The expression of adipogenic genes is decreased in obesity and diabetes mellitus

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Obesity is strongly correlated with type 2 diabetes mellitus, a common disorder of glucose and lipid metabolism. Although adipocytes are critical in obesity, their role in diabetes has only recently been appreciated. We conducted studies by using DNA microarrays to identify differences in gene expression in adipose tissue from lean, obese, and obese-diabetic mice. The expression level of over 11,000 transcripts was analyzed, and 214 transcripts showed significant differences between lean and obese mice. Surprisingly, the expression of genes normally associated with adipocyte differentiation were down-regulated in obesity. Not all obese individuals will become diabetic; many remain normoglycemic despite profound obesity. Understanding the transition to obesity with concomitant diabetes will provide important clues to the pathogenesis of type 2 diabetes. Therefore, we examined the levels of gene expression in adipose tissue from five groups of obese mice with varying degrees of hyperglycemia, and we identified 88 genes whose expression strongly correlated with diabetes severity. This group included many genes that are known to be involved in signal transduction and energy metabolism as well as genes not previously examined in the context of diabetes. Our data show that a decrease in expression of genes normally involved in adipogenesis is associated with obesity, and we further identify genes important for subsequent development of type 2 diabetes mellitus.

Obesity is a strong risk factor for the development of type 2 diabetes mellitus, a disease characterized by insulin resistance, relative insulin hyposecretion, and hyperglycemia. In fact, over 80% of individuals with type 2 diabetes mellitus are obese. However, only 10% of obese individuals are diabetic (1). It is still unclear what determines which obese, nondiabetic individuals will transition to diabetes.

The Obese (*ob*) mouse represents a well-studied model of obesity (2, 3). These animals are markedly hyperphagic because of a nonsense mutation in the gene encoding leptin, a satiety factor secreted by adipocytes (4). Despite extreme obesity, C57BL/6J (B6) *ob*/*ob* mice have only mild, transient hyperglycemia (5). We recently introgressed the *ob* mutation into the BTBR mouse strain and obtained severely diabetic mice (6). Together, these animals provide us with a murine model in which obesity is present with or without diabetes.

DNA microarrays have been successfully used in the analysis of aging and caloric restriction (7), cancer classification (8, 9), and in other diseases (10). We used this method to assess the changes in gene expression associated with obesity and diabetes in our model system. By assessing the level of expression of thousands of genes, we sought to gain insight into the molecular events important in the development of obesity and diabetes.

Here, we report the application of DNA array technology to obesity and diabetes. White adipose tissue from obese mice showed decreased expression of genes important in adipocyte differentiation, as compared with lean controls. Obese animals that progressed to overt diabetes showed further changes in genes involved in signal transduction and energy metabolism. We define a subset of genes whose expression changes with obesity and those genes whose expression further changes in the progression to type 2 diabetes mellitus.

Materials and Methods

Animals. BTBR, B6, and B6-*ob*/+ mice were purchased from the The Jackson Laboratory and bred at the University of Wisconsin Animal Care Facility. Mice were housed on a 12-h light-dark cycle and had ad libitum access to regular chow (Purina no. 5008) and water. All protocols were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee.

Sample Preparation. Epididymal fat pads were isolated from 14-wk-old mice after a 4-h fast, and snap frozen in liquid nitrogen. Total RNA was isolated by using TriReagent (Molecular Research Center, Cincinnati). cDNA was prepared from equal amounts of total RNA pooled from at least four animals by using Superscript Choice System (GIBCO/BRL) with a primer containing oligo(dT) and T7 RNA polymerase promoter sequences. Biotinylated cRNA was synthesized from purified cDNA by using the Bioarray High Yield RNA Transcript Labeling Kit (Enzo Biochem). cRNA was purified by using RNeasy columns (Qiagen, Chatsworth, CA), and quantified by UV spectrometry.

Microarrays. Samples for DNA microarray hybridization were prepared as previously described (11). All experiments were performed at the Academic User Center at Affymetrix, Inc. (Santa Clara, CA). Hybridization to murine 11K arrays was performed for 16 h at 45°C. Microarrays were washed according to Affymetrix protocols and immediately scanned on a GeneArray Scanner.

Data Analysis. All data sets were normalized to total fluorescence, which represents the total amount of cRNA hybridized to a microarray. The threshold for determining the significance of a change in the level of gene expression was made by using an algorithm that requires both a significant absolute and -fold change (11). Identification of genes associated with obesity was determined by selecting only those genes that significantly increased or decreased in every comparison. Expression levels of all genes present on all arrays are provided in the supplemental materials (http://www.biochem.wisc.edu/attie/supplemental_data/).

Gene expression levels that changed with diabetes were identified by linear regression performed on five groups of animals with increasing hyperglycemia. The correlation between expression levels and rank order of the five groups of animals was determined. This method was preferred to several clustering methods because it focuses specifically on linear trends (12, 13).

Abbreviations: B6, C57BL/6J; SREBP, sterol responsive element binding protein; CISH, cytokine-inducible SH2-containing protein; SH2, Src homology 2; PPARγ, peroxisome proliferator activator receptor γ; C/EBPα, CCAAT/enhancer binding protein α.

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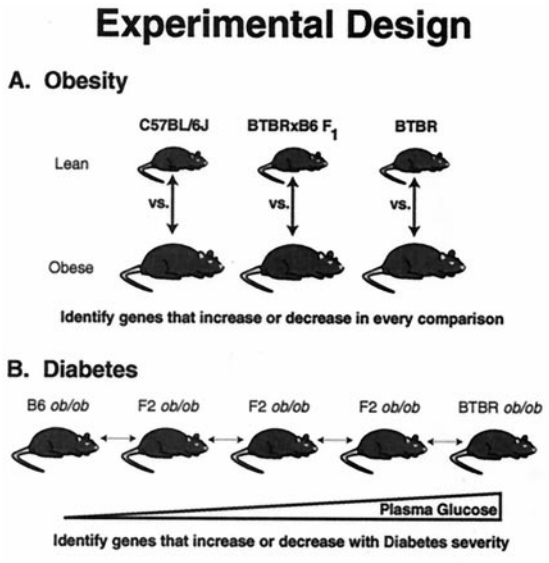

Fig. 1. Experimental design. (*A*) Three sets of lean and obese mice ($n = 3–4$) were compared in a pairwise fashion to identify genes important in the development of obesity. (*B*) Five sets of mice, including B6 *ob*/*ob*, three sets of F_2 *ob*/*ob* mice with increasing hyperglycemia, and BTBR *ob*/*ob* mice, were analyzed to detect genes whose expression levels strongly correlated with the onset of diabetes. Each group of mice was derived from three to four mice with differing genetic backgrounds but similar fasting blood glucose levels.

Similar genes were observed to change when regressions were performed with average fasting glucose levels of the group of animals instead of the rank order. Genes that correlated with $r > 0.95$ or $r < -0.95$ and had at least three absolute expression levels within the limits of detection were selected.

Results

The strains of lean mice, C57BL/6J, BTBR, and BTBR × B6 F_1 (BtB6 F_1) all have normal fasting glucose levels (14). The *ob* allele causes extreme obesity, but only mild, transient hyperglycemia in the B6 background (fasting plasma glucose 210 ± 30.7 mg/dl) as previously described (6). In contrast, introgression of *ob* into BTBR animals caused both obesity and severe diabetes, with fasting plasma glucose levels of 549 ± 24.5 mg/dl. Obese animals derived from a cross between BTBR and B6 (F_2 *ob*/*ob*) showed a large range of fasting glucose levels (150–750 mg/dl). Therefore, this F_2 population was a means of obtaining obese mice exhibiting different degrees of diabetes.

Gene Expression in Lean and Obese Mice. We compared the gene expression profile of adipose tissue from lean and obese animals in several mouse strains (Fig. 1*A*). We assessed the mRNA levels of >11,000 genes. About 10% of the assessed genes showed significant changes in gene expression in either direction. We then compared the gene expression change in each independent comparison and selected only those genes whose expression significantly increased or decreased in every comparison. The ability to detect important alterations in mRNA levels was

Table 1. Genes with decreased expression with obesity in each mouse strain

Accession no.	Description	Fold	Adipogenesis
Hormones and signal transduction			
x72862	β-3-adrenergic receptor	−10.5	Increase
aa500440	GTP-binding protein (Gαi-1)	−2.3	Decrease
aa529056	Guanine nucleotide binding protein 11	−1.6	
u02602	Thyroid stimulating hormone receptor	−1.5	
X61431	Diazepam-binding inhibitor	−2	
AF009246	Ras-related protein (DEXRAS1)	−2.9	
Mitochondrial			
aa245912	Similar to succinate dehydrogenase	−2	
aa466050	Similar to cytochrome *c*1	−1.8	
aa667872	Similar to ubiquinol-cytochrome *c* Reductase core protein 2	−3.4	
aa733351	Similar to ATP synthase E chain	−2.1	
W42043	Branched-chain amino acid aminotransferase	−2.8	
W41817	Cytochrome *c* oxidase, subunit VIIIa	−2.7	
U07235	Aldehyde dehydrogenase (ALDH2)	−3	
D16215	Flavin-containing monooxygenase	−2.8	
aa270965	Mitochondrial enoyl-CoA hydratase	−2.8	
M60798	SOD-1	−2.2	
Lipid metabolism			
D29016	Squalene synthase	−1.8	
aa271471	ATP citrate-lyase mRNA	−2.9	Increase
d50430	Glycerol-3-phosphate dehydrogenase	−2.5	Increase
X51905	Lactate dehydrogenase-B	−3.3	Increase
M21285	Stearoyl-CoA desaturase	−2.5	Increase
109192	Pyruvate carboxylase	−2.1	Increase
Y00516	Aldolase A	−2.5	Increase
AA080172	Phosphoenolpyruvate carboxykinase	−5.3	Increase
W29562	3T3-L1 lipid binding protein	−2.3	Increase
X95279	Spot14	−4.6	Increase
aa197973	Similar to biotin carrier protein of methylmalonyl-CoA carboxyl-transferase	−2.4	
Transcription factors			
AA068578	add1/SREBP	−2.7	Increase
Secreted proteins			
AA106347	Angiotensinogen precursor	−8.1	Increase
W36455	Adipsin	−8.3	Increase
D00466	Apolipoprotein E gene	−2.4	
m60579	Complement component C2	−3.2	
Others			
U63146	Retinol-binding protein (RBP)	−2.5	Increase
AA049662	Retinol-binding protein (RBP)	−2.2	Increase
W14367	Retinol-binding protein (RBP4)	−1.8	Increase
AA154594	Similar to branching enzyme	−2.6	
W85270	Inorganic pyrophosphatase	−2	
W13498	Glycogen phosphorylase	−2.5	
AF012431	D-dopachrome tautomerase (Ddt)	−1.9	
L31783	Uridine kinase	−2.7	
U38940	Asparagine synthetase	−4	
X51703	Ubiquitin	−2.7	
aa688469	Osteogenesis imperfecta (oim)	−3.3	
ab004048	Neuronatin	−2.8	
m30844	B2 protein	−11.2	
U19596	Cdk4 and Cdk6 inhibitor p18	−2.7	
X14061	β-globin complex	−2.6	
W82026	Scr3, ssRNA BP	−2.1	
W83919	Elongation factor Tu	−2.3	
M73483	Glutathione *S*-transferase	−5.8	
M96827	Ob/ob haptoglobin	−2.1	
AA059700	β-2 microglobulin (B2m)	−3.2	
ET61037	TI-225	−2.5	

Fold changes are approximate, as the level of gene expression may have been outside the linear range of detection in one of the comparisons. Genes listed as ''similar to'' show sequence similarities with $E < 5 \times 10^{-5}$ (40). Changes in gene expression during adipocyte differentiation are listed when known (15).

Table 2. Genes with increased expression with obesity

Accession no.	Description	Fold	Adipogenesis
Cytoskeleton and ECM			
X54511	Myc basic motif homologue-1	15	
d00472	Cofilin	1.7	
m86736	Acrogranin	2.7	
u08020	FVB/N collagen pro-α-1	2.1	Decrease
u27340	Sulfated glycoprotein (Sgp1)	3.1	
x56123	Talin	7.7	
W10936	L-34 galactoside-binding lectin	4.5	
AA003323	Similar to filamin A	4.5	
X99347	LPS-binding protein	1.9	
X14425	Profilin	2.1	
X75285	Fibulin-2.	2.2	
120276	Biglycan (Bgn)	2.1	
D13664	Osteoblast specific factor 2 (OSF-2)	3.1	
Lysosomal			
aa255186	Similar to cathepsin S precursor	5.6	
X94444	Preprocathepsin K.	4.5	
AA106931	γ-IFN inducible lysosomal thiol reductase (GILT)	6	
M65270	Cathepsin B	3.5	
AA116604	Cathepsin Z precursor (Ctsz)	3.9	
AA107895	Cathepsin D	2.1	
AA146437	Cathepsin S precursor	7.7	
ab009287	Macrosialin	6.6	
AA000961	Preprolegumain	3.9	
AA117064	Vacuolar adenosine triphosphatase	1.9	
Immune/complement genes			
aa711625	Similar IFN-α induced protein	2.8	
ET62967	Complement C1q precursor	1.4	
m22531	Complement C1q β chain	2.6	
J05020	High affinity IgE receptor	2.5	
m14215	Fc γ receptor	4.8	
W41745	Fc receptor (Fcer1γ)	8.1	
139357	Migration inhibitory factor (Mif)	1.6	
u19482	C10-like chemokine	3.3	
Z11974	Macrophage mannose receptor	3.7	
X67469	AM2 receptor	1.7	
L20315	MPS1	5.4	
X91144	P-selectin glycoprotein ligand	2.8	
Z16078	CD53 gene exon 7	2.2	
Cytoskeleton and ECM			
W11011	Nedd8	1.4	
W08269	Pigment epithelium-derived factor	2.3	
AA097711	Tropomyosin (TM-4)	2.2	
U72680	Ion channel homolog RIC	3	
AA096813	Cysteine proteinase	2.2	
M73741	α-B2-crystallin gene	2.8	
W15873	Similar to Tctex1	1.5	
Cell proliferation			
U44426	D52 (mD52)	5.2	
X06368	c-fms proto-oncogene	2.5	
J05261	Mouse protective protein (Mo54)	2.6	
AA050703	Defender against cell death 1	1.7	
Adipose-specific genes			
M93275	ADRP	4.2	
u18812	Leptin	3	
Membrane proteins			
W64897	Phosphatidylinositol transfer protein	1.8	
u37226	Phospholipid transfer protein	3.4	
AA031158	Brain acid-soluble protein 1	4.5	
AA108956	Similar to human membrane protein	1.8	
AF026124	Schwannoma-associated protein	3.9	
AA108330	Astrocytic phosphoprotein	1.9	
Others			
u69135	UCP2	4.3	
u29539	Retinoic acid-inducible E3 protein	3.4	
U59807	Cystatin B (Stfb)	4.4	
K02236	Metallothionein II (MT-II)	2.6	
M38337	Milk fat globule membrane protein E8	3.1	
M73706	Ferritin large subunit	1.9	
W75072	Creatine kinase B	3.4	
W83564	5-lipoxygenase-activating protein	3.4	
AA106783	Poly A binding protein	1.8	
x84797	Similar to human hematopoietic specific protein 1	4	
X61940	Growth factor-inducible immediate early gene (3CH134)	2.6	

Fold changes are approximate, as the level gene expression may have increased from levels below the linear range of detection in one of the comparisons. Changes in gene expression during adipocyte differentiation are listed as in Table 1.

increased not only by having three independent comparisons, but also through the elimination of strain background as a variable; the genes identified changed regardless of strain. This screening process led to the identification of 136 genes (1.2%) whose expression consistently increased across every comparison and 78 genes (0.7%) with consistently decreased expression levels. These numbers were significantly greater than the number expected to change in the same direction in all three comparisons, by random chance, 3 and 2 genes, respectively ($P << 0.001$, χ^2). We reconfirmed the change in gene expression in 16 genes by semiquantitative reverse transcription-PCR and/or Northern blots and observed qualitative agreement between the methods for all 16 genes (data not shown).

We were surprised to note that many of the changes in gene expression are the opposite of those previously shown to characterize adipocyte differentiation (Table 1) (15). Several mRNAs that encode proteins involved in lipid metabolism were decreased. These transcripts included: ATP-citrate lyase, glycerol 3-P dehydrogenase, stearoyl CoA desaturase, and fatty acid binding protein. Adipocyte-specific mRNAs, such as spot14 and adipsin, decreased 4.3- and 8.6-fold respectively. However, leptin mRNA increased, consistent with previous reports in *ob/ob* mice (4). Furthermore, genes involved in energy metabolism also showed marked decreases, including aldolase, lactate dehydrogenase, and pyruvate carboxylase. Other markers of adipocyte differentiation not directly involved in energy metabolism also showed decreases. For example, the expression of β-3 adrenergic receptor, angiotensinogen, and apolipoprotein E all increase during adipogenesis but showed large decreases in the comparisons of adipose tissue from lean and obese mice.

Another surprising result was the number of genes encoding mitochondrial enzymes whose expression decreased with obesity. Several mRNAs encoding proteins involved in the electron transport chain, such as cytochrome *c*1 and cytochrome *c* oxidase, decreased. The mitochondrial enoyl-CoA hydratase and aldehyde dehydrogenase 2 also showed 2.2- and 2.8-fold decreases, respectively. In contrast, uncoupling protein 2 (UCP2) increased 4-fold in adipose tissue from obese animals.

Large increases in gene expression were observed in genes coding for cytoskeletal and extracellular matrix proteins (Table 2). Expression of type I collagen decreased during adipocyte differentiation, but we observed a 2.1-fold increase in the

Table 3. Genes correlated with the development of hyperglycemia

Accession no.	Description	*r* value	Fold	Accession no.	Description	*r* value	Fold
Signal transduction				Transcription factors			
M34397	IL-3 receptor-like protein	−0.955	−3.5	W87135	Single stranded DNA binding protein p9	0.973	2.2
aa097386	Similar to CAM-like protein kinase	−0.982	n/a	af003866	Pale ear (ep wild type allele)	−0.982	−2.1
U58889	SH3-containing protein (SH3P3)	−0.970	n/a	AB001990	Dcra	−0.985	−1.4
u35124	Nonreceptor tyrosine phosphatase	−0.968	n/a	m11943	Int-1 proto-oncogene	0.956	4
d31943	Cytokine inducible SH2-containing protein	−0.971	−2.2	u67840	Dlx5	−0.988	n/a
u60528	Guanylin precursor gene	0.963	n/a	Z32675	Hairless protein	−0.956	−1.9
aa217487	Similar to mouse pim-1 protein kinase	−0.972	n/a	AJ002366	Transcription factor TFIIH, 62 kD subunit	−0.968	n/a
m64689	flk-2	0.972	n/a	U41285	Dishevelled-3 (Dvl-3)	−0.951	−1.5
C79373	Similar to phosphatase inhibitor-2	0.952	1.5	L38607	BF-2 transcription factor	0.994	n/a
aa105135	Similar to P53-binding protein	−0.982	−2.4	aa710439	BACH1	−0.986	n/a
aa467011	Similar to LMW G-protein	0.955	n/a	W83286	Similar to *H. sapiens* RNA polymerase II	0.967	1.4
d83266	vav-T	−0.958	n/a	D14336	RNA polymerase I associated factor	−0.972	−1.5
W91283	Similar to human ras-like protein	0.989	1.6	Others			
C81377	Similar to rat activated c-raf oncogene	0.960	n/a	aa711217	Similar to NADH-ubiquinone oxidoreductase	0.960	1.4
u70324	Fyn(T)	0.965	n/a	aa521794	Similar to cytochrome *c* oxidase	−0.961	−1.6
X72862	β-3-adrenergic receptor	0.951	n/a	M36660	NAD(P)H menadione oxidoreductase	−0.958	n/a
101695	Calmodulin-dependent phosphodiesterase	−0.960	−1.2	af015284	Selenoprotein W (mSelW)	−0.992	n/a
x04648	IgG1/IgG2β Fc receptor (FcR)	−0.961	−3.3	M99054	Acid phosphatase type 5 gene	−0.960	−1.5
Secreted				aa543785	Thymic dendritic cell-derived factor 1	0.985	1.5
M33960	Plasminogen activator inhibitor (PAI-1)	0.962	2.2	J02809	Neural specific calmodulin-binding protein	−0.954	n/a
D38580	VNSP I(vomeronasal secretory protein I)	−0.962	n/a	aa061099	Ribonuclease HI	−0.980	−3.3
Protein synthesis and processing				C78741	Poly(A) binding protein II (mPABII)	−0.951	−1.4
aa036204	Similar to human 40S ribosomal protein S24	0.971	1.7	AA230943	Similar to mouse Sm-B	−0.952	−2.1
D12907	47-kDa heat shock protein (HSP47)	−0.966	−1.6	aa544831	Similar to renin-binding protein	−0.973	−2.5
125913	Chaperonin	0.999	1.5	U12564	129 defensin-like gene 4C-2	0.971	n/a
C77806	Similar to rat carboxypeptidase B gene	−0.960	n/a	c79315	Similar to *M. musculus* tex292	0.971	1.6
aa270493	Similar to deoxyhypusine synthase	−0.959	−2.4	L06234	Dihydropyridine-sensitive calcium channel	−0.969	n/a
aa611449	Similar to *Homo sapiens* HSPC183	−0.962	−2.6	U62021	Neuronal pentraxin 1 (NPTX1)	−0.951	−2.1
AA105758	MDj10	−0.974	−2.2	aa560507	Similar to antiquitin	−0.984	−1.6
Cytoskeletal and ECM				z72000	BTG3	0.959	2.1
M25825	tctex-1	−0.959	−1.9	af003346	Ubiquitin-conjugating enzyme UbcM2	0.982	1.7
C77864	Similar to Chinese hamster for β tubulin	−0.991	n/a	AA072822	Testosterone 15-α-hydroxylase	−0.957	n/a
aa111610	Similar to tuftelin-interacting protein 10	0.950	n/a	aa547057	Similar to HT Protein	−0.979	−1.4
AA138226	Similar to rat clathrin light chain (LCB3)	0.956	1.1	Metabolism			
m75720	α-1 protease inhibitor 3	0.955	n/a	AA146156	Keratinocyte lipid binding protein (Klbp)	0.986	8.6
C76274	Similar to *Mus musculus* ligatin (Lgtn)	−0.952	n/a	AA120674	Similar to *Homo sapiens* acylphosphatase 2	0.964	2.3
aa204573	Similar to human spindle pole body protein	−0.972	−1.5	U21489	Long-chain acyl-CoA dehydrogenase	−0.963	−1.6
				aa592828	Aldo-keto reductase AKR1C1	0.977	2.2
				ET63206	Fructose-1,6-bisphosphatase	0.960	n/a

r values were calculated using the rank order of the five sets of animals with increasing hyperglycemia. Fold changes represented by n/a mean that the mRNA level increased from, or decreased to, an expression level outside the linear range of detection.

expression of procollagen I. We observed increases in profilin, talin, and actin-binding protein mRNAs consistent with the need to remodel fat pads as adiposity increased. Changes in genes encoding extracellular matrix proteins included increases in the expression levels of cofilin, galactose-binding lectin, and the proteoglycan biglycan. Cathepsins are lysosomal proteases also implicated in tissue remodeling. Increases in expression of cathepsins B and D, as well as cathepsin K, S, and Z precursors, were observed with obesity.

The expression level of certain nuclear proteins and transcription factors was also altered. The gene encoding the Myc basic motif homologue-1 showed a 3.0-fold increase in obese animals. Of particular note, the sterol responsive element binding protein ADD1/SREBP demonstrated a 2.7-fold decrease in expression. SREBP positively regulates many genes coding for lipogenic enzymes, and its down-regulation is consistent with the decrease in expression of the lipogenic enzymes mentioned above (16, 17).

Gene Expression in Obese and Obese-Diabetic Mice. We determined which genes increased or decreased with hyperglycemia across five sets of mice: B6 *ob/ob* mice, three sets of F_2 *ob/ob* mice with increasing hyperglycemia (mean fasting glucoses of 299, 337, and 410 mg/dl), and BTBR *ob/ob* mice (Fig. 1*B*). By generating an F_2 *ob/ob* population from the parental strains, we created mice with intermediate levels of hyperglycemia ranging from very mild to severe. This range of phenotypes allowed us to assess which genes demonstrate a dose-dependent change in gene expression with increasing hyperglycemia.

The degree of correlation between the severity of diabetes and gene expression levels was evaluated by linear regression. In white adipose tissue, there were 34 genes (0.3%) whose expression positively correlated ($r > 0.95$) and 58 genes (0.5%) whose expression negatively correlated with diabetes ($r < -0.95$) (Table 3). As before, we reconfirmed the change in expression for selected genes. The fold change was calculated from the expression levels of the groups with the lowest and highest blood glucose.

Many genes that code for signal transduction proteins had expression levels that correlated with the development of diabetes. Genes similar to both Raf and Ras increased with diabetes, as did Fyn. Many signal transduction molecules use Src homology (SH) 2 and SH3 domains, and two such proteins, SH3-

containing protein (SH3P3) and cytokine-inducible SH2-containing protein (CISH), decreased with diabetes.

Our analysis also identified several genes encoding proteins involved in protein phosphorylation and dephosphorylation, important mediators of many signaling pathways, including those activated by insulin. In particular, PTPK1, a nonreceptor protein tyrosine phosphatase, decreased to undetectable levels as hyperglycemia increased. The expression of Flk-2, a class III receptor tyrosine kinase, increased from undetectable levels with the onset of diabetes. Conversely, CAM-like protein kinase and pim-1 protein kinase decreased significantly with hyperglycemia whereas a phosphatase inhibitor-2-like protein increased with elevated plasma glucose. Expression of vav-T, an SH3 domain-containing G-protein exchange factor, decreased 3.3-fold.

We also observed that many transcription factor mRNA levels changed with worsening diabetes. BF-2, a winged helix transcription factor, and int-1 increased 2- and 4-fold, respectively. Other transcription factors, such as Dlx5, *Disheveled* (Dvl3), and *Pale Ear* (ep) decreased with diabetes.

The expression of several genes involved in energy metabolism changed with hyperglycemia. Klbp, a lipid binding protein, increased 7.3-fold whereas long-chain acyl-CoA dehydrogenase decreased 1.6-fold. AKR1, an aldo-ketoreductase, and fructose-1,6 bis-phosphatase both increased. Interestingly, the β-3 adrenergic receptor decreased 90% in obesity as previously reported (18), but was positively correlated with increasing plasma glucose. Genetic variations in the the β-3 adrenergic receptor have previously been associated with type 2 diabetes mellitus (19).

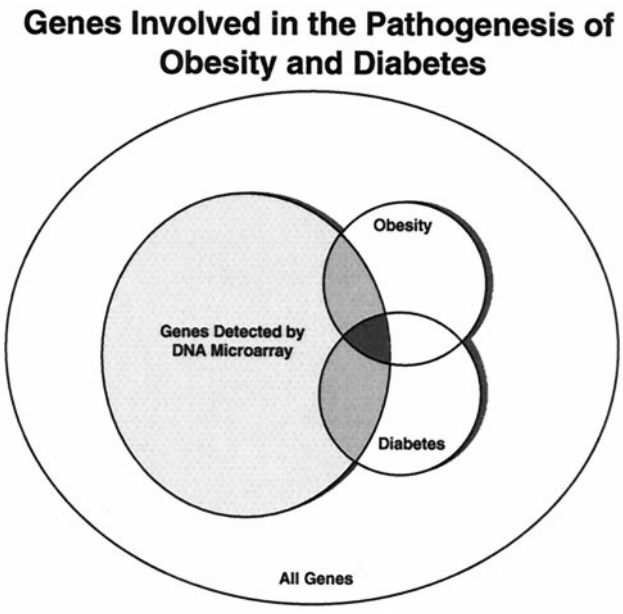

Fig. 2. Venn diagram demonstrating the relationship between genes involved in the development of obesity and those involved in type 2 diabetes mellitus.

Discussion

We used DNA microarrays to identify changes in gene expression in obesity and type 2 diabetes mellitus. By using different strains of mice, we were able to identify those genes whose expression changed regardless of strain background and are therefore most likely to be relevant in obesity and diabetes. Of the over 11,000 genes examined, over 200 showed consistent changes with obesity.

We were able to identify 88 genes whose level of expression in adipose tissue strongly correlated with the progression from normoglycemic obesity to obesity concomitant with diabetes. Of the genes that were evaluated by our analysis, the expression of some changed with obesity alone, whereas the expression of others was identified as important in diabetes alone (Fig. 2). A smaller subset of these genes, including the β-3 adrenergic receptor, demonstrated changes in expression in both diabetes and obesity.

Our data show the reversal of many of the changes in gene expression characteristic of adipocyte differentiation. Cornelius *et al*. organized the changes in gene expression observed in adipogenesis into five groups: hormone signaling and action, lipogenesis and lipolysis, cytoskeletal and extracellular, secreted, and proteins of unknown function (15). We observed changes in mRNA levels of 18 of those genes, distributed across four of the five classes. The expression levels of all but one of the genes changed in the opposite direction from the changes observed during adipocyte differentiation (Tables 1 and 2).

Adipogenesis is promoted by the coordinated expression of many transcription factors (20, 21). We observed a significant decrease in SREBP mRNA, an important regulator of lipogenic enzymes. Other transcription factors important to adipogenesis include the peroxisome proliferator activator receptor γ (PPARγ), and CCAAT/enhancer binding proteins α, β, and δ (C/EBP α,β,δ). Although they did not meet the strictest criteria we used to select genes because of low abundance, both PPARγ2 and C/EBPα did show consistent decreases in all three comparisons of lean vs. obese adipose tissue.

Dedifferentiation, or loss of adipocyte phenotype, has been observed in response to tumor necrosis factor α and transforming growth factor β (22). The molecular mediators of this process have been identified as PPARγ and C/EBPα (23). Dedifferentiation of 3T3-L1 adipocytes includes a suppression of PPARγ and C/EBPα, as well as other lipogenic enzymes. Furthermore, C/EBPα is important in maintaining adipocytes in the fully differentiated state (24, 25). Our data show similar changes in expression of both PPARγ and C/EBPα, as well as decreased SREBP expression in obesity. The simultaneous decrease in mRNAs for genes that are characteristic of mature adipocytes and transcription factors critical in the maintenance of terminally differentiated fat cells leads us to conclude that some degree of dedifferentiation has taken place in the adipose tissue of these animals.

Mutations in the gene encoding leptin represent only one model of obesity in mice. Human obesity is thought to be a leptin-resistant state (26). Our studies do not allow us to infer whether a similar dedifferentiation process occurs in human obesity.

Changes in gene expression in adipose tissue alone might not be sufficient to cause diabetes. Alterations in muscle, liver and pancreatic β-cells are probably also required. However, recent studies in mice that lack white adipose tissue show that adipocytes play an important role in the development of diabetes. For example, transgenic mice lacking adipose tissue because of disruption of transcriptional regulation by C/EBPs and Jun develop hyperglycemia and hyperinsulinemia, two hallmarks of type 2 diabetes (27). Similarly, adipocyte-specific overexpression of a constitutively active form of SREBP leads to dramatic loss of white adipose tissue and subsequent development of diabetes in mice (28).

The changes in gene expression that we observed are provocative, in that the onset of diabetes in our model system correlated with alterations in the expression of many mRNAs coding for signal transduction proteins that have been previously implicated in diabetes. For example, Fyn mRNA levels increased with hyperglycemia, and Fyn has recently been implicated in the compartmentalization of insulin signaling through its interaction with c-Cbl (29, 30). Similarly, we observed an increase in mRNA for Flk-2, a tyrosine kinase that promotes hematopoiesis through

interactions with Grb2 and Shc, two important mediators of both insulin signaling and hematopoiesis (31).

The expression of many transcription factors correlated with diabetes. Expression of BF-2 and int-1 both increased with diabetes. BF-2 has been described in the context of neuronal development and belongs to the same family as HNF3, an important adipogenic transcription factor (32). Int-1 is a proto-oncogene involved in the proliferation of mammary tumors (33). A decrease in mRNA levels was observed for the transcription factors Dishevelled-3 (Dvl-3), Dlx5, and Pale Ear (ep). No association between hyperglycemia and Dishevelled-2 or Dlx5 has been previously reported. The potential role of Pale Ear in diabetes progression is intriguing. Mutations in the orthologous human gene cause Hermansky-Pudlak syndrome in humans, a rare disorder associated with impaired vesicular transport (34), a critical process in insulin-stimulated glucose uptake (35).

The mRNA levels of another protein implicated in vesicular transport, tctex-1, decreased with diabetes. Tctex-1 constitutes one of the three light chains in cytoplasmic dynein (36). It plays an important role in many aspects of membrane and vesicular transport (37, 38). Impaired ability of cells to translocate glucose transporter-containing vesicles to the plasma membrane in response to insulin would result in insulin resistance, an important contributor to the development of type 2 diabetes mellitus.

In conclusion, genetically obese mice exhibited changes in gene expression consistent with dedifferentiation of adipose tissue. Further alterations in gene expression were observed with the development of diabetes. These changes provide valuable new molecular insights into the pathogenesis of obesity and type 2 diabetes mellitus.

Note. While this manuscript was in review, Soukas *et al.*, published a study that also profiled gene expression in wild-type and leptin-deficient mice (39). In both studies, many of the genes associated with adipocyte differentiation showed a decrease in expression with leptin deficiency. This similarity was especially true of genes involved lipid metabolism.

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