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Global comparison of gene expression between subcutaneous and intramuscular adipose tissue of mature Erhualian pig

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ABSTRACT. Adipose tissue plays an important role in energy metabolism and related diseases. The content of intramuscular fat significantly influences the pork quality. In this study, the whole gene expression of dorsal subcutaneous (s.c.) adipose tissue and intramuscular (i.m.) adipose tissue isolated from longissimus dorsi muscle tissue were compared using Affymetrix Gene-Chip microarray technology. The result revealed that 1228 genes were more highly expressed in s.c. adipose tissue, whereas 965 genes were higher expressed in i.m. adipose tissue. We found that the s.c. adipose tissue had a stronger capacity of lipid metabolism and fatty acid metabolism compared with i.m. adipose tissue, and angiopoietin-like-4, neuronatin, neuron-derived orphan receptor-1 alfa, and chloride intracellular channel 5 may play important roles in the regulation of fat deposition between i.m. and s.c. adipose tissues.

Key words: Subcutaneous adipose tissue; Intramuscular adipose tissue; Lipid metabolism; Fatty acid metabolism

INTRODUCTION

In livestock production, the content of intramuscular fat (IMF) plays an important role in the sensory quality of pork and is implicated in consumer acceptance (Fernandez et al., 1999; Webb and O'Neill, 2008). The aim of modern pig breeding is to produce lean meat with a reasonable IMF content. However, it is well documented that IMF content has decreased significantly as a result of long-term intensive selection for increased leanness and thin back fat (Hermesch et al., 2000). A decrease in back fat will lead to a reduction in IMF content, since there is a moderate positive correlation between IMF content and back fat thickness (Lo et al., 1992; Suzuki et al., 2005). This correlation is unfavorable, but reasonable, since both subcutaneous (s.c.) and intramuscular (i.m.) adipocytes share the same regulatory process of adipogenesis. Therefore, to produce lean pork with a reasonable IMF content remains a challenge for modern pig breeding research.

The profiles of adipocyte differentiation and metabolism might be different according to their locations. The difference of various adipose depots, such as s.c., i.m., visceral, and perirenal adipose tissue, has been compared in humans (Vohl et al., 2004) and cows (Pickworth et al., 2011). The growth and differentiation of adipocytes are different between fat and muscle tissues (Sun et al., 2004; Hausman and Hausman, 2006). Intramuscular adipocytes different from those in other depots because that they are distributed among muscle fibers. The IMF content might be influenced by muscle fiber. Adipocyte membrane interactions with the extracellular matrix and with neighboring myofibroblast might trigger a variety of responses within the adipocytes (Katz et al., 2000). Intramuscular adipogenesis can be specifically inhibited by myostatin, which is a muscle-specific secreted peptide (Rebbapragada et al., 2003). However, the whole gene comparison between i.m. and s.c. adipose tissues in mature pigs has not been done *in vivo*. The *in vitro* culture system might have erased such important signal responses, whereas the *in vivo* experiment can reflect the physiological process of the organism.

In this study, we conducted a transcriptional comparison between i.m. and s.c. adipose tissues using mature Erhualian pigs in order to investigate the molecular differences between the two fat tissues. The results can facilitate deeper insight into the specific regulatory mechanisms of i.m. adipogenesis.

MATERIAL AND METHODS

Tissue samples

Male Erhualian pigs at 7 months of age and about 60 kg were used in this experiment. The pigs were from Erhualian protected areas in Chang Zhou City, China. All procedures of the experiments were done according to "The Instructive Notions with Respect to Caring for Laboratory Animals", enacted by the Ministry of Science and Technology of the People's Republic of China. The pigs were exsanguinated after electric stunning. Dorsal s.c. adipose tissue and longissimus dorsi (LD) muscle tissue were separated immediately from the junction of the thoracic and lumbar vertebrae after sabotage, and stored in liquid nitrogen until RNA extraction and i.m. adipose tissue segregation. The i.m. adipose tissue was carefully segregated using ophthalmology tweezers and scalpels to avoid muscle fibers contamination. The isolation was completed on ice in 5 min in order to decrease the RNA degradation.

RNA isolation

Total RNA was extracted from the s.c. and i.m. adipose tissues using Trizol reagent (Invitrogen, Carlsbad, USA), according to the manufacturer description. The absorbance values at 260 and 280 nm were checked to assess the RNA concentration and purity using the NanoDrop1000 Spectrophotometer (Thermo, USA). The A_{260}/A_{280} ratio was evaluated for protein impurities in the samples. The RNA integrity was checked by electrophoresis on 2% agarose gels (m/v).

Microarray assay

The gene chip of the Porcine Genome Array (Affymetrix, Santa Clara, USA), which includes 23,937 probes, containing 23,256 transcripts of 20,201 *Sus scrofa* genes, was used in the experiments. The total RNAs of s.c. and i.m. adipose tissues were individually hybridized with gene chips. Briefly, in the first-strand cDNA synthesis reaction, 10 mg total RNA was used for reverse transcription using a T7-oligo(dT) promoter primer. Then, the double-stranded cDNA was synthesized from the first-strand cDNA using Rnase H. After purification of the resulting DNA, an *in vitro* transcription reaction was done to produce biotin-labeled cRNA using the MEGA Script T7 Kit (Ambion, Inc., USA). After the biotin-labeled cRNA had been cleaned up and fragmented, the cRNA was hybridized to the probe array at 45°C for 16 h. Thereafter, the probe array was washed and stained on the fluidics station, and the microarrays were scanned using the GeneChip Scanner 3000 (Affymetrix). The Affymetrix Micro Array Suite 5.0-Specific Terms GCOS version 1.4 was used for the quantity analysis of the hybridization. The gene expression levels that had ≥2-fold difference between s.c. and i.m. adipose tissues were checked and further analyzed. The microarray assay was carried out by Capital Bio Corporation. The Molecule Annotation System (http://bioinfo.capitalbio.com/mas) was used to analyze the differentially expressed genes, using the Kyoto encyclopedia of genes and genomes (KEGG) public pathway resource and gene ontology (GO) consortium.

Real-time reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed to confirm the microarray results. Total RNA was extracted from s.c. and i.m. adipose tissues as described above and total RNA was reverse transcribed using a revere transcription kit (TaKaRa, Dalian, China) according to the manufacturer protocols. The expression levels were checked for 13 genes: fatty acid synthase (FAS), leptin (LEP), adiponectin (ADIPOQ), peroxisome proliferator-activated receptor gamma (PPAR-γ), fatty acid-binding protein 5 (FABP5), fatty acid-binding protein 4, adipocyte (FABP4), lipoprotein lipase (LPL), glucose phosphate isomerase (GPI), cyclin D2 (CCND2), insulin-like growth factor 2 (IGF2), neuron-derived orphan receptor-1 alfa (NOR-1α), triosephosphate isomerase 1 (TPI1), and transducer of ERBB2, 1 (TOB1). The β-actin gene (ATCB) was used as the invariant control. Primers were designed using Primer Premier 5.0 and are shown in Table 1. RT-PCR was performed using the Fast Start Universal SYBR Green Master (TaKaRa) with a 20-μL reaction system, according to the manufacturer protocol, in an ABI 7300 instrument. The thermal conditions of RT-PCR were as follows: 95°C for 30 s, followed by 40 cycles of degenerating at 95°C for 5 s, and annealing and extension at 60°C for 1 min. The melting-curve analysis was performed in order to monitor the specificity of production. All

experiments were repeated three times. The gene expression levels in the s.c. and i.m. adipose tissues were analyzed with the $2^{-\Delta\Delta CT}$ method.

Table 1. Primer sequences for RT-PCR.

Gene symbol	Accession No.	Product size (bp)	Primer sequence (5' to 3')
LPL	NM_214286	104	F: CCAATGGAGGCACTTT R: ATGGGAGCACTTCACG
FAS	NM_001099930	191	F: CATTCGGTGCGTCCTGGTG R: AGGCGTGCTCCGTCTGCTT
GPI	NM_214330	146	F: GAGTGGCGAATGGAAAGG R: TGGAGACGAACCAGACCC
CCND2	NM_214088	155	F: TTACCTGGACCGCTTCTTG R: GAGGCTTGATGGAGTTGTCG
TOB1	NM_001123205	130	F: CTCCCTTTGGTCACTCTG R: CTGCGGCCACTATTCTT
ADIPOQ	NM_214370	103	F: GCTGTACTACTTCTCCTTCCACATCA R: CTGGTACTGGTCGTAGGTGAAGAGT
FABP4	XM_001927334	126	F: CAGGAAAGTCAAGAGCACC R: ATGATACATTCCACCACCAA
FABP5	NM_001039746	132	F: GCACCAGTCCGCTTAT R: TTCCCACTCCTACTTCCT
IGF2	NM_213883	167	F: GTGGCATCGTGGAAGAGTGC R: CCAGGTGTCATAGCGGAAGAAC
LEP	NM_213840	155	F: ATGCGGTGTATTCTGGTTG R: AGAGCCCTCAAGTCACTCA
PPARG	NM_214379	204	F: ATTCCCGAGAGCTGATCCAA R: TGGAACCCCGAGGCTTTAT
TPI1	NM_001037151	117	F: GAACGGGCGAAAGAACA R: TGCCTGGCGAAGTCAAT
NOR-1	NM_214247	127	F: TTTTGGACGATGCTATCC R: ACAACCCTTGGCTGTTC
ACTB	DQ845171	220	F: TGCTGTCCCTGTACGCCTCTG R: ATGTCCCGCACGATCTCCC

Statistical analysis

Data are reported as means ± SE. Differences of mRNA expression levels were analyzed by the independent-samples *t*-test (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Differentially expressed genes

After quantifying all hybridization spots, the signal intensity was plotted logarithmically. Figure 1 shows the scatter plot of microarray signals of s.c. and i.m. adipose tissues. It shows that the expression levels of many genes were different between the two samples. According to the statistics, 60.08% (14,493/24,123) probes were checked out in the s.c. adipose tissue sample, and 65.44% (15,787/24,123) gene probes were inspected in the i.m. adipose tissue sample. The comparison of the two samples revealed that 1281 probes, representing 1228 transcripts, including 234 known genes (Table 2), were up-regulated (≥2 folds) in s.c. adipose tissue, whereas 1076 probes, representing 965 transcripts, including 172 recognized genes (Table 3), were up-regulated in i.m. adipose tissue (≥2 folds).

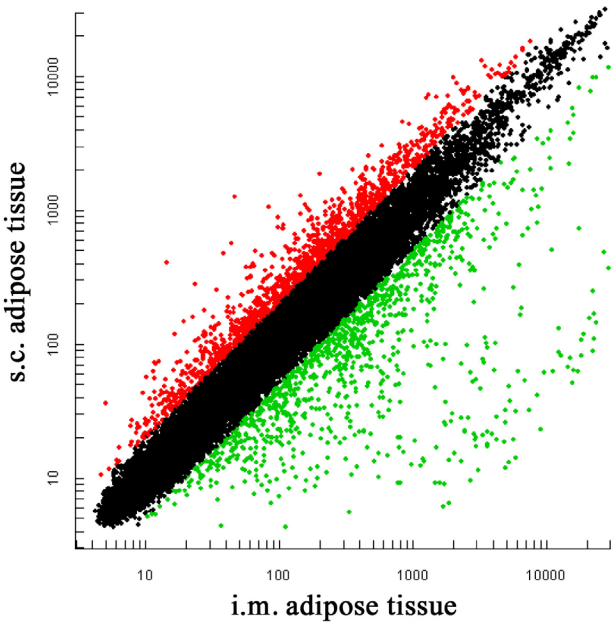

Figure 1. Log-Log Scatter Plot of subcutaneous (s.c.) and intramuscular (i.m.) adipose tiusse. Red replaces higher expressed genes in s.c adipose tissue. Green replaces the higher expressed genes in i.m adipose tissue.

Results of GO and KEGG analyses

In order to clarify the different biological patterns of the two samples, significantly different genes in the s.c. and i.m. adipose samples were individually analyzed by GO and KEGG of the criterion $P < 0.05$.

The GO results (Table 4) showed that of the highly expressed genes in the s.c. sample, 49.25% were for biological processes, 35.15% for molecular function, 14.10% for cellular components, and 1.504% for other items; correspondingly, 49.16% were for biological processes, 31.76% for molecular function, 18.17% for cellular components, and 1.014% for other items in the i.m. sample. With regards the biological processes, the highly expressed genes in the s.c. adipose sample (Figure 2A) significantly belonged to fatty acid β-oxidation, fatty acid metabolism, lipid metabolism, and fatty acid oxidation. Corresponding, the highly expressed genes in the i.m. adipose sample (Figure 2B) were mainly implicated in negative regulation of fat cell differentiation, DNA methylation, glycolysis, and positive regulation of histone acetylation.

The genes that were significantly expressed in the s.c. and i.m. samples were analyzed in KEGG (Figure 3). Figure 3A shows that the highly expressed genes in s.c. fat belong to glycolysis/gluconeogenesis, fatty acid metabolism, fatty acid elongation in mitochondria, glycerolipid metabolism, adipocytokine signaling pathway, Janus kinase/signal transducer and activator of transcription signaling pathway, and insulin signaling pathway. The highly expressed genes in i.m. fat were involved in the mitogen-activated protein kinase cascades signaling pathway, Wingless and INT-1 signaling pathway, adipocytokine signaling pathway, cell communication, cell cycle, insulin signaling pathway, and transforming growth factor-β signaling pathway (Figure 3B).

Table 2. Highly expressed genes in subcutaneous adipose tissue.

Gene title	Gene symbol	Representative public ID	Ratio
Retinol binding protein 7	RBP7	CN155390	27.037
Lysozyme	LYZ	NM_214392.1	12.564
Steroidogenic acute regulatory protein	STAR	NM_213755.1	12.445
Guanylate binding protein 1	GBP1	CO950381	9.214
Phosphoenolpyruvate carboxykinase 1	CH242-37G9.2	BX676168	9.069
Secretogranin V	SCG5	M23654.1	7.572
Amelogenin	AMELX	NM_213906.1	7.162
Interleukin 15	IL15	NM_214390.1	6.873
Regulator of G-protein signaling 1	RGS1	AF139837.1	6.562
Adipose differentiation-related protein	ADRP	AY550037.1	6.416
Kallikrein	KLKB1	NM_214074.1	5.951
C-type lectin domain family 5, member A	CLEC5A	NM_213990.1	5.523
Aquaporin 3	AQP3	CK451710	5.445
Secretory leukocyte peptidase inhibitor	SLPI	NM_213870.1	5.438
Microseminoprotein, beta	MSMB	NM_213852.1	5.113
Apolipoprotein E	APOE	NM_214308.1	4.818
Sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone-preferring, member 1	SULT2A1	BI402591	4.796
Aldo-keto reductase family 1, member C4	AKR1C4	BI184598	4.743
Porcine inhibitor of carbonic anhydrase	PICA	NM_213847.1	4.733
Ficolin	LOC396881	NM_213868.1	4.717
Carboxylesterase	CES3	NM_214246.1	4.674
Lipoprotein lipase	LPL	AY686760.1	4.652
Glycerol kinase	GK	CK457408	4.63
Ameloblastin	AMBN	NM_214037.1	4.601
CD86 molecule	CD86	NM_214222.1	4.437
Adiponectin, C1Q, and collagen domain containing	ADIPOQ	AY589691.1	4.417
Angiopoietin-like 4	ANGPTL4	BI183736	4.393
Acyl-Coenzyme A dehydrogenase, long chain	ACADL	NM_213897.1	4.374
Glycoprotein nmb	GPNMB	CN153410	4.29
Ras homolog gene family, member F	RHOF	CK455476	4.079
Fructose 1,6-bisphosphatase	FBP	NM_213979.1	4.054
Microsomal glutathione S-transferase 1	MGST1	NM_214300.1	4.023
Peptidoglycan recognition protein L	pPGRP-LB	NM_213738.1	3.911
Occludin	OCLN	CF368080	3.844
Chemokine ligand 2	CCL2	NM_214214.1	3.831
Growth hormone receptor	GHR	X54429.1	3.828
Phospholipase A2, group VII	PLA2G7	BQ603958	3.815
Insulin induced gene 1	INSIG1	BP454285	3.714
Vascular endotheial growth factor 2	LOC414908	BI360137	3.635
Paraoxonase 3	PON3	BX667193	3.583
CD247 molecule	CD247	CF367898	3.558
MyoD family inhibitor domain containing	MDFIC	BF075680	3.531
Secreted phosphoprotein 1	SPP1	NM_214023.1	3.529
Aquaporin 11	LOC100127151	CN070334	3.51
GTP binding protein overexpressed in skeletal muscle	GEM	Z80109.1	3.478
Malic enzyme 1, NADP(+)-dependent, cytosolic	ME1	CN163851	3.461
3-beta-hydroxysteroid dehydrogenase/delta-5-delta-4 isomerase	3B-HSD	CO946466	3.46
Prostaglandin reductase 1	PTGR1	NM_214385.1	3.446
Phosphogluconate dehydrogenase	PGD	CN159092	3.413
Monoglyceride lipase	MGLL	CN028971	3.387
Fatty acid synthase	FAS	CF180911	3.354
Methylmalonyl CoA epimerase	MCEE	CN166359	3.343
Guanine nucleotide binding protein, alpha inhibiting activity polypeptide 1	GNAI1	U11249.1	3.343
Enoyl Coenzyme A hydratase 1, peroxisomal	ECH1	BI183989	3.321
Fatty acid binding protein 7, brain	FABP7	CK463743	3.273
Complement component C9	LOC100037951	CF362312	3.265

Continued next on the page

Table 2. Continued.

Gene title	Gene symbol	Representative public ID	Ratio
Lipase, hormone-sensitive	LIPE	AY686758.1	3.24
Adiponectin receptor 2	ADIPOR2	CN156813	3.215
MHC class II DR-alpha	SLA-DRA	AY285933.1	3.212
Ubiquitin carboxyl-terminal esterase L1	UCHL1	CO947028	3.199
Glutathione peroxidase 3	GPX3	BX671405	3.194
Protein phosphatase 1 catalytic subunit alpha isoform	LOC733611	BP157767	3.183
Microsomal glutathione S-transferase 3	MGST3	CK466828	3.18
Flavin containing monooxygenase 1	FMO1	NM_214064.1	3.175
3-oxoacid CoA transferase 1	OXCT1	NM_213938.1	3.151
Transketolase	TKT	CN163555	3.142
Dehydrogenase/reductase member 4	DHRS4	NM_214019.1	3.139
Chemokine ligand 4	CCL4	NM_213779.1	3.134
Protein phosphatase 2, regulatory subunit A, beta isoform	PPP2R1B	CN160205	3.122
Carboxylesterase 1	CES1	CF365558	3.055
Feline leukemia virus subgroup C cellular receptor family, member 2	FLVCR2	CF177239	3.053
Topoisomersae II	TOPOII	NM_213884.1	3.027
1-acylglycerol-3-phosphate O-acyltransferase 1	SBAB-649D6.6	BG608754	3.025
ADP-ribosylation factor-like protein 4A	LOC595121	BF194181	3.024
Chemokine receptor 4	CXCR4	NM_213773.1	2.977
Cell death-inducing DNA fragmentation factor-like effector a	LOC100127171	BX675760	2.976
NAD(P)H dehydrogenase, quinone 1	NQO1	BQ601005	2.966
Ribosomal protein, large, P1	RPLP1	AY550065.1	2.954
Glutamyl aminopeptidase	ENPEP	NM_214017.1	2.939
Cytochrome b5 type B	CYB5B	CN154133	2.923
Peroxisome proliferator-activated receptor gamma	PPARG	AB097926.1	2.917
Lipase A	LIPA	CO986683	2.909
Cystatin B	CSTB	CN164516	2.908
Cytochrome P450, family 27, subfamily A, polypeptide 1	CYP27A1	CN153890	2.9
Chemokine receptor 1	CCR1	NM_001001621.1	2.892
2,4-dienoyl CoA reductase 1	DECR1	BQ602989	2.875
Renin binding protein	RENBP	D83766.1	2.857
Cytochrome b5 type A	CYB5A	NM_001001770.1	2.84
Lectin, galactoside-binding, soluble, 3	LGALS3	BX676137	2.837
PRA1 family protein-like protein	LOC595127	CF793094	2.836
Adrenomedullin	ADM	NM_214107.1	2.819
N-acetylneuraminate pyruvate lyase	NPL	NM_214071.1	2.811
Cystathionase	LOC733654	CN165749	2.805
G protein-coupled receptor 120	GPR120	BI402064	2.781
Coagulation factor V	F5	NM_214120.1	2.778
Translocator protein	TSPO	NM_213753.1	2.759
Killer cell lectin-like receptor subfamily K, member 1	KLRK1	NM_213813.1	2.758
v-myc myelocytomatosis viral oncogene homolog	MYC	NM_001005154.1	2.737
Solute carrier family 11, member 1	SLC11A1	U55068.1	2.725
Cell division cycle 2, G1 to S and G2 to M	CDC2	AJ687786	2.725
Hypothetical	LOC100152232	CF363286	2.724
Integral membrane protein	ITM2A	CK462331	2.722
Crystallin, zeta-like 1	CRYZL1	CN163225	2.718
Glutathione S-transferase omega	GSTO1	NM_214050.1	2.712
MHC class I antigen 1	SLA-1	AB105382.1	2.707
NK-lysin	NKL	BX672894	2.707
Glutathione reductase	GSR	CK461867	2.695
Quinoid dihydropteridine reductase	QDPR	BM189976	2.694
Branched chain keto acid dehydrogenase E1, beta polypeptide	BCKDHB	CO943570	2.677
Karyopherin alpha 2	KPNA2	BG382957	2.656
Elastase, neutrophil expressed	ELANE	NM_214109.1	2.65
Cellular disintegrin precursor	ADAM-9	AJ681165	2.638
CD1 antigen	CD1.1	NM_213831.1	2.635
BTG family, member 3	BTG3	BQ600276	2.629

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Table 2. Continued.

Gene title	Gene symbol	Representative public ID	Ratio
Haptocorrin	LOC396873	BX675338	2.625
Laminin, beta 1	LAMB1	CN155839	2.605
GTP-binding protein SAR1a	LOC595115	CK449626	2.58
POT1 protection of telomeres 1 homolog	POT1	BF703953	2.578
Zona pellucida binding protein	ZPBP	NM_214106.1	2.571
MHC class II histocompatibility antigen SLA-DRB1	SBAB-591C4.1	AB016750.1	2.569
Paraoxonase 2	PON2	CO951896	2.556
Leptin	LEP	AF052691.1	2.55
Lymphocyte antigen 96	LY96	BX918583	2.546
Peroxiredoxin 5	PRDX5	NM_214144.1	2.542
Acyl-CoA synthetase short-chain family member 2	ACSS2	AW483183	2.539
Phytanoyl-CoA 2-hydroxylase	PHYH	CB475937	2.526
Electron-transfer-flavoprotein, beta polypeptide	ETFB	CN158539	2.524
Tyrosine 3/5-monooxygenase activation protein, zeta polypeptide	YWHAZ	CN153545	2.507
Acyl-Coenzyme A oxidase 1, palmitoyl	ACOX1	BP447334	2.504
Long-chain 3-ketoacyl-CoA thiolase	LCTHIO	NM_213966.1	2.497
Progesterone receptor membrane component 1	PGRMC1	NM_213911.1	2.48
Cyclin B2	CCNB2	CK451027	2.479
Metallothionein	MT1A	NM_001001266.1	2.478
Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	SULT1A1	NM_213765.1	2.47
Lectin, galactoside-binding, soluble, 8	LGALS8	BF080287	2.461
Annexin A1	ANXA1	CO944050	2.46
Solute carrier family 26, member 11	SLC26A11	CK461723	2.44
Epoxide hydrolase	EPHX1	NM_214355.1	2.435
P450 oxidoreductase	POR	L33893.1	2.424
Propionyl Coenzyme A carboxylase, beta polypeptide	PCCB	NM_213901.1	2.423
DAZ associated protein 2	DAZAP2	BP169027	2.423
IgG heavy chain	LOC396781	NM_213828.1	2.422
Chemokine ligand 9	CXCL9	BX914993	2.422
Scavenger receptor class B, member 1	SCARB1	NM_213967.1	2.415
Hyaluronan synthase 3	HAS3	NM_001001268.1	2.41
DBF4 homolog	DBF4	CF796296	2.407
Fatty acid binding protein 4, adipocyte	FABP4	AU059657	2.403
Clathrin, light chain	CLTA	BQ600136	2.396
BH3 interacting domain death agonist	BID	BX923313	2.394
Soluble epoxide hydrolase	LOC414425	NM_001001641.1	2.39
Branched chain keto acid dehydrogenase E1, alpha polypeptide	BCKDHA	CF792961	2.389
Ubiquitin-conjugating enzyme E2, J1	UBE2J1	CF790105	2.384
Non-histone protein HMG2	HMGB2	NM_214063.1	2.384
Goosecoid protein	GCS	Y17718.1	2.382
D-aspartate oxidase	DDO	CO942555	2.381
Sterol-C4-methyl oxidase-like	SC4MOL	NM_213752.1	2.37
MHC class II, DQ alpha	SBAB-591C4.5	AY285927.1	2.364
Lactate dehydrogenase B	LDHB	U07180.1	2.358
Hypothetical protein LOC100153293	LOC100153293	BI184480	2.356
Granzyme H	GZMH	BX923569	2.355
Protein S (alpha)	PROS1	CN154806	2.35
CD59 molecule, complement regulatory protein	CD59	NM_214170.1	2.349
Thyroid hormone receptor beta 1	C-ERBA-B1	BX668616	2.349
Heme binding protein	LOC414409	CN159822	2.348
Transmembrane BAX inhibitor motif containing 6	TMBIM6	CK451891	2.336
Rh protein	RH	NM_214378.1	2.335
Glutathione peroxidase 4	GPX4	BI183078	2.327
Malate dehydrogenase 1, NAD	MDH1	NM_213874.1	2.326
Lysosomal 9kDa H+ transporting ATPase V0 subunit e	LOC733646	CB472326	2.322
Thioltransferase	GLRX1	NM_214233.1	2.319
Sirtuin 3	SIRT3	CF365373	2.316
Nitrogen fixation 1-like protein	LOC100156145	CF360660	2.313

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Table 2. Continued.

Gene title	Gene symbol	Representative public ID	Ratio
CCAAT/enhancer binding protein , alpha	CEBPA	AF103944.1	2.313
Superoxide dismutase 1, soluble	SOD1	CO992469	2.296
Ring finger protein 114	RNF114	CO939849	2.296
Cyclin B	CCNB1	BX671487	2.271
Citrate synthase	CS	C94952	2.261
FK506 binding protein 7	FKBP7	CN153356	2.26
Six transmembrane epithelial antigen of the prostate 1	STEAP1	NM_214305.1	2.252
Phosphate regulating endopeptidase homolog, X-linked	PHEX	AJ316616.1	2.247
Cytochrome P450, family 39, subfamily A, polypeptide 1	CYP39A1	CN154029	2.236
Neuronatin	NNAT	CK466245	2.234
Myosin VI	MYO6	NM_214021.1	2.226
Inhibitor of DNA binding 2	ID2	CK466212	2.226
Caspase 8, apoptosis-related cysteine peptidase	CASP8	AJ663595	2.225
Fc fragment of IgG, low affinity IIIb, receptor	FCGR3B	AF372455.1	2.223
Hydroxysteroid (17-beta) dehydrogenase 4	HSD17B4	NM_214306.1	2.222
Proteoglycan 1 precursor-like	LOC100049692	CK464602	2.203
NADPH oxidase heavy chain subunit	GP91-PHOX	NM_214043.1	2.195
B-cell translocation gene 1, anti-proliferative	BTG1	CB481644	2.193
Heme oxygenase 1	HMOX1	X60677.1	2.192
Glycoprotein, alpha-galactosyltransferase 1	GGTA1	L36535.1	2.191
Fatty acid binding protein 5	FABP5	BI400074	2.188
Caveolin 2	CAV2	BF191227	2.188
Cytochrome P450 2A19	CYP2A19	AY280866.1	2.182
Insulin-like growth factor binding protein 3	IGFBP3	AJ657291	2.181
Mitogen-activated protein kinase-activated protein kinase 5	MAPKAPK5	BI400519	2.177
Man9-mannosidase	MAN1A	NM_213885.1	2.165
Interleukin 1 receptor antagonist	IL1RN	BF441608	2.165
H2A histone family, member Z	H2AFZ	CN154655	2.163
Acyl-CoA synthetase long-chain family member 1	ACSL1	BI118904	2.161
Eukaryotic translation elongation factor 1 alpha 1	EEF1A1	CO994537	2.159
Protein kinase inhibitor gamma	PKIG	NM_214371.1	2.146
Protein kinase C theta	LOC100152637	CN163635	2.134
Integrin, beta 2	ITGB2	NM_213908.1	2.127
CD74 antigen	CD74	CO994913	2.126
Solute carrier family 35, member A3	SLC35A3	CK462997	2.118
Uteroferrin	UF	NM_214209.1	2.111
ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4	ST8SIA4	BF712001	2.105
Aldehyde reductase	ALR1	NM_213890.1	2.098
Cellular FLICE-like inhibitory protein	C-FLIP	AY533020.1	2.089
Isocitrate dehydrogenase 3 (NAD+) beta	IDH3B	BX667411	2.078
Fibrinogen-like 2	FGL2	BP433439	2.069
Phosphoenolpyruvate carboxykinase 2	PCK2	CF180618	2.067
Peroxisomal D3,D2-enoyl-CoA isomerase	PECI	CN160253	2.065
Platelet/endothelial cell adhesion molecule	PECAM1	NM_213907.1	2.061
Hypothetical protein LOC100152540	LOC100152540	CN164967	2.055
Cyclin-dependent kinase 5	LOC733700	CF175515	2.054
HUS1 checkpoint homolog	HUS1	CO946905	2.051
Spermidine/spermine N1-acetyltransferase 1	SAT1	NM_214358.1	2.048
Sphingosine-1-phosphate receptor 5	S1PR5	CF175881	2.044
Peroxiredoxin 6	PRDX6	NM_214408.1	2.043
Interferon, gamma-inducible protein 30	IFI30	CK456242	2.041
Polo-like kinase 2	PLK2	CN159550	2.04
Ig gamma 2b chain constant region	IGG2B	M81771.1	2.039
RAD18 homolog	RAD18	CK461304	2.038
Hypothetical protein LOC100153935	LOC100153935	NM_214005.1	2.027
Hypothetical protein LOC100158166	LOC100158166	BX671182	2.02
Notch homolog 4	SBAB-649D6.4	CF360567	2.018
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	CITED1	BI183561	2.014
Spermidine/spermine N1-acetyltransferase family member 2	SAT2	CN162887	2.012
Galactose mutarotase	GALM	NM_214406.1	2.004
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 4	B4GALT4	CO956759	2.002

Table 3. Highly expressed genes in intramuscular adipose tissue.

Gene title	Gene symbol	Representative public ID	Ratio
Uroplakin 3	UPK3A	CK452028	0.5
Prepro-beta-defensin 1	PBD-1	NM_213838.1	0.497
Sorbin polypeptide	LOC396719	AF396456.1	0.497
Cytochrome P450, family 7, subfamily B, polypeptide 1	CYP7B1	CK455462	0.496
Carboxypeptidase E	CPE	CD571929	0.495
Calpastatin	CAST	AJ583408.1	0.493
Growth factor receptor-bound protein 10	GRB10	CF795733	0.491
CD9 molecule	CD9	NM_214006.1	0.491
Glycoprotein hormones, alpha polypeptide	CGA	NM_214446.1	0.488
Sarcoglycan, gamma	SGCG	CK456888	0.484
Integrin, beta 3	ITGB3	NM_214002.1	0.482
Thy-1 cell surface antigen	THY1	BX676685	0.481
Thrombomodulin	THBD	BX676135	0.475
NADH dehydrogenase 1, subcomplex unknown, 1, 6 kDa	NDUFC1	CK460082	0.474
Insulin-like growth factor binding protein 5	IGFBP5	NM_214099.1	0.474
Cyclin D2	CCND2	NM_214088.1	0.469
Follistatin	FST	NM_001003662.1	0.464
Calcium/calmodulin-dependent protein kinase II gamma	CAMK2G	U72972.1	0.463
NDRG2	LOC780431	BI182567	0.462
Complement component 7	C7	CO949473	0.462
Heat shock 105 kDa/110 kDa protein 1	HSPH1	CO993113	0.458
Odd homeobox 1 protein	OB1	NM_213792.1	0.447
Insulin-like growth factor 2	IGF2	NM_213883.1	0.446
Myocyte enhancer factor 2A	MEF2A	BX917896	0.445
Glutathione peroxidase 2	GPX2	CF365816	0.439
Thrombospondin 1	THBS1	BQ601960	0.438
Biglycan	BGN	BF193177	0.433
Protein phosphatase 1, regulatory subunit 12A	PPP1R12A	BI183395	0.431
EGF-like-domain, multiple 8	SBAB-649D6.3	BE232302	0.429
Beta-defensin 2	PBD-2	NM_214442.1	0.424
Splicing factor, arginine/serine-rich 11	LOC733656	CK464903	0.42
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	AF017079.1	0.415
LIM and cysteine-rich domains 1	LMCD1	CA779262	0.412
Inter-alpha inhibitor H4	ITIH4	NM_001001537.1	0.412
Bone morphogenetic protein receptor, type IB	BMPR1B	CO950299	0.412
ATG4 autophagy related 4 homolog D	ATG4D	CK453932	0.411
Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	IKBKG	BX925233	0.404
P311 protein	P311	BQ598577	0.4
Activating transcription factor 4	ATF4	CF792678	0.397
Discoidin domain receptor tyrosine kinase 1	DDR1	CK457958	0.396
CD209 molecule	CD209	BI399887	0.391
Complement component 3	C3	NM_214009.1	0.379
Cofilin 2	CFL2	BM083222	0.374
Neuropeptide Y receptor Y1	NPY1R	NM_214288.1	0.374
Prostamide/PG F synthase	LOC100134955	CF179637	0.373
TIMP metallopeptidase inhibitor 1	TIMP1	NM_213857.1	0.368
Fibronectin	FN1	BF709509	0.367
Vascular smooth muscle alpha-actin	ACT-4	BX670904	0.366
Tenascin C	TNC	NM_214230.1	0.364
Nebulin-related anchoring protein	NRAP	CK459919	0.352
Insulin-like growth factor binding protein 2	IGFBP2	NM_214003.1	0.347
Chemokine ligand 2	CXCL2	BF078671	0.345
Isocitrate dehydrogenase 2 ($NADP^+$), mitochondrial	IDH2	CN159777	0.344
TrkB protein	TRKB	BP171877	0.339
PROCR-like	LOC654289	BX922318	0.339
Protein kinase inhibitor alpha	PKIA	NM_214204.1	0.338
Carnitine palmitoyltransferase 1B	CPT1B	AY181062.1	0.338
Collagen, type VI, alpha 1	COL6A1	CN162503	0.336
Serpin peptidase inhibitor, clade E, member 1	SERPINE1	NM_213910.1	0.335
Nicotinamide phosphoribosyltransferase	NAMPT	BX666697	0.33
Destrin	DSTN	D90053.1	0.327

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Table 3. Continued.

Gene title	Gene symbol	Representative public ID	Ratio
Cadherin 1, type 1, E-cadherin	CDH1	BQ604786	0.324
Stanniocalcin 1	STC1	BI400766	0.323
Calcineurin A protein	LOC396603	BI118300	0.318
Phospholamban	PLN	NM_214213.1	0.316
Parathyroid hormone-like hormone	PTHLH	NM_213916.1	0.315
Calcium/calmodulin-dependent protein kinase II delta	CAMK2D	NM_214381.1	0.305
Glucose phosphate isomerase	GPI	NM_214330.1	0.296
TEA domain family member 4	TEAD4	CF176015	0.293
Heat shock protein 70.2	SBAB-707F1.4	NM_213766.1	0.289
Sarcoplasmic/endoplasmic-reticulum Ca(2+) pump gene 2	SERCA2	X15073.1	0.284
Claudin 7	CLDN7	CK450245	0.272
Dickkopf homolog 3	DKK3	CO949346	0.267
Mitogen-activated protein kinase 12	MAPK12	BI360380	0.265
Zinc finger, AN1-type domain 5	ZFAND5	CN160422	0.264
Collagen, type I, alpha 1	COL1A1	AF201723.1	0.263
CD55 molecule, decay accelerating factor for complement	CD55	NM_213815.1	0.256
Maternally expressed 3	MEG3	CK451038	0.255
Stearoyl-CoA desaturase 5	SCD5	CN153640	0.251
Filamin A, alpha	FLNA	CN166104	0.243
Heat shock protein 70	HSP70	X68213.1	0.243
Monoamine oxidase B	MAOB	NM_001001864.1	0.241
Triosephosphate isomerase 1	TPI1	CN160134	0.23
Transferrin	TF	BX919174	0.23
Chloride intracellular channel 5	CLIC5	BX666230	0.229
Solute carrier family 16, member 3	SLC16A3	BX676033	0.226
Tenascin-X	SBAB-514B12.2	CF359969	0.221
Feline leukemia virus subgroup A receptor	LOC100155620	BE014165	0.22
Fibromodulin	FMOD	CN163410	0.215
Smooth muscle protein 22-alpha	SM22A	CK466398	0.213
Calcium channel, voltage-dependent, alpha 2/delta subunit 1	CACNA2D1	NM_214183.1	0.208
ATPase, Na^+/K^+ transporting, beta 1 polypeptide	ATP1B1	CO950644	0.206
Dnaj homolog, subfamily A, member 4	DNAJA4	NM_214339.1	0.199
Neuron-derived orphan receptor-1 alfa	NOR-1	NM_214247.1	0.197
C1q and tumor necrosis factor related protein 3	C1QTNF3	BQ599486	0.194
Phospholipase C, delta 4	PLCD4	NM_214052.1	0.192
Myosin regulatory light polypeptide 9	MYL9	CK455118	0.189
Transforming growth factor, beta 3	TGFB3	NM_214198.1	0.187
Protein phosphatase 1, regulatory subunit 14A	PPP1R14A	NM_214337.1	0.184
Cardiac muscle alpha actin 1	ACTC1	CO939491	0.169
Pyruvate kinase, muscle	PKM2	CN166623	0.168
Transducer of ERBB2, 1	TOB1	BQ598689	0.165
ATPase inhibitory factor 1	ATPIF1	AJ604725	0.165
Integrin beta 1 binding protein 2	ITGB1BP2	BX924523	0.154
Keratin 8	KRT8	BX667006	0.153
Phosphoglucomutase 1	PGM1	AF091607.1	0.15
Four and a half LIM domains 3	FHL3	NM_213946.1	0.149
Pleiotrophic factor beta	PTF-BETA	D89546.1	0.148
Chemokine ligand 21	CCL21	AY312067.1	0.142
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	CILP	U83114.1	0.141
Collagen, type VI, alpha 3	COL6A3	BI182335	0.136
Calponin 1, basic, smooth muscle	CNN1	NM_213878.1	0.129
Collagen, type VIII, alpha 1	COL8A1	AF054891.1	0.124
Tropomyosin 2	TPM2	CF180239	0.122
Proteolipid protein 1	PLP1	BQ601666	0.122
Amylase, alpha 2B	AMY2B	NM_214195.1	0.116
Tumor necrosis factor receptor superfamily, member 12A	TNFRSF12A	BF710490	0.114
Bridging integrator 1	BIN1	CN162285	0.098
Fat-inducing transcript 1	FIT1	CF180497	0.09
Unc-45 homolog B	UNC45B	CN069994	0.087
Reticulon 2	RTN2	CF179996	0.077
Na,K-ATPase alpha 2 subunit	LOC396828	BX673191	0.077

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Table 3. Continued.

Gene title	Gene symbol	Representative public ID	Ratio
Oculocutaneous albinism II	OCA2	NM_214094.1	0.063
Myogenic factor 6	MYF6	AY188502.1	0.063
ADP-ribosyltransferase 3	ART3	AJ291435.1	0.051
Titin	TTN	CF359670	0.047
Calpain 3	CAPN3	AF148955.1	0.045
Tropomyosin 1	TPM1	CN165926	0.042
Adenylosuccinate synthase like 1	ADSSL1	CK456903	0.038
Titin-cap	TCAP	BM190107	0.032
Troponin C	TNNC1	CK449959	0.032
Phosphofructokinase, muscle	PFKM	CK466479	0.028
Muscle-specific intermediate filament desmin	LOC396725	NM_001001535.1	0.026
COX8H protein	COX8H	BX921027	0.024
Myosin, heavy chain 7, cardiac muscle, beta	MYH7	U75316.1	0.019
Epidermal growth factor	EGF	NM_214020.1	0.018
Troponin I	TNNI1	NM_213912.1	0.015
Skeletal alpha actin	LOC100154254	BM190097	0.013
Myosin, light chain 1, alkali; skeletal, fast	MYL1	NM_214374.1	0.011
Calsarcin 1	LOC733663	CF178743	0.011
Popeye domain containing 3	POPDC3	CF180347	0.011
Myosin light chain, phosphorylatable, fast skeletal muscle	MYLPF	AJ604745	0.007
Myosin, heavy chain 4, skeletal muscle	MYH4	AB025260.1	0.007
Xin actin-binding repeat containing 1	XIRP1	BI596265	0.007
Ryanodine receptor 1	RYR1	M91451.1	0.007
Beta actin	LOC396797	L20459	0.007
Troponin C type 2	TNNC2	NM_001001862.1	0.006
Myosin binding protein H	MYBPH	BX676336	0.006
Creatine kinase, muscle	CKM	AF165173.1	0.006
Peroxisome proliferator activated receptor gamma, coactivator 1 alpha	PPARGC-1	AB106108.1	0.006
Myosin light chain 2V	MLC2V	NM_213791.1	0.006
Creatine kinase, mitochondrial 2	CKMT2	BX667443	0.006
Xin actin-binding repeat containing 2	XIRP2	NM_214396.1	0.005
Sarcolipin	SLN	BX676059	0.005
Phosphorylase, glycogen, muscle	PYGM	CF179951	0.005
Enolase 3	ENO3	AJ301332	0.005
Myoglobin	MB	NM_214236.1	0.005
Cardiac ankyrin repeat protein	CARP	NM_213922.1	0.005
Actin-binding Rho activating protein	ABRA	BX667447	0.005
Troponin T type 3	TNNT3	AB176599.1	0.004
Phosphoglycerate mutase 2	PGAM2	BX667605	0.004
Troponin I	TNNI2	AJ604638	0.004
Alpha-actinin-2-associated LIM protein	LIM	NM_001001637.1	0.004
Myozenin 1	MYOZ1	BX675224	0.003
Troponin T type 1	TNNT1	AB118908.1	0.003
Small muscle protein, X-linked	SMPX	BX664815	0.002
Myotilin	MYOT	BM190434	0.002
Adenosine monophosphate deaminase 1	AMPD1	AJ604865	0.002

Table 4. Gene Ontology results of differentially expressed probes of subcutaneous (s.c.) and intramuscular (i.m.) (Figure 2 B) adipose tissues.

Classification	Content (%)	
	s.c. adipose tissue	i.m. adipose tissue
Biological process	49.25	49.16
Molecular function	35.15	31.76
Cellular component	14.10	18.07
Other items	1.50	1.01

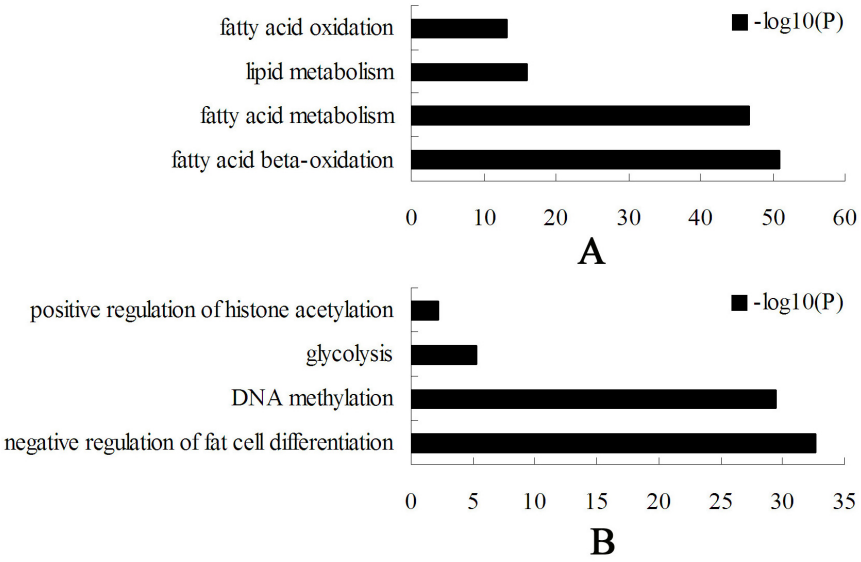

Figure 2. Biological process analyzed of genes that changed two-fold ≥ 2 folds in subcutaneous (**A**) and intramuscular adipose tissue (**B**) by Gene Ontology analysis (P values < 0.05).

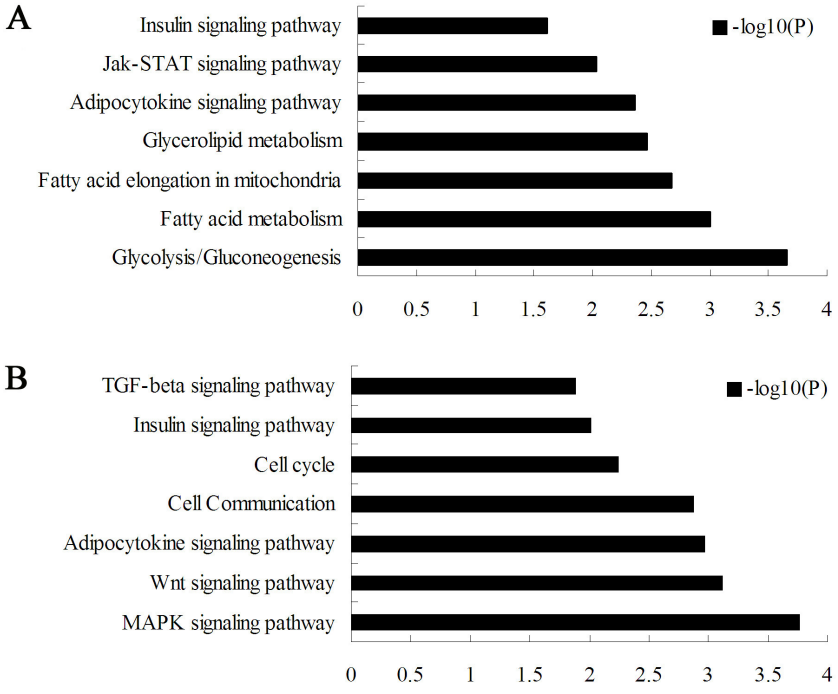

Figure 3. Significant pathways analyzed of genes that changed two-fold ≥ 2 folds in subcutaneous (**A**) and intramuscular adipose tissue (**B**) by KEGG analysis (P values < 0.05).

RT-PCR

In order to validate the microarray chips results, 13 randomly selected genes were analyzed using RT-PCR. As Figure 4A showed, FAS, LEP, ADIPOQ, PPAR-γ, intramuscular FABP5, FABP4, and LPL were more highly expressed in s.c. adipose tissue. On the contrary, GPI, CCND2, IGF2, NOR-1, TPI1, and TOB1 were more highly expressed in i.m. adipose tissue. The RT-PCR results were in accordance with the chips results (Figure 4B).

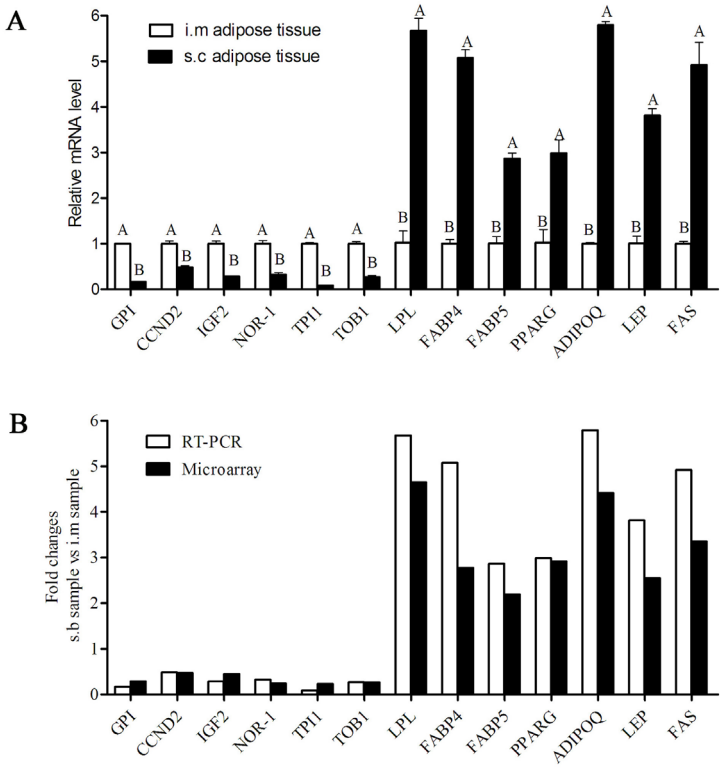

Figure 4. Microarry results conformed by RT-PCR. **A.** RT-PCR results of the genes selected. **B.** Comparison between RT-RCR and microarry results.

DISCUSSION

From the GO biological process analysis, we found that fatty acid oxidation, fatty acid β-oxidation, fatty acid metabolism, and lipid metabolism occurred in higher levels in s.c. adipose tissue. The KEGG results revealed that fatty acid elongation in mitochondria, fatty acid metabolism, and glycerolipid metabolism were significantly more highly expressed in s.c. adipose tissue. These results imply that s.c. adipose tissue has different abilities of lipid metabolism and fatty acid metabolism compared with i.m. adipose tissue.

LPL and FAS are the rate-limiting enzymes of triglyceride accumulation in adipose tissue (Weinstock et al., 1997; Ranganathan et al., 2006). FABP4 and FABP5, which are strongly

associated with fat accumulation, are key mediators of lipid metabolism and intracellular transport (Gorbenko et al., 2006; Ma et al., 2010). Hormone-sensitive lipase (HSL), catalyzing the triglycerides and diglycerides to form glycerol and fatty acids, is a key enzyme in the lipolysis process (Haemmerle et al., 2002; Holm, 2003). FABP4 regulates metabolism by affecting the activities of HSL and PPAR-γ, and has a higher expression in the NIH 3T3-L1 induction process (Gorbenko et al., 2006). Apolipoprotein E (APOE), which can improve the content of free fatty acid and triglyceride in adipose tissue (Huang et al., 2006), had a 4.8-fold higher expression than that in i.m. adipose tissue. In this study, LPL, FAS, FABP4, FABP5, HSL, and APOE were more highly expressed in s.c. adipose tissue, which implied that s.c. adipose tissue has a stronger lipogenic and lipolytic capacity. This higher level of lipogenic metabolism in s.c. tissue was coincidence with a previous report, in that s.c. adipocytes exhibit stronger lipogenesis in the process of preadipocyte differentiation to mature adipocytes in neonatal pigs (Zhou et al., 2010), as well as in mature adipoctyes of 210-day female pigs (Gardan et al., 2006).

Enoyl coenzyme A hydratase 1 peroxisomal (ECH1) and 2,4-dienoyl CoA reductase 1, mitochondrial (DECR1) are the rate-limiting enzymes in β-oxidation of fatty acid (Castro-Chavez et al., 2003). Our results revealed that the expression levels of HSL, ECH1, and DECR1 in s.c. adipose tissue were 3.24-, 3.321-, and 2.875-fold of those in i.m. adipose tissue, respectively. In the s.c. depot, ADIPOQ and adiponectin receptor 2 (ADIPOR2) are both highly expressed. ADIPOR2 positively regulates energy dissipation and fatty acid oxidation by activation of the PPAR-α pathway (Yamauchi et al., 2007). Thus, s.c. adipose tissue was more sensitive to adiponectin, and had a stronger ability of energy dissipation. Therefore, the high expression of ECH1, DECR1, ADIPOQ, and ADIPOR2 may imply that the s.c. adipose tissue also has a stronger capacity of fatty acid β-oxidation compared with i.m. adipose tissue. Adipose differentiation-related protein (ADRP) increases the uptake of long-chain fatty acids (Gao and Serrero, 2000) and its expression can increase the accumulation of neural lipid droplets (Londos et al., 1999). Our results also showed a high level of ADRP in s.c. adipose tissue. These implicate that s.c. adipose tissue has higher abilities for fatty acid uptake compared with i.m. adipose tissue.

In summary, the s.c. adipose tissue had a stronger ability for lipid metabolism and fatty acid metabolism than i.m. adipose tissue. Besides the biological process differences in different fat depots, angiopoietin-like-4 (ANGPTL4), neuronatin (NNAT), neuron-derived orphan receptor-1 alfa (NOR-1), and chloride intracellular channel 5 (CLIC5) may also participate in the metabolism differences between s.c. and i.m. adipose tissues.

ANGPTL4 and NNAT were highly expressed in s.c. adipose tissue compared with i.m. adipose tissue. ANGPTL4 is strongly up-regulated in the differentiation of 3T3-L1, and regulates the deposition of lipid and energy homeostasis (Lei et al., 2011) by suppressing fat accumulation (Mandard et al., 2006) and advancing fatty acids oxidation (Backhed et al., 2007). Compared with i.m. adipose tissue, the expression level of ANGPTL4 in s.c. adipose tissue was about 3.4-fold higher. The imprinted gene NNAT can promote adipogenesis in 3T3-L1 cells by enhancing the phosphorylation of cyclic AMP-response element-binding protein (Suh et al., 2005). NNAT expression is also influenced by leptin as a hypothalamic target (Vrang et al., 2010). The research of NNAT is mainly in the nervous system, and little about it in adipose tissue is known.

NOR-1 and CLIC5 were highly expressed in i.m. adipose tissue compared with s.c. adipose tissue. NOR-1, as a nuclear receptor of the unclear receptor 4A, regulates the metabolism of glucose and lipid in skeletal muscle, liver, and adipose tissues (van Tiel and de Vries,

2012), and is a target of β-adrenergic signaling in skeletal muscle (Pearen et al., 2006). NOR-1 inhibits adipogenesis of 3T3-L1 or 3T3-F442A pre-adipocytes (Chao et al., 2008), and NOR-1 gene transcription is regulated by liver X in adipocytes (Kumar et al., 2009). The specific function of CLIC5 is poorly understood (Bradford et al., 2010). Recent research found that CLIC5 had higher expression in lean-type pig than in obese-type pig, and was negatively related with i.m. fat content (Li et al., 2010). CLIC5 inhibits the differentiation of adipocytes and promotes the proliferation of 3T3-L1 (Li et al., 2010). NOR-1 and CLIC5 were significantly more highly expressed in i.m. adipose tissue. Therefore, ANGPTL4, NNAT, NOR-1, and CLIC5 can be candidate genes for the difference in lipid metabolism between s.c. and i.m. adipose tissues.

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