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MAJOR ARTICLE

Greater IL-6, D-dimer, and ICAM-1 Levels Are Associated With Lower Small HDL Particle Concentration in the Multicenter AIDS Cohort Study

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Objective. Low HDL cholesterol (HDL-C) is common in people living with HIV infection, which is associated with inflammation, and correlates with greater cardiovascular disease (CVD) risk. Particles of HDL are HDL subfractions, and in some general population studies, higher small HDL particle number (HDL-P) has been associated with lower CVD risk. The objective of this study was to determine whether HIV serostatus and systemic inflammation were associated with small HDL-P in the Multicenter AIDS Cohort Study (MACS).

Method. The MACS is composed of HIV-infected and HIV-uninfected men. Separate linear regression analyses were conducted to evaluate the associations between outcomes (small HDL-P, large HDL-P, total HDL-P, and HDL size) and variables of interest (interleukin-6 [IL-6], D-dimer, and intercellular adhesion molecule-1 [ICAM-1] levels), with adjustment for other CVD risk factors.

Results. The study population included 553 HIV-infected (88.1% on current ART) and 319 HIV-uninfected men. The mean age was 52.7 years for HIV-infected men and 55.3 years for HIV-uninfected men. In separate models of the study population, higher log IL-6 was associated with lower total and small HDL-P ($P < .01$ for both), independent of HIV serostatus and CVD risk factors. Similar results were seen with ICAM-1. Positive HIV serostatus was associated with lower small and total HDL-P, adjusted for inflammatory markers.

Conclusions. Greater systemic inflammation and HIV infection both were associated with lower atheroprotective small HDL-P. This may be a potential mechanism contributing to increased cardiovascular risk among HIV-infected people.

Key words. HDL-C; inflammation; lipoprotein particles.

INTRODUCTION

Individuals infected with HIV are at higher risk for cardiovascular disease (CVD) than the general population [1, 2]. Part of this risk is explained by an increased prevalence of traditional CVD risk factors, such as smoking and dyslipidemia [3]. In particular, HIV infection is associated with decreased HDL cholesterol (HDL-C) [4], with a slight increase in HDL-C after initiation of HAART [5]. Although low HDL-C is associated with increased CVD risk, pharmaceutical therapies to raise HDL-C in the general population have been unsuccessful in CVD prevention [6, 7].

The mechanisms of the protective effects of HDL, both in regards to function and types of HDL, are not completely understood. A major atheroprotective function of HDL is reverse cholesterol transport, a process mediated by small HDL particles [8], and HIV negatively alters reverse cholesterol transport [9]. The composition of HDL also is altered in the setting of HIV infection. Earlier in the highly active antiretroviral therapy (HAART) era, a previous analysis in the Multicenter AIDS Cohort Study (MACS) showed that HIV-infected men on HAART and untreated HIV-infected men had lower total HDL particle concentrations (HDL-P) compared to HIV-uninfected men [10].

One hallmark of both untreated and treated HIV infection is inflammation [11, 12]. In the SMART study of HIV-infected

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individuals with CD4 T cell counts of > 350 cells/mm^3, increased IL-6, a systemic inflammatory marker, and D-dimer, a coagulation marker, were associated with mortality [13]. Moreover, greater IL-6 and ICAM-1, an endothelial cell activation marker, were associated with a higher prevalence of coronary stenosis in the MACS [14]. Inflammation also alters the composition of HDL-associated proteins and their function. With regards to HDL particles, greater levels of the inflammatory markers, interleukin-6 (IL-6), D-dimer, and intercellular adhesion molecule-1 (ICAM-1), were associated with lower total and small HDL-P in untreated HIV-infected individuals in a cross-sectional study [11]. Compared to large HDL, small HDL has a higher capacity to accept cholesterol from the periphery, can further decrease endothelial adhesion molecule expression, and has a greater ability for taking up oxidized lipids [15].

The objectives of this cross-sectional study were to determine the relationships among HIV infection, the inflammatory markers IL-6, D-dimer, and ICAM-1, and the outcome of interest, small HDL-P, in the MACS.

METHODS

Study Population

The MACS is an ongoing, prospective cohort of HIV-infected and HIV-uninfected men at risk for HIV infection recruited from 4 sites: Baltimore, MD/Washington, DC; Los Angeles, CA; Chicago, IL; and Pittsburgh, PA. Enrollment periods occurred between 1984–1985, 1987–1991, 2001–2003, and 2010–present. The MACS participants undergo a semiannual standardized interview, physical examination, and laboratory testing. For the current study, analyses were limited to demographic and laboratory measurements from the visit most recently prior to the computed tomography (CT) scan, approximately 6 months, in the MACS Cardiovascular Ancillary Study conducted between 2010 and 2013—the period of time during which HDL particle measurements were performed [16]. All men in the MACS signed informed consent, and the study was approved by Institutional Review Boards at each site.

Briefly, inclusion criteria for the MACS Cardiovascular Ancillary Study were as follows: participants active in the MACS were aged 40 to 70 years, weighed < 300 pounds, and had no history of previous cardiac surgery or percutaneous coronary intervention [14]. Participants for whom inflammatory and fasting lipid particle measurements were missing were excluded from the study.

Lipoprotein and Inflammatory Marker Measurements

Lipoprotein particle concentrations and sizes from plasma samples, which were frozen at -70°C and subsequently thawed, were measured using a nuclear magnetic resonance (NMR) spectroscopic assay (LipoScience, Inc., Raleigh, NC) [10]. Measured fasting lipoprotein parameters included in this study were small HDL-P, large HDL-P, total HDL-P, and HDL size. Small and large HDL are subfractions of HDL that can be assessed by NMR [17]. The size of HDL, measured in nanometers, represents mean HDL particle size, because small HDL and large HDL have different diameter ranges [18]. Intra-assay coefficients of variation (% CV) for small HDL-P, large HDL-P, total HDL-P, and HDL size are 4.1%, 5.6%, 1.2%, and 0.5%, respectively, and the inter-assay % CVs are 3.7%, 5.9%, 1.5%, and 0.6%, respectively [18].

Chemiluminescent ELISA (R&D Systems, Minneapolis, MN) was used to measure serum IL-6 (inter-assay % CV 7–12%) and ICAM-1 (inter-assay % CV 3.3–7%), and a Stago STA-R analyzer (Parsippany, NY) was used to measure plasma D-dimer (inter-assay % CV 4–17%) [14]. These assays were measured in the laboratory of Russell Tracy, PhD, at The University of Vermont, Colchester, VT.

Measurements of Additional Covariates

Data from the semiannual study visit closest to the CT scan in the MACS Cardiovascular Ancillary Study were used. These variables include age, systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, HDL-C, lipid-lowering medication use, body mass index (BMI), smoking status (current, former, and never), and smoking pack-years. Lipid-lowering medications included statins, fibrates, and niacin. Blood samples from the semiannual MACS visit were utilized to measure glucose. In addition, the following HIV-infection–related variables were used: history of an AIDS-defining malignancy or opportunistic infection, current and nadir CD4+ T cell count, current viral load, and duration of ART use.

Statistical Methods

Comparisons of variables between HIV-uninfected and HIV-infected participants were measured using a *t* test for normally distributed continuous variables, a Wilcoxon rank sum test for nonnormally distributed continuous variables, and a χ^2 test for categorical variables.

Linear regression models were created to study the associations between lipoprotein particle concentrations and inflammatory markers. The primary outcome of interest was small HDL-P, and secondary outcomes included total HDL-P, large HDL-P, and HDL size. Covariates that did not have a normal distribution were natural log (ln) transformed, including IL-6 and D-dimer. Viral load was categorized as undetectable, low detectable (above the detectable threshold for the assay to < 1000 copies/mL), and high detectable (≥1000 copies/mL). Natural log IL-6, ln D-dimer, and ICAM-1 were standardized using their standard deviations (SD). As a result, the coefficients in the linear analyses represent the change in the outcome per inflammatory marker SD.

Separate models were constructed for each covariate of interest, ln IL-6, ln D-dimer, and ICAM-1, in order to investigate the individual relationship between each inflammatory marker and lipoprotein measurement. Because some of the covariates of interest potentially could be mediators and not confounders, we created both minimally and fully adjusted models. In the minimally adjusted models, the covariates were age, race, study center, study cohort (pre-2001 versus post-2001), and HIV serostatus. In the fully adjusted models, the following covariates were included: systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, use of lipid lowering medications, BMI, smoking (current, former, and never), and pack-years of smoking. Interaction terms between HIV serostatus and inflammatory markers were added to the models.

In the models limited to HIV-infected participants, the following covariates were added to the fully adjusted models: history of an AIDS-defining malignancy or opportunistic infection, current and nadir CD4+ T cell count, current viral load, and duration of ART use.

RESULTS

Study Population Characteristics

One thousand six men were eligible for inclusion in the analysis. Of these men, 872 men (553 HIV-infected, 319 HIV-uninfected) did not have any missing values for either inflammatory markers or fasting lipoprotein particles and were included in the analyses. Men infected with HIV were younger and had a lower BMI than HIV-uninfected men, but systolic blood pressure was similar between the 2 groups (Table 1). Men infected with HIV also tended to have higher triglyceride levels and lower HDL-C levels. Among HIV-infected men, the current mean CD4+ T cell count was 599 cells/mL, and the proportion of men with an undetectable HIV RNA concentration was 75.8%; 88.1% of HIV-infected men were currently on HAART, among whom 50% were on a protease inhibitor (PI)-based regimen. Men infected with HIV had higher median IL-6 ($P <$ .01) and ICAM-1 ($P <$ .01) levels than HIV-uninfected men. No significant difference in D-dimer level was noted between HIV-infected and HIV-uninfected men. Total, large, and small HDL-P were significantly lower in HIV-infected men than in HIV-uninfected men (Figure 1). Among HIV-infected men, those who were viremic had higher median IL-6 ($P <$ .01), D-dimer ($P <$ .04), and ICAM-1 ($P <$ .01) levels than those with undetectable HIV RNA. In addition, significantly lower mean total and small HDL-P levels ($P <$ .01 for both) were observed in viremic men than in HIV-infected men with undetectable HIV RNA. A lower mean large HDL-P also was noted in viremic men, although this was not a statistically significant finding (P = .08).

Small HDL-P, Large HDL-P, Total HDL-P, and HDL Size

Associations with small HDL-P concentration.

Each SD higher in ln IL-6 was significantly associated with a 0.6 μmol/L lower small HDL-P in the minimally adjusted model (P = .003). This association persisted in the fully adjusted model ($P <$ .001). The interaction between HIV serostatus and ln IL-6 was not significant in the fully adjusted model.

Natural log D-dimer was not significantly associated with small HDL-P in the minimally adjusted model (P = .21). However, the association was significant in a fully adjusted model ($P <$ .001), with each SD greater ln D-dimer associated with a 0.9 μmol/L lower small HDL-P. The interaction between HIV serostatus and ln D-dimer was not significant in the fully adjusted model.

Similarly, a greater ICAM-1 level was associated with a lower small HDL-P level in both the minimally adjusted model and in the fully adjusted model ($P <$ .001). The interaction between HIV serostatus and ICAM-1 was not significant in the fully adjusted model.

Associations with large HDL-P concentration.

In the minimally adjusted model, each SD greater ln IL-6 was associated with a 0.4 μmol/L lower large HDL-P (P = .04). However, this association did not persist after adjusting for traditional CV risk factors (P = 0.31). The interaction between HIV serostatus and ln IL-6 was not significant in the fully adjusted model.

Similarly, no associations were noted between ln D-dimer or ICAM-1 and large HDL-P in either the minimally adjusted models or the fully adjusted models. Interactions between HIV serostatus and the inflammatory marker of interest were not significant in the fully adjusted models.

Associations with total HDL-P concentration.

In a minimally adjusted model that included both HIV-infected and HIV-uninfected participants, each SD higher ln IL-6 was significantly associated with a 1.3 μmol/L lower total HDL-P ($P <$ .001; Table 2). After adjusting for traditional CV risk factors, each SD greater in ln IL-6 was associated with a 1.4 μmol/L lower total HDL-P ($P <$ .001). The interaction between HIV serostatus and ln IL-6 was not significant in the fully adjusted model.

Similarly, in a minimally adjusted model, a greater ln D-dimer level was associated with lower total HDL-P ($P <$ .001). However, this association was not present after the addition of traditional CV risk factors. The interaction between HIV serostatus and ln D-dimer was significant in the fully adjusted model only and was negative (β coefficient, -1.15; P = .02), which suggested that the magnitude of the inverse relationship between D-dimer and total HDL-P was greater in HIV-infected men than in HIV-uninfected men.

For a greater ICAM-1 level, lower total HDL-P was observed in the minimally adjusted model ($P <$ .001) and after adjusting for CV risk factors ($P <$ .001). The interaction between HIV serostatus and ICAM-1 was not significant in the fully adjusted model.

Associations with HDL particle size.

No significant associations were seen between ln IL-6 and HDL size, and the interaction between ln IL-6 and HDL size was not significant in the fully adjusted model.

Table 1. Participant Characteristics[a]

Characteristics	HIV Infected (N = 553)	HIV Uninfected (N = 319)	*P* value
Age (years)	52.7 (6.6)	55.3 (7.2)	< .001
Race			.001
African-American (%)	33.8	25.9	
White (%)	53.3	65.7	
Hispanic (%)	12.9	8.3	
Body mass index (kg/m^2)	26.0 (4.8)	27.4 (5.0)	< .001
Systolic blood pressure (mmHg)	126.8 (15.3)	128.0 (14.6)	0.27
Fasting glucose (mg/dL)	103.8 (29.2)	100.8 (33.6)	0.17
Smoking status			.005
Current (%)	31.6	21.8	
Former (%)	43.1	52.0	
Never (%)	25.3	26.2	
Smoking pack years†	5.5 (0.0, 22.4)	0.5 (0.0, 20.8)	.06
Cholesterol lowering medication use at time of visit (%)	35.6	31.0	0.18
Antihypertensive medication use at the time of visit (%)	35.1	31.6	0.29
Diabetes medication use at the time of visit (%)	9.6	8.2	0.49
Total cholesterol (mg/dL)	188.6 (42.4)	194.2 (35.1)	.04
HDL-C (mg/dL)	48.5 (16.2)	53.9 (15.9)	< .001
LDL-C (mg/dL)	107.6 (36.2)	116.3 (31.0)	< .001
Triglycerides (mg/dL)	174.1 (130.2)	123.9 (68.2)	< .001
IL-6 (pg/mL)[b]	1.6 (1.0, 2.5)	1.3 (0.9, 2.1)	.001
D-dimer (μg/mL)[b]	0.17 (0.11, 0.27)	0.20 (0.13, 0.30)	.04
ICAM-1 (ng/mL)[b]	260.8 (218.0, 316.3)	229.1 (196.0, 273.4)	< .001
HIV-infection–related variables			
History of AIDS defining malignancy or opportunistic infection (%)	14.7		
Current CD4+ T cell count (cells/mL)[b]	599 (419, 766)		
Nadir CD4+ T cell count (cells)[b]	286 (173, 408)		
Current HIV RNA detectable (%)	24.2		
Current HIV RNA among participants with detectable viral load (copies/mL)[b]	247 (43, 4920)		
Current ART use	88.1		
Duration of ART use (years)	9.2 (6.1, 12.2)		
ART type among current ART users			
PI-based regimen (%)	50		
NNRTI (without PI) (%)	44		
NRTI (%)	1		
Other (%)	5		

Abbreviations: ART, antiretroviral therapy; ICAM-1, intracellular adhesion molecule-1; IL-6, interleukin-6; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a]Continuous variables with normal distribution are presented as mean (standard deviation), and categorical variables are presented as number (percent).

[b]Continuous variables with nonnormal distribution are presented as median (interquartile range).

Each SD greater ln D-dimer was associated with .06 nm greater HDL size in the minimally adjusted model ($P < .001$) and .05 nm greater HDL size in the fully adjusted model ($P = .01$). The interaction between ln D-dimer and HDL size was not significant in the fully adjusted model.

No association between ICAM-1 and HDL size was noted in the minimally adjusted model. However, after adjusting for traditional CV risk factors, each SD greater ICAM-1 was associated with a .06 nm greater HDL size ($P < .001$). The interaction between ICAM-1 and HDL size was not significant.

Associations Between HIV Serostatus and HDL Particle Measurements

In a fully adjusted model without an inflammatory variable, HIV positive serostatus was associated with 1.7 lower μmol/L total HDL-P (Table 3). In a fully adjusted model with IL-6, HIV serostatus independently was associated with 1.3 lower μmol/L total HDL-P. Similar results were seen with HIV serostatus and total HDL-P in the models that included D-dimer and ICAM-1.

Positive HIV serostatus was associated with a 1.1 lower μmol/L small HDL-P in a fully adjusted model without an inflammatory variable. Positive HIV serostatus also was independently associated with 0.9 μmol/L lower small HDL-P in a fully adjusted model

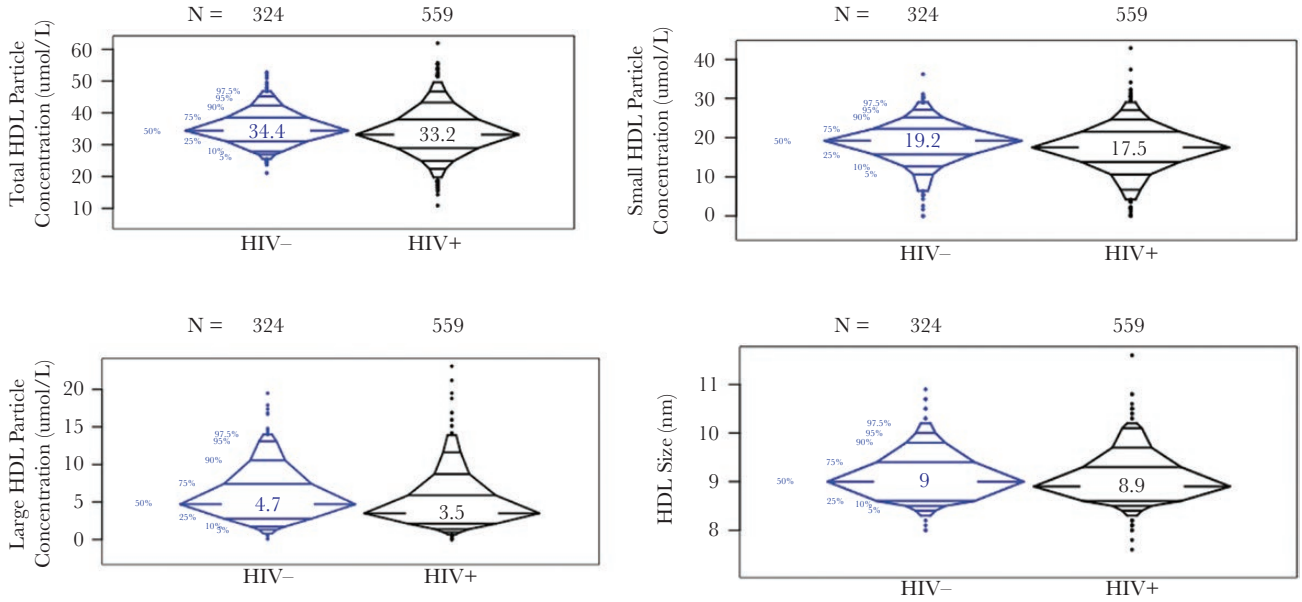

Figure 1: Lower Small, Large, and Total HDL-P in HIV-infected Men Compared With HIV-uninfected Men The median in each unadjusted plot is represented by the middle number, with outliers represented as points.

with IL-6. Similar results were seen with HIV serostatus and small HDL-P in models that included D-dimer and ICAM-1.

Positive HIV serostatus was associated with both lower HDL-P and HDL size in separate models without inflammatory variables. In addition, positive HIV serostatus was independently associated with lower large HDL-P and HDL size in separate models that included ln IL-6, ln D-dimer, and ICAM-1.

Table 2. Associations Between Lipoprotein Concentrations and Inflammatory Markers[a]

	Minimally adjusted model[b]	Fully adjusted model[c]
Total HDL particle concentration (μmol/L)		
Ln IL-6	-1.3 (-1.8, -0.8); $P < .001$	-1.3 (-1.8, -0.9); $P < .001$
Ln D-dimer	-0.9 (-1.3, -0.4); $P < .001$	.02 (-0.8, 0.8); $P = 0.962$[d]
ICAM-1 (ng/mL)	-1.5 (-2.0, -1.1); $P < .001$	-1.6 (-2.0, -1.1); $P < .001$
Small HDL particle concentration (μmol/L)		
Ln IL-6	-0.6 (-1.0, -0.2); $P = .004$	-0.7 (-1.1, -0.3); $P < .001$
Ln D-dimer	-0.4 (-1.0, 0.3); $P = 0.25$[d]	-0.9 (-1.3, -0.5); $P < .001$
ICAM-1 (ng/mL)	-1.0 (-1.4, -0.6); $P < .001$	-0.9 (-1.3, -0.5); $P < .001$
Large HDL particle concentration (μmol/L)		
Ln IL-6	-0.4 (-0.8, .01); $P = .06$[d]	0.1 (-0.1, 0.4); $P = 0.27$
Ln D-dimer	0.1 (-0.2, 0.3); $P = 0.54$	0.1 (-0.1, 0.3); $P = 0.36$
ICAM-1 (ng/mL)	0.1 (-0.2, 0.3); $P = 0.59$	.04 (-0.2, 0.3); $P = 0.75$
HDL size (nm)		
Ln IL-6	-.04 (-0.1, .02); $P = 0.17$[d]	.01 (-.05, .07); $P = 0.78$[d]
Ln D-dimer	.05 (.01, .08); $P = .011$	.05 (.02, .08); $P = .005$
ICAM-1 (ng/mL)	-.03 (-0.1, .05); $P = 0.51$[d]	.06 (.03, 0.10); $P < .001$

Abbreviations: ICAM-1, intracellular adhesion molecule-1; IL-6, interleukin-6; ln, natural log.

[a]Values are presented as beta coefficient (95% confidence interval); *P* value. Each beta coefficient represents a change in the outcome per inflammatory marker standard deviation.

[b]Minimally adjusted models included the following covariates: age, study center, study cohort (pre- or post-2001), HIV serostatus, and an interaction term between HIV serostatus and the inflammatory marker.

[c]Fully adjusted models included all of the variables from the minimally adjusted models and the following covariates: systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, use of lipid lowering medications, body mass index, smoking (current, former, and never), and pack-years of smoking.

[d]Models in which the interaction term between HIV serostatus and the inflammatory marker was significant.

Table 3. Associations of HIV Serostatus With HDL Particle Concentrations and Size, With and Without Inflammatory Markers, in Fully Adjusted Models[a]

	Beta coefficient (95% confidence interval) of HIV positive serostatus
Total HDL particle concentration	
Without inflammatory marker	-1.7 (-2.6, -0.7); $P = .001$
With Ln IL-6	-1.3 (-2.2, -0.3); $P = .01$
With Ln D-dimer	-1.7 (-2.7, -0.8); $P < .001$
With ICAM-1	-1.1 (-2.1, -0.2); $P = .02$
Small HDL particle concentration	
Without inflammatory marker	*-1.1 (-1.9, -0.3); P = .008*
With Ln IL-6	*-0.9 (-1.7, -0.1); P = .03*
With Ln D-dimer	-1.2 (-2.0, -0.4); $P = .003$
With ICAM-1	-0.83 (-1.64, -.02); $P = .044$
Large HDL particle concentration	
Without inflammatory marker	-1.2 (-1.7, -0.8); $P < .001$
With Ln IL-6	-1.2 (-1.7, -0.8); $P < .001$
With Ln D-dimer	-1.2 (-1.7, -0.8); $P < .001$
With ICAM-1	-1.2 (-1.7, -0.7); $P < .001$
HDL size	
Without inflammatory marker	-0.1 (-0.2, -.02); $P = .006$
With Ln IL-6	-0.1 (-0.2, -.04); $P = .003$
With Ln D-dimer	-0.1 (-0.2, -.03); $P = .006$
With ICAM-1	-0.1 (-0.2, -0.1); $P = .001$

Abbreviations: ICAM-1, intracellular adhesion molecule-1; IL6, interleukin-6; ln, natural log.

[a]Values are presented as beta coefficient (95% confidence interval); *P* value. The models include the following covariates: age, study center, study cohort (pre- or post-2001), HIV serostatus, an interaction term between HIV serostatus and the inflammatory marker, systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, use of lipid lowering medications, body mass index, smoking (current, former, and never), and pack-years of smoking.

HIV-infected Participants Analyses

To determine whether the direction and strength of associations noted above between inflammatory markers and HDL particle parameters also were present in an analysis of only HIV-infected men, models that were adjusted for HIV infection specific factors, including history of an AIDS defining malignancy or opportunistic infection, current and nadir CD4+ T cell count, current viral load, and duration of ART use, were created. Similar to the results from the whole study population, each SD greater ln IL-6 was associated with a 1.5 μmol/L lower total HDL-P ($P < .001$) in a model of HIV-infected participants only that was adjusted for traditional CV risk factors and HIV-infection–specific variables (Table 4). For the model that included ln IL-6, high detectable viral load was significantly associated with a 2.8 lower μmol/L total HDL-P ($P = .017$). In separate models, greater D-dimer and ICAM-1 levels were associated with lower levels of total HDL-P ($P = .004$ and $P < .001$, respectively). For the model that included ln D-dimer, high detectable viral load was significantly associated with a 3.32 lower μmol/L total HDL-P ($P = .006$).

Likewise, each SD greater ln IL-6 was associated with a 0.5 μmol/L lower small HDL-P ($P = .04$). Greater ln D-dimer and ICAM-1 levels also were associated with lower small HDL-P ($P < .001$, for both). No significant associations were noted between inflammatory markers and large HDL-P in separate analyses restricted to HIV-infected participants. Greater ln IL-6 and ICAM-1 levels were associated with greater HDL size ($P = .001$, for both).

In addition, we created fully adjusted models that were adjusted for HIV-specific variables, except viral load, and that were restricted to aviremic, HIV-infected men. Each SD greater ln IL-6 was associated with a 1.7 μmol/L lower total HDL-P ($P < .001$; Table 5). Greater ln D-dimer and ICAM-1 levels also were associated with lower total HDL-P ($P < .05$ for both).

Each SD greater ln IL-6 was not associated with a significantly lower small HDL-P, however. On the other hand, greater ln D-dimer and ICAM-1 levels were associated with lower small HDL-P ($P < .001$ for both). In separate analyses, no significant associations were noted between each inflammatory marker and large HDL-P. Each SD greater ICAM-1 was associated with greater HDL size ($P = .002$).

DISCUSSION

Our study is the largest in the current literature to measure the associations between inflammatory markers and HDL particle concentrations in a population of HIV-infected and HIV-uninfected men. We found that greater IL-6 and ICAM-1 levels were associated with both lower total and small HDL-P and a greater D-dimer level was associated with lower small HDL-P, after controlling for traditional CV risk factors in the MACS. In addition, HIV positive serostatus was associated with lower total, small, and large HDL-P concentrations, after adjusting for inflammatory variables. The results indicate that both greater systemic inflammation and HIV infection are independently associated with lower atheroprotective small HDL-P, which is relevant because of the heightened CVD risk in the setting of HIV infection.

A hallmark of HIV infection, inflammation affects HDL composition and function and perturbs HDL metabolism by decreasing paraoxonase, an antioxidant protein that attaches to HDL [19], and cholesteryl ester transfer protein, an enzyme that permits movement of cholesterol from HDL to lipoproteins [20]. As a result, HDL is less able to prevent LDL oxidation. In addition, inflammation decreases HDL-C and proteins and other factors involved in HDL function, such as cholesterol esters and apolipoprotein A-1 [20].

By including both viremic and treated HIV-infected patients, the present study expands on prior studies of HDL-P and inflammation that included only participants with untreated HIV infection. In aviremic, HIV-infected men, we found that both greater levels of D-dimer and ICAM-1, but not IL-6, were associated with lower small HDL-P, and greater levels of D-dimer, ICAM-1, and IL-6 were associated with lower total HDL-P. In the context of untreated HIV infection, IL-6, D-dimer, and ICAM-1 all have previously been found to be

Table 4. HIV-infected Participant Analyses[a]

N = 559	
Total HDL particle concentration (μmol/L)	
Ln IL-6	-1.5 (-2.1, -0.9); $P < .001$
Ln D-dimer	-0.9 (-1.6, -0.3); $P = .004$
ICAM-1 (ng/mL)	-1.6 (-2.2, -1.0); $P < .001$
Small HDL particle concentration (μmol/L)	
Ln IL-6	-0.5 (-1.0, -.03); $P = .04$
Ln D-dimer	-1.0 (-1.5, -0.5); $P < .001$
ICAM-1 (ng/mL)	-0.9 (-1.4, -0.4); $P < .001$
Large HDL particle concentration (μmol/L)	
Ln IL-6	0.2 (-0.1, 0.5); $P = 0.2$
Ln D-dimer	-.02 (-0.3, 0.3); $P = 0.92$
ICAM-1 (ng/mL)	0.1 (-0.2, 0.3); $P = 0.69$
HDL size (nm)	
Ln IL-6	0.1 (.03, 0.1); $P = .001$
Ln D-dimer	.04 (-.002, 0.1); $P = .06$
ICAM-1 (ng/mL)	0.1 (.03, 0.1); $P = .001$

Abbreviations: ICAM-1, intracellular adhesion molecule-1; IL-6, interleukin-6; ln, natural log.

[a]Values are presented as beta coefficient (95% confidence interval); *P* value. The models included the following covariates: age, study center, study cohort (pre- or post-2001), systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, use of lipid lowering medications, body mass index, smoking (current, former, and never), pack-years of smoking, history of an AIDS defining malignancy or opportunistic infection, current and nadir CD4+ T cell count, viral load (undetectable, low detectable [> 0 to < 1000 copies/mL] and high detectable [≥1000 copies/mL] and duration of antiretroviral therapy use.

associated inversely with total and small HDL-P. Although this suggests that greater systemic inflammation, coagulation, and endothelial cell activation are related to lower small and total HDL-P, whether this is a causal relationship is unknown [11].

Table 5. HIV-infected Aviremic Participant Analyses[a]

N = 424	
Total HDL particle concentration (μmol/L)	
Ln IL-6	-1.7 (-2.5, -1.0); $P < .001$
Ln D-dimer	-1.0 (-1.8, -0.2); $P = .02$
ICAM-1	-1.6 (-2.3, -0.9); $P < .001$
Small HDL particle concentration (μmol/L)	
Ln IL-6	-0.5 (-1.2, 0.1); $P = 0.1$
Ln D-dimer	-1.3 (-2.0, -0.7); $P < .001$
ICAM-1	-1.1 (-1.7, -0.6); $P < .001$
Large HDL particle concentration (μmol/L)	
Ln IL-6	-.02 (-0.3, 0.3); $P = 0.89$
Ln D-dimer	-0.1 (-0.5, 0.2); $P = 0.50$
ICAM-1	0.1 (-0.2, 0.4); $P = 0.62$
HDL size (nm)	
Ln IL-6	.04 (-.004, 0.1); $P = .074$
Ln D-dimer	.02 (-.03, 0.1); $P = 0.46$
ICAM-1	0.1 (.02, 0.1); $P = .004$

Abbreviations: ICAM-1, intracellular adhesion molecule-1; IL-6, interleukin-6; ln, natural log.

[a]Values are presented as beta coefficient (95% confidence interval); *P* value. The models included the following covariates: age, study center, study cohort (pre- or post-2001), systolic blood pressure, antihypertensive medication use, diabetes medication use, fasting glucose, use of lipid lowering medications, body mass index, smoking (current, former, and never), pack-years of smoking, history of an AIDS defining malignancy or opportunistic infection, current and nadir CD4+ T cell count, and duration of antiretroviral therapy use.

Furthermore, both treated and untreated HIV-infected individuals have decreased HDL antioxidant ability and less HDL remodeling compared to HIV-uninfected individuals [21].

Previously, HDL-C has been shown to be lower in both untreated and treated HIV-infected individuals, compared to HIV-uninfected individuals in the MACS [10]. Total HDL-P also has been found to be lower in both untreated and treated HIV-infected individuals. In addition, lower large and small HDL-P have been observed in untreated HIV-infected individuals compared to HIV-uninfected individuals [11]. Unlike prior studies, the present study included treated and viremic HIV-infected participants as well as HIV-uninfected participants.

In studies, including VA-HIT and JUPITER, greater total HDL-P was associated with decreased CVD events [7, 22]. In addition, in the SMART study, lower baseline total, small, and large HDL-P were associated with increased CVD risk [23]. We observed both an inverse association between HIV serostatus and small, total, and large HDL-P. Of note, in the JUPITER study, the highest tertile of total HDL-P (> 34.9 μmol/L) had an adjusted hazard ratio of 0.6 for incident CVD events, compared to the lowest tertile of total HDL-P (≤ 29.7 μmol/L; *P* trend = .01) [7]. To put this into perspective, in our study, a 1.1 μmol/L lower total HDL-P was seen for every SD higher in ln IL-6 in the fully adjusted model. Thus, the findings in the study could explain a part of the increased risk of CVD in HIV-infected individuals.

Small dense HDL has antioxidant, anti-inflammatory, and atheroprotective characteristics. Compared to large HDL, small HDL has an increased ability to accept cholesterol and oxidized phospholipids from LDL [24]. A caveat to the clinical use of HDL-P measurements is that studies of associations between HDL particle concentration and CVD have shown discrepant findings in the general population [8]. Some studies have found an inverse relationship between large HDL particles and CVD [25], whereas others have found an inverse relationship between small HDL particles and CVD [8].

Strengths of our study include a large sample size and a well-characterized cohort. A limitation of our study is that it has a cross-sectional design and so no conclusion can be made about a causal relationship between HDL-P, IL-6, D-dimer, and ICAM-1. In addition, because the MACS is composed of male participants, the study cannot be generalized to women.

In conclusion, greater IL-6, D-dimer, and ICAM-1 levels were associated with lower small HDL-P, and greater IL-6 and ICAM-1 levels were associated with lower total HDL-P in a group of male participants regardless of HIV serostatus. Similar associations between inflammatory markers and HDL particle parameters, in terms of direction and strength, were seen in HIV-infected participants alone, and, in addition, significant associations were seen between inflammatory markers and greater large HDL-P and HDL size in the HIV-infected participant group. Future research directions include

determining whether HDL-P and size are predictors of coronary artery disease progression in HIV-infected individuals. In particular, specific areas of interest are whether decreased small HDL-P has a specific role in CVD pathogenesis in HIV-infected individuals and whether interventions that target small HDL-P might mitigate the increased CVD risk in this patient population.

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