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Treatment of HIV infection with a raltegravir-based regimen increases LDL levels, but improves HDL cholesterol efflux capacity

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Abstract

Background—Persons infected with HIV often have altered lipid profiles that may be affected by antiretroviral therapies (ART). Traditional lipid measurements may be insufficient to assess cardiovascular disease (CVD) risk in this population.

Methods—We report results from 39 ART-naïve participants in a substudy of A5248, a single-arm study of raltegravir, emtricitabine/tenofovir administration. Samples were collected at baseline, 12, 24 and 48 weeks after ART initiation. We performed advanced lipid phenotyping using nuclear magnetic resonance spectroscopy (Liposcience, Raleigh, NC, USA) for lipid particle size and number, and examined high-density lipoprotein (HDL) function measuring reverse cholesterol transport using J774 macrophages.

Results—We report significant increases in total cholesterol (13 mg/dl; $P<0.001$) and low-density lipoprotein (LDL; 8 mg/dl; $P=0.03$), with no change in triglycerides and without an increase in LDL particle number ($P>0.1$ all time points). HDL levels were increased over baseline levels at all time points ($P<0.003$), but reached a peak at week 12 and subsequently declined. HDL particle numbers also increased from baseline ($P<0.002$) and HDL function improved at week 48 (7% increase in efflux capacity; $P<0.001$). Oxidized LDL (oxLDL) levels decreased by week 12, but rose subsequently, and were not different from baseline at later time points.

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Disclosure statement

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Conclusions—HDL increases were associated with increases in beneficial HDL particles and HDL cholesterol efflux capacity, which may reduce future CVD events. Persistent inflammation in these HIV+ participants, may be a cause or consequence of oxLDL levels, and may contribute to declining levels of HDL over time. [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00660972) NCT00660972.

Introduction

Inflammation alters lipid concentration, composition and function (by cholesterol efflux capacity [CEC]) [1,2]. Increased markers of immune activation and inflammation are hallmarks of HIV infection, and are predictors of morbidity in this population [3]. The drivers of inflammation in HIV disease have been incompletely explored [3], but may include pro-inflammatory lipids [4,5]. Infection with HIV and subsequent treatment with combination antiretroviral therapy (ART) can be associated with increases in serum triglycerides and low-density lipoprotein (LDL) [6]. We have reported that plasma levels of oxidized LDL (oxLDL) are higher in HIV-infected persons compared to levels in uninfected donors [5], and that levels of oxLDL, but not LDL, are related to markers of monocyte activation that predict mortality [7] and cardiovascular disease (CVD) progression [8,9] in HIV+ persons. We have also found that ART-treated HIV+ individuals with benign lipid profiles based on standard measurements, had pro-atherogenic profiles when assessed by nuclear magnetic resonance (NMR) spectroscopy [10], suggesting that traditional analyses of cholesterol concentration may be insufficient to assess CVD risk.

Studies aimed at the longitudinal characterization of lipid phenotypes and inflammatory markers in persons initiating ART are of interest [11,12]; we obtained plasma samples from participants in the AIDS Clinical Trials Group (ACTG) study A5248(13-15), to characterize longitudinally lipid particle size, number, and function after initiation of a raltegravir (RAL)-based regimen. We hypothesized that RAL-based ART would improve lipid function (CEC) and lipid profiles (particle number, size and density) while reducing viral replication.

Methods

Study design

This is an exploratory substudy of A5248 [13,14] where ART-naïve subjects ($n=39$) with plasma HIV RNA (VL) 10–300,000 copies/ml were treated for 48 weeks with RAL (400 mg twice daily) and emtricitabine/tenofovir disoproxil fumarate (FTC/TDF; 200 mg/300 mg once daily). This study was approved by institutional review boards at all participating sites, CONSORT information has been provided [13,14], and is registered with [Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00660972) (NCT00660972).

Sample collection

Blood samples were collected in EDTA-containing tubes at baseline/entry and weeks 12, 24 and 48 following ART initiation, were centrifuged, and plasma was frozen at -80°C until thawed and analysed in batch. Samples from an age and gender-matched HIV-uninfected group were collected retrospectively from healthy volunteers at the National Heart, Lung, and Blood Institute (NHLBI; $n=13$). Sample size calculations for all our analyses were based on the percent change/difference in CEC, our primary outcome. We hypothesized a 10%

improvement in CEC over 48 weeks of follow-up and calculated the sample size based upon the within-subject change. Our sample size of 39 patients had more than 95% power to detect this 10% change with significance for both one-tailed and two-tailed analyses. Furthermore, hypothesizing a difference of 20% between patient and control population, our sample size of 13 controls provided us approximately 85% power to detect this difference with significance.

Plasma assays

We performed advanced lipid phenotyping using NMR spectroscopy (Liposcience, Raleigh, NC, USA) for lipid concentration, lipid particle size and number, and examined HDL function by measuring reverse cholesterol transport ('cholesterol efflux') using a J774 macrophage system (NHLBI) [15]. Levels of oxLDL were measured by ELISA (Mercodia, Uppsala, Sweden).

Statistical methods

We compared plasma lipid concentrations, NMR indices, and cholesterol efflux at baseline, 12, 24 and 48 weeks in HIV+ donors and in HIV- donors ($n=13$) to provide context for our findings. Normality was assessed by skewness and kurtosis. Pairwise comparisons between changes in a given variable between visits were performed using paired t-tests or Wilcoxon signed-rank tests based on the normality of distribution. Changes in variables over time among the visits were assessed using ANOVA for parametric variables and by Kruskal-Wallis test for non-parametric data. Differences in variables between the HIV-infected and HIV-uninfected groups were assessed by either a Student's t-test or Mann-Whitney U test depending on normality of the variable, for continuous variable and by χ^2 test for categorical variables. P -values <0.05 were determined to be significant. All statistical analyses were performed using R version 3.0.3 (3 June 2014) and STATA 12 (StataCorp LP, College Station, TX, USA).

Results

A total of 39 HIV+ and 13 HIV- donors were included in the analyses; demographic information on participants is included in Table 1. Advanced lipid phenotyping by NMR revealed that baseline levels of several lipid concentrations and particle numbers were significantly different in HIV+ donors compared with HIV- uninfected donors (Table 2). Initiation of ART resulted in significant increases in total cholesterol (TC; +13.7 mg/dl from 126.1 mg/dl to 139.8 mg/dl; $P<0.05$, week 24) and LDL (+11.6 mg/dl; from 78.5 mg/dl to 90.1 mg/dl; $P<0.05$, week 48), but no significant change in triglycerides at any time point. Though this treatment-associated LDL increase was not associated with a significant increase in total LDL particle number ($P>0.1$ for all time points), the large sized LDL particle numbers were increased at 12 weeks (from 181 to 262.2; $P<0.01$) and at week 48 approached levels in HIV-uninfected donors (270.7 versus 358.8, $P=0.1$). Plasma levels of oxLDL decreased significantly at week 12 (42.48 to 39.38; $P<0.02$), subsequently increased, and were not different from baseline at week 24 or 48 ($P>0.5$ or 0.9 ; Figure 1A). Baseline levels of oxLDL were directly related to LDL concentration ($r=0.48$, $P=0.005$) and particle

number ($r=0.33$, $P=0.001$), small LDL particle number ($r=0.44$, $P=0.012$) and VLLDL particle number ($r=0.54$, $P=0.001$). These relationships persisted at week 48.

HDL levels increased following ART initiation (+7.2 mg/dl, week 12, $P<0.001$; +6.2 mg/dl, week 24, $P<0.01$ and +6.3 mg/dl, week 48, $P<0.01$) and was accompanied by an increase in both total and beneficial HDL particle number (Table 2). This improvement in HDL particle composition corresponded with a significant rise in CEC (+0.08 units, at 48 weeks; $P<0.001$, Figure 1B) and at 48 weeks, we report a non-significant difference between HIV+ and HIV– donors (0.85 versus 0.91, $P=0.2$). Among baseline values, CEC was related to HDL cholesterol ($r=0.49$, $P=0.004$) large HDL particles ($r=0.6$, $P=2.4e^{-004}$) and HDL size ($r=-0.355$, $P=0.018$). The relationships between CEC and HDL cholesterol ($r=0.49$, $P=0.023$) and large HDL particles ($r=0.6$, $P=0.022$) were maintained at week 48. While HDL levels and cholesterol efflux values improved with ART, HDL concentration, and both total and large HDL particle numbers were significantly lower at all time points in HIV+ participants compared to levels in HIV– donors (Table 2). Somewhat surprisingly, based on previous work [12] we did not see a relationship between CEC and viraemia at baseline ($r=-0.3$, $P=0.09$) or over time.

Discussion

We have reported that traditional lipid measurements are insufficient to assess CVD risk in ART-treated HIV+ persons [10] and demonstrate here that RAL plus TDF/FTC favourably changed lipid composition and function, a finding that would not be evident measuring traditional lipid concentrations alone. We also report persistent increases in TC and LDL over 48 weeks of RAL-based ART, without a significant increase in total LDL particle number, suggesting that the LDL concentration increase was not associated with an atherogenic/small LDL particle number increase. Further, LDL levels were significantly lower in HIV+ donors at baseline compared to levels in the HIV– donors, and the LDL increases may represent improvement in overall metabolism [16]. Levels of oxLDL transiently decreased at 12 weeks, then rose. oxLDL levels are related to sCD14 levels [5] and decreases in oxLDL are related to improvement in carotid intima media thickness [17] and coronary plaque volume [18]. Thus, increasing LDL levels in a setting of chronic immune activation may result in increased LDL modification, perpetuating inflammation and enhancing CVD risk.

We also report that HDL particle number and HDL-function increased following ART initiation, however, HDL concentration and both total and large HDL particle numbers remained significantly lower in HIV– infected donors compared with HIV-uninfected donors. If the impact of cholesterol efflux improvement demonstrated here is comparable to what was reported in the Dallas Heart Study [19], this would translate into an approximately 10% decrease in future CVD events (A Rohatgi, personal communication, [University of Texas Southwestern, Dallas, TX, USA](#)). Furthermore, because NMR-derived characteristics of lipoproteins relate to cholesterol efflux [15], NMR spectroscopic measurement of lipid composition may more fully capture the lipid profile in ART-treated HIV-infection than do traditional lipid panels. As several studies, including at least one clinical end point trial (REPRIEVE: NCT02344290), are now targeting lipid perturbations and inflammation in

ART-treated HIV-infected persons, a finer characterization of lipid composition may be of value.

This study was limited by small size, and due to a lack of other potentially relevant information, including lifestyle data (smoking and dietary intake, for example), we cannot be certain the HIV+ and HIV– donors are perfectly matched. This work does provide systematic characterization of lipid particle number, size and CEC longitudinally in HIV-infection following initiation of RAL-based ART, extending previous findings [11,12] that explored the role of ART initiation on these indices.

In the parent trial, viral replication was suppressed rapidly [13], and immunological indices improved, but remained higher than levels in HIV-uninfected donors [14,20]. Several contributors may be involved in the modulation and modification of lipids in ART-treated HIV infection, including ART [6], persistent viral replication products (for example, Nef) [12], alterations in lipid metabolism [16], and chronic inflammation and immune activation [2]. Further investigation into these complex relationships, and their modulation, is warranted.

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NTF and MPP performed experiments. DX and AAJ performed statistical analyses. All authors contributed to experimental design, data analysis and writing of the manuscript.

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Figure 1. Antiretroviral therapy results in changes in lipoprotein composition and function

Plasma samples were collected at entry and weeks 12, 24 and 48, following antiretroviral therapy initiation. Advanced lipid phenotyping was performed using NMR spectroscopy for lipid concentration, lipid particle size and number. Levels of oxidized LDL (oxLDL) were measured by ELISA. **(A)** We measured changes in total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and oxLDL from baseline to week 48. **(B)** We also measured concurrent changes in levels of high-density lipoprotein (HDL) cholesterol, HDL particle number (HDL-p) and cholesterol efflux capacity (CEC) [15]. Vertical bars represent standard error of the mean (SEM).

Table 1

Characteristics of the donor groups

Characteristics	HIV-infected donors (<i>n</i> =39, baseline)	HIV-uninfected donors (<i>n</i> =13)
Median age, <u>years</u> (range)	44 (23–58)	46 (23–58)
Males, <u><i>n</i> (%)</u>	35 (90)	10 (77)
Race		
White, <u><i>n</i> (%)</u>	18 (46)	10 (77)
African-Americans, <u><i>n</i> (%)</u>	11 (28)	2 (15)
Hispanic (regardless of race), <u><i>n</i> (%)</u>	7 (18)	
Multiple, <u><i>n</i> (%)</u>	2 (5)	1 (8)
Clinical values		
Median plasma HIV RNA, copies/ml (range)	37,490 (6,644–619,795)	
Median CD4 ⁺ T-cell count, cells/μl (range)	259 (150–599)	

Table 2

Lipid characteristics of the study groups

Lipid characteristics	Patients (n=39)					Controls (n=13)
	Baseline	12 weeks	24 weeks	48 weeks		
Cholesterol efflux capacity	0.77 ±0.1 ^a	0.82 ±0.11 ^b	0.81 ±0.13 ^c	0.85 ±0.17 ^d		0.91 ±0.14
Lipid panel						
Total cholesterol	126.1 ±28 ^e	131.2 ±20.8 ^a	139.9 ±31.4 ^b	143.5 ±31.7 ^d		163.4 ±31.1
HDL cholesterol	34.5 ±12 ^e	41.7 ±11.7 ^a	40.7 ±9.3 ^e	40.8 ±11.3 ^a		56.7 ±14.9
LDL cholesterol	78.5 ±23.9 ^b	78.4 ±20.3 ^b	87 ±28.1 ^d	90.1 ±29.1 ^d		95.7 ±34.9
Triglycerides	83.7 ±34.9 ^d	82.8 ±44.5 ^d	95.2 ±46.2 ^d	93.8 ±43.9 ^d		93.3 ±40.3
HDL particle size	9.09 ±0.54 ^d	9.10 ±0.46 ^d	8.98 ±0.51 ^d	9.03 ±0.52 ^d		9.27 ±0.64
Total HDL particle number	23.23 ±5.79 ^e	26.83 ±4.37 ^e	27.35 ±4.97 ^e	27.73 ±5.71 ^a		35.72±3.99
Small HDL particle number	12.88 ±5.87 ^b	14.5 ±4.43 ^d	15.18 ±6.31 ^d	15.71 ±6.91 ^d		16.98 ±9.88
Medium HDL particle number	4.92 ±3.66 ^c	6.56 ±5.15 ^b	7.08 ±5.93 ^d	6.93 ±5.86 ^d		11.77 ±8.06
Large HDL particle number	3.76 ±2.32 ^c	4.30 ±2.66 ^d	3.72 ±2.1 ^b	3.79 ±2.46 ^b		6.22 ±3.85
LDL particle size	20.44 ±0.67 ^b	20.73 ±0.54 ^d	20.75 ±0.64 ^d	20.63 ±0.64 ^d		20.94 ±0.68
Total LDL particle number	926.7 ±345.6 ^d	871.3 ±295.9 ^d	952.8 ±341.4 ^d	1,037.2 ±392.3 ^d		1,034.9 ±412.3
Very large LDL particle number	1,013.9 ±324.8 ^d	963.2 ±280.3 ^d	1,037.6 ±323.6 ^d	1,115.3 ±366.8 ^d		1,111.7 ±366.7
Large LDL particle number	181 ±135.9 ^a	262.2 ±121.1 ^b	262 ±137.1 ^b	270.7 ±177.1 ^d		358.8 ±204.7
Small LDL particle number	500.8 ±306.5 ^d	401 ±240.4 ^d	412.5 ±254.4 ^d	505.4 ±332.7 ^d		416.9 ±281.8

^a $P<0.0001$;^b $P<0.05$;^c $P<0.01$;^d P non-significant;^e $P<0.0001$. All P -values obtained by either a Student's t -test or Mann-Whitney U test depending on the normality of the variable. P -values represent a comparison between all the parameter values at different visits for values from HIV+ versus HIV- donors. HDL, high-density lipoprotein, LDL, low-density lipoprotein.