High-Density Lipoprotein Particles and Markers of Inflammation and Thrombotic Activity in Patients with Untreated HIV Infection

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Background. Untreated human immunodeficiency virus (HIV) infection is associated with changes in blood lipids, inflammation, thrombotic activity, and increased risk for cardiovascular disease.

Methods. We studied high-density lipoprotein particle (HDLp) concentrations and inflammatory (high-sensitivity C-reactive protein [hsCRP] and interleukin [IL] 6), endothelial activation (E-selectin and soluble intercellular adhesion molecule-1 [sICAM-1]), and thrombotic (fibrinogen and D-dimer) biomarkers in a group of 32 untreated HIV-infected and 29 uninfected persons. Differences in the levels of blood lipids and biomarkers by HIV status were examined before and after adjustment for age, sex, race/ethnicity, smoking status, body mass index, and the presence of hepatitis C.

Results. HIV-infected participants, compared with uninfected participants, had lower HDL cholesterol (HDLc) levels (−26%) and HDLp numbers (−21%), with reductions in large (−50%) and small (−20%) HDLp, specifically (P < .01 for all). A trend was present for higher total cholesterol (P = .01) and triglyceride levels (P = .11) among individuals with HIV infection. Levels of IL-6, sICAM-1, and D-dimer were 65%–70% higher in HIV-infected participants (P < .02 for all). Covariate adjustment did not diminish these associations. For HIV-infected participants, total and small HDLp (respectively) tended to correlate inversely with levels of IL-6 (P = .01 and P = .02), sICAM-1 (P < .01 for both) and D-dimer (P = .03 and P < .01).

Conclusions. Persons with untreated HIV infection have lower HDLp (primarily large and small HDLp) and higher IL-6, sICAM-1, and D-dimer levels, and the relationship of these markers to HIV-mediated atherosclerotic risk requires further study.

Human immunodeficiency virus (HIV) infection, independent of antiretroviral therapy (ART) use, may increase risk for atherosclerotic cardiovascular disease (CVD) via adverse changes in blood lipids, inflammation, and thrombotic activity [1]. High-density lipoprotein cholesterol (HDLc) is inversely correlated with coronary heart disease in the general population, and HIV seroconversion leads to decreases in HDLc levels that do not completely revert with ART initiation [2–5]. In addition to promoting cholesterol efflux from lipid-filled macrophages (foam cells), HDLc also possesses several anti-inflammatory and antithrombotic properties that may protect against injury to endothelial surfaces [6].

Traditional measures of the amount of cholesterol in plasma from a particular class of lipoprotein, such as HDLc or low-density lipoprotein cholesterol (LDLc), are commonly used in clinical practice to assess CVD risk. Several studies have shown that new methods for assessing the size and number of lipoprotein particles provide additional information regarding CVD risk beyond assessment of total cholesterol (TC), HDLc, and LDLc [7–13]. In the Veteran Affairs High-Density Lipoprotein Intervention Trial within the general population, estimates of HDL and LDL particle concentrations (HDLp and LDLp, respectively), not conventional...
HDLc and LDLc measures, were associated with CVD event risk before and after treatment with gemfibrozil [8].

The Strategies for Management of AntiRetroviral Therapy (SMART) trial demonstrated a 60% increased relative risk for CVD events with a strategy of CD4+ cell count-guided interruption of ART, and adverse changes in HDLc after stopping ART may explain some of the excess CVD risk [14, 15]. Further analyses of SMART data demonstrated markers of inflammation (IL-6) and thrombotic activity (D-dimer) at baseline were strongly associated with CVD and mortality risk [16]. Furthermore, IL-6 and D-dimer levels increased after discontinuation of ART, and this increase was associated with increases in HIV RNA levels [16]. In addition, baseline HDLp, but not LDLp, predicted CVD risk in SMART [17]. The relationship between HDLp and markers of inflammation and thrombotic activity has not, to our knowledge, been reported in HIV-infected persons. We characterized differences in specific HDLp concentrations between HIV-infected participants who were not receiving ART and uninfected control subjects and examined associations between HDLp and levels of IL-6, D-dimer, and other biomarkers among HIV-infected participants.

**METHODS**

**Study design.** The protocol was preapproved by the Hennepin County Medical Center (HCMC) Human Subjects Research Committee, and participants were enrolled from March 2007 through June 2008 after giving signed informed consent. Exclusion criteria included ART use within the previous year, known atherosclerotic CVD, pregnancy, current or active bacterial infection, recent hospitalization (within 1 month), systemic vasculitis, and active or ongoing alcohol abuse or illicit drug use (excluding marijuana). Participants were recruited through informational flyers and referrals from patients and health care providers at an urban HIV clinic (HCMC, Minneapolis, MN). The HIV-uninfected control group was recruited in the same way, and efforts were made to enroll participants who were similar to the HIV-infected group with regard to age, sex, race/ethnicity, smoking status, and the presence of diabetes mellitus (DM).

Study participants presented for a single visit at HCMC, where a peripheral blood sample was obtained. Participants were instructed to fast and avoid alcohol during the 8-h period before the visit. Framingham 10-year CVD risk score was calculated using an online calculator provided by the National Heart Lung and Blood Institute [18].

**Laboratory markers.** Blood samples were centrifuged within 1 h after collection and frozen at −70°C until analysis. Specimens were tested at HCMC clinical laboratory for HIV antibody (for HIV-uninfected participants) and HIV RNA level (for HIV-infected participants), and serological testing was performed to detect hepatitis B and C virus and to determine serum TC, LDLc, HDLc, and triglyceride (TG) levels. All samples were handled in a fully blinded fashion, such that laboratory investigators had no knowledge of participant HIV status.

Lipoprotein particle size and concentration was estimated using an automated proton nuclear magnetic resonance (NMR) spectroscopic assay (LipoScience), as previously described [7]. HDL particle size (diameter in nm) was calculated from the weighted average of subclass concentrations. HDL subclass concentrations in nanometer of particles per liter (nmol/L) were obtained from the measured amplitudes of distinct lipid methyl group NMR signals. HDLp subclasses were categorized by particle diameter as large (8.8–13.0 nm), medium (8.2–8.8 nm), and small (7.3–8.2 nm) [7].

Two inflammatory markers (high-sensitivity C-reactive protein [hsCRP] and interleukin-6 [IL-6], 2 endothelial activation markers (soluble intercellular adhesion molecule-1 [sICAM-1] and E-selectin), and 2 thrombotic markers (fibrinogen and D-dimer) were measured by the Laboratory for Clinical Biochemistry Research at the University of Vermont. These markers were chosen because they have a high degree of laboratory and biological reproducibility, have been associated with CVD and all-cause mortality in the general population, and allow us to build on the findings from the SMART study [16, 19–28]. IL-6 was measured with Chemiluminescent Sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems); hsCRP with an NBIi nephelometer, N Antiserum to Human CRP (Siemens Diagnostics); siCAM-1 with ELISA from Parameter Human siCAM-1 Immunoassay (R&D Systems); E-selectin with a Colorimetric Sandwich ELISA (R&D Systems); fibrinogen levels with a BNII nephelometer, N Antiserum to Human Fibrinogen (Siemens Diagnostics); and D-dimer levels with immunoturbidimetric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago).

**Statistical analyses.** Descriptive statistics are reported as means values with standard deviations (SDs) and median values with interquartile ranges (IQRs). Student t test for independent groups and the χ² test for categorical variables were used to compare characteristics of the HIV-infected and HIV-uninfected groups. The summaries by group of lipids and biomarkers are presented as untransformed median values with IQRs. For comparison of the lipids and biomarkers between the HIV-infected and HIV-uninfected groups, the relative percentage difference (with 95% confidence interval) was obtained by exponentiating the mean difference on the natural log scale using generalized linear models. Those models were repeated with adjustment for age, sex, race/ethnicity, smoking status, body mass index, and hepatitis C. Hepatitis C virus infection was chosen over injection drug use (IDU) in covariate models, because 69% of persons with prior IDU had hepatitis C virus infection; current hepatitis C virus infection is more likely to...
be a confounder than is IDU, given potential implications for inflammation; and to limit the number of covariates in this small study. Regression coefficients were similar with and without inclusion of IDU in statistical models. Comparisons of IL-6, sICAM-1, and D-dimer levels with HDL measures were then restricted to HIV-infected persons, and correlations were assessed using nonparametric rank tests for nonnormal distribution of data. The level of statistical significance was defined as \( P < .05 \), and all analyses were conducted with R statistical software (version 2.8.1).

**RESULTS**

**Study sample.** We enrolled 32 HIV-infected and 29 HIV-uninfected participants. The demographic and clinical characteristics of the participants are presented in Table 1. HIV-infected participants were similar to HIV-uninfected participants with respect to age, sex, race/ethnicity, and DM. A higher percentage of the HIV-infected group than of the control group had a history of IDU, and a trend existed for a greater presence of hepatitis C virus infection and smoking. Use of a statin or blood pressure-lowering medication (beta-blocker, thiazide, calcium channel blocker, or angiotensin-converting enzyme inhibitor) was present in 1 and 6 HIV-infected participants, respectively, and in 2 and 3 HIV-uninfected participants, respectively. The majority of HIV-infected participants (84%) had not received ART, and the 5 patients with prior ART use had a mean duration of HIV infection of 14 years (median duration, 13 years) and had discontinued ART for at least 2 years. Most HIV-infected participants (56%) had CD4\(^+\) cell counts that were above the threshold for initiating ART, based on current guidelines (350 cells/mm\(^3\)) [29].

**Lipid and biomarker differences by HIV status.** HIV-infected participants had lower HDLc and TC and higher triglyceride levels, compared with HIV-uninfected control subjects (Table 2). All HDLp measures, except medium HDLp, were lower among HIV-infected participants than they were among HIV-uninfected participants. Levels of IL-6, sICAM-1, and D-dimer, but not of hsCRP, E-selectin, and fibrinogen, were statistically significantly higher in the HIV-infected group than

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-Infected Group (n = 32)</th>
<th>HIV-Uninfected Group (n = 29)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (±SD)</td>
<td>40.0 (9.6)</td>
<td>40.6 (10.8)</td>
<td>.78</td>
</tr>
<tr>
<td>Male sex</td>
<td>88 (28)</td>
<td>90 (26)</td>
<td>.79</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td>.32</td>
</tr>
<tr>
<td>White</td>
<td>44 (14)</td>
<td>48 (14)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>31 (10)</td>
<td>41 (12)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>25 (8)</td>
<td>10 (3)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>59 (19)</td>
<td>41 (12)</td>
<td>.16</td>
</tr>
<tr>
<td>Injection drug use</td>
<td>38 (12)</td>
<td>14 (4)</td>
<td>.04</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>34 (11)</td>
<td>14 (4)</td>
<td>.06</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (2)</td>
<td>7 (2)</td>
<td>.92</td>
</tr>
<tr>
<td>Body mass index, mean value (±SD)</td>
<td>26.0 (5.1)</td>
<td>27.8 (4.5)</td>
<td>.15</td>
</tr>
<tr>
<td>Systolic blood pressure, mean mmHg (±SD)</td>
<td>127.7 (13.9)</td>
<td>126.7 (12.5)</td>
<td>.77</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean mmHg (±SD)</td>
<td>77.0 (11.3)</td>
<td>74.5 (7.8)</td>
<td>.31</td>
</tr>
<tr>
<td>Framingham 10-year CVD risk, mean % (±SD)</td>
<td>4.8 (4.6)</td>
<td>3.5 (4.7)</td>
<td>.27</td>
</tr>
<tr>
<td>No. of patients with score &lt;10%</td>
<td>27</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Duration of infection, mean years (±SD)</td>
<td>6.5 (6.6)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Prior AIDS</td>
<td>7 (2)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Prior ART use</td>
<td>16 (5)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>HIV RNA level, mean log_10 copies/mL (±SD)</td>
<td>4.15 (0.73)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>CD4(^+) cell count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean cells/mm(^3) (±SD)</td>
<td>391 (182)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Median cells/mm(^3) (IQR)</td>
<td>382 (255–514)</td>
<td>...</td>
<td></td>
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</tbody>
</table>

**NOTE.** Data presented are no. (%) of patients, unless otherwise indicated. An attempt was made to match the HIV uninfected group with the HIV infected group on the basis of age, sex, race/ethnicity, smoking status, and presence of diabetes. Body mass index was calculated the weight in kg divided by the square of height in meters. ART, antiretroviral therapy; CVD, cardiovascular disease; HIV, human immunodeficiency virus; SD, standard deviation.
they were in the control group. The percentage difference between HIV-infected and uninfected groups was most striking with respect to HDL (large and small HDLp subclasses, specifically) and for levels of IL-6, sICAM-1, and D-dimer. Adjusting for additional confounders did not have a large influence on estimated differences associated with untreated HIV infection (Figure 1). Thus, after adjustment, HIV-infection was associated with lower levels of total and large HDLp, with a trend present for lower small HDLp levels. In addition, HIV infection remained independently associated with higher levels of IL-6, sICAM-1, and D-dimer.

HDL measures and biomarkers among HIV-infected participants. Correlations between lipid levels (TC, TG, HDLc, and total, large and small HDLp) and biomarkers (hsCRP, IL-6, sICAM-1, and D-dimer) and HIV RNA levels were examined among a group of 32 HIV-infected participants (Table 3). These comparisons were limited to measures that tended to differ between HIV-infected and HIV-uninfected groups (Figure 1). A trend exists for lower TC levels with higher HIV RNA levels, but no other lipid measures were clearly associated with HIV RNA level. Lipid levels did not demonstrate associations with hsCRP levels among HIV-infected participants. Inverse correlations were present between total and small HDLp with IL-6, sICAM-1, and D-dimer levels ($P < .05$ for all except IL-6 and total HDLp). Overall, these associations were stronger for small HDLp. No statistically significant associations were present between large HDLp and levels of IL-6, sICAM-1, or D-dimer. Finally, CD4+ cell counts, before and after square root transformation, were not correlated with any of the HDL measures or biomarker levels.

### DISCUSSION

We compared HDLp levels and markers of inflammation, endothelial activation, and thrombogenesis from HIV-infected participants with relatively preserved immune function who were not receiving ART with HIV-uninfected control subjects, and we characterized the relationship of these measures with one another among the HIV-infected participants. Persons with untreated HIV infection have lower levels of HDLc and HDLp. All stated associations were present between large HDLp and levels of IL-6, sICAM-1, or D-dimer. Finally, CD4+ cell counts, before and after square root transformation, were not correlated with any of the HDL measures or biomarker levels.
Figure 1. Adjusted percentage difference in high-density lipoprotein (HDL) measures and biomarkers by human immunodeficiency virus (HIV) status. For each measure, the percentage difference between the HIV-infected and HIV-uninfected group is plotted with error bars reflecting 95% confidence intervals. The percentage difference was calculated after adjustment for age, sex, race/ethnicity, smoking status, body mass index (calculated as weight in kilograms divided by the square of the height in meters), and hepatitis C virus infection. Results indicate that HIV infection is associated with a decrease in HDL measures and higher levels of interleukin 6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and D-dimer levels. HDLp, high-density lipoprotein particles; hsCRP, high-sensitivity C-reactive protein.

represent another marker of inflammation or a mediator of HIV-related premature atherosclerotic risk in this context requires further study.

Assessment of CVD risk related to blood lipids traditionally consists of measuring HDLc and LDLc levels. In the Multicenter AIDS Cohort Study (MACS), HIV seroconversion led to reductions in TC, HDLc, and LDLc levels [5]. Although initiation of ART among participants in MACS led to substantial increases in TC and LDLc levels, HDLc remained 10 mg/dL below pre-infection levels, which resulted in a lipid profile that resembled the metabolic syndrome [5, 30]. NMR spectroscopy allows further characterization of HDLp size and concentration by

Table 3. Correlation of Lipid Measures with Human Immunodeficiency Virus (HIV) RNA and Biomarkers among HIV-Infected Participants

<table>
<thead>
<tr>
<th>Lipid measurements</th>
<th>Spearman rank correlation coefficient (ρ)</th>
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<tbody>
<tr>
<td></td>
<td>HIV RNA, log10 copies/mL</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>-0.34 (.06)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>0.09 (.64)</td>
</tr>
<tr>
<td>HDLc, mg/dL</td>
<td>-0.21 (.26)</td>
</tr>
<tr>
<td>Total HDL particles, nmol/L</td>
<td>-0.24 (.19)</td>
</tr>
<tr>
<td>Large HDL particles, nmol/L</td>
<td>-0.15 (.43)</td>
</tr>
<tr>
<td>Small HDL particles, nmol/L</td>
<td>-0.22 (.23)</td>
</tr>
</tbody>
</table>

**NOTE.** HDL, high-density lipoprotein; HDLc, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LDLc, low-density lipoprotein cholesterol; sICAM-1, soluble intercellular adhesion molecule-1.
“counting” numbers of lipoprotein particle subclasses, which may be more informative with respect to CVD risk [7–13]. Our finding that levels of total HDLp were lower among HIV-infected participants than they were among HIV-uninfected participants is consistent with additional analyses performed on specimens from the MACS. Using these same methods, in the MACS, total HDLp concentration was lower among 77 HIV-infected, ART-naive persons than it was among a group of 609 uninfected control subjects [31].

We built on the MACS findings by reporting that differences in total HDLp that occur in individuals with HIV infection are primarily attributable to lower levels of large and small HDLp. Small HDLp may be particularly protective in terms of atherosclerotic risk, given anti-inflammatory properties and their preference over larger HDL as initial acceptors of cholesterol from peripheral cells in reverse cholesterol transport [32–34]. With use of these same NMR methods, analyses of lipoprotein particles from HIV-infected persons in SMART have demonstrated that baseline levels of total and small HDLp, but not of LDLp, were independently associated with subsequent risk for CVD events [17]. However, in longitudinal studies of participants from the general population, the clinical consequences of low levels of small HDLp for clinical CVD risk, compared with differences in other HDL subclasses, has been inconsistent [8, 10, 13].

Cohort data, from HIV-infected groups as well as from the general population, have demonstrated increased risk for CVD events and all-cause mortality with elevations in the plasma markers that we studied [16, 20–23, 25, 27, 28]. In a comparison of HIV-infected participants from SMART with matched control subjects from the Coronary Artery Risk Development In Young Adults study (CARDIA) and Multi-Ethnic Study of Atherosclerosis, levels of hsCRP, IL-6, and D-dimer ranged from 50% to over 100% higher in individuals with HIV infection [35]. In SMART, these changes were present for persons receiving or not receiving ART at baseline and were even present among those with HIV RNA levels <400 copies/mL, suggesting that viral suppression alone may not be sufficient to counter the factors driving inflammation in this population. Endothelial activation markers, such as sICAM-1, have also been consistently elevated in cross-sectional comparisons of HIV-infected persons with the general population [36]. These data are consistent with our findings of higher IL-6, sICAM-1, and D-dimer levels in HIV-infected persons. In contrast, we did not identify a statistically significant difference in fibrinogen levels between groups. In the study of Fat Redistribution and Metabolic Change with HIV Infection (FRAM), fibrinogen levels were higher among HIV-infected participants receiving ART and, specifically, among those receiving protease inhibitors, compared with participants from the general population in CARDIA [37]. Our findings and those from the FRAM study suggest that higher fibrinogen levels may be a consequence of ART drug effects rather than of HIV infection per se.

HDL may reduce atherosclerotic disease risk via anti-inflammatory and antithrombotic properties, in addition to reverse cholesterol transport. HDL protects LDL from oxidation and decreases expression of adhesion molecules on endothelial cells (including E-selectin and sICAM-1) [6, 38]. HDL also improves endothelial function via stimulation of nitric oxide synthase activity, enhances endothelium-dependent vasodilation, increases prostacyclin production by endothelial cells, and inhibits endothelial tissue factor expression, all of which down-regulate thrombotic pathways [34, 39, 40]. Small HDLp are primarily responsible for HDL’s anti-inflammatory properties and inhibition of endothelial activation [33, 34]. Among HIV-infected participants, we demonstrate that lower levels of HDLp, and of small HDLp in particular, are inversely correlated with markers of endothelial activation and thrombotic activity. Lower levels of HDLp may be a consequence of HIV infection and/or thrombotic activity, a mediator of endothelial injury and thrombogenesis, or both [34, 39, 41, 42]. Low HDLC levels have been described in many chronic inflammatory states, and inflammation may also change HDLp qualitatively, rendering HDLp less atheroprotective [38, 43, 44].

Limitations of this study include the cross-sectional study design, which resulted in our inability to describe associations for blood lipid and biomarker measures over time. Specifically, we are unable to account for biomarker variations over time, although participants were presumably at a steady state with respect to HIV replication. Although 5 HIV-infected participants were treatment experienced, they had not been receiving ART for >2 years, thereby minimizing influence to blood lipid and biomarker measures associated with recent or current ART use. Furthermore, data from SMART and from the general population suggest levels of these markers measured at a single time point predict subsequent risk for future clinical events [16, 20, 21, 45–50]. Also, comparisons were adjusted for HCV infection but not for IDU, because of the significant overlap in the presence of these variables; accounting for IDU did not change our results. Finally, as a consequence of the small sample size, confidence intervals are wide, and important associations may have been missed.

In summary, untreated HIV infection is associated with lower levels of HDLp, particularly of large and small HDLp. The relationship between small HDLp, inflammation, thrombogenesis, and risk for premature atherosclerosis requires further examination in longitudinal studies involving persons with HIV infection.

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References


12. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002; 90:89–94.


40. Viswambharan H, Ming XF, Zhu S, et al. Reconstituted high-density lipoprotein inhibits thrombin-induced endothelial tissue factor expression through inhibition of RhoA and stimulation of phosphati-
42. Rose H, Hoy J, Woolley I, et al. HIV infection and high density li-
50. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-
reactive protein and low-density lipoprotein cholesterol levels in the predic-