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Endothelial Activation Markers Are Linked to HIV Status and Are Independent of Antiretroviral Therapy and Lipoatrophy

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Abstract

Objectives—To assess the association of inflammatory and endothelial activation biomarkers with the presence of lipoatrophy in HIV-infected subjects and to examine the role of HIV, antiretroviral therapy (ART), and metabolic parameters in endothelial activation and inflammation.

Design—Prospective, cross-sectional study including 4 groups: HIV+ on ART with HIV-1 RNA <1000 copies/mL with and without clinical lipoatrophy, HIV+ ART naive, and healthy controls.

Methods—We measured plasma levels of inflammatory cytokines (tumor necrosis factor- α , soluble tumor necrosis factor receptors I and II, interleukin-6, C-reactive protein, and myeloperoxidase) and endothelial activation markers (soluble intercellular and vascular cell adhesion molecules and von Willebrand factor).

Results—We enrolled 182 subjects. Limb fat and lipoatrophy status were not correlated with endothelial markers. Endothelial markers were higher in HIV+ ART naive when compared with healthy controls and with HIV+ on ART but were similar between HIV+ on ART and healthy controls. Neither endothelial nor inflammatory markers were correlated with HIV duration, CD4 count, lipids, glucose, or specific ART. Strong correlations were found between some inflammatory cytokines and endothelial markers.

Conclusions—There is enhanced endothelial activation in ART naive, whereas HIV+ on ART has similar values to healthy controls. Lipoatrophy did not seem to affect endothelial activation. Results highlight a potential association between heightened inflammation and endothelial activation.

Keywords

cardiovascular disease; endothelial activation; inflammatory cytokines; lipoatrophy

INTRODUCTION

HIV infection carries an increased risk of cardiovascular disease (CVD).^{1–7} The pathogenesis of this increased risk remains incompletely understood, and the extent to which traditional risk

factors, antiretroviral therapy (ART), or HIV itself contribute to this heightened risk remains unclear.^{8–14} Outside of HIV, there is mounting evidence to support the role of chronic inflammation, oxidative stress, and endothelial activation and dysfunction in the development of plaque formation and progression of atherosclerosis.^{15–27} Increased levels of inflammatory markers and evidence of endothelial dysfunction are independently evident within HIV-infected individuals.^{6, 19, 28} Interestingly, studies suggest that HIV-associated fat redistribution, and particularly lipoatrophy, may be a risk factor for CVD.^{29–32} For example, Hadigan et al²⁹ demonstrated that HIV-infected individuals with lipodystrophy have a higher 10-year CVD risk estimate than both Framingham Offspring control subjects and HIV-infected subjects without fat redistribution, when matched for sex, age, and body mass index (BMI). When the type of fat redistribution was taken into account (primary lipoatrophy versus abdominal lipohypertrophy versus mixed lipodystrophy), the subjects with primary lipoatrophy demonstrated the highest 10-year CVD risk estimate.

The cause of this apparent increased CVD risk among HIV-infected individuals with lipoatrophy is unclear, although it may be in part due to the concomitant metabolic abnormalities, such as dyslipidemia, hyperinsulinemia, hypertension, and increased visceral adiposity, as suggested from data both within and outside of HIV.^{29–31, 33–36} Lipodystrophic patients without HIV and lipoatrophic mouse models also demonstrate similar disorders and suggest that adipose tissue functions as an endocrine organ which secretes proteins required for normal homeostasis.^{37–40} Interestingly, adipose tissue implants given to lipodystrophic mice reverse many of their metabolic abnormalities.^{38, 39} In addition, adipose tissue has been shown to produce inflammatory cytokines, such as C-reactive protein (CRP) and interleukin-6 (IL-6), and insulin resistance is also associated with a significant increase in CRP, IL-6, and other acute-phase reactants.^{40, 41}

A dysregulation in the levels of inflammatory cytokines and endothelial adhesion molecules among HIV-infected patients with lipoatrophy may provide a mechanism for the development of endothelial dysfunction and atherosclerosis. In the general population, elevated levels of proinflammatory cytokines, IL-6 and CRP, are associated with subclinical atherosclerosis⁴² and are independently predictive of future cardiovascular events.^{43, 44} In addition, tumor necrosis factor- α (TNF- α) has been implicated in myocardial dysfunction after acute coronary syndromes, and levels of TNF- α have been shown to be higher in patients who experience recurrent myocardial infarction or cardiac death.^{16, 45, 46} The effects of TNF- α are mediated by 2 receptors, tumor necrosis factor receptor-I (TNFR-I) and tumor necrosis factor receptor-II (TNFR-II), and the soluble receptors in plasma can be measured with greater sensitivity and reliability than TNF- α itself.⁴⁷ Myeloperoxidase (MPO), a peroxidase enzyme found in leukocytes and released when leukocytes are activated, has also been established as an independent predictor of early risk of myocardial infarction in patients presenting with chest pain,⁴⁸ and MPO levels are associated with the presence of coronary artery disease and endothelial dysfunction in the general population.⁴⁹ Likewise, levels of 2 circulating adhesion molecules, soluble vascular adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1), and levels of von Willebrand factor (vWF) antigen arise from shedding or proteolytic cleavage from activated endothelial cells and are useful markers for increased activation of endothelial cells in atherosclerosis.^{50–52}

To date, no study has examined the association between inflammatory and circulating adhesion molecules and HIV lipoatrophy. We hypothesized that both inflammatory and endothelial activation biomarkers are increased among HIV-infected subjects with established lipoatrophy when compared with subjects without lipoatrophy. In this study, we measured several plasma inflammatory cytokines, MPO, and markers of endothelial activation. The objectives of this study were (1) to assess the association of inflammatory and endothelial activation biomarker levels with the presence of clinical lipoatrophy in HIV+ subjects on ART, (2) to examine the

role of HIV and ART in the inflammatory process and endothelial activation, and (3) to explore the relationship of inflammatory biomarker levels to limb fat, endothelial activation markers, and metabolic parameters.

METHODS

Study Design/Population

This is a single-site, cross-sectional, observational study conducted at the John T. Carey Special Immunology Unit of University Hospitals, Case Medical Center/Case Western Reserve University in Cleveland, OH. Subjects were enrolled prospectively, and eligible subjects were classified in 1 of 4 groups: HIV+ on ART with clinical lipoatrophy (lipo+ group), HIV+ on ART without lipoatrophy (no-lipo group), HIV+ naive to ART (ART-naive group), and HIV – healthy controls (control group).

Inclusion criteria for all HIV+ groups included HIV infection and ≥ 16 years of age. Subjects were eligible for the lipo+ group if they had established clinical lipoatrophy of at least moderate severity and in at least 2 different areas of the following: face, arms, legs, or buttocks (as self-reported by patients and confirmed by the physician). The no-lipo group had a complete absence of visible lipoatrophy in the face, limbs, and buttocks, and this lack of fat loss was also confirmed by the physician. Additional inclusion criteria for the lipo+ and no-lipo group included receiving highly active ART for ≥ 24 months and an HIV-1 RNA of ≤ 1000 copies/mL at the time of screening. Exclusion criteria for all HIV+ groups included known CVD, diabetes, or current opportunistic infection or other acute inflammatory condition. Healthy controls were chosen and group matched by age and BMI to the HIV+ ART-naive group and were eligible if they had no recent or current infection and no uncontrolled chronic conditions. Exclusion criteria for the healthy control group were the same as those for the HIV-infected group. The number of healthy controls was planned to be at least equal to, but no more than twice, the number of HIV+ naive subjects.

Study evaluations included physical examination, blood pressure, height and weight, anthropometry including standardized hip-to-waist ratio, and blood sampling after at least 8 hours fasting. Limb fat in the lipo+ group was measured via dual-energy x-ray absorptiometry (DEXA) scan. Extensive chart review was conducted for HIV+ subjects, including known duration of HIV infection, ART history, past and present medical diagnoses, current medications, and nadir CD4⁺ cell count. Demographic and social information was collected from both HIV+ subjects and controls, including smoking, alcohol, and drug history and family history of diabetes and premature CVD.

The study was reviewed and approved by the University Hospitals Case Medical Center Institutional Review Board. All subjects or guardians, where appropriate, gave written signed consent before participating.

Metabolic, Inflammatory, and Cardiovascular Tests

Fasting blood was drawn from all subjects for real-time measurements of glucose and lipoprotein profile. In addition, we measured inflammatory cytokines [TNF- α , soluble tumor necrosis factor receptor-I (sTNFR-I), soluble tumor necrosis factor receptor-II (sTNFR-II), IL-6, high-sensitivity C-reactive protein (hsCRP)], endothelial activation markers (vWF, sICAM-1, and sVCAM-1), and the cardiovascular marker MPO. Markers were measured in duplicate and averaged using commercially available enzyme-labeled immunosorbent sandwich assays (Searchlight; Thermo Fisher Scientific, Woburn, MA). The median intra-assay coefficients of variation for TNF- α , sTNFR-I, sTNFR-II, IL-6, hsCRP, vWF, sICAM-1, sVCAM-1, and MPO were 14.5%, 8.8%, 8.6%, 11.7%, 6.9%, 13.3%, 8.0%, 8.7%, and 7.5%,

respectively. The median interassay coefficients of variation for each assay were 9.8%, 10.8%, 6.0%, 10.4%, 4.5%, 9.0%, 4.2%, 13.4%, and 12.6%, respectively. Finally, CD4⁺ cell counts and HIV-1 RNA were measured as markers of HIV disease activity.

Statistical Methods

Descriptive Analyses—Demographics, clinical characteristics, and fasting metabolic parameters are described by study group, and HIV-related characteristics are described for HIV-infected subjects. Continuous measures are described by medians and ranges, and nominal variables are described with percentages.

Pairwise Tests—To address the first objective, HIV+, lipo+ subjects on ART were compared with HIV+ no-lipo subjects for all variables of interest. These 2 groups were then combined into an ART group and compared with the HIV+ ART-naive group.

To address the second objective, similar comparisons were carried out between the HIV+ ART-naive group and the healthy control group (to test the effect of disease only) and the HIV+ subjects on ART and the negative controls. Continuous measures were compared using Wilcoxon rank sum tests and nominal variables using the Fisher exact test as appropriate. The level of significance for all pairwise analyses was set at 0.05.

Correlation Analysis—To meet the third objective, the relationships of each variable to the other variables of interest were estimated within group with Spearman rank correlation coefficients. In the case of the correlations of endothelial markers and inflammatory markers, $P < 0.05$ was considered significant, as this is primarily exploratory. In the case of other metabolic measures, $P < 0.01$ was considered significant, recognizing the large number of correlation coefficients that were estimated.

Regression Analyses—To assess the contribution of HIV-related variables (CD4⁺ cell count, nadir CD4⁺ cell count, and disease duration) and treatment-related variables [length of drug exposure(s)] to endothelial markers, multiple linear regression models were constructed. Additionally, other variables thought to potentially influence the marker value were included, such as age, gender, smoking status, BMI, MPO, TNF- α , and sTNFR-II. Backward elimination methods were employed, leaving variables with P values < 0.25 in the final model.

All analyses were carried out using SAS v. 9.1 (The SAS Institute, Carey, NC).

RESULTS

Study Population

Overall, we enrolled 182 subjects in this study: 82 lipo+, 50 no lipo, 20 antiretroviral (ARV) naive, and 30 controls. Among the lipo+ group, 41 (50%) had isolated lipoatrophy, whereas 37 (47%) had both lipoatrophy and lipohypertrophy. No subjects in the no-lipo or naive groups had isolated lipohypertrophy. Three lipo+ subjects were coinfecting with active viral hepatitis [hepatitis B (1); hepatitis C (2)]. No subjects in either the no-lipo or naive group had concomitant viral hepatitis. Eighty (98%) of the lipo+ subjects and 45 (90%) of the no-lipo group had HIV-1 RNA < 400 copies/mL. Two subjects in the lipo+ group had HIV-1 RNA values of 506 and 959 copies/mL, respectively. Likewise, there were 5 subjects in the no-lipo group with values > 400 , ranging from 512 to 1091 copies/mL. Nineteen subjects in the ART-naive group had measurable HIV-1 RNA values with a median of 54,200 copies/mL (range 514–122,000). One additional ART-naive subject had a HIV-1 RNA value $> 750,000$ copies/mL.

At the time of study evaluation, 40 subjects (49%) among the lipo+ group were taking concomitant medications: 22 were on low-dose prophylactic aspirin; 12 were taking fish oil and/or fibrates; 15 were taking statins or niacin; 17 were on anti-hypertensives; and 7 were on physiologic-replacement trans-dermal testosterone. Among the no-lipo group, 25 (50%) were taking concomitant medications: 4 were on low-dose prophylactic aspirin; 2 were on fish oil and/or fibrates; 14 were on statins or niacin; 12 were on antihypertensives; and 7 were on physiologic-replacement transdermal testosterone. There was only 1 ARV-naïve subject taking a statin for hypercholesterolemia and 1 taking physiologic-replacement transdermal testosterone. No HIV+ subject was receiving thiazolidinediones or growth hormone. Among the healthy controls, 3 were on antihypertensives for preexisting, yet well-controlled hypertension. None of the controls had any medical condition or took any medications known to interfere with the study end points.

Between-Group Comparison of Clinical Characteristics

Lipoatrophy Versus No-Lipoatrophy Groups—As shown in Table 1, gender, race, and BMI were similar between these 2 groups. Age, nadir CD4⁺ cell count, HIV duration, and ART duration of nucleoside/nucleotide analog reverse transcriptase inhibitors and protease inhibitors were higher in the lipo+ group compared with the no-lipo group.

ARV Treated (Regardless of Lipoatrophy Status) Versus ARV Naïve—The lipo+ and no-lipo groups were combined into the HIV+ on ART group and compared with the HIV + ART-naïve group (Table 2). The HIV+ on ART group had significantly more males, whites, older subjects, higher current CD4⁺ cell count, longer HIV duration, and a lower nadir CD4⁺ cell count than the ART-naïve group. BMI and smoking status were similar in the 2 groups.

ART Naïve Versus Healthy Controls—Compared with the healthy controls, the ART-naïve group had more smokers and fewer whites (Table 2). Age, BMI, male gender, and blood pressure were similar between these groups.

ART Treated Versus Healthy Controls—The group of HIV+ on ART had significantly more males, whites, and smokers and a higher median age and blood pressure when compared with the healthy control group (Table 2). BMI was similar between the 2 groups.

Between-Group Comparison of Metabolic Parameters and Plasma Biomarkers

Lipoatrophy Versus No-Lipoatrophy Groups—As shown in Table 1, the median total fasting cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels were similar among the lipo+ and no-lipo groups, but high-density lipoprotein (HDL) was lower and glucose was higher in the lipo+ group. Limb fat in the lipo+ group had a median of 4726 g, which is below the standard normal range of 8–9 kg in normal adults and is consistent with limb fat found in prior studies of HIV-associated lipoatrophy.^{53–55} MPO and sTNFR-I and sTNFR-II were significantly higher in the no-lipo group than in the lipo+ group, but the remainder of the inflammatory markers and all 3 endothelial markers were similar between the 2 groups.

ART Treated (Regardless of Lipoatrophy Status) Versus ART Naïve (Effect of ART)—Total and HDL cholesterol, TG, and glucose levels were higher in the ART group. LDL cholesterol was similar between groups. sTNFR-II and the 2 endothelial markers sVCAM-1 and vWF were significantly higher in the naïve group, whereas sTNFR-I was higher in the treated group.

ART Naïve Versus Healthy Controls (Effect of HIV Disease)—Both total and HDL cholesterol were lower in the ART-naïve group compared with the controls. All endothelial

markers and MPO were higher in the ART-naive group, whereas all inflammatory markers were similar between groups.

ART Treated Versus Healthy Controls—HDL cholesterol was higher and TGs were lower in the control group than the treated group, but glucose and total and LDL cholesterol were similar. sTNFR-I, hsCRP, and MPO were higher, whereas sTNFR-II was lower in the treated group. All 3 endothelial markers and IL-6 and TNF- α were similar between groups.

Correlation Analysis

Correlations were estimated for 4 groups: lipo+, ART treated, ART naive, and HIV healthy controls.

Correlations Among Plasma Biomarkers and Clinical Characteristics—

Correlations between plasma biomarkers, fasting metabolic parameters, and HIV and treatment characteristics were assessed. Limb fat in the lipo+ group was positively correlated with the 2 inflammatory markers hsCRP and IL-6 (both $P < 0.01$) but not with any of the endothelial activation markers. Neither inflammatory, endothelial activation markers nor MPO was correlated with fasting lipids, glucose, CD4⁺ cell count, HIV duration, or specific ART class or duration in any group.

Correlations Among Inflammatory Markers—As expected, IL-6 was positively correlated with hsCRP for all 4 groups and with sTNFR-I for all groups except ART naive. sTNFR-I was positively correlated with sTNFR-II for the lipo+ and ART-treated groups. The P values were ≤ 0.01 for all significant correlations.

Correlations Among Endothelial Activation Markers—The vWF was positively correlated with sVCAM-1 for both the lipo+ and ART-treated groups and with sICAM-1 in the ART-treated group ($P \leq 0.04$).

Correlations Between Inflammatory and Endothelial Activation Markers—

Significant correlations among inflammatory and endothelial activation markers are shown in Table 3. Importantly, the inflammatory marker sTNFR-II was correlated with all 3 endothelial markers in the ARV-treated group and with sVCAM-1 in the naive group. The correlation between sTNFR-II and sVCAM-1 is shown in Figure 1. Due to their similarity, all HIV+ groups have been pooled in the figure for ease in visibility.

Correlations Among MPO and Biomarkers—The MPO levels were positively correlated with sTNFR-II and sVCAM-1 for all the HIV+ groups (all, $P < 0.001$, except ART-naive group, $P = 0.05$), but not for the healthy control group. Likewise, MPO was correlated with IL-6 for the ART-naive group ($P = 0.04$) and with sICAM-1 in the lipo+ group ($P = 0.05$) and the ART-treated group ($P < 0.001$).

Regression Analyses

The relationship of sVCAM-1 to other markers and to measures of disease and treatment was examined in HIV+ treated subjects using linear regression methods. MPO, sTNFR-II, nonnucleoside analog reverse transcriptase inhibitor duration, and nadir CD4⁺ cell count were significantly related to sVCAM-1 ($P = 0.02$, $P < 0.001$, $P = 0.03$, and $P = 0.02$, respectively) in this setting. Of note, limb fat, BMI, smoking, HIV duration, nucleoside/nucleotide analog reverse transcriptase inhibitor duration, and current CD4⁺ cell count were not statistically significant.

DISCUSSION

With the advent of highly active ART, subjects infected with HIV are living decades longer than before. However, with the increased cardiovascular risk defined among those infected with HIV, there are serious implications for quality of life and life expectancy. Moreover, prior reports suggest that those HIV-infected individuals with isolated lipoatrophy may be at an even greater risk of heart disease.^{29–32} In our study, we found no correlation between limb fat and any endothelial or cardiovascular marker in the subjects with clinical lipoatrophy. Likewise, the levels of all 3 endothelial activation markers were similar between the lipo+ group and the no-lipo group. These observations suggest that lipoatrophy does not have an independent effect on endothelial or cardiovascular markers.

In the general population, increasing evidence suggests that chronic inflammation and endothelial dysfunction play an important role in the CVD risk, yet their contribution in HIV infection remains unclear. In general, HIV-infected subjects have decreased inflammation markers after therapy and control of viral replication, although they remain with heightened inflammation. For example, Aukrust et al²⁸ demonstrated a 25% decrease in TNF- α after 78 weeks of starting ART in a study of 40 HIV-infected subjects, although these levels did not normalize. Recent data from the Strategies for Management of Antiretroviral Therapy (SMART) trial have shown that subjects who had an intermittent CD4⁺ cell count–driven interruption of their ART had an increased risk of developing CVD when compared with those who were on continuous therapy.⁵⁶ This finding could be explained by increased immune activation and a heightened inflammatory state in the group whose virus was not continually suppressed with therapy. Recently, the SMART team reported that both D-dimer and IL-6 levels were significantly higher than at the time of SMART 1 month after interruption of ARV and that they correlated with HIV-1 RNA changes. Also, they showed that elevated D-dimer and IL-6 increased the risk of mortality by 12- to 26-fold in the subjects enrolled in the SMART study.⁵⁷ Moreover, Torriani et al¹⁹ found that subjects placed on ART had rapid improvement of endothelial function, regardless of the ART agents used. Taken together, these observations strongly suggest that uncontrolled viremia and heightened inflammation may be the driving force behind endothelial dysfunction. Several of our observations corroborate this hypothesis. First, we showed that despite higher lipid levels, treated subjects with good virologic control had similar endothelial activation markers compared with healthy controls. In contrast, even after controlling for variables such as CD4 count, viral load, and BMI, ART-naïve subjects had higher markers of endothelial activation when compared with either the healthy controls or the treated subjects. Also, the strong correlation found in our study between endothelial markers and some of the inflammation markers is noteworthy.

Indeed, several of the correlations found were notable. Not surprisingly, inflammatory markers were correlated with each other, particularly IL-6 with hsCRP for all groups. Likewise, the endothelial markers were correlated with each other. Importantly, sTNFR-II was correlated with all 3 endothelial markers in the treated group and additionally with sVCAM-1 in the ART-naïve group. These observations suggest a role of inflammation in the enhanced endothelial activation present in HIV and may explain the detrimental effect on CVD incidence seen with the treatment interruption SMART study.⁵⁶

In the general population, MPO is a strong independent predictor of CVD incidence.^{48, 58} In our study, MPO was significantly elevated in both the HIV+ treated and ART-naïve groups when compared with the healthy controls, although the utility of MPO in predicting CVD in HIV population has not been established. Likewise, it is interesting that in our study, MPO was correlated with sTNFR-II and with 2 of the endothelial markers in the HIV+ groups but not in the controls. Thus, the determinant of MPO levels and its relationship with other CVD risks may be different in the HIV+ population and deserves further investigations.

There are limitations to our study. As previously shown, there were significantly more smokers in the HIV+ group than the control group. However, this is indicative of the HIV population as a whole,⁵⁹ and other studies have had difficulty adjusting for the higher smoking rates among the HIV-infected subjects.⁶⁰ Likewise, as expected, the ART-treated group had a longer HIV duration, more concomitant medications, and a worse lipid profile than the ART-naive group. However, it is unlikely that these imbalances significantly affected the results because these factors would be expected to raise the endothelial markers in the ART-treated group but not in the ART-naive group as demonstrated in this study. Moreover, there were more subjects taking antihypertensives in the ART-treated group than the naive group, which has been shown to attenuate levels of some inflammatory markers and endothelial adhesion molecules.⁶¹ However, when subjects on antihypertensives were compared with those subjects not receiving these drugs, there was no difference in the levels of biomarkers (data not shown). In addition, DEXA scan was only performed on those subjects with clinical lipoatrophy secondary to cost; however, physician and patient assessments of clinical lipoatrophy have correlated well with DEXA-measured limb fat.⁶² It is possible that some subjects in the “no-lipoatrophy” group had subclinical lipoatrophy; however, the fact that limb fat did not correlate with endothelial markers in our lipoatrophy group supports our conclusions.

To our knowledge, this is the first study examining the association between inflammatory and endothelial activation biomarkers and lipoatrophy in HIV-infected individuals. Our results demonstrate that lipoatrophy status does not seem to influence endothelial activation, although inflammation seems to be heightened as limb fat decreases. Moreover, our data show that there is enhanced endothelial activation in ART naive, whereas HIV+ on ART have similar values to HIV controls. However, both treated and ART-naive HIV+ subjects have significantly elevated MPO levels compared with HIV controls. The correlation between inflammatory markers and endothelial activation markers in the HIV+ groups suggests that inflammation may play a significant role in endothelial dysfunction seen in HIV. Further studies are needed to confirm these findings and to further define the CVD risk among ART-naive subjects in particular.

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FIGURE 1.

The figure shows the relationship of the concentration of sVCAM-1 with sTNFR-II in subjects with HIV+ compared with HIV controls.

TABLE 1

Clinical Characteristics, Fasting Metabolic Parameters, and Biomarker Levels of Subjects With Lipoatrophy (Lipo+) and No Lipoatrophy (No Lipo)

Median (Range) Unless Otherwise Specified	Lipo+ (n = 82)	No Lipo (n = 50)
Age, yrs *	47.5 (31.6–73.9)	42.0 (16.5–55.1)
No. males (%)	66 (80)	40 (80)
Whites (%)	45 (56)	24 (48)
BMI (kg/m ²)	26.1 (19.0–41.7)	26.3 (18.3–45.3)
Current smokers (%)	36 (44)	24 (48)
Systolic blood pressure (mm Hg) [†]	118 (90–154)	120 (98–160)
Diastolic blood pressure (mm Hg)	80 (60–105)	80 (58–100)
HIV duration (mos) *	153 (26–301)	106 (30–260)
CD4 ⁺ cell count (cells/mm ³)	640 (108–1718)	554 (188–1612)
CD4 ⁺ cell count nadir (cells/mm ³) *	208 (6–964)	143 (8–540)
NNRTI duration (mos)	21 (0–113)	26 (0–95)
PI duration (mos) [†]	45 (0–126)	19 (0–157)
NRTI duration (mos) *	96 (15–228)	73 (0–156)
Limb fat by DEXA (g)	4736 (2135–20,805)	—
Fasting total cholesterol (mg/dL)	182 (103–336)	185 (122–255)
Fasting LDL cholesterol (mg/dL)	113 (30–245)	112 (59–146)
Fasting HDL cholesterol (mg/dL) *	36 (14–98)	47 (24–92)
Fasting TGs (mg/dL)	168 (46–1522)	157 (39–497)
Fasting glucose (mg/dL) *	87 (58–129)	81 (55–124)
TNF- α (pg/mL)	2.1 (0.4–5.0)	4.7 (0.4–4.7)
sTNFR-I (pg/mL) *	620 (185–2088)	745 (441–2066)
sTNFR-II (pg/mL) [†]	238 (74–1247)	330 (97–1340)
hsCRP (mg/mL)	2.73 (2.72–8.46)	2.30 (1.79–2.86)
IL-6 (pg/mL)	2.9 (0.2–71.0)	2.4 (0.6–16.8)
sICAM-1 (ng/mL)	372 (73–1802)	387 (137–1003)
sVCAM-1 (ng/mL)	451 (188–1094)	451 (147–1804)
vWF (U/mL)	17.3 (2.2–249.4)	15.5 (3.7–162.9)
MPO (pg/mL) *	3990 (1022–16,230)	5550 (960–137,700)

NNRTI, nucleoside/nucleotide analog reverse transcriptase inhibitor; NNRTI, nonnucleoside/nucleotide analog reverse transcriptase inhibitor; PI, protease inhibitor

* $P \leq 0.01$.

[†] $P \leq 0.05$.

TABLE 2

Clinical Characteristics, Fasting Metabolic Parameters, and Biomarker Levels for ARV-Treated, ARV-Naive, and HIV Control Groups

Median (Range) Unless Otherwise Specified	ARV Treated (n = 132)	ARV Naive (n = 20)	Healthy Controls (n = 30)
Age, yrs ^{*†}	47.5 (31.6–73.9)	31.2 (20.6–64.4)	39.2 (15.8–64.5)
Males (%) ^{††}	66 (80)	11 (55)	15 (50)
Whites (%) ^{*†§}	45 (56)	4 (20)	18 (60)
BMI (kg/m ²)	26.1 (19.0–41.7)	24.8 (18.2–32.7)	25.9 (18.7–34.0)
Current smokers (%) ^{†§}	36 (44)	11 (55)	2 (7)
Systolic blood pressure (mm Hg) [†]	118 (90–154)	110 (100–130)	110 (90–126)
Diastolic blood pressure (mm Hg) [†]	80 (60–105)	78 (60–84)	70 (50–90)
HIV duration (mos)	153 (26–301)	13 (2–65)	—
CD4 ⁺ cell count (cells/mm ³) [*]	640 (108–1718)	348 (140–760)	—
Nadir CD4 ⁺ cell count (cells/mm ³) [*]	208 (6–964)	284 (52–760)	—
NNRTI duration (mos)	21 (0–113)	0 (0–0)	—
PI duration (mos)	45 (0–126)	0 (0–0)	—
NRTI duration (mos)	96 (15–228)	0 (0–0)	—
Fasting total cholesterol (mg/dL) ^{*§}	184 (103–336)	155 (88–244)	194 (120–286)
Fasting LDL cholesterol	113 (30–245)	102 (58–158)	111 (54–182)
Fasting HDL cholesterol ^{†§}	40 (14–98)	34 (16–57)	53 (28–87)
Fasting TGs (mg/dL) ^{*†}	166 (39–1522)	93 (19–420)	67 (28–1138)
Fasting glucose (mg/dL) [*]	85 (55–129)	77 (55–105)	81 (67–95)
TNF- α (pg/mL)	3.2 (0.4–5.0)	1.0 (0.4–4.7)	1.8 (0.4–85.6)
sTNFR-I (pg/mL) [†]	677 (185–2088)	586 (184–1063)	613 (265.0–1365.0)
sTNFR-II (pg/mL) ^{*†}	294 (74–1340)	600 (155–1270)	412 (76–1070)
hsCRP (mg/mL) [†]	2.56 (1.79–8.46)	1.67 (0.31–16.04)	1.46 (0.53–2.44)
IL-6 (pg/mL)	2.6 (0.2–71.0)	2.8 (0.2–15.2)	2.8 (0.6–8.2)
sICAM-1 (ng/mL)	380 (73–1802)	455 (210–885)	342 (97–577)
sVCAM-1 (ng/mL) ^{†,}	451 (147–1804)	702 (226–2308)	451 (66–1808)
vWF (U/mL) ^{†§}	16.8 (2.2–249.0)	28.9 (4.4–95.0)	12.05 (6.4–200.0)
MPO (pg/mL) ^{†§}	4665 (960–137,700)	5590 (828–20,845)	1684 (1124–10,865)

NRTI = nucleoside/nucleotide analog reverse transcriptase inhibitor; NNRTI = nonnucleoside/nucleotide analog reverse transcriptase inhibitor; PI = protease inhibitor.

* $P \leq 0.01$ (HIV+, ARV treated versus HIV+, ARV naive).

† $P \leq 0.01$ (HIV+, ARV treated versus healthy controls).

‡ $P \leq 0.05$.

§ $P \leq 0.01$ (HIV+, ARV naive versus healthy controls).

|| $P \leq 0.05$.

¶ $P \leq 0.05$.

TABLE 3

Significant Correlations Between Inflammatory and Endothelial Biomarkers by Group

		TNF- α	sTNFRI	sTNFRII	hsCRP	IL-6
sICAM-1	Lipo+	-0.24 [*]	—	—	—	0.35
	ARV-tx	0.032 [†]	—	—	—	0.001
sVCAM-1	Lipo+	—	0.76 <0.001	0.24 0.006	—	—
	ARV-tx	—	—	0.74 <0.001	-0.18 0.038	—
	Naive	—	—	0.51	—	0.50
	HIV-	—	—	0.026	—	0.028
vWF	HIV-	—	—	—	—	0.54
	ARV-tx	—	—	0.21 0.018	—	0.004
	HIV-	0.51 0.006	—	—	0.41 0.03	—

Lipo+, HIV+ antiretroviral treated with lipotrophy subjects; ARV-tx, HIV+ antiretroviral treated regardless of lipotrophy status subjects.

^{*} Spearman correlation coefficient (ρ).

[†] P value.