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Predictors of impaired HDL function in HIV-1 infected compared to uninfected individuals

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Predictors of impaired HDL function in HIV-1 infected compared to uninfected individuals

A thesis submitted in partial satisfaction of the requirements

for the degree Master of Science in Clinical Research

by

Theodoros Kelesidis

2016

ABSTRACT OF THE THESIS

Predictors of impaired HDL function in HIV-1 infected compared to uninfected individuals

by

Theodoros Kelesidis

Masters of Science in Clinical Research

University of California, Los Angeles, 2016

Professor James O. Lloyd-Smith, Chair

Background: HDL function rather than absolute level may be a more accurate indicator for cardiovascular disease (CVD) but it is unclear what drives HDL dysfunction in HIV-1 infection. The objective of this study is to identify factors that may contribute to HDL dysfunction in chronic HIV-1 infection.

Design: Retrospective study of HIV-1 infected males with low overall CVD risk and healthy males with no known CVD risk matched by race to the HIV-1 infected participants.

Methods: We related parameters previously reported to be associated with HDL function to two different measures of HDL function: reduced antioxidant function (oxidized HDL, HDL_{ox}) and HDL-apoA-I exchange (HAE). Multivariable-adjusted linear regression analyses were employed adjusting for false discovery rate (FDR), age, race, body mass index (BMI), CD4 count, viremia, CVD risk, smoking, lipids, apoA-I, albumin.

Results: In multivariate analysis among HIV-1 infected males (n=166) (median age 45 years, median CD4 T cell count 505 copies/ml, 30.1% were viremic), higher BMI, lower apoA-I and

lower albumin were among the most notable correlates of higher HDL_{ox} and lower HAE (impaired HDL function)($p<0.05$). In HIV-1 uninfected participants lower albumin and higher BMI were associated with lower HAE and higher HDL_{ox}, respectively ($p\leq0.05$). HDL_{ox} was inversely related to HAE in HIV-1 infected (but not uninfected) individuals ($p<0.001$).

Conclusion: Increased HDL_{ox} is associated with reduced HDL remodeling in chronic HIV-1 infection. Higher BMI, lower apoA-I and lower albumin were identified as factors associated with abnormal HDL function in chronic HIV-1 infection using two independent methods.

The thesis of Theodoros Kelesidis is approved.

Judith Silverstein Currier

David Elashoff

James O. Lloyd-Smith, Committee Chair

University of California, Los Angeles

2016

DEDICATION

This thesis is dedicated to my wife Maria, my son Christos and my family (Iosif, Chrysanthos, and Evangelia) who supported and encouraged the work presented herein with great patience and care.

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LIST OF ABBREVIATIONS

ABC: abacavir

AIDS: Acquired immunodeficiency syndrome

ANOVA: analysis of variance

ApoA-I: Apolipoprotein A-I

ART: antiretroviral therapy

BIC: Bayesian information criterion

BMI: Body Mass Index

CARE: Center for Clinical AIDS Research and Education

CD4: Cluster of differentiation 4

CI: Confidence Interval

CVD: Cardiovascular disease

DRV/r: darunavir/ritonavir

EDTA: Ethylenediaminetetraacetic acid

EFV/TDF/FTC: efavirenz/emtricitabine/tenofovir disoproxil fumarate

EPR: Electron paramagnetic resonance

FDR: false discovery rate

GFR: Glomerular filtration rate

HAE: HDL-apoA-I exchange

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HDL: high-density lipoprotein

HDLox: oxidized high-density lipoprotein

HDLDI: HDL dynamic index

HIV: human immunodeficiency virus

LDL: low-density lipoprotein

NRTI: Nucleoside reverse transcriptase inhibitors

NNRTIs: Non-nucleoside reverse transcriptase inhibitors

Non-HDL-C: Non High Density Lipoprotein

PI: protease inhibitors

RAL: raltegravir

RNA: Ribonucleic acid

TC: Total Cholesterol

TG: Triglycerides

UCLA: University of California, Los Angeles

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CHAPTER 1. MANUSCRIPT

ABSTRACT

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Conclusion: Increased HDL_{ox} is associated with reduced HDL remodeling in chronic HIV-1 infection. Higher BMI, lower apoA-I and lower albumin were identified as factors

associated with abnormal HDL function in chronic HIV-1 infection using two independent methods.

Key Words: HDL function, Human Immunodeficiency Virus, Inflammation, cardiovascular disease, HDL-apoA-I exchange, HDL remodeling, oxidized HDL

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among HIV-1 infected individuals on effective antiretroviral therapy (ART) [1,2]. However, the exact mechanisms of increased CVD among HIV-1 infected persons remain unclear. High-density lipoprotein cholesterol (HDL-C) is the best independent negative indicator of CVD events[3,4]. HDL function rather than absolute level (HDL-C) may be a more accurate indicator of CVD risk[5,6], and recent studies confirm that CVD is strongly inversely correlated with cholesterol efflux capacity[7]. While HDL performs activities that are CVD-protective, in the setting of inflammation HDL becomes functionally impaired, elevating CVD risk[8]. Inflammation affects HDL by decreasing anti-inflammatory antioxidant factor levels and activity, increasing associated pro-inflammatory proteins, lipid hydroperoxide content and redox activity (HDL_{ox}), reducing cholesterol efflux potential, and diminishing HDL's ability to inhibit LDL oxidation[8]. HIV-1 infected ART-treated individuals have a higher prevalence of dyslipidemia and low HDL-C[9] . HIV-1 infected individuals also have impaired lipoprotein metabolism[10] and HDL_{ox}[11,12] that are associated with CVD[12,13].

Due to the complexity of the HDL particles, measurement of HDL function has been difficult to study extensively in humans[14,15]. Cell-free assays may give more robust measurements of HDL function compared to cell-based assays [16,17] such as cholesterol efflux assays[14] that have several limitations including lack of standardization and significant heterogeneity with regards to types of cells and type of readout that have been used for these assays[16]. ApoA-I, the major protein component of HDL, plays a key role in the promotion of cholesterol efflux [18] and its function is critical to its anti-

atherogenic molecular processes[19]. Modification of apoA-I impairs its ability to exchange on and off HDL, a critical process in reverse cholesterol transport[20,21]. HDL-apoA-I exchange (HAE) is markedly reduced when atherosclerosis is present, or when the subject carries at least one risk factor of CVD[20,21]. A previously described cell-free assay based on electron paramagnetic resonance (EPR) spectroscopy, measures the relative rate of HDL-apoA-I exchange (HAE) and provides a measure of HDL dynamics and the ability of HDL to remodel and release apoA-I (HDL dynamic index - HDLDI)[20,21]. As spin-labeled apoA-I associates with HDL, the EPR spectra's peak amplitude increases due to structural changes in apoA-I from a lipid-free to a lipid-bound conformation[21,22]. The ratio of lipid-free to lipid-bound apoA-I (HDLDI) provides a measure of the relative exchangeability of endogenous apoA-I and the dynamic nature of HDL particles[21,22]. Early studies suggest that lower HDLDI is a CVD-relevant measure of HDL function[21,22]. *In vitro* studies suggest that oxidative modification of apoA-I may impair HDL's ability to mediate cholesterol efflux by inhibiting the remodeling/exchange of apoA-I [20,23]. However, it is unknown whether HDL_{ox} is associated with HDL remodeling *in vivo*. We have developed a novel cell-free fluorometric method that measures HDL associated lipid peroxidation (HDL_{ox}) that offers a reproducible and rapid means of determining HDL function[17]. In certain populations of HIV infected ART treated participants the readout from this assay correlates with measures of subclinical atherosclerosis such as carotid intima media thickness[17] and calcium artery score[24]. We hypothesized that in chronic HIV-1 infection, increased oxidative stress and impaired antioxidant HDL function (as measured by higher HDL_{ox}) are associated with lower HDLDI and this association is independent of other factors

associated with impaired HDL function in HIV uninfected persons (such as elevated body mass index; BMI)[14,15]. There is also limited data regarding predictors of abnormal HDL function in chronic HIV-1 infection including the role of specific classes of antiretrovirals. The objectives of the present analysis were to characterize and evaluate in HIV-1 infected persons anthropometric (such as BMI[15]), biochemical (such as albumin[25]) parameters and comorbidities (such as smoking[26], kidney disease[27], presence of metabolic syndrome[25]) that may have a role in abnormal HDL function (antioxidant, cholesterol efflux; evidence based on HIV uninfected individuals [15,25-27]). Towards this aim, we utilized a cross-section of ART-treated, viremic HIV-1 infected individuals and HIV-1 uninfected healthy controls (matched by age, sex, race). We also compared these changes by ART regimen and hypothesized that raltegravir (RAL) use would be associated with improved HDL function compared to protease inhibitors (PIs). This is in light of prior studies where modest increases in lipids were observed in PI groups compared to decreases with RAL[28]. Finally, since prior data suggest non-nucleoside reverse-transcriptase inhibitors (NNRTIs) have beneficial effect on HDL-C and cholesterol transport[29], we hypothesized that NNRTI use would be associated with improved HDL function compared to PIs.

METHODS

Study Design and Participants

The Center for Clinical AIDS Research and Education (CARE) HDL function study was a cross-sectional study developed to assess determinants of impaired HDL function among HIV-infected patients on stable ART with HIV-1 RNA <200 copies/ml within 6 months of enrollment compared to viremic HIV-1 infected (≥200 copies/ml) and uninfected individuals. The cohort enrolled participants ≥18 years of age males from the University of California, Los Angeles (UCLA) CARE clinic in Los Angeles, California in a single study visit that included biological specimen collection for storage and medical record review. We recruited only males to avoid confounding from sex differences in lipid metabolism[30]. Healthy HIV-1 uninfected ≥18 years of age males with no known dyslipidemia, metabolic and inflammatory comorbidities and no known risk factors for CVD (except for smoking) were additionally recruited in outpatient clinics within UCLA. All individuals enrolled in the study provided written informed consent and the study was approved by the UCLA Institutional Review Board.

Data collection

Sociodemographic characteristics, comorbidities, presence of kidney disease (defined as glomerular filtration rate [GFR]< 60 ml/min/1.73 cm^2), presence of risk factors for CVD (defined as at least one of the following: metabolic syndrome defined by National Cholesterol Education Program criteria[31], diabetes, dyslipidemia, use of lipid lowering medication, hypertension, family history of CVD, Framingham 10-year Coronary Heart

Disease Risk Score ≥6% risk), albumin, lipid profile were abstracted from the medical records for all study participants. In addition, for HIV-1 infected participants data that were also abstracted included duration of HIV-1 infection and ART, current (within 6 months) and nadir CD4+ T lymphocyte counts, plasma HIV-1 RNA levels.

Biomarker and Laboratory Assessment

Plasma lipid analysis

The lipid panel (total cholesterol, HDL-C, and triglycerides) was measured in fasted EDTA-plasma by standard validated clinical assays employing a Beckman DXC, and LDL cholesterol (LDL-C) was calculated by the Friedewald formula. Plasma apoA-I levels were determined by validated nephelometric method as previously described [21,22].

Oxidized HDL

HDL_{ox} was quantified using a previously validated fluorometric biochemical assay that measures HDL lipid peroxidation based on the oxidation of the fluorochrome Amplex Red[12]. To reduce experimental variability and adjust for HDL amount, we normalized the mean fluorescence readout from quadruplicates of each sample (HDL_{ox_sample}) by the mean fluorescence readout from quadruplicates of a pooled plasma control ($HDL_{ox\_control}$) and by concurrent HDL cholesterol concentration level (HDL-C) using the following calculation: “normalized” oxidized HDL ($nHDL_{ox}$) = [HDL_{ox_sample} x 40 (mg/dl)] / [$HDL_{ox\_control}$ x $HDL\text{-}C_{sample}$ (mg/dL)], where 40 mg/dL represents HDL-C of the pooled plasma control. This approach has been validated in clinical studies and has been

shown to reduce experimental variability[11,17,24,32,33].Throughout the results HDL_{ox} is presented as normalized [$nHDL_{ox}$] measure to reflect the adjustment for experimental variability and HDL-C.

HAE assay

HAE assays were performed as previously described [21,22]. Samples were centrifuged to remove apoB-containing lipoproteins and the clarified plasma was mixed with 3 mg/ml spin-labeled apoA-I [21,22] in a 3:1 ratio. Samples were incubated for 15 min at 37°C. Electron paramagnetic resonance (EPR) measurements were performed with a Bruker eScan spectrometer outfitted with a temperature controller (Noxygen). Inter-assay coefficient of variability was 5.3%.

Statistical Analyses

We modeled HDL_{ox} using two different approaches that represented a) a normalized fraction of HDL that is oxidized, and b) a single level of HDL_{ox} with adjustment for level of HDL-C concentration [12,34]. Higher levels of this adjusted measure of HDL function have been associated with worse health outcomes [12,13,34,35]. In addition, we modeled HDLDI using two different approaches that represented a) a normalized to ApoA-I levels fraction of HDLDI and b) a single level of HDLDI with adjustment for apoA-I levels in our statistical models. Our observations were consistent regardless of approach (data not shown). Baseline characteristics were compared using parametric and nonparametric methods as appropriate for the data being evaluated. Pearson's correlation was used to evaluate the association between HDL_{ox} and HDLDI (both log transformed) among all

participants. Multivariate linear regression was used to investigate the predictors of HDL_{ox} and HDLDI. Covariates significant in the univariate analysis ($p<0.05$) were also examined together in multivariate analysis. For each set of hypotheses in multivariate analysis, the false discovery rate (FDR) was controlled at alpha=0.05 using the Benjamini-Hochberg procedure[36]. Statistical hypothesis tests were two-sided with a significance threshold of 0.05 for p values.

Based on our prior published studies on HDL_{ox} among HIV-1 infected participants [17,24,32,33], and using a two-sided, 0.05-level, two-sample t-test with two comparisons, a sample size of 40 individuals per group (HIV-1 versus uninfected individuals), provides at least 80% power to detect differences of at least 0.6 in effect size for HDL_{ox} (expressed as normalized ratio to a pooled plasma control from healthy donors; no units) between groups. All statistical analyses were conducted using JMP Pro 12.01 (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics

Baseline characteristics of the 198 participants are shown in Table 1. Briefly, the median age of HIV-1 infected participants with suppressed viremia on ART (n=116) was 47.5, 67% of them were non-hispanic white, the median CD4 T cell count was 535 copies/ml and the group overall had a low cardiovascular disease risk; only 9% of participants had a 10 year risk of hard coronary heart disease $\geq$6%, 20% were current smokers; 10% had metabolic syndrome. The most common ART regimen was efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/TDF/FTC) (23.7%) followed by TDF/FTC/ darunavir/ritonavir (DRV/r) (16.1%) and TDF/FTC/ raltegravir (RAL) (11.9%). Only 28% of the viremic patients were ART naïve and the rest had virus resistant to ART at the time of the visit. The median age of HIV-1 infected participants with viremia (n=50) was 45 years, and this group overall had a significantly higher CVD risk, lower median CD4 T cell count and higher incidence of coinfections compared to the ART-treated groups ($p<0.05$). There were no differences in other HIV-1 related parameters between the ART-treated and the viremic group ($p>0.05$). The HIV-1 uninfected participants (n=32) were younger (median age of 35 years) compared to HIV-1 uninfected participants ($p<0.001$). Participants were similar across ART groups with the TDF/FTC backbone and they were representative of the full substudy population (Supplemental Table 1).

Comparison of lipids and parameters related to HDL function between groups

We explored differences in parameters previously reported to be associated with impaired HDL functions between study groups. Viremic HIV-1 infected participants had lower albumin, higher prevalence of kidney disease and smoking (all $p<0.001$) and lower HDL ($p<0.05$) compared to ART-treated and uninfected participants (Table 1). HIV-1 infected participants had overall abnormal lipid profile and lower apoA-I levels compared to uninfected persons whereas the ART-treated groups had overall similar lipid profile compared to the viremic group (Table 1)($p>0.05$).

Comparison of HDL function measures between groups

Median HDL_{ox} of viremic subjects, and ART-treated HIV-1 infected subjects was 35% and 17% higher, respectively, compared to uninfected participants ($p<0.001$). Viremic and ART-treated HIV-1 infected subjects had a lower HDLDI compared to uninfected participants ($p<0.01$). Viremic subjects also had a lower HDLDI compared to all the ART-treated groups ($p<0.01$) (Table 1) (Figure 1).

Correlates of HDL remodeling in chronic HIV-1 infection versus uninfected participants

To address our limited understanding of abnormal HDL function in chronic HIV-1 infection, we determined correlates of HDL remodeling among HIV-1 groups. Among HIV-1 infected participants, there was a positive association between age, fasting lipids (total cholesterol, triglycerides), apoA-I, albumin and HDLDI ($p<0.05$). In contrast there was an inverse relationship between HDLDI and BMI, HDL_{ox}, smoking, kidney disease ($p<0.05$). The most notable positive associations were between HDLDI and albumin and

apoA-I (Table 2) whereas lower HDLDI was associated with higher HDL_{ox}, Regarding HIV-1 related parameters, only duration of HIV-1 infection and presence of viremia had a positive association with HDLDI (Supplemental table 2). All associations between HDL_{ox}, apoA-I and HDLDI were attenuated but remained statistically significant ($p<0.05$) after adjusting for FDR and covariates (age, race, BMI, lipids, apoA-I levels, presence of risks factors for CVD, smoking, kidney injury, albumin, viremia, current CD4 T cell count, duration of HIV infection) (Table 3). However, in HIV-1 uninfected participants only albumin demonstrated a moderate positive association with HDLDI (Table 2), even after adjusting for FDR and covariates (age, race, BMI, lipids, apoA-I). These results suggest that apoA-I in HIV-1 infected and albumin in uninfected participants, were the strongest positive correlates of HDL remodeling.

Correlates of impaired antioxidant function in chronic HIV-1 infection versus uninfected participants

We also explored correlates of the above parameters with another measure of HDL dysfunction, impaired antioxidant activity (HDL_{ox}). As expected in both HIV-1 infected and uninfected participants, higher BMI, smoking, kidney disease and lower albumin were associated with higher HDL_{ox} (Table 4). Among HIV-1 infected but not in HIV-uninfected participants, younger age, white race, lower lipids (total cholesterol, triglycerides), lower apoA-I, presence of metabolic syndrome and risk factors for CVD were associated with higher HDL_{ox}. The most notable positive associations were between HDL_{ox} and BMI (Table 4) whereas HDL_{ox} was most strongly negatively associated with albumin and apoA-I. Regarding HIV-1 related parameters, only reduced

duration of HIV-1 infection, lower CD4 T cell count and higher viral load were associated with higher HDL_{ox} (Supplemental table 2). All associations were attenuated for BMI, apoA-I, smoking, albumin after adjusting for FDR and covariates (age, race, BMI, lipids, apoA-I, presence of risks factors for CVD, smoking, kidney injury, albumin, viremia, current CD4 T cell count, duration of HIV infection) but remained statistically significant (Table 5). However, in HIV-1 uninfected participants none of the observed associations between HDL_{ox} and parameters remained significant (Table 5), after adjusting for FDR and covariates (age, race, BMI, lipids, kidney injury, smoking, apoA-I). Overall, our data suggest that smoking, BMI, apoA-I and albumin were among the most notable correlates of HDL dysfunction (using two different measures of HDL function).

Associations of HDL function with ART

We then explored the association of different ART classes with HDL function. Overall, there was no ART class-specific (NRTI, vs. NRRTIs, vs. PIs, vs. RAL) association between HDLDI or HDL_{ox} (Supplemental Tables 1-3). HIV-1 infected participants on ART with the same NRTI backbone (TDF/FTC) who received NNRTIs, PIs or RAL had similar HDLDI and HDL_{ox} levels (Supplemental Table 1). NNRTI use was associated with lower HDL_{ox} in univariate analysis (Supplemental Table 3) but not in multivariate analysis after adjustment for covariates that may affect HDL function (Table 5).

Correlations of measures of HDL function among groups

We determined whether HDL_{ox} correlates with HDL remodeling *in vivo* in chronic HIV-1 infection. Using Pearson correlation (Figure 2) and univariate analysis (Table 2), there

was a significant inverse relationship between HDL_{ox} and HDLDI among all participants ($r=0.50$, $p<0.001$). This association was similar between the viremic ($r=-0.42$, $p=0.003$) and the ART-treated ($r=-0.41$, $p<0.001$) HIV-1 infected participants (Figure 2) but was not present in the uninfected individuals ($r=-0.115$, $p=0.42$). HDL_{ox} correlated with HDLDI (Table 2) in HIV-1 infected groups after adjustment for other clinical factors (Table 3).

DISCUSSION

In this retrospective study of HIV-1 infected males with low overall CVD risk and healthy males with no known CVD risk, we found that chronic HIV-1 infection, despite effective ART, as well as viremia, were associated with impaired HDL function, as determined by two independent measures. The viremic group had approximately 18% mean relative impairment in antioxidant function and 9% relative mean impairment in HDL dynamics (HDLDI) compared to HIV-1 infected participants on effective ART. After correcting for covariates that may affect HDL function, it was determined that the most notable correlates of impaired HDL function were BMI, apoA-I (inversely), and albumin (inversely). There were no consistent differences in both measures of HDL function between the different ART treatment groups. Considering that oxidative modification of HDL (higher HDL_{ox}) has been demonstrated *in vitro* to impair HDL-mediated cholesterol efflux and inhibit HDL remodeling (low HDLDI)[20,23] , it is not surprising that higher HDL_{ox} is correlated with lower HDL remodeling *in vivo* in chronic HIV-1 infection. Overall, our data using novel measures of HDL function, confirm prior evidence that HIV-1 infection is associated with impaired HDL function despite effective ART[17,33,37-39]. HDL oxidation may impair HDL function and HDL dynamics in HIV-1 infection and may have a central role in HIV pathogenesis that both result from and contribute to systemic inflammation of HIV infection.

We addressed the limited understanding of the influence of chronic HIV-1 infection on HDL function by exploring the associations of independent measures of HDL function with covariates that may affect HDL function in uninfected persons[15,25-27]. We found that in HIV-1 infected participants lower apoA-I was independently associated with impaired

HDL dynamics (lower HDLDI) and that higher BMI, lower apoA-I, current smoking, lower albumin, were independently associated with impaired HDL antioxidant function (higher HDL_{ox}). However, in HIV-1 uninfected participants only albumin demonstrated a moderate positive association with HDLDI (but not for HDL_{ox}), after adjusting for covariates. ApoA-I is the major protein in HDL and is known to have a major role in HDL function[6]. Albumin binds to HDL, has antioxidant activity[40] and has previously been shown to affect HDL function [25]. These results parallel our results from a prior small matched cohort study of HIV-1-infected participants with low CVD risk profiles where HDL_{ox} was independently associated with anthropometric parameters of obesity[32]. Smoking is known to affect oxidation of lipoproteins, and lipoprotein metabolism [41] and promotes atherogenesis[42].

We found that compared to uninfected controls, HIV-1 infected males on successful ART and low CVD risk had impaired HDL dynamics and antioxidant function. Initiation of ART among HIV-infected patients incompletely reduces markers of systemic inflammation [43]. HIV-1 infected individuals receiving ART may also have higher oxidative stress compared to HIV-1 infected ART-naïve or healthy subjects due to higher production of free radical species, mitochondrial dysfunction and alterations in antioxidant systems [44,45]. Herein, we showed that HDL_{ox} is associated with HDL remodeling *in vivo* in chronic HIV-1 infection. Prior studies demonstrate that HDL remodeling may be a CVD-relevant measure of HDL function and is associated with cholesterol efflux, one of the main HDL functions [21,22]. *In vitro* studies indicate that oxidative modification of HDL may impair the cholesterol efflux and inhibit HDL

remodeling/exchange of apoA-I [20,23]. Thus, HDL oxidation may impair HDL function and may have a central role in HIV pathogenesis. We previously showed that HDL_{ox} in HIV-1 infected subjects on long term ART and without clinical CVD are i) associated with *in vivo* progression of CVD[12] ii) may stimulate endothelial cells to induce M/M chemotaxis, a measure of HDL function [11,37,46] iii) correlated positively with non-calcified coronary atherosclerotic plaque[13]. Further studies are needed to characterize HDL dynamics, HDL_{ox} and different measures of HDL function including cholesterol efflux in HIV-1 infected males on successful ART.

There are limited data regarding the effect of ART on oxidized lipoproteins, lipoprotein particle number and size and HDL function [47]. Contrary to our original hypothesis, raltegravir did not appear to have more favorable effects on HDL_{ox} and HDLDI than PI treatment. This is consistent with data from our recent prospective study where RAL initiation in ART naïve participants was not associated with favorable effects on HDL_{ox}[48]. We found in unadjusted analysis that NNRTI use was associated with reduced HDL_{ox} (but not HDLDI), consistent with prior data that suggest NNRTI use has beneficial effect on HDL-C and cholesterol transport[29]. However, this relationship did not remain significant in the adjusted analysis. Further studies are needed to confirm the complex effects of ART on measures of HDL function.

The strengths of our study are the careful covariate phenotyping of our study population including novel measures of HDL function. However, there are limitations. Our study is cross-sectional and therefore causality cannot be assessed. Our study may have been

underpowered to detect clinically meaningful associations of ART with measures of HDL function and correlates of HDL function in HIV-1 uninfected participants. Further limitations are also recognized in the context of biochemical assays of HDL function.[11,12,49]. These limitations may compromise the ability to detect differences in measures of HDL function in a population with an overall low CVD risk. The current research focused on cell free assays and the cell-based cholesterol efflux assay was not performed. Our study included only men.

In conclusion, we determined parameters associated with abnormal HDL function using two independent measures of HDL functionality. HIV-1 infected males (even the ones on effective ART with low CVD risk) had impaired antioxidant function and HDL dynamics compared to HIV-1 uninfected males. Higher BMI, lower apoA-I and lower albumin were among the most notable correlates of impaired HDL function among HIV-1 infected participants. There were no notable differences in HDL function among ART treatment groups. We found that HDL_{ox} is associated with HDL remodeling *in vivo* in chronic HIV-1 infection. Thus, larger prospective studies with longer-term treatments are needed to further study and understand the role of HDL function in HIV-1 pathogenesis.

FIGURES

Figure 1: Comparison of measures of HDL function by group. A. Oxidized HDL (HDL_{ox}) B. HDL apoA-I exchange.

Footnote: ART: antiretroviral therapy, HDL-C: high-density lipoprotein cholesterol, HDLDI: HDL Dynamic Index. ***, p<0.001; **, p<0.01 (unpaired T-test)

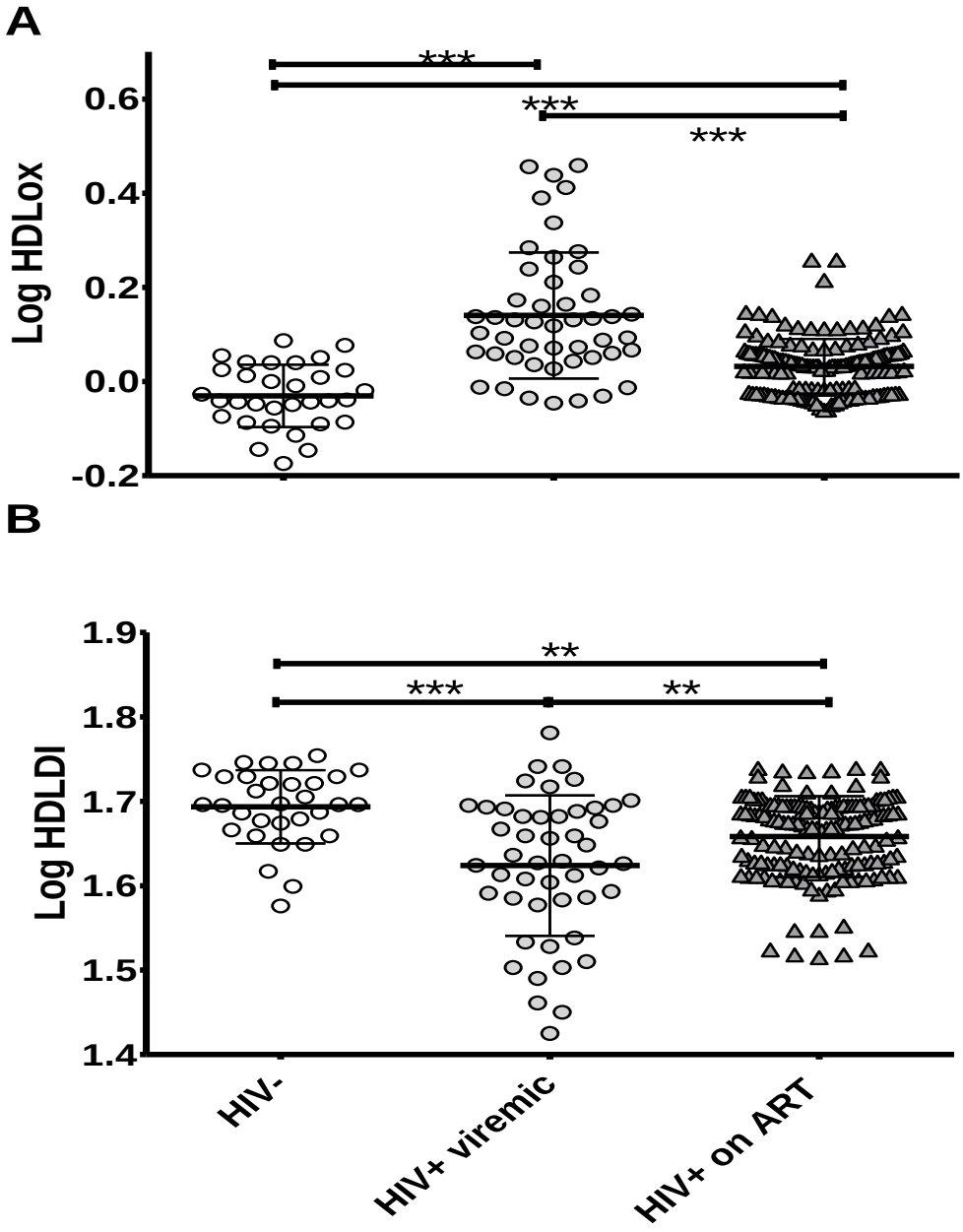
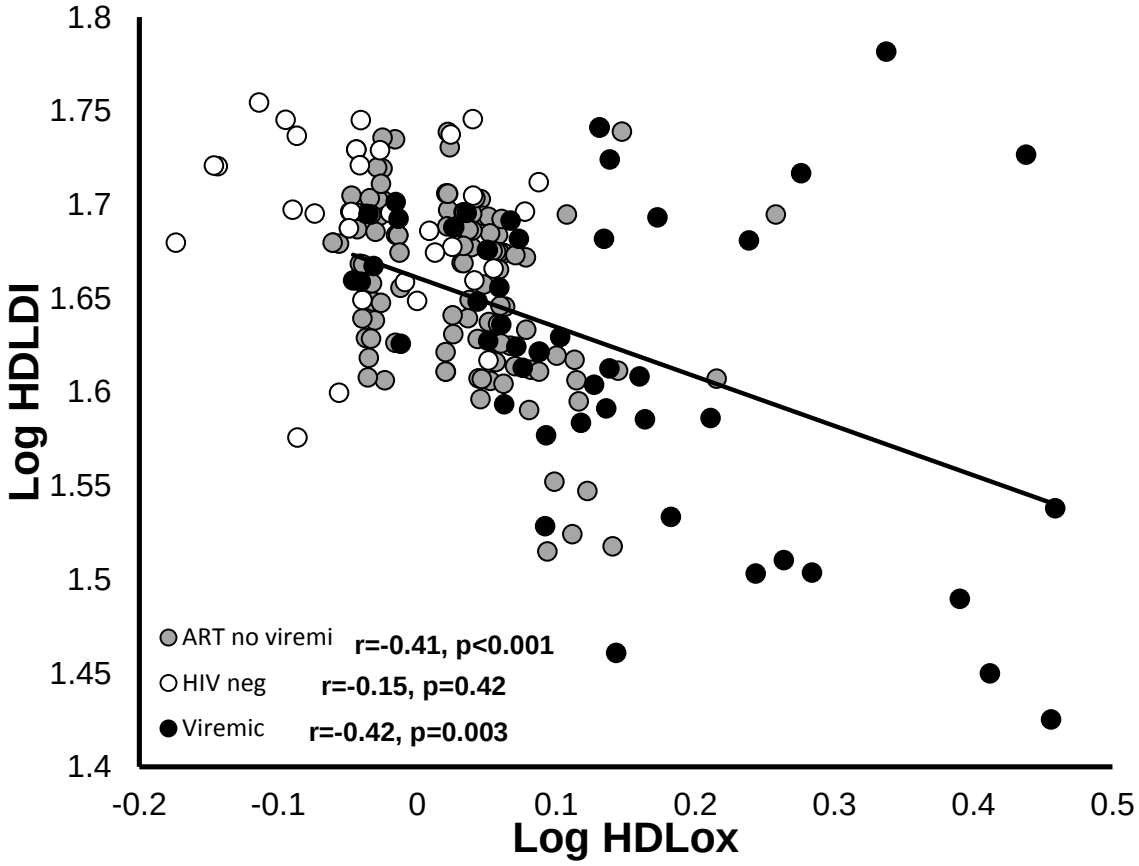


Figure 2: Pearson correlation between different measures of HDL function among different groups. The most notable association between HDL_{ox} and HDLDI was in the HIV-1 infected viremic group (shown).



TABLES
Table 1: Characteristics by group.

	HIV viremic (n=50)	HIV on ART no viremia (n=116)	HIV (-) low CVD risk (n=32)	P value
Demographics				
Age (years)	45 (12.5)	47.5 (15.0)	35 (12.75)	***<0.001***
Race/Ethnicity				0.941
Non-Hispanic White	30 (60%)	78 (67.2%)	20 (63%)	
Hispanic	11 (22%)	22(19.0%)	8 (25%)	
Other	9 (18%)	16 (13.8%)	4 (12%)	
Body Mass Index (BMI) (kg/m^2)	27.8 (5.3)	25.7 (4.6)	26.1 (4.6)	0.215
Current smoker	30 (60%)	23 (19.8%)	12 (38%)	***<0.001***
Kidney disease	23 (46%)	32 (27.6%)	11 (34%)	0.071
Presence of ≥1 Risk factors for CVD (yes/no)[b]	25 (50%)	40 (34.5%)	(-)	***0.036***[a]
Metabolic syndrome	15 (30%)	12 (10.3%)	(-)	***0.002***[a]
> 6% 10-year risk of CVD	15 (30%)	10 (8.6%)	(-)	***<0.001***
HIV related parameters				
Duration of HIV (years)	11 (14.5)	12 (15)	(-)	0.979[a]
Duration of ART (years)	8 (9.5)	7 (12)	(-)	0.611[a]
Current CD4 T cell count (cells/m^3)	511 (343)	535 (327)	(-)	0.998[a]
Nadir CD4 T cell count (cells/m^3)	235 (413)	246 (357)	(-)	0.703[a]
Log Viral load (log_{10} copies/ml)	3.0 (1.94)	(-)	(-)	(-)
Co-infections (%HBV+ and/or HCV+)	9 (18%)	11 (9.5%)	(-)	0.115[a]
% NRTI	36 (72%)	112 (96.6%)	(-)	0.588[a]
% TDF/FTC	25 (50%)	87 (75%)	(-)	0.664[a]
% ABC	7 (14%)	19 (16.4%)	(-)	0.799[a]
% NNRTI	13 (26%)	59 (50.9%)	(-)	0.079[a]
% PI	14 (39%)	46 (39.7%)	(-)	0.845[a]

% RAL	14 (28%)	30 (25.9%)	(-)	0.090[a]
Albumin (gr/dL)	3.9 (1.4)	4.5 (0.45)	4.1 (0.6)	***<0.001***
ApoA-I (mg/dL)	101.4 (42.4)	116.6 (38.0)	170.1 (73.4)	***<0.001***
Lipid Panel				
Total cholesterol [mg/dL]	150 (60.0)	174.5 (52.0)	131 (27)	0.994[a]
Triglycerides [mg/dL]	122.0 (94.0)	132 (110.0)	98 (28)	0.839[a]
HDL cholesterol [mg/dL]	37.0 (16.0)	41.0 (17.0)	43 (14)	0.984[a]
LDL cholesterol [mg/dL]	93.5 (41.8)	100 (44.3)	74 (11)	0.935[a]
Non-HDL cholesterol [mg/dL]	119 (39.0)	129.5 (49.3)	107 (26)	0.991[a]
HDL function measurements				
HDL_{ox} (normalized ratio to pooled control)	1.26 (0.38)	1.08(0.21)	0.91 (0.24)	***<0.001***
HDLDI (%)	42.9 (10.7)	47.1 (7.2)	49.7 (7.1)	***<0.001***

Notes: a: Comparison performed between the two HIV groups (viremia vs. no viremia on ART)

B: except for smoking

C: Median (first – third quartiles) or number (%).

Abbreviations: ABC: abacavir; ApoA-I: Apolipoprotein A-I, ART: antiretroviral therapy, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HDL: high-density lipoprotein; HDL_{ox}: oxidized high-density lipoprotein, HDLDI: HDL dynamic index, HIV: human immunodeficiency virus, LDL: low-density lipoprotein; NRTI: Nucleoside reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PI: protease inhibitors; RAL: raltegravir, TDF/FTC: tenofovir/emtricitabine.

Table 2: Univariate analysis: Predictors of logHDLDI (P <0.10).

		All HIV subjects			HIV-uninfected	
Covariate	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**
Log [Age]	166	***0.001 (0.000, 0.002)***	***0.017***	32	-0.001 (-0.002, 0.001)	0.281
Race (nonwhite vs. white)	166	0.014 (-0.006, 0.035)	0.176	32	-0.013 (-0.048, 0.023)	0.464
Log [BMI] (kg/m^2)	*166*	***-0.135 (-0.271, 0.002)***	***0.054***	32	-0.061 (-0.316, 0.193)	0.625
Lipids	152			31		
Log [TC] (mg/dL)		***0.126 (0.056, 0.195)***	***<0.001***		0.053 (-0.161, 0.268)	0.615
Log [TG] (mg/dL)		***0.049 (0.012, 0.086)***	***0.010***		0.086 (-0.055, 0.227)	0.222
Log [LDL] (mg/dL)		0.029 (-0.034, 0.093)	0.366		0.050 (-0.138, 0.238)	0.593
Log [HDL] (mg/dL)		0.010 (-0.060, 0.081)	0.773		0.039 (-0.153, 0.232)	0.678
Log [Non-HDL-C] (mg/dL)		***0.083 (0.009, 0.156)***	***0.028***		-0.045 (-0.230, 0.141)	0.626
Log [ApoA-I] (mg/dL)	166	***0.318 (0.274, 0.362)***	***<0.0001***	32	0.070 (-0.086, 0.227)	0.368
HDL_{ox}	166	***-0.292 (-0.375, -0.209)***	***<0.0001***	32	-0.098 (-0.338, 0.141)	0.409
Metabolic syndrome (yes/no)	165	-0.006 (-0.019, 0.007)	0.365	32	(-)	(-)
Presence of ≥1 Risk factors for CVD (yes/no)[a]	166	0.000 (-0.009, 0.010)	0.922	32	(-)	(-)
Smoking (yes/no)	165	***-0.023 (-0.032, -0.013)***	***<0.0001***	32	-0.010 (-0.026, 0.006)	0.199
Kidney injury (yes/no)	165	***-0.012 (-0.022, -0.002)***	***0.018***	32	-0.004 (-0.020, 0.013)	0.646
Log [Albumin] (gr/dL)	160	***0.277 (0.160, 0.394)***	***<0.0001***	32	***0.506 (0.150, 0.861)***	***0.007***

Notes: a: except for smoking

Table 3: Multivariate analysis: Predictors of logHDLDl (P <0.10) after adjusting for false discovery rate (FDR) using the Benjamini–Hochberg procedure. Nominal p-values presented. Those with FDR < 0.05 are underlined.

	All HIV subjects (n=149)[1]		**HIV-uninfected (n=32)[2]**	
Covariate1	**Parameter Estimate (95% CI)**	**P value**	**Parameter Estimate (95% CI)**	**P value**
Log [Age]	-0.029 (-0.097, 0.039)	0.447	-0.020 (-0.149, 0.110)	0.758
Race (nonwhite vs. white)	-0.004 (-0.011, 0.003)	0.216	-0.011 (-0.028, 0.005)	0.170
Log [BMI] (kg/m^2)	0.049 (-0.044, 0.141)	0.301	0.161 (-0.140, 0.462)	0.281
Presence of ≥1 Risk factors for CVD (yes/no)[a]	0.002 (-0.004, 0.008)	0.463	(-)	(-)
Smoking (yes/no)	-0.003 (-0.011, 0.005)	0.668	(-)	(-)
Kidney Injury (yes/no)	-0.003 (-0.011, 0.004)	0.395	(-)	(-)
Log [TC] (mg/dL)	0.035 (-0.018, 0.087)	0.123	(-)	(-)
Log [HDL] (mg/dL)	0.008 (-0.057, 0.041)	0.971	(-)	(-)
Log [Albumin] (gr/dL)	0.017 (-0.083, 0.118)	0.730	***0.783 (0.274, 1.292)***	***0.004***
Log [ApoA-I] (mg/dL)	***0.313 (0.263, 0.364)***	***<0.001***	-0.045 (-0.218, 0.129)	0.601
Viremia (yes/no)	-0.002 (-0.010, 0.006)	0.601	(-)	(-)
Log [CD4] (cells/m^3)	-0.016 (-0.044, 0.011)	0.236	(-)	(-)
Log [duration of HIV] (years)	0.014 (-0.003, 0.031)	0.111	(-)	(-)

Notes: a: except for smoking

1. The covariates considered for HIV subjects were age, race (nonwhite vs. white), body mass index, fasting lipid measurements [total cholesterol, HDL-C], apoA-I levels, presence of risks factors for CVD, smoking, kidney injury, albumin, HIV status, viremia (yes/no), current CD4 T cell count, duration of HIV infection. Additional model based on

the above covariates plus HDLox (n=149), gave similar estimates (not shown). 2. The covariates considered for HIV uninfected subjects were age, race (nonwhite vs. white), body mass index, apoA-I levels, albumin.

Table 4: Univariate analysis: Predictors of logHDL_{ox} (P <0.10).

		All HIV subjects			HIV-uninfected	
Covariate	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**
Log [Age]	166	***-0.001 (-0.004,0.001)***	***0.002***	32	-0.002 (-0.004,0.001)	0.175
Race (nonwhite vs. white)	166	***-0.029 (-0.063, 0.004)***	***0.088***	32	0.019 (-0.031, 0.069)	0.441
Log [BMI] (kg/m^2)	166	***0.520 (0.310, 0.732)***	***<0.001***	32	***0.709 (0.419, 1.000)***	***<0.01***
Lipids	152			31		
Log [TC] (mg/dL)		***-0.270 (-0.369, -0.171)***	***<0.001***		0.108 (-0.205, 0.421)	0.487
Log [TG] (mg/dL)		***-0.106 (-0.159, -0.053)***	***0.0001***		0.164 (-0.038, 0.367)	0.108
Log [LDL] (mg/dL)		-0.049 (-0.142, 0.044)	0.296		-0.208 (-0.473, 0.057)	0.119
Log [Non-HDL-C] (mg/dL)		***-0.171 (-0.278, -0.063)***	***0.002***		-0.048 (-0.320, 0.223)	0.721
Log [ApoA-I] (mg/dL)	166	***-0.330 (-0.424, -0.236)***	***<0.001***	32	-0.171 (-0.406, 0.065)	0.150
Metabolic syndrome (yes/no)	165	***0.050 (0.031, 0.070)***	***<0.001***	32	(-)	(-)
Presence of ≥1 Risk factors for CVD (yes/no)[a]	166	***0.026 (0.010, 0.041)***	***0.001***	32	(-)	(-)
Smoking (yes/no)	165	***0.061 (0.047, 0.074)***	***<0.001***	32	***0.044 (0.024, 0.063)***	***<0.01***
Kidney injury (yes/no)	165	***0.019 (0.003, 0.034)***	***0.022***	32	***0.044 (0.024, 0.064)***	***<0.01***
Log [Albumin] (gr/dL)	160	***-0.934 (-1.076, -0.791)***	***<0.001***	32	***-0.969 (-1.471, -0.467)***	***<0.01***

Notes: a: except for smoking

Table 5: Multivariate analysis: Predictors of logHDL_{ox} (P ≤0.05) after adjusting for false discovery rate (FDR) using the Benjamini–Hochberg procedure. Nominal p-values presented. Those with FDR ≤ 0.05 are underlined.

	All HIV subjects (N=149)[1]		HIV-uninfected (N=32)[2]	
Covariate1	**Parameter Estimate (95% CI)**	**P value**	**Parameter Estimate (95% CI)**	**P value**
Log [Age] (years)	-0.012 (-0.108, 0.084)	0.804	-0.053 (-0.215, 0.110)	0.512
Race (nonwhite vs. white)	0.008 (-0.001, 0.0174)	0.090	-0.011 (-0.033, 0.011)	0.314
Log [BMI] (kg/m^2)	***0.131 (0.001, 0.260)***	***0.048***	***0.425 (0.006, 0.855)***	***0.050***
Log [TC] (mg/dL)	-0.066 (-0.137, 0.006)	0.073	0.045 (-0.205, 0.295)	0.712
Log [ApoA-I] (mg/dL)	***-0.162 (-0.234, -0.090)***	***<u>≤0.001</u>***	(-)	(-)
Kidney injury (yes/no)	-0.000 (-0.010, 0.009)	0.952	(-)	(-)
Presence of ≥1 Risk factors for CVD (yes/no)[a]	***0.009 (0.000,0.018)***	***0.047***	(-)	(-)
Smoking (yes/no)	***0.015 (0.004, 0.026)***	***<u>0.010</u>***	(-)	(-)
Log [Albumin] (gr/dL)	***-0.416 (-0.558, -0.274)***	***<u>≤0.001</u>***	-0.469 (-1.070, 0.133)	0.121
Viremia (yes/no)	***0.011 (0.001, 0.022)***	***0.039***	(-)	(-)
Log [CD4] (cells/m^3)	***-0.061 (-0.100, -0.023)***	***<u>0.002</u>***	(-)	(-)
Log [duration of HIV] (years)	***-0.026 (-0.050, -0.001)***	**<u>0.039</u>**	(-)	(-)

Notes: a: except for smoking

1. The covariates considered for HIV subjects were age, race (nonwhite vs. white), body mass index, fasting lipid measurements [total cholesterol], apoA-I, presence of risks factors for CVD, smoking, kidney injury, albumin, viremia (yes/no), current CD4 T cell count. Additional models based on the above covariates plus NNRTI use (n=131) and or HDLDI (n=149), gave similar estimates (not shown). 2. The covariates considered for HIV uninfected subjects were age, race (nonwhite vs. white), body mass index, fasting lipid measurements [total cholesterol] and albumin.

SUPPLEMENTAL TABLES

Supplemental Table 1: Effect of ART Characteristics by group.

Variable	N	All subjects (N=58)	N	NNRTI(N=28)	N	RAL (N=14)	N	PI (N=19)	P value
Age (years)	58	44 (15)	28	44 (12.8)	13	42 (20.5)	17	47 (19)	0.654
Race (white)	58	41 (70.7%)	28	24 (41.4%)	13	7 (12.1%)	17	10 (17.3%)	***0.045***
BMI (kg/m^2)	58	25.3 (4.05)	28	25.26 (4.78)	13	25.68 (4.16)	17	25.2 (4.54)	0.962
Kidney injury	58	14 (24.1%)	28	7 (12.1%)	13	3 (5.2%)	17	4 (6.9%)	***0.023***
Fasting glucose (mg/dL)	55	90 (13)	26	90 (11.5)	13	84 (21.5)	16	91.5 (14.5)	0.388
Lipids	53		26		11		16		
Total cholesterol(mg/dL)		169 (38.5)		170.5 (46.5)		157 (48)		161.5 (60.5)	0.332
Triglycerides (mg/dL)		123 (103)		125 (148.8)		134 (103)		119.5 (104)	0.917
LDL-C (mg/dL)		99 (41)		102 (41.3)		98 (29)		90.5 (51)	0.762
HDL-C (mg/dL)		40 (14.5)		46 (16.5)		39 (10)		39 (11)	***0.075***
Non-HDL-C (mg/dL)		45 (53)		122.5 (51.3)		112.5 (41)		126.5 (64.3)	0.765
Albumin (gr/dL)	56	4.5 (0.4)	27	4.5 (0.4)	13	4.5 (0.45)	16	4.5 (0.65)	0.673
Duration of HIV (years)	58	9.5 (16)	28	12.5 (14.5)	13	8 (17.5)	17	7 (17.5)	0.562
Duration of ART (years)	58	5.5 (11.3)	28	5 (9.3)	13	4 (15.5)	17	7 (14)	0.613
CD4 Nadir (cells/m^3)	41	250 (371.5)	28	215.5 (307.3)	13	450 (363)	17	175 (350.8)	***0.030***
CD4 (cells/m^3)	56	533.5 (359.8)	28	441 (354)	13	629 (355.5)	17	533.5 (324.5)	0.862

Supplemental Table 2: Univariate analysis: HIV-related predictors of logHDLDI in HIV patients (P <0.10).

		HIV all subjects			HIV on ART/no viremia			HIV viremic (naïve, ART failure)	
Covariate1	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**
Log [duration of HIV] (years)	166	***0.033 (0.013, 0.053)***	***0.001***	118	0.011 (-0.012, 0.034)	0.34	48	***0.040 (0.002,0.078)***	***0.038***
Log [duration of ART] (years)	154	0.014 (-0.008, 0.036)	0.201	116	0.007 (-0.014, 0.027)	0.535	38	0.031 (-0.028, 0.090)	0.292
Log (CD4 nadir] (cells/m^3)	128	-0.009 (-0.026, 0.008)	0.287	86	-0.014 (-0.032, 0.003)	0.106	42	-0.010 (-0.045, 0.025)	0.555
Prior AIDS (yes/no)	159	0.003 (-0.007, 0.013)	0.555	112	0.006 (-0.003, 0.015)	0.189	47	0.001 (-0.024, 0.025)	0.958
Viremia (yes/no)	166	***-0.017 (-0.027, -0.010)***	***0.001***	(-)	(-)	(-)	(-)	(-)	(-)
Log[viral load] (copies/mm3)	47	-0.010 (-0.031, 0.011)	0.329	(-)	(-)	(-)	47	-0.010 (-0.031, 0.011)	0.329
Log [CD4] (cells/m^3)	163	-0.010 (-0.050, 0.030)	0.617	118	-0.016 (-0.054, 0.021)	0.391	47	-0.042 (-0.140, 0.056)	0.389
Log [CD4/CD8 ratio]	63	0.023 (-0.010, 0.056)	0.172	118	-0.005 (-0.039, 0.029)	0.769	47	0.015 (-0.071, 0.101)	0.724
Presence of co-infection (HBV/HCV) (yes/no)	166	-0.001 (-0.015, 0.013)	0.892	118	-0.006 (-0.020, 0.009)	0.449	48	0.013 (-0.018, 0.044)	0.412
NRTI use (yes/no)	117	0.004 (-0.021, 0.030)	0.743	117	0.004 (-0.021, 0.030)	0.743	(-)	(-)	(-)
ABC use (yes/no)	117	-0.007 (-0.004, 0.019)	0.186	117	-0.007 (-0.004, 0.019)	0.186	(-)	(-)	(-)
TDF/FTC use (yes/no)	117	0.001 (-0.009, 0.010)	0.835	117	0.001 (-0.009, 0.010)	0.835	(-)	(-)	(-)

NNRTI use (yes/no)	117	0.002 (-0.006, 0.010)	0.640	117	0.002 (-0.006, 0.010)	0.640	(-)	(-)		(-)
PI use (yes/no)	117	0.002 (-0.006, 0.010)	0.597	117	0.002 (-0.006, 0.010)	0.597	(-)	(-)		(-)
RAL use (yes/no)	117	-0.006 (-0.015, 0.004)	0.246	117	-0.006 (-0.015, 0.004)	0.246	(-)	(-)		(-)

Supplemental Table 3: Univariate analysis: HIV-related predictors of logHDL_{ox} in HIV patients (P <0.10)

	HIV all subjects			HIV on ART/no viremia			HIV viremic (naïve, ART failure)		
Covariate1	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**	**N**	**Parameter Estimate (95% CI)**	**P value**
Log [duration of HIV] (years)	166	-0.077 (-0.108, -0.046)	<0.0001	118	-0.029 (-0.057, -0.000)	**0.049**	48	-0.083 (-0.142, -0.024)	**0.007**
Log [duration of ART] (years)	154	-0.025 (-0.059, 0.008)	0.135	116	-0.014 (-0.040, 0.012)	0.287	38	-0.046 (-0.138, 0.046)	0.314
Log (CD4 nadir] (cells/mm3)	128	-0.016 (-0.044,0.013)	0.283	86	0.000 (-0.022, 0.023)	0.972	42	-0.008 (-0.067, 0.050)	0.772
Prior AIDS (yes/no)	159	0.003 (-0.013, 0.019)	0.680	112	-0.004 (-0.015, 0.007)	0.499	47	0.007 (-0.033, 0.047)	0.729
Viremia (yes/no)	166	0.055 (0.040, 0.070)	<0.0001	(-)	(-)	(-)	(-)	(-)	(-)
Log[viral load] (copies/mm3)	47	0.083 (0.060, 0.106)	<0.0001	(-)	(-)	(-)	47	0.083 (0.060, 0.106)	<0.01
Log [CD4] (cells/mm3)	163	-0.140 (-0.202, -0.079)	0.0001	115	-0.054 (-0.100, -0.009)	**0.019**	48	-0.197 (-0.345, -0.050)	0.010
Log [CD4/CD8 ratio]	63	-0.152 (-0.201, -0.103)	<0.0001	115	-0.039 (-0.080, 0.002)	0.065	48	-0.213 (-0.337, -0.090)	**0.001**
Presence of co-infection (HBV/HCV) (yes/no)	166	0.016 (-0.007, 0.039)	0.177	118	0.012 (-0.006, 0029)	0.193	48	-0.002 (-0.052, 0.049)	0.948
NRTI use (yes/no)	(-)	(-)	(-)	117	0.004 (-0.021, 0.030)	0.743	(-)	(-)	(-)
ABC use (yes/no)					-0.003 (-0.017, 0.011)	0.649			
TDF/FTC use (yes/no)					0.002 (-0.010, 0.014)	0.708			

NNRTI use (yes/no)	(-)	(-)		(-)	117	-0.011 (-0.021, -0.001)	**0.034**	(-)	(-)	(-)
PI use (yes/no)	(-)	(-)		(-)	117	0.009 (-0.001, 0.020)	0.090	(-)	(-)	(-)
RAL use (yes/no)	(-)	(-)		(-)	117	0.009 (-0.002, 0.021)	0.116	(-)	(-)	(-)

CHAPTER 2: STATISTICAL APPENDIX

Variables included in statistical analysis

All HDLDI and HDL_{ox} assays were performed in duplicate and the mean was computed and used for analysis. Median and interquartile ranges are reported for continuous variables, and percentages are reported for categorical data. A log transformation was used for substantial skewness in continuous variables as needed. The primary endpoint was HDL function determined by two independent cell free assays, HDLDI and HDL_{ox}. Associations between different HDL function parameters were established by using linear regression and Pearson's correlation coefficient. Parameters that were explored as possible determinants of HDL function included sociodemographic characteristics, non-AIDS comorbidities, albumin, lipid profile, and HIV-related parameters.

Modeling of measures of HDL function

We modeled HDL_{ox} using two different approaches that represented a) a normalized fraction of HDL that is oxidized, and b) a single level of oxidized HDL with adjustment for level of HDL-C concentration [12,34] in our statistical models. In addition, we modeled HDLDI using two different approaches that represented a) a normalized to ApoAI levels fraction of HDLDI and b) a single level of HDLDI with adjustment for apoAI levels in our statistical models. Our observations were consistent regardless of approach.

Comparison of measured variables between study groups

Baseline characteristics were compared using parametric and nonparametric methods as appropriate for the data being evaluated. The Wilcoxon rank sum test, the Kruskal-

Wallis test, the Student t test and analysis of variance (ANOVA) were used to compare continuous variables and the Chi-square or Fisher's exact test were used to compare categorical variables, as needed. In a subset analysis ANOVA was used to compare HDL function in HIV-1 infected individuals well matched by anthropometric, demographic and metabolic characteristics who were on the same ART backbone of nucleoside reverse transcriptase inhibitors (NRTIs) and who were receiving as third agent either NNRTI, PI or RAL. In order to minimize potential confounding due to uncontrolled viremia the comparison of pertinent variables was also done separately in viremic versus ART treated individuals with suppressed viremia. Finally, in order to better characterize HIV-1 related parameters associated with abnormal HDL function and to further minimize potential confounding due to non-HIV related parameters that may affect HDL function, the comparison of HDL function was also done separately in ART treated individuals with suppressed viremia and any risk for CVD versus ART treated individuals with suppressed viremia and no risk for CVD (except for potential smoking).

Association between the two independent measures of HDL function

Pearson's correlation was used to evaluate the association between HDL_{ox} and HDLDI (both log transformed) among all participants.

Linear regression

Linear regression was used to investigate the predictors of HDL_{ox} and HDLDI (continuous outcomes). Normality of distribution of data was explored with normal

quintile plots. The suitability of the regression model was explored with residuals versus fitted values plots (plot of the residuals versus each individual X variable). For non-linearity, non-normality or heteroscedasticity of data log transformations were performed as needed. Representative plots are shown in figures A and B.

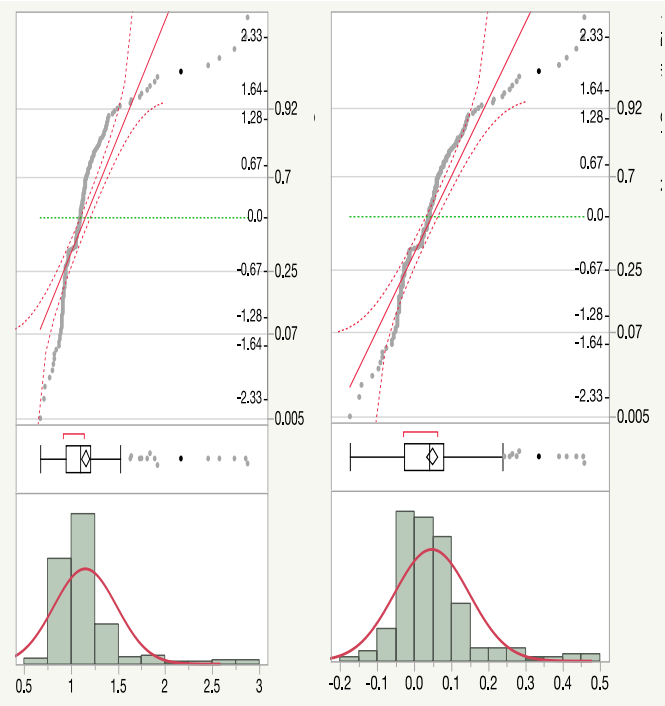


Figure A. Representative normal quantile plot for a continuous variable (HDLox). Log transformation of values gave a more normal distribution of data. Left: Untransformed data. Shapiro-Wilk W test W value=0.707, prob<W <0.0001. Right: Log-transformed data. Shapiro-Wilk W test W value=0.872, prob<W <0.0001.

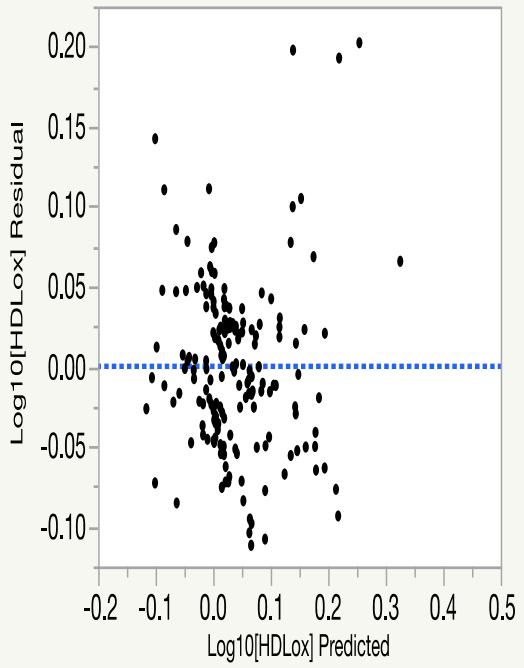


Figure B. Representative Residual by Predicted (Fitted) plot (log HDLox).

Covariates significant in the univariate analysis ($p<0.05$) were also examined together in multivariate analysis. The relative magnitude of association of major parameters with regards to the primary outcomes (HDL_{ox}, HDLDI) and false discovery rate (FDR) was further explored with The Response Screening function in JMP 12.0 and FDR LogWorth by Effect Size plots. When there are large effects, the associated p-values are often very small and visualizing these small values graphically can be challenging. When

transformed to the LogWorth (-log10(p-value)) scale, highly significant p-values have large LogWorths and nonsignificant p-values have low LogWorths. A LogWorth of zero corresponds to a nonsignificant p-value of 1. Any LogWorth above 2 corresponds to a p-value below 0.01.

In the FDR LogWorth by Effect Size plot, the vertical axis is the FDR LogWorth and the horizontal axis is the Effect Size. Generally, larger effects lead to more significant p-values and larger LogWorths. Representative plots for the two primary outcomes (HDLDI, HDL_{ox}) are shown in Figure C.

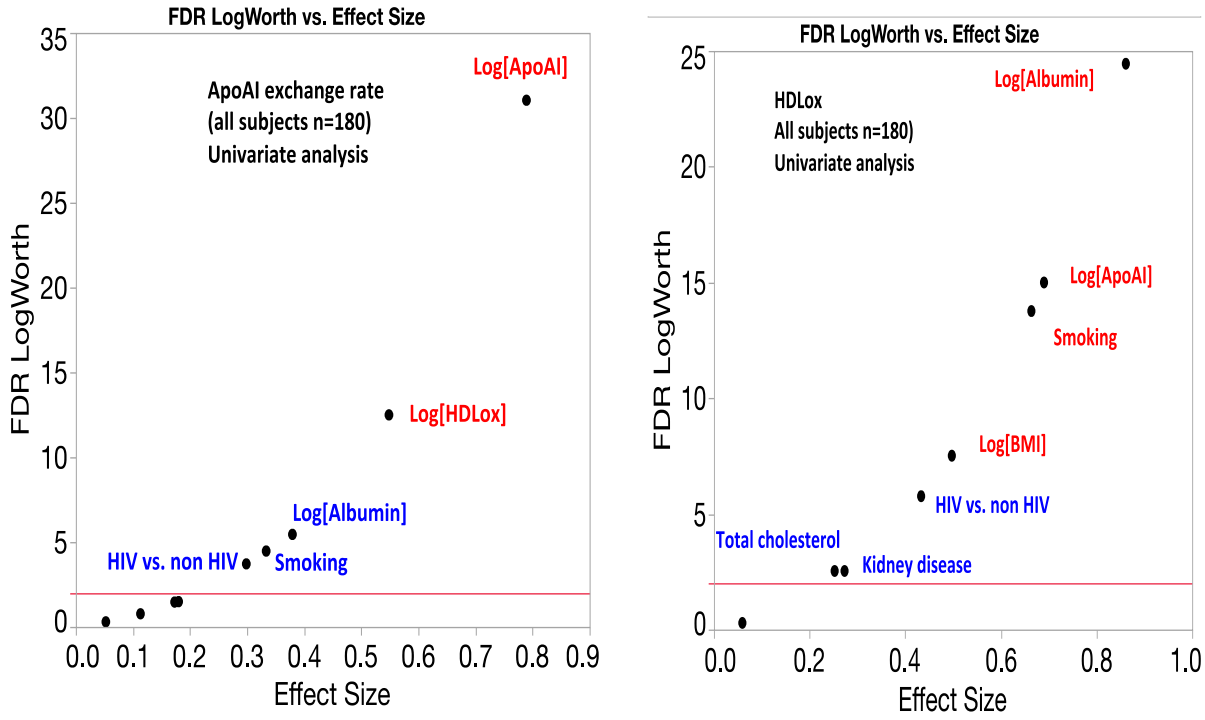


Figure C: Response screening for notable associations between predictors and outcomes. Representative FDR LogWorth versus effect size for HAE (left) and HDLox (right) for all subjects (n=180) with available data in the studied parameters. Variables that had significant association (above red line) with the outcome (despite FDR adjustment) are marked in blue. Variables that also remained significant in multivariate analysis are marked in red.

Initial multivariate models were developed including the following variables: age, race (white versus nonwhite), presence of any CVD risk factor, current smoking, plasma total

cholesterol (log transformed), plasma HDL-C (log transformed), plasma apoA-I (log transformed). For HIV-1 infected persons HIV-1 related parameters such as presence of viremia and current CD4 T cell count were also included in the final multivariate model. For the model building, models were optimized using a forward stepwise approach and selecting the model with a minimum Bayesian information criterion (BIC). Nonlinear effects of continuous variables were evaluated with the use of locally weighted scatterplot smoothing (LOWESS) plots. Collinearity between candidates variables was determined by examination of contingency tables, and multivariate models were then run, including the strongly collinear variables in turn. Interactions between important predictors in final models were evaluated by including interaction terms along with main-effect terms. None of the interactions that were tested was significant, and these interactions are not discussed further in this analysis. Variables that are known a priori to be strongly co-linear and to have interactions were not included together in the final multivariate model (e.g. total cholesterol and LDL-C, triglycerides, non-HDL-C; CD4 T cell count and CD4/CD8 T cell ratio etc). Combinations of remaining variables were then tested, with variables retained in the model as long as the multivariate P value was < .1. For each set of hypotheses in multivariate analysis, the false discovery rate was controlled at alpha=0.05 using the Benjamini-Hochberg procedure [36]. Inference was also guided by nominal p-values as well as consideration of the consistency, direction and magnitude of the effect sizes and confidence intervals. A summary of the p values after Benjamini–Hochberg procedure in the multivariate models of both outcomes (HDLDI, HDL_{ox}) described in Tables 3 and 5 is shown below.

Sample size

Given our prior published studies on HDL_{ox} among HIV-1 infected participants, our sample size calculations to detect differences in effect size of HDL function were based on HDL_{ox}, that is expressed as normalized ratio to a pooled plasma control from healthy donors (no units; e.g. a difference in ratio of 0.1 among two groups indicates a 10% relative difference in HDL_{ox} among the comparison groups)[11,17,24,32,33]. Thus, based on prior studies of HDL function and inflammation in HIV-infected cohorts[17,24,32,33], and using a two-sided, 0.05-level, two-sample t-test with two comparisons, a sample size of 40 individuals per group (HIV-1 versus uninfected individuals), provides at least 80% power to detect differences of at least 0.6 in effect size for HDL_{ox} between groups.

Table: Summary of multivariate analysis and correlates of HDLDI and HDL_{ox} after adjusting for false discovery rate (FDR) using the Benjamini–Hochberg procedure.

Group	All HIV subjects			HIV-uninfected		
P^{FDR}	**<0.05**	**0.05-0.20**	**>0.20**	**<0.05**	**0.05-0.20**	**>0.20**
HDLDI	**HDL_{ox}, apoAI**	(-)	TC, CD4 , duration of HIV	**Albumin**	(-)	Race
HDL_{ox}	**ApoAI Smoking, Albumin, duration of HIV, CD4 count**	BMI, viremia, presence of ≥1 risk factors for CVD	Race, TC,NNRTI use	(-)	BMI	Albumin

Note: Similar models as outlined in Tables 3 and 5 were used for FDR analysis.

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