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Genetic regulation of adipose tissue transcript expression is involved in modulating serum triglyceride and HDL-cholesterol

Satria P. Sajuthi^{1,#}, Neeraj K. Sharma^{2,#}, Mary E. Comeau¹, Jeff W. Chou¹, Donald W. Bowden³, Barry I. Freedman⁴, Carl D. Langefeld¹, John S. Parks⁵, and Swapan K. Das^{2,*}

¹Department of Biostatistical Sciences, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC 27157

²Department of Internal Medicine, Section on Endocrinology and Metabolism, Wake Forest School of Medicine, Winston-Salem, NC 27157

³Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, NC 27157

⁴Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC 27157

⁵Department of Internal Medicine, Section on Molecular Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157

Abstract

Dyslipidemia is a major contributor to the increased cardiovascular disease and mortality associated with obesity and type 2 diabetes. We hypothesized that variation in expression of adipose tissue transcripts is associated with serum lipid concentrations in African Americans (AAs), and common genetic variants regulate expression levels of these transcripts. Fasting serum lipid levels, genome-wide transcript expression profiles of subcutaneous adipose tissue, and genome-wide SNP genotypes were analyzed in a cohort of non-diabetic AAs (N=250). Serum triglyceride (TRIG) and high density lipoprotein-cholesterol (HDL-C) levels were associated (FDR<0.01) with expression level of 1021 and 1875 adipose tissue transcripts, respectively, but none associated with total cholesterol or LDL-C levels. Serum HDL-C-associated transcripts were enriched for salient biological pathways, including branched-chain amino acid degradation, and oxidative phosphorylation. Genes in immuno-inflammatory pathways were activated among individuals with higher serum TRIG levels. We identified significant cis-regulatory SNPs (cis-eSNPs) for 449 serum lipid-associated transcripts in adipose tissue. The cis-eSNPs of 12 genes were nominally associated (p<0.001) with serum lipid level in genome wide association studies in Global Lipids Genetics Consortium (GLGC) cohorts. Allelic effect direction of cis-eSNPs on

* Corresponding author and person to whom reprint requests should be addressed: Swapan K. Das, Ph.D., Section on Endocrinology and Metabolism, Department of Internal Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157, sdas@wakehealth.edu, Telephone: 336-713-6057; Fax: 336-713-7200.

Authors contributed equally

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expression of *MARCH2*, *BEST1* and *TMEM258* matched with effect direction of these SNP alleles on serum TRIG or HDL-C levels in GLGC cohorts. These data suggest that expressions of serum lipid-associated transcripts in adipose tissue are dependent on common cis-eSNPs in African Americans. Thus, genetically-mediated transcriptional regulation in adipose tissue may play a role in reducing HDL-C and increasing TRIG in serum.

Graphical abstract

Keywords

Triglyceride; HDL-cholesterol; Gene expression profiling; Genotype; Expression Quantitative Trait (eQTL); African Americans

1. Introduction

Dyslipidemia is a major contributor to the increased cardiovascular disease and mortality associated with obesity and type 2 diabetes (T2D)^{1, 2}. Identifying the molecular mechanisms contributing to dyslipidemia is crucial for understanding how obesity increases the risk of cardiovascular disease in T2D. Obesity leads to dyslipidemia, characterized by increased serum triglyceride (TRIG) and reduced high density lipoprotein-cholesterol (HDL-C)³. Both hepatic and extra-hepatic tissues play important roles in controlling serum lipid levels⁴. Adipose tissue is the major site of fatty acid storage as TRIG, the largest reservoir of free cholesterol in the body, and also participates in HDL-C biogenesis⁴⁻⁶. Adipose tissue plays a key role in integrating energy metabolism and glucose homeostasis. Abnormal expansion and remodeling of adipose tissue during the progression of obesity leads to insulin resistance and precedes the development of T2D⁷. Dysfunctional adipose tissue in the insulin resistant state impairs adipocyte TRIG storage and HDL lipidation, and can cause reduced circulating HDL-C and increased TRIG^{4, 5}. Identifying the molecular processes, including transcript regulatory mechanisms, involved in triglyceride and cholesterol metabolism in adipose tissue and its involvement in modulating serum lipid levels may provide insight for development of efficient treatment strategies for dyslipidemia, and prevention of cardiovascular mortality. Such studies are limited in the human population⁸⁻¹¹, and have not been done in African Americans.

Serum lipids are modulated by genetic and environmental (e.g. dietary) factors. Genome-wide association studies (GWAS) identified association of genetic polymorphisms (single nucleotide polymorphisms, SNPs) in over 150 loci with serum lipid levels^{12–14}. However, causal genes in many of these loci remain undetermined, and the role of these loci in tissues involved in serum lipid and lipoprotein homeostasis remains unknown. We hypothesized that variation in expression of adipose tissue transcripts is associated with serum lipid and lipoprotein concentrations in non-diabetic African Americans. In this study, a systematic analysis was performed on fasting serum lipid levels, genome-wide transcript expression profiles of subcutaneous adipose tissue, and genome-wide SNP genotypes in a metabolically characterized cohort of non-diabetic African Americans to identify biological pathways and genetic regulatory mechanisms involved in modulating adipose tissue function and regulating blood lipids.

2. Materials and Methods

2.1. Human subjects

This study utilized gluco-metabolic phenotype and multiomic data from 260 unrelated non-diabetic individuals from the “African American Genetics of Metabolism and Expression” (AAGMEx) cohort, previously recruited by our laboratory^{15, 16}. Studies were performed at the Wake Forest School of Medicine (WFSM) Clinical Research Unit. This study was approved by the WFSM Institutional Review Board and all participants provided written informed consent. Clinical, anthropometric, and physiological characteristics of the AAGMEx cohort have been described (Sharma et al., 2016)¹⁶. In brief, participants were healthy, self-reported African Americans residing in North Carolina aged 18–60 years with a body mass index (BMI) between 18 and 42 kg/m². Individuals taking medications that increased bleeding risk or altered insulin sensitivity and/or gene expression in insulin-responsive tissues were excluded. A standard 75-g oral glucose tolerance test (OGTT) was used to exclude individuals with diabetes. Height, weight, waist and hip circumference were measured, body fat was determined, and fasting blood was drawn for DNA isolation and biochemical analyses at the screening visit. During a second visit, subcutaneous adipose tissue (from the abdomen near the umbilicus) biopsies were collected from 256 individuals under fasting conditions using a Bergstrom needle, snap-frozen in liquid N₂ and stored at –80°C.

2.2 Laboratory measurements and physiological phenotyping

Plasma insulin was measured using an immuno-chemiluminometric assay (Invitron Limited, Monmouth, UK), and plasma glucose was measured by glucose oxidase methods (LabCorp, Burlington, NC). Blood samples for lipid profiling were collected in serum separator tubes. Serum TRIG, total cholesterol, and HDL-C concentrations were directly measured by enzymatic colorimetric methods at a CLIA certified laboratory (LabCorp). We note that the automated method (Roche/Hitachi Cobas C system) for direct determination of HDL-C at LabCorp includes PEG-modified enzymes (cholesterol esterase and cholesterol oxidase) that shows selective catalytic activity towards HDL-C, and this method correlates with precipitation-based methods. Low density lipoprotein-cholesterol (LDL-C) cholesterol and

very low density lipoprotein-cholesterol (VLDL-C) concentrations were computed using the Friedewald equation¹⁷.

2.3. Gene expression analysis and genotyping

Extraction of total RNA from adipose tissue was performed using the miRNeasy Mini Kit (Qiagen), and DNA was isolated from whole blood using the Gentra Puregene blood kit (Qiagen). The quantity of isolated nucleic acid samples was determined by ultraviolet spectrophotometry (Nanodrop, Thermo Scientific) and the quality of RNA was determined by electrophoresis (Experion nucleic acid analyzer, BioRad Laboratories, Inc.). Genome-wide expression data were generated using HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, CA) whole genome gene expression arrays for quantitative analyses of transcript expression in adipose samples. Infinium HumanOmni5Exome-4 v1.1 DNA Analysis BeadChips (Illumina) were used to genotype DNA samples based on the manufacturer's recommendations. Additional technical details of standard gene expression analyses and genotyping methods are described in our published studies^{15, 16}. Expression data was submitted to Gene Expression omnibus (GEO id #GSE95674)

2.4. Quality control

Data quality control methods are presented in our previously published studies^{15, 16}. Briefly, measures of gluco-metabolic phenotypes were examined for outliers in a univariate fashion and as correlated pairs. After consideration of the models' fit to these data, the following transformations were used when the trait was the response variable in the model: natural logarithm for TRIG and HDL-C, and square root for total cholesterol, LDL-C, and BMI.

Genome-wide gene expression data (probe level) were extracted using Illumina GenomeStudio V2011.1. Expression level was log₂ transformed, robust multi-array average normalized (RMA, includes quantile normalization)¹⁸, and batch-corrected using ComBat¹⁹. The HumanHT-12 v4 Expression BeadChip includes 47,231 probes annotated to transcripts. Initial analysis included all probes; however, probes for transcripts significantly expressed ($p < 0.05$) in adipose were included in the final analysis. Genotype data were examined to verify sample and SNP quality. Genotype assays of 4,210,443 SNPs passed technical quality filters. The genotypes of 2,296,925 autosomal SNP assays (representing 2,210,735 unique high-quality genotyped SNPs with MAF > 0.01 and HWE-p value $> 1 \times 10^{-6}$) were used in expression quantitative trait loci (eQTL) analyses. Sample sizes in each analysis varied slightly based on available data after quality control.

2.5. Statistical and Bioinformatic analyses

To test for an association between serum lipid traits and expression level, a linear regression model was computed (Software: R(glm)) with the trait (transformed) as the outcome and expression level (log₂) as the predictor. Models included age, gender, and ancestry proportion (admixture) as covariates. As described elsewhere^{15, 16}, admixture estimates used in this analysis was computed using the ADMIXTURE program²⁰. In a secondary analysis, BMI was used as an additional covariate. Benjamini-Hochberg False Discovery Rate (FDR) adjusted p-values were computed by trait for all probes²¹. Expression of a transcript associated with a trait at $p < 0.001$ was considered significant. Probes spanning common SNP

regions, probes representing pseudogenes, and predicted or hypothetical genes (e. g. FLJs, LOCs, MGCs, ORFs) were filtered out from the significant gene lists.

To validate serum lipid-transcript association, we used subcutaneous adipose tissue genome-wide transcript expression data (generated using the HumanHT-12 v4 Expression BeadChip, GEO id #GSE65221) from an independent cohort of 99 non-diabetic European Americans from Arkansas (AREA cohort). Serum lipid and insulin sensitivity of participants in this Arkansas cohort was characterized by our laboratory using a similar protocol²². To compare results in the Arkansas and AAGMEx cohorts, correlation of triglyceride and HDL-cholesterol with adipose transcripts was evaluated using Spearman semi-partial correlation coefficients adjusted for age and gender or age, gender and ancestry proportion (admixture). Additionally, we compared our results with a recently published gene expression study in adipose tissue samples (Affymetrix U219 array) from the Metabolic Syndrome in Men study (METSIM, Finland)¹¹.

To identify cis-expression quantitative trait loci (cis-eQTLs), linear regression model was computed with the log₂ transformed expression values as the outcome and an additive genetic model for the SNP as implemented in the R-package MatrixEQTL²³; age, gender, and African ancestry proportion were covariates as described elsewhere¹⁵. SNPs within $\pm 500\text{kb}$ around the respective expressed transcript were considered for this analysis. Any cis-eSNPs with a false discovery rate (FDR)-corrected p-value (Q-value) < 0.01 (or 1.0%) were considered significant.

Bioinformatic analysis was performed to investigate the enrichment of serum lipid associated transcripts in known biological pathways or gene ontology (GO) categories. Canonical pathway analysis, upstream regulator analysis, and interaction network analysis of serum lipid-associated transcripts (FDR <0.01) were performed using the Ingenuity Pathway analysis program (IPA; <https://analysis.ingenuity.com>, Build version: 377306M; Content version: 27216297). Pathways with a Fisher's exact test p-value ≤ 0.05 were considered significantly enriched in our gene lists. Additional annotations of transcripts were performed by g:GOST Gene group functional profiling analysis (g:Profiler ; <http://biit.cs.ut.ee/gprofiler/index.cgi>). In this analysis, a category with Benjamini-Hochberg FDR p-value <0.05 was considered significant.

2.6. Integration of GWAS data

Cis-eSNPs identified in adipose and muscle of African Americans represented a prioritized set of SNPs providing statistical evidence for genotype-dependent variation in transcript abundance. The Joint analysis of METABOCHIP and GWAS data from the "Global Lipids Genetics Consortium" (GLGC) cohorts (N $>80,000$)¹⁴ were mined to identify the role of putatively functional SNPs in modulating serum triglyceride and HDL-cholesterol. Meta-analyses of SNP association tests in GLGC cohorts are performed using the Stouffer's Z-score method, with weights proportional to the square root of the sample size for each cohort, and described in Willer et al (2013)¹⁴.

3. Results

3.1. Phenotypic characteristics

Serum lipid levels in this non-diabetic African American cohort are summarized in Table 1. Serum LDL-C concentration was >100mg/dl in 129 participants, HDL-C concentrations were 40 mg/dl in 32 participants, and TRIG were 150 mg/dl in 21 individuals. Participants had a broad range of BMIs (28.9 ± 5.6 kg/m²). As expected, BMI was positively correlated with serum TRIG level (Spearman's rho = 0.24, $p = 1.33 \times 10^{-4}$) and inversely correlated with serum HDL-C level (Spearman's rho = -0.39, $p = 1.0 \times 10^{-6}$). The serum TRIG level was inversely correlated with serum HDL-C level ($r = -0.30$, $p = 2.26 \times 10^{-6}$, partial correlation adjusted for age, sex, and BMI). Although participants in this cohort are not severely dyslipidemic, a range of serum lipid concentrations were observed. Thus, the cohort is suitable for identifying biological pathways and genetic regulatory mechanisms involved in modulating adipose tissue function and regulating blood lipids in the absence of other confounding factors (e.g., cardio-metabolic abnormalities) in African Americans.

3.2. Adipose tissue transcript levels are associated with serum triglyceride and HDL cholesterol level

High quality adipose tissue genome-wide expression data from 250 participants was obtained in the AAGMEx cohort and used to evaluate the association of transcript levels with serum lipid levels. Serum TRIG level was associated ($p < 0.001$) with expression level of 1458 adipose tissue transcripts, while serum HDL-C level was associated with expression level of 2089 adipose tissue transcripts (Table-2 and Supplementary Table-1). Expression levels of genes well known for lipoprotein metabolism were positively (e.g. *CETP*, *LPL*) or inversely (e.g. *VLDLR*, *LIPA*) associated with serum-HDL-C level. Total cholesterol and LDL-C was associated ($p < 0.001$) with expression level of only 21 and 114 adipose tissue transcripts, respectively (Figure-1A). No transcript was significantly correlated with total and LDL-C at FDR<1%. Nine participants in AAGMEx were on HMG-CoA reductase inhibitor (statin) therapy, which alters serum lipid levels; thus we reanalyzed the association between adipose transcript and serum lipid levels excluding these subjects. However, results remained consistent; the effect direction of TRIG or HDL-C associated transcripts were completely concordant and effect sizes (β) were strongly correlated ($r^2 > 0.99$) between the overall cohort and the remaining cohort after excluding individuals on statin therapy (Supplementary Figure-1). Studies have shown significant correlation of adipose transcripts with obesity^{22, 24} and we found that serum TRIG and HDL-C levels were correlated with BMI. After adjustment for BMI, adipose tissue transcript level of 135 and 79 genes, respectively, remain significantly associated (FDR<1%) with serum TRIG and HDL-C level (Supplementary Table-2).

3.3. Association of adipose tissue transcript levels with serum triglyceride and HDL cholesterol level in African Americans replicates in independent Caucasian cohorts

Strong concordance was observed between correlations of adipose tissue transcripts and serum lipids in AAGMEx African Americans (N=250) and non-diabetic European Americans (AREA cohort, N=99) data sets²². Among the 580 transcripts significantly correlated with TRIG in both the AAGMEx ($p < 0.001$, Spearman semi-partial correlation

coefficients adjusted for age, gender, and ancestry proportion) and AREA cohort ($p < 0.05$, Spearman semi-partial correlation coefficients adjusted for age and gender), 99.31% showed directional concordance. Similarly, among the 1094 transcripts significantly correlated with HDL-C in both AAGMEx and AREA cohort, 99.26% showed directional concordance. The effect size (ρ) of TRIG or HDL-C associated transcripts was strongly correlated ($r^2 = 0.89$ for TRIG, and $r^2 = 0.91$ for HDL-C) between the AAGMEx and AREA cohorts (Supplementary Figure-2). An additional comparison of our results with a recently published gene expression study in adipose tissue samples (Affymetrix U219 array) from Metabolic Syndrome in Men study (METSIM, Finland, $N = 770$)¹¹, replicated association of 1195 genes (97.4% showed concordance in effect direction) with HDL-C level (Supplementary Table-3). In a more stringent threshold (FDR corrected p -value < 0.01) 626 genes were significantly associated with HDL-C level in both AAGMEx and METSIM cohort with concordant directions of effect.

3.4. Enrichment of salient biological pathways among serum triglyceride and HDL cholesterol associated adipose tissue transcripts

Ingenuity pathway analysis identified enrichment of genes in the triacylglycerol biosynthesis pathway ($p = 0.005 - 2.29 \times 10^{-5}$) among adipose transcripts associated with serum TRIG and HDL-C level (Table-3, and Supplementary Table 4 and 5). Expression levels of several genes in the triacylglycerol biosynthesis pathway in adipose tissue, including Lipin1 (*LPIN1*), alpha/beta hydrolase domain containing 5 (*ABHD5/CGI58*), phospholipid phosphatase 3 (*PLPP3/PPAP2B*) and glycerol-3-phosphate acyltransferase 3, (*GPAT3/AGPAT9*), were negatively correlated with serum TRIG level and positively correlated with serum HDL-C level. Mutations in these genes are known to cause lipodystrophy syndromes and triglyceride storage diseases in humans and animal models^{25, 26}. Thus, the transcript levels of genes involved in the biosynthesis of triglycerides in adipose tissue may determine TRIG and HDL-C levels in serum. Branched chain amino acid (valine, leucine and isoleucine) degradation pathway genes (includes *BCAT1*, *HIBADH*, *HADHB*, *HADHA*, *BCKDHB*, and *MCCCI*), and genes involved in metabolism/degradation of other amino acids/amino acid derivatives (includes Dopamine, Putrescine, Noradrenaline and Adrenaline, Serotonin, Tryptophan degradation genes) were enriched among adipose transcripts modulated with serum TRIG and HDL-C level. Adrenaline and noradrenaline are hormones derived from amino acid tyrosine, and regulate lipolysis in adipose tissue²⁷. Expression of genes involved in degradation of adrenaline and noradrenaline, including several aldehyde dehydrogenase (*ALDH*) and alcohol dehydrogenase (ADH) isoforms, catechol-O-methyltransferase (*COMT*), and monoamine oxidase B (*MAOB*) was associated with serum triglyceride level. Expression level of *COMT* and *ALDHs* were positively correlated, while *MAOB* and *ADHs* were inversely correlated with serum TRIG level.

In concordance with a recent transcriptomic study in 54 Finnish Caucasians⁹, our study in 250 African Americans showed the most significant enrichment of genes involved in mitochondrial function ($p = 4.6 \times 10^{-17}$) including oxidative phosphorylation ($p = 8.29 \times 10^{-21}$) and fatty acid β -oxidation ($p = 1.32 \times 10^{-8}$) among transcripts associated with serum HDL-C level (Table 3, Supplementary Table 5 and 6). Genes in these pathways were positively correlated with serum HDL-C level, suggesting impaired mitochondrial function in

individuals with low serum HDL-C level. Genes in mitochondrial function related pathways were not enriched among transcripts associated with serum TRIG level (Supplementary Table 6 and Figure 1B).

We observed significant enrichment of genes in several inflammatory pathways (e.g. LPS/IL-1 mediated inhibition of RXR function, $p = 7.90 \times 10^{-6}$; Fc γ receptor-mediated phagocytosis in macrophages and monocytes, $p = 9.89 \times 10^{-6}$; integrin signaling, $p = 6.30 \times 10^{-5}$) among transcripts associated with serum TRIG level. These pathways were strongly activated among individuals with higher serum TRIG level and repressed among individuals with high serum HDL-C level (Supplementary Table 4 and Figure 1C).

3.5. Genotype dependent modulation of serum lipid associated transcripts

Expression level of transcripts can be modulated by functional genetic regulatory variants. To identify genotype-dependent variation in expression level of serum lipid-associated transcripts, we integrated genome-wide single nucleotide polymorphism (SNP) genotypes with genome-wide adipose tissue transcript expression profiles from AAGMEx cohort¹⁵ in expression quantitative loci (eQTL) analysis. Intersecting significant cis-eQTL results¹⁵ with data from transcript-serum lipid association analysis, we identified significant ($FDR < 1\%$) cis-regulatory SNPs (cis-eSNPs) for 449 serum lipid-associated transcripts in adipose tissue (Supplementary Table-7). Top cis-eGenes among serum lipid-associated transcripts in adipose tissue of AAGMEx participants includes prostate androgen-regulated mucin-like protein 1 (*PARMI*; best cis-eSNP rs3796683, $p = 3.31 \times 10^{-53}$), and major histocompatibility complex class II DP beta 1 (*HLA-DPBI*; best cis-eSNP rs6925184, $p = 2.09 \times 10^{-37}$) genes (Table-4). These cis-eSNPs are a prioritized set of SNPs that can play a causal role in the regulation of serum TRIG and HDL-C level by modulating expression of key metabolically relevant transcripts in adipose tissue. We thus mined the meta-analysis of GWAS data from the "Global Lipids Genetics Consortium" (GLGC) cohorts¹⁴ to identify association of this subset of eSNPs with serum TRIG and HDL-C levels. We obtained summary statistics for 2308 unique cis-eSNPs from GLGC-GWAS dataset. In this analysis cis-eSNPs for only five genes meet Bonferroni-corrected significance threshold ($p = 2.17 \times 10^{-5}$). On a less stringent threshold, we identified nominal association ($p < 0.001$) of cis-eSNPs of 12 genes, including apolipoprotein B (*APOB*), bestrophin 1 (*BEST1*), dehydrodolichyl diphosphate synthase (*DHDDS*), dehydrogenase/reductase SDR family member 11 (*DHRS11*), gliomedin (*GLDN*), major histocompatibility complex class II DP beta 1 (*HLA-DPBI*), lactamase beta (*LACTB*), membrane-associated ring finger C3HC4-2 (*MARCH2*), macrophage migration inhibitory factor (*MIF*), mitochondrial ribosomal protein S18B (*MRPS18B*), transmembrane protein 258 (*TMEM258*/*C11orf10*), and zinc finger protein 668 (*ZNF668*) genes with serum TRIG or HDL-C (Table-5). Among these genes, allelic effect direction of cis-eSNPs on expression of three genes (*MARCH2*, *BEST1* and *TMEM258*) matched with effect direction of these SNP alleles on serum TRIG and/or HDL-C levels in GLGC cohort. For example, expression of the *MARCH2* gene in adipose tissue is negatively associated with serum HDL-C level ($\beta = -0.35$, $p = 4.0 \times 10^{-5}$). The SNP rs3815783 is a cis-eSNP ($p = 1.50 \times 10^{-5}$) for the *MARCH2* transcript in adipose tissue and the genotype of this eSNP is also associated with serum HDL-C level ($p = 1.57 \times 10^{-10}$) in GLGC cohorts. Thus,

individuals carrying rs3815783_A allele have reduced expression of *MARCH2* in adipose tissue and higher serum HDL-C level.

4. Discussion

This systematic genome-wide transcriptomic analysis identified association of adipose tissue transcript levels with serum lipids in African Americans. Consistent with the known role of adipose tissue, the association was limited to the serum TRIG and HDL-C levels, not with total cholesterol or LDL-C. To our knowledge, this is the first genome-wide transcriptome analysis for serum lipids in African Americans that evaluates adipose tissue transcripts to identify the mechanisms of regulation of serum lipids in an under-investigated population. The majority of the serum TRIG and HDL-C associated transcripts identified in African Americans were also replicated in an independent European American cohort, supporting the physiological relevance of these transcripts in regulation of serum lipids across populations. Both cohorts consisted of generally healthy and non-diabetic individuals. As such, the analyses will not identify gene expression changes that are secondarily driven by hyperglycemia or impaired insulin action in patients with diabetes. Low HDL-C levels and high-TRIG levels are also hallmarks of insulin resistance, and thus insulin resistance could be involved in the mechanisms linking adipose tissue expression to serum lipid levels.

Studies focused on a single gene or genes in one pathway in animal or cell models have advanced our mechanistic understanding regarding the role of adipose tissue in modulating serum lipids and other cardio-metabolic phenotypes. However, consistent with the complex and heterogeneous nature of the serum lipid phenotypes, the present genome-wide analysis in cross-sectional cohorts provides evidence for the involvement of multiple pathways in determining serum TRIG and HDL-C. Consistent with two previous studies in European ancestry individuals^{9, 10}, our study in African Americans showed upregulation of immuno-inflammatory pathway genes and downregulation of oxidative phosphorylation/mitochondrial genes in individuals with low serum HDL-C levels. A study in Pima Indians identified significant negative correlation between adipose tissue macrophage markers and serum HDL-C, independent of obesity²⁸. It can be hypothesized that higher adipose tissue inflammation may cause sequestration of cholesterol in adipocyte and lead to lower serum HDL-C. Expression of transporters that are involved in movement of cholesterol from adipocyte (e.g. ABCA1) to serum may be downregulated by tissue inflammation. In our study, ABCA1 mRNA expression in adipose tissue was positively associated with HDL-C ($\beta = 0.18$, $p = 0.013$) and negatively correlated with TRIG ($\beta = -0.41$, $p = 4.83 \times 10^{-4}$) in African Americans. Compared to wild type mice, adipocyte-specific *ABCA1* knockout mouse showed significantly decreased serum HDL-C level⁵. Genetic regulation (in interaction with dietary factors) of the expression of key transcripts in adipose tissue may determine serum lipid levels; conversely, genetic regulation of transcripts in other tissues (e.g. liver) may determine serum lipid levels, including TRIG and HDL-C, and in turn can shape the adipose tissue transcriptome. Our data suggests that adipose tissue inflammation may affect serum HDL-C level, but the direction of effect or causality cannot be attributed. In fact, HDL-C has been described as a potent anti-inflammatory agent that can suppress proinflammatory cytokine production and cell adhesion molecules²⁹. Thus, an alternative explanation of our observations could be that high serum HDL-C level can limit the recruitment of adipose

tissue macrophages and repress immuno-inflammatory pathway genes. Additional studies will be required to define causal relationships between adipose tissue inflammatory pathway genes and serum HDL-C level. Concurrent analysis of gene expression in other tissues driving serum lipid levels, including liver, would have been useful to determine synergistic interaction between tissues in determining serum HDL-C and TRIG level, but ethical concerns limited us from obtaining such tissue samples from our study volunteers.

Genes involved in metabolism/degradation of amino acids/amino acid derivatives, including branched chain amino acid (valine, leucine and isoleucine; BCAA) degradation, and phenylalanine, tyrosine and tryptophan biosynthesis were enriched among genes associated with serum TRIG and HDL-C level. Individuals with high serum-TRIG and low HDL-C showed downregulation in mRNA level of enzymes involved in degradation of BCAAs, which is likely to increase the serum level of these amino acids. Herman et al (2010) demonstrated a major role for adipose tissue to catabolize BCAA and modulate circulating BCAA level³⁰. However the mechanistic link between BCAA, serum lipid level, and insulin resistance will require further study³¹. Adipocytes have the capacity to produce catecholamines (epinephrine and norepinephrine) and these are important regulators of lipolysis in adipose³². A previous study showed that the level of monoamine oxidase (MAO) enzymes is reduced by one half in the adipose tissue of obese normoglycemic subjects³³. MAO's are involved in multiple biochemical reactions, including degradation of adrenaline and noradrenaline to dihydroxymandelaldehyde. In this study, individuals with high serum-TRIG had low expression of MAOB mRNA in adipose tissue. The mRNA level of enzymes (ADH1A, and ADH1B) involved in further degradation of dihydroxymandelaldehyde to dihydroxyphenylethyleneglycol was downregulated, while catechol-O-methyl-transferase (COMT) mRNA level was upregulated in individuals with high serum-TRIG. We lack serum or adipose tissue metabolite data for AAGMEx participants, a limitation of our study. However, based on the transcript levels of enzymes in this pathway, increased accumulation of endogenous adrenaline, noradrenaline and the intermediate metabolite dihydroxymandelaldehyde in the adipose tissue in the fasting condition is likely in individuals with high serum-TRIG, which may influence the gluco-metabolic phenotype of these individuals.

Genotype variation can lead to gene expression variation, but the converse is biologically impossible. Thus, eSNPs are “causal anchors” in determination of genetically-regulated expression level of transcripts. In eQTL analysis, we integrated genome-wide SNP genotypes with transcript expression profiles from AAGMEx participants and identified significant cis-eSNPs for 449 serum lipid-associated transcripts in adipose tissue. This suggests that variations in the expression level of these transcripts are not a result of altered serum lipid levels, but are rather genetically driven by common polymorphisms that regulate expression of these transcripts in adipose tissue. Additionally, a subset of these cis-eSNPs also showed association with serum TRIG or HDL-C level in a large GWAS study in GLGC cohorts. Apolipoprotein B (APOB) is the main apolipoprotein of chylomicrons, very low density lipoproteins, and low density lipoproteins and is highly expressed in liver and small intestine as long (ApoB100) and short (ApoB48) isoforms, respectively, via RNA editing of the same transcript. The role of APOB in other tissues is not clear; however, Hass et al. (2011) showed that a serum LDL-C associated SNP rs7575840 is a cis-eSNP for the

expression of APOB mRNA in adipose tissue of European Ancestry individuals³⁴. Replicating this finding, the T-allele of rs7575840 was associated with higher expression of APOB in adipose tissue of African American individuals of our AAGMEx cohort. In our study, APOB expression in adipose tissue was positively associated with serum HDL-C level ($\beta = 0.21$, $p = 2.85 \times 10^{-6}$) and inversely associated with serum TRIG level ($\beta = -0.30$, $p = 8.38 \times 10^{-5}$). Many GLGC GWAS-identified serum HDL-C and TRIG level associated SNPs appeared as cis-eSNPs for the APOB expression in adipose tissue of AAGMEx; however, the allelic effect-direction on transcript level was not concordant with their effect-direction on serum lipid levels. Allelic effect direction of cis-eSNPs on expression of three genes (*MARCH2*, *BEST1* and *TMEM258*) matched with effect direction of these SNP alleles on serum TRIG and/or HDL-C levels in GLGC cohort. *MARCH2* is a membrane-bound E3 ubiquitin ligase, which adds ubiquitin to target lysines in substrate proteins, thereby signaling their vesicular transport between membrane compartments in endosome-to-trans-Golgi network. Roles for this gene in HDL-C metabolism are unknown; however, its expression in adipose was consistently (as measured by 3 probes) inversely correlated with serum HDL-C level. A HDL-C-associated SNP rs3815783 ($p = 1.57 \times 10^{-10}$) identified in the GLGC GWAS appears as a cis-eSNP of the *MARCH2* transcript in adipose tissue of the AAGMEx cohort ($p = 1.50 \times 10^{-5}$) and in the European Americans from the GTEx cohort ($p = 6.5 \times 10^{-6}$)³⁵. The minor allele of this SNP may increase serum HDL-C level by reducing the expression of *MARCH2* in adipose. Expression of *TMEM258* (*C11ORF10*) is positively correlated with serum-TRIG and a serum TRIG-associated SNP identified in the GLGC GWAS appears as a cis-eSNP of the *TMEM258* transcript in adipose tissue of the AAGMEx cohort. A recent study in European ancestry individuals identified *TMEM258* (*C11ORF10*) as a cis-eGene of a serum lipid-associated SNP, and a key driver in cardio-metabolic disease-associated gene expression regulatory network³⁶.

5. Conclusion

In conclusion, our data suggests that the derangement of transcript level expression of genes involved in specific biological mechanisms in adipose tissue, namely immuno-inflammatory pathways, oxidative phosphorylation, and metabolism/degradation of amino acids/amino acid derivatives can modulate serum triglyceride and HDL-cholesterol level in African Americans, as well as in other ethnicities. Expressions of serum lipid-associated transcripts are dependent on common genetic regulatory polymorphisms or cis-eSNPs, and genotypes of a subset of these eSNPs are also associated with serum TRIG or HDL-C level. Thus, genetically-mediated transcriptional regulation in adipose tissue may play a role in reducing HDL-C and increasing TRIG in serum. Further studies will be required to define molecular mechanisms of transcriptional dysregulation mediated by these SNPs and biological mechanisms modulated by the target genes of these SNPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

TRIG	Triglyceride or triacylglycerols
HDL-C	high density lipoprotein-cholesterol
LDL-C	low density lipoprotein-cholesterol
cis-eSNPs	cis-regulatory single nucleotide polymorphisms
cis-eQTLs	cis-expression quantitative trait loci
GWAS	genome-wide association studies

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Highlights

- Derangement of transcript level expression of genes in adipose tissue can modulate serum triglyceride (TRIG) and HDL-Cholesterol (HDL-C) level in African Americans.
- In adipose tissue immuno- inflammatory pathway genes were strongly activated among individuals with higher serum TRIG and low HDL-C level.
- Serum HDL-C-associated adipose tissue transcripts were enriched for genes involved in branched chain amino acid and other amino acid catabolism pathway, and mitochondrial oxidative phosphorylation.
- Expressions of serum lipid-associated transcripts in adipose tissue are dependent on common cis-regulatory genetic polymorphisms (cis-eSNPs) and a subset of these eSNPs is also associated with serum TRIG or HDL-C level.
- Genetically-mediated transcriptional regulation in adipose tissue may play a role in reducing HDL-C and increasing TRIG in serum.

Figure-1. Correlation of adipose tissue transcript level with serum lipids in AAGMEx cohort and their enrichment in salient biological pathways

A) Unsupervised hierarchical clustering was performed using PermutMatrixEN (version 1.9.3) software. Dissimilarity matrix based on Euclidean distance between each phenotype was generated using correlation coefficient values of each transcript with phenotypes and seriation under multiple fragment heuristics. This analysis included information for 2800 selected transcripts correlated with at least one lipid phenotype. The hierarchical clustering-based tree was generated using McQuitty's criteria. Intensity of red and green color in heat map indicates degree of positive and negative correlation of transcripts with phenotype, respectively. **B)** Enrichment and **C)** activation of genes in salient biological pathways among Triglyceride and HDL-cholesterol associated transcripts based on ingenuity (IPA) pathway comparison analysis are shown.

Table 1

Demographics of the African American Genetics of Metabolism and Expression (AAGMEx) cohort

Trait	N	Mean ± SD
Gender (M/F)	139/121	
Age (yrs.)	260	40.6±11.5
BMI (Kg/m ²)	260	28.9±5.6
Cholesterol, Total (mg/dL)	259	176.5±36.3
Triglycerides (mg/dL)	259	84.0±43.3
HDL Cholesterol (mg/dL)	259	56.2±15.6
VLDL Cholesterol (mg/dL)	259	16.8±8.6
LDL Cholesterol (mg/dL)	259	103.5±32.3
Fasting Glucose (mg/dl)	260	91.3±9.4
2hr Glucose (mg/dl)	259	100.7±29.6

Table 2

Summary of adipose tissue transcripts associated with serum lipid levels

Serum lipids	Number of associated transcripts							
	p<0.001				q-value<0.01*			
	Total	positively	negatively	Total	positively	negatively	Total	positively
Triglycerides	1458	907	551	1021	662	359	135	109
Total Cholesterol	21	10	11	0	0	0	0	0
HDL Cholesterol	2089	1108	981	1875	994	881	79	52
LDL Cholesterol	114	43	71	0	0	0	0	0

* q-value; FDR corrected p-value

Table 3

Enrichment of HDL-C and TRIG associated adipose tissue transcripts in biological pathways.

Canonical Pathway	p-value	
	HDL-C	TRIG
Oxidative Phosphorylation	8.29E-21	NS
Mitochondrial Dysfunction	4.60E-17	NS
Valine Degradation I	7.70E-08	9.11E-06
Ethanol Degradation II	1.32E-08	1.00E-04
Noradrenaline and Adrenaline Degradation	3.70E-06	5.45E-06
Fatty Acid β -oxidation I	1.32E-08	0.013
Dopamine Degradation	5.44E-04	5.15E-07
Serotonin Degradation	3.43E-05	1.30E-05
Complement System	1.14E-05	8.57E-05
Isoleucine Degradation I	6.77E-07	0.002
Integrin Signaling	2.00E-04	6.30E-05
Toll-like Receptor Signaling	3.15E-04	7.78E-05
IL-8 Signaling	3.72E-04	8.24E-05
Histamine Degradation	6.28E-04	9.89E-05
Tryptophan Degradation X (Mammalian, via Tryptamine)	7.64E-04	9.52E-05
Triacylglycerol Biosynthesis	2.29E-05	0.005
Oxidative Ethanol Degradation III	1.99E-04	0.002
Ketolysis	1.25E-04	0.008
TCA Cycle II (Eukaryotic)	1.78E-05	NS
Leucine Degradation I	6.40E-04	0.002
Glutaryl-CoA Degradation	3.67E-04	0.006
Acetyl-CoA Biosynthesis I (Pyruvate Dehydrogenase Complex)	9.68E-04	NS
LPS/IL-1 Mediated Inhibition of RXR Function	0.011	7.90E-06
Remodeling of Epithelial Adherens Junctions	0.018	4.92E-06
Putrescine Degradation III	0.017	5.38E-06
Fc γ Receptor-mediated Phagocytosis in Macrophages and Monocytes	NS	9.89E-06
phagosome maturation	0.013	9.89E-05
NRF2-mediated Oxidative Stress Response	0.024	1.19E-04
Hepatic Fibrosis/Hepatic Stellate Cell Activation	0.018	1.72E-04
Acute Phase Response Signaling	0.013	3.27E-04
Leukocyte Extravasation Signaling	0.036	4.80E-04
Epithelial Adherens Junction Signaling	NS	7.15E-05
Clathrin-mediated Endocytosis Signaling	0.036	5.37E-04
Role of NFAT in Regulation of the Immune Response	NS	2.73E-04
Xenobiotic Metabolism Signaling	NS	9.33E-04
CD28 Signaling in T Helper Cells	NS	4.74E-04
Type I Diabetes Mellitus Signaling	NS	6.22E-04

Pathways significant at Fisher's exact test p-value < 0.001 in Ingenuity pathway analyses (comparison) are shown. All significant pathways and genes modulated in those are presented in Supplementary Tables 4 and 5.

Table 4

Serum lipid profile associated transcripts are cis-eQTL in adipose tissue

Probe_Id	Entrez Gene_ ID	Symbol	cis-eQTL in Adipose								Association of Adipose Tissue Expression with Serum lipid profiles			
			best cis- eSNP	SNP Chr	SNP Position	A1	A2	MAF	β^*	P [*]	Triglyceride		HDL Cholesterol	
											$\beta^{\#}$	P [#]	$\beta^{\#}$	P [#]
ILMN_1656560	25849	<i>PARM1</i>	rs3796683	4	75971764	T	C	0.366	-0.58	3.31E-53	-0.24	0.002	0.18	6.05E-05
ILMN_1749070	3115	<i>HLA-DPB1</i>	rs6925184	6	33071998	G	C	0.323	0.39	2.09E-37	0.33	0.002	-0.26	5.12E-05
ILMN_1791840	10928	<i>RALBP1</i>	rs16955201	18	9537897	A	G	0.100	-0.30	2.69E-33	0.81	5.27E-05	-0.46	2.13E-04
ILMN_1664303	10553	<i>HTATIP2</i>	rs16906113	11	20384886	T	C	0.398	0.27	6.52E-33	0.42	0.003	-0.29	8.47E-04
ILMN_1725666	2967	<i>GTF2H3</i>	rs6488889	12	124144793	C	T	0.376	0.17	2.65E-32	-0.10	0.633	0.44	6.55E-04
ILMN_1722390	54108	<i>CHRA1</i>	rs4961323	8	141526231	C	T	0.258	-0.15	4.52E-32	0.60	0.023	-0.74	3.21E-06
ILMN_1665033	4883	<i>NPR3</i>	rs918381	5	32788045	C	A	0.248	0.58	4.78E-28	0.12	0.086	-0.19	8.35E-06
ILMN_1807397	4711	<i>NDUFB5</i>	rs78410864	3	179311691	A	G	0.042	-0.80	5.72E-26	-0.17	0.117	0.28	1.79E-05
ILMN_1660635	51110	<i>LACTB2</i>	rs7011101	8	71501474	C	A	0.180	0.36	1.77E-25	-0.23	0.047	0.31	1.11E-05
ILMN_1803676	55556	<i>ENOSF1</i>	rs2847153	18	661647	A	G	0.270	0.27	5.10E-23	-0.47	8.05E-04	0.24	0.005

Top 10 cis-eGenes (FDR<1%) among serum lipid associated (p<0.001) adipose tissue transcripts are shown. Full list of 449 cis-eGenes among lipid associated transcript are shown in supplementary table 7. For eQTL analysis: A1, Minor Allele; A2, Major Allele (as genotyped); MAF, Minor Allele Frequency; β^* , effect size of minor allele (A1); P^{*}, significance in additive model (in MatrixEQTL analysis), P[#] and β^* , significance and effect size of association between serum lipids and transcript expression (in regression analysis adjusted for age, gender and ancestry proportion).

Table 5

Cis-eSNPs of serum lipid level associated transcripts in adipose tissue are associated with serum lipids (triglyceride or HDL cholesterol) in GWAS meta-analysis in subjects from the "Global Lipids Genetics Consortium" (GLGC) cohorts.

SNP	Chr	Position	Association of Adipose tissue transcript level with Genotype (eQTL analysis) in AAGMEX						Gene symbol	Association of Adipose tissue transcript level with serum lipid levels in AAGMEX				Association of genotype with serum lipid levels in GLGC									
			A1		A2		MAF			β		p value*		Probe ID		Triglyceride		HDL cholesterol		Triglyceride		HDL cholesterol	
rs9438549	1	26765541	C	A	0.33	-0.13	1.83E-05	ILMN_1789535	DHDDS	0.44	1.20E-04	-0.33	2.00E-06	C	0.011	0.08	A	0.019	1.38E-04				
rs1429974	2	21300770	T	G	0.24	0.29	5.28E-09	ILMN_1664024	APOB	-0.30	8.38E-05	0.21	2.85E-06	A	0.027	4.57E-07	C	0.019	2.79E-04				
rs2337383	2	21320516	G	A	0.14	0.28	2.70E-05	ILMN_1664024						G	0.016	1.14E-04	A	0.019	3.70E-07				
rs62133262	2	21369926	C	T	0.15	0.28	8.12E-06	ILMN_1664024						C	0.015	0.01	T	0.019	6.78E-04				
rs1076832	6	30419831	A	C	0.13	0.08	2.45E-05	ILMN_1721337	MRPS18B	-1.00	1.41E-04	0.64	6.78E-05	A	0.018	1.19E-04	C	0.003	0.62				
rs9461832	6	33056384	A	G	0.31	0.39	2.54E-36	ILMN_1749070	HLA-DPB1	0.33	2.07E-03	-0.26	5.12E-05	G	0.024	7.21E-04	G	0.017	0.07				
rs6928350	6	33083197	C	G	0.39	0.27	2.78E-17	ILMN_1749070						G	0.034	4.20E-04	G	0.005	0.95				
rs3129294	6	33084671	G	T	0.26	-0.18	3.85E-07	ILMN_1749070						C	0.012	4.73E-04	C	0.003	0.36				
rs2076311	6	33145369	A	C	0.34	0.24	1.52E-13	ILMN_1749070						C	0.025	2.88E-06	A	0.007	0.09				
rs9277934	6	33153528	T	C	0.34	0.24	1.20E-13	ILMN_1749070						C	0.019	3.09E-06	C	0.002	0.88				
rs2072915	6	33162082	T	A	0.34	0.23	5.75E-13	ILMN_1749070						T	0.018	2.36E-06	T	0.000	0.83				
rs421446	6	33174783	C	T	0.40	-0.15	2.76E-06	ILMN_1749070						G	0.014	3.20E-05	A	0.001	0.71				
rs7102974	11	61560035	T	C	0.01	0.41	1.09E-05	ILMN_1786759	TMEM258	0.74	3.32E-04	-0.20	0.12	T	0.064	7.83E-04	C	0.028	0.14				
rs17764935	11	61664757	A	G	0.02	0.19	1.58E-05	ILMN_1718982	BEST1	1.39	1.38E-06	-0.33	0.07	A	0.035	1.12E-04	G	0.016	0.02				
rs2445746	15	51684968	C	T	0.46	0.19	1.11E-05	ILMN_1704376	GLDN	-0.11	0.18	0.17	4.47E-04	C	0.017	7.64E-04	C	0.001	0.80				
rs6494391	15	63406785	T	C	0.36	-0.10	1.87E-05	ILMN_1703335	LACTB	0.64	2.22E-05	-0.21	0.02	T	0.021	5.40E-04	C	0.026	1.26E-05				
rs4774476	15	63407390	T	C	0.33	0.12	9.21E-07	ILMN_1703335						C	0.009	0.08	T	0.020	6.42E-04				
rs1472631	15	63423739	C	T	0.19	0.18	3.83E-09	ILMN_1703335						A	0.012	1.62E-03	G	0.019	1.55E-06				
rs1126309	15	63429062	G	A	0.09	0.18	1.39E-05	ILMN_1703335						A	0.010	0.01	G	0.017	1.14E-05				
rs8037296	15	63516759	G	A	0.17	0.14	1.25E-06	ILMN_1703335						A	0.013	2.40E-04	G	0.017	2.61E-06				
rs1017545	15	63553994	G	A	0.34	0.10	1.82E-05	ILMN_1703335						T	0.016	1.86E-05	C	0.014	1.76E-04				
rs8050894	16	31104509	G	C	0.26	0.05	2.69E-05	ILMN_1739236	ZNF668	1.27	7.62E-05	-0.48	0.02	C	0.024	1.35E-06	G	0.018	2.30E-03				
rs12603276	17	34946547	C	T	0.40	0.16	1.01E-05	ILMN_1756701	DHRS11	-0.02	0.83	0.25	1.83E-05	T	0.010	5.46E-04	C	0.002	0.59				
rs3815783	19	8503025	T	C	0.11	-0.17	1.50E-05	ILMN_1669592	MARCH2	0.48	6.42E-04	-0.35	4.00E-05	G	0.018	4.07E-05	A	0.032	1.57E-10				

SNP	Chr	Position	Association of Adipose tissue transcript level with Genotype (eQTL analysis) in AAGMEx						Gene symbol	Association of Adipose tissue transcript level with serum lipid levels in AAGMEx				Association of genotype with serum lipid levels in GLGC					
			A1		A2		MAF	β		p value*	Probe ID	Triglyceride		HDL cholesterol		Triglyceride		HDL cholesterol	
			A1	A2	MAF	β	p value*	Probe ID		β	p value	β	p value	β	p value	β	p value		
rs1007888	22	24241101	A	G	0.24	-0.15	1.50E-05	ILMN_1807074	MIF	0.31	1.49E-02	-0.27	5.25E-04	C	0.013	6.07E-05	C	0.000	0.65
rs4822451	22	24248166	T	G	0.47	0.13	1.29E-06	ILMN_1807074					T	0.011	7.05E-04	T	0.002	0.31	

in eQTL analysis: A1, Minor Allele; A2, Major Allele (as genotyped in AAGMEx and not always on the same strand as in GLGC); MAF, Minor Allele Frequency; β , effect size of minor allele (A1); p value*, significance in additive model. Joint analysis of METABOCHIP AND GWAS data in GLGC TRIG-GWAS (Willer CJ et al. Nat. Genet. 2013) : A1, effect allele. eSNPs (at FDR<0.01) associated with TRIG or HDL-C (P<0.001) in GLGC cohorts are shown.