Supplemental Materials

Participants

Subcutaneous abdominal adipose tissues were obtained from 6 overweight individuals who participated in a cross-sectional study at the AdventHealth Translational Research Institute (Table 1). All studies were approved by the AdventHealth Institutional Review Board and written informed consent was obtained prior to any procedures.

Body composition and blood analyses

Body composition was measured by dual-energy x-ray absorptiometry (DXA) using a Lunar iDXA Whole-body Scanner (GE Healthcare Lunar, Madison, WI) (1). Fasting blood samples were analyzed in clinical chemistry laboratory at either AdventHealth or onsite at the Translational Research Institute.

Cardiorespiratory fitness

VO_2 max was determined by a cycle ergometer exercise test (1). A metabolic cart was used to measure breath-by-breath VO_2 and VCO_2 (TrueOne 2400, PARVO Medics).

Biopsies

All biopsies were performed after an overnight fast and at least 48 hours after any consumption of alcohol and caffeine and physical activity.

Adipose biopsy. Adipose tissue was obtained under local anesthesia from the subcutaneous abdominal region and immediately snap frozen in liquid nitrogen (2).

Skeletal muscle biopsy. Percutaneous muscle biopsies were obtained from the *vastus lateralis* using the Bergstrom technique (3). During the skeletal muscle biopsy procedure, intermuscular adipose tissue (IMAT) is sometimes collected with the sample. IMAT is carefully dissected from the skeletal muscle under a dissection scope and then snap frozen in liquid nitrogen.

RNA extraction and Microarray

RNA was extracted from ~50mg of adipose tissue using the Qiagen RNeasy Fibrous Tissue Mini Kit (Qiagen Inc, Valencia, CA) (1). RNA purity and quantity were determined using a Biotek Synergy 2 plate reader (BioTek Instruments, Inc. Winooski, VT). Labeling of cRNA and hybridization to Human HT-12 Expression BeadChips (Illumina, San Diego, CA) was performed by the Genomics Core at Sanford Burnham Prebys Medical Discovery Institute (La Jolla, CA).

Bioinformatic Analysis

Analysis of the microarray data were performed as previously described (Vegaaa). Briefly, the BeadChips array expression data were analyzed using a custom-built bioinformatic pipeline, including three seamless connected procedures: 1) raw data preprocessing, 2) differentially expressed gene (DEG) analysis, and 3) the assessment of exercise training remodeling effects. The raw data pre-processing follows the protocol established in beadarray, an R/Bioconductor package designed specifically for Illumine array analysis (4). Raw iDAT files from BeadScan were first read, then filtered to remove non-responding probes. Differences in expression levels across a chip and between chips were then corrected to normalize the signal. Finally, the gene expression values were log2-transformed and quantile normalized for subsequent statistical analysis. The DEG analyses were performed using Limma, an R/Bioconductor software package for gene discovery. Comparisons were made between the abdominal subcutaneous (SAT) intermuscular adipose tissues (IMAT). Once DEG sets were

identified, functional analysis was performed using clusterProfiler, an R/Bioconductor software package for gene set enrichment analysis (GSEA) (5). GSEA was performed on KEGG database (6) to identify significantly enriched biological pathways. Lastly, random forest predictors (7) was applied with split-variable randomization to find genes associated with group assignment and rank them according to their classification significances. The PCA analysis was performed with all DEGs with $p<0.05$ (8586 features). The gene expression data presented in this manuscript has been deposited in the NCBI's Gene Expression Omnibus and are accessible through the accession number GSE139258 and to reviewers using the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139258 with token gnmdyouqvxgbbit.

Supplemental Table 1. Clinical Characteristics

Clinical characteristics	Females (n=6)
Body Weight (kg)	86.3 ± 0.4
BMI (kg/m^2)	35.8 ± 2.5
Fat mass (kg)	40.3 ± 0.9
Lean mass (kg)	43.6 ± 0.9
Waist circumference (cm)	104.4 ± 8.7
Glucose (mg/dL)	93.8 ± 10.9
HbA1C (%)	5.4 ± 0.2
Fasting insulin (uIU/ml)	6.1 ± 3.2
HOMA-IR	1.4 ± 0.6
VO_2 max (ml/kgBW/min)	15.8 ± 1.0
HDL (mg/dL)	53.3 ± 9.9
LDL (mg/dL)	132.5 ± 27.4
Triglycerides (mg/dL)	114.0 ± 64.4

Values are means ± SD. BMI, body mass index; HbA1c, hemoglobin A1c (glycosylated hemoglobin); HOMA-IR; homeostatic model assessment of insulin resistance; BW, body weight.

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