Transcriptional Regulatory Mechanisms in Adipose and Muscle Tissue Associated with Composite Glucometabolic Phenotypes

Carl D. Langefeld1, Mary E. Comeau1, Neeraj K. Sharma2, Donald W. Bowden3, Barry I. Freedman2, and Swapan K. Das2

Objective: Tissue-specific gene expression is associated with individual metabolic measures. However, these measures may not reflect the true but latent underlying biological phenotype. This study reports gene expression associations with multidimensional glucometabolic characterizations of obesity, glucose homeostasis, and lipid traits.

Methods: Factor analysis was computed by using orthogonal rotation to construct composite phenotypes (CPs) from 23 traits in 256 African Americans without diabetes. Genome-wide transcript expression data from adipose and muscle were tested for association with CPs, and expression quantitative trait loci (eQTLs) were identified by associations between cis-acting single-nucleotide polymorphisms (SNPs) and gene expression.

Results: The factor analysis identified six CPs. CPs 1 through 6 individually explained 34%, 12%, 9%, 8%, 6%, and 5% of the variation in 23 glucometabolic traits studied. There were 3,994 and 929 CP-associated transcripts identified in adipose and muscle tissue, respectively; CP2 had the largest number of associated transcripts. Pathway analysis identified multiple canonical pathways from the CP-associated transcripts. In adipose and muscle, significant cis-eQTLs were identified for 558 and 164 CP-associated transcripts (q-value < 0.01), respectively.

Conclusions: Adipose and muscle transcripts comprehensively define pathways involved in regulating glucometabolic disorders. Cis-eQTLs for CP-associated genes may act as primary causal determinants of glucometabolic phenotypes by regulating transcription of key genes.

Introduction

Dysregulation of transcript expression in tissues is linked to the pathophysiology of obesity, insulin resistance, and type 2 diabetes (T2D) (1). The genetic and genomic components of these complex processes are often studied via quantitative endophenotypes. These traits are correlated, and pleiotropy among glucometabolic endophenotypes has been reported. Several transcriptome-wide analyses identified associations of glucometabolic traits (e.g., insulin sensitivity [SI], BMI, percent fat mass, high-density lipoprotein [HDL] cholesterol) with expression levels of transcripts in human muscle, adipose, liver, pancreatic islet, and blood cells (2-5). These studies were successful in defining biological pathways and mechanisms involved in the pathophysiology of T2D and obesity (1,6). However, individual measures are often modestly correlated and may reflect only a portion of the true underlying biological process. Thus, studies focused on single traits may not adequately capture differences in glucometabolic phenotypes between individuals similar in one trait but different in others. For example, two individuals may have the same BMI, but their percent fat mass, waist-to-hip ratio (WHR), and SI can differ substantially, so that each has a different overall metabolic status, translating into differences in disease predisposition (7,8). Approaches that test each endophenotype separately are also liable to reductions in statistical power due to multiple testing penalties. Thus, applying methods that combine correlated endophenotypes into composite phenotypes (CPs) capturing underlying glucometabolic constructs is likely to provide novel insight into pathophysiological and molecular processes involved in these disorders.

1 Department of Biostatistical Sciences, Division of Public Health Sciences, School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA. 2 Department of Internal Medicine, School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA. Correspondence: Swapan K. Das (sdas@wakehealth.edu) 3 Department of Biochemistry, School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA.

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This study applied a factor analysis (FA)-based dimension reduction approach to combine 23 glucose homeostasis, anthropometric, and lipid quantitative traits into a set of uncorrelated glucometabolic dimensions or CPs in African Americans without diabetes. Using the CPs as outcomes, genome-wide transcript expression data from adipose and muscle tissue were analyzed to identify associated transcripts and biological processes that may molecularly define the glucometabolic CPs. The expression quantitative trait locus (eQTL) analysis integrated genome-wide transcript expression and genotype data to identify CP-associated transcripts whose expression levels are influenced by genetic variants.

**Methods**

**Human subjects**

This study was completed at the Wake Forest School of Medicine Clinical Research Unit and was approved by the Wake Forest School of Medicine Institutional Review Board. All participants provided written informed consent. The study utilized glucometabolic phenotype and multi-omic data from 256 unrelated and non-diabetic individuals from the African American Genetics of Metabolism and Expression (AAGMEx) cohort (9). Participants were healthy, self-reported African Americans residing in North Carolina and aged 18 to 60 years with a BMI between 18 and 42 kg/m².

Supporting Information Methods. A frequently sampled intravenous glucose tolerance test (FSIVGT) was performed to evaluate Sf and secretion by minimal model analysis (10). Clinical, anthropometric, and physiological characteristics of the AAGMEx cohort have previously been described (9) and are shown in Table 1. Participants had a broad range of glucometabolic characteristics suitable for capturing the

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**TABLE 1 Summary of 23 anthropometric, obesity, serum lipid, and glucometabolic phenotypes and orthogonal factor loadings in the AAGMEx cohort**

| Phenotype | N | Mean | SD | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|-----------|---|------|----|---------|---------|---------|---------|---------|---------|
| Weight (kg) | 256 | 85.0 | 18.6 | 0.885 | 0.204 | 0.181 | 0.085 | -0.025 | 0.305 |
| Height (cm) | 256 | 171.1 | 10.0 | 0.149 | -0.053 | 0.175 | -0.065 | -0.015 | 0.850 |
| BMI (kg/m²) | 256 | 29.0 | 5.5 | 0.898 | 0.249 | 0.089 | 0.130 | -0.015 | -0.175 |
| Waist (cm) | 254 | 97.0 | 15.0 | 0.880 | 0.255 | 0.246 | 0.151 | -0.043 | -0.026 |
| Hip (cm) | 252 | 107.5 | 12.0 | 0.885 | 0.178 | -0.033 | 0.095 | -0.087 | -0.174 |
| WHR | 252 | 0.92 | 0.08 | 0.385 | 0.205 | 0.444 | 0.147 | 0.043 | 0.160 |
| Fat (%) | 232 | 33.2 | 9.6 | 0.501 | 0.160 | -0.051 | 0.140 | -0.097 | -0.699 |
| Cholesterol total (mg/dL) | 255 | 176.8 | 36.4 | 0.058 | 0.044 | 0.185 | 0.928 | -0.035 | -0.073 |
| TG (mg/dL) | 255 | 84.1 | 43.0 | 0.068 | 0.219 | 0.829 | 0.334 | -0.135 | 0.052 |
| HDL cholesterol (mg/dL) | 255 | 56.1 | 15.7 | -0.406 | -0.133 | -0.475 | 0.241 | 0.203 | -0.123 |
| VLDL cholesterol (mg/dL) | 255 | 16.9 | 8.7 | 0.070 | 0.220 | 0.828 | 0.336 | -0.137 | 0.049 |
| LDL cholesterol (mg/dL) | 255 | 103.8 | 32.3 | 0.237 | 0.055 | 0.215 | 0.816 | -0.099 | -0.034 |
| HbA1c (%) | 256 | 6.6 | 0.7 | 0.070 | 0.417 | 0.052 | 0.308 | -0.277 | 0.216 |
| I0 (mU/L) | 256 | 10.1 | 8.4 | 0.279 | 0.842 | 0.114 | -0.030 | -0.030 | -0.140 |
| I120 (mU/L) | 256 | 62.6 | 78.0 | 0.130 | 0.481 | 0.281 | 0.262 | -0.213 | -0.355 |
| G0 (mg/dL) | 256 | 91.2 | 9.4 | 0.076 | 0.672 | 0.146 | 0.084 | -0.160 | 0.406 |
| G120 (mg/dL) | 255 | 100.8 | 29.8 | 0.058 | 0.484 | 0.144 | 0.342 | -0.356 | -0.113 |
| Matsuda Index | 249 | 6.2 | 6.7 | -0.296 | -0.602 | -0.123 | 0.045 | 0.184 | 0.208 |
| HOMA-IR | 256 | 2.3 | 2.0 | 0.282 | 0.858 | 0.160 | 0.000 | -0.039 | -0.058 |
| SF (× 10^-4, [mU/L]^-1·min^-1) | 233 | 4.0 | 3.3 | 0.429 | -0.163 | -0.212 | 0.189 | 0.540 | 0.324 |
| AIRg (mU/L^-1·min^-1) | 233 | 773.0 | 641.6 | 0.403 | -0.037 | 0.425 | -0.273 | 0.316 | -0.467 |
| D1 | 233 | 2.276.6 | 1,511.5 | -0.079 | -0.209 | 0.021 | -0.097 | 0.868 | -0.130 |
| Sg (min^-1) | 233 | 0.019 | 0.010 | 0.042 | -0.092 | -0.149 | -0.094 | 0.799 | 0.049 |

*aPrincipals component extraction of 23 phenotypes followed by varimax rotation generated orthogonal factor loadings. Factor loadings with absolute value > 0.4 are bolded. Factors denoted as CPs for biological interpretation.

*bPercent fat mass determined by bioelectrical impedance analyzer.

*cFrom 75-g oral glucose tolerance test.

*dFrom insulin modified (0.03 U/kg) FSIVGT; units taken from MINMOD Millennium program.

AAGMEx, African American Genetics of Metabolism and Expression; AIRg, acute insulin response; CP, composite phenotype; D1, disposition index; FSIVGT, frequently sampled intravenous glucose tolerance test; G0, fasting glucose; G120, glucose at 120 min; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; I0, fasting insulin; I120, insulin at 120 min; LDL, low-density lipoprotein; Sf, glucose effectiveness; Sg, insulin sensitivity; TG, tri-glyceride; VLDL, very-low-density lipoprotein; WHR, waist-to-hip ratio.
composite multidimensional structure of these phenotypes. See Supporting Information Methods for laboratory measures and physiological phenotypes as well as gene expression and genotyping.

Statistical analyses

Quality control. Quality control of phenotype, gene expression, and genotype data has been reported (9,11) and is briefly described in the Supporting Information.

Composite phenotype (factor) analysis. Values of the 23 glucometabolic traits likely reflect latent constructs with shared variation. To capture the various dimensions of the glucometabolic traits reflecting these latent constructs, an FA based on the covariance matrix was computed by using principal component extraction and varimax rotation via “PROC FACTOR” in SAS (SAS Institute Inc.); varimax rotation generates orthogonal (independent) factors, denoted here as CPs. All factors with eigenvalues > 1.0 were retained, and the proportion of variance explained was recorded. From these factors, each representing a latent construct, the factor loadings with an absolute value > 0.4 were retained (i.e., < -0.4 or > 0.4), and a linear combination (i.e., weighted mean) of these loadings was computed (Table 1). The resulting scores for factors 1 to 5 were natural logarithm–transformed and standardized (i.e., by subtracting the mean and dividing by the standard deviation [SD]), and the remaining outliers were winsorized (see Supporting Information Methods). Factor 6 did not require natural logarithm transformation and was standardized and winsorized. Thus, the resulting CPs approximately follow a standard normal distribution and were used in subsequent analyses. These six CPs represent six unique dimensions of the glucometabolic domain.

To test for an association between the CPs and expression levels, a linear regression model was computed in which the standardized CPs were modeled as the outcome and the log2 of the transcript expression was the predictor of interest. Models included age, gender, and African ancestry proportion as covariates. Admixture estimates were computed by using the program ADMIXTURE (12) (see online Supporting Information). Expression of a transcript associated with a CP at an uncorrected P < 0.001 was considered for subsequent analyses (e.g., pathway analysis).

Cis-eQTLs. For transcripts associated with one of the six CPs, a cis-eQTL analysis (i.e., within ± 500 kilobase [kb]) around the respective transcript expressed in ≥ 90% of participants) was computed. For each transcript associated with a CP, a linear regression was computed with the log2-transformed expression value as the outcome and the log2 of the transcript expression was the predictor of interest. Models included age, gender, and African ancestry proportion as covariates. Cis-eQTLs with a false discovery rate (FDR)-corrected P value < 0.01 (or 1.0%) were considered significant. See Supporting Information Methods for bioinformatic analysis.

Results

CPs in AAGMEx cohort

The FA identified six orthogonal CPs (eigenvalues > 1.0) that cumulatively explained 74% of the variation in these 23 glucometabolic traits (Figure 1A-1B). Factors 1 through 6 individually explained 34%, 12%, 9%, 8%, 6%, and 5% of the variation. The factor loadings are reported in Figure 1C, and loadings with absolute values greater than 0.4 (i.e., < -0.4 and > 0.4) are highlighted in Table 1.

Factor 1 (CP1) exhibited positive loadings for weight, BMI, waist measurement, hip circumference, percent fat mass, and acute insulin response (AIRG) and exhibited negative loadings for SI and HDL cholesterol. Factor 2 (CP2) exhibited positive loadings for fasting and 2-hour insulin, fasting and 2-hour glucose, and glycated hemoglobin and negative loading for oral glucose tolerance test–derived insulin sensitivity (Matsuda Insulin Sensitivity Index). Factor 3 (CP3) was defined by positive loadings for WHR, serum triglyceride (TG), TG-rich very-low-density lipoprotein cholesterol, and AIRG and negative loading for HDL cholesterol. Factor 4 (CP4) captured a cholesterol dimension independent of the TG-based factor 3, with positive loadings for total and low-density lipoprotein cholesterol. Factor 5 (CP5) exhibited positive loadings for SI, disposition index (DI), and glucose effectiveness. Factor 6 (CP6) was defined by positive loadings for height and fasting glucose and negative loadings for percent fat mass and AIRG. Thus, the six CPs partitioned traits into complex constructs.

Transcripts associated with glucometabolic CPs

Expression levels of 3,994 transcripts in subcutaneous adipose tissue were significantly associated (uncorrected P < 0.001) with at least one of the six CPs (Supporting Information Table S1). CP1 was associated with expression level of 1,925 transcripts in adipose tissue (Table 2). Transcripts most strongly associated included ORM1-like protein 3 (ORMDL3/ORMDL spingolipid biosynthesis regulator 3), transmembrane 7 superfamily member 2 (TM7SF2), and thymocyte nuclear protein 1 (THY1). In humans, the ORMDL3 gene shows highest transcript level expression in liver and adipose tissue. The ORMDL3 expression in adipose tissue was positively associated with SI (β = 0.77, P = 7.01 × 10−8) and negatively associated with BMI (β = −0.86, P = 5.50 × 10−24) in this cohort and was replicated in an independent study in Caucasians (the Metabolic Syndrome in Men cohort, BMI β = −0.429, P = 6.79 × 10−36) (14). In vitro studies in human and mouse cells suggest that downregulation of ORMDL3 increases ceramide, a spingolipid metabolite involved in inflammatory processes and potentially involved in pathophysiology of obesity, insulin resistance, and asthma (15). Among the 1,925 transcripts associated with CP1, 161 were uniquely associated (at a P < 0.001 threshold), while 1,764 were also associated with some of the other CPs (Figure 2). Compared with CP1, CP2 explained a much smaller fraction of total variation (34% vs. 12%) in the 23 glucometabolic phenotypes. However, of the six CPs, expression levels of the highest number of transcripts (3,337 transcripts) were associated with CP2. Fasting insulin levels contributed a high loading (0.842) to CP2 and may have influenced the expression level of adipose transcripts studied after overnight fasting. Transcripts most strongly associated with CP2 included alpha-2-glycoprotein 1, zinc-binding (AZGP1), ubiquitin carboxyl-terminal esterase L1 (UCHL1), and galactosidase, beta 1 (GLB1). The Venn diagram (Figure 3) enumerates the shared transcripts across the CPs. Figure 3 demonstrates the notably larger number of transcripts (1,384 Entrez genes, 41.2%) uniquely associated with CP2. The smallest number of adipose transcripts was associated with CP4 (47, P < 0.001). At a more stringent threshold of FDR-corrected P < 0.01, no adipose transcript remained significantly associated with CP4 or CP5.
Compared with adipose tissue, expression levels of fewer skeletal muscle transcripts were associated with CPs. A total of 929 transcripts in muscle were associated (\(P < 0.001\)) with at least one of the six CPs (Supporting Information Table S2). Among the 299 CP1-associated muscle transcripts, growth factor receptor-bound protein 14 (GRB14), pleckstrin homology-like domain family A member 3 (PHLDA3), and transmembrane protein 192 (TMEM192/FLJ38482) were most strongly associated. The GRB14 transcript level in muscle was positively associated with CP1 (\(\beta = 1.27, P = 6.6 \times 10^{-9}\)). The GRB14 protein interacts with insulin receptors and insulin-like growth factor receptors and likely has an inhibitory effect on receptor tyrosine kinase signaling and, in particular, on insulin receptor signaling; it also may play a role in signaling pathways that regulate growth and glucose metabolism (16). GRB14 knockout mice show improved insulin sensitivity and several genome-wide association studies (GWAS) identified association of SNPs near GRB14 with obesity (WHR, percent fat mass), fasting insulin, and T2D (16-18). As in adipose tissue, the highest number of muscle tissue transcripts (606 transcripts) was associated with CP2 (Table 2). The smallest number of muscle transcripts was associated with CP5 (41 transcripts, \(P < 0.001\)), and only two genes, solute carrier family 25 member 20 (SLC25A20; mitochondrial carnitine/acylcarnitine translocase) and angiopoietin-like 4 (ANGPTL4), remained significantly associated with CP5 at FDR \(P < 0.01\). No muscle transcript remained significantly associated with CP4 or CP6 at FDR \(P < 0.01\).

The expression of a subset of transcripts in both adipose and muscle was associated with CPs. CP1 was associated with 148 transcripts in adipose and muscle tissue (Table 2), with 134 showing directional concordance (increased expression with greater obesity or insulin resistance). For example, expression of GRB14 in both adipose and muscle was positively associated with CP1 (\(\beta = 1.31, P = 1.46 \times 10^{-8}\) in adipose tissue and \(\beta = 1.27, P = 6.6 \times 10^{-9}\) in muscle tissue). Similarly, CP2 was associated with 210 transcripts in adipose and muscle tissue, with 177 showing directional concordance.
Obesity

Pathway enrichment analysis identifies salient biological process linked to glucometabolic CPs

Ingenuity Pathway Analysis identified significant enrichment of biological pathways among genes linked to the transcripts associated with the six CPs. Genes annotated in oxidative phosphorylation and mitochondrial dysfunction pathways were enriched among the first three CPs (CP1-, CP2-, and CP3-associated adipose transcripts) (Figure 4, Supporting Information Table S3). The oxidative phosphorylation pathway was most strongly enriched among CP2-associated adipose transcripts (50 genes, Benjamini-Hochberg [B-H] \( P = 1.0 \times 10^{-15} \)) but was not significantly enriched among CP2-associated muscle transcripts. In adipose tissue, expression of nearly all transcripts in these two pathways was negatively (inversely) associated with CP1, CP2, and CP3. In muscle, genes in oxidative phosphorylation and mitochondrial dysfunction pathways were also enriched among CP1- and CP3-associated transcripts. In contrast to expression in adipose tissue, expression levels of oxidative phosphorylation pathway transcripts in muscle were positively associated with CP1 and CP3 (Supporting Information Table S4). Genes annotated in the eukaryotic initiation factor-2 (eIF2α) signaling, a pathway involved in protein synthesis, were most strongly enriched among adipose tissue transcripts associated with CP1 and CP3 (B-H \( P = 2.51 \times 10^{-7} \) and \( 1.15 \times 10^{-7} \)). The eIF2α signaling pathway was strongly enriched among muscle transcripts associated with CP1, CP2, and CP3 (B-H \( P = 2.0 \times 10^{-8} \) to \( 3.98 \times 10^{-22} \)), and the transcript profile indicated repression of this pathway (activation z score < -2; Figure 5). The expression level of most transcripts in this pathway in adipose and muscle was inversely associated with CP1, CP2, and CP3. Genes annotated in pathways regulating translation and cellular metabolic state based on nutrient availability (e.g., regulation of the eIF4 and p70S6K signaling pathway and the mTOR signaling pathway) were also enriched among adipose and muscle transcripts associated with CP1, CP2, and CP3.

CP-associated transcripts in adipose tissue were also enriched for genes determining fatty acid, amino acid (including branched chain amino acids valine, leucine, and isoleucine), and bioactive amine concentrations (adrenaline, noradrenaline, serotonin, and dopamine). CP1 had the strongest positive loading for BMI. Corroborating our previous findings on obesity (21), CP1-associated transcripts were enriched for the endoplasmic reticulum (ER) stress-induced unfolded protein response pathway (11 genes, B-H \( P = 0.031 \)). CP4-associated adipose and muscle transcripts were not enriched for any biological pathways on Ingenuity Pathway Analysis or for gene ontology categories by DAVID analysis (see online Supporting Information). The CP5-associated transcripts in adipose tissue were only marginally enriched for the triacylglycerol biosynthesis pathway (B-H \( P = 0.038 \)), while CP5-associated transcripts in muscle were enriched for superoxide radical degradation (B-H \( P = 0.003 \)) and triacylglycerol biosynthesis (B-H \( P = 0.049 \)). In adipose tissue, five triacylglycerol biosynthesis pathway genes (GPAM, LPIN1, DGAT2, DGAT1, and ELOVL6) were positively associated with CP5, while in muscle, two genes in this pathway (ABHD5 and PLPP1) were negatively associated. DGAT1 is an ER-localized diacylglycerol O-acyltransferase enzyme and during adipocyte lipolysis it mediates TG synthesis by fatty acid re-estherification; this protects the ER from lipotoxic stress and related adipose tissue inflammation (22).

Adipose tissue transcript profiles for CP2-associated genes displayed repression of the Rho GDP-dissociation inhibitor (activation z score = -3.18) signaling, LXR/RXR activation (z = -2.83), and

Concordance and 33 showing directional discordance. The expression level of genes involved in ribosome function (e.g., RPS17, RPL10A, RPL17, RPL22) and translation initiation (eIF2α and eIF3F) in adipose and muscle tissue was negatively associated with CP2. Expression level of 12 transcripts involved in mitochondrial function in adipose tissue (e.g., ECH1, ETFα, ACOT2, CPT2) was negatively (inversely) associated with CP2, while their expression in muscle was positively associated. Expression of five transcripts (CYP1A1, BCKDHB, PER3, SREBF1, and ANGPTL4) in both tissues was significantly associated with CP5 and exhibited the same effect direction. The angiopoietin-like 4 (ANGPTL4) expression level was negatively associated with CP5 \( (\beta = -0.77, P = 3.27 \times 10^{-5}) \) in adipose and \( \beta = -0.89, P = 2.37 \times 10^{-7} \) in muscle), which captured efficient \( S_I \) upon glucose loading. Among the three FSIVGT-derived glucose homeostasis traits \( (S_I, D_I, \) and glucose effectiveness) that define CP5, ANGPTL4 expression was most strongly associated with \( D_I (\beta = -12.67, P = 5.58 \times 10^{-7}) \) in muscle and \( \beta = -12.5, P = 8.61 \times 10^{-6} \) in adipose tissue). Studies in mouse models showed that ANGPTL4 is a glucocorticoid receptor target gene that promotes lipolysis in adipocytes, inhibits extracellular lipoprotein lipase, and triggers interorgan communication (19). Increased glucocorticoid level during fasting induces ANGPTL4 expression. ANGPTL4-mediated lipolysis in adipocytes activates ceramide synthesis in the liver and induces whole-body insulin resistance by stimulating the activities of the downstream effectors of ceramide, protein phosphatase 2A, and protein kinase \( C_\nu \) (20).

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PPAR signaling ($z = -2.23$) pathways. The LXR/RXR activation and PPAR signaling pathways were also repressed among CP1- and CP3-associated genes in adipose tissue, while transcript profiles for CP5-associated genes indicated significant activation of the PPAR signaling pathway ($z = 2$). The CP2-associated transcripts indicated a strong activation of inflammation-related pathways in adipose tissue, including the Fcγ receptor-mediated phagocytosis in macrophages and monocytes ($z = 4.7$), Tec kinase signaling ($z = 4.24$), integrin signaling ($z = 4.14$), TREM1 signaling ($z = 4.02$), leukocyte extravasation signaling ($z = 3.53$), dendritic cell maturation ($z = 4.33$), and inflammasome pathways ($z = 2.64$). Many of these inflammation-related pathways were also activated among CP1- and CP3-associated adipose transcripts, while CP6-associated transcripts showed repression of these pathways (Figure 4).

**Expression of subset of glucometabolic CP-associated transcripts is dependent on regulatory genetic polymorphisms**

To develop putative causal models, we integrated genotype information (SNPs with minor allele frequency $\geq 0.01$) and gene expression data through eQTL analysis to identify cis-regulatory variants (cis-expression regulatory SNPs [cis-eSNPs]) in modulating the expression of CP-associated transcripts in adipose and muscle tissue. In adipose tissue, significant cis-eQTLs were identified for 558 CP-associated transcripts ($Q < 0.01$; Supporting Information Table S5). In muscle, significant cis-eQTLs were identified for 164 CP-associated transcripts ($Q < 0.01$; Supporting Information Table S6). Twenty-four CP-associated transcripts were cis-expression regulated genes (cis-eGenes) in both adipose and muscle. Among the CP-associated transcripts, TGF beta-inducible nuclear protein 1 (TINP1/NSA2) had the strongest cis-eSNP in adipose tissue (rs6873912, $b = 0.391$, $P = 3.47 \times 10^{-267}$), while Abelson helper integration site 1 (AHI1) had the strongest cis-eSNP in muscle (rs7772705, $b = 0.518$, $P = 1.63 \times 10^{-260}$). Among the adipose cis-eGenes (FDR: 1%), dicarbonyl/L-xylulose reductase (DCXR) was most significantly associated with CP2 ($b = -2.22$, $P = 1.11 \times 10^{-15}$), while among muscle cis-eGenes, prostaglandin D2 synthase (PTGDS) was most significantly associated with CP2 ($b = 1.14$, $P = 1.1 \times 10^{-11}$). The top 10 CP-associated cis-eGenes or genetically regulated transcripts in each tissue, based on average ranking for CP association $P$ value and eQTL $P$ value, are shown in Table 3. The top average ranking

![Figure 3](image-url)
TABLE 2 Summary of adipose and muscle tissue transcripts associated with the six orthogonal glucometabolic phenotype dimensions derived from 23 glucometabolic traits

| Tissue             | Significance threshold | CP1  | CP2  | CP3  | CP4  | CP5  | CP6  |
|--------------------|------------------------|------|------|------|------|------|------|
| N for adipose      |                        | 202  | 246  | 225  | 250  | 230  | 207  |
| Adipose            | $P < 0.001$            | 1,925| 3,337| 1,579| 47   | 245  | 499  |
| N for muscle       | FDR $P < 0.01$         | 1,350| 3,135| 984  | 0    | 0    | 43   |
| Muscle             | $P < 0.001$            | 198  | 241  | 220  | 245  | 225  | 203  |
| N for muscle       | FDR $P < 0.01$         | 198  | 241  | 220  | 245  | 225  | 203  |
| Both tissues       |                        | 198  | 241  | 220  | 245  | 225  | 203  |
| Adipose cis-eGene  | $P < 0.001$ & eQTL $Q < 0.01$ | 265  | 463  | 218  | 7    | 33   | 55   |
| Muscle cis-eGene   | $P < 0.001$ & eQTL $Q < 0.01$ | 61   | 106  | 57   | 6    | 9    | 24   |

$N$ is variable due to either missing phenotype or expression data.

$^a$Values in these rows indicate number of transcripts (probes) significantly associated with each CP at given threshold.

$^b$Expression of number of transcripts in both adipose and muscle tissue is associated ($P < 0.001$ in one tissue and $P < 0.01$ in the other tissue) with a CP. The number in parentheses shows number of transcripts showing same effect direction ($b$) in both tissues.

$^c$Values in these rows indicate number of transcripts (probes) significantly associated with the CP ($P < 0.001$) and are cis-eGenes (FDR < 1% or $Q < 0.01$).

CP, composite phenotype; eGene, expression regulated gene; eQTL, expression quantitative trait locus; FDR, false discovery rate.

Figure 4 Transcripts in subcutaneous adipose tissue are associated with glucometabolic composite phenotypes (CPs) and enriched for salient biological pathways. (A) Heat map shows hierarchical clustering of $-\log_{10} P$ values for 3,994 adipose tissue transcripts (each row indicates a probe for a transcript) associated ($P < 0.001$) with CPs. (B) Enrichment and (C) activation of genes in biological pathways among six CP-associated adipose transcripts based on Ingenuity Pathway Analysis comparison are shown as heat maps. [Color figure can be viewed at wileyonlinelibrary.com]
transcripts included membrane-spanning 4-domains subfamily-A member-6A \((MS4A6A)\) and galectin-related protein \((LGALSL/GRP/HSPC159)\) in adipose and muscle, respectively. The expression of an isoform of \(MS4A6A\) \((NM_022349.2)\) in adipose tissue was positively associated with CP2 \((\beta = 0.93, P = 1.66 \times 10^{-11})\), and common minor alleles (minor allele frequency = 0.33) of SNP rs597982_C were associated with reduced transcript expression \((\beta = -0.45, P = 3.52 \times 10^{-11})\).

**Expression of genes predicted by GWAS for BMI is associated with CPs**

GWASs in large well-powered cohorts identified many loci associated with an increased risk of obesity and other glucometabolic traits. For example, Locke et al. (23) identified 97 genome-wide significant \((P < 5 \times 10^{-8})\) loci for BMI. However, most of these trait-associated SNPs are in the noncoding region of the genome and cannot directly implicate the “culprit gene.” Thus, Locke et al. (23) used data-driven expression prioritization integration for complex traits (DEPICT) (24) to predict and prioritize genes in an expanded set of 511 BMI-associated \((P < 5 \times 10^{-8})\) genomic regions. DEPICT predicted 989 potential causal genes in BMI-associated genomic regions. Among the DEPICT-predicted BMI genes, expression of 127 and 19 genes in adipose tissue and muscle, respectively, were associated with CPs in our AAGMEx cohort (Supporting Information Table S7).

**Discussion**

Existing genome-wide transcriptomic studies have tested the association of transcript levels in tissues with single anthropometric, glucose homeostasis, and lipid traits (2,4,9,14). Some have employed covariate adjustment strategies to account for confounding effects of correlated traits (e.g., S1 adjusted for BMI) (3,9). These strategies cannot fully capture variation across multiple traits simultaneously. Specifically, many of these individual measures are partial manifestations of underlying latent glucometabolic phenotypes, and applying a method that combines correlated

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**Figure 5** Transcripts in skeletal muscle tissue are associated with glucometabolic composite phenotypes (CPs) and enriched for salient biological pathways. (A) Heat map shows hierarchical clustering of \(-\log_{10} P\) values for 929 muscle tissue transcripts (each row indicates a probe for a transcript) associated \((P < 0.001)\) with glucometabolic CPs. (B) Enrichment and (C) activation of genes in biological pathways among six CP-associated muscle transcripts based on Ingenuity Pathway Analysis comparison are shown as heat maps. [Color figure can be viewed at wileyonlinelibrary.com]
TABLE 3 Top glucometabolic CP-associated cis-eGenes in adipose and muscle tissue

| Probe ID  | Symbol | cis-eSNP | Chr | A1 | A2 | MAF  | β  | P                      | CP | β  | P            |
|-----------|--------|----------|-----|----|----|------|----|------------------------|----|----|---------------|
| In adipose tissue |        |          |     |    |    |      |    |                        |    |    |              |
| ILMN_1797731 | MS4A6A | rs597982 | 11  | C  | A  | 0.333 | 0.452 | 3.52 × 10^{-17} | 2  | 0.93 | 1.66 × 10^{-11} |
| ILMN_16740699 | TMM7 | rs2240726 | 7   | G  | A  | 0.294 | 0.120 | 2.16 × 10^{-16} | 2  | −3.39 | 1.74 × 10^{-9} |
| ILMN_1665132 | CD36 | rs3211938 | 7   | G  | T  | 0.100 | 0.710 | 2.11 × 10^{-14} | 2  | −0.74 | 4.33 × 10^{-9} |
| ILMN_2072178 | ECHDC3 | rs200943982 | 10  | T  | C  | 0.401 | 0.281 | 1.94 × 10^{-8} | 2  | −1.12 | 4.85 × 10^{-13} |
| ILMN_2364384 | PPARG | rs3856806 | 3   | T  | C  | 0.075 | 0.754 | 2.81 × 10^{-8} | 2  | −0.95 | 1.68 × 10^{-7} |
| ILMN_1774949 | PI6P | rs2298682 | 21  | G  | A  | 0.317 | 0.254 | 1.75 × 10^{-8} | 2  | −1.47 | 1.52 × 10^{-7} |
| ILMN_1786105 | PCD1 | rs16928023 | 10   | G  | A  | 0.146 | 0.220 | 7.56 × 10^{-10} | 2  | −1.75 | 3.59 × 10^{-10} |
| ILMN_1663538 | CLYBL | rs2281756 | 13  | G  | A  | 0.266 | 0.140 | 5.14 × 10^{-9} | 2  | −2.11 | 3.75 × 10^{-11} |
| ILMN_1720303 | OSTM1 | rs9372177 | 6  | A  | G  | 0.383 | 0.134 | 8.74 × 10^{-10} | 2  | 2.09 | 1.33 × 10^{-9} |
| ILMN_1690982 | DDT | rs79966373 | 22  | G  | C  | 0.178 | 0.302 | 2.81 × 10^{-14} | 2  | −1.25 | 1.32 × 10^{-7} |

Top 10 CP-associated genetically regulated transcripts in each tissue based on average ranking for phenotype association P value and eQTL P value are shown.

Results for most significant association of SNP with transcript level are shown.

Results for most significant association of transcript with CP are shown.

A1, allele 1; A2, allele 2; Chr, chromosome; cis-eSNP, cis-expression regulatory single-nucleotide polymorphism; CP, composite phenotype; eGene, expression regulated gene; eQTL, expression quantitative trait locus; ID, identifier; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

endophenotypes into CPs capturing the underlying glucometabolic construct is more likely to provide novel insight into the pathophysiological and molecular processes involved in T2D and obesity. This study used FA to identify and partition 23 measures of obesity and glucose metabolism into six orthogonal dimensions of glucometabolic CPs. For example, CP1 explained 34% of the variation in the 23 glucometabolic measures in this African American cohort (AAGMEx) and comprehensively captured the obesity and FSIVGT-derived glucose homeostasis phenotypes. Availability of detailed phenotype data for AAGMEx participants enabled us to capture the composite multidimensional structure of glucometabolic CPs. We believe that transcripts associated with the CPs most comprehensively define the repertoire of biological pathways, including novel pathways, involved in the genetic regulation of glucometabolic traits.

Focusing on the top six CPs, a total of 3,994 associated transcripts were identified in subcutaneous adipose tissue; only 929 transcripts in muscle were similarly associated. Thus, transcriptional dysregulation involved in determining glucometabolic phenotypes appears to be more pervasive in adipose tissue. Although CP1 (reflecting a composite obesity–insulin resistance phenotype) explained the largest proportion of variation in the 23 measures, expression levels of the largest number of transcripts in adipose and muscle tissue were most strongly associated with CP2 (reflecting a composite hyperinsulinemic-insulin resistance phenotype). Fasting insulin and the homeostatic model assessment of insulin resistance index had the largest loadings for CP2. Fasting insulin is higher in insulin-resistant subjects, and it alters adipose and muscle tissue gene expression and mediates cross talk between tissues involved in glucose homeostasis (25). Short-term experimental hyperinsulinemia (measured via a 2-hour hyperinsulinemic euglycemic clamp) induced a transcriptional response of 230 genes in the adipose tissue of subcutaneous adipose tissue; only 929 transcripts in muscle tissue were similarly associated. Thus, transcriptional dysregulation involved in determining glucometabolic phenotypes appears to be more pervasive in adipose tissue. Although CP1 (reflecting a composite obesity–insulin resistance phenotype) explained the largest proportion of variation in the 23 measures, expression levels of
Additional studies are required to resolve temporal and mechanistic connections between hyperinsulinemia, obesity, and insulin resistance.

The expression of a subset of transcripts in both adipose and muscle tissue was associated with the six glucometabolic CPs. The inverse correlation of genes in pathways involved in protein synthesis (eIF2 signaling) and the regulation of translation and cellular metabolic states based on nutrient availability (eIF4 and p70S6K signaling pathways and mTOR signaling) with CP1 and CP3 suggests discordant downregulation of these pathways in adipose and muscle tissue. However, CP1- and CP3-associated genes showed discordant regulation of oxidative phosphorylation pathway genes, and CP5-associated genes suggest discordant regulation of the triacylglycerol biosynthesis pathway in adipose and muscle tissue. The enrichment of CP2-associated adipose genes in various inflammation-related pathways supports the tissue-specific activation of these pathways. Some of the glucometabolic CP-associated genes identified here (e.g., ORMDL3 and MS4A6A) are involved in asthma and Alzheimer disease, suggesting common transcriptional mechanisms across diseases. Precise triggers of adipose tissue inflammation are poorly understood (27); our data support the involvement of multiple potential mechanisms. In adipose tissue, PPAR signaling was repressed among CP1- and CP3-associated genes, while transcript profiles for CP5-associated genes indicated significant activation of this pathway. CP5 captures a dimension measuring efficient $S_B$ upon intravenous glucose loading, and CP1 reflects a combined obesity–insulin resistance phenotype. Together, these genome-wide transcriptomic and biological pathway analyses define the repertoire of biological pathways involved in regulating distinct dimensions of obesity and glucose homeostasis. Our study used only adipose and muscle tissue to define transcriptional mechanisms determining CPs. Other metabolic tissues are of interest but are not readily accessible in the clinical setting.

Recent GWAS approaches have successfully identified genetic loci associated with glucometabolic phenotypes; however, identification of precise causal genes in those loci typically remains elusive. Most studies have considered the genes closest to the sentinel SNP as the effector gene. For example, FTO was considered the causal gene in the most significant and highly replicated BMI-associated locus on chromosome 16 (28). Recent studies have refuted this conclusion. Functional genetic analyses, including eQTL and chromatin interaction analysis, suggest that BMI-associated SNPs in the FTO locus contribute to obesity by regulating expression of the IRX3 and IRX5 genes in preadipocytes or brain tissue (29,30). IRX3 is located ~513 kb from the BMI-associated SNPs. In a similar fashion, adipose and muscle transcript levels are key molecular phenotypes associated with composite glucometabolic traits and act proximally to actions of DNA sequence variants. Therefore, the present study focused on identifying transcriptional mechanisms associated with glucometabolic CPs. Our previous studies have shown that a subset of glucometabolic-trait GWAS-identified SNPs are cis-eSNPs (11,31). Herein, we have demonstrated that expression of a subset of CP-associated transcripts is determined by cis-eSNPs and that CP-associated transcripts are among the genes predicted by bioinformatics analysis of GWAS-implicated BMI loci. As an alternative to a GWAS, this approach provides more direct evidence for putative causal genes and novel genetically regulated mechanisms determining glucometabolic phenotypes.

Our data implicate thousands of genes in biological processes determining glucometabolic phenotypes. However, it is likely that a subset of these processes is due to reactive changes in response to primary causal mechanisms. This study cannot conclusively differentiate causal effects from reactive effects based solely on transcriptomic data. Naturally occurring genetic variants, including SNPs in our genome, determine gene expression levels in tissues by controlling transcriptional regulation. Thus, regulatory SNPs may act as primary initiators determining glucometabolic phenotypes via roles in modulation of transcript levels (SNP $\rightarrow$ transcript $\rightarrow$ phenotype) in tissues important for glucose homeostasis. The eQTL analysis in this cohort identified cis-eQTLs for a subset of CP-associated transcripts in adipose and muscle tissue. These CP-associated cis-eGenes may act as key derivers in transcriptional regulatory mechanisms involved in determining glucometabolic phenotypes.

**Conclusion**

Adipose and muscle transcripts associated with CPs comprehensively define the repertoire of biological pathways involved in regulating distinct dimensions of obesity and glucose homeostasis. The cis-eSNPs may act as primary initiators influencing obesity and glucose homeostasis by regulating transcript levels of a subset of genes in adipose and muscle tissue. Further computational analysis and in vitro functional studies will be required to prioritize these genes and validate the causal regulatory role of the key drivers in remodeling transcriptional regulatory networks relevant to glucose homeostasis.

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