

# Antiretroviral Therapy Initiation Is Associated With Decreased Visceral and Subcutaneous Adipose Tissue Density in People Living With Human Immunodeficiency Virus

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**Background.** Adipose tissue (AT) alterations are common in people living with human immunodeficiency virus (PLWH). Decreases in AT density suggest disrupted adipocyte function/hypertrophy. We assessed changes in AT density after antiretroviral therapy (ART) initiation and associations with immunometabolic parameters.

**Methods.** In a prospective randomized clinical trial of ART initiation, L4–L5 abdominal CT scans measured subcutaneous AT (SAT) and visceral AT (VAT) area and density in treatment-naïve PLWH randomized to tenofovir-emtricitabine plus ritonavir-boosted atazanavir, ritonavir-boosted darunavir, or raltegravir. Linear regression models compared week 0 and week 96 levels, and 96-week changes, in SAT and VAT density (in Hounsfield units [HU]). Spearman correlations assessed relationships between AT density and immunometabolic parameters.

**Results.** Of the 228 participants, 89% were male and 44% were white non-Hispanic. Median age was 36 years, baseline HIV-1 RNA was 4.6 log<sub>10</sub> copies/mL, and CD4<sup>+</sup> T-cell count was 344 cells/μL. Over 96 weeks, SAT and VAT HU decreased significantly in all arms. Less dense week 96 SAT and VAT density correlated with higher high-density lipoprotein (HDL) cholesterol and adiponectin ( $r = 0.19$ – $0.30$ ) levels and lower interleukin 6, non-HDL cholesterol, triglyceride, leptin, and homeostatic model assessment of insulin resistance ( $r = -0.23$  to  $-0.68$ ) levels at week 96 after adjusting for baseline CD4<sup>+</sup> T-cell count, HIV-1 RNA, and baseline AT area.

**Conclusions.** Following virologic suppression, lower SAT and VAT density was associated with greater plasma measures of systemic inflammation, lipid disturbances, and insulin resistance independent of AT area, suggesting that changes in AT density with ART may lead to adverse health outcomes independent of AT quantity.

**Clinical Trials Registration.** NCT00851799.

**Keywords.** fat density; HIV; inflammatory biomarkers; antiretroviral therapy; fat gain.

Adipose tissue (AT) disturbances including lipohypertrophy and obesity are common in the setting of treated and untreated human immunodeficiency virus (HIV). Lipohypertrophy or visceral fat accumulation in the abdomen is well described following antiretroviral therapy (ART) initiation, with changes not limited to older ART regimens [1–3]. Abdominal subcutaneous AT (SAT) and visceral AT (VAT) quantities are closely associated with the development of cardiometabolic abnormalities [4, 5],

and increases in visceral fat are associated with greater mortality in people living with HIV (PLWH) [6].

Obesity is associated with changes in AT quality that arise from alterations in tissue vascularity, lipid content, and fibrosis [7]. During weight gain, adipocytes become larger, engorged with lipids, and less dense [8, 9]. With continued adipocyte expansion, inflammation occurs and compensatory mechanisms such as fibrosis are employed to limit further expansion [10]. AT fibrosis and inflammation may be triggered by multiple processes, including infection or tissue injury, and are associated with increased AT density and altered adipocyte function [11]. AT quality can be indirectly assessed by quantifying AT density (in Hounsfield units [HU]) on computed tomography (CT). We have previously shown in PLWH that density from SAT on abdominal CT scans correlates with biopsy-quantified adipocyte size, the gold standard for assessment of AT quality [12]. Importantly, decreases in AT density may be associated with

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cardiovascular risk independent of AT quantity [7, 13], and longitudinal decreases in AT density have been independently associated with increased incidence of and adverse changes in cardiovascular disease (CVD) risk factors [14].

Although AT disturbances have been linked to the use of thymidine analogue nucleoside reverse transcriptase inhibitors (NRTIs) and older protease inhibitors (PIs), weight gain and metabolic complications may be a side effect of all ART, including integrase strand transfer inhibitors (INSTIs) [1, 15]. Limited studies have explored the relationship between AT quality (density) and ART [12]. In this study, we aimed to assess the effects of modern ART initiation on VAT and SAT density, and to determine relationships between AT density and circulating inflammatory and metabolic biomarkers.

## METHODS

A5260s was the cardiometabolic substudy of AIDS Clinical Trial Group (ACTG) protocol A5257, in which treatment-naïve PLWH aged  $\geq 18$  years with HIV type 1 (HIV-1) RNA  $\geq 1000$  copies/mL were randomized in an open-label fashion to receive tenofovir disoproxil fumarate–emtricitabine (TDF/FTC) plus raltegravir (RAL), ritonavir-boosted atazanavir (ATV/r), or ritonavir-boosted darunavir (DRV/r) for at least 96 weeks. The primary objectives of A5260s were to compare changes in carotid intima media thickness and endothelial function between those initiating the randomized regimens [16]. Secondary objectives assessing changes in immune activation markers [17] and body composition [1] have also been published. For this analysis, a subset of participants who had CT data available for interpretation and were virally suppressed (HIV-1 RNA  $< 50$  copies/mL) at week 96 were included. The parent study and substudy were approved by the institutional review boards at participating institutions, and participants provided written informed consent prior to initiation of study procedures.

### Body Composition Measures

Substudy evaluations used for this analysis occurred at weeks 0 and 96. Participants underwent single-slice CT scan at the L4–L5 level to quantify VAT, SAT, and total AT area. Scans were standardized and initially centrally read by blinded personal at LA Biomed (Torrance, California). For this analysis, scans were reinterpreted for SAT and VAT area (cm<sup>2</sup>) and density (HU) at the University of Colorado Anschutz Medical Center by a reader blinded to clinical data and randomized treatment arm, using specialized body composition software (Excelis Visual Information Systems, Boulder, Colorado). Ten percent of images were reanalyzed to assure reproducibility  $\leq 5\%$ . In keeping with reading center standards and similar to other cohorts, AT was identified by a mean attenuation of  $-190$  to  $-30$  HU (more negative = lower density) [13]. VAT was distinguished from SAT by tracing along the fascial plane of the internal abdominal wall [18]. Participants with  $> 1$  scan result at a given study week had their measures averaged for that study week.

### Laboratory Assessment

Blood samples (fasting for  $\geq 8$  hours) were collected at study entry prior to ART initiation and after 96 weeks of treatment. All blood samples were sent to core laboratories without prior thaw for processing. Biomarkers were measured at the University of Vermont Laboratory for Clinical Biochemistry Research (Burlington, Vermont) on batched plasma stored at  $-70^{\circ}\text{C}$ . Tests included high-sensitivity C-reactive protein (hs-CRP) by nephelometry (Siemens BNII Nephelometer, Siemens Healthcare, Indianapolis, Indiana), interleukin 6 (IL-6) using enzyme-linked immunosorbent assay, and D-dimer measured with immunoturbidimetric methods. The lipoproteins GlycA and GlycB were quantified by nuclear magnetic resonance spectroscopy at LipoScience (Raleigh, North Carolina). Serum insulin was measured by commercial testing at Quest Diagnostics by radioimmunoassay. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) index. The HOMA-IR value was obtained using the following calculation:  $\log_{10}(\text{HOMA-IR}) = \log_{10}(\text{glucose [mg/dL]} \times \log_{10}(\text{insulin [IU]} / 405))$ .

### Statistical Analysis

Linear regression models compared changes in SAT and VAT density between treatment arms; all models were adjusted for corresponding baseline AT area. Additional models adjusted for potential confounders (age, race, smoking status, Framingham risk score strata, baseline HIV-1 RNA, and baseline CD4<sup>+</sup> T-cell count). Sex was not included in the initial models as it was addressed in exploratory models (see below). Treatment arms were compared using reverse Helmert contrasts. ATV/r was first compared to DRV/r with a 2.5% significance level. If not significant, the PI/r arms were pooled together and compared with the RAL arm; otherwise, pairwise comparisons were performed. Signed-rank test was used to compare changes in SAT and VAT density. Linear regression models were also used to examine whether AT density changes differed by sex, with results shown in absolute and percentage changes. Spearman correlations evaluated associations between AT density and circulating inflammatory and metabolic biomarkers. Partial Spearman correlations further adjusted for corresponding AT area and other baseline covariates (CD4<sup>+</sup> T-cell count, HIV-1 RNA, and all combined). All treatment arm comparisons were assessed with a 2.5% type I error rate; all other comparisons used a 2-sided 5% significance level.

## RESULTS

### Baseline Characteristics

Of the 334 participants who entered A5260s from 26 ACTG sites in the United States between June 2009 and April 2011, 228 had paired CT scans at week 0 and week 96 (Table 1). Baseline characteristics of participants included in the analysis did not differ from the entire population. Overall, 89% were male, 44% were white non-Hispanic, and 21% were Hispanic. The median baseline age was 36 years, CD4<sup>+</sup> T-cell count 344 cells/ $\mu\text{L}$ ,

**Table 1. Baseline Characteristics by Randomized Treatment Group**

| Characteristic  | ATV/r (n = 75)   | Raltegravir (n = 79) | DRV/r (n = 74)   | Total (N = 228)  |
|---|------------------|----------------------|------------------|------------------|
| Age, y, median (IQR)                                  | 38 (31–45)       | 36 (27–44)           | 35 (28–46)       | 36 (28–45)       |
| Sex, male, No. (%)                                    | 68 (91)          | 70 (89)              | 66 (89)          | 204 (89)         |
| Race/ethnicity, No. (%)                               |                  |                      |                  |                  |
| White non-Hispanic                                    | 38 (51)          | 32 (41)              | 31 (42)          | 101 (44)         |
| Black non-Hispanic                                    | 23 (31)          | 20 (25)              | 25 (34)          | 68 (30)          |
| Hispanic  | 13 (17)          | 19 (24)              | 15 (20)          | 47 (21)          |
| History of smoking, No. (%)                           | 47 (63)          | 41 (52)              | 40 (54)          | 128 (56)         |
| HIV-1 RNA, log <sub>10</sub> copies/mL, median (IQR)  | 4.5 (3.9–5.1)    | 4.5 (4.1–5.1)        | 4.6 (4.1–5.0)    | 4.5 (4.0–5.1)    |
| CD4 <sup>+</sup> T-cell count, cells/μL, median (IQR) | 367 (238–473)    | 331 (175–430)        | 339 (182–459)    | 344 (193–455)    |
| BMI, kg/m <sup>2</sup> , median (IQR)                 | 24.8 (22.4–29.2) | 24.4 (22.2–27.7)     | 24.3 (22.0–27.3) | 24.5 (22.2–27.8) |

Abbreviations: ATV/r, ritonavir-boosted atazanavir; BMI, body mass index; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; IQR, interquartile range.

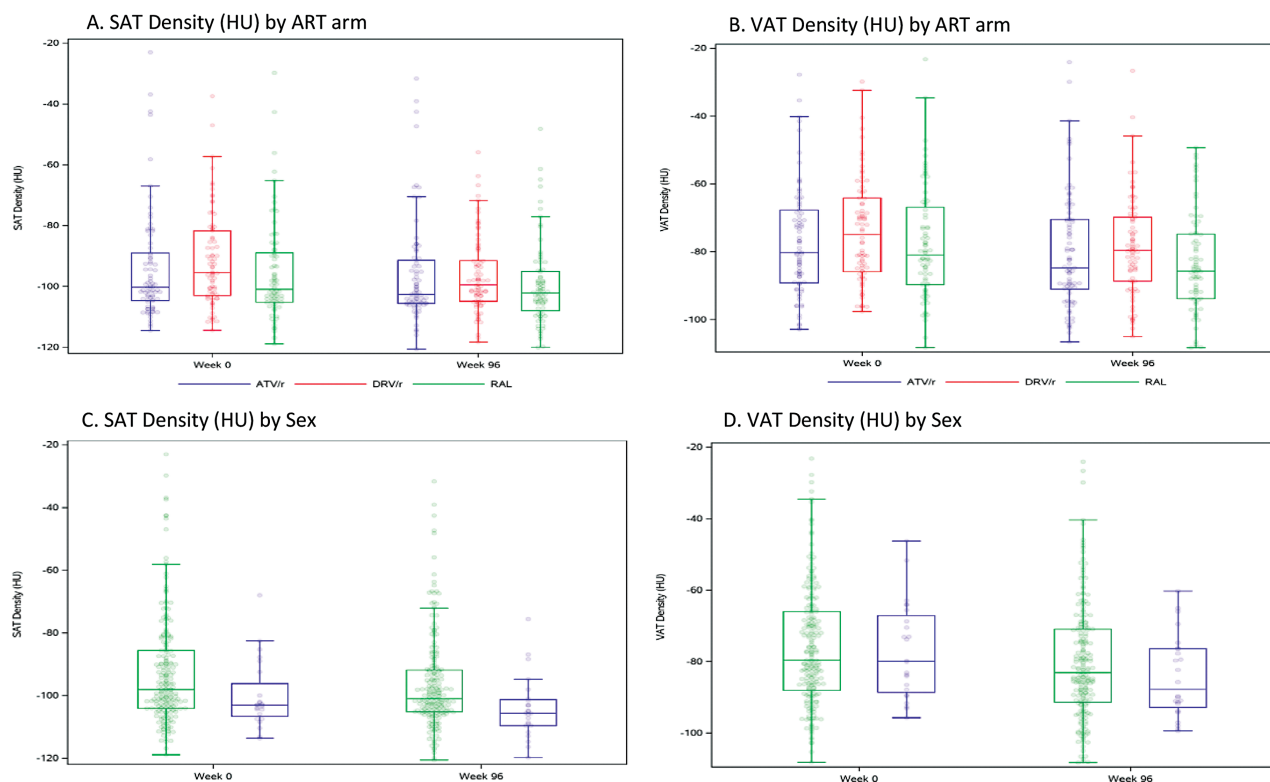
HIV-1 RNA 4.5 log<sub>10</sub> copies/mL, and body mass index (BMI) 24.5 kg/m<sup>2</sup>. At week 96, the median BMI was 25.3 (interquartile range [IQR], 23.1–29.2) kg/m<sup>2</sup>. Overall median SAT density at week 0 was –98.97 HU and was not different between treatment arms ( $P = .15$ ); median VAT density was –79.81 HU, and also did not differ between treatment arms ( $P = .28$ ) (Figure 1).

### Changes in Fat Density

#### Overall Changes and Changes by Treatment Arm

Significant decreases in SAT and VAT density over 96 weeks were observed in all treatment arms ( $P < .01$ ). Overall median

absolute change was –1.9 (IQR, –6.9 to –2.2) HU for SAT density, and –3.2 (IQR, –8.9 to 1.9) HU for VAT density. SAT and VAT changes by treatment arm are shown in Table 2. Although participants on the DRV/r treatment arm had numerically larger decreases in SAT density (percentage change estimate for ATV/r vs DRV/r: 2.2% [95% confidence interval [CI], –2.3% to 6.8%]; RAL vs DRV/r: –0.2% [95% CI, –4.6% to 4.3%];  $P = .26$ ), and participants on RAL had larger decreases in VAT density (percentage change for ATV/r vs RAL: 4.4% [95% CI, –1.8% to 10.7%]; DRV/r vs RAL: 2.9% [95% CI, –3.7% to 8.9%];  $P = .51$ ), the absolute change in SAT and VAT density did not



**Figure 1.** Changes in adipose tissue (AT) density by anatomic depot measured by computed tomographic scan of the abdomen. Subcutaneous AT (A) and visceral AT (B) at week 0 and week 96, by antiretroviral agent; subcutaneous AT (C) and visceral AT (D) at week 0 and week 96, by sex. Abbreviations: ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; HU, Hounsfield units; RAL, raltegravir; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

**Table 2. Absolute and Percentage Changes in Adipose Tissue Density From Week 0 to Week 96, by Treatment Group<sup>a</sup>**

| Change                    | Absolute Change, Median (IQR) | Percentage Change, Median (IQR) | PValue <sup>b</sup> |
|---------------------------|-------------------------------|---------------------------------|---------------------|
| <b>Change in SAT (HU)</b> |                               |                                 |                     |
| ATV/r                     | -1.6 (-5.8 to 3.5)            | -1.5 (-6.2 to 3.4)              | .05                 |
| Raltegravir               | -2.0 (-7.1 to 2.2)            | -2.0 (-6.7 to 2.2)              | .01                 |
| DRV/r                     | -3.2 (-10.5 to 1.0)           | -3.2 (-14.6 to 1.0)             | <.001               |
| Total                     | -1.9 (-6.9 to 2.2)            | -1.9 (-7.9 to 2.1)              | <.001               |
| <b>Change in VAT (HU)</b> |                               |                                 |                     |
| ATV/r                     | -2.3 (-6.5 to 1.5)            | -2.9 (-9.0 to 1.8)              | .004                |
| Raltegravir               | -4.7 (-10.0 to 1.9)           | -6.2 (-12.6 to -2.7)            | <.001               |
| DRV/r                     | -4.0 (-10.5 to 3.0)           | -4.6 (-51.6 to -4.1)            | .003                |
| Total                     | -3.2 (-8.9 to 2.0)            | -3.7 (-12.6 to 2.7)             | <.001               |

Abbreviations: ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; HU, Hounsfield units; IQR, interquartile range; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

<sup>a</sup>Adjusted for baseline adipose tissue area.

<sup>b</sup>P value represents change within treatment.

significantly differ between treatment arms after adjustment for covariates.

Female sex and higher baseline HIV-1 RNA were independently associated with greater declines in SAT density, after adjustment for covariates (Table 3). Only baseline HIV-1 RNA was independently associated with greater declines in VAT density. Differences in VAT or SAT changes by ART type were not seen ( $P = .13$ ), though variability was high.

#### Changes by Sex

At week 0, differences in SAT density were observed by sex, with men having denser SAT than women (median, -98.0 [IQR, -104.1 to -85.7] vs -103.1 [IQR, -106.6 to -96.2], respectively;

between-group  $P = .03$ ), but not VAT (median, -79.7 [IQR, -88.7 to -66.1] for men vs -79.9 [IQR, -88.6 to -67.1] for women;  $P = .95$ ; Figure 1). Decreases in SAT and VAT density ( $P < .001$ ) were observed for both sexes, with women having slightly larger decreases in VAT density (Table 4). In linear regression models adjusting for baseline SAT area, percentage change in SAT density differed somewhat by sex ( $P = .06$ ), suggesting larger percentage decreases in SAT density for women even after adjusting for treatment arm and other covariates. Similar results were observed for changes in VAT ( $P = .26$ ).

#### Associations Between Biomarkers and Changes in Fat Density

Pre-ART (week 0), lower SAT density correlated ( $P < .05$ ) with higher triglyceride, non-high-density lipoprotein (HDL) cholesterol, and leptin levels, higher triglyceride-to-HDL ratio (a marker of insulin resistance), and lower adiponectin concentrations, even after adjusting for baseline CD4<sup>+</sup> T-cell count, HIV-1 RNA, and SAT area. Similar associations for VAT were observed at week 0 (Table 5) after adjusting for baseline CD4<sup>+</sup> T-cell count, HIV-1 RNA, and VAT area. No significant baseline associations were seen between SAT or VAT density and hs-CRP, IL-6, or D-dimer levels. After 96 weeks of ART/during virologic suppression, lower SAT density correlated with higher non-HDL cholesterol and triglyceride levels, triglyceride-to-HDL ratio, and HOMA-IR and with lower adiponectin and HDL cholesterol levels, with similar findings for VAT density. Less dense VAT and SAT density both correlated with higher IL-6 and hs-CRP levels at week 96 (Table 5).

After adjusting for baseline CD4<sup>+</sup> T-cell count, HIV-1 RNA, and SAT area, absolute changes in SAT density correlated with absolute changes in non-HDL cholesterol ( $r = -0.1$ ;  $P = .05$ ), triglycerides ( $r = -0.2$ ;  $P = .004$ ), adiponectin ( $r = 0.2$ ;  $P < .001$ ),

**Table 3. Associations With Changes in Subcutaneous Adipose Tissue and Visceral Adipose Tissue Density<sup>a</sup>**

| Characteristic   | Changes in SAT       |        |                        |        | Changes in VAT       |        |                        |         |
|--|----------------------|--------|------------------------|--------|----------------------|--------|------------------------|---------|
|  | Absolute Change (SE) | PValue | Percentage Change (SE) | PValue | Absolute Change (SE) | PValue | Percentage Change (SE) | P Value |
| Female sex <sup>b</sup>                                | -4.8 (2.4)           | .05    | -5.2 (2.9)             | .08    | -4.0 (2.4)           | .10    | -5.9 (3.8)             | .12     |
| Black non-Hispanic <sup>c</sup> race                   | -0.8 (1.6)           | .63    | -2.3 (2.0)             | .25    | 0.7 (1.8)            | .69    | 1.0 (2.8)              | .73     |
| Hispanic ethnicity <sup>c</sup>                        | -1.9 (1.8)           | .31    | -2.2 (2.2)             | .32    | -3.5 (1.9)           | .07    | -4.6 (3.1)             | .14     |
| Age, y   | -0.1 (0.1)           | .16    | -0.1 (0.1)             | .27    | -0.1 (0.1)           | .25    | -0.1 (0.1)             | .51     |
| Baseline HIV-1 RNA, log <sub>10</sub> copies/mL        | -2.3 (1.1)           | .04    | -2.6 (1.4)             | .06    | -2.7 (1.2)           | .02    | -4.2 (1.9)             | .03     |
| Baseline CD4 <sup>+</sup> T-cell count, cells/ $\mu$ L | -0.0 (0.0)           | .55    | 0.0 (0.0)              | .55    | 0.0 (0.0)            | .68    | 0.0 (0.0)              | .89     |
| PI-based regimen <sup>d</sup>                          | 1.3 (1.4)            | .34    | 1.5 (1.7)              | .39    | 2.4 (1.5)            | .11    | 3.8 (2.4)              | .11     |
| No smoking history <sup>e</sup>                        | 2.4 (1.4)            | .07    | 3.2 (1.7)              | .07    | 1.8 (1.4)            | .23    | 3.1 (2.3)              | .19     |

Results of linear regression are presented as  $\beta$  estimates.

Abbreviations: HIV-1, human immunodeficiency virus type 1; PI, protease inhibitor; SAT, subcutaneous adipose tissue; SE, standard error; VAT, visceral adipose tissue.

<sup>a</sup>Adjusted for baseline SAT and VAT area.

<sup>b</sup>Male sex as reference.

<sup>c</sup>White non-Hispanic as reference.

<sup>d</sup>Raltegravir-based regimen as reference.

<sup>e</sup>Smoking history as reference.

**Table 4. Absolute and Percentage Changes in Adipose Tissue Density From Week 0 to Week 96, by Sex<sup>a</sup>**

| Change                    | Absolute Change, Median (IQR) | Percentage Change, Median (IQR) | PValue <sup>b</sup> |
|---------------------------|-------------------------------|---------------------------------|---------------------|
| <b>Change in SAT (HU)</b> |                               |                                 |                     |
| Male                      | -1.9 (-7.4 to 2.3)            | -1.9 (-9.3 to 2.2)              | <.001               |
| Female                    | -1.6 (-6.8 to 1.3)            | -1.5 (-6.5 to 1.3)              | .05                 |
| Total                     | -1.9 (-6.9 to 2.2)            | -1.9 (-7.9 to 2.1)              | <.001               |
| <b>Change in VAT (HU)</b> |                               |                                 |                     |
| Male                      | -2.9 (-8.7 to 2.3)            | -3.4 (-12.4 to 3.0)             | <.001               |
| Female                    | -4.1 (-11.7 to -1.8)          | -5.2 (-14.3 to -2.1)            | <.001               |
| Total                     | -3.2 (-8.9 to 2.0)            | -3.7 (-12.6 to 2.7)             | <.001               |

Abbreviations: HU, Hounsfield units; IQR, interquartile range; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

<sup>a</sup>Adjusted for baseline adipose tissue area.

<sup>b</sup>P value represents change within sex.

and leptin ( $r = -0.4$ ;  $P < .001$ ) levels, and triglyceride-to-HDL ratio ( $r = -0.2$ ;  $P = .004$ ). After adjusting for baseline CD4<sup>+</sup> T-cell count, HIV-1 RNA, and VAT area, absolute changes in VAT density correlated with absolute changes in oxidized LDL ( $r = -0.1$ ;  $P = .03$ ), HDL cholesterol ( $r = -0.2$ ;  $P = .01$ ), non-HDL cholesterol ( $r = -0.2$ ;  $P = .03$ ), triglycerides ( $r = -0.3$ ;  $P < .001$ ), adiponectin ( $r = 0.3$ ;  $P < .001$ ), and leptin ( $r = -0.4$ ;  $P < .001$ ) levels, and triglyceride-to-HDL ratio ( $r = -0.2$ ;  $P < .001$ ).

## DISCUSSION

In this large, prospective, randomized trial of ART initiation, both SAT and VAT density decreased after 96 weeks of ART, with women having larger decreases in VAT and SAT density in all models. The greatest declines in VAT density occurred

with RAL, followed by darunavir then atazanavir, though variability was high, and these between-arm subset comparisons did not reach statistical significance (nor were they powered to do so). Importantly, both pre- and post-ART, lower AT density correlated with greater disruptions of lipid and insulin-glucose metabolism. Lower AT density on suppressive ART was also associated with higher markers of systemic inflammation (hs-CRP and IL-6) and lower adiponectin and higher leptin levels, indicating poorer adipose tissue function and worse cardiometabolic and inflammatory profiles.

Greater quantity of abdominal AT is associated with greater metabolic dysregulation, including hyperglycemia, hypertriglyceridemia, and low HDL cholesterol [4, 19], and elevated risk of CVD and all-cause mortality among PLWH, even after adjustment for additional risk factors [6, 20]. However, assessments of AT quality/function (including noninvasive assessment of AT quality via CT, as presented here) are fairly novel, with few prior studies reporting results [7, 14, 21–23] and the implications of changes in AT density less well understood. Data from PLWH are particularly scant. We previously reported, using data from an older ACTG ART initiation trial, A5224s, that CT-measured SAT and VAT density decreased following ART initiation, while AT areas increased [12]. Additionally, CT SAT density correlated with biopsy-quantified SAT adipocyte size [12], validating CT AT density as an accurate, noninvasive measure of adipocyte size in PLWH, and opening the door for explorations of clinical implications similar to those explored in this analysis.

We have previously shown that higher baseline HIV-1 RNA levels are the main determinant of VAT gains on ART [12]. Here we extend these observations and show that higher baseline

**Table 5. Partial Correlation Between Adipose Tissue Density and Inflammatory and Metabolic Biomarker Levels<sup>a</sup>**

| Biomarker                             | Week 0           |        |                  |        | Week 96          |        |                  |        |
|---------------------------------------|------------------|--------|------------------|--------|------------------|--------|------------------|--------|
|                                       | SAT Density (HU) |        | VAT Density (HU) |        | SAT Density (HU) |        | VAT Density (HU) |        |
|                                       | Estimate         | PValue | Estimate         | PValue | Estimate         | PValue | Estimate         | PValue |
| hs-CRP (log <sub>10</sub> μg/mL)      | 0.08             | .22    | 0.10             | .13    | -0.10            | .13    | -0.13            | .05    |
| IL-6 (log <sub>10</sub> pg/mL)        | 0.05             | .49    | 0.09             | .18    | -0.16            | .02    | -0.21            | .001   |
| D-dimer (log <sub>10</sub> μg/mL)     | 0.05             | .50    | 0.04             | .52    | -0.04            | .57    | -0.11            | .12    |
| Oxidized LDL (mg/dL)                  | -0.12            | .07    | -0.05            | .42    | -0.09            | .18    | -0.15            | .02    |
| LDL cholesterol (mg/dL)               | -0.10            | .13    | -0.10            | .14    | -0.10            | .13    | -0.09            | .20    |
| HDL cholesterol (mg/dL)               | 0.10             | .15    | 0.03             | .61    | 0.18             | .02    | 0.21             | .002   |
| Non-HDL cholesterol (mg/dL)           | -0.16            | .02    | -0.13            | .06    | -0.22            | .001   | -0.23            | <.001  |
| Triglycerides (mg/dL)                 | -0.15            | .03    | -0.12            | .07    | -0.29            | <.001  | -0.33            | <.001  |
| Triglyceride-to-HDL ratio (mg/dL)     | -0.15            | .02    | -0.12            | .07    | -0.28            | <.001  | -0.32            | <.001  |
| HOMA-IR (log <sub>10</sub> )          | -0.07            | .32    | 0.01             | .91    | -0.17            | .01    | -0.24            | <.001  |
| Adiponectin (log <sub>10</sub> ng/mL) | 0.14             | .04    | 0.21             | .001   | 0.19             | .004   | 0.24             | <.001  |
| Leptin (log <sub>10</sub> pg/mL)      | -0.20            | .002   | -0.25            | <.001  | -0.54            | <.001  | -0.42            | <.001  |

Results of linear regression are presented as β estimates.

Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HU, Hounsfield units; IL-6, interleukin 6; LDL, low-density lipoprotein; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

<sup>a</sup>Adjusted for CD4 T-cell count (μL), human immunodeficiency virus type 1 RNA (log<sub>10</sub> copies/mL), and baseline SAT/VAT area (cm<sup>2</sup>).

HIV-1 RNA was also associated with greater declines in SAT and VAT density, suggesting that that baseline HIV disease severity may directly and persistently affect AT function. The mechanism underlying this observed relationship cannot be definitively determined in this study, but may be due to HIV-related factors such as the presence of HIV-infected immune cells in AT, enhancing local inflammation and causing disruption of AT function (similar to other inflammatory diseases) [24, 25]. Of note, our finding is in line with prior laboratory studies showing that the circulating HIV proteins vpr, nef, and tat mediate detrimental effects on adipose tissue health [26–29]. AT may also represent an important HIV reservoir, with greater HIV-1 RNA in CD4<sup>+</sup> T cells from AT compared to peripheral blood, and greater T-cell activation observed among AT-resident vs circulating immune cells [30]. As such, ART penetration into AT may be an important factor both in minimizing HIV AT reservoir size and optimizing AT function on suppressive ART. In vitro studies have shown that the INSTIs dolutegravir and elvitegravir penetrate AT, whereas NRTI penetration may be more limited, and RAL and bictegravir have not yet been studied in this capacity [31]. Future mechanistic studies will need to be carefully designed to unravel the complex relationship between adipocyte metabolic activity, ART penetration into AT and the impact on the AT HIV reservoir, and the subsequent effects on fat quantity and quality.

Women in our study had larger decreases in SAT density than men following ART initiation. Sex differences in AT density, adipocyte size, and adipokine expression have previously been reported [32, 33]. Progression to obesity is associated with adipocyte hypertrophy in both women and men, and adipocyte hyperplasia (where density remains stable) only in women [34]. Mean adipocyte size is larger in men than women, even after adjusting for BMI [33]. This may reflect reduced capacity of men to expand their SAT depots compared to women, a known sex difference believed to support pregnancy, but could have cardiometabolic implications. Supporting this is the finding that adipocyte size is a major predictor of leptin and adiponectin messenger RNA levels [33]. As the number of women in this study was small, we cannot discern whether greater decreases in AT density on ART contribute to the observed sex differences in cardiometabolic outcomes among PLWH. However, as lower AT density was associated with greater systemic inflammation and adipocytokine disturbances while on suppressive ART in this study, potentially greater cardiometabolic implications for women are suggested by our data.

Consistent with our hypothesis, lower abdominal SAT and VAT density were associated with greater disruptions of inflammatory and metabolic biomarker levels in this cohort. Several potential mechanisms may explain this finding: lower AT density is an indicator of larger adipocytes with larger lipid droplets (reflecting impaired adipogenesis, as seen in obesity), which has been associated with disrupted metabolic activity and

adipokine secretion [35–37]. We hypothesized that RAL might lead to more favorable effects on AT quality (density) compared with the PI arms, given the minimal in vitro effects of RAL on adipocyte differentiation [38]; however, we observed no statistically significant differences in AT density by treatment arm, highlighting the importance of in vivo confirmation of in vitro, hypothesis-generating data. The effects of the INSTI drug class on body composition and metabolism is an area of ongoing research, including recent reports of increased weight gain following switch to INSTI [39–41], but additional studies are needed to investigate the full cardiometabolic impact of these agents. There is a correlation between higher markers of insulin resistance (HOMA-IR and triglyceride-to-HDL ratio) and SAT density at week 96, but most participants (75%) did not have significant insulin resistance at week 96. Taken together, the adverse biomarker profile seen with lower AT density on suppressive ART may provide insight into the pathophysiological associations between hypertrophied adipocytes and cardiometabolic risk factors in PLWH.

Few studies in humans have previously described relationships between AT density and immunometabolic biomarkers [13, 14]. In a large, cross-sectional study from the Framingham Heart Study Third Generation cohort, lower abdominal SAT and VAT density were associated with lower adiponectin and leptin receptor levels and higher leptin levels in both women and men, consistent with greater cardiometabolic risk [21]. Our study observed similar findings, with SAT and VAT density correlating positively with adiponectin levels and negatively with leptin levels before and after virologic suppression, and negatively with IL-6 concentrations following virologic suppression. It is plausible that the imbalances in adipokine (adiponectin and leptin) production seen in correlation with changes in VAT and SAT density result from a combination of chronic immune activation in PLWH on ART [42] and altered AT function, manifested clinically by adipocyte hypertrophy and a proinflammatory state [43, 44].

There are several limitations to our study: First, while we have previously shown that AT density from CT scans accurately reflects adipocyte size in PLWH [12], no AT biopsy specimens were available in this study and fat density was used as a surrogate of adipocyte size/AT quality. Second, no cohort of persons without HIV was available for comparison of changes in SAT and VAT density over time, although given that this was an ART initiation trial and not a longitudinal observational of persons already suppressed on ART, a control group without HIV would not have been easily comparable. Although women had larger decreases in SAT density, our sample consisted mostly of men, limiting generalizability but highlighting the need to further explore potential sex differences in cohorts with larger numbers of women. The range of HU used to identify AT density varies in the literature, which can limit reproducibility of comparisons, though we used values standard for the CT reading center and

comparable to several other large cohorts. Finally, the impact of the changes in AT quality on clinical CVD burden and other outcomes needs to be formally investigated in longer-term studies.

Effective ART has substantially reduced HIV-related morbidity and mortality. As PLWH live longer, an understanding of the relationship between HIV, ART, and contributors to cardiovascular risk is critical. These results help frame the potential interaction between effective ART and AT function, and suggest that earlier and aggressive cardiovascular risk assessment may need to be considered in PLWH.

In conclusion, these data provide a first analysis of both changes in CT-quantified AT density among PLWH initiating modern ART, and relationships between these changes and immunometabolic parameters. We demonstrated that declines in AT density occur with effective ART and are associated with adverse changes in cardiometabolic profiles. Furthermore, measurement of AT density may provide additional insight into AT function beyond BMI and AT quantity alone. Given the associations between less dense AT and increased risk of cardiovascular events in people without HIV, this parameter should be further investigated as a potential risk factor in PLWH initiating ART. Better understanding of the causes of AT-associated inflammation and functional changes in AT on ART are needed to help develop interventions to attenuate cardiovascular risk in PLWH.

## Notes

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