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Metabolic Abnormalities and Viral Replication is Associated with Biomarkers of Vascular Dysfunction in HIV-Infected Children

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Abstract

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Objectives—Human immunodeficiency virus (HIV)-infected children may be at risk for premature cardiovascular disease. We compared levels of biomarkers of vascular dysfunction among HIV-infected children with and without hyperlipidemia to HIV-exposed, uninfected children (HEU) enrolled in the Pediatric HIV/AIDS Cohort Study (PHACS), and determined factors associated with these biomarkers.

Design—Prospective cohort study

Methods—Biomarkers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP1)); coagulant dysfunction (fibrinogen and P-selectin); endothelial dysfunction (soluble intracellular cell adhesion molecule-1 (sICAM), soluble vascular cell adhesion molecule-1 (sVCAM), and E-selectin); and metabolic dysfunction (adiponectin) were measured in 226 HIV-infected and 140 HEU children. Anthropometry, body composition, lipids, glucose, insulin, HIV disease severity, and antiretroviral therapy were recorded.

Results—The median ages were 12.3 y (HIV-infected) and 10.1 y (HEU). Body mass index (BMI) Z-scores, waist and hip circumference, and percent body fat were lower among HIV-infected. Total and non-HDL cholesterol and triglycerides were higher in HIV-infected children. HIV-infected children had higher MCP-1, fibrinogen, sICAM, and sVCAM levels. In multivariable analyses in the HIV-infected children alone, BMI z-score was associated with higher CRP and fibrinogen, but lower MCP-1 and sVCAM. Unfavorable lipid profiles were positively associated with IL6, MCP1, fibrinogen, and P- and E-selectin, whereas increased HIV viral load was associated with markers of inflammation (MCP1 and CRP) and endothelial dysfunction (sICAM and sVCAM).

Conclusions—HIV-infected children have higher levels of biomarkers of vascular dysfunction than do HEU children. Risk factors associated with higher biomarkers include unfavorable lipid levels and active HIV replication.

Keywords

Children; HIV/AIDS; vascular dysfunction; cardiovascular risk factors; biomarkers

INTRODUCTION

Effective antiretroviral (ARV) regimens for human immunodeficiency virus (HIV) infection have increased life expectancy, and many now live for decades with chronic illness.[1] Long-term complications are emerging as the greatest challenges facing HIV-infected individuals. Atherosclerotic cardiovascular disease (CVD) is a leading co-morbidity and cause of mortality among HIV-infected adults.[2] Several studies show that HIV-infected children, compared to healthy peers, have higher rates of CVD risk factors, including dyslipidemia, insulin resistance, obesity and central adiposity.[3–7] HIV infection also results in prolonged chronic inflammation, thereby increasing CVD risk. Exogenous obesity, common among perinatally HIV-infected youth, can also contribute to CVD risk.[8, 9] For perinatally-infected children, these exposures start *in utero* and continue through critical periods of growth, puberty, and development.

Inflammation, now considered the primary mechanism leading to atherosclerosis, can initiate a complex sequence of events that eventually produce detectable arterial changes and symptomatic CVD.[10] A host of cellular pathways are activated through inflammation, with most being initiated through injury to the endothelium.[10] Factors associated with endothelial injury include oxidized cholesterol, hyperglycemia, lifestyle (smoking), and familial/genetic risks.[11] In HIV-infected patients, the effects of chronic immune activation from HIV infection [12, 13] and potential oxidative stress (induced by mitochondrial dysfunction) due to highly active antiretroviral therapy (HAART) also come into play.[14,

15] These factors initiate a cascade of events that can increase inflammation and produce changes in endothelial function and/or coagulation status.

Although HIV-infected children carry risk factors that are associated with premature atherosclerotic CVD, it is currently difficult to ascertain whether the adverse CVD outcomes attributed to HIV infection in adults will be observed as HIV-infected children age. Emerging evidence from large, long-term, and prospective studies on CVD risk in non-HIV healthy children[16, 17] show that risk factors tracked from early childhood are associated with adverse CVD outcomes in adulthood. Studies that show direct evidence of vascular inflammation may provide further proof of increased CVD risk that, in turn, may ultimately lead to new, preventive interventions for these children. C-reactive protein (CRP) is one of the best-studied measures of systemic inflammation and high levels can predict adverse CVD outcomes in adults.[18] A number of other biomarkers are associated with more specific changes in these inflammatory pathways in both HIV-infected and HIV-uninfected populations.[19–21]

We have previously determined that HIV-infected children have increased levels of biomarkers of vascular dysfunction,[22] yet little work has been done to compare these levels to those of HIV-exposed, uninfected (HEU) children or to determine the potential associations of biomarker levels with metabolic factors such as lipids and insulin resistance. We hypothesized that HIV-infected children with hyperlipidemia have higher levels of selected biomarkers associated with vascular inflammation pathways compared to HIV-infected children without hyperlipidemia and HEU children (with and without hyperlipidemia). Furthermore, we sought to determine whether metabolic, anthropometric, and disease- or treatment-specific factors are associated with higher levels of these biomarkers.

METHODS

Study Population and Participants

Participants were enrolled in the Adolescent Master Protocol (AMP), part of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development-supported Pediatric HIV/AIDS Cohort Study (PHACS). Eligible children were between 7 and 16 years and born to HIV-infected mothers. The AMP study has enrolled 451 children with perinatal HIV-infection and 227 HEU. Enrollment began March 2007 at 15 sites in the US and Puerto Rico, and completed recruitment in December 2009. The overall aims of the prospective PHACS study are to determine the impact of HIV infection and ARV therapy on several clinical outcomes in pre-adolescents and adolescents, including growth, nutrition, and cardio-metabolic risk.

The PHACS protocol requires that all HIV-infected children have fasting lipids measured annually. The protocol also requires that repository specimens be drawn at the entry visit on all participants. As these are pediatric patients, the amount of specimen varies across individuals. As of August 2009, there were 357 HIV-infected children enrolled in the study who had fasting lipids at the entry visit and 184 HEU with an entry visit. We determined that 226 of 357 HIV-infected children and 140 of 184 children had an adequate volume of repository specimen to assay for vascular biomarkers. Among the HIV-infected children, we defined hyperlipidemia by the modified National Cholesterol Education Program (NCEP) criteria [total cholesterol >200 mg/dL, LDL-cholesterol >130 mg/dL, triglycerides >110 mg/dL (≤ 9y) or >150 mg/dL (≥10y), or HDL-cholesterol <35 mg/dL][23]. Among the 357 HIV-infected children (all eligible children), 41% met the dyslipidemia definition. In our subsample of 226 (those included in this analysis), 39% met the hyperlipidemia definition.

Our subsample was similar by age, sex, race, CD4 category, and BMI z-score when compared to children who did not have repository samples.

The Institutional Review Boards at all clinical sites and the Harvard School of Public Health (Statistical and Data Management Center) approved the protocol, and informed consent from the parent(s) or guardian(s), and assent from the participants (when appropriate) were obtained.

Data Collection

At the first study visit in AMP, clinical information was collected including age, sex, race, Tanner stage (by physical examination), and family history of diabetes, atherosclerosis, myocardial infarction, and hyperlipidemia. Weight and height were measured and body mass index (BMI) was calculated [weight (kg)/ height^2 (m^2)] and expressed as z-scores.[24] Waist and hip circumferences were measured with a non-stretchable plastic tape measure. Waist and hip circumference were measured according to standard methods.[25] Anthropometric measures were standardized by training sessions conducted by a registered dietitian experienced in anthropometry during the annual PHACS meeting. Percent body fat was calculated from a total body dual-energy X-ray absorptiometry (DXA) scan performed on either a Lunar (General Electric Healthcare, UK) or Hologic (Hologic Inc, Bedford, MA) scanner according to standard methods.[26] Scans were sent to the Body Composition Analysis Center at Tufts University School of Medicine for central analysis and standardization.

Fasting serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, and insulin were measured locally and in real-time at study entry for all HIV-infected children. Non-HDL cholesterol was calculated as the difference between total and HDL-cholesterol. In HEU children, plasma lipid and lipoproteins were measured by standard methods at the Diabetes Research Institute Clinical Chemistry Laboratory at the University of Miami, on a Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN) using manufacturer's reagents and procedures. The homeostatic model assessment of insulin resistance (HOMA-IR) score was calculated: [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5.[27] For HIV-infected children only, concurrent Center for Disease Control (CDC) pediatric HIV disease stage,[28] absolute CD4+ T-lymphocyte cell (CD4) count, plasma HIV-1 RNA by quantitative PCR (viral load), and ARV regimens were recorded.

Biomarkers of Vascular Dysfunction

Fibrinogen and CRP were measured at a central laboratory by nephelometry on a Dade-Behring (Deerfield, IL) auto-analyzer using the manufacturer's reagents and instructions. Intra- and interassay coefficients of variation were 2.6% and 2.7%, respectively, for fibrinogen and 4.4% and 5.7%, respectively, for CRP. Adiponectin was measured by a double-antibody radioimmunoassay (Linco Research, St. Charles, MO), with intra- and inter-assay coefficients of variation both <5%. CRP values greater than 10 mg/dL were not used in the data analysis because high levels could be due to intercurrent infection.

Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), soluble vascular cell adhesion molecule-1 (sVCAM), soluble intracellular cell adhesion molecule-1 (sICAM), soluble E-selectin, and soluble P-selectin were measured by enzyme-linked immunosorbent assay (ELISA) using reagents from R&D Systems (Minneapolis, MN). Intra- and interassay coefficients of variation were, respectively: IL-6, 6.8% and 9.4%; MCP-1 4.0% and <7.5%; sVCAM-1, 5.9% and 10.2%; sICAM-1, 4.8% and 10.1%; E-selectin, 5.0% and 8.8%; and P-selectin, 4.2% and 9.8%.

Statistical Methods

Unadjusted analysis—Using the Kruskal-Wallis test for continuous and Chi-square test for categorical variables, the four study groups were compared by age, sex, and race/ethnicity; Tanner stage; height, weight and BMI z-scores; lipids; and biomarkers of vascular dysfunction. For each biomarker, we evaluated differences between the four study groups by the Wilcoxon rank sum test.

Adjusted analyses—When waist:hip ratio, lipids, and biomarkers of vascular dysfunction were the outcome variable, they were $\log_{10}$ transformed for analysis to normalize the distribution. When lipids were predictor variables, each lipid was categorized into quartiles based on the distribution in the HIV-infected children. Cutoffs were based on the distribution in the HIV-infected to be consistent across models because one set of models included only HIV-infected and another included HIV-infected and HEU (see analyses below).

We evaluated differences between all HIV-infected children and HEU children on anthropometric and lipid outcomes using multivariable general linear regression. Waist:hip ratio, percent body fat, and the lipid outcomes were adjusted for potential confounding by age, race/ethnicity, sex and Tanner stage, while weight, height, and BMI z-score were adjusted for race/ethnicity and Tanner stage only because z-scores are standardized for age and sex.

We compared levels of each biomarker of vascular dysfunction in the four study groups by multivariable linear regression adjusted for sex, age, race/ethnicity, Tanner stage and BMI z-score. Among HIV-infected children only, we determined the association of each metabolic and HIV disease-specific variable including individual lipids, HIV viral load (≤ 400, 400–5,000, and >5,000 copies/mL), CD4 count (<200 and ≥ 200 cells/mm^3), CDC stage (N/A, B, and C), and current use or non-use of each ARV class (protease inhibitor, PI; non-nucleoside reverse transcriptase inhibitor, NNRTI; and nucleoside reverse transcriptase inhibitor, NRTI) separately with each biomarker outcome adjusted for age, sex, race/ethnicity, and BMI z-score. Variables significant at $p \leq 0.1$ or were confounders were retained in the final model. Models were examined for influential points using standardized residuals and assumptions of linearity between age and BMI z-score were evaluated. For presentation, the antilog was taken for each beta coefficient and 95% confidence interval (95% CI) in each model. The interpretation of the antilog is as follows: if the estimate presented for HIV-infected versus HEU was 0.9 in the model of CRP, the interpretation would be that the average CRP in the HIV-infected is 0.9 times the average in the HEU, or 9% lower in the HIV-infected. For categorical variables that were significant at $p \leq 0.05$ on the F-test, we show the Wald p-value for differences between each level versus the reference level. All analyses were performed in SAS 9.1 (SAS Institute, Cary, NC).

RESULTS

Demographic and Clinical Characteristics

In this analysis, we studied a subset of AMP participants including 226 HIV-infected children (89 who met the hyperlipidemia definition) and 140 HEU (40 who met the hyperlipidemia definition). In the HEU, 28.6% met the hyperlipidemia definition. The clinical characteristics of the four groups are shown in Table 1. HIV-infected children were significantly older than HEU and a greater proportion was non-Hispanic black. As expected because of their younger age, HEU children were more likely to be prepubertal (Tanner 1) than HIV-infected children. In the HIV-infected group, 76% had CD4 counts >500 cells/mm^3, 65% had an HIV viral load ≤400 copies/mL, and 72% were on HAART with a

protease inhibitor. The percent that ever used the following medications and the median duration of use are as follows: indinavir (7%, 2.0 yrs);,atazanavir (13%,1.9 yrs); boosted PI (66%; 4.3 yrs); abacavir (36%, 2.4 yrs); and stavudine (79%; 6.2 yrs).

Table 1 also shows differences in anthropometric and metabolic (unadjusted) outcomes between the four groups. HIV-infected children had lower weight, height and BMI z-scores than the HEU; there were no differences between the two HIV groups. HIV-infected children without hyperlipidemia were more likely to have a family member with diabetes than the HEU children and HIV-infected children with hyperlipidemia (23% vs 12%, p=0.004; 23% vs 11%, p=0.09, respectively), although other familial risk factors were similar (atherosclerosis, myocardial infarction, and hypercholesterolemia; data not shown).

Table 2 compares adjusted anthropometric and metabolic parameters potentially associated with vascular inflammation by HIV status. The mean adjusted z-scores were lower in HIV-infected compared to HEU for weight (−0.77 SD), height (−0.76 SD), and BMI (−0.49 SD) ($p<0.001$ for all comparisons). Mean adjusted waist and hip circumferences were each almost 5 cm smaller in the HIV-infected children, although the waist:hip ratio was similar between groups. Total body fat was about 4.7% lower in HIV-infected children. In a similar analysis, after adjusting for age, race, sex, Tanner stage, and BMI z-score, HIV-infected children had 1.05 times (or 5%) higher total cholesterol, 1.08 (or 8%) higher non-HDL cholesterol, and 1.32 (or 32%) higher level of triglycerides than HEU.

Comparisons of Biomarkers of Vascular Dysfunction by HIV Status

Table 3 shows the median (25^{th}, 75^{th}) of the raw (unadjusted) values and comparisons of the biomarkers of vascular dysfunction across all four groups with the pair-wise comparisons between each group. MCP-1 and fibrinogen were the highest in HIV-infected children with hyperlipidemia and there were no differences between the other groups. P-selectin was the lowest among HIV-infected children who did not have hyperlipidemia, but there were no differences among the other groups. sVCAM was highest in the HIV-infected group, regardless of lipid status and E-selectin appeared to be highest in those with hyperlipidemia, regardless of HIV status. Table 4 shows differences between all 4 groups for each biomarker after adjusting for age, sex, race, tanner stage and BMI z-score. HIV-infected children had higher levels of MCP-1, fibrinogen, and sVCAM regardless of lipid status. In addition, sICAM was elevated in HIV-infected children without hyperlipidemia compared to the reference group. The analyses were adjusted for other demographic and clinical factors (data not shown in the Table). We found age was positively associated with CRP, IL-6, and fibrinogen; Hispanic and NHB ethnicity was positively association with fibrinogen; and BMI z-score was positively associated with CRP, IL-6, and fibrinogen.

Correlates of Biomarkers of Vascular Dysfunction in HIV-infected Children

For the HIV-infected children only, we analyzed clinical correlates (including HIV disease-specific measures) of each biomarker of vascular dysfunction in a multivariable model (Table 5). Results for adiponectin are not shown because, other than age and HOMA-IR (known associations), it was not independently associated with any other variables. In general, there were few associations between any of these biomarkers and age and sex, although differences were found by race/ethnicity. Compared to non-Hispanic whites, Hispanics had higher levels of the biomarkers of inflammation (CRP and IL-6) while non-Hispanic blacks had lower levels of MCP-1. Non-Hispanic blacks also had higher levels of fibrinogen, lower levels of P-selectin (measures of coagulant dysfunction and inflammation), and lower levels of sICAM. A higher BMI z-score was associated with higher CRP and fibrinogen and lower MCP-1 and sVCAM. Unfavorable lipid profiles were generally associated with higher levels of these biomarkers of vascular dysfunction. Total

cholesterol was positively associated with P-selectin and E-selectin; LDL-cholesterol was positively associated with fibrinogen; and triglycerides were positively associated with MCP-1. HDL-cholesterol levels were inversely related to IL-6.

Viral load was positively associated with MCP-1 and biomarkers more specific for endothelial dysfunction including sICAM, and sVCAM. Current PI and NNRTI exposures were associated with higher levels of fibrinogen and CRP, respectively. Current NRTI exposure was associated with lower levels of E-selectin. No significant relationships were found for waist or hip circumference, waist:hip ratio, total body fat, HOMA-IR, or CD4 and all biomarkers.

DISCUSSION

Our study shows that biomarkers associated with different pathways of atherosclerosis - inflammation and coagulation and endothelial dysfunction - were higher in HIV-infected children compared HEU children. In the HIV-infected children, elevations of these biomarkers were independent of metabolic factors (hyperlipidemia or insulin resistance), with the exception of the selectins (both P and E), where differences appeared to be influenced strongly by lipid levels. Biomarkers of endothelial dysfunction, sICAM and sVCAM, and biomarkers of inflammation, CRP and MCP-1, were associated with higher HIV viral loads.

Atherosclerosis is considered an inflammatory process.[29] Triggers that can initiate vascular injury include lipids, lipoproteins, angiotensin II, cytokines, glycosylation products, oxidative stress, and infectious agents.[11] This injury results in the activation of nuclear factor-κB (NF-κB) with several pro-inflammatory cytokines released including molecules that increase leukocyte rolling and adherence to the endothelium, leukocyte migration through the endothelium, and recruitment of more inflammatory cells. Activated macrophages secrete several cytokines and growth factors that promote maturation of the atheromatous lesion.

Biomarkers such as hsCRP are independent predictors of future CVD in adults and there is emerging evidence of their utility in children.[18, 30] Other biomarkers that reflect leukocyte adherence, migration, and chemotaxis have also been associated with increased CVD risk in HIV-uninfected populations.[19, 20] We found that CRP and MCP-1, biomarkers associated with inflammation, were associated with increased viral load. In the Strategic Management of Antiretroviral Therapy (SMART) study, hsCRP and IL-6 levels were associated with viral load and CVD all-cause mortality risk in HIV-infected adults.[31] Even in patients with viral suppression, the levels of these biomarkers were about 40–60% higher than in an HIV-uninfected population.[32] However, not all studies have shown that hsCRP levels are associated with adverse CVD events.[33]

HDL-cholesterol and higher triglycerides were associated with biomarkers of inflammation although the HDL effect was diminished in the HIV model when viral load was considered. HDL-cholesterol, thought to be critical in the “reverse transport” of cholesterol from arterial plaques, may also have direct anti-inflammatory effects[34] by decreasing E-selectin[35] (associated with leukocyte tethering and rolling) and limiting expression of vascular adhesion molecules such as VCAM and ICAM.[36] Other studies show that postprandial triglycerides or triglyceride-rich lipoproteins are associated with activation of NF-κB[37] or that very-low-density-lipoproteins (VLDL) can increase expression of leukocyte adhesion factors.[38] We found that triglycerides were associated with higher MCP-1 and E-selectin. The putative role of selectins is to facilitate the tethering and rolling of leukocytes along the endothelium; hyperlipidemia may induce endothelial injury and activate this process. Both

P- and E-selectin levels were associated with hyperlipidemia, even after adjusting for HIV status.

Although a hypercoagulable state is associated with CVD risk, few studies have evaluated biomarkers associated with thrombosis in HIV-infected patients. Fibrinogen is positively associated with mortality in HIV,[31] but whether this translates to increased CVD risk is unclear. PI therapy is associated with increased fibrinogen levels in the Fat Redistribution and Metabolic Change Study (FRAM).[39] We found fibrinogen was positively correlated with LDL-cholesterol levels in HIV-infected children. Fibrinogen may represent coagulation risk, but may also reflect inflammation.

Several studies in adults report associations between endothelial dysfunction markers with HIV disease severity.[40, 41] We found that MCP-1, sICAM, and sVCAM levels were higher in the HIV-infected children compared to HEU and that higher levels were associated with viral load, independent of metabolic status. These findings suggest that HIV itself may cause immune activation and resulting endothelial injury.[41] These biomarkers are associated with all-cause mortality in non-HIV populations[42] and sVCAM levels are associated with increased carotid intima media thickness (cIMT) in HIV-infected adults.[43] The HIV *tat* and *nef* proteins induce VCAM-1, ICAM-1, and MCP-1. ICAM was elevated in HIV-infected children compared to controls and elevations were inversely related to CD4 counts.[44] In addition MCP-1 is proposed to activate viral infection.[45] Treatment interruptions are associated with increased levels of sVCAM, ICAM, and P-selectin,[46] suggesting the influence of viral activity on expression of these biomarkers.

We did not find a strong effect of ARVs on the biomarkers we studied due possibly to the collinearity of ARVs on metabolic outcomes. PI therapy was associated with higher fibrinogen and NNRTI was associated with higher CRP. In cell culture, ARVs can alter endothelial cell mitochondrial DNA thereby increasing production of reactive oxygen species,[47, 48] endothelial cell permeability,[49] and leukocyte adhesion[50]. Thus, ARV therapy could directly or indirectly (through changes in the metabolic profile) increase levels of biomarkers.

Studies on vascular inflammation and structural/functional vascular dysfunction (*i.e.*, vessel compliance, distensibility, and structure) in HIV-infected children have been limited.[51–56] We have recently shown that similar biomarkers are also associated with central adiposity and decreased immune function (lower CD4 counts), although we had limited ability to evaluate the effect of lipids on these biomarkers.[22] Some studies show vascular stiffness (through flow-mediated dilation studies) and cIMT were greater in HIV-infected children than controls[53–55] with differences independent of known CVD risk factors and ARV therapy,[53] while others show an association with ARVs, especially PIs.[52, 54, 55] Longitudinal follow-up in one study revealed cIMT was similar in HIV and controls.[51, 56] Interestingly, some pre-HAART studies in children showed increased coronary artery calcifications,[57] suggesting the contribution of baseline immune activation to CVD risk.

We did find some unexpected results. For instance, BMI was inversely related to sVCAM. In non-HIV cohorts, adiposity is associated with higher levels of these biomarkers.[58] However, in our cohort, we suspect that higher viral loads (sicker children) were associated with lower BMI and, in turn, higher viral loads were associated with many of our biomarkers including sVCAM. We also selected our groups by whether the child met a hyperlipidemia definition and our groups may not represent HIV as a whole. However, the rates of hyperlipidemia in the PHACS cohort as a whole were similar to our study sample. Lastly, we did find that children with hyperlipidemia were more likely to have a family member with diabetes. This is an unusual association and may be a result of reporting bias.

In conclusion, we show that biomarkers associated with vascular inflammatory pathways are increased in HIV-infected children when compared to appropriately matched HEU children. Higher levels of these biomarkers were independently associated with HIV viral load (MCP-1, sICAM and sVCAM) and/or hyperlipidemia (MCP-1, fibrinogen, P-selectin and E-selectin). Our findings provide further evidence that HIV-infected children are at risk for CVD. Since many of these biomarkers were associated with modifiable risk factors, such as hyperlipidemia, interventions to modify these risks should be considered in future studies.

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Table 1

Clinical Characteristics by HIV Status and Hyperlipidemia

Variable	HIV+, hyperlipidemia N=89	HIV+, no hyperlipidemia N=137	HEU, hyperlipidemia N=40	HEU, no hyperlipidemia N=100	Overall P-value
Demographics and Anthropometrics - median (25th, 75th)* **					
Age (y)	12.3[1,2] (10.0, 14.0)	12.3[3,4] (10.0, 14.2)	9.6[1,3] (8.5, 10.8)	10.5[2,4] (8.2, 12.3)	<0.001
Male n, (%)	38 (43)	61 (54)	22 (55)	53 (53)	0.33
Race/ethnicity, n, (%)					<0.001
Non-Hispanic black	60 (67)[1]	109 (80)[2,3]	21 (53)[2]	56 (56)[1,3]	
Hispanic	20 (22)	20 (15)	13 (33)	39 (39)	
Non-Hispanic white/other	9 (10)	8 (6)	6 (15)	5 (5)	
Tanner stage 1, n (%)	22 (25)[1]	37 (27)[2,3]	22 (55)[1,2]	38 (38)[3]	0.004
Weight z-score	0.17[1,2] (−0.58, 1.01)	0.18[3,4] (−0.65, 0.87)	0.97[1,3] (−0.17, 2.6)	0.52[2,4] (−0.21, 1.7)	<0.001
Height z-score	−0.30[1,2] (−1.30, 0.34)	−0.17[3,4] (−1.05, 0.44)	0.30[1,3] (−0.46, 1.0)	0.18[2,4] (−0.48, 1.0)	<0.001
BMI z-score	0.45[1] (−0.32, 1.43)	0.15[2,3] (−0.46, 1.02)	1.1[1,2] (−0.1, 2.4)	0.75[3] (−0.15, 1.7)	0.003
Total Cholesterol (mg/dL)	198[1,2] (164, 218)	158[1,3] (140, 177)	204[3,4] (165, 216)	157[2,4] (142, 172)	<0.001
LDL cholesterol (mg/dL)	113[1,2] (81, 135)	87[1,3] (73, 104)	126[3,4] (95, 136)	90[2,4] (72, 103)	<0.001
HDL cholesterol (mg/dL)	47[1,2] (39, 56)	50[1] (43, 59)	47 (39, 59)	51[2] (44,61)	0.045
Non HDL cholesterol (mg/dL)	146[1,2] (116, 161)	104[1,3] (90, 122)	141[3,4] (124, 165)	103[2,4] (87, 118)	<0.001

Variable	HIV+, hyperlipidemia N=89	HIV+, no hyperlipidemia N=137	HEU, hyperlipidemia N=40	HEU, no hyperlipidemia N=100	Overall P-value
Triglycerides (mg/dL)	151[1,2,3] (107, 186)	75[1,4] (54, 96)	112[2,4,5] (59, 148)	64[3,5] (55, 87)	<0.001
HOMA-IR	1.80 (1.00, 3.70)	1.5 (0.80, 2.95)			0.11
HIV-Specific Disease Characteristics					
CDC Stage, n, (%)					0.32
Stage A, mildly symptomatic	23 (26)	49 (36)			
Stage B, moderately symptomatic	27 (30)	42 (31)			
Stage C, severely symptomatic	27 (30)	30 (22)			
Stage N, not symptomatic	12 (13)	15 (11)			
Absolute CD4 count (cells/mm^3), median (25th, 75th)	750 (530, 995)	704 (502, 902)			0.30
Viral load (copies/mL), median (25th, 75th)	223 (50, 871)	400 (50, 2677)			0.01
≤400 (%)	65 (73)	81 (59)			
401 – 5000 (%)	8 (9)	33 (24)			
>5000 (%)	16 (18)	23 (17)			
HAART-based ARV grouping, n (%)					0.04
HAART with PI	73 (82)	89 (65)			
HAART without PI	11 (12)	25 (18)			
Non-HAART ARV	3 (3)	9 (7)			
No ARV	2 (2)	12 (9)			

[*] Results with the same superscript are significantly different (p≤0.05) from each other

HEU = HIV-exposed, uninfected; IQR = interquartile range; ARV = antiretroviral therapy; CDC = Centers for Disease Control; PI = protease inhibitor; HAART = highly active antiretroviral therapy; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance

Table 2

Adjusted Anthropometric and Metabolic Measures in HIV-Infected Compared to HIV-Exposed but Uninfected (HEU) Children

Outcome*			
Anthropometric Outcomes	**Mean Difference Between HIV-infected and HEU**	**95% CI**	**P-value**
Weight z-score (SD)	−0.77	−1.07, −0.48	<0.001
Height z−score (SD)	−0.76	−1.02, −0.50	<0.001
BMI z−score (SD)	−0.49	−0.76, −0.22	<0.001
Body fat %	−4.67	−7.37, −1.97	<0.001
Waist circumference (cm)	−4.78	−7.84, −1.72	0.002
Hip circumference (cm)	−4.90	−7.88, −1.92	0.001
	HIV-infected relative to HEU* **	**95% CI**	**P-value**
Waist:hip ratio	0.99	0.97, 1.01	0.24
Metabolic Outcomes**			
Total cholesterol	1.05	1.0, 1.11	0.04
LDL-cholesterol	1.03	0.96, 1.11	0.43
HDL-cholesterol	1.00	0.94, 1.05	0.89
Non-HDL cholesterol	1.08	1.01, 1.16	0.02
Total cholesterol/HDL	1.06	1.0, 1.13	0.06
Triglycerides	1.32	1.18, 1.48	<0.001

* Z-scores were adjusted for race/ethnicity and Tanner stage as they are already standardized for age and sex. All other outcomes were adjusted for age, sex, race, and Tanner stage. HEU is reference.

** Waist:hip ratio and all lipids were $\log_{10}$ transformed for analysis and then exponentiated in the table.

*** As an example, the interpretation of the estimate for total cholesterol is that on average the HIV-infected have a 1.05 times greater (or 5% greater) total cholesterol value than the HEU.

BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein

Table 3

Comparison of Biomarkers of Vascular Dysfunction among HIV-Infected with and without Hyperlipidemia and HIV-Exposed, but Uninfected (HEU) Children (Unadjusted)

Biomarker*	Median [25th, 75th] HIV HL N= 89	HIV no HL N=137	HEU HL N=40	HEU no HL N=100	Overall P-value**
Inflammation					
CRP (mg/L)	0.4 [0.20, 1.20]	0.4 [0.20, 1.10]	0.8 [0.30, 2.55]	0.5 [0.20, 2.20]	0.28
IL-6 (pg/mL)	1.1 [0.70, 1.65]	1.0 [0.70, 1.7]	1.2 [0.80, 2.40]	1.15 [0.65, 1.75]	0.33
MCP-1 (pg/mL)	106 [91, 142][1,2,3]	101 [76, 131][1]	97 [73, 125][2]	89 [74, 117][3]	0.004
Coagulant Dysfunction					
Fibrinogen (mg/dL)	381 [339, 421][1,2]	355 [314, 398][1]	360 [311, 415]	340 [292, 391][2]	0.001
P-selectin (ng/mL)	36.9 [27.2, 47.2][1]	29.3 [22.6, 44.9][1,2,3]	40.8 [28.9, 52.2][2]	35.3 [25.5, 51.2][3]	0.005
Endothelial Dysfunction					
sICAM (ng/mL)	280 [168, 341][1]	283 [170, 338]	261 [164, 314]	241 [150, 321][1]	0.12
sVCAM (ng/mL)	766 [659, 952][1,2]	776 [667, 1040][3]	643 [540, 809][1,3]	693 [581, 822][2]	<0.001
E-selectin (ng/mL)	51 [38.8, 64.8][1]	41.3 [27.3, 59.3][1,2,3]	61.7 [45.0, 76.9][2,4]	48.0 [35.9, 68.0][3,4]	<0.001
Metabolic Dysfunction					
Adiponectin (ng/mL)	9.0 [6.5, 11.3]	9.6 [7.6, 12.2]	8.7 [7.3, 11.2]	9.2 [7.8, 11.9]	0.26

*HL = hyperlipidemia; CRP = C-reactive protein; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; sICAM = soluble intracellular cell adhesion molecule-1; sVCAM = soluble vascular cell adhesion molecule-1

**Overall P value from Kruskal-Wallis test for any difference across groups. Results with the same superscript are significantly different (p≤0.05) from each other based on the Wilcoxon test.

Table 4

Multivariable Models of Biomarkers of Vascular Dysfunction among HIV-Infected and HIV-Exposed, but Uninfected (HEU) Children

	Biomarker*: Estimate [95% CI]**							
	Inflammation			**Coagulant dysfunction**		**Endothelial dysfunction**		
Covariate***	**CRP N=350**	**IL-6 N=363**	**MCP-1 N=364**	**Fibrinogen N=365**	**P-selectin N=363**	**sICAM N=355**	**sVCAM N=363**	**E-selectin N=365**
HIV Group								
HIV, HL	0.93 [0.66, 1.3]	1.01 [0.85, 1.3]	**1.2[3] [1.12, 1.4]**	1.1[3] [1.1, 1.2]	1.1 [0.92, 1.2]	1.11 [0.95, 1.3]	**1.1[2] [1.04, 1.3]**	**1.1 [0.97, 1.3]**
HIV, no HL	1.01 [0.73, 1.4]	0.97 [0.79, 1.2]	**1.1[1] [1.01, 1.25]**	1.1 [1.01, 1.1][1]	0.91 [0.8, 1.0]	**1.2[1] [1.0,1.4]**	**1.2[3] [1.1, 1.3]**	**0.90 [0.79, 1.0]**
HEU, HL	1.1 [0.7, 1.8]	1.2 [0.94, 1.6]	**1.06 [0.92, 1.22]**	1.0 [0.97, 1.1]	1.1 [0.92, 1.3]	1.0 [0.85, 1.3]	**0.97 [0.86, 1.1]**	**1.1 [0.93, 1.3]**
HEU, no HL	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	**Ref.**	**Ref.**
P-value	0.85	0.37	0.0015	<0.001	0.073	0.13	<0.001	0.004

* Biomarkers of vascular dysfunction were $\log_{10}$-transformed for analysis. For presentation in the tables and text, the antilog was determined for each beta coefficient and 95% CI in each model. For example, for the outcome CRP in the table above, the estimate for HIV with HL vs. HEU no HL was 0.93. The interpretation is that the average value of CRP in the HIV-infected is 0.93 times that in the HEU or ~ 7% lower in the HIV-infected.

** **Bolded variables are significant at p≤0.05 on the F-test**. The superscript indicates p-values for the differences with the HEU no HL. [1]p≤0.05; [2]p≤0.01; [3]p≤ 0.001.

*** All models were adjusted for age, sex, race/ethnicity, Tanner stage and BMI z-score. Other measures of body composition that were considered for the adjustment: waist and hip circumference, waist:hip ratio, and percent body fat. The number included in each model differs due to missing variables and for CRP values greater than 10 were excluded.

CRP = c-reactive protein; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; sICAM = soluble intracellular cell adhesion molecule-1; sVCAM = soluble vascular cell adhesion molecule-1

Table 5

Multivariable Predictors of Biomarkers of Vascular Dysfunction among HIV-Infected Children

	Biomarker*: Ratio [95% CI]**							
	Inflammation			Coagulant dysfunction		Endothelial dysfunction		
Covariate* **	**CRP N=214**	**IL-6 N=224**	**MCP-1 N=224**	**Fibrinogen N=223**	**P-selectin N=224**	**sICAM N=224**	**sVCAM N=222**	**E-selectin N=225**
Demographic								
Male (vs female)	0.94 [0.7, 1.27]	1.02 [0.84, 1.23]	1.07 [0.97, 1.18]	0.98 [0.93, 1.03]	0.98 [0.85, 1.12]	**0.84[1] [0.71, 0.98]**	1.0 [0.97, 1.1]	1.09 [0.95, 1.24]
Age (y)	1.03 [0.97, 1.1]	1.03 [0.99, 1.07]	0.98 [0.96, 1.00]	1.01 [1.00, 1.02]	0.98 [0.95, 1.0]	0.97 [0.94, 1.00]	1.0 [0.98, 1.0]	0.97 [0.95, 1.00]
Hispanic (vs NHW)	**2.13[1] [1.11, 4.08]**	**1.59[1] [1.05, 2.41]**	0.85 [0.69, 1.05]	1.10 [0.98, 1.24]	0.80 [0.59, 1.07]	0.76 [0.54, 1.08]	1.0 0.86, 1.2]	1.13 [0.85, 1.5]
NHB (vs NHW)	1.17 [0.97, 3.01]	1.40 [0.97, 2.01]	**0.74[2] [0.61, 0.89]**	**1.14[1] [1.03, 1.26]**	**0.73[1] [0.57, 0.94]**	**0.62[2] [0.46, 0.84]**	0.95 [0.81, 1.1]	0.97 0.76, 1.24]
Anthropometric								
BMI Z-score	**1.20[2] [1.05, 1.37]**	1.02 [0.94, 1.11]	**0.95[2] [0.90, 0.99]**	**1.04[3] [1.02, 1.06]**	1.01 [0.95, 1.08]	0.96 [0.90, 1.03]	**0.96[1] [0.92, 1.0]**	0.99 [0.93, 1.05]
Metabolic#								
Total cholesterol	…	…	…	…	**1.29[2] [1.07, 1.56]**	…	…	**1.49[3] [1.24, 1.79]**
LDL cholesterol	…	…	…	**1.11[2] 1.04, 1.20]**	…	…	…	…
HDL cholesterol	…	**0.75[1] [0.56, 0.99]**	…	…	…	…	…	…
Triglycerides	…	…	**1.24[2] 1.07, 1.42]**	…	…	…	…	…
HOMA-IR	…	…	…	…	…	…	…	1.1 [0.9, 1.3]

	Biomarker*: Ratio [95% CI]**							
	Inflammation			Coagulant dysfunction		Endothelial dysfunction		
Covariate* **	**CRP N=214**	**IL-6 N=224**	**MCP-1 N=224**	**Fibrinogen N=223**	**P-selectin N=224**	**sICAM N=224**	**sVCAM N=222**	**E-selectin N=225**
HIV disease specific variables#								
Viral load >5000 copies/mL (vs ≤400)	1.5[4] [0.99, 2.28]	…	**1.23[2] [1.08, 1.14]**	…	…	**1.42[2] [1.14, 1.77]**	**1.5[3] [1.3, 1.6]**	…
PI	…	…	…	**1.12[2] [1.04, 1.21]**	…	…	…	…
NRTI	…	…	…	…	…	…	**0.83[1] [0.69, 0.99]**	…
NNRTI	**1.66[1] [1.09, 2.51]**	…	…	…	…	…	…	…

*Biomarkers of vascular dysfunction were $\log_{10}$-transformed for analysis. For presentation in the tables and text, the antilog determined for each beta coefficient and 95% CI in each model. For example, for the outcome CRP in the table above, the estimate for males vs. females was 0.94. The interpretation is that the average value of CRP in the HIV-infected is 0.94 times that in the HEU or ~9% lower in the HIV-infected.

**Bolded variables are significant at p≤0.05 on the F-test. When the F-test was significant at p≤0.05 then the p values are indicated for differences between the upper level and the lowest level (e.g. highest quartile versus lowest quartile for lipids) [1]p≤0.05; [2]p≤0.01; [3]p≤0.001; [4]p=0.057. For BMI z, the p-value z-score is for the continuous variable.

***All models were adjusted for age, sex, race/ethnicity, and BMI z-score. Other measures of body composition that were considered for the multivariable analysis model included: waist and hip circumference, waist:hip ratio, and percent body fat, HOMA-IR, and CD4 T lymphocyte count. The number included in each model differs due to missing variables and for CRP values greater than 10 were excluded.

#Rows that have empty cells (…) show variables that were not included in the model because their associations were at p>0.10 or were not determined to be a confounder

NHW = Non-Hispanic white; NHB = Non-Hispanic black; BMI = body mass index; PI = protease inhibitor; NRTI = nucleotide reverse transcriptase inhibitor; NNRTI = non-nucleotide reverse transcriptase inhibitor; CRP = c-reactive protein; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; sICAM = soluble intracellular cell adhesion molecule-1; sVCAM = soluble vascular cell adhesion molecule-1