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

4 **individuals**

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13 **Running title:** HDL function in chronic HIV infection

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ABSTRACT

Objective: HDL function rather than absolute level may be a more accurate indicator for

cardiovascular disease (CVD). Novel methods can measure HDL function using patient

samples. The objective of this study is to identify factors that may contribute to HDL

dysfunction in chronic, treated HIV-1 infection.

Design: Retrospective study of HDL function measured in two ways in 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 examined patient level factors associated with two different measures of

HDL dysfunction: reduced antioxidant function (oxidized HDL, HDL_{ox}) and reduced

HDL-apoA-I exchange (HAE), a measure of HDL remodeling, in the HIV infected and

control men. 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.

1   **Results:** In multivariate analysis among HIV-1 infected males (n=166) (median age 45

2   years, CD4 T cell count 505 cells/mm^3, 30.1% were viremic), higher BMI, lower apoA-I

3   and lower albumin were among the most notable correlates of higher HDL_{ox} and lower

4   HAE (p<0.05). In HIV-1 uninfected participants lower albumin and higher BMI were

5   associated with lower HAE and higher HDL_{ox}, respectively (p≤0.05). HDL_{ox} was

6   inversely related to HAE in HIV-1 infected individuals (p<0.001).

7   **Conclusion:** Increased HDL_{ox} correlates with reduced HAE in chronic HIV-1 infection.

8   Higher BMI, lower apoA-I and albumin were identified as factors associated with HDL

9   dysfunction in chronic HIV-1 infection using two independent methods.

10   **Key Words:** HDL function, Human Immunodeficiency Virus, cardiovascular disease,

11   HDL-apoA-I exchange, HDL remodeling, oxidized HDL

12

13   **INTRODUCTION**

14   Cardiovascular disease (CVD) is a major cause of morbidity and mortality among HIV-1

15   infected individuals on effective antiretroviral therapy (ART) [1, 2]. However, the exact

16   mechanisms of increased CVD among HIV-1 infected persons remain unclear. Higher

17   levels of High-density lipoprotein cholesterol (HDL-C) are an important negative

18   indicator of CVD events [3, 4]. HDL function rather than absolute level (HDL-C) may be

19   a more accurate indicator of CVD risk [5, 6], and recent studies confirm that CVD is

20   strongly inversely correlated with cholesterol efflux capacity [7]. While HDL performs

21   activities that are CVD-protective, in the setting of inflammation HDL becomes

22   functionally impaired, elevating CVD risk [8]. Inflammation affects HDL by decreasing

23   anti-inflammatory antioxidant factor levels and activity, increasing associated pro-

3

1   inflammatory proteins, lipid hydroperoxide content and redox activity (HDL_{ox})

2   (independently of HDL levels), reducing cholesterol efflux potential, and diminishing

3   HDL's ability to inhibit LDL oxidation [8]. HIV-1 infected ART-treated individuals have a

4   higher prevalence of dyslipidemia and low HDL-C [9]. HIV-1 infected individuals also

5   have impaired lipoprotein metabolism [10] and HDL_{ox} [11, 12] that have been

6   associated with CVD in some but not all studies [12-14]. We found that HDL_{ox} but not

7   oxidized low-density lipoproteins (LDL_{ox}) was independently and consistently associated

8   with several biomarkers of systemic inflammation and immune activation in both ART-

9   naïve viremic and ART-treated individuals [15]. In a prospective study of 234 HIV-

10   infected ART naïve participants without CVD who were randomized to receive tenofovir-

11   emtricitabine plus atazanavir/ritonavir, darunavir/ ritonavir, or raltegravir (RAL) and

12   achieved plasma HIV-1 RNA <50 copies/ml by week 24 and thereafter HDL_{ox} declined

13   over 96 weeks of ART [14]. Thus, given the emerging role of impaired HDL function in

14   chronic treated HIV-1 infection it is important to understand predictors of HDL

15   dysfunction in chronic HIV-1 infection.

16   Due to the complexity of the HDL particles, measurement of HDL function has been

17   difficult to study in humans [16, 17]. Cell-free assays may give more robust

18   measurements of HDL function compared to cell-based assays [12, 18] such as

19   cholesterol efflux assays [16] that have several limitations including lack of

20   standardization and significant heterogeneity with regards to types of cells and type of

21   readout reported [18]. There is limited data regarding how different measures of HDL

22   function (such as cholesterol efflux, antioxidant function, lipoprotein particle size

23   correlate to each other. ApoA-I, the major protein component of HDL, plays a key role

4

1 in the promotion of cholesterol efflux [19] and its function is critical to its anti-atherogenic

2 molecular processes [20]. Modification of apoA-I impairs its ability to exchange on and

3 off HDL, a critical process in reverse cholesterol transport, that is also a measure of

4 HDL remodeling [21, 22]. HDL-apoA-I exchange (HAE) is markedly reduced when

5 atherosclerosis is present, or when the subject carries at least one risk factor of CVD

6 [21, 22]. A previously described cell-free assay based on electron paramagnetic

7 resonance (EPR) spectroscopy, measures HAE, which provides a measure of HDL

8 dynamics and the ability of HDL to remodel and release apoA-I [21, 22]. As spin-labeled

9 apoA-I associates with HDL, the EPR spectra's peak amplitude increases due to

10 structural changes in apoA-I from a lipid-free to a lipid-bound conformation [22, 23]. The

11 HAE response relative to the maximal detectable HAE response (%HAE) provides a

12 measure of the relative exchangeability of endogenous apoA-I and the dynamic nature

13 of HDL particles [22, 23]. Early studies suggest that lower %HAE is a CVD-relevant

14 measure of HDL function [22, 23]. *In vitro* studies suggest that oxidative modification of

15 apoA-I may impair HDL's ability to mediate cholesterol efflux by inhibiting the

16 remodeling/exchange of apoA-I [21, 24]. However, it is unknown how HDL_{ox} relates to

17 HDL remodeling *in vivo.* We have developed a novel cell-free fluorometric method that

18 measures HDL associated lipid peroxidation (HDL_{ox}) that offers a reproducible and rapid

19 means of determining HDL function [12]. In certain populations of HIV infected ART

20 treated participants the readout from this assay correlates with measures of subclinical

21 atherosclerosis such as carotid intima media thickness [12] and calcium artery score

22 [25]. We hypothesized that in chronic HIV-1 infection, increased oxidative stress and

23 impaired antioxidant HDL function (as measured by higher HDL_{ox}) are associated with

5

lower %HAE and this association is independent of other factors associated with

impaired HDL function in HIV uninfected persons (such as elevated body mass index;

BMI) [16, 17]. 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 [17]), laboratory (such as albumin that binds to

reactive oxygen species and HDL and may affect HDL function [26]) parameters and

comorbidities (such as smoking [27], kidney disease [28], presence of metabolic

syndrome [26]) that may have a role in abnormal HDL function (antioxidant, cholesterol

efflux; evidence based on HIV uninfected individuals [17, 26-28]). Towards this aim, we

utilized a cross-sectional sample of ART-treated, viremic HIV-1 infected individuals and

HIV-1 uninfected healthy controls (matched by 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) within the HIV group. This

is in light of prior studies where more favorable effects on lipids [29] and more

prominent anti-inflammatory effects [30] were observed in RAL groups compared to PI

groups. Finally, since prior data suggest non-nucleoside reverse-transcriptase inhibitors

(NNRTIs) have beneficial effect on HDL-C and cholesterol transport [31], we

hypothesized that NNRTI use would be associated with improved HDL function

compared to PIs.

6

1 **METHODS**

2 **Study Design and Participants**

3

4 The Center for Clinical AIDS Research and Education (CARE) HDL function study was

5 a cross-sectional study developed to assess determinants of impaired HDL function

6 among HIV-infected patients on stable ART with HIV-1 RNA <200 copies/ml within 6

7 months of enrollment compared to viremic HIV-1 infected (≥200 copies/ml) and

8 uninfected individuals. The cohort enrolled participants ≥18 years of age males from the

9 University of California, Los Angeles (UCLA) CARE clinic in Los Angeles, California in a

10 single study visit that included biological specimen collection for storage and medical

11 record review. HIV-1 uninfected ≥18 years of age males with no known dyslipidemia,

12 metabolic and inflammatory comorbidities and no known risk factors for CVD (except for

13 smoking) were additionally recruited in outpatient clinics (such as primary care and

14 general infectious diseases clinics) within UCLA. All individuals enrolled in the study

15 provided written informed consent and the study was approved by the UCLA

16 Institutional Review Board.

17

18 **Data collection**

19 Sociodemographic characteristics, comorbidities, presence of kidney disease (defined

20 as glomerular filtration rate [GFR]< 60 ml/min/1.73 cm2), presence of risk factors for

21 CVD (defined as at least one of the following: metabolic syndrome defined by National

22 Cholesterol Education Program criteria [32], diabetes, dyslipidemia, use of lipid lowering

23 medication, hypertension, family history of CVD, Framingham 10-year Coronary Heart

1   Disease Risk Score ≥10% risk), albumin, lipid profile were abstracted from the medical

2   records for all study participants. In addition, for HIV-1 infected participants data that

3   were also abstracted included duration of HIV-1 infection and ART, current (within 6

4   months) and nadir CD4+ T lymphocyte counts, plasma HIV-1 RNA levels.

5

6   **Biomarker and Laboratory Assessment**

7   *Plasma lipid analysis*

8   The lipid panel (total cholesterol, HDL-C, and triglycerides) was measured in fasted

9   EDTA-plasma by standard validated clinical assays employing a Beckman DXC, and

10   LDL cholesterol (LDL-C) was calculated by the Friedewald formula. Plasma apoA-I

11   levels were determined by validated nephelometric method as previously described [22,

12   23].

13

14   *Oxidized HDL*

15   HDL_{ox} was quantified using a previously validated fluorometric biochemical assay that

16   measures HDL lipid peroxidation based on the oxidation of the fluorochrome Amplex

17   Red [12]. To reduce experimental variability and adjust for HDL amount, we normalized

18   the mean fluorescence readout from quadruplicates of each sample (HDL_{ox}_sample) by

19   the mean fluorescence readout from quadruplicates of a pooled plasma control

20   (HDL_{ox}_control) and by concurrent HDL cholesterol concentration level (HDL-C) using

21   the following calculation: "normalized" oxidized HDL ($nHDL_{ox}$) = [HDL_{ox}_sample x 40

22   (mg/dl)] / [HDL_{ox}_control x HDL-Csample (mg/dL)], where 40 mg/dL represents HDL-C

23   of the pooled plasma control [14, 15]. This approach has been validated in clinical

8

1   studies and has been shown to reduce experimental variability [11, 12, 14, 15, 25, 33,

2   34]. Higher levels of this adjusted measure of HDL function have been associated with

3   worse health outcomes [12, 13, 17, 35]. Throughout the results HDL_{ox} is presented as

4   normalized [$nHDL_{ox}$] measure to reflect the adjustment for experimental variability and

5   HDL-C.

6

7   *HAE assay*

8   HAE assays were performed as previously described [22, 23]. Freshly thawed plasma

9   was mixed 1:4 with PBS (20 mM phosphate, 150 mM NaCl, pH 7.4) and 24% w/v PEG

10   6000 (Sigma) was added to a final concentration of 4%. Samples were centrifuged at

11   13,000 rpm for 10 minutes in a tabletop centrifuge at 4°C to remove apoB-containing

12   lipoproteins. The clarified plasma was then mixed with 3 mg/mL spin-labeled apoA-I in

13   a 3:1 ratio and drawn into an EPR-compatible borosilicate capillary tube (VWR). EPR

14   measurements were performed with a Bruker eScan EPR spectrometer outfitted with

15   temperature controller (Noxygen). Samples were scanned first at 6 °C, incubated for 15

16   minutes at 37 °C, and scanned again at 37 °C. The peak amplitude of the nitroxide

17   signal from spin-labeled apoA-I in the sample (3462–3470 Gauss) was compared to the

18   peak amplitude of a proprietary internal standard (3507–3515 Gauss) provided by

19   Bruker. The internal standard is contained within the eScan spectrometer cavity and

20   does not contact the sample. Since the y-axis of an EPR spectrum is measured in

9

1   arbitrary units, measuring the sample against a fixed internal standard facilitates

2   normalization of sample response. HAE activity was determined by subtracting the

3   sample:internal standard ratio obtained at 6 °C from the sample:internal standard ratio

4   at 37 °C. The baseline spectra of spin-labeled apoA-I in PBS was subtracted from

5   results. Maximum amplitude of spin-labeled apoA-I was determined from spin-labeled

6   apoA-I in a fully lipid-bound conformation and %HAE was determined by dividing the

7   calculated HAE response by the HAE maximum response. All samples were read in

8   triplicate and averaged. HAE was calculated as described [22, 23]. Inter-assay

9   coefficient of variability was 5.3%.

10

11   **Statistical Analyses**

12   Baseline characteristics were compared using parametric (such as ANOVA) and

13   nonparametric methods as appropriate for the data being evaluated. Pearson's

14   correlation was used to evaluate the association between HDL_{ox} and %HAE (both log

15   transformed) among all participants. Multivariate linear regression was used to

16   investigate the predictors of HDL_{ox} and %HAE. Covariates significant in the univariate

17   analysis (p<0.10) were also examined together in multivariate analysis. For each set of

18   hypotheses in multivariate analysis, the false discovery rate (FDR) was controlled at

19   alpha=0.05 using the Benjamini-Hochberg procedure [36]. Statistical hypothesis tests

20   were two-sided with a significance threshold of 0.05 for p values. Based on our prior

21   published studies on nHDLox among HIV-1 infected participants [12, 14, 15, 25, 33, 34],

22   and using a two-sided, 0.05-level, two-sample t-test with two comparisons, a sample

23   size of 40 individuals per group (HIV-1 versus uninfected individuals), provides at least

10

1   80% power to detect differences of at least 0.6 in effect size for HDLox (expressed as

2   normalized ratio to a pooled plasma control from healthy donors; no units) between

3   groups. All statistical analyses were conducted using JMP Pro 12.01 (SAS Institute,

4   Cary, NC).

5

6   **RESULTS**

7   **Baseline characteristics**

8   Baseline characteristics of the 198 participants are shown in Table 1. Briefly, the

9   median age of HIV-1 infected participants with suppressed viremia on ART (n=116) was

10   47.5, 67% of them were non-hispanic white, the median CD4 T cell count was 535

11   cells/mm^3 and the group overall had a low cardiovascular disease risk; only 9% of

12   participants had a 10-year risk of hard coronary heart disease >6%, 20% were current

13   smokers; 10% had metabolic syndrome. All HIV-1 infected persons were men who have

14   sex with men (MSM). The most common ART regimen was

15   efavirenz/emtricitabine/tenofovir disoproxil fumarate (EFV/TDF/FTC) (23.7%) followed

16   by TDF/FTC/ darunavir/ritonavir (DRV/r) (16.1%) and TDF/FTC/ raltegravir (RAL)

17   (11.9%). Only 28% of the viremic patients were ART naïve and the rest had virus

18   resistant to ART at the time of the visit. The median age of HIV-1 infected participants

19   with viremia (n=50) was 45 years, and this group overall had a significantly higher CVD

20   risk, lower median CD4 T cell count and higher incidence of coinfections compared to

21   the ART-treated groups (p<0.05). There were no differences in other HIV-1 related

22   parameters between the ART-treated and the viremic group (p>0.05). The HIV-1

23   uninfected participants (n=32) were younger (median age of 35 years) compared to

24   HIV-1 uninfected participants (p<0.001). Participants were similar across ART groups

11

1 with the TDF/FTC backbone and they were representative of the full substudy

2 population (Supplemental Table 1).

3

4 **Comparison of lipids and parameters related to HDL function between groups**

5 We explored differences in parameters previously reported to be associated with

6 impaired HDL functions between study groups. Viremic HIV-1 infected participants had

7 lower albumin, higher prevalence of kidney disease and smoking ($p<0.001$) and lower

8 HDL ($p<0.05$) compared to ART-treated and uninfected participants (Table 1). HIV-1

9 infected participants had overall abnormal lipid profile and lower apoA-I levels compared

10 to uninfected persons whereas the ART-treated groups had overall similar lipid profile

11 compared to the viremic group (Table 1) ($p>0.05$).

12

13 **Comparison of HDL function measures between groups**

14 Median HDL_{ox} of viremic subjects, and ART-treated HIV-1 infected subjects was 35%

15 and 17% higher, respectively, compared to uninfected participants ($p<0.001$). Viremic

16 and ART-treated HIV-1 infected subjects had a lower %HAE compared to uninfected

17 participants ($p<0.01$). Viremic subjects also had a lower %HAE compared to all the

18 ART-treated groups ($p<0.01$) (Table 1) (Figure 1). Overall, HIV-1 infected persons had

19 higher HDL_{ox} and lower %HAE compared to uninfected participants.

20

21

22

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

2 **participants**

3 To address our limited understanding of abnormal HDL function in chronic HIV-1

4 infection, we determined correlates of HDL remodeling (%HAE assay among HIV-1

5 groups). Among HIV-1 infected participants, there was a positive association between

6 age, fasting lipids (total cholesterol, triglycerides), apoA-I, albumin and %HAE ($p < 0.05$).

7 In contrast there was an inverse relationship between %HAE and BMI, HDL_{ox}, smoking,

8 kidney disease ($p < 0.05$). The most notable positive associations were between %HAE

9 and albumin and apoA-I (Table 2) whereas lower %HAE was associated with higher

10 HDL_{ox}. Regarding HIV-1 related parameters, only duration of HIV-1 infection and

11 presence of viremia had a positive association with %HAE (Supplemental table 2). All

12 associations between HDL_{ox}, apoA-I and %HAE were attenuated but remained

13 statistically significant ($p < 0.05$) after adjusting for FDR and covariates (age, race, BMI,

14 lipids, apoA-I levels, presence of risks factors for CVD, kidney injury, albumin, viremia,

15 current CD4 T cell count, duration of HIV infection) (Table 3). However, in HIV-1

16 uninfected participants only albumin demonstrated a moderate positive association with

17 %HAE (Table 2), even after adjusting for FDR and covariates (age, race, BMI, lipids,

18 apoA-I). These results suggest that apoA-I in HIV-1 infected and albumin in uninfected

19 participants, were the strongest positive correlates of HDL remodeling.

20

21

22

13

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, white race, higher 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 shorter 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, 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).

1 **Associations of HDL function with ART**

2 We then explored the association of different ART classes with HDL function. Overall,

3 there was no ART class-specific (NRTI, vs. NRRTIs, vs. PIs, vs. RAL) association

4 between %HAE or HDL_{ox} (Supplemental Tables 1-3). HIV-1 infected participants on

5 ART with the same NRTI backbone (TDF/FTC) who received NNRTIs, PIs or RAL had

6 similar %HAE and HDL_{ox} levels (Supplemental Table 1). NNRTI use was associated

7 with lower HDL_{ox} in univariate analysis (Supplemental Table 3) but not in multivariate

8 analysis after adjustment for covariates that may affect HDL function (Table 5).

9

10 **Correlations of measures of HDL function among groups**

11 We determined whether HDL_{ox} correlates with HDL remodeling *in vivo* in chronic HIV-1

12 infection. Using Pearson correlation (Figure 2) and univariate analysis (Table 2), there

13 was a significant inverse relationship between HDL_{ox} and %HAE among all HIV-1

14 infected participants (r=0.50, p<0.001). This association was similar between the

15 viremic (r=-0.42, p=0.003) and the aviremic (r=-0.41, p<0.001) HIV-1 infected

16 participants (Figure 2) but was not present in the uninfected individuals (r=-0.115,

17 p=0.42). HDL_{ox} correlated with %HAE (Table 2) in HIV-1 infected groups after

18 adjustment for other clinical factors (Table 3).

19
20 **DISCUSSION**

21 In this cross sectional study of HIV-1 infected males with low overall CVD risk and

22 healthy males with no known CVD risk, we found that chronic HIV-1 infection, despite

23 effective ART, as well as viremia, were associated with impaired HDL function, as

24 determined by two independent measures. Overall, HIV-1 infected persons had higher

1   HDL_{ox} and lower %HAE compared to uninfected participants. The viremic group had

2   approximately 18% mean relative impairment in antioxidant function and 9% relative

3   mean impairment in HDL dynamics (%HAE) compared to HIV-1 infected participants on

4   effective ART. After correcting for covariates that may affect HDL function, it was

5   determined that the most notable correlates of impaired HDL function were BMI, apoA-I

6   (inversely), and albumin (inversely). There were no consistent differences in both

7   measures of HDL function between the different ART treatment groups. In HIV-1

8   uninfected persons, only albumin demonstrated a moderate positive association with

9   %HAE whereas none of the other significant correlates of abnormal HDL function in

10   HIV-1 infected persons were found to correlate with HDL_{ox} and %HAE. The relatively

11   small sample size and the low CVD risk profile of the HIV-1 uninfected group who had

12   normal lipid values may explain these observations and why we did not find an

13   association between HDL_{ox} and %HAE. Considering that oxidative modification of HDL

14   (higher HDL_{ox}) has been demonstrated *in vitro* to impair HDL-mediated cholesterol

15   efflux and inhibit HDL remodeling (low %HAE) [21, 24] , it is not surprising that higher

16   HDL_{ox} is correlated with lower HDL remodeling *in vivo* in chronic HIV-1 infection.

17   Overall, our data using novel measures of HDL function, confirm prior evidence that

18   HIV-1 infection is associated with impaired HDL function despite effective ART [12, 34,

19   37-39]. HDL oxidation may impair HDL function and HDL dynamics in HIV-1 infection

20   and may have a central role in HIV pathogenesis that both result from and contribute to

21   systemic inflammation of HIV infection.

22   We addressed the limited understanding of the influence of chronic HIV-1 infection on

23   HDL function by exploring the associations of independent measures of HDL function

16

1   with covariates that may affect HDL function in uninfected persons [17, 26-28]. We

2   found that in HIV-1 infected participants lower apoA-I was independently associated

3   with impaired HDL dynamics (lower %HAE) and that higher BMI, lower apoA-I, current

4   smoking, lower albumin, were independently associated with impaired HDL antioxidant

5   function (higher HDL_{ox}). However, in HIV-1 uninfected participants only albumin

6   demonstrated a moderate positive association with %HAE (but not for HDL_{ox}), after

7   adjusting for covariates. ApoA-I is the major protein in HDL and is known to have a

8   major role in HDL function [6]. Albumin binds to HDL, has antioxidant activity[40] and

9   has previously been shown to affect HDL function [26]. Albumin represents a very

10   abundant and important circulating antioxidant that attenuates oxidative damage[40].

11   Reactive oxygen species directly contribute to lipid peroxidation and can cause

12   modifications in proteins (including apoA-I). Thus, it is not surprising that in our study

13   lower albumin was independently associated with higher HDL_{ox}. These results parallel

14   our results from a prior small matched cohort study of HIV-1-infected participants with

15   low CVD risk profiles where HDL_{ox} was independently associated with anthropometric

16   parameters of obesity [33]. Obesity and dietary fat can modulate ABCA1-dependent

17   efflux, HDL-mediated activation of endothelial nitric oxide synthase (eNOS) and HDL

18   function [41, 42]. Smoking is known to affect oxidation of lipoproteins, and lipoprotein

19   metabolism and promotes atherogenesis[43].

20

21   We found that compared to uninfected controls, HIV-1 infected males on successful

22   ART and low CVD risk had impaired HDL dynamics and antioxidant function. Initiation

23   of ART among HIV-infected patients incompletely reduces markers of systemic

1 inflammation [44]. HIV-1 infected individuals receiving ART may also have higher

2 oxidative stress compared to HIV-1 infected ART-naïve or healthy subjects due to

3 higher production of free radical species, mitochondrial dysfunction and alterations in

4 antioxidant systems [45]. Herein, we showed that HDL_{ox} is associated with HDL

5 remodeling *in vivo* in chronic HIV-1 infection. Prior studies demonstrate that HDL

6 remodeling may be a CVD-relevant measure of HDL function and is associated with

7 cholesterol efflux, one of the main HDL functions [22, 23]. *In vitro* studies indicate that

8 oxidative modification of HDL may impair the cholesterol efflux and inhibit HDL

9 remodeling/exchange of apoA-I [21, 24]. Thus, HDL oxidation may impair HDL function

10 and may have a central role in HIV pathogenesis. We previously showed that HDL_{ox} in

11 HIV-1 infected subjects on long term ART and without clinical CVD are i) associated

12 with *in vivo* progression of CVD[12] ii) may stimulate endothelial cells to induce M/M

13 chemotaxis, a measure of HDL function [11] iii) correlated positively with non-calcified

14 coronary atherosclerotic plaque[13] iv) independently correlated with several markers of

15 systemic inflammation and immune activation in chronic HIV-1 infection[15]. Further

16 studies are needed to characterize HDL dynamics, HDL_{ox}, different measures of HDL

17 function including cholesterol efflux and mechanisms of HDL dysfunction in HIV-1

18 infected persons on successful ART.

19

20 There are limited data regarding the effect of ART on oxidized lipoproteins, lipoprotein

21 particle number and size and HDL function [46]. Contrary to our original hypothesis,

22 raltegravir did not appear to have more favorable effects on HDL_{ox} and %HAE than PI

23 treatment. This is consistent with data from our recent prospective study where RAL

18

1   initiation in ART naïve participants was not associated with favorable effects on

2   HDL$_{ox}$[47]. We found in unadjusted analysis that NNRTI use was associated with

3   reduced HDL$_{ox}$ (but not %HAE), consistent with prior data that suggest NNRTI use has

4   beneficial effect on HDL-C and cholesterol transport [31]. However, this relationship did

5   not remain significant in the adjusted analysis. Further studies are needed to confirm

6   the complex effects of ART on measures of HDL function.

7

8   The strengths of our study are the careful covariate phenotyping of our study population

9   including novel measures of HDL function. However, there are limitations. Our study is

10   cross-sectional and therefore causality cannot be assessed and potential confounders

11   such as lifestyle changes (especially in viremic patients) may not have been fully

12   accounted for. The small size of our study limits our ability to detect clinically meaningful

13   associations of ART with measures of HDL function and correlates of HDL function in

14   HIV-1 uninfected participants. Further limitations are also recognized in the context of

15   biochemical assays of HDL function [11, 12]. These limitations may compromise the

16   ability to detect differences in measures of HDL function in a population with an overall

17   low CVD risk. The current research focused on cell free assays and the cell-based

18   cholesterol efflux assay was not performed. Finally an important limitation of our study

19   was the inclusion of men only. We recruited only males to avoid confounding from sex

20   differences in lipid metabolism in this pilot study [48].

21

22   In conclusion, we determined parameters associated with abnormal HDL function using

23   two independent measures of HDL functionality. HIV-1 infected males (even the ones

1 on effective ART with low CVD risk) had impaired antioxidant function and HDL

2 dynamics compared to HIV-1 uninfected males. Higher BMI, lower apoA-I and lower

3 albumin were among the most notable correlates of impaired HDL function among HIV-

4 1 infected participants. There were no notable differences in HDL function among ART

5 treatment groups. We found that HDL_{ox} is associated with HDL remodeling *in vivo* in

6 chronic HIV-1 infection. Thus, larger prospective studies with longer-term treatments are

7 needed to further study and understand the role of HDL function in HIV-1 pathogenesis.

8 **Acknowledgments:**

10

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4

5 **FIGURES**

6 **Figure 1: Comparison of measures of HDL function by group.** A. Oxidized HDL

7 (HDL_{ox}) B. HDL apoA-I exchange.

8 Footnote: ART: antiretroviral therapy, HDL-C: high-density lipoprotein cholesterol,

9 %HAE: % HDL apoA-I exchange. ***, $p<0.001$; **, $p<0.01$ (unpaired T-test)

10

11 **Figure 2: Pearson correlation between different measures of HDL function among**

12 **different groups.** The most notable association between HDL_{ox} and %HAE was in the

13 HIV-1 infected viremic group (shown).

14

27

1 **TABLES**

2 **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/m2)	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	7 (14%)	12 (10.3%)	3 (9.4%)	0.471
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]
> 10% 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/m3)	511 (343)	535 (327)	(-)	0.998[a]
Nadir CD4 T cell count (cells/m3)	235 (413)	246 (357)	(-)	0.703[a]
Log Viral load (log10 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]

1

% 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***
%HAE	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, %HAE: 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 log%HAE (P <0.10).

Covariate	N	All HIV subjects Parameter Estimate (95% CI)	P value	N	HIV-uninfected 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/m2)	*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

3

Table 3: Multivariate analysis: Predictors of log%HAE (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.

Covariate1	All HIV subjects (n=149)1		HIV-uninfected (n=32)2	
	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/m2)	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/m3)	-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 (except for smoking), smoking, kidney injury, albumin, HIV status, viremia (yes/no), current CD4 T cell count, duration of HIV infection.

4

1   Additional model based on the above covariates plus HDLox (n=149), gave similar estimates (not shown). 2. The

2   covariates considered for HIV uninfected subjects were age, race (nonwhite vs. white), body mass index, apoA-I

3   levels, albumin. Parsimonious multivariate models with fewer variables (age, BMI, TC, viremia, CD4 T cell count)

4   gave similar results (consistent correlates of primary outcome).

5

1

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

Covariate	N	All HIV subjects Parameter Estimate (95% CI)	P value	N	HIV-uninfected Parameter Estimate (95% CI)	P value
Log [Age]	166	*0.001 (-0.001,0.004)*	*0.102*	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/m2)	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 (-0.171, 0.369)*	*<0.001*		0.108 (-0.205, 0.421)	0.487
Log [TG] (mg/dL)		*0.106, (-0.053, 0.159)*	*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*

3

4   Notes: a: except for smoking

5

6

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.
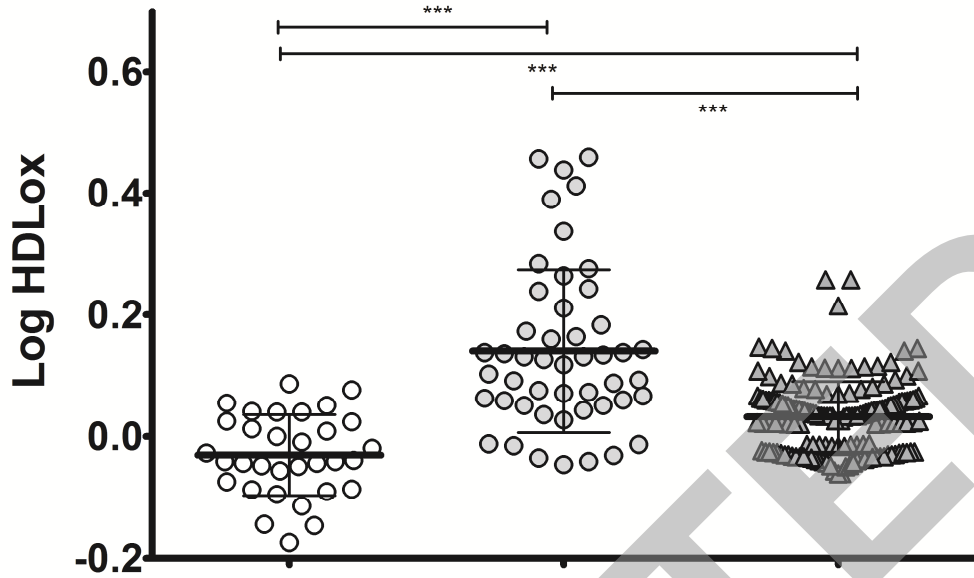
Covariate1	All HIV subjects (N=149)1 Parameter Estimate (95% CI)	P value	HIV-uninfected (N=32)2 Parameter Estimate (95% CI)	P value
Log [Age] (years)	-0.012 (-0.108, 0.084)	0.804	-0.053 (-0.215, 0.110)	0.512
Race (nonwhite vs. white)	0.008 (-0.001, 0.0174)	0.090	-0.011 (-0.033, 0.011)	0.314
Log [BMI] (kg/m2)	*0.131 (0.001, 0.260)*	*0.048*	*0.425 (0.006, 0.855)*	*0.050*
Log [TC] (mg/dL)	0.066 (-0.006, 0.137)	0.073	0.045 (-0.205, 0.295)	0.712
Log [ApoA-I] (mg/dL)	*-0.162 (-0.234, -0.090)*	*<0.001*	(-)	(-)
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)*	*0.010*	(-)	(-)
Log [Albumin] (gr/dL)	*-0.416 (-0.558, -0.274)*	*<0.001*	-0.469 (-1.070, 0.133)	0.121
Viremia (yes/no)	*0.011 (0.001, 0.022)*	*0.039*	(-)	(-)
Log [CD4] (cells/m3)	*-0.061 (-0.100, -0.023)*	*0.002*	(-)	(-)
Log [duration of HIV] (years)	*-0.026 (-0.050, -0.001)*	*0.039*	(-)	(-)

Notes: a: except for smoking

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1   1. The covariates considered for HIV subjects were age, race (nonwhite vs. white), body mass index, fasting lipid

2   measurements [total cholesterol], apoA-I, presence of risks factors for CVD, smoking, kidney injury, albumin, viremia

3   (yes/no), current CD4 T cell count. Additional models based on the above covariates plus NNRTI use (n=131) and or

4   %HAE (n=149), gave similar estimates (not shown). 2. The covariates considered for HIV uninfected subjects were

5   age, race (nonwhite vs. white), body mass index, fasting lipid measurements [total cholesterol] and albumin.

6   Parsimonious multivariate models with fewer variables (age, BMI, TC, viremia, CD4 T cell count) gave similar results

7   (consistent correlates of primary outcome).

A

(Graph A: y-axis "Log HDLox", ranging from -0.2 to 0.6; x-axis groups HIV-, HIV+ viremic, HIV+ on ART; significance bars labeled ***, ***, ***)

B

(Graph B: y-axis "Log HDLDI", ranging from 1.4 to 1.9; x-axis groups HIV-, HIV+ viremic, HIV+ on ART; significance bars labeled **, ***, **)