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

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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 nondiabetic 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 downregulated. 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**

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Received 9 September 2010 and accepted 10 December 2010.

DOI: 10.2337/db10-1270

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1270/-/DC1.

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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 ($AIR_G \times S_I$) 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/m^2)	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	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)
AIR_G (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)	2,468 (2,293)	1,216 (921)[2]	1,451 (1,329)	2,569 (2,394)
S_I ($\times 10^{-4}$ 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 overrepresentation 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

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*	0.91	0.66	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*	0.72	0.63	3.92	7.75	6.46E-03	1.78E-02	8p11.21	Secreted frizzled-related Protein 1
3992	*FADS1*	0.83	0.63	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.

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 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

Continued on facing page

TABLE 4
Continued

Entrez gene ID	Gene symbol	Fold change (insulin-resistant/ insulin-sensitive) Minimum	Fold change (insulin-resistant/ insulin-sensitive) Maximum	q value (%) Minimum	q value (%) Maximum	P value (t test) Minimum	P value (t test) Maximum	Cytoband	Gene name
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.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-039311 (to S.C.E. and S.K.D.) and DK-071349 and DK-080327 (to P.A.K.), a Veterans Administration Merit Grant (to N.R.), and a generous grant from the Sturgis Foundation to the University of Arkansas for Medical

Sciences (UAMS) and the Research Service of the Department of Veterans Affairs. The project was supported in part by award no. 1UL1RR029884 to the UAMS from the National Center for Research Resources.

No potential conflicts of interest relevant to this article were reported.

S.C.E. designed the project, performed human studies, led the analysis of data, and wrote the manuscript. P.A.K. designed the project, performed human studies, analyzed data, and reviewed and edited the manuscript. N.R. designed the project, performed human studies, analyzed data, and reviewed and edited the manuscript. A.Y.-B. and N.K.S. contributed data and reviewed the manuscript. S.K.D. designed the project, researched and analyzed data, and wrote, reviewed, and edited the manuscript.

The authors thank the dedicated staff of the clinical research center at the UAMS for support of the clinical studies and assistance with data management.

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