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Lipoprotein Particle Profiles by Nuclear Magnetic Resonance Spectroscopy in Medically-Underserved HIV-Infected Persons

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Abstract

Background—HIV infection is associated with dyslipidemia and increased risk for cardiovascular events. Few studies have described lipid status in medically-underserved, HIV-infected ethnic minorities, a group that is characterized by health disparities.

Objective—The objective was to characterize the lipid profile of a medically-underserved, largely ethnic minority sample of HIV-infected persons using standard lipid panels and nuclear magnetic resonance (NMR) -derived lipoprotein particle profiles.

Methods—Participants were recruited from a randomized controlled trial of a dietary supplement to manage HIV-related dyslipidemia (N=132). At the initial screening visit, sociodemographic, clinical, and behavioral data were collected, and fasting peripheral venous blood specimens were obtained and lipid status was analyzed using the standard lipid panel and the NMR-derived lipoprotein particle profile.

Results—Using NMR-derived LDL particle cutoffs, a higher percentage of participants was outside the target range (50%) than when standard LDL cholesterol NCEP cutoffs were used (24%). Antiretroviral therapy, especially protease inhibitor-containing regimens, was associated with higher LDL particle concentration.

Conclusion—Substantial numbers of medically-underserved, asymptomatic HIV-infected minorities may be at increased risk for CHD based on NMR-derived lipoprotein values.

Keywords

HIV-1; Highly Active Antiretroviral Therapy; Magnetic Resonance Spectroscopy; Lipids; Lipoproteins LDL; Lipoproteins HDL

Introduction

HIV infection and its treatment are associated with a number of adverse effects. One adverse effect of growing concern is HIV-related dyslipidemia, a condition characterized by abnormal serum lipoprotein parameters that have been associated with the development of premature coronary heart disease (CHD).¹⁻⁴ Emerging data indicate that HIV infection is associated with increased risk for cardiovascular events. The rates of atherosclerosis and acute myocardial infarction are significantly higher in HIV-infected than uninfected persons.⁵⁻⁷ Moreover, ethnic minorities and persons who reside in medically-underserved areas, two groups that are overrepresented among HIV-infected persons, have a higher risk for health outcome disparities related to their infection, including cardiovascular disease.^{7,8} Yet, to date, there have been few studies to characterize the prevalence and correlates of dyslipidemia among medically-underserved, HIV-infected ethnic minorities. Growing evidence suggests that lipoprotein particle subclass, as measured by nuclear magnetic resonance (NMR) spectroscopy, is predictive of CHD risk,⁹⁻¹³ but little is known about this lipid parameter in HIV-infected persons. In this article, we present lipoprotein particle subclass data from a recently completed randomized controlled trial of a dietary supplement to manage HIV-related dyslipidemia in a medically-underserved, largely ethnic minority sample.

Methods

Setting & Sample

The study was conducted between April, 2006 and March, 2009 at three inner-city outpatient HIV/AIDS clinics in Chicago, Illinois that are located in a HRSA-designated medically underserved area. Institutional review board approval was granted by each site, and all participants gave written informed consent before enrollment into the study. Potentially eligible participants either self-referred to the study or were referred by their health care provider. All participants were initially screened over the telephone. They were excluded from the study if they: (a) were younger than 18 or older than 60 years, (b) had a history of coronary artery disease, peripheral vascular disease, stroke or a chronic condition known to influence lipid status (e.g., diabetes mellitus, thyroid disorders, Cushing's Syndrome, familial hyperlipidemia syndrome), (c) had taken glucocorticoids or medications known to have lipid modulatory properties (e.g., statins, fibrates, atypical antipsychotics, such as risperidone and quetiapine) within three months of study entry, or (d) had a current active opportunistic infection or malignancy.

Procedures

Participants who met the screening criteria for the dietary supplement study were instructed to fast after midnight on the day of the first study visit. They were interviewed to obtain sociodemographic, clinical, and behavioral data, and fasting peripheral venous blood specimens were obtained to screen for the presence of dyslipidemia.

Measures

The standard lipid panel was obtained to quantify triglycerides, total cholesterol, and HDL cholesterol; LDL cholesterol levels were calculated using the Friedewald equation. Lipoprotein particle concentration and size were measured using NMR spectroscopy (Liposcience, Inc., North Carolina). Five milliliters (mls) of peripheral venous blood were collected in serum separator tubes which were then centrifuged at 3000 rpm for 15 minutes. The tubes were immediately refrigerated and shipped to Liposcience, Inc. within 96 hours of collection.

Data Analysis

All statistical analyses were performed with SPSS 16.0 (Chicago, IL). Descriptive statistics were used to characterize the sample. Nominal data were analyzed using the chi-square test, while continuous data were analyzed using Pearson's correlation analyses, independent samples t tests, and one-way analysis of variance (ANOVA). A significance level of 0.05 was determined *a priori*.

Results

One hundred thirty-two participants met the initial eligibility criteria for the dietary supplement study and had their blood drawn for the lipid analyses. Demographic and clinical data are shown in Table 1. Participants were predominately male (64.4%), African American (92.4%), and had a mean age of 44.64 years.

We examined the relationships between the standard lipid profile parameters and age, gender, and smoking status. Age significantly correlated with triglyceride concentration ($r = .186$, $p = .003$); no significant correlations were found between age and any other standard or NMR lipid measurements (all p values $> .05$). Smokers had significantly lower triglycerides ($\mu = 127$, $SD = 53.6$) than non-smokers ($\mu = 165$, $SD = 103.4$) ($t = -2.07$, $p = .045$); no significant differences were found for any other standard or NMR lipid measurements. Significant lipid differences were found between men and women (Table 2). Women had significantly lower triglycerides, LDL particle concentration, and significantly larger LDL and HDL particle size (all p values $\leq .05$), however, there were no differences between men and women with respect to current HAART regimen ($p = .50$).

When LDL cholesterol was measured by the standard lipid panel, 83.3% of the sample ($n=110$) met the National Cholesterol Education Program (NCEP) target goal of less than 160 mg/dL (if they had less than two risk factors) or less than 130 mg/dL (if they had two or more risk factors), while 15.9% ($n=21$) had levels that exceeded the target (data missing for one participant).¹⁴ When LDL particle concentration was measured using NMR spectroscopy, only 50% of the sample had levels that met the laboratory's reference cutoffs of "optimal" (< 1000 nmol/L) or "near optimal" (1000-1299 nmol/L). These cutoffs correspond to the 20th and 50th percentiles respectively for LDL particle concentration in the Multi-Ethnic Study of Atherosclerosis (MESA)⁹ (Table 3).

We examined the effects of current highly active antiretroviral therapy (HAART) regimen on LDL particles by categorizing participants into three groups: HAART with protease inhibitor (PI) ($n=68$), HAART without PI ($n=43$), and no HAART ($n=21$). To increase statistical power, we dichotomized the five LDL particle risk categories into optimal (< 1000 nmol/L) and above optimal (≥ 1000 nmol/L) concentrations. The results of the chi-square analysis indicated that significant differences existed among the three groups [χ^2 (2, $N=132$) = 7.514, $p = .023$]. As shown in Figure 1, the number of participants who had elevated LDL particle concentration was 55 (81%) in the HAART with PI group, 28 (65%) in the HAART without PI group, and 11 (52%) in the no HAART group.

Standard and NMR lipoprotein data for the three HAART groups are shown in Table 4. Significant differences in lipoprotein parameters were found for total HDL-C [F (2,129) = 7.048, $p = .001$] and HDL particle concentration [F (2,129) = 8.113, $p < .001$]. Post hoc Bonferroni comparisons indicated that the HAART without PI group had significantly higher HDL-C ($p < .015$) and HDL particle concentration ($p < .014$) than the HAART with PI and no HAART groups. Because gender is associated with HDL values, we performed a chi-square analysis to determine if significant differences existed in the proportions of men and women

in the three HAART groups. No significant differences were found in the gender composition of the three HAART groups [χ^2 (2, N=132) = 1.43, p = .49].

Discussion

This study is the first to characterize NMR-derived lipoprotein particle profiles in a medically-underserved, largely ethnic minority sample of HIV-infected persons, a group that is characterized by health outcome disparities, including higher rates of CHD.^{7,8} NMR spectroscopy quantifies the particle number and size of lipoprotein subclasses. Small dense LDL particles have known atherogenic properties, thus higher numbers of LDL particles theoretically confer greater atherogenic risk than a comparable concentration of LDL cholesterol contained in fewer particles.^{15, 16} Numerous studies have found that LDL particle concentration is predictive of CHD risk.^{9, 11, 17-20} However, the role of LDL particles and their clinical measurement in predicting CHD risk remains unclear. Some studies have found no independent association between LDL particles and CHD risk after adjusting for HDL-C and triglycerides,¹³ while others have raised questions about whether small particles are more atherogenic than large particles. In both the Multi-Ethnic Study of Atherosclerosis (MESA) and Women's Health Study, small and large LDL particles showed similar atherogenic effects.^{9,17}

Using NMR-derived LDL particle cutoffs, 50% of our sample was considered outside the target range and at higher risk for CHD. In contrast, only 34.8% of our sample was identified as outside the target range when standard LDL cholesterol NCEP cutoffs were used. This finding suggests that LDL particle cutoffs may be more sensitive in identifying HIV-infected persons at risk for CHD than standard NCEP LDL cholesterol cutoffs. However, because the NMR cutoffs correspond to percentiles established in the MESA cohort and not have not been conclusively shown to predict coronary events, prospective studies are needed to determine their prognostic significance. Given the accelerated rate of atherosclerosis and increased incidence of acute myocardial infarction in HIV-infected compared with uninfected persons, the need for sensitive markers of CHD risk is urgent.⁵⁻⁷

It remains unclear if the reclassification of CHD risk using NMR-derived lipoprotein concentration is superior to that achieved with the standard lipid profile. In the Women's Health Study (N=27,673), NMR-derived lipoprotein values were not significantly better at predicting incident CVD events over an 11-year follow-up period than the standard lipid profile.¹⁷ Similarly, the European Prospective Investigation in Cancer and Nutrition-Norfolk study (EPIC-Norfolk) (N=25,663) found that the ability of LDL particle concentration to predict fatal and non-fatal CHD during an average six-year follow-up was abrogated after adjusting for HDL cholesterol and triglycerides.¹¹ In contrast, the Cardiovascular Health Study (N=683) found that LDL particle concentration was predictive of subsequent myocardial infarction and angina even when LDL cholesterol was included in the analyses.¹⁸ Additional studies are needed to ascertain whether lipid particle data correlate with CHD indices or are predictive of future cardiac events, and to determine whether relationships are consistent across clinical populations and different ethnic groups.

We found that participants who were not receiving HAART or were on PI-containing HAART regimens had lower HDL particle concentrations than participants on non-PI-containing HAART regimens. While PIs have been associated with atherogenic lipid profiles and increased risk for CHD,^{22,23} few studies have examined the effects of antiretroviral drug classes on NMR-derived lipoprotein profiles. One observational study found that PI-containing HAART regimens were associated with a more atherogenic NMR lipoprotein profile (higher total concentrations of VLDL particles and large LDL particles) than non-PI-containing regimens.²⁴ Another study found that PI-containing regimens were associated with higher

VLDL particle concentration and smaller HDL particles compared with non-PI-containing regimens.²⁵ Additional studies are needed to explicate the contributions, and possible synergistic interactions, of each antiretroviral drug class to changes in the NMR-derived lipid profile.

Conclusion

As HIV infection has become a chronic disease in industrialized countries, morbidity and mortality from cardiovascular disease have been increasing.^{7,26} Our findings suggest that substantial numbers of medically-underserved, asymptomatic HIV-infected minorities may be at increased risk for CHD based on NMR-derived lipoprotein values. Prospective studies are needed to determine if NMR-derived lipoprotein parameters have greater predictive utility than the standard lipid panel in this population.

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List of Abbreviations

HIV	human immunodeficiency virus
CHD	coronary heart disease
NMR	nuclear magnetic resonance
HRSA	Health Resources and Services Administration
ANOVA	analysis of variance
LDL	low density lipoprotein
HDL	high density lipoprotein
NCEP	National Cholesterol Education Program
HAART	highly active antiretroviral therapy
PI	protease inhibitor
VLDL	very low density lipoprotein

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Figure 1.
Participants With LDL Particle Concentrations > 1000 nmol/L by HAART Group

Table 1**Demographic and Clinical Characteristics of the Sample (N=132)**

Age (years)	44.64 (± 6.96)
Gender (n;%)	
-Male	85 (64.4%)
-Female	47 (35.6%)
Ethnicity (n;%)	
-African American	122 (92.4%)
-Caucasian	7 (5.3%)
-Latino/Latina	1 (0.8%)
-Native American	1 (0.8%)
-Mixed	1 (0.8%)
Current smoker	91 (68.9%)
Antiretroviral Regimen (n;%)	
-HAART With PI	68 (51.5%)
-HAART Without PI	43 (32.6%)
-No HAART	21 (15.9%)

Table 2

Effects of Gender on Standard Lipoproteins (mg/dL) and Lipoprotein Particle Concentration (nmol/L) and Size (nm) (N=132)

	Males (n=85)	Females (n=47)	<i>p</i>
Standard Lipids, mg/dL			
-Total Cholesterol	180.6 (41.8)	174 (36.1)	.360
-LDL-C	104.6 (41.2)	100.3 (30.4)	.540
-HDL-C	46.6 (17.2)	50.6 (17.5)	.209
-Triglycerides	148.1 (79.4)	115.4 (55.3)	.006*
NMR concentration, nmol/L			
-LDL	1450 (585)	1266 (471)	.05*
-HDL	30.7 (7.3)	31.1 (7.8)	.733
-VLDL	75.8 (63.7)	46.4 (48.1)	.007*
NMR size, nm			
-LDL	20.9 (1.1)	21.3 (.88)	.009*
-HDL	8.8 (.53)	9.1 (.48)	.015*
-VLDL	54.1 (10.8)	58.1 (14.3)	.077

Table 3

Percentage of Participants in LDL Lipoprotein Concentration (nmol/L) Risk Categories (N=132)

Risk Category	Number (percentage)
< 1000 (optimal)	38 (28.8)
1000-1299 (near or above optimal)	28 (21.2)
1300-1599 (borderline high)	21 (15.9)
1600-2000 (high)	23 (17.4)
> 2000 (very high)	22 (16.7)

Categories correspond to the 20th (< 1000), 50th (<1300), and 80th (<1600) percentiles for a reference population (N=6814) of participants enrolled in the Multi-Ethnic Study of Atherosclerosis.⁹

Table 4

Effects of PI-Containing and Non-PI-Containing HAART Regimens on Standard Lipoproteins (mg/dL) and Lipoprotein Particle Concentration (nmol/L) and Size (nm) (N=132)

	HAART With PI (n=68)	HAART Without PI (n=43)	No HAART (n=21)	<i>p</i>
Standard Lipids, mg/dL				
-Total Cholesterol	181.1 (37.7)	182.7 (43.5)	160.2 (39.9)	.075
-LDL-C	107.0 (37.6)	102.7 (38.5)	91.1 (35.1)	.241
-HDL-C	44.6 (17.1)	55.8 (17.1)	43.2 (13.9)	.001*
-Triglycerides	148.5 (74)	120.9 (65.8)	129.1 (81.4)	.137
NMR concentration, nmol/L				
-LDL	1453 (518)	1351 (581)	1230 (589)	.241
-HDL	29.2 (7.1)	34.4 (7.2)	30.0 (6.6)	<.001*
-VLDL	68.6 (51.9)	61.2 (70.9)	63.0 (63.3)	.805
NMR size, nm				
-LDL	20.9 (.97)	21.2 (1.1)	21.0 (1.1)	.587
-HDL	8.9 (.55)	9.1 (.45)	8.9 (.55)	.139
-VLDL	54.4 (11)	58.6 (14.5)	52.9 (10.4)	.117