Supporting Information

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SI Text

Human Subject Studies. The pioglitazone study included eight NR_d subjects (age 42 ± 5 [SE] yr, BMI 23.1 ± 0.6 [SE] kg/m2) and 17 IRd subjects (age 51 ± 2 yr, BMI 36.4 ± 1.5 kg/m2). The rosiglitazone study included 19 IR_d subjects (age 52 ± 2 yr, BMI 35.6 ± 1.4 kg/m2). The troglitazone study included 11 NR_d subjects (age 48 ± 2 yr, BMI 25.8 ± 1.2 kg/m2) and 17 IR_d subjects (age 50 ± 2 yr, BMI 34.4 ± 1.3 kg/m2). At baseline, each subject underwent a 5 hr 60–80 mU/m2/min hyperinsulinemic-euglycemic clamp (23, 29). After completing the baseline studies, all subjects were treated for 12 wk with pioglitazone (45 mg daily), rosiglitazone (4 mg twice daily), or troglitazone (600 mg daily). Needle biopsies of abdominal subcutaneous adipose tissue were taken in the basal state, before each clamp. Needle biopsies of vastus lateralis skeletal muscle were taken in the basal state (before each clamp) and the insulin-stimulated state (immediately before termination of each hyperinsulinemic-euglycemic clamp). Biopsies were flash-frozen in liquid nitrogen and stored at −80 °C. A schematic of the biopsy, clamp, and TZD treatment study design is shown in Fig. 1*A*. The experimental protocol was approved by the Institutional Review Board for Human Subjects of the University of California, San Diego. The methods used for hyperinsulinemic-euglycemic clamps were performed as described previously (29–32). All subjects were volunteers, generally healthy with normal liver and kidney function. Exclusion criteria were active cardiac, liver, or renal disease or long-term complications from diabetes. None of the nondiabetic subjects were taking medications that alter glucose tolerance. Diabetic subjects taking anti-diabetes medications (sulfonylurea or metformin) were asked to discontinue their medications for at least 2 weeks before initiating their baseline studies. None were previously receiving TZD therapy. All subjects received nutrition counseling regarding diet regulation during the studies.

Hyperinsulinemic-Euglycemic Clamps. Baseline blood samples were drawn and hyperinsulinemic-euglycemic clamps were performed in the morning after a 10 h overnight fast, as described in refs. 29 and 30. The rates of total glucose appearance and disposal (R_d) were calculated from the tracer [3-^{3}H]glucose data using the non-steady-state equations of Steele (31). A distribution volume of 0.19 L/kg and pool fraction of 0.5 were used in the calculations (32). Exogenous glucose infusion (Ginf) rates were used as an approximation for R_d for 10 subjects (5 NR_d and 5 IR_d) because a defective tracer batch prevented calculation of R_d. Ginf rates are approximately equal to R_d when >6 mg/kg per min (8 of these 10 subjects) and slightly over estimate R_d when very low (2 of these 10 subjects). Plasma FFA levels were determined using an acyl-CoA oxidase-based colorimetric kit (NEFA-C) with intra- and inter-assay coefficients of variation (CVs) of 2.4 and 3.3%, respectively. Plasma insulin was measured by a double-antibody technique (33). The intra- and inter-assay CVs were 3.7 and 9.2%, respectively.

Microarray Studies. RNA from 364 adipose tissue and skeletal muscle biopsies was isolated and gene expression patterns in each sample were determined using Affymetix Human Genome U133 Plus 2.0 oligonucleotide microarrays (Affymetrix, Inc., Santa Clara, CA) which include >47,000 transcript features. Total RNA samples were isolated and processed as recommended by Affymetrix (Affymetrix GeneChip Expression Analysis Technical Manual) from 50 to 100 mg of tissue using TRIzol Reagent (Invitrogen). RNA concentrations were adjusted to a final concentration of 1.25 μg/μL. Further RNA processing and microarray analyses were conducted at the University of California Irvine DNA & Protein MicroArray Facility. RNA samples were quality assessed before beginning target preparation/processing steps by running out a small amount of each sample (typically 25–250 ng per well) onto a RNA Lab-On-A-Chip (Caliper Technologies) that was evaluated on an Agilent Bioanalyzer 2100 (Agilent Technologies). Single-stranded, then double-stranded cDNA was synthesized from the poly(A)$^+$ mRNA present in the isolated total RNA (typically 10 μg total RNA starting material each sample reaction) using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen) and poly (T)-nucleotide primers that contained a sequence recognized by T7 RNA polymerase. A portion of the resulting ds cDNA was used as a template to generate biotin-tagged cRNA from an in vitro transcription reaction (IVT), using the Affymetrix GeneChip IVT Labeling Kit. 15 μg of biotin-tagged cRNA was fragmented (average length 100 bases, range 35–200 bases) following prescribed protocols (Affymetrix GeneChip Expression Analysis Technical Manual). 10 μg of fragmented target cRNA was hybridized (45 °C with rotation for 16 h, Affymetrix GeneChip Hybridization Oven 640) to probe sets present on an Affymetrix Human Genome U133 Plus 2.0 array. The GeneChip arrays were washed and stained (SAPE, streptavidin-phycoerythrin) on an Affymetrix Fluidics Station 450, then scanned on a GeneChip Scanner 3000. Hybridization results were quantified and analyzed using GCOS 1.2 software (Affymetrix) using default values (Scaling, Target Signal Intensity = 500; Normalization, All Probe Sets; Parameters, all set at default values). Gene expression levels, expressed as average difference scores, were determined using Affymetrix MAS 5.0 software.Semi-quantitative RT-PCR evaluation of several differentially expressed genes correlated with validated microarray expression data (r_{avg} = 8.83, r_{range} = 0.68–0.98).

Statistical Analyses. Clinical characteristics data were analyzed using Student's *t* test (paired and unpaired, as appropriate) and a statistical cutoff of $P < 0.05$. We applied our Variance Modeled Posterior Inference with Regional Exponentials (VAMPIRE) microarray analysis web suite to the gene expression data to identify significant differences between gene expression profiles from the subject groups. We identified statistically enriched of Gene Ontology (ver. 200704) functional annotation terms in expression data sets using GOby. We used variance-balanced regression and Pearson correlation coefficients to conduct our regression analyses. Variance-balanced regression is a weighted least squares method where the weights are based on the inverse of the estimate of variance, as computed by VAMPIRE. We conducted two regression line comparisons using the F-test statistic for coincidence of two regression lines. We used the Chi-square test statistic where indicated. We corrected for family-wise error associated with multiple comparisons (accounting for the number of probe sets or genes examined) using the stringent Bonferroni corrction at a 5% significance level cutoff (α_{Bonf} = 0.05).

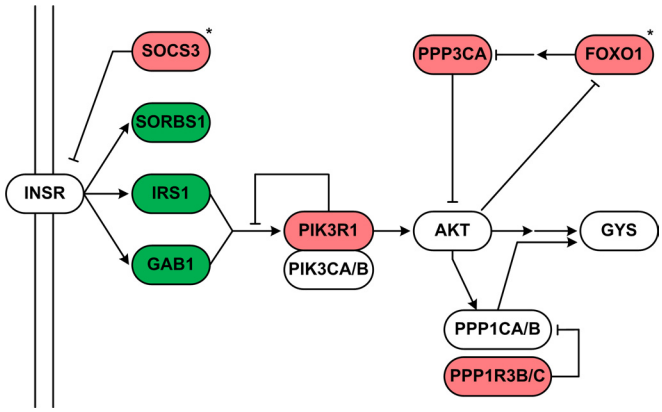

Fig. S1. Overall down-regulation of insulin signaling pathways by insulin-induced gene expression changes in skeletal muscle. An abbreviated schematic of insulin signaling is shown. In response to insulin, genes encoding multiple primary substrates of the insulin receptor are down-regulated and inhibitory signaling components up-regulated. Green ovals indicate genes that were repressed after hyperinsulinemic euglycemic clamp, red ovals indicate genes that were activated after hyperinsulinemic euglycemic clamp, white ovals indicate no insulin-induced expression change ($\alpha_{bonf} = 0.05$). Asterisks indicate insulin-induced gene expression changes that were up-regulated but significantly blunted in IR_d^{all} subjects compared with NR_d subjects ($\alpha_{bonf} = 0.05$). Expression patterns of the remaining genes were not significantly different between IR_d^{all} and NR_d subjects.

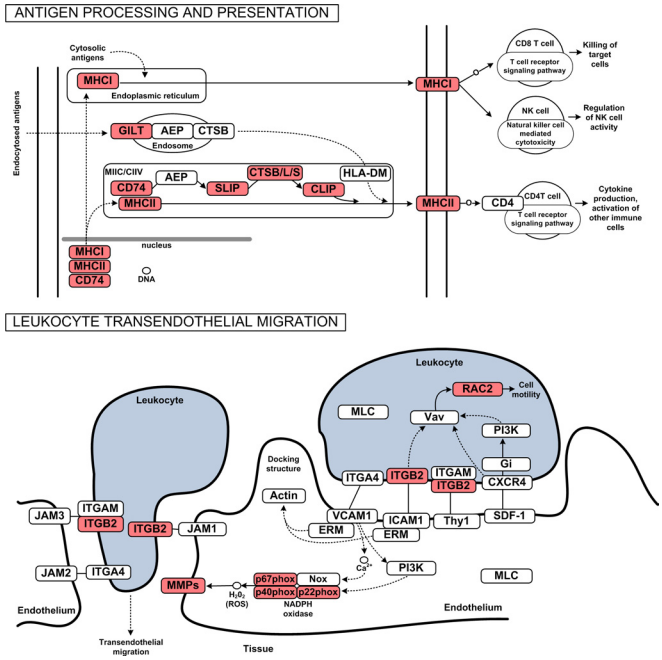

Fig. S2. Post-TZD inflammatory marker overexpression in adipose tissue of nonresponder insulin resistant (IR_d^-) subjects. Schematics of the (*a*) antigen process and presentation and (*b*) leukocyte transendothelial migration pathways highlighting genes that are still overexpressed in IR_d^- subjects after TZD treatment. White ovals indicate genes that are not differentially expressed between NR_d and IR_d^- subjects. Red ovals indicate genes significantly overexpressed in IR_d^- subjects compared with NR_d subjects ($\alpha_{bonf} = 0.05$). Schematics are adapted from KEGG.

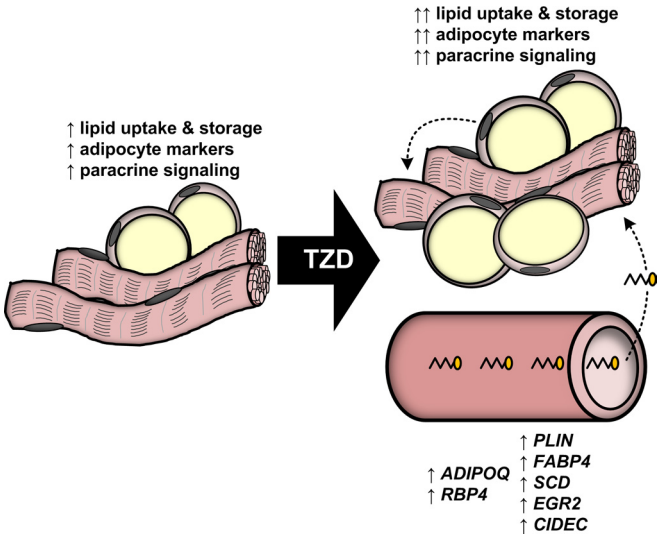

Fig. S3. A model of skeletal muscle adiposity of IR_d^+ subjects. The transcriptional signature of skeletal muscle from IR_d^+ subjects was characterized by over-expression of spcific adipocyte markers, including those involved in lipid uptake/storage and paracrine signaling which were coordinately maintained or further enhanced after TZD-mediated insulin sensitization in IR_d^+ subjects. These post-TZD changes were significantly blunted in IR_d^- subjects, suggesting that increased lipid uptake/storage and myocyte-adipocyte crosstalk are associated with TZD-mediated insulin sensitization.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)
Dataset S7 (XLS)