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

Sudipa Sarkar¹, Sabina Haberlen², Seamus Whelton³, Edward Schneider⁴, Lawrence Kingsley⁵, Frank Palella⁶, Mallory D. Witt⁷, Theodoros Kelesidis⁸, Annabelle Rodriguez⁹, Wendy S. Post²,³, and Todd T. Brown¹,²,‡

¹ Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism, Johns Hopkins School of Medicine, Baltimore, Maryland

² Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland

³ Department of Medicine, Division of Cardiology, Johns Hopkins Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins School of Medicine, Baltimore, Maryland

⁴ Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

⁵ Departments of Infectious Diseases and Microbiology and Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania

⁶ Department of Infectious Diseases, Northwestern University Feinberg School of Medicine, Chicago, Illinois

© The Author(s) 2019. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

© The Author(s) 2019. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Division of HIV Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California

Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, California

Center for Vascular Biology, University of Connecticut Health Center, Farmington, Connecticut

* Corresponding Author:
Sudipa Sarkar, MD MSCI
5501 Hopkins Bayview Circle
Asthma and Allergy Center 3B.74D
Baltimore, MD 21224
Phone: 410-955-3663
Fax: 410-367-2042
E-mail: ssarka19@jhmi.edu

‡ Alternate Corresponding Author:
Todd T. Brown, MD PhD
1830 East Monument Street, Suite 333
Baltimore, MD 21287
Phone: 410-955-3921
Fax: 410-367-2042
E-mail: tbrown27@jhmi.edu
Key Points:

- In HIV-infected men, both untreated and on ART, and HIV-uninfected men, greater IL-6, D-dimer, and ICAM-1 levels are associated with lower small HDL-P, and greater IL-6 and ICAM-1 levels are associated with lower total HDL-P.
Abstract

**Background:** 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. HDL particles are subfractions of HDL, 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).

**Methods:** 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 < 0.01 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 were both associated with lower atheroprotective small HDL-P. This may be a potential mechanism contributing to increased cardiovascular risk among HIV-infected people.

Keywords: Lipoprotein particles, inflammation, HDL-C
Background

HIV-infected individuals are at higher risk for cardiovascular disease (CVD) than the general population.\textsuperscript{1,2} Part of this risk is explained by an increased prevalence of traditional CVD risk factors such as smoking and dyslipidemia.\textsuperscript{3} In particular, HIV infection is associated with decreased HDL cholesterol (HDL-C),\textsuperscript{4} with a slight increase in HDL-C after initiation of HAART.\textsuperscript{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.\textsuperscript{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,\textsuperscript{8} and HIV negatively alters reverse cholesterol transport.\textsuperscript{9} HDL composition is also altered in the setting of HIV infection. Earlier in the 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.\textsuperscript{10}

One hallmark of both untreated and treated HIV infection is inflammation.\textsuperscript{11,12} In the SMART study of HIV-infected individuals with CD4 T cell counts of > 350 cells/mm\textsuperscript{3}, increased IL-6, a systemic inflammatory marker, and D-dimer, a coagulation marker, were associated with mortality.\textsuperscript{18} Moreover, greater IL-6 and ICAM-1, an endothelial cell activation marker, were associated with a higher prevalence of coronary stenosis in the MACS.\textsuperscript{15} Inflammation also alters the composition of HDL-associated proteins and their function. With regards to HDL particles, in a cross-sectional study, 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.\textsuperscript{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.\textsuperscript{13}

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.

\textbf{Methods}

\textbf{Study Population}

The MACS is an ongoing, prospective cohort of HIV-infected and HIV-uninfected men at risk for HIV infection recruited from four 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. MACS participants undergo a semiannual standardized interview, physical examination, and laboratory tests. For the current study, analyses were limited to demographic and laboratory measurements from the visit most recently prior to the CT scan, approximately 6 months, in the MACS Cardiovascular Ancillary Study conducted between 2010 and 2013,\textsuperscript{14} the period of time during which HDL particle measurements were performed. 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 who were ages 40 to 70 years, weighed < 300 pounds, and had no history of previous cardiac surgery or percutaneous coronary intervention.\textsuperscript{15} 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 NMR spectroscopic assay (LipoScience Inc, North Carolina). 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. HDL size, measured in nanometers, represents mean HDL particle size since small HDL and large HDL have different diameter ranges. 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.

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%). 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 Student t test for normally distributed continuous variables, a Wilcoxon rank sum test for non-normally distributed continuous variables, and a chi-squared 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). Ln 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 could potentially 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

1,006 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. HIV-infected men were younger and had a lower BMI than HIV-uninfected men, but systolic blood pressure was similar between the two groups (Table 1). HIV-infected men 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 PI-based regimen. HIV-infected men had higher median IL-6 (P < 0.01) and ICAM-1 (P < 0.01) 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 < 0.01), D-dimer (P < 0.04), and ICAM-1 (P < 0.01) levels than those with undetectable HIV RNA. In addition, significantly lower mean total and small HDL-P levels (P < 0.01 for both) were observed in viremic men than in HIV-infected men with undetectable HIV RNA. A lower
mean large HDL-P was also noted in viremic men, although this was not a statistically significant finding (P = 0.08).

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 = 0.003). This association persisted in the fully adjusted model (P < 0.001). The interaction between HIV serostatus and ln IL-6 was not significant in the fully adjusted model.

Ln D-dimer was not significantly associated with small HDL-P, in the minimally adjusted model (P = 0.21). However, the association was significant in a fully adjusted model (P < 0.001), 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 < 0.001). 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 = 0.04). 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 < 0.001) (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 < 0.001). 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 < 0.001). 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 = 0.02), 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 < 0.001) and after adjusting for CV risk factors (P < 0.001). 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.

Each SD greater ln D-dimer was associated with 0.06 nm greater HDL size in the minimally adjusted model (P < 0.001) and 0.05 nm greater HDL size in the fully adjusted.
model (P = 0.01). 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 0.06 nm greater HDL size (P < 0.001). 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 was independently 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 was also independently associated with 0.9 μmol/L lower small HDL-P, in a fully adjusted model 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.

**HIV-infected participants analyses**
To determine whether the direction and strength of associations noted above between inflammatory markers and HDL particle parameters were also 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, in a model of HIV-infected participants only that was adjusted for traditional CV risk factors and HIV infection specific variables, each SD greater ln IL-6 was associated with a 1.5 μmol/L lower total HDL-P (P < 0.001) (Table 3). 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 = 0.017). In separate models, greater D-dimer and ICAM-1 levels were associated with lower levels of total HDL-P (P = 0.004 and P < 0.001, 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 = 0.006).

Likewise, each SD greater ln IL-6 was associated with a 0.5 μmol/L lower small HDL-P (P = 0.04). Greater ln D-dimer and ICAM-1 levels were also associated with lower small HDL-P (P < 0.001, 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 = 0.001, for both).

In addition, we created fully adjusted models which were adjusted for HIV-specific variables, except viral load, and which 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 < 0.001) (Table 5). Greater ln D-dimer and ICAM-1 levels were also associated with lower total HDL-P (P < 0.05 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 < 0.001 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 = 0.002).

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 (PON), an antioxidant protein which attaches to HDL, and cholesteryl ester transfer protein, an enzyme that permits movement of cholesterol from HDL to lipoproteins. 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.

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 s-ICAM all have previously been found to be inversely associated with total and small HDL-P. While 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. Furthermore, both treated and untreated HIV-infected individuals have decreased HDL antioxidant ability and less HDL remodeling compared to HIV-uninfected individuals.

HDL-C has previously been shown to be lower in both untreated and treated HIV-infected individuals, compared to HIV-uninfected individuals in the MACS. Total HDL-P has also 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. 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. In addition, in the SMART study, lower baseline total, small, and large HDL-P were associated with increased CVD risk. 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 0.01). 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. 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. Some studies have found an
inverse relationship between large HDL particles and CVD, whereas others have found an inverse relationship between small HDL particles and CVD.

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.

**Funding:** This work was supported by the National Institutes of Health [K24 AI120834 to T.T.B. and U01-AI-35042, UM1-AI-35043, U01-AI-35039, U01-AI-35040, and U01-AI-35041] and the National Heart, Lung, and Blood Institute [RO1 HL095129 to W.S.P.].

**References**
1. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med.* 2013;173(8):614-622.

2. Feinstein MJ, Bahiru E, Achenbach C, et al. Patterns of Cardiovascular Mortality for HIV-Infected Adults in the United States: 1999 to 2013. *Am J Cardiol.* 2016;117(2):214-220.

3. Boccara F, Lang S, Meuleman C, et al. HIV and coronary heart disease: time for a better understanding. *J Am Coll Cardiol.* 2013;61(5):511-523.

4. Rose H, Woolley I, Hoy J, et al. HIV infection and high-density lipoprotein: the effect of the disease vs the effect of treatment. *Metabolism.* 2006;55(1):90-95.

5. Riddler SA, Smit E, Cole SR, et al. Impact of HIV infection and HAART on serum lipids in men. *JAMA.* 2003;289(22):2978-2982.

6. Matera R, Horvath KV, Nair H, Schaefer EJ, Asztalos BF. HDL Particle Measurement: Comparison of 5 Methods. *Clin Chem.* 2018;64(3):492-500.

7. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation.* 2013;128(11):1189-1197.

8. Silbernagel G, Pagel P, Pfahler V, et al. High-Density Lipoprotein Subclasses, Coronary Artery Disease, and Cardiovascular Mortality. *Clin Chem.* 2017;63(12):1886-1896.

9. Mujawar Z, Rose H, Morrow MP, et al. Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. *PLoS Biol.* 2006;4(11):e365.

10. Riddler SA, Li X, Otvos J, et al. Antiretroviral therapy is associated with an atherogenic lipoprotein phenotype among HIV-1-infected men in the
11. Baker J, Ayenew W, Quick H, et al. High-density lipoprotein particles and markers of inflammation and thrombotic activity in patients with untreated HIV infection. *J Infect Dis.* 2010;201(2):285-292.

12. Neuhaus J, Jacobs DR, Jr., Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis.* 2010;201(12):1788-1795.

13. Florentin M, Liberopoulos EN, Wierzbicki AS, Mikhailidis DP. Multiple actions of high-density lipoprotein. *Curr Opin Cardiol.* 2008;23(4):370-378.

14. Post WS, Budoff M, Kingsley L, et al. Associations between HIV infection and subclinical coronary atherosclerosis. *Ann Intern Med.* 2014;160(7):458-467.

15. Bahrami H, Budoff M, Haberlen SA, et al. Inflammatory Markers Associated With Subclinical Coronary Artery Disease: The Multicenter AIDS Cohort Study. *J Am Heart Assoc.* 2016;5(6).

16. Rosenson RS, Brewer HB, Jr., Ansell B, et al. Translation of high-density lipoprotein function into clinical practice: current prospects and future challenges. *Circulation.* 2013;128(11):1256-1267.

17. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med.* 2006;26(4):847-870.

18. Kuller LH, Tracy R, Beloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med.* 2008;5(10):e203.

19. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arteriosclerosis Thrombosis and Vascular Biology.* 2001;21(4):473-480.
20. Khovidhunkit W, Memon RA, Feingold KR, Grunfeld C. Infection and inflammation-induced proatherogenic changes of lipoproteins. *J Infect Dis.* 2000;181 Suppl 3:S462-472.

21. Siegel MO, Borkowska AG, Dubrovsky L, et al. HIV infection induces structural and functional changes in high density lipoproteins. *Atherosclerosis.* 2015;243(1):19-29.

22. Kelesidis T, Oda MN, Borja MS, et al. Predictors of Impaired HDL Function in HIV-1 Infected Compared to Uninfected Individuals. *J Acquir Immune Defic Syndr.* 2017;75(3):354-363.

23. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation.* 2006;113(12):1556-1563.

24. Duprez DA, Kuller LH, Tracy R, et al. Lipoprotein particle subclasses, cardiovascular disease and HIV infection. *Atherosclerosis.* 2009;207(2):524-529.

25. Kontush A, Chapman MJ. Antiatherogenic small, dense HDL—guardian angel of the arterial wall? *Nat Clin Pract Cardiovasc Med.* 2006;3(3):144-153.

26. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation.* 2009;119(7):931-939.
| Variable                        | HIV-infected (N = 553) | HIV-uninfected (N = 319) | P value |
|--------------------------------|------------------------|--------------------------|---------|
| Age (years)                    | 52.7 (6.6)             | 55.3 (7.2)               | < 0.001 |
| Race                           |                        |                          | 0.001   |
| 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)               | < 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                 |                        |                          | 0.005   |
| 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)          | 0.06    |
| 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 |
|                                | Value 1       | Value 2       | p-value |
|--------------------------------|---------------|---------------|---------|
| Total cholesterol (mg/dL)      | 188.6 (42.4)  | 194.2 (35.1)  | 0.04    |
| HDL-C (mg/dL)                  | 48.5 (16.2)   | 53.9 (15.9)   | < 0.001 |
| LDL-C (mg/dL)                  | 107.6 (36.2)  | 116.3 (31.0)  | < 0.001 |
| Triglycerides (mg/dL)          | 174.1 (130.2) | 123.9 (68.2)  | < 0.001 |
| IL-6 (pg/mL)†                  | 1.6 (1.0, 2.5)| 1.3 (0.9, 2.1)| 0.001   |
| D-dimer (μg/mL)†               | 0.17 (0.11, 0.27) | 0.20 (0.13, 0.30) | 0.04 |
| ICAM-1 (ng/mL)†                | 260.8 (218.0, 316.3) | 229.1 (196.0, 273.4) | < 0.001 |

**HIV infection related variables**

|                                | Value |
|--------------------------------|-------|
| History of AIDS defining malignancy or opportunistic infection (%) | 14.7  |
| Current CD4+ T cell count (cells/mL)† | 599 (419, 766) |
| Nadir CD4+ T cell count (cells)† | 286 (173, 408) |
| Current HIV RNA detectable (%) | 24.2  |
| Current HIV RNA among participants with detectable viral load (copies/mL)† | 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     |

IL-6 (interleukin-6); intracellular adhesion molecule-1 (ICAM-1)

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

† Continuous variables with non-normal distribution are presented as median (interquartile range).
Table 2: Associations between lipoprotein concentrations and inflammatory markers†

| Lipoprotein particle concentration (μmol/L) | Minimally adjusted model | Fully adjusted model |
|--------------------------------------------|--------------------------|---------------------|
| **Total HDL particle concentration (μmol/L)** |                          |                     |
| Ln IL-6                                    | -1.3 (-1.8, -0.8); P < 0.001 | -1.3 (-1.8, -0.9); P < 0.001 |
| Ln D-dimer                                 | -0.9 (-1.3, -0.4); P < 0.001 | 0.02 (-0.8, 0.8); P = 0.962^ |
| ICAM-1 (ng/mL)                             | -1.5 (-2.0, -1.1); P < 0.001 | -1.6 (-2.0, -1.1); P < 0.001 |
| **Small HDL particle concentration (μmol/L)** |                          |                     |
| Ln IL-6                                    | -0.6 (-1.0, -0.2); P = 0.004 | -0.7 (-1.1, -0.3); P < 0.001 |
| Ln D-dimer                                 | -0.4 (-1.0, 0.3); P = 0.25^ | -0.9 (-1.3, -0.5); P < 0.001 |
| ICAM-1 (ng/mL)                             | -1.0 (-1.4, -0.6); P < 0.001 | -0.9 (-1.3, -0.5); P < 0.001 |
| **Large HDL particle concentration (μmol/L)** |                          |                     |
| Ln IL-6                                    | -0.4 (-0.8, 0.01); P = 0.06^ | 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 | 0.04 (-0.2, 0.3); P = 0.75 |
| **HDL size (nm)**                          |                          |                     |
| Ln IL-6                                    | -0.04 (-0.1, 0.02); P = 0.17^ | 0.01 (-0.05, 0.07); P = 0.78^ |
| Ln D-dimer                                 | 0.05 (0.01, 0.08); P = 0.011 | 0.05 (0.02, 0.08); P = 0.005 |
| ICAM-1 (ng/mL)                             | -0.03 (-0.1, 0.05); P = 0.51^ | 0.06 (0.03, 0.10); P < 0.001 |
Interleukin-6 (IL-6); intracellular adhesion molecule-1 (ICAM-1)

† Values are presented as beta coefficient (95% confidence interval); P value.

* 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.

° 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 (BMI), smoking (current, former, and never), and pack-years of smoking.

^ Models in which the interaction term between HIV serostatus and the inflammatory marker was significant.

‡ Each beta coefficient represents a change in the outcome per inflammatory marker standard deviation.
Table 3: Associations of HIV serostatus with HDL particle concentrations and size, with and without inflammatory markers, in fully adjusted models ††

|                        | Beta coefficient (95% confidence interval) of HIV positive serostatus |
|------------------------|------------------------------------------------------------------------|
| **Total HDL particle concentration** |                                                                       |
| Without inflammatory marker | -1.7 (-2.6, -0.7); P = 0.001                                            |
| With Ln IL-6           | -1.3 (-2.2, -0.3); P = 0.01                                              |
| With Ln D-dimer        | -1.7 (-2.7, -0.8); P < 0.001                                             |
| With ICAM-1            | -1.1 (-2.1, -0.2); P = 0.02                                              |
| **Small HDL particle concentration** |                                                                    |
| Without inflammatory marker | -1.1 (-1.9, -0.3); P = 0.008                                              |
| With Ln IL-6           | -0.9 (-1.7, -0.1); P = 0.03                                              |
| With Ln D-dimer        | -1.2 (-2.0, -0.4); P = 0.003                                              |
| With ICAM-1            | -0.83 (-1.64, -0.02); P = 0.044                                           |
Large HDL particle concentration

| Without inflammatory marker | -1.2 (-1.7, -0.8); P < 0.001 |
|-----------------------------|-------------------------------|
| With Ln IL-6                | -1.2 (-1.7, -0.8); P < 0.001 |
| With Ln D-dimer             | -1.2 (-1.7, -0.8); P < 0.001 |
| With ICAM-1                 | -1.2 (-1.7, -0.7); P < 0.001 |

HDL size

| Without inflammatory marker | -0.1 (-0.2, -0.02); P = 0.006 |
|-----------------------------|-------------------------------|
| With Ln IL-6                | -0.1 (-0.2, -0.04); P = 0.003 |
| With Ln D-dimer             | -0.1 (-0.2, -0.03); P = 0.006 |
| With ICAM-1                 | -0.1 (-0.2, -0.1); P = 0.001 |

†† 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 (BMI), smoking (current, former, and never), and pack-years of smoking.
Table 4: HIV-infected participant analyses°

|                         | Total HDL particle concentration (μmol/L) | Small HDL particle concentration (μmol/L) | Large HDL particle concentration (μmol/L) | HDL size (nm)            |
|-------------------------|-------------------------------------------|------------------------------------------|------------------------------------------|--------------------------|
|                         | Ln IL-6                                   | Ln IL-6                                   | Ln IL-6                                   | Ln IL-6                  |
|                         | -1.5 (-2.1, -0.9); P < 0.001              | -0.5 (-1.0, -0.03); P = 0.04              | 0.2 (-0.1, 0.5); P = 0.2                  | 0.1 (0.03, 0.1); P = 0.001|
|                         | Ln D-dimer                                | -0.9 (-1.6, -0.3); P = 0.004              | -1.0 (-1.5, -0.5); P < 0.001              | -0.02 (-0.3, 0.3); P = 0.92|
|                         |                                          |                                          | ICAM-1 (ng/mL)                            | ICAM-1 (ng/mL)           |
|                         |                                          |                                          | -1.6 (-2.2, -1.0); P < 0.001              | 0.1 (-0.2, 0.3); P = 0.69|
| ICAM-1 (ng/mL)          |                                          |                                          |                                          |                          |
|                         | -1.6 (-2.2, -1.0); P < 0.001              | -0.9 (-1.4, -0.4); P < 0.001              | 0.1 (-0.2, 0.3); P = 0.69                |                          |

Interleukin-6 (IL-6); intracellular adhesion molecule-1 (ICAM-1)

° 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 (BMI), 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 ART use.
Table 5: HIV-infected aviremic participant analyses°

|                        | Total HDL particle concentration (μmol/L) | Small HDL particle concentration (μmol/L) | Large HDL particle concentration (μmol/L) | HDL size (nm) |
|------------------------|------------------------------------------|------------------------------------------|------------------------------------------|---------------|
| Ln IL-6                | -1.7 (-2.5, -1.0); P < 0.001             | -0.5 (-1.2, 0.1); P = 0.1                | -0.02 (-0.3, 0.3); P = 0.89              | 0.04 (-0.004, 0.1); P = 0.074 |
| Ln D-dimer             | -1.0 (-1.8, -0.2); P = 0.02              | -1.3 (-2.0, -0.7); P < 0.001             | -0.1 (-0.5, 0.2); P = 0.50              | 0.02 (-0.03, 0.1); P = 0.46 |
| ICAM-1                 | -1.6 (-2.3, -0.9); P < 0.001             | -1.1 (-1.7, -0.6); P < 0.001             | 0.1 (-0.2, 0.4); P = 0.62               | 0.1 (0.02, 0.1); P = 0.004 |

Interleukin-6 (IL-6); intracellular adhesion molecule-1 (ICAM-1)

° 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 (BMI), 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 ART use.
Figure 1: Lower small, large, and total HDL-P in HIV-infected men compared to HIV-uninfected men. The median in each unadjusted plot is represented by the middle number, with outliers represented as points.
Figure 1

- **Total HDL Particle Concentration (µmol/L)**:
  - N = 324
  - HIV+ vs. HIV-: P = 0.004

- **Small HDL Particle Concentration (µmol/L)**:
  - N = 324
  - HIV+ vs. HIV-: P < 0.001

- **Large HDL Particle Concentration (µmol/L)**:
  - N = 559
  - HIV+ vs. HIV-: P < 0.001

- **HDL Size (nm)**:
  - N = 324
  - HIV+ vs. HIV-: P = 0.134