1.81**
NM_176075.2	peroxisome proliferator-activated receptor gamma, coactivator 1 beta (Ppargc1b)	-1.24	1.17	1.46
NM_033352.1	ATP-binding cassette, sub-family D (ALD), member 2 (Abcd2)	1.48	-1.03	-1.52
Lipid metabolism in WAT				
Fatty acid oxidation				
NM_016999.2	cytochrome P450, family 4, subfamily b, polypeptide 1 (Cyp4b1)	1.48	-1.02	1.49
NM_012940.1	cytochrome P450, family 1, subfamily b, polypeptide 1 (Cyp1b1)	**1.53**	-1.18	1.43
NM_031543.1	cytochrome P450, family 2, subfamily e, polypeptide 1 (Cyp2e1)	**-5.83**	-1.09	**2.04**
NM_031557.2	prostaglandin I2 (prostacyclin) synthase (Ptgis)	**-1.91**	-1.24	**1.53**
Lipid synthesis				
NM_023104.1	acetoacetyl-CoA synthetase (Aacs)	**-1.74**	-1.2	1.44
NM_022193.1	acetyl-coenzyme A carboxylase alpha (Acaca)	**-2.39**	**-1.84**	1.3

Table 4. Expression of fatty acid oxidation and lipid synthesis-related genes (1.5≥fold change) in adipose tissue of normal (NOR), obesity-prone (OP), and obesity-resistant (OR) rats. Differentially expressed genes with a fold change = 1.5 between NOR and OP as well as NOR and OR group are represented, where the OR/OP is the average fold change using SAM two class unpaired test method. Genes showing differential expression of more than 2-fold were shown in bold.

In this study, we first examined the effect of an HFD on alteration of gene expression. Unexpectedly, compared with chow-fed rats, only 3 genes in BAT and 5 genes in WAT showed significant differential expression. This result is comparable with data obtained by Viguerie et al. [31], who found that adipose tissue gene expression was not significant in obese human subjects during low-fat and high-fat hypocaloric diets.

In the current study, a total of 91 (BAT) and 53 (WAT) genes with a 2-fold or more variation between OP and OR groups may be associated with physiological variations, including increased total weight and adipose tissue weight in OP rats. Results obtained from the current study indicated that a higher number of genes in BAT were differentially expressed than in WAT. Enhancement of fatty acid oxidation in adipocytes has been proposed to reduce adiposity and weight loss; therefore, we focused on differential expression of genes involved in lipid metabolism [32]. We found that the majority of these genes were down-regulated in both BAT and WAT of OP rats upon HFD feeding. However, OR rats displayed more resistance to down-regulation of these genes in both BAT and WAT. Down-regulated genes included acyl-CoA synthetase 5, 6 (Acsl5, 6), cytochrome P450 family 2 subfamily e polypeptide 1 (Cyp2e1) in BAT and acetyl-coenzyme A carboxylase alpha (Acaca), acetoacetyl-CoA syntheatase (Aacs), cytochrome P450 family 2 subfamily e polypeptide 1 (Cyp2e1) in WAT (Table 4). Acsls are essential genes for fatty acid degradation through catalysis of ATP-dependent acylation of fatty acids into long-chain acyl CoAs (LCA-CoAs), and the first step in lipid metabolism after fatty acid entry into the cell. LCA-CoAs can then enter the β-oxidation pathway for energy production or undergo further esterification for production of phospholipids, cholesterol esters, and triglycerides [33]. Cyp2e1 catalyzes oxidation of microsomal fatty acids, which can impair mitochondrial function by uncoupling oxidative phosphorylation [34, 35]. Acaca then converts acetyl-CoA to malonyl-CoA, which is used by fatty acid synthase to form palmitic acid. These fatty acids are used in synthesis of triglycerides - the primary source of energy storage. Expression of the Aacs gene was preferentially detected in mature adipocytes, but not in preadipocytes, and increased during adipocyte differentiation [36]. Collectively, distinct differences in expression of fatty acid oxidation and lipogenic genes were observed between OP and OR rat adipose tissues, implying that small differences in genes encoding enzymes for fatty acid oxidation and lipogenesis might lead to significant changes in total lipid accumulated in adipose tissue.

Unexpectedly, we found no significant difference in genes encoding uncoupling proteins (Ucps), even in Ucp1 in BAT (data not shown). Considering the fact that BAT contents in OR rats were slightly lower than OP rats albeit

not significant (Fig. 1E), this result led us to postulate that Ucps may not contributing genes in determining obesity-resistant phenotype at least in the transcriptomic levels. However, protein levels or uncoupling activity of Ucps are not the cases. In our previous study [37], higher protein levels of Ucps in adipose tissues, especially Ucp1 in BAT, were observed in OR rats. Several studies have found that Ucp-1 mediated thermogenesis in BAT was essential for protection against cold but not for maintenance of body weight [38, 39]. Taken together, it remains to be demonstrated whether Ucps are also essential for obesity resistance or only for cold-induced thermogenesis.

We also found no significant difference in expressions of genes encoding adiopokine production between OP and OR rats. All selected adipokine genes, including leptin (Lep), resistin (Retn), adiponectin (Adipoq), retinol binding protein 4 (Rbp4), cathepsin S (Ctss), and apelin (Apln) showed similar expression levels (data not shown). This result is in line with our earlier findings demonstrating that their levels of gene products (proteins) in blood between OP and OR rats showed no significant difference [40].

We observed several genes showing significant variations between the two groups, including genes encoding myosin light chain kinase 2 (Mylk2), and neuronatin (Nnnt), tropomyosin 1 (Tpm1) in BAT, as well as ras-related associated with diabetes (Rrad), nitric oxide synthase 3 (NOS3), and insulin-like growth factor binding protein-2 (Igfbp2) in WAT. Mylk2 is a regulatory target of Ca^{2+}/calmodulin and plays an important role in insulin-stimulated glucose transport in adipocytes [41]. Igfbp2 is the principal binding protein secreted by differentiation of white preadipocytes, suggesting a potential role in the development of obesity. Stephen et al. showed that overexpression of igfbp2 is associated with reduced susceptibility to obesity and improved insulin sensitivity upon an HFD [42]. A research has demonstrated that adipocytes in culture Rrad gene is overexpressed, exhibiting a reduction in the rate of insulin-stimulated glucose uptake [43]. Moreover, overexpression of Rrad in animals potentiates the insulin resistance associated with HFD and alters triglyceride metabolism in muscle [44]. In our study, down-regulation of Mylk2 and Igfbp2, as well as an increased level of Rrad in WAT of OP rats, suggest that OP rats may be of pathological relevance to the increased risk of insulin resistance associated with an HFD. The Nnat gene was originally discovered from a differential display on the developing rat brain. However, Nnat also appears to be abundantly expressed in adipose tissue, and is significantly elevated in adipose tissue of obese Zuker diabetic fatty rats, compared with control lean Zuker rats [45]. Tpm1 is known to associate with microfilaments, also a component of the cytoskeleton in preadipocytes. Thus, Tpm1 affects cell adhesion, and, thereby, cell shape during adipocyte differentiation [46]. Rat adipose tissue expresses two isoforms of Nos, the endothelial isoform (Nos III) and the inducible isoform (Nos II) [47]. NO increases mitochondrial biogenesis, oxidative metabolism, and ATP levels in several cell types [48, 49]. Consistently, compared with wild-type animals, mitochondrial biogenesis and function are markedly decreased in eNos-null mutant ($eNos^{-/-}$) mice [48, 49], with fat accumulation in the abdomen and increased body weight [48, 50]. In this study, decreased Nnat genes as well as increased Tpm1 and Nos3 genes in BAT of OP rats indicate evidence for susceptibility to HFD-induced obesity.

Other significant variations (5>fold changes) between OP and OR groups include genes of troponin T type 3 (Tnnt3), creatine kinase (Ck), actin alpha1 (Acta1), actinin alpha 3 (Actn3), myosin light chain, phosphrylatable, fast skeletal muscle (Mylpf) in BAT, and ribosomal protein L30 (Rpl30) in WAT. All of these genes were found for the first time in adipose tissue of HFD-induced obesity-resistant rats.

It is interesting to mention that some genes encoding muscle-associated proteins (e.g. myosin chain, tropomyosin, and troponin T) were markedly down-regulated in BAT of OP rats, whereas their expressions were highly increased in OR rats mimicking normal diet-fed rats. The molecular mechanism of expression of these muscle-related genes remains a mystery. One possibility for higher expression of these genes is that they would be essential for maintenance of the architecture of cell membranes and other regulatory roles in BAT [46]. This hypothesis may be plausible, considering the fact that BAT cells and muscle myocytes originate from the same progenitor cells [51]. However, it remains to be demonstrated whether higher expression of these genes resulted from higher content of mitochondria in BAT of OR rats.

We found remarkable alterations of a large number of genes encoding metabolic enzymes and some genes coding proteins involved in cell structure and mobility, signal transduction, and developmental processes. However, we failed to find any impairment in expression of genes encoding anti-oxidant enzymes and thermogenesis-related proteins (Ucps) (data not shown). However, our microarray data collectively imply that a large number of genes, particularly those expressed in

BAT, contribute to metabolic regulations directing HFD-induced obesity resistance [52].

In conclusion, major phenotype-based differences in gene expression profiles of adipose tissues were observed between OP and OR rats upon HFD feeding. Changes in gene expression profile associated with lipid metabolism, carbohydrate metabolism, cell structure, transcriptional factors, and signal transduction related genes were exhibited by OP and OR rats. The current study suggests insights into the study of OP and OR rats, untangling some of the molecular events taking place in adipose tissues. These changes provide candidate genes for future investigation of pathogenesis of obesity and type 2 diabetes mellitus.

Abbreviations

Acaca (Acetyl-coenzyme A carboxylase alpha); Acsl5 (Acyl-CoA synthetase long-chain family member 5); Acta1 (Actin alpha1; Actn3, actinin alpha 3); BAT (Brown adipose tissue); CK (Creatine kinase); HFD (High fat diet); Gapdh (Glyceraldehyde-3-phosphate dehydrogenase); Igfbp2 (Insulin-like growth factor binding protein-2); Mylk2 (Myosin light chain kinase 2); Mylpf (Myosin light chain phosphrylatable fast skeletal muscle); NE (Norepinephrine); Nos3 (Nitric oxide synthase 3); OP (Obesity-prone); OR (Obesity-resistant); Rrad (Ras-related associated with diabetes); Rpl30 (Ribosomal protein L30); Tpm1 (Tropomyosin 1); Tnnt3 (Troponin T type 3); Ucp (Uncoupling protein); WAT (White adipose tissue).

Acknowledgements

This research was supported by the SRC program (Center for Food & Nutritional Genomics: grant number 2010-0001888) of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology. JI Joo was a Bain Korea 21 scholarship beneficiary. The authors extend their thanks to Dr. Young Ju Bae of Macrogen Inc. (Seoul, Korea) for her technical assistance in microarray analysis.

References

1 Farmer SR: Transcriptional control of adipocyte formation. Cell Metab 2006;4:263-273.
2 Badman MK, Flier JS: The adipocyte as an active participant in energy balance and metabolism. Gastroenterology 2007;132:2103-2115.
3 Unger RH, Orci L: Diseases of liporegulation: New perspective on obesity and related disorders. FASEB J 2001;15:312-321.
4 Frigolet ME, Torres N, Uribe-Figueroa L, Rangel C, Jimenez-Sanchez G, Tovar AR: White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. J Nutr Biochem 2011;22:118-129.
5 Arner P: Hunting for human obesity genes? Look in the adipose tissue! Int J Obes Relat Metab Disord 2000;24:S57-62.
6 Trayhurn P, Beattie JH: Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. Proc Nutr Soc 2001;60:329-339.
7 Lorente-Cebrian S, Bustos M, Marti A, Martinez JA, Moreno-Aliaga MJ: Eicosapentaenoic acid up-regulates apelin secretion and gene expression in 3t3-l1 adipocytes. Mol Nutr Food Res 2010;54:S104-111.
8 Rajala MW, Scherer PE: Minireview: The adipocyte-at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 2003;144:3765-3773.
9 Trayhurn P: Endocrine and signalling role of adipose tissue: New perspectives on fat. Acta Physiol Scand 2005;184:285-293.
10 Levin BE, Triscari J, Hogan S, Sullivan AC: Resistance to diet-induced obesity: Food intake, pancreatic sympathetic tone, and insulin. Am J Physiol 1987;252:R471-478.
11 West DB, Boozer CN, Moody DL, Atkinson RL: Dietary obesity in nine inbred mouse strains. Am J Physiol 1992;262:R1025-1032.
12 Blundell JE, Cooling J: High-fat and low-fat (behavioural) phenotypes: Biology or environment? Proc Nutr Soc 1999;58:773-777.
13 Ji H, Outterbridge LV, Friedman MI: Phenotype-based treatment of dietary obesity: Differential effects of fenofibrate in obesity-prone and obesity-resistant rats. Metabolism 2005;54:421-429.
14 Jang I, Hwang D, Lee J, Chae K, Kim Y, Kang T, Kim C, Shin D, Hwang J, Huh Y, Cho J: Physiological difference between dietary obesity-susceptible and obesity-resistant sprague dawley rats in response to moderate high fat diet. Exp Anim 2003;52:99-107.
15 Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE: Selective breeding for diet-induced obesity and resistance in sprague-dawley rats. Am J Physiol 1997;273:R725-730.
16 Eberhart GP, West DB, Boozer CN, Atkinson RL: Insulin sensitivity of adipocytes from inbred mouse strains resistant or sensitive to diet-induced obesity. Am J Physiol 1994;266:R1423-1428.
17 Levin BE, Finnegan MB, Marquet E, Sullivan AC: Defective brown adipose oxygen consumption in obese zucker rats. Am J Physiol 1984;247:E94-100.
18 Klaus S, Keijer J: Gene expression profiling of adipose tissue: individual, depot-dependent, and sex-dependent variabilities. Nutrition 2004;20:115-120.

19 Crul T, Testelmans D, Spruit MA, Troosters T, Gosselink R, Geeraerts I, Decramer M, Gayan-Ramirez G: Gene expression profiling in vastus lateralis muscle during an acute exacerbation of copd. Cell Physiol Biochem 2010;25:491-500.
20 Xu RY, Wan YP, Tang QY, Wu J, Cai W: The effects of high fat on central appetite genes in wistar rats: A microarray analysis. Clin Chim Acta 2008;397:96-100.
21 Palou M, Priego T, Sanchez J, Rodriguez AM, Palou A, Pico C: Gene expression patterns in visceral and subcutaneous adipose depots in rats are linked to their morphologic features. Cell Physiol Biochem 2009;24:547-556.
22 Spiegelman BM, Farmer SR: Decreases in tubulin and actin gene expression prior to morphological differentiation of 3t3 adipocytes. Cell 1982;29:53-60.
23 Kim YJ, Park T: Genes are differentially expressed in the epididymal fat of rats rendered obese by a high-fat diet. Nutr Res 2008;28:414-422.
24 Xu RY, Wan YP, Tang QY, Wu J, Cai W: The effects of high fat on central appetite genes in wistar rats: A microarray analysis. Clin Chim Acta 2008;397:96-100.
25 Kim SW, Hwang HJ, Baek YM, Lee SH, Hwang HS, Yun JW: Proteomic and transcriptomic analysis for streptozotocin-induced diabetic rat pancreas in response to fungal polysaccharide treatments. Proteomics 2008;8:2344-2361.
26 Levin BE, Triscari J, Hogan S, Sullivan AC: Resistance to diet-induced obesity: Food intake, pancreatic sympathetic tone, and insulin. Am J Physiol 1987;252:R471-478.
27 Birsoy K, Chen Z, Friedman J: Transcriptional regulation of adipogenesis by klf4. Cell Metab 2008;7:339-347.
28 Mori T, Sakaue H, Iguchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsuki Y, Ogawa W, Hiramatsu R, Kasuga M: Role of kruppel-like factor 15 (klf15) in transcriptional regulation of adipogenesis. J Biol Chem 2005;280:12867-12875.
29 Boyle KB, Hadaschik D, Virtue S, Cawthorn WP, Ridley SH, O'Rahilly S, Siddle K: The transcription factors egr1 and egr2 have opposing influences on adipocyte differentiation. Cell Death Differ 2009;16:782-789.
30 Lopez IP, Marti A, Milagro FI, Zulet Md Mde L, Moreno-Aliaga MJ, Martinez JA, De Miguel C: DNA microarray analysis of genes differentially expressed in diet-induced (cafeteria) obese rats. Obes Res 2003;11:188-194.
31 Viguerie N, Vidal H, Arner P, Holst C, Verdich C, Avizou S, Astrup A, Saris WH, Macdonald IA, Klimcakova E, Clement K, Martinez A, Hoffstedt J, Sorensen TI, Langin D: Adipose tissue gene expression in obese subjects during low-fat and high-fat hypocaloric diets. Diabetologia 2005;48:123-131.
32 Rosen ED, Spiegelman BM: Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847-853.
33 Parkes HA, Preston E, Wilks D, Ballesteros M, Carpenter L, Wood L, Kraegen EW, Furler SM, Cooney GJ: Overexpression of acyl-coa synthetase-1 increases lipid deposition in hepatic (hepg2) cells and rodent liver *in vivo*. Am J Physiol Endocrinol Metab 2006;291:E737-744.
34 Lieber CS: Cyp2e1: From ash to nash. Hepatol Res 2004;28:1-11.
35 Tonsgard JH, Getz GS: Effect of reye's syndrome serum on isolated chinchilla liver mitochondria. J Clin Invest 1985;76:816-825.
36 Yamasaki M, Hasegawa S, Suzuki H, Hidai K, Saitoh Y, Fukui T: Acetoacetyl-coa synthetase gene is abundant in rat adipose, and related with fatty acid synthesis in mature adipocytes. Biochem Biophys Res Commun 2005;335:215-219.
37 Joo JI, Oh TS, Kim DH, Choi DK, Wang X, Choi JW, Yun JW: Differential expression of adipose tissue proteins between obesity susceptible and resistant rats fed a high fat diet. Proteomics 2011, in press.
38 Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP: Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 1997;387:90-94.
39 Sadurskis A, Dicker A, Cannon B, Nedergaard J. Polyunsaturated fatty acids recruit brown adipose tissue: increased UCP content and NST capacity. Am J Physiol 1995;269:E351-360.
40 Choi JW, Wang X, Joo JI, Kim DH, Oh TS, Choi DK, Yun JW: Plasma proteome analysis in diet-induced obesity-prone and obesity-resistant rats. Proteomics 2010;10:4386-4440.
41 Choi YO, Ryu HJ, Kim HR, Song YS, Kim C, Lee W, Choe H, Leem CH, Jang YJ: Implication of phosphorylation of the myosin ii regulatory light chain in insulin-stimulated glut4 translocation in 3t3-f442a adipocytes. Exp Mol Med 2006;38:180-189.
42 Wheatcroft SB, Kearney MT, Shah AM, Ezzat VA, Miell JR, Modo M, Williams SC, Cawthorn WP, Medina-Gomez G, Vidal-Puig A, Sethi JK, Crossey PA: Igf-binding protein-2 protects against the development of obesity and insulin resistance. Diabetes 2007;56:285-294.
43 Moyers JS, Bilan PJ, Reynet C, Kahn CR: Overexpression of rad inhibits glucose uptake in cultured muscle and fat cells. J Biol Chem 1996;271:23111-23116.
44 Ilany J, Bilan PJ, Kapur S, Caldwell JS, Patti ME, Marette A, Kahn CR: Overexpression of rad in muscle worsens diet-induced insulin resistance and glucose intolerance and lowers plasma triglyceride level. Proc Natl Acad Sci USA 2006;103:4481-4486.
45 Suh YH, Kim WH, Moon C, Hong YH, Eun SY, Lim JH, Choi JS, Song J, Jung MH: Ectopic expression of neuronatin potentiates adipogenesis through enhanced phosphorylation of camp-response element-binding protein in 3t3-l1 cells. Biochem Biophys Res Commun 2005;337:481-489.
46 Tong Y, Hara A, Komatsu M, Tanaka N, Kamijo Y, Gonzalez FJ, Aoyama T: Suppression of expression of muscle-associated proteins by pparalpha in brown adipose tissue. Biochem Biophys Res Commun 2005;336:76-83.
47 Mehebik N, Jaubert AM, Sabourault D, Giudicelli Y, Ribiere C: Leptin-induced nitric oxide production in white adipocytes is mediated through pka and map kinase activation. Am J Physiol Cell Physiol 2005;289:C379-387.
48 Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO: Mitochondrial biogenesis in mammals: The role of endogenous nitric oxide. Science 2003;299:896-899.
49 Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, Pisconti A, Brunelli S, Cardile A, Francolini M, Cantoni O, Carruba MO, Moncada S, Clementi E: Mitochondrial biogenesis by no yields functionally active mitochondria in mammals. Proc Natl Acad Sci USA 2004;101:16507-16512.
50 Valerio A, Cardile A, Cozzi V, Bracale R, Tedesco L, Pisconti A, Palomba L, Cantoni O, Clementi E, Moncada S, Carruba MO, Nisoli E: Tnf-alpha downregulates enos expression and mitochondrial biogenesis in fat and muscle of obese rodents. J Clin Invest 2006;116:2791-2798.
51 Farmer SR: Brown fat and skeletal muscle: Unlikely cousins? Cell 2008;134:726-727.
52 Frontera M, Pujol E, Rodriguez-Cuenca S, Catala-Niell A, Roca P, Garcia-Palmer FJ, Gianotti M: Rat brown adipose tissue thermogenic features are altered during mid-pregnancy. Cell Physiol Biochem 2005;15:203-210.