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Gene Expression Variability in Subcutaneous and Omental Adipose Tissue of Obese Men

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We investigated interindividual variability in gene expression in abdominal subcutaneous (SC) and omental (OM) adipose tissue of 10 massively obese men. Affymetrix human U133A microarrays were used to measure gene expression levels. A total of 6811 probesets generated significant signal in both depots in all samples. Interindividual variability in gene expression was rather low, with more than 90% of transcripts showing a coefficient of variation (CV) lower than 23.6% and 21.7% in OM and SC adipose tissues, respectively. The distributions of CV were similar between the two fat depots. A set of highly variable genes was identified for both tissues on the basis of a high CV and elevated gene expression level. Among the set of highly regulated genes, 18 transcripts were involved in lipid metabolism and 28 transcripts were involved in cell death for SC and OM samples, respectively. In conclusion, gene expression interindividual variability was rather low and globally similar between fat compartments, and the adipose tissue transcriptome appeared as relatively stable, although specific pathways were found to be highly variable in SC and OM depots.

Key words: Adipose tissue; Omental; Subcutaneous; Microarrays; Obese men

INTRODUCTION

A higher risk of obesity-related metabolic diseases has been associated with increased adipose tissue mass in the abdominal region (5,24). Using imaging methods, studies have shown that abdominal, and especially visceral or intra-abdominal obesity, in both men and women, is closely associated with a dyslipidemic state that includes hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol levels, elevated apolipoprotein B, a greater proportion of small, dense low-density lipoprotein (LDL) particles, and increased LDL cholesterol to HDL cholesterol ratio (6). This condition is also associated with hyperinsulinemia and insulin resistance (7,24).

Adipose tissue located within the abdominal cavity has been suggested to be functionally and metabolically distinct from that of the subcutaneous compartment (15,23) and a number of differentially expressed genes encoding important functional properties may underlie abdominal obesity-related disorders (23). Many studies have now used microarray profiling of adipose tissue to investigate gene expression in obesity (2–4,16). Analysis of variability in gene expression has been used to examine specific genes that could be related to adipose tissue function. However, so far only animal data are available (2,3), and no large-scale genomic study has been performed to examine the variability of gene expression in human adipose tissue. In this study, we investigated the interindividual variability in gene expression in abdominal subcutaneous (SC) and omental (OM) adipose tissue

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samples from 10 nondiabetic, normolipidemic obese men, using previously established microarrays (23).

SUBJECTS AND METHODS

Patient Selection

The study group included 10 massively obese men undergoing biliopancreatic diversion at the Laval Hospital (Quebec City). This surgical procedure involves bypassing the small intestine and diverting the bile and pancreatic juice to the distal ileum, which produces maldigestion and selective malabsorption essentially for fat and starch (17). Following clinical examination, none of the patients had identified chronic diseases such as cardiomyopathy and endocrine disorders. Body weight was stable at the time of study and no subject had been on a diet or involved in a weight reduction program in the last 6 months. All patients provided informed written consent prior to their inclusion in the study. Adipose tissue samples were obtained at the beginning of the surgery from the abdominal subcutaneous wall (close to the umbi-

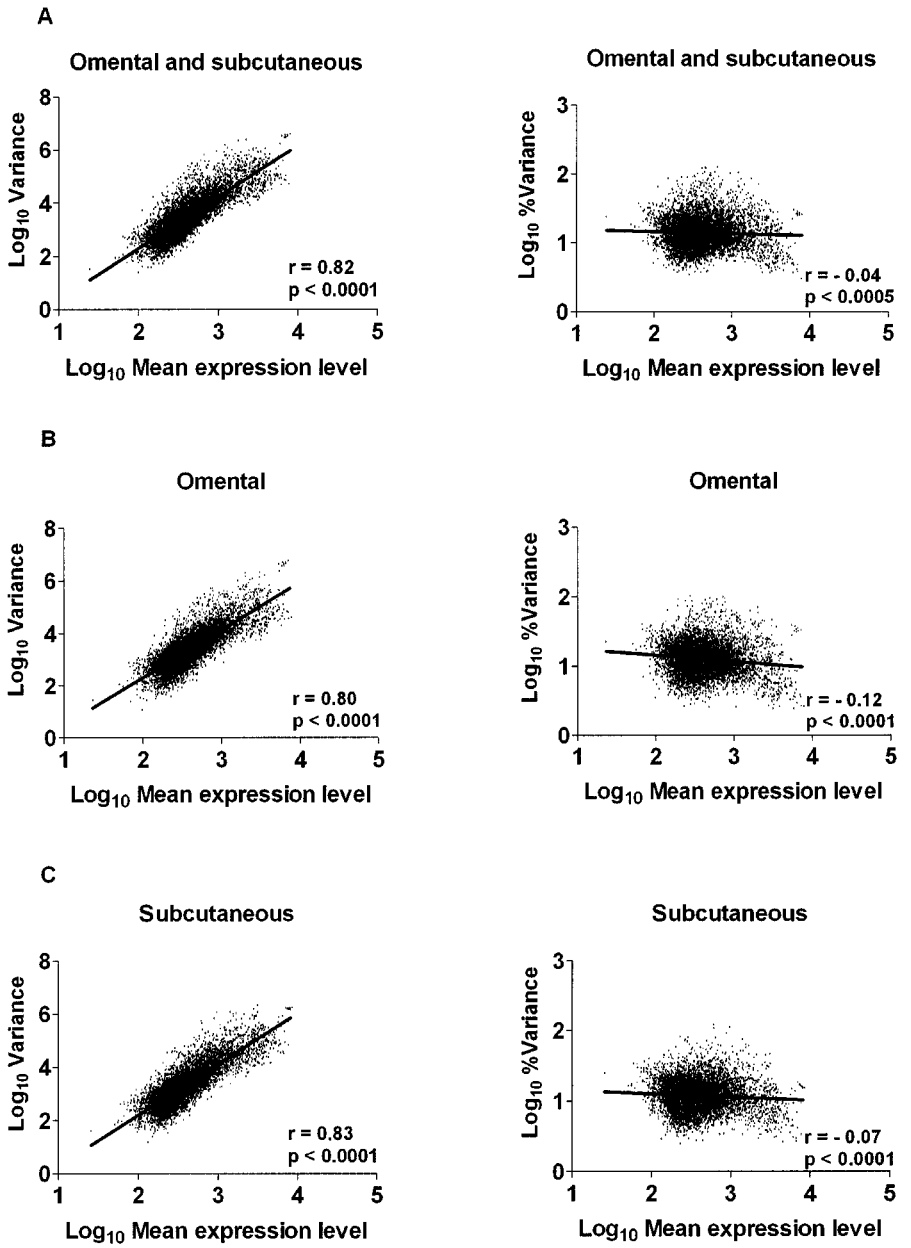

Figure 1. Correlations between gene expression variance or coefficient of variation (% variance) and mean gene expression levels for all 6,811 positive signals regardless of fat depot (A), or in the OM (B) or SC (C) fat compartments.

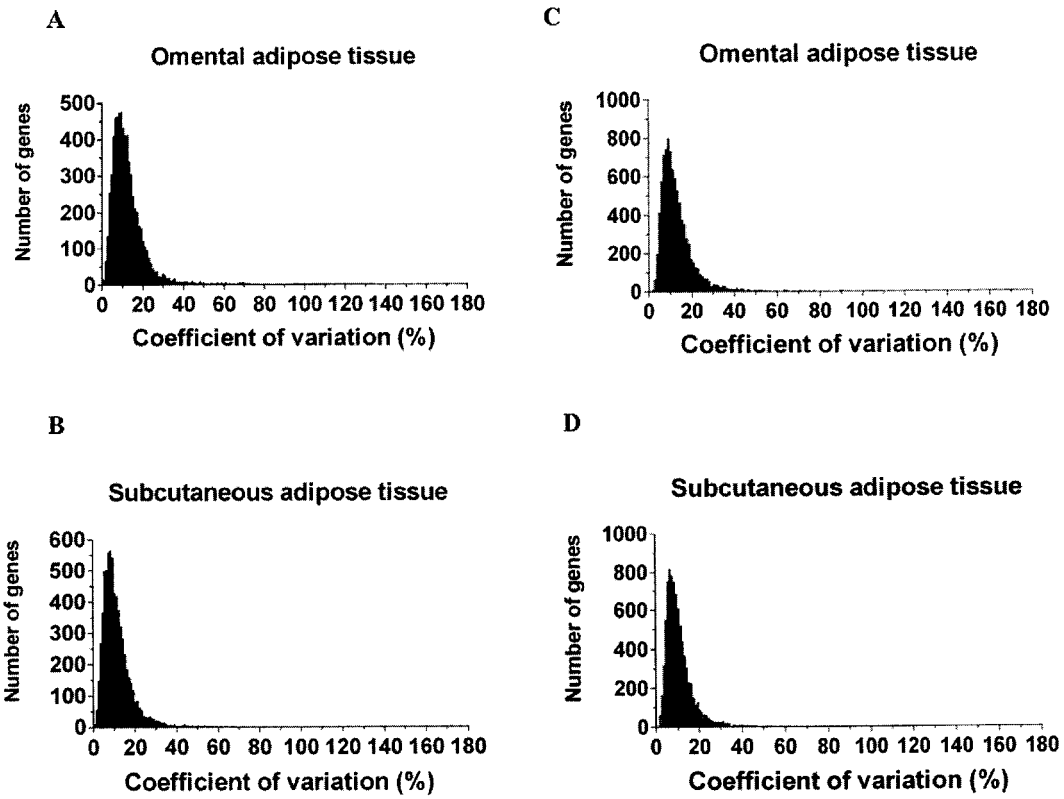

Figure 2. Distribution of coefficients of variation in OM or SC adipose tissue of (A, B) 6,811 probesets that generated significant signal in both fat depots in all 10 subjects, and (C, D) 9,076 and 8,590 probesets that only presented significant signal in at least one and up to nine individuals. Interindividual variability was similar in both sets of transcripts. No difference in CV distribution was observed between fat depots.

licus) and from the greater omentum. Body weight, height, and waist and hip circumferences were measured according to standardized procedures.

RNA Extraction, Reverse Transcription, and Probe Preparation

Adipose tissue samples were homogenized in Trizol reagent and centrifuged to separate the lipid fraction. Total RNA was prepared from the cleared homogenate according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). RNA was repurified using RNEasy mini columns (Qiagen, Hilden, Germany). RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Probes for microarray experiments were prepared using 10 μg of total RNA and hybridized overnight to Affymetrix HG-U133A Gene Chips (Affymetrix, Santa Clara, CA). Nonspecifically bound probe was removed by washing using the Agilent GeneChip Fluidics Station 400. Detection of specifically bound probes was performed by incubating the arrays with a biotinylated anti-streptavidin antibody (Vector Laboratories, Burlingame, CA) prior to staining with SAPE (streptavidin phycoerthryin; Molecular Probes, Eugene, OR). Detailed protocols for probe synthesis and hybridization reactions have been previously described (20). Real-time RT-PCR was used for confirmation with a subset of genes (23).

Array Data Extraction and Analysis

The arrays were scanned using an Agilent Gene Array Scanner and raw data were extracted from scanned images and scaled to 1000 units mean intensity using Microarray Analysis D-Chip software (PM-MM model). A significant signal was considered when the DChip software indicated a "present" call based on the modified algorithm of Microarray Suite analysis software 4 (Affymetrix). Interindividual variance in mean expression level and coefficient of variation (CV) calculations were performed for each transcript from the normalized signal obtained in both fat depots of all 10 subjects of the study (one array per fat sample, for a total of 20 arrays). Nonparametric Spearman rank correlation coefficients were computed to quantify associations between variance and mean expression levels or CV and mean expres-

TABLE 1
LIST OF THE 68 OM ADIPOSE TISSUE TRANSCRIPTS IN UPPER TERTILE OF MEAN EXPRESSION LEVEL AND TOP 2 PERCENTILE OF THE COEFFICIENT OF VARIATION

Probeset	Symbol	Description	Cytogen. Band	Accession	% CV
217739_s_at	PBEF1	Pre-B-cell colony-enhancing factor	7q22.2	NM_005746	100.5
202241_at	TRIB1	Phosphoprotein regulated by mitogenic pathways	8q24.13	NM_025195	95.0
204472_at	GEM	GTP binding protein overexpressed in skeletal muscle	8q13-q21	NM_005261	91.3
202643_s_at	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	6q23	AI738896	86.6
202644_s_at	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	6q23	NM_006290	83.3
202637_s_at	ICAM1	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	AI608725	78.8
202638_s_at	ICAM1	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	NM_000201	46.6
212724_at	RND3	Ras homolog gene family, member E	2q23.3	BG054844	77.6
36711_at	MAFF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	22q13.1	AL021977	76.3
204007_at	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor for (CD16)	1q23	J04162	71.4
202917_s_at	S100A8	S100 calcium binding protein A8 (calgranulin A)	1q21	NM_002964	70.9
203574_at	NFIL3	Nuclear factor, interleukin 3 regulated	9q22	NM_005384	66.0
221541_at	CRISPLD2	Hypothetical protein DKFZp434B044	16q24.1	AL136861	64.2
221477_s_at	MGC5618	Hypothetical protein MGC5618		BF575213	63.3
202388_at	RGS2	Regulator of G-protein signaling 2, 24kD	1q31	NM_002923	63.1
217546_at	MT1M	Metallothionein 1M	16q13	R06655	62.2
200798_x_at	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1q21	NM_021960	61.3
200797_s_at	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1q21	AI275690	39
207574_s_at	GADD45B	Growth arrest and DNA-damage-inducible, beta	19p13.3	NM_015675	61.1
208152_s_at	DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	10q21	NM_004728	60.7
204881_s_at	UGCG	UDP-glucose ceramide glucosyltransferase	9q31	NM_003358	60.1
201325_s_at	EMP1	Epithelial membrane protein 1	12p12.3	NM_001423	60.1
201502_s_at	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	14q13	AI078167	59.5
202672_s_at	ATF3	Activating transcription factor 3	1q32.3	NM_001674	59.1
209193_at	PIM1	Pim-1 oncogene	6p21.2	M24779	59.0
201858_s_at	PRG1	Proteoglycan 1, secretory granule	10q22.1	J03223	56.0
203411_s_at	LMNA	Lamin A/C	1q21.2-q21.3	NM_005572	55.9
212086_x_at	LMNA	Lamin A/C	1q21.2-q21.3	AK026584	51.2
201631_s_at	IER3	Immediate early response 3	6p21.3	NM_003897	54.4
202391_at	BASP1	Brain abundant, membrane attached signal protein 1	5p15.1-p14	NM_006317	53.7
208836_at	ATP1B3	ATPase, Na^+/K^+ transporting, beta 3 polypeptide	3q23	U51478	53.3
207332_s_at	TFRC	Transferrin receptor (p90, CD71)	3q29	NM_003234	53.1
208691_at	TFRC	Transferrin receptor (p90, CD71)	3q29	BC001188	52.5
200800_s_at	HSPA1A	Heat shock 70kD protein 1A	6p21.3	NM_005345	51.9
208470_s_at	HPR	Haptoglobin-related protein	16q22.1	NM_020995	51.7
208151_x_at	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	22q13.1	NM_030881	51.2
209340_at	UAP1	UDP-N-acteylglucosamine pyrophosphorylase 1	1q23.3	S73498	51.1
202581_at	HSPA1B	Heat shock 70kD protein 1B	6p21.3	NM_005346	50.8
200768_s_at	MAT2A	Methionine adenosyltransferase II, alpha	2p11.2	BC001686	49.6
214038_at	CCL8	Chemokine (C-C motif) ligand 8	17q11.2	AI984980	48.6
201466_s_at	JUN	V-jun sarcoma virus 17 oncogene homolog (avian)	1p32-p31	NM_002228	48.0
201739_at	SGK	Serum/glucocorticoid regulated kinase	6q23	NM_005627	47.5
200666_s_at	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	19p13.2	NM_006145	47.5
213629_x_at	MT1F	Metallothionein 1F (functional)	16q13	BF246115	47.0
217165_x_at	MT1F	Metallothionein 1F (functional)	16q13	M10943	41.2
200831_s_at	SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	10q23-q24	AA678241	46.7
212185_x_at	MT2A	Metallothionein 2A	16q13	NM_005953	46.5
200704_at	LITAF	Lipopolysaccharide-induced TNF factor	16p13.13	AB034747	45.7
211456_x_at	LOC645745	Metallothionein 1H-like protein	1q43	AF333388	45.5
208581_x_at	MT1X	Metallothionein 1X	16q13	NM_005952	44.9
202238_s_at	NNMT	Nicotinamide N-methyltransferase	11q23.1	NM_006169	44.6
204419_x_at	HBG2	Hemoglobin, gamma G	11p15.5	NM_000184	44.6
221841_s_at	KLF4	Kruppel-like factor 4 (gut)	9q31	BF514079	44.0
202081_at	IER2	Immediate early response 2	19p13.13	NM_004907	43.9
203649_s_at	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1p35	NM_000300	43.6
220046_s_at	CCNL1	Cyclin L1	3q25.32	NM_020307	43.2
205100_at	GFPT2	Glutamine-fructose-6-phosphate transaminase 2	5q34-q35	NM_005110	43.1
201289_at	CYR61	Cysteine-rich, angiogenic inducer, 61	1p31-p22	NM_001554	42.8

TABLE 1
CONTINUED

Probeset	Symbol	Description	Cytogen. Band	Accession	% CV
215499_at	LOC651423	Similar to mitogen-activated protein kinase kinase 3 isoform A	17q11.2	AA780381	42.3
201473_at	JUNB	Jun B proto-oncogene	19p13.2	NM_002229	42.0
205516_x_at	CIZ1	CDNK1A interacting zinc finger protein	9q34.1	NM_012127	42.0
221651_x_at	IGKC	Immunoglobulin kappa constant	2p12	BC005332	41.3
204745_x_at	MT1G	Metallothionein 1G	16q13	NM_005950	41.3
200881_s_at	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	9p13-p12	NM_001539	41.2
217753_s_at	RPS26	Ribosomal protein S26	12q13	NM_001029	40.7
218520_at	TBK1	TANK-binding kinase 1	12q14.1	NM_013254	40.4
200989_at	HIF1A	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	14q21-q24	NM_001530	39.7
212859_x_at	MT1E	Metallothionein 1E (functional)	16q13	BF217861	39.2

sion levels in SC and OM fat samples either combined or separately. Log-10 transformation for all variables was used to normalize values. SC and OM variances or CVs were compared among fat depots by paired *t*-test. The selection of the most variable genes in SC and OM fat samples was based on the following criteria: 1) probesets that generated significant signal ("present" call) in both depots in all 10 subjects ($n = 6811$); 2) probesets that were in the top 2 percentile of CV for each depot ($n = 136$ for SC and OM); and 3) probesets that generated a mean expression level that was in the upper tertile ($n = 68$ in each depot). In addition, we examined variability in the probesets that generated significant signal ("present" call) in at least one and up to nine individuals.

Biological Pathway Analyses

Cellular pathways related to these transcripts were identified using the Kegg database (http://www.genome.ad.jp/kegg/) and genecards (http://www.genecards.org/). The Ingenuity Pathway Analysis System (Ingenuity® Systems, www.ingenuity.com) was also used to visualize gene expression data in the context of biological pathways. Two input files were uploaded in the Ingenuity Pathway Analysis system considering: 1) probesets highly variable in SC tissues ($n = 68$) and 2) probesets highly variable in OM tissues ($n = 68$). Analyses were performed on both files individually, and a comparison analysis was also performed.

RESULTS

Men of the study were 17.0 to 45.0 years old and were in the morbid obesity range with BMI values ranging from 44.7 to 80.7 kg/m^2. They were characterized by a normal lipid profile, and were slightly hypertensive (9). Of the 22,283 probesets present on the array, significant signal ("present" call) was obtained for 6,811 probesets in both fat compartments of all 10 subjects. A total of 9,076 and 8,590 probesets generated significant signal ("present" call) in at least one and up to nine individuals in OM and SC samples, respectively.

Figure 1 shows the correlations between gene expression variance or CV (% variance) and mean gene expression levels for all 6,811 positive signals, regardless of the fat depot (Fig. 1A), in the OM (Fig. 1B) or SC (Fig. 1C) fat compartments. As expected, highly expressed genes had higher absolute variance in their expression levels as reflected by a positive correlation between mean transcript expression levels and absolute gene expression variance. However, mean gene expression levels were negatively correlated with CV, indicating slightly higher variability at low expression levels. The distribution of CV in gene expression in OM and SC is shown in Figure 2. The left panels show the 6,811 probesets that generated significant signal in both compartments in all 10 fat samples. More than 90% of clones showed a CV lower than 23.6% and 21.7% in OM and SC adipose tissues, respectively. The right panels show CV distributions of genes that generated significant signal ("present" call) in at least one and up to nine individuals in the OM and SC fat samples. More than 90% of clones showed a CV lower than 22.0% and 20.3% in OM and SC samples, respectively. No difference in CV was observed between fat depots in both subsets of transcripts.

Among the 6,811 probesets that generated significant signal ("present" call) in both depots in all 10 subjects, we selected probesets that were in the top 2 percentile of CV in each depot, and then identified the ones (68 probesets in each depot) that were in the upper tertile of mean gene expression level (Tables 1 and 2). Sixty-three genes were obtained in both fat compartments. Selected pathways with highly variable transcripts in SC and OM adipose tissue are shown in Table 3. Some pathways were highly variable in both fat depots, including pathways of hematopoietic cell lineage, the Fc epsilon RI signaling

TABLE 2
LIST OF THE 68 SC ADIPOSE TISSUE TRANSCRIPTS IN THE UPPER TERTILE OF MEAN EXPRESSION LEVEL AND TOP 2 PERCENTILE OF THE COEFFICIENT OF VARIATION

Probeset	Symbol	Description	Cytogen. Band	Accession	% CV
212657_s_at	IL1RN	Interleukin 1 receptor antagonis	2q14.2	U65590	121.4
209875_s_at	SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	4q21-q25	M83248	111.9
209395_at	CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	1q32.1	M80927	107.9
203936_s_at	MMP9	Matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)	20q11.2-q13.1	NM_004994	92.0
208691_at	TFRC	Transferrin receptor (p90, CD71)	3q29	BC001188	79.9
207332_s_at	TFRC	Transferrin receptor (p90, CD71)	3q29	NM_003234	66.3
201952_at	ALCAM	Activated leucocyte cell adhesion molecule	3q13.1	AA156721	78.8
201422_at	IFI30	Interferon, gamma-inducible protein 30	19p13.1	NM_006332	75.5
201850_at	CAPG	Capping protein (actin filament), gelsolin-like	2p11.2	NM_001747	71.1
201847_at	LIPA	Lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	10q23.2-q23.3	NM_000235	64.2
201720_s_at	LAPTM5	Lysosomal-associated multispanning membrane protein 5	1p34	AI589086	62.6
201721_s_at	LAPTM5	Lysosomal-associated multispanning membrane protein 5	1p34	NM_006762	60.7
203523_at	LSP1	Lymphocyte-specific protein 1	11p15.5	NM_002339	62.6
213274_s_at	CTSB	Cathepsin B	8p22	AA020826	61.9
200838_at	CTSB	Cathepsin B	8p22	NM_001908	57.5
200839_s_at	CTSB	Cathepsin B	8p22	NM_001908	44.9
213275_x_at	CTSB	Cathepsin B	8p22	W47179	38.9
219454_at	EGFL6	EGF-like-domain, multiple 6	Xp22	NM_015507	60.8
202803_s_at	ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	21q22.3	NM_000211	59.2
202902_s_at	CTSS	Cathepsin S	1q21	NM_004079	58.2
203337_x_at	ITGB1BP1	Integrin beta 1 binding protein 1	2p25.2	NM_004763	55.4
212737_at	GM2A	GM2 ganglioside activator	5q31.3-q33.1	AL513583	52.5
209122_at	ADFP	Adipose differentiation-related protein	9p22.1	BC005127	51.3
208607_s_at	SAA2	Serum amyloid A2	11p15.1-p14	NM_030754	51.2
205516_x_at	CIZ1	CDKN1A interacting zinc finger protein 1	9q34.1	NM_012127	49.7
202546_at	VAMP8	Vesicle-associated membrane protein 8 (endobrevin)	2p12-p11.2	NM_003761	49.6
200766_at	CTSD	Cathepsin D (lysosomal aspartyl protease)	11p15.5	NM_001909	48.4
202409_at	IGF2	Insulin-like growth factor 2 (somatomedin A)	11p15.5	X07868	48.1
204122_at	TYROBP	TYRO protein tyrosine kinase binding protein	19q13.1	NM_003332	46.8
218520_at	TBK1	TANK-binding kinase 1	12q14.1	NM_013254	46.6
214456_x_at	SAA1	Serum amyloid A1	11p15.1	M23699	46.6
204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	1q23	NM_004106	46.3
221651_x_at	IGKC	Immunoglobulin kappa constant	2p12	BC005332	46.2
201141_at	GPNMB	Glycoprotein (transmembrane) nmb	7p15	NM_002510	46.0
201201_at	CSTB	Cystatin B (stefin B)	21q22.3	NM_000100	45.9
221269_s_at	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3	1p35-p34.3	NM_031286	45.3
203649_s_at	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1p35	NM_000300	42.9
218540_at	THTPA	Thiamine triphosphatase	14q11.2	NM_024328	42.1
209659_s_at	CDC16	CDC16 cell division cycle 16 homolog (S. cerevisiae)	13q34	AF164598	41.9
200831_s_at	SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	10q23-q24	AA678241	41.6
213553_x_at	APOC1	Apolipoprotein C-I	19q13.2	W79394	41.4
213101_s_at	ACTR3	ARP3 actin-related protein 3 homolog (yeast)	2q14.1	Z78330	40.0
202605_at	GUSB	Glucuronidase, beta	7q21.11	NM_000181	39.7
201050_at	PLD3	Phospholipase D family, member 3	19q13.2	NM_012268	39.6
202399_s_at	AP3S2	Adaptor-related protein complex 3, sigma 2 subunit	15q26.1	NM_005829	39.5
202404_s_at	COL1A2	Collagen, type I, alpha 2	7q22.1	NM_000089	39.5
201108_s_at	THBS1	Thrombospondin 1	15q15	BF055462	38.9
201470_at	GSTO1	Glutathione-S-transferase omega 1	10q25.1	NM_004832	38.8
201251_at	PKM2	Pyruvate kinase, muscle	15q22	NM_002654	38.5
200078_s_at	ATP6V0B	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	1p32.3	BC005876	37.9
213515_x_at	HBG1	Hemoglobin, gamma A	11p15.5	AI133353	37.7
217118_s_at	C22orf9	Chromosome 22 open reading frame 9	22q13.31	AK025608	37.6
203382_s_at	APOE	Apolipoprotein E	19q13.2	NM_000041	37.6
201944_at	HEXB	Hexosaminidase B (beta polypeptide)	5q13	NM_000521	37.2
209390_at	TSC1	Tuberous sclerosis 1	9q34	AF013168	37.0
203381_s_at	APOE	Apolipoprotein E	19q13.2	N33009	36.8
201005_at	CD9	CD9 molecule	12p13.3	NM_001769	36.7
218109_s_at	MFSD1	Major facilitator superfamily domain containing 1	3q25.33	NM_022736	36.5

TABLE 2
CONTINUED

Probeset	Symbol	Description	Cytogen. Band	Accession	% CV
203920_at	NR1H3	Nuclear receptor subfamily 1, group H, member 3	11p11.2	NM_005693	36.4
207168_s_at	H2AFY	H2A histone family, member Y	5q31.3-q32	NM_004893	36.4
208998_at	UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	11q13.4	U94592	35.7
207977_s_at	DPT	Dermatopontin	1q12-q23	NM_001937	35.7
203416_at	CD53	CD53 molecule	1p13	NM_000560	35.7
201954_at	ARPC1B	Actin related protein 2/3 complex, subunit 1B, 41 kDa	7q22.1	NM_005720	35.6
201525_at	APOD	Apolipoprotein D	3q26.2-qter	NM_001647	35.4
209183_s_at	C10orf10	Chromosome 10 open reading frame 10	10q11.21	AL136653	35.0
202087_s_at	CTSL	Cathepsin L	9q21-q22	NM_001912	34.9
217753_s_at	RPS26	Ribosomal protein S26	12q13	NM_001029	34.7

pathway, genes involved in glycerophospholipid metabolism, leukocyte transendothelial migration, and the GnRH signaling pathway (PLA2G2A and TFRC). Conversely, several pathways were highly variable only in OM or SC adipose tissue samples. Transcripts related to the Jak-STAT, Wnt, adipocytokine, apoptosis, and MAPK signaling pathways were more variable among OM samples. We also found that pyruvate kinase (PKM2), a transcript related to insulin signaling, glycolysis/gluconeogenesis and type 2 diabetes, was more variable among SC samples. The Ingenuity Pathway Analysis system revealed that 18 transcripts in the SC dataset were involved in lipid metabolism, which was clearly the top function associated with this dataset, whereas 28 transcripts were associated with cell death in OM fat (Table 4).

DISCUSSION

Regional fat distribution accounts for an important part of the association between obesity and related metabolic complications. In the present study, we used SC and OM adipose tissue samples from 10 obese men for microarray hybridizations, and measured expression levels for ~22,200 probesets. We studied the interindividual variability of gene expression in both depots, and attempted to identify highly variable transcripts or pathways in these fat compartments. Interindividual variability in gene expression in both depots in all subjects was rather low. In addition, no difference in the distribution of CVs was observed among fat depots. This provides evidence that gene expression within abdominal OM and SC adipose tissue samples is relatively homogenous, and indirectly suggests that primary characteristics of adipose tissue from both the SC and OM compartments are relatively similar. Several studies have now used microarrays to investigate gene expression profiling of adipose tissue in rodents (2–4,16) and humans (12,15,23). However, no study had examined human adipose tissue gene expression variability. Individual analyses of adipose tissue gene expression within a homogeneous study group or population might help to identify possible new functional links between different genes. This is the largest microarray study of human SC and OM fat performed to date and the first to provide information on the interindividual variability of gene expression in human adipose tissue.

SC and OM fat have been demonstrated as being very different in terms of lipolysis, cytokine secretion, and linking to disease risks such as insulin resistance and dyslipidemia (6,19,22). This wide heterogeneity among individuals could potentially be reflected by different patterns of gene expression in each fat depot. We measured some variability in gene expression in the present analysis. However, the adipose tissue transcriptome appeared as relatively stable, because interindividual variability was rather low, with more than 90% of clones showing a CV lower than 23.6% and 21.7% in OM and SC adipose tissues, respectively, in the 6,811 probsets that generated significant signal in both fat depots in all 10 subjects. Variability in the probesets that were silenced in at least one and up to nine individuals out of 10 in both depots showed similar variability, and no difference in distributions of CVs was observed between fat depots. Interestingly, Boeuf et al. (2) obtained strikingly similar data when analyzing the individual variability of gene expression in subcutaneous white and brown adipose tissue of hamsters. They found that individual variability of gene expression in both types of fats was also low, with more than 80% of clones showing a CV lower than 30%. These results led the authors to conclude that gene expression in adipose tissue was rather robust and stable for animals, under identical environmental conditions. In the present study, gene expression variability was very consistent with that observed by Boeuf et al (2). We suggest that even in human subjects not under controlled physiological, metabolic, and environmental situations, adi-

TABLE 3
SELECTED PATHWAYS WITH HIGHLY VARIABLE TRANSCRIPTS IN OM AND SC ADIPOSE TISSUE, BASED ON THE COEFFICIENT OF VARIATION IN GENE EXPRESSION LEVEL

Omental Adipose Tissue			Subcutaneous Adipose Tissue		
Pathway	No. of Genes	Symbols	Pathway	No. of Genes	Symbols
Aminosugars metabolism	2	UAP1, GFPT2	Aminosugars metabolism	1	HEXB
Antigen processing and presentation	2	HSPA1A, HSPA1B	Antigen processing and presentation	4	IFI30, CTSB**, CTSS, CTSL
Arachidonic acid metabolism	1	PLA2G2A	Arachidonic acid metabolism	1	PLA2G2A
Cell adhesion molecules (CAMs)	1	ICAM1*	Cell adhesion molecules (CAMs)	2	ITGB2, ALCAM
Cell communication	1	LMNA*	Cell communication	3	SPP1, COL1A2, THBS1
Cell cycle	1	GADD45B	Cell cycle	1	CDC16
Fc epsilon RI signaling pathway	2	PLA2G2A, MAP2K3	Fc epsilon RI signaling pathway	2	PLA2G2A, FCER1G
Focal adhesion	1	JUN	Focal adhesion	3	SPP1, COL1A2, THBS1
Glycan structures—biosynthesis 2	1	UGCG	Glycan structures—degradation	2	GUSB,HEXB
Glycerophospholipid metabolism	1	PLA2G2A	Glycerophospholipid metabolism	1	PLA2G2A
GnRH signaling pathway	2	PLA2G2A, MAP2K3	GnRH signaling pathway	1	PLA2G2A
Hematopoietic cell lineage	1	TFRC*	Hematopoietic cell lineage	2	TFRC, CD9
Leukocyte transendothelial migration	1	ICAM1*	Leukocyte transendothelial migration	3	ITGB2, MMP9, TFRC
Linoleic acid metabolism	1	PLA2G2A	Linoleic acid metabolism	1	PLA2G2A
Long-term depression	1	PLA2G2A	Long-term depression	1	PLA2G2A
MAPK signaling pathway	6	GADD45B, HSPA1A, HSPA1B, JUN, PLA2G2A, MAP2K3	MAPK signaling pathway	1	PLA2G2A
mTOR signaling pathway	1	HIF1A	mTOR signaling pathway	1	TSC1
Natural killer cell mediated cytotoxicity	2	ICAM1*, FCGR3B	Natural killer cell mediated cytotoxicity	3	ITGB2, FCER1G, TYROBP
Ribosome	1	RPS26	Ribosome	1	RPS26
Toll-like receptor signaling pathway	4	NFKBIA, MAP2K3, TBK1, JUN	Toll-like receptor signaling pathway	1	TBK1
VEGF signaling pathway	1	PLA2G2A	VEGF signaling pathway	1	PLA2G2A
Wnt signaling pathway	1	JUN	Alkaloid biosynthesis II	1	LIPA
Adipocytokine signaling pathway	1	NFKBIA	Alzheimer's disease	1	APOE*
Apoptosis	1	NFKBIA	ATP synthesis	1	ATP6V0B
B cell receptor signaling pathway	2	NFKBIA, JUN	Bile acid biosynthesis	1	LIPA
Cytokine–cytokine receptor interaction	1	CCL8	Carbon fixation	1	PKM2
Epithelial cell signaling in *Helicobacter pylori* infection	1	NFKBIA	Cholera—infection	1	ATP6V0B
Glutamate metabolism	1	GFPT2	ECM-receptor interaction	3	SPP1, COL1A2, THBS1
Glycosphingolipid metabolism	1	UGCG	Globoside metabolism	1	HEXB
Jak-STAT signaling pathway	1	PIM1	Glutathione metabolism	1	GSTO1
Methionine metabolism	1	MAT2A	Glycerolipid metabolism	1	LIPA
Nicotinate and nicotinamide metabolism	2	NNMT, PBEF1	Glycolysis/gluconeogenesis	1	PKM2
Selenoamino acid metabolism	1	MAT2A	Glycosaminoglycan degradation	2	GUSB, HEXB
T cell receptor signaling pathway	2	NFKBIA, JUN	Insulin signaling pathway	2	PKM2, TSC1
			Metabolism of xenobiotics by cytochrome P450	1	GSTO1
			Neurodegenerative Disorders	1	APOE*

TABLE 3
CONTINUED

Omental Adipose Tissue			Subcutaneous Adipose Tissue		
Pathway	No. of Genes	Symbols	Pathway	No. of Genes	Symbols
			N-Glycan degradation	1	HEXB
			Oxidative phosphorylation	1	ATP6V0B
			Pentose and glucuronate interconversions	1	GUSB
			Porphyrin and chlorophyll metabolism	1	GUSB
			Pyruvate metabolism	1	PKM2*
			Regulation of actin cytoskeleton	2	ITGB2, ARPC1B
			SNARE interactions in vesicular transport	1	VAMP8
			Starch and sucrose metabolism	1	GUSB
			TGF-beta signaling pathway	1	THBS1
			Thiamine metabolism	1	THTPA
			Type II diabetes mellitus	1	PKM2
			Ubiquitin mediated proteolysis	1	CDC16

*Two probesets generated similar results for these genes.

pose tissue gene expression is relatively homogeneous. Our results also indicate that the larger portion of genes in SC and OM adipose tissue have stable expression and suggest that only a few pivotal genes might be responsible for the demonstrated regional differences in adipose tissue physiology and related complications.

Among the set of highly variable transcripts, we found that genes in SC samples were mostly involved in lipid metabolism. We also found that a transcript related to insulin signaling, PKM2, was more variable among SC than OM samples. Insulin increases glucose uptake in muscle and fat, and promotes the storage of substrates in fat, liver, and muscle by stimulating lipogenesis, glycogen and protein synthesis, and by inhibiting lipolysis, glycogenolysis, and protein breakdown. PKM2 is a glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP (13). Kim et al. (14) examined mice with tissue-specific overexpression of LPL and their findings indicated a direct and causative relationship between the accumulation of intracellular fatty acid-derived metabolites and insulin resistance mediated via alterations in the insulin signaling pathway. This phenomenon has been suggested to occur as a means to prevent further fat accumulation in a given tissue, the reduction in insulin action seen in insulin-resistant states affecting metabolic fuel partitioning (8). High variability of SC adipose tissue genes of fat storage and insulin signaling may reflect high variability in the capacity to store fat in this depot in the presence of energy excess.

Cytokines regulate several aspects of adipose tissue metabolism (11,18). Some possibly mediate their responses through activation of the JAK-STAT pathway. We found a transcript related to JAK-STAT pathway that was more variable among OM samples. Another interesting finding of this study is that transcripts related to the MAPK, Wnt, and adipocytokine signaling pathways, and to apoptosis were also more variable among OM samples. These transcripts were PIM1, NFKBIA, JUN, PLA2G2A, HSPA1A, HSPA1B, GADD45B, and MAP2K3. Transcripts related to these cellular processes are involved in cell cycle, apoptosis, growth, proliferation, fate determination, development, immunity, and ubiquitin-mediated proteolysis. The proto-oncogene PIM1 has been shown to prevent the normal process of apoptosis, acting as a cell survival factor. GADD45B is involved in cell cycle arrest, apoptosis, signal transduction, and cell survival. The human HSPA multigene family encodes several highly conserved proteins that are expressed in response to heat shock and a variety of other stress stimuli, including oxidative free radicals and toxic metal ions (21). At the same time, we found that among highly variable genes in OM adipose tissue samples, 28 transcripts showing high variability were

TABLE 4
GENES ASSOCIATED WITH THE TOP FUNCTION IN THE OM AND SC DATASETS CONTAINING HIGHLY VARIABLE TRANSCRIPTS

Category/Process	Genes
Omental adipose tissue: cell death	
Cell death	ATF3, CYR61, DNAJB1, EMP1, GADD45B, HIF1A, HSPA1A, HSPA1B, ICAM1, IER3, JUN, JUNB, KLF4, MAP2K3, MCL1, MT1X, MT2A, NFIL3, NFKBIA, PBEF1, PIM1, PRG1, S100A8, SGK, TBK1, TFRC, TNFAIP3, UGCG
Apoptosis	ATF3, CYR61, GADD45B, HIF1A, HSPA1A, HSPA1B, ICAM1, IER3, JUN, KLF4, MAP2K3, MCL1, MT2A, NFIL3, NFKBIA, PBEF1, PIM1, PRG1, S100A8, SGK, TBK1, TFRC, TNFAIP3, UGCG
Killing	HSPA1A, HSPA1B, S100A8
Cell viability	LMNA, MCL1, MT2A, NFKBIA, TNFAIP3, UGCG
Cytotoxicity	FCGR3B, MT2A, TNFAIP3
Survival	CYR61, HIF1A, HSPA1B, JUN, MCL1, NFIL3, NFKBIA, PIM1, UGCG
Colony survival	JUN
Inhibition	HSPA1B, IER3, MCL1
Activation-induced cell death	ICAM1
Subcutaneous adipose tissue: lipid metabolism	
Storage	ADFP, GM2A, LIPA, SCD
Quantity	APOC1, APOE, CTSS, FCER1G, LIPA, NR1H3, PLA2G2A, SCD, UCP2, IL1RN
Synthesis	APOE, CD9, FCER1G, NR1H3, PLA2G2A, SCD
Release	CTSB, FCER1G, IL1RN, PLA2G2A
Modification	APOE, PLA2G2A, SCD, UCP2, ITGB2
Efflux	APOE, NR1H3, SAA1
Hydrolysis	GM2A, HEXB, PLA2G2A
Accumulation	APOE, NR1H3, IL1RN, UCP2, APOC1
Production	APOE, IL1RN, ITGB2, PLA2G2A, NR1H3
Activation	NR1H3
Esterification	APOE, SCD
Oxidation	APOE, SCD, UCP2
Peroxidation	APOE, PLA2G2A
Co-capping	ITGB2
Uptake	APOC1, APOE
Metabolism	APOC1, APOD, IL1RN, PLA2G2A, SCD
Exchange	APOC1
Liberation	PLA2G2A
Degradation	GM2A, PLA2G2A
Desaturation	SCD
Secretion	APOE, SCD
Steroidogenesis	APOE
Transport	ADFP

involved in cell death. These results suggest high interindividual variability in programmed OM fat cell death.

Obesity has been recently suggested as a proinflammatory state (10), and white adipose tissue is no longer considered an inert tissue mainly devoted to energy storage but is emerging as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation. Many of these cellular pathways were highly variable in both fat depots. For example, pathways of hematopoietic cell lineage, the Fc epsilon RI signaling pathway, glycerophospholipid metabolism, and leukocyte transendothelial migration included highly variable genes in both fat compartments. Two main genes were responsible for this finding (PLA2G2A, TFRC). PLA2G2A plays an important role in a variety of cellular processes, including the production of precursors for inflammatory reactions. Furthermore, it is a key enzyme in eicosanoid synthesis and is therefore an interesting candidate gene in the context of inflammation (1). TFRC encodes the transferrin receptor, which plays an important role in controlling cell growth through iron uptake. Both genes are involved in inflammation, proliferation, growth, and oncogenesis. Our results may reflect high variability in inflammatory responses in both fat compartments in obesity.

In summary, our data demonstrated that interindividual variability of gene expression in abdominal SC and OM adipose tissue samples from obese men was rather low. Future studies are required to investigate relations between different phenotypes (such as obe-

sity, insulin, blood lipids) and expression of these transcripts.

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