Global Gene Expression Profiles of Subcutaneous Adipose and Muscle From Glucose-Tolerant, Insulin-Sensitive, and Insulin-Resistant Individuals Matched for BMI

Steven C. Elbein,[1][†] Philip A. Kern,[2] Neda Rasouli,[3,4] Aiwei Yao-Borengasser,[5] Neeraj K. Sharma,[1] and Swapan K. Das[1]

OBJECTIVE—To determine altered gene expression profiles in subcutaneous adipose and skeletal muscle from nondiabetic, insulin-resistant individuals compared with insulin-sensitive individuals matched for BMI.

RESEARCH DESIGN AND METHODS—A total of 62 non-diabetic individuals were chosen for extremes of insulin sensitivity (31 insulin-resistant and 31 insulin-sensitive subjects; 40 were European American and 22 were African American) and matched for age and obesity measures. Global gene expression profiles were determined and compared between ethnic groups and between insulin-resistant and insulin-sensitive participants individually and using gene-set enrichment analysis.

RESULTS—African American and European American subjects differed in 58 muscle and 140 adipose genes, including many inflammatory and metabolically important genes. Peroxisome proliferator–activated receptor γ cofactor 1A (*PPARGC1A*) was 1.75-fold reduced with insulin resistance in muscle, and fatty acid and lipid metabolism and oxidoreductase activity also were down-regulated. Unexpected categories included ubiquitination, citrullination, and protein degradation. In adipose, highly represented categories included lipid and fatty acid metabolism, insulin action, and cell-cycle regulation. Inflammatory genes were increased in European American subjects and were among the top Kyoto Encyclopedia of Genes and Genomes pathways on gene-set enrichment analysis. *FADS1*, *VEGFA*, *PTPN3*, *KLF15*, *PER3*, *STEAP4*, and *AGTR1* were among genes expressed differentially in both adipose and muscle.

CONCLUSIONS—Adipose tissue gene expression showed more differences between insulin-resistant versus insulin-sensitive groups than the expression of genes in muscle. We confirm the role of *PPARGC1A* in muscle and show some support for inflammation in adipose from European American subjects but find prominent roles for lipid metabolism in insulin sensitivity independent of obesity in both tissues. *Diabetes* 60:1019–1029, 2011

The international obesity epidemic has been accompanied by a rapidly increasing prevalence of type 2 diabetes across ethnic groups (1). The connection between obesity and impaired glucose homeostasis is indisputable. Nonetheless, the means by which excessive adiposity induces insulin resistance and glucose intolerance remain controversial. Indeed, although obesity, insulin resistance, metabolic syndrome, and glucose intolerance are highly correlated, most obese individuals do not develop type 2 diabetes, and many obese individuals have entirely normal metabolic profiles (2,3). In contrast, lean individuals may be as insulin resistant as those with type 2 diabetes (4). Among the mechanisms proposed for these paradoxical observations are muscle-centered hypotheses, including impaired muscle glucose transport, impaired muscle mitochondrial numbers or function, and impaired muscle lipid oxidation (5–7). Alternatively, adipose-centered hypotheses have included inflammation (1), oxidative stress (8), endoplasmic reticulum stress (9), impaired adipose lipid metabolism with ectopic lipid deposition (10), and impaired adipogenesis (11).

Gene expression studies of adipose and muscle may illuminate the physiologic mechanisms that result in insulin resistance, particularly when these studies are unbiased and use global transcript profiles. Two previous studies (12,13) of skeletal muscle that compared diabetic or insulin-resistant and control individuals reported only modest changes in individual transcript levels. Both studies proposed general alterations in genes involved in oxidative metabolism and under the control of the transcription factor peroxisome proliferator–activated receptor (PPAR) γ coactivator (PGC)-1α, encoded by the gene PPARG coactivator 1 (*PPARGC1*). Some, but not all, subsequent studies (14,15) support these findings. Studies of adipose tissue have been less consistent. In a recent study (16), 70% of subcutaneous adipose transcripts were correlated with BMI. Because obesity measures, including BMI, also are well correlated with altered glucose homeostasis and whole-body insulin action, distinguishing the molecular changes in subcutaneous adipose that accompany excessive adiposity from those that cause insulin resistance is a significant challenge. For example, in a recent study (9), we found that endoplasmic reticulum stress response was highly correlated with all measures of obesity but was not independently associated with measures of insulin sensitivity (S_I).

With these considerations, we sought an experimental design that would determine the global transcript profiles of subcutaneous adipose and skeletal muscle in insulin-resistant and insulin-sensitive, nondiabetic individuals independent of the confounding effects of obesity. To accomplish this goal, we selected individuals at the tails of the distribution of S_I after adjusting for age, sex, and BMI from a group of 167 study subjects for whom we had both adipose and muscle samples and a direct measure of S_I. The resulting insulin-resistant and insulin-sensitive groups were successfully matched for age, sex, and BMI and included 40 European American and 22 African American individuals to examine potential ethnic differences in gene expression. Genome-wide expression analysis used in this study allowed us an unbiased hypothesis-free analysis of our data. However, based on available literature, we also tested three specific hypotheses: *1*) muscle transcripts downstream of *PPARGC1* (encoding PGC1α) would be reduced in insulin-resistant compared with insulin-sensitive individuals, *2*) adipose would show markers of inflammation independent of obesity, and *3*) a subset of metabolically important genes will be differentially expressed between African American and European American populations.

RESEARCH DESIGN AND METHODS

Individuals were chosen from previous studies for availability of both adipose and muscle biopsy samples and S_I calculated from the insulin-modified, intravenous glucose tolerance test as described previously (9). In addition, individuals who were selected were aged 20–55 years and had a BMI between 19 and 42 kg/m^2. From 184 total study participants, 167 individuals (122 European American and 45 African American) met the inclusion criteria. Individuals were ranked by S_I after adjustment for age, sex, and natural logarithm of BMI for each ethnic group separately. We finally selected 62 individuals for our genome-wide expression analysis, which included 31 individuals (20 European American and 11 African American) from each tail (insulin resistant and insulin sensitive; European American and African American subjects were ranked separately) of the distribution of the standardized residual of S_I. Characteristics of the study sample are shown in Table 1 and Supplementary Fig. 1. All biopsies were obtained in the fasting state, either before the intravenous glucose tolerance test or on a different day. Adipose biopsies were obtained under local anesthesia from abdominal subcutaneous fat by either needle biopsy or by incision, rinsed in saline, and frozen in liquid nitrogen. All muscle samples were obtained from the vastus lateralis by Bergstrom needle biopsy under local anesthesia and frozen in liquid nitrogen. All study participants provided written, informed consent under protocols originally approved by the University of Arkansas for Medical Sciences.

RNA extraction. Total RNA was isolated from adipose using the RNAeasy Lipid Tissue Mini kit (Qiagen, Valencia, CA) and from muscle using the Ultraspec RNA kit (Biotecx Laboratories, Houston, TX). The quantity and quality of the isolated RNA were determined by ultraviolet spectrophotometry and electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), respectively. High-quality RNA with an average RIN (RNA integrity number) of 8.5 and 8.1, respectively, from adipose and muscle was used for genome-wide transcriptome analysis.

Microarray studies. Genome-wide transcriptome analysis and initial array processing was performed by GenUs Biosystems (Northbrook, IL) using Human Whole Genome 4 × 44 k arrays (Agilent Technologies), according to vendor-recommended standard protocol.

Data analysis. Processed arrays were analyzed using the nonparametric Wilcoxon statistic on normalized data in Statistical Analysis for Microarray software (17). Functional annotation of differentially expressed genes were performed by singular enrichment analysis (SEA) and modular enrichment analysis (MEA) using the DAVID version 6.7 functional annotation tool (18,19). We also performed canonical pathway analysis and interaction network analysis for these differentially expressed genes using ingenuity pathway analysis (IPA; version 8.7, available at https://analysis.ingenuity.com). A small number of genes showed a mean 1.5-fold differential expression; thus, we used all probes corresponding to transcripts with the National Center for Biotechnology Information/Entrez identifiers irrespective of fold change for gene-set enrichment analysis (GSEA). The GSEA analysis was performed by GeneTrail (20). A more detailed description of our microarray studies and data analysis methods is presented in the Supplementary Data.

RESULTS

Study subjects. The study populations were well matched on BMI, age, sex, and percentage of fat (Table 1). Consistent with the study design, all measures of insulin sensitivity, including fasting insulin, S_I, and compensatory insulin response (insulin area under curve from an oral glucose tolerance test, acute insulin response to glucose [AIR$_G$]) were significantly different ($P < 0.00001$) between the insulin-resistant and insulin-sensitive groups. Consistent with previous reports (21), disposition index (AIR$_G$ × S_I) in insulin-resistant individuals (Table 1) showed incomplete compensation. As expected with metabolic syndrome, insulin-resistant subjects had increased waist-to-hip ratios (WHRs) and triglyceride levels, which were statistically significant in the European American subset, and elevated fasting and 2-h postchallenge glucose in both European American and the combined populations ($P < 0.01$ in both for 2-h glucose). Hence, the goals of selecting age-, sex-, and BMI-matched subjects discordant for S_I was achieved (Supplementary Fig. 1). Salient ethnicity-specific differences also were noticed in our cohort; insulin-resistant individuals of the European American subset showed higher serum triglyceride levels and lower AIR$_G$ compared with the African American subset, as has been described previously (21,22).

Ethnic differences in expression profiles. Of 41,000 probes available on the Agilent arrays, 30,632 probes were present in African American adipose samples and 30,020 European American adipose samples. In muscle, we observed 26,314 probes expressed in African American and 23,008 in European American samples. Table 2 summarizes the upregulated and downregulated genes for all comparisons in the study. In muscle, 58 genes showed more than a 1.5-fold (African American/European American) differential expression for at least one probe (Supplementary Table 1), including metabolically important genes such as mitogen-activated protein (MAP) 3 kinase 15 (*MAP3K15*), 24-dehydrocholesterol reductase (*DHCR24*), and ceramide kinase (*CERK*).

In adipose, 140 known genes were differentially expressed (Supplementary Table 2), again including many with potential metabolic impact, including vanin1 (*VNN1*), hydroxysteroid 11-β dehydrogenase 1 (*HSD11B1*), glycerol kinase 5 (*GK5*), stearoyl-CoA desaturase 5 (*SCD5*), interleukin 12 receptor, and β 2 (*IL12RB2*). At a 1.5-fold difference (q value <5%), six known genes were differentially expressed (African American/European American) in both muscle and adipose, suggesting likely genetic control. These genes included *CRYBB2*, *GSTT2*, *IL27*, *LRRC36*, *MAPK8IP1*, and *SOS1*. Notably, *MAPK8IP1* is very near a chromosome 11 locus that was recently associated with fasting glucose (23).

Insulin-sensitive versus insulin-resistant expression profiles in muscle. Given the 0.2–0.4% of all transcripts that differed significantly between African American and European American adipose and muscle samples, we focused our primary comparison of insulin-resistant and insulin-sensitive individuals on the combined set (31 insulin-resistant and 31 insulin-sensitive individuals) after including African American and European American samples in a separate permutation group. Among 10 genes that differed between all insulin-resistant and insulin-sensitive individuals were PPARG coactivator 1A (*PPARGC1A*), which has been reported previously (12,24,25), fatty acid desaturase 1 (*FADS1*), hyaluronoglucosaminidase 4, Harvey rat sarcoma viral oncogene (HRAS)-like suppressor 2, proline

TABLE 1
Demographics of the study population

Trait	European American insulin-sensitive subjects	European American insulin-resistant subjects	African American insulin-sensitive subjects	African American insulin-resistant subjects	Combined insulin-sensitive subjects	Combined insulin-resistant subjects	Combined European American subjects	Combined African American subjects
Sex (male/female)	3/17	7/13	3/8	1/10	6/25	8/23	10/30	4/18
Age (years)	40.9 (8.8)	40.7 (8.7)	40.9 (6.5)	36.1 (9.2)	40.9 (8.0)	39.1 (9.0)	40.8 (8.6)	39.0 (8.8)
BMI (kg/m²)	29.6 (5.5)	30.6 (4.0)	32.7 (5.6)	31.3 (5.5)	30.7 (5.7)	30.9 (4.6)	30.1 (4.8)	31.5 (5.2)
Waist (cm)	94.1 (14.9)	99.9 (10.0)	97.3 (14.6)	97.3 (15.5)	95.3 (14.6)	99.0 (12.0)	97.1 (12.8)	97.0 (14.3)
WHR	0.86 (0.08)	0.90 (0.06)*	0.84 (0.15)	0.97 (0.08)	0.85 (0.11)	0.89 (0.07)	0.88 (0.07)	0.86 (0.12)
Systolic blood pressure (mmHg)	118 (9)	125 (10)*	124 (21)	122 (12)	120 (15)	124 (11)	121 (10)[a]	123 (17)
Diastolic blood pressure (mmHg)	73 (6)	76 (7)	80 (15)	76 (7)	76 (10)	76 (7)	75 (6)	78 (12)
Percentage body fat	37.5 (11.1)	37.1 (7.5)	35.6 (10.0)	37.7 (6.3)	36.9 (10.6)	37.3 (7.0)	37.3 (9.3)	35.6 (8.4)
Triacylglycerol (mmol/L)	1.03 (0.45)	1.76 (0.68)[3]	1.09 (0.96)	1.0 (0.58)	1.05 (0.66)	1.49 (0.74)*	1.39 (0.68)	1.08 (0.77)
Total cholesterol (mmol/L)	4.80 (0.96)	4.90 (0.76)	4.50 (1.16)	4.49 (0.91)	4.69 (1.02)	4.76 (0.83)	4.85 (0.85)	4.50 (1.02)
LDL cholesterol (mmol/L)	2.89 (0.91)	2.97 (0.54)	2.40 (1.06)	2.36 (0.86)	2.72 (0.98)	2.75 (0.72)	2.93 (0.74)[a]	2.37 (0.94)
HDL cholesterol (mmol/L)	1.47 (0.38)	1.13 (0.20)[3]	1.60 (0.27)	1.67 (0.39)	1.52 (0.35)	1.32 (0.38)*	1.30 (0.34)[b]	1.63 (0.33)
Fasting glucose (mmol/L)	4.62 (0.41)	4.81 (0.40)	4.54 (0.37)	4.72 (0.58)	4.60 (0.40)	4.78 (0.46)	4.72 (0.41)	4.70 (0.58)
2-h Glucose (mmol/L)	5.33 (1.67)	6.66 (1.26)[2]	4.94 (1.26)	5.72 (1.73)	5.19 (1.53)	6.32 (1.49)[2]	5.99 (1.61)	5.25 (1.56)
Glucose area under the curve (mmol/L)	794 (138)	905 (117)[2]	753 (132)	762 (227)	779 (135)	854 (135)	851 (138)[a]	751 (179)
Fasting insulin (pmol/L)	23.7 (11.6)	65.6 (23.7)[4]	31.8 (21.2)	63.3 (35.8)*	26.7 (15.9)	64.8 (36.0)[5]	45.2 (34.6)	58.3 (72.9)
Insulin area under the curve	19,370 (10,527)	66,777 (37,820)[5]	20,952 (11,234)	75,967 (36,742)[3]	19,950 (10,628)	70,038 (37,099)[6]	43,681 (36,665)	45,847 (35,940)
AIRg (pmol/L)	1,888 (1,951)	4,526 (2,412)[5]	3,463 (2,557)	6,213 (4,375)	2,447 (2,275)	5,125 (3,277)[5]	3,207 (2,544)	4,782 (3,768)
Disposition index	1,907 (1,720)	995 (474)*	3,487 (2,894)	1,619 (1,357)[6]	2,468 (2,293)	1,216 (921)[2]	1,451 (1,329)	2,569 (2,394)
S_i ($\times10^{-4}$ min/[μU/mL])	6.92 (4.4)	1.42 (0.45)[7]	5.86 (2.26)	1.57 (0.58)[6]	6.54 (2.39)	1.47 (0.50)[7]	4.17 (3.28)	3.75 (2.69)
S_i residual	1.51 (0.46)	−1.39 (0.37)[7]	1.46 (0.43)	−1.26 (0.42)[7]	1.49 (0.44)	−1.34 (0.39)[7]	1.53 (0.24)	1.44 (0.30)

Descriptive statistics on two groups, divided by ethnicity and shown together. Differences not marked are not statistically significant. Significant differences are footnoted within each comparison group. Skewed variables were logarithmically transformed to normality to test significance, but are shown on the linear scale. Standard deviations are shown in parentheses. *$P < 0.05$; [2]$P < 0.01$; [3]$P < 10^{-3}$; [4]$P < 10^{-4}$; [5]$P < 10^{-5}$; [6]$P < 10^{-6}$; [7]$P < 10^{-10}$, all refer to insulin resistance vs. insulin sensitivity. Statistically significant differences between European American and African American individuals are shown as [a]$P < 0.05$, and [b]$P < 10^{-3}$.

TABLE 2
Summary of differentially expressed genes for all comparisons

Comparison	n	Tissue	Upregulated genes*	Downregulated genes*
African American/European American	22/40	Muscle	22	36
African American/European American	22/40	Adipose	23	117
Insulin-resistant/insulin-sensitive, all	31/31	Muscle	3	7
Insulin-resistant/insulin-sensitive, European American	20/20	Muscle	17	5
Insulin-resistant/insulin-sensitive, all	31/31	Adipose	38	134
Insulin-resistant/insulin-sensitive, European American	20/20	Adipose	149	172

*Number of genes that met the criteria of q value <5%, fold change either >1.50 (upregulated), or <0.667 (downregulated) and single-point P value from t test of <0.05 for at least one expressed probe. Genes with probes for multiple transcripts are considered significant only when all expressed probes are in same direction. The table includes genes with corresponding Entrez ID numbers but not referring to open reading frames, transcripts of unknown function, or pseudogenes. n, number of samples in each subset.

dehydrogenase, and a frizzled-related protein (SFRP1) (Table 3), none of which showed significant ethnic differences. Notably, single nucleotide polymorphisms (SNPs) near FADS have been associated with fasting glucose, homeostasis model assessment of β-cell function (23), and plasma phospholipids (26). For comparison with previous studies, we performed a secondary analysis of the European American subset (20 insulin-resistant and 20 insulin-sensitive subjects) (Table 2). In the European American subset, 22 transcripts differed, again including PPARGC1A (Supplementary Table 3) with a 1.75-fold reduction in insulin-resistant individuals (single point $P = 7.5 \times 10^{-5}$). Additional transcripts in the European American sample included serpin peptidase inhibitor A5 (3.37-fold increase in insulin resistance), cortexin 3 (2.4-fold increase in insulin resistance), glycerol-3-phosphate dehydrogenase 1 (GPD1), sorting nexin-associated golgi protein 1 (SNX18/SNAG1), and sortilin-related VPS10 domain containing receptor 1 (SORCS1). Thus, consistent with previous observations (12), few transcripts exceeded 1.5-fold enrichment, although more transcripts differed in the smaller subset of European American samples.

Insulin-sensitive versus insulin-resistant expression profiles in adipose. With European American and African American individuals considered together, 172 genes were ≥1.5-fold differentially expressed in subcutaneous adipose for at least one probe (Tables 2 and 4; Supplementary Table 4). Annotation of these top differentially expressed genes using DAVID enrichment analysis indicated over-representation of these genes in important functional categories (Supplementary Table 5). Highly represented categories (Table 4) included lipid/fatty acid/sterol metabolism (11 genes including fatty acid synthase, fatty acid desaturase 1, fatty acid elongase ELOVL5, 17 β hydroxysteroid dehydrogenase, acyl-CoA synthase, acetyl CoA carboxylase A, and apolipoprotein B), insulin action and glucose metabolism (including hexokinase 2, phosphoenolpyruvate carboxykinase 1, glucose transporters SLC2A3, SLC2A4, and phosphofructokinase PFKFB3), angiogenesis (including vascular endothelial growth factor A [VEGFA], tenomodulin, and prokinectin1), and cell-cycle regulation and cell division (including CCND2, CCNB2, CDKN1B, and CAPRIN1). Among genes recently implicated in adipogenesis or insulin resistance of obesity, we found reductions in STEAP4 (27), KLF15 (28), and bone morphogenic protein BMP7 (29). Notably, missing from the list (Table 4 and Supplementary Table 4) of ≥1.5-fold differentially expressed genes were genes implicated in endoplasmic reticulum stress response, classic inflammatory markers including TNFα, adipokine genes, or macrophage markers.

Female subjects were overrepresented in our study, but both the insulin-resistant and insulin-sensitive group had a similar distribution of male and female subjects. However, our sample set is not sufficiently powered to study differential expression between insulin-resistant and insulin-sensitive individuals after stratifying for both sex and ethnicity.

In the European American subset, 149 transcripts for known genes were increased (≥1.5-fold) and 172 were decreased in adipose from insulin-resistant individuals (Supplementary Table 6). Many genes involved in immune response were increased, including transcripts from the interleukin, immunoglobulin, interferon, chemokine, and tumor necrosis factor families (Supplementary Table 6). Among the other genes significantly increased in European American insulin-resistant adipose were resistin (RETN) (30), thrombospondin 1 (THBS1) (31), sphingosine kinase 1 (SPHK1), and cholesterol 25-hydroxylase. Genes reduced in European American insulin-resistant tissue included acyl-CoA dehydrogenases ACADL and ACADM, isovaleryl CoA dehydrogenase, insulin receptor substrate 1 (IRS1), endoplasmic reticulum stress gene SERP1, acetyl-CoA carboxylase genes ACACA and ACACB, and acetyl-CoA synthetase short-chain family member ACSS2. Notably, genes IRS1 and FADS1 are near SNPs associated with either type 2 diabetes or fasting glucose (23,32). Reduced expression of fatty acid metabolism genes among top differentially expressed genes was again prominent by DAVID analysis and additionally showed enrichment of genes related to immune response in the European American subset (Supplementary Table 7). Ingenuity pathway analysis (IPA) of genes differentially expressed in European American insulin-resistant and insulin-sensitive individuals in adipose showed propionate metabolism as the most significant canonical pathway with seven genes (ACADL, ACACB, ACSS2, ACACA, EHHADH, IVD, and ACADM) differentially expressed (≥1.5-fold) in this pathway (Supplementary Table 8). Interaction network analysis using IPA, however, showed a network of genes related to immune response (score = 42, with 25 differentially expressed genes) and a network related to inflammatory response (score = 37, with 24 differentially expressed genes) as most significant (Supplementary Fig. 2).

GSEA. A previous study of muscle found that most transcripts showed modest (<1.5-fold) expression changes but were clustered in biological pathways (12). To identify altered biological pathways, we performed GSEA using GeneTrail (33). GSEA considers all expressed genes by rank without a fold-change threshold and thus may identify altered pathways in which no individual members are

altered by 1.5-fold. We focused primarily on the full dataset but, for comparability with previous studies, also performed analyses using only European American samples. In muscle, GSEA of all samples showed downregulation of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for arginine and proline metabolism, calcium signaling, MAPK and VEGF signaling, inositol phosphate metabolism, and PPAR signaling in the insulin-resistant group, whereas gene ontology classes were remarkable for downregulation of lipid and fatty acid metabolism, carboxylic acid and oxoacid metabolism, regulation of oxidoreductase activity, insulin receptor signaling, and glucose/hexose/monosaccharide transport (Supplementary Table 9). European American samples were similar but showed no prominent role for KEGG pathways or gene ontology terms related to oxidative phosphorylation (data not shown). Unexpectedly, categories related to ubiquitin-protein ligase, ubiquitin cycles, and apoptosis were prominent.

GSEA in subcutaneous adipose identified primary upregulated KEGG pathways for complement and complement cascades, antigen processing and presentation, and natural killer cell–mediated cytotoxicity; top-ranked gene ontology terms likewise supported upregulation of inflammatory pathways related to chemokines, antigen processing, immune response, and inflammatory response in the insulin-resistant group (Supplementary Table 10). Prominent downregulated KEGG pathways in insulin-resistant individuals included propanoate and butanoate metabolism, valine/leucine/isoleucine degradation, tricarboxylic acid cycle, fatty acid metabolism, steroid biosynthesis, insulin signaling pathway, and PPAR signaling pathway. Gene ontology terms provided a similar picture, with terms related to lipid, fatty acid and steroid metabolism all downregulated (Supplementary Table 10). Significant enrichment of the PPAR signaling pathway among genes downregulated in insulin-resistant individuals was consistent with the downregulation of several lipid and carbohydrate metabolism genes including glycogen synthase 2 (GYS2) in adipose of insulin-resistant subjects. GYS-2 is the rate-limiting enzyme in the storage of glycogen in both liver and adipose tissue and is regulated via a PPAR response element present in the first intron (34). Overall, conclusions were similar in the European American subset, although more categories including adipocytokine signaling and transforming growth factor β signaling pathway were present (Supplementary Table 11).

Transcripts differentially expressed in adipose and muscle. A unique aspect of our study was the availability of both adipose and muscle. Transcripts concordantly regulated in adipose and muscle may provide a stronger indication of underlying pathophysiology. Considering only ≥1.5-fold differentially expressed genes in all individuals, only FADS1 shows downregulation in both adipose and muscle of insulin-resistant individuals. We thus tested all samples for concordantly regulated transcripts with at least a 1.25-fold change, a q value <10%, and a nominally significant P value in both tissues (Supplementary Table 12). We identified 14 genes concordantly increased in insulin-resistant subjects, including desert hedgehog homolog (DHH), serpin peptidase inhibitor SERPINA5, calcium binding proteins S100A4 and S100A6, kinensin family member 1B, and activating transcription factor 5. Among 55 genes with reduced expression, in insulin-resistant samples were cell cycle protein CAPRIN1, endoplasmic reticulum stress–related protein SERP1, angiotensin II receptor (AGTR1), STEAP4, protein tyrosine phosphatase PTPN3,

TABLE 3
Insulin-resistant compared with insulin-sensitive transcript patterns in muscle

Entrez ID	Gene symbol	Fold change (insulin-resistant/insulin-sensitive)		q value (%)		P value (t test)		Cytoband	Gene name
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		
23553	HYAL4	2.67		1.04		1.12E-03		7q31.32	Hyaluronoglucosaminidase 4
5997	RGS2	1.61		3.92		1.76E-02		1q31.2	Regulator of G protein signaling 2, 24 kDa
54979	HRASLS2	1.59		0.92		3.57E-04		11q12.3	HRAS-like suppressor 2
83450	LRRC48	0.67		2.88		1.23E-02		17p11.2	Leucine-rich repeat containing 48
10891	PPARGC1A	0.66	0.91	0.88	35.21	3.91E-04	0.21	4p15.2	PGC1α
5625	PRODH	0.66		1.91		3.70E-03		22q11.21	Proline dehydrogenase (oxidase) 1
6422	SFRP1	0.63	0.72	3.92	7.75	6.46E-03	1.78E-02	8p11.21	Secreted frizzled-related Protein 1
3992	FADS1	0.63	0.83	0.61	26.58	7.46E-04	1.12E-01	11q12.2	Fatty acid desaturase 1
3898	LAD1	0.57		3.92		9.01E-03		1q32.1	Ladinin 1
6768	ST14	0.54		3.19		4.38E-03		11q24.3	Suppression of tumorigenicity 14

Transcripts are listed for the analysis of all samples (European and African American) after removing loci of unknown function and pseudogenes. Fold change is the ratio of mean expression for insulin-resistant samples compared with insulin-sensitive samples. Minimum and maximum values for genes with multiple expressed probes in the array are shown. Genes with at least one probe showing 1.5-fold differential expression (at q value ≤5%, t test P value ≤0.05, and all probes in same direction of differential expression are shown.

TABLE 4
Insulin-resistant compared with insulin-sensitive transcript patterns in adipose tissue

Entrez gene ID	Gene symbol	Fold change (insulin-resistant/ insulin-sensitive)		q value (%)		P value (t test)		Cytoband	Gene name
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		
Fatty acid and sterol metabolism									
10400	PEMT		1.93	0.30		4.79E-05		17p11.2	phosphatidylethanolamine N-methyltransferase
1645	AKR1C1	1.13	1.57	0.87	13.94	9.97E-04	4.80E-02	10p15.1	aldo-keto reductase family 1, member C1
6307	SC4MOL		0.66		0.15		7.32E-03	4q32.3	sterol-C4-methyl oxidase-like
57678	GPAM		0.65		0.71		6.66E-03	10q25.2	glycerol-3-phosphate acyltransferase, mitochondrial
3992	FADS1	0.69	0.60	0.71	11.33	2.95E-02	1.78E-01	11q12.2	fatty acid desaturase 1
2194	FASN		0.59		0.87		4.76E-02	17q25.3	fatty acid synthase
60481	ELOVL5	0.83	0.59	0.00	4.55	1.02E-04	1.07E-01	6p12.1	ELOVL family member 5, elongation of long chain fatty acids
51144	HSD17B12	0.79	0.49	0.00	0.87	1.72E-04	5.62E-03	11p11.2	hydroxysteroid (17-β) dehydrogenase 12
31	ACACA		0.46		0.00		1.21E-02	17q12	acetyl-CoA carboxylase α
38	APOB		0.60		0.15		3.29E-02	2p24.1	apolipoprotein B
Carbohydrate metabolism									
6515	SLC2A3	0.87	0.66	0.15	40.39	1.58E-03	2.06E-01	12p13.31	solute carrier family 2 (facilitated glucose transporter),member 3
3099	HK2	0.98	0.66	0.00	56.34	1.40E-05	8.02E-01	2p13.1	hexokinase 2
3632	INPP5A	0.85	0.67	0.00	0.41	2.47E-08	1.95E-03	10q26.3	inositol polyphosphate-5-phosphatase, 40kDa
5105	PCK1	0.62	0.57	0.00	0.00	2.28E-04	1.58E-03	20q13.31	phosphoenolpyruvate carboxykinase 1
5209	PFKFB3	0.84	0.60	0.09	15.30	4.88E-04	1.52E-01	10p15.1	6-phosphofructo-2-kinase
5573	PRKAR1A	0.80	0.60	0.00	0.00	1.21E-08	8.29E-04	17q24.2	protein kinase, cAMP-dependent, regulatory, type I, α
6517	SLC2A4	0.60	0.59	0.00	0.00	1.93E-04	1.97E-03	17p13.1	solute carrier family 2 (facilitated glucose transporter), member 4
3633	INPP5B	0.82	0.57	0.00	0.20	6.25E-06	7.68E-04	1p34.3	inositol polyphosphate-5-phosphatase, 75kDa
2998	GYS2		0.56		0.09		4.80E-02	12p12.1	glycogen synthase 2
5224	PGAM2		0.52		3.76		2.12E-02	7p13	phosphoglycerate mutase 2
Cell-cycle and cell adhesion									
894	CCND2	1.14	1.76	0.20	9.18	1.07E-05	4.02E-01	12p13.32	cyclin D2
9133	CCNB2	1.13	1.60	1.30	25.84	3.73E-02	2.19E-01	15q22.2	cyclin B2
1027	CDKN1B	0.86	0.67	0.00	25.84	1.08E-04	1.72E-02	12p13.1	cyclin-dependent kinase inhibitor 1B
4076	CAPRIN1	0.94	0.66	0.00	15.30	2.13E-07	1.24E-01	11p13	cell cycle associated protein 1
1010	CDH12		2.22	0.41		4.23E-04		5p14.3	cadherin 12, type 2
10752	CHL1	0.72	0.65	0.15	1.54	4.22E-04	5.97E-03	3p26.3	cell adhesion molecule with homology to L1CAM
1004	CDH6	0.95	0.48	0.00	36.23	2.23E-04	6.34E-01	5p13.3	cadherin 6, type 2, K-cadherin
Angiogenesis									
84432	PROK1		2.29	0.53		6.59E-04		1p13.3	prokineticin 1
64102	TNMD		1.84	1.54		3.21E-03		Xq22.1	tenomodulin
25975	EGFL6		2.16	1.30		7.75E-03		Xp22.2	EGF-like-domain, multiple 6
7422	VEGFA	0.67	0.65	0.00	0.00	5.39E-05	3.35E-04	6p21.1	vascular endothelial growth factor A
2321	FLT1	0.88	0.63	0.00	31.92	4.01E-07	1.36E-01	13q12.2	fms-related tyrosine kinase 1
10085	EDIL3		0.59		0.00		2.06E-03	5q14.3	EGF-like repeats and discoidin I-like domains 3

TABLE 4
Continued

Entrez gene ID	Gene symbol	Fold change (insulin-resistant/ insulin-sensitive)		q value (%)		P value (t test)		Cytoband	Gene name
		Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		
Inflammation									
51330	TNFRSF12A		1.58	3.16		3.04E-03		16p13.3	tumor necrosis factor receptor superfamily, member 12A
4982	TNFRSF11B	1.36	1.50	3.76	3.76	4.53E-02	8.55E-02	8q24.12	tumor necrosis factor receptor superfamily, member 11b
7128	TNFAIP3	0.93	0.66	0.09	46.90	5.63E-04	4.79E-01	6q23.3	tumor necrosis factor, α-induced protein 3
79689	STEAP4		0.59		0.00		9.92E-06	7q21.12	STEAP family member 4
Nuclear receptors									
6258	RXRG		0.66		1.87		1.54E-02	1q23.3	retinoid X receptor, γ
157506	RDH10	0.75	0.61	0.00	0.09	4.61E-05	3.75E-04	8q21.11	retinol dehydrogenase 10 (all-trans)
10499	NCOA2		0.49		0.00		1.68E-09	8q13.3	nuclear receptor coactivator 2
Phosphatases									
11122	PTPRT		1.94	0.30		2.40E-04		20q12	protein tyrosine phosphatase, receptor type, T
84919	PPP1R15B		0.66		0.00		1.43E-05	1q32.1	protein phosphatase 1, regulatory (inhibitor) subunit 15B
7803	PTP4A1	0.90	0.66	0.00	36.23	6.25E-05	9.23E-02	6q12	protein tyrosine phosphatase type IVA, member 1
5774	PTPN3	0.82	0.64	0.00	16.73	6.07E-04	5.29E-02	9q31.3	protein tyrosine phosphatase, nonreceptor type 3
5534	PPP3R1	0.89	0.58	0.00	25.84	1.69E-06	1.64E-01	2p14	protein phosphatase 3 (formerly 2B), regulatory subunit B, α isoform
Miscellaneous									
185	AGTR1	0.71	0.66	0.00	0.00	2.57E-07	1.17E-06	3q24	angiotensin II receptor, type 1
28999	KLF15	0.66	0.63	0.09	0.15	3.20E-04	8.77E-04	3q21.2	Kruppel-like factor 15
827	CAPN6		0.59		1.09	1.63E-03		Xq22.3	calpain 6
655	BMP7	0.74	0.54	0.09	3.76	4.50E-04	1.59E-02	20q13.31	bone morphogenetic protein 7
6271	S100A1		0.46		0.09		5.06E-03	1q21.3	S100 calcium binding protein A1

Selected genes from comparison of insulin-sensitive to insulin-resistant subcutaneous adipose from all individuals studied. Fold change is the ratio of mean expression for insulin-resistant samples compared with insulin-sensitive samples. Minimum and maximum values for genes with multiple expressed probes in the array are shown. Genes with at least one probe showing 1.5-fold differential expression (at q value ≤5%), t test P value ≤0.05, and all probes in same direction of differential expression are shown.

transcription factor *KLF15*, drosophila period homolog *PER3*, MAPK *MAPK14*, and possible vesicle transport protein archain 1 (*ARCN1*). Also among the genes concordantly downregulated in insulin-resistant subjects were fatty acid desaturase (*FADS1*), *VEGFA*, and *CDKAL1*, all of which were implicated by association of nearby SNPs with type 2 diabetes (35) or fasting glucose (23).

Replication of expression signals in adipose. We selected 17 transcripts to test using real-time PCR compared with 18S RNA. Most transcripts were selected for a known role in glucose homeostasis or adipocyte growth, but we included *S100A1*, which has no known role. Primers were designed as close to array primers as possible. Adequate cDNA was not available for four European American and two African American samples. Nonetheless, 12 of 17 transcripts showed significant differences either in all samples or the European American or African American subset. Another three of 17 transcripts demonstrated a trend of significant association (INPPA, $P = 0.09$; ACACA, $P = 0.09$; and VEGFA, $P = 0.08$) in the European American subset. The direction of differential expression was the same as the array with all but *S100A1* (Supplementary Table 13). In general, the ratios from real-time PCR were less than those observed in the arrays. Among the reasons for the more modest observations with RT-PCR were difficulty in matching array probe locations and likely splice variants and use of different normalization standards. Nonetheless, most findings served to validate array signals.

DISCUSSION

Obesity is highly correlated with insulin resistance, but both insulin-resistant lean individuals and metabolically healthy obese individuals are well recognized. A recent study (16) also showed a high correlation between expressions of a large number of transcripts in subcutaneous adipose with BMI. Thus, in most published studies the search for insulin sensitivity–mediated changes in adipose tissue gene expression was confounded by BMI. In addition, many published investigations compared subjects with overt type 2 diabetes, where gene expression may have been altered by hyperglycemia, to normal control subjects. Gene expression in some previous studies was influenced by obtaining muscle biopsies during the hyperinsulinemic state at the end of a 2-h euglycemic clamp (12). To examine the role of adipose and muscle gene expression while minimizing the confounders, we matched nondiabetic individuals who were at extremes of insulin sensitivity for BMI and obesity measures and obtained both adipose and muscle biopsies during a fasting state. Additional unique features of the current study were the inclusion of participants of both European and African ancestry, the inclusion of both adipose and muscle tissue from the same individuals, and the relatively large sample size of our study compared with many earlier studies (12,13,36). These features, along with different arrays and probe sets, likely explain the differences between the current study and other reports.

Several conclusions are evident from our study. First, considerably more transcripts differ with S_I in adipose than in muscle tissue, which may suggest a more important role for altered adipocyte transcription in impaired insulin action. Second, although only a small number of transcripts differed significantly between European American and African American participants, many of these transcripts were in pathways likely to contribute to metabolic

differences including phospholipases, immune factors, and fatty acid and ceramide metabolism. Using stringent criteria, only six genes with putative metabolic importance (*CLIC6*, *HSD11B1*, *SERPINA3*, *THBS1*, *TMEM135*, and *TNMD*) showed ethnic difference as well as differential expression in adipose of insulin-resistant individuals. However, using less stringent criteria (single-point $P < 0.05$ for African Americans/European Americans, irrespective of q value), nearly 20% of adipocyte transcripts that were associated with S_I in European American participants also differed between European American and African American samples. A recent study of lymphocytes suggested that levels of key glucose homeostasis transcripts downstream of SREBP1 differed between European American and African American individuals (37). These expression differences may reflect phenotypic differences in insulin action, secretion, and clearance between European American and African American individuals (21,22). Although the African American subset in our study was of a size comparable to previous studies of global transcript levels, a larger study comparing expression patterns in African American and European American individuals matched for obesity measures is needed to determine whether apparent transcript differences result from a combination of small sample size and additional variability and heterogeneity in the African American subset. For example, we observed more differentially expressed transcripts in the smaller European American population than in the full sample set. This may be a result of a type I statistical error; however, an alternative explanation for this paradox is that African American samples introduced heterogeneity and increased the variance, and, thus, fewer transcripts reached significance. In our analysis, comparison between all insulin-resistant and insulin-sensitive individuals were performed after considering African American and European American individuals as separate permutation groups to minimize that error. Differences reported in European American insulin-resistant groups compared with European American insulin-sensitive groups could also be explained by increased visceral fat (indicated by WHR) in European American insulin-resistant groups.

Previous studies in muscle have argued for a key role of oxidative phosphorylation (OXPHOS) (12,13). We also found that *PPARGC1A* (PGC1α) was reduced in insulin-resistant muscle, but of 17 OXPHOS genes that were reported previously, we found nominal evidence ($P < 0.05$) of differential expression in only four genes (*NDUFB5*, *SDHD*, *UCRC*, and *NDUFU2*), and for all of these, gene expression increased ~1.2-fold in insulin-resistant muscle, arguing against decreased mitochondrial oxidation in insulin resistance. This conclusion was supported by GSEAs, which failed to identify either KEGG pathways or gene ontology terms related to oxidative phosphorylation. Instead, in addition to PPAR signaling, we found downregulation of pathways for arginine and proline metabolism, lipid and fatty acid metabolism, and hexose transport. Novel findings included differential expression of genes in categories of ubiquitin-protein ligase and apoptosis.

In addition to inflammatory markers, a large number of genes in adipose are reported to be associated with insulin resistance in humans. In agreement with our previous studies (9), we found no evidence for clustering with insulin sensitivity for 240 transcripts related to endoplasmic reticulum stress. We reviewed the literature and identified 104 transcripts not related to endoplasmic reticulum stress that were reported as associated with insulin resistance

(11,38–46). We searched for evidence of an association in the same direction as previously reported and found support for 50 of these transcripts, many falling into pathways identified in our global analysis. In the analysis of all subjects, genes involved in inflammation were missing from the list of 1.5-fold differentially expressed genes between the adipose of insulin-resistant and insulin-sensitive subjects. However, GSEA of all subject data and analysis of the European American subset indicated the differential expression of genes in inflammatory pathways. Inflammation was supported with upregulation of *CCL2*, *CCL3*, *CD68*, *IL6*, *IL6R*, *MSR1*, *RETN*, *SPP1*, and *TNF*. This result is consistent with a recent finding from Hyatt et al., which showed significantly lower inflammation in African American individuals compared with BMI-matched European American individuals (47). Thus, our study, along with published studies, indicates an important ethnic difference in the pathophysiology of insulin resistance (34). The recently described (48) gene *SPARC* (osteonectin) was modestly increased with insulin resistance (1.2-fold, *q* value <5%). In contrast to our findings in muscle, we found changes in oxidative phosphorylation, with genes *NDUFS1*, *NDUFB5*, and *NRF1* modestly (1.2-fold) downregulated with insulin resistance. Transcription factors *PPARG*, *PPARA*, *CEBPA*, and *CEBPB* were all modestly downregulated, as were insulin signaling genes *SLC2A4* (GLUT4), *IRS1*, *MAP4K4*, and *HK2*. However, as we noted above, alterations in lipid metabolic genes in adipose tissue were most prominent with the downregulation of *SCD* (49), *FADS1*, and *FASN* with insulin resistance (50). In addition to *PPARA*, we found downregulation of *LIPIN1* in adipose tissue of insulin-resistant compared with insulin-sensitive subjects (51). Finally, in contrast to published findings (52), we found that *APP* was 1.2-fold downregulated rather than upregulated with insulin resistance. Thus, we suggest that many proposed associations were the result of obesity rather than primarily associated with insulin resistance.

By testing adipose and muscle in the same individuals at extremes of insulin sensitivity, we were able to identify pathways unique to each tissue but also similar mechanisms in adipose and muscle. We identified 69 genes with concordant expression in insulin-resistant muscle and adipose, of which only 14 genes were increased with insulin resistance. Although many of the 69 concordantly regulated transcripts have no obvious roles in adipose or muscle, genes associated with cell-cycle progression and differentiation are well represented. Concordantly upregulated genes *S100A4* and *S100A6* encode calcium binding proteins that are involved in cell-cycle progression and cell differentiation, *ATF5* interacts with cell cycle genes, and downregulated gene *PPFIBP1* interacts with S100A4. Other concordantly downregulated genes with cell-cycle roles include *CDKAL1*. Interestingly, both *S100A4* and *S100A6* reside near the well-replicated 1q21 region of type 2 diabetes linkage, whereas *CDKAL1* is a well replicated type 2 diabetes gene based on genome-wide association scans (53). Several additional genes are involved in translation initiation, including helicase gene *DDX42*, initiation factor *EIF4B*, and *CAPRIN1*. Also among the concordantly regulated genes were four histone members (increased in insulin resistance) and the chromatin binding factor, transcriptional regulator, and part of the histone deacetylase complex *CTCF*. These findings suggest a prominent role for chromatin modification, translation initiation, and cell-cycle regulation in insulin resistance.

A number of coordinately regulated genes are involved in cellular stress responses, inflammatory pathways, and unfolded protein response including *BCL2A1*, *CEBPB*, *MAPK14*, *SERP1*, *SMG1*, *STEAP4*, and *VCP*. It was surprising that these genes are concordantly expressed in muscle, but it is consistent with a recent description (54) of increased macrophages in insulin resistant muscle. Finally, fatty acid metabolism was prominent in both tissues, with reduced expression in insulin-resistant individuals for *ACAD10*, *FADS1*, and *CYP4V2*. Other interesting concordantly downregulated transcripts include *PER3*, a gene associated with circadian rhythm but of unknown function in adipose and muscle; *AGTR1* encoding the angiotensin 2 receptor; and genes *KLF15* and *CEBPB*, encoding transcription factors primarily known for adipose function. Like *CDKAL1*, genes *VEGFA* and *FADS1* are near well-replicated SNPs associated with type 2 diabetes or fasting glucose (23,35).

The current study included a larger sample than most previous studies, included a biethnic population, and, as noted above, provided the opportunity to compare adipose and muscle in individuals matched for BMI but were discordant for insulin sensitivity. Nonetheless, this cross-sectional study has some limitations. Although larger than most earlier reports, the design of this study was inevitably of limited size relative to studies focused on mapping genetic etiology. Although inclusion of an African American population provided a unique opportunity to define differences among ethnic groups, this population may have added heterogeneity. A cross-sectional study cannot define cause and effect; thus, in common with previous studies we cannot exclude the possibility that some of the differences in expression were the result of impaired insulin action rather than the cause. Insulin signaling affects many downstream networks. In this study, the observed direction of changes of differentially expressed genes in carbohydrate metabolism, cell-cycle regulation, lipid metabolism, and arginine-proline metabolism are consistent with the known functional effect of reduced insulin signaling in insulin-resistant individuals. However, some other differences between insulin-resistant and insulin-sensitive subjects, including the increase in inflammation and reduction in angiogenesis, are less readily explained by altered insulin signaling.

In summary, by comparing BMI-matched individuals at extremes of insulin sensitivity, we show that expression changes are more prominent in adipose than muscle, confirming earlier and smaller array studies of muscle, but we confirm the role for *PPARGC1A* (encoding PGC1) in muscle. Although we indeed found the expected role for inflammation in adipose, the most highly differentially expressed genes suggest a prominent role for pathways involved in fatty acid metabolism and cell-cycle regulation in both adipose and muscle. Although significant differences observed between African American and European American individuals did not overlap with the significant differences observed between insulin-resistant and insulin-sensitive individuals, ethnic differences between metabolically important genes were prominent and deserve further study.

REFERENCES

1. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 2008;93(Suppl. 1):S64–S73
2. Utzschneider KM, Van de Lagemaat A, Faulenbach MV, et al. Insulin resistance is the best predictor of the metabolic syndrome in subjects with a first-degree relative with type 2 diabetes. Obesity (Silver Spring) 2010;18:1781–1787
3. Blüher M. The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. Curr Opin Lipidol 2010;21:38–43
4. Hollenbeck C, Reaven GM. Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. J Clin Endocrinol Metab 1987;64:1169–1173
5. Abdul-Ghani MA, DeFronzo RA. Mitochondrial dysfunction, insulin resistance, and type 2 diabetes mellitus. Curr Diab Rep 2008;8:173–178
6. Morino K, Petersen KF, Dufour S, et al. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. J Clin Invest 2005;115:3587–3593
7. Muoio DM, Koves TR. Skeletal muscle adaptation to fatty acid depends on coordinated actions of the PPARs and PGC1 alpha: implications for metabolic disease. Appl Physiol Nutr Metab 2007;32:874–883
8. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–948
9. Sharma NK, Das SK, Mondal AK, et al. Endoplasmic reticulum stress markers are associated with obesity in nondiabetic subjects. J Clin Endocrinol Metab 2008;93:4532–4541
10. Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). Public Health Nutr 2007;10(10A):1132–1137
11. Yang X, Jansson PA, Nagaev I, et al. Evidence of impaired adipogenesis in insulin resistance. Biochem Biophys Res Commun 2004;317:1045–1051
12. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34:267–273
13. Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003;100:8466–8471
14. Frederiksen CM, Højlund K, Hansen L, et al. Transcriptional profiling of myotubes from patients with type 2 diabetes: no evidence for a primary defect in oxidative phosphorylation genes. Diabetologia 2008;51:2068–2077
15. Skov V, Glintborg D, Knudsen S, et al. Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. Diabetes 2007;56:2349–2355
16. Emilsson V, Thorleifsson G, Zhang B, et al. Genetics of gene expression and its effect on disease. Nature 2008;452:423–428
17. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 2001;98:5116–5121
18. Huang da W, Sherman BT, Tan Q, et al. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007;8:R183
19. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57
20. Keller A, Backes C, Al Awadhi M, et al. GeneTrailExpress: a web-based pipeline for the statistical evaluation of microarray experiments. BMC Bioinformatics 2008;9:552
21. Rasouli N, Spencer HJ, Rashidi AA, Elbein SC. Impact of family history of diabetes and ethnicity on cell function in obese, glucose-tolerant individuals. J Clin Endocrinol Metab 2007;92:4656–4663
22. Smith LM, Yao-Borengasser A, Starks T, Tripputi M, Kern PA, Rasouli N. Insulin resistance in African-American and Caucasian women: differences in lipotoxicity, adipokines, and gene expression in adipose tissue and muscle. J Clin Endocrinol Metab 2010;95:4441–4448
23. Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105–116
24. Mootha VK, Handschin C, Arlow D, et al. Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. Proc Natl Acad Sci USA 2004;101:6570–6575
25. Crunkhorn S, Dearie F, Mantzoros C, et al. Peroxisome proliferator activator receptor gamma coactivator-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 mitogen-activated protein kinase activation. J Biol Chem 2007;282:15439–15450
26. Illig T, Gieger C, Zhai G, et al. A genome-wide perspective of genetic variation in human metabolism. Nat Genet 2010;42:137–141
27. Arner P, Stenson BM, Dungner E, et al. Expression of six transmembrane protein of prostate 2 in human adipose tissue associates with adiposity and insulin resistance. J Clin Endocrinol Metab 2008;93:2249–2254
28. Sue N, Jack BH, Eaton SA, et al. Targeted disruption of the basic Krüppel-like factor gene (Klf3) reveals a role in adipogenesis. Mol Cell Biol 2008;28:3967–3978
29. Tseng YH, Kokkotou E, Schulz TJ, et al. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 2008;454:1000–1004
30. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307–312
31. Varma V, Yao-Borengasser A, Bodles AM, et al. Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. Diabetes 2008;57:432–439
32. Rung J, Cauchi S, Albrechtsen A, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet 2009;41:1110–1115
33. Backes C, Keller A, Kuentzer J, et al. GeneTrail: advanced gene set enrichment analysis. Nucleic Acids Res 2007;35:W186–W192
34. Mandard S, Stienstra R, Escher P, et al. Glycogen synthase 2 is a novel target gene of peroxisome proliferator-activated receptors. Cell Mol Life Sci 2007;64:1145–1157
35. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638–645
36. MacLaren R, Cui W, Simard S, Cianflone K. Influence of obesity and insulin sensitivity on insulin signaling genes in human omental and subcutaneous adipose tissue. J Lipid Res 2008;49:308–323
37. Schisler JC, Charles PC, Parker JS, et al. Stable patterns of gene expression regulating carbohydrate metabolism determined by geographic ancestry. PLoS ONE 2009;4:e8183
38. Sears DD, Hsiao G, Hsiao A, et al. Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. Proc Natl Acad Sci USA 2009;106:18745–18750
39. Parikh H, Carlsson E, Chutkow WA, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med 2007;4:e158
40. Peter A, Weigert C, Staiger H, et al. Individual stearoyl-coa desaturase 1 expression modulates endoplasmic reticulum stress and inflammation in human myotubes and is associated with skeletal muscle lipid storage and insulin sensitivity in vivo. Diabetes 2009;58:1757–1765
41. Palsgaard J, Brøns C, Friedrichsen M, et al. Gene expression in skeletal muscle biopsies from people with type 2 diabetes and relatives: differential regulation of insulin signaling pathways. PLoS ONE 2009;4:e6575
42. Reyna SM, Ghosh S, Tantiwong P, et al. Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. Diabetes 2008;57:2595–2602
43. Lee YH, Tharp WG, Maple RL, Nair S, Permana PA, Pratley RE. Amyloid precursor protein expression is upregulated in adipocytes in obesity. Obesity (Silver Spring) 2008;16:1493–1500

44. Emanuelli B, Eberlé D, Suzuki R, Kahn CR. Overexpression of the dual-specificity phosphatase MKP-4/DUSP-9 protects against stress-induced insulin resistance. Proc Natl Acad Sci USA 2008;105:3545–3550

45. Monroy A, Kamath S, Chavez AO, et al. Impaired regulation of the TNF-alpha converting enzyme/tissue inhibitor of metalloproteinase 3 proteolytic system in skeletal muscle of obese type 2 diabetic patients: a new mechanism of insulin resistance in humans. Diabetologia 2009;52:2169–2181

46. Padmalayam I, Hasham S, Saxena U, Pillarisetti S. Lipoic acid synthase (LASY): a novel role in inflammation, mitochondrial function, and insulin resistance. Diabetes 2009;58:600–608

47. Hyatt TC, Phadke RP, Hunter GR, Bush NC, Muñoz AJ, Gower BA. Insulin sensitivity in African-American and white women: association with inflammation. Obesity (Silver Spring) 2009;17:276–282

48. Kos K, Wong S, Tan B, et al. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. Diabetes 2009;58:1780–1788

49. Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol 2008;19:248–256

50. Sjögren P, Sierra-Johnson J, Gertow K, et al. Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance. Diabetologia 2008;51:328–335

51. Yao-Borengasser A, Rasouli N, Varma V, et al. Lipin expression is attenuated in adipose tissue of insulin-resistant human subjects and increases with peroxisome proliferator-activated receptor gamma activation. Diabetes 2006;55:2811–2818

52. Lee YH, Martin JM, Maple RL, Tharp WG, Pratley RE. Plasma amyloid-beta peptide levels correlate with adipocyte amyloid precursor protein gene expression in obese individuals. Neuroendocrinology 2009;90:383–390

53. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007;39:770–775

54. Varma V, Yao-Borengasser A, Rasouli N, et al. Muscle inflammatory response and insulin resistance: synergistic interaction between macrophages and fatty acids leads to impaired insulin action. Am J Physiol Endocrinol Metab 2009;296:E1300–E1310