Relationships Between Adipose Mitochondrial Function, Serum Adiponectin, and Insulin Resistance in Persons With HIV After 96 Weeks of Antiretroviral Therapy

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Objective: Some antiretroviral therapy (ART) and HIV itself confer metabolic risk, perhaps through altered mitochondrial function and adipokines. In AIDS Clinical Trials Group study A5224s, adipose mitochondrial DNA (mtDNA) levels decreased on ART, and

electron transport chain complex I (CI) and complex IV (CIV) activity decreased. Another study found decreased serum adiponectin on ART with mtDNA mutation m.10398A>G. We hypothesized that decreased adipose tissue mitochondrial function would be associated with lower adiponectin and insulin sensitivity on ART, and m.10398G would influence these changes.

Design: Retrospective analysis of an ART-naive substudy population from A5224s.

Methods: Analyses included adipose mtDNA levels, CI and CIV activity by immunoassay, visceral adipose tissue by computed tomography, and fasting serum glucose at week 0 and week 96 of ART. Fasting insulin and adiponectin were measured from cryopreserved serum using multiplex bead array. Homeostasis model assessment-2 (HOMA2)-IR and HOMA2-%B estimated insulin resistance and β-cell function, respectively. The m.10398A>G mtDNA variant was available from existing genetic data.

Results: Thirty-seven participants had adipose biopsies at week 0 and week 96. Percent decreases in CIV activity and adiponectin were correlated (Spearman rho 0.41; $P = 0.01$); this association persisted after controlling for age, sex, body mass index, or visceral adipose tissue in single-covariate regression. HOMA2-IR correlated with decreased CIV (-0.44; $P = 0.01$) and CI (-0.34; $P = 0.05$) activity. Among 12 non-Hispanic white persons, m.10398G was associated with decreased adiponectin ($P = 0.04$).

Conclusions: Decreased adipose mitochondrial activity correlated with changes in adiponectin and glucose homeostasis on ART. Previous findings that a mtDNA mutation modulates adiponectin levels in persons with HIV were replicated.

Key Words: AIDS, mitochondria, mtDNA haplogroup, adiponectin, insulin resistance

INTRODUCTION

Insulin resistance (IR) and type-2 diabetes mellitus (diabetes) are important problems in persons with HIV infection treated with antiretroviral therapy (ART).[1,2] The pathophysiology of IR and diabetes in this setting is not well understood, but HIV infection does play a role independent of

ART.[3] Mitochondrial function also influences metabolic diseases[4,5] and is altered during HIV infection, both before and after ART initiation.[6–8] Recent data have specifically highlighted the importance of mitochondrial dysfunction in the pathogenesis of IR and diabetes in HIV-uninfected persons.[9–12] Although not considered traditional "mitochondrial toxicities," diabetes and IR among persons with HIV may also be related to mitochondrial dysfunction.[7,13,14] Older nucleoside reverse transcriptase inhibitors (NRTIs) with known adverse mitochondrial effects[7,15,16] are associated with IR[3,17–19] and diabetes[20,21] in cohort and clinical studies. Importantly, even newer NRTIs can adversely impact mitochondrial function. In AIDS Clinical Trials Group (ACTG) study A5224s, adipose tissue mitochondrial DNA (mtDNA) decreased in subjects treated with either tenofovir disoproxil fumarate (TDF), or abacavir (ABC), and mitochondrial complex I (CI) and complex IV (CIV) activity decreased significantly in persons randomized to TDF.[22]

Adiponectin is an adipose tissue-derived peptide mediator of energy balance and metabolism, including insulin sensitivity. In HIV-negative populations, low adiponectin is associated with low HDL cholesterol and high triglycerides,[23] development of diabetes,[24] and myocardial infarction.[25] Adiponectin is typically decreased in HIV infection,[26] with lean ART-treated men having levels similar to obese, insulin-resistant HIV-seronegative men.[27] In untreated HIV infection, low adiponectin is associated with higher plasma HIV RNA levels,[28] perhaps driven by increases in inflammatory cytokines such as tumor necrosis factor-α, which has been shown to suppress adiponectin in vitro.[29] With ART initiation, adiponectin concentrations increase initially,[30,31] but fall below baseline levels with the development of lipodystrophy.[31,32] Indeed, lower adiponectin is associated with subcutaneous lipoatrophy,[33] IR,[34] and subclinical cardiovascular disease[35] in ART-treated HIV-seropositive persons. These observations are consistent with reported connections between mitochondrial function and adiponectin,[36–38] thus mitochondrial dysfunction may be an important contributor to the pathogenesis of hypoadiponectinemia, with downstream effects on IR and diabetes.

Mitochondrial DNA encodes electron transport chain subunits that are critical for energy production, and mitochondrial function influences vascular health.[39] Combinations of single nucleotide polymorphisms (SNPs) within mtDNA allow for categorization of individuals into haplogroups.[40] Haplogroups influence mitochondrial function[41–44] and have been associated with HIV-related outcomes.[45–54] Diabetes-related phenotypes are associated with mtDNA variants in animal models[55] and in HIV-negative populations.[56–59] Insulin resistance has recently been associated with mtDNA variants in HIV/HCV-coinfected Spaniards[49] and in a small subgroup from an ACTG clinical trial.[60] Adiponectin levels were also associated with mtDNA variants in each of these studies, with the latter finding an association between a nonsynonymous mtDNA mutation (m.10398A>G encoding NADH dehydrogenase subunit 3) and greater decrease in adiponectin after ART initiation, suggesting that adiponectin dysregulation may be a novel mechanism by which mtDNA variation influences IR and diabetes in ART-treated persons

with HIV infection. The m.10398G variant is present in approximately 20% of European-ancestry persons, is shared across several mtDNA haplogroups, and has been of particular clinical interest because of known effects on mitochondrial function.[61,62]

ACTG study A5202 was a randomized trial comparing 2 different blinded NRTI regimens [TDF plus emtricitabine (FTC) or ABC plus lamivudine (3TC)] with open-label efavirenz or ritonavir-boosted atazanavir in previously ART-naive participants with HIV.[63,64] A5224s, a substudy of A5202, included metabolic assessments and regional fat quantitation.[65] An additional subgroup of participants agreed to undergo adipose tissue biopsies before ART (week 0) and at week 96. In these participants, adipose mtDNA levels decreased on ART, and adipose mitochondrial electron transport chain CI and CIV activity decreased predominately in those randomized to TDF/FTC-containing regimens.[22] To extend previous findings, we hypothesized that individuals with HIV on ART have a reduction in serum adiponectin and increased IR that corresponds to decreased adipose mitochondrial function, and that these changes will differ by mtDNA mutation m.10398A>G. We report findings of analyses to test this hypothesis using existing data from A5224s, and new data on fasting adiponectin and estimated IR and pancreatic β-cell function.

METHODS

Participants

ART-naive individuals aged 16 years and older with HIV-1 RNA >1000 copies/mL were included in the A5202 parent trial (NCT00118898). Exclusion criteria for the A5224s metabolic substudy were untreated hypogonadism, thyroid disease, diabetes, and the use of growth hormone, anabolic steroids, or glucocorticoids.[65] A5224s participants willing to undergo adipose biopsies were eligible for a preplanned mitochondrial substudy at selected sites. This analysis used data from participants in the mitochondrial substudy with mtDNA SNP data available from genome-wide genotyping and with suppressed HIV RNA in plasma (<50 copies/mL) at week 96. ACTG protocols A5202, A5224s, and A5128 (ACTG Human DNA Repository) were approved by institutional review boards at each study site, and participants provided written informed consent. Genetic and clinical data used in these analyses were deidentified.

Serum Laboratory Measurements

Routine fasting serum lipid and glucose quantification was performed at each site using commercial assays. Serum insulin and adiponectin levels were quantified from cryopreserved fasting serum samples collected at baseline and week 96 using multiplex bead array at the Laboratory for Clinical Biochemistry Research (University of Vermont, Colchester, VT). Homeostasis model assessment 2-IR (HOMA2-IR) and HOMA2-beta (HOMA2-%B) estimates of IR and pancreatic β-cell function, respectively, were determined using published formulas.[66]

Adipose Imaging and Quantitation

Substudy evaluations included single-slice abdominal computed tomographic (CT) scan of the abdomen at the L4–L5 level at baseline and week 96 to quantify central subcutaneous and visceral adipose tissue (VAT). CT scans were read at a central location (Tufts University, Boston, MA) by personnel blinded to participant characteristics and treatment assignment as described previously.[22,65]

Adipose Tissue mtDNA and Oxidative Phosphorylation Protein and Enzyme Activity Quantitation

Subcutaneous adipose tissue from the lower abdomen was obtained by excisional biopsy performed by an experienced physician using local anesthesia and standardized procedures. DNA was extracted from fat frozen in RNAlater using a Qiagen DNA kit. Adipose mtDNA content was measured by quantitative polymerase chain reaction, and levels of NADH dehydrogenase (CI) and cytochrome *c* oxidase (CIV) enzyme activity were determined using commercial immunoassays as described previously.[22,67]

DNA Genotyping and Mitochondrial SNP Determination

DNA was isolated from whole blood using PUREGENE (Gentra Systems Inc., Minneapolis, MN) under the ACTG Human DNA Repository (Protocol A5128). Genome-wide genotyping was performed using the Illumina 1M duo array, and genotype data underwent quality control and imputation methods described previously.[68,69] Available (directly genotyped) mtDNA SNPs were extracted to generate a variant list for each individual relative to the revised Cambridge reference sequence.[70] Because of the previous findings and known functional effects, the SNP NC_012920.1:m.10398A>G (rs2853826; designated "m.10398G") was analyzed.

Statistical Analysis

Simple proportions are used to describe demographic and genetic data. Medians and interquartile ranges are presented for continuous data. Fisher's exact or Pearson χ^2 tests and Wilcoxon rank-sum test were used for comparisons of categorical and continuous covariates, respectively. Because of the small sample size of participants with complete data available, we explored single-covariate multivariate linear regression models to determine whether associations between adipose measures and adiponectin or HOMA were sensitive to inclusion of the following covariates of potential relevance: sex and continuous age, body mass index (BMI), and VAT. SPSS Statistics Premium 24 (IBM Analytics, Armonk, NY) and Stata SE version 10.1 (StataCorp, College Station, TX) were used for statistical analyses. Because of the lack of independence between several of the outcome measures and the exploratory nature of these analyses, they were not adjusted for multiple comparisons.

RESULTS

Of 269 A5224s participants, 56 were included in the mitochondrial substudy. Of these, 47 had a baseline adipose tissue biopsy or serum sample, were coenrolled in A5128 with genetic data available and are included in baseline data presentation (Table 1). A subset of 37 of these who had a baseline adipose tissue biopsy, a second biopsy at week 96, and week 96 plasma HIV RNA <50 copies/mL were included in analyses. The Figure, Supplemental Digital Content, http://links.lww.com/QAI/B256 provides a simplified flow diagram of participant distribution from A5202 to the analysis groups presented here. Of the 47 with baseline adipose tissue or serum and genetic data, the median age was 39 years, 42 were male, 17 were of non-Hispanic white race/ethnicity, and the median CD4 T cell count was 226 cells/mm³ (Table 1). Median baseline BMI was 25.7 kg/m², HOMA2-IR was 0.95, and randomization to TDF/FTC or ABC/3TC-containing arms was evenly distributed. Baseline characteristics among the 37 participants included in final analyses were similar to the overall group (Table 1). Previous analyses confirmed no significant differences between the randomized ART arms in the parent protocol or substudy.[22]

Baseline and 96-Week Changes in Adiponectin and HOMA

Baseline values of adiponectin, HOMA2-IR, and HOMA2-%B are shown in Table 1. After 96 weeks of ART, serum adiponectin levels decreased <1%, while median increases in HOMA2-IR and HOMA2-%B were 30% and 14%, respectively (Wilcoxon sign rank $P = 0.08$ and 0.38; Table 2). Although there are no consensus cutoffs for defining IR, 7 (19%) and 9 (24%) individuals had a HOMA2-IR >2.0 at baseline or week 96, respectively. Adiponectin or HOMA measures at baseline, week 96, or their changes did not differ by randomized ART arm or NRTI (TDF/FTC vs. ABC/3TC; data not shown).

Relationships Between Adipose Tissue Mitochondrial Measures, Serum Adiponectin, and HOMA

Baseline serum adiponectin was not significantly correlated with either HOMA measure at baseline, but was negatively correlated with baseline BMI (Spearman rho = −0.35; $P = 0.02$), and was weakly positively correlated with mtDNA quantity (rho = 0.31; $P = 0.05$) and CIV activity (rho = 0.29; $P = 0.06$; data not shown) in fat. Baseline HOMA2-IR and HOMA2-%B were significantly positively correlated with baseline BMI and VAT (rho = 0.37–0.51; P value <0.01 for all).

Relative (percentage) change in adipose tissue CIV (Spearman's rho = 0.41; $P = 0.01$; Fig. 1A), but not CI (rho = 0.30; $P = 0.08$; Fig. 1B) activity was significantly positively correlated with relative change in serum adiponectin (eg, a decrease in CIV activity correlated with a decrease in adiponectin). This association remained statistically significant in individual models adjusted for single covariates age,

TABLE 1. Baseline Characteristics According to Substudy Participation and Available 96-Week Adipose Biopsies

Variable	N = 47 With Baseline Adipose Biopsies	N = 37 With Baseline and Week 96 Biopsies, and Week 96 Plasma HIV RNA <50 Copies/mL
Age (yrs)	39 (32, 45)	39 (34, 45)
Sex		
Male, N (%)	42 (89)	33 (89)
Female, N (%)	5 (11)	4 (11)
Race/ethnicity, N (%)		
Black, non-Hispanic	9 (19)	4 (11)
White, non-Hispanic	17 (36)	14 (38)
Hispanic	19 (40)	17 (46)
Other*	2 (4)	2 (5)
Randomized ART, N (%)		
ATV/r + ABC/3TC	10 (21)	6 (16)
ATV/r + TDF/FTC	13 (28)	13 (35)
EFV + ABC/3TC	12 (26)	9 (24)
EFV + TDF/FTC	12 (26)	9 (24)
Plasma HIV-1 RNA ($\log_{10}$ copies/mL)	4.7 (4.3, 5.3)	4.8 (4.3, 5.3)
$CD4^+$ T cells/mm^3	226 (70, 312)	212 (81, 288)
Fat mtDNA (copies/cell)	1211 (1015,1686)	1254 (1040, 1588)
Fat complex 1 activity (OD × 10^3/µg)	46 (34, 53)	48 (37, 53)
Fat complex 4 activity (OD × 10^3/µg)	27 (21, 34)	30 (24, 35)
Visceral fat (cm^2)	90.9 (69.7, 124.3)	93.9 (82.8, 131.9)
BMI (kg/m^2)	25.7 (21.6, 29.9)	25.7 (21.7, 30.0)
Adiponectin (ng/mL)	7077 (4877, 10,058)	7587 (4846, 9530)
Glucose (mg/dL)	86 (82, 94)	89 (82, 95)
Insulin (uU/mL)	7.4 (4.7, 12.0)	7.7 (4.9, 12.1)
HOMA2-IR	0.95 (0.59, 1.53)	0.97 (0.66, 1.54)
HOMA2-%B	97.4 (74.5, 140.8)	97.4 (77.2, 143.2)

Data are median (interquartile range) except where noted.

*Includes Asian/Pacific Islander (N = 1) and >1 reported race/ethnicity (N = 1).

%B, beta-cell; 3TC, lamivudine; ABC, abacavir; ATV/r, ritonavir-boosted atazanavir; EFV, efavirenz; FTC, emtricitabine; HOMA, homeostasis model assessment; OD, optical density; TDF, tenofovir disoproxil fumarate.

sex, or baseline BMI or VAT, with *P* values ranging from 0.01 to 0.05. By contrast, relative changes in CIV (Fig. 2A) and CI activity (Fig. 2B) in fat were significantly negatively correlated with relative change in IR measured by HOMA2-IR (rho = −0.44; *P* = 0.01 and −0.34; *P* = 0.05, respectively; eg, a decrease in CI or CIV activity correlated with an increase in HOMA2-IR measured at week 96). These associations were also robust to adjustment for sex or baseline BMI or VAT in single covariate models (*P* value range 0.01–0.03), but less so with adjustment for age (*P* = 0.11 and 0.06,

respectively). Relative changes in CIV were also significantly correlated with changes in HOMA2-%B (rho = −0.36; *P* = 0.04; data not shown). Neither baseline nor week 96 changes in HOMA2-IR and adiponectin were significantly correlated.

Mitochondrial DNA Variants, Adipose Tissue Mitochondrial Function, Serum Adiponectin, and HOMA

Given the small sample sizes of individual race/ethnicity groups, mtDNA haplogroup analyses were not feasible. Based on our previous data[60] and a priori hypothesis, and the relative frequency of m.10398G in persons of European ancestry,[62] this mtDNA SNP was analyzed separately in the subgroup with paired serum adiponectin data and self-reported non-Hispanic white race/ethnicity. Of these 12 participants, 4 with the m.10398G allele had a median absolute *decrease* in serum adiponectin of 1552 ng/mL at week 96 (median relative decrease of 25%). By contrast, 8 participants with the m.10398A allele had a median *increase* in serum adiponectin of 2555 ng/mL (median relative increase of 32%). Despite the small sample size, the between-group difference in absolute adiponectin changes was statistically significant (*P* = 0.04; Fig. 3A), and the median relative change approached significance (*P* = 0.06; Table 2 and Fig. 3B). The difference in absolute adiponectin change remained significant in single-covariate linear regression models adjusted for age, sex, baseline BMI, or VAT (*P*-value range = 0.03–0.05; data not shown). There were no statistically significant differences in CI or CIV activity, or HOMA estimates (Table 2).

DISCUSSION

This study of ART-naive clinical trial participants with relatively low diabetes risk (median HOMA2-IR<1) identified associations between adipose mitochondrial function, adiponectin, and IR after 2 years of TDF/FTC or ABC/3TC-containing ART exposure and HIV suppression. These results suggest that decreased mitochondrial function on contemporary ART regimens corresponds to metabolic profiles associated with future diabetes risk: decreased adiponectin and IR. We also observed an association between serum adiponectin and an mtDNA variant that is highly consistent with that seen in a distinct ART-naive clinical trial population.[60]

In persons without HIV, low adiponectin is associated with cardiovascular disease risk factors[23,71] and disease,[25] and with risk of type 2 diabetes.[72] Adipokines are dysregulated in persons with HIV.[26,27,73] Lean ART-treated men with HIV had low adiponectin levels similar to obese, insulin-resistant men without HIV.[27] Lower adiponectin was also seen in men with HIV compared with age-matched men without HIV in the Multicenter AIDS Cohort Study and was associated with coronary stenosis by CT angiography.[35] We did not observe significant changes in adiponectin in the overall population after 96 weeks of ART. Some earlier studies reported significant increases in adiponectin on ART,[30] but others did not.[74] Differences in ART and/or population metabolic characteristics before ART could contribute to differences in adiponectin levels between studies.

TABLE 2. Ninety-Six–Week Changes in Mitochondrial Complex I and Complex IV Activity, Serum Adiponectin, and HOMA, Overall and by m.10398A>G Among Non-Hispanic White Participants With Paired Biopsies or Serum Specimens

		Non-Hispanic White Participants	
	All Participants*	m.10398G (N = 4)	m.10398A (N = 8†)
Complex I activity (OD × 10³/µg)			
Baseline (N = 37)	48 (37, 53)	49 (37, 60)	49 (38, 55)
Week 96 (N = 36)	36 (24, 51)	37 (31, 57)	49 (35, 59)
Absolute change (N = 36)	−3.4 (−19.1, +5.7)	−4.5 (−18.2, +9.4)	+3.1 (−8.3, +8.0)
% Change (N = 36)	−9 (−41, +18)	−10 (−38, +27)	+7.5 (−14, +22)
Complex IV activity (OD × 103/µg)			
Baseline (N = 37)	30 (24, 35)	34 (27, 39)	26 (21, 34)
Week 96 (N = 36)	21 (15, 27)	24 (21, 32)	26 (18, 35)
Absolute change (N = 36)	−6.1 (−13.3, −0.75)	−7.2 (−13.3, −0.9)	−2.7 (−61, +2.7)
% Change (N = 36)	−24 (−45, −3)	−21 (−40, −3)	−11 (−21, +9)
Adiponectin (ng/mL)			
Baseline (N = 35)	7587 (4846, 9530)	6291 (5075, 9839)	6628 (3809, 8375)
Week 96 (N = 37)	7156 (3601, 9477)	5766 (3524, 8017)	9393 (3222, 12,117)
Absolute change (N = 35)	−101 (−3257, +875)	−1552 (−2849, −525)	+2555 (−527, +3953)‡
% Change (N = 35)	−1 (−37, +9)	−25 (−37, −10)	+32 (−13, +48)§
HOMA2-IR†			
Baseline (N = 35)	0.97 (0.66, 1.54)	0.88 (0.68, 1.18)	0.92 (0.84, 1.71)
Week 96 (N = 35)	1.19 (0.72, 1.65)	0.94 (0.83, 1.18)	1.03 (0.64, 1.46)
Absolute change (N = 33)	+0.16 (−0.23, +0.47)	+0.26 (−0.26, +0.41)	−0.23 (−0.25, −0.20)†
% Change (N = 33)	+30 (−22, +65)	+32 (−15, +56)	−20 (−26, +54)
HOMA2-%B†			
Baseline (N = 35)	97.4 (77.2, 143.2)	87.2 (80.3, 148.9)	132.4 (77.2, 148.1)
Week 96 (N = 35)	109.1 (82.9, 137.5)	81.9 (75.6, 94.1)	105.7 (79.4, 134.9)
Absolute change (N = 33)	+12.2 (−28.3, +29.9)	−8.4 (−73.3, +13.8)	−7.3 (−47.1, +24.2)†
% Change (N = 33)	+14 (−27, +37)	−9 (−42, +17)	+6 (−37, +37)

All data are median (interquartile range). All m.10398G vs. A Wilcoxon rank-sum $P > 0.1$ except where noted.
*Total N for each measure accounting for missing data shown for each row; see also Figure, Supplemental Digital Content, http://links.lww.com/QAI/B256.
†N with paired HOMA and m.10398A = 6.
‡$P = 0.04$ vs. m.10398G.
§$P = 0.06$ vs. m.10398G.
%B, beta-cell; HOMA, homeostasis model assessment; OD, optical density.

Data from cultured adipocytes suggest that adiponectin synthesis is dependent on mitochondrial function, with induction of mitochondrial biogenesis and palmitate-induced mitochondrial dysfunction leading to increased and decreased adiponectin synthesis, respectively.[36,37] Adiponectin also provides protection against mitochondrial dysfunction in multiple model systems,[75–77] and obesity-associated adiponectin depletion in mice led to hepatic mitochondrial dysfunction.[78] Thus, the direction of effects between adiponectin and mitochondrial function has not been fully elucidated yet.

With respect to mtDNA variation and adiponectin, a cross-sectional analysis of Spanish persons with HIV/HCV coinfection first reported an association between European mtDNA haplogroups and serum adiponectin.[49] In that study, subjects belonging to the JT clade (including persons with both m.10398A and G alleles) had significantly lower adiponectin levels while on ART than those belonging to the HV clade (including only the m.10398A allele).[49] A previous study in an earlier ACTG trial population examined short-term (24 week) changes in adiponectin and also found significant differences at baseline and after ART.[60] Non-Hispanic white persons having the m.10398G variant had significantly higher baseline adiponectin and a significantly greater decrease in adiponectin on ART, the same pattern we observed in the present analysis. The m.10398G is a nonsynonymous variant that results in a threonine to alanine amino acid change in the NADH dehydrogenase subunit 3 of CI and alters in vitro mitochondrial measures including mitochondrial matrix pH and calcium concentration.[61] It has also been studied in many human diseases and aging,[79,80] but risk association studies have been complicated and inconsistent. Almost all African-ancestry persons have the m.10398G allele,[62] since the A allele arose on the N mtDNA branch at the time of the out-of-Africa migration of modern European and Asian populations. In persons of European ancestry, ∼19% carry the G allele, and these are distributed across several major haplogroups as noted above.[62] As a result of the lack of variation of m.10398 in Africans, association analyses are not possible within African-ancestry populations. It is not known whether m.10398 allele frequency contributes to ancestry-specific disease risk, whether the variant has
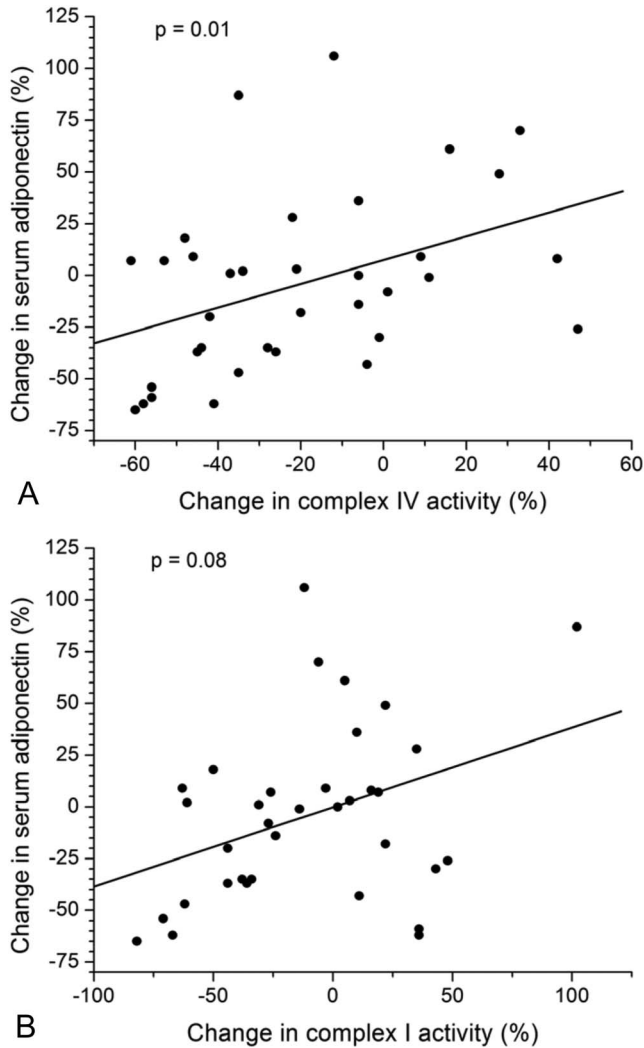
FIGURE 1. Scatterplot of relative (%) change in serum adiponectin vs. relative (%) change in adipose complex IV activity (A; Spearman's rho = 0.41; *P* = 0.01) and complex I (B; Spearman's rho = 0.30; *P* = 0.08) activity (N = 35).
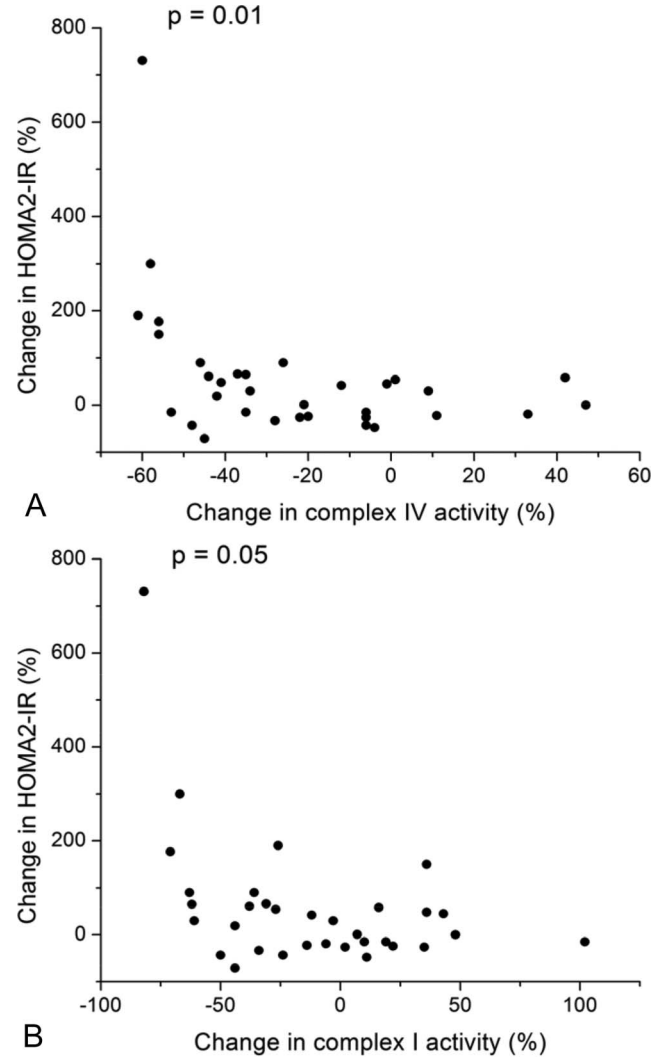

FIGURE 2. Scatterplot of relative (%) change in HOMA2-IR vs. relative (%) change in adipose complex IV (A; Spearman's rho = −0.44; *P* = 0.01) and complex I (B; Spearman's rho = −0.34; *P* = 0.05) activity (N = 33).

different de novo functional implications in African-ancestry populations, or whether interactions with other ancestral variants in mtDNA or nuclear DNA interact to modify its expression or function.

The small sample size of our study precluded haplogroup analyses, limited our capacity to adjust for potential confounders, and may have led to false or missed associations. The m.10398G allele is present in European haplogroups I, J, and K, thus a careful analysis in a larger population might better characterize whether m.10398G is a marker for other variant(s) shared across multiple haplogroups or confined to a single haplogroup. Additional limitations of this study include the fact that most of the participants were male, limiting generalizability of the findings, and the use of self-reported race/ethnicity as the basis of stratification; genetic ancestry was not determined for these analyses. Diabetes was an exclusion from A5224s,

and the overall baseline HOMA2-IR values suggest a low risk of overt diabetes. We did not account for ART changes or interruptions in our analyses, but since we only included data for participants with HIV RNA suppression at week 96, we believe this would have had minimal impact in this subgroup. We cannot yet determine causality between mitochondrial enzyme activity, adiponectin, and IR. Indeed, although correlations between changes in mitochondrial function in fat and adiponectin and IR in the periphery are compelling, the lack of direct correlation between adiponectin and HOMA2-IR or HOMA2-%B in this population suggests alternative or additional mechanisms may be contributing. These data cannot definitively answer this question.

In summary, we show for the first time associations between adipose tissue mitochondrial function, adiponectin, and insulin sensitivity in ART-treated persons with HIV,
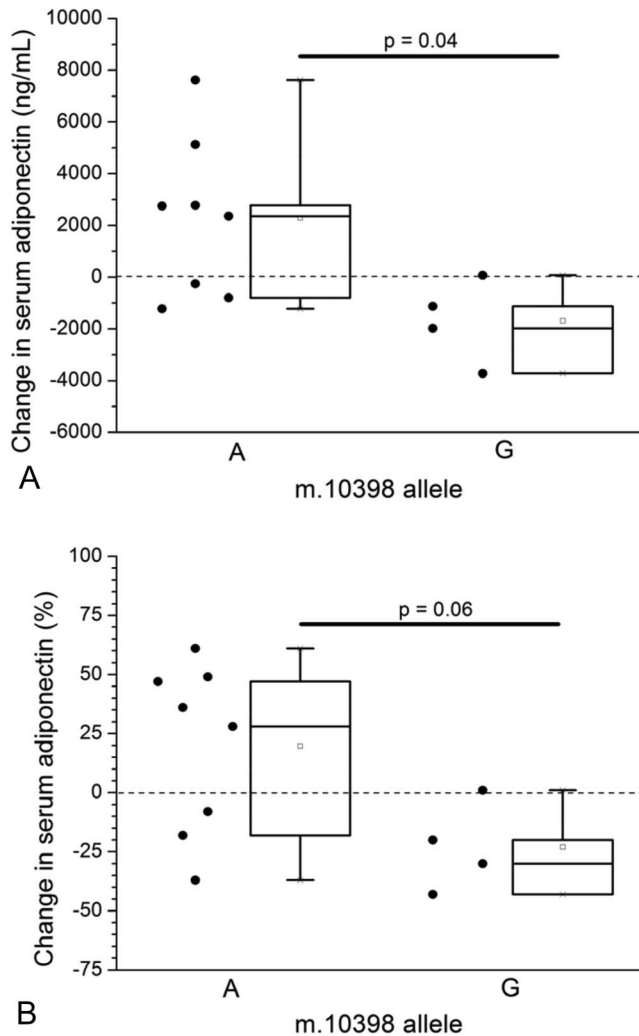
FIGURE 3. Box and dot plots of absolute (A) and relative (%) change (B) in serum adiponectin after 96 weeks of ART among participants with paired data and non-Hispanic white race/ethnicity, by m.10398A>G alleles. Boxes show median and IQR; whiskers show 95th percentiles. Wilcoxon rank-sum *P*-values are 0.04 and 0.06 for (A and B) comparisons, respectively.

which is biologically plausible. Our findings, in combination with results from the previous studies and in vitro data, support the hypothesis that impaired adipose tissue mitochondrial function in ART-treated persons with HIV decreases serum adiponectin and worsens IR. We also found that the m.10398G mtDNA SNP was associated with lower serum adiponectin levels after starting ART,[49] supporting a role for this variant in metabolic complications through an adiponectin-mediated pathway. Next steps will include more detailed mechanistic studies to characterize the mitochondria–adiponectin pathway and its metabolic effects in adipose tissue and peripherally. In addition, future clinical studies can be designed to test adiponectin-targeted and mtDNA genotype-guided therapeutics in HIV-seropositive persons initiating ART to prevent IR and diabetes.

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